CHEMICAL COMPOSITION, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OIL OF LEAVES OF Eugenia involucrata DC.

COMPOSIÇÃO QUÍMICA, ATIVIDADE ANTIMICROBIANA E ANTIOXIDANTE DO ÓLEO ESSENCIAL DAS FOLHAS DE Eugenia involucrata DC.

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ABSTRACT: In the Myrtaceae family, the species Eugenia involucrata DC., popularly known as "cerejeira-do-mato", is traditionally used for the antidiarrheal and digestive action of its leaves. However, no studies were found in the literature regarding its antimicrobial and antioxidant potential. In this context, the objective of the present study was to determine the chemical composition by gas chromatography coupled to mass spectrometry (GC-MS) to evaluate the antimicrobial activity by the broth microdilution technique and the antioxidant activity by the 2,2-diphenyl-1-picryl-hydrazila (DPPH) method of the essential oil of E. involucrata leaves. GC-MS identified 28 compounds, all sesquiterpenes, corresponding to 89.41% of the essential oil. The antimicrobial activity of the essential oil was observed for all Gram-positive bacteria tested (Staplylococcus epidermidis, Enterococcus faecalis, Bacillus subtilis and Staplylococcus aureus) and for yeast Candida albicans. The essential oil presented a reduction capacity of DPPH up to 66.81%, evidencing its antioxidant potential. It is suggested that the antimicrobial and antioxidant action of E. involucrata essential oil is related to the presence of the major compounds, elixene (26.53%), β-caryophyllene (13.16%), α-copaene (8.41%) and germacrene D (7.17%).

KEYWORDS: Myrtaceae. Natural products. Volatile compounds. Hydrodistillation. Biological activities. GC-SM.

INTRODUCTION

The composition of food associated with inadequate processing and storage practices provides ideal conditions for the development of pathogenic microorganisms (FRUTUOSO et al., 2013). These pathogens, once ingested via contaminated food, are a major food safety concern and represent a growing problem in public health (SANTOS et al., 2017). Moreover, the occurrence of a substantial increase of strains with a genetic capacity to acquire and transfer resistance to current antimicrobials has made therapeutic treatment difficult, boosting the pharmaceutical and food industry in the search for alternative antimicrobials (SILVA; FERNANDES JÚNIOR, 2010).

Another factor is a greater demand for “green” products by the consumer, which led to the need to replace chemical additives, with the aim of achieving new preservatives for safer foods (TONGNUANCHAN; SOOTTAWAT, 2014). In addition, naturally occurring antioxidants have gained popularity in the food industry, since synthetic antioxidants, although widely used in food processing, have been questioned with regards to their toxicity to the body (PANIGRAHY; KUMAR; BHATT, 2017). In view of this, natural products, such as plant essential oils, are strong candidates for indication of use in the industry. In addition to their antimicrobial and antioxidant action, they present characteristics that allow a delay in the deterioration and an improvement of the organoleptic quality of the foods.

The species Eugenia involucrata DC. (cerejeira-do-mato) is a Brazilian native plant belonging to the Myrtaceae family, known in traditional medicine for its health benefits. Its leaves are employed in the form of teas, with antidiarrheal and digestive actions (SAUSEN et al., 2009). Research with this genus revealed its therapeutic importance, with antimicrobial and antioxidant activities reported in some species, such as Eugenia caryophyllata (SILVESTRI et al., 2010) and Eugenia jambolana (HAJOORI et al., 2013). However, studies of the antimicrobial and antioxidant activities of the essential oil of E. involucrata leaves are scarce. Studies related to the chemical composition of E. involucrata essential oil
revealed the presence of sesquiterpenes, representing more than 90% of the constituents (CIARLINI; MARANGONI; BOLZAN, 2017; HENRIQUES et al., 1993; MARIN et al., 2008; RAMOS et al., 2006).

Thus, the objectives of the present study were: (1) determine the chemical composition of the essential oil of *E. involucrata* leaves by gas chromatography coupled to mass spectrometry (GC-MS); (2) to evaluate the antimicrobial activity by the broth microdilution technique and (3) to evaluate the antioxidant potential by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.

