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Original Article

# Oleanane-type triterpenoid: an anti-inflammatory compound of the roots *Arrabidaea brachypoda*

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ABSTRACT

*Arrabidaea brachypoda* Bureau, Bignoniaceae, known as "cipó-una", is widely used in traditional medicine in Southeastern and Northeastern Brazil for kidney stones and painful joints. This study was aimed at evaluating the anti-inflammatory proprieties of the oleanane-type triterpenoid 3β-estearioxy-olean-12-ene isolated from the roots of *A. brachypoda*. Carrageenan-induced paw oedema, formalin test and hot plate test were used to investigate the antiinflammatory activity of 3β-estearioxy-olean-12-ene in animals. We observed that 3β-estearioxy-olean-12-ene at doses of 5, 10 and 15 mg/kg *p.o.* demonstrated anti-inflammatory effects, by reduced ($p < 0.05$) paw oedema induced by carrageenan and by decreased ($p < 0.05$) licking time caused by a subplantar injection of formalin. In conclusion, 3β-estearioxy-olean-12-ene, a triterpene isolated from the roots of *A. brachypoda*, demonstrate anti-inflammatory effect in different tests. Thus, it may be useful in the treatment of inflammatory disorders, which supports previous claims of its traditional use.

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## Introduction

The biodiversity of Brazilian is broad and several native Brazilian plant species have a long history of use in traditional medicine. These traditional medicinal plants contain compounds that may not only exhibit a broad range of therapeutic efficacy but also be useful in modern medicine. The Brazilian Cerrado (Neotropical Savanna) is one of the most biogeographically diverse regions of the world (de Medeiros et al., 2013; Agra et al., 2008; Blatt et al., 1998; Mendonça et al., 1998). Many of these plants are commonly used in traditional medicine for the treatment of several ailments (de Medeiros et al., 2013; Blatt et al., 1998; Mendonça et al., 1998), including infectious and inflammatory diseases.

The genus *Arrabidaea* occurs in tropical America from Mexico to Argentina, including Brazilian Cerrado. Species from the genus *Arrabidaea* have been used in traditional medicine for astringent, antioxidant, anti-inflammatory, antimicrobial, antitumor and healing purposes (Zorn et al., 2001; Bolzani et al., 2003; Leite et al., 2006; Martin et al., 2008). The *Arrabidaea brachypoda* Bureau, Bignoniaceae, known as "cipó-una", is widely used in traditional medicine in Southeastern and Northeastern Brazil for kidney stones and painful joints (Alcerito et al., 2002; Rodrigues et al., 2006). Previous study reported that ethanolic extract from *A. brachypoda* roots possess significant anti-inflammatory effects in laboratory animals (da Rocha et al., 2011), supporting the traditional use of this plant in some painful and inflammatory conditions. In addition, because chemical profile of *A. brachypoda* roots extract is established in which flavonoids and triterpenes are the major constituents (da Rocha et al., 2011, 2014).

Based on ethnopharmacological information of the *Arrabidaea brachypoda*, the aim of this work was to evaluate the anti-inflammatory proprieties of 3β-estearioxy-olean-12-ene (**1**), an oleanane-type triterpenoid, isolated from *A. brachypoda* roots ethanolic extract.

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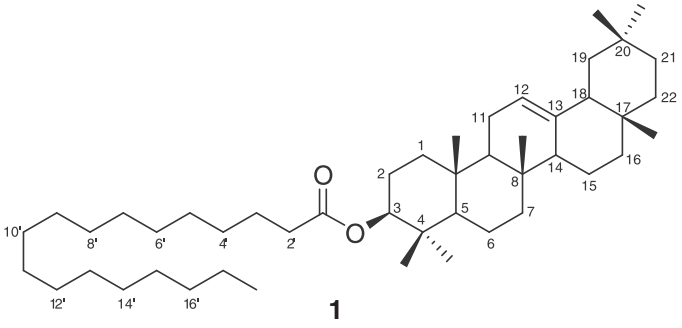

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## Material and methods

### *Plant material*

*Arrabidaea brachypoda* Bureau, Bignoniaceae, roots were collected in Sant'Ana da Serra-João Pinheiro, Minas Gerais, Brazil. Dra. Ana Maria Cristina Teixeira Braga, Department of Botanic of Federal University of Ouro Preto, identified the plant, and a voucher specimen was deposited at the Herbarium of Federal University of Ouro Preto (voucher number 17935).

