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Original Article

Phytochemical prospection and evaluation of antimicrobial, antioxidant and antibiofilm activities of extracts and essential oil from leaves of *Myrsine umbellata* Mart. (Primulaceae)

Prospecção fitoquímica e avaliação da atividade antimicrobiana, antioxidante e antibiofilme de extratos e óleo essencial de folhas de *Myrsine umbellata* Mart. (Primulaceae)

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Abstract

The species *Myrsine umbellata* is a native plant of Brazil, whose barks are traditionally used in herbal medicine to treat liver disorders and combat leprosy. Therefore, the aim of the study was to identify the phytochemical prospection of ethanolic (EE) and acetonic (EA) extracts by colorimetric tests and by gas chromatography coupled to mass spectrometry (GC-MS) of the essential oil (EO) of *M. umbellata* leaves; evaluate the antimicrobial activity in front of standard ATCC strains by the broth microdilution technique; the antioxidant potential by DPPH reduction method and antibiofilm action by crystal violet assay and cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) based on optical density. Phytochemical prospection of EE and EA detected the presence of free steroids, alkaloids, flavonoids (flavones, flavononoids, flavonols and xanthons) and tannins in both extracts (EE and EA) and saponins only in EE. In EO, the majority compounds identified were elixene, caryophyllene (E), spatulenol, d-Cadinene and aromadendrene. EA showed antimicrobial activity with MIC and MBC/MFC values ranging from 3.12 to 100 mg.mL^{-1}, highlighting its efficiency on the Gram-positive strain *S. epidermidis*. EE showed antimicrobial potential in the range of 3.12 to 200 mg.mL^{-1}, and the Gram-negative *E. coli* strain was the most susceptible. However, OE showed bacteriostatic potential against *S.* Typhimurium, *S.* Abaetetuba, *P. aeruginosa*, and *S. epidermidis* strains. The ability to sequester free radicals was evident in EA extract with antioxidant activity of 89.55% and in EE with 63.05%. The antibiofilm potential was observed in EE extract which eradicated the mature biofilm biomass of all tested bacteria with high activity (50% to 84.28%) and EO also showed antibiofilm effect on mature biofilm of UEL enteroaggregative *E. coli*, *S. aureus* and *S.* Enteritidis strains with biomass reduction percentage of 63.74%, 68.04% and 86.19%, respectively. These results indicate the potential of *M. umbellata* extracts and as a source of plant bioactivity for the development of new alternative strategies for the control of planktonic or biofilm-resistant microorganisms.

Keywords: antimicrobial activity, antioxidant activity, biofilm, DPPH, *Myrsine umbellata*.

Resumo

A espécie *Myrsine umbellata* é uma planta nativa do Brasil, tradicionalmente suas cascas são empregadas em fitoterapia no tratamento de afecções do fígado e combate a hanseníase. Portanto, o objetivo do estudo foi realizar a prospecção fitoquímica dos extratos etanólico (EE) e acetônico (EA) por meio de testes colorimétricos e por cromatografia gasosa acoplada à espectrometria de massas (CG-EM) do óleo essencial (OE) das folhas de *M. umbellata*; avaliar a atividade antimicrobiana frente a dozes cepas padrões ATCCs pela técnica de microdiluição em caldo; o potencial antioxidante pelo método de redução de DPPH e ação antibiofilme pela técnica do cristal violeta e a viabilidade celular foi determinada usando 3- (4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio (MTT) com base na densidade óptica. A prospecção fitoquímica dos EE e EA detectou a presença de esteroides livres, alcaloides, flavonoides (flavonas, flavononóis, flavonóis e xantonas) e taninos em ambos os extratos (EE e EA) e de saponinas somente no EE. No OE os compostos majoritários identificados foram elixeno, cariofileno (E), espatulenol, d-cadineno e aromadendreno. O EA apresentou atividade antimicrobiana com valores de CIM e CBM/CFM que variaram de

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3,12 a 100 mg.mL^{-1}, destacando sua eficiência sobre a cepa Gram-positiva *S. epidermidis*. Já EE apresentou potencial antimicrobiano na faixa que variou de 3,12 a 200 mg.mL^{-1}, e a cepa Gram-negativa *E. coli* foi a mais suscetível. Entretanto, OE apresentou potencial bacteriostático frente às cepas *S.* Typhimurium, *S.* Abaetetuba, *P. aeruginosa* e *S. epidermidis.* A capacidade de sequestrar radicais livres foi evidenciada no extrato EA com atividade antioxidante de 89,55% e no EE com 63,05%. O potencial antibiofime foi observado no extrato EE que erradicou a biomassa do biofilme maduro de todas as bactérias testadas com elevada atividade (50% a 84,28%) e OE também apresentou efeito antibiofilme sobre o biofilme maduro das cepas *E. coli* enteroagreativa UEL, *S. aureus* e *S.* Enteritidis com percentual de redução de biomassa de 63,74%, 68,04% e 86,19%, respectivamente. Esses resultados indicam o potencial dos extratos e do OE de *M. umbellata* como fonte de bioativos vegetais para o desenvolvimento de novas estratégias alternativas para o controle de microrganismos resistentes de forma planctônica ou de biofilmes.

Palavras-chaves: atividade antimicrobiana, atividade antioxidante, biofilme, DPPH, *Myrsine umbellata*.

1. Introduction

In recent years there has been a significant increase in bacterial and fungal infections in both humans and animals, due to the selection of microorganisms with high resistance to already commercialized antimicrobials, such as antibiotics and antifungals. In addition to antimicrobial resistance, some bacterial cells are also capable of producing extracellular polymer matrices called biofilms, which provide protection from the external environment and allow better adhesion to surfaces. Therefore, this reduces susceptibility to synthetic antimicrobials, making them more resistant in extreme condition environments. In industries, when biofilms are present on equipment, they cause surface deterioration and/or proliferation of pathogenic microorganisms, generating losses. In the hospital environment, biofilms increase the mortality rate due to diseases caused by the resistance of bacteria to antimicrobials and disinfectants (Bezerra et al., 2017; Frozi et al., 2017).

Therefore, the biological potential of plants is increasingly being studied, through the use of plant extracts and essential oils, which have already shown valuable results, due to the presence of secondary metabolites that are substances with numerous therapeutic properties. In this context, products of plant origin may be a possible solution to the current problem related to synthetic compounds and resistance to pathogens. Furthermore, these natural products can also be an alternative to replace or reduce the amount of synthetic preservatives used in the food and cosmetic industry. Thus, the active principles of plants are being widely studied and patented regarding their antimicrobial, antioxidant, and antibiofilm properties (Gyawali and Ibrahim, 2014; Gonçalves et al., 2015).

