



ISSN: 2230-9926

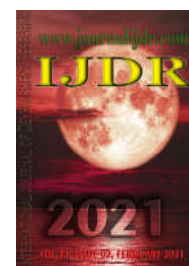
Available online at <http://www.journalijdr.com>

IJDR

International Journal of Development Research

Vol. 11, Issue, 02, pp. 44724-44729, February, 2021

<https://doi.org/10.37118/ijdr.21187.02.2021>



RESEARCH ARTICLE

OPEN ACCESS

PHYTOCHEMICAL PROSPECTION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF LEAVES EXTRACTS FROM *Myrcia palustris* DC

Camila Vogt dos SANTOS¹, Ana Paula MALLMANN¹, Adrieli Gorlin TOLEDO¹, Débora Marina BANDEIRA¹, Lázaro Henrique Soares de Moraes CONCEIÇÃO², Juliana Moço CORRÊA¹ and Fabiana Gisele da Silva PINTO^{1*}

¹Microbiology and Biotechnology Laboratory, Conservation and Management of Natural Resources Program, Universidade Estadual do Oeste do Paraná (UNIOESTE), Rua Universitária, 2069, 85819-110, Cascavel, PR, Brazil; ²Herbarium(HUOP), Universidade Estadual do Oeste do Paraná, Rua Universitária 2069, 85819-110, Cascavel, PR, Brazil

ARTICLE INFO

Article History:

Received 18th December, 2020

Received in revised form

22nd December, 2020

Accepted 09th January, 2021

Published online 28th February, 2021

Key Words:

Water Resources;
Environmental Economics; Beer.

*Corresponding author:

Fabiana Gisele da Silva PINTO

ABSTRACT

The objective of this work was to carry out an investigation regarding phytochemical prospecting of six extracts obtained from leaves of *Myrcia palustris* DC, namely: ethyl acetate (EAE), acetone (AE), ethanolic (EE), methanolic (ME), hexanic (HE) and distilled water (DAE), to evaluate their antimicrobial activity using the broth microdilution technique, with bacteria of medical interest, and detecting the antioxidant potential against the 2,2-diphenyl-1-picryl-hydrazil (DPPH) method. The results demonstrated the presence of compounds from the classes steroids, flavonoids, xanthenes, and tannins. Regarding antimicrobial activity, it was observed that all extracts demonstrated antimicrobial potential against the strains tested, except for the HE, which did not show activity on *K. pneumoniae*. DAE showed the lowest efficiency for most of the strains tested. Regarding antioxidant activity, except from DAE, all others showed antioxidant potential in the highest concentrations tested in the range of 0.1 to 25 mg/mL: EE (82.29%), ME (77.67%), and AE (74.10%) showed greater capture of DPPH radicals. It is suggested that the antimicrobial and antioxidant activities of these extracts is related to the secondary metabolites present, which have already had these biological activities demonstrated in other studies with plant extracts.

Copyright © 2021, Camila Vogt dos SANTOS et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Camila Vogt dos SANTOS, Ana Paula MALLMANN, Adrieli Gorlin TOLEDO, Débora Marina BANDEIRA, Lázaro Henrique Soares de Moraes CONCEIÇÃO et al. 2021. "Phytochemical prospection, antioxidant and antimicrobial activities of leaves extracts from *myrciapalustris* dc.", international journal of development research, 11, (02), 44724-44729.

INTRODUCTION

Natural products have always played an important role in the development of new drugs, cosmetics, and other bioproducts, due to the vast structural diversity and functional groups present in various plant species in the world (Amorim et al., 2019; Bolzani et al., 2016). According to the World Health Organization (WHO), the best source of bioactive molecules is plants, and in this sense, Brazil stands out in the world context for presenting the greatest biodiversity, becoming a great ally of industries, offering raw materials for the discovery of new molecules that can contribute/replace chemicals (Arantes et al., 2016; Carvalho et al., 2014). When investigating new molecules, it is interesting to look for native species, aiming at their valorization, besides emphasizing the importance of the conservation of the Brazilian flora that offers bioactive compounds within its natural resources.

The Myrtaceae family stands out as being plentiful in the country with 20 genera and a thousand species cataloged (Carneiro et al., 2017). This genus has economic appeal as it represents fruit species such as *Psidium guajava* and *Myrciaria cauliflora* (Gressler et al., 2006), among others. Amorim et al. (2019) showed, through biogeographic analyses, that this genus plays an important role in the Atlantic Forest biome for the diversification of the group, after which several transitions occurred in other neotropical regions, especially savanna forests. An important representative of the Myrtaceae family is the genus *Myrcia*, widely used in folk medicine and with effects on glycemic control for *Myrcia multiflora* and the species *Myrcia fallax*, with evidence of activity against cancer cells (Limberger et al., 2004). The species *Myrcia oblongata* has already been described as having antioxidant, acaricide, insecticide, and antimicrobial potential (Santana et al., 2018). There are no reports in the literature on the biological activities of *Myrcia palustris* DC.

