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TESE

Atividade biológica de *Eugenia uniflora* L. (pitanga) e *Psidium cattleianum* S. (araçá) frente as bactérias *Klebsiella pneumoniae* e *Acinetobacter baumannii*

MARCELLE OLIVEIRA GARCIA

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Marcelle Oliveira Garcia

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Tese apresentada ao Programa de Pós-Graduação em Microbiologia e Parasitologia (PPGMPAR) do Instituto de Biologia, Departamento de Microbiologia e Parasitologia da Universidade Federal de Pelotas, como requisito parcial a obtenção do título de Doutora em Ciências Biológicas (área de concentração: Microbiologia).

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Pelotas, 2022

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“Todos os dias sob todos os pontos de vista vou cada vez melhor!”

(Autor desconhecido)

RESUMO

Garcia, Marcelle Oliveira. **Atividade biológica de *Eugenia uniflora* L. (pitanga) e *Psidium cattleianum* S. (araçá) frente as bactérias *Klebsiella pneumoniae* e *Acinetobacter baumannii*.** 2022. 140f. Tese (Doutorado em Ciências Biológicas) – Programa de Pós-Graduação em Microbiologia e Parasitologia, Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, 2022.

Pneumonia é uma infecção pulmonar que envolve caracteristicamente o espaço alveolar e pode ser ocasionada por *Klebsiella pneumoniae* e *Acinetobacter baumannii*. Estas bactérias, na maioria das vezes, apresentam resistência aos antibióticos tornando-se um dos principais desafios para a saúde pública em todo o mundo. Devido a essa problemática é fundamental a busca por novos antibióticos eficazes contra estas bactérias. As plantas medicinais são boas alternativas, como as espécies da família Myrtaceae, *Eugenia uniflora* L. e *Psidium cattleianum* S. que apresentam diversas propriedades biológicas. O objetivo desse estudo foi extrair, caracterizar físico-quimicamente, avaliar a citotoxicidade em células de mamíferos e determinar a atividade antioxidante, antibacteriana e antibiofilme de óleos essenciais (OE) e extratos metanólicos (EM) de *E. uniflora* L. e *P. cattleianum* Sabine contra cepas padrão e isolados clínicos de *K. pneumoniae* e *A. baumannii*. Na análise química do OE de folhas de *E. uniflora* L. (OEE) e *P. cattleianum* S. (OEP) foram encontrados compostos terpenoides, sendo os constituintes majoritários o benzofurano (24,38%) para OEE e α-pineno (24,25%) para o OEP. Ambos os OE testados apresentaram baixo efeito antioxidante sobre o DPPH (2,2, difenil-2-picrilhidridrazil). Quanto às análises microbiológicas, o OE que apresentou melhor resultado foi o OEP contra *A. baumannii*, com concentração inibitória mínima (CIM) e concentração bactericida mínima (CBM) de 13,5 $mg.mL^{-1}$. Enquanto para *K. pneumoniae*, o OE mais ativo foi o OEP, com uma CIM de 121,4 $mg.mL^{-1}$, não apresentando efeito bactericida. Já quando analisada a composição química do EM de *E. uniflora* L. (EME) e *P. cattleianum* S. (PME) foi possível identificar ácidos fenólicos. O PME foi o que apresentou maior atividade antioxidante em todas as concentrações testadas (1 $mg.mL^{-1}$; 0,5 $mg.mL^{-1}$; 0,25 $mg.mL^{-1}$ e 0,125 $mg.mL^{-1}$). Na atividade antibacteriana o EME foi o mais ativo, pois apresentou os menores valores de CIM para a cepa padrão de *A. baumannii* (9,2 $\mu g.mL^{-1}$), enquanto 50% dos isolados apresentaram a mesma concentração de CIM (9,2 $\mu g.mL^{-1}$), sendo que destes 33% mostrou efeito bactericida. O PME apresentou uma porcentagem de inibição de biofilme de 88% e EME de 58,5% frente a cepa padrão, enquanto ambos os extratos apresentaram 88% de inibição de biofilme contra o isolado clínico avaliado. Em relação ao efeito destrutivo do biofilme pré-formado, o EME e PME não apresentaram percentual de inibição frente a cepa padrão de *A. baumannii*. Já frente ao isolado multirresistente, EME destruiu 34% do biofilme pré-formado e PME 42%. EME e PME levaram de 10 minutos até 6 horas, para matar a cepa padrão e isolado multirresistente de *A. baumannii*. Portanto, os resultados apresentados aqui são promissores e demonstram a atividade biológica de OE e EM *E. uniflora* L. e *P. cattleianum* Sabine para uso no desenvolvimento de novos antimicrobianos para o tratamento de doenças causadas por *K. pneumoniae* e *A. baumannii*.

Palavras-chave: resistência; atividade antibacteriana; atividade antibiofilme; araçá; pitanga.

ABSTRACT

Garcia, Marcelle Oliveira. **Biological activity of *Eugenia uniflora* L. (pitanga) and *Psidium cattleianum* S. (araçá) against the bacteria *Klebsiella pneumoniae* and *Acinetobacter baumannii*.** 2022. 140f. Thesis (PhD in Biological Sciences) – Programa de Pós-Graduação em Microbiologia e Parasitologia, Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, 2022.

Pneumonia is a pulmonary infection that characteristically involves the alveolar space and can be caused by *Klebsiella pneumoniae* and *Acinetobacter baumannii*. These bacteria, most of the time, are resistant to antibiotics, becoming one of the main challenges for public health worldwide. Due to this problem, the search for new antibiotics effective against these bacteria is essential. Medicinal plants are good alternatives, such as species from the Myrtaceae family, *Eugenia uniflora* L. and *Psidium cattleianum* S., which have several biological properties. The objective of this study was to extract, characterize physicochemically, evaluate the cytotoxicity in mammalian cells and determine the antioxidant, antibacterial and antibiofilm activity of essential oils (EO) and methanolic extracts (EM) of *E. uniflora* L. and *P. cattleianum* Sabine against standard strains and clinical isolates of *K. pneumoniae* and *A. baumannii*. In the chemical analysis of the EO of leaves of *E. uniflora* L. (OEE) and *P. cattleianum* S. (OEP) terpenoid compounds were found, the major constituents being benzofuran (24.38%) for OEE and α-pinene (24 .25%) for the OEP. Both EO tested showed a low antioxidant effect on DPPH (2,2, diphenyl-2-picrylhydrohydrazyl). As for the microbiological analyses, the OE that presented the best result was the OEP against *A. baumannii*, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 13.5 $mg.mL^{-1}$. While for *K. pneumoniae*, the most active EO was the OEP, with a MIC of 121.4 $mg.mL^{-1}$, showing no bactericidal effect. When the chemical composition of the EM of *E. uniflora* L. (EME) and *P. cattleianum* S. (PME) was analyzed, it was possible to identify phenolic acids. The PME showed the highest antioxidant activity at all concentrations tested (1 $mg.mL^{-1}$; 0.5 $mg.mL^{-1}$; 0.25 $mg.mL^{-1}$ and 0.125 $mg.mL^{-1}$). In the antibacterial activity, EME was the most active, as it presented the lowest MIC values for the standard strain of *A. baumannii* (9.2 $\mu g.mL^{-1}$), while 50% of the isolates presented the same MIC concentration (9. 2 $\mu g.mL^{-1}$), of which 33% showed a bactericidal effect. PME showed a percentage of biofilm inhibition of 88% and EME of 58.5% against the standard strain, while both extracts showed 88% of biofilm inhibition against the evaluated clinical isolate. Regarding the destructive effect of the preformed biofilm, the EME and PME did not show a percentage of inhibition against the standard strain of *A. baumannii*. In contrast to the multidrug-resistant isolate, EME destroyed 34% of the preformed biofilm and PME 42%. EME and PME took from 10 minutes to 6 hours to kill the standard strain and multidrug-resistant isolate of *A. baumannii*. Therefore, the results presented here are promising and demonstrate the biological activity of EO and EM *E. uniflora* L. and *P. cattleianum* Sabine for use in the development of new antimicrobials for the treatment of diseases caused by K. pneumoniae and A. baumannii.

Key-words: resistance; antibacterial activity; antibiofilm activity; araçá; pitanga.

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As Infecções Relacionadas à Assistência à Saúde (IRAS), anteriormente conhecida como infecções nosocomiais ou hospitalares, são aquelas que os pacientes adquirem ao receber atendimento de saúde. Cerca de um em cada 31 pacientes hospitalizados tem pelo menos uma IRAS, sendo assim, essas infecções são um grave problema de saúde pública em todo o mundo. As IRAS mais frequentes são infecções de feridas cirúrgicas, do trato urinário associado à cateter e do trato respiratório inferior, causando pneumonia (DOORDUIJN et al., 2016; HAQUE et al., 2018; CDC, 2010; CDC, 2018; CDC, 2019; WHO, 2011; WHO, 2012).

Pneumonia pode ser definida como uma infecção pulmonar que envolve caracteristicamente o espaço alveolar (TORRES et al., 2021), a maior parte das vezes é ocasionada por bactérias como *Klebsiella pneumoniae* e *Acinetobacter baumannii* (CDC, 2010; CDC, 2019). *K. pneumoniae* são bastonetes Gram-negativos, anaeróbios facultativos, imóveis e encapsulados e estão presentes na microbiota gastrointestinal humana. *A baumannii* são cocobacilos Gram-negativos, ubíquos, aeróbios estritos, imóveis e encontram-se na microbiota da pele humana (KONEMAN, 1997; CDC, 2010).

As infecções ocasionadas por *K. pneumoniae* e *A. baumannii* geralmente ocorrem em ambientes de saúde, em pacientes doentes que precisam de aparelhos respiratórios, cateteres intravenosos, que estão em unidade de terapia intensiva ou aqueles com internações prolongadas ou que fazem tratamento por um longo período com antimicrobianos convencionais, aumentando as chances dessas bactérias desenvolverem resistência a antibióticos e por fim se tornarem multirresistentes (MDR). A resistência bacteriana aos antibióticos tornou-se um dos principais desafios para a saúde pública em todo o mundo (CDC, 2010; CDC, 2019; WHO, 2011). Todavia, a busca por novos antibacterianos eficientes continua, pois é de extrema importância que se encontre novos fármacos ou combinações de medicamentos propostos para uso clínico contra esses micro-organismos (BASSETTI et al., 2019).

As plantas medicinais estão dentre os produtos naturais de grande interesse científico devido à possibilidade de empregá-las como fitofármacos, como também por

apresentarem compostos metabólicos em sua estrutura (NASCIMENTO et al., 2000; PEREIRA; CARDOSO, 2012).

Plantas da família Myrtaceae são encontradas em todo hemisfério sul, mas principalmente na região neotropical e australiana (HEYWOOD et al., 2008; SYTSMA et al., 2004). Apresentam diversas propriedades biológicas, apresentando em sua composição química compostos fenólicos e polifenois, como flavonoides, ácidos fenólicos, taninos, estilbenos, lignanas, cumarinas e tocoferois, lipídios funcionais e carotenóides (DUARTE; PAULL, 2015).

Eugenia uniflora L. é uma espécie nativa do Brasil, conhecida popularmente como pitangueira, pitanga ou pitanga-vermelha. Tem o seu nome derivado do tupi “pi’tãg”, que significa vermelho, devido à cor avermelhada do fruto dessa planta (FOUQUÉ, 1981; VILLACHICA et al., 1996). Encontra-se disseminada, praticamente por todo o território nacional, mais precisamente nos estados da Bahia, Mato Grosso do Sul, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Rio Grande do Sul e Santa Catarina, pois apresenta uma boa adaptação às diferentes condições de solo e clima (LIRA JÚNIOR et al., 2007; FLORA DO BRASIL, 2020). A pitangueira caracteriza-se como um arbusto denso que atinge entre 2 e 4 m, podendo chegar a ter de 6 a 9 m de altura. As folhas possuem as seguintes características morfológicas: são opostas, simples, elípticas e com pecíolo com cerca de 2 mm. Quanto ao fruto da pitanga apresenta formato globoso, sendo achatado nos polos, com 7 a 10 sulcos no sentido longitudinal e coroado com sépalas persistentes. O fruto possui um sabor doce-ácido com um aroma intenso e característico, além disso, há presença de apenas uma semente no fruto, porém, também pode ter o desenvolvimento de duas ou três sementes menores e achatadas (LIRA JÚNIOR et al., 2007; FLORA DO BRASIL, 2020). Várias atividades farmacológicas foram investigadas nessa espécie, incluindo potencial antioxidante, atividade anti-inflamatória, analgésica, antibacteriana e antifúngica (AURICCHIO; BACCHI, 2003; FIGUEIREDO et al., 2019; SOBEH et al., 2019; VICTORIA et al., 2012).

Psidium cattleianum Sabine é conhecido popularmente pelos nomes de araçá, araçá-do-mato, araçá-do-campo, araçá-amarelo, araçá-vermelho, araçazeiro, araçazeiro-da-praia (CORADIN; SIMINSKI; REIS, 2011; RASEIRA et al., 2004). Essa planta apresenta origem brasileira e podem ser encontradas na Bahia e nos estados do Rio Grande do Sul e Santa Catarina (BIEGELMEYER et al., 2011). O araçazeiro é

uma árvore ou arbusto, podendo atingir de 1-9 metros de altura. As flores são axilares, solitárias e brancas e as folhas são coriáceas, brilhantes e aromáticas. Quanto aos frutos possuem forma de bagas globosas, piriformes, ovoides ou achatadas, de coloração amarela ou vermelha quando maduros. A polpa do fruto encontra-se branca, amarela-clara ou vermelha e apresenta diversas sementes pequenas no seu interior (CORADIN SIMINSKI; REIS, 2011; LORENZI et al., 2006). Alguns estudos demonstram que essa espécie é rica em compostos com propriedade antibacteriana (DACOREGGIO; MORONI; KEMPKA, 2019; MEDINA et al., 2011; SCUR et al., 2016).

Sendo assim, antimicrobianos utilizando óleo essencial e extrato de plantas de *E. uniflora* L. e *P. cattleianum* Sabine podem ser novas alternativas terapêuticas para eliminar bactérias como as causadas por *K. pneumoniae* e *A. baumannii*.

1.1 HIPÓTESE

Os óleos essenciais e extratos metanólicos de *Eugenia uniflora* L. e *Psidium cattleianum* Sabine possuem compostos bioativos contra as bactérias *Klebsiella pneumoniae* e *Acinetobacter baumannii*.

1.2 OBJETIVOS

1.2.1 Objetivo Geral

Extrair, caracterizar físico-quimicamente, avaliar a citotoxicidade em células de mamíferos e determinar a atividade antioxidante e antibacteriana de óleos essenciais e extratos metanólicos de *E. uniflora* L. e *P. cattleianum* Sabine contra cepas padrão e isolados clínicos das bactérias *K. pneumoniae* e *A. baumannii*.

1.2.2 Objetivos Específicos

- Extrair e caracterizar físico-quimicamente óleos essenciais e extratos metanólicos de *E. uniflora* L. e *P. cattleianum* Sabine;
- Avalair a atividade antioxidante dos óleos essenciais e extratos metanólicos;
- Determinar *in vitro* a concentração inibitória mínima (CIM) e concentração bactericida mínima (CBM) dos óleos essenciais e dos extratos metanólicos frente a bactérias *K. pneumoniae* e *A. baumannii;*
- Testar os óleos essenciais e os extratos metanólicos quanto ao efeito citotóxico em cultura de células de mamíferos *in vitro*;
- Determinar o tempo de morte frente a *A. baumannii* dos extratos metanólicos de *E. uniflora* L. e *P. cattleianum* Sabine;
- Avaliar a atividade antibiofilme dos extratos metanólicos de *E. uniflora* L. e *P. cattleianum* Sabine quanto a sua capacidade de inibir a formação ou destruir o biofilme formado por *A. baumannii*.

CAPÍTULO II. REVISÃO DE LITERATURA

A revisão de literatura está apresentada na forma de manuscrito de revisão submetido a revista “Rodriguésia” (http:// https://rodriguesia.jbrj.gov.br/).

Myrtaceae family: An update on plant-derived bioactive against bacteria that affect the respiratory system

Família Myrtaceae: Uma atualização sobre bioativos derivados de plantas contra bactérias que afetam o sistema respiratório

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Myrtaceae: bioactives against bacteria

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Abstract: Bacterial infections of the respiratory system are a cause of morbidity and mortality worldwide. Most of these infections respond well to antibiotic therapies, however, several factors cause bacteria to become increasingly resistant, causing a serious public health problem in the world. Due to this problem, new antibiotics have been sought that can replace or enhance the effectiveness of existing drugs. This review is based on original articles obtained by searching major databases in the last years, that reported the potential of essential oils, extracts, and nanotechnology using plants of the Myrtaceae family against bacteria that affect the respiratory system.

Keywords: antibacterial; Myrtaceae; phytochemistry; respiratory infection; therapy.

Resumo: As infecções bacterianas do sistema respiratório são causa de morbidade e mortalidade em todo o mundo. A maioria dessas infecções responde bem às terapias antibióticas, porém, diversos fatores fazem com que as bactérias se tornem cada vez mais resistentes, causando um grave problema de saúde pública no mundo. Devido a este problema, têm-se procurado novos antibióticos que possam substituir ou aumentar a eficácia dos fármacos existentes. Esta revisão é baseada em artigos originais obtidos através de buscas nas principais bases de dados nos últimos anos, que relataram o potencial de óleos essenciais, extratos e nanotecnologia utilizando plantas da família Myrtaceae contra bactérias que afetam o sistema respiratório.

Palavras-chave: antibacteriano; Myrtaceae; fitoquímica; infecção respiratória; terapia.

