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HEALTH SCIENCES

Phytoconstituents, antioxidant and antiglycation activity of *Chrysophyllum cainito* L., *Hancornia speciosa* Gomes and *Plinia glomerata* Berg. fruits

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Abstract: The present study verified the presence of phytoconstituents and evaluated antioxidant (DPPH, FRAP, NO and TBARS tests) and antiglycation (REM test) activities of unconventional wild edible fruits *Chrysophyllum cainito*, *Hancornia speciosa* and *Plinia glomerata*. It was verified the presence of phenolic compounds for all fruits and flavonoids were observed only for *C. cainito*, which presented in its peel the highest total phenols (90.34 µg GAE mg^{-1}) and flavonoids (30.4 µg RE mg^{-1}) content. Sugar concentration was significant for all fruits, where *H. speciosa* showed the highest reducing sugar content (576.12 mg g^{-1}) and *C. cainito* pulp showed the highest total sugar content (858.67 mg g^{-1}). All fruits presented vitamin C and carotenoids, highlighting *P. glomerata* with the best results for ascorbic acid (2260.94 mg 100 g^{-1}) and carotenoids (59.62 µg g^{-1}). Extracts presented antioxidant activity, highlighting *C. cainito* peel that presented 65.64% (DPPH), 231.34 µM TE L^{-1} (FRAP), 49.34% (NO) and 22.56% (TBARS), while in antiglycation evaluation, *P. glomerata* showed evident activity. Therefore, it was possible to determine different phytoconstituents, and antioxidant and antiglycation activities of the fruits. These data provide subsidies for application of these fruits in new studies, to increase knowledge and preservation of these species.

Key words: Ascorbic acid, carotenoids, flavonoids, polyphenols, wild fruits.

INTRODUCTION

Recent studies demonstrate the importance of unconventional species included in human feeding that have been showing to be sources of macro- and micronutrients capable to supply dietary needs. Besides wild fruits may act as functional foods as they possess phytocompounds, vitamins, peptides and sugars physiologically active capable to prevent and/or treat different diseases related to oxidative stress and protein glycation (Liu et al. 2018, Berni et al. 2019, Hegazy et al. 2019). Fruits with excellent color, sweetness and aroma, represent an important source of research to new foods with high nutritional and functional content. Studies of fruits phytocomponents are promising for application and use in food, cosmetic and pharmaceutical markets (Ming 1996, Franzon et al. 2004). Some of these species are *Chrysophyllum cainito* L., *Hancornia speciosa* Gomes and *Plinia glomerata* Berg.

Chrysophyllum cainito belongs to Sapotaceae family and it is commonly known as Star Apple (Morton 1987) (Figure 1a). The fruits are pear-shaped (5-10 cm in diameter), red-purple or pale green, their pulp is smooth, sweet and pleasantly aromatic (Parker et al. 2010). The nutritional analysis of this fruit showed the presence of vitamins as carotene, thiamine, riboflavin, niacin and ascorbic acid.

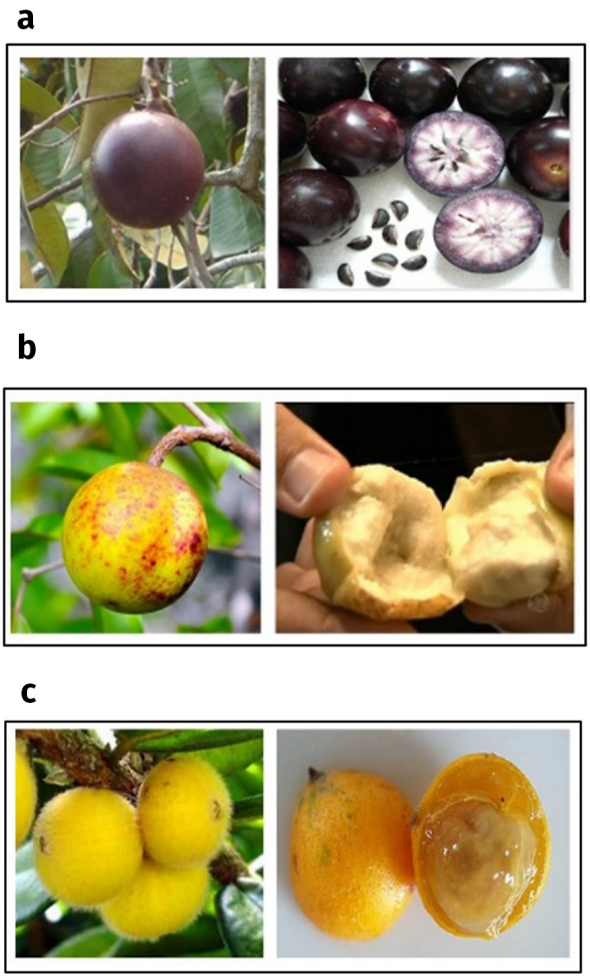

Figure 1. Unconventional wild edible fruits (peel and pulp) *Chrysophyllum cainito* L. (a), *Hancornia speciosa* Gomes (b) and *Plinia glomerata* Berg. (c).