This is a native species found mainly in the states of Paraná, Santa Catarina and Rio Grande do Sul. Among the problems faced by industries today is the search for antioxidants, substances that slow down or inhibit oxidative processes, slowing down the process of food rot. In addition, antioxidants are extremely important in human health, with the function of neutralizing free radicals, which also prevent oxidation in the body at the cellular level; a process related to some diseases, such as Alzheimer's disease (Pastene et al., 2009). The synthetic compounds present in several products, with the objective of preserving food and drugs, can cause harmful effects to animal and human organisms, which makes it necessary to search for natural products with antioxidant actions as alternatives to synthetics (Melo et al., 2011; Santos et al., 2018; Sousa et al., 2007). The improper use of synthetic products also affects the field of medicine, both human and veterinary, because when trying to fight infections caused by microorganisms, high doses of antimicrobials/antifungals are used, resulting in the selection of resistant pathogens (Arantes et al., 2016; Rossi and Andreatzi, 2005). Thus, a plant source antimicrobial can suppress bacterial action in different systems with a low toxic effect and cost, as it is a natural source (Amorim et al., 2019; Souza et al., 2020). Through all the problems exposed, together with the importance of conserving Brazilian flora and the search for new bioactive compounds of interest, this research aims to identify the secondary metabolites present in different plant extracts of *M. palustris* leaves and determine the antimicrobial and antioxidant properties of this species.

MATERIAL AND METHODS

Collecting, drying, and identification of the plant: *M. palustris* leaves were collected at the Ecological Park Paulo Gorski, located in the municipality of Cascavel, Paraná, Brazil (24°57'51.61"S and 53°26'14.80"W). An exsiccate from the plant was taken to the Herbarium UNOP (Thiers, continuously updated) for botanical identification and voucher registration, under the number UNOP 8915. After collection, the leaves were dried at 40°C and ground in a willye knife mill, with a 0.42 mm granulometry membrane and subsequently, the obtained powder was stored in a closed glass jar protected from light, at room temperature, for a maximum of four days (Weber et al., 2014).

Obtaining plant extracts: From the dry leaves of *M. palustris*, plant extracts were prepared according to the methodology proposed by Pandini et al. (2015), with modifications. The dry plant material (10 g) was subjected to extraction with different solvents (100 mL): ethyl acetate (EAE), acetone (AE), ethanol (EE), hexane (HE), methanol (ME) and distilled water (DAE). These liquid preparations were kept on a rotary shaker at 220 rpm for a period of 24 h. Then, they were filtered using Whatman filter paper n° 1 and centrifuged at 5000 rpm for 15 min. The supernatant was collected and subjected to evaporation, apart from the aqueous extract which was stored at 4°C. Finally, crude organic extracts and aqueous extracts were obtained, which were stored in the dark and refrigerated at 4°C. Plant extract yield was calculated using Equation 1:

$$\text{Equation 1 \%} = \frac{\text{extract mass (g)}}{\text{dry and moist vegetable mass (g)}} \times 100$$

Phytochemical prospection: Tests relating to the phytochemical prospection of different plant extracts of *M. palustris* were carried out according to the methodology described by Matos (1997). These tests were based on colorimetric visualization and/or precipitate formation after the addition of specific reagents. The classes of secondary metabolites identified were: saponins from the reaction with distilled water and hydrochloric acid P.A.; steroids and triterpenoids through the Liebermann–Burchard reaction; alkaloids using Dragendorff's reagent; anthocyanidins, anthocyanins, aurones, chalcones, flavonoids, flavones, flavanones, and xanthenes from pH changes in the medium; coumarins by fluorescence reaction with potassium hydroxide; and tannins by reaction with ferric chloride.

Determination of antimicrobial activity

Microorganisms used and inoculum preparation: The antimicrobial activity of plant extracts of *M. palustris* was evaluated following the methodology proposed by Scur et al. (2014), with modifications. The microorganisms used are from the *American Type Culture Collection* (ATCC) and *Cefar Diagnostica* (CCD) collections, with gram positive bacteria: *Bacillus subtilis* (CCD-04), *Enterococcus faecalis* (ATCC 19433), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228); gram negative: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 25933), *Salmonella enterica* Enteritidis (ATCC 13076), *Salmonella enterica* Gallinarum (ATCC 1138), *Salmonella enterica* Typhimurium (ATCC 14028). The microorganisms were recovered in Brain and Heart Infusion (BHI) broth for 24 hours at 36 °C, then seeded in a plate containing Mueller–Hinton (MH) agar and incubated for 24 hours at 36 °C. To carry out the experiments, the concentration of microorganisms was adjusted in 0.85% saline to 1x10⁵ CFU/mL.

Determination of minimum inhibitory concentration (MIC): The tests were performed according to the broth microdilution methodology described by Weber et al. (2014), with modifications. The plant extracts of *M. palustris* were solubilized in 1% Tween. In 96-well microdilution plates, 150 µL of Mueller–Hinton (MH) broth was distributed in all wells. The first well received an additional 150 µL of plant extract at an initial concentration of 200 mg/mL. Then, serial dilution was performed, obtaining concentrations ranging from 200 to 0.09 mg/mL. At the end, 10 µL of inoculum was added to each well and the plate was incubated at 36°C for 24 h. For the positive control, the commercial antibiotic Gentamicin (200 mg/mL) was used. As a negative control, the inoculum was added to the MH broth, without the presence of the extract, to verify the viability of the tested microorganisms. Control of the 1% Tween diluent was also carried out to verify possible interference in the assay. For interpretation of the results, 20 µL of 0.5% triphenyltetrazolium chloride (TTC) was added, acting as a colorimetric developer; the wells that showed red placement were considered negative to inhibiting bacterial growth. The MIC was performed in triplicate and it was possible to determine the lowest concentration of plant extract capable of inhibiting microbial growth.

