In vitro antioxidant and α-amylase inhibitory activity of extracts from peel and pulp of *Chrysophyllum cainito* cultivated in the Mexican southeast

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Summary

Four extracts of *Chrysophyllum cainito* L. were made, namely, ethanolic extract of pulp (EEPC), ethanolic extract of peel (EECC), aqueous extract of pulp (EAPC) and aqueous extract of peel (EACC). The content of phenols and flavonoids, as well as antioxidant activity and in vitro antidiabetic activity, were determined for these extracts. EECC showed a higher content of phenolic compounds and total flavonoids compared to the pulp extracts, specifically 5.16 ± 0.13 g.kg^{-1} (expressed as gallic acid equivalents) and 4.71 ± 0.18 g.kg^{-1} (expressed as catechin equivalents), respectively. Regarding the antioxidant activity, EECC free radical scavenging of 78.8 ± 0.1 % was determined by the 2,2-diphenyl-1-picrilhidrazyl (DPPH) assay and 80.3 ± 0.1 % by the 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. EECC had the highest inhibition index on the digestive enzyme α-amylase (87.7 ± 8.3 %). The *Chrysophyllum cainito* fruit was found to possess a free radical donor capacity and inhibitory activity of amylolytic enzyme, important in control of diabetes mellitus and its complications.

Keywords

Chrysophyllum cainito; phenolic; flavonoid; antioxidant activity; α-amylase

Chrysophyllum cainito L. is an evergreen tree, characteristic by its latex-producing bark, leaves and immature fruits that are harvested twice a year (November to February and April to July) [1]. This tree is native to Central America and is mainly distributed in tropical areas. It is further cultivated throughout Southeast Asia, tropical West Africa and the warmer parts of India for its ornamental value and production of large edible fruits [2]. The fruits are berries, flattened globose, typically measure 5–12 cm in diameter, contain six to 10 lustrous, flattened brown seeds, the fruits remain on the tree even after ripening, even drying on the branches if not harvested [3]. The exocarp or pulp is fleshy, white or lilac, edible, pleasant tasting, sticky from latex, gelatinous and with greenish or purplish coloured leathery epicarp and rubbery latex [1, 3] (Fig. 1). The fruit of *C. cainito* has common names such as "apple star", "caimito", "abiu-roxo" or "abiu-do-Pará". Traditionally, it is consumed fresh and also in preserves and beverages [4, 5].

There are several traditional medicine reports indicating that the bark, leaves, fruits and seeds of the genus *Chrysophyllum* exert a pronounced spectrum of biological activity [4]. Various parts of *C. cainito*, namely, fruits, bark and leaves, have been used for their specific properties, e. g. antitussive, astringent, antihypertensive, antidiabetic, antidiarrheal, anti-inflammatory, antioxidant, wound healing, antibacterial, antidiarrheal, antidiabetic, to control fever, gonorrhea, vesical ca-

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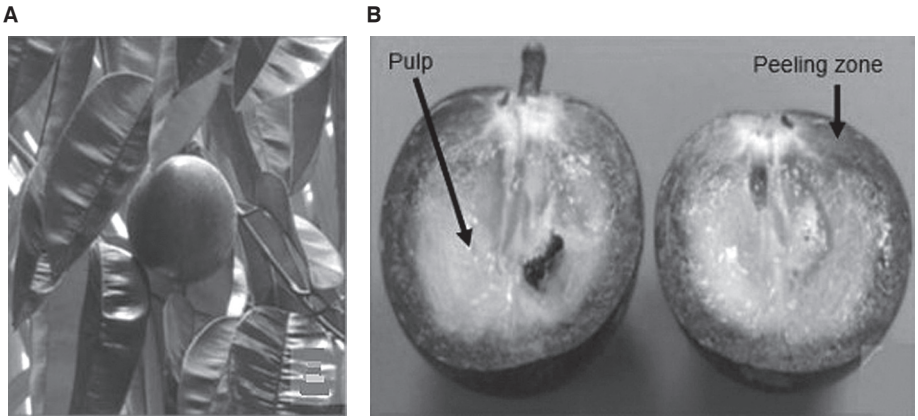

Fig. 1. Fruit of *Chrysophyllum cainito*.
A – exocarp, B – endocarp.

tarrh and abscesses [4–6]. The fruit of *C. cainito* is usable in food, with sweet and pleasant taste. Moreover, it is used in traditional medicine for the treatment of a wide variety of illnesses, however. Scientific studies on the plant are scarce, either in relation to its secondary metabolites, chemical composition or pharmacological activity [4, 6].

