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Bioaccessibility and cytotoxicity of *Euterpe edulis*, *E. oleracea* and *E. precatoria* fruit compounds

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ABSTRACT

The bioaccessibility of bioactive compounds in *Euterpe* spp. fruits influences both their nutritional value and their biological potential. Composition and solubility of bioactive compounds are essential variables in the mechanisms of bioaccessibility and cytotoxicity. This study measured the bioaccessibility and biological properties of compounds in *E. edulis*, *E. oleracea* and *E. precatoria* fruits after *in vitro* digestion. Total phenolic compounds, total anthocyanins, free radical scavenging capacity (DPPH, peroxyl and H_2O_2) and antiproliferative potential on Caco-2 cells were measured. The greatest bioaccessibility of total phenolics (127.35 mg GAE g^{-1}) and total anthocyanins (127.15 mg cyanidin-3-glucoside g^{-1}) was in *E. precatoria*. *In vitro* digestion indicated that the highest scavenging capacity for the oxidative radicals DPPH (307.30 µmol ET g^{-1}), peroxyl (63.94 µmol ET g^{-1}) and H_2O_2 (IC_{50} 0.92 mg mL^{-1}), was also in *E. precatoria* fruits. *Euterpe precatoria* also had the greatest antiproliferative effects (85%), only slightly better than *E. edulis* (83%), but both were much greater than *E. oleracea* (30%). In conclusion, *E. precatoria* has the greatest bioactive potential with an antiproliferative effect on Caco-2 cancer cells comparable to that of *E. edulis*.

KEYWORDS: Juçara; açai; bioactive compound; *in vitro* gastrointestinal digestion; Caco-2 cells

Bioacessibilidade de compostos antioxidantes e citotoxicidade de frutos de *Euterpe edulis*, *E. oleracea* e *E. precatoria*

RESUMO

A bioacessibilidade de compostos bioativos em frutos de *Euterpe* spp. influencia tanto seu valor nutricional quanto seu potencial biológico. A composição e a solubilidade de compostos bioativos são variáveis essenciais nos mecanismos de bioacessibilidade e citotoxicidade. Este estudo mediu a bioacessibilidade e as propriedades biológicas de compostos em frutos de *E. edulis*, *E. oleracea* e *E. precatoria* após digestão *in vitro*. Foram mensurados compostos fenólicos totais, antocianinas totais, capacidade de sequestro de radicais livres (DPPH, peroxil e H_2O_2) e potencial antiproliferativo em células Caco-2. A maior bioacessibilidade de fenólicos totais (127,35 mg GAE g^{-1}) e antocianinas totais (127,15 mg cianidina-3-glicosídeo g^{-1}) foi em *E. precatoria*. A digestão *in vitro* indicou que a maior capacidade de sequestro para os radicais oxidativos DPPH (307,30 µmol ET g^{-1}), peroxil (63,94 µmol ET g^{-1}) e H_2O_2 (CI50 0,92 mg mL^{-1}) também foi observada em frutos de *E. precatoria*. *Euterpe precatoria* também apresentou os maiores efeitos antiproliferativos (85%), apenas ligeiramente superior ao de *E. edulis* (83%), mas ambos foram muito superiores ao de *E. oleracea* (30%). Em conclusão, *E. precatoria* possui o maior potencial bioativo, com um efeito antiproliferativo sobre células cancerígenas Caco-2 comparável ao de *E. edulis*.

PALAVRAS-CHAVE: Juçara; açaí; compostos bioativos; digestão gastrointestinal *in vitro*; células Caco-2

INTRODUCTION

The *Euterpe edulis* Mart., *E. oleracea* Mart. and *E. precatoria* Mart. palms are native to Brazil and found in different regions of the country. Their fruits have important economic potential, are used in the production of juices, ice creams, sweets, jelly, and more. The *E. edulis* is found in the Atlantic Forest and has mainly been economically exploited as palm hearts by family farmers (Carvalho *et al*. 2022). *Euterpe oleracea* and *E. precatoria* are found in the Amazon region, and their fruits are part of a well-developed and valued food item production chain (Cunha Júnior *et al*. 2016).

