Plant volatiles from the Brazilian restinga with bactericidal activity against multiresistant bacteria

Voláteis de plantas da restinga brasileira com atividade bactericida contra bactérias multirresistentes

Gabriele Marques Pinto¹, Juliana Barbosa Succar¹, Cristiane Pimentel Victório¹ *, Maria Cristina de Assis¹

ABSTRACT
This study aimed to evaluate the bactericidal activity of Myrtaceae foliar volatile compounds against strains of Pseudomonas aeruginosa (PAO-1) and Burkholderia cenocepacia (ET-12). Although these bacteria are clinically important to human health, they, along with other bacteria (Escherichia coli, Staphylococcus aureus and Streptococcus agalactiae), also have a high profile of antimicrobial resistance associated with bovine mastitis. The chemical composition of volatiles found in Myrtaceae foliar volatiles was also characterized. To accomplish this, the essential oil was extracted from dry leaves of Eugenia astringens, Eugenia arenaria and Myrrhinum atropurpureum by hydrodistillation. Minimum inhibitory concentration (MIC) was determined by microdilution in broth. Essential oil from E. astringens leaf could inhibit growth the five strains by the Agar diffusion method. MIC demonstrated a reduction in bacterial growth by 74% ± 2.8 (P. aeruginosa), 78.3% ±10.5 (B. cenocepacia), 87.1% ± 1.4 (E. coli), 99 % ±0.02 (S. aureus) and 99.9 ±0.01 (S. agalactiae). The terpene α-pinene was detected in all three species, especially M. atropurpureum and E. astringens. E. astringens showed a high content of (E)-caryophyllene.

Keywords: Bactericidal activity; Multiresistant bacteria; Essential oils; Myrtaceae

RESUMO
O estudo visou avaliação da atividade bactericida de compostos voláteis das folhas de Myrtaceae contra as cepas de Pseudomonas aeruginosa (PAO-1) e Burkholderia cenocepacia (ET-12), de importância clínica em humanos com alto perfil de resistência antimicrobiana e bactérias associadas à mastite bovina (Escherichia coli, Staphylococcus aureus e Streptococcus agalactiae) e a caracterização da composição química dos voláteis. O óleo essencial foi extraído de folhas secas de Eugenia astringens, Eugenia arenaria e Myrrhinum atropurpureum por hidrodestilação. A concentração inibitória mínima (CIM) foi determinada por microdiluição em caldo. O óleo essencial da folha de E. astringens foi capaz de inibir o crescimento das cinco cepas pelo método de difusão em agar. O MIC demonstrou uma redução do crescimento bacteriano em 74% ± 2.8 (P. aeruginosa), 78.3% ±10.5 (B. cenocepacia), 87.1% ± 1.4 (E. coli), 99 % ±0.02 (S. aureus) e 99.9 ±0.01 (S. agalactiae). O α-pineno foi detectado em três espécies, com destaque para M. atropurpureum e E. astringens. E. astringens apresentou alto teor de (E)-cariofileno.

Palavras-chave: Atividade bactericida; Bactérias multirresistentes; Óleos essenciais; Myrtaceae

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INTRODUCTION

Many bacteria are known for their resistance to all classes of antimicrobials and for the ease with which they can acquire new resistance mechanisms (Tafur et al., 2008). Non-fermenting Gram-negative bacteria pose a particular difficulty for the healthcare community because they present maximal multidrug resistance (McGowan, 2006; Muntean et al., 2018). In particular, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Burkholderia cenocepacia* stand out as multidrug-resistant bacteria frequently involved in nosocomial infections with a high mortality rate (Gibson et al., 2003; Zaha et al., 2019). In veterinary medicine, *S. aureus*, *E. coli* and *Streptococcus agalactiae* are etiological agents of bovine mastitis, an intramammary infection (Angeliki et al., 2019). This polymicrobial disease causes considerable economic losses from the reduced quantity and quality of milk, the loss of quarters reserved for lactating cows, the early slaughter of dairy animals, and the high cost of treatment (Heikkilä et al., 2018; Angeliki et al., 2019). The eradication of these bacteria in humans and animals is often unsuccessful, and therapy is generally aimed at decreasing bacterial density (Lutz et al., 2011; Oliver & Murinda, 2012). The increase in bacterial resistance to multiple antimicrobial drugs has spurred the search for new therapeutic alternatives, such as medicinal plants, which represent an important source for obtaining medicines. The antimicrobial activity of plant extracts and metabolites of essential oils of plants has been proven in several studies carried out in countries that have a diversified flora (Holetz et al., 2002; Castro and Firmida, 2011).