Determination of bactericidal concentration (MBC): Before the addition of 0.5% TTC to determine the MIC, 2 µL aliquots were removed from each assay well and transferred individually to petri dishes containing MH agar, which were incubated for 24 hours at 36°C. To determine the MBC, that is, the lowest concentration of plant extracts capable of causing death of the microorganism, the presence/absence of growth of a microbial colony on the plate was verified in the different concentrations of extracts in the MIC test (Scur et al., 2014). The activity of the extracts was classified according to the methodology of Araújo (2010), being defined as: high activity (≤12.5 mg/mL), moderate (12.5–25 mg/mL), low (50–100 mg/mL), and very low (> 100 mg/mL). The tests were performed in triplicate.

Antioxidant activity: The test for the antioxidant activity of the extracts was performed using the DPPH reduction method, proposed by Rufino et al. (2007) and Weber et al. (2014). A calibration curve (0, 10, 20, 30, 40, 50, and 60 µM of DPPH) was performed to obtain the concentration of DPPH in the medium after the reaction with the essential oil, using Equation 2, where y is the concentration of DPPH is absorbance. Then, the plant extracts were solubilized in methanol P.A., obtaining concentrations that varied from 0.1 to 25 mg/mL. Aliquot of 0.1 mL of these extracts was added to 3.9 mL of the DPPH methanolic solution (60 mM) and homogenized in a shaker tube. The absorbance reading was performed in a spectrophotometer (FEMTO, 700 Plus) at 515 nm. As a negative control, 0.1 mL of methanol was added to 3.9 mL of DPPH, and as a positive control, the synthetic antioxidant butylhydroxy-toluene (BHT) was used in concentrations of 0.25 to 1 mg/mL. The methanol P. A. was used to calibrate the apparatus. The percentage of free radical sequestration

(AA%) was expressed by the equation: $AA\% = [(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the negative control and A_1 is the absorbance of the sample. For the calculation of IC₅₀ (concentration at which there is 50% inhibition), was calculated graphically by linear regression of a plot of the antioxidant activities at several extracts concentrations. The tests were performed in triplicate and expressed as mean \pm standard deviation. The IC₅₀ results were analyzed using an ANOVA with Tukey's test ($p < 0.05$) using the statistical software R® version 3.3.2.

The extracts were calculated by equation 4 and classified using the antioxidant activity index (AAI) by Scherer and Godoy (2009). In this index (AAI < 0.5), moderate (AAI 0.5-1.0), strong (AAI 1.0-2.0) and very strong (AAI > 2.0),

Equation 2: $y = 0,0113x - 0,0429$ ($R^2 = 0,995$)

Equation 3: $AA\% = \frac{(A_0 - A_1)}{A_1} \times 100$

Equation 4: $AAI = AA\% \frac{AA\%}{IC_{50}}$

RESULTS AND DISCUSSION

Phytochemical prospection: From the manufacture of *M. palustris* plant extracts with different solvents, the following yield was obtained: DAE (49.52%), ME (19.52%), EE (18.42%), AE (11.54%), EAE (5.72%) and HE (4.95%). Such a yield can be influenced by temperature and extraction time, and also by the choice of solvent, since they have different molecular structures, polarity, and solubility, which influence the vegetable-solvent behavior (Cabana *et al.*, 2013; Fernández-Agulló *et al.*, 2013; Pinelo *et al.*, 2004). Phytochemical prospecting detected the presence of compounds belonging to the classes of saponins, steroids, flavonoids (flavones, flavonols and flavanones), xanthonenes, and tannins. Secondary metabolites such as flavones and flavonones were identified in all extracts (Table 1). The greatest diversity of classes of compounds was observed in EE (7), ME (6), and AE (6), followed by EAE (5), HE (5), and DAE (3), corroborating the literature that reports solvents such as ethanol, methanol, and acetone as being the best vegetable extractors (Cabana *et al.*, 2013; Fernández-Agulló *et al.*, 2013; Souza *et al.*, 2020). The Myrtaceae family has been extensively studied in relation to its chemical composition, being reported as having great potential in accumulating phenolic compounds such as tannins, flavanones, flavones, and flavonols (Takao *et al.*, 2015). However, it is important to emphasize that there are differences in the compounds between species of the same family, genus and even species. This is because the place where the plant is grown and environmental factors such as: temperature, water availability, fertilization, time of collection as well as method of extraction, can interfere with the metabolic pathway of plants, changing the biosynthesis of different compounds in each season of the year (Gobbo-Neto and Lopes, 2007; Morais, 2009). Even though they may have different compounds, due to the different conditions in which each plant is found, as mentioned above, the secondary metabolites found in *M. palustris* extracts have already been identified in other species of the family such as tannins, steroids, saponins, flavonoids, and alkaloids in *Gomidesia affinis*, *Gomidesia spectabilis*, and *Pimenta pseudocaryophyllus* (Paula *et al.*, 2008; Sakita and Aguiar, 2006). As in *M. palustris*, several other species of the genus contain phenolic compounds, represented by flavonoids, flavones, anthocyanins, flavanones, and tannins. These metabolites have been identified in species such as *Myrcia oblongata* (Santana, 2017), *Myrcia bella* (Saldanha, 2010), and *Myrcia hiemalis* (Silva, 2007).