### *Preparation of plant extract, isolation of compound and reference drugs*

To obtain the extract, the roots were dried, powdered (685 g) and extracted with ethanol in Soxhlet equipment for 24 h. The solvent was removed under reduced pressure and then dried with a spray dryer (Büchi Mini Spray Dryer B-290). The yield of the *A. brachypoda* ethanolic extract (AbE) was 11%. To isolate the bioactive compound, AbE was chromatographed on a silica gel (230–400 mesh) column (8 cm × 100 cm) and eluted with crescent polarity mixtures of *n*-hexane/ethyl-acetate and ethyl-acetate/ethanol to give 39 fractions. These fractions were pooled into four groups according to their similarities after the analysis using thin layer chromatography (TLC). Fractions 3–8 were rechromatographed on a silica gel (230–400 mesh) column (8 cm × 100 cm) and eluted with crescent polarity mixtures of *n*-hexane/ethyl-acetate and ethyl-acetate/ethanol to purify the triterpene 3β-stearyloxy-olean-12-ene (**1**). Its structure was determined using spectroscopic techniques (Table 1). The data were compared to those verified in a previous study investigating the chemical structure of this compound (Vieira-Filho et al., 2003).

3β-Estearioxy-olean-12-ene (**1**, AbE-01) was administered in 5, 10, and 15 mg/kg doses after being suspended in vehicle (1% sodium carboxymethylcellulose suspension in distilled water). Indomethacin (10 mg/kg, *p.o.*) and morphine (10 mg/kg, *i.p.*) in vehicle (saline) were used as reference drugs. Test drugs were orally administered in an equivalent volume of 10 ml/kg body weight of the animal.

### *Pharmacological activities*

#### *Animals*

Adult male Wistar rats weighing 180–220 g ($n = 8$) and adult male Swiss mice weighing 28–32 g ($n = 10$) animals, obtained from the Central Animal Facility of the Federal University of Alfenas, were housed under controlled light (12:12 h light–dark cycle; lights on at 6 am) and temperature conditions (23 ± 1 °C) with access to water and food *ad libitum*. The animals were allowed to habituate to the housing facilities for at least 1 week before the experiments began. All experiments were carried out between 8 am and 2 pm in a quiet room and tests were performed by the same visual observer in a double-blind study. The experiments were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of Alfenas (109/2009).

#### *Carrageenan-induced rat paw oedema*

Pedal inflammation in rat was produced as described previously (Vinegar et al., 1969; da Rocha et al., 2011), following an eight o'clock fast with free access to water. Paw oedema was measured with a plethysmometer (Model 7140, Ugo Basile, Italy). The basal volume of the right hind paw was determined before the administration of any drug. After determination of the basal volume, the animals were divided into the experimental groups in such a way that the mean volumes of the different groups were similar. Vehicle (10 ml/kg), 3β-estearioxy-olean-12-ene (doses of 5, 10, and 15 mg/kg) or indomethacin (10 mg/kg) was orally administered 1 h before the *i.pl.* injection of carrageenan (1 mg, 100 μl). The paw volume was measured 1, 2, 3 and 4 h after injection of the inflammatory stimulus. The results are presented as the paw volume (ml) variation in relation to the basal values.

