Revista Brasileira de Farmacognosia (2024) 34:350–357
https://doi.org/10.1007/s43450-023-00489-1

ORIGINAL ARTICLE

Chemical Constituents and Anti-inflammatory, Antinociceptive, and Antioxidant Activities of *Salvia melissiflora* Aerial Parts

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Received: 28 July 2023 / Accepted: 1 November 2023 / Published online: 16 November 2023

Abstract

Chromatographic fractionation of dichloromethane and ethanol extracts of *Salvia melissiflora* Benth., Lamiaceae, aerial parts led to the identification of five known compounds through analyses of NMR data (1D and 2D) and comparison with literature data: oleanolic acid, ursolic acid, *ent*-(5*R*,9*R*)-15,16-epoxy-10*S*-hydroxyclerodan-3,13(16),14-triene-17,12*S*;18,19-diolide (melissiflorine), 7-*epi*-salvianduline A, and rosmarinic acid. The anti-inflammatory and antinociceptive activity of dichloromethane and ethanol extracts was evaluated in mice. The oral administration of ethanol extract reduced the second phase of formalin-induced nociception, lipopolysaccharide (LPS)-induced hyperalgesia, and carrageenan-induced edema in mice. The oral administration of dichloromethane extract also reduced LPS-induced hyperalgesia without altering motor performance of the animals. The anti-inflammatory and antinociceptive effects are probably related to the presence of rosmarinic acid, oleanolic acid, and ursolic acid, respectively. The antioxidant activity of the extracts was evaluated using the ORAC method. The ethanol extract showed activity (TE relative, 2845.2 µmol TE g^{-1}), which can be attributed to the major presence, in this extract, of rosmarinic acid, which showed an antioxidant capacity comparable to that of caffeic acid.

Keywords Clerodanes · Triterpenes · Anti-inflammatory · Antinociceptive · Antioxidant

Introduction

Salvia, Lamiaceae, is represented in Brazil by 70 species, of which 62 are native (Oliveira et al. 2023). It exhibits a remarkable chemical diversity, characterized by its economically valuable volatile oils and diterpenes, triterpenes, and phenolic compounds as non-volatile constituents (Jassbi et al. 2016; Ortiz-Mendoza et al. 2022). *Salvia* species exhibit several therapeutic effects, such as antimicrobial, antiviral, antioxidant, antidepressant, anti-inflammatory, anticholinesterase, antispasmodic, hypoglycemic, antitumor, and cytotoxic activities (Ortiz-Mendoza et al. 2022; Oliveira et al. 2022).

Salvia melissiflora Benth. (syn: *S. paranensis*, *S. platyfrons*) is an aromatic herb or subshrub perennial species that typically reaches a maximum height of 80 cm. It is characterized by its simple leaves arranged in opposite pattern and vibrant red flowers. It is endemic from Brazil, where it is distributed in São Paulo, Paraná, and Santa Catarina states (Oliveira et al. 2023). There are no vernacular names or uses in traditional medicine for this species.

The volatile oil composition of the leaves was previously reported (Kassuya et al. 2009), but there are no other chemical or pharmacological studies on *S. melissiflora*. To address this research gap, a phytochemical investigation was carried on *S. melissiflora* aerial parts, and the extracts were evaluated for their potential anti-inflammatory, antinociceptive, and antioxidant activities.

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Materials and Methods

General Experimental Procedures

Optical rotations were determined using a JASCO PTC-203 polarimeter at room temperature and a wavelength of 589 nm. 1D (^{1}H, ^{13}C) and 2D (*g*HSQC, *g*HMBC) NMR spectra were recorded on Brucker spectrometers (Avance 400 and/or Avance 600) observing ^{1}H at 400 or 600 MHz and ^{13}C at 100 or 150 MHz. $CDCl_3$ or CD_3OD was used as solvents, and chemical shifts were reported in ppm (δ), with coupling constants (*J*) in Hz. TMS served as the internal reference.

