International Journal of Food Science +Technology

Original Article

Phenolic composition, antiproliferative and antiulcerogenic activities of a polyphenol-rich purified extract from açai (*Euterpe oleracea*) fruits

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(Received 7 July 2021; Accepted in revised form 28 August 2021)

Summary The bioactivity and phytochemical composition of a partially purified extract of açai (PPEA), concentrated in phenolic compounds (PC) and without the presence of macronutrients, were investigated. The major PC quantified by UHPLC-DAD-LTQ-Orbitrap MS-MS/MS in the PPEA are anthocyanins. *In vitro*, PPEA showed a cytostatic effect on the K-562 lymphoid leukaemia at a concentration of 40 μg PC mL^{-1}, with a GI_{50} equal to 1.08 μg PC mL^{-1}. *In vivo*, the extract did not promote acute toxicity in mice in any of the doses tested. The extract displayed gastroprotective activity in rats treated orally with 16, 48 and 160 mg PC kg^{-1}, with a significant decrease in the ulcerative lesion index, compared with the negative control. The lack of toxicity and the bioactivity of the PPEA show that this extract is beneficial to health and useful as a commercial food additive containing natural violet colourant, with pharmaceutical and functional potentials.

Keywords Acai, anthocyanins, antiproliferative activity, antiulcerogenic activity.

Introduction

Açai (*Euterpe oleracea* Mart.) is a palm widely distributed in the Amazon basin, where it has the most significant economic importance (Bichara & Rogez, 2011). Açai juice has health benefits associated with its phytochemical composition and high antioxidant capacity (Schauss, 2016). The high concentration of anthocyanins, primarily cyanidin-3-O-glucoside (C-3-G) and cyanidin-3-O-rutinoside (C-3-R), is responsible for the violet colour of açai (Rogez *et al.*, 2011), but minor anthocyanins were also found (Dias *et al.*, 2012). The major non-anthocyanin flavonoids are orientin, homoorientin and taxifolin-deoxyhexose (Dias *et al.*, 2013). Besides, amino acids, fatty acids, minerals and sterols have been identified (Schauss, 2016) and small amounts of lignans, monoterpenoids, norisoprenoids and a quinone derivative have been reported (Yamaguchi *et al.*, 2015).

Açai has significantly higher antioxidant capacity than most dark fruits, or any other fruit or vegetable (Bichara & Rogez, 2011). The bioactivity of açai have been shown by both *in vitro* and *in vivo* studies, including antimicrobial, antioxidant, antiproliferative, anti-inflammatory, anticonvulsant anti-leishmanial and cardioprotective properties, stimulating interest for the production of bioactive extracts (Pacheco-Palencia *et al.*, 2010; Souza-Monteiro *et al.*, 2015; Schauss, 2016; Da Silva *et al.*, 2018). Açai leads increased antioxidant levels in the serum, protects cells from oxidative damage, reduces the formation of reactive oxygen species and downregulates the production of proinflammatory cytokines and chemokines (Schauss, 2016; Magalhães *et al.*, 2020). Collectively, the total

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doi:10.1111/ijfs.15332

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phenolic content and other phytochemical constituents contribute to the total biological activity of *E. oleracea* fruits (Yamaguchi *et al*., 2015; Magalhães *et al*., 2020). However, the large majority of the studies on açai bioactivity were conducted with the pulp or crude extracts and not with purified/enriched extracts; consequently, no conclusions can be made about the exact contributions of phenolic compounds to the bioactivity of açai, and the correlation between bioactivity and phytochemical composition is required. In order to better understand the contribution of phenolic compounds for the bioactivity of açai, the objective of this study was to evaluate the potential *in vitro* antiproliferative and *in vivo* antinociceptive and gastroprotective effects and the phytochemical composition of a partially purified extract of açai (PPEA), which possesses high concentration of phenolic compounds and absence of macronutrients. This study will allow for the evaluation of the advantages of using purified/enriched extracts as food additives with nutraceutical potential rather than crude phenolic extracts or açai pulp.

