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Antioxidant, antimicrobial and allelopathic activities and surface disinfection of the extract of *Psidium cattleianum* sabine leaves

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ABSTRACT

The objective this study was to obtain extracts, using ultrasound and enzymes, of leaves of *Psidium. cattleianum* Sabine, collected in the summer and winter, and evaluate the antioxidant, allelopathic and antimicrobial activities. Photomicrographs of leaf structure were obtained by SEM. The phenolic compounds content (TPC) and the antioxidant activity were determined. The allelopathic activity was verified in bioassays for each of the extracts against the test plant (*Lactuca sativa*). The antimicrobial activity was determined against Gram-positive and Gram-negative bacterial species. It was evaluated the use of extracts as disinfectants. The SEM showed that the extraction form affected the cell structure of the leaves but did not influence the TPC. The highest TPC was found in leaf extracts collected during winter, with values between 123 and 144 mg EAG.g^{-1}. The level of antioxidant activity estimated presented higher value for the leaves collected in the summer, where the IC_{50} presented values of 37 μg mL^{-1}. The results also indicate that all extracts tested show considerable allelopathic activity, with inhibition of growth of more than 50%. The antimicrobial activity can be classified as partially active to very active, where the best results were found against *L. monocytogenes*, with inhibition halos measuring up to 23 mm. It was also verified that the time of collection of leaves interfered in the values found. With regard to surface disinfection, all the extracts tested positive results. Experiments have shown that extracts of *P. cattleianum* Sabine are a source of bioactive compounds, which have antioxidant, allelopathic and antibacterial activity.

1. Introduction

The *Psidium* genus comprehend 100 species, defined as species that produce foodstuffs fruits, lumber, and ornamental ones, with the potential for commercial exploration, considered as medicinal plants, which are used in Brazilian traditional medicine to combat oral, gastrointestinal, urogenital and intestinal inflammations (Brandão, 2003). Among them, the *Psidium cattleianum* Sabine (Myrtaceae) species, commonly known as Cattley guava, is being considered a plant with latent perspectives for the pharmaceutical and food industry due to its potential application as herbal, functional food, among others (Patel, 2012), most probably due to the presence of bioactive compounds, such as phenolic compounds and carotenoids (Medina et al., 2011). It is a Brazilian species that can be found in Bahia, and in the States of Rio Grande do Sul and Santa Catarina, as well as in the neighboring country, Uruguay (Biegelmeyer et al., 2011; Patel, 2012).

The fruits of *P. cattleianum* have a firm and sweet to sub-acid pulp, with a spicy flavor, and are described as being more aromatic than the common guava fruits, *Psidium guajava* (Biegelmeyer et al., 2011; McCook-Russell et al., 2012). It has three or four times more ascorbic acid than citric fruits, and it has a great number of phenolic compounds, such as epicatechin and gallic acid (Alvarenga et al., 2013). Besides its fruits being consumed in natura, experiments with extracts from its leaves have demonstrated antiproliferative activity in cancer cells, as well as antioxidant activity in relation to the radicals DPPH, FRAP, and ABTS. The antioxidant potential of Cattley guava can be explained its capacity to capture the species reactive to oxygen and nitrogen, besides its pulp also having inhibiting potential for O_2, HOCl, and $1O_2$ (Ribeiro et al., 2014).

In Hawaii, since it was introduced, in 1825, as an ornamental garden plant, *P. cattleianum* has become an invading species, which has triggered serious problems (Patel, 2012). Its uncontrollable growth was explained by the production of toxic chemicals by its leaves, as the chlorogenic acid, quercetin, catechin, and ellagic acid (Rezende et al.,

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2003). These molecules, which prevents the growth of other species (Gerlach, 2004), showing allelopathic potential, which is characterized by the positive or negative interference that chemical compounds produced by a plant exert over other organisms (Rice, 1984).

Plants usually produce numerous secondary metabolites, and some of them have allelopathic activity, especially phenolic and terpenoids (Einhellig, 2002), also produced by *P. cattleianum*. Allelopathic substances may provide a competitive advantage for hosting plants through the inhibition of the growth of competitor plants (Kato-Noguchi et al., 2013). Many synthetic agrochemicals are highly toxic to men and other animals, be it due to direct exposition or to the accumulation in the organism after eating contaminated foods. In this sense, allelopathy can be very important, since it enables the identification of compounds, which may serve as basis for the production of more specific herbicides that are also less prejudicial to the environment when compared to the ones being currently used in agriculture (Christoffoleti et al., 1994; Da Silva et al., 2009).

Furthermore, the *P. cattleianum* demonstrated antimicrobial activity for microorganisms *Streptococcus mutans*, *Salmonella* Enteritidis, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Micrococcus luteus* (Souza et al., 2008; Brighenti et al., 2008; Medina et al., 2011; Biegelmeyer et al., 2011; Patel, 2012; Verma et al., 2013; Dos Santos Pereira et al., 2018). These findings are promising considering that the in recent years, more attention has been paid to the production of medicines and natural, where the search for new products of natural origin that present pharmacological properties contributes significantly to the discovery of new substances for different uses. In the case of plants that have relatively high levels of antimicrobial action, they can become sources for new compounds that inhibit the growth of foodborne pathogens (Scur et al., 2016; Biswas et al., 2013).

The leading causes of death worldwide originate from infectious diseases. Bacterial infections, especially in immunocompromised patients, are associated with high mortality and morbidity. As prevention and control strategies for infectious diseases, there is an improvement in public health, especially hygiene and sanitation, the use of antimicrobial agents and safe water initiatives (Soliman et al., 2016). Multi-resistant strains are emerging from the indiscriminate and even inadequate use of synthetic antimicrobials; where the extracts of plants, with antimicrobial potential, aim to delay this process (Weber et al., 2014). In this regard, surfaces carry a small risk of direct transmission of infection but may contribute to secondary cross-contamination by hands and instruments or products that may be contaminated by contact with such surfaces and subsequently contaminate persons or other surfaces.

