



## Essential oil from the leaves of *Eugenia pohliana* DC. (Myrtaceae) alleviate nociception and acute inflammation in mice

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### Abstract

*Eugenia pohliana* DC. (Myrtaceae) is used in folk medicine by communities in Brazil. However, there are no reports on its biological activity. This is the first study to identify the components of *E. pohliana* essential oil (EpEO) and evaluate their antinociceptive and anti-inflammatory activities in an in vivo model at doses of 25, 50, and 100 mg/kg. The essential oil (EO) was obtained by hydrodistillation, and the analysis was performed by gas chromatography coupled with mass spectrometry. Antinociceptive activity was evaluated by writhing tests, tail movement, and formalin (neurogenic and inflammatory pain); naloxone was used to determine the nociception mechanism. Anti-inflammatory activity was assessed by oedema and peritonitis tests. We found that (E)- $\beta$ -caryophyllene (BCP) (15.56%),  $\delta$ -cadinene (11.24%) and  $\alpha$ -cadinol (10.89%) were the major components. In the writhing test, there was a decrease in writing by 42.95–70.70%, in the tail movement, an increase in latency time by 69.12–86.63%, and in the formalin test, there was a reduction in pain neurogenic by 29.54–61.74%, and inflammatory pain by 37.42–64.87%. The antinociceptive effect of EpEO occurs through the activation of opioid receptors. In addition, a reduction in inflammation by 74.93–81.41% was observed in the paw edema test and inhibition of the influx of leukocytes by 51.86–70.38% and neutrophils by 37.74–54.72% in the peritonitis test. It was concluded that EpEO has antinociceptive effect by the opioid pathway, as shown by the inhibitory effect of naloxone, and anti-inflammatory actions, and that its use does not cause hemolytic damage or behavioral change.

**Keywords** Caatinga ·  $\beta$  (E)-caryophyllene · Analgesic · Anti-inflammatory

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## Introduction

Inflammation is the response of the immune system to infections or tissue damage (Matsuda et al. 2019). Excessive or persistent activation can compromise organs and systems, leading to decompensation and organ dysfunction (Hirano et al. 2021). Pain is one of the signs of the inflammatory process; when it is constant or intermittent, it is a debilitating factor that impairs an individual's quality of life (McParland et al. 2021).

Analgesic and anti-inflammatory drugs are used to alleviate disturbances caused by the inflammatory process. However, many drugs have several adverse effects ranging from mild to severe. One of the main analgesics, morphine, can promote respiratory depression, constipation, nausea, vomiting, tolerance, and dependence (Azevedo Neto et al. 2020) whereas non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, can cause skin reactions and gastrointestinal, renal, and cardiovascular complaints (Wongrakpanich et al. 2018). Owing to these limitations, there is a need to seek alternative measures to treat pain and inflammation that may also help in the treatment of diseases (Lee et al. 2021; Russo et al. 2021).

Among natural medicinal products, essential oils extracted from plants have great pharmaceutical relevance (Mondal et al. 2021). Several activities have been described for these oils, such as anti-inflammatory (Han and Parker 2017), antinociceptive (Costa et al. 2020), antimicrobial (Souza et al. 2021), leishmanicidal (Nunes et al. 2021), larvicidal (Silva et al. 2021), antifungal (Kujur et al. 2021), antioxidant, and antiprotozoan (Sampaio et al. 2021). The presence of these activities encourages the discovery of secondary metabolites with pharmacological applications (Jacoby et al. 2021).

Many botanical families contain volatile compounds with medicinal properties, and the Myrtaceae family, which is widely distributed in the Caatinga biome, is a source of essential oils responsible for important health benefits associated (Macedo et al. 2021). One of the largest genera of the Myrtaceae family includes *Eugenia*, which encompasses approximately 1,000 species (Mazine et al. 2014), and contains essential oils with antioxidant properties (Franco et al. 2021) as well as healing (Silva et al. 2018); antimicrobial (Bezerra Filho et al. 2020), anti-inflammatory, and antinociceptive effects (Costa et al. 2020).

Thus, due to the variety of biological activities attributed to the genus, it is expected that the species *Eugenia pohliana* DC. and its essential oil have pharmacological potential; however, to our knowledge, there are still no reports of chemical studies or pharmacological activities of the preparations from this species. Thus, our objective

was to determine, for the first time, the chemical composition of the essential oil of *E. pohliana* leaves and the antinociceptive and anti-inflammatory properties induced by the action of other species of the genus using in vivo models.

