



Chemical characterization, antinociceptive and anti-inflammatory effect of *Lippia lacunosa*, a species used by the Bandeirantes



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ABSTRACT

Ethnopharmacological relevance: *Lippia lacunosa* Mart. & Schauer is an endemic plant from the Serra do Espinhaço mountain range located on the Atlantic plateau, Brazil. It is known as “chá de pedestre” and “rosmaninho” in folk medicine. This species has a characteristic mango aroma and is widely used by the population for flu, colds, sinus infections, coughing, relaxing baths, and foot baths after long walks. It is often confused with and, therefore, used interchangeably with *L. rotundifolia* and *L. pseudothea*.

Aim of the study: This study aimed to increase scientific knowledge on the ethnopharmacological use of *Lippia lacunosa* through the evaluation of the micromolecular composition and anti-inflammatory and antinociceptive activities of the hexane and ethanolic extracts, essential oil, and fractions in mice.

Materials and methods: The chemical profile of *L. lacunosa* extracts and fractions were obtained by chromatographic methods such as Ultra-Performance Liquid Chromatography (UPLC), Gas Chromatography (GC), Column Chromatography (CC), and Thin Layer Chromatography (TLC). Carrageenan-induced paw edema was used to investigate the anti-inflammatory activity in mice. Mechanical allodynia induced by carrageenan and hot plate tests were employed to evaluate the antinociceptive activity.

Results: The main constituents found in the essential oil were the monoterpenes myrcene (13.81%), linalool (6.84%), ipsenone (21.2%), and myrcenone (25.44%); and sesquiterpenes elemol (7.30%) and spathulenol (3.15%). The chromatograph fractionation of essential oil yielded a fraction rich in the main compounds (F33), ipsenone and myrcenone. In experimental models of paw edema and mechanical allodynia induced by carrageenan (600 µg, 30 µL, i.p.), the administration of hexane extract, essential oil (50 or 100 mg/kg, p.o.) or majority fraction (10 mg/kg, p.o.) reduced paw edema. The ethanolic extract (100 mg/kg) reduced mechanical allodynia only in the 2nd h of evaluation. On the other hand, the hexane extract (50 or 100 mg/kg) and essential oil (100 mg/kg), as well as the majority fraction (10 mg/kg), reduced mechanical allodynia throughout the evaluation period. The hexane extract, essential oil, and majority fraction F33 also reduced the heat-induced nociceptive response. Also, majority fraction F33 did not affect the time mice spent in the rota-rod apparatus.

Conclusions: The elucidation of the composition of the essential oil and the demonstration of the activity of *L. lacunosa* in experimental models of acute inflammation and also in models of nociceptive and inflammatory pain can help to increase knowledge on the ancient ethnopharmacological use by the Bandeirantes, aiming at the evaluation of the species as a candidate for herbal medicine or phytopharmaceutical in the treatment of patients with inflammatory and painful conditions.

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1. Introduction

“Chá de pedestre,” “rosmaninho,” and “chá de frade” are popular names that refer to at least three species of the genus *Lippia* (*L. lacunosa*, *L. pseudothea*, and *L. rotundifolia*). They are endemic to Serra do Espinhaço, Minas Gerais - Brazil, and known for many years by the Brazilian population and naturalists (Echternacht et al., 2011; Brandão et al., 2011). They look so much alike that they are used interchangeably by the population and a botanical identification by specialist is needed to differentiate them. Although, in many herbaria, *L. lacunosa* is considered synonymous with *L. pseudothea* and *L. rotundifolia*, there are differences in their chemical composition (Brandão, 2010; Leitão et al., 2008; Meira et al., 2017; Salimena and Silva, 2009).

Lippia lacunosa Mart. & Schauer belongs to the Verbenaceae family and, together with other species of the genus, forms a complex of difficult taxonomic delimitation. It is a monoecious shrub, 0.70–2 m tall, and has tetragonal branches, sessile glandular trichomes, leaves with a leathery blade, and inflorescences with a pink or lilac tube corolla. Its flowers, leaves, and essential oil have a strong, characteristic and pleasant mango odor, related to the presence of myrcene and myrcenone (Leitão et al., 2008; Meira et al., 2017; Salimena and Silva, 2009). The leaves of this plant are widely used by the population for flu, colds, sinusitis, coughing, relaxing baths, and foot baths after long walks (Brandão, 2010; Meira et al., 2017; Pascual et al., 2001). But, despite its wide popular use, few studies have been carried out with the species.

The genus *Lippia* is known for presenting species rich in phenolic compounds such as flavonoids and phenylpropanoids. These substances, in turn, have great potential in pain treatment since they are related to antioxidant and anti-inflammatory activity (Leite et al., 2022a; 2022b). Also, preclinical assays have shown that *Lippia* species exhibit activities in experimental models of inflammation and pain (Ahmed et al., 2004; Monteiro et al., 2007; Mendes et al., 2010; Guilhon et al., 2011; Guimarães et al., 2012; Al-Snafi and Faris, 2013; Oliveira et al., 2014). Experimental models, including paw edema and mechanical allodynia induced by carrageenan and hot plate test, are widely used models for the investigation of anti-inflammatory and analgesic drug candidates.

Thus, investigation related to the folk use of *L. lacunosa*, as well as its chemical constituents, may provide new therapeutic options for the treatment of pain and inflammation. Pain and inflammation, especially of the chronic type, represent a major challenge of modern medicine. Currently available drugs have many adverse effects, especially in the long term. In this sense, new biological targets are being researched and medicinal plants are an excellent option, since they are related to fewer adverse effects and their phytocomplex allows them to act on different targets, often resulting in better activity (Bost et al., 2010; Chopra and Dhingra, 2021; Mahesh et al., 2021; Manion et al., 2019; Newman and Cragg, 2020).

A successful example was the development of Acheflan® from the essential oil of *Cordia verbenacea* in Brazil, an anti-inflammatory for local use that acts to relieve pain associated with inflammation of muscles and tendons (Martim et al., 2021). In this context, the phytochemical study and the evaluation of the anti-inflammatory and antinociceptive activities of the leaves of *L. lacunosa* can help to make the best use of the species and contribute to the research of phytopharmaceuticals or herbal medicines useful in the treatment of pain and inflammation, as well as how to increase scientific knowledge about Brazilian plant species used medicinally by the population and its preservation in the Serra do Espinhaço, Minas Gerais. In our study, the anti-inflammatory and antinociceptive profile of *L. lacunosa* was initially confirmed by demonstrating its effects in an experimental model of paw edema, model of inflammatory pain induced by carrageenan and nociceptive pain induced by heat in mice.

2. Materials and methods

2.1. Plant material

Leaves of *Lippia lacunosa* Mart. & Schauer, Verbenaceae, were collected in São Gonçalo do Rio das Pedras (Serro), Minas Gerais, Brazil (18° 25' 51" S; 43° 28' 55" W), in the ruprestrian fields of Serra do Espinhaço, in September of 2017 and 2018. The specimens were identified by the professor and biologist, Dr. Fátima Regina Gonçalves Salimena, from the Institute of Biological Sciences of the Federal University of Juiz de Fora (UFJF), and the exsiccates were deposited in the herbarium of the Institute of Biological Sciences (ICB) of the Federal University of Minas Gerais (UFMG) under the number BHCB111231. The registration was carried out in the National System of Genetic Heritage and Associated Traditional Knowledge Management (SisGen) (code A30CE2A).

2.2. Extraction

The fresh leaves of *L. lacunosa* were dried in a ventilated oven ($T < 40^{\circ}\text{C}$) and pulverized in a knife mill (Marconi knife mill, model TF 680). The powder (5.0 g) was extracted by maceration with 100 mL of commercial ethanol (EtOH) 92.8° assisted by ultrasound for 20 min. The mixture was centrifuged (Centrifuge, Edutec, model 9004/B), 500 g, using conical Falcon tubes, for 10 min. After decanting, the supernatant was filtered through a simple glass funnel with cotton into a round-bottomed flask with a capacity of 500 mL and the residual material was reserved to further extraction. This procedure was repeated three times. After extraction, solvents were evaporated at 40°C in a rotary evaporator (Buchi rotary evaporator, model R-114, and water bath B-480), under reduced pressure, until the solvents were completely eliminated. The same process was carried out with 100% hexane (Hex). The powder ethanolic and hexane extracts were stored at -20°C until analysis.

