Phytochemical study and antinociceptive effect of the hexanic extract of leaves from *Combretum duarteanum* and friedelin, a triterpene isolated from the hexanic extract, in orofacial nociceptive protocols


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**ABSTRACT**

*Combretum duarteanum* Cambess, Combretaceae, is a plant widely distributed in Northeastern Brazil and, in folk medicine, stems and leaves are used for pain treatment. We investigated the antinociceptive effects of the hexanic extract of leaves from *C. duarteanum* and of friedelin, its main compound, in formalin-, glutamate- and capsaicin-induced orofacial nociception models. In order to isolate friedelin from the hexanic extract, flash chromatography technique was used. Male mice (n = 8/group) were pretreated with hexanic extract, friedelin, morphine or vehicle, before the injection of algogen agents into the right upper lip (perinasal area). The test of formalin-induced orofacial nociception showed that hexanic extract and friedelin significantly reduced nociception (p < 0.001) in both phases of testing. In the glutamate and capsaicin-induced orofacial nociception tests, pre-treatment with hexanic extract produced a significant reduction of orofacial nociception at all doses tested. The results suggest the hexanic extract and friedelin possess antinociceptive effects in models of orofacial nociception in rodents.

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**Introduction**

Pain is defined as a subjective, unpleasant, physical and psychological experience observed as a result of the stimulation of identifiable nerve fibres with defined pathways towards the brain via the spinal cord (Agbaje et al., 2008; Palecek and Willis, 2005). The orofacial region is an area densely innervated by the trigeminal nerve. The latter focuses some of the most common acute, chronic and referred pains, such as migraine, trigeminal neuralgia, post-herpetic neuralgia and teeth pain; however, the precise mechanisms of these pains are still poorly understood (Moalem and Tracey, 2006; Takemura et al., 2006). According to Hargreaves (2011), the management of orofacial pain continues to be a major challenge for medicine.
Currently, numerous therapeutic approaches are being used in order to control orofacial pain. A current approach is the development of new biological compounds from natural products (Siqueira et al., 2010; Venâncio et al., 2011; Guimarães et al., 2013).

Data from the literature show that species of the genus Combretum, Combretaceae, are rich in terpenoids that have a broad spectrum of biological activities including analgesic, anti-inflammatory, antibacterial, anticancer and antiprotozoal (Osborne and Pegel, 1984; Facundo et al., 1993; Pietrovska et al., 2006). Pietrovska et al. (2006) demonstrated that triterpenes isolated from C. leprosum had a significant antinociceptive effect in rodents.

Lima et al. (2012) described several compounds present in genus Combretum including triterpenes, such as friedelin, with important biological activities. Friedelin isolated from Aucuba jonica showed anti-inflammatory activity in acute studies such as carrageenan-induced paw edema, histamine and compound 48/80-induced paw edema (Shimizu and Tommo, 1994). Recently, Antonisamy et al. (2011) showed that friedelin (isolated from Azima tetracantha) possesses potent anti-inflammatory, analgesic and antipyretic properties.

Combretum duarteanum Cambess. is a plant found in Northern and Northeastern Brazil, popularly known as “vaqueta” or “caatinga-branca”, used in folk medicine in the preparation of teas, drinks and food supplement for the treatment of painful conditions, including orofacial pain (Albuquerque et al., 2007; Gouveia et al., 2011). Lately, Gouveia et al. (2011) observed that the ethanolic extract obtained from leaves of the C. duarteanum exhibited an antioxidant, anti-inflammatory and antinociceptive effects. More recently, Lima et al. (2012) demonstrated that the hexane extract obtained from leaves of the C. duarteanum produced strong gastro-protective activity probably due to the presence of two triterpenes and involvement of nitric oxide (NO) and sulfhydryl (SH) pathways. Until now, no data exist concerning the possible orofacial antinociceptive effect of C. duarteanum friedelin.

Thus, the purpose of the present study was to evaluate the antinociceptive effect of the hexanic extract of leaves from C. duarteanum and friedelin, its major compound, in different models of orofacial nociception on rodents.