Despite the publications on other biological activities of *Psidium cattleianum* Sabine fruits (Ribeiro et al., 2014; Dos Santos Pereira et al., 2018), there are few scientific evidences on activities related to the leaves or using water as extraction solvent. Therefore, it is considered important to study the other parts of the plant, as well as the different activities of the aqueous extract of the *P. cattleianum*. Given that, this work aims to evaluate the total phenolic contents and to determine the antioxidant activity, antimicrobial potential against gram-positive and gram-negative bacteria, as well as to test the possibility of using the extracts as surface disinfectants and allelopathic activity of the aqueous extracts of *P. cattleianum* Sabine, obtained with the use of low-frequency ultrasound or enzymes (cellulase complex).

2. Materials and methods

2.1. Plant material and sample preparation

Cattley guava leaves (*Psidium cattleianum* Sabine), yellow morphotype, were collected in the southern region of the State of Santa Catarina (28°19′31.9″ a 28°19′36.5″ S; 49°03′50.3″ a 49°03′51.9″ W), from July to September 2017 (winter) and from December to March 2018 (summer). Leaves were selected according to uniform coloration, ruling out vegetal material with rottenness, injuries and/or defects, as shown in Fig. 1. The vegetal material was sanitized with gauze dampened with distilled water and dried in a forced circulation stove with air at 40 ± 5 °C, to constant mass. After drying, the leaves were manually and separately milled, and sieved in 8 Mesh netting, which finally obtained the samples for extraction.

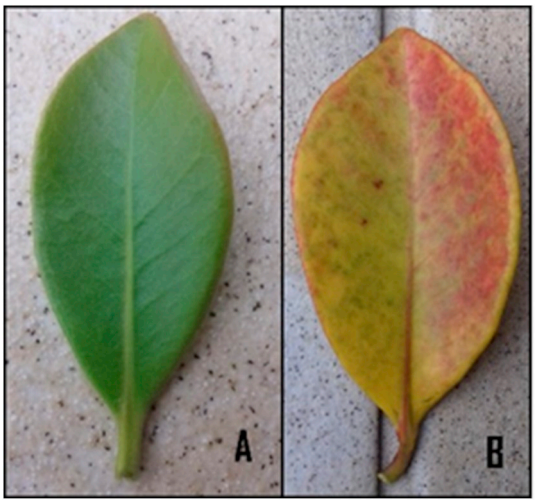

Fig. 1. Features of leaves of *P. cattleianum* Sabine collected in summer (A) and winter (B).

2.2. Preparation and obtaining the extracts

The procedure to obtain the extracts was adapted from Larrauri et al. (1997). For the extraction, 15 g of leaves collected in summer and winter were used, separately, and two method were used of each fraction, WU extraction (water + ultrasound) and WE extraction (water + enzyme), for both seasons, winter and summer, in a total of 4 aqueous extracts.

To obtain WU extracts, 100 mL of distilled water was added to the leaf samples, separately. After that, the mix was taken to an ultrasound bath (70 W) for 3 h. This mixture stayed at rest, in the dark, for other 3 h. The supernatant was filtered with quantitative filter paper ('Whatman' no. 40), and stocked in a volumetric flask of 100 mL, involved in foil paper. To obtain WE extracts, 100 mL of distilled water was added to leaf samples, separately, and 20 μL of a cellulase complex (Novozymes 22086) was added to the mixture. The solution was taken to a bath with orbital agitation at 100 rpm and 45 °C for 6 h. The supernatant was filtered with quantitative filter paper ('Whatman' no. 40) and stored in a volumetric flask of 100 mL, involved in foil paper.

Right after the extraction, the extracts were stored in Eppendorf tubes and kept frozen at −83 °C until its use.

2.3. Scanning electron microscopy (SEM)

In order to verify the influence of the extraction method on the structure of leaves, photomicrographs were obtained using scanning electron microscopy (SEM) (microscope Philips, model XL30). The gold coating was carried out in a BAL-TEC Sputter Coater, model SCD 005, for 120 s on the dried leaves (leaf samples were previously dried at 30 °C for 24 h).

2.4. Determination of total phenolic content (TPC) and antioxidant activity by elimination of radicals by DPPH

The quantification of TPC was carried out by the Folin-Ciocalteu colorimetric method, with modifications (Bonoli et al., 2004). A part of each diluted sample was mixed with 0.5 mL of Folin-Ciocalteu and

agitated for 1 min. 2 mL of sodium carbonate (20%) was added to the mixture, and agitated for 30 s. After a 2 h incubation in darkness, the absorbance at 750 nm was read in relation to prepared white. The standard curve was prepared by solutions of gallic acid in methanol. The concentration of total phenolic compounds was determined in the extracts as equivalents to gallic acid using an equation obtained based on the standard gallic acid graph and expressed in mg of gallic acid equivalent per dry sample gram (mg ECA.g^{-1}). The data were presented as the average of analyses ± standard deviation (SD) of triplicates.

2.5. Antimicrobial activity

The antimicrobial activity of the extracts was evaluated by disc diffusion assay and by determining the minimal inhibitory concentration (MIC), according to Ostrosky et al. (2008) and Oliveira et al. (2016). The tests were performed against Gran positives bacteria (laboratory stock) *Listeria monocytogenes*, *Staphylococcus aureus* and the Gran negatives (laboratory stock) *Salmonella* Enteritidis and *Escherichia coli.* Pathogenic cultures were recovered in BHI and incubated at 36 °C, overnight. Cultures were left at the concentration of 0.5 of the McFarland scale (equivalent to 10^8 Colony Forming Units per milliliter – CFU. ml^{-1}) and diluted in peptone water of casein to the concentration of 10^5 CFU ml^{-1}.

2.5.1. Disc diffusion

The microorganisms were inoculated, via swabs, into plates containing Müller-Hinton Agar. Thereafter, three Whatmann filter paper disks sterile of 6 mm diameter were added to each plate. On the paper disks were added 15 μL of the extracts. For the negative control sterile distilled water was used. Plates were incubated for 24 h at 35 ± 1 °C. The diameters of the inhibition halos were measured with a pachymeter and the result expressed in millimeters (mm). The higher the antimicrobial activity of the extract, the greater the inhibition halo against the tested microorganisms. All the tests were performed in triplicate.