2.3. Essential oil obtention

Essential oil of *L. lacunosa* was obtained through hydrodistillation in a Clevenger apparatus, as described in the Brazilian Pharmacopoeia 6th edition (ANVISA, 2019). Dried and coarsely fragmented leaves (50 g) and 500 ml of ultrapure water (Millipore Direct-Q Water Purifier) were added in a 1000 ml round bottom flask. The extraction time was 120 min from the boiling point of the water and then, essential oil was collected with the aid of anhydrous sodium sulfate (Na_2SO_4) to remove any residual water. The essential oil was stored in a container protected from light, at -20°C until analysis.

2.4. UPLC-UV-DAD analysis

Extracts and essential oil were analyzed by Ultra-Performance Liquid Chromatography (UPLC) in an ACQUITY Ultra Performance system (Waters, USA) with an ultraviolet/diode array detector (UV/DAD) (Waters) and Empower program for data processing (Waters). For this, 5 mg of the ethanolic was solubilized in a methanol High-Performance Liquid Chromatography (HPLC) grade (1.0 mL), and hexanic extract and essential oil were solubilized in acetonitrile HPLC grade (1.0 mL), respectively. The samples were sonicated in an ultrasound bath for 15 min and centrifuged (625 g) for 10 min. The supernatant was filtered through a Millipore membrane (0.22 μm). Chemical profiles were obtained using ultrapure water acidified with 0.1% formic acid (A) and acetonitrile acidified with 0.1% formic acid (B) as mobile phase for 28 min in a reversed phase column (Acquity UPLC® BEH C18, 100 \times 2.1 mm i.d., particle size 1.7 μm), VanGuardTM C18 pre-column (2.1 \times 5 mm, 1.7 μm) at 40°C and 0.3 mL/min flow. The following gradient elution scheme was used: 5–95% B at 0–24 min, 5% B at 24–26 min, and 95% B at 26–28 min.

2.5. Gas Chromatography-mass spectrometry (GC-MS) analysis (hexane extract and essential oil)

Analysis of the hexane extract and the essential oil of *L. lacunosa* was also performed by GC-MS. A GC System 6890N SII was used coupled to a 5973 Network mass spectrometer, both from Agilent Technologies, with an Agilent 122-5532 DB-5MS capillary column (30 m × 250 µm × 0.25 µm), helium as carrier gas in a constant flow of 1 mL/min, temperature programming of 60–290 °C (10 °C/min), 70V, transferline temperature of 280 °C, and ion source 230 °C.

2.6. Fractionation of the essential oil

For fractionation of essential oil of *L. lacunosa* (1.2 g), a 42 cm high and 1.2 cm wide glass chromatographic column was used, filled with adsorbent silica gel 60 (0.063–0.200 mm – Merck) and eluted with solvents and mixtures of increasing polarity: hexane:ethyl acetate, in a continuous flow of 20 drops/min. The 74 fractions obtained were dried, weighed, and monitored by TLC (Thin Layer Chromatography), and finally frozen in a freezer (−22 °C).

2.6.1. Thin layer chromatography

TLC chromatographic profiles were obtained to evaluate the separation of terpenes present in the essential oil and its fractions. The mobile phase used was hexane:ethyl acetate (80:20) and the reagent used to obtain the chromatogram was a solution of sulfuric anisaldehyde with posterior heating at 100 °C (heat gun D26411, DeWalt).

2.6.2. GC-MS analysis (essential oil and fraction)

The essential oil and fraction rich in the main compounds (F33) were also analyzed by GC-MS. The test was performed in a gas chromatograph (Agilent 7890B) equipped with a mass spectrometry detection system (Agilent 5977A -MSD), with a quadrupole mass analyzer. The column used was a capillary type CP – WAX 52 CB (Polyethylene glycol, 30 m × 0.25 mm × 0.25 µm internal diameter). The oven temperature programming started with 60 °C for 5 min and then 25 °C was added per minute until the temperature of 160 °C was reached, remaining for another 5 min. In sequence, 10 °C was added per minute until a final temperature of 240 °C, remaining at this temperature for 5 min, totaling an analytical run of 27 min. The carrier gas used was helium at a constant flow of 1 mL per minute. The injection mode used was with a flow division (split) of 1:10 and injection volume of 1 µL. Data acquisition took place in SCAM mode, using a mass-charge ratio (*m/z*) from 14 to 500. The chromatograph interface with the detector was maintained at 240 °C and electron impact ionization operated at 240 °C was used. The mass analyzer was a simple quadrupole type operated at 150 °C. A volume of 10 µL of essential oil was dissolved in 1.0 mL of hexane, before the sample was injected into the equipment. The mass and fragmentation profile of the peaks found were compared with the spectra bank of the National Institute of Standards and Technology (NIST) library and data from the scientific literature and the ADAMS library (2007), with the calculation of the KOVATS index described below.

Equation for calculating the KOVATS index (KI)

$$KI = \left(\frac{Rt(x) - Rt(n)}{Rt(n+1) - Rt(n)} \right) 100Z + 100N$$

Rt=Retention time

x = Substance to be identified

n = Shorter-chain hydrocarbon (with Rt before Rt(x))

n+1 = Longer-chain hydrocarbon (with Rt after Rt(x))

Z = difference between number of carbon atoms in the longer and shorter chain

N=Number of carbo atoms in the shortest chain standard

2.7. Experimental animals

Male Swiss mice (25–30 g) were used in the experiments. The animals were acclimatized to a room with a 12 h light-dark cycle for at least three days before the experiment. Water and food were provided ad libitum. A room temperature of 27 °C, corresponding to the thermo-neutral zone for mice, was used. The study was approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais (Protocol 277/2017) and carried out according to the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983).

2.8. Drugs

Type IV λ-carrageenan (Sigma, USA), Dexamethasone 21-phosphate disodium (Sigma, USA), Sodium dipyrone (Sanofi Aventis, Brazil), and Phenobarbital (Gardenal®, Sanofi Aventis, Brazil) were used. Sodium chloride 0.9% w/v solution (Sanobiol, Brazil) and Carboxymethylcelulose (CMC, 0.5% w/v in sodium chloride solution) (Sigma-Aldrich, USA) were used. All solutions or suspensions were prepared immediately before each experiment.

The carrageenan suspension was prepared in 0.9% w/v sodium chloride solution the day before the experiment and stored under refrigeration. 30 µL (600 µg of carrageenan) of this suspension was injected via the intraplantar (i.pl.) in the right hind paw of the mice. Dexamethasone, dipyrone, and phenobarbital solutions were prepared immediately before each administration in 0.9% w/v sodium chloride solution. The doses of dipyrone, dexamethasone, and phenobarbital were 500 mg/kg, 5 mg/kg, and 50 mg/kg, respectively. Dipyrone and phenobarbital solutions were administered p.o. in volume of 10 mL/kg. Dexamethasone was administered i.p. in volume of 8 mL/kg.

Samples of ethanol extract, hexane extract, and essential oil were prepared in 0.5% CMC solution and administered immediately by p.o. in volume of 10 mL/kg. The doses of dry ethanol and hexane extracts and essential oil used in the experimental protocols were 25, 50, and 100 mg/kg.

2.9. Paw edema induced by carrageenan

Paw edema was measured with a plethysmometer (Model 7140, Ugo Basile, Italy). The basal volume of the right hind paw was measured before administration of any drug. Next, the animals were divided into the experimental groups, 6 animals in each group, in such a way that the mean paw volumes of the different groups were similar. On the experimental day, carrageenan (600 µg, 30 µL) was injected via the i.pl. route 30 min after p.o. administration of dry ethanol and hexane extracts and essential oil (25, 50, and 100 mg/kg), vehicle or dexamethasone. The paw volume of each animal was again measured at 2, 4, and 6 h after injection of the inflammatory stimulus. The results were expressed as the paw volume change (µl) in relation to the basal values.