This fruit is also used in popular medicine as anti-inflammatory for respiratory system, anti-hypersensitive and in the treatment of diabetes mellitus (Luo et al. 2002, Meira et al. 2014). Phytochemical studies have demonstrated the presence of several bioactive compounds in different plant parts, mainly phenolic compounds, alkaloids, flavonoids, steroids, saponins, tannins, and cardiac glycosides (Oranusi et al. 2015, Doan & Le 2020)

Hancornia speciosa belongs to Apocynaceae family and it is known as Mangaba (Figure 1b), mainly located in the brazilian Cerrado region, with fruits that are generally yellowish when mature, their pulp is eaten fresh and used to make ice cream, juices and jellies by the local population (Rodrigues et al. 2007). This unconventional fruit is rich in vitamin C and it has catechin and proanthocyanidins in the latex obtained from its stem (Ganga et al. 2009, Santos & Silva 2016). In popular medicine, the infusion from the barks and leaves are used against gastric disorders and the latex is used to treat tuberculosis (Sampaio & Nogueira 2006). Studies have showed this plant has active compounds with gastroprotective (Moraes et al. 2008) and hypotensive actions (Silva et al. 2011), potential anti-diabetic (Pereira et al. 2015), and the fruit juice decrease pulmonary edema induced by scorpion venom (Yamashita et al. 2020). According to phytochemical studies performed with this species, different fruit extracts present phenolic acids (gallic acid, chlorogenic acid, vanillic acid, o-coumaric acid and rosmarinic acid) and flavonoids (quercetin, rutin and catechin), being chlorogenic acid and rutin the predominant compounds (Narain et al. 2018, Yamashita et al. 2020).

Plinia glomerata (synonymy: *Eugenia cabelludo* and *Myrciaria glazioviana*) belongs to Myrtaceae family and it is known as "Cabeludinha" due to its hairy appearance (Figure 1c), it is a brazilian native plant widely distributed in the south of Brazil (Serafin et al. 2007). This fruit is rounded, juicy, pleasant and slightly acidic, and it has a yellow color with high ascorbic acid content when mature. Unfortunately, this species is little known in Brazil (Lorenzi 2009). Recent study is highlighting its analgesic and antimicrobial properties (Pacheco-Silva & Donato 2016). Species of this genus showed antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic, antifungal, antibacterial and gastroprotective activities (Borges et al. 2014). Different studies have analyzed phytochemical profile of the fruit extracts of this species and

observed the presence of organic acids mainly flavellagic acid, quinic acid and ascorbic acid; and flavonoids, highlighting dihydroquercetin and quercetin (Fischer et al. 2008, Pereira et al. 2020).

The oxidative stress can cause damage to cell components such as lipids, nucleic acids and proteins, and eventually leads to cell death (Moo-Huchin et al. 2015, Olszowy 2019, Wong et al. 2020, Yan et al. 2020). Protein structures can also be altered through glycation, a non-enzymatic reaction of a sugar with susceptible amino group in the side chains of amino acid residues originating advanced glycation end-products (AGEs) (Yeh et al. 2017, Dil et al. 2019). Both oxidative stress and glycation interfere physiologically promoting chronic and degenerative diseases such as Alzheimer's disease, diabetes and cancer (Cassidy et al. 2020, Luo et al. 2020, Zheng et al. 2020).

Regular consumption of fruits has been associated to prevention of different diseases mainly due to their active compounds diversity (Román et al. 2019, Chaudhary et al. 2020). Among the fruits phytoconstituents, phenolic compounds (flavonoids) and vitamins are highlighted to be compounds capable of scavenging free radicals and inhibiting protein damage caused by sugars and, in this way, acting as antioxidant and antiglycation (Khan et al. 2016, Neha et al. 2019, Khan et al. 2020). Therefore, the present study aimed to verify the presence of these phytoconstituents and evaluate antioxidant and antiglycation activities of *C. cainito*, *H. speciosa* and *P. glomerata* fruits.

MATERIALS AND METHODS

Fruits collection and processing

Fruits were obtained in summer of 2018 from Assis, São Paulo, Brazil. The samples were authenticated and the voucher for each specimen (*C. cainito* nº 01126; *H. speciosa* nº 01125; *P. glomerata* nº 01124) has been deposited in the Department of Biological Sciences, UNESP-Assis herbarium. The fruits were collected, processed and frozen at -18ºC. For the production of extracts, the peel and pulp of *C. cainito*, pulp of *H. speciosa* and whole fruit of *P. glomerata* were used.

Hydroethanolic extract of fruits

The frozen vegetal materials were lyophilized to obtain a dry mass. The dry mass was extracted using 1000 mL ethanol 70% (distilled water) for each 100 g lyophilized fruits in mechanical maceration at room temperature in the dark for 24 hours. After this time, the extract was obtained by vacuum filtration and the vegetal residue was re-extracted twice. The extracts obtained were concentrated in rotary evaporator. The resulting aqueous extract was frozen at -18°C and then lyophilized to obtain the dried hydroethanolic extract. The weight of the dried extracts was used to calculate the yield.

Determination of total phenol and flavonoid content

Total phenols content of extracts was determined using *Folin-Ciocalteu*'s reagent according to the method of Slinkard & Singleton (1977), with some modifications. Gallic acid was used as the standard for dosage. 5 mL of distilled water and 0.25 mL of *Folin-Ciocalteu*'s reagent were added to each 0.5 mL of sample. After 3 minutes, 1 mL of 10% Na_2CO_3 solution was included. The absorbance of all samples was measured at 725 nm using the UV spectrophotometer after incubating at 30ºC for 1 hour. Results were expressed as µg of gallic acid equivalent (GAE) per mg of dry extract. Tests were performed in triplicate**.**

The total flavonoids content of extracts was measured according to the methodology

proposed by Zhishen et al. (1999) with some modifications. In brief, 250 µL of sample solution were mixed with 1.25 mL of distilled water. 75 µL of sodium nitrite 5% were added. After 5 minutes, 150 µL of $AlCl_3$ 10% were added and kept for 6 minutes at room temperature. Then, 0.5 mL of 1 M NaOH were added and the mixture was agitated. The absorbance was measured at 510 nm using UV spectrophotometer. All tests were performed in triplicate and the results were expressed in rutin equivalence (RE).

