

Comparative study of the chemical composition, larvicidal, antimicrobial and cytotoxic activities of volatile oils from *E. puniceifolia* leaves from Minas Gerais and Goiás

Estudo comparativo da composição química, atividade larvicida, antimicrobiana e citotóxica dos óleos voláteis das folhas de *E. puniceifolia* de Minas Gerais e Goiás

Estudio comparativo de la composición química, actividades larvicidas, antimicrobianas y citotóxicas de aceites volátiles de hojas de *E. puniceifolia* de Minas Gerais y Goiás

Received: 08/12/2021 | Reviewed: 08/17/2021 | Accept: 08/19/2021 | Published: 08/22/2021

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Abstract

Eugenia puniceifolia (Kunth) D.C., Myrtaceae, known as “pedra-ume-caá”, is popularly used in the treatment of inflammation, infections, fever, flu, diabetes, and diarrhea. This study aimed to carry out a comparative study of the

chemical composition of volatile oil from *E. punicifolia* leaves collected in Goiás and Minas Gerais, as well as to evaluate the larvicidal activity against *Aedes aegypti* L3 larvae, the antimicrobial activity against bacteria, pathogenic fungi, and environmental, and cytotoxic activity to Balb 3T3 cells (murine fibroblasts). Volatile oils were obtained by hydrodistillation in a Clevenger apparatus and analyzed by Gas Chromatography Coupled to Mass Spectrometry (CG/MS). A total of 60 compounds were identified, the main components found in the leaves of Goiás being Germacrene D, bicyclogermacrene and β -longipenene and in the leaves collected in Minas Gerais they were (Z)-caryophyllene, γ -cadinene, spathulenol, caryophyllene oxide, and α -cadinol. The larvicidal effect was moderate against *Ae. aegypti*, with LC₅₀ of 85.53 $\mu\text{g} / \text{mL}$ for samples from Goiás and LC₅₀ of 91.52 $\mu\text{g} / \text{mL}$ for samples from Minas Gerais. Both oils showed moderate bactericidal activity against *K. rhizophyla* (ATCC 9341), *M. luteus* (ATCC 10240), and *S. aureus* (ATCC 29737). The oils from Goiás (IC₅₀ 706.7 $\mu\text{g} / \text{mL}$) and Minas Gerais (IC₅₀ 160.7 $\mu\text{g} / \text{mL}$) had a lower cytotoxic concentration than the toxic action for larvae and bacteria, evidencing a safety profile and an interesting therapeutic potential, mainly concerning to volatile oil from Goiás. Therefore, the volatile oils from *E. punicifolia* leaves collected in Goiás and Minas Gerais that presented moderate larvicidal activity for *Ae. aegypti* also presented a bactericide activity and less cytotoxicity against murine fibroblasts. This is the first study of the larvicidal, antimicrobial and cytotoxic activity of volatile oils from *E. punicifolia* leaves.

Keywords: Essential oil; Chemodiversity; Medicinal plant; Biological activities; Myrtaceae.

Resumo

Eugenia punicifolia (Kunth) D.C., Myrtaceae, conhecida como “pedra-ume-caá”, é popularmente utilizada no tratamento de inflamações, infecções, febre, gripe, diabetes e diarreia. O objetivo deste trabalho foi realizar um estudo comparativo da composição química do óleo volátil das folhas de *E. punicifolia* coletadas em Goiás e Minas Gerais, bem como avaliar a atividade larvicida contra larvas L3 de *Aedes aegypti*, a atividade antimicrobiana contra bactérias, fungos patogênicos e ambientais, e atividade citotóxica para células Balb 3T3 (fibroblastos murinos). Os óleos voláteis foram obtidos por hidrodestilação em aparelho de Clevenger e analisados por Cromatografia Gasosa Acoplada à Espectrometria de Massa (CG/EM). Foram identificados 60 compostos, sendo os principais componentes encontrados nas folhas de Goiás o Germacrene D, o bicyclogermacrene e o β -longipeneno e nas folhas coletadas em Minas Gerais foram (Z)-cariofileno, γ -cadineno, espatulenol, óxido de cariofileno e α -cadinol. O efeito larvicida foi moderado contra *Ae. aegypti*, com CL₅₀ de 85,53 $\mu\text{g}/\text{mL}$ para amostras goianas e CL₅₀ de 91,52 $\mu\text{g}/\text{mL}$ para amostras mineiras. Ambos os óleos apresentaram potencial bactericida moderado contra *K. rhizophyla* (ATCC 9341), *M. luteus* (ATCC 10240) e *S. aureus* (ATCC 29737). Os óleos de Goiás (IC₅₀ 706,7 $\mu\text{g} / \text{mL}$) e Minas Gerais (IC₅₀ 160,7 $\mu\text{g}/\text{mL}$) apresentaram menor concentração citotóxica do que a ação tóxica para larvas e bactérias, o que pode sugerir um perfil de segurança e potencial terapêutico interessante, principalmente no que diz respeito ao óleo volátil de Goiás. Portanto, os óleos voláteis das folhas de *E. punicifolia* coletadas em Goiás e Minas Gerais apresentaram moderado potencial larvicida para *Ae. Aegypti*, atividade bactericida e menor citotoxicidade contra fibroblastos murinos. Este é o primeiro estudo da atividade larvicida, antimicrobiana e citotóxica de óleos voláteis de folhas de *E. punicifolia*.

Palavras-chave: Óleo essencial; Quimiodiversidade; Planta medicinal; Atividades biológicas, Myrtaceae.

Resumen

Eugenia punicifolia (Kunth) D.C., Myrtaceae, conocida como “pedra-ume-caá”, se usa popularmente en el tratamiento de inflamación, infecciones, fiebre, gripe, diabetes y diarrea. El objetivo de este trabajo fue realizar un estudio comparativo de la composición química del aceite volátil de hojas de *E. punicifolia* recolectadas en Goiás y Minas Gerais, así como evaluar la actividad larvicida frente a larvas de *Aedes aegypti* L3, la actividad antimicrobiana frente a bacterias, hongos patógenos y ambientales, y actividad citotóxica para las células Balb 3T3 (fibroblastos murinos). Los aceites volátiles se obtuvieron por hidrodestilación en un aparato Clevenger y se analizaron mediante cromatografía de gases acoplada a espectrometría de masas (CG MS). Se identificaron un total de 60 compuestos, siendo los principales componentes encontrados en las hojas de Goiás Germacrene D, bicyclogermacrene y β -longipenene y en las hojas recolectadas en Minas Gerais fueron (Z)-cariofileno, γ -cadineno, spathulenol, óxido de cariofileno, y α -cadinol. El efecto larvicida fue moderado contra *Ae. aegypti*, con CL₅₀ de 85,53 $\mu\text{g} / \text{mL}$ para muestras de Goiás y CL₅₀ de 91,52 $\mu\text{g}/\text{mL}$ para muestras de Minas Gerais. Ambos aceites mostraron un potencial bactericida moderado contra *K. rhizophyla* (ATCC 9341), *M. luteus* (ATCC 10240) y *S. aureus* (ATCC 29737). En cuanto a la citotoxicidad, los aceites de Goiás (IC₅₀ 706,7 $\mu\text{g} / \text{mL}$) y Minas Gerais (IC₅₀ 160,7 $\mu\text{g} / \text{mL}$) presentaron una concentración citotóxica menor que la acción tóxica para larvas y bacterias, evidenciando un perfil de seguridad y un interesante potencial terapéutico, principalmente con respecto al aceite volátil de Goiás. Por lo tanto, los aceites volátiles de las hojas de *E. punicifolia* recolectadas en Goiás y Minas Gerais presentaron un potencial larvicida moderado para *Ae. aegypti*, actividad bactericida y menor citotoxicidad frente a fibroblastos murinos. Este es el primer estudio de la actividad larvicida, antimicrobiana y citotóxica de los aceites volátiles de las hojas de *E. punicifolia*.