Various phenolic compounds were identified in the fruit of *C. cainito* including catechin, epicatechin, gallocatechin, epigallocatechin, quercetin, quercitrin, isoquercitrin, micricitrin and gallic acid. The antioxidant activity of these compounds may promote beneficial effects on human health and prevent or treat oxidative stress-associated pathologies [2]. However, not all of its properties are known and more studies are needed to characterize the nutritional and biological properties of this fruit, in order to promote new alternatives for its application [7].

Hence, this study aimed to perform a proximate chemical analysis of freeze-dried pulp and peel of the *C. cainito* fruit and determine the content of chlorogenic acid, phenolic compounds, total flavonoids, as well as to evaluate the antioxidant and antidiabetic activity of aqueous and ethanolic extracts of the pulp and peel of *C. cainito.*

MATERIALS AND METHODS

Sample collection

The fruits of *C. cainito*, purple variety, were collected between the March 2019 and February 2020 in the municipality of Tacotalpa, Tabasco, México. One hundred fruits were harvested at ripeness optimal for consumption. They were washed for 15 min by immersion in an active chlorine solution (50 $\mu g \cdot ml^{-1}$). The pulp and peel were manually removed from the fruit using a stainless steel knife. The pulp and peel were separated to be treated with sodium metabisulfite (0.15 $g \cdot l^{-1}$) as an antimicrobial agent for 5 min, which was removed by centrifugation at 15 000 $\times g$ for 15 min at 10 °C in a centrifuge (Hermle, Gosheim, Germany). Subsequently, the supernatant was obtained and frozen for freeze-drying using a cascade lyophilizer (Labconco, Kansas City, Kansas, USA) at temperature of 40 °C and pressure of 0.140 Pa. The freeze-drying process lasted 72 h until the maximum amount of water was eliminated, then the freeze-dried samples were kept in amber-coloured containers and stored at a temperature of 4 °C for a maximum of two weeks.

Proximate analysis

The proximate analysis was performed according to the methodology proposed by AOAC [8]. Six parameters were determined, namely, moisture, total ash, crude fat, crude protein, crude fibre and nitrogen-free extract.

An amount of 5 g of sample was weighed in an aluminium tray (constant weight) and placed in a drying oven with analogous control HS35-EA (Novatech, Guadalajara, Jalisco, Mexico) during 24 h at 60 °C. When the weight of the sample remained constant, the moisture percentage was calculated.

For the determination of total ash, 5 g of the sample was placed in crucibles (constant weight) and was incinerated in a muffle furnace (Büchi, Flawil, Switzerland) at a temperature of 500–550 °C for 3 h. After this process, it was cooled to estimate the percentage by weight difference.

The percentage of crude fat was determined by

the Soxhlet method in a Goldfisch fat extraction apparatus model 3500100 (Labconco, Kansas City, Missouri, USA), where it was extracted for 3–5 h and then the flask was placed in a drying oven to evaporate the residual hexane.

The percentage of fat was determined by loss of sample weight or by amount of sample removed.

For crude protein determination, 50–100 mg of dry and defatted sample was weighed and analysed by the micro-Kjeldahl method [8], which consisted of three steps: digestion, distillation and titration.

Crude fibre was determined by weighing approximately 2 g of defatted sample, which was boiled for 30 min with 200 ml of 1.25% sulfuric acid (Sigma-Aldrich, St. Louis, Missouri, USA) subsequently washed 3 times with hot distilled water and the solids were separated with Oklahoma stainless steel filter model 5521000 (mesh 74 μm, (Labconco) and 3 further washes were carried out with hot distilled water. Then, 200 ml of 1.25% NaOH (Sigma-Aldrich) were added and sample was boiled for 30 min. Then, it was washed and filtered as indicated above; the solids were dried at 105 °C for 12 h, weighed and incinerated in a muffle furnace at 550 °C for 3 h. After this process they were cooled to estimate the percentage by weight difference.

The nitrogen-free extract content was obtained by subtracting the sum obtained in the analysis of moisture, ash, crude fat, crude protein and crude fibre from 100 % of the sample.

Determination of reducing sugars

The determination of reducing sugars was performed using the 3,5-dinitrosalicylic acid (DNS) (Sigma Aldrich) technique [9], which consists of determining the presence of free carbonyl groups (C=O) of reducing sugars. The procedure is based on a redox reaction, which occurs by DNS to provoking oxidation of sugars and, at the same time, its own endothermic reduction, where one mole of sugar reacts with one mole of DNS, giving rise to a stoichiometric reaction, which allows to know the amount of reducing sugars present in the sample. The standard glucose solutions 1 mg·ml^{-1} was prepared with distilled water, the DNS solution was added and the standard curve was made. Subsequently, the DNS solution was applied to each of the samples (0.1 mg of freeze-dried sample in 100 ml of distilled water) and placed to boil for 10 min, immediately after which water was added and stirred for 15 min. Absorbance was measured at 540 nm in Evolution 220 UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Preparation of extracts