Determination of ascorbic acid (Vitamin C)

For the determination of ascorbic acid, the method of Ballentine (1941) was used with some modifications. A starch solution (1%) was prepared; the titrant solution, consisting of Kl (10 g L^{-1}), KlO_3 (0.54 g L^{-1}) and H_2SO_4 (0.18 M) and a standard solution of ascorbic acid (0.5 g L^{-1}). The sample was prepared weighing 100g of frozen fruit; it was added 50 mL of distilled water and powdered. This mixture was filtered with the aid of a pump, the resulting solution was added to a 100 mL volumetric flask and the volume was filled with distilled water. For the titration, 25 mL of analyte solution or standard solution was added and 500 µL of starch solution 1% in a 125 mL erlenmeyer and the mixture was mixed with the aid of a burette, the titrant solution was slowly added to the erlenmeyer with the analyte solution until a small and permanent alteration to a blue color. The concentration of ascorbic acid in the samples was determined by the following formula: $C_{am} = [(V_{am}xC_p)/V_p]x100$. C_{am} = concentration of ascorbic acid in the sample in mg per 100 g of fruit. V_{am} = volume of titrant used in the sample. C_p = concentration of standard ascorbic acid solution. V_p = titrant volume in standard ascorbic acid solution. The experiment was carried out in triplicate.

Determination of total carotenoids

To determine the total amount of carotenoids, the method of Carvalho et al. (2012) with some modifications was used. Approximately 15 g of the crushed samples plus 3 g of Celite 545 were added to a beaker and 25 mL of pure acetone were added. This mixture was stirred with the aid of a glass stick and then filtrated, the Celite-bound vegetable mass was re-extracted twice or until the solution became colorless. The extraction solution was added to a separatory funnel containing 40 mL of petroleum ether, whereupon the acetone was removed with the slow addition of distilled water, the aqueous phase was discarded. The ether phase was then transferred to a 50 mL volumetric flask containing 15 g of anhydrous sodium sulfate and the volume was filled with petroleum ether. The samples were read at the wavelength of 450 nm in UV-Vis spectrophotometer. The concentration of carotenoids was determined by the following formula: $CC = (AxV_{(mL)}x104)/(A^{1\%}_{1cm}xP_{(g)})$. CC = concentration of β-carotenoids in µg g^{-1}. A = absorbance. $V_{(mL)}$ = Volume of the extract. $P_{(g)}$ = weight of the sample. $A^{1\%}_{1cm}$ = 2592 (extinction coefficient of β-carotenoids in petroleum ether). The experiment was carried out in triplicate.

Reducing and total sugars dosage

The method DNS (3 5-dinitrosalicylic acid) was used according to Bobbio & Bobbio (1995). For which a standard curve of D-glucose was established to quantity of sugar in each sample. The extracts were diluted in water, then centrifuged at 3500 rpm for 20 minutes and an aliquot of the supernatant was removed. In sequence, 500 µL of sample were mixed with 500 µL of the DNS reagent under stirring, then maintained at approximately 100°C for 5 minutes and followed by cooling in an ice bath. In sequence, 8 mL of the $KNaC_4H_4O_6$ ($4H_2O$) at 15.1 g L^{-1} were added and the mixture

absorbance were determined at 540 nm in the spectrophotometer, for reducing sugars. For total sugar determination, it was first necessary to carry out a hydrolysis of extracts. Therefore, to 2.0 mL of the supernatant from the centrifuged extract solution, 2.0 mL of 2N HCl were added, this mixture was heated in boiling water for 10 minutes and 2.0 mL of 2N NaOH were added under stirring, and then cooling in ice bath. After these procedures, a method of determining reducing sugars was carried out. The tests were performed in triplicate.

Determination of antioxidant activity

DPPH radical scavenging

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was determined spectrophotometrically as described by Brand-Williams et al. (1995), with some modifications. Ascorbic acid was used as positive control. In brief, 50 µL of different extract concentrations of each fruit and ascorbic acid sample were mixed with 250 µL of DPPH solution (500 µM), 1 mL of the acetate buffer solution (pH 5.5, 100 mM) and 1.25 mL of ethanol. The mixture was kept for 30 minutes in the dark to perform complete reaction. Finally, the absorbance of each sample was measured at 517 nm by using UV spectrophotometer. Free radical scavenging activity was calculated using following formula: Antioxidant activity (%) = $[(A_{Control}-A_{Sample})/A_{Control}]$ x100. A_{Sample} is the absorbance of the samples and $A_{control}$ is the control absorbance (contain everything except the extract). For the extracts with the highest activity, the EC_{50} was determined.