## Materials and methods

### Herbal material

Leaves of *Eugenia pohliana* DC. were collected in June 2019 in Serra do Catimbau, in the municipality of Buíque, Pernambuco, Brazil (8° 30'57"S 37° 20' 59"W) a region of the Caatinga and registered in the National System of Management of Genetic Heritage and Associated Traditional Knowledge (A08E18B). An exsiccata (number 54805) was deposited in the Herbarium of Professor Vasconcelos Sobrinho (PEUFR) of the Department of Biology of the Federal Rural University of Pernambuco (UFRPE), Recife, Brazil. Then, the plant material was subjected to washing with running water, followed by natural drying in a clean, dry, and insect-free place for 6 h.

### Extraction

To obtain the essential oil from *Eugenia pohliana* DC. leaves (EpEO), the plant material after drying was ground and subjected to hydrodistillation (600 g, 4 h). The essential oil (EO) obtained was weighed, and the yield was calculated in % (m/m) and immediately stored in a dark flask under refrigeration (−5 °C) until used for analysis.

### Chemical composition of essential oil

Operating conditions, the (CG-MS) Shimadzu GCMS-QP2010 model. Nitrogen was used as gas from the GC transported by an Rtx®-5MS silica capillary column (30 mx 0.25 mm × 0.25 µm), at a flow rate of 1 L/min and an inlet pressure of 30 psi. and MS was optimized as follows: 70 eV, extraction gas (He) 13.6 mL.min<sup>-1</sup> and pressure of 53.5 kPa. Using the following temperature program: 100 °C (3 min) to 310 °C (3.5 °C/min). The identification of chemical components was performed by comparing the results obtained through the gas chromatography test coupled to mass spectrometry with results already existing in the literature, specifically in the NIST08 Mass Spectral Library, a mass spectral library of the GC-MS system, Wiley 9th Edition Mass Spectral Ratio Registry™ and other published data (Adams 2007).

## Animals

Male Swiss mice used weighed between 30 and 35 g, aged between 8 and 10 weeks, and were obtained from the bioterium of the Keizo Asami Immunopathology Laboratory (LIKA), Federal University of Pernambuco (UFPE) maintained under standard conditions for 12-h light/dark cycle, at  $22 \pm 2$  °C, with water ad libitum available. The experimental protocols used were approved by the Animal Use Ethics Committee (CEUA) of UFPE, protocol number 0070/2020. On the day of testing, the mice fasted for 6 h before each test.

## Haemolytic potential

Briefly, blood was collected by cardiac puncture in mice and centrifuged (3000 rpm, 10 min) where the supernatant was discarded and obtaining the erythrocyte concentrate (pellet) for the assay. A saline solution of the pellet (2%) was incubated in a microtube together with different concentrations of EpEO (156.25–5000 mg/mL) at room temperature (25 °C). After 30 min of incubation, the samples were centrifuged (3000 rpm, 5 min) and the supernatant collected and the hemoglobin released quantified in a spectrophotometer at 540 nm of absorbance (Jimenez et al. 2003).

## Antinociceptive activity

### Treatments

To evaluate the antinociceptive activity, groups of animals ( $n=6$ ) were treated orally with saline solution (negative control), EpEO (25, 50, or 100 mg/kg *per os*) 1 h before the evaluations, while the reference drug, indomethacin (10 mg/kg/*ip*) and morphine (10 mg/kg/*ip*) 30 min before. In the investigation of the antinociceptive mechanism, the animals were pretreated with a non-selective opioid receptor antagonist (naloxone 2 mg/kg) 30 min before treatment with the highest effective concentration of EpEO (100 mg/kg).

### Acetic acid-induced abdominal writhing test

The abdominal contortion model was performed according to Oliveira et al. (2018). Thirty-six mice were immobilized and received an intraperitoneal nociception-inducing agent (acetic acid 0.85% v/v; 0.1 mL/10 g b.w) and divided into 6 treatment groups. With the aid of a chronometer, the number of contortions performed by each animal was observed for 5 min after the application of acetic acid.

### Tail movement

Initially, the mice were pre-selected where their tails were immersed in a glass container containing distilled water

( $55 \pm 1$  °C) of which, which showed withdrawal reflex before 5 s, were selected and divided into five groups. Twenty-four hours later, treatments were administered, and again the same procedure was performed at times 0, 30, 60, 90, and 120 min. With a stopwatch, the time the animal took to perform a reflex action (tail movement) caused by heat, limited to 20 s, was recorded (Khatun et al. 2015).

### Formalin test

Following the Hunskaar and Hole (1987) protocol, thirty-six animals were randomly divided into 6 groups. Afterward, the mice were immobilized and received formalin (10%, 20  $\mu$ L) in the left hind paw and the licking time by the animal at the application site was recorded during two phases: the first phase (0–5 min) representing neurogenic pain; and second (15–30 min) cytokine-mediated inflammatory pain.

### Investigation of antinociceptive activity mechanisms

A group of mice received pretreatment with naloxone and 30 min later, 100 mg/kg of EpEO was applied. After 60 min, the formalin test was followed.