Material and methods

General experimental procedures

The melting point (m.p.) was measured using a Microquimica MQAPF-301 Model. IR spectra were acquired on a Biorad FTS-3500 GX spectrophotometer. GC-MS analyses were performed on a Shimadzu QP5050A GC-MS system equipped with an AOC-20i autoinjector. The chromatograph was equipped with a J & W Scientific DB-5MS (coated with 5%-phenyl-95%-methylpolysiloxane) fused capillary column (30 m × 0.25 mm × 0.25 μm film thickness). MS were taken at 70 eV with a scan interval of 0.5 s and fragments of 40-500 Da. 1D and 2D NMR data were recorded at 293 K in CDCl3 on a Bruker Avance III 400 NMR spectrometer, operating at 9.4 Tesla, observing 1H and 13C at 400.13 and 100.61 MHz, respectively. The spectrometer was equipped with either a 5-mm multinuclear direct detection probe (1D NMR experiments) or a 5-mm multinuclear inverse detection probe (2D NMR experiments), both with z-gradient. One-bond and long-range 1H-13C correlation from HSQC and HMBC NMR experiments were optimized for an average coupling constant JH-C and JCH of 140 and 8 Hz, respectively. All 1H and 13C NMR chemical shifts (δ) are given in ppm related to the TMS signal at 0.00 ppm as an internal reference, and the coupling constants (J) in Hz. Silica gel 60 (Merck, 230-400 mesh) was used for column chromatography while precoated silica gel plates (Merck, 60 F254, 0.25 mm) were used for analytical TLC. Compounds were visualized by spraying with p-anisaldehyde reagent followed by heating on a hot plate.

Plant material

Leaves of Combretum duarteanum Cambess. (Combretaceae), were collected in the region around Serra Branca, Paraíba, Brazil, in March 2007. The plant was authenticated by Prof. Maria de Fátima Agra from the Federal University of Paraíba, and a voucher specimen was deposited at the Lauro Pires Xavier Herbarium (Voucher Nº 6767).

Extraction and isolation of friedelin

The dried leaves (1 kg) were powdered and extracted with ethanol, stirred and macerated at room temperature for approximately 48 h, and the procedure was repeated three times. The solvent was fully evaporated under reduced pressure, yielding 200 g of ethanol extract. The ethanol extract underwent liquid-liquid partition with hexane, obtaining 65.48 g of hexane extract (HE). Such step was repeated to secure the required quantity for the study (Lima et al., 2013). The hexane extract (1 g) was submitted to column chromatography eluted with CHCl3:MeOH 95:05 (v/v), resulting in thirty fractions of 50 ml each. The eluted fractions were evaluated and pooled according to TLC analysis, affording ten fractions (F-1 to F-10). F-3 was presented as a yellow light amorphous material submitted to recrystallization with methanol, obtaining in the end of the process a white amorphous solid, which was submitted to spectroscopic analysis, such as 1D and 2D NMR experiments, IR and Mass.

Friedelin (1): white amorphous solid, m.p. 259-260°C (lit. 261-262°C, Subhadhirasakul and Pechponsong, 2005); IR (KBr)νmax 1720 cm⁻¹; EIMS m/z (% intensity): 426 ([M]+, 6.47), 411([M-CH3]+), 2.39, 302 (6.13), 273 (4.24), 246 (9.66), 231 (11.87), 218 (14.08), 205 (14.47), 191 (15.22), 179 (15.57), 163 (21.76), 149(10.02), 137 (16.45), 125 (31.80), 123 (40.97),109 (49.73), 95 (64.89), 81 (62.00), 69 (91.43), 55 (100.00); The 1H and 13C NMR data are in agreement with those from literature (Subhadhirasakul and Pechponsong 2005; Almeida 2011).

Animals

Male Swiss mice (27-33 g), 2-3 months of age, were used throughout this study. The animals were randomly housed in cages at 21 ± 2°C on a 12 h light/dark cycle (lights on from 6 a.m. to 6 p.m.) with free access to food (Purina®, Brazil) and water. Before the experiments, the animals were acclimatized to the...
laboratory for at least 1 h prior and were used only once for each test. Experiments were carried out between 9 a.m. and 2 p.m. in a quiet room. All experiments involving the behavioral analysis were carried out by the same visual observer and in a blind manner. Experimental protocols were approved by the Animal Care and Use Committee (CEPA/UFS: 17/10) at the University of Sergipe, and handling procedures were in accordance with the International Association for the Study of Pain (IASP) guidelines for the use of animals in pain research (Zimmermann, 1983). All efforts were made to minimize the number of animals used and their discomfort, and all behavior tests were performed under blind conditions.

