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Research Article

Inhibitory Activity of Red and Yellow Araçá Genotypes Towards Carbohydrate-Hydrolyzing Enzymes: Putative Role of Ellagitannins

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Abstract: *Psidium cattleianum* Sabine (araçá) is a species native to Southeast Brazil that grows under abiotic stress conditions conferring high content of bioactive compounds to its fruits. The presence of these compounds is thought to be responsible for the many health-promoting effects including antioxidant, anti-inflammatory, anti-aging and antidiabetic activities. In this study, we evaluated the inhibitory potential of 10 (red and yellow) araçá genotypes towards carbohydrate-hydrolyzing enzymes (CHEs) using cell-free (*α*-glucosidase, *α*-amylase) and cell-based assays (sucrase). Araçá extracts displayed stronger inhibition towards *α*-glucosidase than *α*-amylase, and only 3 inhibited sucrase activity. The high variability towards the *in vitro* inhibitory CHEs activity was reflected in the total phenolics content with values ranging between 38.9 and 117 mg/100 g. Of the thirty compounds identified by High-Performance Liquid Chromatography-Diode Array Detection-Electrospray Ionization-Tandem Mass Spectrometry (HPLC-DAD-ESI-MS/MS), including caffeic acids (9), organic acids (3) ellagitannins (15) and flavonoids (3), ellagitannins were the most abundant class. Statistical analysis showed ellagitannins were the main discriminators to the CHEs inhibitory activity. In summary, by expanding the panel of red and yellow araçá varieties studied, our results show that not all araçá genotypes inhibit CHE as only YA-23, RA-29, and RA-87 inhibited all 3 CHE which were related to the presence of ellagitannins. Information on the araçá genotypes with greater CHE inhibitory activity allied with the health-promoting effects of ellagitannin-rich foods, can be used to scale-up commercially exploitable genotypes with the aim to develop araçá-containing food formulations targeted to the pre-diabetic population.

***Keywords*:** native fruits, diabetes, phytochemicals, *α*-amylase, *α*-glucosidase, sucrase

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Abbreviations

ASCA	Analysis of Variance-Simultaneous Component Analysis
CHE	carbohydrate-hydrolysing enzymes
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer solution
HHDP	hexahydroxydiphenic acid
IC_{50}	inhibitory concentration at 50%
LC-DAD	liquid chromatography coupled with diode-array detection
LC-MS	liquid chromatography coupled with mass spectrometry detection
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide salt
PBS	phosphate buffer solution
PCA	principal component analysis
SEM	standard error mean
T2DM	type 2 diabetes mellitus
Vit C	vitamin C
Vit E	vitamin E

1. Introduction

Climate changes and the increase of atmospheric carbon dioxide are changing temperature and rainfall patterns worldwide leading to an increase in the intensity, duration and frequency of sunshine and drought periods. In adaptation to these changes, plants adjust their development, including yield and quality of its fruits [1].

Araçá (*Psidium cattleianum* Sabine) from the Myrtaceae family is a species native to Brazil that grows under temperature and water stress conditions in a delimited area in the Southeast of the country. Due to the abiotic conditions in which araçá plants grow, their fruits have high content of bioactive compounds (e.g. carotenoids, anthocyanins and phenolic compounds) thought to be responsible for the many health-promoting effects including antioxidant, anti-aging, anti-inflammatory and anti-diabetic properties [2]. Due to its lower content of carbohydrates, when compared to widely consumed apples [2], araçá fruits are less caloric making it highly appealing to be included in the eating plans of diabetic patients. This is particularly relevant, considering the increasing prevalence of type-2 diabetes (T2DM) worldwide, but even more concerning the rising incidence of diabetes among children and young adults [3] likely to increase the economic burden of T2DM in the near future.

In addition to its lower carbohydrate content, studies have shown that the intake of *Psidium guineensis* Sw clarified juice prepared from commercial frozen pulp, had a positive effect on postprandial glycaemia in healthy individuals after the consumption of carbohydrates [4] thus supporting the *in vivo* anti-diabetic properties of araçá fruits. Other *in vitro* studies conducted on a small panel of red and yellow araçá extracts revealed that compounds present in extracts were able to inhibit carbohydrate hydrolyzing enzymes (CHE) such as α-glucosidase [5-6] and α-amylase [6]. The results observed were partly attributed to the presence of anthocyanins and other non-flavonoid compounds [5-6] that prevented the degradation of starch by salivary and pancreatic α-amylase during the digestive process and subsequent liberation of glucose units by intestinal CHE and absorption by the organism [7]. In spite of the strong association between the presence of bioactive phenolics and flavonoids and the inhibition of CHE observed in single red and yellow araçá fruits [5-6], work on a wider panel of 6 (red and yellow) araçá fruit genotypes over consecutive harvest seasons have shown that the phytochemical composition (e.g. ascorbic acid content, total anthocyanins, phenolics, tannins and total carotenoids) contributing to the antioxidant capacity was mostly influenced by the (red and yellow) genotypes, and less by harvest seasons [8]. The variability in the phytochemical composition reported for the wider panel [8] may explain the subtle differences in total antioxidant activity and the cell-free α-glucosidase and α-amylase inhibitory activities (IC_{50}) observed when single red and yellow accessions were studied [6]. Following the work by Vinholes and colleagues (2018), the authors found that simulated digested extracts of red araçá still retained antioxidant capacity and α-glucosidase inhibitory activity [9]. Considering that during metabolization phenolic compounds lose the molecular traits responsible for their antioxidant capacity [10-11], the α-glucosidase inhibitory activity reported in red araçá digested extracts, and not found in digested extracts of other tropical butiá and pitanga (purple, red and orange) native fruits [9], was attributed

to the release of ellagitannin compounds entangled in the carbohydrate matrix surviving the digestive process [9]. While the link between araçá phenolic composition and the inhibition of CHEs was evidenced in studies using single red and yellow araçá fruits [6] comparative studies on a wider panel of araçá genotypes are still missing. This is relevant as targeting CHE by dietary inhibitors may be an effective strategy to delay the intestinal absorption of glucose, reduce the postprandial blood glucose levels and help manage the postprandial hyperglycemia whilst reducing the side effects caused by treatment with drugs such as acarbose.

In view of this, the main goal herein is to study the *in vitro* inhibition towards hydrolytic enzymes involved in sugar metabolism in an expanded panel of red (6) and yellow (4) araçá genotypes using cell-free and cell-based assays grown in the same geographical region thus minimizing the contribution of environmental conditions (soil, humidity, sun) on the araçá's phytochemical composition. Characterization of the phytochemical profile by liquid chromatography coupled to mass spectrometry detection (LC-MS) and liquid chromatography coupled to diode array detection (LC-DAD) was used to evaluate the impact of phenolic panel and its content on the inhibition of CHE, and statistical analysis was performed to identify the main discriminators on the inhibitory activity.

2. Materials and methods

2.1 *Standards and reagents*

Reagents including phosphate buffer saline solution (PBS, pH 7.0), *α*-amylase (EC number: 3.2.1.1), *α*-glucosidase (EC number: 3.2.1.20), 4-nitrophenyl *α*-D-glucopyranoside (PNP-G), sodium carbonate, caffeic acid, citric acid, ellagic acid and quercetin-3-*O*-glucoside were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was purchased from VETEC (Duque de Caxias, RJ, Brazil), starch, potassium iodide, iodine from Synth (Diadema, SP, Brazil), acarbose (Glucobay®) from Bayer Pharma AG (Leverkusen, Germany) and acetonitrile and formic acid from Chem-Lab NV (Zedelgem, Belgium). Glucose oxidase assay kit was purchased from Megazyme (Bray, Wicklow, Ireland).

2.2 *Araçá fruit samples and preparation of extracts*

Araçá fruits of yellow araçá accessions (YA-102, YA-86 and YA-23), one yellow cultivar (BRS Yacy) and red araçá accessions (RA-29, RA-9, RA-44, RA-93, RA-19 and RA-87) were provided by the Active Germplasm Bank of native fruits of southern Brazil at Embrapa Clima Temperado (Pelotas, 31°40′47″ S, 52°26′24″ W). For each accession or cultivar, completely ripe fruits (approx. 50) were harvested from at least three trees. Fruits (700-800 g) selected based on the uniformity of color, size, and the absence of visible injury, were frozen (-20 °C) within 30 min.

Extracts were prepared using the whole fruit. At least ten fruits from each sample were thawed at room temperature, sliced and homogenized (5 g) with 20 mL of ethanol (95%) for 5 min using an Ultra-Turrax homogenizer. The homogenates were filtered (Whatman no. 4, Darmstadt, Germany), evaporated under reduced pressure, re-dissolved in water (20 mL), freeze-dried (L101, Liobrás, São Carlos, SP, Brazil) and stored in a desiccator, protected from sunlight for a maximum of 4 weeks [9].

2.3 *Inhibition of carbohydrate-hydrolyzing enzymes*

The inhibitory potential of extracts for *α*-glucosidase activity was done as described earlier [9]. An aliquot of 20 µL of fruit extract or 20 µL of water (control) was added to a vial containing 100 µL of PNP-G (3.25 mM) in PBS (pH 7.0). The reaction was initiated by the addition of 100 µL of enzyme (72 mU/mL in PBS, pH 7.0) and vials were incubated at 37 °C for 10 min. The reaction was stopped by adding 600 µL Na_2CO_3 (1 M) and the absorbance at λ = 405 nm was measured by spectrophotometry (Genesys, Thermo, Brooklyn, NY, USA). IC_{50} values were calculated using at least five concentrations (serial dilution) for each extract. The inhibition percentage (I%) for *α*-glucosidase assay was calculated using equation 1:

$$I\% = (A_{control} - A_{sample})/A_{control} \times 100 \quad (1)$$

Where $A_{control}$ is the absorbance of the control (water) reaction, and A_{sample} is the absorbance of the extract tested in

the reaction mixture.