## Anti-inflammatory activity

### Treatments

To evaluate the anti-inflammatory activity, sixty male Swiss mice were used, thirty for each test. Groups of animals ( $n=6$ ) were treated orally with saline solution (negative control), EpEO at concentrations (25, 50 or 100 mg/kg b.w) or indomethacin (20 mg/kg) 1 h before the evaluations.

### Carrageenan-induced paw edema

Following the protocol by Winter et al. (1962), the paw edema test was performed to determine the mean variation of the volume of the paw (%) measured with a plethysmometer, during a predetermined period (0, 60, 120, 180, and 240 min) after the application of the phlogistic agent (15  $\mu$ L of 2% carrageenan) in the plantar region of the left paw. For the results, the formula was used: (percentage change (%)) = ((final paw volume – initial paw volume)/(initial paw volume)  $\times$  100).

### Peritonitis

Carrageenan-induced inflammation of the peritoneum test followed the protocol described by Oliveira et al. (2016). Briefly, 1% carrageenan (0.1 ml, 10 g b.w) was administered intraperitoneally and after 4 h the animals were anesthetized and an application of 2 ml of heparinized PBS was injected

into the peritoneal region followed by a gentle abdominal massage. Then, an aspiration of the peritoneal exudate for the quantification of leukocytes using an automatic analyzer was performed. Additionally, to investigate the role of regulator proteins in the inflammatory response, the quantification of cytokines levels (IL-1 $\beta$  and TNF- $\alpha$ ) in the peritoneal exudate was performed using the Millipore 7-plex kit (Millipore Corp., Billerica, MA, USA) as described by Oliveira et al (2021).

### Adverse effects assessment

To assess possible adverse effects caused by treatment with EpEO, groups of animals ( $n=6$ ) were submitted to open field and elevated plus-maze tests. Treatments were performed using a negative control group (0.9% saline) and EpEO (1000 mg/kg).

### Open field

Following Archer's protocols (1973) Mice from the control and EpEO-treated groups (1000 mg/kg) were positioned in the central part of a circular acrylic arena (75 cm  $\times$  75 cm  $\times$  50 cm) dividing it into four squares vertically and horizontally. Then, for 5 min, the mice were evaluated for the number of crossings (rearings) in the squares and the act of self-cleaning (crossing).

### Elevated plus-maze test

Several solid substances cause anxiety when ingested. Thus, the anxiety level of a mouse submitted to the elevated plus-maze test was evaluated as described by Kraeuter et al. (2019). Both groups of mice are individually allocated in the central part of the platform and recorded the number of entries and the time spent in open and closed arms. The animal's anxiety index was determined using the following equation:  $AI = 1 - [ \{ (\text{time spent in the open arms} / \text{total time in the maze}) + (\text{number of open-arm entries} / \text{total entries in the maze}) \} / 2 ]$ , the closer to 1 the value, the more anxious the animal was during the test.

### Statistical analysis

Data analysis was performed using GraphPad Prism® version 8.0 and expressed as mean values with standard deviation ( $\pm$  SD). Using one-way analysis of variance (ANOVA) we calculated statistically significant differences ( $p < 0.001$ ) followed by Bonferroni or Dunnett's.

## Results and discussion

### Chemical characterization

This is the first report of the chemical composition of the essential oil extracted from *Eugenia pohliana* DC. leaves. The chemical components, their respective retention indices (RI), and relative amounts of EO extracted from *E. pohliana* leaves are shown in Table 1. The EpEO presented a yellowish color, and the chemical characterization showed 38 compounds, comprising 98.73% of the total. The major components were sesquiterpenes: (E)- $\beta$ -caryophyllene (15.56%),  $\delta$ -cadinene (11.24%), and  $\alpha$ -cadinol (10.89%).

The presence of (E)- $\beta$ -caryophyllene (BCP), an odorous bicyclic sesquiterpene, common in plants of this family and has been reported as a component of *Eugenia brejoensis* Mazine species that have antimicrobial activity (Mendes et al. 2018); and *Eugenia calycina* Cambess., which has larvicidal (Silva et al. 2021), cytotoxic, and antimicrobial activity (Sousa et al. 2015). BCP is also found in *Eugenia dysenterica* DC., which has healing activity (Silva et al. 2018); *Eugenia sulcata* Spring ex Mart. which has insecticidal activity (Gonzalez et al. 2014) and *Eugenia egensis* DC. which has cytotoxic and antioxidant properties (Silva et al. 2017).

BCP, a phytocannabinoid, acts as a ligand for cannabinoid receptor-2 (CB-2) (Gertsch et al. 2008), a part of the endocannabinoid system that is involved in cell signaling (Meccariello et al. 2020). When activated, this receptor inhibits mediators of inflammation, contributing to the relief of pain and inflammation (Maayah et al. 2020). Compounds that interact with the endocannabinoid system have shown promise for the treatment of various diseases (Shah et al. 2021). Other activities can be attributed to BCP, such as antimicrobial activity against *Staphylococcus mutans* (Yoo and Jwa. 2018), antiherpetic (Astani et al. 2011), wound healing (Koyama et al. 2019), anti-inflammatory (Brito et al. 2019), and protective effects against ischemic brain injury (Chang et al. 2013).