**MATERIAL AND METHODS**

**Collection and identification of plant material**

The collection of the leaves of *E. involucrata* was carried out in the ecological park Paulo Gorski, located in the municipality of Cascavel in the western region of the state of Paraná, between October 2016 and March 2017. The geographic location was determined using the Global Positioning System as follows: 24° 57’51,4” S 53° 26’00,9” W. The sample of the botanical material was sent for identification by the Herbarium of the State University of the West of Paraná (UNIOESTE) and deposited under number 1650 for registration of the voucher.

**Extraction of essential oil**

The leaves were oven dried with air circulation at 40 °C for between 48 and 72 h. Grinding was achieved using a knife mill, with a 0.42 mm granulometry membrane. From the dry and milled sample, distilled water was added in the ratio of 1:10 (w/v) and the sample was subjected to the hydrodistillation method for 4 h using a Clevenger-type apparatus (WEBER et al. 2014).

The percentage of essential oil yield (%) was calculated by: \[ \% = \frac{EO(w)}{VM (w)} \times 100 \]

where EO is the total extracted essential oil (w) and VM is dry and ground vegetable mass (w). Subsequently, the samples were stored in conical bottom tubes wrapped in foil, under light and refrigeration, at an average temperature of 4 °C until the tests were carried out.

**Chemical analysis of essential oil**

The analysis of the constituents of *E. involucrata* essential oil was performed from the Thermo-Finnigan GC-MS system by the Gas Chromatography coupled to Mass Spectrometry Laboratory, from the State University of Maringá (UEM), Paraná, Brazil. This system consists of a GC FOCUS (Thermo Electron), coupled to a DSQ II mass spectrometer (Thermo Electron) and a TriPlus AS automatic injector (Thermo Electron). Chromatographic separation was performed with a HP-5ms fused silica capillary column (30 m long, 0.25 and 0.25 µm ID of the film, 5% phenyl-95% dimethylpolysiloxane composition). The temperature of the injector was 250 °C. The sample and the alkane standards C7–C28 were injected at a split-ratio of 1:25. The programming of the temperature used was 50 °C maintained for 2 min, a temperature rise to 180° C at a ratio of 2 °C min⁻¹, followed by an increase to 290 °C at a ratio of 5° C min⁻¹. The interface between the GC and MS was maintained at 270 °C and the temperature of the ionization source of the mass spectrometer was 250 °C. The identification of the compounds was accomplished by comparing their retention times with the retention times obtained from the literature and through their retention indices (ADAMS, 2007; BABUSHOK; LINSTROM; ZENKEVICH, 2011; YU et al., 2007).

**Microorganisms used**

The essential oil was tested against the strains of the American Type Culture Collection (ATCC) and Cefar Diagnóstica Cultures Collection (CCCD), with six Gram-negative strains: *Escherichia coli* (ATCC 25922), *Salmonella enterica* Enteritidis (ATCC 13076), *Salmonella enterica* Typhimurium (ATCC 14028), *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 subtilis* subsp. *spizizenii* (CCCD B005); and a yeast *Candida albicans* (ATCC 10231).

**Antimicrobial activity**

The microorganisms were recovered in a Brain Heart Infusion enrichment broth and incubated for 24 h at 36 ± 0.1 °C. After this period, the strains were harvested in a Muller-Hinton (MH) agar medium and incubated for 24 h at 36 ± 0.1 °C. To standardize the inoculum, the strains were diluted in saline solution (0.85%), resulting in a final concentration of $1 \times 10^9$ CFU.mL⁻¹ for bacteria and $1 \times 10^5$ CFU.mL⁻¹ for yeast *C. albicans*.

