Chemical characterization, antioxidant, cytotoxic, and antibacterial activities of *Eugenia uniflora* L. and *Psidium cattleianum* Sabine essential oils against *Klebsiella pneumoniae* and *Acinetobacter baumannii*

Caracterização química, antioxidante, citotóxica e antibacteriana dos óleos essenciais de *Eugenia uniflora* L. e *Psidium cattleianum* Sabine contra *Klebsiella pneumoniae* e *Acinetobacter baumannii*

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ABSTRACT
INTRODUCTION: *Eugenia uniflora* L. and *Psidium cattleianum* Sabine essential oils (EO) can be biologically active and serve as novel sources of antibiotics for *Klebsiella pneumoniae* and *Acinetobacter baumannii* bacteria. METHODS: The EO of *E. uniflora* L. (EOE) and *P. cattleianum* Sabine (EOP) were extracted from de leaves of the plants and chemically characterized by gas chromatography-mass spectrometry (GC-MS). The antioxidant potential was evaluated by the DPPH (2,2- diphenyl-1-picryl-hydrazyl-hydrate) technique and the cytotoxicity evaluated in mammalian VERO cell line by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The antibacterial activity of EOE and EOP was assessed by the broth microdilution method. RESULTS: The major compounds of EOE were benzofuran (24.38%), germacrene B (20.12%), β-elemene (9.33%) and β-cubebene (8.55%), and for EOP were α-pinene (24.25%), β-caryophyllene (20.45%), and eucalyptol (10.43%). Both EO tested showed low antioxidant effect and dose-dependent cytotoxicity in VERO cell line. The EOE was less toxic for the mammalian cells with an IC_{50} (half maximal inhibitory concentration) index of 75.0 $mg.mL^{-1}$. The EOP and EOE were more active against *A. baumannii*, with a minimum inhibitory concentration (MIC) of 14.0 and 56.0 $mg.mL^{-1}$ and a minimal bactericide concentration (MBC) of 14.0 and 112.0 $mg.mL^{-1}$, respectively. CONCLUSIONS: Although EOE and EOP have low antioxidant and antibacterial activity against *A. baumanni* and *K. pneumoniae* new approaches can be applied to improve this effect in vivo.

Keywords: Pitanga, Araçá, Gram-negative bactéria, Myrtaceae.

RESUMO
INTRODUÇÃO: Os óleos essenciais (EO) de *Eugenia uniflora* L. e *Psidium cattleianum* Sabine podem ser biologicamente ativos e servir como novas fontes de antibióticos para as bactérias *Klebsiella pneumoniae* e *Acinetobacter baumannii*. MÉTODOS: Os EO de *E. uniflora* L. (EOE) e *P. cattleianum* Sabine (EOP) foram extraídos das folhas das plantas e quimicamente caracterizados por cromatografia gasosa acoplada à espectrometria de massa (GC-MS). O potencial antioxidante foi avaliado pela técnica DPPH (2,2-difenil-1-picril-hidrazil-hidrato) e a citotoxicidade avaliada na linhagem celular VERO de mamíferos pelo ensaio MTT (3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromide). A atividade antibacteriana de EOE e EOP foi avaliada pelo método da microdiluição em caldo. RESULTADOS: Os principais compostos de EOE foram benzofurano (24,38%), germacreno B (20,12%), β-elemeno (9,33%) e β-cubebeno (8,55%), e para EOP foram α-pineno (24,25%), β-caryophyllene (20,45%), e eucaliptol (10,43%). Ambos os EO testados mostraram baixo efeito antioxidante e citotoxicidade dose-dependente em células VERO. O EOE foi menos tóxico para as células de mamíferos com IC_{50} (concentração inibitória média) de 75,0 $mg.mL^{-1}$.Os EOP e EOE foram mais ativos contra *A. baumannii*, com uma concentração inibitória mínima (MIC) de 14,0 e 56,0 $mg.mL^{-1}$ e uma concentração bactericida mínima (MBC) de 14,0 e 112,0 mg.mL-1, respectivamente. CONCLUSÕES: Embora EOE e EOP tenham baixa atividade antioxidante e antibacteriana contra *A. baumanni* e *K. pneumoniae*, novas abordagens podem ser aplicadas para melhorar este efeito *in vivo.*

Palavras-chave: Pitanga, Araçá, bactérias Gram-negativas, Myrtaceae.

