Emirates Journal of Food and Agriculture. 2022. 34(6): 473-479
doi: 10.9755/ejfa.2022.v34.i6.2874
http://www.ejfa.me/

RESEARCH ARTICLE

Rapid characterization of secondary metabolites in *Caryocar brasiliense* leaf extract and antiradical activity

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ABSTRACT

Leaves of *Caryocar brasiliense*, the gold of the Cerrado, are used in folk medicine to treat diseases of the respiratory tract. The content of total phenols and flavonoids of ethanolic extracts from the leaves of *C. brasiliense* were analysed by the Folin-Dennis reagent and aluminium chloride, respectively, in addition to the antioxidant activity by DPPH and ABTS tests. The coupling of ultra-high-performance liquid chromatography (UPLC) and mass spectrometry allowed the identification of 25 compounds, including tannins, triterpenes, saponins, lipids, and flavonoids. The structures were established by retention time behaviour, extensive analyses of their MS spectra, and by comparison with standards and/or of their MS data with those reported in the literature. Twenty-one compounds are being registered for the first time in *C. brasiliense.* The results suggest the potential of this species as a source of bioactive substances with antioxidant properties.

Keywords: Antioxidants; Flavonoids; LC/MS; Pequi; Tannins

INTRODUCTION

The family Caryocaraceae order Malpighiales has 2 genera and 25 species spread across South and Central America (Fleming et al., 2009). The *Caryocar* genus comprises 16 species in Brazil, among which, *Caryocar brasiliense* stands out, popularly known as pequizeiro (pequi) (Nunes and Gil, 2016).

Caryocar brasiliense is a typical species of the Brazilian Cerrado that has economic value due to its fruits being used in human and/or animal food and in the manufacture of liquors and medical syrups. The extraction of essential oils has been used in the cosmetic and pharmaceutical industry (Oliveira et al., 2017). In folk medicine, pulp oil and leaves are used as a tonic agent against asthma, the flu, colds, and bronchopulmonary diseases (Almeida and Silva, 1994). Some biological activities, such as the antioxidant, antifungal, antitumor, and vasodilator properties, stand out in the literature (Oliveira et al., 2018; Roesler et al., 2008). Previous chemical studies have revealed the presence of secondary metabolites with therapeutic potential, such as flavonoids, tannins, saponins, phenolic acids, and triterpenes (Oliveira et al., 2018; Ascari et al., 2013; Roesler et al., 2008).

The antioxidant activity in food and beverages has aroused the interest of the scientific community, as the intake of these antioxidants helps to neutralise the action of free radicals that are associated with the development of many chronic diseases, such as cancer, inflammation, and cardiovascular diseases (Zehiroglu and Sarikaya, 2019). The search for the identification of biologically active phytochemicals from new natural sources has increased due to their potential use as ingredients in food, pharmaceutical, and cosmetic formulations (Castro-Enríquez et al., 2020). Liquid chromatography coupled with mass spectrometry (LC-MS) proved to be a very useful analytical technique in the characterisation of the chemical constituents of food and medicinal plant extracts (Lucci et al., 2017).

Considering the potential use of the species *C. brasiliense* as a functional food and medicinal plant and the few

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Received: 23 October 2021; **Accepted:** 12 May 2022

reports on the chemical composition of leaves, in this study, we investigated the chemical composition by ultra-performance liquid chromatography coupled with diode array detector and quadrupole time-of-flight mass spectrometry (HPLC-DAD-ESI-MS/MS) in extracts of *C. brasiliense* leaves and its correlation with their in vitro antioxidant activity.

MATERIAL AND METHODS

Reagents

Quercetin, catechin, ellagic acid, gallic acid, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, and potassium persulfate were obtained from Sigma-Aldrich Chemical Co. (Steinheim, Germany). Vitexin, isoquercetin, orientin and isoorientin have been isolated previously (Pessoa et al., 2021). Sodium carbonate and aluminium chloride hexahydrate were purchased from Dinâmica Química (São Paulo, Brazil). All solvents were of analytical grade purity and were supplied from Quimex (Minas Gerais, Brazil) and Merck (Darmstadt, Germany). Ultrapure water was used.