Myrtaceae Juss. is the largest family that belongs to the order Myrtales. It comprises 142 genera and includes more than 5,000 species of trees and shrubs, the occurrence of which has been described in tropical and subtropical regions of the world, with particular diversity in South and Central America and Australia (Chase et al., 2016; Wilson et al., 2005). In Brazil, Myrtaceae is one of the ten richest angiosperm families, and about 50% of species are endemic to Brazil. They are important to fauna for their wild berries (Sobral et al., 2015; Silva & Victório 2021). It is a relevant plant family in the Atlantic Forest domain, quite common in restinga areas and found in several biomes (Souza & Morim, 2008; Arruda & Victório, 2011; Defaveri et al., 2011; Victório et al., 2018; Silva & Victório 2021). The presence of oil aromatic compounds is a chemical feature of this family that presents secretory cavities in leaf parenchyma where terpenoids are produced, stored and secreted (Metcalfe & Chalk, 1983; Arruda & Victório 2011; Victório et al., 2018). Several Myrtaceae species are used in folk medicine as antidiarrheal, antimicrobial, antioxidant, antirheumatic, anti-inflammatory, and cleansing agents (De Souza et al., 2018). Data revealed that *Eugenia astringens*, “araponga” and “abajiru” are abundant in restingas and display gastro-protective activity by significant inhibition of ulcer formation. Also, the aerial parts are widely used in folk medicine to treat infections, inflammation, diabetes and
parasites (Kneip, 2009; Meyre-Silva et al., 2009; Carneiro et al., 2021). *Eugenia arenaria*,
popular names “pintagai-uma” and “camboi”, and *Myrrhinum atropurpureum*, also known as
“pau-ferro”, “carrapato” and “murtilo”, are endemic to the restingas of Rio de Janeiro.
Nonetheless, studies on these species are scarce, and few biological activities have been recorded.
Therefore, the present study evaluated the chemical profile and antimicrobial activity of volatiles
of *E. astringens*, *E. arenaria* and *M. atropurpureum* the from restinga environment against strains
of *B. cenocepacia*, *P. aeruginosa*, *S. aureus*, *E. coli* and *S. agalactiae*.

**MATERIAL AND METHODS**

**Plant material**

Dry leaves of *Eugenia astringens* Cambess (syn *Eugenia rotundifolia* Casar., *Eugenia umbelliflora* O. Berg) (HUNI650), *Eugenia arenaria* Cambess. and *Myrrhinium atropurpureum* Schott (RB 415731) were used to extract essential oils. Leaves of *E. astringens* and *M. atropurpureum* were collected in August 2016 from individuals of the Grumari restinga (23°02’94”S, 43°31’98”W). *E. arenaria* leaves were also collected in August 2016 from the Massambaba restinga (22°55’33”S 42°16’17”W). Both restingas are in Rio de Janeiro state, Brazil. The plants were identified by taxonomist Dr. Marcelo da Costa Souza and deposited at the Herbarium RBR (UFRRJ). Permission for collection was obtained from the Ministry of the Environment (SISBIO/ICMBio, under number 37376-2).