**Minimum inhibitory concentration (MIC)**

The assays for the essential oil were carried out according to the broth microdilution methodology described by Weber et al. (2014). The
essential oil was solubilized in methanol P.A. and diluted in MH broth to the bacterial strains and Roswell Park Memorial Institute broth (RPMI-1640) to C. albicans. In 96-well microdilution plates, 150 μL of MH broth or RPMI-1640 was dispensed. Serial dilutions of the essential oil were performed between concentrations of 7000 and 3.37 μg.mL⁻¹. Finally, 10 μL of inoculum was added to each well and the plates were incubated at 36 ± 0.1 °C for 18–24 h. As positive controls, we used commercial antibiotic gentamicin (200 mg.mL⁻¹) and commercial antifungal nystatin (200 mg.mL⁻¹). As a negative control the inoculum was added to the MH broth, without the presence of the essential oil to prove the viability of the tested microorganism. The triphenyltetrazolium chloride (TTC) at 0.5% was also used as a colorimetric developer. The MIC was measured in triplicate, where it was possible to determine the lowest concentration of essential oil capable of inhibiting microbial growth.

Minimum bactericidal concentration (MBC)/minimum fungicidal concentration (MFC)

The assay methodology of Weber et al. (2014) was performed with modifications. Before the addition of 0.5% TTC to determine the MIC, a 2 μL aliquot of each well of the microdilution plate was removed and plated on the MH agar surface. The plates were incubated at 36 ± 0.1 °C for 18–24 h. The assay was performed in triplicate and to determine the MBC and MFC was observed if there was microbial growth in MH agar, allowing us to verify which was the lowest concentration of the essential oil capable of causing the death of the bacterium/fungus tested.

Antioxidant activity

The antioxidant activity of the essential oil was determined according to the method of reducing DPPH, as proposed by Rufino et al. (2007). Initially, a calibration curve (0, 10, 20, 30, 40, 50 and 60 μM DPPH) was made to obtain the DPPH concentration in the medium after the reaction with the essential oil, with the equation \( y = 0.011x - 0.005 \) (\( R^2 = 0.999 \)), where \( y \) is the concentration of DPPH and \( x \) is absorbance. For this, aliquots of 0.1 mL of essential oil at different concentrations (10, 20, 30, 40 and 50 mg.mL⁻¹) were added to 3.9 mL of methanolic DPPH solution (60 mM) and homogenized in a shaker tube. As a negative control, 0.1 mL of a solution of 50% methanol, 70% acetone and distilled water in a ratio of 2:2:1 (v/v/v), and as a positive control, the synthetic antioxidant BHT (butyl-hydroxy-toluene), was used in different concentrations (0.0312, 0.062, 0.125, 0.25 and 0.5 mg.mL⁻¹). The tests were performed in a spectrophotometer at 515 nm for 1-min reading intervals until stabilization of absorbance. As a result of its white color, methanol was used for the calibration of the spectrophotometer. The percentage of free radical sequestration (AA%) was expressed by the equation: AA% = \( \frac{A_0 - A_1}{A_1} \) × 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 (amount of antioxidant substance required to reduce the initial concentration of DPPH by 50%), the concentrations of the essential oil and BHT were used to obtain the equation of the line with \( R^2 \) greater than 0.80, find the value of IC₅₀ from linear regression. The tests were performed in triplicate and expressed as mean ± standard deviation. The IC₅₀ results were analyzed by the chi-square test of adhesion using the R® statistical program (R DEVELOPMENT CORE TEAM, 2017).

RESULTS AND DISCUSSION

Chemical composition of essential oil of E. involucrata

From the extraction of the essential oil from the leaves of E. involucrata, a total yield of 0.21% was observed. The chemical composition of the essential oil, together with its retention indices, is shown in Table 1. The GC-MS analysis identified 28 compounds, corresponding to 89.41% of the essential oil.

The analysis of chemical components of the essential oil of E. involucrata, demonstrated the presence of hydrocarbon and oxygenated sesquiterpenes in proportions of 83.05% and 6.36%, respectively. The most abundant components in the sample were elixene (26.53%), β-caryophyllene (13.16%), α-copaene (8.41%) and germacrene D (7.17%), all hydrocarbon sesquiterpenes.

