Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Food Funct.*, 2023, **14**, 3994

Brazilian berry waste as a source of bioactive compounds: grumixama (*Eugenia brasiliensis* Lam.) as a case study

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Grumixama, *Eugenia brasiliensis* Lam., is a Brazilian berry little explored commercially and scientifically. However, local small producers market this fruit in the form of frozen pulp, which generates bioresidues, composed of seeds and peels. With the view to propose strategies for valuing grumixama, this study aimed to determine the chemical composition and assess the bioactivities of the hydroethanolic extracts of the whole residue (GR), seed (GS) and peel (GP) fractions of *E. brasiliensis*. From the results, GP had the highest concentration of organic acids (oxalic, malic, ascorbic and citric acids), total tocopherols, condensed tannins, anthocyanins, and other flavonoids. On the other hand, GS showed the highest content of monounsaturated fatty acids and hydrolysable tannins, whereas GR displayed a mixture of compounds detected in each of its parts. Regarding the bioactivities, low extract concentrations were required in two *in vitro* antioxidant assays, namely TBARS (EC_{50} = 0.90–1.34 μg mL^{-1}) and OxHLIA (IC_{50} = 21–65 μg mL^{-1}). Furthermore, GP had the highest inhibition activity of cellular oxidation in the CAA assay (80 ± 0.6%), while GS showed the highest anti-inflammatory activity *via* nitric oxide production inhibition (EC_{50} = 98.0 ± 0.5 μg mL^{-1}). All samples induced cell growth inhibition of the tested tumor cells (GI_{50} = 14.7–186 μg mL^{-1}) besides antibacterial and antifungal effects at low concentrations, but all samples were harmful to normal cells at moderate concentrations (GI_{50} = 145–268 μg mL^{-1}). Therefore, *E. brasiliensis* residue could be a good source of bioactive compounds to be used in several areas. However, additional studies are needed to confirm its safety as well as to unravel the mechanisms behind its biological activities.

Received 29th December 2022,
Accepted 22nd March 2023

DOI: 10.1039/d2fo04107c

rsc.li/food-function

1. Introduction

Brazil is one of the planet's most significant World Environmental Heritage sites, being home to wondrous biodiversity. Around 22% of the world's natural species are found in its vast territory, formed by six very distinct continental biomes.[1] Such biodiversity comprises plants and fruits that have incommensurable bioactive compounds belonging to different chemical classes, such as carotenoids, phenolic compounds, and vitamins, among others. These compounds could be explored for the discovery of valuables for the maintenance of good health.[2] However, most of the Brazilian native species are still poorly known and underexploited,[3] such as some of the 400 species belonging to the *Eugenia* genera (Myrtaceae family). Among them, there is *Eugenia brasiliensis* Lam., native to the Atlantic Rainforest and locally known as grumixameira.[4] This tree can grow up to a height of 10–15 meters, with a trunk diameter ranging from 25 to 40 cm. Its leaves are simple, coriaceous, and glabrous, measuring 6–9 cm in width by 3–5 cm in length. During the months of September to November, the tree produces solitary white flowers on its branches.[5,6]

Amidst the relatively few works found in the literature on *E. brasiliensis*, some address the chemical composition of the essential oil obtained from its leaves, which is mainly composed of α-terpineol, α-pinene, spathulenol, and τ-cadinol.[7,8] Furthermore, grumixameira leaves have been used in folk

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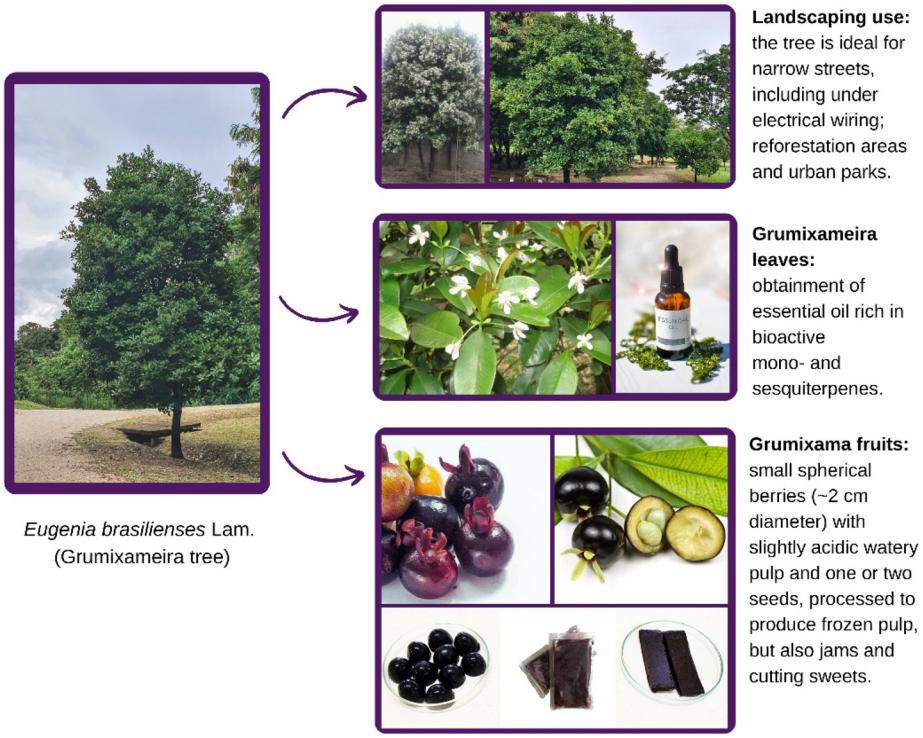

Fig. 1 *Eugenia brasiliensis* Lam.: current applications of the tree, leaves and fruits.

medicine for the treatment of diarrhea, arthritis, and rheumatism.[5,9] However, the *E. brasiliensis* tree also produces small berries (~2 cm diameter) known as grumixama or cereja brasileira (Brazilian cherry), which have a thin peel ranging from white–yellow (γ-variety) and red (β-variety) to dark purple (α-variety) (Fig. 1).[1,4]

E. brasiliensis fruit has interesting potential for commercialization due to its unique and attractive sensorial attributes; however, this species is scarcely explored. Currently, grumixama fruits are marketed as artisanal goods, mainly in the form of frozen pulp (Fig. 1).[1,5,10] As for the scientific knowledge about this exotic fruit, some studies have identified part of its composition in terms of bioactive compounds, including some quercetin derivatives, myricetin, ellagitannin derivatives, and anthocyanins in the edible part and seeds.[1,11,12] Moreover, some authors determined, by chemical methods, the antioxidant activity and, by *in vivo* assays, the anti-inflammatory activity of the extracts obtained from grumixama pulp and seed.[1,2]

To the best of our knowledge, reports on the chemical composition and biological potential of the bioresidues generated by the processing of grumixama are really scarce. With the view to expand its potential and propose strategies for valuing this fruit, the present work aimed to determine the chemical profile of this biomaterial, including the identification and quantification of lipophilic (tocopherols and fatty acids) and hydrophilic compounds (organic acids, polyphenols and anthocyanins), as well as to assess its biological activities, namely antioxidant, anti-inflammatory, anti-proliferative, and antimicrobial activities, by *in vitro* assays. Besides, the bioresidue hepatotoxic potential was investigated on porcine liver cells.