Aqueous and ethanolic extracts were made at 40 g·kg^{-1} of freeze-dried *C. cainito* pulp or peel [10]. The extraction was carried out in 50 ml beakers, in a heated circulator water bath (Novatech) at 40 °C with continuous agitation for 3 h. Then, the extracts were centrifuged at 2 700 ×*g* for 30 min at 10 °C. The supernatants were recovered, the procedure was repeated twice and the extract was filtered through Whatman No. 1 filter paper (Whatman, Maidstone, United Kingdom) under vacuum. The samples were then stored in amber containers at 4 °C for further analysis for a maximum of two weeks. Four extracts were obtained: ethanolic extract of caimito pulp (EEPC), aqueous extract of caimito pulp (EAPC), ethanolic extract of caimito peel (EECC) and aqueous extract of caimito peel (EACC).

Determination of total phenolics content

The total phenolics content (*TPC*) of *C. cainito* pulp and peel was determined by the method described by Kim et al. [11], for which 0.125 ml of the extract previously mixed with 0.625 ml of Folin-Ciocalteu reagent (Sigma Aldrich) and 0.5 ml of 7.5% Na_2CO_3 (Sigma Aldrich) were used. After 45 min of incubation at room temperature while protected from light, absorbance was measured at 760 nm with Evolution 220 UV-Vis spectrophotometer. Phenolics content was calculated using a standard curve constructed with gallic acid (Sigma-Aldrich) as a standard. Results were reported as gallic acid equivalents (GAE) in grams per kilogram of dry extract.

Determination of total flavonoids content

The total flavonoids content (*TFC*) was determined by the method proposed by Barros et al. [12], based on the formation of a flavonoid-aluminium complex. Volumes of 0.5 ml of extracts were added to 2 ml of distilled water and 0.15 ml of 5% $NaNO_2$ (Sigma Aldrich) solution was added, stirred and left to stand for 6 min. Then, 0.15 ml of 10% $AlCl_3$ was added, stirred and left to stand for 6 min and 2 ml of 4% NaOH was added to the mixture and topped up with water until a final volume of 5 ml was obtained. The absorbance of the reaction was measured at 510 nm with a Evolution 220 UV-Vis spectrophotometer. Total flavonoids content was calculated with a standard curve constructed using catechin (Sigma-Aldrich) as a standard. Results were reported as catechin equivalents (CE) in grams per kilogram of dry extract.

Determination of chlorogenic acid

The colorimetric assay for the determination

of chlorogenic acid was performed by the procedure reported by Díaz-Orduño et al. [13]. It consisted of mixing 400 μl of extract with 400 μl of 0.17 mol·l^{-1} urea and 400 μl of 0.1 m·l^{-1} acetic acid (both Sigma Aldrich), This was shaken for 15 s, 400 μl of 0.14 mol·l^{-1} $NaNO_2$ was added and incubated for 2 min at 25 °C, in the absence of light. Finally, 400 μl of 0.5 mol·l^{-1} NaOH was added and centrifuged at 5 000 ×*g* at 25 °C for 10 min. To quantify the concentration, a calibration curve was prepared from 0 μg·ml^{-1} to 250 μg·ml^{-1} of chlorogenic acid (Sigma Aldrich) and absorbance was measured at 510 nm with a Evolution 220 UV-Vis spectrophotometer. Content of chlorogenic acid was expressed as chlorogenic acid equivalents (CAE) in grams per kilogram of dry extract.

DPPH assay

To determine the free radical-scavenging capacity of the extracts, the method proposed by Shimada et al. [14] was used, which is based on the use of 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH). Aliquots of 150 μl of each extract were taken and mixed with 1 350 μl of 0.1 mmol·l^{-1} DPPH (Sigma Aldrich) in ethanol (Sigma Aldrich) The mixtures were mixed with vortex for 20 s and allowed to react for 30 min at 25 °C protected from light. After the reaction was completed, the absorbance was determined on a Evolution 220 UV-Vis spectrophotometer at a wavelength of 517 nm. Ascorbic acid at a concentration of 0.1 mg·ml^{-1} was used as a positive control. All analyses of the extracts were performed in duplicate. Antioxidant activity was expressed as percentage of free radical capture.