α-Amylase inhibition was determined using the method described elsewhere [12-13]. Briefly, 60 µL of araçá extract or acarbose was pre-incubated for 5 min at 37 °C with 50 µL of *α*-amylase (6 U/mL) dissolved in PBS, pH 7.0 and 200 µL of the same buffer. Then, 250 µL starch solution (4 mg/mL in PBS, pH 7.0) was added as the substrate and the mixture incubated for 15 min at 37 °C. The reaction was stopped by adding 50 µL HCl (1 M). Following this, 100 µL of a mixed solution of I_2 and KI (both at 0.005 M) was added to the reaction mixture, and absorbance was measured at 690 nm by spectrophotometry (Genesys, Thermo, Brooklyn, NY, USA). IC_{50} values were calculated using at least five concentrations (serial dilution) for each extract. The inhibition percentage (I%) for the *α*-amylase assay was calculated using equation 2:

$$I\% = (A_{control} - A_{test1}) - (A_{sample} - A_{test2})/(A_{control} - A_{test1}) \times 100 \quad (2)$$

Where $A_{control}$ is the absorbance of 100% of enzyme activity (+ enzyme – inhibitor); A_{sample} is the sample (+ enzyme + inhibitor); A_{test1} is the absorbance of starch due to reducing sugar (– enzyme – inhibitor), and A_{test2} is absorbance of the inhibitor and the starch (– enzyme + inhibitor). Acarbose was used as positive control in both assays.

The sucrase (*α*-glucosidase) inhibitory activity was determined by cell-based assay using Caco-2 cell intestinal model, which expresses sucrase and isomaltase [14]. The method is based on the addition of sucrose to the apical side, which in contact with sucrase is hydrolyzed producing free glucose. The released glucose in the apical and basal sides of Caco-2 cells is used to calculate the efficiency of inhibition of carbohydrate digestion compared to controls (no added extract).

Caco-2 cell culture. Caco-2 cell line was provided by the Rio de Janeiro Cell Bank (Banco de Células do Rio de Janeiro-BCRJ) and were used between passage 40-46. Cells were routinely maintained in a humidified atmosphere of 5% CO_2 plus 95% air and grown in 20% RPMI medium containing 5.55 mM glucose supplemented with 20% fetal bovine serum (FBS, Gibco), 25 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid), 0.1% fungizone and 100 U/L penicillin/streptomycin. Culture medium was changed every 2-3 days and was split every 7 days. For sub-culturing, the cells were removed mechanically and sub-cultured in plastic culture dishes (21 cm^2; Ø 60 mm; Corning Costar, NY, USA) and were used between passage numbers 40 and 46.

Inhibition of sucrase. Cells were seeded (5×10^4 cells/cm^2), grown and differentiated in 6-well plates (Corning® Transwell® polyester membranes, pore size 0.4 µm, Ø:24 mm). After cells reached 100% confluency (5 days) cells were grown for 14-20 days more. The culture medium was removed, and apical and basal chambers were washed 3 times with PBS (2 mL). As araçá extracts of YA-23, RA-29 and RA-87 displayed no cytotoxicity (MTT assay) up to 24 h (Appendix Figure S1a-d) the medium in the apical chamber was replaced with a mixture of 200 µL of extracts of YA-23; RA-29; or RA-87 at 50, 100 and 150 µg/mL, as well as 28 mM sucrose solution in PBS (800 µL) as a substrate for sucrase inhibitory activity assay. Acarbose (50 and 150 µg/mL) was used as a positive control. In the basal chamber, 1 mL of PBS was added instead of the culture medium. The assay plate was incubated at 37 °C in 5% CO_2 atmosphere for 2 h. After incubation, the solution of the apical chamber was collected and dipped in boiling water (100 °C) for 1 min (enzyme inactivation). The liberated extracellular glucose concentration in the cell free solution was measured by the glucose oxidase method (test kit Megazime®) using a SpectraMax Plus plate-reader (Molecular Devices Corp, San Jose, CA, USA).

2.4 *Chemical characterization of extracts by liquid-chromatography platforms*

Freeze-dried extracts were dissolved in water/formic acid (99.5:0.5, v/v) to a final concentration of 20 mg/mL and filtered (0.45 µm) prior to injection. Identification (LC-ESI-MS/MS) and quantification (LC-DAD) of compounds was done as reported elsewhere [9]. Data was expressed as mg/100 g fresh weight. Quantification was achieved based on calibration curves obtained for caffeic acid (2.00-60.00 µg/mL, $r^2 = 0.9981$, limit of detection (LOD) 0.20 µg/mL and limit of quantitation (LOQ) 0.71 µg/mL), citric acid (50-1,500 µg/mL, $r^2 = 0.9897$, LOD = 0.42 µg/mL and LOQ = 1.41 µg/mL), ellagic acid (5-160 µg/mL, $r^2 = 0.9981$, LOD = 0.99 µg/mL and LOQ = 3.32 µg/mL) and quercetin-3-*O*-glucoside (1.5-48 µg/mL, $r^2 = 0.9985$, LOD = 0.36 µg/mL and LOQ = 1.21 µg/mL). The limits of detection (LOD) and quantification (LOQ) were determined according to standardized methodology [15] using equations 3 and 4:

$$LOD = 3.3 \times \sigma/b \quad (3)$$

$$LOQ = 10 \times \sigma/b \quad (4)$$

2.5 *Statistical analysis*

All experiments were done in triplicate (n = 3) and results were expressed as mean ± standard error of the mean (SEM). Dataset of 30 phenolic compounds estimated in 10 araçá samples was processed using Principal Component Analysis (PCA). Prior to analysis, variables were normalized to the total area for each sample. All calculations were made using Matlab R2023a (The MathWorks, Inc., USA). Statistical significance of the differences between two groups of araçá accessions was evaluated using Analysis of Variance-Simultaneous Component Analysis (ASCA) [16]. The significance of the difference between araçá fruits was evaluated using a permutation test with 1,000 permutation. Percentage of the variance explained by the model was used as a quality-of-fit criterion [17]. ASCA and permutation tests were implemented in MATLAB R2023a using the algorithms described earlier [16]. Calibration model for prediction of the α-glucosidase inhibition using phenolic compounds concentrations was calculated using Partial Least Square regression (PLS) and validated using leave-one-out validation. Prior to calculation, decimal logarithm of the concentration estimates was calculated and dataset standardized. Ranking of the variables that contributed most to the model was done using Variable Importance in Prediction (VIP) [18].

3. Results and discussion

3.1 *α-Glucosidase and α-amylase inhibitory activity*

The potential of red and yellow araçá fruit extracts for CHE inhibition was assessed using cell-free α-glucosidase and α-amylase assays. Figure 1 shows the dose-dependent inhibitory response of α-glucosidase (Figure 1a and b) and α-amylase enzymes (Figure 1c and d) to araçá extracts. The IC_{50} values for α-glucosidase and α-amylase enzymes, calculated from curves in Figure 1, are shown in Table 1, with all samples showing IC_{50} values lower than the positive control acarbose. Among all araçá genotypes, YA-23 outperformed the other yellow and all red araçá extracts showing 80% inhibition at 45 µg/mL (Figure 1a). The IC_{50} values of α-glucosidase varied from 60.9 ± 3.20 to 153 ± 13.0 µg/mL. These values were in general three to eight-fold higher than values previously reported [5-6]. Studying ethanolic extracts, Vinholes and colleagues previously reported lower IC_{50} values for red and yellow araçá fruits, with the yellow genotype displaying lower IC_{50} values than the red genotype [5]. In another study, methanolic extracts of red araçá fruits exhibited lower IC_{50} values for the α-glucosidase than the yellow Bicudo accession [6]. The IC_{50} values estimated in this study for the edible fraction of red accessions RA-44 and RA-87 (Table 1) were higher than those reported earlier for red accessions AC-44 and AC-87 fruit parts [6]. The difference between IC_{50} values observed in this study and others previously reported may be multifold, namely to different agricultural araçá fruit harvests [5], different fruit harvesting/collection strategies [5], different fruit sampling approaches as seeds were separated from pulp/peel [6] and different sample extraction protocols [5-6].

Regarding the α-amylase inhibition assays, the IC_{50} values obtained for YA-23, RA-29, RA-9 and RA-87 extracts varied from 111 ± 10 to 149 ± 7.0 µg/mL (Table 1). These values were all higher than that of the positive control (acarbose) and in general 12 to 23-fold lower than that found for crude extracts of araçá pulp-peel [6]. Of note, extracts of yellow YA-86, YA-102 and YACI and red fruits RA-19, RA-44 and RA-93 showed very low inhibition (Figure 1c and d) and IC_{50} values were not estimated.