2. Materials and methods

2.1 Sample preparation

In December 2020, residues of the α-variety of *E. brasiliensis* resulting from the manufacture of frozen pulp were provided by Sitio do Bello – Frutas Nativas, located in Paraibuna, São Paulo, Southeast Brazil. The biomaterial was sealed in plastic bags and maintained at −20 °C until preparation. Three grumixama (G) fruit samples were prepared: whole residue (GR), seeds (GS, 70% of GR), and peel (GP, 30% of GR). The samples were lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) to complete dryness (dry matter yield: 41.7 ± 0.1%, 45.48 ± 0.08%, and 21.5 ± 0.1% for GR, GS and GP, respectively, with respect to the fresh weight (fw) of the sample), reduced to homogeneous powders, and stored at −20 °C until analysis.

2.2 Determination of chemical composition

2.2.1 Fatty acids. Fatty acids from *E. brasiliensis* residues were analysed with lipid fractions obtained by Soxhlet extraction using petroleum ether as a solvent. After solvent removal, the lipid fractions were derivatized as described by Obodai *et al.*[13] Then, fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID, Dani model GC 1000 instrument, Milan, Italy) as described in detail

by Sampaio *et al.*[14] Fatty acids were identified by comparing the relative retention times of the fatty acid methyl ester (FAME) peaks with those of the standards (standard mixture 47885-U, Sigma, St Louis, USA). The results were recorded and processed using the Clarity 4.0.1.7 Software (Informer Technologies, Inc., Solihull, Great Britain) and expressed as the relative percentage of each fatty acid.

2.2.2 Tocopherols. Tocopherol extraction was performed according to an established protocol;[15] analyses were carried out in High Performance Liquid Chromatography (HPLC) (Knauer, Berlin German) apparatus connected to a fluorescence detector (FL-2020, Jasco, Japan) working under conditions determined by the authors. The tocol standard (Matreya LLC, Gap, PA, USA) was used as an internal standard, whereas authentic standards of α-, β-, γ-, and δ-tocopherols (Sigma, St Louis, MO, USA) were used to identify and quantify the detected compounds. The results are expressed in mg per 100 g dw (dry sample).

2.2.3 Organic acids. Organic acid extraction was performed by maceration of dry samples (1.5 g) with metaphosphoric acid (4.5%, 25 mL), and the identification and quantification of the compounds were accomplished using an Ultra-Fast Liquid Chromatograph coupled to a Photodiode Array detector (UFLC-PDA, Shimadzu, 20A series, Kyoto, Japan), working under conditions previously established.[16] The results are expressed as mg per 100 g dw.

2.2.4 Phenolic compounds. Non-anthocyanin phenolic compounds were extracted from the residues by maceration in the hydroethanolic solvent (80%).[17] The extracts were analysed using an HPLC system equipped with a diode array detector coupled to a mass spectrometry detector (HPLC-ESI-Orbitrap-MS, Thermo Scientific), working under the conditions previously described.[18] Compound detection was achieved using a diode array detector (DAD) at the wavelengths of 280, 330, and 370 nm. Compound separation was performed using a Waters Spherisorb S3 ODS-2 C18 column (4.6 × 150 mm; 3 μm; Milford, USA). The system was coupled to an Orbitrap Exploris 120 mass spectrometer (Orbitrap MS, Thermo) with an electrospray ionization (ESI) source operating in the negative mode between the charge mass ratios (m/z) of 100 and 1500. Data acquisition, processing and interpretation were carried out with the Xcalibur® software (ThermoFinnigan, San Jose, CA, USA). For the identification of the compounds, the retention time (R_t), wavelength of maximum absorption (λ_{max}), pseudo-molecular ion ($[M - H]^-$), UV-Vis spectra, mass spectra, and patterns of ion fragmentation (MS^2) were compared with those in the literature and of commercially available standards.

Anthocyanin compounds were recovered from the residues by maceration protected from light using the hydroethanolic solvent (80 : 20, ethanol : water, v/v) acidified with citric acid (0.5%, pH 3). The samples were extracted twice, and the combined extracts were evaporated and lyophilized, re-dissolved in ethanol : water (20 : 80 v/v), and filtered and injected into the HPLC-DAD/MS operating in the positive mode between charge mass ratios 100 and 1500 (m/z) under conditions established by our team.[19] Data acquisition and the compound identification were performed as described above.

The results of non-anthocyanin and anthocyanin phenolic compounds are expressed in mg per g of extract (mg per g E) and mg per g of dry sample (mg per g dw).

2.3 Evaluation of extract bioactivity

2.3.1 Antioxidant activity. The antioxidant potential of the extracts obtained from different parts of the *E. brasiliensis* residue was evaluated by three cell-based assays. The thiobarbituric acid reactive substance assay (TBARS) was applied to determine the anti-lipid peroxidation,[16] whose results are expressed as EC_{50} values (μg mL^{-1}, half-maximal effective concentration to inhibit the lipid peroxidation). The anti-haemolytic activity of the extracts was tested using the oxidative haemolysis inhibition assay (OxHLIA);[20] the results are expressed as IC_{50} values (μg mL^{-1}) for a Δt of 60 min, meaning the extract concentration required to keep 50% of the erythrocytes intact for 60 min compared to those in the negative control (phosphate-buffered saline, PBS). For both assays, Trolox was used as the positive control. Finally, the cellular antioxidant activity (CAA) was tested using a murine macrophage cell line (RAW 246.7). In this assay, the capacity of extracts to prevent the oxidation of intracellular 7′-dichlorodihydrofluorescein (DCFH) was determined according to a methodology previous described.[21] Quercetin was used as the positive control, whereas 7′-dichlorodihydrofluorescein and Dulbecco's modified Eagle's medium (DMEM) were used as the negative control. The results are expressed as the percentage of oxidation inhibition at the maximum concentration tested (2000 μg mL^{-1}).

2.3.2 Anti-inflammatory activity. To assess the anti-inflammatory potential of the extracts, the method of lipopolysaccharide-induced nitric oxide (NO) production with a murine macrophage cell line (RAW 264.7) was used.[22] The results are expressed as EC_{50} values (μg mL^{-1}).

2.3.3 Antiproliferative activity and hepatotoxicity. For the determination of the antiproliferative activity of the samples the following human tumour cell lines were used: gastric cancer (AGS), colon cancer (Caco-2), breast cancer (MCF-7), and lung cancer (NCI-H460) cell lines. To test the hepatotoxicity of the samples, a non-tumour porcine liver primary cell line (PLP2) was used. The evaluation was performed by sulphonamide B (SRB) colorimetric assay.[22] The results are expressed as the extract concentration required to 50% cell growth inhibition (GI_{50} values (μg mL^{-1})).

2.3.4 Antibacterial and antifungal activity. For evaluation of the antibacterial activity of the extracts, the following bacteria were used: *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Yersinia enterocolitica*. Microdilution assays were carried out as previously described.[23] *Aspergillus fumigatus* and *Aspergillus brasiliensis* were used to determine the antifungal activity as previously described.[24]

2.4 Statistical analysis

All assays were performed in triplicate and the results are expressed as mean value ± standard deviation. Statistical ana-

lyses were carried out using the R software (version 11). Differences between two samples were tested using Student's *t*-test, and for three samples, the analyses of variance (ANOVA) was applied followed by Tukey's honest significant difference (HSD) test at the 5% of significance levels using the package laercio.

