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Exploring the bioactive compounds from berry biowaste: *Eugenia involucrata* DC. as a study case

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ABSTRACT

Aiming at valorising the *Eugenia involucrata* residue, the present work aimed in investigating the chemical profile and *in vitro* bioactivities of the residue (EIR) and its individual parts, i.e., seeds (EIS) and peels (EIP). Chromatographic analyses showed that EIP had the highest content of organic acids, tocopherols, and anthocyanins, whereas EIS was the richest in hydrolysable tannins. bis-HHDP-glucoside isomers and cyanidin-3-*O*-galactoside were the main detected phenolic compounds. By the cellular antioxidant activity (CAA) assay, the samples showed similar. antioxidant potential with 71%, 77%, and 80% inhibition for EIP, EIR, and EIS, respectively, while EIS demonstrated the best antioxidant activity by thiobarbituric acid reactive substances (TBARS, IC_{50} = 4.96 μg/mL) and oxidative inhibition assay (OxHLIA, IC_{50} = 40 μg/mL). The samples were able to inhibit the growth of seven bacteria and two fungi. Between the microorganisms tested, all samples showed a strong antibacterial activity (MIC = 0.156 mg/mL) against *Yersinia enterocolitica* and *Pseudomonas aeruginosa* (MIC = 0.156–0.625 mg/mL). Moreover, they also display a high antifungal activity against *Arpergillus brasiliensis* (MIC = 0.625 mg/mL). Furthermore, only EIR and EIS had anti-inflammatory effect *via* NO production inhibition (IC_{50} = 168–259 μg/mL, respectively) and anti-proliferative activity towards four tumour and one non-tumour cell lines. These findings suggest that the biowaste generated during the processing of *E. involucrata* fruit has potential as a source of bioactive compounds.

1. Introduction

Fruit processing produces huge amounts of biowaste worldwide, biomasses with no commercial value but potentially rich in bioactive compounds. Of the 1.4 million metric tons of fruits processed by year in the planet, 25%–30% is converted into waste or by-products (Patra et al., 2022). Thus, to minimize the loss and environmental impact inherent to this monumental biomass, the recovery of phytochemicals and dietary fibres from fruit wastes, which could be upcycled transformed into a new product that can potentially be added to other industrial products, such as functional foods, supplements, besides pharmaceutical and cosmetic ingredients, following the sustainability upcycle concern that has been proposed by several authors (Marcillo-Parra et al., 2021; Spacki et al., 2022). This concept can help reduce waste, make production more resource-efficient, and contribute to the development of new products/ingredients. In general, this industrial chain aims to reduce the cost of production and disposal of waste generated, developing more sustainable products with high added value, which meet the circular economy, transforming valueless waste into a product with added value. In the specific case of berries, highly recommended in the diet due to their health benefits, the industrial processing for the production of juices and frozen pulps generates large

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amounts of biowaste rich in phenolic acids and flavonoids (antioxidant molecules). In this sense, in recent years several studies have been carried out to exploit berry pomaces as functional ingredients for bakery products and pasta, among others, as well as to obtain natural food additives such as colorants and preservatives (Albuquerque, Oliveira, et al., 2020; Venskutonis, 2020).

In this context, the processing of native Brazilian berry fruits, namely of the genus Eugenia, generates a biowaste that could be better valued, however, studies on some of these berries and their residues are scarce in the literature (Sardi et al., 2017). Among the *Eugenia* species, the *Eugenia involucrata* DC. is still little-known and underexplored (Fig. S1). However, some Brazilian environmental recovery programs have encouraged its planting and consider it to have economic value (Barbosa, 2017; Degenhardt et al., 2007; Vieira et al., 2016). Nonetheless, despite being native to Brazil, the species can adapt to other milder temperature regions, such as the Mediterranean climate, being found in Sicily Island, Italy (Mannino et al., 2022).

The *Eugenia involucrata* fruit, locally known as *Cereja brasileira* (Brazilian cherry), *Cereja do Rio Grande* or *Cereja do mato*, is a sweet red-purple berry, with length ranging from 2 to 4 cm and diameter between 1.3 and 2.7 cm, containing 3 to 4 seeds, that is not commonly commercialized *in natura* due to its high perishability. Hence, small local industries exploit *E. involucrata* berries for the production of frozen fruit pulps and other products, such as ice creams, jellies, and liqueurs (Degenhardt et al., 2007).

Regarding the scientific knowledge on the Brazilian cherry fruit, few data on its chemical composition and bioactivities are found in the literature. Some phenolic compounds, such as phenolic acids, flavonols, proanthocyanidins and anthocyanins, have been previously identified by HPLC-DAD-MS/MS, UHPLC-MS/MS, HPLC-PDA, and HPLC-Orbitrap (Girardelo et al., 2020; Infante et al., 2016; Mannino et al., 2022; Nicácio et al., 2017; Schmidt et al., 2020). However, the quantification of these compounds is not completely described. Furthermore, the same authors have reported the high antioxidant activity of this fruit (Girardelo et al., 2020; Infante et al., 2016; Mannino et al., 2022; Nicácio et al., 2017; Schmidt et al., 2020). With respect to the phytochemical and bioactive profiles of the biowaste generated by the industrial processing of the fruit, information is even scarcer. While specific studies outline the characterization of phenolic compounds and antioxidant activity in extracts derived from the peel and seeds of the unprocessed *E. involucrata* fruit (Girardelo et al., 2020; Nicácio et al., 2017), no studies, as of the preparation of this manuscript, have investigated the chemical characteristics and bioactive properties of the residue from industrial *E. involucrata* fruit processing.

Considering all the above, this study aimed to evaluate the potential of *E. involucrata* biowaste as a source of bioactive compounds. For this purpose, the chemical characterization of the whole biowaste (EIR) from the production of its frozen pulp was carried out, as well as that of its fractions, namely seeds (EIS) and peels (EIP), in terms of hydrophilic (organic acids and phenolic compounds) and lipophilic (tocopherols and fatty acids) compounds. Additionally, antioxidant, anti-inflammatory, anti-proliferative, and antimicrobial activities of the extracts were investigated by different *in vitro* assays. The concentration of extracts that can cause liver toxicity was also assessed using porcine liver primary cells.

2. Material and methods

2.1. Sample preparation

Eugenia involucrata residue was supplied by Sitio do Bello - Frutas Nativas®, a local producer of frozen pulp from Brazilian native species, localized in Paraibuna, São Paulo, south-eastern Brazil. Three distinct samples of fruit residue were obtained: (1) the entire *E. involucrata* residue (EIR); (2) the seeds (EIS, equivalent to 92 ± 2% of EIR), and (3) the peels (EIP, equivalent to 8 ± 2% of EIR). These biomaterials were kept frozen until the lyophilization process (FreeZone 4.5, Labconco, Kansas City, MO, USA) conducted until constant weight (dry matter yields of 39 ± 2%, 37.1 ± 0.8%, and 20.1 ± 0.1% for EIR, EIS and EIP, respectively, concerning the fresh weight of the sample), homogenized, and finally stored at −20 °C until analysis.