HPLC separations were conducted on a Waters apparatus equipped with a PDA detector and a semipreparative Nucleosil 100–5 C18 column (250 × 10 mm). Water-to-acetonitrile ratio (60:40, isocratic) was used as mobile phase, with a flow rate of 2 ml min^{-1}, at room temperature. The effluent was monitored over the 210–400 nm range. Silica gel 60 (Merck®, 230–400 mesh) was used for column chromatographic separations (CC), while precoated silica gel 60 GF_{254} plates (Macherey–Nagel®) were used for analytical and preparative TLC. Compounds were visualized by exposure under $UV_{254/366}$ and spraying with a 5% (v/v) H_2SO_4 in ethanol solution, followed by heating. All solvents and drugs used were of analytical or spectroscopic grade, and the mixtures of solvents were prepared as v/v.

Plant Material

Salvia melissiflora Benth., Lamiaceae, were collected in Quatro Barras, Paraná State, Brazil (S 25° 24′ 29.0″, W 49° 59′ 57.98″), in October 2018. The plant was authenticated by Élide P. Santos who deposited a voucher specimen in the herbarium of Federal University of Paraná (UPCB 85.281). The study of this species was registered on SISGEN under number A19F875.

Extraction and Isolation of the Compounds

Dried and powdered aerial parts (174.3 g) were subjected to sequential extraction, at room temperature, using hexanes (Hex), CH_2Cl_2, and EtOH (500 ml, three times each solvent). After solvent removal was obtained the extracts in Hex (1.67 g), CH_2Cl_2 (EDSM, 1.72 g), and EtOH (EESH, 3.90 g). Aliquots of EDSM and EESM were reserved for biological assays.

The remaining EDSM (1.70 g) was fractionated by CC, eluted with several solvents in polarity order (Hex; Hex:CH_2Cl_2 1:1; CH_2Cl_2; CH_2Cl_2:acetone 9:1, 8:2, and 1:1; and finally MeOH), resulting in thirteen pooled fractions (A_{1-13}) after TLC analysis. Fraction A_7 (256.0 mg) was subjected to an additional CC using a mixture of Hex:EtOAc:MeOH (5:4:1), followed by pure EtOAc, yielding seven subfractions ($A_{7.1–7}$). Subfraction $A_{7.4}$ (55.2 mg) was purified by preparative TLC in toluene:EtOAc (3:2), yielding **3** (1.5 mg), **1** + **2** (2.5 mg), and a mixture (19.3 mg) that was purified by HPLC to give **4** (2.3 mg, retention time 17.15 min).

The remaining EtOH extract (3.70 g) was suspended in H_2O:EtOH (9:1) and subjected to extraction with EtOAc and *n*-butanol. The *n*-butanol fraction (MSB, 298.7 mg) was submitted to CC, eluted with mixtures of CH_2Cl_2:MeOH (9:1, 8:2), followed by MeOH, yielding five subfractions (B_{1-5}). Subfraction B_5 (98.7 mg) yielded **5** (23.3 mg) after PTLC in CH_2Cl_2:MeOH (8:2).

Animals

The experiments were conducted using male Swiss mice (25–45 g), obtained from the Federal University of Paraná breeding facility through outbreeding housed under a 12 h/12 h light/dark cycle, with controlled humidity (60–80%), and temperature (22 ± 1 ºC). Food and water were freely available to the animals. The animals were acclimatized to the experiment room, at the same conditions, at least 2 h before testing and were used only once throughout the experiments. The studies were carried out in accordance with the current Brazilian and international guidelines for the care of laboratory animals and were approved by the Institution's Ethics Committee for Animal Use (# 937). The number of animals was the minimum necessary to show consistent effects, and all efforts were made to minimize the number of animals used and their suffering. All the drugs used were of analytical grade.

Formalin-Induced Nociception

Formalin-induced nociception was evaluated as described before (Lomba et al. 2017). The mice were orally treated with the EESM (10, 30 or 100 mg kg^{-1}) 1 h before injection of 20 µl of 2.5% formalin (0.92% formaldehyde), made up in saline, in the right hind paw. Control animals received the same volume of vehicle (Tween 20 1%, *p.o.*) or indomethacin (IND, 5 mg kg^{-1}, *p.o.*). The time that the animals spent licking the injected paw (nociceptive behavior) was recorded in blocks of 5 min for 40 min. Phase I was considered as the time spending executing the nociceptive behavior in the first 5 min and phase II the time spending executing the nociceptive behavior from 15 to 40 min.

