



Antioxidant activity, antibacterial and inhibitory effect of intestinal disaccharidases of extracts obtained from *Eugenia uniflora* L. Seeds

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Abstract

The use of medicinal plants for disease prevention, treatment and cure is an ancient practice used by humanity, and many plants species are used in bioprospecting research. In this context, its stands out *Eugenia uniflora* L., popularly known as pitangueira and belongs to the Myrtaceae family, with a wide geographic distribution and native of Brazil. In view of the therapeutic qualities of the plant and the lack of the studies on its seeds, the present study had as objective to evaluate the phytochemical profile of the extracts of *Eugenia uniflora* L. seeds, from different solvents, as well as their antibacterial activity, antioxidant and its inhibitory effect of intestinal disaccharidases. Results showed a high content of phenolic compounds and total flavonoids, thus characterizing antioxidant activity, also highlighting the best bacteriostatic action for the Gram positive strain of *Staphylococcus aureus* in the ethanolic fraction. Regarding the disaccharidases, a strong inhibitory action was observed for all concentrations, evidencing a antihyperglycemic potential. The present research allowed to concluded that *Eugenia uniflora* L. seeds have promising biological activities for the industrial sector, but a more detailed investigation is needed regarding their bioactive compounds.

Keywords: *Eugenia uniflora* L., antioxidant activity, disaccharidases.

Atividade antioxidante, antibacteriana e efeito inibidor de dissacarídeos intestinais de extratos obtidos das sementes de *Eugenia uniflora* L.

Resumo

A utilização de plantas com fins medicinais para prevenção, tratamento e cura de doenças é uma prática antiga utilizada pela humanidade, sendo que muitas espécies vegetais são usadas para a pesquisa da bioprospecção. Neste contexto, destaca-se a *Eugenia uniflora* L., conhecida popularmente como pitangueira e pertencente à família Myrtaceae, com ampla distribuição geográfica e nativa do Brasil. Diante das qualidades terapêuticas da planta e a falta de estudos sobre suas sementes, o presente trabalho teve como objetivo avaliar o perfil fitoquímico dos extratos das sementes de *Eugenia uniflora* L. a partir de diferentes solventes, bem como sua atividade antibacteriana, antioxidante e seu efeito inibidor de dissacarídeos intestinais. Os resultados mostraram um alto teor de compostos fenólicos e flavonóides totais, caracterizando a atividade antioxidante, destacando também a melhor ação bacteriostática para a cepa Gram positiva de *Staphylococcus aureus* na fração etanólica. Em relação às dissacarídeos, uma forte ação inibitória foi observada para todas as concentrações, evidenciando um potencial anti-hiperglicêmico. A presente pesquisa permitiu concluir que as sementes de *Eugenia uniflora* L. apresentam atividades biológicas promissoras para o setor industrial, mas é necessária uma investigação mais detalhada de seus compostos bioativos.

Palavras-chave: *Eugenia uniflora* L., atividade antioxidante, dissacarídeos.

1. Introduction

Several plants have been used by humanity in all continents to control different diseases and pests, beyond representing an important source of biologically active natural products, many of which constitute a model for the

synthesis of a large number of drugs (Vilegas et al., 2014). Several plants, despite of being consumed as functional and/or nutraceuticals food are used for bioprospecting research, which means the search for chemical products

having biological or pharmacological properties that could be used to treat several diseases.

Vilegas et al. (2014) stated that in the mid 1940s natural products played important role in the manufacture of antibacterial products, such as penicillin, chloramphenicol, neomycin, among others, being this decade considered very important for the antibiotics production.

Yunes and Cechinel (2014) emphasized the importance of ethnobotanical and ethnopharmacological studies on increasing the medicinal plants knowledge and also encourage the sustainable use of the plants biodiversity. In this context the *E. uniflora*, popularly known as pitangueira, which was selected for study, it is already used in the popular medicine with therapeutic qualities. It is already known that essential oils extracted from those plants display antimicrobial and antioxidant properties (Dorman and Deans, 2000; Duarte, 2006; Andrade et al., 2007).

E. uniflora (Mirtalis: Mirtaceae) is a Brazilian native plant having a wide geographic distribution and is adapted to different climatic conditions, ranging from the southwest (Minas Gerais state) to the south (Rio Grande do Sul state) (Bezerra et al., 2000).

The plant leaves have diuretic, anti-depressive, hypoglycemic action and are used in the treatment of digestive disorders, bronchitis coughs and fever (Lima Melro et al., 2019; Scalón et al., 2001; Gentil and Minami, 2005). The fruits are usually used on the jellies, sweets, ice cream, liqueurs and cosmetology production (Scalón et al., 2001).