2.5.2. Minimum inhibitory concentration (MIC)

The extracts of the leaves of Cattley guava that presented antimicrobial activity through the disc diffusion, were submitted to determination of the MIC, in sterile 96-well plate. Serial dilutions (up to 4 times) of the samples of the extracts were performed in BHI to obtain the desired concentration range. 100 μL of a bacterial suspension in BHI was added into each well. Negative control wells (containing the BHI and 200 μL of extract without the bacterial suspension) and positive control wells (containing BHI and the bacterial suspension) and were also prepared. The plates were incubated for 18 h at 37 °C without shaking. After time, 10 μL of 3% resazurin was added and left for another 2 h in the incubator at 37 °C. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract that was able to inhibit the visible growth of the bacteria.

Table 1
Total phenolic content (TPC) and antioxidant activity (IC_{50}) of aqueous extracts of *Psidium cattleianum* Sabine leaves collected in summer and winter.

Leaves	TPC (mg de EGA. g^{-1})		Antioxidant activity DPPH (IC_{50} μg. mL^{-1})	
	WU	WE	WU	WE
Summer	101 ± 0.2^{bA}	121 ± 0.1^{bA}	37.3 ± 0.9^{bB}	160 ± 0.5^{bA}
Winter	144 ± 0.5^{aA}	123 ± 0.1^{aA}	340 ± 1.9^{aA}	208 ± 5.3^{aB}

Tukey Test for TPC and antioxidant activity, separated. Different lowercase letters (in the column) correspond to different experiments ($p < 0.05$), when comparing the seasons when the leaves were collected. Different uppercase letters (in line), correspond to different experiments (p<‘0.05), when comparing extraction methods. WU and WE correspond to extraction methods using water + ultrasound and water + enzyme, respectively.

2.6. Surface disinfection

After testing the antimicrobial activity with all the microorganisms, the bacterium *Listeria monocytogenes* was chosen for this test because it presented the worst performance against the tested extracts. The extracts were obtained as previously described, with only one modification. The extraction cycles were repeated four times for both extracts, WU and WE.

Four surfaces were used: glass, plastic, stainless steel and granite, with 5 cm^2 each. The surfaces were contaminated in an aseptic environment with 0.1 mL of suspension of *L. monocytogenes*. Thirty minutes after contamination the control group (without disinfection) was collected with swabs previously moistened in 0.1% (m/v) peptone water solution, which were transferred to tubes containing 9 mL of 0.1% (p/v) peptone water solution, vortex shaken for 2 min. Serial dilutions was prepared and plated on agar Listeria according to Ottaviani and Agosti (ALOA).

Afterwards, each surface was submitted to the disinfection process by the spray wipe spray technique for each solution, and each surface received the application with the aid of a spray bottle, then it was wiped with sterilized gauze and the disinfectant (extracts), which remained on the surface for 10 min. After the disinfection period, samples were collected After the disinfection period, samples were collected as described for the control. The swabs were immersed in 9 mL of sterile saline solution, homogenized in vortex for 2 min and inoculated 0.1 mL to agar ALOA. The plates, control and post-disinfection were incubated at 37 °C for 24 h in a bacteriological oven. The Colony Forming Units (log CFU. cm^2) were counted, after incubation, (Bambace et al., 2003).

2.7. Allelopathic activity

To evaluate the allelopathic effect of the extracts, we performed germination inhibition and growth biotests, according to Formagio et al. (2010), with some adaptations, using lettuce (*Lactuca sativa* cv. Grands rapids), as a bioindicator plant. Lettuce seeds were obtained commercially, and non-germinated seeds were used for biogermination tests, while seeds that were germinated 24 h before in distilled water were used for growth tests.

For the germination biotests, 10 seeds per replication (plate) were used, as well as 2 replications per treatment, for WU and WE extract for leaves collected in winter and summer, in a total sum of four treatments. The seeds were distributed in Petri dishes (9 cm of diameter) covered with Whatman filter paper no. 2 and damped with 5 mL of solution of the different extracts. 5 mL of distilled water were used in the Control experiment. After putting the seeds on the dishes, they were closed with Parafilm®. Dishes were kept in a BOD oven with 40% humidity and controlled photoperiod, for 5 days. The germination percentage (%G) was obtained by the relation between the number of germinated seeds at the end of the test and the number of seeds added to the dish. The germination index (GI) was obtained by the relation between the sum of germinated plantlets in each count and the number of count days. The mean germination time (MGT) was obtained by the relation between the sum of the multiplication of germinated seeds in each count and the time passed between each count and the number of germinated seeds in each count, and the average speed of germination (ASG) by the relation between 1 and the average germination time.

For the growth biotest, plantlets had their aerial and radicular parts measured with the help of a digital pachymeter. The plantlets that presented 2 mm of radicle were transferred for new Petri dishes (9 cm of diameter), covered with two Whatman filter paper n°. 2 and dampened with 5 mL of the aqueous extracts or distilled water (Control). Petri dishes were incubated in BOD ovens with 40% humidity and controlled photoperiod for 5 days and, having the sizes of plantlets and radicles, the percentage of inhibition of growth (%IG) was calculated in relation to positive control (plants with distilled water).