**Distillation and analysis of volatiles**

Once collected, the plant materials were oven dried for 3–5 days at 50°C, ground, and
stored in the dark at room temperature. Samples between 50 g and 100 g of dry leaves were cut
and used in the distillation of essential oils in a Clevenger-type apparatus during 3 h. Pure essential
oils were diluted in dichloromethane in the proportion of 1%, and then 1.0 µL of the solution was
injected (split 1:20) into an Agilent 7890A gas chromatograph equipped with a flame ionization
detector (GC/FID) and a HP-5MS (5% phenyl-methylpolysiloxane) fused silica capillary column
(30 m x 0.25 mm x 0.25 µm). Hydrogen was used as carrier gas at a flow rate of 1.0 mL/minute.
Oven temperature was programmed from 60 to 240°C at 3°C/minute. Injector temperature was
kept at 250°C and detector temperature at 280°C. The percentage composition was obtained by
normalization. The data presented are the mean of three replicates. Analyses by GC/MS were
performed on an Agilent 5973N mass selective detector coupled to an Agilent 6890N gas
chromatograph fitted with a HP-5MS fused silica capillary column (30 m x 0.25 mm x 0.25 µm).
Helium was used as carrier gas at 1.0 mL/minute. The mass detector was operated in electronic
ionization mode (70 eV) at 3.15 scans/second, with mass range from 40 to 450 u. The transfer
line was kept at 260°C, ion source at 230°C and analyzer at 150°C. Oven temperature program
and injection procedure were the same as noted above. Identification of oil components was
performed by comparing their mass spectra with those from the Wiley Registry of Mass Spectral Data (Wiley, 1994) and by comparing their linear retention indices (LRI) with those from the literature (Adams 2007). LRI were calculated according to Van Den Dool and Kratz (1963) after the injection of a homologous series of hydrocarbons (n-C7-C26) in the same column and conditions as above.

**Bacterial strains used in bactericidal assays**

The bacterial strains used for screening were kindly provided by the Collection of Reference Microorganisms in Sanitary Surveillance - FIOCRUZ-INCQS, Rio de Janeiro, RJ: *Escherichia coli* - INCQS (00219) - ATCC (8739), *Staphylococcus aureus* subsp. *aureus* - INCQS (00039) - ATCC (6538) and *Streptococcus agalactiae* - INCQS (00128) - ATCC (13813). The *Burkholderia cepacia* ET-12 strain (sample J2315 belonging to genovariante IIIa) and *Pseudomonas aeruginosa* strain PAO-1 were assigned by the Department of Microbiology, Immunology and Parasitology of the Univesidade Estadual do Rio de Janeiro (UERJ).

**Preparation of bacterial suspensions**

The bacterial suspensions were prepared in Mueller Hinton II medium (MHII). The cultures were homogenized and placed in an orbital shaker (New Ethics) at 150 rpm at 33°C for 18 h. After this time, the cultures were centrifuged at 4500 rpm for 10 min at 4°C. The supernatants were discarded, and the pellet formed was homogenized. Dilutions in MHII medium from the bacterial pellet that were performed on a photochrometer (Biochrom, model Libra S2) at 680 nm in order to obtain bacterial suspensions with DO680nm = 1.3 which corresponds to 1x10^8 CFU/mL.

**Determination of the Minimum Inhibitory Concentration (MIC) of essential oils against Gram-negative and -positive bacteria**

Minimum Inhibitory Concentration (MIC) of essential oils was determined by disk diffusion in agar and broth microdilution, according to the standards of the Clinical and Laboratory Standards Institute (Wayne, 2012).

**Statistical analysis**

Results were expressed as means±SEM of the values obtained. Statistical analyses were performed using GraphPad Prism 5 software. Statistical differences between groups were determined by Bonferroni’s Multiple Comparison Test.

**RESULTS AND DISCUSSION**

**Determination of bactericidal activity of essential oils by the disk diffusion test**
Antibacterial activity of leaf volatiles was evaluated against Gram-positive and -negative bacteria. Results from the disk diffusion test against the different strains showed that the essential oils of *M. atropurpureum* (50%) and *E. arenaria* (25%) had bactericidal activity only for PAO-1 and ET-12, but no activity against the other strains tested. We observed a greater effect on the bactericidal activity of *E. astringens* leaf volatiles, which could significantly inhibit the bacterial growth of all five strains at a minimum concentration of 25% when compared to the growth of cultures not treated with oils (Table 1).

### Table 1. Effect of leaf volatiles from *Myrtaceae* species, using the disk diffusion test.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Minimum inhibitory concentration of leaf oils (vol/vol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. aeruginosa</em></td>
</tr>
<tr>
<td><em>E. arenaria</em></td>
<td>25%</td>
</tr>
<tr>
<td><em>E. astringens</em></td>
<td>25%</td>
</tr>
<tr>
<td><em>M. atropurpureum</em></td>
<td>50%</td>
</tr>
</tbody>
</table>

*NA- No activity. *Succar et al. 2019*

The disk diffusion technique is one of the cheapest and least labor-intensive antibacterial susceptibility testing methods currently available. However, in the study by Vambe et al. (2018), they report that this procedure has difficulties in providing reproducible results for various plant extracts. This can be attributed to the occurrence of some phytochemicals, especially those that are nonpolar, by failing to diffuse rapidly in polarized agarose gels (Rios et al., 1988). When using the agar diffusion technique, other authors report that irregular diffusion of the lipophilic components of essential oils can occur, resulting in uneven concentrations in agar and causing the formation of regions with variable antimicrobial activity (Setzer et al., 2004; Sokmen et al., 2004). In order to confirm our results, we evaluated bactericidal activity using the microdilution test (MIC).

