Extracts of Leaves of *Ficus auriculata* Lour.: Antioxidant, Antimicrobial and Phytotoxic Activity

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Summary

Ficus auriculata Lour. is a tree of the genus *Ficus*. The aim of this study was to obtain the total phenolic compounds; and to determine the antioxidant (DPPH), antimicrobial (diffusion disc and Minimal Inhibitory Concentration) and phytotoxic activity (in cucumber - *Cucumis sativus* L.) of the extracts. Five extraction methods were used to obtain extracts of young and mature leaves of *F. auriculata*: water/ethanol (M1), water/ethanol/ultrasound (M2), water/ethanol/cellulase complex (M3), water (M4) and water/cellulase complex (M5). The phenolic content for extracts of young leaves was of 30.22 ± 2.99 mg GAE.g^{-1} dry sample (M1), 35.22 ± 0.53 mg GAE.g^{-1} dry sample (M2) and 28.90 ± 0.57 mg GAE.g^{-1} dry sample (M3) and for the mature leaves of 24.42 ± 0.04 mg GAE.g^{-1} dry sample (M2) and 17.13 ± 4.69 mg GAE.g^{-1} dry sample (M1). For the antioxidant activity, the lowest values of IC_{50} (or higher antioxidant activity) occurred for the extracts of young and mature leaves obtained by the M2. The extracts were able to inhibit all the evaluated bacteria, presenting MICs in the range of 21.60–90.32 μg.ml^{-1} for the *Escherichia coli*, 21.60-188.85 μg.ml^{-1} for the *Salmonella enteritidis*, 64.22-188.85 μg.ml^{-1} for the *Staphylococcus aureus* and 76.31-87.82 μg.ml^{-1} for the *Listeria monocytogenes*. The extracts of young and adult leaves presented herbicide potential, occurring suppression of the growth of cucumber plants. The use of the extract obtained in M3 led to the death of the plants. Extracts of *F. auriculata* presented antioxidant and antimicrobial activities, which agree with previous studies linking phenolic compounds to these properties.

Key words

ethanol/water; ultrasound; cellulase complex; extraction; phenolic compounds

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Introduction

Ficus L., with about 750 species, is the richest genus of the family Moraceae, with wide distribution in tropical and subtropical regions of the world (Araújo et al., 2014). *Ficus auriculata* Lour. (popularly known in Brazil as Figueira-de-jardim) is widely distributed in temperate, tropical and subtropical regions, and it is tree of about 4 - 10 m tall and dioecious in nature. It contains abundant amount of white latex in every part of the plant (Gaire et al., 2011). The leaves of *F. auriculata* are edible and also protect against the formation of excessive peroxide levels in palm oil when used during its preparation, presumably due to the its antioxidant properties (Lansky et al., 2008; Shi et al., 2011).

Researchers report the contemporary ethnopharmacological uses of *Ficus* species against cancer and inflammation due to potential antioxidant and antimicrobial activity. Species including *F. benghalensis* L., *F. carica* L., *F. microcarpa* L. and *F. racemosa* L. have been reported. However, the antioxidant and antimicrobial activity, as well as other properties, of most species belonging to genus *Ficus*, as *F. auriculata*, have remain unexamined or lack extensive documentation (Gaire et al., 2011; Shi et al., 2011). Preliminary phytochemical screening of the plant leaf extract revealed the presence of alkaloids, glycosides, flavonoids, terpenoids, tannins and reducing sugar but saponin was found absent in the leaf of *Ficus auriculata* (Thingbaijam et al., 2012).

In developing countries, the use of pesticides has dangerous effects on people and their environment. Scientists have focused on searching for plant compounds to develop bio-pesticides as alternative. The allelopathic potential of plants may develop a healthy and sustainable agriculture by reducing the use of synthetic pesticides. Compounds as phenols, flavonoids, tannins, coumarins, terpenoids, alkaloids and polyacetylenes can provide excellent inhibition of weed seed germination, insect, bacterial and fungal influences (Hamdi et al., 2017). There are no studies of the phytotoxic activity of *F. auriculata*, however, as it contains these compounds, the *F. auriculata* can present phytotoxic activity and being able to be used to obtain a biopesticide.