According to the Nucleus of Food Studies and Research (UNICAMP, 2011), in the Brazilian Table of Food Composition, Pitanga plants have on average 88.3%, 10.2%, 3.2%, 0.9%, 0.4%, 0.2% of moisture, carbohydrates, fibers, proteins, ashes and lipids, respectively and 18 mg of calcium in a 100 g of the fruit pulp, but these values can be modified depending on the plants genetic variability and the growing region.

The seeds are greenish usually small, globose and flattened, usually one per fruit (Bezerra et al., 2000). Seeds are considered fruit residuals not having an industrial application, though it has considerable amount of antioxidant phenolic compounds (Queiroz et al., 2015). However, some seeds have been processed by the industry due to the presence of high nutritional content when processed and into by-products avoiding disposal, minimizing the environmental impact and leading to high-added value in terms of economic, scientific and technological interest (Ferrari et al., 2004).

Due to the presence of antioxidant compounds and the fact that *E. uniflora* seeds are considered a waste product from the fruit pulp industries, this work has two main objectives: i) to characterize the *E. uniflora* seeds extracts chemical profile obtained from different solvents extraction and ii) to evaluate the seed extracts lethal effects on strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphylococcus aureus*, as well as its antioxidant action and the inhibitory effect on intestinal disaccharidases.

2. Materials and Methods

2.1. Plant material

The seeds were collected from the ripe red pulp fruits of *E. uniflora* the urban area of the city of Xaxim-SC, during the fruiting period, between October and November 2016 and 2017.

2.2. Preparation of extracts

The fruit extraction was done by seed maceration, using 120 g of *E. uniflora* crushed seeds. The seeds were submerged in different organic solvents, absolute ethyl alcohol, ethyl acetate, hexane and dichloromethane for five days, in closed glass containers at room temperature (20 to 25°C), at the mass ratio of 1:2 (wt). After extraction, the supernatant was subjected to rotary evaporation, under mild vacuum pressure and temperature controlled to 40°C ± 1 °C and then stored in a freezer, where it remained until the moment of the analysis.

2.3. Identification and quantification of phytocomposites

The identification of chemical components was performed by Gas Chromatography coupled to Mass Spectrometry (GC-MS), where a volume of 1000 µL was injected for each extract in the chromatograph 7890B (Agilend) coupled to a quadripolar mass spectrometer 5977A (Agilend). The injector was maintained at 280°C. Separation of the constituents was performed using a 19091S capillary column, sized 30 m x 250 mm x 0.25 µm. The mobile phase flow (carrier gas He) was adjusted to 1.2 ml.min⁻¹. The GC temperature program was 85°C (held for 4 minutes) to 290°C at a rate of 40°C.min⁻¹ (maintained for 1 minute) and up to 300°C at a rate of 5°C. min⁻¹ (maintained for 15 minutes). The mass spectrometer was operated using electron impact ionization (70 eV) in the range of 50-500 m/z. The temperature of the MS transfer line was set at 150°C and the temperature of the ion source was set at 230°C. The chemical components present in the extracts were identified by comparison with the help of equipment library (Agilent P/N G1033A). The relative amounts of each individual component were calculated using their respective peak areas in the chromatogram.

2.4. Antibacterial activity

The tests were carried out with standard strains of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 25923) and *S. typhimurium* (ATCC 13311). The cultures were first grown in BHI broth for 24 hours in an oven at 37 ± 1°C and peeled in Petri dishes containing PCA agar. After this time, culture suspensions diluted in 0.85% saline were prepared using 10⁸ UFC.mL⁻¹ (CLSI, 2012). The analyzes were performed only with the ethanolic fraction and ethyl acetate diluted in 10% DMSO, obtaining a standard concentration of 20000 µg.mL⁻¹ of each extract, for later serial dilution until reaching 218.75 µg.mL⁻¹. The antibacterial activity of the *E. uniflora* seed extracts was determined by the standard broth microdilution method. For the determination of Minimum Inhibitory Concentration (MIC), 100 µL of BHI, 100 µL of each extract in descending