Palabras clave: Aceite esencial; Quimiodiversidad; Planta medicinal; Actividades biológicas; Myrtaceae.

1. Introduction

Eugenia puniceifolia (Kunth) DC, Myrtaceae, known as Pedra-ume-Caá, Myrtle, Red myrtle, Pitanga-do-campo, is found widely distributed in the Amazon, Cerrado, Caatinga, Atlantic Forest, and Pantanal (Sobral, et al., 2015). It is a shrub with a yellow cylindrical stem with light spots, the leaves are elliptical or opposite lanceolate and petiolate, the flowers are arranged in white panicles. It has ripe fruits that are simple, fleshy, with an intense red color with a glabrous and shiny surface, astringent, indehiscent flavor, with an obovate to an elliptical shape, with two or three seeds (Martins, 1989; Coneglian, 2007; Senra, 2012) Anatomically, it presents secretory cavities with diffuse distribution both on the leaf surface and on the petiole (Lemos, et al., 2019).

The leaves are popularly used in the form of a decoction or aqueous infusion to treat inflammation, fever, flu, diabetes, in alcoholic infusions for the treatment of wounds and infectious diseases, diarrhea, stomach disorders, and as a hypoglycemic (Oliveira, et al., 2005; Milk, et al., 2010; Basting, et al., 2014, Pascual, et al., 2012).

In the Myrtaceae family, there are several species with larvicidal potential for *Aedes aegypti*. Those with $CL_{50} \leq 50$ $\mu\text{g/mL}$ are considered active, such as essential oil of *Psidium guajava* L. (Lima, et al., 2011), *Eucalyptus maculata* Hook (Sarma, et al., 2019), *Baeckea frutescens* L., *Callistemon citrinus* (Crimson Bottlebrush), *Melaleuca leucadendra* (L.) L., *Syzygium nervosa* A. Cunn. ex DC. (An, et al., 2020). Others are classified as having moderate activity, LC_{50} between 50 and 100 $\mu\text{g/mL}$, such as *Syzygium zeylanicum* (L.) DC., *Eucalyptus nitens* Maiden (Govindarajan & Benelli, 2016), *Psidium guajava* L. (Mendes, et al., 2017), *Callistemon linearis* (Schrad. & JCWendl.) Colvill ex Sweet (Sarma, et al., 2019).

Studies evaluating the toxic effect of volatile oil from species of the genus *Eugenia* against L3 larvae of *Ae. aegypti* were verified in the researched literature. The volatile oils from *Eugenia triquetra* O. Berg showed larvicidal activity with a LC_{50} of 64.8 ± 5.6 ppm (Mora, et al., 2010), from *Eugenia patrisii* Vahl showed larvicidal with an IC_{50} of 417 $\mu\text{g/mL}$, *Eugenia piauhiensis* Vellaff. with IC_{50} of 230 $\mu\text{g/mL}$ (Dias, 2013; Dias, et al., 2015), *Eugenia brejoensis* Mazine with LC_{50} of 214.7 ppm, (Silva, et al., 2015), *Eugenia candollena* DC with LC_{50} of 300 $\mu\text{g/mL}$ (Neves, et al., 2017), *Eugenia calycina* Cambess with LC_{50} of 199.3 $\mu\text{g/mL}$ (Silva, 2018), *Eugenia calycina* LC_{50} in 24 and 48 h of 199.3 and 166.4 $\mu\text{g/mL}$ (Silva, et al., 2021). No studies on the larvicidal activity of *E. puniceifolia* were found.

Regarding antimicrobial studies, Araújo (2011) found that the methanol extract of *E. puniceifolia* leaves, collected in Maracanã-Pará, inhibited *Candida albicans* and *Candida parapsilosis* (MIC = 0.625 mg/mL), *S. aureus*, and *S. cereus* (MIC = 0.312 mg/mL). Silva et al. (2020) concluded that the hydroalcoholic extract of *E. puniceifolia* stems bark, collected in Altinho - Pernambuco, was fungistatic for *Cryptococcus neoformans* (MIC= 0.030 $\mu\text{g}/\mu\text{l}$) and fungicide for *Cryptococcus gattii* (MIC = 0.312 $\mu\text{g}/\mu\text{l}$). There are no reports in the researched literature on the evaluation of the antimicrobial activity of the volatile oil of *E. puniceifolia*.

The aim of this study was to carry out a comparative study of the chemical composition of volatile oil from *E. puniceifolia* leaves collected in Minas Gerais and Goiás, as well as to evaluate the larvicidal activity against L3 larvae of *Aedes aegypti*, antimicrobial activity against fungi, and bacteria (pathogenic and environmental), and cytotoxic activity to Balb 3T3 cells (murine fibroblasts).

2. Plant material

2.1 Collect botanical material

The leaves of 10 individuals of *Eugenia puniceifolia* (Kunth) D.C, Myrtaceae, were collected in the morning, in São Gonçalo do Abatê – Minas Gerais (MG) in September 2017, and in Hidrolândia – Goiás (GO) in October 2017. The species was identified by Prof. Dr. José Realino de Paula, an exsiccate was prepared and deposited in the Herbarium of the Federal University of Goiás under the number UFG-48579. The leaves were dried in a hot air oven at approximately 38° C.

2.2 Volatile oil extraction and GC-MS analysis

To extract the volatile oil, the dried botanical material (leaves) from the two regions was ground separately, immediately before each extraction, in a Poli® industrial blender (model LS-08MBR-N), submitted to hydrodistillation in a Clevenger-type apparatus for 3 hours. The volatile oils obtained were desiccated with Na₂SO₄, placed, and stored in a freezer at -22° C until use.

The volatile oils were submitted to chromatographic analysis, in the gas phase, coupled to mass spectrometry (CG/MS) in a Shimadzu GC-MS QP2010A apparatus, with a silica capillary column SBD-5 (30 m×0.25 mm ID, 0.25 m film thickness) (composed of 5% phenyl methylpolysiloxane) and programmed temperature as follows: 60-240 °C at 3 °C/min, then at 280 °C at 10 °C/min, ending with 10 min at 280 °C, with carrier gas with a flow rate of 1 ml/min and split mode at a ratio of 1:20 and the injection port set to 225°C. Operating parameters of the significant quadripolar mass spectrometer: interface temperature 240°C; Electron impact ionization at 70 eV with scan mass range 40-350 m/z at a sampling rate of 1 scan/s. The chemical constituents of the volatile oil were identified by comparing the mass spectra and retention indices with those reported in the literature for the most common components of volatile oils (Adams, 2007). Retention indices were calculated by co-injecting a mixture of hydrocarbons, C₈ - C₃₂, and using the Van Den Dool & Kratz equation (Dool & Kratz, 1963; Adams, 2007).