#### *Formalin test*

A formalin solution (5% in 0.9% saline; 20 μl/paw) was injected into the hind paw plantar surface (*i.pl.*), and the mice were individually placed in transparent observation chambers, as previously described (Santos and Calixto, 1997; Vilela et al., 2010). Oral treatments (*p.o.*) with vehicle, indomethacin or 3β-estearioxy-olean-12-ene were given 1 h prior to formalin injection. Morphine was administrated (*i.p.*) 30 min before the test. The time spent in licking the injected paw was recorded and expressed as the total licking time in the early phase (0–5 min) and late phase (20–30 min) after formalin injection.

#### *Hot plate test*

The hot plate was an electrically heated surface kept at a constant temperature of 50.0 ± 0.5 °C. Mice ($n = 8$ per group) were placed on the heated surface within the Plexiglas walls to constrain their locomotion on the plate, and the latency to a discomfort reaction (licking of the paws or jumping) was recorded 0, 30, 60, and 120 min after 3β-estearioxy-olean-12-ene or morphine (10 mg/kg), where upon the reaction time of 0 min is the start of the test. A cut-off time of 20 s was chosen to indicate complete analgesia and to avoid tissue injury. The latencies for paw licking or jumping were recorded for each animal (Yamamoto et al., 2002).

#### *Statistical analysis*

The data obtained were analysed using GraphPad software programme version 6.0 and expressed as the mean ± S.E.M. Statistically significant differences between groups were calculated by the application of an analysis of variance (ANOVA) followed by the Tukey test. *p*-Values less than 0.05 ($p < 0.05$) were considered significant.

## Results

Fig. 1 shows that carrageenan induced rat paw oedema with compared with the vehicle administration. The 3β-stearyloxy-olean-12-ene significantly inhibited carrageenan-induced rat paw oedema ($F_{4,20} = 30.2$; $p < 0.001$). The inhibitory values of oedema at 3 h post-carrageenan treatment were 47.9, 53.1 and 50.4% for 5, 10 and 15 mg/kg of the compound, respectively. Indomethacin (10 mg/kg) gave a percentage inhibition of 65.2% (Fig. 1). At 4 h post-carrageenan treatment, the inhibitory values of oedema were 47.3, 55.6 and 57.1% for 5, 10 and 15 mg/kg of the compound, respectively. Indomethacin (10 mg/kg) gave a percentage inhibition of 74.8% (Fig. 1).

**Table 1**
NMR spectral data of 3-β(stearyloxy)olean-12-ene (AbE-01).

| Carbon number | AbE-01 ($\delta_C$) | AbE-01 ($\delta_H$) | HMBC | Carbon number | AbE-01 ($\delta_C$) | AbE-01 ($\delta_H$) | HMBC |
|---|---|---|---|---|---|---|---|
| 1 | 37.74 | 1.13 and 1.61 | | 1′ | 173.55 | – | |
| 2 | 22.69 | 1.64 | 3 | 2′ | 34.87 | 2.30 | 1′, 3′, 4′ |
| 3 | 80.51 | 4.52 | 1′, 1, 2, 4, 23, 24 | 3′ | 25.17 | 1.63 | |
| 4 | 36.83 | – | | 4′ | 29.26 | 1.29 | |
| 5 | 55.26 | 0.80 | | 5′ | 29.27 | 1.29 | |
| 6 | 18.35 | 1.43 and 1.56 | | 6′ | 29.48 | 1.26 | |
| 7 | 32.47 | 1.36 and 1.52 | | 7′ | 29.59 | 1.26 | |
| 8 | 41.51 | – | | 8′ | 29.65 | 1.26 | |
| 9 | 47.55 | 1.60 | | 9′ | 29.68 | 1.28 | |
| 10 | 36.94 | – | | 10′ | 29.72 | 1.28 | |
| 11 | 23.61 | 1.86 | 12 | 11′ | 29.72 | 1.26 | |
| 12 | 121.65 | 5.19 | 9, 11, 14, 18 | 12′ | 29.93 | 1.26 | |
| 13 | 145.16 | – | | 13′ | 29.72 | 1.26 | |
| 14 | 41.69 | – | | 14′ | 29.59 | 1.26 | |
| 15 | 26.24 | 0.96 | | 15′ | 29.48 | 1.26 | |
| 16 | 27.04 | 2.20 | 28 | 16′ | 31.93 | 1.26 | |
| 17 | 32.54 | – | | 17′ | 22.69 | 1.26 | |
| 18 | 47.36 | 1.94 | 12 | 18′ | 14.12 | 0.88 | 17′, 16′ |
| 19 | 46.90 | 1.03 and 1.76 | | | | | |
| 20 | 31.10 | – | | | | | |
| 21 | 34.83 | 1.10 and 1.39 | | | | | |
| 22 | 37.24 | 1.22 and 1.46 | | | | | |
| 23 | 28.13 | 0.88 | 3, 4, 5, 24 | | | | |
| 24 | 16.82 | 0.88 | 3, 4, 5, 23 | | | | |
| 25 | 15.57 | 0.96 | 1, 5, 9, 10 | | | | |
| 26 | 16.90 | 0.97 | 7, 8, 9, 14 | | | | |
| 27 | 26.00 | 1.14 | 8, 13, 14, 15 | | | | |
| 28 | 28.43 | 0.84 | 16, 17, 18, 22 | | | | |
| 29 | 33.35 | 0.88 | 19, 20, 21, 30 | | | | |
| 30 | 23.75 | 0.88 | 19, 20, 21, 29 | | | | |