order of concentration and a 5 μL aliquot of the standardized inoculum were added to each of the 96 wells. For the positive control BHI broth and microorganisms were used, in the negative control BHI broth, microorganisms and 10% DMSO, for the white only the BHI culture medium and for the alcoholic extract, ethyl alcohol, BHI and the inoculum were used. Plates were incubated for 24 hours in the oven at $37 \pm 1^\circ\text{C}$. After the incubation time, the MIC was determined by applying 20 μL of 0.5% triphenyltetrazolium chloride (TTC) in all wells. It was considered as MIC the lowest concentration of the extract that inhibited bacterial growth, observed by the lack of red staining of the inoculum. In the wells in which there was no red staining, plating was performed in Petri dishes containing PCA Agar medium, incubating again at $37 \pm 1^\circ\text{C}$ for 24 h, for determination of Minimum Bacterial Concentration (MBC), considered as the lowest concentration of the extract in a study where there was no bacterial growth. The interpretation of the results was done by the visual analysis of the plated microplates, comparing them with the negative control, without the bacterium. The results were expressed by visual analysis of the three replicates.

2.5. Antioxidant activity

The antioxidant activity was determined only for the ethanolic extract of pitanga seeds, through the evaluation of the reduction of DPPH by spectrophotometry, performed according to the methodology described by Mensor et al. (2001). First the extract stock solution ($1\text{mg}\cdot\text{mL}^{-1}$) was prepared by weighing 0.01g of the same dilution in 10 ml of ethanol. From this solution, 50, 30, 20, 10 and 5 $\mu\text{g}\cdot\text{mL}^{-1}$ dilutions were made in absolute ethanol, with final volume of 2.5 mL, and mixed with 1 mL of 0.03 mM DPPH. White was made from the extract with ethanol, so that for each concentration there was a white. In the negative control, only ethanol was placed with DPPH. All analyzes were performed in triplicate and, after 30 minutes of reaction, the absorbance was read in a spectrophotometer at a wavelength of 517 nm. Reduction of the DPPH moiety has been observed by continuously monitoring the decline in absorbance over time. The results were expressed as efficient concentration (EC_{50}). The free radical sequestration rate DPPH or percent inhibition of oxidation was calculated by the Equation 1.

$$\%AA = 100 - \left\{ \frac{(A_{\text{sample}} - A_{\text{white}}) * 100}{A_{\text{control}}} \right\} \quad (1)$$

% AA: Percentage of antioxidant activity

A_{sample}: Absorbance of solution with sample and with the free radical DPPH.

A_{white}: Absorbance of solution with sample without free radical DPPH.

A_{control}: Absorbance of the reference solution of DPPH and ethanol.

From the data obtained, a linear regression graph of extract concentration ($\mu\text{g}\cdot\text{mL}^{-1}$) versus antioxidant activity expressed as mean \pm standard deviation was plotted and EC_{50} was calculated as mean of the replicates.

2.6. Totals phenols

The quantification of phenolic compounds was performed according to the spectrophotometric method of Folin-Ciocalteu, as described by Kosar et al. (2005). The same consists in mixing 10 μL of the already diluted extract in ethanol ($0.01\text{ g}\cdot\text{mL}^{-1}$) with 600 μL of deionized water, followed by 50 μL of Folin reactive, then adding 150 μL of solution of sodium carbonate 20% and incubating for one hour at room temperature and darkness. The absorbance was measured in a spectrophotometer at 760 nm wavelength. The total phenol content was determined by the interpolation of the absorbance of the samples against the standard curve of gallic acid ($2\text{ mg}\cdot\text{mL}^{-1}$), constructed from concentrations of 2 to 0.031 $\text{mg}\cdot\text{mL}^{-1}$ obtained by serial dilution, and expressed as mg of EAG (gallic acid equivalents) per g extract. The tests were performed in triplicate. The absorbance of the sample was compared with the standard curve of gallic acid.

2.7. Quantification of total anthocyanins and total flavonoids

The determination of anthocyanin and total flavonoid contents was performed according to the method described by Francis (1982). In aliquots of 1 g of ethanolic extract, 10 mL of extractive solution 95% ethanol: HCl were added to 1.5 N to adjust the pH of the medium to 2.0, in the proportion of 85:15. Then the mixture (diluted extract) was homogenized for 2 min and transferred to a tube wrapped in foil, resting at 4°C for 24 h. The resulting material was filtered and the ethanol/HCl solution added until the volume of 10 mL. The absorbance was read in a spectrophotometer at a wave-length of 535 nm and the total content of anthocyanins expressed in mg of AT.100 g^{-1} of the analyzed sample (extract). The absorbance values were contrasted with white value (ethanol/HCl solution). The analyzes were performed in triplicate and the calculation was performed according to the equations 2 and 3.