2.10. Mechanical allodynia induced by carrageenan

Mechanical allodynia was measured by using an electronic von Frey apparatus (Model EFF 301, Insight, Brazil). The mice were kept individually in acrylic cages whose floor was a metal grid. The animals were habituated to the experimental apparatus daily, approximately 60 min a day, for three days before the experiments. A hand-held force transducer, fitted with a polypropylene tip (0.5mm²), was gradually pressed on the plantar surface of the right hind paw. The test consisted of evoking a hind paw flexion reflex. The paw withdrawal threshold (PWT) was determined by averaging five measurements. Before the experiments, baseline PWT of each animal was determined. After that, the animals were divided into the experimental groups, 6 animals in each group, in such a way that the mean PWT of the different groups were similar.

On the experimental day, carrageenan (600 go, 30 µl) was injected via the i.p. route 30 min after p.m. administration of ethanol extract and hexane extract and essential oil (25, 50 and 100 mg/kg), vehicle or dexamethasone. The paw withdrawal threshold of each animal was again measured at 2, 4, and 6 h after carrageenan injection. The results were expressed as the absolute PWT (in grams).

2.11. Nociceptive response induced by heat (hot plate model)

A plate temperature (Model EFF361, Insight) was used to measure response latency time according to the method described by [Woolfe and MacDonald \(1944\)](#). The temperature used was 50 °C and the cutting time was 50 s, in order to avoid tissue damage. The latency to lick one of the hind paws or to jump off the plate was determined. The animal was removed from the hot plate immediately after the response. Thirty min after treatments, ethanol and hexane extracts and essential oil were administered po, at doses of 25, 50, and 100 mg/kg or the positive control dipyrone, mice were placed again on the hot plate apparatus to measure the latency pain response.

2.12. Evaluation of motor coordination

The motor coordination of the animals was evaluated by rotating rod (14 rpm), according to the procedure proposed by [Vaz et al. \(1996\)](#). The animals were trained on the apparatus for 3 days before the experiment. On the experimental day, the animals were placed on the rotating rod for 120s and the time they spent on it was measured. The cut-off time was 120s. After determination of the baseline values, ethanol and hexane extracts and essential oil (25, 50, and 100 mg/kg), phenobarbital or vehicle were administered. The animals were tested again in the apparatus 0.5, 2, 4, and 6 h later. Results were expressed as time(s) spent on the rotating rod.

2.13. Statistical analysis

The results were presented as mean ± standard error of the mean (S.E.M.). Both temporal changes and areas under the curves (AUC) were shown. AUC was calculated using the trapezoidal rule with the aid of the GraphPrism 5.0 for Windows software. Differences were evaluated by using one-way or two-way ANOVA followed by Newman-Keuls or Bonferroni post-hoc. A $P < 0.05$ was considered significant. Statistical analysis was conducted using GraphPrism 5.0 for Windows software.

3. Results and discussion

3.1. Chemical composition of *L. lacunosa*

3.1.1. Extracts and essential oil of *L. lacunosa*

UPLC-UV-DAD analysis allowed a qualitative exploratory investigation of the chemical composition of plant derivatives from the leaves of *L. lacunosa* ([Supplementary material](#)). The analysis of the chromatographic profile of the ethanolic extract of *L. lacunosa*, at λ 210 nm, revealed that most of the peaks have retention time (TR) between 8 and 15 min, indicating that the same consists mainly of substances of medium polarity. Some peaks in retention time between 21 and 24 min appeared, also revealing the presence of less polar substances ([Fig. 1 of Supplementary material](#)). It is also possible to identify the presence of flavonoids in peaks with RT between 9 and 11.4 min and close to 12 and 20 min, which present flavonoids with characteristic UV absorption spectra ([Fig. 1 of Supplementary material](#)) ([Mabry et al., 1970; Simões et al., 2017](#)). The major peak has a retention time of 12.6 min, but could not be identified by its ultraviolet spectrum ([Fig. 1 of Supplementary material](#)).

Flavonoids represent one of the most important and diversified groups among secondary metabolites and have already been isolated in several species of *Lippia*: *L. alba*, *L. citriodora*, *L. graveolens*, *L. sidoides*, *L.*

dulcis, *L. nodiflora*, and *L. triphylla* ([Gomes et al., 2011; Leite et al., 2022a; 2022b](#)). Studies carried out with the species *L. gracilis* showed that naringenin has anti-inflammatory and antinociceptive activities ([Guimarães et al., 2012](#)). A fractionation performed with the dichloromethane extract from the leaves of *L. lacunosa* resulted in the isolation of 7 flavonoids (cirsimarin, eupatilin, eupatorin, salvigenin, 3'-O-methyl-eupatorin, 3,7-dimethoxy-5,6,4'-trihydroxyflavone, and 7'-O-methyl-apigenin) and a triterpene (oleanolic acid), which were evaluated for antimycobacterial activity ([Castellar et al., 2011; Leitão et al., 2006](#)). Although the triterpenes are present in several species of *Lippia*, this class of secondary metabolites is not the majority in the species. Low molecular weight terpenes are usually the major components in the genus *Lippia* ([Viccini et al., 2005; Gomes et al., 2011; Leitão et al., 2008; Salimena and Silva, 1991](#)).

The chromatogram of the hexane extract ([Fig. 2 of Supplementary material](#)) shows that the major peaks had a longer retention time between 10 and 27 min, evidencing their lower polarity, as expected for a hexane extract. Some peaks (RT = 11.5 and 12.6 min) are common with the chromatograms of the ethanol extract. For *L. lacunosa* essential oil ([Fig. 3 of Supplementary material](#)), it is possible to observe two prominent peaks, with RT = 12.6 and 14 min, with high absorption in the ultraviolet, indicating high concentration of the respective substances. It should be noted that these peaks are also found in hexane and ethanol extracts. As expected, the *L. lacunosa* essential oil chromatogram shows peaks of low polarity substances, with retention time signals between 17 and 23 min ([Fig. 3 of Supplementary material](#)). As UPLC-UV-DAD is not the best technique for analyzing non-polar mixtures, the analysis of the hexane extract and the essential oil was performed by GC-MS. [Fig. 4 of Supplementary material](#) demonstrates the chemical profile of hexane extract and essential oil obtained by GC-MS. As a result, the chromatographic profile of the hexane extract is more complex than the profile demonstrated for the essential oil. The reason is that hydrodistillation extracts only substances with low molecular weight ([Simões et al., 2017](#)).

In the analysis by GC-MS of the essential oil were identified approximately 23 compound (91.86% of the total), these being terpenes of low molecular weight: 71.08% of monoterpenes and 19.44% of sesquiterpenes. The main monoterpenes identified were β -myrcene, ipnone, myrcenone, and linalool, and the sesquiterpenes, elemol and spathulenol ([Fig. 1](#)). The identification of the substances of *L. lacunosa* occurred by the comparison of fragmentation profiles in NIST and ADAMS libraries. [Table 1](#) shows the main substances identified in the essential oil of *L. lacunosa*. Many of the components identified in this study have already been mentioned in previous studies, for species of the same genus ([Chavan et al., 2010; Gomes et al., 2011; Gomide et al., 2013, 2016; Singulani, 2012; Pascual et al., 2001](#)).

[Leitão et al. \(2008\)](#) elucidated the chemical composition of *L. lacunosa*, presenting the components myrcenone and myrcene as major components in the essential oil extracted from fresh leaves. These two monoterpenes are the components responsible for the yellow color and characteristic odor of mango, which the essential oil of *L. lacunosa* presents. Monoterpenes are the main class of the compounds of the volatile oils found in most *Lippia* species, including *L. lacunosa*, which corroborates the results found in this study ([Castellar et al., 2011; Viccini et al., 2005; Gomes et al., 2011; Gomide et al., 2013, 2016; Leitão et al., 2008; Salimena and Silva, 1991](#)).