1 Introduction

Infections of the respiratory system are one of the most common diseases observed in the population, causing morbidity and mortality worldwide. Most of these infections respond well to antibiotic therapies, however, several factors such as the increase and indiscriminate use of this type of medication, cross-resistance, and lack of new drugs, among others, cause pathogens to develop resistance (Public Health Agency of Canada 2012; Nweze *et al.* 2012).

The emergence of multidrug-resistant (MDR) bacteria increases rates of morbidity, mortality, length of hospital stay, and costs of treating the patient. Thus, bacterial resistance to antibiotics causes a major public health problem (Woolhouse *et al.* 2016). Each year 700,000 people worldwide die of the MDR pathogens infections and if no action is taken, it is estimated that there will be more than 10 million deaths by 2050 (Tillotson & Zinner 2017). Therefore, the search for new antibiotics that are capable of overcoming microbial resistance is critical.

Given these circumstances, bioprospection studies have been developed, aiming to identify plants from which new drugs may be produced, either using essential oils, crude plant extracts, isolating active components, combinations of antibiotics, nanotechnology, and other approaches. Phytochemicals revolve around the research and development (R&D) sector of the pharmaceutical industries as a source of new molecules leading to the development of new novel drugs, and is estimated that 30-50% of modern drugs are based on natural products, especially plants (Boucher *et al.* 2017; Newman & Cragg 2016).

Several species of plants from the Myrtaceae family are used for medicinal purposes, including the treatment of infectious diseases, and it is thought that the underlying mechanism of action is related to the astringent properties of the plants. Here, the essential oils (EO), extracts, and nanoproducts synthesized from plants of the Myrtaceae family used against bacteria causative of respiratory infections will be revised. A search was performed in the PubMed and Science Direct databases for original scientific articles published from May 2015

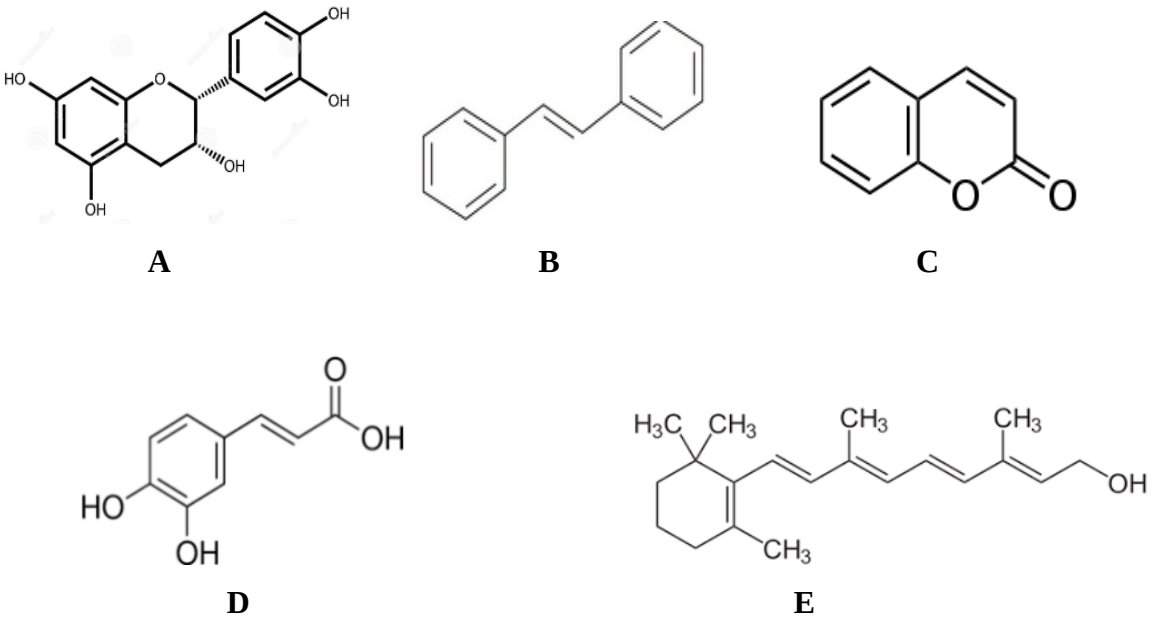
to February 2022, and only studies that had been carried out with plants of the Myrtaceae family that tested this antimicrobial against bacteria involved in respiratory infections were included in this review.

2 Myrtaceae family

Myrtaceae is a family of plants present in the main group of angiosperms, comprising 145 genera and 5,970 species (The Plant List 2013). The species that make up this family are predominantly distributed in the Southern hemisphere and are more found in the Neotropical and Australian regions (Figure 1) (Sytsma *et al.* 2004; Heywood, Brummit & Culham 2007). In Brazil, there are 140 genera within the Myrtaceae family and 6,000 species (Proença *et al.* 2022). This family has several bioactive properties due to its chemical composition, comprising phenolic and polyphenol compounds, such as flavonoids, phenolic acids, tannins, stilbenes, lignans, coumarins and tocopherols, functional lipids and carotenoids (Figure 2) (Duarte & Paull 2015).

Figure 1. Geographic distribution of plants in the Myrtaceae family.

The Myrtaceae family consists of several species, among them: *Eucalyptus globulus* Labill. (eucalyptus), *Eugenia uniflora* L. (pitanga), *Campomanesia adamantium* (Cambess.) O. Berg (guabiroba), *Melaleuca alternifolia* (Maiden & Betche) Cheel (tea tree), *Psidium guajava* L. (guava), *Psidium cattleianum* Sabine (araçá), *Syzygium cumini* (L.) Skeels (jambolan), *Syzygium aromaticum* (L.) Merr. & L. M. Perry (clove) (The Plant List 2013).



A **B** **C**

D **E**

Figure 2. Molecular representation of the main compounds present in the chemical composition of plants in the Myrtaceae family. A: Flavonoid; B: Stilbene; C: Coumarin; D: Phenolic acid; E: Carotenoid.

3 Bioactive compounds from plants of the Myrtaceae family in the treatment of bacteria that cause respiratory infection

3.1 Essential oils

Essential oils (EO) are natural volatile compounds present in plants, presenting more than 3,000 secondary metabolites. Among these metabolites, about 500 are volatile compounds, including mono and sesquiterpenes, terpenoids, aldehydes, and phenols (Schelz, Molnar & Hohmann 2006). Some of these constituents have proven biological properties, such as anti-inflammatory effects (Lazarini *et al.* 2018) and antibacterial (Schelz, Molnar & Hohmann 2006).

Throughout the text and in Table 1 we observe the chemical constituents and activity of essential oils of different species of the Myrtaceae family against bacteria that cause respiratory infection.

3.1.1 Genus *Eucalyptus*

3.1.1.1 *Eucalyptus globulus* Labill.

Within the Myrtaceae family is the genus *Eucalyptus*, popularly known as eucalyptus, a name that represents more than 700 species worldwide. Luís *et al.* (2016) when investigating EO of *Eucalyptus globulus* found in their chemical composition, through gas chromatography coupled with mass spectrophotometry (GC-MS), 45 constituents, the main ones being 1.8-cineol (eucalyptol) (63.81%), α-pinene (16.06%) and aromadendrene (3.68%). Salem *et al.* (2018) found 67 volatile constituents in the EO of *E. globulus*, in which they found differences depending on the plant stage since eucalyptol (13.23%) was observed in the vegetative stage, while in the full flowering stage (32.19%) and fruiting stage (37.82%) p-cymene was found as the major compound, as observed in Table 1. In this same study, the antibacterial activity of *E. globulus* and *E. radiata* Hook. EO against several microorganisms were tested, in which *E. globulus* EO showed activity against *A. baumannii* ATCC 17978 with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 4µL.mL^{-1}, while the EO of *E. radiata* present MIC and MBC of 8µL.mL^{-1} against this bacterium.

The combination of *E. globulus* EO with conventional antibiotics (cefoperazone, piperacillin, ciprofloxacin, tetracycline, chloramphenicol, and gentamicin) was evaluated and can be used as a combined antibiotic therapy against *A. baumannii* and the authors identified that the best result of the fractional inhibitory concentration index (FICI) was that presented in the combination of chloramphenicol with *E. globulus* EO, in which they found modal values of 0.12 (*A. baumannii* ATCC 17978) and 0.09 (*A. baumannii* ATCC 19606), followed by the combination of the same antibiotic with the EO of *E. radiata* finding 0.12 against *A. baumannii*

ATCC 17978 and 0.06 for *A. baumannii* ATCC 19606. Salem *et al.* (2018) observed that the *E. globulus* EO, in the MIC, values of concentrations of 4 $mg.mL^{-1}$ were identified for *S. aureus* ATCC 6816 in all the tested phases of the plant and for methicillin-resistant *S. aureus* (MRSA) found a lower MIC of 2 $mg.mL^{-1}$ (vegetative stage) and 4 $mg.mL^{-1}$ in the other phases. For *K. pneumoniae* CIP 104727, the MIC was 4 $mg.mL^{-1}$ in all EO of the different parts of the plant tested. The antibacterial activity of this EO can occur due to its chemical composition, since 1.8-cineol with p-cymene can act in synergy, potentiating its effect (Veras *et al.* 2012). In the checkerboard test when testing the EO of *E. globulus* with ampicillin they found partial synergism with a fractional inhibitory concentration index (FICI) of 0.53 $\mu g.mL^{-1}$ compared to MRSA and FICI of 1$\mu g.mL^{-1}$ compared to *K. pneumoniae* CIP 104.727, showing additivity (Salem *et al.* 2018).

Table 1. Chemical constituents and activity of essential oils of different species of the Myrtaceae family against bacteria that cause respiratory infection.

Genus	Species	Part of the plant	Majority EO compound (Evaluation method)	Bacteria	Antibacterial activity MIC/MBC	Reference
Eucalyptus	*E. radiata*	Leaves and small branches	Limonene (GC-MS)	*P. aeruginosa* ATCC 27853	32 / 32 µg.mL^{-1}	Luís et al. (2016)
				K. pneumoniae ATCC 13883	16 /16 µg.mL^{-1}	
				A. baumannii ATCC 17978	8 / 8 µg.mL^{-1}	
				A. baumannii ATCC 19606	8 / 8 µg.mL^{-1}	
				P. aeruginosa PA 08	16 / 16 µg.mL^{-1}	
				P. aeruginosa PA 12/08	32 / 32 µg.mL^{-1}	
				K. pneumoniae KP 08	16 /16 µg.mL^{-1}	
				P. aeruginosa ATCC 27853	32 /32 µg.mL^{-1}	
	E. globulus		1.8-cineole (Eucalyptol)	*K. pneumoniae* ATCC 13883	16 / 16 µg.mL^{-1}	
				A. baumannii ATCC 17978	4 /4 µg.mL^{-1}	
				A. baumannii ATCC 19606	8 / 8 µg.mL^{-1}	
	E. globulus		Eucalyptol	*P. aeruginosa* PA 08	32 / 32 µg.mL^{-1}	
				P. aeruginosa PA 12/08	32 / 32 µg.mL^{-1}	
				K. pneumoniae KP 08	32 / 32 µg.mL^{-1}	
				S. aureus ATCC 6816	VS FFS FS	

			(CG-MS)		4 / ND mg.mL^{-1}	4 / ND mg.mL^{-1}	4 / ND mg.mL^{-1}	
		Aerial parts: Vegetative stage (VS); Full flowering stage (FFS); Fruiting stage (F)		MRSA	2 / ND mg.mL^{-1}	4 / ND mg.mL^{-1}	4 / ND mg.mL^{-1}	Salen et al. (2018)
				K. pneumoniae CIP 104727	4 / ND mg.mL^{-1}	4 / ND mg.mL^{-1}	4 / ND mg.mL^{-1}	
					EuHN		EuB	
				A. baumannii ATCC 19606	1 / 2 µg.mL^{-1}		1.41 / 2 µg.mL^{-1}	
	E. camaldulensis	Leaves	Spatulenol (GC-MS)	*A. baumannii* ATCC BAA 747	1 / 1 µg.mL^{-1}		1 / 1 µg.mL^{-1}	Knezevic et al. (2016)
				A. baumannii ATCC 13420	1 / 2 µg.mL^{-1}		1 / 1.41 µg.mL^{-1}	
				20 isolades of *A. baumannii*	0.5 - 2 / 0.7 - 4 µg.mL^{-1}		0.1 – 1.4 / 0.7 – 2 µg.mL^{-1}	
				MRSA	1 / 2 µg.mL^{-1}			
				ESBL – Cs –Kp	0.50 / 0.50 µg.mL^{-1}			
		Leaves	Terpinen-4-ol (GC-MS e GC-FID)	ESBL – CR – Kp	0.25 / 0.25 µg.mL^{-1}			
				CR – Ab	0.25 / 0.25 µg.mL^{-1}			
				CR – Pa	1 / 1 µg.mL^{-1}			
	M. alternifolia Cheel.			MSSA ATCC 29213	1 / 2 µg.mL^{-1}			Oliva et al. (2018)
Melaleuca				MRSA NCTC 12493	4.42 / 17.6 mg.mL^{-1}			
		Aerial part	α-carene (GC-MS)	*K. pneumoniae* ATCC 700603	8.84 mg.mL^{-1} / -			
				S. aureus	2.21 / 4.42 mg.mL^{-1}			
		Leaves	Limonene 1.8 – cineole	*K. pneumonie* ATCC 13883	62,5 mg.mL^{-1} / -			

	M. leucadendra L.		Viridiflorol α-pinene	*P. aeruginosa* ATCC 27853	31,2 mg.mL^{-1} / -	Bautista-Silva et al. (2020)
				S. aureus ATCC25923	31,2 mg.mL^{-1} / -	
Syzygium	*S. aromaticum* L.	Clove buds	3-allylguaiacol (GC-MS)	MRSA NCTC 12493 *K. pneumoniae* ATCC 700603	0.21 / 0.21 mg.mL^{-1} (allmicroorganisms)	Imane et al. (2020)
Rhodamnia	*R. dunetorum*	Leaves	Caryophyllene epoxide (GC-MS / HP-SMS) α-pinene (GC-MS / DB-17MS)	*S. aureus* *Haemophilus influenzae* ATCC 49247 *S. aureus* ATCC 29213 *S. pneumoniae* ATCC 49619	1024 µg.mL^{-1} / ND (all microorganisms)	Houdkova et al. (2018)
Eugenia	*E. jambolana*	Leaves	α-pinene (GC-MS)	*S. aureus* ATCC 25923 *P. aeruginosa* ATCC 25853 *S. aureus* AS 358 *P. aeruginosa* PA 03	128 µg.mL^{-1} / ND ND ND ND	Pereira et al. (2017a)
	E. uniflora	Leaves	Isofuran-germacrene	*S. aureus* ATCC 25923 *P. aeruginosa* ATCC 25853 *S. aureus* AS 358 *P. aeruginosa* PA 03	256 µg.mL^{-1} / ND ND ND ND	Pereira et al. (2017b)
Pimenta	*P. dioica* L.	Leaves and berries	PDL (GC-MS) PDB (GC-MS)	*A. baumannii* ATCC 19606	PDL PDB	Ismail et al. (2020)

	β-mircene	β-mircene		0.69 µg.mL^{-1}	0.86 µg.mL^{-1}
			14 clinical isolates MDR *A. baumannii*	0.52 a 5.18 µg.mL^{-1}	0.52 a 5.18 µg.mL^{-1}
	PRL	PRB		PRL	PRB
			A. baumannii ATCC 19606	1.03 µg.mL^{-1}	1.03 µg.mL^{-1}
P. racemosa	β-mircene	β-mircene			
			14 clinical isolates MDR *A. baumannii*	0.52 a 5.18 µg.mL^{-1}	0.69 a 5.87 µg.mL^{-1}

MIC: Minimum Inhibitory Concentration;MBC: Minimum Bactericidal Concentration; GC-MS: Gas Chromatography coupled to Mass Spectrometry; GC-FID: Gas Chromatography with Flame Ionization Detector; EuHN: E. *camaldulensis* fromHerceg Novi; EuB: *E. camaldulensis*from Bar; MRSA: Methicillin resistant *S. aureus*; ESBL-Cs-Kp:Carbapenem sensitive *K. pneumoniae*; ESBL-CR-Kp: Carbapenem resistant *K. pneumoniae*; CR-Ab: Carbapenem resistant *A. baumannii*; CR-Pa: Carbapenem resistant *P. aeruginosa*; MSSA: Methicillin sensitive *S. aureus*; VS: Vegetative stage; FFS: Full flowering stage; FS: Fruiting stage; PDL: *P. dioica* (L.) leaves; PDB: *P. dioica* (L.) berries; PRL: *P. racemosa* leaves; PRB: *P. racemosa* berries; ND: not determined.

3.1.1.2 *Eucalyptus radiata* Hook.

In *E. radiata* EO, 72 compounds were observed, of which the majority are limonene (68.51%), α-terpineol (8.60%), and α-terpinyl acetate (6.07%) (Luís *et al.* 2016). In the same study, the authors tested the antibacterial potential of the EO of *E. globulus* and *E. radiata* with standard strains: *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *A. baumannii* ATCC 17978, *A. baumannii* ATCC 19606 and three more clinical isolates: *P. aeruginosa* PA 08, *P. aeruginosa* PA 12/08 and *K. pneumoniae* KP 08, it was possible to observe in the MIC test that the EO of *E. radiata* showed better antibacterial activity, showing to be bactericidal, as it was analyzed that the MIC values were lower when compared to the EO of *E. globulus* against *P. aeruginosa* PA 08 and *K. pneumoniae* KP 08. Furthermore, Luís *et al.* (2016) tested the combination of conventional antibiotics (cefoperazone, piperacillin, ciprofloxacin, tetracycline, chloramphenicol, and gentamicin) combined with EO *E. radiata* finding modal values of 0.12 against *A. baumannii* ATCC 17978 and 0.06 for *A. baumannii* ATCC 19606.