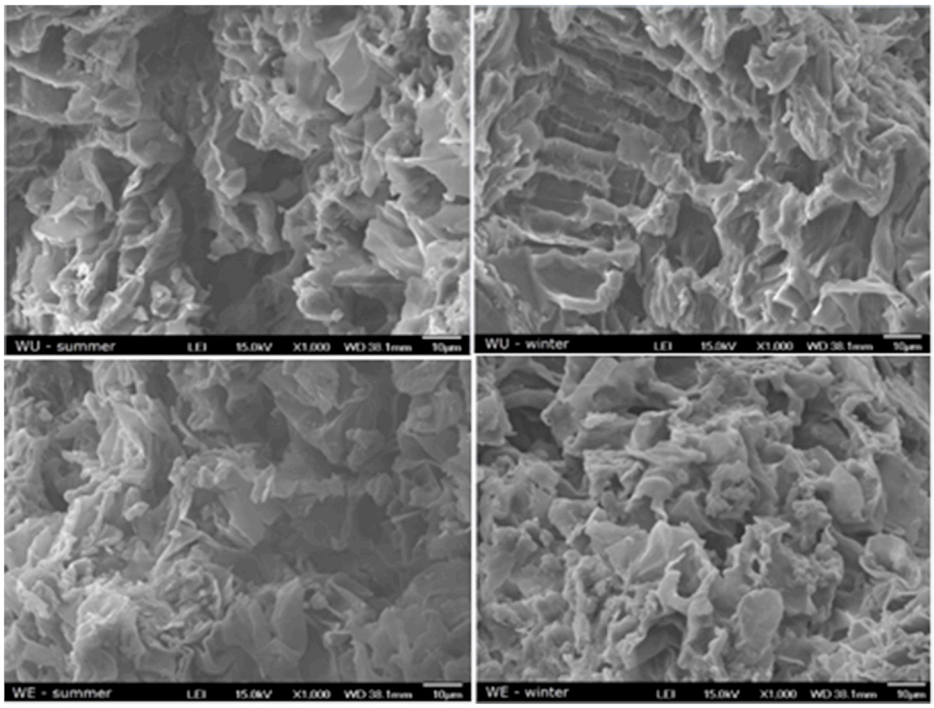

Fig. 2. SEM of the leaves submitted to extraction using ultrasound (WU – summer and WU – winter) and the enzyme complex (WE – Summer and WE – winter).

2.8. Statistical analysis

The statistical analysis of experimental results was performed using the software Statistica® 10.0 (Statsoft Inc.), performing the Tukey Test, with 95% of reliability.

3. Results and discussion

3.1. Total phenolic content and antioxidant activity of the extracts of *Psidium cattleianum sabine leaves*

The results obtained in the determination of the total phenolic content (TPC) expressed as equivalents to gallic acid (EGA) per g of dry vegetal material, and the antioxidant activity (IC_{50}) in relation to DPPH radical, expressed in ($\mu g.mL^{-1}$) of the aqueous extracts of *Psidium cattleianum* Sabine leaves, are shown in Table 1.

For TPC values, it was verified that, both for leaves collected in summer and in winter, there was no statistically significant difference ($p < 0.05$) between WU and WE extract, which shows that the form of extraction did not affect TPC. However, when one verifies the season in which *Psidium cattleianum* Sabine leaves were collected, one notices that it affected the total phenolic content. For the WU extraction, it was verified a statistically significant difference ($p < 0.05$) in TPC values for the extracts obtained from the leaves collected in summer and winter, with higher TPC values for the ones collected in winter. The same behavior was observed for WE extractions. Chen et al. (2007), when determined the TPC in aqueous extracts of *Psidium gaujaya* L. leaves, have observed values of 154.36 ± 2.97 μg of $EAG.g^{-1}$. Similar values were also observed by Qian and Nihorimbere (2004), in which the TPC value found was 511.6 ± 6.2 μg of $EAG.g^{-1}$.

Gobbo-Neto and Lopes (2007) highlight that there is an influence of harvest season for leaves of a species on the TPC, as well as on its active constituents. It was reported seasonal variation on the contents of almost all classes of secondary metabolites, such as phenolic acids, flavonoids, coumarins, and saponins. Annual, monthly, and even daily temperature variations are one of the factors exerting higher influence of the development of each species, affecting, thus, the production of secondary metabolites. Lower temperatures, a typical characteristic of winter, significantly affects the level of secondary metabolites (Harris, 2009). The low winter temperature has also other characteristics that determine its positive influence in relation to the production and composition of secondary metabolites. The hydrological stress can be mentioned, considering that, in this season, rain is less abundant, which can increase evapotranspiration in species. There are many reports of these conditions leading to an increase in the production of many metabolite types (Harris, 2009).

Alvarenga et al. (2016) have assessed the chromatographic profile of yellow cattley guava leaves and observed the prevalence of compounds such as quercetin, quercitrin, and isoquercitrin, all part of the flavonoid class, and ellagic acid, as well as lower amounts of catechin, chlorogenic acid and kaempferol. Such compounds can be correlated to the studies aforementioned, suggesting that the higher phenolic content presented in *Psidium cattleianum* Sabine leaves collected in winter may have happened due to relations and factors similar to the ones discussed.

Regarding the antioxidant activity, the extracts ability in capturing free radicals, expressed in the value of IC_{50}, it was verified that, for extracts obtained through the WU method, there was a statistically significant difference ($p < 0.05$) in IC_{50} values, when compared leaves collected in winter and summer, with higher antioxidant activity for extracts of leaves collected in summer. The same behavior happens for extracts obtained by the WE method. When comparing extraction methods, for a same season, it was verified that there was statistically significant difference ($p < 0.05$) for extracts WU and WE, indicating that the extraction method may affect the availability of molecules with this activity in the extract. For summer leaves, the WU extraction presented higher antioxidant activity, and for winter leaves, the WE extraction presented higher antioxidant activity.

For IC_{50} values lower than 50 $\mu g\,mL^{-1}$, the extract is considered very active, for values from 50 to 100 $\mu g\,mL^{-1}$, it is considered moderately active, for values between 100 and 200 $\mu g\,mL^{-1}$, it is considered slightly active, and is considered inactive for values above 200 $\mu g\,mL^{-1}$ (Reynertson et al., 2005). Therefore, the extract obtained by the WU method for summer leaves can be considered very active, the extract obtained by the WE method for summer leaves can be considered slightly active, and

Table 2
Antimicrobial activities (in mm) of aqueous extracts of *Psidium cattleianum* Sabine leaves collected in summer and winter.

Leaves	Method of extraction	Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	*L. monocytogenes*	*E. coli*	*S.* Enteritidis
Summer	WU	12.5 ± 1.9^{aA}	18 ± 1.0^{aA}	9.5 ± 0.0^{aA}	11.2 ± 0.0^{aA}
	WE	18.2 ± 0.0^{aA}	23.1 ± 0.1^{aB}	15.2 ± 0.1^{aB}	13.9 ± 0.0^{aB}
Winter	WU	10.7 ± 1.0^{aA}	18.5 ± 1.1^{aA}	15.4 ± 0.1^{bA}	12.7 ± 1.0^{aA}
	WE	17.2 ± 2.0^{aB}	18.7 ± 1.2^{bA}	17 ± 1.1^{aA}	17.5 ± 0.1^{bB}

Zone of inhibition determined by agar disc diffusion. Results presented as means ± standard deviation. WU and WE correspond to extraction methods using water + ultrasound and water + enzyme, respectively. Different lowercase letters correspond to different experiments ($p < 0.05$), when comparing the seasons when the leaves were collected, for the same extraction method. Different uppercase letters correspond to different experiments ($p < 0.05$), when comparing extraction methods, for the same season.

the extracts obtained by WE and WU methods for winter leaves can be considered inactive.

