Extracts of branches and fruits of *Ficus auriculata* Lour: antioxidant, antimicrobial and phytotoxic activities

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Summary

The present study aimed to obtain the phenolic compounds from *Ficus auriculata* Lour fruits and branches and to determine the antioxidant and antimicrobial activities and phytotoxicity. For this purpose, five extraction methodologies were used, containing water and ethanol as solvents, low-frequency ultrasound and a cellulases complex. The antioxidant activity was determined by eliminating the DPPH radical. Antimicrobial activity was determined against Gram-positive and Gram-negative bacteria, through disk diffusion and minimum inhibitory concentration. The extracts were also used for tests of phytotoxicity in cucumber (*Cucumis sativus* L.). The contents of total phenolic compounds showed up to 16.16 mg GAE·g^{-1} and 22.56 GAE·g^{-1} for the extracts obtained from the branches and by aqueous and enzymatic extraction. The highest antioxidant activity was found in the extract of the branches obtained with the mixture of ethanol and water and the use of ultrasound, with an IC_{50} value of 190.57 μg.mL^{-1}. Extracts from branches and fruits showed antimicrobial activity against all tested microorganisms, with inhibition halos of up to 17 mm and minimum inhibitory concentrations of 25.5 μg.mL^{-1} to 122.25 μg.mL^{-1}. The extracts showed phytotoxicity against cucumber seeds, with a 30% reduction in growth compared to the control. The extracts from the branches and fruits of *F. auriculata* have biological potential in relation to the tested activities and the difference in these was demonstrated according to the form of extraction.

Key words

phenolic compounds, ethanol, ultrasound, celluloses, water

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Received: February 13, 2020 | Accepted: June 15, 2020

Introduction

Plants of the genus *Ficus* (Moraceae), known as fig trees, grow in tropical and subtropical regions. In Brazil, approximately 58 species are recognized, among them, the *Ficus auriculata* Lour, which is a dioecious tree, with a height of six to nine meters. This tree originates from Asian countries (Chaudhary et al., 2012), and is widely used for shading.

In several species of *Ficus*, phytochemical investigations revealed the presence of phenolic compounds, such as triterpenes, flavonoids and anthocyanins, responsible for beneficial activities against helminths and bacteria and recommended in cases of inflammation and gastric disease (Abdel-Hameed, 2009). Additionally, studies have reported the existence of antioxidant and antimicrobial activity in *Ficus benghalensis* L., *Ficus carica* L., *Ficus microcarpa* L.f., *Ficus racemosa* L., *Ficus drupacea* L. and *F. auriculata* Lour. (Shi et al., 2011; Thingbaijam et al., 2012; Yessoufou et al., 2015).

There are few studies on the benefits of *F. auriculat*a to human health due to its phytochemical compounds. Despite the intensive use of fruits and leaves by the Asian population in the treatment of diarrhoea, cuts, cholera and verminous, no one, to the best of our knowledge, has studied the properties of the branches. Moreover, although *F. auriculata* is an exotic tree, it grows easily in Brazil but has not been widely used for nutritional purposes or scientific research (Yessoufou et al., 2015). The benefits mentioned above can be obtained naturally through alimentation, as a nutritional supplement or as medicine. It can also be used to extend the shelf life of foods and cosmetics, as phytotherapics against diseases caused by oxidative stress, or in the formulation of new drugs (Abdel-Hameed, 2009).

Besides the health benefits, some chemical compounds produced by the secondary metabolism of plants (terpenoids, alkaloids, phenols, flavonoids, tannins, and coumarins) have phytotoxic activity. In other words, they can modify the growth of other plants, and can therefore potentially be used in the control of weeds in agriculture as a less aggressive herbicide, thereby reducing environmental contamination (Gatti et al., 2004).

In order to obtain these compounds, it is necessary to extract the phenolic compounds from the vegetal tissues. Extraction is an important step in phytochemical processing for the discovery of bioactive components of plant materials. The selection of a suitable extraction technique is also important for the quality control of herbal products (Dhanani et al., 2017). The use of non-conventional technologies, such as ultrasound, the use of enzymes and the use of harmless solvents, has advantages such as the reduction of time and energy spent during the process (Barba et al., 2016).

In this context, the objective of this study was to obtain the fruit and branch extracts of *F. auriculata* using different methods of extraction and to quantify the phenolic compounds content, as well as to determine the antioxidant and antimicrobial activity, and to assess the phytotoxic effect of the extracts.