1 INTRODUCTION

Traditional medicine has been used since ancient times and it continues to play a fundamental role in health care, especially in primary health care. At an earlier time in the nineteenth century, more than 80% of Medicine products were formulated from plants, and in some countries, and are extensively integrated into the public health system (Shinwari; Qaiser, 2011, WHO, 2015). Medicinal plants are used as the plainest medication resource in traditional and complementary medicine worldwide. Furthermore, they are among the natural products of great scientific interest due to the possibility of using them as phytopharmaceuticals and presenting metabolite compounds in their chemical composition (Nascimento et al., 2000; Pereira; Cardoso, 2012; WHO, 2015). Secondary metabolite agents include essential oils (EO), which are naturally occurring volatile substances produced by plants, made up of many biologically active molecules (Kavoosi et al., 2013).

Plants of the Myrtaceae family, such as *Eugenia uniflora* L., are plants native to South America, found in Brazil (popularly known as “pitanga”), Argentina, Uruguay, and Paraguay (Consolini; Sarubbio, 2002), while *Psidium cattleianum* Sabine is a native Brazilian species (popularly known as “araçá”) that can be found in states from Bahia and Rio Grande do Sul, as also is found in Uruguay (Pereira et al., 2018). These two species are used for therapeutic purposes and have antioxidant (Victoria et al., 2012; Castro et al., 2014; Scur et al., 2016; Figueiredo et al., 2019) and antimicrobial activity (Scur et al., 2016; Bona et al., 2014; Soliman et al., 2016; Sobeh et al., 2016).

Gram-negative bacteria as *Acinetobacter baumannii* and *Klebsiella pneumoniae* are at high risk in hospital environments in many parts of the world. In developing countries, infections caused by these pathogens have been challenging over the past two decades because of their high morbidity and mortality rates, as well as their prolonged hospital stay. *A. baumannii* and *K. pneumoniae* have been identified as the leading causes of previously effective multi-drug resistant (MDR) infections, making treatment difficult (de Angelis et al., 2014; Oduro-Mensah et al., 2016; Singh; Manchanda, 2017). Antimicrobial resistance is a serious threat to public health worldwide because MDR strains account for approximately 50% of nosocomial infections worldwide. This can lead to rising costs, treatment failure, mortality, and reduce drug effectiveness and available treatment alternatives (Rice, 2008; ECDC, 2013; WHO, 2018).

In this study, we extracted the EO from the leaves of species *E. uniflora* L. and *P. cattleianum* Sabine from South of Brazil, and evaluate the chemical composition,

antioxidant, cytotoxic, and antibacterial activity against *K. pneumoniae* and *A. baumannii* bacteria, commonly involved in the community and nosocomial infections.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The leaves of *E. uniflora* L. (pitanga) and *P. cattleianum* Sabine (araçá) were collected in the orchard of the Agricultural Center of Palma, Federal University of Pelotas, Capão do Leão (31°48'13"S e 52°30'30"W). The leaves were harvested in April 2018 in the morning, during the autumn season. The samples were identified and the plant material stored in the Herbarium PEL at the Institute of Biology, Botany Department of the Federal University of Pelotas, Capão do Leão, Rio Grande do Sul, and identified by the following numbers of exsiccating: *P. cattleianum* Sabine (PEL N ° 26970) and *E. uniflora* L. (PEL N ° 26971). The plant's names where been checked through the website The Plant List (2013).

2.3 ESSENTIAL OILS (EO) EXTRACTION

To extract the EO, the leaves were dried in an oven with air circulation and later crushed in a knife mill. The EO extraction was carried out according to the Brazilian Pharmacopoeia (Brazil, 2010), using the steam drag hydro distillation process with the aid of the Clevenger apparatus (100g/4h). The yields of the two EO were calculated from the dry weight of the plant. The oils were named; EO of *E. uniflora* L. (EOE) and EO *P. cattleianum* Sabine (EOP).

2.4 CHEMICAL COMPOSITION OF EOE AND EOP

The chemical characterization of the EO was performed in gas chromatography coupled to mass spectrometry (GC-MS), brand Shimadzu QP2010, equipped with a split/splitless splitter. A Rtx-5MS Restek (30 m × 0.25 mm × 0.25 microns) capillary column was used under the following chromatographic conditions: helium gas carrier obtained by electron impact fragments to a power of 70 eV rate of 1.2 mL/min, 1:50 split flow, and the volume of the injected sample of 1 µL. Programmed oven temperature: initial temperature of 40°C with a heating ramp of 5°C/min to 280°C and remained stable at this temperature for 10 min, totaling 58 min of running, with the injector and interface temperature. The composts were analyzed using the NIST08 spectral library as the reference standard.