3. Results and discussion

3.1 Chemical composition

The different parts of the *E. brasiliensis* residue were evaluated in terms of organic acid contents. The results are presented in Table 1. The *E. brasiliensis* residue showed in its composition four organic acids. Citric acid was the most abundant in all samples, followed by malic acid, and only traces of ascorbic acid were found. The GP sample showed the highest organic acid concentration. Few data on the chemical composition of the *E. brasiliensis* fruit are found in the literature. Some studies reported ascorbic acid in the grumixama pulp (18.75 mg per 100 g fw), peel (10.55 mg per 100 g fw), and seed (3.82 mg per 100 g fw),[11] besides citric acid in the whole fruit (1.16 mg per 100 g fw).[10]

3.1.1 Tocopherols. The tocopherol profiles of the *E. brasiliensis* extracts were characterized by HPLC and the results are presented in Table 1. Two tocopherol isomers were found in the grumixama residues, namely α- and γ-tocopherols. α-Tocopherol was detected in a higher content in GP; on the other hand, GR and GS showed a higher γ-tocopherol content. In general, GP was richer in tocopherols than GS. To the best of our knowledge, this is the first report on the tocopherol composition of *E. brasiliensis* fruits and by-products. For a future investigation, the determination of tocotrienols is recommended, as this potent class of bioactive compounds has not yet been determined in grumixama and in its residues.

Table 1 Organic acid and tocopherol composition of *E. brasiliensis* residue

	GR	GS	GP
Organic acids (g per 100 g dw)			
Oxalic acid	0.27 ± 0.01^{b}	0.19 ± 0.01^{c}	0.59 ± 0.02^{a}
Malic acid	1.03 ± 0.02^{b}	0.88 ± 0.01^{c}	1.23 ± 0.02^{a}
Ascorbic acid	tr	tr	tr
Citric acid	2.46 ± 0.02^{b}	2.05 ± 0.03^{c}	3.7 ± 0.1^{a}
Total organic acids	3.77 ± 0.03^{b}	3.1 ± 0.1^{c}	5.5 ± 0.2^{a}
Tocopherols (mg per 100 g dw)			
α-Tocopherol	0.62 ± 0.01^{b}	0.227 ± 0.006^{c}	2.58 ± 0.03^{a}
γ-Tocopherol	1.00 ± 0.06^{a}	0.74 ± 0.03^{b}	0.98 ± 0.02^{a}
Total tocopherols	1.62 ± 0.06^{b}	0.97 ± 0.04^{c}	3.56 ± 0.06^{a}

tr – traces; dw – dry weight; oxalic acid ($y = 1 \times 10^{7}x + 231\,891$; $r^2 = 0.9999$); malic acid ($y = 950041x + 6255.6$, $r^2 = 0.9998$; ascorbic acid ($y = 4 \times 10^{7}x + 1 \times 10^{6}$; $r^2 = 0.9909$); citric acid ($y = 1 \times 10^{6}x + 10\,277$; $r^2 = 0.9997$). Defferent letters on the same line mean a significant difference between the samples by the Tukey's HDS test ($p < 0.05$).

Table 2 Fatty acid composition of *E. brasiliensis* residue (GR), seeds (GS), and peels (GP)

	GR	GS	GP
Lipid content (g per 100 g dw)	1.75 ± 0.06^{b}	2.05 ± 0.05^{a}	1.29 ± 0.08^{c}
Fatty acids (%)			
Tridecanoic acid (C13:0)	nd	3.555 ± 0.001	nd
Tetradecanoic acid (C14:0)	nd	5.05 ± 0.04	nd
Pentadecanoic acid (C15:0)	nd	5.988 ± 0.002	nd
Palmitic acid (C16:0)	27.0 ± 0.8^{b}	22.89 ± 0.01^{c}	32.8 ± 0.7^{a}
Palmitoleic acid (C16:1)	nd	5.304 ± 0.002	nd
Heptadecenoic acid (C17:1)	nd	4.837 ± 0.002	nd
Stearic acid (C18:0)	9.5 ± 0.3^{c}	11.825 ± 0.004^{b}	18.7 ± 0.4^{a}
Oleic acid (C18:1n9)	19.6 ± 0.6^{a}	11.989 ± 0.004^{c}	17.59 ± 0.09^{b}
Linoleic acid (C18:2n6)	38.89 ± 0.04^{a}	25.23 ± 0.01^{b}	24.4 ± 0.1^{c}
α-Linolenic acid (C18:3n3)	5.01 ± 0.03^{b}	3.336 ± 0.001^{c}	6.53 ± 0.03^{a}
SFA	$\mathbf{36.5 \pm 0.5^{c}}$	$\mathbf{49.31 \pm 0.02^{b}}$	$\mathbf{51.5 \pm 0.03^{a}}$
MUFA	$\mathbf{19.6 \pm 0.6^{b}}$	$\mathbf{22.13 \pm 0.01^{a}}$	$\mathbf{17.56 \pm 0.09^{c}}$
PUFA	$\mathbf{43.9 \pm 0.1^{a}}$	$\mathbf{28.56 \pm 0.01^{c}}$	$\mathbf{30.9 \pm 0.2^{b}}$

nd – not detected; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated acids; different letters on the same line mean a significant difference between the samples by the Tukey's HDS test ($p < 0.05$).

3.1.2 Fatty acids. The fatty acid profiles of grumixama residues are shown in Table 2. Regarding lipid content, as expected, GS had the highest content among the samples. In the literature, only data on the lipid content of the whole edible part of purple grumixama can be found. According to the report of Teixeira *et al.*,[12] the lipid content of the edible part of *E. brasiliensis* fruits varies from 0.16 to 0.26 g per 100 g fw. These authors verified that both lipid content and nutritional value vary largely according to the region of production.[12]

Regarding the fatty acid profiles, a total of ten compounds were detected in the residue samples; however, GR and GP showed only five fatty acids. The seed presented the richest diversity of fatty acids and, until the publication of this study, no data on the fatty acid composition of the grumixama fruit was found. Other residues from berries belonging to the Myrtaceae species such as pitanga seeds (*Eugenia uniflora* L.),[25,26] jabuticaba pomace (*Myrciaria cauliflora* Mart.),[27] feijoa seeds (*Feijoa sellowiana* Berg.),[25] and cagaita seeds (*Eugenia dysenterica* DC)[28] have presented palmitic and linoleic acids as major fatty acids, which was also verified in our *E. brasiliensis* residue samples. However, the SFA content found in GS was lower than that described for Andean blackberry (*Rubus glaucus* Benth.) seed (82.11%). Likewise, Cape gooseberry (*Physalis peruviana* L.) had a richer linoleic acid composition (72.42%) than that found in the grumixama samples, in addition to a lower SFA content (12.87%).[29]

GR exhibited the highest percentage of PUFA among the samples, which surpassed the SFA content. The dietary fatty acid composition is a critical determinant of plasma lipid concentrations and, therefore, has a significant impact on the risk of developing coronary heart disease. A diet that is high in SFA can raise total cholesterol and LDL-C levels. Conversely, substituting SFA with PUFA can lead to a decrease in such levels.[30,31]

All grumixama extracts presented elevated levels of linoleic and α-linoleic acids, indicating that the fruit residue may serve

as an alternative source of essential fatty acids to supplement cell maintenance. This finding is particularly noteworthy, as essential fatty acids play a crucial role in maintaining optimal health and preventing chronic diseases.[30,31] Therefore, the incorporation of grumixama residue into one's diet may lead to significant health benefits.

3.1.3 Phenolic compounds. The phenolic profiles of *E. brasiliensis* residues were determined *via* LC-MS analysis. A total of thirty-four compounds were detected, thirty of them in the negative mode and four in the positive mode, as shown in Table 3. The tentative identification of each compound was achieved considering its chromatographic characteristics, namely, the mass-to-charge ratio (m/z), MS^2 fragmentation (m/z), retention time (Rt, min) and maximum absorbance (λ_{max}, nm). These data were compared to data from the literature and with spectrograms of commercial standard compounds.