Material and methods

The branches and fruits of *F. auriculata* were collected in an urban area of the municipality of Pinhalzinho - SC - Brazil (Latitude: 26° 50' 53" S, Longitude: 52° 59' 31" W, elevation: 515 m) in January and February 2016. Only ripe fruits were harvested. For branches, those with the same diameter (1 ± 0.1 cm) and similar visual characteristics were selected. The samples (approximately 1 kg each) were placed in plastic packaging and immediately taken to the Bioprocess Laboratory, at the State University of Santa Catarina, Pinhalzinho Campus, located 3 km from the collection site. The fruits and branches were washed with distilled water and dried in a hot air circulating oven at 45°C for 72 h. Afterwards, they were ground, sieved (8 Mesh) and stored at 8°C in the dark (Barba et al., 2016) for up to 48 h.

Obtaining extracts of branches and fruits

Five methods were used to obtain the extracts, while always keeping the samples protected from light to avoid degradation. The first method (B-EW- branch / F-EW- fruit) consisted of mixing 0.5 g of the sample in 40 mL of ethanol/water (50:50, $v \cdot v^{-1}$). After this, the mixture was left to stand for 2 h. The second method (B-EWU- branch / F-EWU- fruit) used a solution with the same proportion of sample and ethanol/water as the first method, but the mixture was left to stand for 1 h, and was subsequently subjected to a further one-hour ultrasonic bath (135W, 40 KHz). For the third method (B-EWC- branch / F-EWC- fruit), 0.5 g of each sample was mixed with a 40 ml ethanol/water solution and was left to stand for 1 h. Then, 20 µL of the cellulase complex (NS 22086 - Novozymes - activity of 1000 BHU (2).g^{-1}) was added and the mixture was left to stand for one more hour at 45°C and 100 rpm. In the fourth method (B-W- branch / F-W- fruit), 0.5 g of each sample was mixed with 40 mL of distilled water and left to stand for 2 h. The fifth method (B-WC- branch /F-WC- fruit) consisted of mixing 0.5 g of the samples and 40 mL of distilled water. The mixture stood for 1 h and then 20 µL of the cellulase complex was added and left for another 1 h at 45°C and 100 rpm. All extracts were filtered with Whatman No. 40 filter paper and stored in polypropylene tubes in the dark (Barba et al., 2016).

Determination of total phenolic compounds

Total phenolic compounds were determined by spectrophotometry using the Folin-Ciocalteau reagent (Shi et al., 2011). The amount of 0.1 mL of each extract was diluted with 6 mL of distilled water and 0.5 mL of Folin-Ciocalteau reagent and 2 mL of a Na_2CO_3 solution (99.5%) were added. The mixture was shaken manually for 30 s and incubated for 2 h at 25°C in the dark. The absorbance was read at 720 nm and the results were expressed in mg of gallic acid equivalent (mg GAE) per gram (g) of sample (dry basis). The calibration curve was obtained with different concentrations of gallic acid (25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275 and 300 $\mu g \cdot mL^{-1}$).

Antioxidant activity (effect of elimination of DPPH radical)

The antioxidant activity was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl radical) method, based on the methodology of Oldoni et al. (2016). The amount of 0.3 mL of the extract was mixed with 2.7 mL of DPPH (40 $\mu g \cdot mL^{-1}$) methanolic solution and incubated for 1 h in the absence of light at 25°C. The absorbance was measured in duplicate at 517 nm after 1, 5 and 10

min, and then every 10 min up to 60 min for each of the extracts. As reference standards (blank), nothing but methanol was added to the plant extracts at the same sample concentrations. The greater the consumption of DPPH by the sample over time, the greater its antioxidant activity. For the inhibitory concentration, IC_{50}, 50 µL of various concentrations of extracts were added to 5 mL of a solution of DPPH (in methanol). These were incubated at room temperature for 30 min, followed by an absorbance reading and a comparison to blank at 517 nm.