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Euterpe oleracea has received considerable attention from the scientific community because its fruits are described as "superfoods" because of high antioxidant activity and potential anti-inflammatory effects (Silva *et al*. 2019b). Also, *E. edulis* (traditionally used for palm hearts) fruits have also been found to be potential "superfood" because they also have the same bioactive compounds as those found in *E. oleracea*, including ascorbic acid, tocopherols, carotenoids, anthocyanins, flavonoids, and phenolic acids (Jamar *et al*. 2018; Leite *et al*. 2018).

Studies of the antioxidant composition of foods seldom address the bioavailability of antioxidants in the human body due to the high cost, long study duration, and ethical constraints of human nutritional studies (Minekus *et al*. 2014). Thus, *in vitro* assays using cell cultures and gastrointestinal digestion methods are preferred because they generate rapid results regarding cytotoxicity in cancer cells and the bioaccessibility of bioactive compounds. *In vitro* gastrointestinal digestion methods simulate *in vivo* physiological conditions with respect to enzymes, digestive salts, pH, and digestion time. They can also test the bioaccessibility of food matrices, i.e., the amount of a compound released from a food matrix considered available for absorption in the intestine (Minekus *et al*. 2014). Although *in vitro* methods simulate the three phases of human digestion (oral, gastric, and intestinal), only after the intestinal phase can a bioaccessible sample be obtained.

Studies involving cell cultures investigate cytotoxicity on cancer cells of bioactive compounds in the bioaccessible fraction of a sample. This refers to their ability to make *in vitro* cancer cells non-viable. The Caco-2 model is a cell line derived from human colon adenocarcinoma and is extensively employed in cytotoxicity research. These cells fall into the category of type 1 human intestinal cells and exhibit spontaneous differentiation in standard culture conditions, thus enhancing their *in vitro* utility (Panse and Gerk 2022).

Composition and solubility of extract compounds from plants are important for the mechanisms of bioaccessibility and cytotoxicity. Thus, studies must determine antioxidant composition in each species and how it becomes bioavailable for absorption and metabolism by the body. This requires evaluating antioxidant activity in both solvent extracts and *in vitro* digestion systems.

Here, we examined the biological properties of the pulp of the fruits of three species of palm (*E. edulis*, *E. oleracea* and *E. precatoria*) to determine bioaccessibility of antioxidant compounds using an *in vitro* digestion process. We also measured the antioxidant properties of ethanolic extracts from fresh fruit pulp. Finally, we tested the *in vitro* digested pulps for cytotoxic effects on Caco-2 human colon adenocarcinoma cells.

MATERIALS AND METHODS

Plant material

Fruits of *E. edulis*, *E. oleracea*, and *E. precatoria* were directly obtained from producers in their respective places of origin. *Euterpe edulis* was acquired in the city of Barra do Turvo, São Paulo, Brazil (24°55'37"S 48°28'26"W), *E. oleracea* in Igarapé-Miri, Pará, Brazil (1°58'37''S 48° 57'34''W), and *E. precatoria* in Feijó, Acre, Brazil (8°10'14''S 70°21'30''W), and in Boca do Acre, Amazonas, Brazil (8°44'26''S 67°23'3''W). The records obtained through the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* (SISGEN), *Conselho de Gestão do Patrimônio Genético* (CGEN), Ministry of the Environment, Brazil, for the use of the native species used in this study are A9B82C6 (*E. edulis*), A9C7182 (*E. oleracea*) and AC37753 (*E. precatoria*).

After harvesting, ripe fruits were selected of the same age (based on color) and washed where they were harvested. Fruits were dried in a forced-air circulation oven for 24 h at 50°C, followed by packaging in polyethylene bags and shipment via airmail to the laboratory. In the laboratory, fruit pulp was manually removed, ground, and stored in a freezer (-18°C) until the time of the assays.

Preparation of extracts

Samples of *Euterpe* fruit pulps were extracted using ethanol: water (80:20, v/v), in a 1:10 (w/v) ratio. After an initial vortex mixing for 1 min, extracts were placed in an ultrasonic bath (Unique, USC-2850 a) for 20 min at room temperature (25 ± 5ºC), then cooled to 4º and centrifuged at 20,000 g for 20 min in a benchtop centrifuge (MPW 350-350R). Extracts were filtered through qualitative filter paper and transferred to test tubes to be stored at -18°C until analysis.

