Atividades antinociceptiva, anti-inflamatória e cicatrizante do óleo essencial das folhas de *Eugenia uniflora* L. (pitanga) cultivada no Nordeste do Brasil

Antinociceptive, anti-inflammatory, and wound healing activities of the essential oil from the leaves of *Eugenia uniflora* L. (pitanga) cultivated in Northeastern Brazil

Actividades antinociceptivas, antiinflamatorias y cicatrizantes del aceite esencial de las hojas de *Eugenia uniflora* L. (pitanga) cultivadas en el noreste de Brasil

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RESUMO
Este trabalho descreve a composição química, a toxicidade aguda e os efeitos antinociceptivo, anti-inflamatório e cicatrizante do óleo essencial das folhas de *Eugenia uniflora* L. cultivada no Nordeste do Brasil. O óleo essencial apresentou como constituintes majoritários selina-1,3,7(11) - trien-8-ona (33,92%), selina-1,3,7(11) - trien-8-ona epóxido (29,31%), germacreno B (8,88%) e (E)-cariofileno (5,62%). Verificou-se que o óleo essencial não apresentou toxicidade aguda na dose máxima de 2.000 mg/kg. No ensaio da formalina, o óleo apresentou atividade antinociceptiva, com redução do tempo de lambida em 92,01% (fase neurogênica) e 85,01% (fase inflamatória), tendo como mecanismos o sistema opioide, colinérgico, adenosinérgico e canais de potássio sensíveis ao ATP. No teste inflamatório, o óleo essencial reduziu o edema de pata, com redução dos níveis de TNF-α e IL-1β. A EOU acelerou a cicatrização em 98,8% em 14 dias. Os resultados sugerem que o óleo essencial tem potencial para o desenvolvimento de agentes analgésicos, anti-inflamatórios e cicatrizantes a partir de fontes naturais.
**Palavras-chave:** pitangueira, óleo essencial, myrtaceae, selina-1,3,7(11)-trien-8-ona, ferimentos, Brasil.

**ABSTRACT**

This work describes the chemical composition, acute toxicity, and antinociceptive, anti-inflammatory, and healing effects of the essential oil from the leaves of *Eugenia uniflora* L. cultivated in Northeastern Brazil. The essential oil presented as the major constituents selina-1,3,7(11)-trien-8-one (33.92%), selina-1,3,7(11)-trien-8-one epoxide (29.31%), germacrene B (8.88%) and (E)-caryophyllene (5.62%). It was found that the essential oil did not show acute toxicity at the maximum dose of 2,000 mg/kg. In the formalin assay, the oil showed antinociceptive activity, with a reduction in licking time by 92.01% (neurogenic phase) and 85.01% (inflammatory phase), having as mechanisms the opioid, cholinergic, adenosinergic system and ATP-sensitive potassium channels. In the inflammatory test, the essential oil reduced paw edema, with reduced levels of TNF-α and IL-1β. The EEOU accelerated healing by 98.8% in 14 days. The results suggest that essential oil has the potential for the development of analgesic, anti-inflammatory, and healing agents from natural sources.

**Keywords:** pitangueira, essential oil, myrtaceae, selina-1,3,7(11)-trien-8-one, wounds, Brazil.

**RESUMEN**

Este trabajo describe la composición química, la toxicidad aguda y los efectos antinociceptivos, antiinflamatorios y curativos del aceite esencial de las hojas de *Eugenia uniflora* L. cultivadas en el noreste de Brasil. El aceite esencial presentado como constituyentes principales selina-1,3,7(11)-trien-8-ona (33,92%), epóxido de selina-1,3,7(11)-trien-8-ona (29,31%) , germacreno B (8,88%) y (E)-cariofileno (5,62%). Se encontró que el aceite esencial no mostró toxicidad aguda a la dosis máxima de 2000 mg/kg. En el ensayo de formalina, el aceite mostró actividad antinociceptiva, con una reducción del tiempo de lamido en un 92,01% (fase neurogénica) y un 85,01% (fase inflamatoria), teniendo como mecanismos el sistema opioide, colinérgico, adenosinérgico y los canales de potasio sensibles a ATP. En la prueba inflamatoria, el aceite esencial redujo el edema de la pata, con niveles reducidos de TNF-α e IL-1β. La EEOu aceleró la curación un 98,8% en 14 días. Los resultados sugieren que el aceite esencial tiene potencial para el desarrollo de agentes analgésicos, antiinflamatorios y cicatrizantes a partir de fuentes naturales.