**Formalin-induced orofacial nociception**

The orofacial test was performed according to previous studies (Clavelou et al., 1995; Luccarini et al., 2006). Mice were pretreated (orally, p.o.) with HE at doses of 100, 200 and 400 mg/kg, friedelin (50 mg/kg, p.o.), vehicle (Tween 800.2%) or morphine (5 mg/kg) 60 min before the administration of formalin 2%. The possible involvement of the opioid system was evaluated by the administration of naloxone (1.5 mg/kg, i.p.), a non-selective opioid antagonist, 30 min before the administration of treatment with the highest dose of HE or friedelin.

The induction of nociception was performed through subcutaneous injection of formalin (20 µl, 2%) into the upper lip (perinasal area) of mice according to what was described by Luccarini et al. (2006), with adaptations (Quintans-Júnior et al., 2010). The first phase (neurogenic phase) occurs within the first 0-5 min after the administration of the nociceptive substance and this phase is followed by a latency period of about 10 min. Then, a second phase (inflammatory phase) occurs within 15-40 min after the administration of the nociceptive substance. Animals were observed individually in mirrored chambers (30 × 30 × 30 cm) to allow an unobstructed view of the orofacial region; the nociceptive behavior assessed mirrored chambers (30 × 30 × 30 cm) to allow an unobstructed view of the orofacial region; the nociceptive behavior assessed.

**Glutamate- and capsaicin-induced orofacial nociception**

The orofacial pain was induced by glutamate or capsaicin in mice as described earlier (Quintans-Júnior et al., 2010). Mice (n = 8, per group) were injected with 40 µl of glutamate (25 mM) or capsaicin (20 µl, 2.5 µg) subcutaneously into the right upper lip (perinasal area), using a 27 gauge needle. Capsaicin was dissolved in ethanol, dimethyl sulfoxide and distilled water (1:1:8). In pilot studies, rodents manifested pain-related face-rubbing behavior following the capsaicin injection with high intensity at 10-20 min period. Therefore, pain quantification was performed at this period by measuring the time (s) that animals spent face-rubbing the injected area with fore- or hindpaws. HE (100, 200 or 400 mg/kg, p.o.), friedelin (50 mg/kg, p.o.) or vehicle (Tween 800.2%) were given to animals as described for formalin test, 1 h before the local injection of glutamate or capsaicin. Morphine (5 mg/kg, i.p.), administered 1 h before the algogen, was included as a positive control. An additional group received a similar volume of capsaicin vehicle (data not shown).

**Evaluation of the motor activity (Rota-rod test)**

To investigate the possible interference of C. duarteum or friedelin in the motor activity of the animals, we used the rotarod apparatus according to method described Quintans-Júnior et al. (2010). Initially, the mice able to remain on the rotarod apparatus (AVS®, Brazil) for more than 180 s (9 rpm) were selected 24 h before the test. Then, the selected animals were divided into five groups (n = 8, per group) and were pretreated systemically (p.o.) with HE or friedelin at the same doses of formalin test. The control group received vehicle (Tween 800.2%) and standard group received diazepam (DZP, 3 mg/kg, i.p.). After 60, 120 and 240 min, each animal was submitted to the rotarod apparatus and the time that the animals remained on the rotating rod was registered. The maximum time of stay of the animal on the rotarod was 180 s.

**Statistics analysis**

Data obtained were expressed as mean and standard error of the mean (mean ± SEM) and the differences among the groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey’s test. In all cases, differences were considered significant if p < 0.05. All statistic analyses were performed using Graph Pad Prism 5.0 (Graph Pad Prism Software Inc., San Diego, CA, USA). The percentage of inhibition by an antinociceptive agent was determined using the following formula (Reanmongkol et al., 1994):

\[ \text{Inhibition %} = 100 \times \frac{\text{control} - \text{experiment}}{\text{control}}. \]

**Drugs and reagents**

The drugs and reagents used in this study were morphine sulphate (Dimorf-Cristalia, Brazil), glutamate (Sigma, USA), capsaicin (Sigma, USA), naloxone (Res. Biochemicals Inc., USA), diazepam (União Química, Brazil) and formaldehyde (Merck, USA). The vehicle was 0.2% Tween 80 (Sigma, USA) dissolved in saline solution (NaCl 0.9%).