In vitro gastrointestinal digestion and bioaccessibility

In vitro digestion of pulp treated as described above was performed following the protocol of the COST Action INFOGEST FA1005 network (Brodkorb *et al*. 2019). This process includes oral, gastric, and intestinal phases, employing synthetic saliva, gastric, and intestinal fluids including their respective enzymes (amylase, pepsin, bile salts, and pancreatin). After digestion, the tubes containing the bioaccessible fraction (intestinal phase) were centrifuged and the supernatant was collected and stored in a freezer (-18°C) until bioaccessibility analysis.

Antioxidant activity

The choice of antioxidant activity models was based on their relative effectiveness in simulating the ability of compounds in the fruit pulps to neutralize free radicals, which are known to cause oxidative stresses and cellular damage. These models can provide an important measure of the antioxidant activity of ethanolic extracts and samples after *in vitro* gastrointestinal digestion (Seon *et al*. 2020).

DPPH free radical scavenging was applied in three replicates to the fruit pulp (ethanol extract and intestinal phase) (Baliyan *et al*. 2022). Absorbance readings were performed on a UV-visible spectrophotometer (Shimadzu, UV-1800) at 517 nm. Results are expressed in µmol Trolox equivalent (TE) per gram of dry sample, calculated through a calibration curve (y = -350.3x + 167.69, R^2 = 0.9975), where "y" is the variable analysed (DPPH free radical scavenging) and "x" is the value of the spectrophotometer reading.

Peroxyl radical scavenging capacity (ROO•), the "ORAC" (Oxygen Peroxyl Radical Absorbance Capacity) method, was applied to the ethanol extract and intestinal phase, in three replicates, in a 96-well microplate using a SpectraMax M3 microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) in fluorescence mode, with emission at 528 nm and excitation at 485 nm. As above, results were expressed in TE per gram of dry sample, from calibration curves (y = 4E-05x - 28.542, R^2 = 0.9923), where "y" is the variable analysed (peroxyl radical scavenging capacity) and "x" is the value of the spectrophotometer reading.

H_2O_2 scavenging activity in the fruit pulp (ethanol extract and intestinal phase) was determined in three replicates by measuring the oxidation of lucigenin according to the methodology applied by Pleh *et al*. (2021), using a SpectraMax M3 microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA) in luminescence mode after a 5 min incubation period. Results are expressed as IC_{50} (mg mL^{-1}). The term IC_{50} is is the concentration of a substance required to reduce biological activity by 50%, which in this case is the concentration of the sample extract needed to eliminate 50% of the reactive species H_2O_2.

Total phenolic compounds (TPC) and total anthocyanins (TAC)

The TPC of the fruit pulp was measured in three replicates by the Folin-Ciocalteu spectrophotometric method (ethanol extract and intestinal phase) (Michiu *et al*. 2022) using a UV-spectrophotometer at 760 nm. Results are in mg gallic acid equivalents (GAE) per gram of dry sample, calculated by calibration curves (y = 77.983x - 2.6162, R^2 = 0.9995), where "y" is the variable analysed (TPC) and "x" is the value of the spectrophotometer reading.

TAC (ethanol extract and intestinal phase) was determined using the differential pH method in pH 1.0 buffer (0.025 M KCl) and pH 4.5 buffer (0.4 M $C_2H_3NaO_2$) (Lee *et al*. 2008). Absorbance was measured with a UV-spectrophotometer at 510 and 700 nm. TAC values were expressed in mg cyanidin-3-glucoside per gram of dry sample.

Caco-2 cell viability (MTT method)

Human colon adenocarcinoma intestinal cells (Caco-2) from the American Type Culture Collection (ATCC® HTB-37™) were cultured followed by viability assay using the MTT assay (Dolghi *et al*. 2021). This assay is based on the ability of reductase enzymes in the mitochondria of viable cells to reduce the salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) to formazan, whose production is proportional to the number of viable cells.