2.3 Larvicidal activity

The larvicidal tests were performed at the Insect Biology and Physiology Laboratory (IPTSP/UFG) in a biological chamber with a temperature of 25 °C ± 1 °C, relative humidity of 85% ± 5% and a 12-hour photophase (Silva et al., 2003). The tests were carried out in serially decreasing dilutions from 100 to 20 ppm, in 50 mL polystyrene containers containing 25 mL of solution and twenty L3 larvae of *Ae. Aegypti* and later, the mortality events were quantified after 24 hours of exposure, with all tests being performed in triplicate, and having water and surfactant with negative controls. To assess larvicidal activity, the following criteria were used: LC₅₀ ≤ 50 µg / mL is considered active, between 50 and 100 µg / mL, moderate activity, between 100 and 750 µg / mL of efficient or effective activity, and LC₅₀ ≤ 750 µg / mL, inactive (Komalamisra, et al., 2005, Neves, et al., 2017; Silva, et al., 2021).

2.4 Antimicrobial activity

The volatile oils of the leaves were tested against 14 strains of bacteria: *Bacillus cereus* (ATCC 14579), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Escherichia coli* (ATCC 8739), *Kocuria rhizophila* (ATCC 9341), *Micrococcus luteus* (ATCC 10240), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), *Salmonella sp.* (ATCC 19430), *Salmonella sp.* (ATCC 14028), *Enterobacter aerogenes* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 70063) and 14 fungal strains: *Aspergillus brasiliensis* (ATCC 16404), *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida glabrata* (ATCC 90050), *Candida*

krusei (ATCC 34135), *Candida krusei* (ATCC 6258), *Candida metaparapsilosis* (ATCC 96143), *C. ortoparasilosis* (ATCC 96141), *Candida parapsilosis* (ATCC 22019), *Candida tropicalis* (ATCC 750), *Cryptococcus gattii* (ATCC 24065), *Cryptococcus neoformans* (ATCC 28957), *Cryptococcus neoformans* (ATCC 90112) and *Saccharomyces cerevisiae* (ATCC 9763).

The antibacterial assay was done by microdilution method (Clinical and Laboratory Standards Institute M7A6 [CLSI], 2012) utilizing 96-well microtiter plates to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The serial concentrations varying from 1024 to 2 µg/mL. The bacterial suspensions were adjusted with Mueller Hinton medium in the concentration of 1.0×10^5 CFU/mL. *E. puniceifolia* volatile oils were dissolved in a 1% dimethyl sulfoxide solution (Merck KGaA, Germany) containing 0.1% polysorbate-80 starting from 2048 µg/mL in Mueller Hinton broth. (100 µL) medium with bacterial inoculum (1.0×10^5 CFU/well) was mixed in the same volume with 10 concentrations of volatile oil varying from 2048 µg/mL to 4 µg/mL. The microplates were incubated for 24 h, at 37° C. The lowest concentration without visible microbial biomass growth under the optical microscope was defined as the concentrations that completely inhibited bacterial growth. Minimum bactericidal concentration was determined by 20 µL serial subcultivation in microtiter plates containing Agar Mueller Hinton for 24 h. The lowest concentration without visible microbial growth was defined as MBC, indicating the death of 99.5% of the original inoculum. Streptomycin (Sigma P7794) and ampicillin (Panfarma, Belgrade, Serbia) were utilized as positive controls (1 mg/mL in sterile saline solution). A solution of 1% dimethyl sulfoxide was utilized as a negative control. Tests were done in triplicate.

The classification for antimicrobial activity criteria were MIC < 100 µg/mL (good antimicrobial activity); MIC between 100-500 µg/mL (moderate antimicrobial activity); MIC between 500-1000 µg/mL (weak antimicrobial activity), and MIC above 1000 µg/mL (inactive) (Holetz, et al., 2002).

2.5 Cytotoxicity assay by neutral red dye incorporation method

Initially, Balb/c 3T3 cells (murine fibroblasts) were distributed in 96-well plates at a density of 3×10^3 cells/100 µL/well, so that the peripheral wells were filled with culture medium, being cultured for 24 hours for adhesion. Then, the culture medium was removed from the plates and 50 µL of complete medium was added to each well. Subsequently, the oils from the leaves collected in Goiás and Minas Gerais were prepared at a concentration twice the desired concentrations and added to the corresponding wells. In parallel, another plate was also prepared separately, in which the cells were exposed to 8 different concentrations of Sodium Lauryl Sulfate (SLS) (CASRN 151-21-3), covering the range from 100 to 6.8 µg/mL, as per described in protocol N°. 129 of the OECD (2010).

$$\text{Cell viability (\%)} = \frac{(\text{Average ABS corresponding to each concentration} - B)}{(\text{Negative control ABS} - \text{Blank corresponding to the negative control})}$$

After calculating the cell viability values, the 50% inhibitory concentration of cell growth (IC₅₀) was determined for the substance evaluated and for the positive control (SDS), so that the LD₅₀ value was estimated using the equation described in Protocol N°. 129 of the OECD:

$$\log \text{LD}_{50} \text{ (mg/kg)} = 0.372 \log \text{IC}_{50} \text{ (}\mu\text{g/mL)} + 2.024 \text{ (ICCVAM, 2006)}$$

According to the literature, volatile oils with CC₅₀ < 10 µg/mL are highly toxic, > 10 < 100 µg/mL are toxic, > 100 < 1000 µg/mL moderately toxic and > 1000 µg/mL non-toxic (Lima et al., 2012; Andrade et al., 2018; Fernandes et al., 2021a).

2.6 Statistical analysis

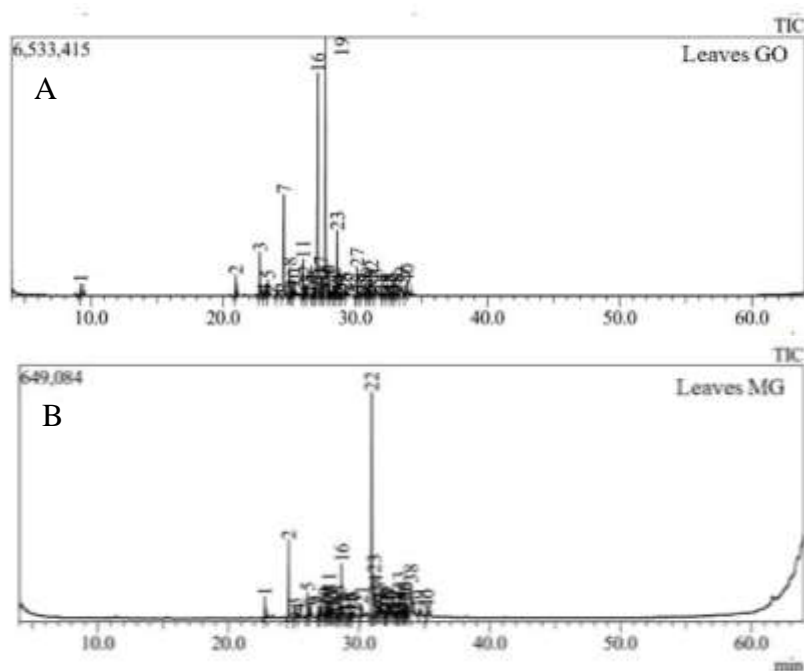
The results were expressed as Mean \pm Standard Deviation of the viability inherent to each concentration evaluated, and the mean of three independent experiments, carried out in sextuplicate, was analyzed. IC₅₀ values were obtained through non-linear regression, and analyzes were conducted using the GraphPad Prism 5.0 software.