Mechanical Hyperalgesia

Mechanical hyperalgesia was assessed using an electronic Von Frey apparatus (Insight, Brazil) as described previously

(Lomba et al. 2021) with an adaptation for mice. First, the mice were placed in individual clear Plexiglas boxes (9.5 × 6.5 × 9.5 cm) on an elevated wire mesh platform for adaptation. The mesh floor allowed the tip of the apparatus to stimulate the midplantar region of the right hind paw using a disposable polypropylene tip (0.5 mm diameter). This tip was connected to a force transducer and digital display that allowed determination of the mass (in g) applied. Increasing force was applied to the paw, until the occurrence of paw withdrawal response. The mechanical threshold was calculated as the average value of three similar withdrawal responses, with a maximum of five stimulations with an interval of 15 s between them. After the determination of the basal withdrawal threshold, the animals were treated with EESM (10, 18, and 30 mg kg^{-1}), EDSM (7.5 mg kg^{-1}), the vehicle (Tween 20 1%), or the positive control IND (5 mg kg^{-1}) by oral route. After 1 h, animals received 20 µl injection of *E. coli* lipopolysaccharide (LPS, 0111:B4, 100 ng/paw, Sigma-Aldrich, USA) into the right hind paw. The occurrence of mechanical hyperalgesia was determined as the difference between the average mechanical threshold observed prior to any treatment (baseline) and 1, 3, and 5 h after the injection of LPS.

Carrageenan-Induced Edema

Carrageenan-induced hind paw edema is the most commonly used animal model to evaluate the anti-inflammatory potential of pharmacological substances (Winter et al. 1962). Edema was measured as described before (Lomba et al. 2017). Paw thickness (in µm) was measured using a digital micrometer (Metal Great Tools Co., MT-045B, China) before any treatment. The mice were then treated with EESM (100 mg kg^{-1}) or vehicle (Tween 20, 1%) or the positive control dexamethasone (DEX, 5 mg kg^{-1}, Sigma-Aldrich, USA) orally. One hour after oral treatment, the animals received carrageenan (Cg, 300 µg) suspended in 20 µl of sterile 0.9% saline into the right hind paw. Contralateral paw received only saline and was used as control.

Motor Performance

Mice were previously selected based on their ability to remain on a rotarod apparatus (Ugo Basile, Italy) that rotated at 22 rotations per minute for at least 60 s (one of three trials). Animals were treated with vehicle or EDSM (7.5 mg kg^{-1}) by oral route. Positive control group received diazepam (5 mg kg^{-1}, subcutaneously). Three hours after EDSM treatment or 30 min after diazepam treatment, the animals were subjected to the rotarod test as previously described (Lomba et al. 2017).

Antioxidant Assay

The antioxidant capacity of the samples was determined using the oxygen radical absorbance capacity (ORAC) assay, employing fluorescein as the fluorescent probe and AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride) as a free radical source. The experiments were carried on 96-well plates (Prior et al. 2003). Various dilutions of the samples (5 to 500 µg ml^{-1}) were prepared in a phosphate buffer/DMSO solution (99:1). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard, at concentrations of 12.5–200 µmol l^{-1}. The measurements were performed using a fluorescent filter (excitation λ, 485 nm, and emission λ, 528 nm) in a microplate reader, monitoring the reaction at 37 °C every 2 min for a period of 70 min. Results were expressed as µmol of Trolox equivalent (TE) per gram of dried extract (µmol TE g^{-1}). Caffeic acid, chlorogenic acid, quercetin, isoquercetin, and rosmarinic acid were utilized as positive controls, while the solvent was the negative control. All experiments were conducted in triplicate.

Statistical Analysis

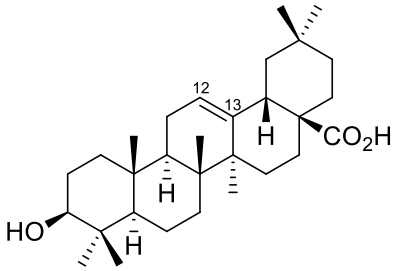
Data are expressed as mean ± the standard deviation or mean ± standard error of the mean. The data were analyzed using one-way or two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni's test. The data obtained were analyzed using the GraphPad Prism software version 9.0. P values less than 0.05 ($p < 0.05$) were considered significant.