Ferric Reducing Antioxidant Power (FRAP)

FRAP of each extracts was determined as described by Benzie & Strain (1999), with slight modifications. Briefly, in the dark the FRAP reagent was prepared with 25 mL of acetate buffer (300 mM / pH 3.6), 2.5 mL of TPTZ (10 mM) in HCl solution (40 mM) and 2.5 mL of $FeCl_3$ (20 mM). In sequence, 90 µL of different concentration of samples were mixed to 270 µL ultrapure water and 2.7 mL FRAP reagent. The mixture was incubated in a water bath at 37ºC for 30 minutes. After cooling, the samples and control (ascorbic acid) were read with absorbance at 595 nm in UV-visible spectrophotometer. A standard calibration curve was ploted using Trolox, thus the results were expressed in µM Trolox equivalent (TE). The determinations were performed in triplicate.

Lipid peroxidation inhibition

Lipid peroxidation inhibition was evaluated through TBARS assay described by Costa et al. (2012). Dried egg yolk was homogenized (1% w/v) in PBS buffer (20 mM/pH 7.4). 1 mL of the resulting homogenate was sonicated and mixed with 0.1 mL of the sample at concentrations of 250, 500 and 1000 µg mL^{-1} or positive control (Trolox 140 µg mL^{-1}). Lipid peroxidation was induced adding 0.1 mL of AAPH solution (0.12 M) and maintaining for 30 minutes at 37° C. After cooling at room temperature, 0.5 mL of trichloroacetic acid (15%) and 0.5 mL of thiobarbituric acid (0.67%) were added and heated at 97° C for 15 minutes. Samples were centrifuged at 1200 rpm for 10 minutes and absorbance of the supernatant was determined at 532 nm. Results were expressed as percentage of TBARS formed by AAPH (lipid peroxidation positive control).

Nitric Oxide scavenging activity

Evaluation of nitric oxide sequestering capacity (NO) was performed according to Marcocci et al. (1994). A mixture containing 320 µL of the sample diluted at different concentrations (250, 500 and 1000 µg mL^{-1}) and 360 µL of NPS (25 mM PBS, pH 7.4) was incubated at 37° C for 2 hours in dark. Then, 215 µL of Griess reagent were added

and the absorbance was determined at 540 nm. The values were submitted to an equation obtained by linear regression of a calibration curve of sodium nitrite previously performed in the same test conditions and the results were expressed as formed nitrite concentration (μM mL^{-1}).

Evaluation of antiglycation activity

Relative electrophoretic mobility (REM)

To determine antiglycation activity, Ledesma-Osuna et al. (2008) method was adapted. For this purpose, 600 μL of BSA (30 mg mL^{-1}) was mixed with fruit extract solution (10 mg mL^{-1}) or 600 μL of Aminoguanidine (AMG) (22.1 mg mL^{-1}) used as control. In sequence, 600 μL of Ribose (200 mg mL^{-1}) and 1.5 mL of potassium phosphate buffer (0.01M, pH 8.0) were added; the reaction mixture was incubated for 2 days at 40°C. Then, samples were dialyzed to remove unbound sugar and salts, and kept frozen at -20°C until use. Treatments were performed in duplicates. Aliquots of the dialyzed samples were analyzed by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) with running gel 8% and stacking gel 4%. After completion of the electrophoresis, gels were stained with Coomassie Brilliant Blue (R-250). Activity was determined as a function of the distance between BSA with different treatments versus untreated BSA.

High performance liquid chromatography (HPLC-PDA)

Chromatographic analysis were performed on high performance liquid chromatography (analytical, binary) Simadzu® consisting of two LC-10AD pumps equipped with a DGU-20A3R degasser, a SIL-10A automatic sampler a SPD-M10A photodiode array detector (PDA). Chromatograms were obtained in reverse phase, using a Phenomenex Luna-C18 column with dimensions 250x4.5 mm and 5 μm particle size. First, 30 mg of extracts were dissolved into methanol 95% (water) (less viable volume), then injected in a C18 cartridge and eluted with 3 mL of methanol 95% (water), 1 mL of this solution was concentrated in rotary evaporator. Thus, the concentrated samples were dissolved into 95% methanol (water) at the concentration extract of 10 mg mL^{-1} and filtered with syringe filter with pore size of 0.45 μm. In sequence, the gradient elution mode was used, the composition of the mobile phase being varied from 5% to 100% methanol in water over 40 minutes. The acquisition range used in the PDA varied from 190 to 800nm.

RESULTS

Hydroethanolic extract of fruits

The hydroethanolic extraction using 100 g dry mass of each fruit resulted in 3000 mL of hydroethanolic extract for each evaluated species. The extracts obtained were concentrated in rotary evaporator and the resulting aqueous extract was then lyophilized. This process resulted in dried extracts and the yields were 4.56 g *C. cainito* pulp and 3.27 g *C. cainito* peel; 7.86 g *H. speciosa* pulp; and 4.23 g *P. glomerata* fruit.

Total phenols and flavonoids, total and reducing sugars

Table I shows the values of reducing and total sugars, total phenols and flavonoids in the different evaluated fruit extracts. The highest total and reducing sugars concentration was observed for the *C. cainito* pulp extract (858.67±11.80 mg g^{-1}) and for the *H. speciosa* fruit extract (576.12±7.97 mg g^{-1}), respectively. According to this analysis, total sugars represented more than 50% of the dry mass of the extracts, being

Table I. Dosage of reducing and total sugars, total phenols and flavonoids of *H. speciosa, P. glomerata* and *C. cainito* fruit extracts.