The method of determining antioxidant activity was quantified mainly to indicate the antioxidant potential of phenolic compounds isolated in extracts and various substances (Borges et al., 2011). Bernal et al. (2013) have found a well-stablished positive correlation between the intensity of solar radiation, higher in summer, and the production of phenolic compounds. In the case of flavonoids and phenylpropanoids, specially, the protection against photo-destruction performed by these metabolites when absorbing and/or dissipating solar energy hampers the damage to more internal tissue by UV-B radiation.. This correlation can be one of the reasons why, even with lower phenolic content values, aqueous extracts of leaves collected in summer have presented higher antioxidant activity than the ones collected during winter. Qian and Nihorimbere (2004), when studying the aqueous extract of *Psidium guajava* L., obtained the IC_{50} of $130 \pm 1.0\ \mu g\ mL^{-1}$. While Leite et al. (2014), when determining the IC_{50} of hydroalcoholic extracts of *Psidium guajava* L. leaves, obtained the value of $268.40\ \mu g\ mL^{-1}$.

Fig. 2 shows the SEM of the leaves submitted to extraction using ultrasound (WU – summer and WU – winter) and the enzyme complex (WE – Summer and WE – winter).

Differently from that obtained for TPC, it was observed that there was a difference between WU and WE extract, which shows that the form of extraction did affect the cellular structure of leaves. For the WU extraction, the formation of cavities in the vegetal structure was verified, which possibly occurred due to the phenomenon of cavitation. US waves induce alternative cycles of compression and rarefaction of the material. Furthermore, when sonication is carried out in a liquid medium, thousands of gaseous cavitation bubbles are formed and part of them collapse which induce alteration of surface tension and viscosity. A fountain of little bubbles moves very fast through material leading to formation of microscopic channels, elongation and flattening of the cells (Fernandes et al., 2008; Nowacka et al., 2012). It is known that the US treatment is connected with free radical generation (Kohno et al., 2011), what can change both chemical composition and antioxidative potential of dried herbs. Santacatalina et al. (2014) suggested, the mechanical stress affected by application of US may contribute in releasing of oxidative enzymes and intracellular compounds into the solvent and therefore resulting in phenolic degradation.

For the WE extraction, the occurrence of hydrolysis of the plant structure was observed. Since the cell wall is composed of different polysaccharides bound to a structural protein, mixtures of enzymes and complexes with multiple activity are more efficient than isolated enzymes (Jordan and McAuliffe, 2018). The enzymatic complex used is composed of cellulases, which are enzymes classified as glycosyl hydrolases and degraded the plant cell wall, hydrolyzing oligosaccharides, polysaccharides and recognizing the α-1,4 bonds between glucose molecules (Raven and Evert, 2001). Miron et al. (2013) compared enzyme-assisted extraction with conventional extraction of phenolic compounds from lemon balm. Cellulase, pectinase and endo-β-1, 4-xylanase were used to degrade the cell wall and release the phenolic compounds. The results indicated that enzymatic assisted extraction showed extracts with higher total phenol contents and antioxidant activity compared to the traditional method.

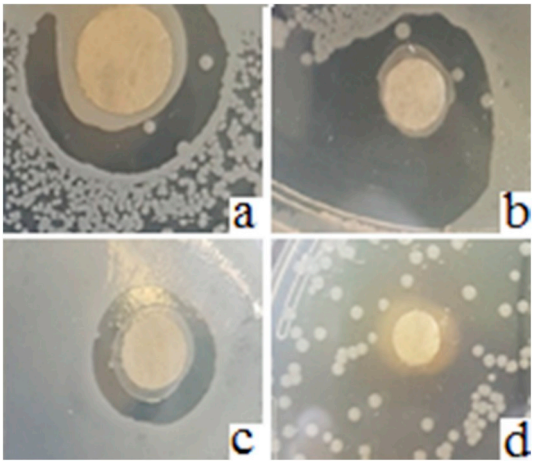

Fig. 3. Zones of inhibition that was determined by agar disc diffusion, for all tested microorganisms using of extract. *A* corresponds to the best zone of inhibition against *S. aureus*, *B* the best zone of inhibition against *L. monocytogenes*, C the best zone of inhibition against *E. coli* and D the best zone of inhibition against *S.* Enteritis.

3.2. Antimicrobial activity of the extracts of Psidium cattleianum sabine leaves

Staphylococcus aureus is considered an important human pathogen, especially when related to the wide range of clinical infections caused. It is one of the leading causes of infective endocarditis and bacteremia, as well as cutaneous, osteoarticular and soft tissue, pleuropulmonary and device related infections (Tong et al., 2015; Wertheim et al., 2005). *Listeria monocytogenes* is a relevant foodborne pathogen in public health, responsible for outbreaks of listeriosis often associated to the consumption of ready to eat dairy, meat and fishery products. Listeriosis is a serious disease that can lead to death and mainly affect the elderly, children and immunocompromised individuals (Jarvis et al., 2016; Jordan and McAuliffe, 2018). *Escherichia coli* is a common cause of diarrheagenic illness globally, of complicated and uncomplicated urinary tract infections, and a leading cause of bacteremia and neonatal meningitis (Poolman, 2016). *Salmonella* Enteritidis is one of the important sources of salmonellosis cases and outbreaks. These illnesses have been attributed to eggs and egg-containing foods more than any other food (Howard et al., 2012).

The antimicrobial activities (in mm) of aqueous extracts of *Psidium cattleianum* Sabine leaves collected in summer and winter are shown in Table 2. The classification establishes that extracts with halos of inhibition <9 mm are classified as inactive; from 9 to 12 mm are partially

Table 3
Antibacterial activity of aqueous extracts of *Psidium cattleianum* Sabine leaves expressed as minimum inhibitory concentrations (MICs), in μg.mL^{-1}.

Leaves	Method of extraction	Gram-positive bactéria		Gram-negative bacteria	
		S. aureus	*L. monocytogenes*	*E. coli*	*S.* Enteritidis
Summer	WU	12.6	12.6	6.3	12.6
	WE	15.1	15.1	30.2	7.6
Winter	WU	18	18	36	18
	WE	15.4	15.4	30.7	15.4

WU and WE correspond to extraction methods using water + ultrasound and water + enzyme, respectively.