In the family of Myrtaceae, the species of the genus Eugenia present as the predominant chemical compounds from the group of sesquiterpenes, and to a lesser extent, it is observed the compounds from the group of monoterpenes (CARNEIRO et al., 2017; HENRIQUES et al., 1993; MARIN, et al. 2008; RAMOS et al., 2006). However, in this species, no compounds belonging to the monoterpenes were found.
In the literature, the chemical constituents of the essential oil of leaf *E. involucrata* were identified by several authors. Ramos et al. (2006) found 27 chemical compounds, considering found compounds in the majority: bicyclogermacrene (19%), globulol (14%), epi-globulol (8%) and γ-elemene (7.2%). Henriques et al. (1993) characterized 93.5% of the essential oil, with viridiflorene (36.2%), β-caryophyllene (23.1%) and germacrene D (6%) as the major compounds. More recently, Ciarlini, Marangoni and Bolzan, (2017) detected 88.79% of the compounds present in the oil *E. involucrata*, the most abundant being β-elemene (48.41%), bicyclogermacrene (22.96%), caryophyllene (13.94%) and germacrene D (4.02%). Considering these previous studies, the presence of some compounds in common in the essential oils of *E. involucrata* was discovered, however, because it was a mixture, it was observed that the chemical profile differs in quantity, number of compounds and molecular configuration. This variation may be related to climatic factors (temperature, relative air humidity, exposure to ultraviolet radiation and wind regime), geographic location (altitude, habitat and air pollution), soil composition, plant organ, age and stage of the cycle.

**Table 1.** Chemical composition of the essential oil of *E. involucrata* leaves obtained by hydrodistillation and analyzed by GC-MS.

| Nº | Compound                  | Area(%) | RT       | RI     | RI*     |
|----|---------------------------|---------|----------|--------|---------|
| 1  | δ-Elemene                 | 4.29    | 25.97    | 1331   | 1337    |
| 2  | α-Cubebene                | 0.25    | 26.49    | 1343   | 1345    |
| 3  | Cyclosativene             | 0.21    | 27.43    | 1364   | 1367    |
| 4  | α-Copaene**               | 8.41    | 27.74    | 1371   | 1376    |
| 5  | β-Bourbonene              | 0.42    | 28.07    | 1378   | 1381    |
| 6  | β-Elemene                 | 4.95    | 28.35    | 1385   | 1390    |
| 7  | α-Gurjunene               | 0.48    | 29.08    | 1401   | 1409    |
| 8  | Caryophyllene**           | 13.16   | 32.80    | 1489   | 1492    |
| 9  | β-Gurjunene               | 0.81    | 30.05    | 1424   | 1431    |
| 10 | Aromadendrene             | 0.70    | 30.38    | 1432   | 1440    |
| 11 | α-caryophyllene           | 3.45    | 31.11    | 1449   | 1459    |
| 12 | Allo-Aromadendrene        | 2.82    | 31.29    | 1454   | 1465    |
| 13 | γ-Muurolene               | 1.04    | 31.97    | 1470   | 1476    |
| 14 | Germacrene D**            | 7.17    | 32.19    | 1475   | 1480    |
| 15 | Elixene**                 | 26.53   | 32.80    | 1489   | 1492    |
| 16 | α-Muurolene               | 0.85    | 32.96    | 1493   | 1498    |
| 17 | γ-Cadinene                | 0.53    | 33.53    | 1507   | 1513    |
| 18 | β-Cadinene                | 6.00    | 33.75    | 1512   | 1518    |
| 19 | 1,4-Cadinadiene           | 0.06    | 34.31    | 1526   | 1528    |
| 20 | α-Cadinene                | 0.11    | 34.48    | 1531   | 1533    |
| 21 | α-Calacorene              | 0.11    | 34.64    | 1535   | 1540    |
| 22 | Germacrene B              | 0.70    | 35.32    | 1552   | 1551    |
|    | **Hydrocarbon sesquiterpenes** | 83.05 |         |        |         |
| 23 | Spathulenol               | 2.57    | 36.05    | 1570   | 1576    |
| 24 | Caryophyllene oxide       | 0.75    | 36.24    | 1575   | 1581    |
| 25 | Globulol                  | 0.52    | 36.40    | 1579   | 1583    |
| 26 | IsoSpathulenol            | 1.85    | 38.06    | 1621   | 1628    |
| 27 | τ-Cadinol                 | 0.46    | 38.60    | 1635   | 1638    |
| 28 | α-Cadinol                 | 0.21    | 39.09    | 1648   | 1652    |
|    | **Oxygenated Sesquiterpenes** | 6.36  |         |        |         |
| 29 | NI                        | 1.75    | 32.49    | 1482   |         |
| 30 | NI                        | 4.25    | 33.27    | 1500   |         |
| 31 | NI                        | 1.07    | 34.05    | 1520   |         |
|    | None indentified          | 7.07    | 34.05    | 1520   |         |
|    | **Total**                 | 96.48   | 34.29    | 1520   |         |