For the extraction of compounds that may present antioxidant, antimicrobial and phytotoxic activities several techniques may be employed, from traditional techniques to new extraction techniques, many of which are considered eco-friendly. In recent years, several novel techniques, such as enzymatic extraction and ultrasonic-assisted extraction have been used for extraction of phenolic compounds from plants instead of conventional technique (Deng et al., 2017). Obviously, the method selected for the extraction affects both the yield and the chemical composition of the extract, and hence, its bioactivity (Sánchez-Gómez et al., 2017).

Extraction procedures using water as solvent meet the requirements of the so-called "green chemistry" (Sánchez-Gómez et al., 2017). Ultrasonic-assisted extraction has become more and more popular because it is a simple and eco-friendly method. This method utilizes acoustic cavitation to disrupt plant tissues and increase mass transfer, obtaining benefits like higher efficiency, shorter extraction time and less power consumption than the conventional extraction techniques (Deng et al., 2017). Enzymes are one of the common biocatalysts and have been widely utilized to improve the extraction yields of targeted compounds from various sources. Besides, enzyme assisted extraction is considered as one of the green processes, environment friendly and require less energy (Bhotmange et al., 2017).

Therefore, the purposes of this study are to compare the efficiency of methods of extraction using ethanol/water, water, cellulase complex and ultrasound in the obtaining of phenolic compounds of the young and mature leaves of *F. auriculata* and to determine the antioxidant, antimicrobial and phytotoxic activities by *in vitro* assays.

Materials and methods

The leaves of *F. auriculata* (young and mature) were collected in January and February 2016 from Pinhalzinho, Santa Catarina, Brazil (south region, latitude: 26°50'53"S, longitude: 52°59'31"W, altitude: 515 m, season of the year: summer). Leaves were washed with distilled water and dried in an oven at 45°C for 72 h. The leaves were powdered and sieved (apertures of 8 Mesh). The samples were stored under refrigeration (8°C) in the absence of light (Barba et al., 2016).

Preparation of extracts

Five methods were used to obtain the extracts of the leaves of *F. auriculata*, with samples always protected from light. Method 1 (M1): 0.5 g of leaf sample and 40 ml of ethanol-water solution (50:50, $v.v^{-1}$) were mixed. The mixture was allowed to stand for 2 h. Method 2 (M2): 0.5 g of leaf sample and 40 ml of ethanol-water solution (50:50, $v.v^{-1}$) were mixed. The mixture was allowed to stand for 1 h, and after, left over 1 h in the ultrasound bath (frequency of 50/60 Hz and ultrasonic power of 135 Watts RMS). Method 3 (M3): 0.5 g of leaf sample and 40 ml of ethanol-water solution (50:50, $v.v^{-1}$) were mixed. The mixture was allowed to stand for 1 h. A cellulase complex, 20 µL (NS22086-Novozymes, activity 1,000 BHU(2).g^{-1}, optimum temperature of 45 – 50°C and optimum pH 5.0 – 5.5), was then added and the mixture was left for another hour in a bath with orbital stirring at 45°C and 100 rpm. Method 4 (M4): 0.5 g of leaf sample and 40 ml of distilled water were mixed. The mixture was allowed to stand for 2 h. Method 5 (M5): 0.5 g of leaf sample and 40 ml of distilled water were mixed. A cellulase complex, 20 µL (NS22086-Novozymes), was then added and the mixture was left for another hour in a bath with orbital stirring at 45 °C and 100 rpm. The extracts were filtered (Whatman n° 40 filter paper) and stored in tubes in the absence of light to avoid oxidation and degradation (Vatai et al., 2009; Paes et al., 2014; Barba et al., 2016).

Determination of total phenolic compounds

Extracts were evaluated for their total phenolic compounds with Folin-Ciocalteau reagent (Shi et al., 2011). A 0.1 ml aliquot of each extract was diluted with distilled water (6 ml). After, 0.5 ml of Folin-Ciocalteau reagent was introduced into the tube. After addition of 2 ml Na_2CO_3 and stirring for 30 s the mixture was incubated for 2 h in ambient temperature (25°C). The absorbance was measured at 720 nm. The results were expressed as mg of gallic acid equivalent per g of dry sample. The calibration curve was obtained from solutions with different concentrations of gallic acid (25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300 $\mu g.ml^{-1}$).