Plant material

Leaves of *C. brasiliense*, randomly obtained from three specimens, were collected in the municipality of Gurupi – TO (11°44'21" S; 49°05'25" W) in March 2018. A voucher (number 26019) of this species was deposited in the HURB herbarium at the Federal University of Recôncavo da Bahia (UFRB). A procedure for accessing the genetic heritage was carried out and the project was registered with SisGen (register A10589B).

Preparation of *C. brasiliense* leaves extract

Leaves of *C. brasiliense* were dried at room temperature and protected from light. Then, they were crushed in a blender. The powdered leaves (30 g) were subjected to extraction by maceration with ethanol 99.5%. The material extracted was filtered and concentrated in a rotary evaporator and dried to a constant weight, yielding 3.8 g of crude extract.

Total phenol content

The content of total phenols in the extract was quantified using the Folin-Dennis reagent (Singleton et al., 1999). Briefly, 0.5 mL of the extract solution (0.1 mg mL^{-1}) was mixed with 2.5 mL of the Folin-Dennis reagent. After 5 min, 2.0 mL of 5% sodium carbonate solution was added to the reaction mixture. After 2 h, the absorbance was read at 760 nm (visible UV spectrophotometer T60, PG instruments, UK). A standard curve was constructed using gallic acid (0–0.022 mg mL^{-1}) as a reference substance. The result was expressed in milligram equivalents of gallic acid (mg AGE g^{-1} of extract).

Total flavonoid content

The flavonoid content in the extract was quantified using aluminium chloride (Meda et al., 2005). Briefly, 2.0 mL of the extract solution (0.1 mg mL^{-1}) was mixed with 2.0 mL 2% aluminium chloride solution. After 30 min, the absorbance was read at 415 nm (visible UV spectrophotometer T60, PG instruments, UK). A standard curve was constructed using quercetin (0–0.020 mg mL^{-1}) as a reference substance. The result was expressed in milligram equivalents of quercetin (mg QE g^{-1} of extract).

Antioxidant activity

ABTS$^{\bullet+}$ Cation Radical Decolourisation Assay

The sequestering activity of the radical cation $ABTS^{+}$ was determined according to the methodology described by Chen et al. (2011). Briefly, 7 mM ABTS (5 mL) in water and 2.45 mM (88 µL) potassium persulfate reacted to form 5 mL of the $ABTS^{\bullet+}$ radical. The mixture was stored in the dark at room temperature for 16 h to allow cation radical formation. Radical solution was adjusted by diluting with methanol to yield an absorbance of about 0.700 at 734 nm. Then, 2.7 mL of the $ABTS^{\bullet+}$ solution was added to 0.3 mL of the sample at concentrations ranging from 1 to 10 µg mL^{-1}. After incubation at room temperature for 6 min, absorbance was measured at 734 nm (visible UV spectrophotometer T60, PG instruments, UK). Ascorbic acid was used as a standard.

DPPH$^{\bullet}$ Free radical scavenging assay

The DPPH free radical assay was measured according to Sousa et al. (2007), with modifications. Briefly, 2.7 mL DPPH methanol solution (40 µg mL^{-1}) was mixed with 0.3 ml of different concentrations of extracts (1 to 40 µg mL^{-1}). After 30 min, the absorbance of the remaining DPPH was measured at 517 nm (visible UV spectrophotometer T60, PG instruments, UK). Ascorbic acid was used as a standard. For both radicals, the percent inhibition was defined through the equation 1:

$$\% \text{ inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (1)$$

Where $A_{control}$ is the absorbance of reagent, and A_{sample} is the absorbance of the reagent + extract. From the % inhibition, an IC_{50} was obtained.

Characterisation of compounds

Sample preparation

An aliquot of the extract (10 mg) was solubilised under agitation in acidified water (pH 2 with HCl, 2 mL) and passed through the SPE C-18 cartridge that was preconditioned with ultrapure water and methanol (6 mL each). The cartridge was washed with 10 mL ultrapure water and eluted with 10 mL methanol. The extract was dissolved in methanol and filtered through a 0.45 µm for analysis in UPLC-QTOF-MS.