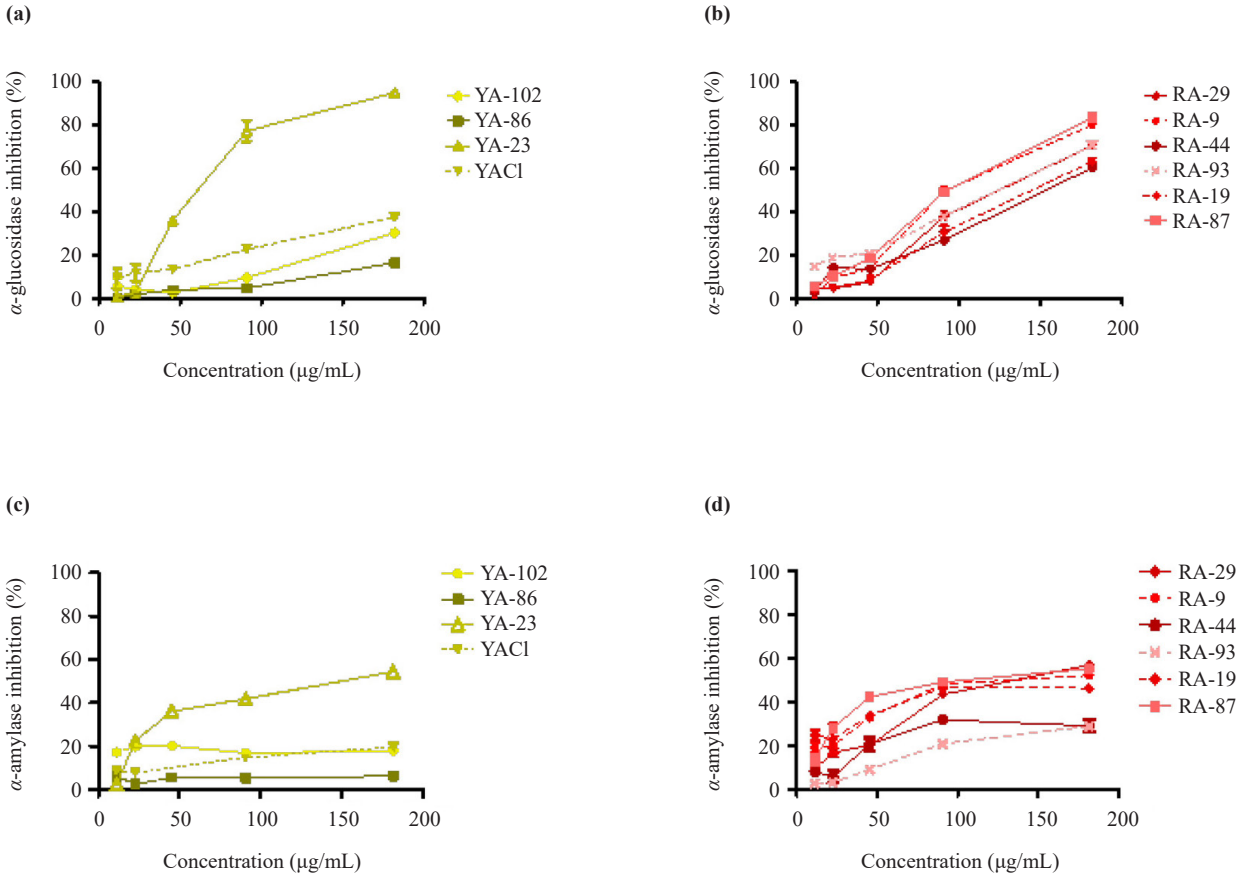

Figure 1. Araçá fruit extracts inhibition of α-glucosidase for yellow (a) and red (b) genotypes and α-amylase for yellow (c) and red (d) genotypes. YA-102: yellow araçá accession 102; YA-86: yellow araçá accession 86; YA-23: yellow araçá accession 23; YACI: cultivar BRS Yaci; RA-29: red araçá accession 29; RA-9: red araçá accession 9; RA-44: red araçá accession 44; RA-93: red araçá accession 93; RA-19: red araçá accession 19 and RA-87: red araçá accession 87. Results are expressed as mean ± standard error mean (SEM) of triplicate experiments (n = 3)

Overall, YA-23, RA-29, RA-9 and RA-87 araçá extracts showed higher inhibitory activity towards α-glucosidase than α-amylase (Table 1). This is in agreement with the work in crude pulp-peel araçá extracts [6] and in other fruits from Macaronesia region [19] suggesting that compounds in these extracts may act as CHE inhibitors suitable for diabetes management and treatment. Ideally, such compounds should possess high inhibitory activity towards α-glucosidase avoiding the release of glucose into circulation, and mild inhibitory activity towards α-amylase avoiding the accumulation of undigested starch, a source of gas in the intestine, causing gastrointestinal discomfort (diarrhea, abdominal pain and flatulence) [20]. Even though the reasons behind the differences between IC_{50} values observed in our study and others may be related to sample preparation approaches, i.e. analysis of the whole fruit or separation of seeds from pulp/peel [6], and extraction protocols [5-6], results obtained in 10 araçá genotypes highlight the high variability in the inhibition of CHE.

Table 1. IC_{50} values (µg/mL) determined for *α*-glucosidase and *α*-amylase inhibition of araçá (yellow and red genotypes) ethanolic extracts and positive controls. Results are expressed as mean ± standard error mean (SEM) of triplicate experiments (n = 3)

Araçá	*α*-glucosidase		*α*-amylase	
	IC_{50} (µg/mL)			
Yellow				
102	-	-	-	-
86	-	-	-	-
23	60.9	± 3.2	148	± 7.0
BRS Yaci	-	-	-	-
Red				
29	125	± 7.6	135	± 0.5
9	93.7	± 6.4	132	± 15.4
44	153	± 13.0	-	-
93	125	± 6.7	-	-
19	145	± 4.3	-	-
87	93.7	± 4.9	111	± 10.2
Positive control				
Acarbose	414	± 20.2	20.1	± 0.2

(-) not possible to calculate IC_{50} values

3.2 *Sucrase inhibitory activity*

In view of the strong inhibitory activity of YA-23, RA-29, RA-9 and RA-87 extracts in cell-free assays (Table 1) these were further studied with respect to sucrase inhibition by cell-based assays using Caco-2 cells. Caco-2 cells monolayer is a suitable and widely used model to investigate the absorption, transport, and metabolism of compounds due to its similarity to enterocytes as it expresses most of the morphological and functional characteristics of the small intestine such as *α*-glucosidase (sucrase and isomaltase) and lactase activities on the apical membrane [21]. However, as previous studies had already shown red and yellow araçá extracts with anti-proliferative and cytotoxic effects [22-23] preliminary viability assays were carried out in YA-23, RA-29, RA-9 and RA-87 genotypes up to 72 h (Appendix). With the exception of RA-9 that displayed cytotoxicity at low concentrations (Appendix Figure S1) YA-23, RA-29, and RA-87 were screened for their sucrase inhibitory activity.

The inhibitory effect of YA-23, RA-87 and RA-29 extracts on sucrase activity measured in apical and basal sides of Caco-2 monolayers is shown in Figure 2. As shown, YA-23, RA-29 and RA-87 araçá extracts tested under non-cytotoxic concentrations inhibited sucrase activity and were even more effective in the inhibition of sucrase than acarbose, a widely prescribed drug with hypoglycemic effect. The yellow araçá genotype (YA-23) showed the highest inhibitory activity against the enzyme in the apical side of monolayer, reaching 67.6% inhibition at the lowest concentration tested (50 µg/mL) and 76.7% at the highest concentration (Figure 2a). Curiously, this extract was more effective than the purified extract of *Eucommia ulmoides* Oliv. leaves (IC_{50} of 70 µg/mL) [24]. RA-29 displayed values higher than 50% inhibition for all tested concentrations, while RA-87 displayed 50% inhibition only at higher concentrations (100 and 150 µg/mL, Figures 2b and 2c). All araçá extracts showed higher sucrase inhibition than the isolated feruloylated arabinoxylan mono- and oligosaccharides from corn bran and wheat aleurone (IC_{50} of 1,030 and 1,280 µg/mL, respectively) [25]. The inhibition of sucrase in the apical side of Caco-2 monolayer may result in decreased glucose transport across the monolayer [14, 26] and can be responsible for reduced glucose levels in the basal side.