2.2. Determination of the chemical composition

2.2.1. Tocopherols

Tocopherol isomers present in the samples were extracted by consecutive centrifugation using methanol and hexane as solvents, following the protocol described by Barros et al. (2013). The identification and quantification were performed in a High-Performance Liquid Chromatography (Knauer, Berlin, Germany) system connected to a fluorescence detector (HPLC-FL 2020; Jasco, Japan). To identify and quantify the compounds, authentic standards of the α-, β-, γ- and δ-tocopherol isoforms were used. To correct any interferences in the quantification, Tocol was employed as an internal standard. Results were expressed as mg/100 g dry weight (dw).

2.2.2. Fatty acids

The crude lipid fractions of the samples were obtained by Soxhlet extraction with petroleum ether (120 °C/7 h), which were further transesterified and analysed by gas chromatography with flame ionization detection (GC-FD), as described by Sampaio et al. (2021). The fatty acid methyl esters (FAME) were identified by comparing their retention time with the standard (standard mixture 47885-U, Sigma, St. Louis, MO, USA). Results were expressed in relative percentages of each fatty acid.

2.2.3. Organic acids

Organic acids present in the samples were extracted with metaphosphoric acid (4.5% *w/v*), identified and quantified in an ultra-fast liquid chromatography system coupled to a photodiode array detector (UFLC-PDA, Shimadzu, 20A series, Nakagyo Ward, Kyoto, Japan), as previously described by Barros et al. (2013). For quantification, calibration curves constructed with oxalic ($y = 1 \times 10^7x + 231{,}891$; $R^2 = 0.9999$), malic ($y = 950041x + 6255.6$; $R^2 = 0.9998$), ascorbic ($y = 4 \times 10^7x + 1 \times 10^6$; $R^2 = 0.9909$), and citric ($y = 1 \times 10^6x + 10{,}277$; $R^2 = 0.9997$) acids were used. Results were expressed in mg/100 g dw.

2.2.4. Non-anthocyanin phenolic compounds

Hydroethanolic extracts (80:20 ethanol:water, *v/v*) were obtained from each sample (33 g/L) by solid-liquid extraction at room temperature, as described by (Albuquerque, Pereira, et al., 2020). The extracts were analysed in an HPLC-ESI-Orbitrap-MS system working in the condition previous described (Bessada et al., 2016). Compound detection was performed using a diode array detector (DAD) at the wavelengths of 280, 330 and 370 nm. The system coupled to Orbitrap Exploris 120 mass spectrometer (Orbitrap MS, Thermo, Walthan, MA, USA) with an electrospray ionization (ESI) source working in the negative mode. For identification, retention time (Rt), wavelength of maximum absorption (λ_{max}), pseudomolecular ion ($[M\text{-}H]^-$), UV–Vis spectrum, mass spectrum and patterns of the ion fragmentation (MS^2) were compared with literature and commercially available standard compounds. Data acquisition, processing and interpretation were carried out by Xcalibur® software (Thermo Finnigan, San Jose, CA, USA). For quantification, authentic standards of gallic acid ($y = 45933x - 19{,}932$, $R^2 = 0.9969$, limit of detection (LOD) = 1.05 μg/mL and limit of quantification (LOQ) = 4.41 μg/mL), ellagic acid ($y = 30262x - 32{,}276$, $R^2 = 0.9986$; LOD = 0.41 μg/mL and LOQ = 1.22 μg/mL), quercetin-3-*O*-rutinoside ($y = 23794x - 46{,}683$, $R^2 = 0.9998$, LOD = 0.18 μg/mL and LOQ = 0.65 μg/mL), quercetin-3-*O*-glucoside ($y = 28555x + 3032.3$, $R^2 = 0.9998$; LOD = 0.21 μg/mL; LOQ = 0.71 μg/mL) and luteolin-6-*C*-glucoside ($y = 27772x - 11{,}351$, $R^2 = 0.9987$, LOD = 0.51 μg/mL and LOQ = 2.02 μg/mL) were used. Results were expressed in

mg/g of extract (E) and mg/g dw.

2.2.5. Anthocyanins

The samples were extracted in the same conditions described above but using solvent acidified with citric acid until pH 3 (Albuquerque, Pereira, et al., 2020). Anthocyanins were tentatively identified and quantified using an HPLC-DAD-MS/MS system working in the condition previously established by Gonçalves et al. (2017). For quantification, an authentic standard of cyanidin 3-*O*-glucoside ($y = 135478x - 3{,}000{,}000$, $R^2 = 0.9986$, LOD = 0.25 μg/mL; LOQ = 0.83 μg/mL) was used. The results were expressed in mg/g E and mg/g dw.

2.3. Assessment of the bioactivities of the hydroethanolic extracts

2.3.1. Antioxidant activity

The hydroethanolic extracts obtained in section 2.2.4 were evaluated regarding their antioxidant activity through three cell-based assays, which measured: i) the thiobarbituric acid reactive substance (TBARS) formation inhibition capacity (Corrêa et al., 2015), with results expressed as IC_{50} values (μg/mL), which means the half concentration required to inhibit the lipid peroxidation; ii) the oxidative haemolysis inhibition capacity (OxHLIA) (Lockowandt et al., 2019), with results expressed as IC_{50} values (μg/mL) for a 60 min Δt, meaning the extract concentration able to promote a 60 min haemolysis delay; and iii) the cellular antioxidant activity (CAA) (de la Fuente et al., 2022), with results expressed as the percentage of inhibition at the maximum concentration tested (2 mg/mL) in murine macrophage cells (RAW 246.7). Trolox was used as positive control for the TBARS and OxHLIA assays, whereas quercetin was used for the CAA assay.

2.3.2. Antimicrobial activity

A microdilution method and the rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay were performed as described by (de la Fuente et al., 2022) to assess the antimicrobial activity of the extracts. For antibacterial activity evaluation, four Gram-negative bacteria were tested, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Yesinia enterocolitica*, as well as three Gram-positive bacteria, namely *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*. The antifungal activity was evaluated against *Aspergillus brasiliensis* and *Aspergillus fumigatus*. The results were expressed as minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC) for bacteria and fungi, respectively, following protocols previously established by the group (de la Fuente et al., 2022). As positive controls, Ampicillin was and Streptomycin at 1 mg/mL were used for bacteria, likewise Ketoconazole for fungi.

2.3.3. Anti-inflammatory activity

Different concentrations of the extracts (6.25–400 μg/mL) were tested regarding their ability to inhibit lipopolysaccharide (LPS)-induced nitric oxide (NO) production by murine macrophage cell line (RAW 264.7) commercially acquired from European Collection of Authenticated Cell Cultures (ECACC) (Corrêa et al., 2015). The results were expressed as IC_{50} values (μg/mL).