$$\text{Total Anthocyanins} \left(\frac{\text{mg}}{100\text{g}} \right) = Fd * \frac{Abs}{98.2} \quad (2)$$

$$Fd = (100) / \left(\frac{m}{V} \right) \quad (3)$$

Fd: dilution factor

Abs: absorbance at 535 nm,

M: mass (g)

V: volume (mL)

The total flavonoid content was quantified according to the same methodology used for analysis of anthocyanins, according to Francis (1982) by the pH single method, the difference only in the wave-length for absorbance reading, which was made at 374 nm and the calculation performed according to the equations 4 and 5:

$$\text{Total Flavonoids} \left(\frac{\text{mg}}{100\text{g}} \right) = Fd * \frac{Abs}{76.6} \quad (4)$$

$$Fd = (100) / \left(\frac{m}{V} \right) \quad (5)$$

2.8. *In vitro* screening for inhibition of disaccharidases

The activity of disaccharidases maltase, sucrase and lactase were determined according to methodology described by Pereira et al. (2011), with modifications. Ethanolic extracts were prepared at the dilutions of 250, 500 and 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ and acarbose at the dilutions of 20, 40 and 80 $\mu\text{g}\cdot\text{mL}^{-1}$, both in 1 wt % Tween 80. To obtain the intestinal homogenate, normal Wistar rats were used, and for euthanasia, the protocols were evaluated by the Ethics Committee on the Use of Animals (CEUA 004/2017). A small intestine segment of the animal was removed, homogenized in saline and centrifuged at 2000 rpm. Subsequently, the supernatant was used for the *in vitro* measurement of the activity of the disaccharidases and determination of total proteins, incubating 10 μL of the same with 10 μL of the extract in the different concentrations for 5 minutes, adding 10 μL of the substrate (maltose, lactose or sucrose) and incubating for another 30 minutes in a water bath at 37°C. After this, 250 μL of the glucose-oxidase buffer was then added, according to the manufacturer's recommendations and incubated for a further 10 minutes in a 37°C water bath. Subsequently, the spectrophotometer was read at 505 nm. The Proteins were quantified by the method of Lowry et al. (1951) and the assays performed on six replicates and conducted together with the respective controls. The values were expressed as enzymatic activity (U) per milligram of protein and the calculations based on the methodology of Pereira et al. (2011).

2.9. Statistical analysis

The results were expressed as the mean of six replicates \pm standard deviation (E.P.M.). The comparison of activity between the different concentrations of the ethanolic

extracts of *E. uniflora* seeds was analyzed by unidirectional ANOVA followed by Tukey's test, using STATISTICA 7.0 software, considering $P < 0.05$ as significant.

3. Results and Discussions

3.1. Income from extracts

The technique used to obtain the dried extracts was the same used for all solvents. The percent yield of the extractions and polarity index of the solvents used are shown in Table 1. From the results obtained, it was observed that the solvents with higher polar indexes provided higher extraction yields, showing lower concentration of apolar compounds present in the vegetable matrix. It suggests that ethanol, due to its polarity, may have facilitated the solubilization of more polar compounds, hence leading to increased yield.

3.2. Phytochemical analysis by GC-MS

The analysis of *E. uniflora* seeds extracts, submitted to GC-MS analysis, allowed to verify the predominance of sesquiterpenes (α -muurolene, δ -cadinene, T-muurolol, α -cadinol, caryophyllene and 6-isopropenyl-4, dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol) belonging to terpene compounds and phytosterol (β -sitosterol), to a lesser extent fatty acids (palmitic acid and linoleic acid) and phenol (pyrogallol) in the different fractions, according to their respective solvent, as shown in Table 2.

Santos et al. (2015), analyzing the pitanga seeds extracts chemical composition, found three major components belonging to the sesquiterpenes group (germacrone,

Table 1. Yields of dried extracts from 120g of seed, with different solvents.

Solvent	Polarity*	Yield (%)	(g)
Hexane	0.00	0.19	0.23
Dichloromethane	3.10	0.28	0.34
Ethyl acetate	4.40	2.98	3.58
Ethanol	5.20	8.90	10.68

*Solvent polarity index in ascending order; Source: Souza et al. (2014).

Table 2. Chemical constituents of the extracts of *E. uniflora* seeds and their percentage, according to the retention time.