### Determination of the minimum inhibitory concentration (MIC) of essential oils from *Myrtaceae* species against multiresistant strains

The determination of the minimum inhibitory concentration was carried out only with *E. astringens* essential oil by the broth microdilution methodology (Table 2).

### Table 2. Bactericidal properties of *Eugenia astringens* leaf volatiles

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>MIC (CFU/mL)</th>
<th>Control</th>
<th>25%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>2.1 x 10^9±0.3</td>
<td>4.5 x 10^9±1.5**</td>
<td></td>
</tr>
<tr>
<td><em>Burkholderiacecnopecacia</em></td>
<td>1.0 x 10^1±0.1</td>
<td>3.5 x 10^9±0.3**</td>
<td></td>
</tr>
<tr>
<td><em>Echerichia coli</em></td>
<td>2.9 x 10^9±0.9</td>
<td>2.2 x 10^9±0.2**</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>2.5 x 10^4±0.5</td>
<td>8.3 x 10^3±4.2***</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.4 x 10^7±0.8</td>
<td>1.7 x 10^3±1.3***</td>
<td></td>
</tr>
</tbody>
</table>

MIC= Minimum inhibitory concentration (vol/vol%), CFU/mL= Colony Forming Units/mL. The results represent mean ± SEM of three experiments performed in triplicate. *p<0.05, **p<0.01 and *** p<0.001 were obtained after comparison with untreated control.
*P. aeruginosa* and *B. cenocepacia* showed more resistance to treatment with the essential oil of *E. astringens* when compared to the other strains tested. These data are in agreement with those found in the literature, which report the greater tolerance of *Pseudomonas* sp. to inhibition of bacterial growth compared to other species by essential oils of plants (Raut & Karuppayil, 2014).

To better demonstrate the inhibitory action of *E. astringens* essential oil, **Figure 1** gives the percentage of growth inhibition of bacterial cultures treated with 25% essential oil when compared to untreated cultures taken as 100%.

Reductions of 74% (*P. aeruginosa*), 78.3% (*B. cenocepacia*), 87.1% (*E. coli*), 99% (*S. aureus*) and 99.9% (*S. agalactiae*) of bacterial mass were observed in cultures of the five strains treated with essential oils. The treatment was more efficient for Gram-positive strains, reducing bacterial growth by 90%. These data were in agreement with several other reports where essential oils were shown to be more active towards Gram-positive than Gram-negative bacteria (Marino et al., 2002; Chorianopoulos et al., 2004; Gutierrez et al., 2008; Tiwari et al., 2009). Gram-negative bacteria are less susceptible to the action of antibacterials because they have an outer membrane that consists of phospholipids, proteins, and lipopolysaccharides (LPS), limiting the diffusion of hydrophobic compounds across the plasma membrane (Burt, 2004; Bassolé & Juliani, 2012). Since essential oils are typically lipophilic, they can accumulate in the lipid bilayer. This accumulation of lipids in the outer membrane of the bacterial cell reduces its diffusion and insertion into the inner membrane.

**Figure 1.** Percentage of inhibition of bacterial growth under *Eugenia astringens* leaf volatiles. The results represent mean ± SEM of three experiments performed in triplicate.

The affinity of essential oils for lipid compounds in the bacterial inner membrane causes structural changes making it more permeable to protons and ions. Therefore, damage to the membrane causes functional changes, such as selective permeability, enzymatic activity and energy generation promoted by the decrease of proton force (Bakkali et al., 2008).
Phytochemical analysis of leaf oils

Hydrodistillation of the dry leaves of *E. astringens, E. arenaria* and *M. atropurpureum* gave essential oils in yields of 2.06±0.25, 5.85±0.85 and 4.03±0.67 μL/g, respectively.