3. Results

3.1 Volatile oils

The yields of volatile oils from leaves collected in Hidrolândia-GO and São Gonçalo do Abatê-MG were 0.21, and 0.26%, respectively. Through GC-MS analysis (Figure 1), 42 (99.66%) compounds were identified in volatile oil from Goiás samples and 38 (97.05%) compounds in volatile oil from Minas Gerais samples (Table 1).

Figure 1. Chromatograms of volatile oils from *E. puniceifolia* leaves collected in Hidrolândia - Goiás (A) and São Gonçalo do Abatê - Minas Gerais (B).



Source: Authors.

In volatile oil from GO, 89.23% of sesquiterpene hydrocarbons, 9.19% of oxygenated sesquiterpenes hydrocarbons and 1.24% of monoterpene hydrocarbons were identified. As for the volatile MG oil, 59.46% oxygenated sesquiterpenes and 37.59% of sesquiterpenes hydrocarbons were found (Table 1).

Table 1. Chemical constituents of volatile oils from *E. puniceifolia* leaves collected in Hidrolândia-GO and São Gonçalo do Abatê-MG.

Compound	KI	Goiás	Minas Gerais
(Z) β -ocinene	1032	0.15	-
(E) β -ocinene	1044	1.09	-
δ -elemene	1335	1.74	-
Isoledene	1374	-	2.47
α -Ylangene	1373	3.71	-
β -bourbonene	1387	0.20	-
β -cubebene	1387	0.13	-
β -elemene	1389	1.31	-
sibirene	1400	0.18	-
β -longipinene	1400	9.42	-
(Z)-caryophyllene	1408	-	8.92
β -caryophyllene	1417	-	1.41
α -copaene	1430	-	0.33
γ -elemene	1434	3.09	-
α -guaiene	1437	0.58	-
aromadendrene	1439	0.54	-
α -humulene	1452	3.20	2.72
allo aromadendrene	1458	0.96	1.05
Trans muurola3.5-diene	1451	0.16	-
(E) 9-epi caryophyllene	1464	0.21	1.38
γ -muurolene	1478	0.69	-
germacrene D	1484	21.34	1.62
β -selinene	1489	1.94	-
cis β -guaine	1492	1.28	-
viridiflorene	1496	1.17	-
γ -amorphene	1995	-	1.29
bicyclogermacrene	1500	26.73	2.99
α -muurolene	1500	-	0.84
(E,E) α -farnesene	1505	0.50	0.55
α -bulnesene	1509	-	0.58
δ -amorphene	1511	5.99	1.43
γ -cadinene	1513	0.76	6.27
δ -cadinene	1522	-	0.54
zonarene	1528	0.17	-
γ -cuprenene	1532	-	0.58
Trans-cadina 1,4-diene	1533	0.37	-
α -cadinene	1537	0.28	0.57
α -calacorene	1544	0.11	0.69
Germacrene B	1559	2.47	1.36
palustrol	1567	0.26	-
Spathulenol	1577	0.78	26.84

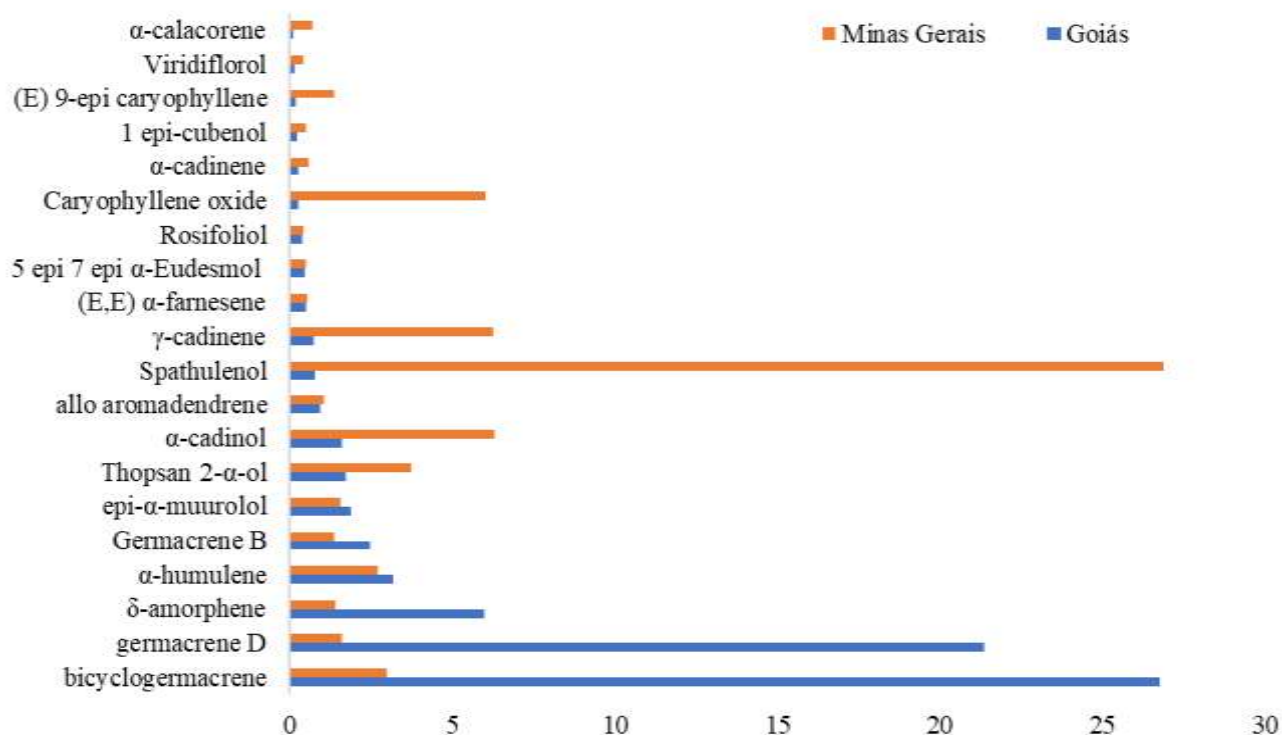
Caryophyllene oxide	1582	0.29	6.02
Thopsan 2-α-ol	1586	1.71	3.74
Globulol	1590	1.26	-
Viridiflorol	1592	0.15	0.41
Salvial-4(14)-em-1-one	1593	-	1.9
cubeban-11-ol	1595	-	0.52
Rosifoliol	1600	0.40	0.42
5 epi 7 epi-α-Eudesmol	1607	0.45	0.49
humulene epoxide II	1608	-	1.56
1 epi-cubenol	1627	0.23	0.50
Cis-Cadin 4-em-7-ol	1635	0.18	-
allo aromadendrene epoxide	1639	-	3.41
Caryophylla 4(12),8(13) dien 5α-ol	1639	-	0.85
epi-α-cadinol	1638	-	1.78
epi-α-muurolol	1640	1.87	1.56
α-muurolol	1644	-	1.44
α-cadinol	1652	1.61	6.28
cadalene	1675	-	0.62
eudesma-4(15),7dien-1β-ol	1687	-	1.12
Monoterpenes hydrocarbons		1.24	0.00
Sesquiterpenes hydrocarbons		89.23	37.59
Oxygenated Sesquiterpenes hydrocarbons		9.19	59.46
Total identified		99.66	97.05

Source: Authors.