ABTS assay

The method of Pukalskas et al. [15] was used. A 2 mmol·l^{-1} stock solution of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid cation radical ($ABTS^{\cdot+}$) was prepared by dissolving 54.8 mg of ABTS (Sigma Aldrich) in 50 ml of phosphate buffered saline (PBS; 0.01 mol·l^{-1}, pH 7.4). The $ABTS^{\cdot+}$ was obtained by reacting 10 ml of the ABTS stock solution with 40 μl of 70 mmol·l^{-1} $K_2S_4O_8$ (Sigma Aldrich) prepared 16–17 h prior to use. To study the antioxidant compounds, 7 ml of the $ABTS^{\cdot+}$ solution was diluted in 52 ml of PBS to an absorbance of 0.8 ± 0.03 measured at a wavelength of 734 nm. Measurement of the antioxidant activity of the samples was performed by mixing 10 μl of the extract and 990 μl of diluted $ABTS^{\cdot+}$ in 2 ml microtubes and then reading the absorbance at 734 nm after 6 min. Ascorbic acid at a concentration of 0.1 mg·ml^{-1} was used as a positive control. Antioxidant activity was expressed as percentage of free radical capture.

Inhibition of α-amylase

To determine the in vitro α-amylase activity of the extracts, the method Dineshkumar et al. [16], based on α-amylase (EC 3.2.1.1) inhibition was employed. A volume of 200 μl of starch (Maizena, Glen Cove Starch Manufacturing, Whasburn, Illinois, USA) was added in tubes and incubated in a heated circulator water bath at 100 °C for 5 min and subsequently incubated at 37 °C for 5 min. A volume of 200 μl of 50% dimethyl sulfoxide (DMSO; Sigma Aldrich), 200 μl of various fruit extracts dissolved in water or 95% ethanol depending on the type of extract, 200 μl of porcine pancreatic α-amylase (2 U·ml^{-1}), 100 μl of buffer 0.5 mol·l^{-1} Tris-HCl (Sigma Aldrich) with 0.01 mol·l^{-1} NaCl (Sigma Aldrich), pH 6.9 and 500 μl of 0.1% DNS were added. The mixtures were allowed to react for 10 min at 100 °C and allowed to cool to at 25 °C during 15 min. Acarbose (Bayer, Leverkusen, Germany) at a concentration of 150 µg·ml^{-1} was used as a positive control. The absorbances of the mixtures were determined using Evolution 220 UV-Vis spectrophotometer at a wavelength of 540 nm.

Experimental design and data analysis

The study was performed in two phases. In the first phase, the proximate chemical composition and the content of total phenolics, flavonoids and chlorogenic acid were determined. In the second phase, the antioxidant and antidiabetic activity of each type of *C. cainito* extract (EEPC, EAPC, EECC and EACC) was evaluated, which constituted the response variables.

For the interpretation of the results, ANOVA was applied for a completely randomized design ($P \leq 0.05$). In order to establish significance of the differences between treatments, Tukey's mean comparison technique was applied at $P \leq 0.05$. For this, Prism 5 software (GraphPad, San Diego, California, USA) was used.

Tab. 1. Results pf proximate analysis of freeze-dried caimito peel and pulp.

Parameter	Peel [%]	Pulp [%]
Moisture	1.6 ± 0.2 ^{a}	3.6 ± 0.1 ^{b}
Ash	3.2 ± 0.4 ^{a}	1.9 ± 0.2 ^{b}
Crude fat	22.0 ± 2.8 ^{a}	8.6 ± 1.8 ^{b}
Crude protein	3.3 ± 0.1 ^{a}	3.3 ± 0.1 ^{a}
Crude fibre	45.7 ± 8.9 ^{a}	7.4 ± 4.4 ^{b}
Nitrogen free extract	24.2 ± 0.2 ^{a}	75.3 ± 0.2 ^{b}

Different letters in superscript in the same row indicate significant statistical differences ($P < 0.05$).

RESULTS AND DISCUSSION

Proximate analysis

The freeze-dried pulp of *C. cainito* presented a higher moisture content compared to the peel (Tab. 1). In the freeze-dried peel, a higher fat and fibre content of 22.0 % and 45.7 %, respectively, was found, with a significant statistical difference between both fruit parts. The results showed that C. *cainito* peel had higher fibre content (45.7 ± 8.9 %) compared to the pulp. PARKER et al. [17] reported that the covering peel of fruits is important mainly for insoluble fibre, which should be consumed in greater amounts. The protein content reported in the present study was 3.3 ± 0.1 % and 3.3 ± 0.1 % for peel and pulp, respectively. These results indicated that the fruit contained acceptable amounts of protein to help to avoid protein malnutrition, often occurring in third-world countries. The high ash content in the peel can promote, upon consumption, peristalsis and provide minerals to the body [17].