Antimicrobial activity by disc diffusion methods and minimal inhibitory concentration (MIC)

The antimicrobial activity of the extracts was evaluated by the disc diffusion method and the minimum inhibitory concentration (MIC) determination in accordance with the method developed by Ostrosky et al. (2008). The tests were performed against Gram-positive bacteria: *Staphylococcus aureus* Rosenbach 1884 and *Listeria monocytogenes* (E. Murray et al. 1926) Pirie, 1940 and Gram-negative bacteria: *Escherichia coli* (Migula, 1895) Castellani and Chalmers, 1919 and *Salmonella enterica* serovar Enteritidis. The cultures were recovered in BHI (Brain Heart Infusion), incubated at 36°C and kept at the concentration of 0.5 according to the Mc Farland scale (equivalent to 10^8 CFU·mL^{-1}) and diluted in peptone casein water to the concentration of 10^5 CFU·mL^{-1}.

The microorganisms were inoculated onto plates containing Müller-Hinton agar. Then, three 6 mm diameter Whatman sterile filter paper disks were added to each plate and to them 15 µL of each plant extract was added. Sterile distilled water was used for the negative control. Plates were incubated at 35 ± 1°C for 24 h. The diameters of inhibition halos were measured with a pachymeter and the result was expressed in millimetres (mm). The greater the inhibition halo, the greater the antimicrobial activity of the extract. All assays were performed in triplicate.

The extracts that showed antimicrobial activity by the disc diffusion technique were submitted to MIC in sterile 96-well plates. Serial dilutions were performed at BHI and 100 µL of the bacterial suspension in BHI was added to each well. Additionally, positive control wells (containing BHI and bacterial suspension) and negative control wells (containing BHI and 200 µL of extracts without the bacterial suspension) were prepared. The plates were incubated at 37°C for 18 h. After this, 10 µL of 3% resazurin solution was added and the mixture was kept at 37°C for 2 h. Values below 100 µg.mL^{-1} are considered significant, values between 100 and 625 µg.mL^{-1} are considered moderate and values greater than 625 µg.mL^{-1} are considered low (Kuete, 2010; Tchinda et al., 2017).

Phytotoxic activity of the extracts of the branches and fruits of *Ficus auriculata*

The phytotoxic activity was determined using cucumber (*Cucumis sativus* L.). Firstly, the seeds were prepared in plastic cups and 26.1 g of commercial substrate (Sphagno peat moss, expanded vermiculite, dolomitic limestone, agricultural gypsum and NPK fertilizer; pH of 5.5 ± 0.5; electrical conductivity of 0.7 ± 0.3 mS cm^{-1}; density of 145 kg m^{-3}; water-retention capacity of 55% and moisture of 50%) was added to each beaker, simulating a conventional plantation. Thirty-three plants were used, including the triplicate for each of the extracts and the triplicate of the control (water only applied). The application of extracts and water began seven days after sowing and continued for seven days, always at the same time of day. For each application, a manual sprayer containing 5 ml of each extract diluted in 15 ml of water was used, that is, 20 ml per application. After seven days of application, the evaluation of the plants was carried out by measuring their height (cm) and visual characteristics. These were assessed according to the scale of the European Weed Research Community (EWRC), which consists of the visual evaluation index and its description of plant phytointoxication, as follows: 1 = no damage; 2 = minor changes (discoloration, deformation); 3 = small changes in many plants, such as chlorosis and shading; 4 = strong discoloration or reasonable deformation and no necrosis; 5 = necrosis of some leaves, accompanied by deformation in leaves and shoots; 6 = reduction in plant size, leaf shading and necrosis; 7 = more than 80% of the leaves destroyed; 8 = extremely severe damage, leaving small green areas on the plants; 9 = plant death (EWRC, 1964).

Statistical analysis

For all experiments, the results were expressed as the mean ± standard deviation. The statistical significance ($P < 0.05$) was obtained by the Tukey test using Statistica® 13.3 software.

Results

Total phenolic compounds of the extracts of the branches and fruits of *Ficus auriculat*a Lour.

Figure 1 shows the results of total phenolic compounds of the extracts of the branches and fruits of *F. auriculata*. It was verified that for the extracts of the branches, the highest contents of phenolic compounds were B-EW (16.16 ± 3.88 mg GAE·g^{-1}), B-EWU (13.87 ± 2.02 mg GAE·g^{-1}) and B-EWC (8.93 ± 4.08 mg GAE·g^{-1}), and the results showed no statistically significant difference ($P > 0.05$). The difference between the phenolic compounds content for the fruit extracts F-EWU (19.56 ± 0.20 mg GAE·g^{-1}) and F-EWC (22.56 ± 2.75 mg GAE. g^{-1}), was not statistically significant ($P > 0.05$). The results showed that the ethanol/water mixture led to higher extractions compared to the extraction methods using only water and that for the fruits, and the use of the enzymatic complex (F-EWC) potentiated the extraction of phenolic compounds.