The major compounds present in Goiás leaves were bicyclogermacrene (26.73%), germacrene D (21.34%), β -longipinene (9.42%), and δ -amorphene (5.99%). In the volatile MG oil were spathulenol (24.53%), (Z)-caryophyllene (8.92%), α -cadinol (6.28%), and γ -cadinene (6.27%) and caryophyllene oxide (5.57%).

Both oils share 20 equal compounds (Figure 2), but in different concentrations according to the place of collection, such as bicyclogermacrene which in Goiás has 26.73% and Minas Gerais 2.99%, germacrene D with 21.34 % and 1.62%, respectively, and Spathulenol with 0.78% and 26.84%.

Figure 2. Ratio of compounds that both oils share.



Source: Authors.

3.2 Antimicrobial activity

The volatile oils from the leaves showed moderate bactericidal potential against *K. rhizophyla* (ATCC 9341), *M. luteus* (ATCC 10240), and *S. aureus* (ATCC 29737), with the volatile oil from the leaves of Minas Gerais with a MIC of 256 $\mu\text{g/mL}$ for the three strains. The volatile oil from Goiás had a MIC of 128 $\mu\text{g/mL}$ for *K. rhizophyla*, and 256 $\mu\text{g/mL}$ for *M. luteus* (Table 2). Antifungal activity was not verified.

Table 2. Evaluation of the *in vitro* antibacterial activity of volatile oil from *E. puniceifolia* leaves collected in Goiás and Minas Gerais.

Bacteria	Goiás MIC / MBC (µg/mL)	Minas Gerais MIC / MBC (µg/mL)
Gram positive		
<i>Bacillus cereus</i> ATCC 14579	>1024 (>1024)	>1024 (>1024)
<i>Bacillus subtilis</i> ATCC 6633	>1024 (>1024)	>1024 (>1024)
<i>Kocuria rhizophyla</i> ATCC 9341	128 (>1024)	256 (>1024)
<i>Micrococcus luteus</i> ATCC 10240	256 (>1024)	256 (>1024)
<i>Staphylococcus aureus</i> ATCC 25923	>1024 (>1024)	>1024 (>1024)
<i>Staphylococcus aureus</i> ATCC 29737	>1024 (>1024)	256 (>1024)
<i>Staphylococcus aureus</i> ATCC 6538	>1024 (>1024)	>1024 (>1024)
<i>Staphylococcus aureus</i> ATCC 29213	>1024 (>1024)	>1024 (>1024)
<i>Staphylococcus epidermidis</i> ATCC 12228	>1024 (>1024)	>1024 (>1024)
Gram negative		
<i>Escherichia coli</i> ATCC 25922	>1024 (>1024)	>1024 (>1024)
<i>Escherichia coli</i> ATCC 8739	>1024 (>1024)	>1024 (>1024)
<i>Pseudomonas aeruginosa</i> ATCC 27853	>1024 (>1024)	>1024 (>1024)
<i>Salmonella</i> sp. ATCC 19430	>1024 (>1024)	>1024 (>1024)
<i>Salmonella</i> sp. ATCC 14028	>1024 (>1024)	>1024 (>1024)
<i>Enterobacter aerogenes</i> ATCC 13048	>1024 (>1024)	>1024 (>1024)
<i>Klebsiella pneumoniae</i> ATCC 70063	>1024 (>1024)	>1024 (>1024)
Fungi	CIM / CFM (µg/mL)	CIM / CFM (µg/mL)
<i>Aspergillus brasiliensis</i> ATCC 16404	>1024 (>1024)	>1024 (>1024)
<i>Candida albicans</i> ATCC 90028	>1024 (>1024)	>1024 (>1024)
<i>Candida glabrata</i> ATCC 90030	>1024 (>1024)	>1024 (>1024)
<i>Candida glabrata</i> ATCC 90050	>1024 (>1024)	>1024 (>1024)
<i>Candida krusei</i> ATCC 34135	>1024 (>1024)	>1024 (>1024)
<i>Candida krusei</i> ATCC 6258	>1024 (>1024)	>1024 (>1024)
<i>Candida metaparapsilosis</i> ATCC 96143	>1024 (>1024)	>1024 (>1024)
<i>Candida ortoparasilosis</i> ATCC 96141	>1024 (>1024)	>1024 (>1024)
<i>Candida parasilosis</i> ATCC 22019	>1024 (>1024)	>1024 (>1024)
<i>Candida. tropicalis</i> ATCC 750	>1024 (>1024)	>1024 (>1024)
<i>Cryptococcus gatti</i> ATCC 24065	>1024 (>1024)	>1024 (>1024)
<i>Cryptococcus neoformans</i> ATCC 28957	>1024 (>1024)	>1024 (>1024)
<i>Cryptococcus neoformans</i> ATCC 90112	>1024 (>1024)	>1024 (>1024)
<i>Saccharomyces cerevisiae</i> ATCC 9763	>1024 (>1024)	>1024 (>1024)

MIC (Minimum Inhibitory Concentration) and a - MBC (Minimum Bactericidal Concentration). Source: Authors.

3.3 Larvicidal Activity

As for the toxicity of the volatile oil to *Ae. aegypti* in the third stage, it was possible to observe that the samples of volatile oils from the leaves collected in Goiás had moderate larvicidal activity against *Aedes* larvae, presenting an LC₅₀ of 85.53 ± 5.32 µg/mL and LC₉₀ of 129.60 ± 9.85 µg/mL. The volatile oil from Minas Gerais had a LC₅₀ of 91.52 ± 6.24 µg/mL and a LC₉₀ of 123.86 ± 10.88 µg/mL. Values were obtained through non-linear regression, with confidence intervals ($\alpha = 0.05$).

3.4 Cytotoxicity assay

At the concentrations tested, volatile oils showed moderate cytotoxicity against normal fibroblasts (Balb/c 3T3) with IC₅₀ 160.7 µg/mL for volatile oil from Minas Gerais and IC₅₀ 706.7 µg/mL for Goiás compared to the positive control, Lauril Sodium Lauryl Sulfate (SLS), which is toxic with an IC₅₀ of 37.08 µg/mL (Table 3 and 4).

According to the Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests (OECD, 2010), for the assay to be considered acceptable, at least one of the evaluated concentrations must present cytotoxicity > 0 % and ≤ 50% of cell viability. Oil from Goiás showed cell viability ≤ 50% at concentrations below 0.18 mg/mL and that from Minas Gerais at concentrations below 0.37 mg/mL. Given these parameters, it was verified that the dose-response curves (Table 4 and Figure 3) have values that are following the acceptability criteria.