Results and Discussion

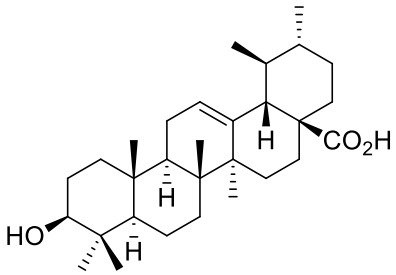
Phytochemical Study

The dichloromethane extract from aerial parts of *S. melissiflora* furnished four known compounds (**1**–**4**), while the ethanol extract yielded **5** as main constituent. These compounds were identified by analyses of their NMR data and comparison with literature.

Compounds **1** and **2** were isolated in mixture. Their NMR data revealed typical signals of triterpenes. The basic skeleton was determined by analyzing the chemical shifts of the carbons of the double bond, which indicate the presence of triterpenes belonging to the oleanane (δ_C 143.8, C-13, and 122.2, C-12) and ursane types (δ_C 138.3, C-13, and 125.7, C-12) (Olea and Roque 1990). Analyses of HSQC and HMBC spectra led to identification of oleanolic acid (**1**) and ursolic acid (**2**) (Dais et al. 2017).

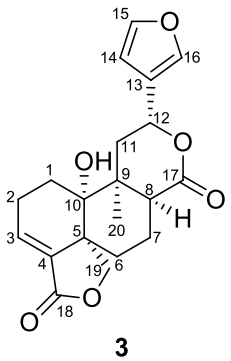


1



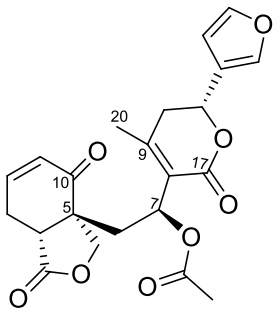
2

Compound **3** exhibited characteristic signals of a clerodane diterpenoid in its NMR data. Signals for a monosubstituted furan ring (δ_H 7.45, H-16; 7.39, H-15; 6.40, H-14), a γ-lactone (δ_H 4.37 and 4.30, H-19; δ_C 170.5, C-18; 71.6, C-19), a δ-lactone (δ_H 5.65, H-12; δ_C 174.8, C-17; 71.7, C-12), a methyl group (δ_H 1.28, H-20), a trisubstituted double bond (δ_H 6.90, H-3; δ_C 136.3, C-3; 132.0, C-4), and an oxygenated quaternary carbon (δ_C 73.8, C-10) were observed. After a comprehensive analysis of the HSQC and HMBC correlations, it was definitively established that compound **3** is a pentacyclic clerodane, characterized by a *cis* junction between the rings at C-8 and C-9, with an α-disposition of C-20, which is a common feature in *neo*-clerodane diterpenoids (Li et al. 2015; Fragoso-Cerrano et al. 2019). This specific compound corresponds to the structure previously identified as *ent*-(5*R*,9*R*)-15,16-epoxy-10*S*-hydroxyclerodan-3,13(16),14-triene-17,12*S*;18,19-diolide (Almanza et al. 1997; Bisio et al. 2015), and we propose the common name melissiflorine.



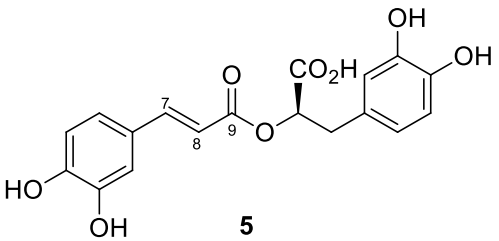
3

The NMR data of compound **4** were similar to those of **3**, indicating a clerodane diterpenoid. However, the ^{1}H and ^{13}C NMR spectra of **4** exhibited additional signals that could be attributed to an α, β-unsaturated ketone (δ_C 197.5, C-10) and an acetyl group (δ_H 2.02; δ_C 169.5). Additionally, the methyl group at C-20 (δ_H 2.09) appeared to be more deshielded in comparison with the same methyl group in **3**. These findings suggested the presence of a double bond between C-8 and C-9 and a cleavage of the bond between C-9 and C-10, resulting in a tetracyclic rearranged *seco*-clerodane. HSQC and HMBC correlations confirmed the structure of **4**, a compound known as 7-*epi*-salvianduline A (Ortega et al. 1991).