In three replicates, Caco-2 cells were seeded in 96-well plates at the uniform density of 10^4 cells $well^{-1}$, and after an adhesion period of 24 h, the medium was changed and the cells were treated with increasing concentrations of fruit sample extracts (ethanol extract and intestinal phase; 0.00, 1.25, 1.56, 2.50, 6.25, 12.50, and 25.00 µg mL^{-1}) in 200 µL of Dulbecco's Modified Minimum Essential Medium (DMEM). After 4 h incubation, 20 µL of MTT (5 mg mL^{-1}) were added, followed by incubation in the dark for 3 h at 37°C. The medium containing MTT was completely removed and the formazan produced was solubilized in DMSO (100 µL $well^{-1}$). Spectrophotometer absorbance readings were at 570 nm and the results were expressed as a percentage of cell viability. No cytotoxicity was observed in the ethanolic extract samples (cell viability ranged from 88% to 100%).

Statistical analyses

Analysis of variance (ANOVA) was used to compare the response variables (antioxidant activity, DPPH and ORAC, total phenolic content - TPC, total antioxidant capacity -TAC, H_2O_2 sequestration capacity, and cell viability of Caco-2 cells -MTT assay) among the predictor variable which were species (*E. edulis*, *E. oleracea*, and *E. precatoria*) and the sample type (pulp *in vitro* digestion, and fresh pulp in ethanol extract). For the analysis of DPPH, ORAC, TAC and TPC, the data, with eight independent observations per group, were subjected to analysis of variance, and the significant effects identified by the F test ($P < 0.05$) were compared by the Tukey test, using the statistical software Sisvar (Ferreira 2011). The results regarding H_2O_2 sequestration and cell viability were expressed as mean ± standard deviation.

RESULTS

Bioaccessibility of total phenolic compounds (TPC) and total anthocyanins (TAC)

TPC content varied widely among the three palm species, both in the ethanolic extract and after *in vitro* digestion, with *E. precatoria* showing higher values (Table 1). The TPC content of the ethanolic extract from *E. precatoria* (79.27 mg GAE g^{-1}) was about six times greater than that of *E. edulis* (12.27 mg GAE g^{-1}) and nearly eight times greater than *E. oleracea* (10.40 mg GAE g^{-1}). In *in vitro* digestion, *E. precatoria* also had the highest TPC content (127.35 mg GAE g^{-1}) compared to *E. edulis* (80.76 mg GAE g^{-1}) and *E. oleracea* (77.45 mg GAE g^{-1}). TPC content in *E. edulis* and *E. oleracea* were similar, both in the ethanolic extract and after *in vitro* digestion.

Table 1. Average by species of total phenolic compounds (TPC) and total anthocyanins (TAC) of fruit pulps of the genus *Euterpe* obtained from ethanolic extract and *in vitro* digestion.

***Euterpe* spp.**	**TPC (mg GAE g^{-1})**	
	Ethanol extract	***In vitro digestion***
E. edulis	12.27 ^{b,B}	80.76 ^{b,A}
E. oleracea	10.40 ^{b,B}	77.45 ^{b,A}
E. precatoria	79.27 ^{a,B}	127.35 ^{a,A}
SE	0.85	1.10
CV (%)	5.89	2.73
SMD	3.36	4.34
***Euterpe* spp.**	**TAC (mg cyanidin-3-glucoside g^{-1})**	
	Ethanol extract	***In vitro* digestion**
E. edulis	7.08 ^{b,B}	75.41 ^{b,A}
E. oleracea	8.03 ^{b,B}	74.46 ^{b,A}
E. precatoria	186.89 ^{a,A}	127.15 ^{a,B}
SE	1.37	0.74
CV (%)	4.36	1.14
SMD	5.41	3.62

SE: standard error; CV: coefficient of variation; SMD: significant minimum difference; means followed by the same lowercase letter in the column do not differ significantly from each other by Tukey's test ($P < 0.05$); means followed by the same uppercase letter in the row do not differ significantly from each other according to Tukey's test ($P < 0.05$); the averages were obtained from eight independent observations, with each observation analysed in triplicate.