UPLC-DAD-ESI-QTOF-MS/MS analysis

Analysis of the extract was performed using a XEVO-G2XSQTOF mass spectrometer (Waters, MA, USA) connected to the ACQUITY UPLC system (Waters, MA, USA) via electrospray ionisation (ESI) and DAD analytical detector (Waters Acquity) at a wavelength of 200-400 nm. For analysis of the sample, a BEH C-18 Waters column (2.1 mm × 50 mm, 1.7 μm); mobile phase: H_2O with 0.1% formic acid (solvent A) and CH_3CN with 0.1% formic acid (solvent B); flow rate**,** 0.4 ml/min, and the injection volume was 5.0 μl. Gradient elution: 0**-**5 min, 5**-**10% B; 5**-**9 min, 10-95% B. Negative mode, sensitivity mode, capillary voltage, 3.0 kV; sample cone voltage 40 V, source temperature, 100°C; desolvation temperature, 450°C; cone gas flow rate, 50 L/h; and desolvation gas (N2) flow rate, 800 L/h. Leucine-enkephalin (10 ng ml^{-1}) was used as a reference substance for calibration. All data acquisition and analyses were controlled using Waters MassLynx software (Waters MA, USA).

Statistical analyses

The analyses were performed in three repetitions, and the obtained results were expressed as mean ± standard deviation from the mean. For statistical analysis, the program Microsoft Excel (Microsoft Corp., Redmond, WA) was used.

RESULTS AND DISCUSSION

Identification of the main metabolites of EtOH extract from *C. brasiliense* leaves

The ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS) in negative mode was used to identify the compounds from the *C. brasiliense* ethanolic extract. The extract and standard chromatograms are shown in Fig. 1. The compounds tentatively or unambiguously identified in the extract are listed in Table 1. Some known compounds (**1, 3, 4, 6, 9,** and **11**) present in leaf extract were identified by comparison with authentic standards according to retention times, ultraviolet (UV) data and mass spectra. Identification of the other compounds was proposed by matching maximum UV absorptions, pseudomolecular ion mass values, and MS/MS fragmentation patterns with data published in the literature. A total of 25 identified compounds were classified as ellagitannins (**2, 5,** and **6**), flavonoids (**1, 3, 4, 7, 8, 9, 10, 11, 12, 13,** and **14**), triterpenes (**17, 18, 19, 20, 21, 24,** and **25**), lipids (**15, 17,** and **23**), and saponin (**16**).

Ellagitannins

The mass spectrum of ellagitanins produce characteristic fragment ions with *m/z* $[M-H-170]^-$, $[M-H-170-162]^-$, and $[M-H-302]^-$, which indicate the loss of gallic acid, the galloylglucose group, and the hexahydroxydiphenoyl (HHDP) group. Peak **2** (RT 1.19 min, $\lambda_{máx}$ 267 nm) was identified as HHDP-galloyl-hexoside with a $[M-H]^-$ ion at *m/z* 633.0735 in negative ESI mode (Chang et al., 2019). The MS^2 spectrum of compound **2** produced characteristic fragments at *m/z* 463 (loss of gallic acid) and *m/z* 301 (loss of galloylhexoside). Peak **5** (RT = 2.12 min, $\lambda_{máx}$ 217 and 273 nm) yielded $[M-H]^-$ at *m/z* 953.0961, a doubly charged ion at *m/z* 476 $[M-2H]^{2-}$, and the characteristic product ions in *m/z* 301 and *m/z* 169, being identified as chebulagic acid (Avula et al., 2013). Peak **6** (RT 2.22 min, $\lambda_{máx}$ 253 and 366 nm) yielded a $[M-H]^-$ ion at *m/z* 300.9992, the identity was confirmed as ellagic acid by comparison with the standard. Ellagic acid (**6**) was previously identified in leaf extracts of *C. brasiliense* (Oliveira et al., 2018); however, this is the first report of the occurrence of compounds **2** and **5** in *C. brasiliense*.