**Palavras clave:** pitangueira, aceite esencial, myrtaceae, selina-1,3,7(11)-trien-8-ona, heridas, Brasil.
1 INTRODUCTION

In recent decades, interest in medicinal and aromatic plants and the products obtained from them has increased, as they contain important agents capable of assisting in the treatment and cure of various diseases, contributing to the improvement of human health (Abreu et al. 2015; Aqeel et al. 2023). Essential oils are important secondary metabolic products extracted from different parts of plants, being used as raw material in the perfumery, food, and pharmaceutical sectors (Kant and Kumar, 2022).

Brazil is a country rich in plant diversity with a higher rate of endemism, which has contributed to the development of therapeutic alternatives by identifying secondary metabolites active for several diseases that affect human health, presenting great potential economically. The botanical species belonging to the Myrtaceae family represent most of this botanical richness, with 121 genera represented by approximately 5,800 species of evergreen trees or shrubs with edible fruits distributed in tropical and subtropical areas (Macedo et al. 2021). In addition to having fruits of commercial interest to the food industry, the leaves of the plants are rich in essential oils, which has been attracting the interest of industries, since several members of this family are indicated in ethnobotanical research for the cure of several human diseases, some of these already scientifically proven (Abreu et al. 2015).

_Eugenia uniflora_ Linn (Myrtaceae) popularly known as “Pitangueira”, is a fruitful harbored species cultivated in Brazil. The species is recognized as a medicinal plant in ethnobotanical studies, the leaves being used in infusions to treat diarrhea, stomach pain, colic, worms, fever, flu, cough, bronchitis, anxiety, high blood pressure, diabetes, flu, cough, sore throat, tooth inflammation, and headache (Magalhães et al. 2019; Fidelis et al. 2022). Previous investigations related to the composition of _E. uniflora_ essential oil revealed the richness of compounds. In the essential oil of the leaves, it was possible to find cruzerene, p-cymene, and α-terpinene (Weyerstahl, 1988). The compounds spathulenol, β-caryophyllene, germacrene B, germacrene D, γ-elemene, β-selinene, and β-elemene were found in the fruits, and in seline-1,3,7(11)-trien-8-one and seline-
1,3,7(11)-trien-8-one epoxide were found in the essential oil of fruit pulps (Soares et al. 2015). These components may vary depending on the different climatic and environmental conditions associated with the cultivation of the species (Fidelis and others 2022).

Several biological activities have already been described for essential oil from *E. uniflora* leaves cultivated in different geographical regions, such as antifungal and antibacterial (Santos et al. 2018), antioxidant (Costa et al. 2020), antinociceptive (De Jesus et al. 2023), anti-Leishmania (Rodrigues et al. 2013) and anti-inflammatory (de Jesus et al. 2023). However, no study has investigated the antinociceptive and anti-inflammatory effects of the essential oil from the leaves of this species cultivated in Northeast Brazil.

Thus, this study aimed to obtain and investigate the chemical composition of essential oil from the leaves of *E. uniflora* cultivated in Northeastern Brazil, acute toxicity, antinociceptive activity and mechanism of action, anti-inflammatory and healing activities in an animal model in mice.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

The leaves of *Eugenia uniflora* L. were collected at the experimental station of the Instituto Agronomy de Pernambuco - IPA in Itapirema, in the city of Goiana, Pernambuco, northeastern Brazil (7°33'45"S, 35°0'0"W), in September de 2020. Fertile samples collected were identified by Prof. Dr. Maria Rita Cabral Sales de Melo by comparison with authentic samples and descriptions reported in books, being deposited exsiccate of plant material in the Herbarium Professor Vasconcelos Sobrinho of the Federal Rural University of Pernambuco (UFRPE) (Voucher No. PEUFR55818).

2.2 ESSENTIAL OIL EXTRACTION

Clean fresh leaves, free of pests or microorganisms, were washed with distilled water and subsequently crushed. The essential oil extraction was carried out in triplicate, using hydrodistillation for 4 hours at a temperature of
approximately 100°C, in a Clevenger-type apparatus, using 300 g of the crushed material in each extraction, being added to the flask, with water in the proportion of 1:10 % (w/w) (European Pharmacopoeia, 2020). After hydrodistillation, excess water was removed with Anhydrous Sodium Sulfate (Na₂SO₄), and the yield calculation was obtained based on the amount of plant material used in the process and the mass obtained % (w/w), in triplicate. The essential oil obtained was stored in an amber bottle, kept under refrigeration at -4 °C, and protected from light.