The family Primulaceae contains 58 genera and nearly 2,600 species. The genus Myrsine L. (synonym *Rapanea* Aubl.) comprises about 300 species with a pantropical distribution, with 25 species recorded among all regions of Brazil (Ricketson and Pipoly, 1997; Freitas, 2020).Species within this genus show antibacterial properties: *R. parviflora* (A.Dc.) Mez (Suffredini et al., 2007) and *Rapanea sp*. (Montovani et al., 2009), antifungal: *R. melanophloeos* (L.) Mez (Ohtani and Hostettmann, 1992), *M. umbellata* Mart., and *M. coriacea* (Sw.) R.Br. ex Roem. & Schult. (Miranda et al., 2015), anti-inflammatory: *M. guianensis* (Aubl.) Kuntze (Ospina et al., 2001), antioxidant: *M. guianensis* (Ospina et al., 2001), and *M. coriacea* (Miranda et al., 2015), insecticidal: *M. umbellata*, and *M. coriacea* (Miranda et al., 2015) and anthelmintic: *M. africana* L., and *R. melanophoeos* (L.) (Githiori et al., 2002).

The species *Myrsine umbellata* Mart. is a Brazilian native arboreal plant, popularly known as capororoca and its distribution is wide with occurrence form in all regions of Brazil. The barks presented medicinal applications in diuretic treatment, urinary tract infections, liver problems and also as a remedy for the treatment of leprosy (Farias et al., 1993; Lorenzi, 2009; Freitas, 2020). The biological activities of the leaves of this species are still scarce, so far with reports addressing only its antifungal and insecticidal potential (Miranda et al., 2015).

Studies with plant bioactives have great value since they are an alternative to the use of synthetic products in the pharmaceutical and food industries, and also to the problems faced with biofilm formation and antimicrobial-resistant pathogens. Given this, research with plant extracts and essential oil from native Brazilian plant species is relevant for the scientific validation and appreciation of the regional flora.

This study aims to determine the chemical composition, evaluate the antimicrobial, antioxidant and antibiofilm potential of plant extracts and essential oil from *Myrsine umbellata* leaves against standard strains of clinical importance.

2. Materials and Methods

2.1. Collection and identification of the leaves of M. umbellata

M. umbellata leaves were collected in the Paulo Gorski Ecological Park, located in Cascavel, Paraná, Brazil (24º57'51.61'S and 53º26'14.80'W) between the months of August 2019 and March 2020, in the morning, in the early hours of the sunny day and with temperature around 20ºC, humid subtropical climate, between late winter and early autumn, phenological state of the vegetative plant and collection time around one hour. The plant was identified by Lázaro Henrique Soares de Moraes Conceição, exsiccate was delivered to the Herbarium of the State University of Western Paraná (UNOP) for botanical identification and registration UNOP 10731.

2.2. Obtaining of plant extracts

The leaves of *M. umbellata* were dried in an air oven at 40°C for 72 hours, and subsequently ground in a Willey-type knife mill to a particle size of 0.42 mm. Plant extracts were prepared according to the methodology proposed by Pandini et al. (2015) and Santana et al. (2021), with

modifications. Dried plant material (10 g) was extracted with different solvents (100 mL): ethanol P. A. (EE) and acetone P. A. (EA). These liquid preparations were kept in a rotary shaker at 220 rpm for 24 hours. After this period, the solutions were filtered using Whatman nº1 filter paper and then centrifuged at 5000 rpm for 15 minutes. Then, the supernatant was collected and submitted to rotoevaporation in a rotary evaporator, for total elimination of solvents, obtaining the organic raw extracts at the end of the process. The acetonic (EA) and ethanolic (EE) extracts were stored away from the light in a freezer at 4ºC. The yield of the plant extracts was calculated by the expression: Percentage (%) = (extract mass (g) / dried and ground vegetable mass (g)) x 100.

2.3. Extraction of essential oil

The essential oil of *M. umbellata* (OE) was obtained by the hydro distillation method using the Clevenger type equipment, according to the methodology proposed by Weber et al. (2014), with modifications. Distilled water 1:10 (w/v) was added from the dried and ground sample and the solution was hydro distilled for 5 hours. After extraction, the essential oil was stored in conical bottom tubes surrounded by aluminum foil, shielded from light and at an average temperature of 4°C until the experiment was carried out. The percentage yield of the essential oil (%) was calculated by the equation: %= (Total extracted essential oil (grams) / dried and ground vegetable mass (grams)) x 100.

2.4. Chemical Analysis of Essential Oil- Gas Chromatography coupled to Mass Spectrometry (GC-MS)

The analysis of Gas Chromatography coupled to Mass Spectrometry (GC-MS) was performed by the Laboratory of Gas Chromatography coupled to Mass Spectrometry - COMCAP, of the State University of Maringá (UEM), Paraná, Brazil. For the analysis of the EO constituents of *M. umbellata* was performed from the CG-EM Thermo-Finnigan system, composed of a gas chromatograph GC FOCUS (Thermo Electron), coupled to a mass spectrometer DSQ II (Thermo Electron) and an automatic injector Triplus AS (Thermo Electron). Chromatographic separation was performed with an HP-5ms fused silica capillary column (30 m long, 0.25 and 0.25 a film ID; 5% phenyl-95% dimethyl polysiloxane composition). The temperature of the injector was degrees 250ºC. Samples and alkali patterns were injected with a split rate of 1:25. The temperature programming used was 50ºC maintained for 2 minutes, increasing the temperature to 180ºC at a ratio of 2ºC. min^{-1} and followed by an increase to 290ºC at a ratio of 5ºC. min^{-1}. The interface between GC and MS was maintained at 270ºC and the ionization source temperature of the mass spectrometer was 250ºC. The identification of the components was made by comparing the retention times with those obtained in the literature for the same compounds analyzed using the retention index (RI) (Adams, 2007; Babushok et al., 2011; Yu et al., 2007).

2.5. Phytochemical prospecting of extracts

The phytochemical tests of the EA and EE plant extracts of *M. umbellata* were performed 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.

2.6. Microorganisms used

The experimental trials with EA, EE and EO were conducted at the Laboratory of Microbiology and Biotechnology (LAMIBI) of the State University of Western Paraná (UNIOESTE), Cascavel, PR, BR. Different strains from the American *Type Culture Colletion* (ATCC) collection and the Cefar Diagnostic Culture (CCCD) collection were used for the assay, with seven Gram-negative strains: *Escherichia coli* (ATCC 25922), *Salmonella* enteritidis (ATCC 13076), *Salmonella* Typhimurium (ATCC 14028), *Salmonella* Abaetetuba (ATCC 35640), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 25933) and *Klebsiella pneumoniae* (ATCC 13883); four gram-positive: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 19433), *Staphylococcus epidermidis* (ATCC 12228) and *Bacillus subtillis* (CCD-04); a yeast *Candida albicans* (ATCC 10231) (Scur et al., 2016).