3.1.1.3 *Eucalyptus camaldulensis* Dehnh.

Eucalyptus camaldulensis is also a species within the genus *Eucalyptus* that has biological properties, in addition to being tested against MDR strains, such as *A. baumannii* (Jazani et al., 2012). Knezevic *et al.* (2016) evaluated two types of EO of *E. camaldulensis* collected from two coastal areas of Montenegro, Europe - Herceg Novi (EuHN) and Bar (EuB). In these OE 43 compounds were identified, of these, the most representative were spatulenol (EuHN - 18.90%/EuB - 21.39%), krypton (EuHN - 7.59%/EuB - 12.15%), p-cymene (EuHN - 5.35%/EuB - 7.56%) and 1.8-cineole (EuHN - 7.62%/EuB - 1.95%). Antibacterial activity was performed against three standard strains: *A. baumannii* ATCC 19606, *A. baumannii* ATCC BAA747, *A. baumannii* NCTC 13420, and twenty more *A. baumannii* MRD isolated from

clinical and outpatient wounds. In this study, they found that MIC values for the reference bacteria ranged from 1 to 2 μl.mL^{-1} and for isolates from 0.5 to 2 μl.mL^{-1} for both tested EO. In addition, the authors found a synergism in the combination of EO *E. camaldulensis* with ciprofloxacin showing FICI values below 0.5 μL.mL^{-1} against two *A. baumannii* isolates (Aba-4914 and Aba-5055) and an effect additive against Aba-6673. When the EO was tested with gentamicin for Aba-4914, it obtained synergism, decreasing the concentrations of the antibiotic, as was shown in the combination of the EO and the antimicrobial polymyxin B, which showed a synergistic potential against three multi-resistant microorganisms (Knezevic *et al.* 2016).

3.1.2 Genus *Melaleuca*

3.1.2.1 *Melaleuca alternifolia* (Maiden & Betche) Cheels

The species of *Melaleuca alternifolia* is used as a topical antiseptic and anti-inflammatory agent (Saller *et al.* 1998). The EO extracted from this plant has antibacterial activity against Gram-positive and Gram-negative bacteria (Carson *et al.* 1995; Cox *et al.* 2000). Oliva *et al.* (2018) identified through the GC-MS technique three main constituents in the EO of *M. alternifolia*, namely: terpinene 4-ol (35.4%), eucalyptol (15.2) and α-pinene (12.4%). While Imane *et al.* (2020) found, through the same analysis, terpinene 4-ol (13.5%) and α-pinene (13.1%), however, in smaller quantities, with α-carene (17.4%) being the compound majority found in the EO in this study. In determining the antimicrobial activity, the following microorganisms were used: *S. aureus* methicillin-sensitive (MSSA) ATCC 29213, MRSA - clinical isolate (skin), extended-spectrum beta-lactams *K. pneumoniae* carbapenem-sensitive (ESBL-CS-Kp) - clinical isolate (urine), carbapenem-resistant *K. pneumoniae* (ESBL-CR-Kp) - clinical isolate (urine), carbapenem-resistant *A. baumannii* (CR-Ab) - clinical isolate (sputum) and carbapenem-resistant *P. aeruginosa* (CR-Pa) - clinical isolate (bronchoalveolar lavage). The test results showed that *M. alternifolia* EO was active, showing MIC and MBC

values of 0.25 µg.mL^{-1} for CR-Ab and ESBL- CR-Kp, while for ESBL-CS-Kp it was 0.50 µg.mL^{-1} and for MRSA MIC of 0.50 µg.mL^{-1} and MBC of 2.0 µg.mL^{-1}. The EO was active against MSSA ATCC 29213 with MIC and MBC of 1.0 µg.mL^{-1}and 2.0 µg.mL^{-1}, respectively, and CR-Pa MIC of 1.0 µg.mL^{-1} and MBC of 1.0 µg.mL^{-1}, however, it was possible to notice that the EO was shown to be bactericidal against all the tested bacteria (Oliva *et al.* 2018).

Imane *et al.* (2020) also obtained antibacterial activity against the microorganisms evaluated. The MIC found against MRSA NCTC 12493 was 4.42 mg.mL^{-1} and against *S. aureus* isolate it was 2.21 mg.mL^{-1}, both showing to be bactericidal. When evaluating the same EO against *K. pneumoniae* ATCC 700603 it was possible to find a MIC of 8.84 mg.mL^{-1}. From the results observed, it is noted that the EO of *M. alternifolia* is promising to be used in the treatment of infections caused by Gram-negative MDR microorganisms, taking into account that several infections, such as hospital pneumonia, are caused by this bacteria (mainly *A. baumannii* and *K. pneumoniae*), in addition, this EO can be used inhalable, as local therapy in the case of respiratory infections (Ekren *et al.* 2018; Li *et al.* 2016; Oliva *et al.* 2018).

Oliva *et al.* (2018) in the checkerboard assay tested some antibiotics amikacin, oxacillin, cefazolin, vancomycin, and rifampicin for MSSA (ATCC 29213) and MRSA, while for the other bacteria the combination of EO with amikacin, meropenem, and colistin was evaluated. The results showed a synergistic effect in subinhibitory concentrations of the combinations of the EO of *M. alternifolia* and the antimicrobials amikacin, oxacillin, and cefazolin against the two Gram-positive bacteria, as well as when tested with amikacin, meropenem, and colistin against all Gram-negative microorganisms.

3.1.2.2 *Melaleuca leucadendra* (L.) L.

The chemical characterization of EO *M. leucadendra* was analyzed by GC–MS and revealed 45 compounds, accounting for 99.73% of the total oil composition. Monoterpenoids

dominated the EO (77.43%), with four primary compounds: α-pinene (9.06%), Limonene (32.00%), 1,8-cineole (17.32%), and viridiflorol (14.89%) (Bautista-Silva *et al.*, 2020).

M. leucadendra EO achieved a broad spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria. Among the microorganisms tested by Bautista-Silva *et al.* (2020), *K. pneumoniae* ATCC 13883 was one of those with the highest MIC value (62,5 $mg.mL^{-1}$), while the EO showed the lowest antibacterial activity (31,2 $mg.mL^{-1}$) against *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC25923. The authors evaluated the activity of the EO of *M. leucadendra* against the tested strains (exponential phase) during the different periods, in which it was possible to observe that there was a reduction in cell viability, decreasing bacterial growth for *K. pneumoniae* at concentrations below MIC (62,5 $mg.mL^{-1}$).

3.1.3 Genus *Syzygium*

3.1.3.1 *Syzygium aromaticum* (L.) Merr. & L. M. Perry

Imane *et al.* (2020) in their research also evaluated the OE of cloves, as it is popularly known, however, receive the scientific name of *Syzygium aromaticum* (The Plant List 2013). In the chemical characterization of the EO of *S. aromaticum*, they found 3-allyl guaiacol (42.6%), eugenol acetate (15.9%), and caryophyllene (15.5%) as the three main compounds. This OE obtained antibacterial activity against MRSA NCTC 12493, *K. pneumoniae* ATCC 700603, and an isolate of *S. aureus* with MIC and MBC of 0.21 $mg.mL^{-1}$ (Imane *et al.* 2020) (Table 1).

3.1.4 Genus *Pimenta*

The genus *Pimenta* belongs to the Myrtaceae family and has several medicinal purposes. *Pimenta dioica* (L.) Merr. and *Pimenta racemosa* (Mill.) J. W. Moore are the most recognized

species within this genus, as they have pharmacological effects due to their rich composition of essential oil (Chaverri & Cicció 2015; Ismail *et al.* 2020).

3.1.4.1 *Pimenta dioica* (L.) Merr. and *Pimenta racemosa* (Mill.) J. W. Moore

Ismail *et al.* (2020) tested EO extracted from leaves and berries of *P. dioica* and *P. racemosa*, finding β-myrcene as the main constituent in the chemical composition of *P. dioica* leaves (44.1%), 1.8-cineol (18.8%) and limonene (11.7%), while EO extracted from the berry of the same species showed β-myrcene (13.9%), limonene (4.6%) and β- linalool (3.6%) as the majority, being similar compounds, although β-myrcene and limonene have a lower percentage in their composition. These authors tested the four OE against the standard strain of *A. baumannii* ATCC 19606 and fourteen MDR clinical isolates of *A. baumannii*, showing that the EO tested from *P. dioica*, extracted from leaves and berry, presented MIC which ranged from 0.51 to 5.2 $\mu g.mL^{-1}$ against the fifteen microorganisms evaluated, thus, the EO of this plant showed a stronger antimicrobial potential in terms of lower MIC values when compared to the other EO tested.

The *P. racemosa* EO was also analyzed by Ismail *et al.* (2020), they identified three main compounds, β-myrcene, limonene, and β-cis-ocimene in the EO extracted from leaves and berry, however, in different amounts of 39.6%, 15.5% and 2.8% for one and 42.3%, 14.3% and 4.6%, respectively. All EO tested by the authors showed a bactericidal effect after 24 h incubation, both EO prepared with leaf and berry *P. racemosa* exhibited the same bactericidal activity at 2.08 and 2.76 µg.mL-1, respectively, although the EO *P. racemosa* presented a less pronounced action when compared with the EO *P. dioica* (Ismail *et al.* 2020).

3.1.5 Genus *Rhodamnia*

3.1.5.1 *Rhodamnia dumetorum* (DC.) Merr. & L. M. Perry

Rhodamnia dumetorum is a plant species originally from Cambodia and in the study by (Houdkova *et al.* 2018) the chemical characterization of EO extracted from leaves of this plant was evaluated using GC-MS equipped with two capillary columns of different polarity HP-5MS and DB-17MS. In addition, a flame ionization detector (FID) coupled to a quadrupole selective mass detector, in which 72 constituents were identified, equivalent to 91.37% (HP-5MS) and 90.48% (DB-17MS) of its total content. The major volatile compounds were as follows: caryophyllene epoxide (33.29%/4.51%), α-pinene (26.09%/73.53%) and humulene-1,2-epoxide (2.48%/0.39%) (Table 1). Antibacterial activity was performed against bacteria related to respiratory tract infections (*Haemophilus influenzae* ATCC 49247, *S. aureus* ATCC 29213, and *Streptococcus pneumoniae* ATCC 49619). Concentration values of >1,024 $\mu g.mL^{-1}$ were found against all tested microorganisms. The *R. dumetorum* EO showed moderate cytotoxicity (IC_{50} 1.98 ± 1.17 $\mu g.mL^{-1}$) against pulmonary fibroblast cells (MRC-5) (Houdkova *et al.* 2018).

3.1.6 Genus *Eugenia*

3.1.6.1 *Eugenia jambolana* Lam.

Pereira *et al.* (2017a) evaluated the EO of *Eugenia jambolana* (EjEO) and found 26 compounds in its composition (98.93%), with α-pinene (48.09%) and nerolidol (8.73%) as the majority constituents. In the same study, the authors analyzed the antibacterial activity of EjEO against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC25853, and isolates of *S. aureus* SA 358 and *P. aeruginosa* PA 03. They observed that OE had a better effect against the strain of *S. aureus* (128 $\mu g.mL^{-1}$), which can be evaluated by *in vivo* assays. In the technique of modulation

of antibiotic activity, by direct contact, the combination of EjEO with the antimicrobials amikacin or gentamicin showed an increase in MIC when tested with *S. aureus*, obtaining an antagonistic effect, while in the gaseous contact method with the association of same oil with amikacin or erythromycin against *P. aeruginosa* reduced the halos, thus, having a synergistic activity (Pereira *et al.* 2017a). When assessing EO with ciprofloxacin and norfloxacin using the same technique, however, with exposure to red and blue LED (Light Emitting Diodes) there was an increase in the halo, indicating synergism. Phototherapy combined with the use of EO may be an option to reduce the excessive use of antimicrobials, as the application of LED lights showed a positive effect against Gram-positive and Gram-negative microorganisms (Pereira *et al.* 2017a; Wagner 2011).

3.1.6.2 *Eugenia uniflora* L.

Eugenia uniflora is a species native to Brazil, popularly known as pitangueira, pitanga or pitanga-vermelha. It is spread, practically all over the national territory (Fouqué 1981; Villachica 1996; Mazine *et al.* 2022). Pereira *et al.* (2017b) chemically characterized the EO of *E. uniflora* (EuEO) and found isofuran-germacrene (65.80%) as the main compound, followed by germacra-3,7,9-trien-6-one (16.19%) and β-element (4.47%). In the antibacterial assay using the broth microdilution technique for *S. aureus* ATCC 25923 obtained a MIC of 256 $\mu g.mL^{-1}$, however, it is noted that in the test of bacterial resistance modulation by direct contact performed for the same microorganism, when EuEO was combined with commercial antimicrobials (amicanine and gentamicin) there was a reduction in the concentration of the antibiotic, resulting in synergism. Contrary to what was seen for *P. aeruginosa*, which presented antagonism when tested the combination of EuEO with amikacin and erythromycin (Pereira *et al.* 2017b). The antagonism resulting from the combination of EuEO over aminoglycosides against *P. aeruginosa* may occur due to the presence of a complex barrier system formed by

the membrane (phospholipids, lipopolysaccharides, and proteins) that allow a high degree of impermeability to antimicrobials (Lambert; Joynson & Forbes 2001).

EO is a viable alternative to antibiotics in the fight against microorganisms (Table 1). The antimicrobial activity of these EO seems to be stronger than the sum of their separate components, demonstrating the occurrence of a synergy between the numerous constituents present in their chemical composition. Therefore, the use of EO extracted from plants is a research target, as there is a need to find substances that are not resistant to antibiotics, as they have specific antimicrobial agents and, therefore, could be used in the treatment of a variety of infections, contributing to the reduction of existing bacterial resistance.

3.2 Extracts

Natural extracts are a set of chemical compounds with biological activities from parts of the plant, such as the leaves, stems, fruits, or roots of medicinal plants. These extracts have important biological properties, such as antioxidant, antifungal, antibacterial, and antiparasitic activity, among others (Chakraborty *et al.* 2014; Njimoh *et al.* 2015). Several studies analyzed chemical compounds and the antibacterial activity of extracts made from different plants of the Myrtaceae family (Table 2).

Table 2. Chemical compounds and antibacterial activity of extracts from different species of the Myrtaceae family against microorganisms involved with respiratory infection.

Genus	Species	Part of the plant	Extraction method	Major extract compound (Evaluation method)	Bacterias	Antibacterial activity MIC/MBC	Reference
Syzygium	*S. cumini*	Pulp	Dynamic maceration	Caffeic acid (HPLC)	*S. aureus* MTCC-740	0.5 $mg.mL^{-1}$	Singh et al. (2016)
					K. pneumoniae sub. sp. *pneumoniae* MTCC-109	2 $mg.mL^{-1}$	
					MRSA	2 $mg.mL^{-1}$	
	S. praecox	Leaves	Ultrasonic	Flavonoids and terpenoids	*S. aureus* ATCC 6538	-	Panda et al. (2020)
					17 isolates	-	
Eucalyptus	*S.aromaticum* L.	Leaves	Maceration	ND	*S. aureus*	0.39 /3.1 $mg.mL^{-1}$	Moradi; Hadi; Bazargani et al. (2020)
	E. camadulensis	Leaves	Maceration	ND	*P. aeruginosa*	0.78 / 6.25 $mg.mL^{-1}$	
Eugenia	*E. brasiliensis* Lam.	Pulp	Ultrasonic	Catechin (LC-MS/MS)	*S. aureus* ATCC 25923	62.5 / 500 $\mu g.mL^{-1}$	Lazarini et al. (2018)
					S. aureus MRSA ATCC 33591	250 / 500 $\mu g.mL^{-1}$	

							P. aeruginosa ATCC 27853	250 / 500 µg.mL^{-1}					
				ET	EO	EE		ET	EO	EE			
	E. tinifolia Lam. (ET) *E. orbiculata* Lam. (EO) *E. elliptica* Lam. (EE)	Leaves	Maceration	Kaempferol and Quercetin (HPLC)	Quercetin and epigalocatequin (HPLC)	Quercetin (HPLC)	*P. aeruginosa* ATCC 27853	19.5 µgFW.mL^{-1} / ND	19.5 µgFW.mL^{-1} / ND	19.5 µgFW.mL^{-1} / ND			
							K. oxytoca ATCC 43086	9.7 µgFW.mL^{-1} / ND	4.9 µgFW.mL^{-1} / ND	39.1 µgFW.mL^{-1} / ND			
				Caryophyllene oxide (HF1)				CEE	HF	DF	AcF	AqF	
Campomanesia	*C. adamantium*	Leaves and flowers	Dynamic maceration followed by fractionation	Isoaromadendrene (HF2/2)			*S. aureus* ATCC 6538	250 µg.mL^{-1}/ ND	31.25 µg.mL^{-1}/ ND	250 µg.mL^{-1}/ ND	500 µg.mL^{-1}/ ND	>1000 µg.mL^{-1}/ ND	Sá et al. (2018)
				Octadecanoic acid									