Materials and methods

Animals

Adult male Wistar/Uni albino rats (150–250 g), 8 weeks old, were used for all gastric ulcer related experiments, while adult male Swiss albino mice (25–30 g), 8 weeks old, were used for the acute toxicity assays and antinociception evaluation. The animals were used in the experiments after a minimum period of seven days of adaptation to the vivarium, with a light-dark cycle of 12 h, controlled temperature (20 ± 2 °C) and water and food provided *ad libitum*. Before all experiments, the animals were fasted for a period of 12 h with free access to water. Studies were carried out following the principles and guidelines adopted by the National Council for Control of Animal Experimentation (CONCEA) and were approved by the Ethics Committee on Animal Use of the Federal University of Para (CEUA/UFPA, number 2306280720).

Preparation of the purified extract

The PPEA used in this study was kindly provided by the company Amazon Dreams (Belém, PA, Brazil). *E. oleracea* fruits were collected in the state of Pará, north region of Brazil. The PPEA was produced by a patented process licensed by both Amazon Dreams and Federal University of Pará (PI 1003060) that consisted of a lyophilised extract, obtained after the application of suitable techniques for the extraction with 1:0.5 (w/v) of fresh fruits/ water during 150 s followed by microfiltration using diatomaceous earth and purification (adsorption on acrylic macroporous resins and desorption with ethanol 75%) of the phenolic compounds present in the *E. oleracea* fruits (Rogez *et al*., 2009). The solvent was evaporated using an industrial vacuum concentrator following a semi-industrial lyophilisation. The final extract obtained after desorption was free of fatty acid, proteins and fibres (< 1% dry matter) and enriched in phenolic compounds.

UHPLC analysis of the phenolic compounds

The profiles of the anthocyanins (Dias *et al*., 2012) and non-anthocyanin flavonoids (Dias *et al*., 2013) of the PPEA were analysed in an ultra-high performance liquid chromatography-diode array detector-linear ion-trap quadrupole-Orbitrap mass spectrometry (UHPLC-DAD-LTQ-Orbitrap MS) system. Instrumental parameters and more detailed description can be found in Methods S1. For both methods, the compounds were identified by comparison with reference compounds from retention times, MS and MS/MS analysis. The results are expressed as mg phenolic compounds (PC) g^{-1} dry extract (DE).

Antiproliferative activity

For the *in vitro* screening, the following human tumour cell lines, provided by the National Cancer Institute (USA), were selected: U251 (Glioma), MCF-7 (breast), NCI-ADR/RES (ovarian expressing multiple drugs resistance phenotype), 786–0 (kidney), PC-3 (prostate), HT-29 (colon), K-562 (lymphoid leukaemia). A non-tumoural cell line (HaCaT, immortalised human keratinocyte) was also used. The antiproliferative activity of PPEA was carried out as described by Banzato *et al*. (2020). The extract was tested at concentrations of 0.04, 0.4, 4.0 and 40 μg quantified PC mL^{-1}, in triplicate and incubated for 48 h. Doxorubicin chloride was used as a positive control. The cell growth was determined by spectrophotometric reading of absorbance at 540 nm on a VersaMax microplate reader (Molecular Devices, Sunnyvale, USA). The concentrations that induce 50% growth inhibition (GI_{50} value) and 100% (total) growth inhibition (TGI value) were determined through nonlinear regression analysis of the concentration-response curves for each cell line using ORIGIN 8.0 software.

Acute toxicity evaluation

Experimental groups of male Swiss mice ($n = 3$) were treated with PPEA orally (po) (160 and 320 mg quantified PC kg^{-1}) and intraperitoneally (ip) (16, 48 and 160 quantified mg PC kg^{-1}). The general effects were observed by keeping the animals free walking on a flat

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surface for 4 h, evaluating possible manifestations related to the motor system and muscle tone, autonomic nervous system, final state of consciousness and final disposition. All animals were examined twice daily for a period of 14 days. This test was based on the OECD 423/2002 – OECD guidelines for the testing of chemicals, with modifications (OECD, 2002).

Antinociceptive activity

The antinociceptive activity was determined by the acetic acid-induced abdominal writhing test (Spindola *et al*., 2010). Experimental groups of male Swiss mice ($n = 6$) were treated (po) with either saline solution (0.9%; 10 mL kg^{-1}; negative control), indomethacin (30 mg kg^{-1}; positive control), or with PPEA (16, 48 and 160 mg quantified PC kg^{-1}). One hour after the oral treatments, the animals received acetic acid (0.8%, 10 mL kg^{-1} ip).