4

The ^{1}H NMR spectrum of **5** showed signals characteristic of two trisubstituted aromatic rings (δ_H 7.02–6.25), and an olefinic bond (δ_H 7.50 and 6.25, $J = 15.9$ Hz, H-8, H-7; δ_C 169.3, C-9), suggesting a caffeoyl-derivative. The remaining data supported the identification of rosmarinic acid (Kuhnt et al. 1994).



5

Antinociceptive and Anti-inflammatory Activity Studies

Formalin-induced nociception is characterized by two phases: an initial phase (phase I), which is believed to be neurogenic, and a second phase (phase II), which starts approximately 15 min after the injection of formalin and

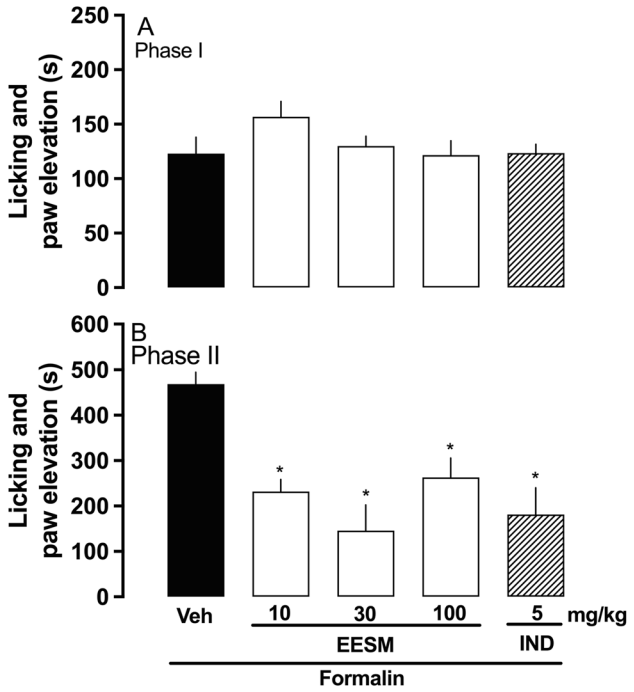


Fig. 1 Effect of ethanol extract of *Salvia melissiflora* (EESM) on formalin-induced nociception. Mice were treated with indicated doses of EESM, or the appropriate vehicle (Veh), or indomethacin (IND; 5 mg $kg.^{-1}$) orally 1 h before an intraplantar injection of 2.5% formalin. Formalin-induced nociceptive behavior was evaluated in phase I (**A**) or II (**B**) as described before. The data are expressed as the mean ± standard error of mean of the change in time spent licking the paw (in seconds) ($n = 5–7$). The data were analyzed using one-way ANOVA followed by Bonferroni's post hoc test. Symbols denote significant differences compared with the control Veh-treated group ($^{*}p < 0.05$)

lasts till the end of the experiment and is considered to be an inflammatory phase. The treatment of the mice with 10, 30, or 100 mg kg^{-1} EESM did not change the first phase of formalin-induced nociception but significantly reduced the second phase (Fig. 1A and B). Similarly, the non-selective, non-steroidal anti-inflammatory drug IND also reduced only the second phase of formalin-induced nociceptive behavior. Additionally, EESM dose dependently reduced the LPS-induced mechanical hyperalgesia. The lowest dose used (10 mg kg^{-1}) did not change the LPS-induced mechanical hyperalgesia, while higher doses, 18 mg kg^{-1} and 30 mg kg^{-1}, reduced and completely abolished LPS-induced hyperalgesia (Fig. 2A). As expected, IND also reduced this response (Fig. 2A). This extract also showed anti-inflammatory activity. EESM 100 mg kg^{-1} significantly reduced the carrageenan-induced edema (Fig. 2B), particularly 5 h after Cg injection. The steroidal anti-inflammatory drug DEX significantly reduced edema formation at time points 3 and 5 h after Cg injection (Fig. 2B). As mentioned above, the main compound found in EESM was rosmarinic acid (**5**).