2.3 CHEMICAL ANALYSIS OF THE ESSENTIAL OIL

The identifications of the essential oil components were performed in an Agilent 5975C (Series GC/MSD), equipped with a DB-5 column, following the chromatographic conditions described by Barbosa et al. (2020). Quantitative analyses were performed under the same conditions described for the gas chromatography associated with mass spectrometry GC-MS in Thermo TraceGC Ultra equipment equipped with a VB-5 column. The identifications made by GC-MS were made by comparing the mass spectra obtained by the equipment with those from the equipment’s libraries (MassFinder 4, Dr. Hochmuth scientific consulting, Hamburg, Germany); NIST08 Mass Spectral Library (ChemSW Inc. Fairfield, CA, USA); Wiley Registry™ of Mass Spectral Data 9th Edition (Wiley, Hoboken, NJ, USA). It was also compared with the spectra published by Adams (2007) and their retention rates calculated by co-injection of the essential oil sample with a solution of C9–C30 hydrocarbon standards, calculated by the equation of Van den Dool and Kratz (1963). Quantifications GC was performed in triplicate to obtain standard deviation.

2.4 EXPERIMENTAL MODEL IN VIVO AND ETHICAL PROCEDURES

The experimental procedures were carried out in albino Swiss mice (8-10 weeks old, 30-32 g) obtained from the central vivarium of the Keizo Asami Institute - Lika/UFPE. The animals were acclimatized in cages with a solid polypropylene bottom (size: 18 cm x 34 cm x 41 cm) with wooden bedding, under
standard environmental conditions of 22 ± 3°C, light-dark cycle 12/12, and fed with the standard ration and drinking water ad libitum. The animal experimentation protocol was carried out following the recommendations of the National Council for the Control of Animal Experimentation in Brazil (CONCEA) and the Council of International Organizations of Medical Sciences (CIOMS). All experiments were approved by the Ethics Committee on Animal Use (CEUA) of the UFPE, under nº 0099/2021.

2.4.1 Acute Toxicity Test and Median Lethal Dose

The acute toxicity study to assess safety was carried out by guideline 423 of the Organization for Economic Co-operation and Development (OECD, 2002). First, Swiss albino mice (female) were divided into groups (n = 3) and orally treated by gavage with 100 µL of the following essential oil doses: 5, 50, 300, and 2000 mg/kg. The animals were observed at 30, 60, 120, 180, and 240 minutes after oral treatment and daily for 14 days. The weights of animals and ingested food were recorded daily, as well as possible signs of alteration, such as tremors, convulsions, salivation, piloerection, hyperactivity, and bleeding, among other toxicity indicators. Mortality was also assessed at 14 days and LD$_{50}$ was calculated.

2.4.2 Antinociceptive Activity

The evaluation was performed according to the methodology of Tjølsen et al. (1992). Six experimental groups (n = 6) of male mice were used, with the following treatments: vehicle (saline solution 0.9%, w/v; p.o.), essential oil Eugenia uniflora (EOEu) (50, 100, and 200 mg/kg; p.o.) and Indomethacin (20 mg/kg; p.o.); Morphine group (10 mg/kg) received treatment via intraperitoneal (i.p.). After 30 min (for morphine) or 60 minutes (for the other groups), nociception was induced by intraplantar (i.pl.) injection of 2.5% (v/v) formalin (20 µL) in the left hind paw. After the formalin injection, the animals were transferred to an observation chamber (15 cm in diameter and 20 cm in height) equipped with a mirror inclined at 45° below the chamber and observed from 0 to 5 min (first
phase, neurogenic phase) and 15 to 30 min (second phase, inflammatory phase) about the time spent in seconds (s) licking or biting the paw that received the nociceptor agent.