Fruits	Sugar		Total Phenols[II]	Total Flavonoids[III]
	Reducing[I]	Total[I]		
C. cainito/Peel	334.40±4.34	605.64±9.04	90.34±1.79	30.4±1.24
C. cainito/Pulp	306.02±10.44	858.67±11.80	72.37±1.51	21.45±0.35
H. speciosa	576.12±7.97	794.77±11.96	48.29±0.81	ND
P glomerata	574.05±4.82	659.20±8.78	60.62±2.10	ND

[I] - Results expressed in mg per g of dry extract;
[II] - Total phenols expressed in µg gallic acid equivalent (GAE) per mg of dry extract;
[III] - Total flavonoids expressed in µg rutin equivalent (RE) per mg of dry extract;
ND = Not detected.

represented mainly by reducing sugars. In relation to the total phenols and flavonoids, the extracts of *C. cainito* peel presented the highest values (90.34±1.79 µg GAE mg^{-1} and 30.4±1.24 µg RE mg^{-1}, respectively). *H. speciosa* and *P. glomerata* fruit extracts presented results only for the total phenols content (48.29±0.81 µg GAE mg^{-1} and 60.62±2.10 µg GAE mg^{-1}, respectively).

Determination of ascorbic acid (vitamin C) and total carotenoids

Results of ascorbic acid (vitamin C) and carotenoids determinations of *C. cainito*, *H. speciosa*, and *P. glomerata* fruits are presented in Table II, which highlights *P. glomerata* with the highest values for both ascorbic acid (2260.94±1.45 mg 100 g^{-1}) and carotenoids (59.62±1.06 µg g^{-1}). Both phytoconstituents were observed in the *C. cainito* pulp extract, presenting values of vitamin C (33.64±0.87 mg 100 g^{-1}) and carotenoids (1.85±0.56 µg g^{-1}). For the *H. speciosa* pulp extract, the vitamin C (102.55±1.21 mg 100 g^{-1}) and carotenoids (8.40±0.41 µg g^{-1}) were also determined.

Antioxidant activity

DPPH radical scavenging

Table III shows values of antioxidant activity of the different fruit extracts evaluated by the DPPH test. The *C. cainito* species (peel extract at the concentration of 1000 µg mL^{-1}) presented the highest activity (65.64±1.39 %) with an EC_{50} of 379.87 µg mL^{-1}. *P. glomerata* species presented the highest activity (46.54±2.17 %) at the same concentration and EC_{50} of 1008.23 µg mL^{-1} was observed. *H. speciosa* presented no antioxidant activity at different concentrations tested.

Ferric reducing antioxidant power test (FRAP)

Results of antioxidant activity evaluation by the FRAP test are presented in Table IV. *C. cainito* peel extract presented the highest observed value (231.34±3.41 µM TE) at the concentration of 1000 µg mL^{-1}. *H. speciosa* and *P. glomerata* fruit extracts presented activity at the same concentration (20.11±3.60 µM TE and 107.89±0.90 µM TE, respectively).

Lipid peroxidation inhibition

Results of TBARS test for the different evaluated fruits are presented in Table V. A maximum lipid peroxidation inhibiting activity of 22.56±1.67% was observed for the *C. cainito* extract (peel). *H. speciosa* presented a lipid peroxidation inhibition of 2.23±0.78% at the concentration of 1000 µg mL^{-1}. *P. glomerata* extract presented 11.50±2.37% inhibition at the same concentration.

Table II. Determination of ascorbic acid and total carotenoids content of *C. cainito* and *H. speciosa* pulp, and *P. glomerata*.

Compound	*C. cainito*	*H. speciosa*	*P. glomerata*
Ascorbic acid[a]	33.67±0.87	102.55±1.21	2260.94±1.45
Carotenoids[b]	1.85±0.56	8.40±0.41	59.62±1.06

[a] - Ascorbic acid in mg per 100 g of fruit; [b] - Carotenoids in µg per g of fruit.

Table III. Antioxidant activity by DPPH radical scavenging method (%) of *C. cainito* and *P. glomerata* fruit extracts and ascorbic acid.

Concentration (*µg mL^{-1}*)	*C. cainito*		*P. glomerata*	Ascorbic acid[I]
	Peel	Pulp		
50	3.56±1.03a	ND	ND	ND
75	10.62±1.22b	ND	3.53±1.17a	ND
100	20.45±0.96c	ND	8.45±1.56a	ND
250	28.67±2.16c	3.57±0.97a	18.34±0.73b	ND
500	60.34±0.60d	4.45±1.53a	30.34±1.56c	ND
1000	65.64±1.39d	8.24±1.86b	46.54±2.17d	94.62±1.54
EC_{50} (µg mL^{-1})	379.87		1008.23	

[I] - Ascorbic acid (150 µg mL^{-1}). Values are expressed as mean±SE. Same letters within the same column indicate no significant differences among samples by Tukey test (α≤0.05). ND = Not detected.

Table IV. Ferric reducing antioxidant power (FRAP) test results of *H. speciosa, P. glomerata*, *C. cainito* fruit extracts and ascorbic acid, expressed in µM of Trolox equivalent (TE).

Concentration (µg mL^{-1})	*C. cainito*		*H. speciosa*	*P. glomerata*	Ascorbic acid[I]
	Peel	Pulp			
100	15.34±0.25a	ND	ND	ND	ND
250	50.12±5.11b	ND	ND	ND	ND
500	120.23±4.91c	ND	ND	43.07±2.04a	ND
1000	231.34±3.41d	20.43±1.41	20.11±3.60	107.89±0.90b	124.65±3.76

[I] - Ascorbic acid (150 µg mL^{-1}); Values are expressed as mean±SE. Same letters within the same column indicate no significant differences among samples by Tukey test (α≤0.05). ND = Not detected.