In the present study, differences were observed in the concentrations of the monoterpenes myrcene (13.81%) and myrcenone (25.44%), when compared to the results reported in the literature. [Leitão et al. \(2008\)](#) reported for the same substances a similar yield of 11.9% and 25.41%, respectively, and [Gomide et al. \(2013\)](#) found a content of 15.20% for myrcene and 35% for myrcenone. The same group later found 19.47% myrcene and 58.57% myrcenone ([Gomide et al., 2016](#)). It should be noted that the essential oil of *L. lacunosa* used by Leitão and Gomide was extracted from fresh leaves. On the other hand, these differences in the concentrations of the essential oil constituents of *L. lacunosa* may be

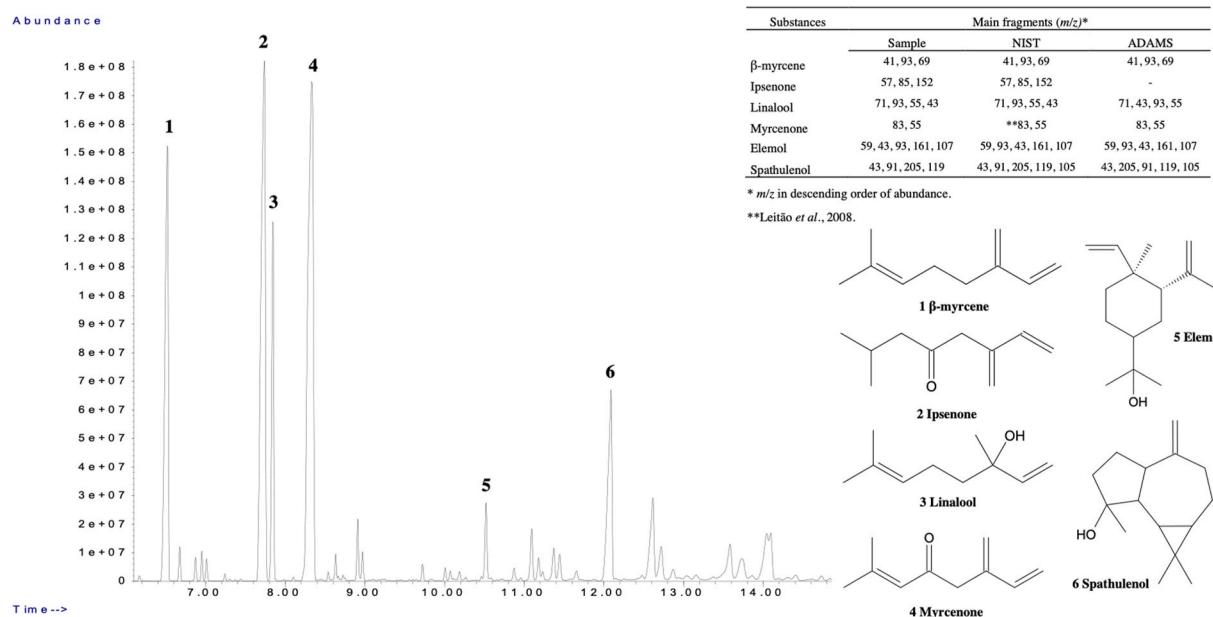


Fig. 1. Chromatographic profile obtained by GC-MS of *L. lacunosa* and its main fragments. Chromatographic conditions: item 2.6.2.

Table 1

Essential oil chemical composition of *Lippia lacunosa* leaves, determined by gas chromatography-mass spectroscopy (GC-MS)^a, retention time and relative % of area under each peak.

Substances	RT (min)	(%)
MONOTERPENES		
1 β-myrcene	6.48	13.81
2 α-phellandrene	6.68	1.12
3 o-cymene	6.95	0.44
4 β-phellandrene	7.01	0.35
5 Ocymene	7.24	0.11
6 γ-terpinene	7.30	0.03
7 Ipsenone	7.74	21.2
8 β-linalool	7.85	6.84
9 Myrcenone	8.33	25.44
10 4-terpineol	8.54	0.14
11 α-terpineol	8.63	0.42
12 Ocymenone	8.91	0.96
13 Geranyl acetate	10.01	0.22
SESSQUITERPENES		
14 β-cariophyllene	10.52	1.54
15 α-cariophyllene	10.87	0.29
16 α-curcumene	11.09	1.16
17 γ-elemene	11.37	0.82
18 Elemol	12.09	7.30
19 Spathulenol	1.62	3.11
20 Cariophyllene oxide	12.72	1.20
21 γ-eudesmol	13.59	1.79
22 β-eudesmol	14.05	2.23
23 α-eudesmol	14.09	1.34
Total		91.86

^a Methodology see item 2.6.2.

associated with echographic factors, which have already begun to be investigated but require further studies (Gomide et al., 2013, 2016; Leitão et al., 2008; Meira et al., 2017).

The concentrations of essential oil constituents can vary depending on several factors: soil type, climate, altitude, time of plant collection, and rainfall (Meira et al., 2017; Soares and Tavares-Dias, 2013). In the genus *Lippia*, the secretion of essential oils has been associated with the presence of trichomes and the constituents are very unstable in the presence of light, heat, and humidity; consequently, the time of harvesting the plant material can directly or indirectly influence the quantitative and qualitative variations of the essential oil (Gomide et al.,

2013, 2016, 2022b).

Hybridization is also an important factor to be considered in the diversification of the flora of the rupestrian fields, since most species of the Espinhaço Range have a restricted distribution and are marked by a high rate of endemism, perhaps the highest among Brazilian plant formations (Rapini et al., 2008; Salimena, 2010). This fact is corroborated by Viccini et al. (2005), who, when studying 14 species of the genus *Lippia*, endemic to the Serra do Espinhaço, observed hybridity in the species *L. lacunosa* and *L. rotundifolia* (Viccini et al., 2005; Meira et al., 2017). Possibly, *L. lacunosa* was also described by Saint-Hilaire as a pedestrian tea, since both are found in the same natural and endemic environment, the rocky fields of Serra do Espinhaço.

3.1.2. Fractionation of essential oil

Because it is a less complex matrix than the hexane extract and demonstrated important anti-inflammatory and antinociceptive activity in this study, *L. lacunosa* essential oil was the material selected for fractionation and further phytochemical investigation to identify the active substances and select chemical markers for the quality control.

Fractionation of essential oil of *L. lacunosa* by column chromatography (CC) on silica gel yielded 74 fractions, with a gradient of polarity ranging from hexane to ethyl acetate. The fraction F33 obtained (Fig. 5 of Supplementary material), containing ipsenone and myrcenone, showed the best fractionation yield (4.65%) and was rich in the major components of the essential oil (Table 1).

Furthermore, fraction 20 (0.44% fractionation yield) was characterized by GC-MS as ipsenone, and fraction 46 (0.80% fractionation yield) was identified as myrcenone. Thus, F33 was chosen for the evaluation of anti-inflammatory and antinociceptive activities based on the presence of the major components of the essential oil of *L. lacunosa* and on the good yield obtained (Fig. 6 of Supplementary material).

3.2. Anti-inflammatory and antinociceptive activity

Initially, the activity of ethanolic and hexane extracts and essential oil from *L. lacunosa* leaves in an experimental model of acute inflammation (carrageenan-induced paw edema) was evaluated. Edema induced by carrageenan, a mixture of polysaccharides extracted from marine algae of the Rhodophyceae family and composed of sulfated D-galactose, is a widely used model for the investigation of anti-

inflammatory drug candidates (Morris, 2003).

In the present study, the control group that received carrageenan showed marked paw edema, which remained throughout the evaluation period, 2, 4, and 6 h. The pre-treatment of the animals with the ethanolic extract of *L. lacunosa*, at the doses evaluated, 25, 50, and 100 mg/kg, did not significantly reduce paw edema in the intervals evaluated in this protocol (Fig. 2A). Although the average values of paw volume for the doses of 50 and 100 mg/kg did not differ statistically from the values of the group that received carrageenan when compared at each evaluation moment, when performing a global evaluation (area under the curve), a significant reduction was verified (Fig. 2B).

A reduction of paw edema by pre-treatment of animals with the hexane extract was observed in the 4th and 6th h, dose of 50 mg/kg, and in the 2nd, 4th, and 6th h, dose of 100 mg/kg (Fig. 2). For the essential oil, a reduction of paw edema was observed in the 4th h, 50 mg/kg dose, and also in the 2nd, 4th, and 6th h, 100 mg/kg dose (Fig. 2). Dexamethasone, used as a control in the experiment, reduced the formation

of paw edema in this model in the evaluated periods (Fig. 2). All decreases were statistically significant.