To verify the antinociceptive action of essential oil *E. uniflora* (EOEu) on the opioid, cholinergic, adenosinergic, and ATP-sensitive potassium channels (K-ATP) systems, the pharmacological antagonist's Naloxone (5 mg/kg; i.p), Atropine (5 mg /kg; i.p.), Caffeine (10 mg/kg; i.p.) and Glibenclamide (10 mg/kg; i.p.), respectively, were used, being injected 30 min before treatment of the groups with essential oil (200 mg/kg, p.o.) (De Lavor et al., 2018). After treatments, 2.5% formalin (20 µL/paw) was injected into the right hind paw of mice. Paw licking time was recorded, in seconds, from 0 to 5 min (neurogenic phase) and from 15 to 30 min (inflammatory phase) after formalin administration.

**2.4.3 Anti-Inflammatory Activity**

The anti-inflammatory effect of essential oil *Eugenia uniflora* (EOEu) was performed through the λ-carrageenan-induced paw edema according to the methodology of Ou et al. 2019. The male mice were divided into five groups (n = 6), being treated orally (p.o.) by gavage with 100 µL of vehicle (saline solution 0.9%, w/v; p.o.), essential oil *Eugenia uniflora* (EOEu) (50, 100 and 200 mg/kg; p.o.) or indomethacin (20 mg/kg; p.o.). After 1 hour, the animals received 1% λ-carrageenan (w/v) (20 µL/paw) in the right hind paw and saline solution (0.9%, w/v), (20 µL/paw) in the left paw. The thickness of the right and left rear paws of each animal was measured using a caliper at 1, 2, 3, and 4 hours after injecting λ-carrageenan. Inhibition of edema was calculated by (right paw – left paw) and expressed in mm.

Four hours after λ-carrageenan inoculation the animals were euthanized with excessive doses of ketamine (300 mg/kg; i.p.) and xylazine (30 mg/kg; i.p.), and the paws were removed and weighed. A part of the right hind paw was weighed and macerated in 1 mL of cold sterile saline solution, containing 0.5% bovine serum albumin (BSA), 0.1M phenylmethylsulfonyl fluoride (PMSF), and 10 mM ethylenediaminetetraacetic acid (EDTA). Supernatants were collected to
determine TNF-α and IL1-β concentrations using Mouse Cytokines ELISA Kit, High Sensitivity in ELISA, Thermo Fisher (Waltham, USA) according to the manufacturer’s instructions.

2.4.4 Wound Healing Activity

Female mice (weight ± 30g) were randomly distributed into 3 groups (n=12). Each mouse was anesthetized with a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally. After the loss of the animals’ reflexes, the back was shaved, and antisepsis was performed with 2% chlorhexidine. An 8 mm diameter circular skin wound was made on the dorsal surface with the help of a biopsy punch. Each group of animals was treated with a single local application of 100 µL of 0.9% saline solution (negative control), Dersani® (positive control), or essential oil E. uniflora (EOEu). The clinical evolution of the wounds was evaluated daily, and its results were evaluated in the respective periods of 3, 7, and 14 days. The wounds were photographed, and the areas of the lesions were calculated using the ImageJ 2.0 software (National Institutes of Health), determining the area and degree of contraction of the wound, using the respective equations: A= π. R. r, where “A” = area (mm²), “R” = largest radius, and “r” = smallest radius (Mulisa et al., 2015). The initial area corresponds to the day of injury induction (D0) and the final area corresponds to the day of euthanasia (D3, D7, and D14).

2.5 STATISTICAL ANALYSIS

The data were analyzed using the GraphPad Prism® software (version 8.4.3; San Diego California USA). Statistical analysis was done using one-way ANOVA or two-way ANOVA followed by post-hoc Tukey. The data were expressed in means ± SD. P-values < 0.05 were considered statistically significant.
3 RESULTS AND DISCUSSION

3.1 EXTRACTION AND CHARACTERIZATION OF ESSENTIAL OIL

The essential oil obtained from leaves of *E. uniflora* L. (EOEu) showed a yield of 1.12±0.06% (w/w), with 96.63% of its chemical constituents being identified, the components with the highest levels being selin-1,3,7(11)-trien-8-one (33.92%), seline-1,3,7(11)-trien-8-one epoxide (29.31%), germacrene B (8.88%) and (E)-caryophyllene (5.62%) (Table 1).