For the assays, the microorganisms were recovered in enrichment broth with Brain Heart Infusion (BHI) and incubated for 24 hours at 36± 1ºC. After this period, the strains were striated in Muller Hinton Agar (MH) and incubated at 36± 1ºC for 24 hours. To standardize the inoculum the strains were diluted in saline solution (0.85%), resulting in the final concentration of $1x10^5$ CFU. mL^{-1} for bacteria and $1x10^6$ $CFU.mL^{-1}$ for *C. albicans* yeasts. The inoculum was compared by turbidity with the reading pattern 0.5 on the scale McFarland and the absorbance of suspension spectrophotometer (A600 = 0.3) (Scur et al., 2016).

2.7. Testing of antimicrobial activity

2.7.1. Minimum Inhibitory Concentration (MIC)

The assays were performed according to the broth microdilution methodology described by Weber et al. (2014), Pandini et al. (2018) and CLSI (2015), with modifications. The EA, EE and EO of *M. umbellata* were solubilized in methanol P.A.. In 96-well microdilution plates were distributed in the first column 300 µl of the solution of vegetable extract or essential oil with MH broth and from the second column 150 µl of MH broth per well for bacteria and broth of the Roswell Park Memorial Institute (RPMI) for yeast *C. albicans*. Then, serial dilutions were performed, obtaining concentrations ranging from 200 to 0.09 $mg.mL^{-1}$ for plant extracts and from 7000 to 1.7 $µg.mL^{-1}$ for essential oil. At the end, 10 µl of inoculum were added to each well and the plates were incubated at 35± 1ºC within 18-24 hours. For the positive control, the commercial antibiotic Gentamicin and the commercial antifungal Nystatin were used in the same concentrations of the tests. As a negative control, the inoculum was added in broth of MH (bacteria) and RPMI (yeast), without the presence of EA and EE and OE to verify the viability of the microorganism tested. It was also performed the sterility control of extracts and essential oils solubilized in methanol P.A. which consisted of the solution of extract

or oil diluted in methanol and MH (bacteria) and RPMI (yeast) broth without the presence of inoculum. Finally, the diluent was controlled with methanol and MH (bacteria) and RPMI (yeast) broth and inoculum to verify if there was interference of the diluent in the test. Trifeniltetrazole chloride (TTC) from 20 µl to 0.5% (35± 1ºC for 15 min.) was used as a colorimetric indicator of microbial metabolism, in the presence of the red color was considered negative evidence of inhibition effect of plant extracts and essential oil. Three experiments of triplicate MIC were carried out, being possible to determine the lowest concentration of EA, EE and OE capable of inhibiting microbial growth.

2.7.2. Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

For the trial the methodology of Weber et al. (2014) was performed, with modifications. Before the addition of the TTC 0.5% to determine the MIC, an aliquot of 2 µl was removed from each well of the microdilution plate and sown on the surface of the MH agar. The plates were incubated at 35± 1ºC within 18-24 hours. The trial was performed in triplicate, and to determine the MBC/MFC it was observed if there was microbial growth in MH agar, allowing to verify the lower concentration of plant extracts and essential oil capable of causing the death of the bacterium/fungus tested.

2.8. Antioxidant activity

The antioxidant activity of EA, EE and EO were performed according to the method of reduction of the free radical 2,2-diphenyl-1-picril-hydrazyl (DPPH), proposed by Rufino et al. (2007) and Pandini et al. (2015). Initially, a calibration curve (0 to 60 µM DPPH) was performed to obtain the concentration of DPPH in the medium after the reaction with plant extracts and essential oil. Then, the plant extracts and the essential oil were solubilized in methanol P. A., obtaining concentrations ranging from 0.1 to 15 $mg.mL^{-1}$. An aliquot of 0.1 mL of these plant extracts and essential oil was added to 3.9 mL of methanol solution of DPPH (60 µM) and homogenized in tube stirrer. Methanol P. A. added to DPPH was used as a negative control, and the synthetic antioxidant BHT (butyl-hydroxy-toluene) (0.10 to 1.0 $mg.mL^{-1}$) was used as a positive control. The tests were measured in a spectrophotometer at 515 nm at 1 minute reading intervals until absorbance stabilization. All concentrations were defined from the pre-test. As white, methanol was used for the calibration of the spectrophotometer. The percentage of free radical sequestration (AA%) was expressed by the equation: AA%= ((A0-A1)) / A1 x 100, where A0 is the absorbance of negative control and A1 is the absorbance of the sample. For the calculation of IC50 (amount of antioxidant substance needed to reduce the initial DPPH concentration by 50%), the concentrations of the treatments were used to obtain the straight line equation with R^2 greater than 0.80, and thus found the IC50 value, from a linear regression. The tests were performed in triplicate and expressed with mean standard deviation. The IC50 results were analyzed using ANOVA with Tukey test ($p<0.05$) using the SISVAR 2011 statistical program. Then, the extracts were related to the antioxidant activity index (AAI) calculated by the equation AAI= AA%/ IC50 and classified according to the AAI into low (AAI <0.5), moderate (AAI between 0.5 and 1.0), strong (AAI between 1.0 and 2.0) and very strong (AAI > 2.0) according to the methodology proposed in Scherer and Godoy (2009).

2.9. Antibiofilm activity

2.9.1. Microorganisms

Different strains from the *American Type Culture Collection* (ATCC) collection were used for the assay, Gram-negative strains: *Escherichia coli* (ATCC 25922), *Escherichia coli* enteroagreativa UEL, *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* Typhimurium (ATCC 14028) and *Salmonella* Enteritidis (ATCC 13076); Gram-positive strains: *Staphylococcus aureus* (ATCC 25923), are the main etiological bacterial agents responsible for a number of infectious diseases and show greater resistance to antimicrobials. Negative control was used BHI supplemented with glucose 1% and inoculum of the tested strains; and as interference control of color of EA, EE, EO and BHI in the different concentrations of these diluted in dimethyl sulfoxide (DMSO) and BHI. And the control of the diluent DMSO to verify if there was interference of the same in the test.