Another compound present in the composition of EpEO was  $\delta$ -cadinene, found in the oil of *Eugenia caryophyllata* Thunb. (Wang et al. 2021) which showed antinociceptive and anti-inflammatory activities (Taher et al. 2015) and *Eugenia brasiliensis* Lam., which showed significant antimicrobial activity (Silva et al. 2019). This compound has demonstrated acaricidal activity against *Psoroptes cuniculi* (Guo et al. 2017).

The oxygenated sesquiterpene  $\alpha$ -cadinol is a termiticide (Morikawa et al. 2014) reported in the composition of *E. brejoensis* (Mendes et al. 2018) and *Eugenia pyriiformis* Cambess. (Durazzini et al. 2019). To date, there

**Table 1** Chemical composition of the essential oil obtained from the leaves of *E. Pohliana* (EpEO)

Compone	RI <sup>a</sup>	RI <sup>b</sup>	%	Compone	RI <sup>a</sup>	RI <sup>b</sup>	%
$\alpha$ -pinene	931	932	0.34	$\gamma$ -cadinene	1515	1513	3.37
$\beta$ -pinene	973	974	0.67	$\delta$ -cadinene	1526	1522	11.24
Limonene	1027	1024	0.06	trans-cadina-1,4-diene	1534	1533	0.13
$\delta$ -elemene	1337	1335	3.19	$\alpha$ -cadinene	1539	1537	1.02
$\alpha$ -cubebene	1349	1348	0.11	$\alpha$ -Calacorene	1544	1544	0.70
$\alpha$ -ylangene	1371	1373	0.25	Elemol	1550	1548	0.12
$\alpha$ -copaene	1375	1374	0.62	Germacrene B	1558	1559	0.52
$\beta$ -elemene	1392	1389	3.06	Palustrol	1568	1567	0.78
$\alpha$ -gurjunene	1410	1409	1.73	Spathulenol	1578	1577	1.69
$\beta$ - (E) -caryophyllene	1421	1417	12.56	Globulol	1585	1590	2.57
$\alpha$ -humulene	1454	1452	3.09	Guaiol	1593	1600	2.41
Allo-aromadendrene	1461	1458	1.50	Ledol	1604	1602	1.48
trans-candina-1(6),4-diene	1474	1475	1.63	1,10-di-epi-cubenol	1616	1618	0.74
$\gamma$ -muurolene	1477	1478	1.34	1-epi-cubenol	1629	1627	1.53
$\alpha$ -amorphene	1481	1483	4.51	tau.-Muurolol	1645	1644	10.81
$\beta$ -selinene	1487	1489	1.36	$\alpha$ -cadinol	1659	1652	10.89
Bicyclogermacrene	1498	1500	8.13	Shyobunol	1692	1700	0.10
$\alpha$ -pinene	931	932	0.34	eudesm-7(11)-en-4- $\alpha$ -ol	1697	1700	0.10
$\beta$ -pinene	973	974	0.67	$\gamma$ -cadinene	1515	1513	3.37
				Total			98.73

% Area of compost relative to EpEO

<sup>a</sup>RI retention rate determined

<sup>b</sup>RI retention index specialized literature

are no reports of antinociceptive and anti-inflammatory activities for these species, but other pharmacological activities have been described, such as antimicrobial activity in *E. brejoensis* (Bezerra Filho et al. 2020) and *E. pyriformis* (Souza et al. 2021).

Other species of the Myrtaceae family with biological activity contain  $\alpha$ -cadinol, such as *Myrcia tomentosa* (Aubl.) DC. which has antimicrobial activity (Sa et al. 2017), *Eucalyptus occidentalis* Endl., which has shown repellent and insecticidal activity (Bande-Borujeni et al. 2018) and *Plinia trunciflora* (O.Berg) Kausel, which has antimicrobial activity against yeasts and bacteria (Lago et al. 2011).

### Hemolytic activity

Haemolysis generally occurs by lysis of erythrocytes, potentially leading to hemolytic anemia. A variety of substances can induce hemolysis, making assessment of this activity extremely important (Barros et al. 2016). Thus, EpEO had a hemolysis rate in the range of 0.74 and 2.2% at the concentrations tested. These data show low toxicity, as they do not present significant hemolytic activity (Jimenez et al. 2003).