Antimicrobial activity: There was a variation in the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts according to the extracting solvent and the tested microorganism. Thus, all extracts, apart from HE and DAE, showed antimicrobial activity for all tested strains. DAE showed less efficiency compared to the strains tested (Table 2),

and the low antimicrobial activity can be explained by the low dissolution/affinity of the chemical compounds present in the plant with water in the extraction method (Table 1). EE and ME extracts showed the highest antimicrobial activities when compared to the other extracts, with MIC and MBC concentrations ranging from 1.56 to 25 mg/mL between gram negative and positive strains, presenting activity classified as high. Phytochemical prospecting of both extracts reported the presence of the same compounds (steroids, flavones, flavonols, xanthonenes, flavanones, and tannins), justifying the similar antimicrobial activity presented by them. Tannins were present in both extracts, which may have contributed to greater antimicrobial activity since they were identified only in them. According to Mello (2001), tannins have three mechanisms of action that make them bactericidal and/or fungicidal, the first is the inhibition of enzyme synthesis, the second acts on cell membranes, modifying their metabolism, and the third involves a complexation of tannins and metal ions which decreases microbial cell availability. The extracts of AcOH and Hex showed a similar profile of antimicrobial activity, considered low-high for all bacteria, with MIC and MBC concentrations ranging from 3.12 to 100 mg/mL. The justification for its similar antimicrobial activity can be attributed to the chemical compounds present, which are the same, apart from the saponins found only in AE (Table 1).

EAE showed low to moderate antimicrobial activity for all tested microorganisms, the extract being less efficient after DAE, however, the presence of the same groups of phytochemicals from other extracts (steroids, flavones, flavonols, xanthonenes, and flavanones) were revealed. Despite this, phytochemical prospecting (Table 1), as it is a qualitative method, does not allow quantifying these groups, that is, the presence and absence of such compounds is detected, following the colorimetric methodology applied in this study, which probably justifies extracts containing the same class of phytochemicals exhibiting different antimicrobial behaviors (Amorim *et al.*, 2019; Pandini *et al.*, 2015). In addition, the presence of these groups of phytochemicals in low amounts was probably insufficient to significantly inhibit the tested microorganisms. Another relevant feature is that not only the quantity, but also the synergistic action, when used in combination in the same extract, can have additive or synergistic effects on the microorganism (Amorim *et al.*, 2019; Pandini *et al.*, 2015).

When comparing this study with others already reported, it was possible to verify that the EE extract showed better antimicrobial potential, similar to other studies, as in Nene *et al.* (2016) who, when evaluating extracts of *Myrcia bella* obtained antimicrobial activity for *S. aureus*; and in Souza *et al.* (2020) where, in addition to the EE, the ME of the leaves of *Zanthoxylum caribaeum* L. also exhibited antimicrobial capacity. Although few studies on antimicrobial activity of plant extracts of the genus *Myrcia* have been reported, several studies have demonstrated activity of extracts from species belonging to the Myrtaceae family on different microorganisms. The ME of *Eucalyptus globulos*, *Eucalyptus maculata*, and *Eucalyptus viminalis* significantly inhibited the growth of gram positive microorganisms: *E. faecalis* and *S. aureus* (Takahashi *et al.*, 2004). The EE of *Psidium guajava* inhibited the growth of gram positive and negative bacteria such as *S. aureus* and *P. mirabilis* (Gonçalves *et al.*, 2005). The efficiency of plant extracts from *Myrciaria cauliflora* and *Syzygium cumini* has already been demonstrated against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. Tiphymurium*, *S. aureus*, and *B. subtilis* by Bona *et al.* (2014). The antimicrobial activity of secondary metabolites from several plants has already been demonstrated, so it is suggested that the potential of *M. palustris* is related to the presence mainly of tannins and flavonoids in these extracts. The compounds belonging to the class of flavonoids (flavones, flavonols, xanthonenes, and flavanones) can act on microorganisms by three mechanisms: causing perforation and reducing the fluidity of the plasma membrane; causing inhibition of topoisomerase, resulting in the inhibition of nucleic acid synthesis, and/or inhibiting energy metabolism; these in turn cause irreversible damage to cells (Cushnie and Lamb, 2011; Samy and Gopalakrishnakone, 2010; Sher, 2009).

Table 1. Phytochemical prospection of aqueous and organic solvent extracts from leaves of *Myrciapalustris* DC.

Metabolite classes	<i>Myrciapalustris</i> extracts					
	EE	ME	EAE	AE	HE	DAE
Saponins	+	-	-	+	-	+
Steroids	+	+	+	+	+	-
Triterpenoids	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-
Anthocyanins	-	-	-	-	-	-
Anthocyanidins	-	-	-	-	-	-
Flavones	+	+	+	+	+	+
Flavonols	+	+	+	+	+	-
Xanthonnes	+	+	+	+	+	-
Chalcones	-	-	-	-	-	-
Aurones	-	-	-	-	-	-
Flavanones	+	+	+	+	+	+
Condensed Tannins	+	+	-	-	-	-
Coumarins	-	-	-	-	-	-

+ Presence of the compound; - absence of compound. EE: ethanol extract; ME: methanol extract; EAE: ethyl acetate; AE: acetone extract; HE: hexane extract; and DAE: distilled water.

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts from the leaves of *Myrciapalustris* DC.