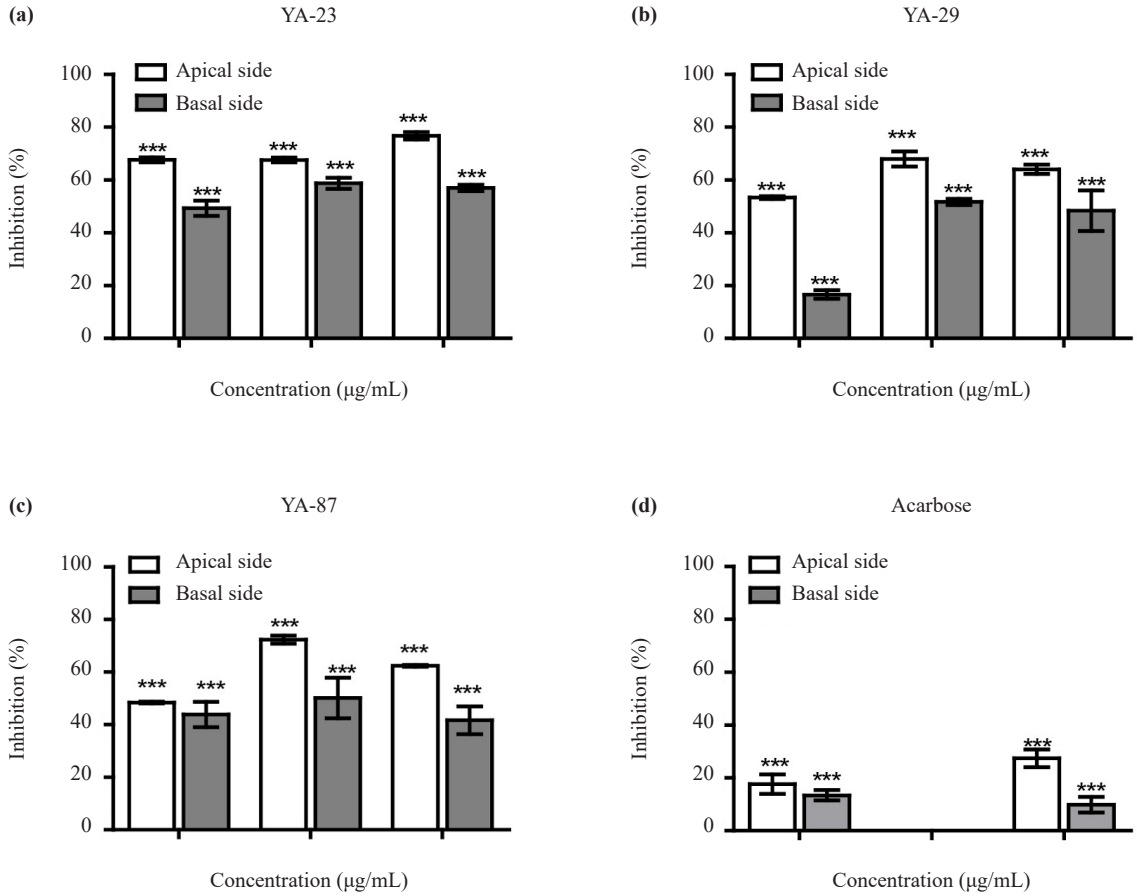

Figure 2. Sucrase inhibitory activity of different accessions of araçá fruit extracts on apical and basal sides of Caco-2 monolayer. YA-23: yellow araçá accession 23 (a); RA-29: red araçá accession 29 (b); RA-87: red araçá accession 87 (c); and acarbose (d). Results are expressed as mean ± standard error mean (SEM) of triplicate experiments (n = 3). Two-way ANOVA was used for comparison with Bonferroni post-test; *** $p < 0.001$ versus control

3.3 *Characterization of composition in araçá extracts and statistical analysis*

To further understand the distinct inhibitory activity displayed by both red and yellow araçá fruits, extracts were characterized by LC-based approaches. Identification of compounds was carried out by LC-ESI-MS based on the retention time (RT), UV absorption maxima and mass spectrometric data against authentic standards and data available in the literature for *Psidium* and other species. A representative LC-MS chromatogram of red and yellow araçá extracts is shown in Appendix Figure S2. Compounds identified **(**listed in Appendix Table S1) were numbered by their order of elution in reverse-phase column and further quantified by LC-DAD against calibration curves (as detailed in the Experimental section).

Table 2. Content of phenolic compounds (mg/100 g fresh weight) in ethanolic extracts of yellow and red araçá genotypes. Results are expressed as mean values of triplicates (n = 3). Values correspond to mean ± S.E.M.

Peak	Compounds	Concentration (mg/100 g fresh weight)									
		Yellow genotypes				Red genotypes					
		YA-102	YA-86	YA-23	BRS-Yaci	RA-29	RA-9	RA-44	RA-93	RA-19	RA-87
	Caffeic acid derivatives [1]										
1	Caffeic acid hexoside	0.012 ± 0.001	0.0110 ± 0.0001	0.0210 ± 0.0001	0.0120 ± 0.0002	0.0100 ± 0.0005	0.0100 ± 0.0001	0.0120 ± 0.0001	0.0100 ± 0.0001	-	0.0110 ± 0.0001
2	4-O-caffeoylquinic acid	-	0.010 ± 0.001	0.012 ± 0.001	-	0.011 ± 0.001	-	0.0110 ± 0.0002	0.0090 ± 0.0006	-	-
3	Caffeic acid hexoside derivative	-	-	0.021 ± 0.002	-	-	-	-	-	-	-
4	Caffeoylquinic isocitrate	-	0.009 ± 0.001	0.0190 ± 0.0004	0.0130 ± 0.0004	0.010 ± 0.001	0.008 ± 0.001	-	0.0110 ± 0.0003	-	0.011 ± 0.001
6	Caffeic acid hexoside derivative	0.0130 ± 0.0004	-	0.0210 ± 0.0004	0.0090 ± 0.0004	-	0.009 ± 0.001	-	0.0100 ± 0.0003	-	0.010 ± 0.001
8	4,5-*O*-dicaffeoylquinic acid	0.0240 ± 0.0002	0.031 ± 0.001	0.0330 ± 0.0002	0.019 ± 0.001	0.028 ± 0.002	0.0200 ± 0.0004	0.033 ± 0.001	0.0310 ± 0.0004	0.0230 ± 0.0001	0.0220 ± 0.0002
9	4,5-*O*-dicaffeoylquinic acid isomer	0.0210 ± 0.0001	0.029 ± 0.001	0.0290 ± 0.0002	0.020 ± 0.001	0.018 ± 0.001	0.0110 ± 0.0002	0.013 ± 0.001	0.0120 ± 0.0004	0.0120 ± 0.0002	0.0090 ± 0.0002
10	Caffeic acid galloyl-hexoside	0.0140 ± 0.0001	0.0120 ± 0.0001	0.023 ± 0.0001	0.0090 ± 0.0001	0.0090 ± 0.0001	0.0080 ± 0.0003	0.0090 ± 0.0001	0.0090 ± 0.0001	0.0110 ± 0.0001	0.0120 ± 0.0002
11	Caffeic acid hexoside derivative	0.0190 ± 0.0002	0.0190 ± 0.0001	0.029 ± 0.001	0.0090 ± 0.0005	0.018 ± 0.001	0.018 ± 0.001	0.019 ± 0.001	0.020 ± 0.001	0.0130 ± 0.0001	0.0230 ± 0.0005
	Sub-total	0.103 ± 0.001	0.121 ± 0.003	0.208 ± 0.002	0.091 ± 0.007	0.104 ± 0.008	0.084 ± 0.003	0.097 ± 0.006	0.112 ± 0.002	0.0590 ± 0.0004	0.098 ± 0.001
	Organic acids [2]										
5	Isocitric acid	18 ± 1	4.1 ± 0.1	15.8 ± 0.4	2.6 ± 0.2	5.8 ± 0.5	4.3 ± 0.3	10. ± 2	5.73 ± 0.03	9 ± 1	13.6 ± 0.2
7	Citric acid	6.88 ± 0.04	6.2 ± 0.7	7.4 ± 0.1	6 ± 1	7 ± 1	6.7 ± 0.4	7.6 ± 0.1	7.1 ± 0.1	3.7 ± 0.2	4.4 ± 0.1
12	Vanillic acid di-hexoside	3.0 ± 0.1	3.4 ± 0.2	3.67 ± 0.04	1.6 ± 0.1	2.8 ± 0.3	-	2.41 ± 0.04	2.2 ± 0.2	2.4 ± 0.1	3.14 ± 0.02
	Sub-total	28 ± 10	13.6 ± 0.7	26.9 ± 0.2	9.2 ± 0.3	16 ± 2	11.0 ± 0.4	20 ± 2	15.1 ± 0.2	15 ± 1	21.1 ± 0.2
	Ellagitannins [3]										
13	HHDP-Glc	1.63 ± 0.03	1.9 ± 0.2	5.6 ± 0.1	1.7 ± 0.2	5.0 ± 0.2	6.9 ± 0.6	1.5 ± 0.1	5.6 ± 0.3	1.13 ± 0.04	4.8 ± 0.3
14	Unknown compound	1.36 ± 0.02	-	3.1 ± 0.3	-	-	7.9 ± 0.5	-	3.9 ± 0.2	-	-
15	Unknown compound	-	1.4 ± 0.2	0.47 ± 0.02	0.7 ± 0.1	8.4 ± 0.1	-	1.7 ± 0.1	3.2 ± 0.1	1.8 ± 0.1	-