### Antinociceptive activity

#### Acetic acid-induced writhing test

The acetic acid-induced writhing model, although not specific, is a simple and sensitive test that is the standard for evaluating candidate drugs with antinociceptive action (Hunnskaar and Hole 1987). EpEO reduced writhing in animals by 42.95%, 52.94%, and 70.70% when treated with doses of 25, 50, and 100 mg/kg, respectively. The standard drugs morphine and indomethacin promoted a reduction of 98.83% and 85.88%, respectively, when compared to the control group (Fig. 1).

The genus *Eugenia* has shown positive results in tests of writhing induced by acetic acid; for example, the methanolic extract of *Eugenia uniflora* L. leaves lead to 60% and 74% reduction in writhing at concentrations of 100 and 200 mg/kg, respectively. The EO from *E. caryophyllata* leaves inhibited 89.6% of abdominal writhing induced by acetic acid in mice in the treatment with a concentration of 100 mg/kg (Taher et al. 2015), which was attributed to the presence of BCP as a major compound in its chemical composition (Wang et al. 2021).

**Tail Immersion**

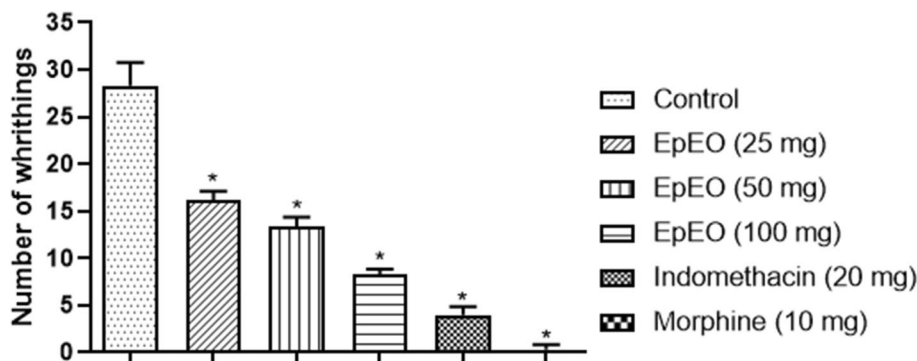
The tail-immersion test is a highly sensitive test for opioid drugs and is ideal for the evaluation of drugs that act on the central nervous system (Oliveira et al. 2018; Khatun et al. 2015). EpEO promoted greater analgesic action after 90 min of administration, with an increase in latency time by 86.63–69.12% compared to control (Fig. 2). Treatment with 100 mg/kg of EpEO or morphine showed an antinociceptive action above 50% at assessment time points. These results

indicate that EpEO has a central analgesic effect similar to that of morphine (Kotlinska et al. 2013).

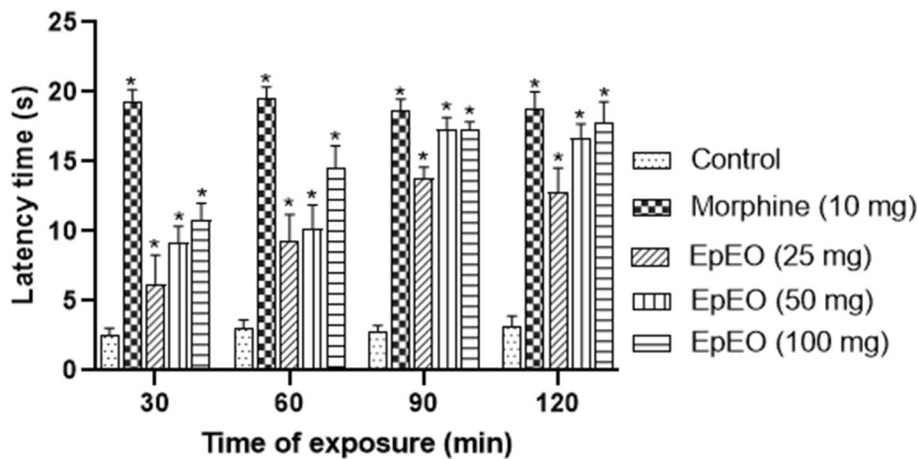
**Formalin test**

In the formalin-induced nociception test, EpEO showed antinociceptive action at all doses and during both phases of the test. A significant reduction ( $p < 0.001$ ) in time spent licking the paw was achieved; 61.74%, 65.5%, and 29.54% in the first phase (neurogenic pain) and in 64.87%, 64.3%,