2.5 DPPH RADICAL SCAVENGING ASSAY

To evaluate the antioxidant activity of EOE and EOP, the *in vitro* capture technique of the free radical DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) (Sigma-Aldrich®) was used, according to Pellati et al. (2004). In this study, four concentrations of OE were tested (1 $mg.mL^{-1}$; 0.5 $mg.mL^{-1}$; 0.25 $mg.mL^{-1}$ and 0.125 $mg.mL^{-1}$), which were mixed with an ethanol solution (2.7 mL in the concentration of 0.06 mM) containing the radical DPPH (300 µL). After 15 min of incubation at room temperature, absorbance readings were made using a spectrophotometer (Bel Photonics model UV-M51) at 517 nm. Rutin was used as a standard in the same concentrations as the EO. The test was performed in triplicate. The values were expressed as the percentage of inhibition of DPPH absorbance (% inhibition) concerning the control values without the EO. The % inhibition was calculated according to equation 1.

Equation 1: $\% \ inhibition = (A_{(DPPH)} - A_{(EO)} / A_{(DPPH)}) \ x \ 100$

2.6 MINIMAL INHIBITORY CONCENTRATION (MIC)

Two standard strains were used for the microbiological assays, *Acinetobacter baumannii* ATCC 19606 and *Klebsiella pneumoniae* ATCC 700603, both provided by the Oswaldo Cruz Foundation Microorganisms Collection (FIOCRUZ). The broth microdilution technique was used to determine the MIC according to the Clinical and Laboratory Standard Institute (CLSI, 2017). For the assays, a sterile polystyrene-96 microwell plate (Kasvi®) was used. The culture medium was Brain Heart Infusion (BHI, Acumedia®) with emulsifying agent Tween 80 (Synth, TW80) 1%. The concentrations of oils tested range from 1.7 to 224 $mg.mL^{-1}$. As a negative control, 50 µL of BHI broth plus TW80 was used, and as a positive control, 50 µL of BHI broth plus TW80 with 50 µL of the bacterial suspension was used. For the bacterial inoculum, *A. baumannii* and *K. pneumoniae* were cultured in tubes containing BHI, intending to reach 0.5 of optical density at 630 nm (DO_{630}), and 50 µL of these inoculums were added to 4950 µL of BHI broth. Subsequently, 50 µL of this suspension was added to all wells of the plate, except those with the negative control, resulting in final concentrations of $3x10^4$ $UFC.mL^{-1}$. The experiment was performed in triplicate and the microplate was incubated at 37°C for 24 h. After incubation, 20 µL of 2,3,5–Triphenyl-Tetrazolium-Chloride P.A. (CTT, Dinâmica®) at 0.5% was added to all the wells and the plate was incubated for 20 min at 37°C. After the incubation period, the plates were under observation to verify if there would be any change of color.

2.7 MINIMAL BACTERICIDE CONCENTRATION (MBC)

From the MIC results, the MBC was determined (CLSI, 2017), which is defined as the lowest concentration of EO where visible growth in the subculture can be observed. Thus, 5 µL were removed from each well of the MIC assay. After 24 h, the samples under incubation that inhibited bacterial growth were put on BHI agar plates and incubated at 37°C for 24 h. The absence of bacterial growth on the agar plates indicates that the EO tested have bactericidal activity, while colony growth indicates bacteriostatic activity.