Hydrolysable tannin derivatives were the most abundant compounds in *E. brasiliensis* residues, as seventeen identified components belong to this class. Compounds **1**, **2**, **6**, and **10** showed the same pseudomolecular ion ($[M - H]^-$ at m/z 783), according to their MS fragmentation: at m/z 633, m/z 481 and m/z 301 characteristics of ellagic acid were detected. Previously, a compound with such mass spectrum characteristics was identified as bis-HHDP-glucose in purple and yellow grumixama.[12] Compounds **3**, **4**, **21**, **23**, **24** ($[M - H]^-$ at m/z 935) revealed two ion fragments during the ionization, the first was at m/z 633 after the loss of 302 u, possibly by the loss of the HHDP group, and the other was at m/z 301, which can be explained by disjoint of a gallic acid molecule (170 u) and a hexoside unit (162 u); therefore, these compounds were tentatively identified as galloyl-bis-HHDP-glucose isomers.[12] Compound **5** ($[M - H]^-$ at m/z 453) gave off two ions in MS^2 at m/z 313 and at m/z 169. In the *E. brasiliensis* pulp, this compound was identified as hydroxymethoxyphenyl-galloyl-hexoside.[32] Compound **7** ($[M - H]^-$ at m/z 1569), showed the detachment of six daughter ions in MS^2, the spectrometric characteristics of this peak were similar to those of sanguiin H-10 identified in raspberry fruits.[33] To the best of our knowledge, this is the first time that this compound is detected in *E. brasiliensis*. Compounds **8** and **9**, with the same pseudomolecular ion ($[M - H]^-$ at m/z 785) and similar spectrometric characteristics, release three ion fragments. Teixeira *et al.*[12] detected a similar compound in yellow grumixama, identified as digalloyl-HHDP-glucose, but did not find it in the purple variety. These authors also detected a compound with the same mass spectrum as that of compound **11** ($[M - H]^-$ at m/z 933) and identified it as castalagin/vescalagin.[12] Mass spectrometry characteristics of compounds **14** and **15** ($[M - H]^-$ at m/z 937) coincided with those of trigalloyl-HHDP-glucoside, previously described by our group in a report on *Castanea sativa* Mill. flowers.[34] Compound **19** ($[M - H]^-$ at m/z 433) released a unique ion fragment at m/z 301, equivalent to ellagic acid after yielding a pentoside unit (132 u); therefore, this compound was tentatively identified as ellagic acid pentoside. Compound **22** presented a doubly charged pseudomolecular ion ($[M - 2H]^{2-}$ at m/z 865, which was tentatively identified as ellagic acid pentoside.

Indeed, hydrolysed tannins are the main phenolic compounds studied in the *E. brasiliensis* fruit.[5,12] GS showed the highest number of these compounds (17 compounds with a total of 104 mg per g E), followed by GR (17 compounds, 85 mg per g E), whereas GP presented 13 compounds with a total of 13 mg per g E. Teixeira *et al.*[12] detected ten and seven ellagitannins, in grumixama fresh pulps from yellow and purple varieties, respectively. According to their results, castalagin/vescalagin was the most abundant compound detected by HPLC-ESI-MS/MS. In our study, the digalloyl-HHDP-glucose isomers were the most abundant in the GS and GR samples, followed by sanguiin H-10. In contrast, hydroxymethoxyphenyl-galloyl-hexoside was the most representative hydrolysable tannin detected in GP.

Regarding condensed tannins, seven compounds were detected. A procyanidin tetramer was found in compound **12** ($[M - H]^-$ at m/z 1153), which showed fragmentation behaviour in MS^2 similar to the one attributed to the compound identified in *Prunus avium* L. leaves.[35] Compounds **25** and **26**, with the same precursor ion ($[M - H]^-$ at m/z 865), exhibited similar mass spectrum parameters to those of a B-type procyanidin trimer elucidated in grape pomace.[36] Compound **27** ($[M - H]^-$ at m/z 863, showed fragmentation similar to that of compounds **25** and **26**, except for having 2 u less than the others. Therefore, this compound was identified as a single A-type like procyanidin trimer.[36] Compounds **30** ($[M - H]^-$ at m/z 861) showed 4 u less than compounds **25** and **26**; in previous studies, this compound has been identified as a double A-type linked procyanidin trimer.[36] Compounds **28** and **29** ($[M - H]^-$ at m/z 1017) revealed the presence of a galloyl group in their composition. According to a study carried out with grape pomace, this compound was tentatively identified as monogalloylated type B procyanidin.[36] As far as we know, this is the first study reporting the presence of such compounds in *E. brasiliensis*.

Previously, Teixeira *et al.*[12] have detected only one (*epi*)catechin(*epi*)catechin ($[M - H]^-$ at m/z 577) when analysing purple and yellow grumixama seeds. However, this compound was not found in our residue extracts. In general, GP showed the highest condensed tannin content, with a total of 27 ± 1 mg per g E, while monogalloylated B-type procyanidin trimer isomer II (19 ± 1 mg per g E) was the most abundant compound. On the other hand, only a procyanidin tetramer (1.27 ± 0.01 mg per g E) was detected in GS. Only two procyanidins were found in GR, namely monogalloylated B-type procyanidin trimer isomer II (0.155 ± 0.004 mg per g E) and a procyanidin tetramer (9.4 ± 0.3 mg per g E). Such a low concentration of condensed tannins in GR is probably due to the small percentage of peel in this sample.

Regarding the flavonoid compounds found in the *E. brasiliensis* residue, three quercetin derivatives were detected and identified as quercetin-3-*O*-glucoside and quercetin-*O*-hexoside (compounds **13** and **17** ($[M - H]^-$ at m/z 463), respectively), and quercetin-3-*O*-rutinose (compound **16** $[M - H]^-$ at m/z 609), with reference to the standard compounds used in the quantification. The presence of quercetin-3-*O*-glucoside

Table 3 Phenolic profile of hydroethanolic extracts of residue (GR), seeds (GS), and peels (GP) of *E. brasiliensis*. Part (1): tentative identification of the compounds detected. Part (2): quantification of phenolic compounds in each sample