The i.p. carrageenan injection induces local inflammation, resulting from the action of several mediators such as bradykinin, serotonin, histamine, cytokines, chemokines, eicosanoids, and reactive oxygen species. Carrageenan-induced edema, then, is the result of vasodilation and increased vascular permeability, which culminate in fluid leakage into the interstitium. The migration of neutrophils to the inflammatory site occurs with consequent production of several inflammatory mediators mentioned (Salvemini et al., 1996). The results showed that the hexane extract and the essential oil of *L. lacunosa* markedly reduced the paw edema of the animals, at doses of 50 and 100 mg/kg. Other research groups have evaluated the anti-inflammatory activity of other species of *Lippia*.

Studies carried out with the essential oil of the leaves of *L. gracilis* Shauer demonstrated anti-inflammatory activity: at a dose of 50–200 mg/kg in paw edema and peritonitis models (Mendes et al., 2010) and at

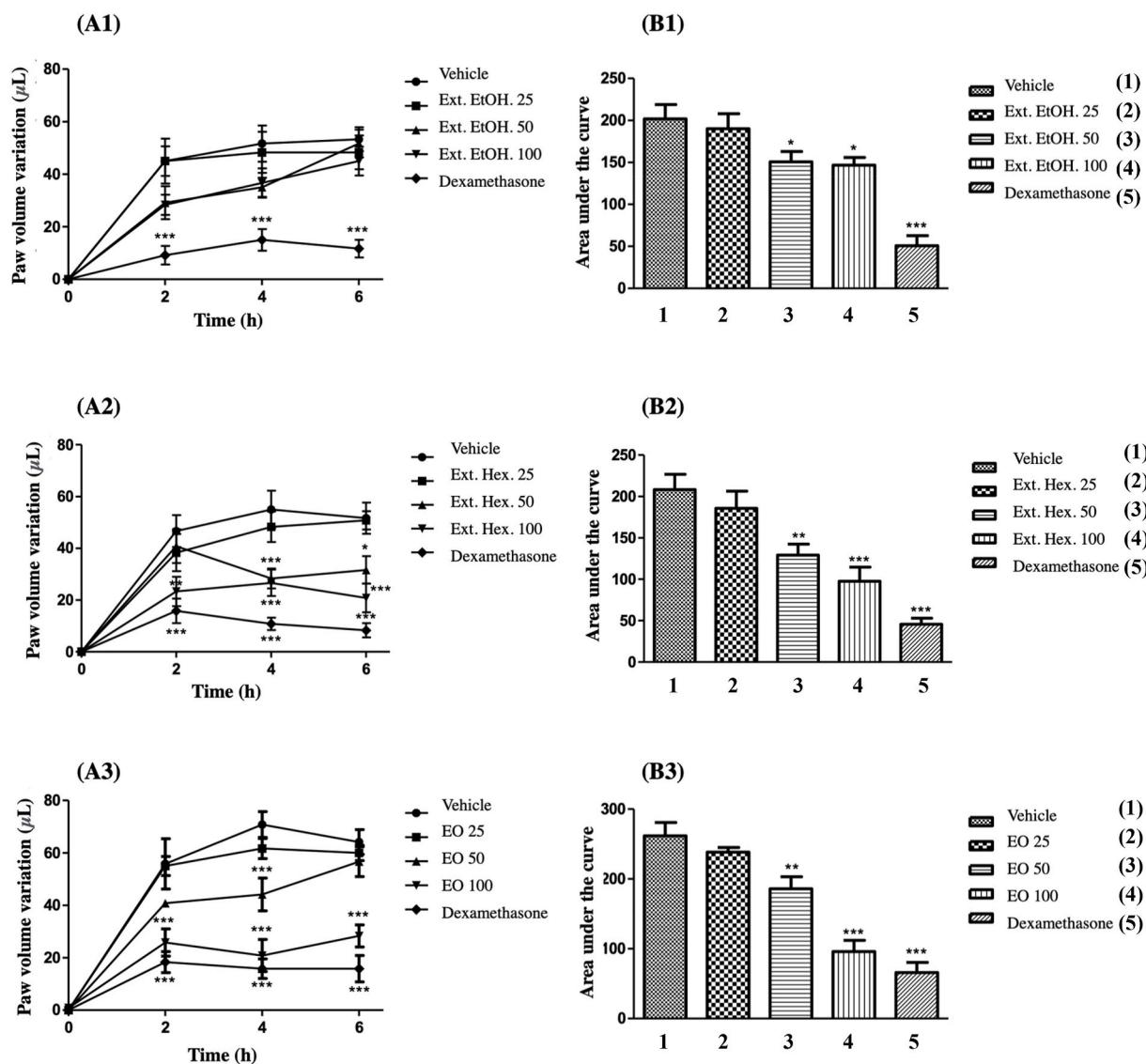


Fig. 2. Effects induced by *L. lacunosa* derivatives or dexamethasone (5 mg/kg, ip, - 30 min) on the paw edema induced by injection of carrageenan (600 µg, 30 µL, i.p.). Paw edema was evaluated 2, 4 and 6 h after carrageenan injection. (A) Temporal course and (B) area under the curve: A1, B1 (Ethanolic extracts - Ext. EtOH; 25, 50 and 100 mg/kg, po, - 30 min); A2, B2 (Hexane extracts - Ext. Hex; 25, 50 and 100 mg/kg, po, - 30 min); A3, B3 (Essential oils - EO; 25, 50 and 100 mg/kg, po, - 30 min).

Note: *, ** and *** indicate statistically significant differences in relation to the control group ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively). $n = 6$. The results were presented as mean \pm standard error of the mean (S.E.M.). Differences were evaluated by using one-way or two-way ANOVA followed by Newman-Keuls or Bonferroni post-hoc.

a dose of 100–400 mg/kg in hot-plate tests in carrageenan-induced inflammation in mice (Guimarães et al., 2012). Topical application of essential oil extracted from leaves of *L. sidoides*, at doses of 1 and 10 mg/ear in a single application, significantly reduced edema (Monteiro et al., 2007) and doses of 1, 5, 10, 50, and 100 mg/kg (po) of essential oil from *L. sidoides* leaves were tested in mice, in a model of ethanol-induced gastric injury, and inhibition of gastric lesions was observed for doses of 10, 50, and 100 mg/kg (Monteiro et al., 2007). Furthermore, the ethanolic extract of aerial parts of *L. dulcis*, at doses of 50, 100, 200, and 400 mg/kg, showed significant anti-inflammatory activity, reducing carrageenan-induced paw edema in rats (PÉREZ et al., 2005). Other studies have shown that the administration of methanolic extract of leaves of *L. nodiflora*, at doses of 200 and 400 mg/kg (po), in rats, reduced the anti-inflammatory activity in an experimental model of paw edema induced by carrageenan (Ahmed et al., 2004); and at the doses of 100 and 200 mg/kg (po) of aqueous extract of *L. nodiflora* leaves, anti-inflammatory activity in mice was confirmed in an experimental

model of carrageenan-induced paw edema (Al-Snafi and Faris, 2013).

Considering that the development of carrageenan-induced paw edema depends on the action of several inflammatory mediators, we can suggest that the anti-inflammatory activity of the hexane extract and the essential oil of *L. lacunosa* may be related to the reduction in the production of inflammatory mediators and the accumulation of inflammatory cells, mainly neutrophils. In a study of another species of *Lippia*, in mice, it was demonstrated that the essential oil of the leaves of *L. gracilis* inhibited the migration of leukocytes to the peritoneal cavity (17.4, 29.6, and 38.4% at doses of 50, 100, and 200 mg/kg, po, respectively), in an experimental model of carrageenan-induced peritonitis (Mendes et al., 2010).

The activity of *L. lacunosa* derivatives was also demonstrated in an experimental model of inflammatory pain, carrageenan-induced mechanical allodynia. The i.p. injection carrageenan induces the formation of paw edema and the development of mechanical allodynia, a marked sensitization of nociceptors (Gregory et al., 2013; Posadas et al., 2004).