2.8 MAMMALIAN CELLS CYTOTOXICITY

The evaluation of the cytotoxic effect of EOE and EOP used the kidney epithelial cells extracted from monkeys (VERO – ATCC CCL-81) cells from the cell bank of the Laboratory of Virology and Immunology of the School of Veterinary Medicine, Federal University of Pelotas, using the method described by Picoli et al. (Picoli et al., 2015). These cells were grown in Minimum Essential Medium (E-MEM, Sigma-Aldrich®) supplemented with fetal bovine serum (SFB, Gibco®). An amount of $3x10^4$ cells per well were seeded in 96-well microplates (Kasvi®) and grown for 24 h at 37°C in an atmosphere of 5% CO_2 until the formation of the monolayer. The concentrations tested of EO were 1.7 to 224 $mg.mL^{-1}$. After the incubation, the culture medium was removed, and the cell viability was evaluated by measuring the reduction of 1 $mg.mL^{-1}$ soluble MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphe-nyltetrazolium bromide] (Sigma-Aldrich®). Fifty µL of the MTT were added and the plates were incubated at 37°C for 4 hours. After removal of the cell supernatant, the crystals were solubilized by the addition of 100 µL of dimethyl sulfoxide (DMSO, Sigma-Aldrich®) in each well, then they were manually shaken and incubated at 37°C for 15 min. The absorbance of each well was read on a microplate reader (Thermo plate®) at a wavelength of 540 nm. All assays were performed independently at least three times in triplicate, and results were expressed as the percentage of cell growth inhibition in comparison with the negative control (non-treated). The cytotoxic activity was assessed using half-maximal inhibitory concentration (IC_{50}), able to inhibit 50% of cell growth, which was assessed through a non-linear regression model. The EO selectivity index (SI) was calculated $SI = IC_{50}/MIC$. The higher the SI value, the less toxic the compound is to the cell.

2.9 STATISTICAL ANALYSIS

Statistical analysis was carried out by two-way analysis of variance (ANOVA) using a probability value of $p<0.05$ using the GraphPad Prism 8.2.0 software. For cytotoxicity

assay, Dunnett's post-test was conducted to identify significant differences between the negative control and the means of different treatments. The IC_{50} values were assessed through a non-linear regression model.

3 RESULTS

3.1 CHEMICAL COMPOSITION OF EO

The specimens collected of *E. uniflora* L. and *P. cattleianum* Sabine presented a differentiated composition for their essential oils, and the oil yield was 0.1 and 0.4% for EOE and EOP, respectively. The chemical analysis, using GC-MS, identified 20 compounds in the EOE. Four of them were the major constituents: benzofuran (24.38%), germacrene B (20.12%), β-elemene (9.33%), and β-cubebene (8.55%) (Figure 1A, 1B, 1C and 1D). Nineteen compounds were found in the EOP, and α-pinene (24.25%), β-caryophyllene (20.45%), and eucalyptol (10.43%) were identified as the main ones (Figure 1E, 1F, and 1G). Table 1 shows all the components identified in both EO, and these components (Figure 2) belong to the class of terpenes, with monoterpenes and sesquiterpenes being the dominant components; among the sesquiterpenes present, the non-oxygenated ones were the most frequent.

3.2 DPPH RADICAL SCAVENGING ASSAY

Both EOE and EOP demonstrated a low effect on DPPH, which remained in its oxidized form when compared to its standard. The EOE showed a higher percentage of inhibition in the concentrations of 1 $mg.mL^{-1}$ (8.1%), 0.5 $mg.mL^{-1}$ (8.7%) and 0.125 $mg.mL^{-1}$ (6.6%), while in the concentration of 0.25 $mg.mL^{-1}$ it obtained a lower inhibition value (-3.9%). The EOP showed a percentage of inhibition of 8.1%, 6.9%, 5% and 4.8% in the following tested concentrations: 1 $mg.mL^{-1}$, 0.5 $mg.mL^{-1}$, 0.25 $mg.mL^{-1}$ and 0.125 $mg.mL^{-1}$.

3.3 ANTIBACTERIAL ACTIVITY (MIC AND MBC)

In the broth microdilution assay, EOE and EOP were more active against the *A. baumannii* species. The EOP presents the lowest MIC and MBC value observed, 14.0 $mg.mL^{-1}$. The EOE had a MIC of 56.0 $mg.mL^{-1}$, being bactericidal at the concentration of 112.0 $mg.mL^{-1}$ (Table 2). When the EOE and the EOP were tested against *K. pneumoniae*, higher concentrations were active. The EOE had MIC of 112.0 $mg.mL^{-1}$ and MBC of 224.0 $mg.mL^{-1}$, while the EOP had a MIC of 112.0 $mg.mL^{-1,}$ and no bactericidal activity was

observed under concentrations tested in the present study (MBC > 224.0 mg.mL^{-1}) (Table 2).

