

Full Length Research Paper

Isolation and anti-inflammatory effects of maturin acetate from the roots of *Psacalium peltatum* (Asteraceae)

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Anti-inflammatory effects of maturin acetate (MA) isolated from the roots of *Psacalium peltatum* were investigated using *in vitro* and *in vivo* models. Inhibitory effects of increasing concentrations of MA on the production of pro-inflammatory cytokines (TNF- α and IL-1 β) by lipopolysaccharide (LPS)-activated peritoneal macrophages were measured *in vitro*. MA at 75 μ g/ml concentration inhibited TNF- α production by 80% and IL-1 β by more than 85% ($p < 0.01$). MA inhibited inflammation by 75% when applied topically to 12-O-tetradecanoylphorbol-13-acetate (TPA) induced, dose-dependent acute ear edema. Myeloperoxidase enzyme levels were also reduced in the inflamed tissue. MA showed a significant inhibition level of 58.95% at the onset of carrageenan-induced inflammation (1 h) and the effect persisted up to 5 h. Thus, MA shows high cytokine production and marked anti-inflammatory effects.

Key words: *Psacalium peltatum*, maturin acetate, pro-inflammatory cytokines, 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced acute ear edema, myeloperoxidase enzyme, carrageenan-induced inflammation.

INTRODUCTION

The biological activity of natural plant products has motivated extensive worldwide research to determine the pharmaceutical applications of their active compounds. *Psacalium peltatum* (Kunth) Cass. (synonyms: *Cacalia peltata* Kunth, *Senecio peltiferus* Hemsley) is an endemic medicinal plant, member of the matarique complex, which grows in central Mexico. The matarique medicinal plant complex consists of different plant species that are

grouped under the same common name, among them are *Acourtia thurberi*, *Psacalium decompositum*, *Psacalium sinuatum* and *Psacalium palmeri* (Linares and Bye, 1987). These plants share certain features, such as morphological characteristics of the employed plant and the same medical application. In Mexico, *P. peltatum* roots have traditionally been macerated in alcohol to treat conditions that cause inflammation such as wounds, skin

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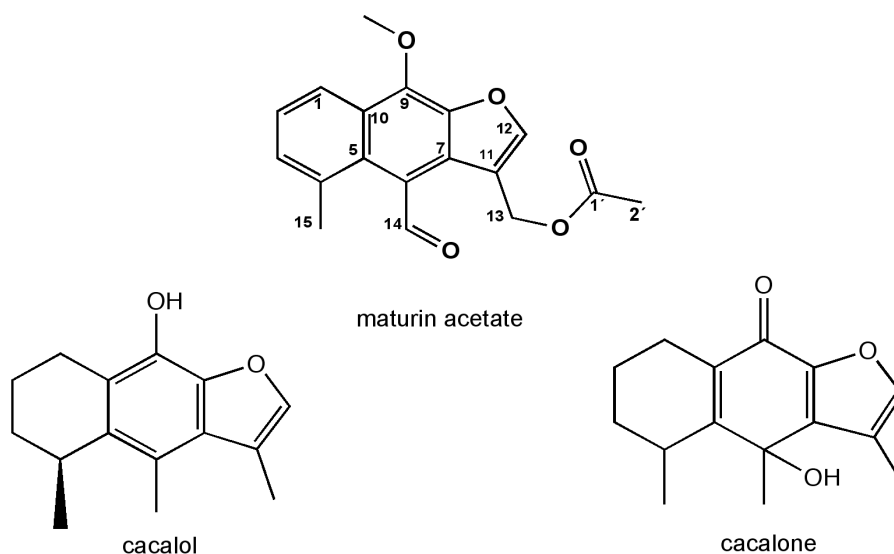


Figure 1. Structure of matorin acetate, cacalol and cacalone.

ulcers and rheumatism (Bye et al., 1995). The anti-diabetic effects of *P. peltatum* roots have also been studied (Contreras-Weber et al., 2002, 2005). The furanoeremophilane-type sesquiterpene named matorin acetate (MA) isolated from *P. peltatum* is similar to cacalol and cacalone isolated from *P. decompositum* (Figure 1). These two latter compounds are known to inhibit edema, showing a dose-dependent anti-inflammatory effect when tested in *in vivo* models (Jimenez-Estrada et al., 2006). Further investigations have shown that cacalone in a natural mixture with epicalalone exhibits higher anti-inflammatory effect than cacalol using an *in vivo* 12-O-tetradecanoylphorbol-13-acetate (TPA) model (Acevedo-Quiroz et al., 2008). The present work reports the anti-inflammatory effects of the bioactive compound MA isolated from *P. peltatum* using *in vitro* and *in vivo* models.