Antiulcerogenic activity

The ethanol-induced ulcer model (Vendramini-Costa *et al*., 2014) was applied. The groups ($n = 8$) of Wistar rats were treated orally with saline solution (0.9%, 10 mL kg^{-1}; negative control), carbenoxolone (200 mg kg^{-1}; positive control) or with PPEA (16, 48 and 160 mg quantified PC kg^{-1}). After 60 min, all animals received 1 mL of absolute ethanol orally. Finally, 60 min after the administration of the ulcerogenic agent, the animals were euthanised by deepening anaesthesia (using ketamine and xylazine at 300 and 30 mg kg^{-1}, respectively), the stomachs were removed, opened along the greater curvature and washed. The lesion count and the ulcerative lesion index (ULI) were calculated as described by Vendramini-Costa *et al*. (2014). Gastroprotection (%) was calculated using the mean ULI, as follows: (ULI negative control-ULI treatment/ULI negative control)*100.

Participation of sulfhydryl substances, prostaglandins and nitric oxide in gastric cytoprotection

To evaluate the mechanisms of action for the gastroprotective effects, a dose of 64 mg quantified PC kg^{-1} was used, which is the effective dose of phenolic compounds in PPEA that causes 50% gastroprotection (ED_{50}). A more detailed description of the experiments can be found in Methods S1.

Determination of the gastric secretions

The experiment to determine the gastric secretions was performed according to an optimised methodology (Monteiro *et al*., 2013). Three groups of animals were used ($n = 8$). All of the animals were anesthetised with thiopental sodium (50 mg kg^{-1}, ip) and trichotomised in the abdominal region. Saline solution (0.9%, 10 mL kg^{-1}), cimetidine (100 mg kg^{-1}), or PPEA (64 mg quantified PC kg^{-1}) was administered to the duodenum. The hydrogen ion concentration (mEq[H^+] mL^{-1} (4 h) $^{-1}$) was determined by titration of the gastric juice.

Statistical analysis

The experimental data were analysed using the analysis of variance (ANOVA) module at significance levels of $P < 0.01$, $P < 0.001$ and $P < 0.0001$. The Tukey test was then used to compare sets of data with the same levels of significance. Statistical analyses were performed using the GraphPad Prism version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results and discussion

In order to evaluate the contribution of phenolic compounds to the potential antiproliferative, antinociceptive and gastroprotective effects of açai, a purified extract of açai concentrating 163.13 mg PC g^{-1} DE was used. The PPEA is basically composed by a higher concentration of phenolic compounds, without the presence of other metabolites or macronutrients such as lipids, protein and fibre, thus avoiding possible interference of these constituents in the results.

Identification and quantification of phenolic compounds

The phenolic compounds identified by UHPLC-DAD-LTQ-Orbitrap MS-MS/MS in the PPEA used in this study are shown in Table S1. A total of 37 compounds were identified based on retention times, high-resolution mass spectral data and MS/MS fragmentation compared with the literature and reference compounds. In addition, 16 of them were quantified. The quantified polyphenols present in the PPEA were divided into anthocyanins (142.94 mg g^{-1} DE) and non-anthocyanins (20.19 mg g^{-1} DE). C-3-G and C-3-R were shown to be the major anthocyanins, representing approximately 88% of the total quantified phenolic content, of which 40% and 60% were C-3-G and C-3-R, respectively. The PPEA presented twice higher concentration of anthocyanins than the hydroethanolic extracts from six genotypes of açai reported by Torma *et al*. (2017), which obtained total anthocyanin values ranging from 9.15 to 64.83 mg g^{-1} DE and higher non-anthocyanin content compared with the hydroethanolic extract from açai (1.23 mg g^{-1} DE) reported by Costa *et al*. (2021). Another study found from freeze-dried açai a total anthocyanin content of 3.19 mg g^{-1} DE, of which 36.7% was C-3-G, 60.5% was C-3-R and 2.8% were others anthocyanins (Schauss *et al*., 2006). In agreement, the predominance of C-3-R and C-3-G were also previously described in

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commercial and non-commercial freeze-dried açai (Dias *et al*., 2012, 2013; Xiong *et al*., 2020; Costa *et al*., 2021).