**Results and discussion**

The present study evaluates, in an unprecedented manner, the antinociceptive effect of hexanic extract (HE) obtained from Combretum duarteum Cambess, Combretaceae, and friedelin (1), a triterpene, by formalin-, glutamate- and capsaicin-induced orofacial nociception models in mice. For the first time, we demonstrate that the systemic administration, by oral route, of the HE and friedelin (100% from GC) doses which did not produce any motor performance alteration produced consistent antinociceptive effects in different models of orofacial nociception.

The isolated compound obtained was a white amorphous solid with m.p. 259-260°C. The MS spectrum showed a molecular ion peak at m/z 426 M+. The IR spectrum showed an intense band at 1720 cm⁻¹ consistent with a six membered ring.
skeleton containing a carbonyl group. The $^1$H NMR revealed signals for seven singlet methyls at $\delta$ 1.18 (H-28), 1.05 (H-27), 1.01 (H-26), 1.00 (H-30), 0.95 (H-29), 0.87 (H-25) and 0.72 (H-24), a doublet methyl at $\delta$ 0.88 (d, $J$ = 6.6 Hz, H-23), a methine proton at $\delta$ 2.25 (q, $J$ = 6.8 Hz, H-4), and methane protons at $\delta$ 2.39 (ddd, $J$ = 13.7, 5.2, 2.0 Hz, H-2) and $\delta$ 2.29 (ddd, $J$ = 13.7, 7.0, 1.0 Hz, H-2), respectively. No vinylic proton signal was observed. The remaining proton signals were at $\delta$ 1.2-2.00. These $^1$H NMR data showed that the isolated compound seemed to be friedelin (Subhadhirasakul & Pechpong, 2005). The $^{13}$C NMR spectrum showed a total of thirty carbons, among them a ketone carbon at $\delta$ 213.2 was observed. The remaining 29 carbons showed signals of having chemical shifts between 6.8 and 59.5 ppm. The $^1$H and $^{13}$C NMR data of the isolated compound were identical to those in the literature identified for friedelin (Subhadhirasakul & Pechpong, 2005; Almeida et al., 2011). Therefore, the isolated compound was identified as the pentacyclic triterpene known as friedelin (1). The confirmation of the structure of the isolated compound was supported by HMBC and HSQC experiments, and comparison to literature data (Subhadhirasakul & Pechpong, 2005; Almeida et al., 2011).

The formalin test is a valuable tool widely used in studies of pain in animals and it is a reliable model for study of the behavior of the intensity coding of orofacial nociceptive stimulation and counter-irritation phenomena, as well as for testing the effect of analgesic drugs (Luccarini et al., 2006). During the orofacial formalin test, two distinct phases due to different mechanisms of nociception are produced: the first phase is associated to the direct stimulation of C-nociceptors and it corresponds to the neurogenic pain acutely sensitive to drugs interacting with the opioid system, whereas the second phase reflects integration between nociceptors and spinal and brainstem signaling, and corresponds to inflammatory pain, being inhibited by cyclooxygenase (COX) inhibitors and supposed to depend on central neural changes induced by afferent generated activity during the initial phase in the local inflammatory response. Face rubbing due to formalin injection into the upper lip (perinasal region), has been mentioned as a specific nociceptive response (Dallel et al., 1995; Raboisson and Dallel, 2004). It was observed that all doses tested of HE and friedelin were able to significantly decrease ($p < 0.001$) the time that the animal kept displaying rubbing the orofacial region, in both phases of formalin test, in comparison with the control group (vehicle) (As shown in Table 1). As expected, naloxone reversed the analgesic effect of morphine. However, it did not alter the analgesic activity of the HE in a higher dose, and friedelin.

Beforehand, Antonisamy et al. (2011) had actually demonstrated that friedelin does not act through opioid pathways. Conversely, Gouveia et al. (2011) suggested that ethanolic extract (EE) from leaves of the C. duarteanum produced antinociceptive effect by the enchacement of the opioid pathway. Perhaps, EE contains compounds, different from friedelin, that contribute to the analgesic profile, such as what was shown by Lima et al. (2012). Those results suggest that the analgesic effect may act both by inhibiting the release of substance P and by inhibiting the arachidonic acid cascade reducing the production of inflammatory molecules, such as serotonin, histamine, bradykinin and/or prostaglandins (Luccarini et al., 2006). Another possibility for the inconsistence in results is probably related to the different types of models of nociception evaluated, and also because of the different areas studied, since the orofacial nociception involves the trigeminal system which presents complex and unique physiological characteristics compared to the spinal nociceptive system (Conti et al., 2003).