Antioxidant activity (scavenging effects on DPPH radical)

The DPPH radical scavenging activity of extracts was measured by the DPPH method described by Oldoni et al. (2016) and Shi et al. (2011), with slight modifications. The reaction medium consisted of 0.3 ml of the extract and 2.7 ml of DPPH solution in methanol (concentration 40 $\mu g.ml^{-1}$). The solution was mixed and incubated in the absence of light at room temperature. Subsequently, the absorbance was measured in 1 min, 5 min, 10 min and then every 10 min

until 60 min for each extracts, in duplicate, using a spectrophotometer at 517 nm. Gallic acid was used as positive control. To obtain the IC_{50} value, 50 µl of various concentrations of the extracts were added to 5 ml a DDPH solution (in methanol) 0.0004%. This was incubated at room temperature for 30 min after which absorbance was read against blank at 517 nm (Annan and Houghton, 2008).

Antimicrobial activity

The antimicrobial activity of the extracts, in different concentrations, 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 Gram-positive bacteria (laboratory stock) *Staphylococcus aureus*, *Listeria monocytogenes* and the Gram-negative bacteria (laboratory stock) *Escherichia coli* and *Salmonella enterica* serotype Enteritidis, pathogenic bacteria of importance in the food industry. 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.

Disc difusion

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

Minimum inhibitory concentration (MIC)

The extracts of the leaves of *F. auriculata* that presented antimicrobial activity through the disc diffusion, were submitted to determination of the MIC, in sterile 96-well plate. Serial dilutions (up to four times) of the samples of the extracts were performed in BHI to obtain the desired concentration range. Into each well 100 µl of a bacterial suspension in BHI was added. Positive control wells (containing BHI and the bacterial suspension) and negative control wells (containing the BHI and 200 µl of extract without the bacterial suspension) were also prepared. The plates were incubated at 37°C for 18 h 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 extract that inhibited the visible growth of the bacteria.

Phytotoxic activity

Phytotoxic activity study was performed using cucumber (*Cucumis sativus* L.) (Bastos et al., 2017). Seeding was carried out in plastic trays containing 15 cells, totaling 2.4 liters per tray. In each cell, 1 seed was seeded in 26.1 g of commercial substrate (peat, expanded vermiculite, dolomitic limestone, agricultural gypsum and traces of NPK fertilizer, pH 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 maximum humidity of 50%), simulating a conventional plantation. A total of 33 plants were used, with the triplicate for each of the extracts and the triplicate of control (water only applied). The initial application of extracts and water (control) was seven days after sowing (Zeynep et al., 2014) and continued for seven days, always at the same time of day (between 9:00 and 10:00 am). For each application, a manual sprayer containing 5 ml of each extract mixed with 15 ml of water was used, totaling 20 ml per application. On the 7th day of application, after 6 h of the last spray, the evaluation of the plants was performed by measuring the height (cm) and the visual characteristics, according to Index of evaluation and its description of plant phytotoxification of EWRC (1964): 1 – No damage; 2 – Small changes (discoloration, deformation) visible in some plants; 3 – Small changes (chlorosis and wrinkling) visible in many plants; 4 – Strong discoloration or reasonable deformation, without necrosis; 5 – Necrosis of some leaves, accompanied by deformation in leaves and shoots; 6 – Reduction in size of the plants, wrinkling and necrosis of leaves; 7 – More than 80% of damaged leaves; 8 – Extremely serious damage, leaving small green areas on the plants, and 9 – Death of the plant.

Statistical analysis

Assays were performed in triplicate and the results were expressed as average with standard deviation. The significance ($p < 0.05$) was obtained by the ANOVA test and Tukey test by using Statistica® 10.0 software (StatSoft, 2010).

Results and discussion

Phenolic compounds

Phenolic compounds are having wide bioactivity including antioxidant properties/activity. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties (Thingbaijam et al., 2012). The results of phenolic compounds in the extracts of the young and mature leaves of *F. auriculata*, obtained by different methods, are shown in Figure 1.

The contents of phenolic compounds in extracts of young leaves obtained by the extraction methods M1 (30.22 ± 2.99 mgGAE.g^{-1} dry sample), M2 (35.22 ± 0.53 mgGAE.g^{-1} dry sample) and M3 (28.90 ± 0.57 mgGAE.g^{-1} dry sample) were equal to each other ($p > 0.05$) and differed statistically ($p < 0.05$) from the content of