Other minor anthocyanins identified were pelargonidin-3-glucoside, peonidin-3-glucoside and peonidin-3-rutinoside. These were also found in açai by Dias *et al*. (2012); Schauss *et al*. (2006) and Xiong *et al*. (2020). The major non-anthocyanin compounds were orientin, homoorientin, vitexin, isovitexin, rutin, scoparin, taxifolin deoxyhexose or its isomer, catechin and simple phenols such as vanillic acid and protocatechuic acid. They have also been characterised and reported in other studies with açai (Schauss *et al*., 2006; Dias *et al*., 2013; Costa *et al*., 2021). The content of homoorientin, orientin and vitexin in PPEA (5.60 ± 0.08, 3.33 ± 0.06 and 0.55 ± 0.02 mg g^{-1} DE, respectively) were found to be higher compared with the content previously described in another hydroethanolic extract from açai (0.12, 0.05 and 0.05 mg g^{-1} DE, respectively) (Costa *et al*., 2021).

Acute toxicity evaluation

In this study, a general screening for potential acute toxicity effects of the PPEA was performed, assessing its actions on the final state of consciousness, final disposition, exploratory capacity, motor coordination, muscle tone, motor reflexes and its effects on the central and autonomic nervous system. Neither systemic toxicity or death were observed in the animals treated (po) with the extract at doses 160 and 320 mg quantified PC kg^{-1} (corresponding to about 1000 and 2000 mg DE kg^{-1}), or in the animals treated with PPEA (ip) at doses of 16 and 48 mg quantified PC kg^{-1} (corresponding to about 100 and 300 mg DE kg^{-1}, respectively) during the 14 days of observation. However, animals treated ip with the extract at the highest dose of 160 quantified PC kg^{-1} (corresponding to about 1000 mg DE kg^{-1}), exhibited the characteristic purple colouration of anthocyanins in the skin and extremities 10 min after administration, which remained for 24 h. Neither death nor any other clinical signs of systemic toxicity were observed. There were no modifications in parameters such as locomotion, exploratory behaviour or aspect of the hair in any of the groups.

Antiproliferative activity

The data in Fig. 1 show that the PPEA produced a cytostatic effect on the lymphoid leukaemia cell line (K-562) at concentrations higher than 0.04 μg quantified PC mL^{-1}. For the other cell lines studied, no antiproliferative activity was produced with the concentrations of PPEA used. The concentrations of PPEA that reduced K-562 cell proliferation by 50% (GI_{50}) and 100% were 1.08 and 23.9 μg quantified PC mL^{-1}, respectively.

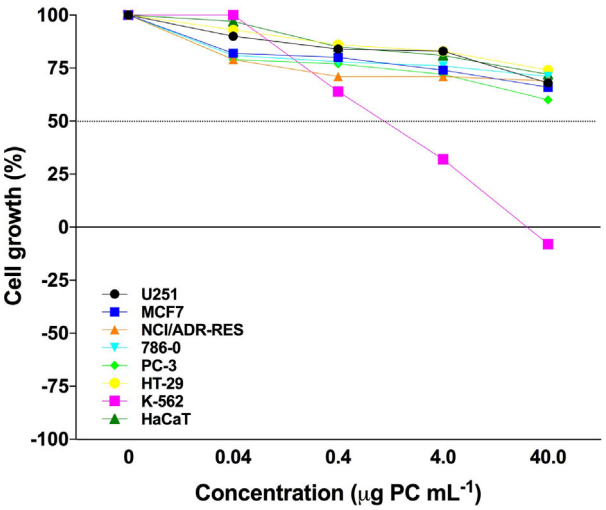

Figure 1 Effect of different concentrations of partially purified extract of açai (PPEA) in the growth of Human tumour cell lines: U251 (Glioma), MCF-7 (breast), NCI-ADR/RES (ovarian expressing phenotype multiple drugs resistance), 786–0 (kidney), PC-3 (prostate), HT-29 (colon) and K-562 (lymphoid leukaemia); Non-tumour cell line: HaCaT (immortalised human keratinocyte).