Antioxidant activity of the extracts against the radical DPPH•

The results of antioxidant activity against the DPPH radical are shown in Table 1. The extractive method that produced extracts with lower activity was B-WC/F-WC (water and enzymatic complex) as the higher values indicate lower antioxidant activity. For the extracts of the branches, the best result was for the B-EWU method, followed by B-EWC, B-EW, B-W and B-WC. The differences between all the means were statistically significant ($P < 0.05$). For the extracts obtained from the fruits, the highest antioxidant activities were for F-EW and F-EWU, showing no statistically significant difference ($P > 0.05$), followed by extracts obtained in F-EWC, F-W, F-WC, for which the difference between the means was statistically significant ($P < 0.05$). The highest antioxidant activity was found in extracts obtained by ethanol/water and the use of ultrasound (B-EWU).

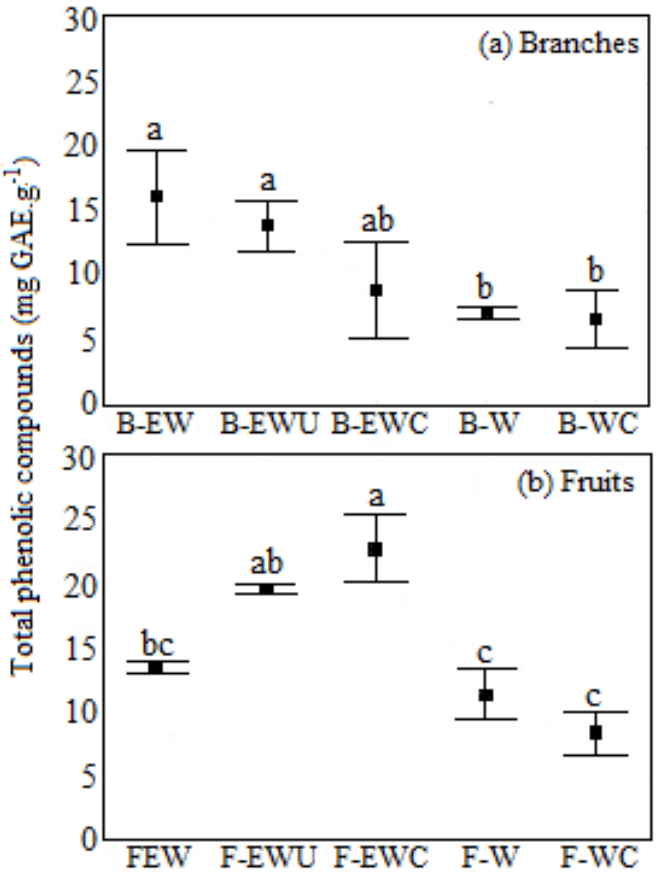

Figure 1. Total phenolic compounds of the extracts of the branches (a) and fruits (b) of *Ficus auriculata* Lour. obtained by five different methods. Values of means ± standard deviation. Equivalent means with different lowercase letters represent statistically different means ($P < 0.05$) for each fraction of the plant by the Tukey test

Table 1. The IC_{50} values for the DPPH radical elimination assays using the extracts of the branches and fruits of *Ficus auriculata* Lour

Extraction Method	IC_{50} values for the DPPH radical elimination assay ($\mu g.mL^{-1}$)	
	Branches	Fruits
B-EW/F-EW	220.39±3.32^{c}	302.77±2.48^{a}
B-EWU/F-EWU	190.57±4.25^{a}	296.05±5.69^{a}
B-EWC/F-EWC	203.99±2.89^{b}	332.56±8.54^{b}
B-W/F-W	281.04±1.26^{d}	364.78± 6.39^{c}
B-WC/F-WC	317.76±3.42^{e}	423.64±4.67^{d}
Gallic Acid*	21.66±0.19	

Note: * gallic acid used as a standard as reference; IC_{50} is the concentration of antioxidant (extract) required to obtain 50% of the neutralizing capacity of the radical; the different lowercase letters in the column are significantly different ($P < 0.05$), by the Tukey test; B = branch, F = fruits, E = ethanol, W = water, U = ultrasound and C = cellulase complex

Antimicrobial activity against microorganisms of importance in food

The extracts were tested for their activity against pathogenic bacteria. Table 2 shows the means of the diameters of inhibition halos. The extracts active against *E. coli* were B-EW, B-W and F-EW and the partially active extracts were B-WC and F-EWU. Only B-EWC was active against *S.* Enteritidis and B-EW, B-EWU and F-EWU were partially active. With respect to the *S. aureus*, the active extract was B-EWC and the partially active ones were B-EW, B-W, F-EW and F-EWU. For *L. monocytogenes*, the active extracts were F-EWC and F-W and the partially active ones were B-EWU, B-EWC, B-W and F-EW.