2.3.4. Antiproliferative activity

The antiproliferative activities of the extracts were evaluated by the sulforhodamine B colorimetric assay (de la Fuente et al., 2022). Different concentrations (6.25–400 μg/mL) of each sample were tested on four human tumour cell lines, more specifically gastric cancer (AGS), colon cancer (Caco-2), breast cancer (MCF-7) and lung cancer (NCI–H460), commercially acquired from the Leibniz-Institute DSMZ – German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany, and on a non-tumour primary cell culture obtained from porcine liver cells (PLP2). The results were expressed as GI_{50} values (μg/mL), which means the extract concentration able to inhibit 50% of cell proliferation.

2.4. Statistical analysis

All analyses were performed in triplicate and the results are expressed as mean ± standard deviation. Statistical differences ($p < 0.05$) between two samples were assessed by Student's *t*-test and, for three samples, the analyses of variance (ANOVA) was used for detecting significant differences ($p < 0.05$) between then; besides, the Tukey's HSD test was applied to discriminate the samples. All statistical tests were performed in R software (version 4.0.3, R Project for Statistical Computing, https://www.r-project.org/). The package "Laercio" was used to carry out Tukey's HSD test.

3. Results and discussion

3.1. Chemical composition

3.1.1. Organic acids

The composition of the *E. involucrata* residue in terms of organic acids is presented in Table 1. A total of five organic acids were detected, among which citric acid was the major component. Only traces of ascorbic and fumaric acids were detected in the samples. All compounds were found in the greatest concentration in EIP, reason why this residue had the highest concentration of total organic acids (11.8 g/100 g dw); on the other hand, ES showed the lowest content (5.68 g/100 g dw). The organic acid composition of EIR was similar to that of EIS, since both samples did not show a significative difference ($p > 0.05$) in their malic and citric acid tenors. This outcome was somehow expected due to the high content of seeds in the residue. In the literature, few data regarding the chemical composition of *E. involucrata* are found. However, according to Mannino et al. (2022), only citric acid was identified in an ethanolic extract obtained from a mixture of pulp and peel of Brazilian cherry, although its content was not determined.

3.1.2. Tocopherols

The samples were evaluated regarding their tocopherol composition. According to the data presented in Table 1, only two tocopherol isomers, namely α- and γ-tocopherol, were detected in the extracts. The α-tocopherol content found in EIP was 2-fold its γ-tocopherol tenor; however, an opposite tendency was verified for samples EIS and EIR. In general, EIP showed the highest and EIR the lowest concentration of tocopherols (6.2 and 1.1 mg/100 g, respectively). Vitamin E (tocopherols and tocotrienols) is important for the protection of cell membranes against lipoperoxidation, being also essential for reproduction, so much that there is the recommendation intake of at least 12 mg/day to prevent erythrocyte haemolysis (Busso et al., 2021). Considering the levels found in *E. involucrata* residues, EIP could be explored as a source of vitamin E. To the best of the authors' knowledge, this is the first study

Table 1
Organic acid and tocopherol composition of *E. involucrata* residue (EIR), seeds (EIS), and peels (EIP).

	EIR	EIS	EIP
Organic acids (g/100 g dw)			
Oxalic acid	0.59 ± 0.01^{b}	0.46 ± 0.01^{c}	0.90 ± 0.04^{a}
Malic acid	0.49 ± 0.05^{b}	0.43 ± 0.04^{b}	1.04 ± 0.03^{a}
Ascorbic acid	tr	tr	tr
Citric acid	5.2 ± 0.1^{b}	4.8 ± 0.1^{b}	10.0 ± 0.2^{a}
Fumaric acid	tr	tr	tr
Total organic acids	6.25 ± 0.03^{b}	5.68 ± 0.02^{c}	11.9 ± 0.2^{a}
Tocopherols (mg/100 g dw)			
α-Tocopherol	0.59 ± 0.02^{b}	0.26 ± 0.01^{c}	4.3 ± 0.1^{a}
γ-Tocopherol	0.95 ± 0.02^{b}	0.85 ± 0.05^{b}	1.98 ± 0.04^{a}
Total tocopherols	1.53 ± 0.03^{b}	1.1 ± 0.1^{c}	6.2 ± 0.1^{a}

Different letters on the same line indicate significant differences ($p < 0.05$) between samples by a Tukey's HDS test. Tr – traces; dw – dry weight.

reporting the tocopherol composition of individual parts of the *E. involucrata* berry. However, Barzotto et al. (2019) verified that the leaves of the Brazilian cherry have a high content of α-tocopherol (87.9 mg/100 g dw), a concentration value much higher than the ones found in our samples.

3.1.3. Fatty acid profile

Before the determination of fatty acids, the lipid contents of the samples were determined by Soxhlet. The fat values found are shown in Table 2. EIS and EIR had a similar fat content ($p > 0.05$), higher than the one registered for EIP. The composition of the lipid fraction of each sample was analysed by GC-FID and their fatty acid profiles are presented in Table 2. In a total of 15 fatty acids were detected in the samples, being linoleic acid the most abundant, followed by palmitic and oleic acids. Such profiles corroborate literature data that describe the above mentioned fatty acids as the predominant ones in other berry pomaces (Campalani et al., 2020; Vašeková, 2020).

The samples were discriminated by the percentage of each fatty acid found in their composition. For example, EIP showed the highest percentage of palmitic and α-linolenic acids; on the other hand, the lowest amount of linoleic and oleic acids was found in this sample. Whereas EIS has the largest amount of linoleic acid. The fatty acid profile of EIR varied according to the composition of its fractions. In terms of fatty acid classes, around fifty per cent (45.5%–49.6%) of the fatty acids found in the samples were classified as polyunsaturated fatty acids (PUFA), whereas 37.5%–38.2% were saturated fatty acids (SFA) and the remainder were monounsaturated fatty acids (MUFA). EIP was richer in PUFA than EIS; on the contrary, it had the lowest amount of MUFA. Regarding the percentage of SFA, no significant difference was observed between EIP and EIS.

Table 2
Fatty acid composition of *E. involucrata* residue (EIR), seeds (EIS), and peels (EIP).