Previous studies with açai have demonstrated that the fruit's polyphenols reduced the proliferation of leukaemic cell HL-60 and human colon cell HT-29 (Del Pozo-Insfran *et al*., 2006; Pacheco-Palencia *et al*., 2010). The anthocyanins fraction of the açai pulp extract at 10.7 μM of total soluble phenolics reduced HL-60 cell viability by 82%, while the equivalent concentration of the fraction of phenolic acids and açai pulp reduced proliferation by 62%. The mechanism of the antiproliferative effect and pro-apoptotic induction activity of açai polyphenols against HL-60 cells was based on the activation of caspase-3 in a dose- and time-dependent manner (Del Pozo-Insfran *et al*., 2006). The monomeric and polymeric fraction of an anthocyanins extract (100 μg C-3-G equivalents mL^{-1}) produced from açai pulp inhibited the growth of HT-29 cells by 95.2% and 92.3%, respectively. The GI_{50} of the monomeric and polymeric anthocyanins fractions were 12.1 μg C-3-G equivalents mL^{-1} and 14.4 μg C-3-G equivalents mL^{-1}, respectively (Pacheco-Palencia *et al*., 2010). Açai pulp presented a cytotoxic effect for HT-29 cells, with a GI_{50} of 10.2 μg of gallic acid equivalents mL^{-1} (Pacheco-Palencia *et al*., 2008). The PPEA studied here presented a GI_{50} of 1.08 μg quantified PC mL^{-1} on K-562, which is ten times less than the values for the anthocyanin fractions and the açai pulp on HT-29, for which we did not detect any effect at the maximum tested concentration of 40 μg quantified PC mL^{-1} (about 250 μg DE of PPEA mL^{-1}).

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This activity may be due to the polyphenol's antioxidant capacity in the extract, which directly neutralises the reactive oxygen species, promoting an increase in the oxygen radical absorption capacity of the cells that can lead to genetic mutations, genomic instability and carcinogenesis in different organs (Yamaguchi *et al.*, 2015; Schauss, 2016). Many berry extracts were also assessed for potential antiproliferative activity against cancer cells (Dai *et al.*, 2009). The cytotoxic effect of anthocyanin-containing blackberry extracts and pure-C-3-G on HL-60 cells is shown by Dai *et al.* (2009). The anthocyanins have demonstrated positive anticancer effects by targeting inflammation, oxidative stress and apoptotic signalling pathways including inflammatory PI3K/Akt/mTOR pathway, Bax/Bcl-2/caspases as apoptosis modulators and NF-κB/Nrf2 as oxidative stress mediators in cancer dysregulated metabolism (Fakhri *et al.*, 2020).

Therefore, the results suggest that inhibition of the growth of K-562 cells by PPEA might be associated with flavonoids, particularly anthocyanins because anthocyanins are the primary components in PPEA. Our data corroborate other studies on antiproliferative properties of açai. However, further investigation is needed to determine the mechanism of the antiproliferative activity against K-562 cells here observed.

Antinociceptive activity

The potential antinociceptive effect of the PPEA was evaluated using the acetic acid-induced abdominal writhing model in mice. Pre-treatment with PPEA at doses of 16, 48 and 160 mg quantified PC kg^{-1} did not reduce the number of abdominal writhes. In the control group, previously treated with saline solution (10 mL kg^{-1} po), the injection of acetic acid (0.8% in saline solution 0.9% ip) induced 27.5 ± 4.38 abdominal writhes during the 20 min of experimental evaluation (Fig. 2). Indomethacin, the positive control in this experiment, reduced the number of abdominal writhes by 85.45% (4.0 ± 4.8). Most of the studies evaluating the antinociceptive activity of açai were conducted with its oil or crude extracts (ethanolic or hydroalcoholic) from the flowers, stalks and fruits, with all of them presenting pain relief effects (revised in Magalhães *et al.*, 2020). However, we can conclude that different from the ethanolic and aqueous extracts from different parts of açai plant, the PPEA did not present antinociceptive activity, suggesting that phenolic compounds might not be responsible for this antinociceptive effect.

Antiulcerogenic activity

The PPEA provided significant protection of the gastric mucosa at the highest dose administered, inhibiting the ULI in the experimental model of an ethanol-induced ulcer in rats (Fig. 3). In fact, at 160 mg quantified PC kg^{-1}, the extract reduced the ULI by 76%, differing significantly from the treatment with saline solution (the negative control) ($P < 0.05$). Carbenoxolone, a drug that increases mucus production, reduced ULI by 87.1% at 200 mg kg^{-1}.