Table 1. The composition chemical of the essential oil of *Eugenia uniflora* (EOEu) cultivated in northeastern Brazil, by GC-MS, highlighting the significant components in bold.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compounds</th>
<th>RI&lt;sup&gt;b&lt;/sup&gt;</th>
<th>RI&lt;sup&gt;c&lt;/sup&gt;</th>
<th>%</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β-elemene</td>
<td>1389</td>
<td>1391</td>
<td>3.45</td>
<td>0.35</td>
</tr>
<tr>
<td>2</td>
<td>(E)-caryophyllene</td>
<td>1417</td>
<td>1419</td>
<td>5.62</td>
<td>0.41</td>
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<tr>
<td>3</td>
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<td>1434</td>
<td>1433</td>
<td>2.43</td>
<td>0.23</td>
</tr>
<tr>
<td>4</td>
<td>α-humulene</td>
<td>1452</td>
<td>1453</td>
<td>0.32</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>β-chamigrene</td>
<td>1489</td>
<td>1475</td>
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<td>0.02</td>
</tr>
<tr>
<td>6</td>
<td>germacrene D</td>
<td>1480</td>
<td>1481</td>
<td>0.85</td>
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</tr>
<tr>
<td>7</td>
<td>β-selinene</td>
<td>1489</td>
<td>1486</td>
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<tr>
<td>8</td>
<td>curzerene</td>
<td>1499</td>
<td>1496</td>
<td>3.41</td>
<td>0.31</td>
</tr>
<tr>
<td>9</td>
<td>selina-3,7(11)-diene</td>
<td>1545</td>
<td>1542</td>
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<td>0.02</td>
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<tr>
<td>10</td>
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<td>1557</td>
<td>8.88</td>
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<tr>
<td>11</td>
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<td>1577</td>
<td>1578</td>
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<tr>
<td>13</td>
<td>viridiflorol</td>
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<td>1592</td>
<td>0.53</td>
<td>0.02</td>
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<td>1632</td>
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<td>1746</td>
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<td>1.35</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>96.63</td>
<td></td>
</tr>
</tbody>
</table>

Component<sup>a</sup> = Essential oil constituents listed in order of elution from a 30 m nonpolar capillary column VB-5, GC-FID detector; RI<sup>b</sup> = Adams (2007) specialized literature retention rates; RI<sup>c</sup> = Retention Index calculated against a series of C9-C30 n-alkanes. % = Relative area of the EOEu component; SD = Standard Deviation.

Source: Elaborate by the authors.

Similar yields were found in studies with the same species, with values from 0.22 to 1.68% (Cipriano et al. 2023) and 0.8 to 3.1% (Costa et al. 2020). *Eugenia* species found in South America show high chemical variability and
diverse biological activities in their essential oils, these variations can be attributed to geographic occurrences or seasonality. The variation in the chemical composition of the essential oil of *E. uniflora* cultivated in Brazil has been reported. Costa et al. (2020) observed that the chemical composition of the species is strongly influenced by seasonal, environmental, ecological characteristics, geographic variations, among others, with approximately four different chemical types being found in the country, namely: (1) selin-1,3,7(11)-trien-8-one and selin-1,3,7(11)-trien-8-one epoxide; (2) selin-1,3,7(11)-trien-8-one, selin-1,3,7(11)-trien-8-one epoxide and caryophyllene oxide; (3) curzerene; and (4) germacrene B, curzerene and β-caryophyllene. The chemical composition of EOEu obtained in the Northeast of Brazil varies between the described chemotypes, where further studies must be carried out to evaluate the environmental conditions on the production of these constituents.

3.2 ACUTE TOXICITY TEST

Acute toxicity testing is an important aspect of evaluating the safety of substances, including essential oils. These tests are typically carried out following standard guidelines, such as those provided by the Organization for Economic Co-operation and Development (OECD) and the US Environmental Protection Agency (EPA), to assess the potential harmful effects of a substance after a single or short-term exposure. The LD50 value indicates the substance’s acute toxicity (Adnyana et al., 2023; Gupta et al., 2024).