**Figure 4** compares the main components of leaf volatiles from Myrtaceae species used in the agar disk diffusion method. **Table 3** presents the chemical composition of leaf volatiles identified in *E. astringens* and used in continuous testing to evaluate bactericidal activity.

The sesquiterpenes represented the main fraction of leaf volatiles of *E. astringens* accounting for 51.1%, as characterized by a high percentage of oxygenated monoterpenes. *M. atropurpureum* species were mainly composed of monoterpenes (76.7%), the most abundant among them being α-pinene (35.5%), 1,8 cineol (20.8), and (24.8%) of sesquiterpenes. *E. arenaria* essential oil was rich in sesquiterpenes, including (E)-caryophyllene, α-muurolene, caryophyllene oxide, 1,10-di-epi-cubenol and apodophyllene, the main component in oils.

Studies by Victorio et al. (2011) for the species *M. atropurpureum* showed proportions similar those noted above between mono- (80%) and sequisterpenes (17%), although the collection was carried out in April, 2009, a time of year characterized by different climatic events in Brazil. The representativeness of the components was the same in that α-pinene and 1,8 cineole were the main monoterpenes, considering the relative area.

**Figure 4.** The major leaf volatiles: mono- and sesquiterpenes from leaves of *Eugenia astringens, E. arenaria* and *Myrrhinium atropurpureum* collected in Brazilian restingas

Previous studies have gathered data for the analysis of *E. astringens* volatiles (Magina et al. 2009; Defaveri et al. 2011; De Souza et al. 2018). In the present report, we highlight the presence of the major components α- and β-pinenes (**Figure 4**) in accordance with *E. astringens* oils from leaves collected in October in Santa Catarina, southern Brazil, although the amount is much smaller, 11.2 and 13.2% (Magina et al. 2009). In comparison with *M. atropurpureum* volatiles, the same α-pinene component was found in large amounts. The difference really lies in
the concentration of β-pinene, which is about 22% higher in *E. astringens*, and the presence of high concentrations (20.8%) of 1,8-cineole in *M. atropurpureum*. Among *E. astringens* volatiles, this study highlights the presence of glenol+globulol (14.6%) and volatiles from the selinene group, such as selin-11-en-4α-ol (5.3%) (Table 3).

Unlike *E. astringens* and *M. atropurpureum*, *E. arenaria* showed concentrations of α- and β-pinenes and 1.8 cineol below 8%, while in the set of volatiles of *E. arenaria*, α-pinene (7.9%) stands out. *E. astringens* and *E. arenaria* have similar concentrations of (*E*)-caryophylene (8.8% and 8.3%, respectively), and all evaluated species have close values of caryophyllene oxide (*E. arenaria* – 10.2%, *E. astringens* - 7.3, and *M. atropurpureum* – 7.9%) (Figure 4).

Based on other studies of antimicrobial activity with volatiles from Myrtaceae species, the most promising essential oils contain such components as α-pinene, 1,8-cineole, α-terpineol, and β-caryophyllene that show strong antimicrobial properties and are commonly cited (Bakkali et al., 2008; Hyun-Jun & Su-Kyun, 2018; Allenspach & Steuer, 2021; Santos et al., 2021). Among the essential oils studied, the set of volatiles from *E. astringens* had better bactericidal responses, with (*E*)-caryophylene and α-pinene being the volatile components in greatest quantities (Figure 4). However, synergistic activity between the phytochemicals of essential oils is generally responsible for the biological activity, not necessarily the most abundant component.