Chemical constituents	Hexane		Dichloro	E. acetate	Ethanol
	RT	%	%	%	%
Pyrogallol	7.122	6.07	5.47	4.11	4.27
α -muurolene	7.646	3.75	3.47	7.37	3.60
δ -cadinene	7.728	9.58	8.92	9.00	9.13
Isopropenyl*	7.811	10.11	9.51	7.90	9.71
T-muurolol	8.144	5.98	5.58	5.63	5.76
α -cadinol	8.144	5.98	5.58	5.63	5.76
Palmitic acid	8.915	2.67	2.52	2.12	3.05
Linoleic acid	9.360	3.89	2.91	1.76	3.66
β -sitosterol	15.725	13.15	12.39	12.54	12.80
Caryophyllene	7.361	3.05	2.75	2.86	2.93

%; Peak area; RT: Retention time (min); *6-isopropenyl-4, dimethyl-1,2,3 5 6,7,8,8a-octahydro-naphthalen-2-ol; Source: authored by the author.

furanodiene and γ -elemene). In the analyzes carried out by Luzia et al. (2010), 58.06% of the unsaturated fatty acids present in the lipid fraction of *E. uniflora* seeds were found by gas chromatography, and oleic acid (omega-9) average 38.29% and linoleic acid (omega-6) 13.41%. The palmitic acid, which is a saturated fatty acid, presented 34.09%.

Bagetti et al. (2009) also analyzed pitanga seeds and reported that those seeds form purple, red and orange fruits had predominance of linoleic and palmitic acid. Victoria et al. (2012), found in the pitanga leaves essential oil sesquiterpenes are the major components.

The phytochemical groups found in the present study corroborate with the literature, differentiating only in its constituents.

Geographic location, cultivation form, climatic conditions, age of the plant material, period, storage conditions and solvent used, and selective pressures in pitanga plants can influence the plant chemical composition and the concentrations of compounds. The time of the year at which the pitanga fruits are sampled is one of the most important factors in determining the quantity and the variation of the active constituents, since those constituents are not constant during the year and may vary in certain months.

β -sitosterol was the chemical substance found in large quantities in the extracts.

According to the scientific literature phytosterols help to reduce cholesterol, help prevent cardiovascular diseases and other diseases related to oxidative stress, have a strong anti-inflammatory action, help fight rheumatoid arthritis and prevent benign prostatic hyperplasia (Al-Okbi, 2014; Cabral and Klein, 2017; Scapinello et al., 2019; Wang et al., 2015). However, sesquiterpenes act as plant phytoalexins, an antibiotic produced in response to microbial infections (Vizzotto et al., 2010). This characteristic may be associated with the antibacterial action of pitanga seeds against certain bacteria strains.

3.3. *In vitro* antibacterial activity

When evaluating the *in vitro* antibacterial activity of *E. uniflora* seeds on strains of *E. coli*, *P. aeruginosa*, *S. aureus* and *S. typhimurium*, positive results were obtained for the two extracts used, against all strains analyzed, however, the ethyl acetate fraction inhibited the bacteria at a high concentration (20,000 $\mu\text{g.mL}^{-1}$).

The best results were found in the fraction obtained with the ethanol as solvent, inhibiting the bacteria from the concentration of 875 $\mu\text{g.mL}^{-1}$ for *S. aureus*, presenting more effective MIC values (Table 3). Possibly, this occurred due to the higher percentage of chemical compounds present in the extract, especially polar ones, as reported by other authors (Miranda et al., 2015; Santos et al., 2016).

Table 3. Antibacterial activity of ethanolic extract and ethyl acetate of *E. uniflora* seeds by the MIC technique against ATCC strains.

Extract	Concentration $\mu\text{g.mL}^{-1}$	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhimurium</i>
Ethyl acetate	20,000	Ac	Ac	Ac	Ac
	10,000	*C	*C	*C	*C
	5,000	*C	*C	*C	*C
	2,500	*C	*C	*C	*C
	1,750	*C	*C	*C	*C
	875	*C	*C	*C	*C
	437.5	*C	*C	*C	*C
	218.7	*C	*C	*C	*C
	+	*C	*C	*C	*C
	-	*C	*C	*C	*C
	BB	Ac	Ac	Ac	Ac
Ethyl alcohol	20,000	Ac	Ac	Ac	Ac
	10,000	Ac	*C	Ac	Ac
	5,000	Ac	*C	Ac	*C
	2,500	*C	*C	Ac	*C
	1,750	*C	*C	Ac	*C
	875	*C	*C	Ac	*C
	437.5	*C	*C	*C	*C
	218.7	*C	*C	*C	*C
	+	*C	*C	*C	*C
	-	*C	*C	*C	*C
	BB	Ac	Ac	Ac	Ac
ALE	*C	*C	*C	*C	

*C: bacterial growth; Ac: absence growth; (+): positive control; (-): negative control; BB: white; ALE: ethyl alcohol; Source: authored by the author.