RT: Retention time; RI: Values calculated retention indices; RI*: Retention index values from NIST/EPA/NIH Mass Spectral Library, version 2.0 d, april 2005; **Majority compounds; NI: Not identified.
vegetative, genetic diversity, seasonality, circadian rhythm, water availability, nutrients, protection against pathogens, among others (GOBBO-NETO; LOPES, 2007). Thus, research aimed at standardizing the chemical constituents of essential oils should be performed to verify the environmental influences on the metabolic production of these compounds and for their possible safe use by the market.

Antimicrobial activity

Table 2. Minimal inhibitory concentration (MIC), minimum bactericidal concentration/minimum fungicidal concentration (MBC/MFC) essential oil of E. involucrata against pathogenic microorganisms.

| Microorganisms               | MIC (µg.mL\(^{-1}\)) | MBC/MFC (µg.mL\(^{-1}\)) |
|------------------------------|-----------------------|---------------------------|
| **Gram-positive**            |                       |                           |
| S. aureus (ATCC 25923)       | 875                   | 7000                      |
| S. epidermidis (ATCC 12228)  | 875                   | 1750                      |
| E. faecalis (ATCC 19433)     | 7000                  | 7000                      |
| B. subtilis (CCCD B005)      | 875                   | 1750                      |
| **Gram-negative**            |                       |                           |
| S. Typhimurium (ATCC 14028)  | -                     | -                         |
| S. Enteritidis (ATCC 13076)  | -                     | -                         |
| E. coli (ATCC 25922)         | -                     | -                         |
| K. pneumoniae (ATCC 13883)   | -                     | -                         |
| P. mirabilis (ATCC 25933)    | -                     | -                         |
| P. aeruginosa (ATCC 27853)   | -                     | -                         |
| **Yeast**                    |                       |                           |
| C. albicans (ATCC 10231)     | 1750                  | 3500                      |

(-) No activity

The results concerning the antimicrobial activity of the essential oil of the leaves of E. involucrata are described in Table 2. The inhibitory and bactericidal effects of the oil were verified for all Gram-positive bacteria tested and for yeast C. albicans. MIC values ranged from 875–7000 µg.mL\(^{-1}\) and those from CBM from 1750–7000 µg.mL\(^{-1}\). The bacteria most susceptible to oil action were S. epidermidis and B. subtilis, with MICs of 875 and CBM of 1750 µg.mL\(^{-1}\).

Due to the unique characteristics of the essential oils, it is believed that the antimicrobial activity of the oils is attributed to several cellular mechanisms. The hydrophobic profile of the compounds present in the oil is the main characteristic that proves its antimicrobial potential. Its role in irreversibly disrupting microbial cell membrane lipids makes the membrane permeable and promotes the loss of internal cellular content (ions, glucose and ATP), leading to the death of the microorganism. In addition, the oils can also alter the enzymatic systems involving energy production and the synthesis of structural compounds (BURT, 2004; DJILANI; DICKO, 2012).

Although no reports of the antimicrobial activity of E. involucrata essential oil were found in the literature, many species with antimicrobial properties were observed within the genus Eugenia. Stefanello et al. (2008) tested the essential oil of the leaves of Eugenia chlorophylla, proving its antimicrobial properties against S. aureus (MIC=1000 µg.mL\(^{-1}\)). Lago et al. (2011) reported the antimicrobial potential of Eugenia uniflora essential oil against S. epidermidis (MIC=7500 µg.mL\(^{-1}\)). Ogunwande et al. (2005) demonstrated the inhibitory effect of E. uniflora essential oil for Gram-positive B. cereus (MIC=39 µg.mL\(^{-1}\)) and S. aureus (MIC=156 µg.mL\(^{-1}\)) and Gram-negative P. aeruginosa (MIC=625 µg.mL\(^{-1}\)) and E. coli (MIC=625 µg.mL\(^{-1}\)) at significantly low concentrations.