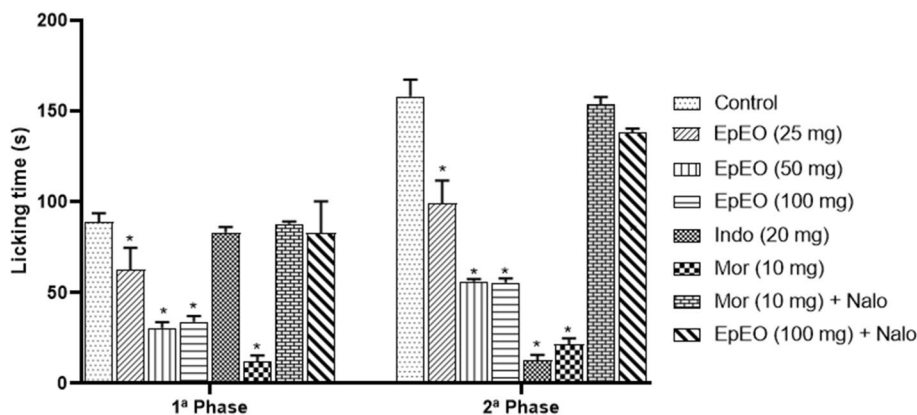
**Fig. 1** Effect of *Eugenia pohliana* essential oil (EpEO) on abdominal contortion induced by acetic acid. Legend: EpEO: *Eugenia pohliana* essential oil. Values represent the mean  $\pm$  SEM. \* $p < 0.001$  compared with Control, one-way ANOVA followed by Dunnett's Test



**Fig. 2** Effect of *Eugenia pohliana* essential oil (EpEO) on tail-immersion assay. Legend: values represent the mean  $\pm$  SEM. \* $p < 0.001$  compared with control, one-way ANOVA followed by Dunnett's test



**Fig. 3** Effect of *Eugenia pohliana* Essential Oil (EpEO) on both phases of the formalin assay. Legend: values represent the mean  $\pm$  SEM. \* $p < 0.001$  compared with control, one-way ANOVA followed by Dunnett's test



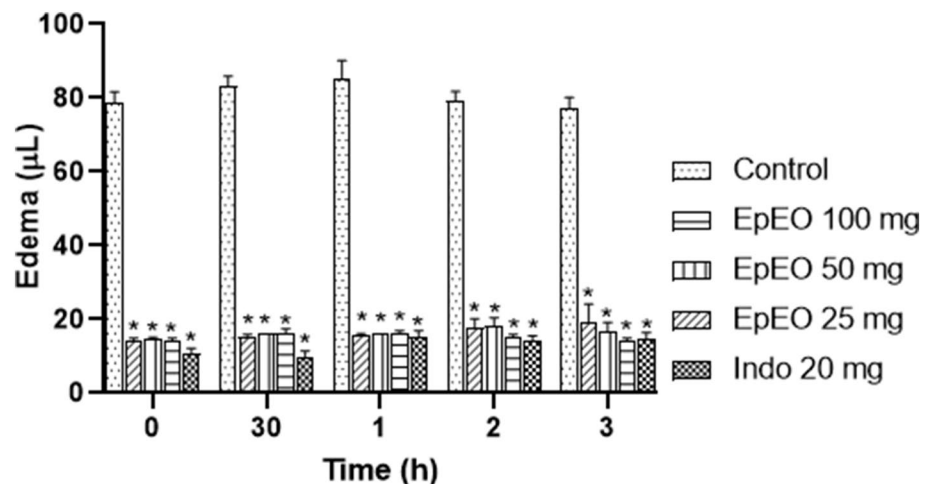
and 37.42% in the second phase (inflammatory pain) at doses of 100, 50, and 25 mg/kg, respectively, compared to the control (Fig. 3). Morphine, the standard drug, reduced licking time in both phases (86.5–86.3%), while indomethacin was effective only in the second phase of the test (92%) when compared to the control.

The EO of *Eugenia candolleana* DC. leaves, with composition dominated by BCP, inhibited the licking time after formalin injection by up to 55.65% in the first phase; however, the results were more significant in the second phase, with inhibition of up to 96.7% at a dose of 100 mg/kg (Guimarães et al. 2009). According to the literature, BCP is among the major compounds of this species (Neves et al. 2017). Santos et al. (2020) reported that acetone extract from *Myrciaria floribunda* (H. West ex Willd.) O. Berg fruits reduced licking time by up to 86.52% in the treatment with 100 mg/kg in the first phase and 82.58% in the second phase. (Silva Barbosa et al. 2020) described the chemical composition of the EO of *M. floribunda* fruit peels as dominated by BCP and  $\delta$ -cadinene (Silva Barbosa et al. 2020).

Commercially obtained BCP alone has a significant effect on inflammatory pain in the formalin test; however, it does not have any treatment effect on neurogenic pain (Klauke et al. 2014). Thus, the significant results in the inflammatory pain phase of EpEO treatment may be related to the presence of BCP.

To investigate the mechanism of action and antinociceptive activity using the formalin test, we pretreated the rats with naloxone, a non-selective opioid receptor antagonist, followed by treatment with EpEO 100 mg/kg or morphine. The action of EpEO was almost entirely inhibited, similar to morphine, in the two phases of the formalin test (Fig. 3). These results suggest that the antinociceptive effect of EpEO occurs through the activation of opioid receptors (Lewanowitsch et al. 2006), as suggested by the tail-flick test.