The analgesic and anti-inflammatory activity of rosmarinic acid (**5**) is well documented. Using similar models, Boonyarikpunchai et al. (2014) showed that rosmarinic acid (**5**), isolated from *Thunbergia laurifolia* Lindl., Acanthaceae, also significantly reduced acetic acid-induced writhing, while 100 mg kg^{-1} additionally inhibited the first and second phases of formalin-induced nociception and carrageenan-induced edema. This study also showed that rosmarinic acid (**5**) reduced heat-induced nociception, an effect that was blocked by naloxone, suggesting that rosmarinic acid (**5**) is effective against inflammatory pain and has an effect dependent of opioid release. However,

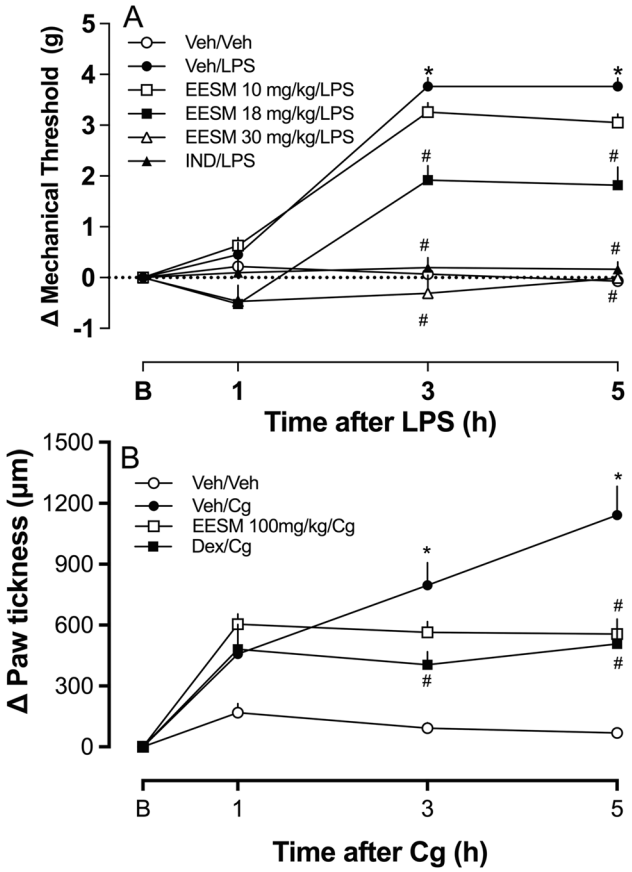


Fig. 2 Effect of ethanol extract of *Salvia melissiflora* (EESM) on LPS-induced hyperalgesia and Cg-induced edema. Mice were treated with indicated doses of EESM, or the appropriate vehicle (Veh), or indomethacin (IND; 5 mg kg^{-1}), or dexamethasone (DEX, 1 mg kg^{-1}) orally 1 h before an intraplantar injection of 100 ng lipopolysaccharide (LPS, **A**) or 300 µg carrageenan (Cg, **B**). The mechanical threshold and the paw thickness were evaluated prior to any injection (0) and 1, 3, and 5 h after LPS (**A**) or Cg (**B**) injection, respectively. The data are expressed as the mean ± standard error of mean of the change in the mechanical threshold (in grams) or the paw thickness (in µm) ($n = 6–8$). The data were analyzed using repeated measures two-way ANOVA followed by Bonferroni's post hoc test. Symbols denote significant differences compared with the control Veh/Veh-treated group ($^{*}p < 0.05$) or compared with the Veh/LPS or Veh/Cg group ($.^{\#}p < 0.05$)