					(HF2/6)	*S. aureus* ATCC 25923	250 µg.mL^{-1}/ ND	62.5 µg.mL^{-1}/ ND	250 µg.mL^{-1}/ ND	500 µg.mL^{-1}/ ND	>1000 µg.mL^{-1}/ ND
					Cubenol (HF9/3/1/2/1)						
					(GC-MS)	*S. aureus* ATCC 29213	1000 µg.mL^{-1}/ ND	500 µg.mL^{-1}/ ND	250 µg.mL^{-1}/ ND	500 µg.mL^{-1}/ ND	1000 µg.mL^{-1}/ ND
						K. pneumoniae ATCC 700603	>1000 µg.mL^{-1}/ ND	>1000 µg.mL^{-1}/ ND	>1000 µg.mL^{-1}/ ND	>1000 µg.mL^{-1}/ ND	>1000 µg.mL^{-1}/ ND
						P. aeruginosa SPM1	>1000 µg.mL^{-1}/ ND	500 µg.mL^{-1}/ ND	>1000 µg.mL^{-1}/ ND	>1000 µg.mL^{-1}/ ND	>1000 µg.mL^{-1}/ ND
Psidium	*P. guayaquilense* Landrun & Cornejo	Leaves	Soxhlet	Phenolic compounds		*S. aureus* ATCC 25923	50 µg.mL^{-1}/ ND				Rondon et al. (2017)

P. rostratum MC Vaugh						
P. guajava	Leaves	Dynamicmaceration	ND	*S. aureus* ATCC 25923	1250 / 1250 μg.mL^{-1}	Valle et al. (2015)
				P. aeruginosa ATCC 27853	- / -	
				K. pneumoniae ATCC BAA 1705	- / -	
				K. pneumoniae (CRE)	- / -	
				K. pneumoniae (ESβL)	- / -	
				P. aeruginosa (MβL)	- / -	
				A. baumannii (MβL)	- / -	

					MRSA 1	1250 / 1250 μg.mL^{-1}		
					MRSA 2	625 / 1250 μg.mL^{-1}		
					MRSA 3	1250 / 1250 μg.mL^{-1}		
					MRSA4	625 / 625 μg.mL^{-1}		
		Leaves	Maceration	Phenolic compounds Saponin Steroids (HPLC)	*S. aureus*	ND		Chakraborty et al. (2018)
	P. guava	Fruits	Ultrasonic	(+) – catechin (HPLC)	*S. aureus* CMCC(B) 26003	1250 / 2500 mg.mL^{-1}		Fu; Lu; Zhou (2016)
					P. aeruginosa ATCC 27853	312.5 / 312.5 mg.mL^{-1}		
	P. cattleianum S.	Leaves	WU - Ultrasonic WE – Dynamic maceration	Phenolic compounds	*S. aureus*	WU Verão – 12.6 μg.mL^{-1} Inverno – 18 μg.mL^{-1}	WE Verão – 15.1 μg.mL^{-1} Inverno – 15.4 μg.mL^{-1}	Dacoreggio; Moroni; Kempka (2019)
					P. aeruginosa ATCC 27853	312.5 / 312.5 mg.mL^{-1}		
Myrtus	*M. communis*L.	Leaves	Soxhlet	1.1.8a-trimethylocta-hydro-2,6-naphthalenedione (GC-MS)	*P. aeruginosa* ATCC 9027	- / -		Mir et al. (2020)
					S. aureus	9.7 μg.mL^{-1} / 0.3 mg.mL^{-1}		

					K. pneumoniae	- / -	
Callistemon	*C. citrinus* Skeels	Leaves	Soxhlet	ND	MRSA ATCC 33591	125 / 250 µg.mL^{-1}	Shehabeldine et al. (2020)
					MSSA ATCC 25923	62.5 / 250 µg.mL^{-1}	

MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; HPLC: High Performance Liquid Chromatography; LC-MS/MS: Liquid chromatography coupled to mass spectrometry; GC-MS: Gas Chromatography coupled to Mass Spectrometry; ET: *E. tinifolia* Lam.; EO: *E. orbiculata* Lam.; EE: *E. elliptica* Lam.; MRSA: Methicillin resistant *S. aureus*; CEE: Crude ethanolic extract; HF: Hexanic extract; DF: Dichloromethane; AcF: Ethyl acetate extract; AqF: Aqueous extract; *K. pneumoniae* (CRE): Carbapenem resistant *K. pneumoniae*; *K. pneumoniae* (ESβL): extended spectrum β-lactamase-producing *K. pneumoniae*; *P. aeruginosa* (MβL): metallo-β-lactamase *P. aeruginosa*; *A. baumannii* (MβL):metallo-β-lactamase *A. baumannii* ;WU: extraction method with water and ultrasound; WE: water and enzyme extraction method;MRSA: methicillin resistant *S. aureus*; MSSA: methicillin sensitive *S. aureus*; ND: not determined.

3.2.1 Genus Syzygium

3.2.1.1 *Syzygium cumini* (L.) Skeels

Syzygium cumini, also known by the common names jambolão, plum java, and black plum is native to tropical Asia, mainly India (Singh *et al.* 2016). Singh *et al.* (2016) evaluated the ethanolic extract of *S. cumini* and through the HPLC technique (High-Performance Liquid Chromatography) found several phenolic compounds in their chemical composition, namely: caffeic acid (65.6 $mg.mL^{-1}$), gallic acid (41.4 $mg.mL^{-1}$), synaptic acid (21.3 $mg.mL^{-1}$), delphinidin (20.2 $mg.mL^{-1}$) and quercetin acid (14.9 $mg.mL^{-1}$). In the evaluation of the antibacterial activity of the extract against pathogenic strains, the MICs varied between 0.5 and 2 $mg.mL^{-1}$ against *S. aureus* (MTCC-740), *K. pneumoniae* (MTCC-109), and an MRSA isolate (Table 2). The extract of *S. cumini* showed greater inhibitory potential, MIC equal to 0.5 $mg.mL^{-1}$ compared to the reference strain of *S. aureus*, while for the other bacteria it reached a MIC of 2 $mg.mL^{-1}$.

3.2.1.2 *Syzygium praecox* (Roxb.) Rathakr. & N.C.Nair

Panda *et al.* (2020) investigated the antibacterial activity of the *S. praecox* extract prepared with the leaves of the plant and acetone as a solvent, in which they found terpenoids and alkaloids as major phytochemicals in their chemical characterization, but this extract was not able to inhibit *Staphylococcus* MDR isolates, as well as *S. aureus* strain ATCC 6538.

3.2.2 Genus *Eucalyptus*

3.2.2.1 *Eucalyptus camadulensis* Dehnh.

Moradi; Hadi & Bazargani (2020) investigated the antibacterial effect of the extract of *S. aromaticum* and *Eucalyptus camadulensis* against *P. aeruginosa* (isolated from a patient with

cystic fibrosis) and the authors obtained bactericidal activity with MIC/MBC of 0.78/6.25 $mg.mL^{-1}$ and 0.39/3.1 $mg.mL^{-1}$, respectively.

3.2.3 Genus *Myrtus*

3.2.3.1 *Myrtus communis* L.

Myrtus communis is native to the Mediterranean region, including other countries in the Middle East, such as Jordan, Iraq, and Saudi Arabia (Mir *et al.* 2020). Mir *et al.* (2020) identified in the ethanolic extract of leaves of this species a total of 50 constituents in its chemical composition, using the GC-MS technique, the dominant compounds being the following: 1.1.8a-trimethylocta-hydro-2.6-naphthalenedione (27.6%), pyrogallol (9.1%) and 1.8-cineole (3.9%). The antibacterial effect of the extract was evaluated against *P. aeruginosa* ATCC 9027 and isolates of *S. aureus* e *K. pneumoniae*, in which the standard strain tested and *K. pneumoniae* were resistant to the extract, only the isolate of *S. aureus* was inhibited (MIC of 9.7 $\mu g.mL^{-1}$). In addition, the authors analyzed the MICs found for several antimicrobials (colistin, vancomycin, tetracycline, and levofloxacin) alone and in combination with the ethanolic extract of the leaf of *M. communis*. Where found a MIC of 0.61 $\mu g.mL^{-1}$ from the plant extract against *S. aureus*.

3.2.4 Genus *Eugenia*

3.2.4.1 *Eugenia brasiliensis* Lam.

Eugenia brasiliensis receives the popular name of "grumixama", "grumixameira" and "Brazilian cherry". This species has several varieties, but the most common is the yellow fruit (Silva *et al.* 2014; Teixeira *et al.* 2015). The ethanol extract of *E. brasiliensis* submitted content of total phenolic compounds of 389.88 ± 3.48 mg of GAE/g and in the LC-MS/MS, catechin,

elagitanine, flavonoids, and anthocyanins were identified (Lazarini *et al.* 2018). The grumixama extract showed a better antibacterial effect against *S. aureus* ATCC 25923 (MIC of 62.5 μg.mL^{-1}) when compared with MRSA ATCC 33591 and *P. aeruginosa* ATCC 27853 obtained MIC of 250 μg.mL^{-1} and the extract proved to be bactericidal for all microorganisms tested with MBC of 500 μg.mL^{-1}. This extract did not present toxic effects on *Galleria mellonella* larvae at doses of 0.025 g/kg, therefore, the ethanol extract of *E. brasiliensis* should be further investigated for its safety in therapeutic use, as the natives report that this plant is effective in treating many diseases, including inflammatory and infectious (Lazarini *et al.* 2018; Pietrovski *et al.* 2010; Silva *et al.* 2014).

3.2.4.2 *Eugenia elliptica* Lam., *Eugenia orbiculata* Lam. and *Eugenia tinifolia* Lam.

Ramhit *et al.* (2018) researched extracts of plants endemic to Mauritania (Africa). The extracts of *Eugenia elliptica*, *Eugenia orbiculata* and *Eugenia tinifolia* submitted significant differences in phenolic compounds, flavonoids, and proanthocyanidins. In the chemical characterization using the HPLC technique, two flavonoids were found in *E. tinifolia* (kaempferol and quercetin) and *E. orbiculata* only quercetin, as well as the polyphenol epigallocatechin. In the antibacterial assays, it was analyzed that all the extracts presented activity against the tested microorganisms. The three extracts of the genus *Eugenia* present a MIC of 19.5 μg of fresh weight (FW).mL^{-1} against *P. aeruginosa* ATCC 27853. When tested against *Klebsiella oxytoca* ATCC 43086, this bacterium was more sensitive to extracts of *E. orbiculata* (MIC = 4.9 μgFW.mL^{-1}) and *E. tinifolia* (MIC = 9.7 μgFW.mL^{-1}). The extracts showed MIC values lower than at least one of the tested antibiotics (amoxicillin, chloramphenicol, and tetracycline) against microorganisms. The difference in the effect of extracts and conventional antimicrobials may be in the penetrating power and levels of active compounds that interfere with the bacteria, which can lead to death (Ramhit *et al.* 2018).

3.2.5 Genus *Campomanesia*

3.2.5.1 *Campomanesia adamantium* (Cambess.) O. Berg

Campomanesia adamantium is a plant found in Brazil, native to the Cerrado biome, popularly known as "guabiroba-do-campo" (Lima *et al.*, 2011). Sá *et al.* (2018) evaluated the antimicrobial activity of several extracts of *C. adamantium*: crude ethanolic extract (CEE), hexane (HF), dichloromethane (DF), ethyl acetate (AcF), and aqueous (AqF). The HF extract was fractionated, resulting in fractions HF1, HF2/2, HF2/6, and HF9/3/1/2/1 that were analyzed by GC-MS. Caryophyllene oxides (HF1), isoaromadendrene (HF2/2), octadecanoic acid (HF2/6), and cubenol (HF9/3/1/2/1 were the chemical compounds found. All extracts tested showed antibacterial activity. Of all the extracts evaluated, HF was the one with the best activity against *S. aureus* ATCC 6538 with MIC = 31.25 $\mu g.mL^{-1}$, afterward the MIC for *S. aureus* ATCC 25923 was 62.5 $\mu g.mL^{-1}$ and for the clinical isolate of *P. aeruginosa* SPM1, MIC = 500 $\mu g.mL^{-1}$. The other extracts obtained higher MIC values, including when tested against *K. pneumoniae* ATCC 700603 (MIC >1,000 $\mu g.mL^{-1}$), in which the extract was less active.

3.2.6 Genus *Callistemon*

3.2.6.1 *Callistemon citrinus* (Curtis) Skeels

Callistemon citrinus popularly known as bottlebrush belongs to the Myrtaceae family and is widely distributed in Australia, South America, and tropical Asia, but can also be found worldwide (Oyedeji *et al.* 2009). Shehabeldine *et al.* (2020) evaluate the crude extract of *C. citrinus* against MRSA ATCC 33591 and MSSA ATCC 25923, and found, respectively, a MIC of 125 $\mu g.mL^{-1}$ and 62.5 $\mu g.mL^{-1}$, while both presented MBC of 250 $\mu g.mL^{-1}$. However, MIC values revealed a bacteriostatic activity for the crude extract against MSSA, and bactericidal activity against MRSA (Shehabeldine *et al.* 2020).

3.2.7 Genus *Psidium*

3.2.7.1 *Psidium guayaquilense* Landrum & Cornejo and *Psidium rostratum* McVaugh

The species *Psidium guayaquilense* and *Psidium rostratum*, both are named guayabas and come from the province of Ecuador. These species were evaluated by (María *et al.* 2018), in which the quantification tests of total phenolic compounds. They found 941.97 ± 30.69 mg of GAE/g of dry extracts for the ethanolic extract of *P. guayaquilense*, while for the extract of *P. rostratum* it was 591.34 ± 24.31 mg of GAE/g of dry extracts. Regarding antibacterial activity, the two extracts were effective against *S. aureus* ATCC 25923 with a MIC of 50 $\mu g.mL^{-1}$.

3.2.7.2 *Psidium guajava* L.

Within the genus *Psidium*, there is the specie *P. guajava* (guava). Valle *et al.* (2015) evaluated ethanolic extracts of this species in the Philippines and observed the antibacterial effect against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC BAA-1705, *K. pneumoniae* carbapenem-resistant (CRE), *K. pneumoniae* producer of extended-spectrum β-lactamase (ESβL), *A. baumannii* metallo-β-lactamase (MβL), *P. aeruginosa* MβL, MRSA 1 (wound isolate), MRSA 2 (wound isolate), MRSA 3 (blood isolate) and MRSA 4 (sputum isolate). In the disk diffusion method, only MRSA isolates were sensitive to the extract, the inhibition halos ranged from 13 to 18 mm (Valle *et al.* 2015). While Chakraborty *et al.* (2018) analyzed the effect of *P. guajava* ethanolic extract against 10 clinical MRSA isolates and 10 non-clinical MRSA isolates and found that the zone of inhibition in a non-clinical MRSA culture was 29.69 ± 0.78 mm when for clinical MRSA isolates was 24.73 ± 0.55 mm. The results of the study by Valle *et al.* (2015) for the antibacterial activity using the guava ethanolic extract showed action only against *S. aureus* ATCC 25923 and against all MRSA. The extract did not present activity against the other microorganisms. The lowest MIC values

(625 µg.mL^{-1}) were found against MRSA 1 and MRSA 4. The extract tested against MRSA 4 was bactericidal at the same concentration of MIC, however, for MRSA 1 it needed a higher concentration (2,500 µg.mL^{-1}) to inhibit the bacteria growth (Valle *et al.* 2015).

Fu; Lu & Zhou (2016) when testing the phenolic compounds in methanolic extract of *P. guava* found 6 constituents: catechin (391.93 ± 15.08 mg.kg^{-1}), quercetin (122.23 ± 10.14 mg.kg^{-1}), gallic acid (99.15 ± 1.62 mg.kg^{-1}), epicatechin (58.43 ± 4.70 mg.kg^{-1}), luteolin (51.39 ± 3 mg.kg^{-1}) and kaempferol (38.06 ± 2.00 mg.kg^{-1}). When testing the methanolic extract of guava against microorganisms, obtained the best results against *P. aeruginosa* ATCC 27853, as it reached a lower MIC and MBC value (312.5/312.5 mg.mL^{-1}) when compared to bacteria *S. aureus* CMCC(B)26003 (1,250/2,500 mg.mL^{-1}). The authors evaluated the compounds found in the extract separately and analyzed that the polyphenol catechin, the major constituent in the extract, presented MIC and MBC of 1.25 mg.mL^{-1} against *S. aureus* and 2.5 mg.mL^{-1} against *P. aeruginosa*. The compound with the best antibacterial activity, when tested separately, was the gallic acid against *S. aureus*, having activity at a concentration of 0.63 mg.mL^{-1} (MIC/MBC), a constituent also found in the extract of *P. guava* (Fu; Lu & Zhou 2016).

3.2.7.3 *Psidium cattleianum* Sabine

Psidium cattleianum is popularly known by the names of araçá, araçá-do-mato, araçá-do-campo, yellow araçá, red araçá, araçazeiro, araçazeiro-da-praia (Coradin, Siminski & Reis 2011; Raseira *et al.* 2004). This plant has Brazilian origin and can be found in Bahia and the states of Rio Grande do Sul and Santa Catarina (Biegelmeyer *et al.* 2011). Many studies demonstrate the use of the species *P. cattleianum* in several areas (Dacoreggio, Moroni & Kempka 2019; Medina *et al.* 2011; Scur *et al.* 2016), however, there are few studies evaluating *P. cattleianum* mainly against bacteria associated with respiratory infections, especially MDR.

In the Dacoreggio, Moroni & Kempka (2019) study, was obtained the aqueous extracts of *P. cattleianum* leaves, harvested in winter and summer. They used two methods, WU extraction (water + ultrasound) and WE extraction (water + enzyme - cellulase complex). Regarding the number of total phenolics, it was observed that there was no statistically significant difference ($p<0.05$), considering how the extracts were obtained, however, the phenolic content presented differences concerning the season in which the leaves were collected. Values of 101 mg of EGA.g^{-1} (WU) were observed in the extract that the leaves were harvested in the summer and a higher content of phenolic compounds in those harvested in the winter (WU – 144 mg of EGA.g^{-1}). The same was observed in the extract with WE extraction, in the summer they found 121 mg of EGA.g^{-1}, while in the winter it was123 mg of EGA.g^{-1} of phenolic compounds. The outliers of the number of phenolic compounds present in each extract can vary based on several environmental factors, such as the temperature difference presented in the seasons (Dacoreggio, Moroni & Kempka 2019).