Peak	Compounds	Concentration (mg/100 g fresh weight)									
		Yellow genotypes				Red genotypes					
		YA-102	YA-86	YA-23	BRS-Yaci	RA-29	RA-9	RA-44	RA-93	RA-19	RA-87
16	Di-HHDP-Glc	2.1 ± 0.1	0.60 ± 0.02	3.2 ± 0.1	-	2.4 ± 0.1	2.0 ± 0.1	-	2.0 ± 0.2	1.15 ± 0.04	4.2 ± 0.1
17	Castalagin/Vescalagin isomer	2.3 ± 0.1	-	15.6 ± 0.1	5.1 ± 0.5	14.6 ± 0.1	9.7 ± 0.5	6.7 ± 0.3	10.9 ± 0.2	-	9.3 ± 0.3
18	Castalagin/Vescalagin isomer	9.3 ± 0.3	10.8 ± 0.8	11.4 ± 0.3	2.4 ± 0.1	11.2 ± 0.2	5.2 ± 0.3	3.3 ± 0.1	7.9 ± 0.2	3.4 ± 0.1	6.0 ± 0.2
19	HHDP-tri-galloyl-Glc isomer	9.8 ± 0.1	6.2 ± 0.7	4.8 ± 0.3	3.56 ± 0.02	6.3 ± 0.1	4.4 ± 0.4	3.5 ± 0.2	4.6 ± 0.1	1.6 ± 0.1	5.5 ± 0.1
20	HHDP-tri-galloyl-Glc isomer	5.0 ± 0.1	4.2 ± 0.4	5.5 ± 0.3	3.7 ± 0.3	11.8 ± 0.1	7.1 ± 0.7	9.1 ± 0.3	7.3 ± 0.2	2.7 ± 0.2	10.5 ± 0.2
21	HHDP-tri-galloyl-Glc isomer	5.6 ± 0.2	7.1 ± 0.8	7.4 ± 0.4	4.7 ± 0.4	-	6.8 ± 0.7	4.7 ± 0.6	7.2 ± 0.2	0.8 ± 0.1	3.2 ± 0.2
22	HHDP-tri-galloyl-Glc isomer	4.7 ± 0.1	3.1 ± 0.2	4.2 ± 0.1	3.2 ± 0.3	-	9.6 ± 0.8	8.4 ± 0.4	5.4 ± 0.2	1.8 ± 0.2	2.8 ± 0.1
23	Galloyl-di-HHDP-Glc isomer	7.9 ± 0.2	5.7 ± 0.4	6.5 ± 0.1	-	14.5 ± 0.1	16.5 ± 1.1	9.3 ± 0.3	12.1 ± 0.2	2.5 ± 0.1	9.2 ± 0.2
24	Galloyl-di-HHDP-Glc isomer	2.7 ± 0.2	-	1.7 ± 0.1	-	-	-	-	-	-	-
25	HHDP-Glc isomer	12.6 ± 0.2	8.0 ± 0.4	10.7 ± 0.1	8.4 ± 0.8	15.9 ± 0.3	14.6 ± 1.4	14.2 ± 0.7	12.6 ± 0.2	4.6 ± 0.1	15.9 ± 0.3
26	HHDP-tri-galloyl-Glc isomer	-	-	1.16 ± 0.01	-	-	-	-	-	-	-
27	Galloyl-di-HHDP-Glc isomer	-	5.0 ± 0.2	1.55 ± 0.01	7.1 ± 0.3	9.1 ± 0.1	6.1 ± 0.8	10.2 ± 0.7	6.54 ± 0.04	2.7 ± 0.1	8.1 ± 0.1
	Sub-total	65 ± 1	54 ± 3	83 ± 2	40 ± 4	100 ± 1	97 ± 8	73 ± 3	90 ± 2	24.1 ± 0.4	79.4 ± 0.9
	Flavonoids [4]										
28	Taxifolin hexoside	0.182 ± 0.002	0.0610 ± 0.0004	0.100 ± 0.001	0.1010 ± 0.0002	0.161 ± 0.002	0.170 ± 0.005	0.122 ± 0.006	0.141 ± 0.002	0.0430 ± 0.0002	0.0410 ± 0.0005
29	Quercetin-glucoronide	0.093 ± 0.003	0.0390 ± 0.0002	0.0300 ± 0.0002	0.0320 ± 0.0002	0.071 ± 0.001	0.072 ± 0.004	0.159 ± 0.005	0.073 ± 0.003	0.032 ± 0.004	0.0230 ± 0.0002
30	Eriodictyol hexoside	0.0820 ± 0.0005	0.0320 ± 0.0002	0.041 ± 0.001	0.061 ± 0.002	0.049 ± 0.002	0.021 ± 0.002	0.032 ± 0.002	0.032 ± 0.003	0.013 ± 0.001	0.0220 ± 0.0004
	Sub-total	0.36 ± 0.01	0.132 ± 0.001	0.171 ± 0.001	0.19 ± 0.01	0.2810 ± 0.0002	0.26 ± 0.01	0.31 ± 0.02	0.25 ± 0.01	0.088 ± 0.005	0.086 ± 0.001
	Total	93.5 ± 0.6	68 ± 4	109 ± 2	50 ± 6	117 ± 2	108 ± 8	93 ± 2	105 ± 2	39 ± 2	101 ± 1

[1]Quantified by caffeic acid; [2]Quantified by citric acid; [3]Quantified by ellagic acid; [4]Quantified by quercetin-3-*O*-glucoside

Thirty compounds belonging to four classes were identified (as shown in Table 2) including caffeic acid derivatives, organic acids, ellagitannins and flavonoids. Of these, compounds 5, 7, 8-11, 13, 18-20, 25, 28-30 were common to all araçá samples, while caffeic acid derivatives (1-4, 6, 8-11) and vanillic acid di-hexoside (12) are reported for the first time in araçá. The total content of phenolic compounds estimated in araçá fruit extracts ranged from 38.9 to 117 mg/100 g of fresh weight (Table 2). Our findings are in agreement with the results reported by Teixeira and colleagues who investigated 6 genotypes over 6 harvest seasons reported that genotype had a stronger influence than the harvest season on the variability on the phytochemical composition of araçá fruits, and hence on the antioxidant capacity [8]. Ellagitannins were the predominant class in both yellow and red araçá extracts ranging from 62% to 89% of total compounds followed by organic acids, caffeic acid derivatives and flavonoids (Table 2). Our results show that, in general, red genotypes contained higher amounts of ellagitannins than yellow genotypes, except for RA-19 that contained the lowest amount among all genotypes studied (24.1 mg/100 g). Even though the content of phenolic compounds found in this study is consistent with previous reports [27] and only slightly smaller than those reported for crude methanolic extracts of red and yellow araçá genotypes [6], expansion to a broader panel of yellow (4) and red (6) genotypes carried out for the first time showcases the variability of phenolic content that can occur among red and yellow araçá genotypes and overlooked in previous studies.

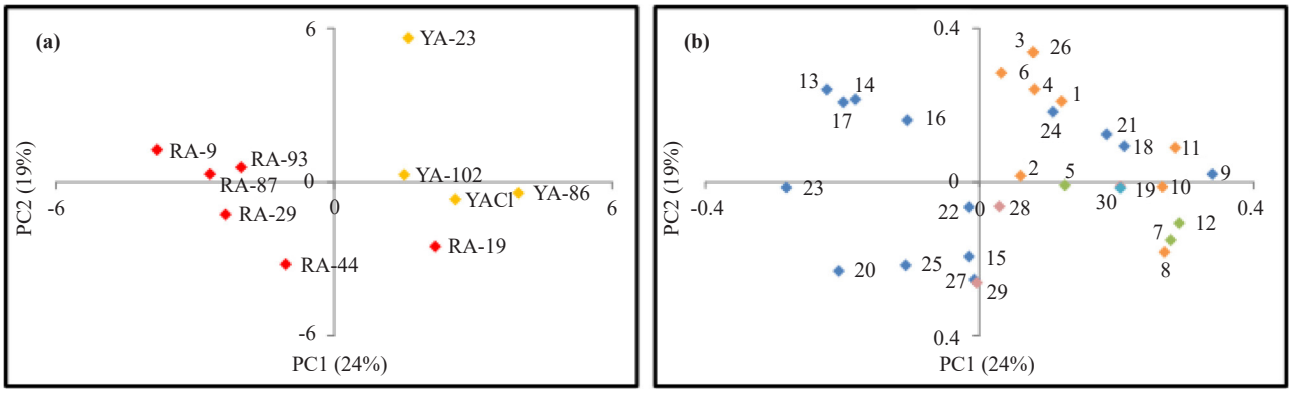

Figure 3. PCA score (a) and loading (b) plots of principal components 1 and 2 obtained using phenolic com-position data set (compounds are numbered according with Table 2) of one araçá cultivar (YACI: BRS Yaci; yellow genotype) and nine araçá accessions (YA-102, YA-86 and YA-23 yellow genotypes and RA-29, RA-9, RA-44, RA-93, RA-19 and RA-87 red genotypes). In loading plots (b) caffeic acid derivatives shown as orange diamonds, organic acids as green diamonds, ellagitannins as blue diamonds and flavonoids as magenta diamonds

Principal Component Analysis (PCA) allowed the discrimination of araçá genotypes (Figure 3) based on the phytochemical composition (Table 2). The first two principal components account for 43% of the data variance. Araçá extracts formed two separate clusters along the PC1 according to the color of the fruits except for red accession RA-19 (Figure 3a). Separation along PC1 axis of all yellow genotypes and red genotype RA-19 was attributed to the higher concentration of caffeic acid derivatives, organic acids, vanillic acid, some ellagitannins (18, 19, 21, 24, 26) and two flavonoids (28, 30) in these extracts (Figure 3b). The remaining red genotypes were distinguished by their higher concentration of ellagitannins (13-17, 20, 22, 23 and 25) (Figure 3b). Sample distribution along PC2 axis was related to the differences in composition of individual accessions and cultivar independently of fruit color. As identified by PCA two groups of araçá fruits were considered: one comprising all yellow accessions and one red accession RA-19, and the other comprising red accessions. Statistical significance of the differences between the two groups of araçá accessions was evaluated using Analysis of Variance-Simultaneous Component Analysis (ASCA). According to the permutation test, the difference between the two groups of araçá accessions was significant (p-values of 0.009). Variables that contributed most to the difference between the two groups were citric acid (7), 4,5-*O*-dicafeoylquinic acid isomer (9), caffeic acid galloyl-hexoside (10), caffeic acid hexoside derivative (11) and vanillic acid di-hexoside (12) present at higher concentration in yellow accessions and RA-19 group, and hexahydroxydiphenoyl (HHDP)-tri-galloyl-Glc isomer (20) and Galloyl-di-HHDP-Glc isomer (23) present at higher concentration in red accessions group. Further calibration

model for the prediction of *α*-glucosidase inhibition using phenolic compounds was calculated using Partial Least Square regression (Appendix Table S2). Analysis of the contribution of the phenolic compounds (variables) to the model can be estimated using Variable Importance in Prediction (VIP) scores reflecting the relative importance of the variable for predicting a response. As VIP values are scaled so that the average of their squared values is equal to 1, variables with a VIP value larger than 1 are considered important. The VIP scores revealed that compounds 19 (HHDP-tri-galloyl-Glc isomer), 30 (eriodictyol hexoside), 9 (4,5-*O*-dicafffeoylquinic acid isomer), 20 (HHDP-tri-galloyl-Glc isomer), 24 (galloyl-di-HHDP-Glc isomer), 18 (castalagin/vescalagin), 1 (caffeic acid hexoside), 6 (caffeic acid hexoside derivative) and 23 (galloyl-di-HHDP-Glc isomer) contributed the most to the inhibition of *α*-glucosidase in extracts.