In the acute toxicity test, EOEu did not cause mortality or behavioral changes at doses up to 2,000 mg/kg, indicating toxicological safety. However, studies must be carried out to investigate its subacute and chronic effects. In an acute toxicity test in mice, a single oral administration of the essential oil obtained from *E. uniflora* leaves at different doses (10, 50, 100, and 200 mg/kg) did not cause mortality. Furthermore, single administration of the essential oil from the leaves did not induce any signs of toxicity in mice, such as weight change or loss of appetite (Victoria et al., 2012).
3.3 EVALUATION OF THE ANTINOCICEPTIVE EFFECT

In the formalin-induced nociception test, the EOEu at doses of 50, 100, and 200 mg/kg reduced licking time in the first phase (neurogenic) by 26.6%, 54.39%, and 92%, respectively. The second phase (inflammatory) promoted significantly reduced licking time in 28.98%, 57.59%, and 85%, in doses of 50, 100, and 200 mg/kg, respectively. Morphine (10 mg/kg) reduced the nociceptive response both in the first (68.6%) and the second phase (97.65%), while indomethacin (20 mg/kg) produced a low antinociceptive effect in the first phase (0.39%) and a significant reduction in the second phase (53.24%) (Figure 1).

The EOEu showed an antinociceptive effect in the neurogenic (first phase) and inflammatory (second phase) phases, with a dose-dependent effect. The data were like the findings of the other authors who observed that the essential oil of E. uniflora chemotype curzerene does not present acute oral toxicity and produces an antinociceptive effect (De Jesus et al., 2023). Future investigations should be carried out to analyze most components present in EOEu, to elucidate which compounds alone or synergistically are responsible for the antinociceptive effect of the essential oil.

Figure 1. Effects of essential oil of *Eugenia uniflora* (EOEu 50, 100, and 200 mg/kg), Indomethacin (20 mg/kg), and Morphine (10 mg/kg) in the first (0-5 min) and second (15-30 min) phase in formalin-induced nociception (2.5% v/v; intraplantar).

All results were expressed as the mean ± SD (standard deviation) (n=6). abcdefDifferent lowercase letters mean statistical difference, one-way ANOVA with post-hoc Tukey. (p < 0.05).

Source: Elaborate by the authors.
In a previous study, oral administration of essential oil from leaves and terpenoids isolated from *E. uniflora* inhibited abdominal constrictions induced by oral acetic acid at a dose of 200 mg/kg by 48% and increased the latency time in the hot plate test. Isolated furanosequiterpenes were considered responsible for the antinociceptive effect found (Amorin et al., 2009). In our study, EOEu showed a dose-dependent nociception inhibition effect in both phases, which significantly decreased the duration of reaction time (licking) as the dose of essential oil increased. The group pre-treated with indomethacin showed a reduction in nociception only in the second phase (inflammatory) when compared to the control, which was already predicted due to the anti-inflammatory potential of indomethacin. Furthermore, as expected, morphine significantly decreased licking time in the first phase and showed a notable reduction in licking time in the second phase.

The activity observed in the first phase of the licking time is typical of an action in the central nervous system on opioid receptors, reducing pain. Morphine, as it is an opioid drug, also acts in the second phase, characterized by the emergence of a local inflammatory process, where mediators such as prostaglandins are produced. These mediators are inhibited by anti-inflammatory drugs, such as acetylsalicylic acid, indomethacin, and dexamethasone (Shibata et al., 1989). Therefore, the results suggest that one of the mechanisms of action of the constituents of EOEu is probably related to the inhibition of cyclooxygenase with a reduction in prostaglandins. The formalin test also indicated a possible anti-inflammatory activity of EOEu due to the reduction of nociception in the second (inflammatory) phase, subsequently confirmed by the paw edema test induced by carrageenan.

The investigation of the possible mechanisms by which EOEu produces an antinociceptive effect shows that the antagonist naloxone (Figure 2), atropine (Figure 3), Glibenclamide (Figure 4), and Caffeine (Figure 5) altered the antinociceptive effect of EOEu (200 mg/kg) in the first phase but did not alter it in the second phase.
Figure 2. Effects of essential oil of *Eugenia uniflora* (EOEu 200 mg/kg) on opioid receptors in the first (0-5 min) and second (15-30 min) phases of nociception induced by formalin (2.5% v/v; intraplantar).

![First Phase](chart1.png)

![Second Phase](chart2.png)

All results were expressed as the mean ± SD (standard deviation) (n=6). abc Different lowercase letters mean statistical difference, one-way ANOVA with post-hoc Tukey. (p < 0.05).

Source: Elaborate by the authors.

Figure 3. Effects of essential oil of *Eugenia uniflora* (EOEu 200 mg/kg) in the cholinergic system in the first (0-5 min) and second (15-30 min) phases of nociception induced by formalin (2.5% v/v; intraplantar).