**Table 3.** Chemical composition of volatiles of leaves of *Eugenia astringens* collected in Grumari restinga, Brazil.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>LRI exp</th>
<th>Relative area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-thujene</td>
<td>927</td>
<td>0.2</td>
</tr>
<tr>
<td>α-pinene</td>
<td>934</td>
<td>23.6</td>
</tr>
<tr>
<td>n.l.</td>
<td>942</td>
<td>0.4</td>
</tr>
<tr>
<td>camphene</td>
<td>948</td>
<td>0.3</td>
</tr>
<tr>
<td>β-pinene</td>
<td>976</td>
<td>31.1</td>
</tr>
<tr>
<td>myrcene</td>
<td>991</td>
<td>0.4</td>
</tr>
<tr>
<td>limonene</td>
<td>1027</td>
<td>1.6</td>
</tr>
<tr>
<td>1,8-cineol</td>
<td>1029</td>
<td>0.2</td>
</tr>
<tr>
<td>γ-terpinene</td>
<td>1057</td>
<td>0.2</td>
</tr>
<tr>
<td>terpinolene</td>
<td>1088</td>
<td>0.3</td>
</tr>
<tr>
<td>endo-fenchol</td>
<td>1113</td>
<td>0.1</td>
</tr>
<tr>
<td>camphene hydrate</td>
<td>1146</td>
<td>0.1</td>
</tr>
<tr>
<td>borneol</td>
<td>1164</td>
<td>0.1</td>
</tr>
<tr>
<td>terpinen-4-ol</td>
<td>1176</td>
<td>0.2</td>
</tr>
<tr>
<td>α-terpineol</td>
<td>1190</td>
<td>2.6</td>
</tr>
<tr>
<td>bornyl acetate</td>
<td>1285</td>
<td>0.1</td>
</tr>
<tr>
<td>trans-pinocarvylacetate</td>
<td>1299</td>
<td>5.7</td>
</tr>
<tr>
<td>α-cubenene</td>
<td>1348</td>
<td>0.5</td>
</tr>
<tr>
<td>α-copaene</td>
<td>1374</td>
<td>0.4</td>
</tr>
<tr>
<td>α-gurjunene</td>
<td>1407</td>
<td>0.72</td>
</tr>
<tr>
<td>(<em>E</em>)-caryophyllene</td>
<td>1417</td>
<td>8.8</td>
</tr>
<tr>
<td>β-gurjunene</td>
<td>1429</td>
<td>0.3</td>
</tr>
<tr>
<td>aromadendrene</td>
<td>1446</td>
<td>2.2</td>
</tr>
<tr>
<td>trans-muurola-3,5-diene</td>
<td>1448</td>
<td>0.4</td>
</tr>
</tbody>
</table>
α-humulene 1451 1.1
allo-aromadendrene 1458 0.8
trans-cadina-1(6),4-diene 1471 0.6
β-selinene 1483 2.1
valencene 1485 0.2
α-selinene 1492 0.4
γ-cadinene 1513 0.7
trans-calamene +zoarene 1521 5.7
trans-cadi-1,4-diene 1530 1.6
n.i. 1557 1.2
spathulenol 1577 5.6
caryophyllene oxide 1580 7.2
glenol+globulol 1581 14.6
viridiflorol 1590 0.7
cubeban-11-ol 1591 0.2
rosifoliol 1600 1.4
humulene epoxide II 1607 0.2
n.i. 1611 0.5
1,10-di-epi-cubenol 1614 0.4
n.i. 1621 0.5
1-epi-cubenol 1626 4.6
n.i. 1631 0.6
cariofila-4(12),8(13)dien-5α or 5β-ol 1635 1.4
epi-a-cadinol + epi-a-muurolol 1641 3.7
α-muurolol 1646 0.7
n.i. 1650 0.1
selin-11-en-4α-ol 1653 5.3
neo-intermedeol 1656 0.4
n.i. 1668 0.5
muskatone 1676 0.1
n.i. 1677 0.5
apodophyllene 1709 0.9
n.i. 1767 0.2

| Monoterpenes | 32.7 |
| Diterpenes    | 5.8  |
| Sesquiterpenes| 51.1 |

Yield (μL/g)b 2.06±0.25

a LRIexp: experimental linear retention indices (calculated according to Van den Dool & Kratz 1963).
b Succar et al. 2019, n= 3. Values show media from 3 analyses and 3 injections. *The results represent means ± standard error of the phytochemical analysis of three samples of essential oils. **n.i. - no identified.

CONCLUSION

Data showed that most antimicrobial activity of the tested volatile oils appears to derive from terpenoids, particularly monoterpenes and sesquiterpenes. A bioautography study would be necessary to confirm the volatile compounds involved in the antimicrobial action. Results found using E. astringens leaf volatiles demonstrated the potential to exert beneficial antibacterial effect and could be a natural source, especially when used in combination with antibiotics to enhance their activity. However, further studies are in order to determine their mode of action and probable toxicological effects in order to optimize their use.
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