Part (1) Identification

Peak	RT (min)	Λ_{max} (nm)	$[M \pm H]^{\pm}$ (m/z)	MS^2 (m/z)	Tentative identification	Ref.
1	4.19	280	783	633(21), 481(10), 301(100)	bis-HHDP-glucose isomer I	12
2	4.33	280	783	633(21), 481(10), 301(100)	bis-HHDP-glucose isomer II	12
3	4.52	280	935	633(25), 301(100)	Galloyl-bis-HHDP-glucose isomer I	12
4	4.78	280	935	633(25), 301(100)	Galloyl-bis-HHDP-glucose isomer II	12
5	5.12	277	453	313(15), 169(100)	Hydroxymethoxyphenyl-galloyl-hexoside	32
6	5.68	280	783	633(21), 481(10), 301(100)	bis-HHDP-glucose isomer III	12
7	6.16	278	1568	1265(100), 1103(8), 933(16), 783(5), 633(17), 301(72)	Sanguiin H-10	33
8	6.54	281	785	785(100), 633(5), 483(17), 301(25)	Digalloyl-HHDP-glucose isomer I	12
9	7.01	281	785	785(100), 633(5), 483(7), 301(38)	Digalloyl-HHDP-glucose isomer II	12
10	7.42	278	783	633(21), 481(10), 301(100)	bis-HHDP-glucose isomer IV	12
11	8.09	280	933	915(5), 633(8), 451(24)301(7)	Castalagin/vescalagin	12
12	10.28	281	1153	865(6), 863(17), 577(61), 575(17), 289(42), 287(10)	Procyanidin tetramer	36
13	11.68	342	463	301(100)	Quercetin-3-*O*-glucoside	DAD-MSn
14	12.41	280	937	637(100), 467(12), 301(8)	Trigalloyl-HHDP-glucoside isomer I	34
15	12.87	280	937	637(100), 467(2), 301(4)	Trigalloyl-HHDP-glucoside isomer II	34
16	13.86	355	609	301(100)	Quercetin-3-*O*-rutinoside	DAD-MSn
17	14.29	351	463	301(100)	Quercetin-*O*-hexoside	DAD-MSn
18	14.99	282	1251	1083(100), 781(13), 601(4), 301(13)	Punicalagin gallate	36
19	15.51	362	433	301(100)	Ellagic acid pentoside	36
20	16.14	348	447	285(100)	Luteolin-*O*-hexoside	36
21	16.57	282	935	633(25), 301(100)	Galloyl-bis-HHDP-glucose isomer III	12
22	17.22	368	865	733(35), 564(9), 301(100), 169(18)	Ellagic acid pentoside	DAD-MSn
23	18.56	276	935	633(25), 301(100)	Galloyl-bis-HHDP-glucose IV	12
24	20.14	275	935	633(25), 301(100)	Galloyl-bis-HHDP-glucose V	12
25	22.58	282	865	739(100), 713(21), 695(35), 577(11), 575(9), 449(14), 451(7), 289(5), 287(2)	B-type Procyanidin trimers isomer I	36
26	23.51	282	865	739(100), 713(21), 695(35), 577(11), 575(9), 449(14), 451(7), 289(5), 287(2)	B-type Procyanidin trimers isomer II	36
27	25.25	287	863	847(32), 713(12), 695(54), 577(100), 533(11), 467(26), 453(4), 289(4), 287(8)	Single A-type linked PAC trimers	36
28	28.57	281	1017	891(52), 865(100), 729(21), 695(9), 577(35), 575(5), 407(9), 289(5), 287(6)	Monogalloylated B-type PAC trimers isomer I	36
29	29.54	287	1017	891(52), 865(100), 729(21), 695(9), 577(35), 575(5), 407(9), 289(5), 287(6)	Monogalloylated B-type PAC trimers isomer II	36
30	34.46	288	861	843(33), 735(100), 709(47), 693(65), 691(18), 575(55), 573(25), 421(19), 411(15), 289(11), 287(8), 285(5)	Double A-type linked PAC trimers	36
Anthocyanin compounds						
31	24.21	520	465	303(100)	Delphinidin-3-*O*-glucoside	37
32	25.23	517	449	287(100)	Cyanidin-3-*O*-galactoside	37
33	27.14	515	449	287(100)	Cyanidin-3-*O*-glucoside	37
34	28.77	516	419	287(100)	Cyanidin-3-*O*-arabinoside	37

Part (2) Quantification

	Quantification in term of dry extract (mg per g E)			Quantification in term of dry samples (mg per g dw)		
Peaks	GR	GS	GP	GR	GS	GP
1	4.78 ± 0.2*	6.53 ± 0.05*	nd	0.85 ± 0.03*	1.01 ± 0.01*	nd
2	3.1 ± 0.1^{b}	3.8 ± 0.01^{a}	nd	0.56 ± 0.01*	0.589 ± 0.002*	nd
3	4.8 ± 0.2^{b}	5.8 ± 0.1^{a}	1.11 ± 0.01^{c}	0.85 ± 0.02^{a}	0.90 ± 0.02^{a}	0.318 ± 0.002^{b}
4	8.2 ± 0.1^{b}	8.63 ± 0.02^{a}	1.15 ± 0.01^{c}	1.47 ± 0.02^{a}	1.340 ± 0.003^{b}	0.331 ± 0.002^{c}
5	0.405 ± 0.005*	nd	1.81 ± 0.005*	0.072 ± 0.001*	nd	0.520 ± 0.001*
6	0.4 ± 0.01^{c}	13.12 ± 0.03^{a}	0.93 ± 0.01^{b}	0.071 ± 0.001^{c}	2.036 ± 0.005^{a}	0.269 ± 0.002^{b}
7	13.53 ± 0.2^{b}	16.2 ± 0.1^{a}	1.32 ± 0.01^{c}	2.42 ± 0.04^{a}	2.51 ± 0.02^{a}	0.380 ± 0.004^{b}
8	6.4 ± 0.1^{b}	8.6 ± 0.1^{a}	1.41 ± 0.02^{c}	1.14 ± 0.02^{b}	1.33 ± 0.02^{a}	0.404 ± 0.006^{c}
9	15.8 ± 0.1^{b}	18.6 ± 0.1^{a}	1.28 ± 0.02^{c}	2.82 ± 0.01^{b}	2.88 ± 0.01^{a}	0.367 ± 0.005^{c}
10	8.7 ± 0.1*	9.6 ± 0.1*	nd	1.55 ± 0.01*	1.49 ± 0.01*	nd
11	6.4 ± 0.1*	4.59 ± 0.04*	nd	1.14 ± 0.02*	0.71 ± 0.01*	nd