Flavonoids

Eleven different flavonoids belonging to three subclasses of flavonoids (flavan-3-ols, flavonols, and flavones) were identified. Peaks **1**, **3**, **4**, **9**, and **11** were identified to be catechin, isoorientin, orientin, vitexin, and isoquercitrin, respectively, by comparing their retention times and UV and MS data with those of the standards (see supplementary material). Catechin (**1**) and vitexin (**9**) were previously reported in the leaves of *C. brasilense* (Oliveira et al., 2018).

Peak **7** (RT 2.46 min, $\lambda_{máx}$ 266 and 347 nm) showed a deprotonated molecule ion at *m/z* 463.0882 $[M-H]^-$. In the MS^2 spectrum of compound 7, the product ions at m/z 301 and *m/z* 300 corresponded to the loss of a hexose unit (162 Da) and the radical ion aglycone, respectively, suggesting that compound 7 was quercetin-hexoside (Geng et al., 2007).

Peak **8** (RT 2.49 min, $\lambda_{máx}$ 269 and 346 nm) displayed a $[M-H]^-$ ion at *m/z* 599.1052. In the MS^2 spectra, the product ion at *m/z* 447 $[M-H-152]^-$ was produced by loss of a galloyl group. In addition, the characteristic product ion of quercetin at *m/z* 301 was observed. Thus, compound **8** was identified as quercetin-galloyl-deoxyhexoside (Gu et al., 2013).

Peak **10** (RT 2.55 min, $\lambda_{máx}$ 266 and 347 nm) presented $[M-H]^-$ ion at *m/z* 431.0975 and a product ion *m/z* 311, corresponding to $[M-H-120]^-$. The loss of 120 Da can be associated with to the loss of moieties from the *C*-linked glucose. Compound **10** was identified as isovitexin (Abad-Garcia et al., 2008).

Peak **12** (RT 2.65 min, $\lambda_{máx}$ 269 and 347 nm) provided a mass spectrum with a deprotonated molecule ion at *m/z* 599.1045 $[M-H]^-$. The fragment ions at *m/z* 447 can be

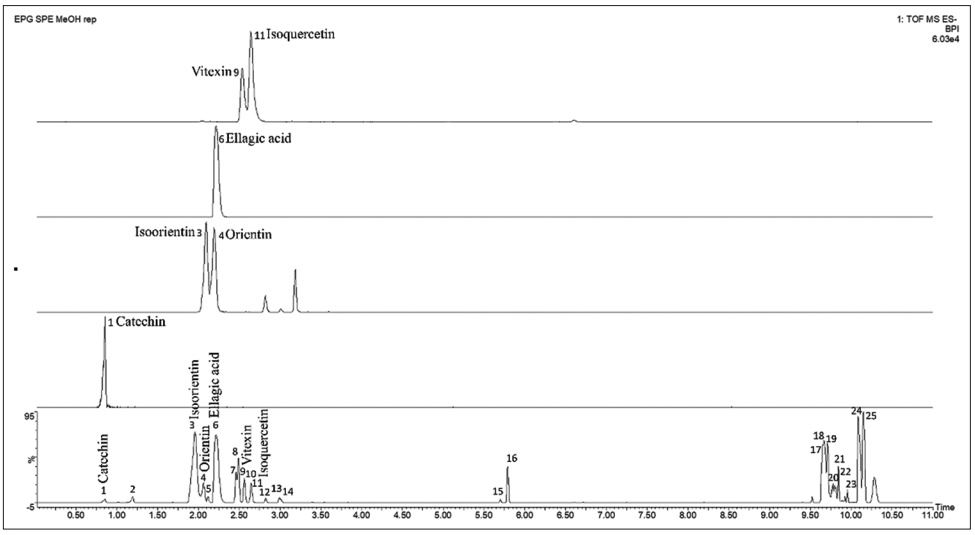

Fig 1. Chromatograms of EtOH extracts of *Caryocar brasiliense* leaves and standards.

justified as [M-galloyl-H]$^-$ and *m/z* 169, which was proposed to represent [gallic acid-H]$^-$. These data identified **12** as luteolin-galloyl-hexoside (Latté et al., 2002).