Both in the ethanolic extract and after *in vitro* digestion, and similar to TFC, *E. precatoria* (186.89 mg cyanidin-3-glucoside g^{-1}) had greater TAC contents than *E. edulis* (7.08 mg cyanidin-3-glucoside g^{-1}) and *E. oleracea* (8.03 mg cyanidin-3-glucoside g^{-1}; Table 1). *In vitro* digestion, TAC content (127.15 mg cyanidin-3-glucoside g^{-1}) was greater in *E. precatoria* than *E. edulis* (75.41 mg cyanidin-3-glucoside g^{-1}) and *E. oleracea* (74.46 mg cyanidin-3-glucoside g^{-1}), indicating greater bioaccessibility in the first.

Pulp TAC values were much greater after *in vitro* digestion (Table 1), especially for *E. edulis* and *E. oleracea*. This increase was most pronounced in *E. edulis*, which was 10 times greater than the ethanolic extract.

Antioxidant activity and bioaccessibility

In the ethanolic extract, *E. edulis* had the highest DPPH antioxidant activity (34.62 µmol TE g^{-1}), while *E. precatoria* was lowest (4.47 µmol TE g^{-1}) (Table 2). However, after *in vitro* digestion, *E. precatoria* had the highest DPPH antioxidant activity (307.30 µmol TE g^{-1}), compared to *E. edulis* (181.86 µmol TE g^{-1}) and *E. oleracea* (143.87 µmol TE g^{-1}.

With the ORAC method, *E. precatoria* had the highest peroxyl radical scavenging capacity (175.87 µmol TE g^{-1}), followed by *E. edulis* (11.32 µmol TE g^{-1}) and *E. oleracea* (4.31 µmol TE g^{-1}) (Table 2). With *in vitro* digestion the peroxyl radical scavenging capacity increased for *E. edulis* (39.27 µmol TE g^{-1}) and *E. oleracea* (31.48 µmol TE g^{-1}) but decreased for *E. precatoria* (63.94 µmol TE g^{-1}).

Table 2. Average by species of DPPH radical scavenging capacity, peroxyl radical scavenging capacity (ORAC) and hydrogen peroxide (H_2O_2) scavenging capacity of fruit pulps of the genus *Euterpe* obtained from ethanolic extract and *in vitro* digestion.

***Euterpe* spp.**	**DPPH (µmol TE g^{-1})**	
	Ethanol extract	***In vitro* digestion**
E. edulis	34.62 ^{a,B}	181.86 ^{b,A}
E. oleracea	17.06 ^{b,B}	143.87 ^{c,A}
E. precatoria	4.47 ^{c,B}	307.30 ^{a,A}
SE	0,96	7,49
CV (%)	17.23	9.23
SMD	3.79	29.49
***Euterpe* spp.**	**ORAC (µmol TE g^{-1})**	
	Ethanol extract	***In vitro* digestion**
E. edulis	11.32 ^{c,B}	39.27 ^{b,A}
E. oleracea	4.31 ^{c,B}	31.48 ^{c,A}
E. precatoria	175.87 ^{b,A}	63.94 ^{a,B}
SE	2.56	1.07
CV (%)	4.23	3.76
SMD	12.55	5.24
***Euterpe* spp.**	**H_2O_2* (IC_{50}, mg mL^{-1})**	
	Ethanol extract	***In vitro* digestion**
E. edulis	n.d.	4.11 ± 0.06
E. oleracea	n.d.	1.08 ± 0.05
E. precatoria	16.18 ± 2.64	0.92 ± 0.02

SE: standard error; CV: coefficient of variation; SMD: significant minimum difference; means followed by the same lowercase letter in the column do not differ significantly from each other by Tukey's test ($P < 0.05$); means followed by the same uppercase letter in the row do not differ significantly from each other according to Tukey's test ($P < 0.05$); the averages were obtained from eight independent observations, with each observation analysed in triplicate; * mean ± standard deviation of three replicates.; n.d.: values not detected by the analysis method.

Only *E. precatoria* was able to scavenge the H_2O2 radical, with an IC_{50} of 16.18 mg mL^{-1} (not measurable in the other two species, Table 2). With *in vitro* digestion, all species had measurable H_2O_2 radical inhibition. *Euterpe precatoria* had the greatest inhibitory capacity, with the lowest IC_{50} (0.92 mg mL^{-1}), followed by *E. oleracea* (1.08 mg mL^{-1}) and *E. edulis* (4.1 mg mL^{-1}).