2.9.2. Bacterial biofilm activity test

The test was performed in 96-well microplates (flat bottom) with polystyrene surface. To standardize the inoculum, the strains were diluted 1:100 (10^8 CFU/mL) in saline solution (0.85%), the turbidity of the bacterial suspension was adjusted as the reading pattern on the scale of 0.5 McFarland, then transferred an aliquot of 50 µl in 4,950 mL of BHI broth supplemented with glucose 1% resulting in a final concentration of 0.5 X 10^6 $CFU.mL^{-1}$. In each well of the microplates were distributed 180 µl of BHI medium supplemented with 1% glucose and 20 µl of bacterial inoculum for biofilm formation and these were incubated for 24 hours at 37± 1ºC until reaching the late exponential phase (24 hour) to allow cell fixation and biofilm formation. The evaluation of biofilm formation was performed by reading the optical density (OD) of 570 nm using an microplate reader. Based on the OD produced by biofilms, bacterial strains were classified into the following categories: OD ≤ ODc = no biofilm producer; ODc < OD ≤2X ODc = weak biofilm producer; 2X ODc < OD ≤ 4X ODc= moderate biofilm producer; 4X ODc <OD = strong biofilm producer. The plates that presented strong biofilm production in 24 hours of formation were used for the assay. After the incubation period, the culture medium was removed and the wells washed once with buffered phosphate saline solution (PBS 1X), and then added 150 µl of a new BHI medium containing EA, EE (200 to 1.56 $mg.mL^{-1}$) and OE (7000 to 54.68 $µg.mL^{-1}$). The material was incubated again at 37± 1ºC for 24 h (Stepanović et al., 2007).

2.9.3. Quantification of biofilm biomass by crystal violet

After incubation, the medium was gently aspirated, and the wells were washed three times with PBS 1X. Then, the wells received 150 µl of methanol P.A. for 20 minutes to fix the sample, after the methanol was removed 150 µl

of 2% crystal violet was added for 15 minutes. Then the wells were washed in 1X PBS until the total dye output. After drying, 150 µl of 95% ethanol was added and left in contact for 30 minutes, then the contents of the wells were transferred to a new plate and the optical density reading (OD) was performed at 570 nm using Biotek microplate reader, Epoch model. The data were calculated in general mean OD of biofilm biomass quantified by staining using crystal violet (Christensen et al., 1985; Bandeira et al., 2022). The percentage of eradication of biofilm was determined by the equation: Percentage of eradication (%): ((OD negative control – OD experimental) / OD negative control) X 100 in the Microsoft Excel 2010 program. The values of eradication percentages were classified as follows: (< 50%) indicate low antibiofilm activity and (> 50%) high antibiofilm activity (Sandasi et al., 2008; Famuyide et al., 2019).

2.9.4. Biofilm cell viability test

The activity of the extracts and the oil on the biofilm was measured by the reduction (%) of the absorbance value when compared with the negative control, since the sample tested is a biofilm producer. As a criterion to express the results of the antibiofilm potential of EA, EE and OE of *M. umbellata*, both the biofilm biomass test by crystal violet assay and the cell viability by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test (MTT) were determined from the MIC concentrations, 2X MIC and 4X MIC of each tested bacterial strain. After the methanol was removed, a 180 µl aliquot of BHI and 20 µl of MTT were added to each well, and the plates were incubated in the dark at 37ºC for 2 hours. After, the medium was removed and added 150 µl of DMSO for 15 minutes. The supernatants were then transferred to a new plate and the optical density was measured at 570 nm in Epoch-type Biotek microplate reader. The cell viability data of mature biofilms were expressed in general mean OD and the percentage of cell viability (CV%) was determined by the equation: (OD experimental /OD negative control) x 100 (Jia et al., 2011; Famuyide et al., 2019). The values of the percentage of cell viability were classified as: (< 50%) indicate low cellular activity and (> 50%) indicate high cellular activity (Sandasi et al., 2008).

3. Results and Discussion

3.1. Yield, chemical composition of essential oil and phytochemical prospecting of plant extracts

Based on obtaining plant extracts from *M. umbellata* leaves with different solvents, the following yields were obtained: EE (13.26%) EA (8.39%) of EO from *M. umbellata* leaves. The yield of different plant extracts can be influenced by several factors, such as temperature, extraction time, solid-solvent ratio, and most importantly the choice of the solvent extracted, which, due to its chemical characteristics (e.g. different solubility and polarity), influence its yield (Fernández-Agulló et al., 2013).

Phytochemical prospection of *M. umbellata* plant extracts revealed the presence of compounds belonging to the classes of free steroids, alkaloids, flavonoids (flavones, flavanols, xanthons, and flavanonols) and tannins in both extracts (EE, EA), and saponins only in EE (Table 1).

Although there are no reports in the literature on phytochemical prospecting of plant extracts from *M. umbellata* leaves, several secondary metabolites from different species of *Myrsine* genus have already been identified and isolated, such as flavonoids (*M. seguinii* H. Lév.; *M. coriacea*) (Zhong et al., 1997; Alves et al., 2012), terpenes (*R. lancifolia* (Mart.) Mez; *R. guianensis* Aubl.) (Januário et al., 1992), saponins (*R. melanophloeos*) (Ohtani and Hostettmann, 1992), tannins (*M. coriacea*) (Alves et al., 2012), and triterpenoids (*M. africana* L.; *R. melanophloeos* L.) (Manguro et al., 1997; Ohtani and Hostettmann, 1992).

In the literature, the phytochemical prospecting study of organic extracts of *Myrsine coriacea* leaves was reported and the classes of compounds were identified as: flavones, flavonols, xanthons, tannins, saponins, and phenolic compounds (Alves et al., 2012), confirming the results obtained in our study, since these classes of compounds were also identified in the extracts of *M. umbellata*.

Differences between compounds found in species of the same family, genus, and species, depending on the location of the plant and regional environmental factors such as seasonality, temperature, climatic factors, pH, soil, water availability, collection time, extraction method, choice of solvent and its polarity characteristics, among others, which are key in the extraction of bioactive compounds with biological potential (Fernández-Agulló et al., 2013; Toledo et al., 2020).

The chemical composition of the EO of *M. umbellata*, along with its retention indices, is expressed as shown in Table 2. Thirty-six compounds, corresponding to 93.05% of the essential oil, were identified by GC-EM. The analysis of the chemical components of *M. umbellata* essential oil demonstrated the presence of the compounds: elixen

Table 1. Phytochemical prospection of secondary metabolites present in ethanolic and acetonic extracts of *M. umbellata* leaves.

Secondary metabolites	EE	EA
Saponins	+	-
Free Steroids	+	+
Triterpenoid	-	-
Alkaloids	+	+
Antocyanins	-	-
Antoyanidinis	-	-
Flavones	+	+
Flavanols	+	+
Xanthones	+	+
Chalconas	-	-
Auronas	-	-
Flavanonols	+	+
Condensed tannins	+	+
Coumarins	-	-

(+)presence; (-)absence, EE: ethanolic extract; EA: acetonic extract.