It is observed that in most studies on the action of essential oils, Gram-positive bacteria are more susceptible than Gram-negative bacteria (BURT, 2004; LAGO et al., 2011; STEFANELLO et al., 2008). According to the literature, Gram-positive strains are more susceptible to Gram-negative strains (S. Typhimurium, S. Enteritidis, E. coli, K. pneumoniae, P. mirabilis and P. aeruginosa), which were resistant to the oil concentrations tested.

One of the explanations for this fact is related to the presence of an outer phospholipid membrane that surrounds the Gram-negative cell wall. This membrane contains hydrophilic lipopolysaccharides that act as a barrier to
macromolecules and hydrophobic compounds, thus providing greater tolerance to some antimicrobial compounds, such as those found in essential oils (PANDEY et al., 2016). In addition, the periplasmic space contains enzymes capable of breaking molecules and the membrane contains efflux pumps capable of removing compounds considered harmful to the bacterial cell (BURT, 2004; TADEG et al., 2005). Therefore, as Gram-positive bacteria do not contain this additional barrier, although they present a cell wall with greater thickness, it is not as complex, being composed of lipophilic ends of lipoteichoic acids, which facilitates the direct contact of the essential oil with the bacterial cell (TONGNUANCHAN; SOOTTAWAT, 2014).

As in our study, some authors have proved the antifungal potential of Eugenia species. Stefanello et al. (2008) tested the essential oil of E. chlorophylla, proving its antifungal properties of C. albicans, with a MIC of 500 μg.mL⁻¹. This was also observed by Lago et al. (2011), who in his study demonstrated the inhibition of C. albicans by the essential oil of E. uniflora in the concentration of 1800 μg.mL⁻¹.

The antifungal action found in many essential oils is related to the presence of sesquiterpenes. Since the oil has lipophilic characteristics, this allows it to penetrate the fungal cell wall, interfering with the action of enzymes involved in its synthesis, and also to establish a pH gradient through the cytoplasmic membrane and block energy production, causing membrane changes and changing the fungus morphology (DJILANI; DICKO, 2012; PANDEY et al., 2016).

Table 3. Percentage of antioxidant activity of the essential oil of the leaves of E. involucrata by the DPPH method.

| Sample          | Concentration (mg.mL⁻¹) |
|-----------------|-------------------------|
|                 | 10 | 20 | 30 | 40 | 50 |
| Essential oil   |    |    |    |    |    |
| BHT             | 14.91±0.33 | 25±0.66 | 35.96±1.6 | 51±1.19 | 66.81±2.16 |
| BHT             | 18.8±49 | 67.28±64 | 88.62±45 | 94.59±41 |

(-) Not tested; BHT (commercial synthetic antioxidant Butylhydroxytoluene); Percentage of DPPH radical sequestration was expressed as mean ± standard deviation.

Table 4. Value of IC₅₀ of the essential oil of the leaves of E. involucrata by the DPPH method.

| Sample          | IC₅₀ (mg.mL⁻¹) |
|-----------------|---------------|
| Essential oil   | 38.6±1.11     |
| BHT             | 0.094±0.01    |

BHT (commercial synthetic antioxidant Butylhydroxytoluene); Values of IC₅₀ (Concentration of E. involucrata leaves extract necessary to reduce 50% of the DPPH radical) expressed as mean ± standard deviation; Different letters in the same column express significant differences (p <0.05); Equation (Allows to estimate expected value of variable y, given the values of variable x); R² (Coefficient of determination of a linear statistical model that explains the percentage of relation of observed values).
It is suggested that the antioxidant activity of the *E. involucrata* essential oil is attributed mainly to its major components: elixene, β-caryophyllene, α-copaene and germacrene D, and the potential of β-caryophyllene and germacrene D, as excellent antioxidants (CARNEIRO et al., 2017; HEMALATHA et al., 2015). Generally, the potential of the essential oils corresponds to the phenolic content. However, some essential oils have antioxidant behavior according to the chemical structure of their components, as is the case for some terpenes (AMORATI; FOTI; VALGIMIGLI, 2013; PANIGRAHY; KUMAR; BHATT, 2017).