Present data are similar to those published by HERNÁNDEZ SÁNCHEZ et al. [18], who analysed *C. cainito* and reported moisture, ash and protein percentages of 9.2 %, 2.3 % and 2.2 %, respectively. In another study, the proximate composition of fresh pulp of *C. cainito* included contents of moisture (75.9 ± 4.3 %), fibre (2.3 ± 0.2 %), protein (1.9 ± 0.2 %) and ash (0.6 ± 0.1 %) [19]. The low content of moisture found in the present study in both parts of the fruit could be associated with the water removal process during freeze-drying, which can extract more than 95 % of water content from food, while maintaining the physical and physicochemical properties related to its quality [20]. On the other hand, the high lipid content in the peel may represent not only the fat, but also the content of extractable substances such as fat-soluble vitamins and natural pigments, which could be concentrated during the freeze-drying process [21].

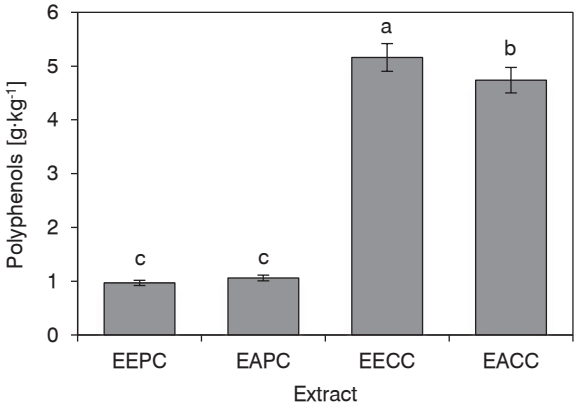

Fig. 2. Total phenolics content of *C. cainito* peel and pulp extracts.

Different letters above columns indicate significant statistical differences ($P < 0.05$).
EEPC – ethanolic extract of pulp, EECC – ethanolic extract of peel, EAPC – aqueous extract of pulp, EACC – aqueous extract of peel.

Reducing sugars

Reducing sugars in pulp and peel of *C. cainito* were contained at 207 ± 0.57 g·kg^{-1} and 210 ± 1.99 g·kg^{-1} dry weight, respectively, with no significant differences between the two parts of the fruit ($P < 0.05$). In the ripening phase of a fruit, the content of reducing sugars such as glucose and fructose increases significantly. This is due to the breakdown of starch into monomers during fruit ripening [1]. The results obtained in this study for reducing sugars analysis are similar to the data published by WILLIAMS and BENKEBLIA [1], who reported an increase in reducing sugars during ripening of *C. cainito* from 103.24–245 g·kg^{-1} fresh weight (green variety) and from 83.13 g·kg^{-1} to 260.74 g·kg^{-1} fresh weight (purple variety), with an increase during ripening.

Total phenolics content

Fig. 2 shows that the EECC had a higher *TPC* (5.2 ± 0.13 g·kg^{-1}), which was statistically different from *TPC* found in EACC (4.7 ± 0.05 g·kg^{-1}). It was also observed that the contents of phenolics found in the aqueous and ethanolic extracts of *C. cainito* pulp were not statistically significantly different from each other. EECC of *C. cainito* showed the highest *TPC*, compared to the other analysed extracts, although lower than the values reported by KUBOLA et al. [22], who analysed *C. cainito* pulp and reported *TPC* of 17.88 g·kg^{-1}. In another study, NINGSIH et al. [23], reported *TPC* of 132.36 ± 0.09 g·kg^{-1} in an ethanolic extract of fresh *C. cainito* pulp. DA ROSA et al. [2] reported for a methanolic extract of *C. cainito* peel *TPC* of 0.039 ± 2.10 g·kg^{-1} and for pulp 0.01 ± 0.05 g·kg^{-1} expressed as tannic acid equivalents. Based on these results, it can be inferred that the peel is the part of *C. cainito* with the highest content of phytochemicals, representing an excellent source of phenolic compounds, which confer the specific purple colour in this variety, making it attractive to the consumer [24, 25], It is important to mention that these compounds have unique chemical structures and are potentially useful due to their pharmacological properties when consumed periodically [26].

In all the studies discussed, alcoholic extracts

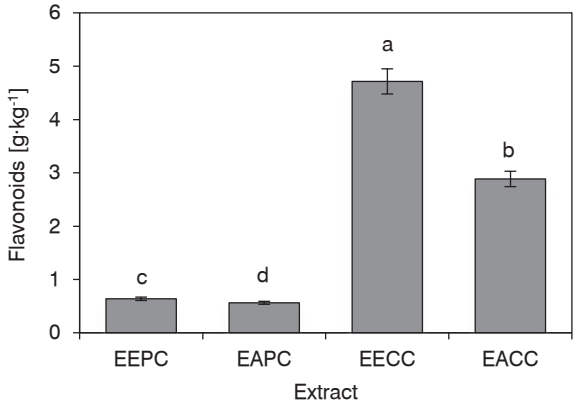

Fig. 3. Total flavonoids content of *C. cainito* peel and pulp extracts.