Table 2. Chemical composition of the essential oil of the leaves of *M. umbellata* obtained by hydrodistillation and analyzed by CG-EM.

Number	Composed	Area (%)	TR	IR	IR*
1	α-Thujeno	0.15	8.87	924	928
2	α-Pinena	2.42	9.20	930	936
3	Myrcene	0.29	12.10	989	989
4	m-Cimeno	0.24	14.02	1023	1022
5	D-Limonene	0.17	14.31	1028	1030
6	Terpineno-4-ol	0.19	23.99	1179	1177
7	δ-Elemeno	0.97	34.20	1334	1337
8	α-Cubebene	0.62	34.97	1346	1351
9	α-Ylangene	0.20	36.34	1367	1370
10	Isoledene	0.15	36.45	1369	1373
11	α-Copaeno	1.70	36.74	1373	1376
12	β-Elemeno	1.22	37.66	1387	1390
13	α-Gurjuneno	1.29	38.70	1404	1408
14	Caryophyllene, (E) **	8.91	39.40	1415	1420
15	γ-Elemeno	2.82	40.18	1428	1436
16	Aromadendrene **	5.12	40.58	1434	1440
17	α-Humulene	3.14	41.59	1451	1453
18	Alloaromadendrene	0.46	41.88	1456	1460
19	γ-Muuroleno	4.07	42.93	1473	1476
20	Germacrene D	2.85	43.19	1477	1481
21	β-Selineno	2.58	43.64	1484	1486
22	Viridifloren	2.23	43.88	1488	1492
23	Elixeno [2] **	15.62	44.13	1492	1492
24	α-Muuroleno	1.18	44.39	1496	1498
25	γ-Cadinene	2.65	45.19	1510	1513
26	δ-Cadinene **	6.30	45.58	1517	1520
27	Selina-3,7(11)-diene	0.59	46.80	1538	1540
28	Germacrene B	4.06	47.76	1554	1551
29	Nerolidol, (E)-	0.46	48.17	1561	1561
30	Espatulenol **	6.95	48.82	1572	1576
31	Globulol	3.59	49.34	1581	1582
32	Globulol, epi-	3.22	49.81	1589	1585
33	α-Cadinol, epi-	0.98	52.60	1639	1595
34	α-Muurolol, epi -	1.12	52.71	1641	1638
35	α-Muurolol	0.35	52.88	1644	1641
36	α-Cadinol	2.03	53.33	1652	1643
	NI	1.10	34.00	1330	1652

TR: Retention Time; IR: Values of Calculated Retention Indices. IR*: Values of Retention Indices found in the literature. **Compound Majorities; NI: Not identified.

(15.62%), caryophyllene (E) (8.91%), spatulenol (6.95%), d-Cadinene (6.30%), and aromadendrene (5.12%), all of these sesquiterpenes were more abundant in the sample (as shown in Table 2).

In the family Primulaceae, the species of the genus *Myrsine* predominantly present the compounds of the sesquiterpenes group, and in less quantity monoterpenes are observed (Sardans et al., 2010; Luna et al., 2014).

Sesquiterpenes were identified as major compounds in the leaf essential oil as well as in fruits of *M. coriacea*, *M. venosa* A.DC., *M. lessertiana* A.DC., and *M. sandwicensis* A.DC. (Luna et al., 2014; Sardans et al., 2010). Corrêa (2017) found eighteen substances in the essential oil of *M. parvifolia* A.DC. leaves considering as majorities: caryophyllene oxide (14.4%), β-caryophyllene (12.6%), and γ-muurolene (7.9%).

The phytochemical profile of essential oils can differ among species of the same genus in quantity, number of compounds, and molecular configuration, according to variation in climatic factors, geographical location, soil composition, plant organ, age and stage of the vegetative cycle, genetic diversity, seasonality, circadian rhythm, water availability, nutrients, protection against pathogens, among others (Gobbo-Neto and Lopes, 2007).

3.2. Antimicrobial activity

Regarding antimicrobial activity in the microdilution broth assay, the EA, EE, and EO of *M. umbellata* leaves were tested for their ability to inhibit growth (Minimum Inhibitory Concentration- MIC) or cause death (Minimum Bactericidal Concentration- MBC/ Minimum Fungicidal Concentration- MFC) of microorganisms (as shown in Table 3). The MIC and MBC/MFC of the plant extracts were classified according to Araújo (2010) and Pandini et al. (2015), with the activity classified as: high (<12.5 $mg.mL^{-1}$), moderate (12.5 to 25 $mg.mL^{-1}$), low (50 to 100 $mg.mL^{-1}$) and very low (>100 $mg.mL^{-1}$). On the other hand, the essential oil followed the classification proposed by Sartoratto et al. (2004) and was considered as high (<100 $\mu g/mL^{-1}$), moderate (100 to 500 $\mu g/mL^{-1}$), low (500 to 1000 $\mu g/mL^{-1}$) and very low (>1000 $\mu g/mL^{-1}$). Antimicrobial activity was observer to vary according to the type of extraction, solvents used, and microorganisms tested.

Both plant extracts of *M. umbellata* showed antimicrobial potential when tested against the twelve standard pathogenic strains (ATCCs). On the other hand, EO presented only bacteriostatic potential against *S.* Typhimurium, *S.* Abaetetuba, *P. aeruginosa* and *S. epidermidis* strains, and did not show antimicrobial activity against the other strains tested (as shown in Table 3).

Regarding the antimicrobial activity of the plant extracts of *M. umbellata* leaves against standard ATCC strains ATCC it was observed that EA showed the best antimicrobial activity when compared to EE and EO, with MIC and MBC/MFC values ranging from 3.12 to 100 $mg.mL^{-1}$, classified as high to moderate and moderate to low, respectively, highlighting their efficiency on the Gram-positive strain *S. epidermidis* with MIC of 3.12 $mg.mL^{-1}$ and MBC of 25 $mg.mL^{-1}$. Subsequently, EE showed the second-best antimicrobial activity being classified with high and moderate MIC, and moderate, low, and very low MBC. This extract was effective for the Gram-negative *E. coli* strain (MIC of 3.12 $mg.mL^{-1}$ and MBC of 12.5 $mg.mL^{-1}$). The antimicrobial potential of EA and EE extracts against standard ATCC strains may be related to their phytochemical profile, mainly by the presence of flavonoid, alkaloid, tannin and saponin classes. The steroids present in both extracts tested are terpenic compounds, and although the mechanism of action is not fully elucidated, it is believed that they break the cell membrane of microorganisms by interaction with lipophilic compounds (Silva et al., 2014).