Table 4
Disinfection power (log CFU.cm^{-2}) of aqueous extracts of *Psidium cattleianum* Sabine leaves against *Listeria monocytogenes* in glass, plastic, stainless steel and granite surfaces.

Leaves	Method of extraction	*Listeria monocytogenes* (log CFU.cm^{-2})			
		Glass	Plastic	Stainless steel	Granite
Summer	WU	0.00	0.00	0.00	0.00
	WE	0.00	0.60	0.00	0.00
Winter	WU	0.00	0.00	0.00	0.00
	WE	0.00	0.00	0.00	0.00
Control		1.38	3.09	3.25	1.78

WU and WE correspond to extraction methods using water + ultrasound and water + enzyme, respectively.

active, from 13 to 18 mm are active and, >18 mm are very active (Oliveira et al., 2016).

For the tested bacteria (Gram-positive and Gram-negative), aqueous extracts of *Psidium cattleianum* Sabine leaves that showed antimicrobial activity can be classified as partially active to very active, that is to say, none of the extracts tested was classified as inactive. In general, it observed that Gram-positive bacteria presented less resistance against the extracts tested when compared to Gram-negative bacteria. This fact may be related to bacterial cell structure, since the Gram-positive bacteria present a single layer in the cell wall, whereas the Gram-negative bacteria present an extra layer of lipopolysaccharides and proteins in the cell wall that form a barrier of permeability to antimicrobial agents (Forsythe, 2013).

Fig. 3 shows the best zones of inhibition that was determined by agar disc diffusion, for all tested microorganisms.

Regarding the season, the WE extract was statistically different ($p < 0.05$) when compared to the two microorganisms *S.* Enteritidis and L. *monocytogenes*; whereas for the WU extract only *E. coli* showed a statistically different in relation to the two seasons. For *S. aureus*, independent of the collection season, there was no statistical difference in relation to WU and WE extract. When comparing the extracts WU and WE for the same season, it verified that the results varied according to the microorganism used. For *S.* Enteritidis the results showed a significant difference ($p < 0.05$), independent of the season, which shows that the extraction mode affected the content of the compounds responsible for the antimicrobial activity. For *E. coli* and *L. monocytogenes*, the results presented significant difference, only in the summer season. While for *S. aureus* the results presented significant difference only during the winter season.

Gobbo-Neto and Lopes (2007) highlight that seasonal variation on the contents of almost all classes of secondary metabolites, such as phenolic acids, flavonoids, coumarins, and saponins. Annual, monthly, and even daily temperature variations are one of the factors exerting higher influence of the development of each species, affecting, thus, the production of secondary metabolites. Alvarenga et al., 2016 have assessed the chromatographic profile of yellow cattley guava leaves and observed the prevalence of compounds such as quercetin, quercitrin, and isoquercitrin, all part of the flavonoid class. It is known that the action of flavonoids on bacterial cells occurs through the formation of complexes between the cell wall and proteins, causing their rupture (Taguri et al., 2004). Flavonoids having the 2OH groups in their rings are less active against microorganisms than those with hydroxyl groups; this finding confirms the idea that the membrane is the target. The ability to complex with soluble and extracellular proteins in bacterial cell walls is probably due to their activity. However, microbial membranes can also be broken by lipophilic flavonoids. (Cowan, 1999; Sato et al., 1996). On the other hand, the inhibition of nutrient transport and the formation of complexes between the tannins and the bacterial cell wall acts to prevent microbial growth (McSweeney et al., 2001). Finally, the rupture of lipophilic compounds from microbial membranes, causing the death of microorganisms, is associated with the mechanism of action of the triterpenoids (Tepe et al., 2004).

The antibacterial activity of aqueous extracts of *Psidium cattleianum* Sabine leaves expressed as minimum inhibitory concentrations (MICs) shown in Table 3. The antibacterial activity of plant extracts can be considered significant when MIC values are lower than 100 μg/ml, moderate when $100 < MIC \leq 625\ \mu g\ mL^{-1}$ and low when $MIC > 625\ \mu g\ mL^{-1}$ (Kuete, 2010; Tchinda et al., 2017).

The MIC values ranged from 6.28 μg mL^{-1} to 35.95 μg mL^{-1} and considered significant values. Therefore, the obtained MIC values are very important when considering importance of the tested bacteria. Scur et al. (2016) when investigating the antimicrobial activity and phytochemical screening of extracts of *P. cattleianum* Sabine reported that for the aqueous extract the MIC ranged from 6.25 to 50 mg mL^{-1} for Gram-negative bacteria and 6.25–12.5 mg mL^{-1} for Gram-positive bacteria, which shows that the result was three times higher than that stated in the literature.

Regarding the disinfection of surfaces, all the extracts tested presented significant positive results against *Listeria monocytogenes* on the four surfaces tested, as can be shown in Table 4. All extracts tested inhibited the growth of *L. monocytogenes* as compared to the control sample. Only the WE - summer extract did not present null count against the tested plastic surface. However, as the control sample presented values of 3.09 (log CFU.cm^{-2}), it was observed that it was efficient in the reduction of 2.49 (log CFU.cm^{-2}) of the count.

Table 5
Germination percentage (%G), germination speed index (GI), medium germination time (MGT), and average speed of germination (ASG) of Lactuca sativa cv. Grands rapids seed in relation to aqueous extracts of *Psidium cattleianum* Sabine leaves collected in summer and winter.