Caco-2 cell viability (MTT method)

To determine Caco-2 cell viability results, it was necessary to exclude the cytotoxic effects originating from the enzymes used in the *in vitro* digestion. After conducting preliminary assays using various concentrations of digested samples (0.00, 1.25, 1.56, 2.50, 6.25, 12.50, and 25.00 µg mL^{-1}), it was concluded that the lack of viability of Caco-2 cells treated with concentrations of 6.25, 12.50, and 25.00 µg mL^{-1} of digested samples was primarily due to the action of the digestive enzymes employed, rather than the presence of bioaccessible compounds. Therefore, only the digested samples at concentrations of 1.25, 1.56, and 2.5 µg mL^{-1} were considered for analysis, while the 0.00 µg mL^{-1} concentration, without the addition of samples, served as the control.

At 1.25 μg mL^{-1} concentration, Caco-2 cell viability was minimally reduced by *E. edulis* (87%) and *E. precatoria* (96%) but remained unaffected when treated with *E. oleracea* (100%). Similarly, at a concentration of 1.56 μg mL^{-1}, a slight reduction in cell viability was observed in Caco-2 cells treated with *E. edulis* (84%), *E. oleracea* (96%), and *E. precatoria* (94%). However, at the highest tested concentration (2.5 μg mL^{-1}), cell viability was reduced by *E. edulis* and *E. precatoria* extracts (17% and 14%, respectively), while relatively higher cell viability (70%) was observed under *E. oleracea* extracts (Figure 1).

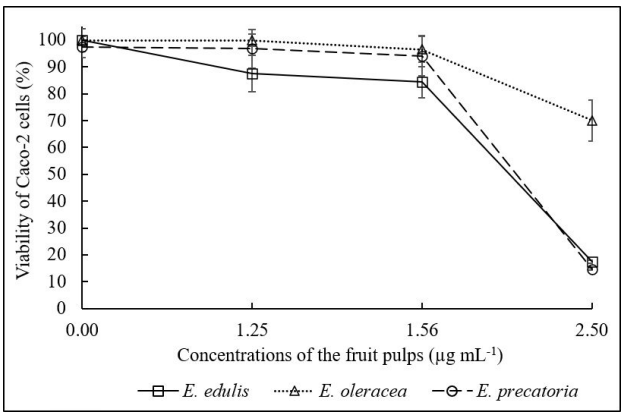

Figure 1. Viability of Caco-2 cells (%) treated with concentrations of the fruit pulps of three species of the genus *Euterpe* obtained from *in vitro* digestion. The data are reported as the mean ± standard deviation from eight independent observations, with each observation analysed in triplicate (data not statistically analysed).

DISCUSSION

In this study, we evaluated the bioaccessibility of antioxidant compounds in the pulps of *Euterpe edulis*, *E. oleracea* and *E. precatoria* following *in vitro* gastrointestinal digestion, as well as their antiproliferative effects on human Caco-2 colon adenocarcinoma cells. We found that *E. precatoria* had the highest bioaccessibility of total phenolics and total anthocyanins, together with the strongest radical scavenging activities. Although *E. edulis* and *E. oleracea* had lower bioaccessibility in antioxidant assays, both *E. precatoria* and *E. edulis* had strong antiproliferative effects while *E. oleracea* had weaker effects on Caco-2 cells. Thus, fruit from *E. precatoria* has superior bioactive potential, and that *E. edulis* also represents a promising source of cytotoxic agents, with medicinal potential.

Variability in bioaccessibility of compounds in fruits of the three *Euterpe* spp. is likely to be due to digestion. That is, the diverse composition of fruit pulp of these three species after *in vitro* digestion results in there lease of bound phenolic compounds, leading to the formation of a variety of new phenolic compounds through chemical bonds with other compounds, such as fibers and proteins, similar to the results in Schulz *et al*. (2017). However, in that study, bioavailability depended on the nature of the phenolic compound, due to the chemical complexity of phenols and transformations that occur during digestion, along with interactions with other dietary components. Again, in that study, an important portion of phenolic compounds are in glycosylated, esterified, or polymerized forms that are hydrolysed during gastrointestinal digestion. This process leads to structural changes in phenols and the formation of phenolic derivatives through the partial degradation of other combined forms or interactions with other compounds released during digestion, such as minerals, fibers, or proteins, leading to increased bioaccessibility (see Dantas *et al*. 2019 for similar biochemistry in other species).