Peak **13** (RT 2.83 min, $\lambda_{máx}$ 268 and 347 nm) presented [M-H]$^-$ ion at *m/z* 433.0777 and a product ion *m/z* 301, corresponding to [M-H-132]$^-$. The loss of 132 Da can be associated with a pentosyl group. Compound **13** was identified as quercetin-pentoside (Geng et al., 2007). Flavonols **7** and **13** were previously identified by mass spectrometry in the hydroalcoholic extract of *C. brasiliense* leaves (Oliveira et al., 2018).

Peak **14** (RT 3.00 min, $\lambda_{máx}$ 270 and 340 nm) showed an ion referring to the deprotonated molecule in *m/z* 583.1099 [M-H]$^-$ and the characteristic product ions in *m/z* 431 [M-galloyl-H]$^-$, *m/z* 284, and *m/z* 169 [gallic acid-H]$^-$, being identified as kaempferol-galloyl-deoxyhexoside (Gu et al., 2013). This is the first report of the occurrence of *O*-galloyl-flavonoids **8**, **12** and **14** in *C. brasiliense*.

Triterpenes and saponin

Peak **16** (RT 5.79 min) was identified as soyasaponin Ba through the adduct ion in *m/z* 1003.5388 [M+HCOO]$^-$ and deprotonated molecular ion in *m/z* 957.5096 [M-H]$^-$. The characteristic product ion in *m/z* 795 [M-H-146]$^-$ suggests the loss of a rhamnose (Shiraiwa et al., 1991).

The UV spectrum of peaks **18** (RT 9.71 min), **24** (RT 10.09 min), and **25** (RT 10.15 min) showed $\lambda_{máx}$ around 300 nm, characteristic of cinnamic acid derivatives. The values of *m/z* 633.3659 correspond to coumaroyltormentic acid [M-H]$^-$ (**18**), and two isomers (**24** and **25**), coumaroylalphitolic acid [M-H]$^-$, both represented by ions at *m/z* 617.3851 (Wu et al., 2013). Peaks **19** (RT 9.76 min), **20** (RT 9.78 min), **21** (RT 9.80 min), and **22** (RT 9.84 min) were identified as isomers of ursolic acid/oleanolic acid and hydroxy-ursolic acid (Wu et al., 2013). Ursane and oleanan triterpenes have anti-tumour, anti-inflammatory, and antioxidant properties that can contribute to the medicinal properties of pequi leaves (Dalla Vechia et al., 2009). Saponins and triterpenes were previously isolated from other species of the genus *Caryocar* (Ascari et al., 2013). These kinds of triterpenes are being reported for the first time in *C. brasiliense*.

Other compounds

Peak **15** (RT 5.70 min) gave the [M-H]$^-$ at *m/z* 327.2150 and was identified as trihydroxy-octadecadienoic acid (Yang et al., 2013). Peak **17** (RT 9.67 min) was observed at *m/z* 555.2728 and identified as phosphatidylglycerol (22:6) (Hsu and Turk, 2008). Peak **23** (RT 9.96 min) with a [M-H]$^-$ ion at *m/z* 540.3014 was identified as phosphatidylcholine (20:5) (Nguyen et al., 2020). Free fatty acids, lysophospholipids and glycerophospholipids have been implicated in plant defence responses to biotic and abiotic stress (Cho et al., 2012). This is the first report of the identification of these lipids in leaves of *C. brasiliense*.

Antioxidant activity

Excessive reactive oxygen species (ROS) can cause oxidative damage to macromolecules when the body's antioxidant defences are overloaded, as observed during the aging process and in the presence of disease (Salehi et al., 2018). Oxidative stress is markedly implicated in the pathophysiology of many disorders, such as neurodegenerative diseases, cancer, and cardiovascular diseases (Baineni et al., 2016). Enzymatic and non-enzymatic antioxidant systems in the body regulate the balance between ROS and antioxidants. Alternatively, exogenous antioxidants, obtained from the ingestion of fruits and vegetables, were related to the balance between

Table 1: Compounds identified in Caryocar brasiliense extract using UPLC-DAD-QTOF-ESI-MS/MS to date