MATERIALS AND METHODS

Sample preparation and phytochemical analysis

Roots of *P. peltatum* were collected from the pine-oak forest at Mineral del Chico, Hidalgo, Mexico, [20° 09' 55" N and 98° 45' 08" W]. A voucher specimen was deposited at the National Herbarium (MEXU 1138692) of the Institute of Biology, UNAM, México. Air-dried and powdered roots (4.381 kg) of *P. peltatum* were sequentially extracted with n-hexane by thorough maceration (3 times × 2 l) at room temperature. The extract was filtered and concentrated. After the removal of solvent in vacuum, it afforded an oil residue. The n-hexane *P. peltatum* root extract (6.0 g) was fractionated by column chromatography over silica gel and eluted with n-hexane, ethyl acetate, and mixtures of these solvents. The collected fractions and compounds were monitored by thin layer

chromatography (TLC) on 0.2 mm pre-coated silica gel 60 F₂₅₄ plates (E. Merck), with the hexane/ethyl acetate (8:2) solvent system.

Compounds were observed under ultra violet (UV) light and by spraying with 1.0% ceric sulfate/H₂SO₄ solution. The isolated compound was recrystallized in acetone-hexane to yield a pure compound. The structure was examined using infrared spectra (IR), ¹H, ¹³C nuclear magnetic resonance (NMR) spectra and mass spectra (MS). Uncorrected melting points (m.p.) were determined on a Fisher Jones apparatus. Infrared radiation (IR) was recorded with a Nicolet fourier transform infrared spectroscopy (FT-IR) SX spectrometer using chloroform as solvent. ¹H, ¹³C NMR spectra were measured with a Varian Gemini-2000 and a Varian VXR-300 (200 MHz) spectrometer. Chemical shifts are reported in ppm relative to tetramethylsilane (Me₄Si, δ = 0) in CDCl₃ as solvent and J values in Hz.

Electron impact mass spectra were measured with a Jeol JMS-AX 505 HA spectrometer. MA was detected and quantified by high-performance liquid chromatography (HPLC). Sample solutions were prepared by dissolving the MA and *P. peltatum* root extract in methanol. A Synergi Polar-RP column (150 × 2.00 mm, 4 μm particle size; Phenomenex) was used. The mobile phase consisted of deionized water (A) and acetonitrile HPLC grade (B). Gradient elution was performed starting with 70A/30B and changing the composition to 100B after 25 min. The detection wavelength, flow rate and column temperature were set at 254 nm, 0.2 ml/min, and 40°C, respectively. For all solutions (samples and standard), 10 μl were injected. A sample of 1.00 mg of the compound was dissolved in 5.00 ml methanol and serial dilutions of this stock solution with methanol were done to obtain calibration curves. Within the concentration range injected (200.0 to 2 μg/ml), the detector response was linear (R² ≥ 0.9948), with a detection limit of less than 0.002 μg/ml (data not shown).

Experimental animals

Male ICR-CD1, BALB/c mice weighing 25 to 30 g and Wistar rats weighing 180 to 200 g were provided by the Instituto de Fisiología

Celular, UNAM, and approved by the Animal Care and Use Committee (NOM-062-ZOO-1999). All animals were housed under a 12 h light-dark cycle at $24 \pm 2^\circ\text{C}$, humidity $50 \pm 5\%$ with *ad libitum* access to food and water. All experiments were carried out using six animals per group.