When it comes to the branches, B-W was active against *E. coli* and B-EWC was active against *S.* Enteretidis. For the fruits, the highest inhibition halo occurred for F-W against *L. monocytogenes*, classified as active. B-WC and F-WC showed the lowest activity against the bacteria and B-W and F-W led to the largest inhibition halos. Both the ethanol/water extracts and the water-only extracts showed antimicrobial activity.

The MIC results are shown in Table 3. The values of antimicrobial activity ranged from 20.83 $\mu g.mL^{-1}$ (F-EW against *E. coli*) to 122.25 $\mu g.mL^{-1}$ (F-EWU for *S. aureus*). Most of the extracts can therefore be classified as having significant antimicrobial activity since 55% of the results were superior to 100 $\mu g.mL^{-1}$, 35% of the extracts showed no activity against the bacteria studied and 10% of the extracts showed moderate activity.

Phytotoxic activity of extracts of *Ficus auriculata* (herbicide potential)

The mean values of the growth of cucumber plants are shown in Table 4. The lower heights of the plants occurred in the extracts B-EW, F-EW, B-EWU, F-EWU, B-EWC and F-EWC, with a mean reduction of 30% in growth compared to the control, and showing a statistically significant difference ($P > 0.05$).

Figures 2 and 3 present the visual appearance of the cucumber plants sprinkled with the extracts. Based on the EWRC classification, the plants that were sprayed with the extracts B-EW/F-EW, B-EWU/F-EWU and B-EWC/F-EWC, for branches (Figures 2a, 2b and 2c) and for fruits (Figures 3a, 3b and 3c), were classified in index 9 as phytointoxication because they presented a reduction in size and damage to the roots, which resulted in the death of all plants, demonstrating that these extracts had bioherbicide potential. For the extracts obtained in B-W/F-W and B-WC/F-WC, the branches (Figures 2d and 2e) and fruits (Figures 3d and 3e) showed no damage to the plants and could be classified in index 1.

Table 2. The antimicrobial activity by disc diffusion method (halo in mm) of the extracts of the branches and fruits of *Ficus auriculata* Lour

Extraction Method	Gram-negative bacteria		Gram-positive bacteria	
	Escherichia coli	*Salmonella* Enteritidis	*Staphylococcus aureus*	*Listeria monocytogenes*
B-EW	14.33±4.93	13.33±1.15	13.33±1.15	NA
B-EWU	NA	10.33±1.53	9.00±0.00	11.00±0.00
B-EWC	NA	17.00±0.00	15.50±0.71	12.50±2.12
B-W	17.00±0.00	NA	11.00±0.00	12.00±0.00
B-WC	10.00±0.00	NA	NA	NA
F-EW	14.00±3.46	NA	10.50±0.71	11.00±7.07
F-EWU	11.00±0.00	11.50±3.51	12.00±3.54	NA
F-EWC	NA	NA	NA	14.00±0.00
F-W	NA	NA	NA	15.50±2.12
F-WC	NA	NA	NA	NA

Note: NA: extracts that did not present antimicrobial activity (no halo formation); B = branch, F = fruits, E = ethanol, W = water, U = ultrasound and C = cellulase complex

Table 3. The antimicrobial activity of extracts *Ficus auriculata* Lour. expressed as minimum inhibitory concentrations (in $\mu g.mL^{-1}$) of phenolic compounds

Extraction Method	Gram-negative bacteria		Gram-positive bacteria	
	Escherichia coli	*Salmonella* Enteritidis	*Staphylococcus aureus*	*Listeria monocytogenes*
B-EW	25.50	50.50	50.50	ND
B-EWU	ND	43.36	ND	86.72
B-EWC	ND	55.82	55.82	111.65
B-W	NA	ND	NA	NA
B-WC	NA	ND	ND	ND
F-EW	20.83	ND	41.66	41.66
F-EWU	NA	61.12	122.25	ND
F-EWC	ND	ND	ND	NA
F-W	ND	ND	ND	NA
F-WC	ND	ND	ND	ND