3.4 CYTOTOXICITY TEST ON MAMMALIAN CELL

As shown in Figure 3, both EO tested exhibited a concentration-dependent cytotoxic activity. The results showed that the EOE was less toxic in mammalian cells when compared to EOP. The IC_{50} values, calculated based on cell viability, were established as 6.0 and 75.0 mg.mL^{-1} from EOP and EOE, respectively. EOP and EOE treatment with concentrations of 3.5 mg.mL^{-1} and 56.0 mg.mL^{-1} present ~60% of viable cells. Statistical analyses demonstrated a significant reduction in the percentage of viable cells in concentrations ≥ 3.5 mg.mL^{-1} from EOP, and ≥ 14.0 mg.mL^{-1} from EOE, compared with non-treated cells (p<0.0001). Under the MIC values determined from *A. baumannii* the EOE and EOP treatments presented 58% (MIC= 56.0 mg.mL^{-1}) and 14% (MIC= 14.0 mg.mL^{-1}) of mammalian cells viable, respectively. For *K. pneumoniae*, the MIC values determined presented 21% (MIC= 112.0 mg.mL^{-1}) of viable cells from EOE and 0.6% (MIC= 112.0 mg.mL^{-1}) from EOP. The IC_{50} and MIC (mg.mL^{-1}) values are the two variables used to calculate the SI (SI = IC_{50}/MIC), which is a measure of the safe of EO. In this study, EOE presents SI range from 0.4-1.3, and EOP 0.05-0.7 (Table 2).

4 DISCUSSION

The chemical composition of the EO of the *E. uniflora* L. and *P. cattleianum* Sabine species have been reported previously and identify a greater number of terpenes and terpene derivatives (Victoria et al., 2012; Figueiredo et al., 2019; Pino et al., 2001; Biegelmeyer et al., 2011). In our study, we evaluate the chemical composition of EOE and EOP by GC-MS and the identified compounds belong in most of the terpenes class (monoterpenes and sesquiterpenes). These compounds are volatile constituents that are a diverse group of organic compounds, usually with a low molecular weight (<250 Da) and high vapor pressure under environmental conditions, which diffuse rapidly through the gas phase and within biological systems (Pereira et al., 2018). The profile of compounds found in each plant species is variable, as it depends on many factors, such as local climatic and environmental conditions, soil variations, season, geographic location, geology, stage of the vegetative cycle, part of the plant, time of collection and the method used to obtain the EO (Alves et al., 2008; Viuda-Martos et al., 2008).

Regarding the antioxidant activity, some studies prove that terpenoid compounds show activity in the elimination of the radical DPPH (Yu et al., 2011; Zouari et al., 2011). Figueiredo et al. (2019) tested five EOE and they all showed the ability to sequester the free radical with an inhibition percentage range from 30.3-45.1%. Other authors found antioxidant activity when studying EO and attributed this result to compounds as germacrene B, which is a strong antioxidant due to its extra cyclic methylene portion, and β-caryophyllene, which also can eliminate free radicals as determined in the DPPH test (Victoria et al., 2012; Dorman et al., 2000). However, despite finding similar constituents in the chemical composition of EOE, a low effect on DPPH was evaluated in our research. Scur et al. (Scur et al., 2016) evaluated EOP and it showed a low elimination of DPPH radicals when tested at concentrations of 50 $mg.mL^{-1}$ (4%), 75 $mg.mL^{-1}$ (8.5%) and 100 $mg.mL^{-1}$ (16.2%). The same was found when testing this EO in the present study, as it was possible to verify a low percentage of DPPH inhibition, however, we use lower concentrations of the EO (0.125 to 1 $mg.mL^{-1}$). The antioxidant activity of Myrtaceae fruits reported by other authors may be related to the presence of phenolic compounds, mainly flavonoids (Medina et al., 2011), as these are one of the main components responsible for the antioxidant capacity of natural products (Podsedek, 2007). Essential oils from *E. uniflora* L. and *P. cattleianum* Sabine species have not been widely explored, and so more studies must be carried out to determine their antioxidant activity.