However, the flavonoids (flavones, flavonols, xanthons and flavonols) found in EE and EA are hydroxylated phenolic

Table 3. Minimum Inhibitory Concentration (MIC) and Bactericidal Concentration (MBC) or Minimum Fungicidal Concentration (MFC) of ethanolic and acetonic extracts and essential oil of *M. umbellata* leaves in front of different standard strains.

Strains	MIC/MBC	($mg.mL^{-1}$)	EO
	EE	EA	
Gram-positive			
Staphylococcus aureus (ATCC 25923)	12.5/200	25/100	-/-
Staphylococcus epidermidis (ATCC 12228)	12.5/200	3.12/25	437.5/-
Enterococcus faecalis (ATCC 19433)	6.25/25	6.25/25	-/-
Bacillus subtilis (CCC B005)	6.25/25	3.12/50	-/-
Gram-negative			
Salmonella enterica Typhimurium (ATCC 14028)	6.25/25	12.5/25	875/-
Salmonella enterica Enteritidis (ATCC 13076)	12.5/100	6.25/12.5	-/-
Salmonella enterica Abaetetuba (ATCC 35640)	12.5/25	6.25/12.5	875/-
Escherichia coli (ATCC 25922)	3.12/25	3.12/12.5	-/-
Klebsiella pneumoniae (ATCC 13883)	3.12/25	12.5/50	-/-
Proteus mirabilis (ATCC 25933)	3.12/25	3.12/50	-/-
Pseudomonas aeruginosa (ATCC 27853)	3.12/25	3.12/50	218.75/-
Yeast	CIM/CFM		
Candida albicans (ATCC 10231)	6.25/25	12.5/50	-/-

(-) No activity. EE: ethanolic extract; EA: acetonic extract; EO: essential oil.

substances and their mode of antimicrobial action happens by forming complexes with extracellular and soluble proteins, which, when binding to the bacterial wall, cause irreversible damage to the cells. Additionally, they can cause perforation and reduced fluidity of the plasma membrane and finally inhibit nucleic acid synthesis and/ or inhibit energy metabolism (Cushnie and Lamb, 2011).

The alkaloids identified in EE and EA are nitrogenous substances that inhibit the action of Gram-negative bacteria that cause cell lysis and morphological changes in microorganisms (Fumagali et al., 2008). Tannins, also identified in all the extracts tested, are water-soluble phenolic compounds and polar solvents, which, when precipitating proteins, have antifungal and antimicrobial effects, and their mode of action may be related to their ability to inhibit microbial adhesins and bacterial enzymes. They can also act on the cell membranes of microorganisms, hindering the entry of ions essential to microbial metabolism (Loguercio et al., 2005).

As for the saponins present only in the extract EE, the amphiphilic behavior, and the ability to form complexes with membrane steroids, proteins, and phospholipids, allows their antimicrobial actions, and can alter the permeability of bacteria cell membranes, even leading to their destruction (Gyawali and Ibrahim, 2014).

Finally, this was the first report on the antimicrobial potential of *M. umbellata* extracts against standard ATCC strains. In the literature, antimicrobial activity of plant extracts of Myrsine species has been demonstrated with the ability to inhibit bacterial growth of *S. aureus*, which reinforces the results found in the present study (Montovani et al., 2009).

3.3. Antioxidant activity

The antioxidant capacity of EE, EA, and EO of *M. umbellata* was determined by DPPH-free radical sequestration assay. The commercial antioxidant butylated hydroxytoluene (BHT) was used as a positive control. Concentrations ranging from 0.1 to 15 mg.mL^{-1} and for extracts and EO and BHT control ranging from 0.1 to 1.0 mg.mL^{-1} were tested. The results expressed refer to the concentration of 1.0 mg.mL^{-1}, which showed the most expressive results (as shown in Table 4).

EA at the concentration of 1.0 mg.mL^{-1} showed the highest percentage of DPPH radical sequestration when compared to the others, with an antioxidant percentage of 89.55% and IC50 value of 0.47 mg.mL^{-1}. Followed by EE with an antioxidant percentage of 63.05%, with IC50 value of 0.59 mg.mL^{-1}. EO in turn showed the lowest percentage of radical sequestration with an antioxidant percentage of 10.46% and IC50 value of 0.03 mg.mL^{-1}. These data demonstrate the need for a higher concentration of essential oil to sequester the same amount of DPPH radicals when compared to BHT and other extracts.

According to the classification proposed by Scherer and Godoy (2009), extracts from *M. umbellata* plants showed a strong antioxidant activity index (AAI) for EA (AAI 1.90), moderate for EE (AAI 0.59), and weak for *M. umbellata* EO (AAI 0.03). The literature reports the antioxidant activity of *M. coriacea* extracts with high antioxidant activity, which corroborates the results found in this study (Miranda et al., 2015).

Studies on the antioxidant potential of plant extracts and essential oil of *M. umbellata* have not been reported in the literature, with this being the first report. The excellent antioxidant activity of these extracts can be explained by the presence of phenolic compounds, mainly flavonoids, which are great antioxidants and have an ideal structure for radical sequestration and reduction of the redox potential of the medium (Jing et al., 2012).

3.4. Evaluation of the activity of plant extracts and essential oil on bacterial biofilm

The activity of EE, EA, and EO extracts was evaluated on standard bacterial biofilms. Statistical analysis of biomass and cell viability data was performed using ANOVA and Tukey tests when data were in normality, and Kruskal-Wallis and Dunn tests when data were not in normal distribution. There was no significant difference between the different concentrations tested (MIC, 2X MIC and 4X MIC); therefore, the results expressed refer to the action of MIC on the biofilm. Therefore, the results expressed refer to the action of the MIC on the mature biofilm, where statistically significant differences were observed when compared to the treatment and control groups by the t-test.

The results showed that EE had the best antibiofilm activity (Table 5), as it showed biofilm disrupturion rates for all the tested bacterial strains. The percentage of biofilm eradication ranged from 50% to 84.28%, being most efficient against the biofilm formed by *S. aureus*. The phytochemical compounds saponins, free steroids, alkaloids, flavonoids, and tannins present in EE demonstrate potential for the eradication of biofilm biomass already formed. These compounds act by disaggregating consolidated colonies, dehydrating the cell wall, preventing nutrient replacement, and breaking the structure of already formed biofilms (Cushnie and Lamb, 2011; Nuño et al., 2018).

Table 4. Percentage sequestration of radical DPPH and IC_{50} value by DPPH assay of plant extracts and essential oil of *M. umbellata* leaves.