Table 3. Cell viability values obtained after 48 hours of exposure of Balb/c 3T3 cells to the substance, obtained using the neutral red incorporation method as described in OECD Protocol No. 129. The estimated IC₅₀ values for the test substance are also described.

Concentration (mg/mL)	Cell viability (%)	IC ₅₀ (µg/mL)	Cell viability (%)	IC ₅₀ (µg/mL)
	Minas Gerais	Minas Gerais	Goiás	Goiás
5.00	0.0±0.0		0.1±0.1	
2.50	0.0±0.0		1.5±0.32	
1.25	0.23±0.02		20.17±4.26	
0.75	12.9±0.21	160.7	40.2±6.07	706.7
0.37	29.1±1.51		65.23±3.18	
0.18	51.38±1.87		87.7±1.15	
0.09	70.66±1.94		94.7±1.05	
0.04	88.09±0.96		93.97±1.31	

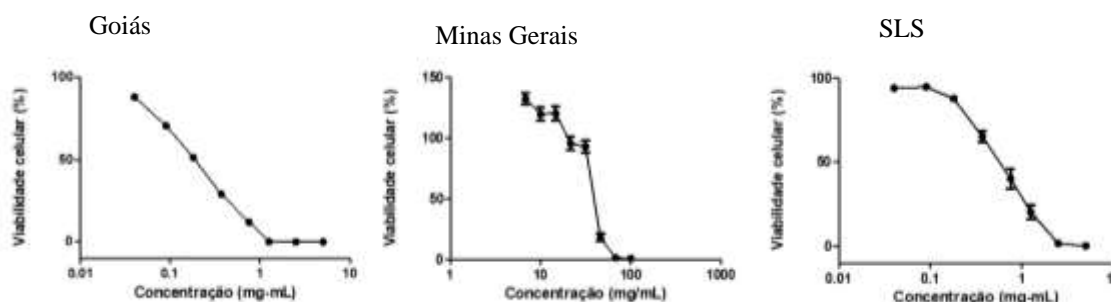
Source: Authors.

Table 4. Cell viability values obtained after 48 hours of exposure of Balb/c 3T3 cells to the Sodium Lauryl Sulfate (SLS) positive control, obtained using the neutral red incorporation method, as described in Protocol No. 129 of the OECD.

Material	Concentration (µg/mL)	Cell viability (%)	IC ₅₀ (µg/mL)
SLS (Positive Control)	100	0.63± 1.08	37.08
	68.1	1.15 ± 2.03	
	46.4	17.85 ± 7.53	
	31.6	92.96 ± 11.74	
	21.5	95.71 ± 13.58	
	14.7	120.38 ± 14.33	
	10.0	119.93 ± 12.97	
	6.8	132.57 ± 10.88	

Source: Authors.

Figure 3: Cell viability of Balb/c 3T3 cells after 48 hours of exposure to volatile oil GO, MG and the positive control of the assay (Sodium Lauryl Sulfate -SLS), using the neutral red incorporation method.



Source: Authors. - software GraphPad Prism 5.0.

4. Discussion

The volatile oil yields for specimens from Hidrolândia - GO and São Gonçalo do Abaté - MG were 0.21 and 0.26%, respectively for leaves. In the literature, the yield of oils from *E. puniceifolia* leaves ranged from 0.79% and 0.82% in mountainous regions of Pernambuco (PE) (Oliveira, et al., 2005), 0.18% in Macaé, Rio de Janeiro (RJ) (Ramos, et al., 2010), 0.58, 1.42 and 0.84% of the aerial parts of three specimens collected in different locations in the city of Carolina – Maranhão (MA) (Fernandes, et al., 2021b) and 0.26 and 0, 14% of the leaves of 2 specimens collected in Magalhães Barata, Pará (PA) (Franco, et al., 2021). Variations in volatile oil yields of the same species in different regions of Brazil can be attributed to climatic, geographical, ecological, and physiological factors (Oliveira, et al., 2005, Fernandes, et al., 2021).

The predominant class of terpenes in the GO sample were sesquiterpenes (89.23%), while for those from MG it was oxygenated sesquiterpenes (59.46%). In the literature, there was a prevalence of the class of sesquiterpene hydrocarbons (including oxygenated ones) in volatile oils from the leaves of *E. puniceifolia* in Amazonas (AM) (70.6 and 65.8%) (Maia, et al., 1997), Rio de Janeiro (55.5 and 44%) (Ramos, et al., 2010), Pará (70.70%) (Pereira, et al., 2010), Maranhão (60.2%) (Fernandes, et al., 2021b), Pará (88.4 and 71.83%) (Franco, et al., 2021). However, there was a predominance of monoterpene hydrocarbons in Pernambuco (66 and 76.6%) (Oliveira, et al., 2005) and in two specimens from Maranhão (81.8 and 86.2%) (Fernandes, et al., 2021b).

The major compounds in the volatile oil of *E. puniceifolia* in GO, germacrene D (21.34%) and bicyclogermacrene (26.73%), are derived from germacrene. Geracrene D was found in volatile oils of *E. puniceifolia* from MA (5.3%) (Fernandes, et al., 2021), PA (5.39%) (Pereira, et al., 2010), RJ (1.9 %) (Ramos, et al., 2010), PA (2.05%) (Franco, et al., 2021), as well as in MG (1.62%). Its isomer germacrene B was also found in samples of Pernambuco (PE) (1.3%) (Oliveira, et al., 2005), PA (0.8%) (Pereira, et al., 2010), RJ (1.9%) (Ramos, et al., 2010), MA (16.3%) (Fernandes, et al., 2021b) and PA (1.1%) (Franco, et al., 2021). Bicyclogermacrene was identified in the samples of PA (8.75%) (Pereira et al., 2010), MA (7.0%) (Fernandes et al., 2021b) and PA (9.88 and 5.86%) (Franco, et al., 2021) and MG (2.99%).

In the present work, it was verified in the sample of MG β - caryophyllene (1.41%) and its isomers (Z)-caryophyllene (8.92%), caryophyllene oxide (5.57%), and α -humulene (2.72%) and the GO sample the caryophyllene oxide (0.29%) and α -humulene (3.2%). β -caryophyllene was identified as the majority compound in volatile oils from leaves collected in Caracará and Itacoatiara - AM (32.9 and 23.6%) (Maia, et al., 1997), in Serra Negra and Brejo da Madre de Deus - PE (22.7 and 16.2 %) (Oliveira, et al., 2005), in Macaé-RJ (6.5%) (Ramos, et al., 2010), in Maracanã-PA (9.87%) and in the aerial parts of *E. puniceifolia* in Carolina - MA (3.2 to 13.21%) (Fernandes, et al., 2021b) and Magalhães Barata-PA (13.11 and 11.47%) (Franco, et al., 2021).