Table 3 *(Contd.)*

Part (2) Quantification

Peaks	Quantification in term of dry extract (mg per g E)			Quantification in term of dry samples (mg per g dw)		
	GR	GS	GP	GR	GS	GP
12	9.4 ± 0.3*	0.182 ± 0.001*	nd	1.69 ± 0.05*	0.0283 ± 0.0002*	nd
13	1.22 ± 0.02^{ab}	1.27 ± 0.01^{a}	1.14 ± 0.02^{b}	0.219 ± 0.004^{b}	0.197 ± 0.001^{c}	0.328 ± 0.007^{a}
14	6.8 ± 0.1^{b}	8.5 ± 0.1^{a}	0.59 ± 0.01^{c}	1.21 ± 0.01^{b}	1.32 ± 0.01^{a}	0.170 ± 0.004^{c}
15	0.5 ± 0.03^{b}	0.76 ± 0.01^{a}	0.8 ± 0.01^{a}	0.090 ± 0.005^{c}	0.118 ± 0.001^{b}	0.229 ± 0.004^{a}
16	4.05 ± 0.04^{b}	2.96 ± 0.03^{b}	12 ± 1^{a}	0.725 ± 0.006^{b}	0.46 ± 0.01^{c}	3.4 ± 0.2^{a}
17	1.12 ± 0.01^{b}	0.96 ± 0.01^{c}	1.78 ± 0.01^{a}	0.201 ± 0.001^{b}	0.149 ± 0.002^{c}	0.512 ± 0.003a
18	3.4 ± 0.1^{a}	3.2 ± 0.1^{a}	0.41 ± 0.01^{b}	0.61 ± 0.02^{a}	0.50 ± 0.02^{b}	0.119 ± 0.003c
19	0.71 ± 0.01^{a}	0.64 ± 0.01^{b}	nd	0.126 ± 0.002*	0.099 ± 0.001*	nd
20	0.76 ± 0.01^{b}	0.63 ± 0.02^{c}	1.81 ± 0.01^{a}	0.136 ± 0.001^{b}	0.098 ± 0.003^{c}	0.522 ± 0.003^{a}
21	0.62 ± 0.01^{b}	0.524 ± 0.003^{c}	1.16 ± 0.03^{a}	0.112 ± 0.001^{b}	0.0814 ± 0.0005^{c}	0.334 ± 0.008^{a}
22	nd	nd	0.351 ± 0.002	nd	nd	0.101 ± 0.001
23	0.194 ± 0.001*	nd	0.5 ± 0.01*	0.0346 ± 0.0001*	nd	0.143 ± 0.004*
24	nd	nd	0.57 ± 0.01	nd	nd	0.164 ± 0.004
25	nd	nd	1.36 ± 0.04	nd	nd	0.39 ± 0.01
26	nd	nd	1.35 ± 0.03	nd	nd	0.387 ± 0.008
27	nd	nd	1.34 ± 0.02	nd	nd	0.385 ± 0.005
28	nd	nd	1.56 ± 0.04	Nd	nd	0.45 ± 0.01
29	0.155 ± 0.004*	nd	19 ± 1*	0.028 ± 0.001*	nd	5.4 ± 0.2*
30	nd	nd	2.77 ± 0.01	nd	nd	0.797 ± 0.003
31	2.85 ± 0.07^{b}	2.597 ± 0.006^{c}	10.9 ± 0.5^{a}	0.57 ± 0.01^{b}	0.477 ± 0.001^{b}	3.5 ± 2^{a}
32	10.7 ± 0.2^{b}	5.4 ± 0.1^{c}	59 ± 0.6^{a}	2.14 ± 0.05^{b}	0.99 ± 0.02^{c}	19.2 ± 0.2^{a}
33	2.044 ± 0.006^{c}	2.280 ± 0.006^{b}	2.53 ± 0.05^{a}	0.410 ± 0.001^{b}	0.419 ± 0.001^{b}	0.81 ± 0.02^{a}
34	1.993 ± 0.003^{c}	2.332 ± 0.003^{b}	3.62 ± 0.08^{a}	0.400 ± 0.001^{b}	0.428 ± 0.001^{b}	1.16 ± 0.02^{a}
THT	85 ± 1^{b}	110.5 ± 0.8^{a}	13 ± 0.2^{c}	15.2 ± 0.2^{b}	17.2 ± 0.1^{a}	3.85 ± 0.05^{c}
TCT	9.4 ± 0.3b	0.182 ± 0.001^{c}	27 ± 1^{a}	1.69 ± 0.05^{b}	0.0283 ± 0.0002^{c}	7.8 ± 0.3^{a}
TF	7.2 ± 0.1^{b}	5.8 ± 0.1^{b}	17 ± 1^{a}	1.28 ± 0.01^{b}	0.90 ± 0.01^{b}	4.8 ± 0.2^{a}
TPC-na	101 ± 2^{b}	115 ± 1^{a}	57 ± 2^{c}	18.1 ± 0.3^{a}	18.1 ± 0.1^{a}	16.4 ± 0.5^{b}
TA	17.5 ± 0.3^{b}	12.6 ± 0.1^{c}	77 ± 1^{a}	3.52 ± 0.06^{b}	2.31 ± 0.02^{c}	24.6 ± 0.4^{a}

nd – not detected; PAC – procyanidin; THT – Total hydrolysable tannins; TCT – Total condensed tannins; TF – Total flavonoids; TPC-na – Total phenolic compounds non-anthocyanin; TA – Total anthocyanin; different letters on the same mean difference means significant difference between the sample by the Tukey HSD test ($p < 0.005$). * on the same line means a significant difference between the samples by the Student's *t*-test ($p < 0.05$).

has been reported in trace amounts in fresh purple grumixama, but has not been detected in its seeds.[12] However, in the present study, this compound was found in a relative amount in GS (8.5 ± 0.1 mg per g E) and at lower concentrations in GR and GP (1.22 ± 0.02 and 1.14 ± 0.02 mg per g E, respectively). Compound **20** ($[M - H]^-$ at *m*/*z* 447) released the fragment ion at *m*/*z* 285, characteristic of luteolin/kaempferol after the detachment of a hexoside unit (162 u). Considering RT and λ_{max}, this compound was identified as luteolin-*O*-hexoside. As far as we know, this is the first time that this compound is detected in *E. brasiliensis*. GP showed the highest concentration of luteolin-*O*-hexoside, followed by GR.

Regarding the anthocyanins, four compounds were detected and identified in each part of the *E. brasiliensis* residue. The first anthocyanin detected, compound **31** ($[M + H]^+$ at *m*/*z* 465), released a mass fragment at 303 *m*/*z*, characteristic of the aglycone delphinidin after the loss of the hexoside molecule (162 u). According to the previous elucidation of the chemical profile of *E. brasiliensis*, this anthocyanin was tentatively identified as a delphinidin-3-*O*-glucoside.[11,12,37] Compounds **32** and **33**, with the molecular ion $[M + H]^+$ at *m*/*z* 449, showed the same fragmentation behaviour $[M + H - 162]^+$, revealing an anthocyanidin of cyanidin (*m*/*z* 287); however, these compounds showed retention times, and according to the literature, the first cyanidin hexoside was identified as cyanidin-3-*O*-galactoside and the other one as cyanidin-3-*O*-glucoside.[11,12,37] The last anthocyanin compound detected, compound **34** ($[M + H]^+$ at 419 *m*/*z*), also released a molecule of cyanidin, suggesting the loss of a pentose unit (132 u); furthermore, this compound was tentatively identified as a cyanidin-3-*O*-arabinoside.[37] In contrast to what found in previous studies,[11,12,37] cyanidin-3-*O*-galactoside was the most abundant compound found in the grumixama samples, followed by delphinidin-3-*O*-glucoside, whereas cyanidin-3-*O*-glucoside was the minor compound detected. As for the amount of anthocyanins, as expected, GP presented the highest content among the samples, followed by GR, while a low content of anthocyanins was found in GS. Other authors have not reported the presence of anthocyanins in the seed.[12] Nonetheless, the presence of anthocyanins in our seed residue extract may be explained by the contact of this part of the fruit with the corresponding peel during the depulping process.

The total anthocyanin content in GR (3.52 ± 0.06 mg per g dw) was higher than that described in the literature for the grumixama residue produced by the same supplier (1.04 mg per g dw), but using different extraction methods.[38] Machado

et al.[38] applied an extraction process by Soxhlet using ethanol as the solvent for compound recovery. In addition, the fruit composition, and consequently the composition of its residue, can be varied according to the climatic interferences of the different years of production. The variation in the grumixama composition in different years has been reported, as well as variation according to geographic regions.[12]

Regarding anthocyanins in the grumixama peel, the total of compounds recovered in this work (24.6 ± 0.4 mg per g dw) was lower than the value described in the literature for peels taken from whole fruits (48.37 mg per g dw).[11] This difference may be a result of the pulp processing, which may promot the extraction of intrinsic liquids from the fruit peel, and consequently, the transfer of anthocyanins to the fruit pulp. One must also consider that the extractive method used by Nascimento *et al.*[11] was ultrasound extraction with a mixture methanol and formic acid, which was likely more effective for anthocyanin recovery than the method employed in our study.