Concentration (mg.mL^{-1})	BHT	EE	EA	EO
1.0	97.20±0.30	63.05±0.55*	89.55±0.58*	10.46±0.23*
IC_{50}	0.46±0.00	1.44±0.02	0.47±0.00	15.21±2.23
AAI	2.11	0.59	1.90	0.03

Absorbance mean (%) standard deviation. *Expresses significant difference between extracts and essential oil when compared to BHT (Tukey test (p<0.05). IC50: 50% inhibition concentration of DPPH; AAI: Antioxidant Activity Index. BHT: butylated hydroxytoluene; EE: ethanolic extract; EA: acetonic extract; EO: essential oil.

Table 5. Effect of *M. umbellata* ethanolic extract on eradication and viability of biofilms of standard bacteria.

Microorganisms	EE	CV	MTT
		ERADICATION	VIABILITY
Staphylococcus aureus (ATCC 25923)	CONTROL	3.88±0.08	0.32±0.06
	MIC	0.61±0.29*	0.10±0.05*
	%	84.28	31.25
Pseudomonas aeruginosa (ATCC 27853)	CONTROL	3.49±0.17	0.28±0.04
	MIC	1.17±0.12*	0.20±0.05*
	%	66.48	71.43
Escherichia coli (ATCC 25922)	CONTROL	1.74±0.11	0.20±0.06
	MIC	0.87±0.06*	0.75±0.09*
	%	50.00	NI
Escherichia coli enteroagreativa UEL	CONTROL	3.42±0.14	0.29±0.03
	MIC	1.16±0.22*	1,34±0,04*
	%	66.08	NI
Salmonella Typhimurium (ATCC 14028)	CONTROL	1.78±0.15	0.37±0.02
	MIC	0.44±0.10*	2.10±0.09*
	%	75.28	NI
Salmonella Enteritidis (ATCC 13076)	CONTROL	2.68±0.12	0.35±0.02
	MIC	0.90±0.03*	2.16±0.07*
	%	66.42	NI

Average of optical density ± standard deviation. CV= Crystal Violet. EE= ethanolic extract. NI= not inhibition. *Values differ by t test ($p>0.05$). % eradication = data are expressed as percentage of eradication of biofilm biomass at MIC concentration (%). MTT= 3- (4,5-dimethylthiazol-2-yl) -2,5- diphenyltetrazolium. % Viability = data are expressed as percentage of cell viability of biofilms at MIC concentration (%).

In the literature it has been reported that plant extracts with antibiofilm potential can act by breaking down bacterial cell communication (Trentin et al. 2013). In addition, the polarity of the plant extract can also influence the disruption of the already stablished biofilm biomass, interfering with the penetration of the substance into the cell matrix, and cell-cell communication strategies (quorum sensing) of bacteria (Zheng and Stewart, 2002).

EO showed moderate antibiofilm potential (Table 6), eradicating the biomass of biofilms formed from of *S. Enteritidis* (86.19%), *S. aureus* (68.04%) and enteroaggregative *E. coli* (UEL) (63.74%) strains. These results can be explained by the disruption of essential oil constituents with the microbial cell, through passive diffusion of the component molecule through the cell wall of Gram-positive bacteria or through the outer membrane of Gram-negative bacteria. Therefore, essential oils are typically lipophilic compounds, which allows their passage through the cell wall, where they accumulate in the bacterial cytoplasmic membrane, causing damage to the structure of the different layers of polysaccharides, fatty acids, and phospholipids, increasing permeability. The expansion and increased fluidity of the cytoplasmic membrane can cause a breakdown of integrity, consequently generating loss of small intracellular components, such as hydrogen, potassium and sodium, which is associated with a decrease in membrane potential, intracellular pH and ATP pool, caused by this damage to the ionic gradient that occurs between the inside and outside of the cell. In addition, high concentrations of essential oils or exposure over long periods of time can cause further damage to the cytoplasmic membrane, promoting the loss of macromolecules, such as DNA and proteins, a factor strictly related to cell death (Bakkali et al., 2008; Hammer and Carson, 2011).

However, it was observed EO caused an increase in biomass for the established *S.* Typhimurium biofilm. This result may occur due to the overproduction of exopolysaccharide, which is responsible for protecting the metabolically active bacteria embedded in the biofilm community. Studies demonstrate that some essential oil compounds can eliminate only the cells closest to the biofilm interface (Selim et al., 2014; Kifer et al., 2016).

EA showed low antibiofilm activity (as shown in Table 7), with eradication percentage of less than 50% against all established biofilms, except the biofilm of the gram-negative strain *S.* Enteritidis, with inhibition percentage of more than 50, breaking the biomass by 67.16%. Furthermor, it was observed that EA promoted an increase in the biofilm biomass of *E. coli* and *S.* Typhimurium, probably because the phytochemical compounds failed to break down the polymeric matrix of the biofilm and there was no rupture of the biomass when stained with crystal violet. For the other biofilms tested, this extract showed low biomass eradication.

Although the phytochemical compounds of EA showed the presence of classes of metabolites with proven

Table 6. Effect of *M. umbellata* essential oil on the eradication and viability of biofilms of standard bacteria.

Microorganisms	OE	CV	MTT
		ERADICATION	VIABILITY
Staphylococcus aureus (ATCC 25923)	CONTROL	3.88±0.08	0.32±0.06
	MIC	1.24±0.05*	1.76±0.00*
	%	68.04	NI
Pseudomonas aeruginosa (ATCC 27853)	CONTROL	3.49±0.17	0.28±0.04
	MIC	3.32±0.01	0.43±0.01*
	%	4.87	NI
Escherichia coli (ATCC 25922)	CONTROL	1.74±0.11	0.20±0.06
	MIC	1.02±0.12*	0.23±0.08
	%	41.38	NI
Escherichia coli enteroagreativa UEL	CONTROL	3.42±0.14	0.29±0.03
	MIC	1.24±0.13*	2.04±0.00*
	%	63.74	NI
Salmonella Typhimurium (ATCC 14028)	CONTROL	1.78±0.15	0.37±0.02
	MIC	2.14±0.09*	0.89±0.01*
	%	NI	NI
Salmonella Enteritidis (ATCC 13076)	CONTROL	2.68±0.12	0.35±0.02
	MIC	0.37±0.04*	1.09±0.03*
	%	86.19	NI

Average of optical density ± standard deviation. CV= Crystal Violet. OE= essential oil. NI= not inhibition. *Values differ by t test (p>0.05). % eradication = data are expressed as percentage of eradication of biofilm biomass at MIC concentration (%). MTT= 3- (4,5-dimethylthiazol-2-yl) -2,5- diphenyltetrazolium % Viability = data are expressed as percentage of cell viability of biofilms at MIC concentration (%).