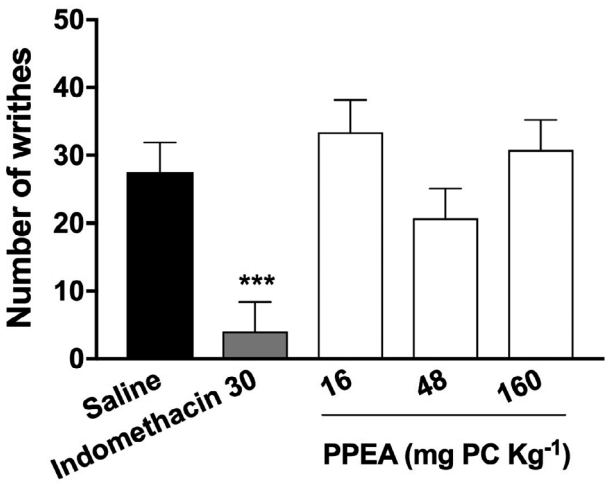

Figure 2 Acetic acid-induced abdominal writhing (0.8% in saline solution) in pretreated mice (60 min) orally with saline solution (10 mL kg^{-1}), indomethacin (30 mg kg^{-1}) and partially purified extract of açai (PPEA, 16, 48 and 160 mg PC kg^{-1}). The results are expressed as the mean±standard deviation (ANOVA: $F(4,25) = 41.77$. Tukey test ****$P < 0.0001$).

Several studies showed attenuation of the ethanol-induced stomach lesions in rats by flavonoids, such as anthocyanin-enriched extracts of strawberry (*Fragaria spp.*) (Alvarez-Suarez *et al.*, 2011) and extract of mangosteen (*Garcinia mangostana),* which have cinnamic acid derivatives and flavonoids (Nainwal *et al.*, 2010). Phenolic compounds, especially flavonoids, but also C-3-G have been reported to prevent or reduce gastric lesions induced by different ulcerogenic agents (Li *et al.*, 2008; Cury *et al.*, 2020; Serafim *et al.*, 2020). The mechanisms of action responsible for the antiulcerogenic activity of flavonoids are the elimination of free radicals, the neutralising capacity of reactive oxygen species, the transition of metal ion chelators, the inhibition of oxidant enzymes, the increase in protein and non-protein antioxidants and the reduction of lipid peroxidation (Serafim *et al.*, 2020). In order to establish the cytoprotective mechanism of the antiulcerogenic activity of PPEA, we performed a series of experiments blocking different protective factors and asking if PPEA would still display its gastroprotective effects. For that, a dose of 64 mg quantified PC kg^{-1} was used, which is the ED_{50} for the antiulcerogenic effect.

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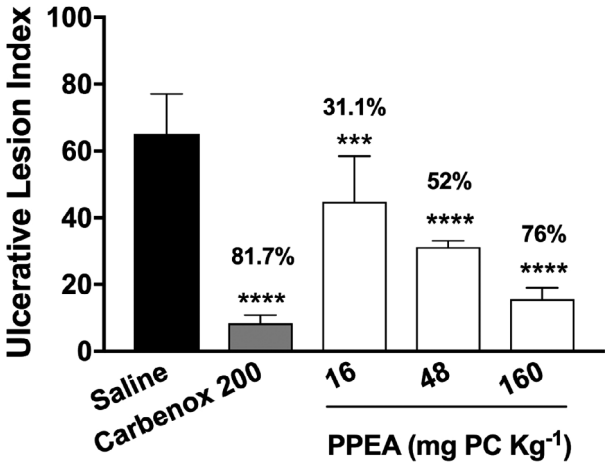

Figure 3 Effect of the oral administration of a saline solution (10 mL kg^{-1}), carbenoxolone (carbenox, 200 mg kg^{-1}) and partially purified extract of açai (PPEA, 16, 48 and 160 mg PC kg^{-1}) in ethanol-induced gastric ulcers in rats. The results are expressed as the mean followed by their respective standard deviations and % gastroprotection. (ANOVA: $F(4,35) = 58.36$. Tukey test ***$P < 0.001$; ****$P < 0.0001$).

Participation of sulfhydryl substances in gastric cytoprotection

The PPEA (64 mg quantified PC kg^{-1}), when administered to animals previously treated with NEM, resulted in an increase in ULI and the disappearance of the gastroprotective activity, with an ulcerative inhibition index of 7.4% and 30.7%, respectively (Fig. 4a). Sousa *et al*. (2018) observed strong participation of the endogenous sulfhydryl (SH) in the gastroprotective effect with *Pilosocereus gounellei* hypothesised to be due to flavonoids present in the extract. In a previous study, the gastroprotective effect of a flavonol diglucoside extract from *Equisetum palustre* might partially be through endogenous SH (Yesilada & Gurbuz, 2010). Usually, the damage growth is accompanied by a decrease of the concentration of mucosal NP-SH compounds (Vendramini-Costa *et al*., 2014; Serafim *et al*., 2020). These data suggest that the gastric mucosa cytoprotection mechanism of PPEA is related to the participation of SH compounds in this experimental model.