![First Phase](chart3.png)

![Second Phase](chart4.png)

All results were expressed as the mean ± SD (standard deviation) (n=6). abc Different lowercase letters mean statistical difference, one-way ANOVA with post-hoc Tukey. (p < 0.05).

Source: Elaborate by the authors.
Figure 4. Effects of essential oil of *Eugenia uniflora* (EOEu 200 mg/kg) in the ATP-sensitive potassium channels (K-ATP) systems in the first (0-5 min) and second (15-30 min) phases of nociception induced by formalin (2.5% v/v; intraplantar).

All results were expressed as the mean ± SD (standard deviation) (n=6). abc Different lowercase letters mean statistical difference, one-way ANOVA with posthoc Tukey. (p < 0.05).

Source: Elaborate by the authors.

Figure 5. Effects of essential oil of *Eugenia uniflora* (EOEu 200 mg/kg) on the adenosinergic system in the first (0-5 min) and second (15-30 min) phase in formalin-induced nociception (2.5% v/v; intraplantar).

All results were expressed as the mean ± SD (standard deviation) (n=6). abc Different lowercase letters mean statistical difference, one-way ANOVA with post-hoc Tukey. (p < 0.05).

Source: Elaborate by the authors.

In short, it can be stated that EOEu has an antinociceptive effect at the central and peripheral levels, having as mechanisms of action the pathways opioid, cholinergic, adenosinergic system, and ATP-sensitive potassium channels (K+ATP). The antinociceptive effect of EOEu can be attributed to chemical components, which can act synergistically through different mechanisms. Researchers observed that the essential oil of *Croton sonderianus* has an antinociceptive effect, with ATP-sensitive potassium channels as a mechanism of action, presenting spatulenol and (E)-caryophyllene in common
with EOEu (Santos et al., 2005). The (E)-caryophyllene is a potent antinociceptive acting centrally via the opioid pathway. Studies should be carried out to investigate the possible antinociceptive mechanisms of compounds isolated from EOEu (Paula-Freire et al., 2014).

3.4 ANTI-INFLAMMATORY ACTIVITY

The results show all EOEu doses (50, 100, and 200 mg/kg) significantly inhibited edema, showing a reduction in the total inflammatory response (AUC) by 78.13%, 84.49%, and 94.64%, about the vehicle group. Indomethacin (20 mg/kg) significantly reduced AUC by 65.28% when compared to the control group, demonstrating an anti-inflammatory effect at all time intervals (Figure 6). These findings allow us to infer that EOEu acts to inhibit edema progression (decreased production of histamine, serotonin, and prostaglandins) (Antonisamy et al., 2010).
Figure 6. Effect of essential oil of *Eugenia uniflora* (EOEu) and Indomethacin (20 mg/kg) on carrageenan-induced paw edema in mice. A) Results are expressed differences between paw volume (mm) throughout treatment and (B) Total edema responses, measured as Area Under Curve (AUC) of treated groups.

All results were expressed as the mean ± standard deviation (SD) (n=6). abcDifferent lowercase letters mean statistical difference, two-way ANOVA (A), and one-way ANOVA with post-hoc Tukey (B). (p < 0.05).
Source: Elaborate by the authors.

The anti-inflammatory effects of the essential oil of other *Eugenia* species have already been reported in the literature. *Eugenia stipitata* essential oil was tested on paw edema induced by carrageenan and the results showed a significant reduction in the volume of paw edema, with inhibition of 88.66%, 91.3%, and 96.94% at concentrations of 40 mg/kg, 100 mg/kg, and 250 mg/kg, respectively (Costa et al., 2020). In tests with *Eugenia gracillima*, groups treated with doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg significantly reduced the volume of paw edema with an inhibition rate of 98.13%-98.20% when compared to the control group (Guedes et al., 2023). Likewise, treatment with *Eugenia*
pohliana essential oil promoted a 74.93%-81.4% reduction in carrageenan-induced paw edema (Nascimento et al., 2022).

A significant reduction in TNF-α and IL-1β levels was observed in the EOEu-treated groups and all analyses showed a dose-dependent relationship. At doses of 50, 100, and 200 mg/kg, the oil reduced the levels of TNF-α by 71%, 73.51%, and 81.84% and the levels of IL-1β were reduced by 48.86%, 62.56%, and 72.37% about the vehicle (Figure 7).