In vitro determination of cytokine production in peritoneal macrophages

To obtain peritoneal macrophages, 8-week-old male BALB/c mice were killed by cervical dislocation. Peritoneal cells were obtained by lifting the peritoneal wall and injecting 4 ml of sterile phosphate buffered saline (PBS). The peritoneum was then massaged, and the fluid (approximately 4 ml) was drawn into a syringe. Cells were washed twice and re-suspended in Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma) containing 10% fetal bovine serum (FBS) (GIBCO) and 100 $\mu\text{g/ml}$ gentamycin (GIBCO); later the concentration was adjusted to 3×10^6 cell/ml. Cell viability was determined as described in Badisa et al. (2003). Absorbance was measured on a microplate reader at 515 nm. Cytokines IL-1 β and TNF- α were measured by enzyme-linked immunosorbent assay (ELISA) in the supernatant of peritoneal macrophages. These cell supernatants used for cytokine determination were prepared as follows: macrophages were washed twice, adjusted to a density of 10^6 cells/ml, and cultured at 37°C in 5% CO_2 for 24 h in RPMI complete medium either alone or with 2 $\mu\text{g/ml}$ LPS in the absence or the presence of increasing doses of maturated acetate (MA). After 24 h, samples were centrifuged at 2,500 rpm for 20 min at 18°C and the supernatant collected and assayed for IL-1 β (range of detection 63 to 4000 pg/ml) and TNF- α (range of detection 16 to 2000 pg/ml) using an ELISA kit (Peptotech) in a format of sandwich.

In vivo TPA-induced acute ear edema assay and myeloperoxidase (MPO) activity

The assay involving TPA-induced acute ear edema in mice was based on the method described by Merlos et al. (1991) modified by Jimenez-Estrada et al. (2006). Increasing concentrations of MA and indomethacin as positive control were dissolved in ethanol:acetone (1:1). The degree of neutrophil infiltration was indirectly quantified by determination of ear myeloperoxidase (MPO) activity (Bradley et al., 1982; Suzuki et al., 1983). Enzyme activity was determined by colorimetry at a wavelength of 450 nm, using a BIOTEK micro plate reader ELx808. Activity was expressed as absorbance increase and enzyme inhibition percentages.

In vivo carrageenan-induced rat paw edema assay

The assay involving carrageenan-induced rat paw edema was based on the method described by Winter et al. (1962), with certain modifications. Rats were administered sodium naproxen (Sigma) and MA at 100 mg/kg i.p. Sodium naproxen was used as positive control because it is widely accepted as a non-steroidal anti-inflammatory drug (NSAID) and is dose-related (Delporte et al., 2005). The 100 mg/kg MA dose was chosen as it is high enough to evidence anti-inflammatory effects; this has been shown in ibuprofen (In-Tae et al., 2004) and diclofenac (Ojewole, 2005). When there is no prior evidence of activity, natural extracts are commonly used at this concentration (Bolanle et al., 2012; Arawawala et al., 2012; Prakash Babu et al., 2011). Before treatment, the mean volume (from 3 or 4 measurements) of the

right paw of each animal was determined (V_0 , basal volume and V_t , edema at different time) using a Plethysmometer 7159, Ugo Basile. These individual records were used to calculate the variation of edema ($V_t - V_0$) in each group. Percentages of inhibition (I%) were determined using the following formula: $I\% = 100 - [B \times 100] / A$, where A is the mean variation of edema ($V_t - V_0$) in the control group and B is the ($V_t - V_0$) in the groups treated with MA and sodium naproxen.

Statistical analysis

Data obtained from animal experiments were expressed as mean values \pm standard error (\pm SEM). Statistical differences between the treated and control groups were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. Values of $P \leq 0.05$ (*) and $P \leq 0.01$ (**) were considered to be statistically significant (Tallarida and Murria, 1981).

RESULTS AND DISCUSSION

Medicinal plants and their products have been used for many centuries to treat different kinds of acute and chronic inflammatory diseases such as wound-healing, edema and rheumatoid arthritis (Gayathri et al., 2007). MA has been isolated from the roots of Mexican species such as *P. beamanii* (Perez et al., 2004), *P. radulifolium* (Garduño and Delgado, 2003), *Roldana angulifolia* (Arciniega et al., 2006), *Trichilia cuneata* (Doe et al., 2004) and from the South African species *Senecio digitalifolius* (Bohlmann and Zdero, 1978), *Senecio lydenburgensis* (Bohlmann et al., 1979) and *Senecio affinis* (Bohlmann and Bapuji, 1982).