The authors, when testing the antibacterial activity of the aqueous extract of *P. cattleianum*, obtained MIC concentrations ranging from 12.6 to 18 μg.mL^{-1} against *S. aureus*. The two extracts showed lower MIC values than the extracts made with leaves collected in the summer season (WU= 12.6 μg.mL^{-1} and WE= 15.1 μg.mL^{-1}) (Dacoreggio, Moroni & Kempka 2019).

Traditional medicine has been accepted as an alternative form of health care and the development of microbiological resistance to available antibiotics has led scientists to investigate the antimicrobial activity of medicinal plants. Many extracts from different plants of the Myrtaceae family have been tested for their antimicrobial activities, as their antimicrobial agents are increasingly potent against multidrug-resistant bacteria. Therefore, medicinal plants and extracts made from them are often recognized as a source of new drugs and complementary

medicines for synthetic drugs and their versatile applications against microorganisms that cause respiratory tract infections.

3.3 Nanotechnology

Nanotechnology has been applied in several areas. As a delivery system has been investigated to contribute to the control and release of drugs, improve the effectiveness and selectivity of drugs, in addition to assisting in the treatment of infectious diseases (Flores *et al.* 2011; Gupta & Gupta 2005). Table 3 shows nanoparticles synthesized with extracts from different plant species of the Myrtaceae family.

Asghar *et al.* (2020) investigated the antibacterial activity of the synthesis of chitosan functionalized silver nanoparticles (CS-AgNPs) using ethanolic buds extract of *S. aromaticum* against resistant miro-organisms such as vancomycin resistance *S. aureus* (VRSA) LT 4312 and MRSA LT 0531, finding a MIC of 64 $\mu g.mL^{-1}$. Nickel oxide nanoparticles (NiO-NPs) have also been suggested as an antibacterial agent, therefore, Saleem *et al.* (2017) synthesized NiO-NPs with extract of leaves of *Eucalyptus globulus* (ELE) presenting an average size of NiO-NPs with 19 nm. The antimicrobial activity of the NiO-NPs synthesized was tested with 1 mM NiNO3 and ELE with distilled water (1:8 v/v) using the good diffusion technique against the clinical isolate of *P. aeruginosa* ESβL (48 and 64), MSSA (MS-2 and MS-6) and MRSA (MR-10 and MR-31), in which they found zones of inhibition that varied between 13 to 15 mm. While the MIC presented against all microorganisms was 0.8 $mg.mL^{-1}$ and MBC was 1.6 $mg.mL^{-1}$. In addition, the combination of the nanoparticle and the ELE showed inhibition of biofilm formation depending on the tested dose. The antibiofilm concentrations tested were 0, 0.1, 0.2, 0.4, 0.8 and 1.6 $mg.mL^{-1}$ of NiO-NPs. The best results were obtained for the MRSA isolate (32, 62, 72, 73, 76, and 83% inhibition, respectively). The results found by Saleem *et al.*

(2017) are positive, allowing NiO-NPs associated with *E. globulus* extract to be applied against bacterial infections, to protect human health from pathogenic microorganisms.

Although there are already some studies on the green synthesis of silver nanoparticles, there is currently no alternative treatment for infection with MDR microorganisms. Thus, Wintachai *et al.* (2019) investigated the potential of silver nanoparticles synthesized with ethanolic extract of *Eucalyptus critriodora* leaves as an inhibitor of *A. baumannii* MDR infection. The spherical size of the nanoparticle was in the range of 8 to 15 nm. Antibacterial assays (MIC) were performed against clinical isolates of *A. baumannii* MDR (n=10), in which the MIC and MBC values varied between 0.05 to 0.18 $\mu g.mL^{-1}$ and 0.36 to 0.72 $\mu g.mL^{-1}$, respectively. A reference strain of *A. baumannii* ATCC 19606 was used, which obtained MIC and MBC of 0.09 and 0.36 $\mu g.mL^{-1}$. The antibiofilm activity of the silver nanoparticle associated with *E. critriodora* extract was analyzed against 5 clinical isolates of *A. baumannii* MDR plus the standard strain, in parallel with colistin. When testing 1/8 to 1/2 of the MIC (0.012-0.045 $\mu g.mL^{-1}$) of silver nanoparticles, the best result for the reduction in biofilm formation was the one presented in 1/8 MIC (0.012 $\mu g.mL^{-1}$). The silver nanoparticle synthesized with the *E. critriodora* extract did not show significant cytotoxicity at the maximum concentration of 0.72 $\mu g.mL^{-1}$ when tested against the human lung epithelial cell line (A549). The authors also analyzed that the clinical isolates of *A. baumannii* MDR in A549 cells were sensitive when treated with concentrations varying from 1/8 to 1/2 MIC (0.012-0.045 $\mu g.mL^{-1}$), then, after checking the results nanoparticles synthesized with the ethanolic extract of *E. critriodora* may be a potential alternative therapy to reduce respiratory infections, such as those caused by *A. baumannii* MDR (Wintachai *et al.* 2019).

Hashemi *et al.* (2020) prepared iron (ZVINPs) and silver (AgNPs) nanoparticles, in which the biosynthesis of both was using aqueous extract of *Feijoa sellowiana* fruit. Through the HPLC chromatography method, five phenolic acids were detected in the extract: catechin 1

(188.5 mg.g^{-1} of extract), gallic acid 2 (18.5 mg.g^{-1} of extract), caffeic acid 3 (3.2mg.g^{-1} of extract), rutin 4 (15.8mg.g^{-1} of extract) and p-coumaric acid 5 (4.7mg.g^{-1} of extract). The authors investigated the antibacterial activity of the nanoparticles against pathogenic bacteria (*S. aureus* ATCC 29213; *A. baumannii* ATCC 29606; *K. pneumonia* ATCC 700603 and *P. aeruginosa* ATCC 27853) and clinical isolates from the same species. The tested concentrations of each of the nanoparticles ranged from 125 to 0.25 μg.mL^{-1} of AgNPs and 30 to 0.15 μg.mL^{-1} of ZVINPs. ZVINPs showed the best antibacterial potential against three standard strains tested (*A. baumannii* ATCC 29606; *K. pneumonia* ATCC 700603 and *P. aeruginosa* ATCC 27853), while for *S. aureus* ATCC 29213 AgNPs had a more pronounced action reaching a MIC of 2 μg.mL^{-1}. Both nanoparticles proved to be bactericidal against the strains evaluated (Hashemi *et al.* 2020).

Hashemi *et al.* (2020) also tested the antibacterial activity of nanoparticles against clinical isolates of *S. aureus, A. baumannii, K. pneumoniae,* and *P. aeruginosa*, in which AgNPs showed better activity against *A. baumannii* (3.5 μg.mL^{-1}) and *S. aureus* (4 μg.mL^{-1}), while against *P. aeruginosa* and *K. pneumoniae*, ZVINPs present a MIC of 15 μg.mL^{-1} for both bacteria. The two nanoparticles were bactericidal, however, the lowest concentrations of MBC found were for the ZVINPs nanoparticle. The mechanism of action of silver nanoparticles synthesized with the *F. sellowiana* extract can be explained due to the presence of phenolic compounds in the extract reacting with the silver nanoparticles and forming complex, fighting microorganisms (Ebrahimzadeh *et al.* 2019; Hashemi *et al.* 2020).

Ali *et al.* (2015) performed the green synthesis of silver nanoparticles (AgNPs) with an aqueous extract of *E. globulus* (ELE), in which a solution was carried out with both (1:4 v/v) and, then, irradiated with micro-waves. The ELEAgNPs were approximately 1.9 to 4.3 nm in size with microwave treatment and 5-25 nm without treatment. The ELEAgNPs were approximately 1.9 to 4.3 nm in size with microwave treatment and 5-25 nm without treatment.

ELEAgNPs were evaluated for antibacterial activity against *P. aeruginosa* ESβL, MRSA (MR-6), and MSSA (MS-6). In the good diffusion test, when ELEAgNPs were tested, the zones of inhibition were greater ranging from 19 to 21 mm, when compared to the values tested only with ELE (8-10 mm). The concentrations found in MIC and MBC with ELEAgNPs against MRSA (27/30 $\mu g.mL^{-1}$), MSSA (30/33 $\mu g.mL^{-1}$), while for *P. aeruginosa* ESβL it was 27/36 $\mu g.mL^{-1}$. The authors performed antibiofilm activity with a concentration of 30 $\mu g.mL^{-1}$, showing 82 ± 3% and 81 ± 5% biofilm inhibition, respectively, against *S. aureus* and *P. aeruginosa*. This inhibition can occur due to the polyphenol compounds present in the chemical characterization of the *E. globulus* leaf extract, which can capture the iron in the medium, causing microorganisms to die (Ali *et al.* 2015).

The formulation of iron nanoparticles (FeNP) synthesized with aqueous extract of *E. robusta* leaves with various concentrations of iron salt, in the proportion of 1:1 was evaluated by Vitta *et al.* (2020). As for the quantification of phenolic and flavonoid compounds, *E. robusta* extract showed, respectively, 158.47 ± 0.64 mg gallic acid (GAE)/g extract, 131.12 ± 4.49 (mg quercetin (QE)/g extract, while FeNP showed 98.21 ± 10.34 mgGAE/g and 40.54 ± 6.87 mg QE/g. The antibacterial activity through the agar diffusion method evaluated the FeNP obtained under various forms of synthesis in the following concentrations (FeNP I = 0.01 $g.mL^{-1}$ extract + 1 mM [Fe]; FeNP II = 0.01 $g.mL^{-1}$ extract + 5 mMe [Fe] and FeNP III = 0.005 $g.mL^{-1}$ +0.005 mM [Fe]) against *P. aeruginosa* and *S. aureus* and it was noticed that as the size of the nanoparticle decreased, the values of the inhibition halos. It is believed that the compounds found in the chemical composition of *E. robusta* extract contributed to the antibacterial potential, in addition to the fact that the size of the nanoparticle interfered in the mechanism of action, because the smaller the particle, the greater the power of penetration into the bacteria, causing it degrades and is eliminated. Thus, nanoparticles are promising for application as an antibacterial in the clinical area (Vitta *et al.* 2020).

Table 3. Antibacterial activity and characteristics of nanoparticles synthesized with extract from different species of the Myrtaceae family against bacteria involved with respiratory infection.

Genus	Species	Partoftheplant	Nanoparticle	Size	Biologicalorganisms	Antibacterialactivity MIC/MBC	Reference
Eucalyptus	*E. globulus*	Leaves	Nickel oxide	19 nm	MSSA (MS-02)	0.8/1.6 mg.mL^{-1}	Saleem et al. (2017)
					MSSA (MS-06)	0.8/1.6 mg.mL^{-1}	
					P. aeruginosa – 48	0.8/1.6 mg.mL^{-1}	
					P. aeruginosa – 64	0.8/1.6 mg.mL^{-1}	
					MRSA – 10	0.8/1.6 mg.mL^{-1}	
					MRSA - 31	0.8/1.6 mg.mL^{-1}	
		Leaves	Silver	1 to 9 nm (microwave treatment)	*P. aeruginosa* (ESβL)	27 / 36 µg.mL^{-1}	Ali et al. (2015)
				5 to 25 nm (no treatment)	*S. aureus* MRSA (MR-6)	27 / 30 µg.mL^{-1}	
					S. aureus MSSA (MS-6)	30 / 33 µg.mL^{-1}	
	E. critriodora	Leaves	Silver	8 to 15 nm	*A. baumannii* ATCC 19606	0.09 / 0.36 µg.mL^{-1}	Wintachai et al. (2019)
					10 isolates*A. baumannii*	Rangedfrom	

						0.05 – 0.18 µg.mL^{-1}/ 0.36 - >0.72	
	E. robusta	Leaves	Iron	0.8 nm	*S. aureus*	ND	Vitta et al. (2020)
					P. aeruginosa	ND	
Syzygium	*S. aromaticum* L.	Clovebuds	Silver	2 nm	VRSA LT 4312	64 µg/mL^{-1} / ND	Asghar et al. (2020)
					MRSA LT 0531		
Feijoa	*F. sellowiana*	Fruit	Silver	ND	*S. aureus* ATCC 29213	2 µg/mL^{-1} / 70 µg/mL^{-1}	Hashemi et al. (2020)
					A. baumannii ATCC 29606	9 µg/mL^{-1} / 70 µg/mL^{-1}	
					K. pneumonia ATCC 700603	9 µg/mL^{-1} / 70 µg/mL^{-1}	
					P. aeruginosa ATCC 27853	4.5 µg/mL^{-1} / 140 µg/mL^{-1}	
					S. aureus	4 µg/mL^{-1} / 62.5 µg/mL^{-1}	
					A. baumannii	3.5 µg/mL^{-1} / 112.5 µg/mL^{-1}	

			K. pneumonia	32 µg/mL^{-1} / 250 µg/mL^{-1}
			P. aeruginosa	62.5 µg/mL^{-1} / 250 µg/mL^{-1}
			S. aureus ATCC 29213	4 µg/mL^{-1} / 7.5 µg/mL^{-1}
			A. baumannii ATCC 29606	2 µg/mL^{-1} / 15 µg/mL^{-1}
			K. pneumonia ATCC 700603	7.5 µg/mL^{-1} / 15 µg/mL^{-1}
			P. aeruginosa ATCC 27853	4 µg/mL^{-1} / 7.5 µg/mL^{-1}
	Iron	ND	*S. aureus*	7.5 µg/mL^{-1} / 15 µg/mL^{-1}
			A. baumannii	15 µg/mL^{-1} / 60 µg/mL^{-1}
			K. pneumonia	15 µg/mL^{-1} / 60 µg/mL^{-1}
			P. aeruginosa	15 µg/mL^{-1} / 60 µg/mL^{-1}

MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; MRSA: Methicillin resistant *S. aureus*; MSSA: Methicillin sensitive *S. aureus*; *P. aeruginosa* (ESβL): extended spectrum β-lactamase-producing *P. aeruginosa*; VRSA: Vancomycin resistant *S. aureus*; ND: not determined.

4 Conclusions

Due to the great problem of bacterial resistance that is increasing with each passing year, it becomes more difficult to contain the microorganisms that cause respiratory tract infections that end up becoming MDR, one of the causes being the indiscriminate and excessive use of conventional medicines that the market offers. Thus, there is a real need and urgency to develop new antimicrobials that serve as a strategy for conventional antibiotics, making it possible to control and eliminate, especially MDR bacteria, or else, antibacterial agents that enhance the action of existing drugs.

This review shows the studies carried out, in the last years, with plants of the Myrtaceae family, presenting their chemical composition and antibacterial activity. Species of this family have several constituents that have antimicrobial activity and can be used in the therapy of bacteria that cause respiratory infections. In addition, through the literature, it was possible to observe, that there are few studies with plants of the Myrtaceae family in the clinical area and mainly being tested against pathogens involved with a respiratory infection and that are MDR. Hence, it is essential to encourage the scientific community to continue research in search of new effective therapeutic agents, so that they are used mainly in the clinical application against the microorganisms that cause respiratory tract infections since studies shown with species of the Myrtaceae family demonstrated promising results.

5 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.

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CAPÍTULO III. CHEMICAL CHARACTERIZATION, ANTIOXIDANT, CYTOTOXIC, AND ANTIBACTERIAL ACTIVITIES OF *Eugenia uniflora* L. AND *Psidium cattleianum* Sabine ESSENTIAL OILS AGAINST *Klebsiella pneumoniae* AND *Acinetobacter baumannii*

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

***Eugenia uniflora* L. and *Psidium cattleianum* Sabine essential oils as antibiotics: *in vitro* studies.**

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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 bacteria; Myrtaceae.

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.

Materials and methods

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).

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).

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.

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

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.

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.

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.

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.

Results

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).