Note: MIC determination was only performed for extracts showing antimicrobial activity using the disc diffusion method. ND: the antimicrobial activity was not determined because it did not present inhibition halo. NA: extracts that did not present antimicrobial activity by the MIC. B = branch, F = fruits, E = ethanol, W = water, U = ultrasound and C = cellulase complex

Table 4. Height of *Cucumis sativus* plants using *Ficus auriculata* Lour extracts as a bioherbicide

Extraction Method	Plant growing (cm)	
	Branches	Fruits
B-EW/F-EW	2.7±0.7 ^{cA}	2.5±0.4 ^{bA}
B-EWU/F-EWU	2.5 ±0.7 ^{cA}	2.4±0.8 ^{bA}
B-EWC/F-EWC	2.3±0.3 ^{cA}	2.5±0.5 ^{bA}
B-W/F-W	7.3±0.7 ^{bB}	7.0±0.6 ^{aB}
B-WC/F-WC	7.0±1.2^{abB}	7.7±0.9 ^{Ab}
Blank	9.0±0.6 ^{a}	

Note: the different lowercase letters in the column are significantly different for the extracts and the different capital letters in the column are different for the fractions of *F. auriculata* Lour (P <0.05) by the Tukey test; B = branch, F = fruits, E = ethanol, W = water, U = ultrasound and C = cellulase complex

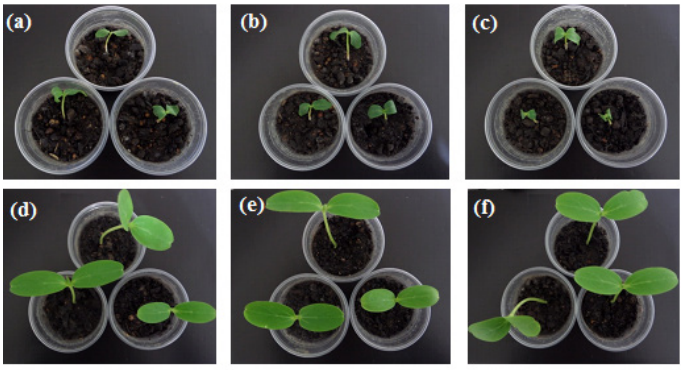

Figure 2. Phytotoxicity assays of B-EW (a), B-EWU (b), B-EWC (c), B-W (d) and B-WC (e) extracts of *Ficus auriculata* Lour branches and control (f) in *Cucumis sativus* L.

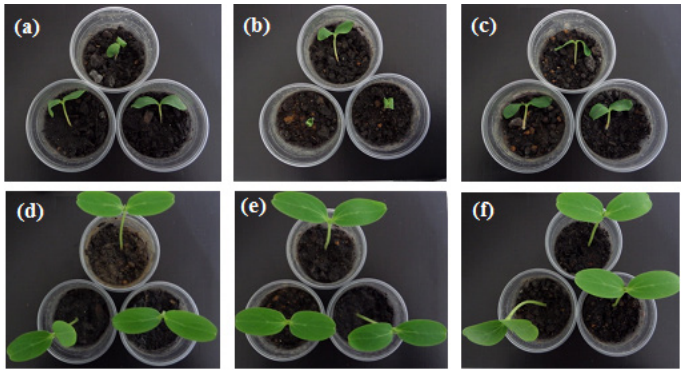

Figure 3. Phytotoxicity assays of F-EW (a) F-EWU (b) F-EWC (c) F-W (d) and F-WC (e) extracts of *Ficus auriculata* Lour fruits and control (f) in *Cucumis sativus* L.

Discussion

The higher contents of phenolic compounds were obtained using the ethanol/water combination as a solvent when compared to the use of water only. Several factors make the ethanol/water combination more efficient in extracting the compounds compared to pure water. Water and ethanol have a polarity difference, and when mixed, a change occurs in the dielectric constant of the water, increasing the extraction capacity. The ethanol is less polar than water and its characteristics lead to the extraction of polyphenols with lower polarities, whereas the water extracts the more polar polyphenols, broadening the range of extracted compounds (Bodoira et al., 2017). Furthermore, differences in the chemical structure of phenolic compounds also play an important role with respect to their solubility in solvents of different polarity. Therefore, the type of extraction solvent may have a significant impact on the yield of extracting polyphenols from plant tissues, as was the case in the present study (Złotek et al., 2016).