Different letters above columns indicate significant statistical differences ($P < 0.05$).
EEPC – ethanolic extract of pulp, EECC – ethanolic extract of peel, EAPC – aqueous extract of pulp, EACC – aqueous extract of peel.

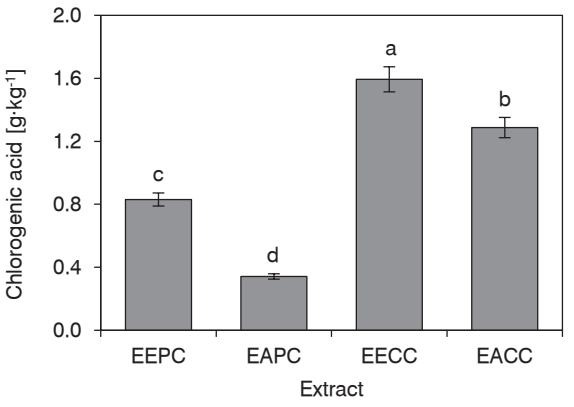

Fig. 4. Chlorogenic acid content of *C. cainito* peel and pulp extracts.

Different letters above columns indicate significant statistical differences ($P < 0.05$).
EEPC – ethanolic extract of pulp, EECC – ethanolic extract of peel, EAPC – aqueous extract of pulp, EACC – aqueous extract of peel.

showed the highest *TPC* values. This may be due to the fact that solvents with intermediate polarity are usually mostly used for the extraction of phytochemicals. Ethanol is a solvent with intermediate polarity that has been used for the extraction of phenolic compounds, flavonoids, catechols and tannins from plant materials [27, 28].

Flavonoids content

Ethanolic extracts of the two parts of the fruit showed higher flavonoid content compared to aqueous extracts (Fig. 3). EECC showed the highest *TFC* compared to all the extracts evaluated ($P < 0.05$). The results of this study showed that EECC of *C. cainito* had the highest *TFC* (4.71 ± 0.18 g·kg^{-1} sample, expressed as CE). However, these values are lower than those reported by Ningsih et al. [5], who found in the ethanolic extract of leaves *TFC* of 8.62 ± 0.10 g·kg^{-1}, expressed as quercetin equivalents (QE). The differences found in phytochemical content could be explained by the fact that parts where a higher *TFC* was found were leaves [29]. On the other hand, a previous study reported a flavonoids content in ethanolic extract of fresh pulp of 0.52 ± 0.01 g·kg^{-1} (expressed as QE) [23]. The present results, regarding the higher *TFC* in *C. cainito* peel, could be associated with the fruit freeze-drying process, which could concentrate the phytochemicals and lead to different results.

Several studies suggested that dietary intake of flavonoids can reduce the risk of diabetes, hypertension and tumours of the breast, colon, lung, prostate and pancreas [25]. This could be associated with their antioxidant and protective activity against oxidative damage.

Chlorogenic acid

Fig. 4 shows that ethanolic extracts of the two fruit parts showed higher chlorogenic acid content compared to aqueous extracts. The ethanolic peel extract of *C. cainito* showed the highest content of this particular phenolic acid among all the extracts evaluated ($P < 0.05$). EECC of *C. cainito* showed a higher chlorogenic acid content of 1.59 ± 0.05 g·kg^{-1} (expressed as CAE), compared to the other extracts analysed. It can be observed in Fig. 2 that EECC also showed the highest *TPC*. In view of the results, it can be inferred that chlorogenic acid is one of the polyphenols found in the highest proportion in the ethanolic extract of *C. cainito* peel. The results on chlorogenic acid content obtained in this study are similar to those reported by Kubola et al. [22], who analysed *C. cainito* pulp and found a chlorogenic acid content of 1.99 ± 0.01 g·kg^{-1}. These results demonstrate that this fruit contains an important amount of chlorogenic acid, which is representative of hydroxycinnamic acids, and is found in foods mainly as an ester with quinic acid and caffeic acid [30]. Dietary polyphenols are beneficial to human health by exerting several biological effects such as free radical scavenging, metal chelation, modulation of enzyme activity and alteration of signal transduction pathways. Chlorogenic acid contains neighbouring hydroxyl groups in an aromatic residue and exhibits antimutagenic, carcinogenic, antioxidant and antidiabetic activi-