Nascimento et al. (2006) reported that the ethanolic fraction has a greater capacity for bacterial inhibition, since it obtains a better extraction of the polar constituents of the plants, which have a synergism between the active principles, reflecting in this antibacterial action.

Gram negative microorganisms have an outer membrane composed of lipoproteins, phospholipids, proteins and lipopolysaccharides, besides the cell wall, giving them greater resistance to the aggression of antibiotics and plant extracts, due to their greater complexity. However, Gram positives do not have this outer layer, therefore, they are more sensitive to chemical compounds (Teneva et al., 2016).

Probably, the strains of *S. aureus* used in the analyzes, were inhibited with a lower concentration of extract due to their cellular structure, since these bacteria are Gram positive, however, it will be necessary to analyze with different strains of Gram positive bacteria to confirm this hypothesis.

In the studies conducted by Victoria et al. (2012), the essential oil of *E. uniflora* leaves showed biological activity in Gram positive bacteria, corroborating the present study. This activity has also been observed in other essential oils (Lago et al., 2011; Burt, 2004).

The MBC tested by plating was performed with the highest concentrations (20,000 $\mu\text{g}\cdot\text{mL}^{-1}$), which did not change the color of the wells (biological activity) for the two extracts. Depending on the plant species and the bacterial strains tested, this action can be classified as bacteriostatic or bactericidal. In the present study, in all PCA plaques there was bacterial growth, characterizing the seeds of *E. uniflora* as being bacteriostatic for the studied microorganisms, that is, the extracts only inhibited its growth, but did not kill the microorganisms.

Due to the existence of just few studies carried out with seed extracts of the plant under study, the results obtained in these analyzes were compared with experiments from other parts of the plant.

The use of *Eugenia* species for the treatment of infectious diseases is well known in popular medicine (Hussein et al., 2003). *Eugenia dysenterica* DC, used for kidney and bladder infections, for diabetes and other diseases (Palhares, 2003). In a study carried out by Mendonça et al. (2016) with ethanolic extracts of leaves and stems of pitangueira, the results also demonstrated antimicrobial activity against strains of *E. coli*, *S. aureus* and *Pseudomonas* sp.

Becker et al. (2017) identified in the oils of *E. uniflora* leaves twelve compounds, among them sesquiterpenes α -muurolene, δ -cadinene and caryophyllene, evidencing bactericidal activity for *S. typhimurium*. The antibacterial activity performed by terpenes and derivatives has been described by researchers involving several plant species, but it is important to note that this activity is not always related to the major compounds. There are also studies that report the bioactivity of some extracts as being the product of the interaction of several compounds, occurring a synergism between them (Vilegas et al., 2014; Casanova and Costa, 2017).

3.4. Antioxidant potential and total phenol content

The antioxidant activity by the DPPH method and the total phenol content by Folin-Ciocalteu found in the extracts of the pitanga seeds are presented in Table 4, where the EC_{50} value is calculated by the reduction of 50% of the initial concentration of DPPH in the presence of antioxidant substances. It is worth noticing that the lower the value of EC_{50} , the greater the free radical capture, and therefore the higher the antioxidant activity of the analyzed extract. The results obtained (Table 4), can be considered satisfactory when compared with data from other species described in the literature.

Results lower than those observed in the present study, regarding the amount of phenolic compounds, were pointed out by Luzia et al. (2010), when analyzing extracts of the *E. uniflora* seeds obtaining a concentration of 75.64 mg of gallic acid equivalents per gram of pitanga extract. The EC_{50} was 30.72 $\text{mg}\cdot\text{mL}^{-1}$. The difference in total phenol concentration can be explained by the chemical composition of the seeds that, depending on the plant stress level and due to external factors, produces different secondary metabolites. Another aspect observed is the solvent used, the higher the polarity of the extraction solvent, the greater the amount of phenolic compounds extracted (Santos et al., 2016).

Ascorbic acid is used as a positive control in the analysis of antioxidant activity. Therefore, it can be used as comparative. Reynertson et al. (2005), obtained EC_{50} of 19.6 $\mu\text{g}\cdot\text{mL}^{-1}$ in extracts of the pitanga pulp and compared that result with EC_{50} of 18.3 $\mu\text{g}\cdot\text{mL}^{-1}$ of commercial ascorbic acid, suggesting that the results were similar.