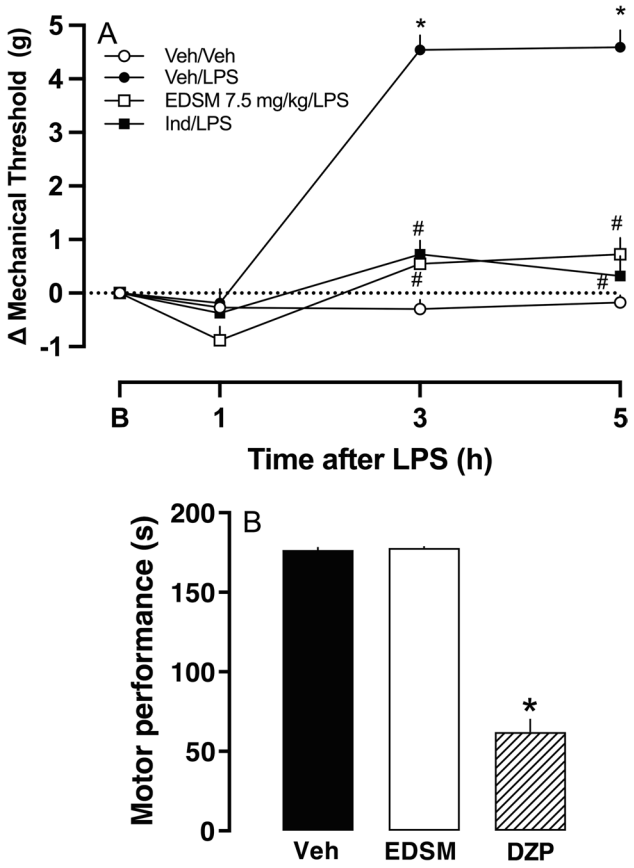


Fig. 3 Effect of ethanol extract of *Salvia melissiflora* (EESM) on hyperalgesia and motor performance. Mice were treated with dichloromethane extract (EDSM) (7.5 mg/kg, orally), or indomethacin (IND; 5 mg kg^{-1}, orally), or the appropriate vehicle (Veh) before an intraplantar injection of 100 ng lipopolysaccharide (LPS, **A**). In a separate experiment, the animals were only treated with the same dose of EDSM, the appropriate vehicle (Veh), or diazepam (DZP, 5 mg kg^{-1}, subcutaneously). The mechanical threshold was evaluated prior to any injection (0) and 1, 3, and 5 h after LPS (**A**). The motor performance was evaluated 1 h after the administration of EDSM or Veh or 15 min after the administration of DZP. The data are expressed as the mean ± standard error of mean of the change in the mechanical threshold (in grams) or time spent in the rotarod apparatus (in seconds) ($n = 6–8$). The data were analyzed using repeated measures two-way ANOVA followed by Bonferroni's post hoc test (A) or one-way ANOVA followed by Bonferroni's post hoc test (**B**). Symbols denote significant differences compared with the control Veh/Veh- or Veh-treated group ($^{*}p < 0.05$) or compared with the Veh/LPS ($.^{\#}p < 0.05$)

other mechanisms may be involved since the isolated compound was effective in reducing carrageenan-induced edema, which is not reduced by opioid drugs. Our results show that EESM, which contains rosmarinic acid (**5**), at the doses used in the present study was effective mostly in the inflammatory pain and edema. We did not observe a reduction in the first phase of formalin-induced pain, suggesting that the extract, at the doses used, is not acting through opioid release. The highest dose of EESM we tested was 100 mg kg^{-1} and, therefore, the amount of rosmarinic acid (**5**) in the extract is certainly lower than that used by Boonyarikpunchai et al. (2014) to reduce formalin-induced nociception. These authors only observed a reduction in the first phase of formalin-induced pain at 100 mg kg^{-1} (or higher dose) of rosmarinic acid (**5**). They suggested that higher doses of rosmarinic acid (**5**) may be acting through central mechanisms. These results suggest that, at higher doses, EESM could also have central analgesic effects. Other mechanisms for rosmarinic acid (**5**) to reduce inflammatory pain and edema have been proposed involving the cholinergic systems, the L-arginine-nitric oxide pathway, and the reduction in the release of inflammatory cytokines particularly in neuropathic pain (Guginski et al. 2009; Rahbardar et al. 2017, 2018; Areti et al. 2018; El Gabbas et al. 2019; Ma et al. 2020; Borgonetti and Galeotti 2022). The effect of rosmarinic acid (**5**) on formalin-induced nociception and LPS-induced mechanical hyperalgesia cannot be attributed to motor impairment since even high doses of this compound did not alter the motor performance of the animals, as showed in previous studies (Yu et al. 2022). Therefore, the anti-inflammatory and antinociceptive effect of EESM was expected and confirmed by these studies and is related, at least in part, to the presence of rosmarinic acid (**5**).

Differently from EESM, EDSM was only tested in the inflammatory hyperalgesia since the evidences in the literature suggested that oleanolic acid (**1**) and ursolic acid (**2**) are mainly related to the blockage of the release of inflammatory mediators. EDSM also significantly reduced the LPS-induced mechanical hyperalgesia observed at 3 and 5 h after the injection of LPS in the hind paw (Fig. 2A) without interfering with the motor performance of the animals (Fig. 3B). As mentioned before, oleanolic acid (**1**) and ursolic acid (**2**) were the main triterpenes found in EDSM and are also commonly found in other *Salvia* species (Jassbi et al. 2016; Ortiz-Mendonza et al. 2022).