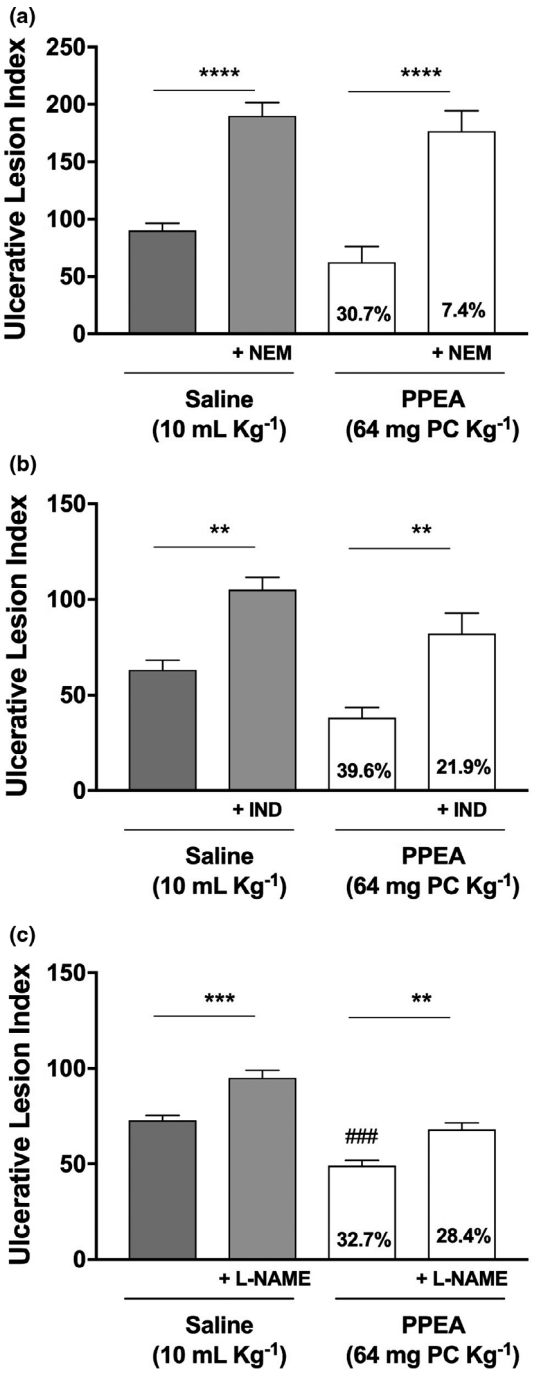

Figure 4 Effect of the oral administration of PPEA (64 mg PC kg^{-1}) and saline solution (10 mL kg^{-1}), as a negative control, on the ULI (mean±standard deviation) and gastroprotection. Protection against ethanol-induced stomach ulcers in rats pretreated with (a) N-ethylmaleimide (NEM, an inhibitor of sulfhydryl compounds) given by subcutaneous injection (10 mg kg^{-1}), (ANOVA: $F(3,28) = 23.44$); (b) indomethacin (a prostaglandin synthesis inhibitor) (10 mg kg^{-1}, ip), (ANOVA: $F(3,28) = 15.34$; (C) L-nitro-arginine methyl ester (L-NAME, an inhibitor of NO synthesis) given by intravenous injection (5 mg kg^{-1}), (ANOVA: $F(3,28) = 32.99$. Tukey test **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$). Gastroprotection data related to the respective control groups (saline + pre-treatment versus PPEA + pre-treatment, saline only versus PPEA only). *compared with its respective control groups, as described above. # compared to control saline only.