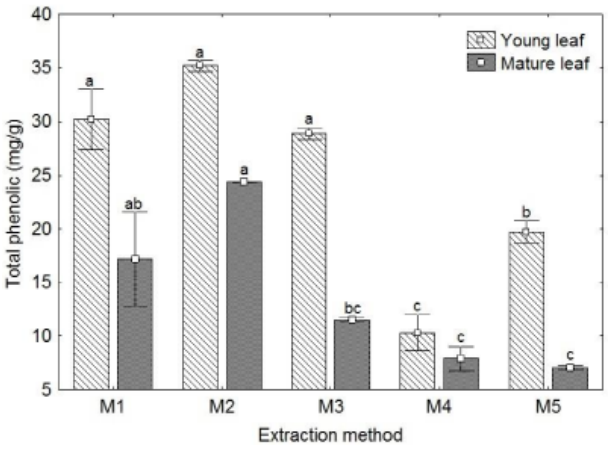

Figure 1. Levels of total phenolic compounds in young and mature leaves of *Ficus auriculata* of five extraction methods. Values are mean of duplicates determinations ± standard deviation. The equal bars with different lowercases are significantly different ($p < 0.05$). For total phenolic compounds, results are expressed as mg of gallic acid equivalent per g of dry sample

phenolic compounds obtained by M4 (10.27 ± 1.78 mgGAE.g^{-1} dry sample) and M5 (19.73 ± 1.17 mgGAE.g^{-1} dry sample). The phenolic content in extracts of mature leaves obtained by M2 (24.42 ± 0.04 mgGAE.g^{-1} dry sample) was statistically equal (p> 0.05) to the content obtained by M1 (17.13 ± 4.69 mgGAE.g^{-1} dry sample) and different from the others (M3= 11.50 ± 0.28 mgGAE.g^{-1} dry sample, M4= 7.87 ± 1.21 mgGAE.g^{-1} dry sample and M5 = 7.07 ± 0.16 mgGAE.g^{-1} dry sample). Additional research is necessary to determine the specific chemical constituents of the leaf extracts. Extraction starts when solvent molecules penetrate the plant matrices, causing the cytoplasm layer to be exposed directly to the solvent, bringing about the dissolution of the bioactive compounds. The mixture of ethanol and water at different concentrations is used as an extraction solvent and ethanol concentration affects the dielectric constant of the mixture hence altering the energy required to deteriorate the attraction of water molecules to the target molecules (Chiang et al., 2017). Ethanol-water mixtures (55-75% ethanol) are the most suitable solvents for simultaneous extraction of the most important phytochemicals (Zekovic et al., 2017).

Thingbaijam et al. (2012) determined the content of phenolic compounds of methanolic extracts of leaves of *F. auriculata*, obtained in 72 h of extraction and subjected to removal of the solvent in a rotary evaporator. The value obtained (21.404 mg GAE. g-1 dry sample) was similar to values of M5 (young leaf extract), M1 and M2 (mature leaf extracts), and lower than the highest value found in the present study (M1, M2 and M3 - young leaf extracts). Shi et al. (2011) determined the content of phenolic compounds and flavonoids of young leaves of seven *Ficus* species. The content of total phenolic compounds and flavonoids in the ethanolic extract of *F. auriculata* was approximately 9 mg.g^{-1} and 3 mg.g^{-1}, respectively. The difference of results for the other extracts can be justified by the maturity of the leaves, the drying and extraction methods as well, as the geographical origin of the samples.

The total phenolic content is significantly different among the three vegetal materials, following the order: leaves > peels > pulps. Leaves possess the strongest antioxidant potential and pulp the weakest one. These facts may be explained by the highest amounts of phenolic compounds occurring in leaves (Sirisha et al., 2010). Part of the chemical composition of *F. auriculata* is kaempeferol, quercetin, myricetin, betulinic acid, lupeol, stigmasterol, bergapten, scopoletin, ß-sitosterol-3-O-ß-D-glucopyranoside, myricetin and quercetin-3-O-ß-D-glucopyranoside (Salem et al., 2013).

Antioxidant activity

Oxidative stress is characterized by an increased production of free radicals, which include reactive oxygen species (ROS), reactive nitrogen species (NOS), carbon-centered and sulfur-centered radicals. ROS, such as superoxide anion (O_2^-), hydroxyl (•OH), peroxyl radical (ROO•) and hydrogen peroxide (H_2O_2), can induce oxidative degradation of proteins, unsaturated fatty acids, carbohydrates and nucleic acids (Zekovic et al., 2017). Excessive oxidative stress is implicated in the development of several chronic diseases, such as diabetes, cardiovascular diseases, cancer and ageing diseases. Many risk factors, such as cigarette smoking, irradiation and environmental pollutants, may induce excessive oxidative stress in human body, and the intake of antioxidants is regarded as a preventative method to reduce the negative effects induced by oxidative stress. Fruits, vegetables, cereals, spices and herbs are the main sources of natural antioxidants and many of them have displayed strong free radical scavenging abilities and anti-inflammatory activities (Xu et al., 2017).