In this work, phytochemical analysis of dried n-hexane *P. peltatum* root extract (6 g) by silica gel column chromatography revealed MA as the root's main constituent. Fractions eluted with hexane afforded a mixture of β -sitosterol/stigmasterol (15 mg) (Voutquenne et al., 1999). Fractions eluted with hexane/ethyl acetate (99:1) yielded maturated acetate (12 mg) (Correa and Romo, 1966) and MA (2.34 g) in the form of yellow needles (Figure 1). MA recrystallized from acetone-hexane. Crystallographic analysis of MA allowed the unambiguous assignment of the structure (Rojano-Vilchis, 2012). The ^{13}C NMR data from MA have not been reported before and were unequivocally assigned as: ^{13}C NMR (75.4 MHz, CDCl_3) δ 121.03 (C-1), 130.24 (C-2), 124.77 (C-3), 130.36 (C-4), 133.44 (C-5), 122.05 (C-6), 129.38 (C-7), 142.41 (C-8), 141.78 (C-9), 125.42 (C-10), 116.44 (C-11), 148.94 (C-12), 58.48 (C-13), 193.45 (C-14), 26.62 (C-15), 61.10 (O-CH $_3$ -9), 170.56 (C-1') 21.00 (C-2') ppm. In the present work, MA was directly obtained from the n-hexane root extract without undergoing the procedure of preparing an acetylated product with acetic anhydride in pyridine from maturated acetate, as has been the case in previous studies (Correa and Romo, 1966). Chromatographic conditions of isolated MA were

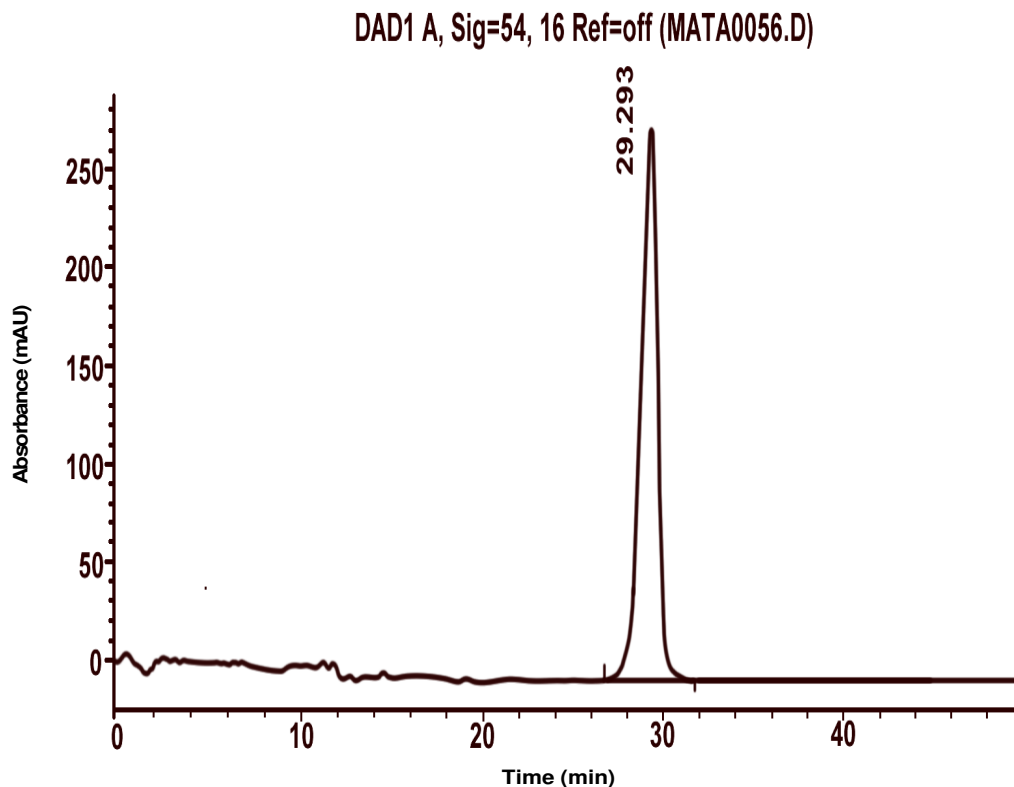


Figure 2. HPLC profile of maturin acetate.

optimized by HPLC to evaluate compound purity in the shortest period possible. MA showed one peak, with retention time of 29.29 min and with concentration of 228.75 mg/g in the n-hexane *P. peltatum* root extract. (Figure 2).