As shown in Table 3, GP contains a high amount of anthocyanin compounds. From a nutraceutical point of view, the frequent consumption of the whole grumixama fruit could virtually contribute to a good health maintenance, as a strong body of evidence suggests that consuming berries rich in anthocyanin compounds can provide benefits to mental health, improving cognitive function and memory,[39,40] as well as reducing the risks of developing anxiety and depression.[41] Additionally, due to their antioxidant and anti-inflammatory properties, such compounds have the potential to prevent cardiovascular diseases and cancer.[39,40]

In addition, the phenolic analysis of the samples showed that GS is richer in hydrolysable tannins, whereas the highest concentration of flavanols, condensed tannins, and anthocyanins was found in GP. Consequently, GR showed a high amount of phenolic non-anthocyanins (101 ± 2 mg per g E and 18.1 mg per g dw) and hydrolysable tannins (85 ± 1 mg per g E and 15.2 mg per g dw), and a moderate amount of anthocyanins (17.5 ± 0.3 mg per g E and 3.52 ± 0.06 mg per g dw).

3.2 Bioactivities of the extracts

3.2.1 Antioxidant activity. The hydroethanolic extracts obtained from the *E. brasiliensis* residues were evaluated regarding their capability to inhibit lipid oxidation, delay the oxidative haemolysis, and inhibit the cellular oxidation, and the results are shown in Table 4. In general, the extracts showed a high capacity to inhibit lipid peroxidation (EC_{50} = 0.90–1.34 μg mL^{-1}), all of them being more efficient than the positive control (Trolox EC_{50} = 5.8 μg mL^{-1}). However, GS and GR had the better activities, without significant differences between their EC_{50} values, and GP had the worst performance (EC_{50} = 1.34 μg mL^{-1}). Regarding the ability to prevent the oxidative haemolysis, GS was the most effective sample and showed similar antioxidant activity to that of Trolox. GP required the highest concentration to exert sufficient antioxidant activity to preserve 50% of the erythrocytes, whereas GR needed an moderate concentration. For the CAA assay, the results were contrary to those obtained by the other antioxidant methods. The GP extract, at a concentration of 2 mg mL^{-1}, was able to promote 80 ± 0.6% inhibition of oxidation. At the lowest concentration tested (125 μg mL^{-1}), this extract showed 18% inhibition activity. The GS extract had a lower antioxidant capacity (31 ± 3.2%).

Table 4 Bioactivities of hydroethanolic extracts obtained from the whole residue (GR), seeds (GS) and peels (GP) of *E. brasiliensis*

	Samples		
Bioactivity assays	GR	GS	GP
Antioxidant activity			
TBARS (EC_{50}, μg mL^{-1})	0.93 ± 0.03^{b}	0.90 ± 0.04^{b}	1.34 ± 0.04^{a}
OxHLIA (IC_{50}, μg mL^{-1})	29 ± 1^{c}	21.6 ± 0.6^{b}	65 ± 2^{a}
CAA (% inhibition)	61 ± 4^{b}	31 ± 1^{c}	80 ± 6^{a}
Anti-inflammatory activity (EC_{50}, μg mL^{-1})			
RAW 264.7	279 ± 1*	98.0 ± 0.5*	>400
Anti-proliferative activity (GI_{50}, μg mL^{-1})			
AGS	28 ± 3^{b}	28 ± 2^{b}	75 ± 4^{a}
Caco-2	44 ± 2^{b}	14.7 ± 0.3^{a}	67 ± 3^{a}
MCF-7	52 ± 3^{b}	47 ± 4^{b}	73 ± 5^{a}
NCI-H460	71 ± 3^{b}	62 ± 1^{b}	186 ± 4^{a}
Hepatotoxicity (GI_{50}, μg mL^{-1})			
PLP2	153 ± 14^{b}	145 ± 22^{b}	268 ± 22^{a}

Control used: Trolox for TBARS (IC_{50} = 5.8 ± 0.6 μg mL^{-1}) and OxHLIA (IC_{50} = 21.8 ± 0.2 μg mL^{-1}); quercetin for CAA (95% inhibition); dexamethasone for anti-inflammatory activity (EC_{50} = 16 ± 1 μg mL^{-1}); and ellipticine for AGS (GI_{50} = 1.23 ± 0.03 μg ml^{-1}), Caco-2 (GI_{50} = 1.21 ± 0.03 μg ml^{-1}), MCF-7 (GI_{50} = 1.02 ± 0.02 μg ml^{-1}), NCI-H460 (GI_{50} = 1.01 ± 0.01 μg ml^{-1}), and PLP2 (GI_{50} = 1.4 ± 0.1 μg ml^{-1}). In each line, different letters or an asterisk (*) indicate significant differences ($p < 0.05$) between samples by a Tukey HSD test or a Student's *t*-test, respectively.

Our results are in agreement with previous studies that evaluated the antioxidant activity of the seed and edible parts of *E. brasiliensis* fruits using other chemical methods, such as 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene bleaching, oxygen radical absorbance capacity (ORAC), superoxide anion, and hypochlorous acid assays, and the high antioxidant capacity of this fruit and its seeds have been validated.[2,3,42] Moreover, in a clinical trial, the ingestion of grumixama juice (10 mL per kg body weight), rich in cyanidin 3-*O*-glucoside and quercetin, besides ellagitannin derivatives, was efficient in increasing the antioxidant capacity of the plasma.[43]

The antioxidant potential of several South American fruits, including Andean blackberry (*Rubus glaucus* Benth.), tomato (*Solanum betaceum* C.) and Andean berry (*Vaccinium meridionale* Swartz), is closely linked to their anthocyanin and ellagitannin contents. Additionally, studies indicate that their antioxidant potential is associated with numerous health benefits.[29,44]

3.2.2 Anti-inflammatory activity. The samples were evaluated regarding their ability to inhibit the pro-inflammatory mediator NO formation. The results are presented in Table 4. Only GS and GR showed anti-inflammatory activity, as no activity was observed for GP in the maximum concentration tested (400 μg mL^{-1}). GS showed the highest anti-inflammatory action (EC_{50} = 98 μg mL^{-1}), probably due to its phenolic

composition that is rich in ellagitannin derivatives. Other studies detected the anti-inflammatory activity of the other parts of *E. brasiliensis*. For example, the hydroethanolic extract of its pulp (200 μg mL^{-1}) has shown effectiveness in the decreased NF-kB activation and TNF-α release *in vitro* assay using the RAW 267 cells; besides, in a mouse model, a low dose of the extract (3 mg kg^{-1}) was enough to decrease TFN-α levels, and a higher dose of the same extract (30 mg kg^{-1}) exerted anti-edematogenic effects.[1] The hydroethanolic and ethyl acetate extracts of grumixama leaves containing quercetin derivatives, catechin, galangin, and apigenin were able to reduce inflammation in mice with pleurisy induced by carrageenan.[9]

3.2.3 Anti-proliferative activity. The antiproliferative potential of the grumixama residue samples were tested against four human-tumour cells and the results are shown in Table 4. In general, all samples inhibited the proliferation of malignant cells at low concentrations. The seed extract (GS) showed the greatest activity against the Caco-2 cells and had similar activity to that of GR on the AGS, MCF-7 and NCI-H460 cells. For all cell lines, a higher concentration of GP was required, indicating lower antiproliferative potential for this sample. Among the cell lines evaluated, the Caco-2 and AGS cells were more sensitive to *E. brasiliensis* residue extracts (GI_{50} = 28–75, and 14.7–67 μg mL^{-1}, respectively), whereas higher concentrations were required to reduce NCI-H460 cell development (GI_{50} = 62–186 μg mL^{-1}). The results herein reported suggest that both the *E. brasiliensis* whole fruit and bioresidues could be better explored for the screening of new therapeutic alternatives against tumour illness. This hypothesis can be supported by results reported by Teixeira *et al.*,[45] which showed that a single ingestion of *E. brasiliensis* juice (10 mL per kg body weight) by health women can produce metabolites able to inhibit the proliferation of breast adenocarcinoma cells (MDA-MB-231 cells). Furthermore, the main phenolic compounds found in the *E. brasiliensis* residues, namely anthocyanins and ellagitannins, can be responsible for such anti-proliferative activity.[3,45] This was indicated by the researchers who reported the anticancer potential of the Andean berry (*Vaccinium meridionale* Swartz), a native fruit from South America. The authors found a correlation between the action of *V. meridionale* against colon cancer cells and its phenolic and anthocyanin composition.[44]