	EIR	EIS	EIP	*p*-value
Lipid content (g/100 g dw)	2.3 ± 0.2^{a}	2.7 ± 0.2^{a}	1.8 ± 0.1^{b}	0.0157
Fatty acids (relative %)				
Undecanoic acid (C11:0)	0.63 ± 0.02^{b}	0.71 ± 0.01^{a}	0.45 ± 0.02^{c}	0.0012
Lauric acid (C12:0)	0.83 ± 0.04	0.91 ± 0.04		0.1650
Tridecanoic acid (C13:0)	0.29 ± 0.02^{b}	0.23 ± 0.01^{b}	0.54 ± 0.05^{a}	0.0041
Myristic acid (C14:0)	0.73 ± 0.07^{ab}	0.56 ± 0.05^{b}	1.9 ± 0.1^{a}	0.0267
Palmitic acid (C16:0)	25.1 ± 0.7^{ab}	23.0 ± 0.6^{b}	26.22 ± 0.19^{a}	0.02505
Palmitoleic acid (C16:1)	nd	nd	1.41 ± 0.19	–
Margaric acid (C17:0)	0.54 ± 0.04	0.57 ± 0.05	0.47 ± 0.05	0.2496
Stearic acid (acid C18:0)	7.67 ± 0.06^{a}	7.99 ± 0.08^{a}	5.16 ± 0.09^{b}	<0.0001
Oleic acid (C18:1n9)	14.7 ± 0.2^{a}	14.02 ± 0.07^{a}	11.7 ± 0.4^{b}	0.0029
Linoleic acid (C18:2n6)	37.1 ± 0.4^{b}	40.1 ± 0.2^{a}	32.9 ± 0.2^{c}	0.0003
α-Linolenic acid (C18:3n3)	8.1 ± 0.1^{b}	7.3 ± 0.2^{b}	16.3 ± 0.3^{a}	<0.0001
Eicosanoic acid (C20:0)	1.9 ± 0.1^{b}	2.38 ± 0.04^{a}	1.21 ± 0.01^{c}	0.0007
Docosanoic acid (C22:0)	1.84 ± 0.07^{b}	1.6 ± 0.1b	2.17 ± 0.04^{a}	0.0216
Docosadienoic acid (C22:2)	0.4 ± 0.04	0.42 ± 0.01	0.33 ± 0.02	0.0719
Tricosanoic acid (C23:0)	0.24 ± 0.01^{b}	0.29 ± 0.02^{a}	0.17 ± 0.02^{c}	0.0143
Fatty acid classes				
SFA	39.7 ± 0.7^{a}	38.2 ± 0.4^{ab}	37.5 ± 0.1^{b}	0.0391
MUFA	14.7 ± 0.2^{a}	14.02 ± 0.07^{a}	13.1 ± 0.2^{b}	0.0057
PUFA	45.6 ± 0.5^{b}	47.8 ± 0.4^{ab}	49.46 ± 0.09^{a}	0.0058

Different letters on the same line indicate significant differences ($p < 0.05$) between samples by a Tukey's HDS test. SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

3.1.4. Phenolic compounds

According to the data shown in Table 3, hydrolysable tannins and flavonols were the only classes of compounds detected in the samples. In all samples, a phenolic acid was detected in the **Compound 11** ([M-H]$^{-}$ at *m/z* 433) that revealed, after the loss of a pentoside unit (−132 u), a product ion equivalent to an ellagic acid molecule (301 *m/z*). According to mass spectrometry characteristics and data from the literature, this compound was tentatively identified as an ellagic acid pentoside (Zhang & Zhu, 2015). A total of 9 compounds were tentatively identified as hydrolysable tannins, more specifically described as following. **Compounds 1**–**4** showed UV spectra coherent with galloyl and hexahydroxydiphenoyl (HHDP) derivatives. **Compounds 1** and **2**, presented a singly charged pseudomolecular ion [M-H]$^{-}$ at m/z 783 and daughter ions at m/z 481 and 301, which together with their early elution allowed their identification as bis-HHDP-glucose isomers (i.e., pedunculagin) isomers, characteristics of, which were confirmed in the study of the chemical composition of *Eugenia brasiliensis* Lam. (Teixeira et al., 2015). **Compounds 3** and **4** ([M-H]$^{-}$ at *m/z* 785, fragment ions at *m/z* 633, 481 and 301) coincided with a digalloyl-HHDP-glucose isomer, according to the study carried out by Teixeira et al. (2015). **Compounds 5**–**7** were tentatively described as castalagin/vescalagin ([M-H]$^{-}$ at *m/z* 933) owing to their mass spectrum characteristics that were similar to such compounds found in other Eugenia spp. fruits (Teixeira et al., 2015; Tong et al., 2014). **Compound 10** ([M-H]$^{-}$ at *m/z* 939), showed fragment pattern at *m/z* 631, 469, 169; previously, a compound with the same characteristics has been identified as pentagalloyl glucose in *Myrciaria jaboticaba* (Vell.) Berg. (Albuquerque, Pereira, et al., 2020). Finally, **compound 14** ([M-H]$^{-}$ at *m/z* 935) revealed two ion fragments during the ionization, namely at *m/z* 633 and *m/z* 301 (−170), possibly for the discharge of HHDP group (302 u), a gallic acid molecule (170 u) and a hexoside unit (162 u); therefore, these peaks were tentatively identified as galloyl-bis-HHDP-glucose isomers (Teixeira et al., 2015). To the best of our knowledge, this is the first study reporting the identification and quantification of these hydrolysable tannins in *E. involucrata*.

Regarding flavonols, three quercetin derivatives were detected as **Compounds 8, 7**, and **14**. The first one ([M-H]$^{-}$ at *m/z* 609) had a fragmentation behaviour that suggested the loss of a rutinosyl group ([M-H-308]); hence, this identification as quercetin-3-*O*-rutinoside was confirmed by data from previous studies on *E. involucrata* fruit (Girardelo et al., 2020; Mannino et al., 2022). The second quercetin derivative ([M-H]$^{-}$ at *m/z* 463) in MS^{2} revealed the detachment of a hexoside unit (-162 u). Finally, the last compound ([M-H]$^{-}$ at *m/z* 505) in MS^{2} fragment released at *m/z* 301 ([M-H-42-162]$^{-}$, with loss of an acetylhexoside moiety, was tentatively assigned as a quercetin-3-*O*-acetyl-glucoside (Silva et al., 2021). **Compounds 12** and **13** corresponded to kaempferol derivatives (λ_{max} around 334 nm, and MS^{2} fragment at *m/z* 285). **Compound 12** ([M−H]$^{-}$ at *m/z* 593) presented a pseudomolecular ion [M−H]$^{-}$ at m/z 593 that released fragments at *m/z* 285 ([M−H−308]$^{-}$, loss of a rutinosyl moiety). **Compound 13** was characterised as kaempferol-*O*-glucoside based on its fragmentation pattern similar to **Compound 8**. According to previous studies, this last compound has been found in *E. involucrata* leaves (Cipriani et al., 2022).

Concerning anthocyanin compounds, five compounds were detected. **Compounds 16** and **18**, with the same pseudomolecular ion ([M-H]$^{+}$ at *m/z* 465), released a fragment ion at *m/z* 303, revealing an aglycone delphinidin after the loss of hexoside unit (162 u); therefore, considering the Rt of each compound, they were tentatively assigned as delphinidin-3-*O*-glucoside and delphinidin-3-*O*-galactoside, as earlier described by other authors (Girardelo et al., 2020; Schmidt et al., 2020). Likewise, **Compounds 17** and **19** ([M-H]$^{+}$ at *m/z* 449), with a unique MS^{2} ion at

Table 3
Phenolic profile of *E. involucrata* residue (EIR), seed (EIS), and peel (EIP) extracts. The retention time (Rt); – wavelength of maximum absorption (λ_{max}); pseudomolecular ion ([M-H] $^{-/+}$), fragmentation pattens (MS^2) are presented.