The intrapodal administration formalin induced an nociceptive behaviour of licking the paw during the test (Fig. 2B). The 3β-stearyloxy-olean-12-ene at doses of 5–15 mg/kg *p.o.* had a significant activity compared to the control in both the early ($F_{5,60}$ = 34.3; $p$ < 0.001; Fig. 2A) and late phases ($F_{5,60}$ = 25.4; $p$ < 0.001, Fig. 2B) of the formalin test. The reference drug, indomethacin, suppressed only the late phase of the formalin test, while morphine inhibited both phases of the painful stimulus.

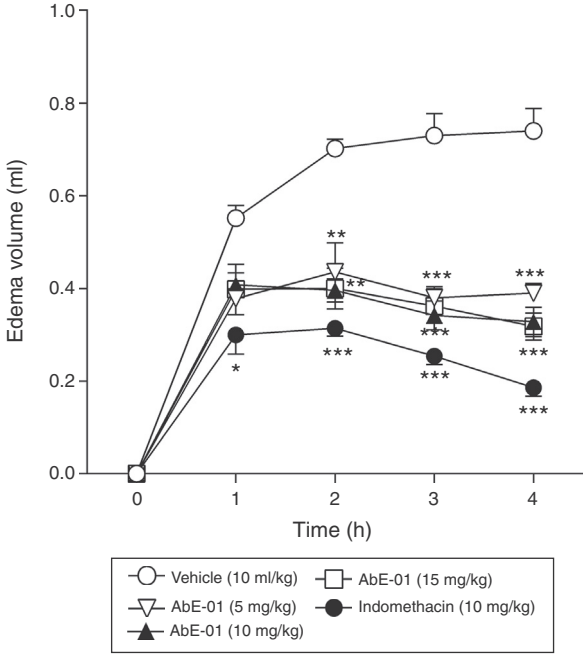


**Fig. 1.** Effects of the administration of the 3-β-stearyloxy-olean-12-ene (AbE-01; 5, 10 and 15 mg/kg, *p.o.*) or indomethacin (5 mg/kg, *p.o.*) on rat paw oedema induced by intraplantar carrageenan injection (1 mg/paw). Each point represents the mean ± S.E.M. The asterisks denote the significance levels when compared with the control group: $^{*}p < 0.05$, $^{**}p < <0.01$, $^{***}p < 0.001$.