Studies suggest that the antioxidant potential of phenolics derives from the number and position of hydroxyl groups in their structure (Cao et al., 1997).

According to the results obtained, it can be inferred that the seeds of *E. uniflora* analyzed have a large amount of phenolic compounds and a relevant antioxidant activity.

Using the linear relation $y = 1.9444x + 3.6766$, which showed a correlation coefficient of 0.9249, obtained through the standard curve for the EC_{50} calculation (Figura 1), the antioxidant activity was determined. Analyzing the Figure 1, it was observed that there was an increase in DPPH inhibition with increase of concentration of the extract. These results indicate that seeds employed in this work have antioxidant chemicals capable of capturing free

Table 4. Determination of antioxidant activity and total phenolic compounds of ethanolic fractions of *Eugenia uniflora* L. seeds.

Determination	Ethanolic extract
EC_{50} ($\mu\text{g}\cdot\text{mL}^{-1}$)	23.81 \pm 0.40
Total phenolic compounds ($\text{mg}\cdot\text{g}^{-1}$ extract)	166.19 \pm 0.02

EC_{50} is defined as the concentration sufficient to obtain 50% of the maximum effect estimated at 100% mg gallic acid equivalents per g extract.

radicals, aiming at the prevention of diseases resulting from oxidative stress.

In conducting research with total anthocyanins, a low value was obtained in the samples ($4.19 \pm 0.82 \text{ mg} \cdot 100\text{g}^{-1}$ extract), since they are compounds classified as pigments of plants and are present in low concentrations in the seeds.

As for the concentration of flavonoids, an amount of $18.18 \pm 0.409 \text{ mg} \cdot 100\text{g}^{-1}$ of extract was found in the fractions studied. Flavonoids are substances capable of lowering blood pressure and cholesterol levels, reducing the risk of coronary problems (Raven, 2014). This fact can confirm the use of leaves and seeds of the pitangueira, by popular medicine, as a coadjuvant in the treatments of hypertension (Gentil and Minami, 2005). Pereira et al. (2011) reported the inhibitory effect of flavonoids on α -glucosidase activity, suggesting its antidiabetic potential. The literature also states that antioxidant activity is directly related to phenolic and flavonoid total contents (Cabral et al., 2009).

3.5. Inhibition *in vitro* of disaccharidases

In vitro analyzes of the enzymatic inhibition by the ethanolic extract of *E. uniflora* seeds presented promising results. In comparison with the control, the activity of the disaccharidases was inhibited by the extract in all concentrations tested.

As shown in Figure 2, the enzyme lactase was inhibited at the three concentrations (250, 500 and 1000 $\text{mg} \cdot \text{mL}^{-1}$), with acarbose having no effect on it. According to Gomis (2008), this is due to the fact that the medicine does not induce lactose intolerance, suggesting therefore that the extract can cause this effect, requiring *in vivo* analyzes to prove the effect.

In relation to the sucrase enzyme, it was observed that its action was inhibited by the extracts in the respective dilutions, by acarbose in all tested concentrations (20, 40 and 80 $\mu\text{g} \cdot \text{mL}^{-1}$), as depicted in Figure 3. The same was observed for maltase (Figure 4), hence evidencing the possible antihyperglycemic potential of the seeds of *E. uniflora* when compared to the drug acarbose.

In the literature it was not found studies on inhibition of disaccharidases by *E. uniflora* seeds, however, Arai et al. (1999) showed the potential of different fractions from ethanolic (70%) extract of *E. uniflora* leaves in inhibit the α -glucosidases. Other species presented important antidiabetic effects, as shown by Wang et al. (2012), when investigating the anti-enzymatic action of ethanolic extracts of *Camellia sinensis* L., finding a positive correlation between phenolic content and inhibitory activity of disaccharidases. Similar results were report by Liu et al. (2018) that found alpha-glucosidase inhibitory activity with phenolics from *Eugenia jambolana* seeds.

