Phytochemical and Antinociceptive Properties of *Matayba elaeagnoides* Radlk. Barks

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A mixture of triterpenes named lupeol (1), α-amyris (2), β-amyris (3), and β-sitosterol (4) has been isolated from the hexane fraction of *Matayba elaeagnoides*. In addition, scopoletin (5), umbelliferone (6), 3β-O-d-glycopyranosyl-sitosterol (7) and betulin (8) were isolated from the chloroform fraction. All the structures were identified by spectroscopic techniques in accordance with literature data. The extracts (hydroalcoholic and methanolic) and some fractions (hexane, chloroform, ethyl acetate and butanol) exerted promising antinociceptive effects in mice. In addition, we have tested the pure compound betulin (8). When analyzed against induced pain using the writhing test (3–10 mg kg⁻¹, i.p.), betulin showed a dose-dependent effect with a calculated ID₅₀ value of 7.74 (6.53–9.17) mg kg⁻¹ [17.5 (14.7–20.7) μmol kg⁻¹] and a maximal inhibition (MI) of 58.3% in relation to the control group. When evaluated in the formalin test (3–10 mg kg⁻¹, i.p.), this compound inhibited both phases of pain (neurogenic and inflammatory pain), with calculated ID₅₀ values of 18.3 (17.7–18.9) and 8.3 (7.7–8.9) mg kg⁻¹ [41.5 (38.4–42.7) and 18.8 (17.6–19.9) μmol kg⁻¹] and maximal inhibition of 40.8 and 64.9% for the first and second phases, respectively. Using the same models, this compound was several times more active than two clinically used drugs, namely aspirin and paracetamol, suggesting that its main active principle is related to the antinociceptive effect found for the chloroform fraction of *M. elaeagnoides* barks.

Key words: *Matayba elaeagnoides*, Antinociceptive, Betulin

Introduction

Sapindaceae consists of a large family of plants, with occurrence and distribution in distinct areas of the world, mainly in the tropical and subtropical regions (Ferrucci, 2000; Guarim-Neto *et al.*, 2000). Several species have demonstrated different compound classes such as saponins, terpenoids and flavonoids, which exhibit especially diuretic, stimulant, expectorant, anti-helmintic and anticancer properties (Arisawa *et al.*, 1984; Mesquita *et al.*, 2005). *Matayba elaeagnoides*, known as “camboatá, camboatá or cuvantá”, is frequently used in folk medicine as an anti-inflammatory agent and to combat liver cancer (Lorenzi, 2000; Ribeiro, 2004). Recently, it was reported that it presents positive effects against inflammatory processes and antiplasmodial activity *in vitro* against *Plasmodium falciparum*, associated with the presence of matayosides A–D (Mineo *et al.*, 2004). This study extends previous work carried out on this plant and describes the phytochemical analysis and *in vivo* antinociceptive activity against two pain models in mice (acetic acid and formalin) using the crude extract, fractions and a pure compound isolated from *M. elaeagnoides* bark.

Material and Methods

Plant material

Barks of *M. elaeagnoides* (Sapindaceae) were collected in Caçador Santa Catarina, Brazil in May 2004 and identified by Dr. Ademir Reis (Department of Botany, Universidade Federal de Santa Catarina, Brazil). A voucher specimen was deposited at Barbosa Rodrigues Herbarium (Itajaí, SC, Brazil) under number MTS 001.
**Phytochemical analysis**

Air-dried material from *M. elaeagnoides* (2.6 kg, bark) was cut into small pieces and macerated with methanol at room temperature for 10 d. After filtration, the solvent was removed by rotary evaporation under reduced pressure, yielding the respective methanolic extract (ME). Additionally, a hydroalcoholic extract (HE) was prepared by maceration with an ethanol/water mixture (1:1) and stored until pharmacological test. The methanolic extract (220.0 g) was then suspended in MeOH/water (9:1) and successively partitioned with hexane, chloroform, ethyl acetate and butanol, furnishing the respective fractions. The hexane fraction (6.85 g) was chromatographed on a silica gel column eluted with a hexane/acetone mixture with increasing polarity. The chloroform (1.2 g) and ethyl acetate (3.6 g) fractions were chromatographed separately on a silica gel column eluted with a mixture of chloroform/methanol with increasing polarity. Similar fractions, which showed positive reaction with anisaldehyde/sulfuric acid reagents and short-wave UV light, were combined and re-chromatographed as described above, yielding a mixture of terpenes, steroids and coumarins. The compounds were identified based on their spectral data as lupeol (1), α-amyrin (2), β-amyrin (3), β-sitosterol (4), scopoletin (5), umbelliferone (6), 3β-O-d-glycopyranosyl-sitosterol (7) and betulin (8). The purity of all isolated substances was examined by thin layer chromatography (TLC) using Merck silica gel pre-coated aluminum plates, with a layer thickness of 200 μm, with several solvent systems of different polarities.

**Animals**

Swiss mice (25–35 g), housed at (22 ± 2) °C under a 12 h light/12 h dark cycle and with access to food and water *ad libitum*, were acclimatized to the laboratory for at least 1 h before testing. For each experiment, one group of 6–8 animals was used. The experiments reported here were carried out in accordance with the current ethical and care guidelines for the care of laboratory animals and the investigation of experimental pain in conscious animals (Zimmermann, 1983). The experiments were approved by the local Ethics Committee of this institution (number 412/06-UNIVALI). The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the drug treatments.