Microorganisms	CIM/CBM (mg/mL)					
	EE	ME	AE	HE	EAE	DAE
Gram (+)						
<i>B. subtilis</i>	6.25/12.5	6.25/12.5	12.5/50	3.12/25	6.25/12.5	200/-
<i>E. faecalis</i>	6.25/6.25	12.5/12.5	50/50	12.5/25	12.5/25	-/-
<i>S. aureus</i>	1.56/1.56	3.12/3.12	12.5/12.5	25/50	12.5/25	50/50
<i>S. epidermidis</i>	1.56/1.56	3.12/3.12	6.25/12.5	25/50	25/50	-/-
Gram (-)						
<i>E. coli</i>	6.25/12.5	6.25/12.5	12.5/50	25/50	25/50	100/-
<i>K. pneumoniae</i>	6.25/25	3.12/12.5	12.5/25	-/-	25/100	-/-
<i>P. aeruginosa</i>	1.56/1.56	1.56/1.56	6.25/6.25	100/100	50/50	50/100
<i>P. mirabilis</i>	1.56/3.12	1.56/3.12	6.25/6.25	12.5/50	25/50	-/-
<i>S. Enteritidis</i>	3.12/6.25	6.25/6.25	3.12/6.25	12.5/50	12.5/25	-/-
<i>S. Gallinarum</i>	6.25/12.5	6.25/12.5	12.5/25	25/50	25/100	-/-
<i>S. Typhimurium</i>	6.25/12.5	6.25/25	100/100	25/50	25/100	-/-

High: < 12.5 mg/mL; Moderate: 12.5–25 mg/mL; low: 50–100 mg/mL; very low: > 100 mg/mL; -: Not detected; EE: ethanol extract; ME: methanol extract; EAE: ethyl acetate; AE: acetone extract; HE: hexane extract; and DAE: distilled water.

Table 3: Percentage of DPPH radical scavenging and IC₅₀ value of the plant extracts of *Myrciapalustris* DC.

Concentration (mg/mL)	Control	<i>Myrciapalustris</i> extracts					
	BHT	EE	ME	AE	HE	EAE	DAE
25	-	-	-	-	69.49±0.5	65.17±0.4	19.49±0.1
20	-	-	-	-	55.80±0.4	52.38±0.2	15.77±0.3
15	-	-	-	-	47.90±0.9	40.77±0.8	12.20±0.0
10	-	-	-	-	33.03±0.7	33.03±1.8	7.73±1.9
5	-	-	-	-	22.02±0.4	21.13±2.0	2.52±0.0
1	98.75±0.2	82.29±0.5	77.67±0.8	74.10±0.6	-	-	-
0.80	-	77.67±0.3	70.23±0.3	53.42±0.8	-	-	-
0.50	77.23±0.1	-	-	-	-	-	-
0.40	-	62.79±0.9	51.63±0.7	38.98±0.8	-	-	-
0.25	53.48±1.3	-	-	-	-	-	-
0.20	-	47.91±1.1	32.18±0.3	30.80±0.6	-	-	-
0.10	38.03±0.2	32.88±0.3	24.10±0.3	25.89±0.3	-	-	-
0.05	20.35±3.0	-	-	-	-	-	-
IC ₅₀	0.28±0.0	0.29±0.0	0.48±0.0	1.48±0.5	16.83±0.1	18.42±0.2	60.38±2.4
IAA	3.52±0.2	0.82±0.5	1.61±0.8	0.5±0.6	0.041±0.5	0.03±0.4	0.032±0.1

(-) Not tested; BHT (commercial synthetic antioxidant Butylhydroxytoluene), EE: ethanol extract; ME: methanol extract; EAE: ethyl acetate; AE: acetone extract; HE: hexane extract; and DAE: distilled water. Values of IC₅₀ (Concentration of *E. involucrata* leaves extract is necessary to reduce 50% of the DPPH radical) expressed as mean ± standard deviation; IAA: antioxidant activity index.

The high potential of EE and ME extracts can be attributed to the presence of tannins, which already have antimicrobial action demonstrated in the literature (Simões *et al.*, 2007). The mechanism of antimicrobial action of tannins involves the inhibition of bacterial enzymes and/or the ability to combine with the substrates of these enzymes. In addition, they modify the metabolism due to the action on the cell membrane and are based on the tannin and metal ions complex, resulting in the reduction of essential ions for microbial metabolism.

Furthermore, they can cause bacterial colonies to disintegrate, resulting in the inhibition of microbial growth (Doss *et al.*, 2009; Scalbert, 1991). Among the group of steroids, a compound common to the extracts AE, DAE, and EE was the presence of saponins (Table 1), which probably contributed to the antimicrobial activities, acting on the cell membrane and increasing permeability (Desoti *et al.*, 2011; Simões *et al.*, 2004). It is important to note that the bacteria on which the extracts had a better inhibition performance were *S. Gallinarum* and *S. Enteritidis*.

These are of great importance for the poultry sector, as they are present in the birds' accommodation environment and are a vehicle for contamination of breeding stock and consequently, the eggs produced. In a study by Hwang *et al.* (2020), *Salmonella* were evaluated for their prevalence in an avian production system as well as the meteorological factors associated with contamination, such is the importance of this bacterial group in confined systems for birds.