Nitric Oxide (NO) scavenging activity

Table VI shows the values of NO scavenging activity of the fruit extracts. The antioxidant activity by nitric oxide test showed a maximum activity of 49.31±1.75% and an EC_{50} of 1032.48 µg mL^{-1} for the *C. cainito* peel extract. A maximum NO scavenging activity of 38.79±2.14% was observed for *P. glomerata* in this study. The *H. speciosa* extract did not present activity for this test and no scientific literature on this activity was reported for this species.

Table V. Thiobarbituric acid reactive substances (TBARS) formation inhibition (%) *in vitro* by *C. cainito*, *H. speciosa* and *P. glomerata* fruit extracts, and Trolox.

Concentration (μg mL^{-1})	*C. cainito*		*H. speciosa*	*P. glomerata*	Trolox[I]
	Peel	Pulp			
75	0.62±0.54a	ND	ND	ND	ND
100	1.71±1.11a	1.64±0.25a	ND	ND	ND
250	6.64±0.98b	5.48±0.16b	ND	3.29±0.59a	ND
500	14.67±1.47c	10.45±0.84c	ND	6.45±3.12b	ND
1000	22.56±1.67d	18.9±2.29d	2.23 ± 0.78	11.50±2.37c	62.86±2.11

[I] - Trolox (150 μg mL^{-1}); Values are expressed as mean±SE. Same letters within the same column indicate no significant differences among samples by Tukey test (α≤0.05). ND = Not detected.

Table VI. Antioxidant activity by the Nitric Oxide scavenging method (%) of *C. cainito* and *P. glomerata*, and rutin.

Concentration (μg mL^{-1})	*C. cainito*		*P. glomerata*	Rutin[I]
	Peel	Pulp		
75	4.64±0.93a	ND	ND	ND
100	10.92±2.12b	ND	ND	ND
250	27.76±1.46c	5.70±2.21a	4.04±0.93a	ND
500	32.86±0.87c	17.80±1.43b	29.30±2.77b	ND
1000	49.34±1.75d	18.51±0.60b	38.79±2.14c	98.68±4.53
EC_{50} (μg mL^{-1})	1033.24			

[I] - Rutin (1000 μg mL^{-1}); Values are expressed as mean±SE. Same letters within the same column indicate no significant differences among samples by Tukey test (α≤0.05). ND = Not detected.

Evaluation of antiglycation activity

Relative electrophoretic mobility (REM)

The extracts with the highest antioxidant activity observed in the previous analyses were evaluated and Figure 2 shows the electrophoretic profiles of these different extracts. It is possible to observe that the electrophoretic pattern of the *P. glomerata* extract (PG) with BSA and Ribose showed similarity with control, glycation inhibitor (AMG), thus suggesting an antiglycation activity of this extract. However, *C. cainito* and *H. speciosa* extracts did not show activity in the evaluation methodology employed.

High performance liquid chromatography (HPLC-PDA)

HPLC-PDA screening of *C. cainito*, *H. speciosa* and *P. glomerata* extracts presented in Figure 3 (a, b and c, respectively) showed a chromatographic profile with a wide range of metabolites detected, indicating that the extraction methods were efficient in extracting polyphenolic compounds. The spectral scanning range varied from 200-600 nm and eluted peaks were obtained in the UV region. These peaks suggest the presence of typical flavonoid characterized by absorption bands in Figure 3 (a', b' and c'), recognized as Band II (~240-290 nm) attributed to the A-ring and the Band I (~300-390 nm) attributed to the B-ring.

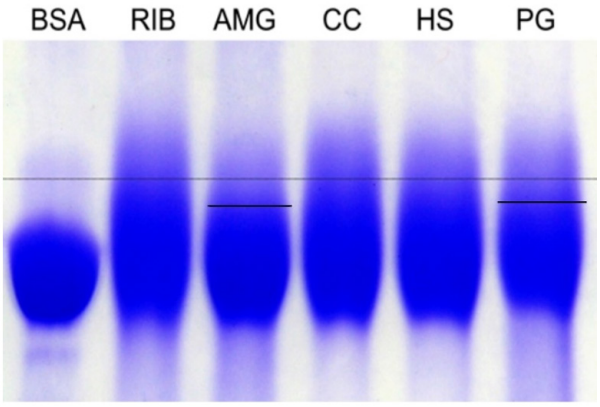

Figure 2. Relative electrophoretic mobility (REM). Results of *C. cainito* (Peel), *H. speciosa* and *P. glomerata* fruit extracts (10 mg mL^{-1}). BSA: BSA without treatment. RIB: BSA and ribose. AMG: BSA, ribose and aminoguanidine. CC: BSA, ribose and *C. cainito* extract. HS: BSA, ribose and *H. speciosa* extract. PG: BSA, ribose and *P. glomerata* extract.

DISCUSSION

In the determination of total phenols and flavonoids for the evaluated species, it was possible to observe the presence of these phytoconstituents in all fruit extracts. The phenol and flavonoid values observed for *C. cainito* are similar to those found in studies conducted by Luo et al. (2002) with fruits of this species. They demonstrated the presence of phenolic compounds, among them different flavonoids, but only confirmed the antioxidant activity of these compounds by DPPH test. The observed values for *H. speciosa* are in agreement with the study of Assumpção et al. (2014) that indicated the presence of phenolic compounds in this species fruit and study carried out by Ferreira et al. (2007) using *H. speciosa* leaf ethanolic extract demonstrated the presence of phenolic compounds and rutin flavonoid. In addition, they also demonstrated that phenolic compounds in this extract exhibit vasodilatory activity. The results observed for *P. glomerata* extract corroborate with study carried out by Fischer et al. (2008) that demonstrated the presence of phenolic compounds in leaf extracts, as well as their antinociceptive activity. Bagattoli et al. (2016) also demonstrated the presence of phenolic compounds in fruit extracts and observed anticancer activity.