Leaves	WU	WE
	%G	
Summer	75 ± 21.2^{aA}	75 ± 7.1^{bA}
Winter	40 ± 14.1^{bA}	50 ± 0.0^{cA}
Control	100 ± 0.0^{aA}	100 ± 0.0^{aA}
GI		
Summer	7.4 ± 3.1^{bA}	3.9 ± 1.2^{aA}
Winter	3.1 ± 2.0^{bB}	16 ± 0.7^{bA}
Control	15.3 ± 0.0^{aA}	15.3 ± 0.06^{bA}
MGT (day)		
Summer	5.1 ± 0.2^{aA}	5.5 ± 1.8^{aA}
Winter	5.4 ± 0.2^{aA}	5.4 ± 0.1^{aA}
Control	4.6 ± 0.0^{aA}	4.6 ± 0.0^{aA}
ASG (day^{-1})		
Summer	0.19 ± 0.0^{aA}	0.19 ± 0.1^{aA}
Winter	0.19 ± 0.0^{aA}	0.18 ± 0.0^{aA}
Control	0.22 ± 0.0^{aA}	0.22 ± 0.0^{aA}

Tukey Test for %G, GI, MGT and ASG, separated. Different lowercase letters (in the column) correspond to different experiments ($p < 0.05$), when comparing the seasons when the leaves were collected and control. Different uppercase letters (in line), correspond to different experiments ($p < 0.05$), when comparing extraction methods. WU and WE correspond to extraction methods using water + ultrasound and water + enzyme, respectively.

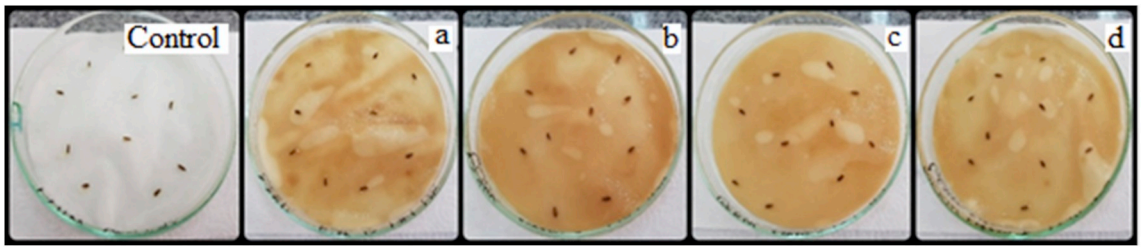

Fig. 4. Germination of *Lactuca sativa cv*. Grands rapids seeds in aqueous extracts of *Psidium cattleianum* Sabine leaves collected in summer, winter, and the control experiment, after 24 h. A = WU extract of summer leaves, B = WE extract of summer leaves, C = WU extract of winter leaves, and D = WE extract of winter leaves. WU and WE correspond to the extraction methods using water + ultrasound and water + enzyme, respectively. In the control experiment, distilled water was used for wetting.

It is known that the chemical composition of the surface influences bacterial adhesion and proliferation. In materials with different functional groups in their chemical composition, there is a difference in the number of cells adhered due to the fact that the bacteria-surface interaction depends on the hydrophobicity and loading of the material (Katsikogianni and Missirlis, 2004). Silva et al. (2008) when studying the adhesion to and viability of *L. monocytogenes* on food contact surfaces reported that the results show different patterns of adhered cell viability for the different surfaces. The results also demonstrated that the percentage of viable cells on the surface of glass and polypropylene was close to 100%, despite the lower extent of adhesion. Cells adhered to granite exhibited reduced viability despite the high number of adhered cells. Beresford et al. (2001), investigating the adhesion of *Listeria monocytogenes* to seventeen different materials approved for food use, representing metals, rubbers and polymers, reported surprise that there was no difference in the degree of fixation that occurred instantaneously or after 2 h. The adhesion of *L. monocytogenes* to abiotic surfaces is a phenomenon dependent on several factors, which demonstrates the importance of a better understanding of microbial viability, since adherent bacteria that remain viable are the true culprits for postprocess contamination, as well as the search for new disinfectant agents that can derail the adhered cells.

3.3. Allelopathic activity of the leaf extracts

The evaluation of allelopathic and the observation of changes in seed germination rates shows the toxic and/or cytotoxic action of the tested extracts or substances (Luz et al., 2012). In Table 5, are presented the results of the germination percentage (%G), germination speed index (GI), medium germination time (MGT) and average speed of germination (ASG) of *Lactuca sativa* cv. Grands rapids seeds submitted to aqueous extracts of *Psidium cattleianum* Sabine leaves collected in summer and winter.

Regarding the %G for WU extracts, it was verified a statistically significant difference ($p < 0.05$) between the extract of winter leaves and the rest, with a 60% inhibition of germination. For WE extracts, there was a statistically significant difference ($p < 0.05$) among all three extracts, summer leaves, winter leaves, and control. When comparing WU and WE extracts, for the same season, it can be verified that the results did not show significant differences, which demonstrates that the extraction manner did not affect the content of compounds responsible for germination. Thus, the influence of germination happened due to the season when the leaves were collected, with a 25%–60% variation in germination inhibition, when compared to the control experiment (100%).

For the GI, while summer leaves did not show significant difference ($p > 0.05$) in relation to the type of extract used, winter leaves have shown a higher and statistically different germination speed index ($p < 0.05$) when the WE extract was used, that is, for this last one, the speed of seed germination can by faster. However, about the season, the WU extract was statistically different ($p < 0.05$) when compared both seasons and the control group, while for the WE extract, only summer leaves have shown a statistically different value in relation to the other season and the control. It can be concluded that the reduction of GI can create a delay in the germination process, but not necessarily, after 24 h, a lower %G and consequent increase in the inhibition percentage. In relation to MGT and ASG, it is observed that the values found were not statistically different ($p > 0.05$) regarding the extract used, WU and WE, as well as the seasons and the control experiment. That is, regardless of the extract of season when leaves collected, *Psidium cattleianum* Sabine leaves will present stable germination speed and average time.

Table 6
Growth inhibition percentage (%IG) for *Lactuca sativa cv*. Grands rapids in aqueous extracts of *Psidium cattleianum* Sabine leaves collected in summer and winter.

Leaves	%IG	
	WU	WE
Summer	57.1 ± 4.9^{aA}	58 ± 0.4^{aA}
Winter	51.6 ± 2.4^{aA}	55.3 ± 1.3^{aA}
Control	0.0 ± 0.01^{bA}	0.00 ± 0.01^{bA}

Tukey Test with 95% reliability. Different lowercase letters (in the column) correspond to different experiments ($p < 0.05$), when comparing the seasons when the leaves were collected and control. Different uppercase letters (in line), correspond to different experiments ($p < 0.05$), when comparing extraction methods. WU and WE correspond to extraction methods using water + ultrasound and water + enzyme, respectively. In the control experiment, distilled water used for wetting.