Antioxidant activity: The antioxidant capacity of *M. palustris* plant extracts was determined by decreasing the absorbance at 515 nm, using the DPPH sequestration test. The extracts that showed higher DPPH radical scavenging, when compared to each other, were EE (82.29%), ME (77.67%), and AE (74.10%), in their highest tested concentration (1 mg/mL) and IC₅₀ values of 0.29, 0.48, and 1.48 mg/mL, respectively. HE (65.17%) and EAE (69.49%) had antioxidant activity rates below 70%, even at their highest tested concentration (25 mg/mL), and IC₅₀ values of 16.83 and 18.42, respectively. Apart from DAE (19.49%), all other *M. palustris* extracts showed antioxidant activity considered high: EE (82.29%), ME (77.67%), AE (74.10%), HE (65.17%), and EAE (69.49%), although they did not reach the percentage of the synthetic antioxidant BHT, at 98.75% (1 mg/mL) and an IC₅₀ value of 0.28 mg/mL. This means that more plant extracts are needed to sequester the same amount of DPPH free radicals when compared to the control. The DAE cannot be considered a good antioxidant since, in its highest concentration (25 mg/mL), it had a very low antioxidant capacity, of 19.49% and an IC₅₀ value of 60.38 mg/mL (Table 3). Thus, it was observed that the ability to sequester free radicals depends on the concentration tested and the extractor solvent utilized (Rufino *et al.*, 2007; Scherer and Godoy, 2009; Weber *et al.*, 2014). When comparing our data with those in the literature, we prioritized studies involving the same method used in this research; the DPPH method is widely used and consists of capturing this free radical, resulting in a decrease in absorbance (Ruffino *et al.*, 2007). Studies on the antioxidant potential of leaf extracts from species of the Myrtaceae family are scarce; the focus of this study in Brazil is edible fruits. However, the antioxidant potential of leaf infusions of some species of this family was observed in *Psidium laruoteanum* and *Psidium australe* which revealed an interesting source of natural antioxidant associated with the presence of phenolic compounds in the extracts (Takao *et al.*, 2015). The plant extracts of *M. palustris* showed strong antioxidant activity for EE and ME and moderate for EAE and EA and the other HE and DAE showed index, following the classification proposed by Scherer and Godoy, (2009). Within the genus *Myrcia*, the AE (IAA = 8.5), ME (IAA = 4.7), and HE (IAA = 4.0) of the mature leaves of *Myrciasplendens* showed antioxidant activity considered high (Pontes *et al.*, 2018). Other species such as *Myrcia tomentosa* (IAA = 4.1), *Myrcia bella* (IAA = 3.9), and *Myrcia lingua* (IAA = 3.9) also showed antioxidant activity, considered to be very high (Takao *et al.*, 2015).

The antioxidant activity of *M. palustris* can be attributed to the phenolic compounds present in each extract, which have proven antioxidant activity (Aquino *et al.*, 2017). However, it is possible to observe differences in the antioxidant capacity between the extracts of *M. palustris* and also, when compared with other species. This is because although they all have phenolic compounds, which can be in different amounts and/or molecular form, interfering in the ability to sequester DPPH free radicals from each extract (Aquino *et al.*, 2017; Pontes *et al.*, 2018; Takao *et al.*, 2015). In all extracts of *M. palustris*, phenolic compounds were found, which are widely distributed in nature and can act as antioxidants in several ways. One of them is related to its ability to donate hydrogen or electrons, since its structure allows it to support an unpaired electron. In addition, they can repair the damage to molecules attacked by free radicals and block the spread of free radicals in lipid oxidation (Sucupira *et al.*, 2012). This class is represented by a wide variety of compounds, among them are flavonoids, presenting under many variations such as, flavonols, flavones, flavanones, and anthocyanins (Morais *et al.*, 2009; Silva *et al.*, 2010; Takao *et al.*, 2015). Flavonoids have a great antioxidant capacity due to their carbonic skeleton, which favors the stabilization of free radicals (Aquino *et al.*, 2017).

Tannins also represent phenolic compounds, found in many plant species, which are molecules that act in the process of stabilizing free radicals. The presence of this compound in EtOH and MeOH may justify its high antioxidant potential, since they were identified only in these extracts (Aquino *et al.*, 2017; Bernardes *et al.*, 2011; Paiva *et al.*, 2002).

Conclusion

The yield of plant extracts varied according to the solvents used, being DAE (49.52%), ME (19.52%), EE (18.42%), AE (11.54%), EAE (5.72%), and HE (4.95%). Phytochemical prospecting detected the presence of steroids, flavonoids (flavones, flavonols, and flavanones), xanthenes, and tannins. The antimicrobial activity with the best inhibition performance was observed in the extracts EAE, AE, EE, and ME showing activity for all strains tested. The antioxidant potential was established for all extracts, except DAE, with an emphasis on EE with 82.29% DPPH radical sequestration. *Myrcia palustris* DC combines important characteristics for biotechnological applicability, as it has proven antimicrobial activity and antioxidant potential, which can be used as raw material in different industrial applications when incorporating or developing new products.

Acknowledgements

Coordination for Improvement of Personnel Higher Education (CAPES). Araucária Foundation and Brazilian National Council for Scientific and Technological Development (CNPq).