Based on the results here presented, the inhibitory activity observed for YA-23, RA-29, RA-9 and RA-87 (Table 1) can be attributed to ellagitannins as these predominate in araçá extracts YA-23, RA-29, RA-9 and RA-87 (Table 2). Statistical analysis carried out on the extract's composition (Section 3.3.) identified several compounds contributing to *α*-glucosidase inhibitory activity in araçá fruits (and shown in Table S2) that include galloyl- and HHDP-containing compounds together with caffeic acid derivatives. The yellow genotype YA-23 contains high amounts of caffeic acid hexoside and Castalagin/Vescalagin, residual in the remaining yellow genotypes, which could account for the highest alpha-glucosidase inhibition among the yellow genotype (YA-23). However, the contribution of other compounds (not identified in this study) cannot be excluded nor the synergistic and additive effect of these to the overall alpha-glucosidase inhibition. In fact, previous studies conducted with *in vitro* simulated digested extracts of pomegranate, another ellagitannin-rich food [28], had already assigned ellagitannins, namely castalagin/vescalagin isomers, also present in araçá fruit genotypes (Table 2), with *α*-glucosidase inhibition properties [29]. The authors attributed the reminiscent high *α*-glucosidase inhibitory activity in *in vitro* digested extracts to the ability of ellagitannins to survive the digestive process [29]. In another study, Camu-camu (*Myrciaria dubia*) seed extract with high content of castalagin (17) and vescalagin (18) displayed strong inhibition of *α*-glucosidase and *α*-amylase (above 82%) at low concentrations (1 μg/mL) [30]. The strong inhibition of castalagin and vescalagin and other ellagitannins seems to be related to the presence of a hexahydroxydiphenoyl (HHDP) unit at the 4-*O*- and 6-*O*-glucose positions crucial for enzyme interaction and protein binding changing the enzyme conformation [29], as vescalin and castalin compounds lacking this unit in their structure were inactive towards CHE [31]. Ellagitannins enzymatic inhibition mode of action is likely related to their protein binding properties (association and precipitation) that change the enzyme conformation and reduce the catalysis velocity and the accessibility to the active site of the substrate [29]. Nevertheless, the contribution of other compounds, such as caffeic acid hexoside (1), citric acid (7), and caffeoyl quinic acids derivatives also present in araçá genotypes (Table 2), to the inhibition of CHE in araçá fruits cannot be excluded as these compounds have previously been associated with *α*-glucosidase and *α*-amylase inhibitory activity [26, 32]. Likewise, the contribution of other phenolic compounds such as caffeic acid hexoside (compound 1), identified in all genotypes (except for RA-19, Table 2) and reported as a main constituent of *Geoffroea decorticans* extract had previously shown high *α*-glucosidase inhibition [33].

For instance, caffeoyl quinic acids derivatives, including 4-*O*-caffeoylquinic and 4,5-*O*-dicaffeoylquinic acids (compounds 2 and 8, respectively) present in all genotypes (Table 2), were previously found to reduce the catalytic activity of the enzyme by establishing hydrophobic interactions thus altering its molecular conformation and, in this way, able to inhibit the *α*-glucosidase [32]. In a similar manner, citric acid (compound 7), identified in all genotypes (Table 2), was also reported with the ability to inhibit the pancreatic porcine *α*-amylase enzyme activity with an IC_{50} of 0.91 mM [26].

In this study, investigation on the inhibitory activity towards CHE on a wide panel of red and yellow araçá fruits genotypes shows that not all araçá fruits possess anti-diabetic properties. To this date, studies advocating to the potential anti-diabetic properties of araçá fruits [5-6, 11] had mainly focused on individual red and yellow genotypes preventing from obtaining a comprehensive view on the variability of araçá fruits inhibitory activity towards CHE and the real anti-diabetic properties. Our study shows that of the 6 red and 4 yellow araçá fruit genotypes screened, all red araçá extracts and one yellow exhibited strong inhibition towards *α*-glucosidase, where only 4 exhibited *α*-amylase inhibitory activity (Table 1) and just 3 (YA-23, RA-29, and RA-87) araçá genotypes exhibited inhibition towards sucrase activity (Figure 2). The high variability in the CHE inhibitory activity was reflected in the phenolic content. Chemical characterization by advanced LC-MS screening approaches showed that araçá extracts with higher inhibitory activity towards CHE were particularly rich in ellagitannins (Table 2). Statistical analysis identified galloyl and HHDP-containing compounds

together with caffeic acid derivatives as key contributors to *α*-glucosidase inhibitory activity in araçá fruits (Appendix Table S2). The yellow genotype YA-23 contains high amounts of caffeic acid hexoside and Castalagin/Vescalagin and residual in the remaining yellow genotypes, which could account for the highest alpha-glucosidase inhibition among the yellow genotypes. This is in accordance with recent reported findings conducted on purified galloyl-based polyphenols with free and unfree galloyl moieties where the authors found that both free galloyl and intramolecularly-linked galloyl (HHDP) groups contributed to *α*-glucosidase enzyme inhibition [34].

Based on the findings from this study, our results suggest that, allied with its low sugar content, the inclusion and ingestion of certain araçá fruits rich in ellagitannins in the eating habits of (pre)diabetic patients may turn out to be a valuable nutritional strategy to help manage hyperglycemia status and keeping the postprandial glucose levels low in (pre)diabetic patients. While the link between araçá phenolic composition (and antioxidant capacity) with the inhibition of CHE was evidenced in previous studies, though most of the research has been devoted to low molecular weight compounds such as Vit C, Vit E, carotenoids, phenolic acids and flavonoids. To date, very little interest has been devoted to unveil the role of high molecular weight compounds on the inhibition of CHE. However, the innovative findings described by Vinholes and colleagues were pivotal showing that after *in vitro* digestion, araçá fruits but not pitanga and butiá, still retained antioxidant capacity and the ability to inhibit alpha-glucosidase activity [9]. This was attributed to the high content of ellagitannins (identified by advanced LC-MS) which entangled in the carbohydrate matrix of araçá survived the digestive process, unlike low molecular weight flavonoids are readily metabolized with a marked decrease in their antioxidant capacity [10-11]. Upon reaching the intestine, ellagitannins containing the HHDP moiety are metabolized by gut microbiota with formation of urolithins as already shown by *ex vivo* studies on individual ellagitannins [35]. The ability of ellagitannin-rich araçá fruits to inhibit CHE as shown in this study is in agreement with others on pomegranate another ellagitannin-rich fruit [29]. Even though the nutraceutical effect of each of the ellagitannins is not yet fully understood, they occur as mixtures in fruits suggesting that ellagitannins may exert their health benefits through a combined and synergistic effect able to ameliorate metabolic disease [36-37].

The health-promoting effects of ellagitannin-rich foods such as araçá fruits goes beyond the obvious glucose lowering (anti-hyperglycemic) effect attributed to the inhibition of CHE as urolithins, the intestinal metabolites of ellagitannins, not only have high antioxidant capacity [28] and hypertensive properties [37] but have been shown to bind to estrogen receptors and thus key players in the gut-brain-endocrine interactome and valuable nutritional alternatives in most hormone/endocrine-dependent diseases (cardiovascular disorders, osteoporosis, muscle health, neurological disorders, and cancers of breast, endometrium, and prostate) [38]. In addition to this, the ingestion of ellagitannin-rich foods may be associated with a more diverse microbiota ecology and improved gut functionality as (ellagi)tannins have been found to modulate the growth of *Gram*(+) and *Gram*(-) bacteria [39-40] thus ultimately shaping the profile of microbiota-derived end products in circulation. In fact, micromolar levels of other (poly)phenol microbial metabolites have been shown to reduce the release of pro-inflammatory cytokines in endothelial cell cultures grown under normo [41] and even under hyperglycemic conditions [42].

The CHE inhibiting potential displayed by YA-23, RA-29 and RA-87 araçá genotypes, contributes to the valorization of native tropical fruits which are currently farmed locally in family orchards [43]. This allied with its resistance to diseases and pests, and its ability to adapt and grow under abiotic conditions, makes the YA-23, RA-29, and RA-87 araçá fruits highly desirable raw material in the development of functional foods [44]. Even though ellagitannins were shown to survive the digestive process, the microencapsulation of araçá pulp to maintain the fruits sensorial characteristics and to increase the bioavailability of bioactive compounds are surely worth exploring not just by Food (Bio)technology industry but also by pharmaceutical companies.