Table 1. IC_{50} values for DPPH radical scavenging assays of young and mature leaves extracts of *Ficus auriculata* L. using gallic acid as reference.

Method of extraction	IC_{50} values of each free radical (DPPH) scavenging assay (µg.ml-1)	
	Young leaf	Mature leaf
M1	232.54 ± 1.28b	212.83 ± 2.38b
M2	182.87 ± 2.32a	153.77 ± 1.48a
M3	268.76 ± 4.87c	214.65 ± 1.57b
M4	392.98 ± 3.80d	383.63 ± 6.49c
M5	417.33 ± 5.53e	434.67 ± 5.33d
Gallic acid	21.66 ± 0.19	

The IC_{50} values were obtained by linear regression analysis. Values are mean of triplicate determinations ± standard deviation. The different lowercases letters, in column, are significantly different by Tukey test (p <0.05).

The results of IC_{50} values for DPPH radical scavenging assays of young and mature leaves extracts of *Ficus auriculata* using gallic acid as reference are shown in Table 1.

It was verified that, for the extracts of young leaf and mature leaf, the lowest values of IC_{50} occurred for the extracts obtained by the M2 method (ethanol/water and ultrasound) and these were statistically different (p<0.05) from the IC_{50} for the extracts obtained by the other methods. Thus, these extracts presented higher antioxidant activity. There was a significant difference (p<0.05) between all extraction methods for the extracts of young leaves, whereas for the extracts of mature leaves, M1 and M3 were statistically the same (p< 0.05). The lowest antioxidant activity was obtained for the extract obtained by M5 (water and enzymatic complex) for both leaves.

The extracts obtained from the young leaves by M1, M2 and M3 presented the same phenolic compounds content, and the extracts obtained from the mature leaves by M1 and M2 presented the same phenolic compounds content (Figure 1). However, the antioxidant activity against the DPPH radical was better with the extract obtained by M2 for both leaves. Due to its low toxicity and economic accessibility, aqueous ethanol has been commonly employed to extract antioxidants from plants. According to the principle of similarity and intermiscibility, when polarities of solvent and solute are similar, the solute is easily dissolved from plant cells. This enhancement of antioxidant compounds could be attributed to cavitation and thermal effects of the ultrasound technique, which cause disruption of the cell wall and intensification of mass transfer (Xu et al., 2017). In study of Shi et al. (2011) the IC_{50} for *F. auriculata* resulted in 290 µg.ml^{-1}; higher value than the lowest values found in the present study (182.87 µg.ml^{-1} for young leaves extracts and 153.77 µg.ml^{-1} for mature leaves extracts, Table 1). Ficus-derived antioxidant activity has been cited as a reason for its effectiveness in alleviating symptoms of streptozotocin-mediated diabetes in rats. The leaves also protect against the formation of excessive peroxide levels in palm oil when used during its preparation, presumably due to the its antioxidant properties (Lansky et al., 2008).

Antimicrobial activity

Escherichia coli is the predominant species of human intestinal microflora and some animals. Some strains are pathogenic when present in foods in high amounts, and can cause diarrhea, hemorrhagic colitis and Hemolytic Uremic Syndrome (Ismail et al.,

2016). *Salmonella* spp. are the main micro-organisms responsible for foodborne diseases in the world. Among the 2,500 serotypes of *Salmonella*, the main ones involved in toxiinfections are *S. enteritidis* and *S. typhimurium* (Fardsanei et al., 2016). *Listeria monocytogenes* is a facultative intracellular bacterium that can cause listeriosis, a serious disease transmitted primarily by food. Despite its low incidence, it presents a high degree of health risk, since it causes changes in the central nervous system (meningitis, encephalitis) and even abortion (Jalali and Abedi, 2008). *Staphylococcus aureus* inhabits the nasal mucosa and the skin of humans. This microorganism is responsible for a wide variety of diseases, including skin infections and food intoxication. Food intoxication is due to contamination of food by the thermoresistant exotoxins (enterotoxins) produced by the bacteria (Jans et al., 2017). The antimicrobial activities (in mm) of young and mature leaves extracts of *Ficus auriculata* are shown in Table 2. A classification scheme where the extracts with halos of inhibition < 9 mm were classified as inactive; from 9-12 mm partially active, from 13-18 mm active, and > 18 mm very active (Oliveira et al., 2016).