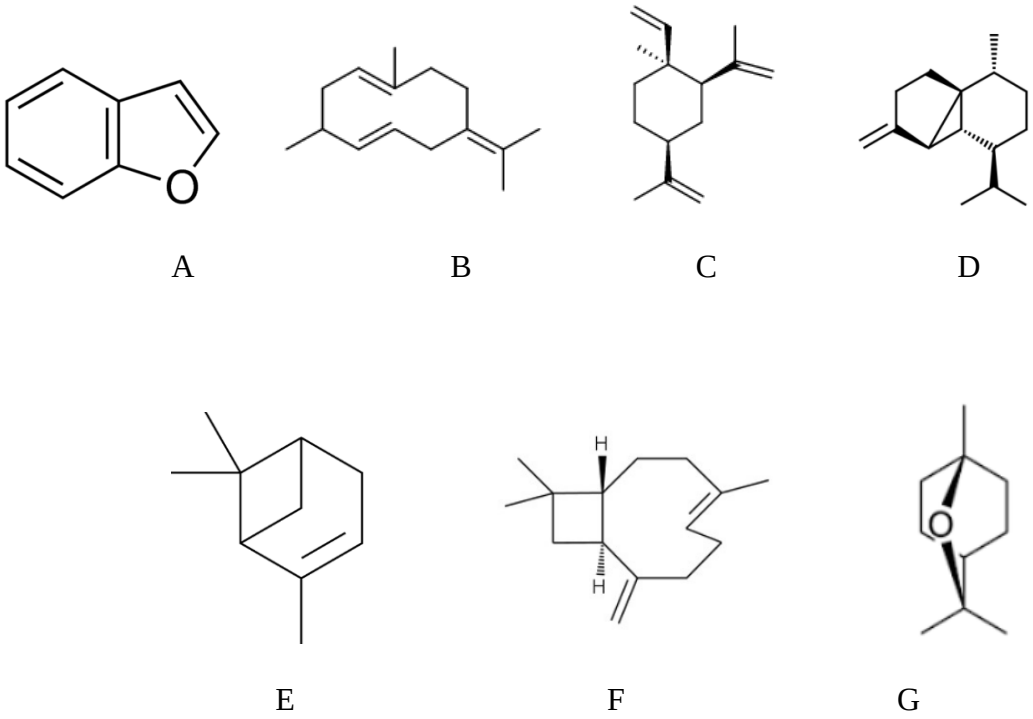
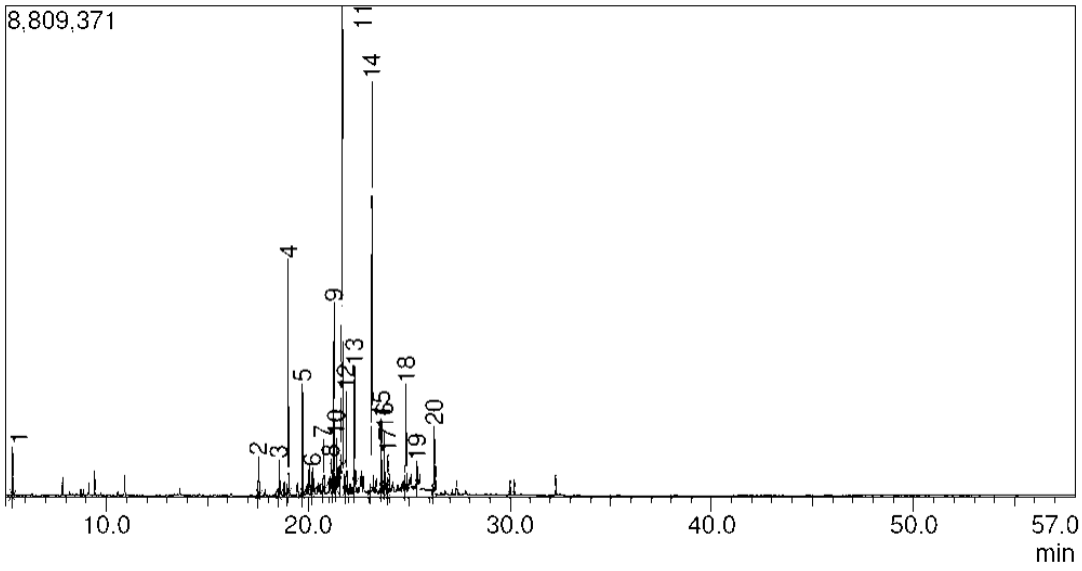
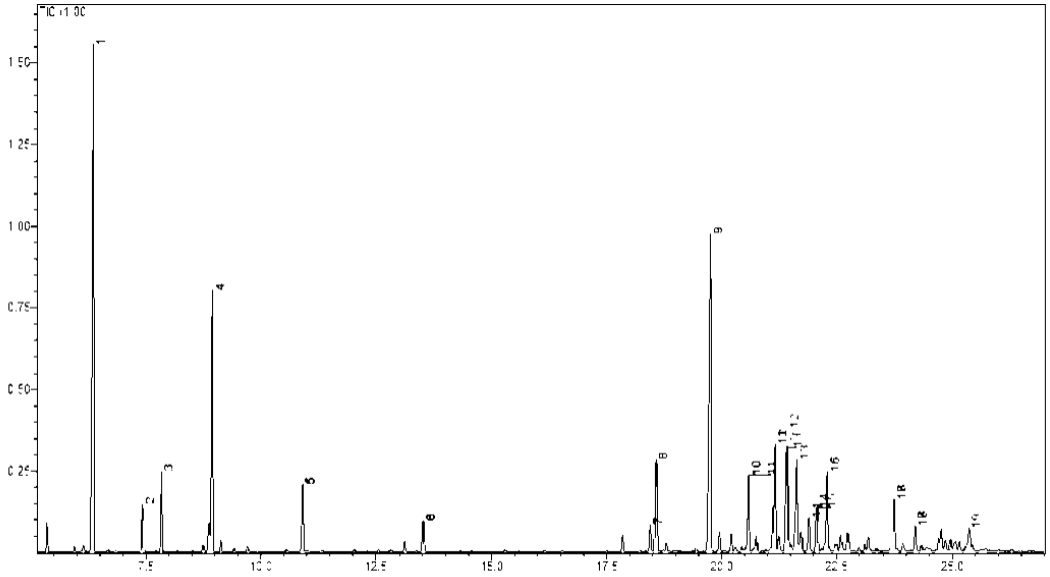


Figure 1. Molecular representation of the major compounds found in *E. uniflora* L. (EOE) and *P. cattleianum* Sabine (EOP). A: benzofuran, 6–ethenyl–4,5,6,7–tetrahydro; B: germacrene B; C: β-elemene; D: β-cubebene E: α-pinene; F: β-caryophyllene and G: eucalyptol.

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.



(EOE)



(EOP)

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

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.

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 $mg.mL^{-1}$.

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).

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.

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).

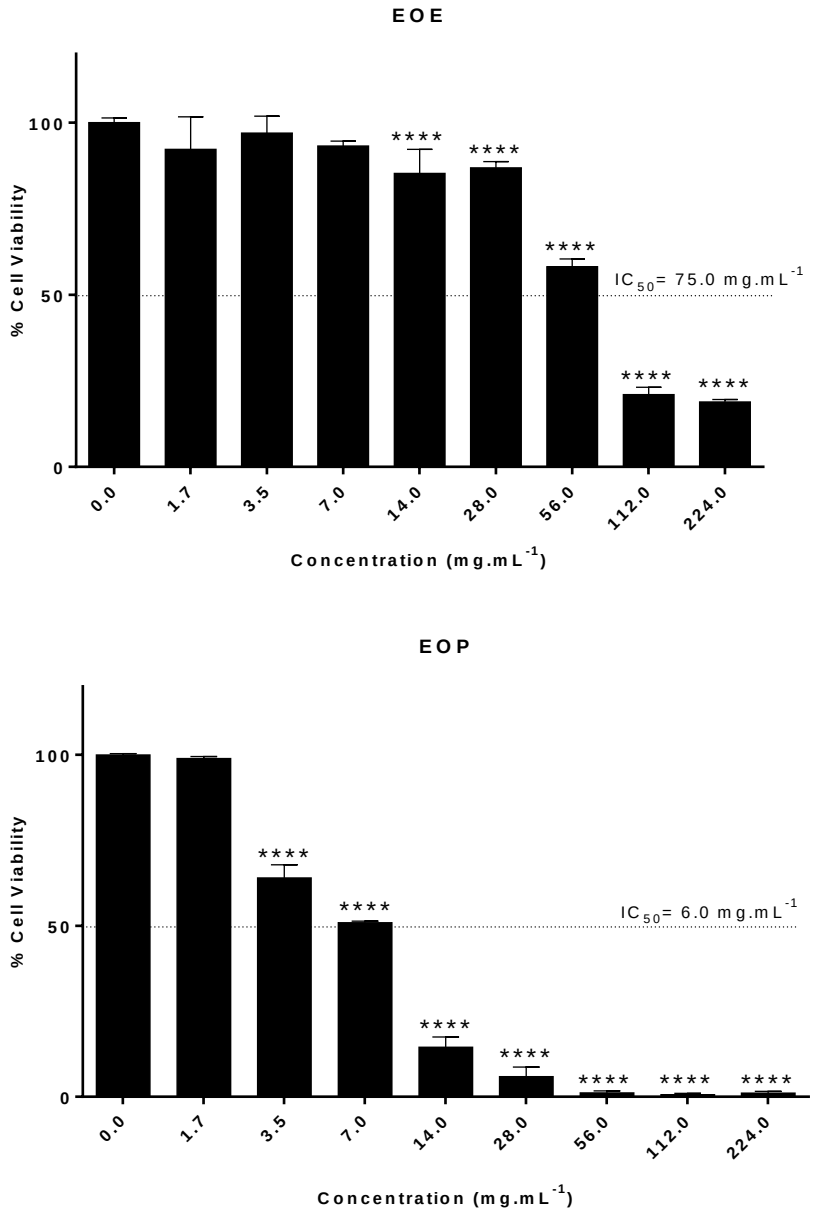


Figure 3. Cytotoxicity effect of EOE and EOP at 1.7–224.0 mg.mL^{-1} against VERO cell line. Cell proliferation was investigated by MTT assay. The half-maximal inhibitory concentration (IC_{50}) values are shown for each EO. EOP: essential oil of *P. cattleianum* Sabine. EOE: Essential oil of *E. uniflora* 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).

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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CAPÍTULO IV. PLANTS OF THE MYRTACEAE FAMILY: PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT, ANTIBACTERIAL, AND ANTIBIOFILM ACTIVITY AGAINST MULTI-DRUG RESISTANT (MDR) *Acinetobacter baumannii*

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Plants of the Myrtaceae family: phytochemical analysis, antioxidant, antibacterial, and antibiofilm activity against multi-drug resistant (MDR) *Acinetobacter baumannii*

Medicinal plants against *Acinetobacter baumannii*

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ABSTRACT

Myrtaceae family plants are rich in bioactive compounds, presenting biological properties. This work aimed to evaluate the chemical composition, antioxidant, antibacterial, and antibiofilm activity of methanolic extracts of *Eugenia uniflora* L. and *Psidium cattleianum* Sabine (EME and PME), against *A. baumannii* ATCC® 19606™ and six multidrug-resistant (MDR) isolates. As result, the EME presented gallic acid and the PME caffeic acid as the major compounds. EME and PME have antioxidant and antibacterial activity, with MIC_{50}=18.5 $\mu g.mL^{-1}$ and MIC_{90}=36.9 $\mu g.mL^{-1}$ for both extracts. EME presented bactericidal activity against the ATCC® 19606™ and five MDR isolates, while PME was bactericidal against three MDR isolates. Additionally, both extracts killed the bacterial cells after 10 min of treatment. The PME and EME have antibiofilm activity, with a biofilm inhibition percentage of 58.5% to 88% and biofilm disruption of 34% to 42% (p<0.05). These results highlight that EME and PME can potentially be a natural antioxidant, antibacterial and antibiofilm agents against *A. baumannii* that could be applied in the development of new antibiotics or disinfectants. To the best of the authors' knowledge, this is the first report, on the antibacterial and antibiofilm activity of EME and PME against this important nosocomial pathogen.

Keywords: araçá, extract, inhibition, phenolic compounds, pitanga

1 INTRODUCTION

Acinetobacter baumannii is a species of bacterium that has the form of Gram-negative coccobacilli, they are ubiquitous and opportunistic (Ma & McClean 2021). *A. baumannii* cause nosocomial infections, such as bacteremia, pneumonia, urinary tract infection, meningitis, and wound infection (Khalil et al. 2021, Ma & McClean 2021). This pathogen is considered to be multi-drug resistant (MDR), causing great concern worldwide, as they can disperse in hospital environments, leading to nosocomial outbreaks (Jain et al., 2019). The World Health Organization (WHO) has determined *A. baumannii* as a critical priority for the search and development of new drugs, proving to be a major public health problem due to its MDR (Moubareck & Halat 2020, Tacconelli et al. 2018).

Medicinal plants have bioactive compounds in their chemical composition, presenting activity against MDR bacteria (Kuete 2010, Nascimento et al. 2000). *Eugenia uniflora* L. is a species native to Brazil and is popularly named “Pitanga” (Fouqué 1981, Villachica 1996). Several biological properties of *E. uniflora* L. have already been explored, for example, antioxidant, antibacterial, and antifungal activity (Figueiredo et al. 2019, Garcia et al. 2021, Victoria et al. 2012). *Psidium cattleianum* Sabine is also a Brazilian species, popularly known by the name of "Araçá” (Coradin et al. 2011, Raseira et al. 2004). This plant is rich in phenolic compounds and some studies have already proven antioxidant and antibacterial activity (Dacoreggio et al. 2019, Garcia et al. 2021, Medina et al. 2011, Scur et al. 2016).

Due to the diversity of plants of the *Eugenia* and *Psidium* genera in southern Brazil and the biological activity presented by the species *E. uniflora* L. and *P. cattleianum* Sabine, the objective of this work was to evaluate the chemical composition, antioxidant, antibacterial, and antibiofilm activity of *E. uniflora* L. and *P. cattleianum* Sabine methanolic extracts (EME and PME) against *A. baumannii* ATCC® 19606™ and MDR isolates of *A. baumannii*.

2 MATERIALS AND METHODS

2.2 Plant material

The fruits of *E. uniflora* L. and *P. cattleianum* Sabine were collected in the Centro Agropecuário da Palma, Universidade Federal de Pelotas (UFPel), Capão do Leão, RS, Brazil (31°48'13"S and 52°30'30"W). The fruits were harvested during the month of February 2017. Samples of plant material were identified and stored in the PEL Herbário do Instituto de Biologia, Departamento de Botânica, UFPel, and identified by the following numbers of exsiccate: *Psidium cattleianum* Sabine (PEL N° 26970) and *Eugenia uniflora* L. (PEL N° 26971).

2.3 Preparation of the extracts

The fruits were stored at a temperature of -70°C, lyophilized, macerated, and then a methanolic extract was prepared. The extraction was carried out in two phases, and in phase A, 5 g of the sample of each of the macerated fruits was weighed and then 40 mL of methanol (Synth®) was added. This mixture was stirred in a shaking incubator model TE-420 (Tecnal) at 138 rpm for 2 h and then left to rest for 15 min at room temperature. Then, 10 mL of methanol was added to the flask and stirred under the same conditions for another 1 h. In phase B, the extracts obtained in phase A were filtered through filter paper into a flask with a capacity of 500 mL and then evaporated in a rotary evaporator model Q344B (Quimis) at 60°C. After the solvent was completely evaporated, 10 mL of distilled water was added and then stirred in an ultrasonic bath model USC - 1850 (Unique) for 10 min. Then, 5 mL of 0.1 $mol.L^{-1}$ barium hydroxide and 5 mL of 5% zinc sulfate were added, manually stirred, and then left to rest for 20 min. Subsequently, it was centrifuged in a centrifuge model ct 5000r (Cientec) at 500 rpm for 15 min. The intermediate phases were removed, filtered, placed in an amber bottle, and then stored under refrigeration at -20°C until used.

2.4 Determination of total phenolic compounds

The total phenolic content was quantified using extracts made from freeze-dried fruits of *E. uniflora* L. and *P. cattleianum* Sabine, using the method described by (Scaglioni et al. 2014). Ferulic acid was used as a reference standard and quantification was performed using the Folin-Ciocalteu reagent technique. The test was performed in triplicate.

2.5 Chromatography of the extracts

The identification of phenolic acids in the extracts was performed using reference standards obtained from Sigma-Aldrich, namely: caffeic, chlorogenic, p-coumaric, ferulic, gallic, p-hydroxybenzoic, protocatechuic, syringic, and vanillin acids. An aliquot of the extracts was added in methanol:water (1:1). This mixture was used for injection into a liquid chromatograph (Shimadzu, Tokyo, Japan, CLASS-M10A), together with a UV detector (HPLC-UV), using the same conditions determined by Scaglioni et al. (2014).

2.6 Analytical parameters

Phenolic determination was based on its detection and quantification limits, linearity, repeatability, and recovery (Ribani et al. 2004), for chromatographic analysis, using different standards (Table I). The limit of detection (LOD) was calculated in signal-to-noise ratio at a ratio of 3:1. The limit of quantification (LOQ) was established as three times the LOD (Scaglioni et al. 2014).

2.7 Antioxidant activity

The extracts were evaluated for their antioxidant activity by the *in vitro* free radical capture method DPPH (2.2, diphenyl-2-picrylhydro-hydroxyl). Four different EME and PME concentrations (1 $mg.mL^{-1}$; 0.5 $mg.mL^{-1}$; 0.25 $mg.mL^{-1}$ and 0.125 $mg.mL^{-1}$) were mixed with

an ethanol solution (2.7 mL at a concentration of 0.06 mM) containing the DPPH radical (300 µL). After a resting period at room temperature (15 min), a spectrophotometer model UV-M51 (Bel Photonics) at 517 nm was used to take absorbance readings. The assay was performed in triplicate and rutin (Sigma-Aldrich®) was used as a standard at the same concentrations as the extracts (Garcia et al. 2021). Results were determined as percent inhibition of DPPH absorbance (% inhibition) relative to control results without extracts. The % inhibition was calculated according to equation 1.

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

Table I. Calibration parameters for the determination of phenolic acids by HPLC-UV* in extracts of *P. cattleianum* Sabine and *Eugenia uniflora* L.

N	Phenolic acid	T.R. (min)	Analytical curve	Linearity ($\mu g.mL^{-1}$)	% RSD	r^2	LOD ($\mu g.mL^{-1}$)	LOQ ($\mu g.mL^{-1}$)
1	Gallic	5.16	$y = (0.741\text{-}0.754) \times 10^5$	0.36 – 3.6	3.8	0.998	0.12	0.36
2	Protocatechuic	6.71	$y = (0.475 – 0.867) \times 10^5$	0.81 – 8.1	3.1	0.999	0.27	0.81
3	Chlorogenic	8.17	$y = (0.455 – 1.798) \times 10^5$	1.23 – 12.3	1.9	0.999	0.41	1.23
4	p-Hydroxybenzoic	9.4	$y = (0.480 – 0.989) \times 10^5$	0.96 – 9.6	1.5	0.999	0.32	0.96
5	Caffeic	10.9	$y = (0.824 – 0.203) \times 10^5$	0.72 – 7.2	1.7	0.999	0.24	0.72
6	Syringic	12.21	$y = (0.903 – 4.186) \times 10^5$	0.75 – 7.5	1.8	0.999	0.25	0.75
7	Vanillin	13.8	$y = (1.265 – 2.199) \times 10^5$	0.54 – 5.4	1.7	0.999	0.18	0.54
8	p-Coumaric	17.92	$y = (1.512 – 1.119) \times 10^5$	0.57 – 5.7	2.1	0.999	0.19	0.57
9	Ferulic	22.05	$y = (1.405 – 2.354) \times 10^5$	0.75 – 7.5	2.6	0.999	0.25	0.75

*Analysis technique: High Performance Liquid Chromatography, Ultraviolet. N: corresponding number of phenolic acid; R.T.: retention time; RSD: relative standard deviation; r^2: coefficient of determination; LOD: detection limit; LOQ: limit of quantification.