Table 1 Antioxidant capacity of *Salvia melissiflora* extracts

Sample	ORAC assay (μmol TE g^{-1})
EDSM (CH_2Cl_2 extract)	202.6 ± 38.10
EESM (EtOH extract)	2845.2 ± 2.70
Caffeic acid[a]	2.85 ± 0.03[b]
Chlorogenic acid[a]	2.65 ± 0.03[b]
Isoquercetin[a]	5.15 ± 0.09[b]
Quercetin[a]	5.60 ± 0.08[b]
Rosmarinic acid[a]	3.00 ± 0.05[b]

[a]positive experimental control; [b]data of pure compounds are expressed as relative Trolox equivalent. The values are average of triplicate assays ± standard deviation

Several studies have shown that extracts from different plants that contain oleanolic acid (**1**) and ursolic acid (**2**) possess anti-inflammatory and antinociceptive activity, including the reduction of mechanical hyperalgesia (Azevedo et al. 2016; Kuraoka-Oliveira et al. 2020). Therefore, the presence of these compounds as main compounds in EDSM justifies the antinociceptive effect of EDSM. Ursolic acid (**2**) was also effective in neuropathic pain reducing mechanical and thermal hyperalgesia (Bhat et al. 2016), thermal pain, and carrageenan-induced edema formation acting as COX-2 inhibitors and/or glutamate receptors antagonist (Silva et al. 2017; Rodrigues et al. 2012). Additionally, Qasaymeh et al (2023) also showed that both oleanolic acid (**1**) and ursolic acid (**2**) inhibited the release of TNF-α and IL-12 *in vitro*. Due to this possible effect in glutamate receptors, we investigated if EDSM, at the doses used in the present study, could affect the motor performance of the animals, a common effect observed in drugs that affect the glutamatergic system. We did not observe any effect on the motor performance of the animals, suggesting that the antinociceptive effect observed in the mechanical threshold evaluation is not a bias due to a motor impairment but does not exclude the possibility that the EDSM could be acting through both, blocking inflammatory mediators release and as NMDA antagonists. However, it is important to mention that previous studies also have shown that oleanolic acid (**1**) and ursolic acid (**2**) modulate the activities of several cytochrome P450 (CYP) enzymes suggesting that the consumption of herbal medicines containing these triterpenes can cause important pharmacological interactions (Kim et al. 2004).

Antioxidant Assay

The antioxidant activity of the extracts from *S. melissiflora* was evaluated using the ORAC-FL method and measured in µmol Trolox equivalent (TE) per gram of dry extract (µmol TE g^{-1}). Extracts with TE > 800.0 µmol TE g^{-1} are considered to have antioxidant activity (Prior et al. 2003). Among the extracts tested, only EESM showed antioxidant activity (Table 1). This result aligns with the findings of the phytochemical study, which identified rosmarinic acid (**5**) as the major constituent in this extract. In our assay, **5** showed antioxidant capacity comparable to that of caffeic acid (Table 1). Rosmarinic acid (**5**) is a well-known compound with powerful antioxidant properties, including scavenging free radicals, activating enzymes, inhibiting lipid peroxidation, and protecting DNA. These properties enable it to effectively counteract oxidative stress-induced damage (Huang et al. 2009; Guan et al. 2022) and contribute to the anti-inflammatory and antinociceptive properties of this extract.

Acknowledgements The authors are grateful to Élide P. Santos for collection and identification of the plant.

Author Contribution Conceived and designed the phytochemical analysis and analyzed the data: CSO and MEAS Performed phytochemical analysis: CSO. Conceived and designed the pharmacological experiments and analyzed the data: FLB, CAE, and ARZ. Performed pharmacological experiments: FLB and CAE. Conceived and designed the antioxidant assay and analyzed the data: TEBR and MJS. Wrote the paper: CSO, MEAS, and ARZ.

Funding This study was financially supported by CAPES (finance code 001), FAPESP (process numbers 06/06079–4, 09/05992–6, 15/03726–8, and 21/09693–5), and CNPq (process numbers #311166/2016–4, #304266/2017–5, and #427859/2018–2) for.

Declarations

Conflict of Interest The authors declare no competing interests.

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