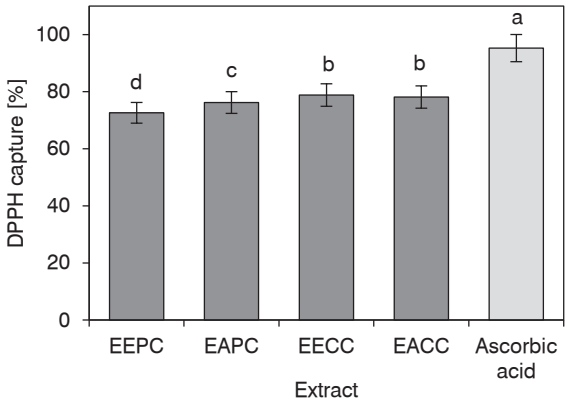

Fig. 5. Percentage of DPPH capture by extracts of *C. cainito* peel and pulp.

Different letters above columns indicate significant statistical differences ($P < 0.05$).
EEPC – ethanolic extract of pulp, EECC – ethanolic extract of peel, EAPC – aqueous extract of pulp, EACC – aqueous extract of peel.

ties in vitro. Therefore; the presence of this acid could play a potential role in the treatment of diabetes [30–32]. The antioxidant capacity of this acid generally depends on the number of hydroxyl groups and the influence of the carboxylate group on the hydrogen donating capacities [33].

DPPH assay

Fig. 5 shows that the highest percentage of free radical scavenging was obtained with the aqueous and ethanolic peel extracts of *C. cainito*, with no significant differences between the two extracts. On the other hand, the aqueous pulp extract revealed higher free radical scavenging compared to the ethanolic extract ($P < 0.05$). EECC and EACC showed free radical scavenging of 78.8 % and 78.1 %, respectively, which was lower than published by Kubola et al. [22] who reported free radical scavenging of 94.4 % for methanolic extract of *C. cainito* pulp. In another study, in which an ethanolic extract of leaves of *C. cainito* was analysed, scavenging percentage of 62.2 % was found [5]. It can be observed (Fig. 2–4) that EECC and EACC revealed higher *TPC*, *TFC* and chlorogenic acid content compared to the other extracts evaluated. Then it can be inferred that the correlation between antioxidant activity and total phenolics and flavonoids contents has been observed again, similar to other studies [34, 35]. The antioxidant activity of flavonoids depends on the functional groups of their basic structure. Several mechanisms of antioxidant activity, such as free radical scavenging and metal ion chelating, are affected by the configuration, substitution and total number of functional hydroxyl groups [5, 36]. Hence, these compounds should be evaluated by at least two measurement systems that can address the different mechanisms [26]. The DPPH method is commonly used for aqueous or organic extracts with hydrophilic and lipophilic compounds, while the ABTS method is generally indicated to evaluate the antioxidant activity of hydrophilic compounds [25]. Differences in percentages of free radical scavenging determined by the DPPH system were observed in the studies mentioned above. Such differences can be explained by the fact that each plant possesses diverse content of secondary metabolites and their concentration is highly correlated to the parameters of location, crop density, growing conditions and solar radiation. Therefore, as higher is the crop stress, higher will be the concentration of secondary metabolites [37].

ABTS assay

Fig. 6 shows that the highest antioxidant activity was determined for EEPC, showing significant differences among the evaluated extracts. On the other hand, EACC revealed higher antioxidant activity compared to EECC, with significant statistical differences between the two types of extracts ($P < 0.05$). EEPC of *C. cainito* showed the highest percentage of free radical reduction of 80.3 %, suggesting that the higher content of hydrophilic antioxidant compounds was present in the pulp extract compared to the other evaluated extracts [33]. These results are similar to those published

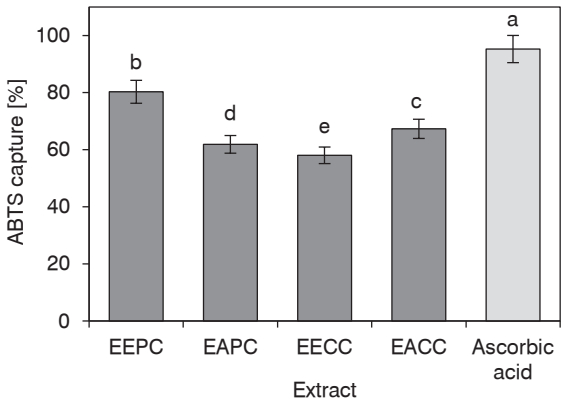

Fig. 6. Percentage of ABTS capture by extracts of *C. cainito* peel and pulp.