REFERENCES

- Amorim B, Vasconcelos TNC, Souza G, Alves M, Antonelli A, Lucas E 2019. Advanced understanding of phylogenetic relationships, morphological evolution and biogeographic history of the mega-diverse plant genus *Myrcia* and its relatives Myrtaceae: Myrteae. *Molecular Phylogenetics and Evolution* 138: 65-88.
- Aquino VVF, Costa JGM, Angélico EC, Medeiros RS, Araújo MF, Rodrigues OG 2017. Metabólitos secundários e ação antioxidante de *Croton heliotropiifolius* e *Croton blanchetianus*. *Acta Brasiliensis* 1: 7-10.
- Arantes VP, Santos LF, Diniz KS, Silva GO, Costa GM 2016. Estudo comparativo da atividade antibacteriana de extratos vegetais de *Senna spectabilis*, *Rosmarinus officinalis* e *Eugenia uniflora* frente à cepa padrão de *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6528 e *Streptococcus pyogenes* ATCC 19615. *Arq Cienc Saúde UNIPAR* 20: 151-158
- Bernardes NR, Glória LL, Nunes CR, Pessanha RR, Muzitano MF, Oliveira DB 2011. Quantificação dos teores de taninos e fenóis totais e avaliação da atividade antioxidante dos frutos de Aroeira. *Vértices* 13: 117-128.
- Bolzani VS 2016. Biodiversidade, bioprospecção e inovação no Brasil. *Ciência e Cultura*.
- Bona EAM, Pinto FGS, Fruet TK, Jorge TCMJ, Moura AC 2014. Comparação de métodos para avaliação da atividade antimicrobiana e determinação da concentração inibitória mínima em extratos vegetais aquosos e etanólicos. *Arq Inst Biol* 81: 218-225.
- Cabana R, Silva LR, Valentao P, Vitorro CI, Andrade PB 2013. Effect of diferente extraction methodologies on the recovery of bioactive metabolites from *Satureja parvifolia* Phil. *Epling Lamiaceae*. *Ind Crops Prod* 48: 49-56.
- Carneiro NS, Alves CCF, Alves JM, Egea MB, Martins CHG, SILVA TS, Bretanha LC, Balleste MP, Micke GA, Silveira EV, Miranda MLD 2017. Chemical composition, antioxidant and antibacterial activities of essential oils from leaves and flowers of *Eugenia klotzschiana* Berg Myrtaceae. *An Acad Bras Cienc* 89: 1907-1915.
- Carvalho AF, Silva DM, Silva TRC, Scarcelli E, Manhani MR 2014. Avaliação da atividade antibacteriana de extratos etanólicos e de ciclohexano a partir das flores de camomila *Matricaria chamomilla* L.. *Rev Bras Pl Med* 16: 521-526.
- Cushnie TPT, Lamb AJ 2011. Recent advances in understanding the antibacterial properties of flavonoids. *Int J Antimicrob Agents* 38: 99-107.