When the enzymatic complex was used, the content of phenolic compounds, mainly for fruits (F-EWC), was higher. The cellulase, xylanase and glycosyl hydrolases, which comprise the complex used, promote the partial hydrolysis of the polysaccharides that structure the cell wall, increasing its permeability and the consequent diffusion of the compounds, which explains the content of phenolic compounds obtained for F-EWC. According to Manian et al. (2008), the main contributors of the phenolic compounds present in *Ficus* spp. may be derivatives of pelargonidin, flavonoids and high molecular mass tannins such as quercetin, myricetin, kaempferol, gallic and ellagic acids.

Both the shells and fruits of the *Ficus* species have oxalic acid, citric acid, malic acid, shikimic and fumaric acid. However, the fruits contain a greater amount of chlorogenic acid and psoralen, while the shells have a higher content of quercetin 3-O-rutinoside, which means that the total phenolic content for each of the plant materials can be formed by different compounds and in different amounts (Sirisha et al., 2010).

For the antioxidant activity, the extracts obtained with the ethanol/water combination and ultrasound presented the best results. In addition to the extraction characteristics already mentioned for the ethanol/water combination, the use of ultrasound enhances the extraction as it modifies the physical and chemical properties of the materials to be hydrolyzed. This is due to the propagation and interaction of sound waves that disturb the cell wall of the plant, facilitating the release of extractable compounds, increasing the solvent transport from the continuous phase to the plant cells (Dhanani et al., 2017).

The results for the antioxidant activity were similar to those obtained by Shi et al. (2011). The authors determined the antioxidant activity of dry leaves ethanolic extracts of seven species of *Ficus* (*F. virens* var. *sublanceolata* (Miq.) Corner, *F. auriculata*, *F. vasculosa* Wall. ex. Miq., *F. callosa* Wild., *F. virens* Verins, *F. racemosa* L. and *F. oligodon* Miq.). The IC_{50} values for the extracts of *F. auriculata* of 0.29 mg·mL^{-1} (290 µg·mL^{-1}) were lower than those found for B-EW, B-EWU, B-EWC and B-W and close to the values of F-EW and F-EWU.

The biologically active compounds generally occur at very low concentrations in plants. The extraction yield and extract quality may be affected by the extraction methods, phytochemical nature, particle size, composition, solvent nature and presence of interfering substances (Nile et al., 2017). Therefore, there may be a difference between extracts with higher content of phenolic compounds and extracts with higher antioxidant activity, according to the extraction method used, as was the case in the present study.

The antioxidant activity or elimination activity of free radicals may be related to the nature of the phenolic compounds that contributes to electron transfer and the ability to release hydrogen. Also, some extracts have the capacity to neutralize hydroxyl radicals, and may be directly related to the prevention of the propagation of the lipid peroxidation process. They may also be linked to the scanning function of active oxygen species, reducing the reaction rates in the chain, as was the case in the study conducted by Manian et al. (2008) with extracts of *F. bengalensis*, *F. racemosa* and green tea (*Camellia sinensis* (L) O. Kuntz). The greater the molecular mass of phenolic compounds, the greater the ability to neutralize free radicals, which is, for instance, the characteristic of the ellagic acid (molecular weight of 302.197 $g.mol^{-1}$) present in the *Ficus* species.

In the study of antimicrobial activity, 55% of the extracts showed significant activity and these results can be attributed to the compounds that are present in *Ficus* species, such as phenolic acids, tannins, coumarins, flavonoids and isoflavonoids, which are known for their antimicrobial properties (Sagar et al., 2012). The effect of phenolic compounds on bacterial growth depends on the dose tested and on the cell structure of the microorganism, especially the cell membrane. Phenolic compounds have the ability to bind to bacterial cell membranes, and some may interact with lipids and proteins, altering membrane permeability (Pacheco-Ordaz et al., 2018).

When the MIC was determined against Gram-positive and Gram-negative bacteria, B-EW/F-EW, B-EWU/F-EWU, B-EWC/F-EWC, i.e. the methods containing the ethanol-water mixture, showed a greater effect against the bacteria studied. The highest values of phenolic compounds was obtained in these extracts. The phenols may be in the form of phenolic acids, tannins, flavonoids and isoflavonoids, which are commonly present in *Ficus* species and have activity against pathogenic bacteria (Sagar et al., 2012).

