In vitro antiglycation and antioxidant properties of
Eugenia pyriformis leaves and fruits

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ABSTRACT
Eight phenolic compounds were isolated from Eugenia pyriformis leaves fraction by semi-preparative HPLC and characterized by Nuclear Magnetic Resonance (NMR) and mass spectrometry (ESI-MS). Five compounds were isolated and identified for the first time in E. pyriformis species, while this is the first report of the accumulation of isoquercitrin, quercitrin, and the aglycone quercetin in its leaves. E. pyriformis leaves and fruits extracts, as well as the compounds isolated from the leaves most active fraction, were evaluated for their antiglycation and antioxidant activities. The mixture of myricetin-3-O-(2”-O-galloyl)-x-L-rhamnoside and myricetin-3-O-(4”-O-galloyl)x-L-rhamnoside showed the highest antiglycation activity. These results suggest that this species is a promising source of bioactive compounds. Further studies to investigate the inhibition of the glycation process in vivo are necessary to evaluate its use in the treatment and/or prevention of advanced glycation end-products (AGEs)-associated diseases.

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1. Introduction
Brazil is recognized for its high diversity of fruit species, in which the Myrtaceae family stands out for its large number of species presenting nutritional, commercial, and therapeutic...
properties (Pereira et al. 2012). Eugenia pyriformis Cambess leaves have been used in folk medicine due to their antimicrobial activities, and its fruits are commercialized in different forms. Its fruits present high antioxidant potential attributed to the presence of phenolic compounds (such as gallic acid and quercetin) (Klein et al. 2018), and studies describe that E. pyriformis leaves essential oils are rich in terpenoids (Apel et al. 2004). Even though E. pyriformis presents great economic potential for exploration, few reports in the literature describe the extraction, isolation and biological properties of compounds from its leaves and fruits.

Peng et al. (2011) report that, since oxidative stress accelerates the advanced glycation end-products (AGEs) formation, antioxidant and antiglycation properties can be correlated. AGEs are macromolecules generated by the reaction of aldehyde groups of reducing sugars with amino groups of proteins. This reaction results in Schiff bases that are subsequently rearranged to Amadori products (Maillard reaction). In vivo, their accumulation can be associated with the development of some pathogenic processes, such as diabetes (Peng et al. 2011). In addition, the glycation reaction with proteins, such as collagen, has been reported for causing changes in the dermal cells, such as the reduction of skin elasticity and increase of the appearance of wrinkles (Freitas et al. 2020). Phenolic compounds, which have been vastly investigated for their antioxidant properties, were also described as valuable natural products to prevent the AGEs formation and consequent health complications (Fraige et al. 2018).

Therefore, in this study, the antiglycation and antioxidant activities of E. pyriformis leaves and fruits extracts were evaluated. A bio-guided fractionation was performed, leading to the isolation of the bioactive compounds present in the most active extract.

2. Results and discussion

2.1. Antioxidant assays

To determine the antioxidant capacity of E. pyriformis leaves and fruits, the ethanol: water (70:30 v/v) and ethyl acetate (EtOAc) extracts obtained from these organs were subjected to both the 2,2-diphenyl-1-picrylhydrazyl (DPPH*) and peroxyl radical (ROO*) scavenging assays methods. The results represented by the EC_{50} are shown in Table S1 (Supplementary Material). All leaves extracts presented higher antioxidant activity compared to fruit ones. The EC_{50} values obtained for the leaves’ hydroethanolic and EtOAc leaves extracts in the scavenging of DPPH* radical method were 18.8 ± 0.4 and 8.4 ± 0.4 µg mL^{-1}, respectively. Using the peroxyl radical scavenging assay, even higher antioxidant results were obtained: 6.4 ± 0.2 and 4.9 ± 0.4 µg mL^{-1} for the hydroethanolic and EtOAc extracts, respectively. Assays based on ROO* scavenging are considered more biologically relevant as they reflect the physiological media since these radicals are present in the human organism (Fraige et al. 2018). Our results are in accordance with the literature since a previous study of plants from the Southern Brazilian region reported that E. pyriformis leaves presented the highest antioxidant activity (Salvador et al. 2011).

2.2. Antiglycation assay

The antiglycation activities of E. pyriformis extracts were also evaluated. All leaves extracts showed higher antiglycation activity than fruits extracts, presenting values ranging from
38 to 61% of inhibition of the AGEs formation (Figure S1A, Supplementary Material). These results show that these extracts could be promising sources of compounds to prevent health disorders related to glycation reaction and contribute to treat skin ageing (Chinchansure et al. 2015; Fraige et al. 2018; Freitas et al. 2020).

On the other hand, no significant inhibition of the AGEs formation was observed for both fruit extracts: 18% and 2% for the EtOAc and hydroethanolic ones, respectively. Peng et al. (2011) related that the higher potential to inhibit the AGEs formation might be caused by the ability of some antioxidant compounds to scavenge free radicals formed during glycation reaction, such as polyphenolics and flavonoids. Therefore, in this case, a possible lower concentration of antioxidant compounds present on fruit extracts can contribute to minimizing the AGE inhibitory activities.