Fig. 4 shows the germination of *Lactuca sativa cv*. Grands rapids seeds in aqueous extracts of *Psidium cattleianum* Sabine leaves collected in summer and winter and in the control experiment, after 24 h.

Hister et al. (2016) reported that the aqueous extract of *Psidium cattleianum* leaves have presented a drop in seed germination in relation to the negative control, as well as the extracts that had a concentration of 75 $g.L^{-1}$ of dry leaves presented a partial to total inhibition of germination. Tur et al. (2012) also verified that extracts of fresh and dry *Lonchocarpus campestris* leaves (Mart ex. Benth) have acted differently over the germination of *L. sativa* seeds. The extract of fresh leaves did not affect germination, on the other hand, the extract of dry leaves reduced germination, which was sharper in higher concentrations of 8%. Allelopathic compounds, since they interfere in cell division, membrane permeability, and enzyme activation, are considered as germination and growth inhibitors (Ali et al., 2014). Thus, the reduction in seed germinability shows a cytotoxic effect that may be caused by the allelopathic action of negative influence by the extract on *L. sativa* seeds.

The results of growth biotests shown in Table 6, expressed through the percentage of growth inhibition in relation to the positive control (plant with distilled water).

For the %IG, when compared WU and WE extracts for a same season (in line), it is verified that, for both extracts, there was no statistical difference ($p > 0.05$) among extraction methods, being possible to use

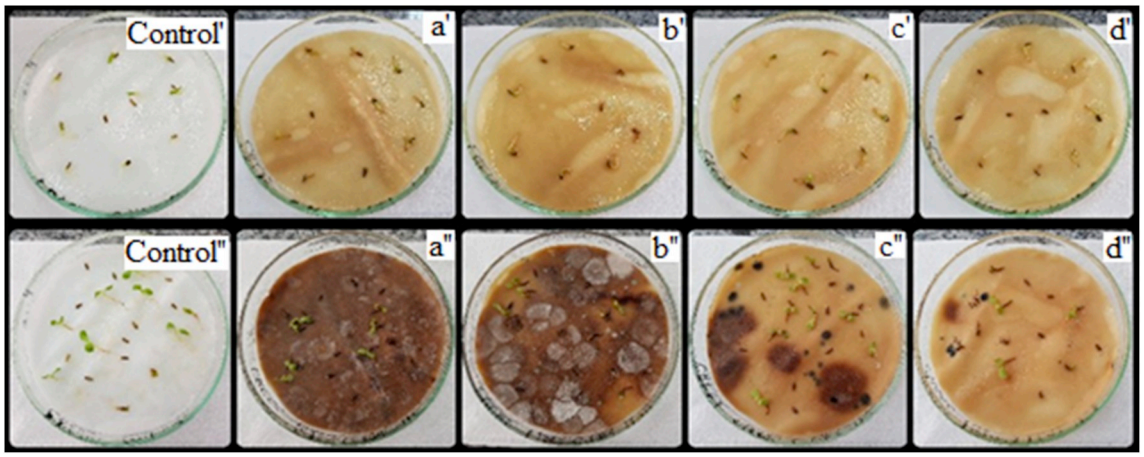

Fig. 5. Growth biotests of *Lactuca sativa cv.* Grands rapids plantlets treated with WU and WE extracts of winter and summer leaves, collected in the 1st and 5th day of growth. A′ and C′ correspond to the 1st day of growth in WU extract of summer and winter leaves, respectively. B′ and D′ correspond to the 1st day of growth in WE extract of summer and winter leaves, respectively. A″ and C″ correspond to the 5th day of growth in WU extract of summer and winter leaves, respectively. B″ and D″ correspond to the 5th day of growth in WE extract of summer and winter leaves, respectively. WU and WE correspond to extraction methods using water + ultrasound and water + enzyme, respectively.

the ultrasound and the enzyme complex both to obtain the extract. When compared WU and WE extracts separately and between seasons (in column), it is verified that, for both extracts, there was no statistical difference ($p > 0.05$) between extract of summer leaves and winter ones. Therefore, the results showed a 50% plant growth inhibition, demonstrating the allelopathic effect of *Psidium cattleianum* Sabine leaves. In many studies, it is observed a lower effect of extracts on germination when compared to initial development, given that the germination process uses reserves of the seed itself (Maraschin-Silva and Aqüila, 2005). However, it was observed, in this case, that the values were close in both biotests. Reports from other studies point out that the effect commonly caused by extracts on initial growth is the reduction in root sizes (Aqüila, 2000), considering that this effect was observed in the treatments performed.

Fig. 5 shows the growth biotests for *Lactuca sativa cv.* Grands rapids plantlets treated with WU and WE extract of winter and summer leaves, collected in the 1st and 5th day of growth.

It can be observed that the allelopathic influence on lettuce plantlet growth happened in the abnormality specially in the radicular system, in which roots presented necrosis, damages and even lack of roots. The plants radicular system is the most sensitive one to the action of allelochemicals, given that its elongation depends on cell divisions that, if inhibited, compromise its normal development (Hoffmann et al., 2007). The presence of abnormality in the roots can be considered a good parameter for the registry of plantlet abnormality, since this organ is more sensitive to the allelopathic activity than the aerial part. According to Pires and Oliveira (2001), effects such as darkening and stiffening are secondary effects of allelopathy in response to changes happening at the cell level, an effect observed in many allelopathy studies.

4. Conclusions

The results showed that *Psidium cattleianum* Sabine leaves have shown a content of total phenolic compounds that present antioxidant, antimicrobial activity and allelopathic activity. Generally, the extraction methods used, with the assist of ultrasound and enzymes, did not affect the tested activities; however, the season when the leaves were collected had influence. In relation to the phenolic content, leaves collected in winter have higher values, while summer leaves had lower antioxidant activity. For the zone of inhibition, the best results were found against *L. monocytogenes* bacteria, with inhibition halos measuring up to 23 mm. For the minimum inhibitory concentrations (MIC), all the extracts tested showed significant activity. Regarding the disinfection of the surfaces, all the tested extracts presented positive results against *L. monocytogenes* on the four surfaces tested. Besides that, extracts from all tested samples and extractions showed considerable allelopathic activity, which suggests that leaf extracts can be tested as bioherbicides. The results highlight the significance of this plant, native in the South of Brazil.

Conflicts of interest

The authors declare that they have no conflict of interest.

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