The extracts used in *E. coli* and *S.* Enteretidis showed the lowest MIC values, which means that a smaller amount of extract is able to inhibit the growth of these bacteria which are classified as Gram-negative. Gram-positive bacteria are more resistant than Gram-negative when treated with extracts of *F. auriculata*. There are some bioactive compounds that have the ability to interact with the cell wall and plasma membrane and cause their rupture, such as isoflavonoids, terpenoids and ellagic acids, inhibiting bacterial growth (Kuete et al., 2009). Variation in MIC results of plant extracts against bacteria may be related to the concentration of active principles present in each plant species, the profile of the solvent employed and the type of bacterial strains tested, among other factors.

Similarly, Gaire et al. (2011) evaluated the antibacterial activity of extracts of the *F. auriculata* shell and found inhibition halos with an extract obtained using *n*-hexane as solvent, 3.20 ± 0.09 mm against *E. coli* and 7.80 ± 0.36 mm against *S. aureus*. In extracts using chloroform, the authors obtained 1.40 ± 0.03 for *E. coli* and 7.30 ± 0.21 mm for *S. aureus*, while for methanolic extracts the results were 4.5 ± 0.15 mm and 6.9 ± 0.03 mm for *E. coli* and *S. aureus*, respectively. These are lower results than those found in this study.

The extracts of *F. auriculata* presented phytotoxic potential in the tested plant, which may be related to the higher content of phenolic compounds found in the extracts B-EW/F-EW, B-EWU/F-EWU and B-EWC/F-EWC. The B-W/F-W and B-WC/F-WC extracts from the fruits showed lower amounts of these compounds and, consequently, showed a low phytotoxicity potential. Some phenolic compounds that have allelopathic activities are derivatives of benzoic acid, cinnamic acid, flavonoids, tannins and coumarins (Ladhari et al., 2013).

The inhibitory effect may have occurred through a variety of mechanisms such as reduction of mitotic activity in the roots, reduced rate of ion absorption, inhibition of photosynthetic respiration and enzymatic action. It may also have occurred because the roots are generally the first to absorb phytotoxins from the environment (Ladhari et al., 2013). Studies have shown that any plant tissue can contribute to phytotoxic effects, depending on the number of secondary metabolites produced by plants, as well as on the culture conditions and the species (Gatti et al., 2004). Still, the decrease in growth may be a consequence of the early inhibition of photosynthetic efficiency (Puig et al., 2018). Allelopathic inhibitions are the result of more than one chemical agent (phenols, terpenes, alkaloids, fatty acids, peptides, cyanogenic glycosides, saponins and tannins), and will depend on their concentration, composition and interaction.

Puig et al. (2018) studied the herbicidal potential of *Eucalyptus globulus* Labill aqueous extracts against the species of *Lactuca sativa* and *Agrostis stolonifera*, and concluded that the spray treatment of the plants with the extract reduced the aerial and root biomass of the adult lettuce plants. By HPLC analysis, a total of eight phenolic compounds (chlorogenic, two derivatives of p-cumparic acid, ellagic acid, hyperoside, rutin, quercitrin and kaempferol) and five other low molecular weight organic acids (citric, malic, chiquinic, succinic and fumaric) in *E. globulus* extracts were detected, which are also found in extracts of *F. auriculata*.

Conclusion

The extracts of the branches and fruits of *Ficus auriculata* Lour. presented a high content of phenolic compounds and presented antioxidant activity against the radical DPPH. The extracts presented antimicrobial activity against Gram-negative and Gram-positive bacteria of importance in the food industry. As for the phytotoxic potential, the extracts presented allelopathic effects in cucumber plants, indicating the possibility of use as a bioherbicide. With respect to the extraction methods, there were differences between the activities, with more satisfactory responses obtained by using the ethanol/water and enzymatic complexes for the extraction of the compounds. The extracts of *Ficus auriculata* Lour can be used in processes that require antioxidant, antimicrobial and phytotoxic activities.

Acknowledgments

The work described in this article was supported by the Biolab - UDESC. This study was financed in part by the *Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina* (FAPESC) and *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) - finance code 001. This study is registered in the *Sistema Nacional de Patrimônio Genético e Conhecimento Tradicional Associado* (AFF9195).

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