Peak	Rt (min)	λ_{max} (nm)	[M-H]$^{-/+}$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	References
Non-anthocyanin phenolic compounds						
1	4.73	281	783	633 (21),481 (10), 301 (100)	bis-HHDP-glucose isomer I	Teixeira et al. (2015)
2	5.11	281	783	633 (21),481 (10), 301 (100)	bis-HHDP-glucose isomer II	Teixeira et al. (2015)
3	5.65	272	785	785(100), 633(11), 483(3), 301(48)	Digalloyl-HHDP-hexose isomer I	Teixeira et al. (2015)
4	6.15	272	785	785(100), 633(5), 483(7), 301(38)	Digalloyl-HHDP-hexose isomer II	Teixeira et al. (2015)
5	7.82	278	933	915 (5),633 (15),451 (34),301 (10)	Castalagin/ vescalagin isomer I	Teixeira et al. (2015), Tong et al. (2014)
6	8.87	278	933	915 (5),633 (8),451 (24)301 (7)	Castalagin/ vescalagin isomer II	Teixeira et al. (2015), Tong et al. (2014)
7	10.15	278	933	915 (8),633 (12),451 (23)301 (15)	Castalagin/ vescalagin isomer III	Teixeira et al. (2015), Tong et al. (2014)
8	13.11	355	609	301 (100)	Quercetin-3-*O*-rutinoside	Schmidt et al. (2020)
9	14.37	351	463	301 (100)	Quercetin-3-*O*-glucoside	Schmidt et al. (2020)
10	15.04	274	939	631 (31),469 (66),169 (100)	Pentagalloyl glucose	Albuquerque et al. (2020)
11	15.51	362	433	301 (100)	Ellagic acid pentoside	Zhang and Zhu (2015)
12	15.94	346	593	285 (100)	Kaempferol-3-*O*-rutinoside	Schmidt et al. (2020)
13	17.31	348	447	285 (100)	Kaempferol hexoside	Schmidt et al. (2020)
14	18.21	345	505	301 (100)	Quercetin-3-*O*-acetyl-glucoside	Silva et al. (2021)
15	22.53	277	935	633(25), 301(100)	Galloyl-bis-HHDP-glucose	Teixeira et al. (2015)
Anthocyanins						
16	24.44	519	465	303 (100)	Delphinidin-3-*O*-glucoside	DAD-MS
17	25.75	514	449	287 (100)	Cyanidin-3-*O*-galactoside	Flores et al. (2012)
18	26.91	507	465	303 (100)	Delphinidin-3-*O*-galactoside	Girardelo et al. (2020), Schmidt et al. (2020)
19	27.30	511	449	287 (100)	Cyanidin-3-*O*-glucoside	Girardelo et al. (2020), Schmidt et al. (2020)
20	28.83	515	419	287 (100)	Cyanidin-3-*O*-arabinoside	Girardelo et al. (2020), Schmidt et al. (2020)

m/z 287 ([M-H-162]$^+$), according to their elution times, were described as cyanidin-3-*O*-galactoside and cyanidin-3-*O*-glucoside, respectively (Flores et al., 2012; Girardelo et al., 2020; Schmidt et al., 2020). **Peak 20** ([M-H]$^+$ at *m/z* 419) showed a fragment ion at m/z 287, pointing to the loss of a pentoside molecule (132 u). Other studies on the phenolic compounds of *E. involucrata* have identified this compound as cyanidin-3-*O*-arabinoside (Girardelo et al., 2020; Schmidt et al., 2020).

In the composition of EIS, fourteen phenolic compound non-anthocyanins (TPC-na) were found, whereas in EIP only thirteen were detected. The phenolic profile of the whole residue (EIR) contained the same fifteen TPC-na found in EIS. According to Table 4 data, digalloyl-HHDP-hexose isomer I was the main hydrolysable tannin (HT) found in EIR, followed by bis-HHDP-glucose isomer I. In the seed fraction (EIS), the most representative compounds of this class were the digalloyl-HHDP-hexose isomer I, bis-HHDP-glucose isomer I and the digalloyl-HHDP-hexoside isomer II. EIP showed the lowest amount of HT (1.81 mg/g E, equivalent to 0.69 mg/g dw) among samples; its main compound belonging to this class, the bis-HHDP-glucose isomer I, was quantified with a content of 0.39 mg/g E, equivalent to 0.150 mg/g dw. On the other hand, EIS had the highest amount of HT (7.7 mg/g E and 1.34 mg/g dw). Some studies have proven, through *in vitro* and *in vitro* assays, that the main HT compounds found in *E. involucrata* have protective effects against mutagenicity (Okuda & Ito, 2011; R. M. Silva et al., 2016).

Regarding the flavonols, EIP was the richest sample in this compound class. Quercetin-3-*O*-rutinoside was the most abundant compound in all samples, followed by quercetin-3-*O*-glucoside. These results are in line with the ones reported by Schmidt et al. (2020) in their investigation on the phenolic composition of the edible part (pulp + peel) of *E. involucrata* fruits harvested in three different regions of Brazil. According to the authors, the content of quercetin-3-*O*-rutinoside ranged between 0.207 and 0.478 mg/g dw, whereas the quercetin-3-*O*-glucoside concentrations were of 0.223–0.369 mg/g dw. Thereby, it is worth mentioning that the concentration of quercetin derivatives may undergo significant variations depending on the place of cultivation (Schmidt et al., 2020). In the same study, the concentration of kaempferol-3-*O*-rutinoside varied between 0.0391 and 0.042 mg/g dw; nonetheless, no significant difference were found between fruits harvested in the different zones (Schmidt et al., 2020). Other compounds, which were detected in our samples, namely proanthocyanidins [procyanidin B and C, (*epi*)gallocatechin derivatives, among others] and phenolic acids (coumaric acid derivative), have been detected in the edible part of *E. involucrata* (Girardelo et al., 2020; Mannino et al., 2022; Schmidt et al., 2020).

As expected, EIP had the highest concentration of anthocyanins (12.0 mg/g and 3.67 mg/g dw); however, this value was lower than the one previously reported for the whole edible part (6.71–8.79 mg/g dw) (Schmidt et al., 2020). Interestingly, 1.43 mg/g dw of anthocyanins were determined in the seeds, probably due to the processing, where the intrinsic content of the peel may have migrated to the other parts of the fruit, leading to a decrease in the concentration of such compounds in the corresponding peel sample. The concentration of each anthocyanin in EIR was similar; yet, in EIS and EIP cyanidin-3-*O*-galactoside was the most abundant compound. Such results are different from those described by Schmidt et al., 2020 for the anthocyanin profile of the whole edible part of *E. involucrata*. According to the authors, delphinidin-3-*O*-glucoside was the major anthocyanin (3.842–7.523 mg/g dw) found in the hydromethanolic extract of the whole berry, followed by cyanidin-3-*O*-glucoside (1.549–2.317 mg/g dw). Differently, Girardelo et al. (2020) found that a cyanidin-3-*O*-glucoside corresponded to 79% of the total anthocyanin present in their ethanolic extract of *E. involucrata* fruit. One must observe that several factors can influence the phenolic composition in plant tissue extracts, such as plant/fruit cultivation conditions, extraction solvent used, extraction method, and employed analytical techniques, among others (Albuquerque, Oliveira, et al., 2020).