Compound	RT (min)	λ (nm)	$[M-H]^-$	$[M-H]^-$ Calculated	Molecular Formula	Error (ppm)	Fragments (m/z)	Tentative Identification	Reference
1	0.85	280	289.0721	289.0717	$C_{15}H_{14}O_6$	1.38	245.0803, 203.0712	Catechin[a]	Oliveira et al., 2018
2	1.19	267	633.0735	633.0733	$C_{27}H_{22}O_{18}$	0.31	300.9777	Galloyl-HHDP -hexoside	Chang et al., 2019
3	1.96	268, 347	447.0931	447.0932	$C_{21}H_{20}O_{11}$	-0.22	327.0864, 357.0516	Isoorientin[a]	Pessoa et al., 2021
4	2.07	268, 347	447.0931	447.0932	$C_{21}H_{20}O_{11}$	-0.22	327.0399	Orientin[a]	Pessoa et al., 2021
5	2.12	273	953.0961	953.0901	$C_{41}H_{30}O_{27}$	6.29	476.0377, 300.9877, 169.0001	Chebulagic acid	Avula et al., 2013
6	2.22	253, 366	300.9992	300.9990	$C_{14}H_6O_8$	0.66	283.9973, 257.0065, 229.0155	Ellagic acid[a]	Santisteban et al., 2019
7	2.46	266, 347	463.0888	463.0882	$C_{21}H_{20}O_{12}$	1.29	300.0170	Quercetin-hexoside	Geng et al., 2007
8	2.49	269, 346	599.1052	599.1042	$C_{21}H_{20}O_{12}$	1.66	447.0872, 300.0123	Quercetin-galloyl -deoxyhexoside	Gu et al., 2013
9	2.50	268, 347	431.0975	431.0984	$C_{21}H_{20}O_{10}$	-2.08	311.0440	Vitexin[a]	Pessoa et al., 2021
10	2.55	266, 347	431.0975	431.0984	$C_{21}H_{20}O_{10}$	-2.08	311.0440	Isovitexin	Abad-Garcia et al., 2008
11	2.56	347	463.0870	463.0882	$C_{21}H_{20}O_{12}$	-2.59	300.0146	Isoquercitrin[a]	Pessoa et al., 2021
12	2.65	269, 347	599.1045	599.1042	$C_{21}H_{20}O_{12}$	0.50	447.0849, 169.0007	Luteolin-galloyl-hexoside	Latté et al., 2002
13	2.83	268, 347	433.0777	433.0776	$C_{20}H_{18}O_{11}$	0.23	301.0160	Quercetin-pentosise	Geng et al., 2007
14	3.00	270, 340	583.1099	583.1093	$C_{28}H_{24}O_{14}$	1.02	431.0915, 284.0792, 169.0007	Kaempferol-galloyl -deoxyhexoside	Gu et al., 2013
15	5.70		327.2150	327.2176	$C_{18}H_{32}O_5$	-7.94	309.2083	Trihydroxy octadecadienoic acid	Yang et al., 2013
16	5.79		957.5096	957.5065	$C_{48}H_{78}O_{19}$	3.23	795.4664	Soyasaponin Ba	Shiraiwa et al., 1991
17	9.67		555.2772	555.2728	$C_{28}H_{45}O_9P$	7.92	393.1464	Phosphatidylglycerol (22:6)	Hsu & Turk 2008
18	9.71		633.3659	633.3644	$C_{35}H_{54}O_{10}$	2.36		Coumaroyltormentic acid	Wu et al., 2013
19	9.76		455.3515	455.3531	$C_{30}H_{48}O_3$	-3.51	427.1560	Ursolic acid/oleanolic acid isomer	Wu et al., 2013
20	9.78		455.3515	455.3531	$C_{30}H_{48}O_3$	-3.51	397.2471	Ursolic acid/oleanolic acid isomer	Wu et al., 2013
21	9.80		455.3515	455.3531	$C_{30}H_{48}O_3$	-3.51		Ursolic acid/oleanolic acid isomer	Wu et al., 2013
22	9.84		471.3470	471.3480	$C_{30}H_{48}O_4$	-0.2.21		Hydroxy-Ursolic acid	Wu et al., 2013
23	9.96		540.3014	540.3096	$C_{28}H_{48}NO_7P$	-2.22	480.3048, 255.2199	Phosphatidylcholine (20:5)	Nguyen et al., 2020
24	10.09	306	617.3851	617.3848	$C_{39}H_{54}O_6$	0.48	501.2280	Coumaroylalphitolic acid	Wu et al., 2013
25	10.15	306	617.3851	617.3848	$C_{39}H_{54}O_6$	0.48	501.2280	Coumaroylalphitolic acid	Wu et al., 2013

[a]Compared with authentic standard.

free radicals and antioxidant status, helping to control stress and minimising the risk of diseases (Kumar et al., 2016).