For the tested bacteria (Gram-positive and Gram-negative), extracts of young and mature leaves that showed antimicrobial activity can be classified as partially active to active. The extracts active against *E. coli* were obtained by M1 and M5 from young leaves and by M1, M2 and M4 from mature leaves. The active extracts against *S. enteritidis* were M1, M2, M3 and M4 from young leaves and M2 from mature leaves. It was evidenced that the extraction method, when used young leaves, may vary in relation to the solvent and the use of ultrasound. For the *S. aureus*, it is verified that the extracts of young leaves obtained by M1 and M4 are considered active and by M3 from mature leaves. For *L. monocytogenes*, the active extracts were also obtained by M1 and M4 from both leaves. Extracts with ethanol/water and extracts with only water as solvents presented antimicrobial activity, demonstrating that the compounds extracted from the young and mature leaves of *F. auriculata* have antimicrobial activity. Gaire et al. (2011) carried out the extraction of compounds from the dry bark of *F. auriculata,* using hexane, chloroform and methanol as solvents. For *E. coli*, the halos of inhibition were 3.2 ± 0.09 mm, 1.4 ± 0.03 mm and 4.5 ± 0.15 mm; and for *S. aureus*, the halos of inhibition were 7.8 ± 0.36 mm, 7.3 ± 0.21 mm and 6.9 ± 0.03 mm, with hexane, chloroform and methanol, respectively. These values were lower than the values obtained in the present study.

Flavonoids and isoflavonoids, which may be present especially in the extracts of young leaves, are considered one of the classes responsible for the antimicrobial activity of plants, mainly due to the ability of these compounds to complex the bacterial cell wall,

Table 2. Antimicrobial activities (in mm) of young and mature leaves extracts of *Ficus auriculata* L.

Method of extraction	Gram-negative bacteria		Gram-positive bacteria	
	Escherichia coli	*Salmonella enteritidis*	*Staphylococcus aureus*	*Listeria monocytogenes*
		Young leaf		
M1	13.67 ± 2.08	13.00 ± 0.00	15.00 ± 0.00	15.33 ± 2.08
M2	11.50 ± 0.71	15.50 ± 3.54	12.00 ± 1.73	12.00 ± 0.00
M3	11.00 ± 0.00	14.00 ± 0.00	11.00 ± 0.00	NA
M4	NA	15.00 ± 0.00	14.00 ± 1.41	16.00 ± 0.00
M5	13.00 ± 0.00	NA	NA	NA
		Mature leaf		
M1	15.50 ± 3.54	11.00 ± 0.00	NA	15.00 ± 0.00
M2	14.00 ± 1.41	17.00 ± 0.00	NA	9.50 ± 0.71
M3	NA	NA	13.00 ± 0.00	NA
M4	13.00 ± 1.41	NA	NA	15.00 ± 0.00
M5	NA	NA	10.00 ± 0.00	NA

Zone of Inhibition was determined by agar disc diffusion. Results were presented as mean ± standard deviation. NA: extract that does not have antimicrobial activity.

Table 3. Antibacterial activity of extracts of *Ficus auriculata* L. expressed as minimum inhibitory concentrations (MIC), in $\mu g.ml^{-1.}$

Method of extraction	Gram-negative bacteria		Gram-positive bacteria	
	Escherichia coli	*Salmonella enteritidis*	*Staphylococcus aureus*	*Listeria monocytogenes*
		Young leaf		
M1	47.21	188.85	188.85	87.82
M2	55.03	55.03	110.05	NA
M3	90.32	NA	90.32	Unrealized
M4	Unrealized	NA	64.22	NA
M5	30.83	Unrealized	Unrealized	Unrealized
Mature leaf				
M1	21.60	21.60	Unrealized	NA
M2	38.15	152.60	Unrealized	76.31
M3	Unrealized	Unrealized	71.89	Unrealized
M4	49.22	Unrealized	Unrealized	NA
M5	Unrealized	Unrealized	NA	Unrealized

The determination of MIC was only performed for the extracts that showed antimicrobial activity using the agar diffusion method. Unrealized: the antibacterial activity was not determined because it did not present inhibition halo. NA: extract that does not have antibacterial activity.