Table 4
Phenolic compounds content of *E. involucrata* residue (EIR), seed (EIS), and peel (EIP) extracts.

Peak	Content (mg/g extract, E)			Content (mg/g dw)		
	EIR	EIS	EIP	EIR	EIS	EIP
1	1.26 ± 0.01^{b}	1.49 ± 0.01^{a}	0.39 ± 0.01^{c}	0.259 ± 0.002^{a}	0.254 ± 0.002^{a}	0.150 ± 0.004^{b}
2	0.75 ± 0.01*	nd	0.2328 ± 0.0003*	0.154 ± 0.002*	nd	0.0896 ± 0.0001*
3	1.48 ± 0.01^{b}	2.64 ± 0.03^{a}	0.25 ± 0.001^{c}	0.305 ± 0.001^{b}	0.449 ± 0.005^{a}	0.0961 ± 0.0001^{c}
4	0.776 ± 0.005^{b}	1.42 ± 0.01^{a}	0.27 ± 0.02^{c}	0.159 ± 0.001^{b}	0.241 ± 0.001^{a}	0.105 ± 0.006^{c}
5	0.72 ± 0.01^{b}	0.9 ± 0.01^{a}	0.149 ± 0.001^{c}	0.147 ± 0.001^{b}	0.153 ± 0.002^{a}	0.0574 ± 0.0005^{c}
6	0.29 ± 0.01^{a}	0.31 ± 0.01^{a}	0.1411 ± 0.0003^{b}	0.060 ± 0.002^{a}	0.053 ± 0.002^{b}	0.0543 ± 0.0001^{b}
7	0.56 ± 0.01*	0.83 ± 0.01*	nd	0.115 ± 0.002*	0.141 ± 0.001*	nd
8	1.21 ± 0.02^{b}	0.96 ± 0.02^{c}	1.35 ± 0.02^{a}	0.248 ± 0.004^{b}	0.164 ± 0.004^{c}	0.521 ± 0.009^{a}
9	1.04 ± 0.02^{a}	0.96 ± 0.01^{b}	0.9 ± 0.02^{b}	0.214 ± 0.003^{b}	0.163 ± 0.002^{c}	0.347 ± 0.007^{a}
10	0.093 ± 0.001^{a}	0.088 ± 0.001^{b}	0.072 ± 0.001^{c}	0.0191 ± 0.0002^{b}	0.0150 ± 0.0001^{c}	0.0275 ± 0.0004^{a}
11	0.036 ± 0.001^{b}	0.029 ± 0.002^{b}	0.21 ± 0.002^{a}	0.0074 ± 0.0001^{b}	0.0050 ± 0.0003^{c}	0.081 ± 0.001^{a}
12	0.797 ± 0.002^{b}	0.746 ± 0.00^{c}	0.818 ± 0.001^{a}	0.1636 ± 0.0004^{b}	0.1270 ± 0.0002^{c}	0.3149 ± 0.0005^{a}
13	0.148 ± 0.002^{a}	0.139 ± 0.001^{b}	0.112 ± 0.002^{c}	0.0303 ± 0.0003^{b}	0.0236 ± 0.0002^{c}	0.043 ± 0.001^{a}
14	0.57 ± 0.001*	0.607 ± 0.001*	nd	0.1171 ± 0.0002*	0.1033 ± 0.0002*	nd
15	0.104 ± 0.001^{a}	0.099 ± 0.001^{a}	0.088 ± 0.001^{b}	0.0214 ± 0.0002^{b}	0.0169 ± 0.0002^{c}	0.0341 ± 0.0004^{a}
16	2.240 ± 0.001^{b}	1.737 ± 0.001^{c}	2.55 ± 0.01^{a}	0.5501 ± 0.0001^{b}	0.3574 ± 0.0002^{c}	0.777 ± 0.005^{a}
17	2.301 ± 0.003^{b}	1.909 ± 0.005^{c}	4.99 ± 0.08^{a}	0.5652 ± 0.0006^{b}	0.393 ± 0.001^{c}	1.53 ± 0.01^{a}
18	2.241 ± 0.002^{a}	1.743 ± 0.002^{b}	2.37 ± 0.01^{a}	0.5504 ± 0.0004^{b}	0.3588 ± 0.0002^{c}	0.722 ± 0.002^{a}
19	2.242 ± 0.003^{a}	1.741 ± 0.001^{c}	2.32 ± 0.01^{b}	0.5505 ± 0.0005^{b}	0.3583 ± 0.0004^{c}	0.706 ± 0.001^{a}
20	2.265 ± 0.001^{b}	1.763 ± 0.001^{c}	2.348 ± 0.006^{a}	0.5564 ± 0.0002^{b}	0.3628 ± 0.0003^{c}	0.715 ± 0.001^{a}
THT	6.04 ± 0.06^{b}	7.67 ± 0.09^{a}	1.60 ± 0.03^{c}	1.24 ± 0.01^{a}	1.33 ± 0.03^{b}	0.61 ± 0.01^{c}
TF	3.76 ± 0.04^{a}	3.41 ± 0.04^{b}	3.18 ± 0.04^{c}	0.772 ± 0.001^{b}	0.581 ± 0.006^{c}	1.23 ± 0.02^{a}
TPC-na	9.84 ± 0.09^{b}	11.2 ± 0.1^{a}	4.99 ± 0.08^{c}	2.02 ± 0.02^{a}	1.92 ± 0.04^{a}	1.92 ± 0.03^{a}
TA	9.049 ± 0.08^{b}	7.156 ± 0.009^{c}	12.0 ± 0.1^{a}	2.223 ± 0.002^{b}	1.473 ± 0.002^{c}	3.67 ± 0.02^{a}

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. Nd – not detected; THT – Total hydrolysable tannins; TF – Total flavonols; TPC-na – Total non-anthocyanin phenolic compounds; TA – Total anthocyanins.