- Desoti VC, Maldaner CL, Carletto MS, Heinz AA, Coelho MS, Piatí D, Tiunan TS 2011. Triagem fitoquímica e avaliação das atividades antimicrobiana e citotóxica de plantas medicinais nativas da região oeste do estado do Paraná. *Arq Ciênc Saúde UNIPAR* 15: 3-13.
- Doss A, Mubarak HM, Dhanabalan R 2009. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. *Indian J Sci Technol* 2: 41-43
- Fernández-Agulló A, Pereira E, Freire MS, Valentão P, Andrade PB, González Álvarez JÁ, Pereira JÁ 2013. Influence of solvent on the antioxidant and antimicrobial properties of walnut *Juglans regia* L. green husk extracts. *Ind Crops Prod* 42: 126-132.
- Gobbo-Neto L, LOPES NP 2007. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Quim Nova* 30: 374-381.
- Gonçalves AL, Filho AA, Menezes H 2005. Estudo comparativo da atividade antimicrobiana de extratos de algumas árvores nativas. *Arq Inst Biol* 72: 353-358.
- Gressler E, PIZO MA, Morellato P 2006. Polinização e dispersão de sementes em Myrtaceae do Brasil. *Revista Brasil Bot* 29: 509-530.
- Hwang D, Rothrock MJ, Pang H, Guo M, Mishra A 2020 Predicting *Salmonella* prevalence associated with meteorological factors in pastured poultry farms in southeastern United States. *Science of The Total Environment* 713: 136-167.
- Limberger RP, Sobral M, Henriques AT 2004. Óleos voláteis de espécies de *Myrcia* nativas do Rio Grande do Sul. *Quim Nova* 27: 916-919.
- Matos FJ 1997. A fitoquímica experimental. Fortaleza: UFC, 141 p
- Melo PS, Bergamaschi KB, Tiveron AP, Massarioli AP, Oldoni TLC, Zanús MC, Pereira GE, Alencar SM 2011. Composição fenólica e atividade antioxidante de resíduos agroindustriais. *Cienc Rural* 41: 1088-1093.
- Morais SM, Cavalcanti ESB, Costa SMO, Aguiar LA 2009. Ação antioxidante de chás e condimentos de grande consumo no Brasil. *Rev Bras Farmacogn* 19: 315-320.
- Nene RV, Cardoso CRP, Teixeira JR, Faustino PAS, Carvalho MR, Madaleno LL, Frigieri MC 2016. Atividade antimicrobiana de *Myrcia bella*, *Arrabidaea brachypoda* e *Hymenaea courbaril*. *Cienc Tecnol* 8.
- Paiva SR, Heringer AP, Figueiredo MR, Kaplan MAC 2002. Taninos condensados de espécies de plumbaginaceae. *Floresta Ambient* 9: 153-157.
- Pandini JA, Pinto FGS, Scur MC, Alves LFA, Martins CC 2015. Antimicrobial, insecticidal, and antioxidant activity of essential oil and extracts of *Guarea kunthiana* A. Juss. *J Med Plants Res* 9: 48-55.
- Pastene E, Gómez M, Speisky H, Nuñez-Vergara L 2009. Un sistema para la detección de antioxidantes volátiles comúnmente emitidos desde especias y hierbas medicinales. *Quim Nova* 32: 482-487.
- Paula JA, Paula JR, Bara MTF, Rezende MH, Ferreira HD 2008. Estudo farmacognóstico das folhas de *Pimenta pseudocaryophyllus* Gomes L.R. Landrum – Myrtaceae. *Rev Brasil Farmacogn* 18: 265-278.
- Pinelo M, Rubilar M, Sineiro J, Núñez MJ 2004. Extraction of antioxidant phenolic from almond hulls *Prunus amygdalus* and pine sawdust *Pinus pinaster*. *Food Chem* 85: 267-273.
- Pontes FC, Abdalla VCP, Imatomi M, Fuentes LFG, Gualtieri SCJ 2018. Antifungal and antioxidant activities of mature leaves of *Myrcia splendens* Sw. DC. *Braz J Biol* 1-6.
- Rossi F, Andreazzi DB 2005. Resistência bacteriana: interpretando o antibiograma, 1a ed., São Paulo: Atheneu.
- Ruíno MSM, Alves RE, Brito ES, Morais SM, Sampaio CG, Pérez-Jiménez J, Saura-Calixto FD 2007. Metodologia Científica: Determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. *Embrapa* 127: 1-4.
- Sakita MN, Aguiar OT 2006. Triagem fitoquímica e aspectos botânicos de *Gomidesia affinis* Camb. *Legr. e Gomidesia spectabilis* dc. *Berg. Biol* 6: 817-820.
- Saldanha LL 2010. Prospecção química e avaliação das atividades antioxidantes e alelopática de *Myrcia bella* Cambess. Dissertação de Mestrado, Universidade Estadual Paulista, Botucatu, Brasil.
- Samy RP, Gopalakrishnakone P 2010. Therapeutic potential of plants as antimicrobials for drug discovery. *Evid Based Complement Alternat Med* 7: 283-294.
- Santana CB 2017. Composição química, atividade antimicrobiana, inseticida e antioxidante do óleo essencial e extratos de *Myrcia oblongata* DC. Dissertação de Mestrado, Universidade Estadual do Oeste do Paraná, Cascavel, Brasil.
- Santana CB, Souza JGL, Coracini MDA, Walerius AH, Soares VD, Costa WF, Pinto FGS 2018. Chemical composition of essential oil from *Myrcia oblongata* DC and potential antimicrobial, antioxidant and acaricidal activity against *Dermanyssus gallinae* DEGEER, 1778. *Biosci J* 34: 996-1009.
- Santos C, Galaverna RS, Angolini CFF, Nunes VVA, Almeida LFR, Ruiz ALTG, Carvalho JE, Duarte RMT, Duarte MCT, Eberlin MN 2018. Antioxidative, antiproliferative and antimicrobial activities of phenolic compounds from three *Myrcia* species. *Molecules* 23: 986-998.
- Santos JAS, Sena TJO, Santos KBS, Costa MLA, Santos KCB, Santos AF 2018. Estudo do potencial antioxidante de *Anacardium occidentale* L. e determinação de seus compostos fenólicos. *Diversitas Journal* 3: 455-474.
- Scalbert A 1991. Antimicrobial properties of tannins. *Phytochem* 30: 3875-3883.
- Scherer R, Wagner R, Duarte MCT, Godoy HT 2009. Composição e atividades antioxidantes e antimicrobiana dos óleos essenciais de cravo-india, citronela e palmarosa. *Revista Brasileira Plantas Mediciniais* 11: 442-449.
- Scur MC, Pinto FGS, Bona EAM, Weber LD, Alves LFA, Moura AC 2014. Ocorrência and antimicrobial resistance of *Salmonella* serotypes isolates recovered from poultry of Western Paraná, Brazil. *Afr J Agric Res* 9: 823-830.
- Sher A 2009. Antimicrobial activity of natural products from medicinal plants. *J Med Sci* 7: 72-78.
- Silva JG, Souza IA, Hígino JS, Siqueira-Junior JP, Pereira JV, Pereira MSV 2007. Atividade antimicrobiana do extrato de *Anacardium occidentale* Linn. em amostras multiresistentes de *Staphylococcus aureus*. *Rev Bras Farmacogn* 17: 572-577.
- Silva MLC, Costa RS, Santana AS, Koblitz MGB 2010. Compostos fenólicos, carotenoides e atividade antioxidante em produtos vegetais. *Semin Cienc Agrar* 31: 669-682.
- Simões CMO *et al.* 2007. Farmacognosia: da planta ao medicamento. 6. ed. Porto Alegre UFRGS; Florianópolis Editora UFSC.
- Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR 2004. Farmacognosia: da planta ao medicamento in Portuguese. 5. ed. Porto Alegre UFRGS, Florianópolis Editora UFSC.
- Sousa CMM, Silva HR, Vieira-JR GM, Ayres MCC, Costa CLS, Araújo DS, Cavalcante LCD, Barros EDS, Araújo PBM, Brandão MS, Chaves MH 2007. Fenóis totais e atividade antioxidante de cinco plantas medicinais. *Quim Nova* 30: 351-355.
- Souza JGL, Pinto FGS, Toledo AG, Alves LFA, Alves DS 2020. Biological activities and phytochemical screening of leaf extracts from *Zanthoxylum caribaeum* L. Rutaceae. *Biosci J* 36: 223-234.
- Sucupira NR, Silva AB, Pereira G, Costa JN 2012. Métodos para determinação da atividade antioxidante de frutos. *UNOPAR Cienc Biol Saúde* 14: 263-269.
- Takahashi T, Kokubo R, Sakaino M 2004. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculata*. *Lett Appl Microbiol* 39: 60-64.
- Takao LK, Imatomi M, Gualtieri SCJ 2015. Antioxidant activity and phenolic content of leaf infusions of Myrtaceae species from Cerrado Brazilian Savanna. *Braz J Biol* 75: 948-952.
- Weber LD, Pinto FGS, Scur MC, JGL, Costa WF, Leite CW 2014. Chemical composition and antimicrobial and antioxidant activity of essential oil and various plant extracts from *Prunus myrtifolia* L. *Urb. Afr J Agric Res* 9: 846-853.