To evaluate the antioxidant potential of pequi leaf extracts, we quantified the content of total phenols and total flavonoids and evaluated the sequestering activity against DPPH and ABTS radicals, as described in Table 2. The leaf extract of *C. brasiliense* showed similar content of phenolic substances compared to other species of the genus (Yamaguchi et al., 2017). The total phenol content of the

Table 2: Total phenol, flavonoids, and antioxidants activities of *C. brasiliense*

Sample	Total phenol (mg AGE g^{-1})	Flavonoids (mg QE g^{-1})	DPPH (IC_{50} µg mL^{-1})	ABTS (IC_{50} µg mL^{-1})
Extract EtOH	239.01±14.65	86.23±3.49	10.49±0.2	1.71±0.3
Ascorbic acid			2.1±0.0	2.8±0.2

leaves was higher than that quantified in the fruits of pequi, 239.01 mg GAE g^{-1} compared to 209 g GAE kg^{-1} of extract, respectively (Roesler et al., 2008). The content of secondary metabolites can vary according to some factors, such as the organ of the plant, place of collection, and solvent extractor. Phenolic compounds, especially flavonoids, are considered powerful antioxidants due to their ability to directly scavenge radicals, chelate metals, and inhibit xanthine oxidase, among others (Machado et al., 2008).

The DPPH radical assay consists of a fast, simple, and sensitive method of assessing the antioxidant activity of natural compounds (Bao et al., 2018). The EtOH extract from *C. brasiliense* leaves showed promising free radical scavenging activity, with an IC_{50} of 10.49 µg mL^{-1}, however, other values were reported for hydroalcoholic extracts of leaves of *C. brasiliense* obtained in ethanol-water (70:30) and ethanol-water (25:75) with EC_{50} of 4.6 µg mL^{-1} and (Oliveira et al., 2018) and 58 µg mL^{-1} (Paula-Júnior et al., 2006), respectively. Roesler et al. (2008) reported similar activity of pequi fruit extract with an IC_{50} of 9.44 µg mL^{-1}. The ethanolic extract of leaves of *Caryocar coriaceum* showed greater antiradical activity with an IC_{50} of 3.24 µg mL^{-1} (Duavy et al., 2012), similar to the extract of *Caryocar villosum* fruit with an IC_{50} of 7.81 µg mL^{-1} (Yamaguchi et al., 2017). The *C. brasiliense* leaf extract was more reactive against the radical cation ABTS (Table 2), and a similar result has been reported for extracts of *C. villosum* fruit (Yamaguchi et al., 2017). This result may be related to the lower selectivity towards hydroxyl groups presented by the radical ABTS when compared to DPPH (Bao et al., 2018). The antioxidant activity observed in this study may be related to the flavonoids and tannins identified in the analysis by UPLC-QTOF-MS, such as ellagic acid and the derivatives of quercetin, luteolin, and apigenin (Tian et al., 2021).

CONCLUSIONS

In this study, we reported the identification of 25 substances in the ethanolic extract of leaves from *C. brasiliense*. Of these substances, 21 were reported for the first time in this species. Additionally, we reported the high content of phenols and flavonoids, as well as promising free radical scavenging activity. These results suggest that *C. brasiliense* leaves are a source of bioactive substances with antioxidant properties that may be related to the medicinal properties attributed to this species.

ACKNOWLEDGEMENTS

The authors would like to thank CNPq, Capes and CENAPESQ – UFRPE

Author's contributions

ECT: Methodology
ARCJ, MGC and ROF: Analysis of results and article writing
SSC and TMSS: UPLC/MS Analysis

Supplementary material

Chromatograms and mass spectra of the standards are available at: https://www.ejfa.me

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