Cytokine synthesis is a primary mode of response of macrophages to inflammatory stimuli by bacterial products including LPS (Cheng et al., 2008). TNF- α is a cytokine with very diverse biological activities playing a role in various physiological and pathological phenomena, such as infection, inflammation, immunomodulation, cancer, cachexia and lethal septic shock. IL-1 β is among the most potent and multifunctional cell activators. IL-1 β is a key mediator in the series of host responses to infection and inflammation known as acute-phase response, and plays a particularly important role in the induction of acute-phase proteins whose synthesis is increased during inflammation. IL-1 β has been studied regarding its pathogenetic role in rheumatoid arthritis, vasculitis, Alzheimer disease, diabetes and autoimmune diseases in general (Mire-Sluis et al., 1998).

As shown in Figure 3, LPS-activated macrophages produced considerable levels of the two cytokines TNF- α and IL-1 β . The treatment of cells with MA induced a concentration-dependent inhibition of the production of

the two pro-inflammatory cytokines by LPS-stimulated macrophages. 75 μ g/ml of MA resulted in 80% inhibition of TNF- α and in more than 85% inhibition of IL-1 β ($p < 0.01$). These results confirm previously reported data, indicating that many medicinal plant extracts contain anti-inflammatory substances which act as inhibitors of pro-inflammatory responses (Calixto et al., 2004; Grabley et al., 1999; Tan et al., 2004).

Inflammation is a rapid local response to tissue damage with edema formation. Edema is characterized by reddening and swelling of the skin at the damage site (Ahamed et al., 2007). In this study, topical administration of MA and indomethacin significantly decreased acute ear edema induced by TPA at all administered doses compared with control groups ($p < 0.01$). Edema inhibition percentage (EI%) by indomethacin was 89.19% at a dose of 0.46 mg/ear, and MA showed its maximum inhibition level (75.50%) at a dose of 0.56 mg/ear. MA and indomethacin showed a dose-dependent anti-inflammatory effect and ID₅₀ values of 0.40 mg/ear ($r^2 = 0.99$) and 0.097 mg/ear ($r^2 = 0.97$), respectively (Table 1). MA showed a greater anti-inflammatory effect (75.50% edema inhibition) than cacalol (45.5% edema inhibition) in the same experimental model (Jimenez-Estrada et al., 2006). The TPA-induced acute ear edema response is