TPC levels were greater in the *in vitro* digestion than in ethanolic extraction, suggesting bioaccessibility is increased through gastrointestinal digestion. Even though the ethanolic extracts of *E. edulis* and *E. oleracea* had shown lower TPC contents than *E. precatoria*, *in vitro* digestion revealed that these two species had a substantial increase in TPC bioaccessibility (approximately 550% and 650%, respectively, for *E. edulis* and *E. oleracea*), which was much higher than *E. precatoria* (approximately 60%). However, *E. precatoria* was the species that had the highest TPC content in both the ethanolic extract and *in vitro* digestion, indicating that this species is a richer source of TPC and is more bioaccessible compared to the other two studied species.

Although TAC values in the pulp of *E. precatoria* was highest here, both in the ethanolic extract and *in vitro* digestion, *E. oleracea* is still an excellent source of polyphenols and highly biologically active anthocyanins. Due to these characteristics, freeze-dried *Euterpe* spp. samples have been used to obtain isolated standards of cyanidin-3-glucoside and cyanidin-3-rutinoside, as they are the two most abundant anthocyanins in *Euterpe* spp. (Silva *et al*. 2019a). One must remember genetic variation and environmental conditions of plant cultivation contribute to the variation in TAC levels among plants, including the species studied here, giving distinct profiles of bioactive compounds among individuals and species (Taiz *et al*. 2021).

Flavonoid compounds, including anthocyanins, have increased solubility when exposed to the gastric and intestinal conditions of digestion, influenced by pH and enzyme activity, and which affects their ability to form chemical bonds and new compounds, thereby influencing bioaccessibility (Dias *et al*. 2021; Lucas-Gonzalez *et al*. 2016). This indicated that the digestive process efficiently enhances the bioaccessibility of these compounds. Flavonoids bound to high molecular weight compounds, such as proteins, can be released by digestive enzyme action and can bind to other compounds to form new molecular structures (Dias *et al*. 2021). However, as noted by other authors, the bioaccessibility of phenolic compounds in food matrices is variable and dependent on factors such as plant species and the chemical nature of the compounds (Schulz *et al*. 2017). Thus, bioaccessibility must

be tested, such as through *in vitro* digestion, as we did here and recommend for further study.

The peroxyl radical scavenging capacity of *E. precatoria* was higher than that of *E. edulis* and *E. oleracea*, indicating its greater antioxidant potential. On the other hand, the increased ORAC antioxidant activity of *E. edulis* and *E. oleracea* suggests that *in vitro* digestion was able to solubilize reactive antioxidant compounds to scavenge peroxyl radicals, in contrast to *E. precatoria*. These results are consistent with those found for TAC, which had higher increases in bioaccessibility in *E. edulis* and *E. oleracea* when compared to *E. precatoria*.

DPPH antioxidant activity increased after *in vitro* digestion in all species. However, while *E. precatoria* had the lowest antioxidant activity (in the ethanolic extraction), it had the highest ability to neutralize the DPPH radical after *in vitro* digestion. The lower antioxidant activity in the ethanolic extract of *E. precatoria* suggests lower solubility of the antioxidant compounds, making them less available for reaction. However, during digestion, the compounds in the *E. precatoria* sample may have been converted into more soluble and reactive compounds, resulting in higher antioxidant activity of the digested sample (Dutra *et al*. 2017; Schulz *et al*. 2017). This indicates greater bioaccessibility of the bioactive compounds in *E. precatoria* fruits.