In the determination of total and reducing sugars concentration, it was demonstrated that all fruit extracts presented these phytoconstituents, and over 50% of the dry extract masses of *H. speciosa* and *P. glomerata* were composed reducing sugars. These results are in accordance with study carried out by Fernandes et al. (2003) that identified the presence of total and reducing sugars in extracts of different varieties of fruit, concluding that this is a common characteristic of pulpy fruits.

In determination of ascorbic acid (vitamin C) and total carotenoids, the *C. cainito* fruit extracts (peel and pulp) showed concentration of ascorbic acid similar to study conducted by Oranusi et al. (2015), that obtained 43.54 mg ascorbic acid per 100 g of pulp of this species. In this study, total carotenoids were quantified; however, there are no results in the recent literature. *H. speciosa* fruit extract presented levels of ascorbic acid similar to those observed in study carried by Moura et al. (2002), where the content of 139.64 mg ascorbic acid per 100 g of pulp was obtained. Results of total carotenoids of *H. speciosa* fruits are inexistent in the recent literature. Similarly, there are no data on content of ascorbic acid and carotenoids of *P. glomerata* fruit extract.

In the evaluation of antioxidant activity, the DPPH sequestration method was used for the first time to determine the antioxidant potential of *C. cainito* fruit extracts (peel and pulp) due to the fact that there are no scientific data using this fruit parts separately. However, evaluating the ethyl acetate fraction of methanolic extract of the whole fruit, Luo et al. (2002) found an IC_{50} (22 µg mL^{-1}). In another study, Ningsih et al. (2016) used

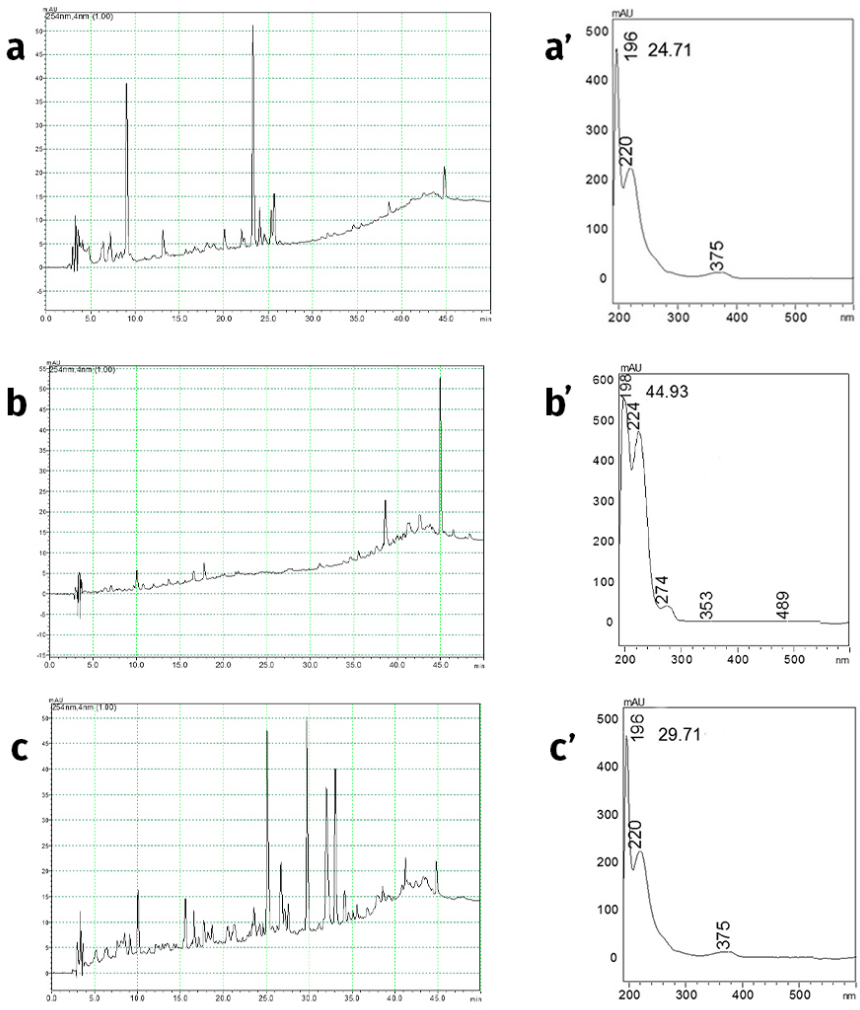

Figure 3. Chromatographic profile of *C. cainito* (a), *H. speciosa* (b) and *P. glomerata* (c) hydroethanolic extracts obtained in HPLC-PDA. Elution System: A (Methanol) and B (Water) Gradient: 5–100% A in B in 40 min. Phenomenex ® Luna C18 column (250 × 4.6 mm id. 5 µm). HPLC, modular binary Simadzu®, flow 1.0 mL min^{-1}. λ = 190 – 800 nm. Injection volume: 20 µL. a', b' and c': Maximum absorption bands in the UV region illustrated for flavonoids.

the hydroethanolic leaf extract and obtained 91.08% of antioxidant activity for the DPPH test. *H. speciosa* pulp hydroethanolic extract presented no antioxidant activity evaluated by the DPPH test at different concentrations tested. However, antioxidant activity evaluation of the methanolic and acetonic extract of the mangaba fruit was performed by Assumpção et al. (2014) and Schiassi et al. (2018) obtaining a high value of EC_{50} (3050 g of fruit per g of DPPH and 2681.91 g of fruit per g of DPPH, respectively). *P. glomerata* extract showed antioxidant activity by the DPPH test and it was demonstrated in study carried by Bagattoli et al. (2016) where methanolic extract of the fruit peel presented antioxidant compounds with an EC_{50} of 15.9 µg mL^{-1}.