3.2. Bioactivities of the hydroethanolic extracts

3.2.1. Antioxidant activity

The antioxidant activities of the hydroethanolic extracts were determined by three based-cell methods and the results are shown in Table 5. In the TBARS assay, the extracts showed a high potential to inhibit lipid peroxidation; EIS was able to inhibit TBARS formation by 50% with in a lower concentration than the one required for the positive control (Trolox). On the other hand, the IC_{50} values found for EIP and EIR were more than 2-fold the EIS's value. Nonetheless, all analysed fractions of *E. involucrata* residue exhibited better lipid oxidation inhibition values than the ones found for other berry species' residues, such as *Euterpe edulis* peel (IC_{50} = 204 μg/mL) (Garcia et al., 2019), *Morus nigra* seed (IC_{50} = 23 μg/mL), and *Vinis vinifera* seed and pomace (IC_{50} = 168 and 49.6 μg/mL, respectively) (Gómez-Mejía et al., 2021; Peixoto et al., 2018). Concerning the OxHLIA assay, no sample showed better activity than the positive control. However, the required concentration of EIS was less than twice that of EIP. Likewise observed in the TBARS assay, the EIP extract concentration capable of preventing oxidative haemolysis of 50% of erythrocytes from sheep blood was the highest among samples, being more than 2-fold the EIS extract concentration required. Comparing such results with those reported for other berries, EIS and EIR were more effective than the *V. vinifera* waste ($IC_{50\ \Delta t\ 60\ min}$ = 70 μg/mL), whereas only EIS showed higher activity than the *M. nigra* seed extract ($IC_{50\ \Delta t\ 60\ min}$ = 46 μg/mL) (Gómez-Mejía et al., 2021) and the *E. edulis* peels extract ($IC_{50\ \Delta t\ 60\ min}$ = 42 μg/mL) (Garcia et al., 2019). Surprisingly, for the CAA assay, data did not follow the same trend observed in the other two assays, as there were no significant differences between the *E. involucrata* analysed extracts. At the maximum concentration tested (2 mg/mL), the extracts showed percentages of inhibition (71%–80%) lower than the one found for quercetin (95% inhibition at 0.3 μg/mL). In the study by Mannino et al. (2022), the concentration of *E. involucrata* fruit extract required to inhibit 50% of cellular oxidation in HepG2 cells was equal to 530 μg of fresh weight per mL cell medium.

Other studies have reported investigation of the antioxidant capacities of *E. involucrata* fruit extracts by manifold chemical methods, such as ABTS, DPPH, FRAP, ORAC, and deoxyribose assays, and all of them concluded that this fruit has a promissory antioxidant potential, at least

Table 5
Antioxidant, anti-inflammatory and anti-proliferative activities of *E. involucrata* residue (EIR), seed (EIS), and peel (EIP) extracts.

	EIR	EIS	EIP	Positive control
Antioxidant activity				Trolox
TBARS (IC_{50}, μg/mL)	10.2 ± 0.4^{b}	4.96 ± 0.09^{c}	11.1 ± 0.7^{a}	5.8 ± 0.6^{d}
OxHLIA (IC_{50}, μg/mL)[1]	50±2^{b}	40±2^{c}	99±5^{a}	21.8 ± 0.2 Quercetin
CAA (% inhibition at 2 mg/mL)	77±6^{a}	80±4^{a}	71±6^{a}	95±5^{b}
Anti-inflammatory activity				Dexamethasone
NO-production inhibition ($IC_{50,}$ μg/mL)	259 ± 3*	168 ± 2*	>400	16 ± 1
Anti-proliferative activity ($GI_{50,}$ μg/mL)				Ellipticine
AGS (gastric cancer)	56 ± 4*	88 ± 3*	>400	1.23 ± 0.03
Caco-2 (colon cancer)	61±5^{b}	26.7 ± 0.3^{c}	82±7^{a}	1.21 ± 0.03
MCF-7 (breast cancer)	173 ± 4*	114 ± 6*	>400	1.02 ± 0.02
NCI–H460 (lung cancer)	214 ± 12*	77 ± 1*	>400	1.01 ± 0.01
PLP2 (porcine liver cells)	233 ± 11*	172 ± 4*	>400	1.4 ± 0.1

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. [1] IC_{50} values calculated for a 60 min Δ*t*.

in vitro (Girardelo et al., 2020; Infante et al., 2016; Nicácio et al., 2017). However, as far as we know, this is the first study to evaluate the antioxidant capacity of the *E. involucrata* biowaste.

3.2.2. Anti-inflammatory activity

Through an *in vitro* assay, the extracts were tested regarding their capacity to reduce the production of the pro-inflammatory mediator NO using an LPS-induced inflammation assay. As presented in Table 5, EIS was the most effective in reducing the inflammatory process, whereas no activity was detected in the maximum concentration tested (400 μg/mL) for the EIP extract. Likely due to the residue composition, EIR showed lower activity than EIS. In an *in vivo* study with carrageenan-induced neutrophil migration in mice, the supplementation with the ethanolic extract of pulp and seeds of *E. involucrata* (500 mg/kg in a single dose) did not show anti-inflammatory activity, whereas the leaf extract was able to reduce 40% of the neutrophil migration (Infante et al., 2016). Therefore, new studies are necessary to elucidate the anti-inflammatory potential of *E. involucrata* fruits and biowastes.

3.2.3. Antiproliferative activity

The extracts' concentrations required to inhibiting 50% of the growth of the tumour cell lines and non-tumour cell line tested are presented in Table 5. EIR and EIS, at low extract concentrations (GI_{50} = 26.7–214 μg/mL), had a harmful effect towards all human tumour cell lines assessed. On the other hand, EIP only exhibited inhibitory activity on Caco-2 cells. In general, EIS had the highest activity on Caco-2 (GI_{50} = 26.7 μg/mL) and the lowest on MCF-7 (GI_{50} = 114 μg/mL), whreas EIR showed the best and worst activity on AGS and NCI–H460 lines (GI_{50} = 56–214 μg/mL, respectively. Similar to our findings, Girardelo et al. (2020) found that their seed ethanolic extract inhibited the grow of adenocarcinoma tumour cell line (PANC-1) (GI_{50} = 645 μg/mL), unlike their pulp + peel extract. These authors deduced that the anti-proliferative activity of the seed extract was a consequence of the pro-antioxidant effect of the main phenolic compounds detected in it, namely epicatechin, catechin and ellagic acid. Indeed, the cytotoxicity of the plant extracts can be intrinsically correlated with their chemical composition, mainly in terms of phenolic compounds. In this sense, a deeper study, with purified extracts, can help to better describe the implication of the phytochemicals present in the samples with the extracts' antiproliferative activities (Albuquerque et al., 2021).

Regarding the anti-proliferative activity on healthy cells (PLP2), EIS and EIR extracts showed moderate toxicity (GI_{50} = 172–233 μg/mL); however, the extract concentrations required to inhibit the proliferation of tumour cells were lower than such values. Furthermore, our EIP extract was not noxious to normal cells in the maximal concentration assessed (400 μg/mL). In the study carried out by Girardelo et al. (2020), the ethanolic extracts obtained from the *E. involucrata* seed and whole fruit did not show cytotoxicity on a human umbilical vein endothelial cell line (HUVEC) at a concentration range of 1–1000 μg/mL. However, no other study was found in the literature that reports the toxicity of products derived from *E. involucrata*; therefore, further studies are needed to determine the safety of this fruit and residues.