**Fig. 4** Responses of different concentrations of *Eugenia pohliana* essential oil (EpEO) to paw oedema induced by carrageenan. Legend: values represent the mean  $\pm$  SEM. \* $p < 0.001$  compared with control, one-way ANOVA followed by Dunnett's Test



## Anti-inflammatory activity

### Carrageenan-induced paw oedema

As previously mentioned, there are no studies on the biological activities of *E. pohliana*; however, based on evidence of anti-inflammatory action of the genus *Eugenia* in addition to the expressive action of the treatment with EO in the second phase (inflammatory phase) of the formalin test, we investigated the anti-inflammatory potential using the paw edema test to screen for this possible effect. Indeed, treatment with EpEO promoted a reduction of 74.93–81.4% in paw oedema induced by carrageenan, while indomethacin inhibited it by 81.1% at 3 h when compared to control (Fig. 4). The activity of EpEO and indomethacin remained constant until the end of the test.

Similarly, Costa et al. (2020) described that an *Eugenia stipitata* McVaugh EO had an anti-inflammatory effect on paw oedema, reducing it by up to 96.94% after treatment with 250 mg/kg. According to Sobeh et al. (2019), the paw edema test with the methanolic extract of *E. uniflora* leaves reduced edema by 32%. In this study, the inhibition potential for Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) was determined. The results show significant inhibition, with IC<sub>50</sub> values of 5.63  $\mu$ g/mL for COX-1 and 0.18  $\mu$ g/mL for COX-2. The inhibition of these enzymes prevents the production of inflammatory substances and the action of anti-inflammatory NSAIDs (Trinh et al. 2021). Mesquita et al. (2017) reported that BCP is a major volatile chemical extracted from *E. uniflora*.

### Peritonitis

After confirming the anti-edematogenic effect of the EpEO treatment, the carrageenan-induced peritonitis test was performed as a model of acute inflammation. Oral administration of EpEO promoted a significant reduction ( $p < 0.001$ )

**Table 2** Effect of *Eugenia pohliana* essential oil (EpEO) on leukocyte migration and neutrophil migration in peritoneal exudation in carrageenan-induced

Treatments	Dose (mg/kg)	Leukocytes ( $10^5$ /ml)	Inhibition (%)	Neutrophils ( $10^5$ /ml)	Inhibition (%)
Control	–	$8.1 \pm 0.9$	–	$5.3 \pm 0.5$	–
Indomethacin	20 mg/kg	$1.7 \pm 0.6^*$	79.01	$1.5 \pm 0.3^*$	71.69
EpEO	100 mg/kg	$2.4 \pm 0.6^*$	70.38	$2.4 \pm 0.3^*$	54.72
	50 mg/kg	$3.2 \pm 0.5^*$	60.50	$2.7 \pm 0.3^*$	49.06
	25 mg/kg	$3.9 \pm 0.5^*$	51.86	$3.3 \pm 0.2^*$	37.74

Values represent the mean  $\pm$  SEM

\* $p < 0.001$  compared with Control, one-way ANOVA followed by Dunnett's Test

of 51.86–70.38% and 37.74–54.72% in the influx of leukocytes and neutrophils, respectively, at all doses tested. Indomethacin inhibited 79.01% of leukocyte migration by 71.69% of neutrophils (Table 2).

Lazarini et al. (2020) showed that oral treatment with ethanolic extract of *Eugenia selloi* B.D.Jacks. fruits promoted a decrease in neutrophils by 58% and 70% at concentrations of 3 and 10 mg/kg, respectively. A methanolic extract of *E. uniflora* leaves (100 mg/kg) reduced the number of leukocytes to  $4.84 \pm 1.97 \times 10^6$  mL compared to untreated animals ( $10.04 \pm 2.64 \times 10^6$  mL) (Sobeh et al. 2019). BCP, the main compound of EpEO, reduces the peritoneal migration of neutrophils at a dose of 100  $\mu$ L (Brito et al. 2019). Such anti-inflammatory effects may be related to its ability to activate CB-2 receptors, as the absence of these receptors suppresses the inflammatory response in animal models (Gertsch et al. 2008).