4. Conclusions

In the present study, a large panel of red and yellow araçá fruit extracts was investigated towards the inhibition of carbohydrate-hydrolyzing (CHE) enzymes. Of 10 araçá genotypes studied, YA-23, RA-9, RA-87 and RA-29 showed the lowest IC_{50} values, and only YA-23, RA-29 and RA-87 displayed strong inhibition towards sucrase activity. By expanding the panel of red and yellow araçá genotypes to include a wider revealed that not all araçá genotypes are able to inhibit CHE. Inhibition values were partially attributed to high content of ellagitannins. Based on the LC-DAD-ESI-MS/MS data, 10 compounds were described for the first time in araçá extracts with ellagitannins as the most

abundant class. Improved knowledge on which araçá genotypes display stronger inhibition of CHE and thus higher anti-hyperglycemic effect provide novel information on the potential genotypes to be commercially exploited as raw material in the development of functional foods (e.g. juices, nectars, yoghurts and ice creams) for the management of T2DM onset in pre-diabetic population. In parallel, given the great adaptability to the growing climate changes of native araçá fruit and being typically cultivated on a small-scale, valorisation of specific araçá genotypes with the best performance to the inhibition of CHE, will surely contribute to the sustainability of local and regional economy.

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Conflict of interest

The authors declare no competing financial interest.

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Appendix: Inhibitory activity of red and yellow araçá genotypes towards carbohydrate-hydrolyzing enzymes: Putative role of ellagitannins

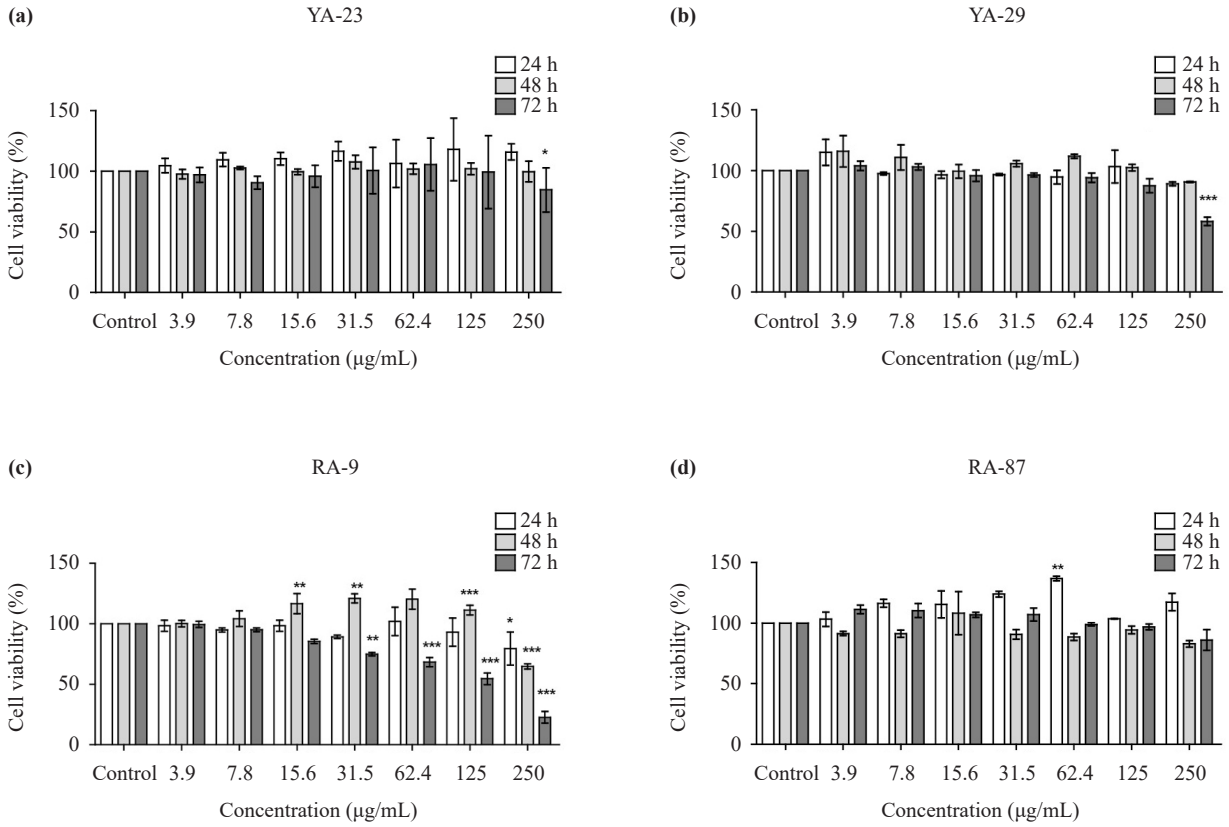

Figure S1. Cytotoxicity of araçá fruit extracts genotypes on non-tumorigenic Hacat cells after 24, 48 and 72 h (MTT assay) namely (a) yellow araçá accession 23 (YA-23), (b) red araçá accession 29 (RA-29), (c) red araçá accession 9 (RA-9) and (d) red araçá accession 87 (RA-87). Results are expressed as mean values of two independent experiments in duplicate (n = 4) ± standard deviation (SD) and are presented as the percentage of the control. Two-way ANOVA was used for comparison with Bonferroni post-test; * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ versus control

A.1 *Cell viability assay*

The viability of non-tumorigenic immortalized human keratinocytes cells (HaCaT) after exposure to araçá fruit extracts was assessed by the dehydrogenases-dependent 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction. Hacat cells were cultivated and maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco) containing 0.1% fungizone and 100 U/L penicillin/streptomycin and supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco). Hacat cells were seeded at a density of 5×10^3 cells/well in 96 well plastic cell cultures (Nest) and experiments performed 24 hours after the initial seeding. Samples YA-23, RA-29, RA-9 and RA-87 were tested at concentrations of 3.9, 7.8, 15.6, 31.3, 62.5, 125 and 250 µg/mL and experiments were carried out at three different incubation times: 24, 48 and 72 h. One hour and forty-five minutes before the end of the experiment 50 µL MTT (0.5 mg/mL) was added. At the end of the experiment the medium was removed and the purple insoluble formazan, produced by viable cells, was dissolved in 100 µL of dimethylsulphoxide and quantified by measuring the absorbance at 492 nm (using a SpectraMax Plus plate reader). Cell viability was calculated as follows:

$$\text{Cell viability } (\%) = \frac{\left(A_{\text{control}} - A_{\text{sample}}\right)}{A_{\text{control}}} \times 100$$

where $A_{control}$ was measure of the formazan formed in negative control cells and A_{sample} was the measure of the formazan formed after extracts exposure.

A.2 *HPLC-ESI-MS^n screening*

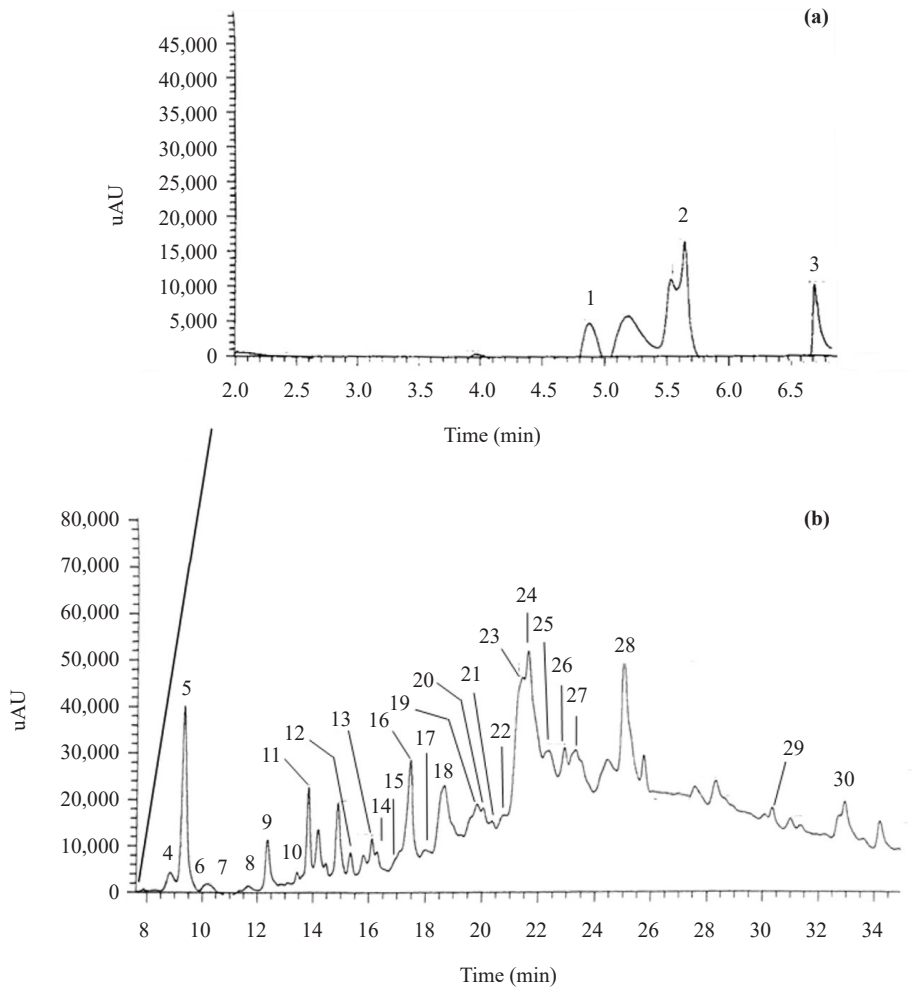

Figure S2. Representative LC-MS chromatogram of araçá accession YA-23. Compounds were numbered as identified Table 1. (a) LC-MS chromatogram showing retention time from 2 to 6.9 minutes and (b) retention from 7 to 34 minutes