In our study, we demonstrated that the EOP and EOE present a concentration-dependent cytotoxic activity in mammalian VERO cells. Here, we observed that the EOE was less toxic (IC_{50}= 75.0 $mg.mL^{-1}$) in comparison to EOP (IC_{50}= 6.0 $mg.mL^{-1}$). Additionally, in relationship to pharmacological safety, the EOE showed a higher SI value. Previous studies conducted with EO of plants of the genus *Eugenia* (*E. egensis*, *E. flavescens*, *E. polystachya* and *E. patrissi*), found that the EO presented cytotoxicity against HCT-116 cell line (da Silva et al., 2013). Pinto et al. (2019) studied the EO of cloves (*E. caryophyllus*) and its main component, eugenol, and these showed low toxicity in mammalian cells (VERO). Considering EOE and EOP cytotoxicity on VERO cells, the pharmacological effects of OE are dose-dependent and could be optimized to avoid adverse effects. Possibility of cytotoxicity evaluation in other cell lines, and alternatives such as nanoencapsulation have many advantages for the delivery because they can increase OE compound's interaction with tissues and cells, bioavailability, and OE targeting consequently resulting in increased efficacy and decreased adverse effects. Thereby, these approaches can allow the use of lower concentrations of treatment reducing the cytotoxicity.

Essential oils are rich in secondary metabolites that have antimicrobial activity. These compounds include those of the terpene class since the lipophilic character of most of these constituents become bound to the biomembranes of living organisms, allowing the fluidity and permeability of membranes to increase, which may lead to the death of the microorganism (Tepe et al., 2004; Mulyaningsih et al., 2010; Krstin et al., 2015). Sobeh et al. (2016) evaluated the antibacterial effect of EOE against several Gram-positive and Gram-negative bacteria, including *K. pneumoniae* ATTC 700603, in which it had a MIC and MBC concentration >10 $mg.mL^{-1}$.

About the EOP, no research was found shows the activity of this EO against *A. baumannii*, since most of the existing studies with the EO of this species are with other clinical and food bacteria, and fungi (Castro et al., 2014; Scur et al., 2016; Soliman et al., 2016). The EOP was tested previously by Scur et al. (2016) against *K. pneumoniae* ATCC 13883, and obtained MIC and MBC in the highest concentration tested (200 $mg.mL^{-1}$). In our study, we obtained lower MIC values from EOP (MIC= 112.0 $mg.mL^{-1}$), in comparison at this previous study, however, EOP was not bactericidal (MBC > 224.0 $mg.mL^{-1}$). Thus, as far as we know, this is the first study to assess the potential of EOP against *A. baumannii*, and here the MIC/MBC values were 14.0 $mg.mL^{-1}$. However, the EOP has an IC_{50}= 6.0 $mg.mL^{-1}$, and under the MIC value only 14% of mammalian cells present viability. Therefore, the data obtained in this investigation suggest that the EOP deserves further studies, especially against clinical isolates of *A. baumannii* to possibly be used as an alternative in the treatment of infections.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

Marcelle Oliveira Garcia: Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Kamila Furtado da Cunha**: Data curation, Investigation, Validation. **Suzane Olachea Allend:** Data curation, Investigation, Validation. **Mirian Elert da Silva:** Investigation. **Ivandra Ignês Santi:** Data curation, Investigation, Validation, Visualization, Writing - review & editing. **Rogério Antonio Freitag:** Methodology. **Silvia de Oliveira Hübner:** Methodology. **Daiane Drawanz**

Hartwig: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - review & editing, Visualization, Supervision, Funding acquisition.

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Figure 1. Molecular representation of the major compounds found in *E. uniflora* L. (EOE) and *P. cattleianum* Sabine (EOP). A: benzofuran, 6–ethenyl–4,5,6,7–tetrahydro; B: germacrene B; C: β-elemene; D: β-cubebene E: α-pinene; F: β-caryophyllene and G: eucalyptol.