2.3. Fractionation of E. pyriformis EtOAc leaves extract

In order to identify the compounds responsible for the high antiglycation inhibition observed on the EtOAc leaves extract, a fractionation in a C18 column chromatography was performed. Six fractions were obtained (A–F) and were submitted to the antiglycation assay (Figure S1B, Supplementary Material). Fractions B, C, and F inhibited more than 50% of AGEs formation. Since fraction C presented the higher AGEs inhibition results (56.9%), it was submitted to the isolation procedure to determine the compounds responsible for the antioxidant and antiglycation activities.

2.4. Isolation and characterization of the compounds

The isolation of the compounds present in fraction C from E. pyriformis leaves was performed by semi-preparative HPLC–DAD (Figure S2, Supplementary Material). The NMR analyses of the isolated peaks were compared with the literature to confirm the presence of compounds myricetin-3-O-α-L-rhamnoside (myricitrin, 1) (Zhang et al. 2003), quercetin-3-O-β-D-glucoside (isoquercitrin, 2) (Zhu et al. 2013), quercetin-3-O-β-D-galactoside (hyperoside, 3) (Zhu et al. 2013), quercetin-3-O-α-L-rhamnoside (quercitrin, 4) (Zhang et al. 2003), myricetin-3-O-(2′′-O-galloyl)-α-L-rhamnoside (5) (Lee et al. 2020), myricetin-3-O-(4′′-O-galloyl)-α-L-rhamnoside (6) (Gadetskaya et al. 2017), quercetin-3-O-(2′′-O-galloyl)-α-L-rhamnoside (7) (Lee et al. 2020) and quercetin (8) (Gabrielska et al. 2006), as shown in Figure S3. NMR signals description can be found in the Supplementary Material (S2). Compounds 2 and 3, and 5 and 6 were obtained as mixtures. These compounds were also analysed by ESI-MS/MS in negative ionization mode, and the fragments observed are described in Table S2 (Supplementary Material).

This study reports compounds 1, 3, and 5–7 in E. pyriformis species for the first time. Compounds 2 and 4 have been putatively annotated by High Resolution Mass Spectrometry in a study comprising E. pyriformis fruits residues (Rodrigues et al. 2021), and our study confirms their presence in E. pyriformis leaves by isolation and NMR analyses. In addition, this is the first report of compound 7 in the Eugenia genus. The aglycone quercetin (8) has been previously reported in the fruits (Haminiuk et al. 2014; Rodrigues et al. 2021), and we report for the first time its accumulation in E. pyriformis leaves.
2.5. Antioxidant and antiglycation assays of isolated compounds

The isolated compounds were subjected to the antioxidant and antiglycation assays (Figure S3, Supplementary Material). On the ROO$^*$ assay, the highest activity was obtained for compounds 1 ($EC_{50} = 1.05 \pm 0.05 \mu g \text{ mL}^{-1}$) and 4 ($EC_{50} = 1.05 \pm 0.10 \mu g \text{ mL}^{-1}$), which did not differ statistically compared to the positive control (gallic acid, $EC_{50} = 0.91 \pm 0.07 \mu g \text{ mL}^{-1}$). Compounds 4–7 presented the highest antioxidant activity in the DPPH$^*$ assay.

The highest glycation inhibitions were observed for compound 4 ($67.14 \pm 0.55\%$) and for the mixture of compounds 5 and 6 ($68.25 \pm 0.72\%$), which did not differ statistically. In addition, this mixture presented promising results in the antioxidant assays, with $EC_{50} = 4.79 \pm 0.30 \mu g \text{ mL}^{-1}$ to DPPH$^*$, and $2.21 \pm 0.04 \mu g \text{ mL}^{-1}$ to ROO$^*$.

The basic structure of flavonoids can contribute to trapping the oxidative free radicals generated during the process of AGEs formation due to the resonance mechanism, and can contribute to decreasing the final levels of AGEs (Peng et al. 2011). These properties can be observed on the flavonoid skeleton of all isolated compounds.

3. Experimental

See Supplementary Material (S1).

4. Conclusions

The present study showed that Eugenia pyriformis leaves have higher antioxidant and antiglycation activities than its fruits, which guided our study towards the leaves bioactive compounds. Eight flavonoids were isolated, in which five of them were identified for the first time in $E$. pyriformis species. Promising results considering the antioxidant and antiglycation properties were obtained for the mixture of myricetin-3-O-(2"'-O-galloyl)-α-L-rhamnoside and myricetin-3-O-(4"'-O-galloyl)-α-L-rhamnoside, and for the pure compound quercetin-3-O-α-L-rhamnoside. Thus, the results demonstrated a correlation between the antioxidant and antiglycation activities. These results open new possibilities for further evaluation of $E$. pyriformis as a therapeutic approach for preventing complications related to AGEs formation. In addition, the isolated compounds could be considered promising candidates for the development of technological applications in pharmaceutical sciences.

Disclosure statement

No potential conflict of interest was reported by the authors.

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