The greater DPPH radical scavenging capacity of *E. precatoria* after digestion may also be related to the chemical nature of the extracted compounds. Each species can contain different antioxidants with varying chemical and structural properties that can affect their absorption and metabolism by the body (Verruck *et al*. 2018; Melo *et al*. 2020). The antioxidant activity of digested samples tends to be higher than that found in ethanolic extracts. This means that, even if the ethanolic extract has relatively low antioxidant activity, the compounds can be transformed during digestion, resulting in increased antioxidant activity due to the facilitated extraction during the digestive process (Yao *et al*. 2021; Hu *et al*. 2023). The *in vitro* digestion efficiently solubilized and released antioxidant compounds from the samples under study, explaining both the expression of the inhibitory activity of the *E. edulis* and *E. oleracea* samples and the increase in the inhibitory activity of *E. precatoria*. Also, it is important to note that antioxidant activity results can vary depending on the methodology.

Euterpe edulis had lower bioaccessibility of bioactive compounds and antioxidant activity compared to *E. precatoria*. However, *E. edulis* had a similar antiproliferative effect. This suggests that other antioxidant compounds in the pulp of *E. edulis* fruits can contribute to this effect, such as carotenoids and vitamin C, or compounds with anti-inflammatory properties, like unsaturated fatty acids (Bilawal *et al*. 2021). Additionally, further research is needed to identify the complete profile of active compounds and elucidate the mechanisms underlying the observed antiproliferative effect in *E. edulis*.

Euterpe oleracea is the most widely commercially exploited species in the açaí industry due to its popularity, consumer acceptance, availability, and ease of cultivation. Furthermore, this species has been extensively researched and recognized as a rich source of antioxidant compounds, such as anthocyanins and flavonoids (Silva *et al*. 2019b). These compounds are known to play a protective role against the proliferation of Caco-2 cells (Avila-Sosa *et al*. 2019; Nascimento *et al*. 2022).

The inhibitory effect on Caco-2 cells by *E. precatoria* is consistent with the results of TPC and TAC and antioxidant activity. Thus *E. precatoria* is the best of these three species as a source of bioaccessible bioactive compounds with higher antioxidant activity. The greatest protective and anti-proliferative effect against Caco-2 cells of *E. precatoria* is associated with its superior bioaccessible profile. The lack of cytotoxicity in the ethanolic extract samples could be attributed to the low bioaccessibility of the bioactive compounds in these undigested samples, and consequently, their data were not presented.

E. oleracea has been the subject of studies on the prevention of human colon cancer. This is due to the binding of phenolic compounds to macronutrients, such as dietary fiber, which causes most of them to pass through the small intestine and colon, where they become more bioactive and bioavailable, thanks to interactions with the intestinal microbiota, which also benefits from the processing of polyphenols by bacterial strains (Nascimento *et al*. 2022; Van Der Merwe 2021; Afrin *et al*. 2020). For example, the study by Dias *et al*. (2014) showed that the signalling pathways associated with reduced growth of colon cancer cells were triggered by the polyphenols in *Euterpe* spp. pulp.

Different colon cancer cell lines treated with *Euterpe* spp. pulp extract showed increased expression of pro-apoptotic genes, that is, genes that stimulate programmed cell death. *E. edulis* can have cytotoxic potential equal to or greater than the values of other native Brazilian fruits, such as *Euterpe* spp. itself (Reis *et al*. 2020). In that study with *E. edulis* in an *in vivo* study using an animal model, the number of lesions resulting from colon cancer was reduced due to the addition of *E. edulis* pulp to the diet. The results of this study suggest that the fruits of *E. precatoria* and *E. edulis* can be considered interesting options to be included in the açaí industry, but the fruits of *E. precatoria* stand out due to their higher bioaccessibility of compounds with bioactive activity.

CONCLUSIONS

Both the ethanolic extract and the bioaccessible fraction from *in vitro* digestion of the *E. precatoria* fruit pulps had higher levels of total phenolic compounds (TPC), total anthocyanins (TAC), and antioxidant capacity than *E. edulis* and *E. oleracea*. Fruit pulps of *E. precatoria* and *E. edulis* both efficiently inhibited the growth of Caco-2 cells. The results of this study

indicate that *E. precatoria* palm fruit possesses the greatest nutritional and antiproliferative potential, but that *E. edulis* has a bioactive potential comparable to *E. oleracea*.

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