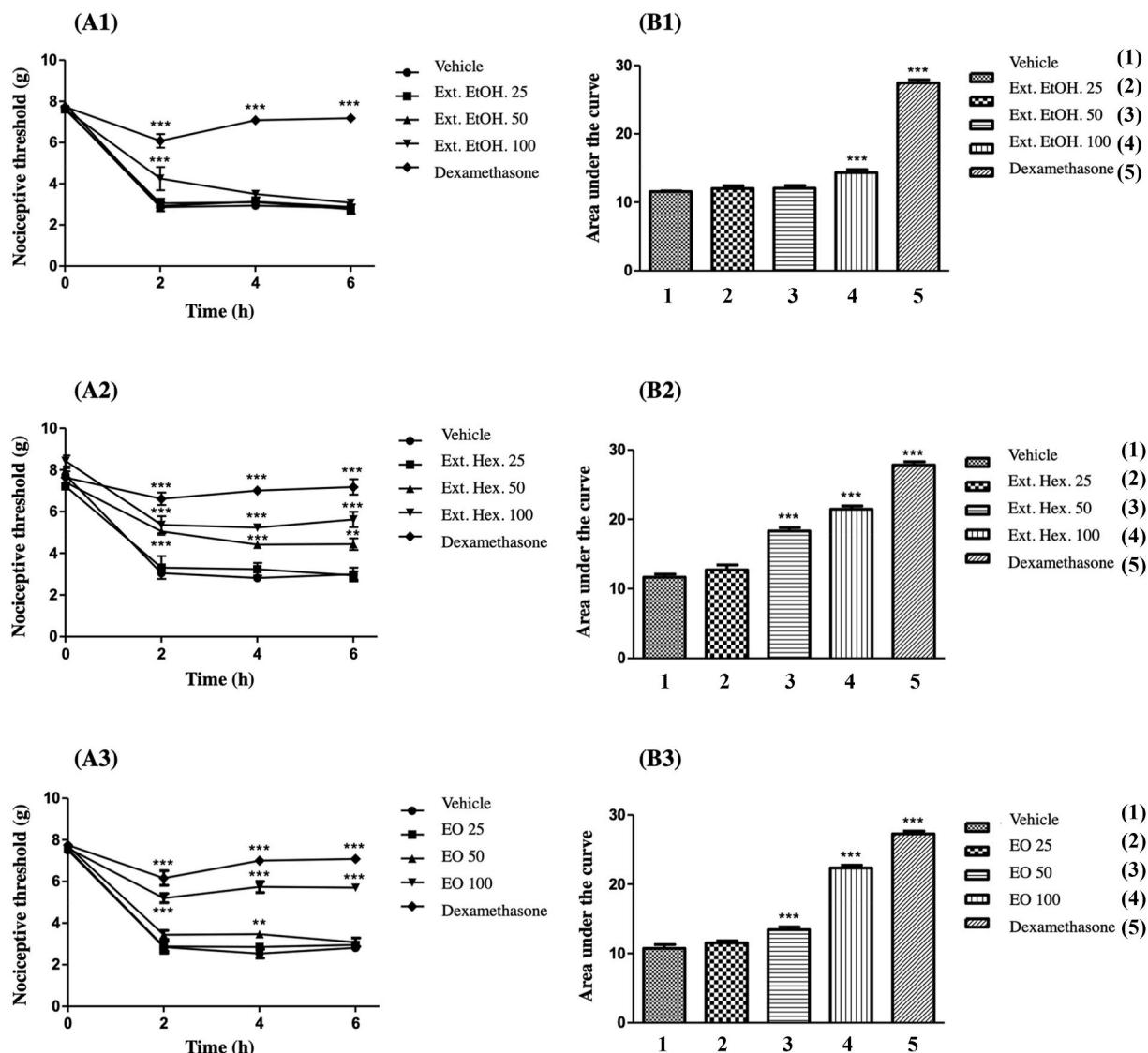


Fig. 3. Effects induced by *L. lacunosa* derivatives or dexamethasone (5 mg/kg, ip, - 30 min) on the mechanical allodynia induced by carrageenan injection (600 µg, 30 µl, i.pl.). Nociceptive threshold was evaluated 2, 4 and 6 h after carrageenan injection. (A) Temporal course and (B) area under the curve: A1, B1 (Ethanolic extracts - Ext. EtOH; 25, 50 and 100 mg/kg, po, - 30 min); A2, B2 (Hexane extracts - Ext. Hex; 25, 50 and 100 mg/kg, po, - 30 min); A3, B3 (Essential oils - EO; 25, 50 and 100 mg/kg, po, - 30 min).

Note: *, ** and *** indicate statistically significant differences in relation to the control group ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively). $n = 6$. The results were presented as mean \pm standard error of the mean (S.E.M.). Differences were evaluated by using one-way or two-way ANOVA followed by Newman-Keuls or Bonferroni post-hoc.

In the present study, the control group that received carrageenan showed a statistically significant decrease in the nociceptive threshold, which was maintained throughout the evaluation period of 2, 4, and 6 h.

The ethanolic extract of *L. lacunosa* reduced mechanical allodynia, at a dose of 100 mg/kg, only in the first evaluation, corresponding to 2 h (Fig. 3). The hexane extract, at doses of 50 and 100 mg/kg, also reduced mechanical allodynia in the evaluations performed on the 2nd, 4th, and 6th h after carrageenan injection (Fig. 3). The essential oil, at a dose of 50 mg/kg, also reduced mechanical allodynia in the 4th h evaluated and, at a dose of 100 mg/kg, we observed a reduction in mechanical allodynia in the 2nd, 4th, and 6th h after carrageenan injection (Fig. 3). Dexamethasone, used as a control in the experiment, reduced mechanical allodynia induced by i.pl. of carrageenan in the evaluated periods (Fig. 3). All the decreases were statistically significant.

The antinociceptive activity of other *Lippia* species in experimental pain models has also been demonstrated. The essential oil from the leaves of *L. gracilis* was tested in mice at doses of 10, 30, and 100 mg/kg (po) and the results showed a significant reduction in the number of abdominal writhes in the acetic acid-induced writhing model and the duration of the licking behavior of the paw injected with formalin (Guilhon et al., 2011). Another study investigated the antinociceptive activity of the methanolic extract of the aerial parts of *L. nodiflora*, in mice, at doses of 250 and 500 mg/kg (po), in an experimental model of abdominal writhing induced by acetic acid, with expressive results of 61% and 78% in the inhibition of writhing (Ahmed et al., 2004).

Thus, in view of the previous results indicating the remarkable activity, mainly, the hexane extract and essential oil of *L. lacunosa* in an experimental model of inflammatory pain, we investigated the effect of these mixtures in an experimental model of pain provoked by thermal stimulation. In the hot plate model, the thermal stimulus causes a nociceptive behavior in mice by direct activation of nociceptors (Le Bars et al., 2001). At the temperature of 50 °C, used in the experiment, activation of transient receptor potential cation channel (TRP) receptors occurs, essential for the initial processing of the nociceptive response (Tominaga, 2007). Some drugs, such as opioid analgesics and tricyclic antidepressants, are able to increase latency for nociceptive behavior (Loh et al., 1976; Rosland et al., 1988).

The results of the experiment showed that the control group (vehicle) had an average latency of approximately 15 s. The group treated with the hexane extract of *L. lacunosa*, at doses of 50 and 100 mg/kg, increased the latency for nociceptive behavior in the evaluated model (Fig. 4). The administration of *L. lacunosa* essential oil at a dose of 100 mg/kg, but not at a dose of 50 mg/kg, also increased the latency of nociceptive behavior in the hot plate model (Fig. 4). The group treated with dipyrone, the positive control of the experiment, markedly increased the latency of nociceptive behavior. Thus, the activities of the hexane extract and essential oil from the leaves of *L. lacunosa* suggest actions in the nociceptive processing in the Central Nervous System

(CNS) or in the mechanisms of direct activation of nociceptors sensitive to thermal stimuli.

In previous studies the antinociceptive activity of other species of *Lippia* were also presented. Mice were submitted to the hot plate model, after oral administration of essential oil from the leaves of *L. gracilis*, at doses of 10, 30, and 100 mg/kg, showing significant antinociceptive activity at all doses tested (Guilhon et al., 2011). The analgesic activity of the methanol extract of *L. gracilis*, in mice, was investigated in a hot plate model. Oral administration of doses of 100, 200, and 400 mg/kg increased latency for nociceptive behavior (Guimarães et al., 2012). The antinociceptive activity of the ethanol extract of the aerial parts of *L. origanoides* was tested in a hot plate model in mice, at doses of 10, 30, and 100 mg/kg (p.o) and the authors demonstrated that the administration of this extract increased the latency for the nociceptive behavior (Oliveira et al., 2014). This corroborates the results of the present study, since the hexane extract and essential oil from the leaves of *L. lacunosa* also increased the latency of the nociceptive behavior in the referred experimental model.