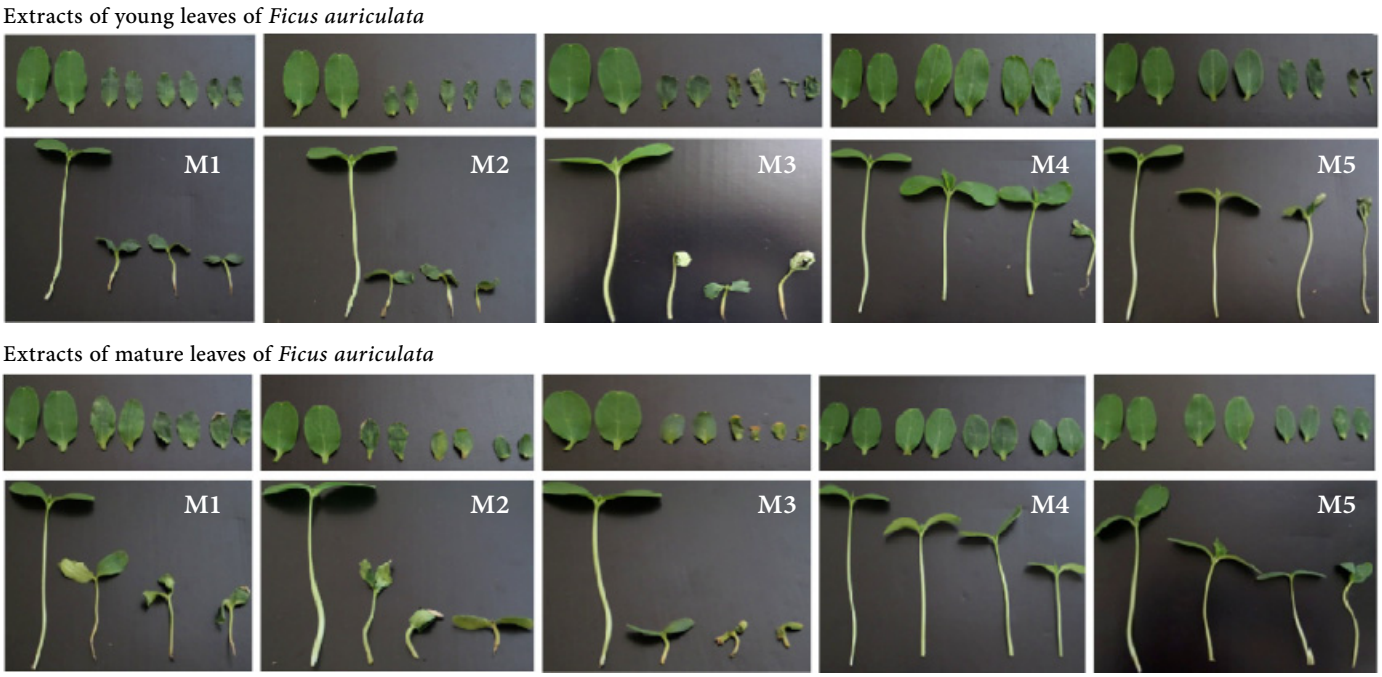

Figure 2. Phytotoxic activity of extracts of young and mature leaves of *Ficus auriculata* obtained by different methods. The first plant of each image is the control plant (only sprayed with water) and the others are the triplicate of the same extract.

and consequently to inhibit bacterial growth (Kuete et al., 2008). Furthermore, several coumarins were isolated from several different *Ficus* spp. and multiple flavonoids have been identified from *Ficus* spp. stems, leaves, and roots (Lansky et al., 2008).

The antibacterial activity of extracts of *Ficus auriculata* expressed as minimum inhibitory concentrations (MIC) are shown in Table 3.

The antibacterial activity of plant extracts can be considered significant when MIC values are lower than 100 $\mu g.ml^{-1}$, moderate when 100 < MIC ≤ 625 $\mu g.ml^{-1}$ and low when MIC > 625 $\mu g.ml^{-1}$ (Kuete and Efferth, 2010; Tchinda et al., 2016). The MIC values ranged from 21.60 $\mu g.ml^{-1}$ to 188.85 $\mu g.ml^{-1}$ and are considered significant and moderate values. Only four results of MIC were considered moderate, being them: M1 for extract of young leaves against *S. enteritidis* and *S. aureus,* M2 for extract of young leaves against *S. aureus* and M2 for extract of mature leaves against *S. enteritidis*.