In the hot plate test, treatment with 3β-stearyloxy-olean-12-ene at doses of 5–15 mg/kg increased the latency time as compared to the control group at 30 ($F_{4,39}$ = 6.60; $p$ < 0.001), 60 ($F_{4,39}$ = 7.36; $p$ < 0.001) and 120 min ($F_{4,39}$ = 11.22; $p$ < 0.001). As expected, administration of the vehicle did not induce any antinociceptive effect. Morphine significantly increased the latency time (Fig. 3).

## Discussion

Traditional medicine for the treatment of various diseases is becoming more popular. Therefore, the present study was aimed at evaluating the scientific basis for the traditional use of *A. brachypoda* Bureau, Bignoniaceae, using *in vivo* inflammatory models. The results of this investigation demonstrate that the triterpene 3β-estearioxy-olean-12-ene from *A. brachypoda* exerts anti-inflammatory effects in animals.

For the screening of compounds possessing anti-inflammatory activity, carrageenan-induced paw oedema is a model widely employed and has frequently been used to assess the anti-oedematogenic effect of natural products (Mendes et al., 2010). The development of oedema induced by carrageenan is a biphasic event. The early phase (1–2 h) is mainly mediated by histamine, serotonin and bradykinin. The late phase is sustained by the release of prostaglandins and nitric oxide, with a peak at 3 h, and is produced by inducible isoforms of cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) (Brito and Antonio, 1998; Seibert et al., 1994). Previous oral treatment with this 3β-stearyloxy-olean-12-ene was effective in reducing the oedematogenic response evoked by carrageenan in late phase. This reduction may be caused by inhibition of one or more intracellular signalling pathways involved in mediating the inflammatory response (Thomazzi et al., 2010).

The formalin test is a model that consists of two distinct phases. The first phase, or neurogenic phase (immediately after

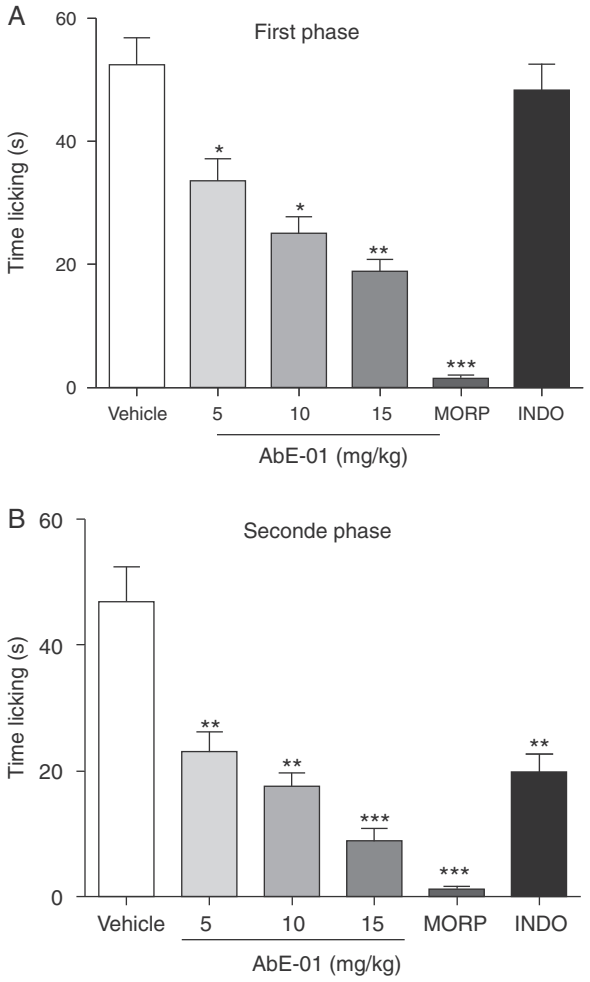


**Fig. 2.** Effects of 3-β-stearyloxy-olean-12-ene (AbE-01) given by oral route on the licking induced by formalin in mice. Animals were pretreated orally with vehicle, AbE-01 (doses 5, 10, and 15 mg/kg), indomethacin (INDO; 5 mg/kg) or morphine (MORP; 10 mg/kg) prior to formalin. The total time spent licking the hind paw was measured in the first (Panel A) and second (Panel B) phases after intraplantar injection of formalin. Each point represents the mean ± S.E.M. The asterisks denote the significance levels when compared with the control group: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