To evaluate whether an important pathway is involved with the analgesic profile of HE and friedelin, we investigated them in glutamate-induced orofacial nociception test. Glutamate, which is an excitatory neurotransmitter, present in both central and peripheral terminals of trigeminal and dorsal root ganglion neurons, has been used as a pharmacological tool in studies of orofacial nociception in animals for testing the effect of analgesic drugs, such as natural products or new drugs (Quintans-Júniior et al., 2010). Primary afferent fibers are sensitized by nerve or tissue injury or by response to noxious stimuli, it occurs by the release of amino acids from the central and spinal afferents trigeminal (Keast and Stephensen, 2000; Lam et al., 2005). Now, we showed that pretreatment with HE or friedelin promoted a significant decrease ($p < 0.001$) in the time the animals kept rubbing the orofacial region behavior as compared to the control group. Those results suggest a possible interaction with the glutamatergic system. Hence, the nociceptive response caused by glutamate seems to involve peripheral, spinal, and supra-spinal sites, and its action is mediated by NMDA (N-methyl-D-aspartate) and non-NMDA receptors (Lam et al., 2005; Fundytus, 2001; Beirith et al., 2002).

Capsaicin (8-methyl-N-vanillyl-6-trans-nonenamide) is the strong ingredient in hot chili peppers of the Capsicum genus, and it selectively activates the unmyelinated C-fibre class of nociceptors (Caterina et al., 1997). Capsaicin is used as a pharmacological tool for the study of nociception because when applied to skin, muscle and other tissues it produces inflammation, It activates and sensitizes the trigeminal and spinal small-diameter nociceptive afferents, as well as dorsal horn neurons (Hu et al., 2005; Lam et al., 2009). Table 2 shows that all doses tested of HE and friedelin produced a significant decrease ($p < 0.001$) in nociceptive behavior induced by capsaicin, in comparison to the control group (vehicle). This finding may be related to a possible inhibition of substance P release or due to a direct block of its receptor neurokinin-1 (NK-1). The tonic activation of NK-1...
receptors through the administration of the NK-1 receptor antagonist SR14033 blocked the second phase of the orofacial formalin test in rodents (Raboisson & Dallelo, 2004). Waning et al. (2007) demonstrated that capsaicin-sensitive receptor potential vanilloid transient 1 (TRPV1), which plays an important role in pain transduction, is a Ca²⁺ influx channels involved in cell migration. Additionally, Honda et al. (2008) suggests that TRPV1 receptor mechanisms in mouse facial skin responses influence the nociceptive cutaneous noxious thermal and mechanical stimulus, inducing neuroplastic changes in the caudal subnucleus (Vc) neurons and C1-C2. All nociceptive tests conducted in the present study have a motor response as indicative of nociceptive behavior (rubbing the orofacial region - face regional). However, drugs that interfere with motor activity or induce sedation may give false-positive nociceptive results in these tests. Thus, we performed the rota-rod test, which provides subsidies for a good index for neurological deficits including sedation, muscle relaxant and decreased motor activity (Pu et al., 1995; Novas et al., 1998; Quintans-Júnior, 2010). We did not register any significant alteration in motor performance for HE and friedelin (data not shown).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Painful response</th>
<th>% inhibition</th>
<th>Painful response</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>81.5 ± 6.6</td>
<td>-</td>
<td>87.8 ± 9.4</td>
<td>-</td>
</tr>
<tr>
<td>HE</td>
<td>100</td>
<td>37.6 ± 5.3c</td>
<td>53.8</td>
<td>65.3 ± 13.0b</td>
<td>25.6</td>
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<tr>
<td>HE</td>
<td>200</td>
<td>29.2 ± 1.8c</td>
<td>64.2</td>
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<tr>
<td>HE</td>
<td>400</td>
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<td>52.6</td>
<td>48.6 ± 6.9c</td>
<td>44.6</td>
</tr>
<tr>
<td>HE + NAL</td>
<td>1.5 + 400</td>
<td>26.0 ± 2.3c</td>
<td>68.1</td>
<td>19.0 ± 9.5c</td>
<td>78.4</td>
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<tr>
<td>Friedelin (1)</td>
<td>50</td>
<td>33.5 ± 8.1c</td>
<td>58.9</td>
<td>21.9 ± 7.7c,d</td>
<td>75.1</td>
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<tr>
<td>Friedelin + NAL</td>
<td>1.5 + 50</td>
<td>29.8 ± 7.3c</td>
<td>63.4</td>
<td>28.4 ± 5.4c,d</td>
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<td>Morphine</td>
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<td>88.3</td>
<td>19.1 ± 2.0c</td>
<td>78.2</td>
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<td>MOR + NAL</td>
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<td>70.6 ± 4.3</td>
<td>13.3</td>
<td>81.6 ± 5.8c</td>
<td>7.1</td>
</tr>
</tbody>
</table>