In the present study, high levels of total phenols, flavonoids and β -sitosterol were found, substances that may evidence the possible inhibitory action of disaccharidases, corroborating with the aforementioned literature. Plants have been widely used as a method of diabetes control due to their hypoglycemic and antioxidant potentials (Oliveira et al., 2017). Barbosa-Filho et al. (2005)

collected 224 plants for study, citing ten families that presented hypoglycemic action, among them the Myrtaceae family. Recently, Sobeh et al. (2019) also found a robust anti-diabetic activity in streptozotocin-diabetic rats with

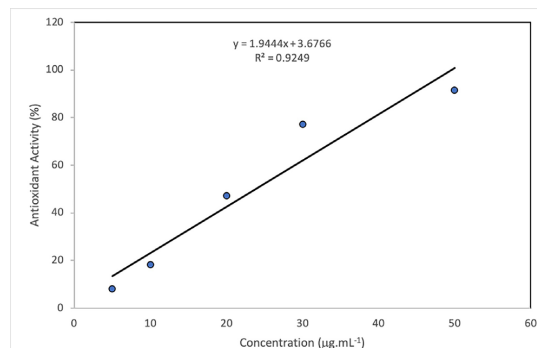


Figure 1. Percentage of DPPH inhibition as a function of the concentration of ethanolic fraction of pitanga seeds. Source: authored by the author.

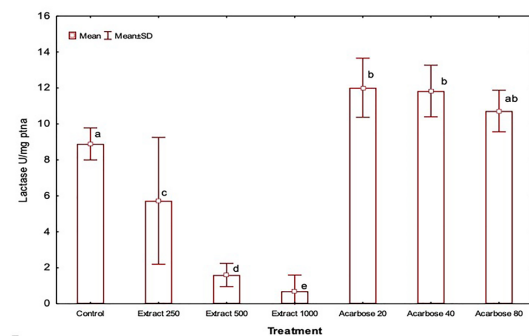


Figure 2. Activity of the lactase enzyme for the different concentrations of ethanolic extract ($\text{mg} \cdot \text{mL}^{-1}$) of the *E. uniflora* seeds. Means followed by the same letter do not differ statistically from each other by the Tukey test at 5% probability.

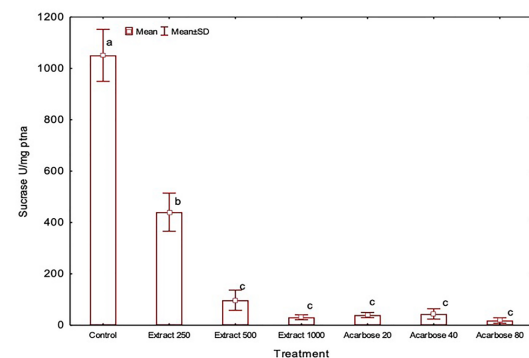


Figure 3. Activity of the sucrase enzyme for the different concentrations of ethanolic extract ($\text{mg} \cdot \text{mL}^{-1}$) of the *E. uniflora* seeds. Means followed by the same letter do not differ statistically from each other by the Tukey test at 5% probability.

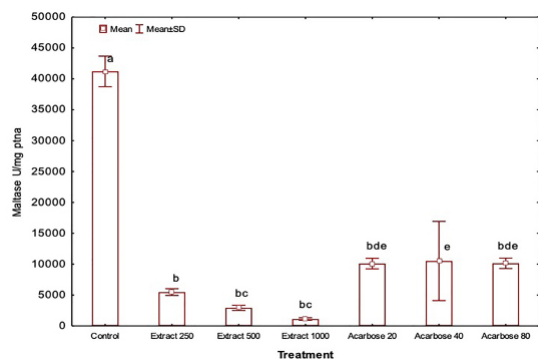


Figure 4. Activity of the maltase enzyme for the different concentrations of ethanolic extract ($\text{mg}\cdot\text{mL}^{-1}$) of the *E. uniflora* seeds. Means followed by the same letter do not differ statistically from each other by the Tukey test at 5% probability.

E. uniflora leaves extract. It should be noted that, in order to evidence a possible antidiabetic action, it would be necessary to isolate the chemical substance β -sitosterol and perform *in vivo* analyzes.

4. Conclusions

Results obtained in this work indicated a strong antioxidant activity, which can be correlated to the phenolic content present in the identified chemical substances. Fatty acids, such as omega 6 and omega 9, and phytosterols are at high concentration, which may suggest the ability of seeds to reduce cholesterol, help prevent cardiovascular diseases and the strong anti-inflammatory activity, due to the presence of β -sitosterol as a major component in the fractions studied.

In relation to the antibacterial activity, the ethanolic extract acted on the Gram positive bacterium with more efficiency, inhibiting its growth at lower extract concentrations, demonstrating a possible bacteriostatic action.

As for the inhibition of the enzymes lactase, sucrase and maltase, the ethanolic extracts showed great activity, however, could induce lactose intolerance.

The results obtained in this study corroborate the use of pitangueira by popular medicine for the control of several illnesses, and the results described, stimulate the continuity of studies on the plant, opening the possibility for future applications in the pharmaceutical industry.

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