The γ -cadinene (6.27%), δ -amorphene (1.43%), δ -cadinene (0.54%) and α -cadinene (0.28%) found in the MG sample and the δ -amorphene (5.99%), γ -cadinene (0.76%) and α -cadinene (0.57%) in the GO sample were also found in AM (4.5 and 6.0% - δ -cadinene) (Maia, et al., 1997), RJ (1.1% - γ -cadinene) (Ramos, et al., 2010), MA (1.5% - δ -cadinene and 1.5% - γ -cadinene) (Fernandes, et al., 2021b) and PA (0.63% γ -cadinene, 1.76 and 4.01% δ -cadinene and 0.05% α -cadinene) (Franco, et al., 2021). α -Cadinol, an oxygenated sesquiterpene identified in volatile oil from MG (6.28) and in GO (1.61%), was also identified in the RJ sample (10%) (Ramos, et al., 2010) and PA (1.91 and 2.44%) (Franco, et al., 2021).

The isomers of δ -elemene (1.74%), β -elemene (1.31%) and γ -elemene (3.09%) are present in the volatile oil of GO and are commonly identified in the oil from the leaves of *E. puniceifolia* in AM (5.3 to 7.9% - γ -elemene) (Maia, et al., 1998), in RJ (1.61 and 6.2% - δ -elemene and β -elemene) (Ramos, et al., 2010), in PA (1.69% - γ -elemene) (Pereira, et al., 2010), in MA (0.8, 4.3 and 3.3% - δ -elemene, β -elemene and γ -elemene) (Fernandes, et al., 2021b), and PA (14.2 and 3.5% - δ -elemene, 25.12 and 2.38% β -elemene and 4.22% and γ -elemene) (Franco, et al., 2021).

Higher concentrations of β -caryophyllene may be related to water stress and collection time after 4 pm (Silva, et al., 2016). The production of caryophyllene oxide is related to the exposure of β -caryophyllene to atmospheric air after the extraction of volatile oil (Barros, et al., 2009). β -elemene and its isomers are by-products of germacrene and arise due to acidic and heating conditions at the time of extraction (Barros, et al., 2009). The hydrodistillation itself induces the formation of cadinol isomers due to the reaction of water with cadinyl cation, on the other hand, the exit of a proton from the cadinyl cation leads to the synthesis of α and γ -muurulene (Barros, et al., 2009; Steele, et al., 1998).

Volatile oil, after extraction, is prone to oxidative damage, chemical transformations, or polymerization, or isomerization reactions due to processing and storage conditions, temperature, light, and oxygen availability (Turek & Stintzing, 2013). The variability in yield and chemical composition of volatile oils from plants of the same species is intrinsically related to geographic location, ecological factors such as biome, plant health, soil type, available nutrients, water stress, predation and herbivory, genetic and physiological aspects (flowering, fruiting, reproductive rest and age) in addition to atmospheric parameters such as climate, temperature, insolation, and precipitation are related to the chemical diversity of volatile compounds, as well as seasonal aspects and harvest time (Gobbo-Neto & Lopes, 2007; Cruz, et al., 2014; Verma, et al., 2014).

Both pure volatile oils had moderate bactericidal potential against *K. rhizophyla* (ATCC 9341), *M. luteus* (ATCC 10240), and *S. aureus* (ATCC 29737), with the volatile oil from Goiás having a MIC of 256, 512 and 256 $\mu\text{g/mL}$ and oil from Minas Gerais with a MIC of 128, 256, respectively. Antifungal activity was not verified.

The literature reports the moderate bactericidal effect of the volatile oil of the leaves species of the genus *Eugenia* for *S. aureus* (ATCC 25923) such as *Eugenia brasiliensis* Lam. (MIC 152.2 and MBC 624.9 $\mu\text{g/mL}$), *Eugenia umbelliflora* O. Berg. (MIC of 119.2 and MBC 477.0 $\mu\text{g/mL}$) (Magina, et al., 2009) *E. brejoensis* (MIC 128 $\mu\text{g/mL}$) for *S. aureus* ATCC 29213 (Bezerra Filho, et al., 2020). Weak activity for *S. aureus* ATCC 25923 *E. beaurepaireana* (MIC 1110 $\mu\text{g/mL}$) (Magina, et al., 2009), *Eugenia involucrata* (MIC 875 $\mu\text{g/mL}$) for *S. aureus* (ATCC 25923) (Toledo, et al., 2020). The oil from the stems, leaves, and flowers of *E. chlorophylla* showed moderate effect for *K. rhizophila* ATCC 9341 (MIC 500 $\mu\text{g/mL}$) and *S. aureus* ATCC 8538 (MIC 500 $\mu\text{g/mL}$) and weak for *S. aureus* ATCC 25923 (MIC 1000 $\mu\text{g/mL}$) (Stefanello, et al., 2011).

The β -caryophyllene sesquiterpene showed good activity against several bacteria such as *B. cereus* MTCC 1307 (MIC between 3 and 14 $\mu\text{g/mL}$), *E. coli* MTCC 732 (MIC 9 $\mu\text{g/mL}$), *B. subtilis* MTCC 6910 (8 $\mu\text{g/mL}$), *S. aureus* MTCC 7405 (3 $\mu\text{g/mL}$), *P. aeruginosa* MTCC 4302, *P. citrinum* MTCC 7124 (MIC of 7 $\mu\text{g/mL}$), *A. niger* MTCC 2196, *R. oryzae* MTCC 1987 (6 $\mu\text{g/mL}$), *T. reesei* MTCC 3929 (4 $\mu\text{g/mL}$) and *K. pneumoniae* MTCC 7028 (14 $\mu\text{g/mL}$) (Dahham, et al., 2015).

The larvicidal activity of volatile oil from GO and MG was considered moderate with LC_{50} of 85.53 and 91.52 $\mu\text{g/mL}$, respectively. There are no reports in the literature on the larvicidal activity of *E. puniceifolia*. In the Myrtaceae family, there are

several species with larvicidal activity for *Aedes aegypti*, considered active with $LC_{50} \leq 50$ $\mu\text{g/mL}$, such as *Psidium guajava* L. (Lima, et al., 2011), *Eucalyptus maculata* Hook (Sarma, et al., 2019), *Baeckea frutescens* L., *Callistemon citrinus* (Crimson Bottlebrush), *Melaleuca leucadendra* (L.) L., *Syzygium nervosa* A. Cunn. ex DC. (An, et al., 2020). Other species showed moderate activity, LC_{50} between 50 and 100 $\mu\text{g/mL}$, such as *E. triquetra* O. Berg (LC_{50} of 64.8 ± 5.6 ppm) (Mora, et al., 2010), *Syzygium zeylanicum* (L.) (Govindarajan & Benelli, 2016), *Eucalyptus nitens* Maiden (Costa, et al., 2017), *Psidium guajava* L. (Mendes, et al., 2017), *Callistemon linearis* (Schrad. & JCWendl.) Colvill ex Sweet (Sarma, et al., 2019). And efficient activity with LC_{50} between 100 and 750 $\mu\text{g/mL}$ as *Eugenia patrisii* Vahl (IC_{50} of 417 $\mu\text{g/mL}$), *Eugenia piauhiensis* Vellaff. (LC_{50} of 230 $\mu\text{g/mL}$) (Dias, 2013; Dias, et al., 2015), *Eugenia brejoensis* (LC_{50} of 214.7 ppm) (Silva, et al., 2015), *Eugenia candollena* DC (LC_{50} of 300 $\mu\text{g/mL}$) (Neves, et al., 2017), *Eugenia calycina* (LC_{50} of 199.3 $\mu\text{g/mL}$) (Silva, 2018) and *Eugenia calycina* (LC_{50} of 266.8 and 312.1 $\mu\text{g/mL}$) (Silva, et al., 2021).