Figure 7. Effect of essential oil of Eugenia uniflora (EOEu) and Indomethacin (20 mg/kg) on TNF-α (A) and IL-1β (B) carrageenan-induced paw edema in mice.

All results were expressed as the mean ± SD (standard deviation) (n=6). Different lowercase letters mean statistical difference, one-way ANOVA with posthoc Tukey. (p < 0.05).

Source: Elaborate by the authors.

Based on the literature, to our knowledge, this EOEu chemotype has not yet been evaluated in anti-inflammatory trials (Maiolini et al., 2023). However, extracts and fractions of E. uniflora have demonstrated anti-inflammatory properties. The infusion of fresh leaves was analyzed in the carrageenan-induced paw edema model and showed a significant effect at doses of 150 and 300 mg/kg
(Schapoval et al., 1994). The aqueous fraction also significantly reduced IL-1β and TNF-α levels. Furthermore, the methanolic extract of *E. uniflora* reduced carrageenan-induced paw edema by 32% (Sobeh et al., 2019). These data demonstrate the high potential of this species as a source of anti-inflammatory compounds.

The effect of EOEu may be related to the richness of bioactive with anti-inflammatory action, such as selina-1,3,7(11)-trien-8-one epoxide (Soares et al., 2014). The anti-inflammatory effects of essential oil from other *Eugenia* species have been evidenced in previous studies (Costa et al., 2020; Nascimento et al., 2022). The in vivo anti-inflammatory activity of the EOEu, evidenced in this study, is reported for the first time, evidencing the novelty of this work.

### 3.5 WOUND HEALING ACTIVITY

Wound healing activity is an essential aspect of natural product research, especially in the context of essential oils. Essential oils are known to possess various properties that can contribute to the wound healing process, such as antimicrobial, anti-inflammatory, and antioxidant effects (Almadani et al., 2021).

A statistical difference was observed in the wound area regression in the group treated with EuOE compared to the negative control (saline) and positive control (Dersani) groups (Figure 8A). On the 3rd day of the experiment, the degrees of wound contraction were: OEEu = 47.1%; Negative Control (saline) = 13.7%; Positive Control (Dersani) = 19.5%. On the 7th day, the macroscopic retraction observed in the group treated with EuOE (71.7%) was statistically significant (p<0.05) when compared to the Negative Control (32.1%) and Positive Control (56.7%) groups. On the 14th day, the groups presented the following values of degree of contraction: OEEu = 98.8%; Negative Control (saline) = 87.9%; Positive Control (Dersani) = 91.5% (Figure 8B). The superior wound healing effects of EuOE compared to saline and Dersani (positive control) highlight its efficacy.
Figure 8. The wound healing effect of essential oil *E. uniflora* (EOEu). **A)** shows wound healing activity of the negative control, positive control, and EOEu groups on days 0, 3, 7, and 14. **B)** shows the change in wound size in mm² on days 0, 3, 7, and 14.

All results were expressed as the mean ± standard deviation (SD). 

**abc** Different lowercase letters mean statistical difference, one-way ANOVA with post-hoc Tukey. (p < 0.05).

Source: Elaborate by the authors.

Essential oils have been widely studied for their therapeutic properties, including healing activity. Studies have shown that these mixtures promote wound healing, as they are antimicrobial, anti-inflammatory, antioxidant, and stimulating cell proliferation, with these properties being essential for promoting tissue regeneration and the formation of an extracellular matrix suitable for healing. These effects are attributed to the complex chemical composition of essential oils, which often contain compounds such as terpenes, phenols, and aldehydes, known for their antimicrobial and anti-inflammatory properties (Khezri et al., 2019; Ahmadi et al., 2019). Further research is needed to better understand the mechanisms of action of essential oils and to optimize their use in wound care.

### 4 CONCLUSIONS

The essential oil from *E. uniflora* was found to contain major compounds such as selina-1,3,7(11)-trien-8-one, selina-1,3,7(11)-trien-8-one epoxide, germacrene B, and (E)-caryophyllene. It exhibited antinociceptive activity through...
both central (neurogenic) and peripheral (inflammatory) mechanisms. Additionally, the essential oil showed significant anti-inflammatory and healing activities. These findings suggest that the essential oil of *E. uniflora* L. is rich in bioactive compounds with potential applications in human and veterinary health. It could be utilized in pharmaceutical and biotechnological formulations.
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