Phytotoxic activity

Chemicals with allelopathic activity belong to different categories of secondary compounds such as phenols, benzoic and cinnamic acid derivatives, flavonoids, tannins, coumarins, terpenoids, alkaloids and polyacetylenes. They have the potential to either inhibit or stimulate the growth of plants and this can be by different mechanisms and acting on different sites of action. Various factors in this effect as season, changing concentrations, population, interactions of allelochemical components (antagonistic and synergistic) can be involved (Ladhari et al., 2013, Hamdi et al., 2017).

For all plants of cucumber sprayed with extracts of the *F. auriculata* young and mature leaves higher suppression in the growth was verified when compared to the control (sprayed with water - height of 12.1 ± 0.4 cm). When the extracts of the young leaves were sprayed, the highest suppression occurred for M1 (height of 3.3 ± 0.9 cm), M2 (height of 2.3 ± 0.6 cm) and M3 (height of 4.0 ± 1.0 cm) extracts, with statistically equal results ($p > 0.05$). When the extracts of the mature leaves were sprayed, the highest suppression occurred with M3 extract (height of 1.4 ± 0.2 cm), statistically different ($p < 0.05$) from the other extracts (M1 = 4.7 ± 0.5 cm, M2 = 2.9 ± 1.1 cm, M4 = 8.6 ± 1.9 cm and M5 = 8.0 ± 1.1 cm) and control. Comparing the extracts, for the same method, it was verified that only M3 differed statistically ($p < 0.05$), with higher suppression of growth when the mature leaf extract was used.

For the extracts M1, M2 and M3 obtained from the young leaves and extracts M1 and M2 obtained from the mature leaves, the content of extracted phenolic compounds was higher than for the other extracts. However, it should be noted that all the extracts affected the growth of the plants, which demonstrates the action of the phenolic compounds present in the extracts. The enhanced plant growth or, on the contrary, plant growth inhibition could be the result of a positive or negative balance between nutrient and phenolic compound concentration (Sánchez-Gómez et al., 2017). The presence of some putative allelochemicals such as glucosylated quercetin, reported by Salem et al. (2013), might explain the results observed (Sánchez-Gómez et al., 2017).

In Figure 2 are shown the cucumber plants sprayed with the extracts obtained by the methods M1 to M5, in 7 days of growth.

The extracts obtained from young leaves by methods M1, M2, M4 and M5 caused the reduction of the size and the formation of wrinkles. However, there was no necrosis of the leaves. For the extract obtained by M3, it is observed that the plants died. M1, M2, M4 and M5 presented phytointoxication index of 6 and M3 presented phytointoxication index of 9. The plants sprayed with the extracts of the mature leaves, obtained from M1, M2 and M3, presented reduction of the size, formation of wrinkles, necrosis in some leaves and extremely serious damage, leaving small green areas on the plants. For extracts obtained by M4 and M5, plants showed no damage, but had a reduction in size. M1, M2 and M3

presented phytointoxication index of 8 and M4 and M5 presented phytointoxication index of 2.

When plants are under allelopathic stress, activities of antioxidant enzymes, such as superoxide dismutase, catalase and peroxidase, will immediately be induced for plants autoprotection. Generally, reactive oxygen species (ROS) production and antioxidant enzymes induction are in well balance, any oxidative stress that breaks the balance could harm the plant growth (Zhu et al., 2017). It is quite possible that allelochemicals may produce more than one effect on the cellular processes responsible for reduced plant growth. However, the details of the biochemical mechanism through which a particular compound exerts a toxic effect on the growth of plants are not well known (Ladhari et al., 2013).

Conclusion

The extracts obtained from young and mature leaves of *Ficus auriculata* presented considerable capacity to scavenge the DPPH free radicals, presented capacity to inhibit the growth of Gram-positive and Gram-negative bacteria important of food industry and presented phytotoxic activity, suggesting the possibility of its use as herbicide. The best results for antioxidant, antimicrobial and phytotoxic activities were obtained for the extracts of young leaves. As for the extraction method, there were differences in relation to the extracts for different activities investigated, with the best results for the extracts obtained by ethanol/water, ethanol/water/ultrasound and ethanol/water/cellulase complex. In conclusion, *Ficus auriculata* can be considered a potential source of antioxidant, antimicrobial and phytotoxic molecules.

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