Table S1. Phenolic compounds identified in red and yellow araçá extracts. Compounds were assigned based on their retention time (RT), UV and mass spectrometric data from authentic standards and data available in literature for *Psidium* and other species. Compounds were numbered according to their order of elution

Peak	RT (min)	Compound	UV (nm)	$[M-H]^-$ (*m/z*)	fragments (*m/z*)	Ref.
1	4.68	Caffeic acid hexoside (detected as formic acid adduct)*	262, 322	387	MS^2[387]: 341 MS^3[341]: 179	[45]
2	5.72	4-*O*-caffeoylquinic acid*	259, 298	353	MS^2[353]: 191, **173**, 111 MS^3[173]: 111	[46]
3	6.61	Caffeic acid hexoside derivative (detected as formic acid adduct)*	259, 301	415	MS^2[415]: **369**, 341 MS^3[369]: **341**, 179	
4	7.90	Caffeoylquinic isocitrate*	247, 280, 346, 361	353	MS^2[353]: 173, 111, MS^3[173]: 111	[47]
5	8.30	Isocitric acid	247, 343	191	MS^2[191]: 173, 111 MS^3[173]: 111	
6	10.06	Caffeic acid hexoside derivative*	289, 325	457	MS^2[457]: **341**, 179 MS^3[341]: **179**, 161	[48]
7	10.51	Citric acid	277	191	MS^2[191]: 173, 111	**
8	11.74	4,5-*O*-dicaffeoylquinic acid*	265	515	MS^2[515]: 173 MS^3[173]: 111	[45]
9	12.90	4,5-*O*-dicaffeoylquinic acid isomer*	271	515	MS^2[515]: **173**, 341 MS^3[173]: 111	[45]
10	13.10	Caffeic acid galloyl-hexoside*	268	511	MS^2[511]: 341	
11	13.73	Caffeic acid hexoside derivative*	277	689	MS^2[689]: 515 MS^3[515]: 173	
12	15.23	Vannilic acid di-hexoside (detected as formic acid adduct)*	262, 300	537	MS^2[537]: 491, 329, 167 MS^3[329]: 167	[49]
13	15.99	HHDP-Glc	259, 285	481	MS^2[481]: 463, **319**, 301 MS^3[319]: 301	[50] [51]
14	16.99	Unknown	265, 280	978	MS^2[978]: 932, **860**, 803, 581	[52]
15	17.08	Unknown	265, 280	978	MS^2[978]: **932**, 915, 870, 569	[52]
16	17.98	di-HHDP-Glc	259, 285	783	MS^2[783]: 481, **301**, 275 MS^3[301]: 257	[50] [51]
17	18.78	Castalagin/vescalagin	259, 282	933	MS^2[933]: 889, **631**, 569	[50]
18	19.20	Castalagin/vescalagin	259, 282	933	MS^2[933]: **631**, 451	[50]
19	19.59	HHDP-tri-galloyl-Glc isomer	259, 283	951	MS^2[951]: **906**, 605 MS^3[906]: **783**, 763, 744, 605	[50]
20	19.83	HHDP-tri-galloyl-Glc isomer	259, 280	951	MS^2[951]: **906**, 783, 605	[50]
21	20.76	HHDP-tri-galloyl-Glc isomer	277	951	MS^2[951]: **906**, 783, 605 MS^3[906]: **783**, 763, 744, 605, 480, 301, 273	[50]
22	21.21	HHDP-tri-galloyl-Glc isomer	274	951	MS^2[951]: 906	[50]
23	21.95	Galloyl-di-HHDP-Glc isomer	280	935	MS^2[935]: **633**, 571, 301 MS^3[633]: 615, 480, 329, 365	[51]

Table S1. (cont.)

Peak	RT (min)	Compound	UV (nm)	[M-H]$^-$ (*m/z*)	fragments (*m/z*)	Ref.
24	22.27	Galloyl-di-HHDP-Glc isomer	283	935	MS^2[935]: **633**, 571, 301 MS^3[633]: **615**, 589, 571, 419, 329, 299, 275	[51]
25	22.63	HHDP-Glc isomer		481	MS^2[481]: 463, **319**, 301 MS^3[319]: 301	[50] [51]
26	23.07	HHDP-tri-galloyl- Glc isomer	280	951	MS^2[951]: 906, **783** MS^3[783]: **605**, 389	[50]
27	23.38	Galloyl-di-HHDP-Glc isomer	280	935	MS^2[935]: **633**, 419, 329, 301	[51]
28	24.60	Taxifolin hexoside	286, 349	465	MS^2[465]: **437**, 303, 275, 190 MS^3[437]: 275	[51] [53] [54]
29	30.71	Quercetin-glucoronide	289, 355	477	MS^2[477]: 301	[51] [54] [55]
30	33.25	Eriodictyol hexoside	295, 343	449	MS^2[449]: 287 MS^3[287]: 269, **181**, 167, 153	[51] [54]

*Compounds identified for the first time in araçá; **Authentic Standard

Compound 1 ([M-H]$^-$ ion at *m/z* 387) was tentatively identified as caffeic acid hexoside based on the LC-MS^2 spectrum analysis which gave origin to an ion at *m/z* 341 corresponding to the loss of 46 Da (formic acid). LC-MS^3 analysis of fragment *m/z* 341 generates a fragment with *m/z* 179 (caffeic acid) and the neutral loss of a hexoside residue of 162 Da (hexose) [45].

Compound 2 ([M-H]$^-$ ion at *m/z* 353) was tentatively assigned as 4-*O*-caffeoylquinic acid based on the LC-MS^2 spectrum analysis gave origin to a base peak fragment at *m/z* 173 corresponding to quinic acid and neutral loss of 179 Da (caffeic acid) which indicates the presence of a caffeoyl moiety bonded to quinic acid at the position 4 according to [46].

Compound 3 ([M-H]$^-$ ion at *m/z* 415) was tentatively identified as a caffeic acid hexoside derivative. LC-MS^2 spectrum displayed the fragment ion at *m/z* 369 due to the loss of formic acid [M-H-46] and MS^3 analysis resulted in a fragment with 341 Da (caffeic acid hexoside) resultant of a neutral loss of 28 Da most likely from an ethyl residue.

Compound 4 was identified as caffeoylquinic isocitrate according to the fragmentation pattern reported [47].

Compound 5 was tentatively identified as isocitric acid and **compound 7** was identified as citric acid using a standard compound.

Compound 6 ([M-H]$^-$ ion at *m/z* 457) was tentatively identified as a caffeic acid hexoside derivative. The LC-MS^2 spectrum showed a base peak ion at *m/z* 341 (caffeic acid hexoside) resulting from the neutral loss of 116 Da which may correspond to a malic acid or a deoxypentose residue [48]. LC-MS^3 analysis of fragment *m/z* 341 generates a fragment with *m/z* 179 (caffeic acid) and the neutral loss of a hexoside residue of 162 Da (hexose).

Compounds 8 and **9** ([M-H]$^-$ ion at *m/z* 515) were tentatively identified as 4,5-*O*-dicaffeoyl quinic acid and 4,5-*O*-dicaffeoyl quinic acid isomer, respectively, as the LC-MS^2 analysis gave to the fragment ion at *m/z* 173 as base peak and also the presence of ion at *m/z* 111 which are characteristic of quinic acid substituted at position 4-OH [45-46].

Compound 10 ([M-H]$^-$ ion at *m/z* 511) was tentatively identified as caffeic acid galloyl-hexoside as the LC-MS^2 spectrum displayed the fragment ion at *m/z* 341 and the neutral loss of 170 Da which may correspond to the neutral loss of gallic acid.

Compound 11 ([M-H]$^-$ ion at *m/z* 689) was tentatively identified as caffeic acid derivative, based on the LC-MS^2 base peak at *m/z* 515 resulting from a neutral loss of 174 Da and LC-MS^3 base peak at *m/z* 173 (quinic acid substituted at position 4-OH) and neutral loss of 342 Da corresponding to the neutral loss of caffeic acid hexoside.

Compound 12 was identified as vannilic acid di-hexoside based on the fragmentation pattern reported [49].

Compound 13 to 30 were reported as araçá components and were identified based on the fragmentation pattern reported by previously [50-51, 55].

Table S2. Set of phenolic compounds related to the inhibition parameters based on Variable Importance in Prediction (VIP)

Compound name	Compound number	VIP
Caffeic acid hexoside	1	0.62
Caffeoyl quinic acid hexoside derivative	6	0.68
Caffeic acid hexoside derivative	9	1.19
Castalagin/Vescalagin isomer	18	0.76
Trisgalloyl HHDP-glc isomer	19	1.52
Trisgalloyl HHDP-glc isomer	20	1.00
Galloyl-bis-HHDP-glc	23	0.70
Galloyl-bis-HHDP-glc	24	0.80
Eriodictiol hexoside	30	1.32

Relationship between a set of phenolic compounds and inhibition values was further evaluated using PLS regression. Calibration model for the prediction of the α-glucosidase inhibition included 9 compounds with root mean square errors of 1 and 22 and R^2 of 0.9997 and 0.7474 for calibration and validation data, respectively. Selection of the variables was done using parameter variable Importance in Prediction (VIP) as described earlier [56].