In order to validate the results obtained in the nociception protocols, the effects induced by the hexane extract and essential oil on the motor activity of the animals were also evaluated. It is worth mentioning that, in order to reduce the number of animals used, the present validation was not performed for the ethanol extract, as it did not show promising results in the anti-inflammatory tests.

3.2.1. Fraction rich in the main compounds ipsenone and myrcenone (fraction 33)

As demonstrated, the essential oil showed good yield and less complexity in the chemical constitution of its matrix, when compared to the hexane extract. The essential oil also obtained expressive anti-inflammatory activity, in an experimental model of paw edema, and antinociceptive activity, in the models of inflammatory pain induced by carrageenan and nociceptive pain induced by heat. Therefore, it was selected for the biological tests. This fraction is composed of myrcenone and ipsenone. Myrcenone is a substance found in some *Lippia* species, such as *L. javanica* (Viljoen et al., 2005), *L. lacunosa*, *L. rotundifolia*, and *L. pseudothea* (Castellar et al., 2011; Gomide et al., 2013, 2016; Singulani, 2012; Leitão et al., 2006, 2008). Regarding the anti-inflammatory and antinociceptive activities, there are no studies, to date, on the isolated monoterpenes myrcenone. Ipsenone has also been found in other species of the genus *Lippia*, such as *L. multiflora*, *L. triplinervis*, and *L. javanica* (Lamaty et al., 1990; Leitão et al., 2011; Viljoen et al., 2005). In fact, a study with the essential oil of *L. multiflora* demonstrated analgesic, antipyretic, and anti-inflammatory activities (Abena et al., 2003).

Our results, thus, demonstrated that F33 (myrcenone and ipsenone) significantly reduced the paw edema of the animals in the 2nd, 4th, and 6th h (dose of 10 mg/kg) and, similarly to the response presented by

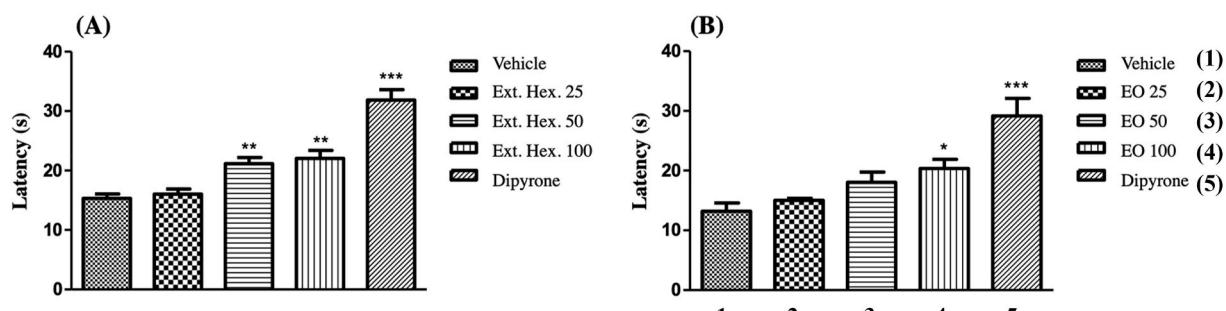


Fig. 4. Effects induced by the hexane extract of *L. lacunosa* leaves (Ext. Hex; 25, 50 and 100 mg/kg, po, - 30 min) and essential oil of *L. lacunosa* leaves (OE; 25, 50 and 100 mg/kg, po, - 30 min) or dipyrone (500 mg/kg, po, - 30 min) on the nociceptive response induced by heat (hot plate model, 50 °C). Note: *, ** and *** indicate statistically significant differences in relation to the control group ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively). n = 6. The results were presented as mean \pm standard error of the mean (S.E.M.). Differences were evaluated by using one-way or two-way ANOVA followed by Newman-Keuls or Bonferroni post-hoc.

dexamethasone, used as positive control of the experiment. For the 5 mg/kg dose, a reduction in edema was observed only in the 4th h in the temporal course analysis (Fig. 5).

Riella et al. (2012) evaluated the anti-inflammatory activity using doses of 10, 30, and 100 mg/kg of thymol, a major component of a fraction of the essential oil of *L. gracilis*, in an experimental model of paw edema induced by carrageenan (Riella et al., 2012). The results showed that thymol significantly reduces edema at a dose of 100 mg/kg. Nine phenolic compounds (phenolic acids and flavonoids) and 22 terpenes (monoterpenes and sesquiterpenes) from *L. graveolens* and *L. palmeri* were detected and the fractions were studied, proving their anti-inflammatory activity in *in vitro* assays using RAW 264.7 macrophage cells (Leyva-López et al., 2016). Myrcene, one of the main constituents of the essential oil of the leaves of *L. lacunosa*, was tested in the lipopolysaccharide (LPS)-induced pleurisy model, at doses of 50, 100, 200, and 400 mg/kg, in mice. The results showed inhibition of leukocyte migration (33% and 22%), neutrophils (36% and 18%), mononuclear macrophages (25% and 18%), and eosinophils (64% and 51%), at a dose of 50 mg/kg and 400 mg/kg, respectively (Souza et al., 2003). Therefore, considering that carrageenan-induced edema involves the production of several inflammatory mediators, this can suggest that the anti-inflammatory activity of F33 may be related to the reduction in the production of inflammatory mediators and the accumulation of inflammatory cells.

It was also observed that a fraction rich in ipsenone and myrcenone, only at a dose of 10 mg/kg, reduced mechanical allodynia in the 2nd, 4th, and 6th h after carrageenan injection (Fig. 5). Dexamethasone, used as a positive control, reduced mechanical allodynia induced by i.p. of carrageenan in the periods evaluated (Fig. 5). The control group that received carrageenan showed a statistically significant decrease in the nociceptive threshold, which was maintained throughout the evaluation period (2, 4, and 6 h).

In this sense, studies of some components present in the essential oil of *L. lacunosa*, such as myrcene and linalool, have already demonstrated

anti-inflammatory activity. The activity of myrcene, isolated from the essential oil of lemongrass (*Cymbopogon citratus*), was investigated in a mouse model of abdominal writhing induced by acetic acid. In this study, the authors observed significant inhibition of nociception at doses of 20 and 40 mg/kg (s.c.) (Rao et al., 2011). Linalool, at doses from 25 to 75 mg/kg, also showed antinociceptive activity in the model of visceral pain induced by acetic acid in mice (Peana et al., 2003). The antinociceptive effects of the essential oil of *Ocimum basilicum* L. leaf and its main component, linalool, were tested in models of orofacial nociception induced by formalin, glutamate, and capsaicin in mice, at doses of 50, 100, and 200 mg/kg, (ip), presenting significant results (Venâncio et al., 2010).

Then, the effect of the fraction in the experimental model of pain provoked by thermal stimulus was also investigated. The administration of F33 at doses of 5 and 10 mg/kg significantly increased the latency of nociceptive behavior in the hot plate model (Fig. 6). The group treated with dipyrone, the positive control of the experiment, increased the latency of nociceptive behavior in a statistically significant way (Fig. 6). The reduction in the nociceptive response induced by heat also suggests that F33 may inhibit nociceptive processing in the CNS or the mechanisms of direct activation of nociceptors by thermal stimuli.

The activity of myrcene (*C. citratus*) was also investigated in a hot plate model in mice. In this study, the authors observed significant inhibition of nociception at doses of 10 and 20 mg/kg (i.p.). Furthermore, the antinociceptive effect of myrcene was attenuated by the opioid antagonist naloxone (Rao et al., 2011). Caryophyllene oxide, a substance also present in the essential oil of *L. lacunosa*, demonstrated antinociceptive activity in mice, at doses of 12.5 and 25 mg/kg, using the hot plate test (Chavan et al., 2010).