2.8 Minimal Inhibitory Concentration (MIC) and Minimal Bactericide Concentration (MBC)

For the MIC and MBC determination, a standard strain of *A. baumannii* ATCC® 19606™ and six MDR isolates of *A. baumannii* (Ab 02, Ab 03, Ab 10, Ab 13, Ab 15, and Ab 47) collected from patients were used. The standard strain was provided by the Fundação Oswaldo Cruz (FIOCRUZ). The MDR strains belong to the Laboratório de Bacteriologia e Bioensaios (LaBBio), UFPel, Capão do Leão, RS, Brazil and were kindly provided to us by the Laboratório de Microbiologia do Hospital Escola, UFPel, Pelotas, RS, Brazil. Bacterial strains evaluated in this study were previously characterized, are genetically different and all classified as strong biofilm formers (de Freitas et al. 2020). For the MIC determination assay, the broth microdilution method was used (CLSI 2018), using microplates with 96 wells. Serial dilutions of the extracts were performed, in which the concentrations tested ranged from 0.3 to 36.9 $\mu g.mL^{-1}$. The experiments were performed in triplicate and the experiment was repeated three times. The plates were incubated at 37 ºC for 24 hours. After incubation, 20 µL of 0.02% 7-hydroxy-3H-phenoxazine-3-one (Resazurin, Sigma-Aldrich®) were added to all wells and after that, it was observed if there was a color change, showing bacterial growth. From the results of the MIC, the determination of the MBC was carried out, following guidelines from (CLSI 2018), to observe whether the action of the extract was bactericidal or bacteriostatic. The MIC_{50} and MIC_{90} values of both extracts were investigated, which represent the concentrations necessary to inhibit 50% and 90% of the bacteria.

2.9 Time-kill Assay

The EME and PME were evaluated for the time of kill *A. baumannii* ATCC® 19606™ and Ab 02, following the protocol described by Allend et al. (2022), with modifications. Briefly, the extracts were prepared in BHI broth at 1×MIC concentration and a bacterial suspension

(1×10^6 CFU.mL^{-1}) was added, and the microtubes were incubated at 37°C. The samples were evaluated at times 0 h; 10 min; 20 min; 30 min; 1 h; 2 h; 4 h; 6 h; 8 h and 24 h. The 100 µL aliquots were removed from the microtube and diluted (1:10) in saline. From the dilutions, drop counts were performed, in which 2 µL of each dilution were added to MacConkey agar plates (Kasvi®) and then incubated for 24 h at 37°C. The number of viable colonies was enumerated and expressed as Log.CFU.mL^{-1}.

2.10 Antibiofilm activity: biofilm inhibition

The biofilms formed by *A. baumannii* ATCC® 19606™ and the Ab 02 MDR isolate were exposed to EME and PME before inducing biofilm formation, following the protocol of Halicki et al. (2020), with modifications. In 96-well microplates, 20 µL of bacterial suspension (1×10^6 CFU.mL^{-1}) were added to 180 µL of Muller Hinton (MH) broth (Kasvi®) with an extract concentration of 1×MIC. Controls were included: MH broth only with extract 1×MIC concentration, biofilm formation controls (positive control), and MH broth only (negative control). The microplates were incubated for 24h at 37°C. The next steps of washing, fixing, staining, and quantifying the biofilm were performed according to (Stepanović et al. 2007).

2.11 Antibiofilm activity: disruption of the biofilm

In this assay, the formation of the bacterial biofilm was first induced, and the adhered cells were treated with different concentrations of EME and PME to evaluate the activity in disruption of the bacterial biofilm mature. Then, 20 µL of bacterial suspension (1×10^6 CFU.mL^{-1}) in 180 µL of MH broth was then added to the 96-well microplates, then the plates were incubated for 24 h at 37°C, for biofilm formation. After the incubation period, the contents of the wells were removed and washed with 0.9% saline to eliminate non-adhered cells. Subsequently, 100 µL of MH broth containing the 1×MIC concentrations of EME and PME

extracts. The microplates were incubated for 24 h at 37°C and after the steps of washing, fixation, and staining the biofilm quantification was performed by optical density (OD) at 540 nm determination (Stepanović et al. 2007).

2.12 Statistical analysis

For statistical analysis, two-way analysis of variance (ANOVA) was used, followed by Tukey's multiple comparisons test, using GraphPad Prism software version 8.0.1. Values of $p<0.05$ were considered statistically significant. The experiments were performed in triplicate, and the data were demonstrated according to to mean values and standard deviation.

3 RESULTS

3.1 Phenolic compounds

The determination of the total phenolic compounds of EME and PME showed results of 1.72 mg ferulic acid.100 g^{-1} of total phenols for EME and 7.1 mg ferulic acid.100 g^{-1} for PME.

3.2 Chemical characterization of methanolic extracts

Analyzes of EME and PME demonstrated different phenolic acids (Table II). The EME presents gallic acid (2.95 $mg.g^{-1}$) and protocatechuic acid (2.08 $mg.g^{-1}$) as major compounds, followed by coumaric acid (0.075 $mg.g^{-1}$). PME present three phenolic acids in its composition, namely, caffeic acid (0.775 $mg.g^{-1}$), chlorogenic acid (0.625 $mg.g^{-1}$) and gallic acid (0.2 $mg.g^{-1}$).

Table II. Chemical composition of *E. uniflora* L. and *P. cattleianum* Sabine methanolic extracts obtained by HPLC-UV.

Phenolic acids	EME (mg.g^{-1})	PME (mg.g^{-1})
Gallic	2.95	0.2
Protocatechoic	2.08	-
Chlorogenic	-	0.625
p-Hydroxybenzoic	-	-
Caffeic	-	0.775
Syringe	-	-
Vanillin	-	-
p-Cumaric	0.075	-
Ferulic	-	-

EME: *Eugenia unflora* L. methanolic extract; PME: *Psidium cattleianum* Sabine methanolic extract.

3.3 Antioxidant activity

When evaluating the antioxidant activity, EME presents the highest percentage in all concentrations tested when compared to PME. For the EME, was observed that there was greater capture of the DPPH free radical, with an inhibition percentage of 44.6% (1 mg.mL^{-1}), 29.7% (0.5 mg.mL^{-1}), 20.4% (0.25 mg.mL^{-1}) and 13.7% (0.125 mg.mL^{-1}). PME showed a low antioxidant effect, as the inhibition values were 15.7%, 10.4%, 10.9% and 5.6%, respectively, at concentrations of 1 mg.mL^{-1}, 0.5 mg.mL^{-1}, 0.25 mg.mL^{-1} and 0.125 mg.mL^{-1}.

3.4 Minimal Inhibitory Concentration (MIC) and Minimal Bactericide Concentration (MBC)

The results obtained in MIC and MBC assays are shown in Table III. Both extracts showed antibacterial activity against the standard strain *A. baumannii* ATCC® 19606™ and all MDR isolates tested. The EME presents a MIC range from 9.2 to 18.5 $\mu g.mL^{-1}$ and PME from 18.5 to 36.9 $\mu g.mL^{-1}$. When we evaluated the bactericidal activity, the EME presented MBC ranging from 18.5 to 36.9 $\mu g.mL^{-1,}$ and the PME had MBC of 36.9 $\mu g.mL^{-1}$. When we analyze the results for the MDR strains of *A. baumannii*, the EME had a better activity for all tested isolates, the same can be observed for the standard strain of *A. baumannii* ATCC® 19606™ (Table III). The lowest MIC values (9.2 $\mu g.mL^{-1}$) were found for isolates Ab 13, Ab 15, and Ab 47, however, only Ab 13 and Ab 15 were killed, with an MBC of 18.5 $\mu g.mL^{-1}$ and 36.9 $\mu g.mL^{-1}$, respectively. PME showed antibacterial activity, where for three isolates (Ab 02, Ab 10, and Ab 47) it was bacteriostatic and for the other isolates, it was bactericidal at the highest concentration evaluated (36.9 $\mu g.mL^{-1}$). As for the concentration of extracts necessary to inhibit bacterial growth, 18.5 $\mu g.mL^{-1}$ was found as MIC_{50} and 36.9 $\mu g.mL^{-1}$ as MIC_{90} for both extracts.

3.5 Time-kill assay

Figure 1 shows the result of the time to kill the standard strain *A. baumannii* ATCC® 19606™ (Figure 1a) and the MDR isolate Ab 02 (Figure 1b) when treated with EME and PME, with a reduction of 99.9% (≥3 log10) of the total number of $CFU.mL^{-1}$ in the original inoculum using 1×MIC and presented as a statistical difference against the untreated cells (standard curve). *A. baumannii* ATCC® 19606™ was killed in 6 hours of treatment, and Ab 02 isolate in 10 minutes, for both extracts.

Table III. MIC and MBC of EME and PME against standard strains of *A. baumannii* and clinical isolates of *A. baumannii*.

Microorganisms	MIC/MBC (μg.mL^{-1})	
	PME	**EME**
A. baumannii ATCC® 19606™	18.5 / *	9.2 / 36.9
Ab 02	36.9 / *	18.5 / 18.5
Ab 03	36.9 / 36.9	18.5 /18.5
Ab 10	36.9 / *	18.5 / 36.9
Ab 13	18.5 / 36.9	9.2 /18.5
Ab 15	18.5 / 36.9	9.2 / 36.9
Ab 47	18.5 / *	9.2 / *

PME: *P. cattleianum* Sabine methanolic extract; EME: *E. uniflora* L. methanolic extract; *There were no MBC values in the concentration ranges tested.

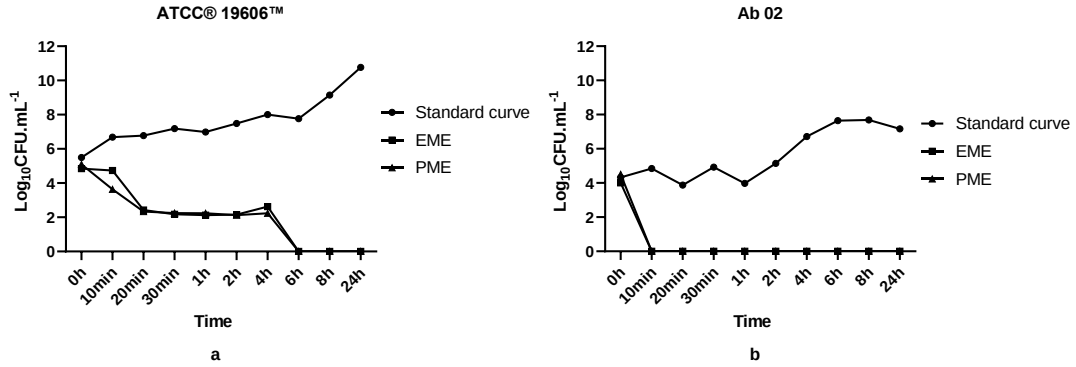

Figure 1. Time-kill curve for *A. baumannii* ATCC® 19606™ (a) and isolate Ab 02 (b). Treatment with 1× MIC of EME (ATCC® 19606™ = 9.2 μg.mL^{-1} and Ab 02 = 18.5 μg.mL^{-1}) and 1× MIC of PME (ATCC® 19606™ = 18.5 μg.mL^{-1} and Ab 02 = 36.9 μg.mL^{-1}). The standard curve represents the growth of the untreated bacteria.

3.6 Antibiofilm activity

EME and PME showed inhibition of biofilm formation in *A. baumannii* ATCC® 19606™ and isolate Ab 02 (Figure 2a and b). PME was more active against *A. baumannii* ATCC® 19606™ when compared to EME ($p<0.05$, Figure 2a) since the PME presents a biofilm inhibition percentage of 88% and EME of 58.5%. Additionally, when we evaluated Ab 02, the biofilm inhibition percentage was 88% for both extracts, EME and PME ($p<0.05$, Figure 2a). The growth control (bacteria untreated) presents an OD_{540nm} of 0.78 from ATCC® 19606™ and 1.26 from Ab 02 (Figure 2b). Bacterial biofilm was inhibited by the addition of 9.2 $\mu g.mL^{-1}$ of EME and when treated with 18.5 $\mu g.mL^{-1}$ of PME, presenting an OD_{540nm} for ATCC® 19606™ of 0.1 and 0.06, respectively ($p<0$ .05, Figure 2b). In the treatment of Ab 02, for both extracts, the OD_{540nm} reached 0.19 ($p<0$ .05, Figure 2b).

Regarding the biofilm disruption activity in ATCC® 19606™, the EME and PME did not present any inhibition percentage ($p>0.05$, Figure 2c). However, when we analyze the OD_{540nm} values, significant disruption was observed for ATCC® 19606™ (Figure 2d). The EME and PME treatment reached an OD_{540nm} of 0.3 and 0.4, respectively, different from untreated bacteria ($OD_{540nm}=0.8$) ($p<0.05$). In Ab 02, EME disrupted 34% of formed biofilm and PME 42% ($p<0.05$, Figure 2c). The isolate Ab 02, when evaluating the OD_{540nm}, presented values of 0.5 to PME and 0.6 to EME, a significant difference in comparison to untreated bacteria ($OD_{540nm}=1.3$) ($p<0.05$, Figure 2d).

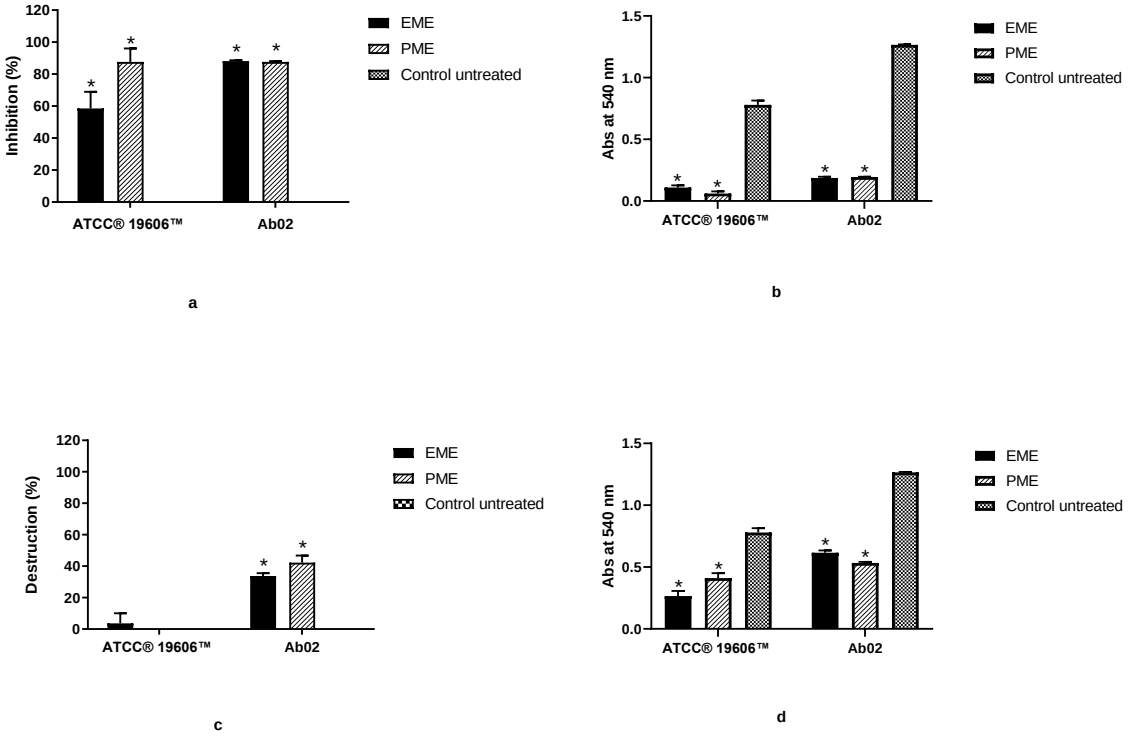

Figure 2. Antibiofilm activity of EME and PME (1× MIC) against *A. baumannii* ATCC® 19606™ and isolate Ab 02. Biofilm inhibition and disruption values are present in percentage (a and c) and optical density (OD_{540nm}) (b and d). Data are expressed as means ± SD from three independent experiments, analyzed by two-way ANOVA followed by Tukey's multiple comparison test. (*p<0.05 compared to control untreated).

4 DISCUSSION

Antibiotic resistance requires the search and development of new antibiotics and disinfectants that are effective in inhibiting or killing MDR bacteria cells and biofilms. Medicinal plants of the Myrtaceae family have promising biological properties against MDR pathogens (Garcia et al. 2021, Kuete 2010, Valle et al. 2015). In this context, our study showed that methanolic extracts of *E. uniflora* L. and *P. cattleianum* Sabine (EME and PME, respectively) have antibacterial and antibiofilm activity against a standard strain of *A. baumannii* and MDR isolates.