Costa et al (2022) reported the effects of essential oil from *Psidium glaziovianum* Kiaersk. P. promoted a reduction of inflammatory cytokines and attributed the presence of monoterpenes in its composition such as 1,8-cineole,  $\alpha$ -pinene, and  $\beta$ -pinene. In our study, treatment with EpEO (25, 50 and 100 mg/kg *per os*) promoted a significant reduction ( $p < 0.001$ ) in TNF- $\alpha$  levels (51.27, 55.57 and 66.58%) and IL-1 $\beta$  (51.40, 60.16 and 73.64%) (Fig. 5). As expected, the indomethacin group reduced TNF- $\alpha$  and IL-1 $\beta$

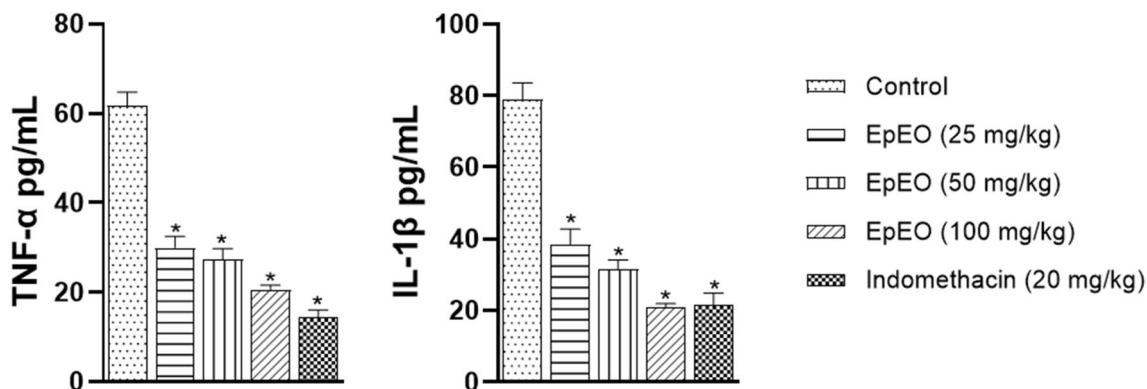
by 76.59 and 72.71%, respectively. The hypothesis of anti-inflammatory action is possibly related to the presence of  $\beta$ -caryophyllene (Aguilar-Ávila et al. 2019) in the composition of EpEO. These findings confirm the anti-inflammatory action of EpEO in a dose-dependent.

## Adverse effects assessment

### Open field and elevated plus-maze test

In general, analgesic agents have direct side effects on the central nervous system (CNS). To test for these side effects, we used the open field and elevated plus maze tests, which have become a standard for measuring anxiety, sedation, and activity not only in rodents but also in several other animals (Prut and Belzung 2003). The absence of the animal's exploratory behavior indicates that the substance tested has sedative and depressant activity (File and Wardill 1975; Mujumdar et al. 2000).

Thus, to investigate the occurrence of this possible effect in the treatment with EpEO, we tested the animals in the open field and elevated plus maze test after a dose of 1000 mg/kg, which is 10 times greater than the therapeutic dose used in our tests. Treatment with EpEO in the open field test showed no significant differences in rearing ( $20 \pm 2$ ) and crossing ( $58 \pm 12$ ). However, diazepam



**Fig. 5** TNF- $\alpha$  and IL-1 $\beta$  levels in peritoneal exudate from mice treated with saline (control) or *Eugenia pohliana* essential oil (EpEO). Legend: values represent the mean  $\pm$  SEM. \* $p < 0.001$  compared with control, one-way analysis of variance (ANOVA) followed by Dunnett's test



resulted in an absence of the animals' exploratory behavior with values of  $8 \pm 1$  for rearing and  $37 \pm 6$  for crossing. The control group showed rearing of  $25 \pm 3$  and crossing of  $75 \pm 7$ .

In the LCE test, treatment with EpEO did not promote anxiety symptoms, presenting an anxiety index of 0.54, which was not significant when compared with the control (0.41). Diazepam, on the other hand, had an anxiety index of 0.09, indicating that its neurological activity caused anxiety in the animals.

Thus, our results revealed that EpEO treatment did not affect exploratory activity and anxiety. In contrast, diazepam at 1 mg/kg showed significant values in relation to the control. Based on these results, EpEO can be considered as a possible herbal medicine candidate for the treatment of pain and inflammation.

## Conclusion

The essential oil of *Eugenia pohliana* leaves showed biological activity and reduced inflammatory and non-inflammatory pain as well as acute inflammatory processes. Antinociceptive activity has a mechanism of action via the opioid pathway, the same pathway as morphine. Regarding the chemical components present in EpEO, the large presence of BCP may be the underlying reason for its activity in relieving pain and inflammation. BCP is a CB-2 ligand that acts in the regulation of inflammatory mediators. Additionally, EpEO showed no effect on behavior and its hemolytic potential was not significant; thus, it is a promising candidate for the development of new analgesic and anti-inflammatory molecules.

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**Author contributions** ALD, JBG, WKC, AMO performed the methodologies. WKC, AMO analyzed and interpreted the results. BOV performed the essential oil extraction. ALD, WKC, AMO wrote the article. JCROFA, DMAFN performed the analysis and chemical characterization of essential oil. AMO, MVS performed a critical review of the final version of the article. MTSC, THN, AMO, MVS contributed reagents, materials and analytical tools.

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## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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