According to Govindarajan and Benelli (2016) and Benelli et al. (2018), γ -elemene showed larvicidal effect against *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus* with LC_{50} of 10.53, 11.29, and 12.18 $\mu\text{g/mL}$, in addition to low toxicity of aquatic larvivorous organisms (*Anisops bouvieri*, *Diplonychus indicus*, *Poecilia reticulata* *Gambusia affinis*) with LC_{50} ranging from 829.14 to 2326.27 $\mu\text{g/mL}$.

Sesquiterpenes derived from germacrene are related to larvicidal and bactericidal activity, such as Germacrene D, which demonstrated good larvicidal activity against *Aedes aegypti* and *Culex quinquefasciatus* ($LC_{50} = 18.8$ and 21.3 $\mu\text{g/mL}$, respectively) (Govindarajan, 2010), the bicyclogermacrene isomer against *Anopheles subpictus*, *Aedes albopictus* and *Culex tritaeniorhynchus* exhibited an LC_{50} of 10.3, 11.1, 12.5 $\mu\text{g/mL}$ and LC_{90} of 20.9, 22.14, 24.2 $\mu\text{g/mL}$, respectively (Govindarajan & Benelli, 2016). The volatile oil from the leaves of *Cryptocarya infectoria* (Blume) Miq. rich in germacrene D (55.5%) and bicyclogermacrene (11.4%) showed good activity for *Aedes aegypti* with LC_{50} of 18.04 and LC_{90} of 39.12 $\mu\text{g/mL}$ (Chau, et al., 2020).

In the literature, there are reports of larvicidal and bactericidal activity for caryophyllene isomers. Nararak, et al. (2019), evaluated the irritating and repellent effect of Caryophyllene oxide at concentrations 0.1; 0.25; 0.5 and 1.0% v/v for *A. aegypti* and *Anopheles minimus* and found an avoidance response between 86-96% for *Anopheles minimus* and 22.0% - 59.3% for *Aedes aegypti* at concentrations 0.5 and 1%, showing greater repelling and irritating effect compared to the DEET reference standard (N,N-diethyl-3-methylbenzamide) which repelled 0 - 9% and 5.5 - 54.2%, respectively. The β -caryophyllene showed weak larvicidal activity with an LC_{50} of 298.4 $\mu\text{g/mL}$ and LC_{90} of 1227.3 $\mu\text{g/mL}$ for *Aedes aegypti* (Nogueira Sobrinho, et al., 2021). α -humulene is already reported in the literature for having good larvicidal action against *Anopheles subpictus* ($LC_{50} = 6.19$ $\mu\text{g/mL}$), *Aedes albopictus* (LC_{50} 6.86 $\mu\text{g/mL}$) and *Culex tritaeniorhynchus* (LC_{50} 7.39 $\mu\text{g/mL}$) (Govindarajan & Benelli, 2016), *Armigera Helicoverpa* ($LC_{50} = 20.86$ $\mu\text{g/ml}$) (Benelli, et al., 2018).

The volatile oils of *E. puniceifolia* from both regions showed moderate cytotoxicity against Balb/c 3T3 (normal fibroblasts) with IC_{50} 706.7 $\mu\text{g/mL}$ and IC_{50} of 160.7 $\mu\text{g/mL}$ for oils from Goiás and Minas Gerais, respectively, compared to the positive control with IC_{50} 37.08 $\mu\text{g/mL}$. There are no reports in the literature on the cytotoxic evaluation of volatile oil from *Eugenia puniceifolia*, but Galeno, et al. (2014) found that the aqueous extract of *E. puniceifolia* leaves showed low toxicity at concentrations of 50, 25 and 12 $\mu\text{g/mL}$ for fibroblasts (3T3-L1 cell), compared to cells treated with doxorubicin at a concentration of 5 $\mu\text{g/mL}$, and according to Costa, et al. (2016), the hydroalcoholic extract did not reduce the viability of human neutrophils (*in vitro*) at concentrations of 0.1-1000 $\mu\text{g/mL}$.

In the work by Sousa, et al. (2015), the oil from *E. calycina* leaves showed moderate cytotoxicity with CC_{50} 137.4 $\mu\text{g/mL}$ for HeLa cells, already in the work by Silva, et al. (2021) showed an IC_{50} of 266.8 for HeLa cells, and an IC_{50} of 312.1 $\mu\text{g/mL}$ for Vero cells within 24 h of exposure. Some oils have a toxic effect on MRC5 (human fibroblast) cells, such as oil

from the leaves of *E. flavescens* DC. (IC₅₀ 14.0 µg/mL), *E. patrisii* Vahl (IC₅₀ 18.1 µg/mL) (Silva et al., 2017) and *E. uniflora* (IC₅₀ between 10.27 and 14.95 µg/mL) (Figueiredo, et al., 2019).

The volatile oil collected in Goiás showed larvicidal activity against *Ae. aegypti* (IC₅₀ 91 µg/mL) and antimicrobial in *K. rhizophyla* (IC₅₀ 128 µg/mL) and *M. luteus* (IC₅₀ 256 µg/mL), concentrations lower than the toxic action observed in murine fibroblasts (IC₅₀ 706.7 µg/mL), thus showing an interesting therapeutic potential. The volatile oil from Minas Gerais also showed larvicidal activity at a lower concentration (IC₅₀ 85 µg/mL) than the toxic action for fibroblasts (IC₅₀ 160.7 µg/mL).

5. Conclusion

It was found as the major compounds of the volatile oil from *E. puniceifolia* leaves collected in the Goiás the bicyclogermacrene, germacrene D, β-longipinene and δ-amorphene and collected from Minas Gerais, (Z)-caryophyllene, α-cadinol, γ-cadinene, and caryophyllene oxide. Both oils showed moderate activity to L3 larvae of *Ae. aegypti*, with LC₅₀ of 85.53 and 91.52 µg/mL, for Goiás and Minas Gerais, respectively. The oils showed moderate activity against gram-positive bacteria, *K. rhizophyla* (ATCC 9341), *M. luteus* (ATCC 10240), and *S. aureus* (ATCC 29737), and moderate cytotoxicity in murine fibroblasts (Balb/c 3T3), thus these oils can be well tolerated against the biological system, however, further studies are needed to assess their toxicity *in vivo*. Although the oils have different concentrations of the majors and compounds, the biological activities evaluated in this study were similar, which suggests the synergistic action of the compounds in common. This is the first study of larvicidal, antimicrobial, and cytotoxic activity of volatile oils from *E. puniceifolia* leaves.

Acknowledgements

The authors would like to thank the Natural Products Research Laboratory (LPPN), Faculty of Pharmacy of the Federal University of Goiás (UFG), the Laboratory of Insect Biology and Physiology of the Tropical Pathology and Public Health Institute (IPTSP/UFG), the Laboratory of Teaching and Research in In vitro Toxicology (ToxIn/UFG), to the Toxicopharmacological Research and Studies Nucleus (NEPET/UFG).

Declaration of interest

No potential conflict of interest was reported by the authors.

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