Finally, the motor activity of the mice was also evaluated in the rotating rod, observing the statistically significant difference, in order to validate the results obtained in the nociception protocols. F33 at doses of 2.5, 5.0, and 10.0 mg/kg did not change the time the animals remained on the rotating rod (Fig. 6). However, this time was reduced by the

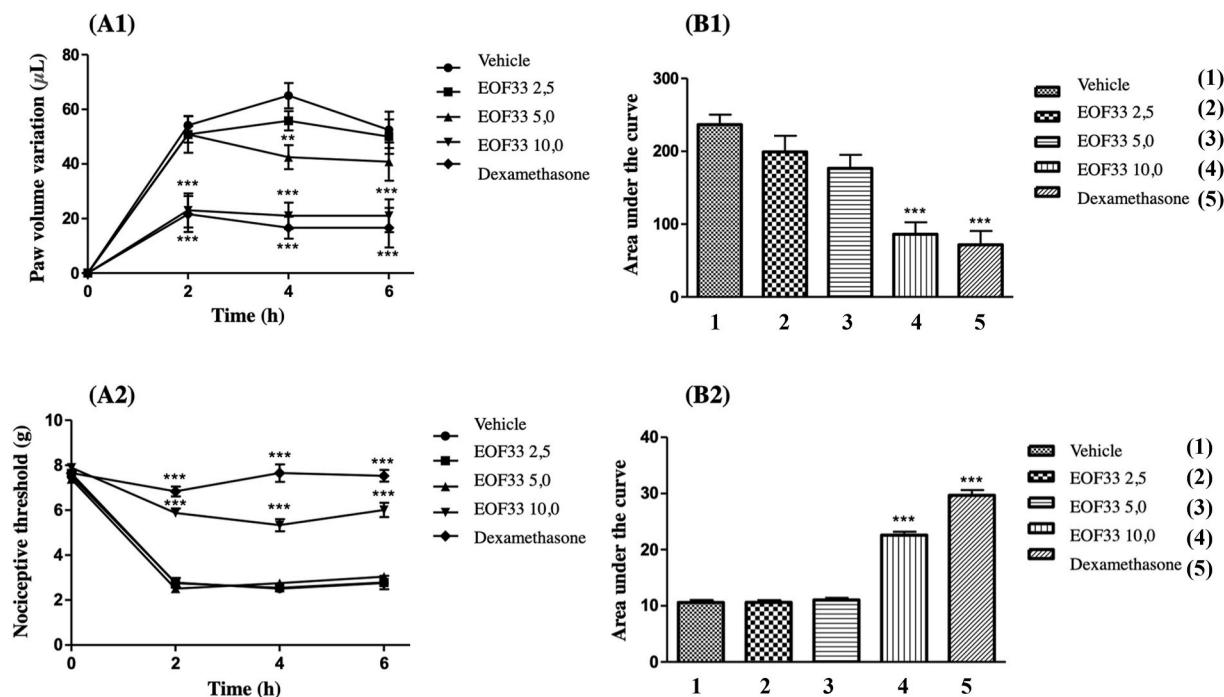


Fig. 5. Effects induced by the fraction 33 isolated from the essential oil of *L. lacunosa* (F33; 2.5, 5.0 and 10.0 mg/kg, po, -30 min) or dexamethasone (5 mg/kg, ip, -30 min) on the paw edema (A1 and B1) or on the mechanical allodynia induced by carrageenan injection (600 µg, 30 µl, i.pl.). (A) Temporal course and (B) area under the curve: A1, B1 (paw edema); A2, B2 (nociceptive threshold).

Note: ** and *** indicate statistically significant differences in relation to the control group ($p < 0.01$ and $p < 0.001$, respectively). $n = 6$. The results were presented as mean \pm standard error of the mean (S.E.M.). Differences were evaluated by using one-way or two-way ANOVA followed by Newman-Keuls or Bonferroni post-hoc.

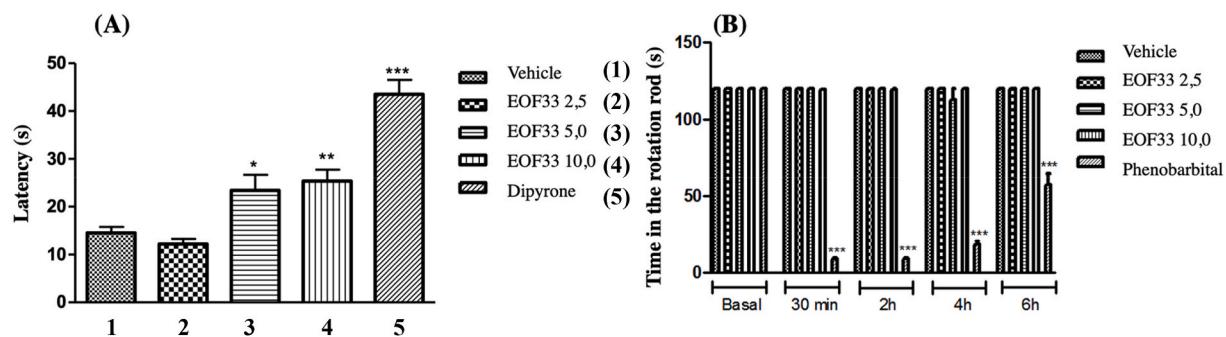


Fig. 6. Effects induced by the fraction 33 isolated from the essential oil of *L. lacunosa* (F33; 2.5, 5.0 and 10.0 mg/kg, po, - 30 min) and the essential oil of *L. lacunosa* leaves (OE; 25, 50 and 100 mg/kg, po, - 30 min) or dipyrone (500 mg/kg, po, - 30 min) on the nociceptive response induced by heat (hot plate model, 50 °C) (A); or by phenobarbital (50 mg/kg, po, - 30 min) on the time spent by the animals on the rota-rod (14 rpm, 120 s cutoff time) (B).

Note: *, ** and *** indicate statistically significant differences in relation to the control group ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively). $n = 6$. The results were presented as mean ± standard error of the mean (S.E.M.). Differences were evaluated by using one-way or two-way ANOVA followed by Newman-Keuls or Bonferroni post-hoc.

administration of phenobarbital, at a dose of 50 mg/kg, used as a positive control in this protocol (Fig. 6). Since F33 did not change the time the mice spent on the rotating rod, it is very unlikely that the attenuation of nociceptive behavior in the evaluated models was a result of impaired motor activity or muscle relaxation. Thus, this indicates that the reduction of the nociceptive behavior, in the different experimental models of pain, resulted from a genuine antinociceptive activity of F33.

4. Conclusions

In the present study, different plant derivatives were obtained from the leaves of *L. lacunosa*. From the analysis of its essential oil, it was noticed that the major components are monoterpenes (71.08%), identified as myrcene (13.81%), linalool (6.84%), ipsenone (21.2%), and myrcenone (25.44%), in addition to the sesquiterpenes elemol (7.30%) and spathulenol (3.11%). This study also shows, for the first time, that the hexane extract, the essential oil, and the F33 fraction of *L. lacunosa* present anti-inflammatory and antinociceptive activities in the different experimental models evaluated in mice, which places *Lippia lacunosa* as a promising candidate for an herbal medicine for inflammation and pain diseases. Also, this study increase knowledge on the popular use of an important species described by the Bandeirantes in Brazil.

Ethical approval

The project was approved by the Ethics Committee on Animal Use at Universidade Federal de Minas Gerais (UFMG), Brazil, under protocol number 277/2017.

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CRediT authorship contribution statement

Gizzelle Delfino Araújo Ladeira: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Thais Magalhães Acácio:** Methodology, Investigation. **Felipe Fernandes Rodrigues:** Methodology, Investigation. **Juliana Mendes Amorim:** Methodology, Investigation, Formal analysis, Writing – review &

editing. **Gustavo Pereira Cosenza:** Methodology, Investigation. **Maria Jose Nunes De Paiva:** Methodology, Investigation. **Renes Resende Machado:** Conceptualization, Methodology, Writing – review & editing, Supervision. **Rachel Oliveira Castilho:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Abbreviation

ANOVA	One-Way Analysis of Variance
CC	Column chromatography
CMC	Carboxymethylcellulose
CNS	Central nervous system
DAD	Diode Array Detector
F33	Fraction rich in the main compounds
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
NIST	National Institute of Standards and Technology
TLC	Thin Layer Chromatography
TRP	Transient receptor potential cation channel
UFMG	Universidade Federal de Minas Gerais
UPLC	Ultra-Performance Liquid Chromatography
UV	Ultraviolet

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jep.2023.116473>.

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