For the cytotoxicity on human-tumour cells, a primary culture from porcine liver cells was also tested and the results are shown in Table 4. The samples showed the same behaviour above mentioned for tumour cell lines. No significant difference was detected between the hepatotoxicity of GS and GR, which were more toxic than GP. Although the samples showed hepatotoxic potential at moderate concentrations (GI_{50} = 145, 153 and 268 μg mL^{-1} for GS, GR and GP, respectively), the concentrations necessary to inhibit the growth of the tested tumour cells were lower (GI_{50} = 14.7–62, 28–71 and 67–186 μg mL^{-1} for GS, GR and GP, respectively) than the toxic concentrations.

In an animal model assay using *Galletia mellonella* larvae, the hydroethanolic extract of *E. brasiliensis* seed showed low toxicity (2.5 g kg^{-1}).[46] On the other hand, the pulp extract, by the same assay, showed no toxicity in a concentration range from 0.62 to 10 g kg^{-1}.[1] The distinct toxicity degrees found in different parts of *E. brasiliensis* may be explained by the particular chemical composition of each plant part.[1] For a better understanding and description of the toxicity of extracts obtained from the residues of *E. brasiliensis*, further studies are necessary.

3.2.4 Antimicrobial activity. The samples were evaluated regarding their potential to inhibit the growth of seven bacteria and two microfungi, the results are presented in Table 5.

Table 5 Antibacterial and antifungal activities of hydroethanolic extracts obtained from the whole residue (GR), seeds (GS), and peels (GP) of *E. brasiliensis*

	Samples						Control			
	GR		GS		GP		Streptomycin		Ampicillin	
Antibacterial activity (mg mL^{-1})	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive bacteria										
Staphylococcus aureus	0.3125	>20	1.25	>20	0.625	>20	0.0078	0.0078	0.156	0.156
Bacillus cereus	0.3125	1.25	0.156	0.625	0.078	0.3125	0.0078	0.0078	nt	nt
Listeria monocytogenes	2.5	>20	2.5	>20	0.3125	>20	0.0078	0.0078	0.156	0.156
Gram-negative bacteria										
Escherichia coli	*5*	20	2.5	10	5	20	0.1	0.1	0.156	0.156
Salmonella enterica	1.25	>20	1.25	>20	>20	>20	0.0078	0.0078	0.156	0.156
Pseudomonas aeruginosa	*1.25*	>20	2.5	>20	1.25	>20	nt	nt	0.625	0.625
Yersinia enterocolitica	0.078	0.3125	0.156	0.625	0.625	2.5	0.0078	0.078	0.156	0.156

							Ketoconazole	
Antifungal activity (mg mL^{-1})	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus fumigatus	1.25	>20	0.625	>20	0.3125	>20	0.5	1.0
Aspergillus brasiliensis	1.25	>20	0.625	>20	1.25	>20	0.0625	0.125

MIC – minimal inhibitory concentration; MBC or MFC – minimum bactericidal or fungicidal concentrations, respectively.

All samples showed antibacterial activity on bacteria tested, except GP that was not able to inhibit the growth of *S. enterica* at maximal concentrations used (20 mg mL^{-1}). In general, *B. cereus* and *L. monocytogenes* were more sensitive to the GP extract (MIC = 0.078, and 0.3125 mg mL^{-1}, respectively), whereas GR was more harmful to *S. aureus* and *Y. enterocolitica* (MIC = 0.3125 and 0.078 mg mL^{-1}, respectively), and GS showed the best activity to inhibit *E. coli* (MIC = 2.5 mg mL^{-1}). In addition to their bacteriostatic activity, all samples showed bactericidal action on *B. cereus*, *E. coli*, and *Y. enterocolitica.* For the other microorganisms, no bactericidal activity was observed at the maximum concentration evaluated. Unfortunately, no studies addressing the antibacterial activity of *E. brasiliensis* residues were found in the literature to compare with our results. The antibacterial activity of grumixama pulp extracts has also been confirmed on *S. aureus*, *B. cereus*, and *P. aeruginosa*.[42,47] On the other hand, the pulp extract did not show antibacterial activity against *L. monocytogenes* and *E. coli*.[42]

Regarding the antifungal activity of the samples, the data presented in Table 5 show that all of them were able to inhibit the fungal growth; however, none of them showed a fungicidal effect in the maximal concentration tested (20 mg mL^{-1}). GP was the most efficient one on *A. fumigatus* (MIC = 0.3125 mg mL^{-1}), while GS had the best activity against *A. brasiliensis* (MIC = 0.625 mg mL^{-1}).

Although the antifungal activity of the *E. brasiliensis* plant and fruit has been described in previous studies, to the best of our knowledge, this is the first time that its fruit residues are evaluated for antifungal potential. The extracts obtained from the seeds and leaves of *E. brasiliensis* have shown inhibitory action against *Candida albicans* (MIC = 15.62 and 31.25 μg mL^{-1}, respectively), but its pulp extract did not exhibit such biological activity.[46]

4. Conclusion

According to our findings, the bioresidue of *E. brasiliensis* berries is composed of a greater amount of seeds than peels. When analysed individually, the seeds had a higher content of hydrolysable tannins, while the peel fraction was richer in organic acids, tocopherols, condensed tannins, and anthocyanin. In general, the residue has a chemical composition based on the mixture of the two fractions studied. Hydroethanolic extracts obtained from each part of the residue showed promissory biological potential, requiring low concentrations to exert antioxidant, antiproliferative, and antimicrobial activities. In addition, GS and GR showed anti-inflammatory properties. Therefore, the *E. brasiliensis* fruit residue could be exploited to produce high added-value additives, such as natural bifunctional (colorant-preservative) ingredients for food products, cosmetics, nutraceuticals, and pharmaceuticals, following the circular bioeconomy concept and, not less importantly, stimulating the Grumixama production chain.

Author contributions

B. R. Albuquerque: investigation, formal analysis, and writing – original draft; T. C. Finimundy: investigation, methodology, and writing – review & editing; J. Pinela: methodology and writing – review & editing. T. C. P. S. Pires: methodology and writing – review & editing; F. Mandim: methodology and writing – review & editing; J. Vaz: methodology and writing – review & editing. R. C. G. Corrêa: writing – review & editing; M. B. P. P. Oliveira: conceptualization, supervision, and writing – review & editing; L. Barros: conceptualization, methodology, supervision, writing – review & editing, and funding acquisition.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2020). National funding by FCT, P.I., through the individual scientific employment program-contract for the contracts of J. Pinela (CEECIND/01011/2018) and L. Barros (CEEC Institutional) and the research grants of B. R. Albuquerque (SFRH/BD/136370/2018 and COVID/BD/152908/2022) and F. Mandim (SFRH/BD/146614/2019) are gratefully acknowledged. Thanks to the MICINN for the Juan de la Cierva Formación contract for T. C. S. P. Pires (FJC20120-045405-I). R. C. G. Corrêa is a research grant recipient of the Cesumar Institute of Science, Technology, and Innovation (ICETI).

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