Recent studies on the antioxidant activity of the essential oils of *E. involucrata* leaves demonstrated an antioxidant potential of 6.41% using the methodology of β-carotene/linoleic acid (CIARLINI; MARANGONI; BOLZAN, 2017), that is, an activity about 10 times lower than that found in this study. According to the authors, different techniques for the extraction of volatile compounds may reflect the antioxidant potential of the species.

Although few studies exist focusing on the leaves of *E. involucrata*, the fruit was much researched as to its antioxidant potential, due to its use in the form of juices, liqueurs and jellies (LORENZI, 2009), Marin et al. (2008), Infante et al. (2016) and Nicácio et al (2017) confirmed the antioxidant potential of *E. involucrata*. In addition, Carneiro et al. (2017) and Infante et al. (2016) evidenced antioxidant activity in the essential oils of four other species: *Eugenia klotzschiana*, *Eugenia brasiensis*, *Eugenia myrcianthes* and *Eugenia leitonii*, thus demonstrating the potential of this genus.

Finally, it is suggested that studies related to the method of collection, drying and extraction of essential oil may contribute to further clarification regarding its antioxidant action, since these factors directly influence the active principles that make up the essential oil (GOBBO-NETO; LOPES, 2007; CIARLINI; MARANGONI; BOLZAN, 2017).

**CONCLUSIONS**

In the characterization of essential oil of *E. involucrata*, 28 compounds were identified, all sesquiterpenes. The compounds found in most of the sample were elixene (26.53%), β-caryophyllene (13.16%), α-copaene (8.41%) and germacrene D (7.17%), very common within this genus, inferring the antimicrobial and antioxidant potential of the essential oil.

The essential oil of *E. involucrata* leaves presented antimicrobial activity for all Gram-positive bacteria (*S. epidermidis, E. faecalis, B. subtilis* and *S. aureus*) and for yeast *C. albicans*. Their ability to sequester free radicals was also reported, demonstrating antioxidant activity of up to 66.81%.

It is hoped that these results will contribute to further clarification of the biological potential of this essential oil, allowing future scientific validation research to provide subsidies for plant bioengineering to make possible the international standardization of the compounds present in essential oils, optimizing its potential for biological and commercial applications.

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**RESUMO**: Na família Myrtaceae, a espécie *Eugenia involucrata* DC. popularmente denominada “cerejeira-do-mato” é conhecida tradicionalmente pela ação antidiarreica e digestiva de suas folhas. Contudo, na literatura não foram encontrados trabalhos referentes ao seu potencial antimicrobiano e antioxidante. Neste contexto, o objetivo do presente estudo foi determinar a composição química por cromatografia gasosa acoplada a espectrometria de massas (CG-EM) e avaliar a atividade antimicrobiana pela técnica de microdiluição em caldo e a atividade antioxidante pelo método do 2,2-difenil-1-picril-hidrazila (DPPH) do óleo essencial das folhas de *E. involucrata*. A CG-EM identificou 28 compostos, todos sesquiterpenos, correspondendo a 89,41% do óleo essencial. A atividade antimicrobiana do óleo essencial foi observada para todas as bactérias Gram-positivas testadas (*Staphylococcus epidermidis, Enterococcus faecalis, Bacillus subtilis* e *Staphylococcus aureus*) e para a levadura *Candida albicans*. O óleo essencial apresentou capacidade redutora de radicais DPPH de até 66,81%, evidenciando sua potencialidade antioxidante. Sugere-se que a ação antimicrobiana e antioxidante do óleo essencial de *E. involucrata* esteja relacionada à presença dos compostos majoritários, elixeno (26,53%), β-cariofileno (13,16%), α-copaeno (8,41%) e germacrene D (7,17%).

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