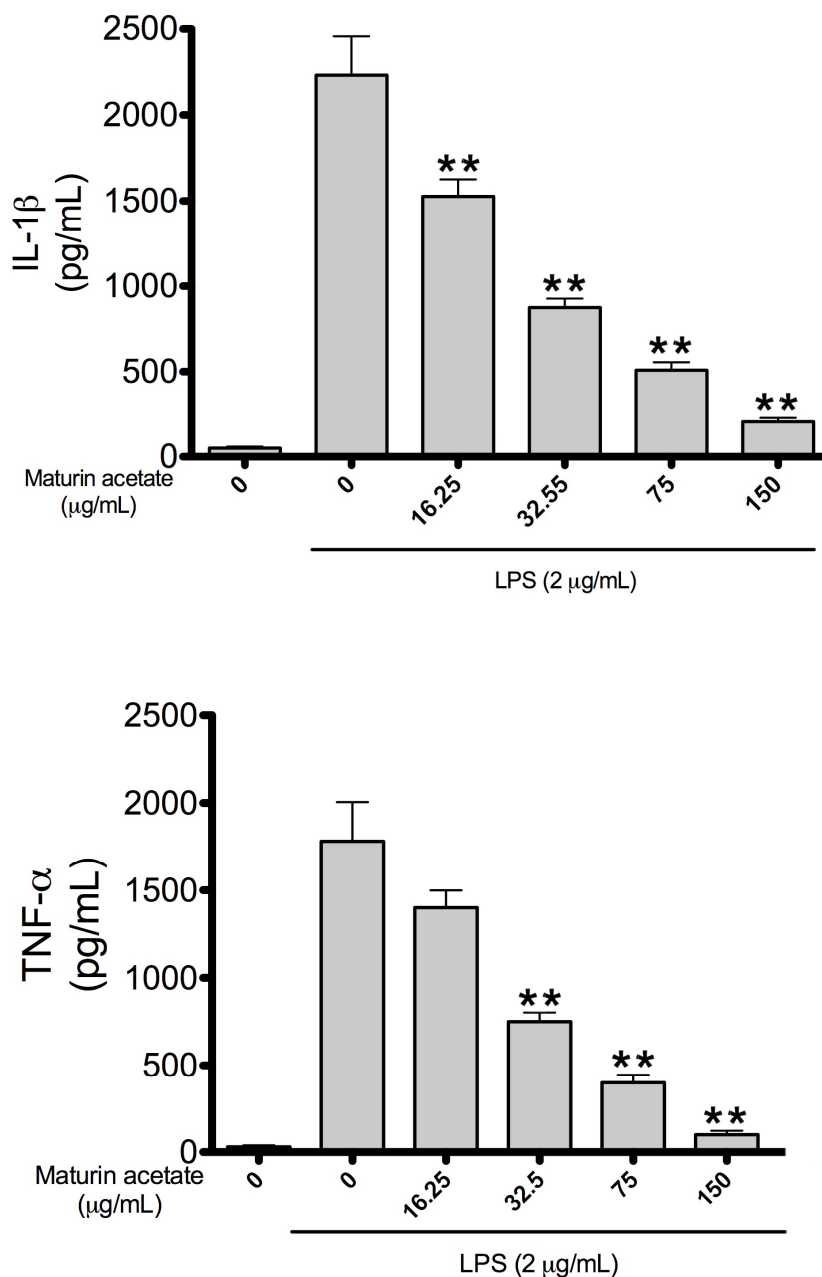


Figure 3. Effect of matorin acetate on LPS-induced cytokine secretion. Peritoneal macrophages (1×10^6 /ml) were plated in 24-well plates and stimulated with LPS (2 μg/ml) in the absence or presence of the indicated concentrations of compound for 24 h. At the end of the culture period, culture medium was collected for the determination of IL-1β, TNF-α by ELISA kits. Results are expressed as mean ± SEM of cytokine concentration (μg/ml). (**p < 0.01) versus LPS-stimulated cells.

associated with an increase in myeloperoxidase (MPO) concentration resulting from chemotaxis of neutrophils in the inflamed tissues (Smith, 1994). MA at doses of 0.31, 0.42 and 0.56 mg/ear reduced MPO activity (Figure 4).

Topical application of MA significantly reduced MPO levels in ear homogenates, indicating that neutrophil migration control participates in the observed topical anti-inflammatory activity. Anti-inflammatory effect observed

Table 1. Anti-inflammatory effects of increasing concentrations of indomethacin and maturin acetate on TPA-induced mouse ear edema.

Substance	Dose (mg/ear)	Weight of ears (mg)	Edema inhibition (%)	ID ₅₀ (mg/ear)
Control	-	16.24±0.86	-	0.097, r ² =0.97
	0.05	10.53±1.04**	35.15	
	0.15	7.10±1.34**	56.29	
Indomethacin	0.46	1.57±0.33**	89.19	
	-	15.35±0.42	-	
Control	0.31	11.04±0.23**	25.71	
	0.42	6.78±1.55**	54.41	
Maturin acetate	0.56	3.64±0.39**	75.50	
	-	-	-	

The weight of ears was represented as the mean ± SEM (standard error of mean) of six animals. **p < 0.01.