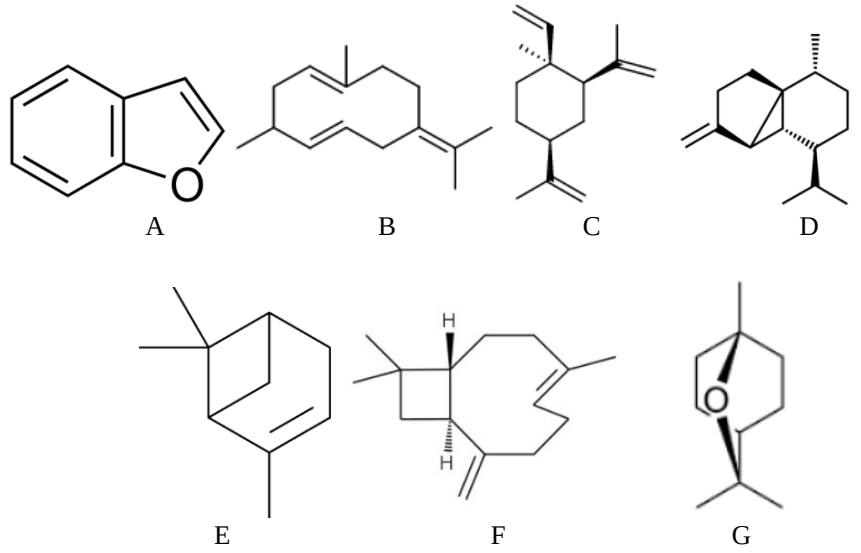
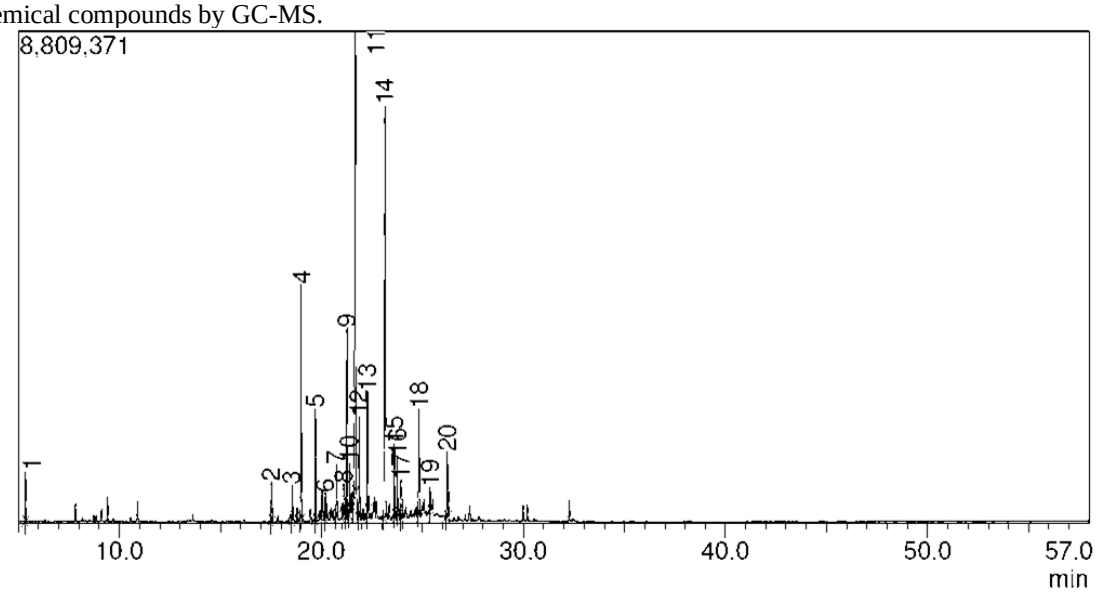
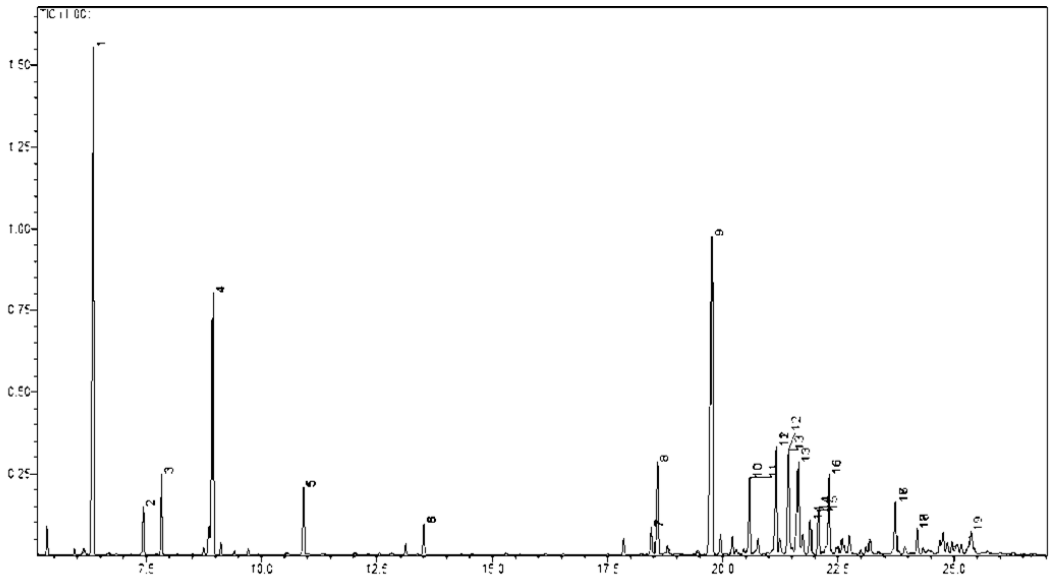


Figure 2. Chromatograms depicting the peaks of (A) *E. uniflora* L. (EOE) and *P. cattleianum* Sabine (EOP) chemical compounds by GC-MS.