N = 8, per group.

Table 1
Effect of HE, friedelin or morphine (MOR) in formalin-induced orofacial nociception, in presence and absence of naloxone (NAL), in rodents.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Glutamate test</th>
<th></th>
<th>Capsaicin test</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Painful response</td>
<td>% inhibition</td>
<td>Painful response</td>
<td>% inhibition</td>
</tr>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>105.3 ± 13.2</td>
<td>-</td>
<td>98.5 ± 9.1</td>
<td>-</td>
</tr>
<tr>
<td>HE</td>
<td>100</td>
<td>53.6 ± 8.1b</td>
<td>49.1</td>
<td>40.1 ± 7.2b</td>
<td>59.3</td>
</tr>
<tr>
<td>HE</td>
<td>200</td>
<td>48.3 ± 7.5b</td>
<td>54.1</td>
<td>34.9 ± 6.8b</td>
<td>64.6</td>
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<tr>
<td>HE</td>
<td>400</td>
<td>43.5 ± 11.0b</td>
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<td>26.5 ± 8.1b</td>
<td>73.1</td>
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<tr>
<td>Friedelin (1)</td>
<td>50</td>
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<td>61.9</td>
<td>29.6 ± 8.3b,c</td>
<td>69.9</td>
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<tr>
<td>Morphine</td>
<td>5</td>
<td>19.5 ± 7.7b</td>
<td>81.5</td>
<td>14.3 ± 2.3b</td>
<td>85.5</td>
</tr>
</tbody>
</table>

n = 8, per group.

Table 2
Effect of HE, friedelin or morphine in glutamate- or capsaicin-induced orofacial nociception in rodents.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Glutamate test</th>
<th></th>
<th>Capsaicin test</th>
<th></th>
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<tbody>
<tr>
<td></td>
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<td>% inhibition</td>
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</tr>
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<td>-</td>
<td>98.5 ± 9.1</td>
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<tr>
<td>HE</td>
<td>100</td>
<td>53.6 ± 8.1b</td>
<td>49.1</td>
<td>40.1 ± 7.2b</td>
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<td>73.1</td>
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<td>61.9</td>
<td>29.6 ± 8.3b,c</td>
<td>69.9</td>
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<tr>
<td>Morphine</td>
<td>5</td>
<td>19.5 ± 7.7b</td>
<td>81.5</td>
<td>14.3 ± 2.3b</td>
<td>85.5</td>
</tr>
</tbody>
</table>

n = 8, per group.

*Values represent mean ± S.E.M.

*p < 0.05 (one-way ANOVA and Tukey’s test), significantly different from control.

*p < 0.001 (one-way ANOVA and Tukey’s test), significantly different from control.

*p < 0.05 (one-way ANOVA and Tukey’s test), significantly different from HE 400 mg/kg group.
Conclusions

It can be concluded that the antinociceptive profile of EH and friedelin probably occurs via central and peripheral pathways, modulating the inflammatory and neuropathic pain, but without opioid system involvement. The friedelin seems, at least in part, to contribute to the explanation of the antinociceptive properties of C. duarteanum extract. Furthermore, the antinociceptive action demonstrated in the present study supports, at least partly, the ethnomedical use of this medicinal plant.

Authors’ contributions

JSSQ, TTS, SSA, AGSC, SMR, LJQJr contributed with pharmacological tests. EVC, JFT, CSE, AB and MSS contributed with the isolation and characterization of friedelin and phytochemistry study.

Conflicts of interest

The authors declare no conflicts of interest.

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