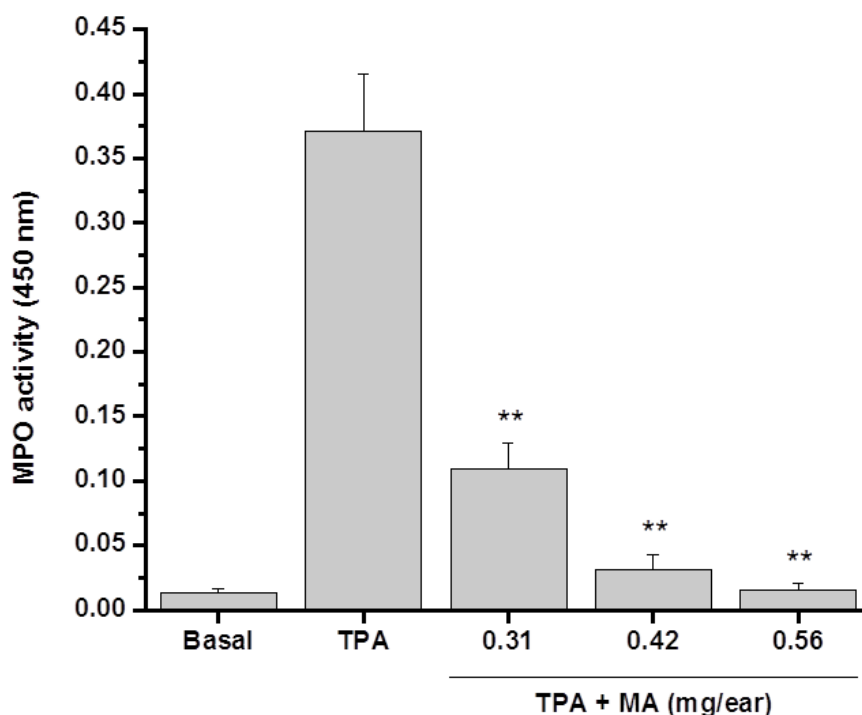


Figure 4. Measurement of myeloperoxidase (MPO) activity using neutrophil infiltration within the dermis, treated with TPA alone and TPA + MA. Values are expressed as mean ± SEM of absorbance at 450 nm. Each bar represents the mean of six replicates. The asterisk denotes the significance levels when compared with controls (** p < 0.01).

in TPA ear model was confirmed using the carrageenan-induced rat paw edema assay. Before injection of carrageenan in the paw edema assay, the basal paw volume values (V_0) ranged from 0.69 to 1.00 ml (0.87 ± 0.04 ml) and the mean variations of edema ($V_t - V_0$) ± standard error of mean (SEM) in the control group (N = 6) were 1 h (0.42 ± 0.10 ml), 3 h (0.98 ± 0.12 ml) and 5 h (1.06 ± 0.06 ml). MA showed significant (p < 0.05)

inhibition of 58.95% of the inflammation induced by carrageenan at the beginning of the inflammatory process (1 h), and the effect persisted up to 5 h (Table 2). Administration of MA inhibited edema starting from the first hour along all phases of inflammation, which was probably due to inhibition of different aspects and chemical mediators of inflammation (Ahamed et al., 2005). The development of edema in the rat paw after the

Table 2. Anti-inflammatory effects of sodium naproxen and maturin acetate (100 mg/kg) on carrageenan-induced paw edema.

Substance	Edema inhibition (%)		
	1 h	3 h	5 h
Sodium naproxen	4.70±24.9	49.91±12.7 *	41.6±10.9 *
Maturin acetate	58.95±12.4 *	35.08±14.6 *	35.22±6.1

Each value is represented as the mean ± SEM (standard error of mean) of six animals. *p < 0.05.

injection of carrageenan has been described as a biphasic event. The early phase (2.3 to 3 h) of inflammation is due to the release of vasoactive amines such as histamine and serotonin. The later phase (4.5 to 6 h) is due to the activation of kinin-like substances such as prostaglandins induced by cyclooxygenase, proteases and lysosomes (Olajide et al., 2000), producing an edema dependent on the mobilization of neutrophils (Vinegar et al., 1987; Hwang et al., 1996).

Present results indicate that MA reduces the exudation of the acute inflammation process and the early phase inflammatory response related to the release of pro-inflammatory mediators such as histamine and serotonin. This edematous response was reduced significantly in rats pretreated with indomethacin, a compound known to be a cyclooxygenase inhibitor.

Conclusions

Maturin acetate (MA) was isolated and quantified as the main constituent of the n-hexane *P. peltatum* root extract and exerted an important anti-inflammatory effect due to the modulation of macrophage responses and cytokine production. In addition, MA suppressed LPS-induced pro-inflammatory IL-1 β and TNF- α production by mouse peritoneal macrophages acting as *in vitro* anti-inflammatory agent. Furthermore, MA showed anti-inflammatory effects in experimental animal models of acute inflammation generated with TPA and carrageenan. MA displayed an effective anti-inflammatory response similar to the indomethacin, which has been found to be effective in the treatment of rheumatic conditions as a cyclooxygenase inhibitor limiting prostaglandin biosynthesis.

In this study, MA significantly reduced MPO levels by a mechanism related to the inhibition of neutrophil migration induced by carrageenan. MA showed a significant percentage of inhibition (58.95%) at the beginning of the inflammatory process (1 h) and the effect persisted up to 5 h, in comparison to sodium naproxen (4.70%). The present analysis confirms some of the beneficial effects ascribed in traditional medicine to the roots of *P. peltatum*. The anti-inflammatory effects produced by MA

may be therapeutically used for diverse inflammatory diseases. Further experiments are required to determine to what extent this sesquiterpenoid compound and the *P. peltatum* root extract can be used as therapeutic agents with anti-inflammatory activity.

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