In our research, the EME and PME showed bacteriostatic and bactericidal activity against the Gram-negative bacteria *A. baumannii* ATCC® 19606™ and MDR isolates. EME presents the lowers MIC (9.2 to 18.5 μg.mL^{-1}) and MBC (18.5 to 36.9 μg.mL^{-}1) values, in comparison to PME. The EME had activity for all tested isolates and the lowest MIC values (9.2 μg.mL^{-1}) were found for three isolates (Ab 13, Ab 15, and Ab 47). However, only Ab 13 and Ab 15 were killed, with an MBC of 18.5 μg.mL^{-1} and 36.9 μg.mL^{-1}, respectively. Previous studies evaluating EME, obtained from leaves and pulps, showed antibacterial activity against Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC® 15442™ and *Shigella sonnei* ATCC® 11060™), with MIC of 10 and 156 μg.mL^{-1}, respectively (Bouzada et al. 2009). Lazarini et al. (2018) evaluated *Eugenia brasiliensis* Lam. against *Pseudomonas aeruginosa* ATCC® 27853™, finding a MIC of 250 μg.mL^{-1}.

Here, the EME presents a MIC from 18.5 to 36.9 μg.mL^{-1}, and an MBC of 36.9 μg.mL^{-1}. Our results of antibacterial activity showed that PME could inhibit the growth of the standard strain (*A. baumannii* ATCC® 19606™), and three tested isolates (Ab 02, Ab 10, and Ab 47), proving to be bacteriostatic. The bactericidal activity was shown only against three isolates (Ab 03, Ab 13, and Ab 15) (Table III). PME prepared from the leaves of the plant presents antibacterial activity at a concentration of 312.5 mg.mL^{-1} against *P. aeruginosa* ATCC® 27853™, being bactericidal at the same concentration (Dacoreggio et al. 2019). However, Valle et al. (2015) found different results when testing *P. guajava* leaf extract against Gram-negative bacteria, that had no effect against these pathogens, including MDR isolates of *A. baumannii*, being active only against Gram-positive bacteria. Similar results were observed when investigating the activity of extracts from *P. cattleianum* Sabine, which activity against Gram-positive bacteria (Zandoná et al. 2020). Some authors have reported in previous studies that Gram-positive bacteria are more susceptible to antimicrobials (Snoussi et al. 2018, Teneva et al. 2016). Gram-negative bacteria strains have virulence mechanisms, becoming more resistant

to various drugs due to efflux pumps (Davin-Regli et al. 2021). Gram-negative bacteria, in the cell wall, have an outer membrane (OM) composed of lipoproteins, phospholipids, proteins, and lipopolysaccharides, which provide these pathogens with greater resistance to antibiotics and plant extract activity (Lazzarotto-Figueiró et al. 2021).

A plant extract is considered active against a microorganism when the MIC concentrations found are below 100 $\mu g.mL^{-1}$, with moderate effect when $100 \leq CIM \leq 625$ $\mu g.mL^{-1}$ and weak antibacterial activity when concentrations are above 625 $\mu g.mL^{-1}$ (Djeussi et al. 2016, Kuete 2013). Thus, as both extracts present MIC_{50}=18.5 $\mu g.mL^{-1}$ and MIC_{90}=36.9 $\mu g.mL^{-1}$, have been considered active against ATCC® 19606™ and MDR isolates. In EME and PME, phenolic compounds were observed in their chemical composition and some studies argue that these constituents are responsible for the antimicrobial activity, mainly small phenolic compounds, called phenolic acids, which penetrate the plasmatic membrane (Ikigai et al. 1993), causing hyper acidification in the membrane interphase (Vattem et al. 2005).

As for the dead time that the EME and PME extracts take to kill *A. baumannii* ATCC® 19606™ and the MDR isolate, in our study, it was found 6 h and 10 min, respectively. Shetty et al. (2021) analyzed aqueous (AGuE) and ethanolic (EGuE) extracts of *P. guajava* (guava) against periodontal bacteria (*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*), at 6 h the authors observed a statistically significant decrease in *P. gingivalis* colonies in both AGuE and EGuE. The bacteriostatic activity was observed for 4-6 hours in both types of guava extracts, with no statistically significant difference in colony counts over the same period. Mordmuang et al. (2019) evaluated the ethanolic extract of *Rhodomyrtus tomentosa*, a plant from the Myrtaceae family, against *S. aureus* Newbould (ATCC® 29740™), which they obtained as a result that 1× MIC of the extract (16 $uL.mL^{-1}$) inhibited bacterial growth in 8 h.

The total phenolic content found in our study was 1.72 mg of ferulic acid.100 g-1 for EME and 7.1 mg ferulic acid.100 g^{-1} for PME. Our values were lower than those found previously in PME (463 mg gallic acid.100 g^{-1}), pitanga red (210 mg gallic acid. 100 g^{-1}), and pitanga orange (179 mg gallic acid.100 g^{-1}) (Bagetti et al. 2011). Celli et al. (2011) analyzed that the number of phenolic compounds from crude extracts of red and purple varieties of Brazilian cherries ranged, considering the stages of fruit ripening.

The differences in amounts of constituents found in plant extracts may vary due to several conditions, which may be associated with geographic location, time of year the material was collected, cultivation method, climatic conditions, age of the plant material, period, storage conditions, as well as other influencing factors are the extraction method, as well as the chosen solvent, thus varying the quantification of concentrations and compounds found in the chemical composition of each plant (Lazzarotto-Figueiró et al. 2021).

Aqueous and acetone-prepared extracts of *P. cattleianum* Sabine showed high levels of phenolic compounds, values ranging from 402.7 to 768.2 mg gallic acid.100 g^{-1} for different plant genotypes (Medina et al. 2011). Dacoreggio et al. (2019) did not find a statistically significant difference when they evaluated extracts of araçá leaves collected in winter and summer, about the composition of phenolic compounds, despite observing a significant difference when the extraction method was changed.

As for the phenolic acids investigated in the extracts of araçá and pitanga, major constituents were found in each of the extracts, in the EME gallic acid, followed by protocatechoic and coumaric acid, while the predominant ones in the PME were, respectively, caffeic, chlorogenic and gallic acid, as presented in Table II. Mallmann et al. (2020) analyzed non-extractable phenolic compounds (NEPC) and extractable phenolic compounds (EPC) from different araçá genotypes, in which they found gallic acid and ellagic acid as the only compounds in both fractions.

Medina et al. (2011) found gallic acid as the major phenolic compound when investigating aqueous and acetone extracts of the specie *Psidium cattleianum* Sabine. Gallic acid also was found by us in PME, despite being in smaller quantities. However, Zandoná et al. (2020) quantified phenolic acids from araçá leaves extracted by aqueous infusion (Al), pressurized liquid extraction system with water (PLE-W), ethanol (PLE-E), and 1:1 water:ethanol ratio combination (PLE -W:E) and in their results found vanillic acid as the main constituent in all extracts tested, but values found for chlorogenic acid and gallic acid were lower than those found in this study.

Even with discrepancies in the values found in the chemical composition of each of the studies, it was possible to analyze which phenolic compounds were present in the extracts, in which the presence of these constituents allows for a strong antioxidant activity (Albuquerque et al. 2021). Our extracts showed antioxidant activity, in which EME had the highest antioxidant inhibition of 44.6%, 29.7%, 20.4% and 13.7%, while PME had the lowest antioxidant effect (15.7%, 10 .4%, 10.9% and 5.6%) in the respective concentrations of 1 $mg.mL^{-1}$, 0.5 $mg.mL^{-1}$, 0.25 $mg.mL^{-1}$ and 0.125 $mg.mL^{-1}$.

Extracts of *P. cattleianum* Sabine extracted with acetone rich in phenolic compounds showed a better antioxidant effect (DDPH technique), exhibiting percentages of inhibition ranging from 35.3% to 45.3% (red araçá) about the species with yellow color (ranging from 19.7% to 34.6%) (Medina et al. 2011). The hydroalcoholic extract of *P. cattleianum* Sabine leaves obtained the free radical scavenging capacity (IC_{50}=15.9 $\mu g.mL^{-1}$) obtained by the DPPH method (Alvarenga et al. 2013). Celli et al. (2011) analyzed in the extracts of fruits of the species of *E. uniflora* L. a decrease in the capture of the free radical DPPH in the two varieties of the fruit, depending on the stage of fruit development. These results agree with the results observed in the quantification of phenolic compounds by the same authors.

Bagetti et al. (2011) performed the DPPH sequestration method and observed *E. uniflora* L. methanolic extracts with purple coloration showed higher antioxidant activity than extracts prepared with red and orange fruits. In our work, we verified that there was a greater capture of the DPPH free radical in the tested concentrations of EME, when we correlated these values with those analyzed between the content of phenolic acids found, this suggests that there was greater antioxidant activity in the EME, the same results analyzed by Bagetti et al. (2011). Phenolic compounds can act by scavenging free radicals, in addition to inhibiting the growth of MDR pathogens (Toledo et al. 2023), as can be observed in our results of antibacterial activity (Table III) and in the time-kill results (Figure 1) reported here, as both extracts present activity against *A. baumannii* ATCC® 19606™ as well as the MDR isolates.

The ability of bacteria to form biofilms allows resistance to the microorganism to survive in the environment, even under adverse conditions (Jamal et al. 2018). The activity of EME and PME in inhibiting biofilm formation shows that both extracts present activity against the biofilm formation for *A. baumannii*. Based on the following criteria, inhibition values that are above 50% represent a good activity, on the other hand, if this inhibition is between 0 and 49%, it consists of a bad activity on the ability to inhibit biofilm formation (Famuyide et al. 2019, Sandasi & Leonard 2008). Thus, our results show that both extracts have good antibiofilm activity, with biofilm inhibition percentages ranging from 58.5% to 88%. Famuyide et al. (2019), testing extracts of the *Eugenia* species (*E. erythrophylla*, *E. natalitia*, *E. woodii, E. umtamvunensis,* and *E. zeyheri*) observed that they prevented biofilm formation for *P. aeruginosa* with values above 50%. On the other hand, only the extracts of *E. erythrophylla* and *E. natalitia* inhibited the biofilm formation of the Gram-negative bacterium *Escherichia coli*. Lakshmana et al. (2020) observed a reduction in biofilm formation when testing ethanolic extract of the *Psidium* genus (80.0%) against *Staphylococcus aureus*.

In our research, we observed that PME was not able to disrupt biofilm formed for *A. baumannii* ATCC® 19606™, and EME destroyed only 4% of the biofilm formed by this strain. In isolate Ab 02, the PME presented a disruptive activity (42%) when compared to the EME (34%). Famuyide et al. (2019) verified the effect of *Eugenia* genus extracts against *P. aeruginosa*, after 24 h and 48 h of pre-formed biofilm and reported that all extracts could not destroy the biofilm and only *E. natalitia* and *E. zeyheri* destroyed the biofilm formed by *E. coli* after 24 h.

Previous studies have found that inhibiting biofilm formation on surfaces is easier than eradicating pre-formed biofilms (Famuyide et al. 2019, Sandasi et al. 2011). The presence of an extracellular polymeric matrix allows a strong adherence of the pathogen to surfaces, protecting against the activity of antimicrobials and the activity of efflux pumps, which launch the drugs out of the cell (Jamal et al., 2018). However, it is believed that the inhibition of biofilm formation analyzed when testing our extracts must have occurred due to the presence of phenolic compounds in the chemical composition, as these provide an antibiofilm effect (Payne et al. 2013, Quave et al. 2012), and may present several mechanisms of activity, in which these constituents interrelate with bacterial proteins and cell wall structures, leading to the destruction of cytoplasmic membranes, reduction of membrane fluidity, inhibition of nucleic acid synthesis, cell wall synthesis or energy metabolism (Gyawali & Ibrahim 2014).

In conclusion, the results presented here suggest that EME and PME can be significant sources of natural antioxidants, antibacterial, and antibiofilm. Taken together, these findings indicate the potential of EME and PME as promising agents against a human pathogenic bacterium and its biofilm, thus denoting the need for further studies *in vivo* conducted to evaluate the safety and effectiveness of EME and PME as an alternative to control of *A. baumannii*.

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6 AUTHOR CONTRIBUTIONS

Garcia MO and Hartwig DD provided study design, study guidance, data analysis, and critical manuscript editing. Allend SO, Cunha KFda, and Seixas Neto ACP contributed to the study design, carrying out experiments, and data analysis. Santi IIde, Rodrigues MHP, and Furlong EB carried out experiments and analyzed data. All authors contributed in part to writing and editing the manuscript and approved the final version.

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FIGURES SESSION

Figure 1. Time-kill curve for *A. baumannii* ATCC® 19606™ (a) and isolate Ab 02 (b). Treatment with 1× MIC of EME (ATCC® 19606™ = 9.2 μg.mL^{-1} and Ab 02 = 18.5 μg.mL^{-1}) and 1× MIC of PME (ATCC® 19606™ = 18.5 μg.mL^{-1} and Ab 02 = 36.9 μg.mL^{-1}). The standard curve represents the growth of the untreated bacteria.

Figure 2. Antibiofilm activity of EME and PME (1× MIC) against *A. baumannii* ATCC® 19606™ and isolate Ab 02. Biofilm inhibition and disruption values are present in percentage (a and c) and optical density (OD_{540nm}) (b and d). Data are expressed as means ± SD from three independent experiments, analyzed by two-way ANOVA followed by Tukey's multiple comparison test. (*p<0.05 compared to control untreated).

TABLES SESSION

Table I. Calibration parameters for the determination of phenolic acids by HPLC-UV* in extracts of *P. cattleianum* Sabine and *Eugenia uniflora* L.

Table II. Chemical composition of *E. uniflora* L. and *P. cattleianum* Sabine methanolic extracts obtained by HPLC-UV.

Table III. MIC and MBC of EME and PME against standard strains of *A. baumannii* ATCC® 19606™ and MDR isolates.

CONSIDERAÇÕES FINAIS

- OEE e OEP apresentam compostos terpenoides em sua composição química, enquanto EME e PME apresentam ácidos fenólicos;
- Ambos os OE testados apresentaram baixo efeito antioxidante. Já entre os extratos, o PME apresentou capacidade antioxidante em todas as concentrações testadas;
- No ensaio de citotoxicidade em células VERO foi possível analisar que quanto menores as concentrações do OE, mais alta foi a viabilidade celular. O OEP foi o que apresentou o menor valor de IC_{50} em relação ao OEE, sendo menos tóxicos para as células de mamíferos;
- Quanto a atividade antibacteriana o OEP mostrou-se bactericida contra *A. baumannii* ATCC® 19606™ e bacteriostática frente a *K. pneumoniae* ATCC® 700603™. Já para os extratos, o EME foi o mais potente contra a cepa padrão de *A. baumannii*, como também para a maioria dos isolados, nos quais mostrou efeito bactericida;
- O OEP apresentou os maiores valores de SI, que indica o quanto um composto é ativo contra o micro-organismo sem causar dados à viabilidade das células de mamíferos;
- Quanto a atividade antibiofilme, o PME foi mais ativo contra *A. baumannii* ATCC® 19606™ quando comparada ao EME. O PME apresentou uma porcentagem de inibição de biofilme de 88% e EME de 58,5%;
- A porcentagem de inibição do biofilme foi de 88% para ambos os extratos, EME e PME, quando avaliado frente ao isolado Ab 02;
- Em relação à atividade de ruptura do biofilme na cepa padrão *A. baumannii* ATCC® 19606™, o EME e PME não apresentaram percentual de inibição. Já no isolado Ab 02, EME rompeu 34% do biofilme formado e PME 42%;
- *A. baumannii* ATCC® 19606™ e o isolado Ab 02 levaram respectivamente, 6 horas e 10 minutos, para serem eliminados após tratamento com ambos extratos de EME e PME;

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ANEXOS

Anexo A – Patente

INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

07/04/2022 870220030233 17:14

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Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 10 2022 006745 7

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PETICIONAMENTO ELETRÔNICO

Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 07/04/2022 às 17:14, Petição 870220030233

Petição 870220030233, de 07/04/2022, pág. 1/26

Dados do Pedido

Natureza Patente: 10 - Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade (54): USO DO EXTRATO DE Eugenia uniflora L. (PITANGA) NA PREPARAÇÃO DE MEDICAMENTO ANTIBIÓTICO E ANTIBIOFILME PARA TRATAR INFECÇÕES CAUSADAS POR Acinetobacter baumannii MULTIRRESISTENTE

Resumo: A presente invenção traz o uso do extrato de Eugenia uniflora L. (pitanga) na preparação de medicamento de ação antibiótica e antibiofilme para tratar infecções causadas por Acinetobacter baumannii resistente à múltiplas drogas. A. baumannii é um patógeno de importância clínica, frequentemente associado a infecções relacionadas à assistência em saúde (IRAS), que apresenta resistência a múltiplas drogas e capacidade de formação de biofilme, limitando e dificultando a terapêutica. Esse fato justifica a busca por novas alternativas capazes de tratar as infecções causadas por este patógeno. O extrato obtido de frutos da espécie Eugenia uniflora L. (Pitanga), possui efeito antibiótico e antibiofilme frente a A. baumannii multirresistente, sendo uma opção para uso em medicamentos para tratar infecções causadas por este patógeno.

Figura a publicar: 1

PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 07/04/2022 às 17:14, Petição 870220030233

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Documentos anexados

Tipo Anexo	Nome
Comprovante de pagamento de GRU 200	Comprovante.pdf
Resumo	RESUMO.pdf
Reivindicação	REIVINDICACOES.pdf
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Desenho	FIGURAS.pdf
Procuração	PROCURAÇÃO_DIGITAL.pdf

Acesso ao Patrimônio Genético

☑ Declaração Positiva de Acesso - Declaro que o objeto do presente pedido de patente de invenção foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético Brasileiro, realizado a partir de 30 de junho de 2000, e que foram cumpridas as determinações da Lei 13.123 de 20 de maio de 2015, informando ainda:

Número da Autorização de Acesso: A50A1A0
Data da Autorização de Acesso: 15/04/2020

Declaração de veracidade

☑ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.

PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 07/04/2022 às 17:14, Petição 870220030233