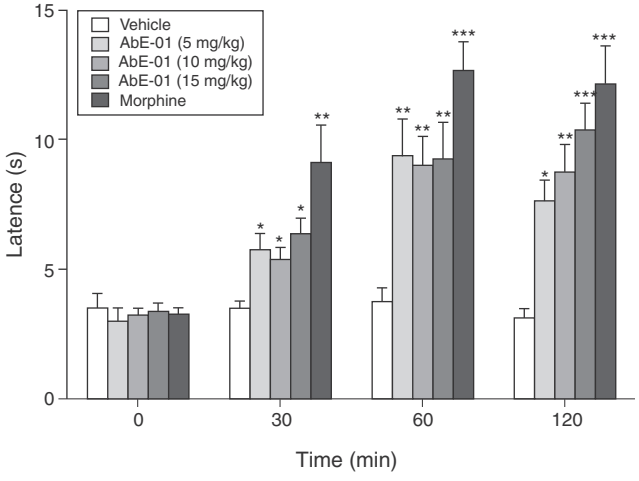


**Fig. 3.** Effects of 3-β-stearyloxy-olean-12-ene (AbE-01) administered orally in the hot plate test. Animals were pretreated orally with vehicle, morphine (Morp; 10 mg/kg), AbE-01 (doses 5, 10, and 15 mg/kg), prior to the tests at 50 °C. Each point represents the mean ± S.E.M. The asterisks denote the significance levels when compared with the control group: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

formalin injection), seems to be caused by the direct effect of formalin on sensory C-fibres. The second phase, or inflammatory phase (starting approximately 20 min after injection), is associated with the development of an inflammatory response and the release of nociceptive mediators (Abbott et al., 1995; Davidson and Carlton, 1998) A reduction of the late phase behavioural response to an *i.p.* formalin injection was observed, demonstrating the anti-inflammatory activity produced by 3β-stearyloxy-olean-12-ene. The results obtained from carrageenan-induced rat paw oedema also confirmed this effect. Similar to other substances that act on the central nervous system (CNS), 3β-stearyloxy-olean-12-ene inhibited both phases of the formalin test in a manner similar to that of morphine. Moreover, the results of this test are in agreement with those obtained from the hot plate test, confirming the central antinociceptive effect of the compound.

Phytochemical analyses of the *roots of A. brachypoda* extract indicated that the large majority of its constituents were triterpenes (da Rocha et al., 2011). In this regard, the anti-inflammatory activity is a common property of many triterpenoids (Safayhi and Sailer, 1997). The anti-inflammatory effects of triterpenes (*i.e.* oleanolic and ursolic acids) have been attributed to various mechanisms including: inhibition of lipoxygenase and cyclooxygenase activities, inhibition of elastase and inhibition of complement activity, possibly through the inhibition on C3-convertase of the classical complement pathway (Singh et al., 1992). In addition, previous works shown that the basic carbon skeleton has no influence on the anti-inflammatory activity, and the presence of a C-28 or C-30 carboxylic group and an alcoholic group at C-28 increases the activity in carrageenan-induces oedema (Recio et al., 1995), however, the mechanism of action of the 3β-stearyloxy-olean-12-ene is unknown.

In the present study, we demonstrated the efficacy of 3β-estearioxy-olean-12-ene, a triterpene isolated from the roots of *A. brachypoda*, in different anti-inflammatory tests. Thus, it may be useful in the treatment of inflammatory disorders, which supports previous claims of its traditional use.

## Author contributions

CQR, FCV, FVS-C and GPC participated in study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content. WV, AG-P and MHS participated in study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, administrative, technical, or material support, and study supervision.

## Conflicts of interest

The authors declare no conflicts of interest.

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