**Writhing test**

The abdominal constriction, induced by intraperitoneal injection of acetic acid (0.6%), was carried out according to the procedures described previously by Collier *et al.* (1968) and Souza *et al.* (1998) with minor modifications. The animals were pretreated with extract, fractions or compound 8 (3–10 mg kg⁻¹, i.p.) 30 min before the acetic acid injection. Control animals received a similar volume of 0.9% (10 ml kg⁻¹, i.p.). All experiments were carried out at (23 ± 2) °C. After challenge, pairs of mice were placed in separate boxes and the number of constrictions of the abdominal muscles and stretching were counted cumulatively over a period of 20 min. Antinociceptive activity was expressed as the reduction of the number of abdominal constrictions comparing control and pre-treated animals.

**Formalin-induced nociception**

The procedure used was essentially similar to that previously described (Hunskaar and Hole, 1987). Animals from the same strain were used and 20 μl of 2.5% formalin solution (0.92% formaldehyde), made up in a phosphate-buffered solution (137.0 mM NaCl, 2.7 mM KCl and 10 mM phosphate buffer), were injected intraplantarly in the right hindpaw. After injection, the time spent licking the injected paw was measured and considered as indicative of pain. The initial nociceptive scores normally peaked 5 min after formalin injection (first phase) and 15–30 min after formalin injection (second phase), representing the neurogenic and inflammatory pain, respectively. In an attempt to investigate the possible antinociceptive action 3–10 mg kg⁻¹, i.p., of compound 8 were used.

**Statistical analysis**

The results are represented as mean ± S.E.M., except for the ID₅₀ values (*i.e.*, the dose that reduced responses by 50% in relation to control values), and presented as geometric mean and their respective 95% confidence limit. The ID₅₀ values were determined by linear regression GraphPad. Statistical significance between groups was calculated by means of analysis of variance followed by Newman-Kuels’ multiple comparison tests. *P*-values less than 0.05 were considered as indicative of significance.
Results and Discussion

Recently, many plants have received special attention as sources of new antinociceptive agents (Calixto et al., 1998; Patocška, 2003; Oliveira et al., 2005; Lima-Junior et al., 2006; Abdel, 2006). Despite its common use in folk medicine to treat various pathologies associated with dolorous processes, it was observed a lack of literature on experimental studies concerning the analgesic properties of *M. elaeagnoides*. On the other hand, some plants belonging to the *Matayba* genus have exhibited important antiplasmodial activity (Mesquita et al., 2005).

Table I shows that hydroalcoholic and methanolic extracts and different fractions caused the pronounced effect when analyzed against the writhing model in mice. As can be observed, hydroalcoholic and methanolic extracts as well as hexane, chloroform, ethyl acetate and butanolic fractions showed antinociceptive activity, with MI values of 71.1, 54.6, 46.4, 46.4, 66.0 and 25.7%, respectively, at 10 mg kg\(^{-1}\), i.p. Considering that hexane and chloroform fractions demonstrated a better chromatographic profile and a good antinociceptive effect, they were separately chromatographed on a silica gel column eluted with a hexane/acetone gradient, monitored by TLC, yielding lupeol (1), \(\alpha\)-amyrin (2), \(\beta\)-amyrin (3), \(\beta\)-sitosterol (4), umbelliferone (6), 3\(\beta\)-O-d-glycopyranosyl-sitosterol (7) and betulin (8), which were directly compared with authentic samples and spectroscopic data (IR, \(^1\)H NMR and \(^{13}\)C NMR). Although the ethyl fraction presented promising antinociceptive activity (Table I), we obtained a limited quantity of material for separation and purification of other active principles. However, the chromatographic profile by TLC demonstrated the strong presence of phenolic compounds when revealed with specific reagents, whose studies will be continued for further publications.

Table I. Antinociceptive activity of extracts and fractions of *M. elaeagnoides* against acetic acid-induced abdominal constrictions in mice, in comparison with aspirin and paracetamol (10 mg kg\(^{-1}\), i.p.).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MI (%)</th>
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<tbody>
<tr>
<td>Methanolic extract</td>
<td>54.6 ± 2.8</td>
</tr>
<tr>
<td>Hydroalcoholic extract</td>
<td>71.1 ± 1.9</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>46.4 ± 1.5</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>66.0 ± 0.6</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>46.4 ± 0.4</td>
</tr>
<tr>
<td>Butanolic fraction</td>
<td>25.7 ± 2.4</td>
</tr>
<tr>
<td>Paracetamol(^a)</td>
<td>38.0 ± 1.0</td>
</tr>
<tr>
<td>Aspirin(^a)</td>
<td>35.0 ± 2.0</td>
</tr>
</tbody>
</table>

\(^a\) Bresciani et al. (2003).

It is well documented that 1–7 present distinct pharmacological properties particularly anti-inflammatory and analgesic effects and for this reason, they were not included in this study (Santos et al., 1995; Delira et al., 2003; Patocška, 2003; Otsuki et al., 2005; Oliveira et al., 2005; Lima-Junior et al., 2006; Reyes et al., 2006; Meotti et al., 2006).

However, the antinociceptive profile of 8, to the
that Matayba arborescens L. var. S.E.M. of six experiments. Subsequently, it was found that Adiantum cuneatum also inhibited the neurogenic pain induced by the formalin test. Each column represents mean ± S.E.M. of six experiments. Significance levels when compared with the control group: **p < 0.01; *p < 0.05.

(Hunskaar et al., 1985; Souza et al., 1998). By antagonizing the formalin-induced hindpaw oedema was observed suggesting an associated anti-inflammatory effect (Fig. 3). The factor by which this compound inhibits such an inflammatory oedema induced by irritants could be the modulation of the liberation or blocking of B2 and prostaglandin receptor systems (Corrêa and Calixto, 1993).

For the first time, the extract, fractions and pure compounds obtained from M. elaeagnoides that produced antinociception against the acetic acid-induced visceral nociceptive response were distinguished, supporting the ethnomedical use of this plant. Experimental studies are currently in progress in order to determine the other active principles present in this plant.

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