(EOE)



(EOP)

Table 1. Chemical characterization of the essential oils of *E. uniflora* L. (EOE) and *P. cattleianum* Sabine (EOP) obtained by GC-MS.

EOE				EOP			
Peak	**Chemical composition**	**RT**	**Area (%)**	**Peak**	**Chemical composition**	**RT**	**Area (%)**
1	o-Xylene	5.360	1.23	**1**	α- Pinene	6.358	24.25
2	δ - Elemene	17.541	1.43	**2**	β- Pinene	7.432	1.72
3	α- Copaene	18.568	1.26	**3**	β- Myrcene	7,832	2.77
4	β- Elemene	19.006	9.33	**4**	Eucalyptol	8.944	10.43
5	β- Caryophyllene	19.717	4.33	**5**	β- Linalool	10.913	2.86
6	Aromadendrene	20.203	1.11	**6**	(-) - α- Terpineol	13.522	1.32
7	Alloaromadendrene	21.761	1.80	**7**	α- Ylangene	18.448	1.25
8	γ – Selinene	21.123	1.25	**8**	α- Cubebene	18.578	4.33
9	β - Cubebene	21.280	8.55	**9**	β - Caryophyllene	19.764	20.45
10	β - Selinene	21.404	1.73	**10**	α- Caryophyllene	20.580	3.59
11	Benzofuran, 6 – ethenyl – 4,5,6,7 – tetrahydro	21.685	24.38	**11**	Germacrene D	21.149	5.33
12	NI	21.890	3.83	**12**	β - Selinene	21.415	5.12
13	δ – Cadinene	22.296	4.66	**13**	NI	21.629	4.88
14	Germacrene B	23.164	20.12	**14**	NI	21.893	1.48
15	Spathulenol	23.618	2.66	**15**	γ – Muurolene	22.068	1.97
16	Globulol	23.765	2.02	**16**	(+) - δ – Cadinene	22.291	3.34
17	NI	23.951	2.01	**17**	Caryophylenne oxide	23.739	2.60
18	NI	24.842	4.29	**18**	(+) - Ledol	24.207	1.18
19	NI	25.377	1.13	**19**	NI	25.373	1.11
20	NI	26.239	2.88				

EOE: essential oil of *E. uniflora* L.; EOP: essential oil of *P. cattleianum* Sabine; RT: retention time; NI: not identified.

Table 2. Antibacterial activity, IC_{50} and SI from essential oils of *E. uniflora* L. (EOE) and *P. cattleianum* Sabine (EOP).

	A. baumannii			*K. pneumoniae*			IC_{50} (mg.mL^{-1})
	MIC	MBC	SI	MIC	MBC	SI	VERO
EOE	56.0	112.0	1.3	112.0	224.0	0.7	75.0
EOP	14.0	14.0	0.4	112.0	>224.0	0.05	6.0

MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericide Concentration; EOE: essential oil of *E. uniflora* L.; EOP: essential oil of *P. cattleianum* Sabine. The selectivity index is the ratio between the half-maximal inhibitory concentration (IC_{50}) value of the essential oils obtained for VERO cells and the MIC value (SI = IC_{50}/MIC). Data are expressed as means ± SD from three independent experiments.

Figure 3. Cytotoxicity effect of EOE and EOP at 1.7–224.0 mg.mL^{-1} against VERO cell line. Cell proliferation was investigated by MTT assay. The half-maximal inhibitory concentration (IC_{50}) values are shown for each EO. EOP: essential oil of *P. cattleianum* Sabine. EOE: Essential oil of *E. uniflora* L.. Data are expressed as means ± SD from three independent experiments, analyzed by two-way ANOVA followed by Dunnett's multiple comparison test. **** $p < 0.0001$ compared with negative control (non-treated = 0.0).