Different letters above columns indicate significant statistical differences ($P < 0.05$).
EEPC – ethanolic extract of pulp, EECC – ethanolic extract of peel, EAPC – aqueous extract of pulp, EACC – aqueous extract of peel.
ABTS - 2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulfonic acid).

by LEE et al. [27], who reported a free radical scavenging percentage by the ABTS assay of 97.1 % in ethanolic extract of *Annona muricata*. Other studies reported a free radical scavenging capacity similar to this study, using the ABTS method, in fruits of *Phyllanthus emblica* [38] and *Citrus limon* 'Baladi' [38] (90 % and 63 %, respectively).

Inhibition of α-amylase

Fig. 7 shows that EECC inhibited the in vitro α-amylase activity most strongly, by 87.7 %, followed by EACC with 85.3 % inhibition. No significant differences between the peel extracts were observed. However, the obtained values were higher than those found with EEPC and EAPC (69.7 % and 56.2 %, respectively. When comparing the inhibitory activity between pulp extracts prepared using various solvents, the ethanolic extract presented a higher percentage of inhibition with a statistically significant difference between the two types of extracts ($P < 0.05$).

It is important to highlight that EECC in the present study had higher contents of *TPC*, *TFC* and chlorogenic acid compared to the other evaluated extracts (Fig. 2–4). Wide diversity of natural flavonoids and antioxidants play a pivotal role in lowering blood glucose and exhibit hypoglycemic and antihyperglycemic activities through various mechanisms of action comprising autooxidation of glucose, increasing serum insulin levels, increasing tissue sensitivity to insulin action, activation of NADPH oxidase, electron transport system of mitochondria and decreasing the activity and/or inhibition of hydrolysing enzymes [23, 39]. Flavonoids may act against diabetes mellitus by inhibiting α-amylase activity in the small intestine [40].

Inhibition of enzymes such as α-amylase is an important strategy to reduce postprandial blood glucose levels by slowing down glucose absorption in the small intestine [41]. α-Amylase is one of the enzymes involved in carbohydrate metabolism, in the hydrolysis of 1,4-glycosidic linkages of starch and glycogen, where the enzyme degrades complex oligosaccharides to monosaccharides, which is the form of carbohydrates to be transported from intestine into blood circulation [23].

In another study performed with 10% ethanolic extract of *A. heterophyllus* stems, 58% inhibition of α-amylase was reported [42]. Such concentration is much higher than the concentration used in the present study, however, different plants and types of plant material were studied. Based on these results, it was suggested that *C. cainito* is a medicinal fruit with antioxidant and amylolytic enzyme inhibitory potential [23].

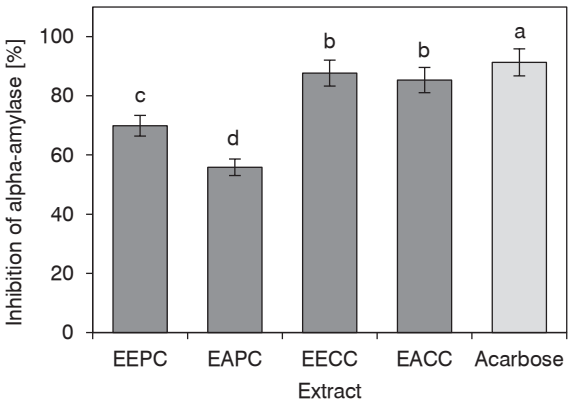

Fig. 7. Percentage of inhibition of α-amylase by *C. cainito* peel and pulp extracts.

Different letters above columns indicate significant statistical differences ($P < 0.05$).
EEPC – ethanolic extract of pulp, EECC – ethanolic extract of peel, EAPC – aqueous extract of pulp, EACC – aqueous extract of peel.

CONCLUSIONS

The ethanolic extract of *C. cainito* peel (EECC) showed a higher content of *TPC*, *TFC* and chlorogenic acid, in addition to a strong antioxidant potential and higher α-amylase inhibitory activity, when compared to the other extracts. It is also suggested that *C. cainito* peel is a source of natural antioxidants that may decrease premature aging, in addition to the inhibition of α-amylase activity that may serve as a practical therapeutic approach to reduce postprandial hyperglycemia, diabetes mellitus and its complications. Hence, regular consumption of herbs and fruits such as *C. cainito* represent an advantage of reduced costs in comparison with chemically synthesized medications, as well as less side effects such as flatulence, diarrhea, fatigue and stomach discomfort. However, to the above, it is important to carry out complementary studies to determine the profile of the analytes related to the activities found in *C. cainito* peel. Natural antioxidants, particularly in fruits and vegetables such as *C. cainito*, have gained increasing interest among consumers and the scientific community, as epidemiological studies indicated that frequent consumption of natural antioxidants is associated with a lower risk of chronic non-communicable cardiovascular diseases and cancer.

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