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Participation of prostaglandins in gastric cytoprotection
The antiulcerogenic activity of the extract administered after pre-treatment with indomethacin was 21.9%, whereas, without prior pre-treatment, the gastroprotection was 39.6%, indicating the participation of prostaglandin synthesis in the protective effect of PPEA (Fig. 4b). The effect herein observed may be due to the presence of flavonoids or other polyphenols, such as anthocyanins, which in previous studies have been shown to affect prostaglandin production in different models (Nainwal *et al*., 2010; Serafim *et al*., 2020). The possible mechanism that could justify the gastroprotective effects of phenolic compounds is the inhibition of cyclooxygenase (COX), which is involved in the production of proinflammatory mediators, such as prostaglandins. Anthocyanins decrease COX-2 expression and, in the other hand, are less active in COX-1, which is mainly responsible for the synthesis of cytoprotective prostaglandins in the gastrointestinal tract (Schauss *et al*., 2006).

Participation of nitric oxide in gastric cytoprotection
The gastroprotective effect of PPEA was not altered by blocking nitric oxide activity (a protective effect of 32.7% compared with 28.4% in animals pre-treated with L-NAME), demonstrating involvement of this pathway, at least in part, in the observed cytoprotection effect (Fig. 4c). In accordance, a study evaluating the gastroprotective effects of the ethanol extract from *Pilosocereus gounellei* also demonstrates the participation of NO in the gastric protection due to flavonoids present in the extract (Sousa *et al*., 2018).

Determination of the gastric secretions
In the pylorus ligation model, PPEA did not promote any significant alterations in the volume of secretions, pH, or in the hydrogen ion concentration at a dose of 64 mg quantified PC kg^{-1} when compared with the control group. As expected, the positive control Cimetidine significantly reduced the secretion volume and the gastric acidity by 33% and 62%, respectively (Table 1). These results suggest that the anti-secretory property was not involved in the antiulcerogenic activity of the extract.

Table 1 Effect of the intraduodenal administration of the partially purified extract of *E. oleracea* (ED_{50}, 64 mg PC kg^{-1}) by volume, pH and hydrogen ion concentration of gastric contents in the pylorus ligation model

Treatment (mg kg^{-1})	Volume Secretion (mL)[a]	pH[b]	[H+] (mEq mL^{-1} (4 h)$^{-1}$)[c]
Saline (10 mL kg^{-1})	3.16 ± 0.15	3.12 ± 0.12	0.0226 ± 0.0019
Cimetidine (100)	2.12 ± 0.11*	5.40 ± 0.34**	0.0086 ± 0.0012**
PPEA (64)	2.97	3.07	0.0189

Duncan's test *$P < 0.05$, **$P < 0.001$, significantly different from groups treated with saline; PPEA: partially purified extract of açai.
[a]ANOVA: $F(2,16) = 3.7479$, $P < 0.05$.
[b]ANOVA: $F(2,21) = 30.908$ $P < 0.001$.
[c]ANOVA: $F(2,20) = 15.107$, $P < 0.001$.

Conclusions

Our study suggests that the partially purified extract of açai (PPEA), high in phenolic compounds presents antiproliferative and gastroprotective effects and does not present acute toxicity. For the first time, we can suggest that the phenolic compounds present in the PPEA has an important contribution to the bioactivity linked to antiproliferative activity and gastroprotective effect of the *E. oleracea*, as this extract was absent of other compounds commonly found in extracts and pulp, for example fatty acids, amines and others. Further investigations are required to determine the individual contribution of the other compounds present in açai to its bioactivity. The results herein described confirm the nutraceutical potential of PPEA and supports its use as a food additive containing natural violet colourant. In this sense, further studies could evaluate the mechanism of action and the potential of açai to prevent side effects of other medication regimens (such as anticancer chemotherapy or radiotherapy) to the gastric mucosa.

Acknowledgements

The authors are grateful to CAPES, CNPq, FAPESPA and the Amazon Fund of Banco Nacional do Desenvolvimento Econômico e Social all from Brazil, for their financial support. The authors thank the technical support offered by Sirlene V. Tinti and Ana L.T.G. Ruiz from the CPQBA, University of Campinas (UNICAMP) and the MASSMET platform of UCLouvain.

Author contribution

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Conflicts of interest

The authors declare that no conflicts of interest exist.

Ethical approval

All procedures performed in the studies involving animals were in accordance with the Ethical Principle for Animals adopted by the National Council for Control of Animal Experimentation (CONCEA) and was approved by the Ethic Committee on Animal Use of the Federal University of Para (CEUA/UFPA, number 2306280720).

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15332.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Methods S1. Materials and methods.

Table S1. Phenolic compounds (PC) identified and quantified in the partially purified extract of *E. oleracea* by UHPLC-DAD-LTQ-Orbitrap MS-MS/MS.

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