3.2.4. Antimicrobial activity

The antimicrobial activity of the extracts was tested on seven bacteria and two fungi, and the results found are shown in Table 6. In general, all extracts were able to inhibit bacterial growth (MIC = 0.156–20 mg/mL) for all bacteria tested, with the greatest inhibitions values registered against *Y. enterocolitica* (MIC = 0.156–0.625 mg/mL). For Gram-negative bacteria, the samples showed similar inhibition capacities, except towards (1) *P. aeruginosa*, against which EIP was more efficient, requiring half the extraction concentration of the other samples; and (2) *S. enterica*, for which a higher concentration of EIP was necessary. For Gram-positive bacteria, EIR had the best antibacterial activity towards *B. cereus* and *S. aureus*, followed by EIS. For L. *monocytogenes*, the same MIC was required for all tested extracts. The high activity of EIR can be explained by the synergetic action of the bioactive compounds, namely hydrolysable tannins, flavonoids, and anthocyanins, found in the seeds and peels (Tables 1–4). The synergic effect between flavonoids with ellagitannins have been described in literature. For instance, the combination of quercetin, kaempferol, myricetin derivatives, with punicalagin and ellagic acid derivatives was able to reduce up to 20 times in comparison to the activity of individual compounds of the minimal concentration to inhibit 50% of the growth of *S. aureus* (Tomás-Menor et al., 2015). Synergetic effect between resvestratol and kaempferol was also confirmed against food-borne pathogenes, namely *B. cereuls*, *S. aureus*, and *E. coli* (Skroza et al., 2019). EIR and EIS showed in their composition high amount of hydrolysable tannins, including bis-HHDP-hexoside, digalloyl-HDDP--glucoside, and galloyl-HHDP-hexoside, compounds that have demonstrated high bacterial and antifungal activity (Brighenti et al., 2021; Olchowik-Grabarek et al., 2022). While α-tocopherol also shows moderate activity in resistant bacteria (Bergonzi et al., 2021). The bactericidal activity of the extracts only was confirmed, at the maximum concentration tested in this assay (20 mg/mL), on *E. coli*, *Y. enterocolitica*, *B. cereus* and *L. monocytogenes*; in the latter, only EIS and EIP were able to promote bacterial death.

Regarding antifungal activity, samples had the same antifungal potential and were able to inhibit the growth of *A. brasiliensis* and *A. fumigatus* at low concentrations (MIC = 0.625–1.25 mg/mL, respectively); nevertheless, no fungicidal activity was detected at the maximum concentrations tested (20 mg/mL). The results found in our

Table 6
Antimicrobial activity of *E. involucrata* residue (EIR), seed (EIS), and peel (EIP) extracts.

	EIR		EIS		EIP		Positive Controls			
							Streptomycin		Ampicillin	
Antibacterial activity	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria										
Escherichia coli	5	20	5	20	5	20	0.01	0.01	0.156	0.156
Pseudomonas aeruginosa	1.25	>20	1.25	>20	0.625	>20	0.0625	0.0625	0.625	0.625
Salmonella enterica	2.5	>20	2.5	>20	5	>20	0.0078	0.0078	0.156	0.156
Yersinia enterocolitica	0.156	0.625	0.156	0.625	0.156	0.625	0.0078	0.0078	0.156	0.156
Gram-positive bacteria										
Bacillus cereus	0.625	2.5	2.5	10	5	20	0.0078	0.0078	nt	nt
Listeria monocytogenes	5	>20	5	20	5	20	0.0078	0.0078	0.156	0.156
Staphylococcus aureus	0.156	>20	5	>20	10	>20	0.0078	0.0078	0.156	0.156
							Ketoconazole			
Antifungal activity	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC		
Aspergillus brasiliensis	0.625	>20	0.625	>20	0.625	>20	0.0625	0.125		
Aspergillus fumigatus	1.25	>20	1.25	>20	1.25	>20	0.5	1		

MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration; MFC – minimum fungicidal concentration; nt – not tested.

study indicate that *E. involucrata* residue and its fractions have antimicrobial potential. However, in the study performed by Sardi et al. (2017), the hydroethanolic extracts obtained from pulp, seed and leaves of this species, did not show antifungal activity against *Candida albicans* at the maximum concentration tested by the authors (2 mg/mL).

4. Conclusion

Eugenia involucrata DC. is a Brazilian native plant still under explored economical and scientifically; however, the dissemination of novel information on attesting the potentialities of its edible fruit is essential for the species preservation and best valorization. Our results evidence that the biowaste generated from the industrial processing of *E. involucrata* berries, composed by seed and peel, is indeed rich in bioactive compounds, such as tocopherols, hydrolysable tannins and anthocyanins. To better understand the action mechanism of the compounds found in each fraction and the synergic effect between them, further studies with isolated compounds are necessary. Furthermore, the residue and its different fractions have interesting biological potentials, such as antioxidant, anti-proliferative, anti-inflammatory, and antimicrobial properties. Considering these multifaceted biological potentials, the *E. involucrata* residue emerges as a promising resource for the development of innovative products. For instance, the antioxidant properties could be harnessed in the formulation of therapeutic medicines aimed in preventing or treating oxidative stress-related disorders. The anti-proliferative effects open avenues for developing pharmaceuticals targeting abnormal cell growth, while the antimicrobial attributes suggest applications in the production of natural preservatives. Moreover, the residue's potential as a source of natural colorants adds another dimension to its versatility. The extraction of pigments from the residue could lead to the development of eco-friendly and sustainable natural colorants for various industries, such as food and textiles. Therefore, the *E. involucrata* residue hold potential to be better explored as a source of new products. However, it is essential to highlight that additional research, including *in vivo* tests, is essential to determine the safety of consuming products derived from this fruit residue. Furthermore, it is worth noting that the production and processing of *E. involucrata* is currently limited. Therefore, promoting the cultivation and commercialization of this species becomes essential in this context. In this manner, this study not only advocates for the sustainable utilization of residues from the processing of *E. involucrata* fruit but also plays a role in disseminating the largely unexplored potential of this fruit.

CRediT authorship contribution statement

Bianca R. Albuquerque: Writing – original draft, Investigation, Conceptualization. **Tiane C. Finimundy:** Methodology, Investigation. **José Pinela:** Methodology, Investigation. **Tânia C.S.P. Pires:** Methodology, Investigation. **Ricardo C. Calhelha:** Writing – review & editing, Conceptualization. **Josiana Vaz:** Methodology, Investigation. **Rúbia C. G. Corrêa:** Writing – review & editing. **M. Beatriz P.P. Oliveira:** Writing – review & editing, Conceptualization. **Lillian Barros:** Writing – review & editing, Supervision, Project administration.

Declaration of competing 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2023.103532.

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