Table 7. Effect of *M. umbellata* on the eradication and viability of biofilms of standard bacteria.

Microorganisms	EA	CV	MTT
		ERADICATION	VIABILITY
Staphylococcus aureus (ATCC 25923)	CONTROL	3.88±0.08	0.32±0.06
	MIC	2.07±0.26*	0.86±0.09*
	%	46.65	>100
Pseudomonas aeruginosa (ATCC 27853)	CONTROL	3.49±0.17	0.28±0.04
	MIC	3.20±0.04	0.52±0.02*
	%	8.31	NI
Escherichia coli (ATCC 25922)	CONTROL	1.74±0.11	0.20±0.06
	MIC	1.79±0,21	0.79±0.01*
	%	NI	NI
Escherichia coli enteroagreativa UEL	CONTROL	3.42±0.14	0.29±0.03
	MIC	1.91±0.10*	0.55±0.01*
	%	44.15	NI
Salmonella Typhimurium (ATCC 14028)	CONTROL	1.78±0.15	0.37±0.02
	MIC	2.58±0.04*	0.62±0.10*
	%	NI	NI
Salmonella Enteritidis (ATCC 13076)	CONTROL	2.68±0.12	0.35±0.02
	MIC	0.88±0.06*	1.42±0.01*
	%	67.16	NI

Average of optical density ± standard deviation. CV= Crystal Violet. EA= acetonic extract. *Values differ by t test (p>0.05). % eradication = data are expressed as percentage of eradication of biofilm biomass at MIC concentration (%). MTT= 3- (4,5-dimethylthiazol-2-yl) -2,5- diphenyltetrazolium % Viability = data are expressed as percentage of cell viability of biofilms at MIC concentration (%).

antibiofilm action in the literature, this action was not evidenced in the biofilms tested in our study. Probably, these substances are present in lower concentrations, or even have a synergistic effect between them, which provides an increase in the biomass of the biofilm formed, activating genes that will produce an excess of cellular matrix. In addition, there may be a low penetration of these compounds, or even an increase in the cell membrane efflux pumps, which expel these antimicrobial agents from the bacterial cells (Jamal et al., 2018).

Regarding cell viability, it was evaluated by the MTT colorimetric assay, which is based on the ability of viable cells to metabolically reduce the MTT salt by mitochondrial enzyme into a blue-purple formazan crystal that accumulates in the cell cytoplasm. EE was able to reduce the cellular activity of the mature biofilm of *S. aureus* strain in 31.25%. For the other strains the extract had a non-toxic behavior with high viability (Table 5). The percentage of cell viability of the OE and EA extract was high for all strains tested (Table 6 and 7). The reduction assay using MTT allowed showing that the plant extracts and essential oil of *M. umbellata* contain bacteriostatic potential against mature biofilms. It is emphasized that these caused only the rupture, destabilizing the biofilm matrix, allowing partial removal, but not promoting total eradication.

Studies show that for therapeutic efficacy of antimicrobials towards mature biofilms, the concentration of essential oil and plant extracts administered should be higher than the MIC value between consecutive doses. Reports in the literature suggest that after a certain period of time, tissues contain different concentrations of antimicrobials, lower than the MIC value, and this value has been referred to as subinhibitory concentrations. Although the subinhibitory concentrations of antimicrobial agents are ineffective in killing bacteria, they have the ability to alter various characteristics such as the physical structures of cell surfaces, chemical nature and can also modify the expression of different virulence factors (Wojnicz and Jankowski 2007; Pompilio et. al., 2010). It is reported that the lethal dose of antimicrobial needed to kill bacteria from biofilms should be up to 1000 times higher than the dose required to kill bacteria in their planktonic form (Melchior et al. 2006).

This is the first scientific report on the ability of plant extracts and essential oil of *Myrsine* species to eradicate or cause disruption of biofilms. However, research is being conducted with plant extracts and essential oils of other species on antibiofilm activity, such as carried out by Bazargani and Rohloff (2015), who tested the antibiofilm activity of OE and extracts from *Mentha piperita* leaves and *Coriandrum sativum* and *Pimpinella anisum* seeds against *S. aureus* and *E. coli* biofilms. They identified that *C. sativum* oil showed the highest antibiofilm activity in both bacteria tested. Famuyide et al. (2019) evaluated the antibiofilm potential of EA from *Eugenia* leaves from South Africa and *Syzygium* species and showed good to poor potential to destroy the biofilms formed.

In summary, the results of the antibiofilm potential tests indicated EE and OE of *M. umbellata* were effective in eradicating biomass and caused damage to the cellular activity of mature biofilms. However, there is a need for future studies in order to individually identify the compounds of this plant responsible for this eradication and its molecular mechanism of action against antimicrobial resistant bacteria.

4. Conclusion

The prospection of plant extracts of *M. umbellata* demonstrated the presence of classes of compounds such as saponins, free steroids, alkaloids, flavonoids (flavones, flavanols, flavanonols and xanthons) and tannins, and the EE showed a greater diversity of compounds (8 classes). Thirty-eight compounds were identified in the essential oil composition, all of them sesquiterpenes, with the main compounds being: elixen (15.62%), caryophyllene (E) (8.91%), spatulenol (6.95%), d-cadinene (6.30%), and aromadendrene (5.12%).

EE and EA showed antimicrobial activity for all tested ATCC strains. EA showed the best antimicrobial activity, highlighting its efficiency on the Gram-positive *S. epidermidis* strain. EE showed antimicrobial potential and the Gram-negative *E. coli* strain was more susceptible. However, EO showed only bacteriostatic potential against *S.* Typhimurium, *S.* Abaetetuba, *P. aeruginosa*, and *S. epidermidis* strains. The ability to sequester free radicals was evidenced in EA, which demonstrated an antioxidant activity of 89.55% and EE 63.05%. EO showed a low antioxidant potential with an activity of 10.46%.

Regarding antibiofilm potential, EE eradicated the biofilm biomass of all tested strains and showed high antibiofilm activity (50% to 84.28%). EO also showed antibiofilm effect on enteroaggregative *E. coli* (UEL), *S. aureus*, and *S.* Enteritidis strains, with percentage of mature biofilm biomass breakdown of 63.74%, 68.04%, and 86.19%, respectively.

The results suggest that the leaves of *M. umbellata* can be considered a source for the production of natural phytotherapy with possible uses in different industrial sectors. Therefore, this study is a valuable contribution to future bioassays to identify and isolate the plant bioactives responsible for the high antimicrobial, antioxidant or antibiofilm potential of these extracts and essential oil.

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