The fruit extracts evaluated by the FRAP test demonstrated that all of them present iron ion reduced power, confirming their antioxidant activity. The results observed for *C. cainito* are in agreement with the studies carried by Oguntoyinbo et al. (2015) that evaluated *C. albidum* (African star apple) ethanolic extract, a species of the same *C. cainito* genus, and obtained an antioxidant activity of 0.39±0.01 µmol Fe^{+2} g^{-1} of dry mass. However, the result found for this species extract was reported for the first time in this study. Results for the *H. speciosa* species are similar to those observed by Rufino et al. (2010) that demonstrated in their study that hydroethanolic extract of *H. speciosa* fruit presents an antioxidant activity

in iron ion reducing, corroborating with the results obtained in this study. *P. glomerata* also showed a significant reduction of iron ion, which is characteristic of the genus as demonstrated in study conducted by Sacchet et al. (2015), that evaluated *P. trunciflora* fruit aqueous extract and demonstrated antioxidant potential.

The antioxidant test determined by the lipid peroxidation showed that the *C. cainito* fruit extract (peel and pulp) significantly decreased the lipid peroxidation promoted by AAPH (77.44%). In the scientific literature there are no reports of this effect for this species; however, study with the genus carried by Philippe et al. (2010) evaluated *C. perpulchrum* (yellow star apple) root methanolic extract and obtained a lipid peroxidation inhibition of 64.40%. These results are presented for the first time due to the fact that in the recent literature there are only reports evaluating TBARS test for both *in vivo* and *in vitro* with other species of Apocynaceae family (Conrad et al. 2013, Vale et al. 2015, Dogra 2016). Similarly, there are no studies with *P. glomerata* in the recent literature. However, study performed by Sacchet et al. (2015) found capable compounds to reduce lipid peroxidation evaluating *P. trunciflora* fruit aqueous extract, species of the *Plinia* genus.

C. cainito extracts demonstrated a NO scavenging potential showing antioxidant activity for the fruit extracts. However, there is no recent literature on evaluation of this test evaluation for *C. cainito*. Studies performed by Ma et al. (2004) and Partap & Pandey (2012) have demonstrated that Sapotaceae family species present compounds with antioxidant activity by NO scavenging test. Similarly, the *P. glomerata* fruit extract presented NO scavenger potential, but there are no data of this effect in the literature. However, studies performed by Jagetia & Baliga (2004) with species of the same family of *P. glomerata* demonstrated that the hydroethanolic extract of the *Eugenia jambolana* seed at the concentration of 1000 μg mL^{-1} presented an activity of 64.80±0.87%. Results that can corroborate with those found in the present study.

The extracts antiglycation evaluation conducted in the present study was performed for the first time with Relative Electrophoretic Mobility, using bovine serum albumin (BSA) as standard protein and ribose as sugar. When a combination of BSA and sugar occurs it is possible to observe modification in SDS/PAGE since the protein migration becomes shorter than the control (non-glycated) possibly due to the covalent binding between protein and sugar (Kańska & Boratyński 2002, Ledesma-Osuna et al. 2008). *P. glomerata* species, showed an antiglycation activity similar to that observed for control AMG. This effect can be related to the antidiabetic activity reported in studies performed by Borges et al. (2014) and Fujita et al. (2015), that showed that species of the *Plinia genus* present anti-hyperglycemic action and act in the prevention of diabetes-related diseases.

In the high performance liquid chromatography (HPLC-PDA) analysis, the results of *C. cainito* extracts are in agreement with the study carried out by Luo et al. (2002) that evaluated methanolic extract of this fruit species and observed a variety of phenolic compounds, mainly flavonoids. However, for the *H. speciosa* and *P. glomerata*, there are no studies in the current scientific literature showing the chromatographic profile (HPLC) of the hydroethanolic extract of the fruits species and this information was first demonstrated in this study.

CONCLUSION

According to the results obtained in the present study, it was possible to verify the presence

of different phytoconstituents (phenolic compounds, flavonoids, vitamin C, carotenoids and sugars) in *C. cainito*, *H. speciosa* and *P. glomerata* fruits that can be correlated to the antioxidant and antiglycation activities observed. *C. cainito* peel presented the highest values of total phenols and flavonoids. *H. speciosa* presented the highest reducing sugar content and *C. cainito* pulp the highest total sugar content. *P. glomerata* showed the best result for ascorbic acid and carotenoids. For the antioxidant activity, *C. cainito* peel presented the highest activity in all methods employed (DPPH, FRAP, NO and TBARS), and for the antiglycation evaluation, *P. glomerata* showed the most evident activity. These data contribute to food security, nutritional and functional characterization, and can provide sources of new active compounds for application in pharmaceutical, food and cosmetic industries.

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