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Isolation and identification of compounds with antinociceptive action from *Ipomoea pes-caprae* (L.) R. Br.

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This study describes the isolation and identification of several constituents from *Ipomoea pes-caprae* (L.) R. Br., a medicinal plant frequently employed in folk medicine of many countries as a remedy against several diseases, including inflammation and pain. Our results demonstrate that some of these compounds, such as glochidone, betulinic acid, α- and β-amyrin acetate, isoquercitrin, etc. showed pronounced antinociceptive properties in the writhing test and formalin test in mice. These data confirm our previous work concerning the antinociceptive action of the hydroalcoholic extract of *I. pes-caprae* and justify, at least in part, the popular use of this plant for the treatment of dolorous processes.

1. Introduction

Ipomoea pes-caprae (L.) R. Br. (Convolvulaceae) is a medicinal plant commonly used in folk medicine of many countries to treat a variety of ailments, including inflammations, gastrointestinal problems, diuretic disorders, dolorous processes, etc. [1–3]. In Brazil, it is popularly known as "salsa-da-praia", "pé-de-cabra" or "batateira-da-praia", growing in abundance on Brazil's Atlantic coast as a vine plant [4]. Pharmacological studies have shown that some extracts and pure substances obtained from *I. pes-caprae* exhibit different types of activity, such as antispasmodic [1, 5], antiinflammatory [6, 7], insulinogenic and hypoglycemic [3]. An extract of petroleum ether which was denominated IPA was clinically effective on dermatitis caused by venomous jellyfishes [8]. Such action was confirmed by Pongprayoon et al. [9], which showed that IPA is able to neutralize the toxic effects of different crude jellyfish venoms.

We have recently evaluated the analgesic activity of some extracts obtained from *I. pes-caprae* and verified that they exert potent and dose-related antinociceptive action in different models of pain in mice [10]. Therefore, the present study was conducted in order to isolate and identify the main active compounds present in this plant. Such compounds were assessed in the formalin test and writhing test in mice. In addition, the results of some well-known analgesic drugs, such as aspirin, paracetamol, indomethacin and dipyrone, were included for comparison.

2. Investigations, results and discussion

The promising antinociceptive effects shown by a crude hydroalcoholic extract obtained from *I. pes-caprae* [10] encouraged us to determine and identify the main phytoconstituents which could be responsible for these pharmacological actions. Thus, we prepared a crude methanolic extract and partitioned it with solvents of increasing polarity in order to obtain different fractions of distinct constituents in order to separate selectively the active principles present in this plant [11]. In this context, the ethyl acetate fraction was selected in a first step for phytochemical analysis, since it exhibited significant antinociceptive action and presented the best yield. Usual chromatographic procedures (CC, TLC and GC) led to the isolation of fifteen compounds, which were identified on the basis of their spectral data and comparison with authentic samples.

Some of them were evaluated as antinociceptive in two classical models of pain in mice. Because the analgesic effects of compounds **1**, **2** [12], **6**, **7** [13], **8** [14], **10** [15] and **13** [16] were previously reported, they were not included in this study.

Table 1 indicates the antinociceptive action of some constituents isolated from *I. pres-caprae* against acetic acid-induced abdominal constriction. As can be observed, the ethyl acetate fraction, glochidone (**3**) and betulinic acid (**11**) significantly inhibited the number of constrictions in relation to the control group (10 mg/kp, i.p.), with inhibition rates of 81.0, 75.5 and 88.1%, respectively. The mixture of α-amyrin acetate (**4**), β-amyrin acetate (**5**) and isoquercitrin (**12**) exhibited moderate activity, with inhibition rates of 54.4 and 34.5%, respectively. In the same procedure, aspirin and paracetamol, two well-known clinically used drugs [17], were less active than the constituents of *I. pres-caprae*, except compound **12** (Table 1).

When analysed in the formalin test, a model in which two distinct phases of pain can be distinguished (neurogenic and inflammatory) [18, 19], it was possible to confirm the antinociceptive action of these compounds. Table 2 shows that the ethyl acetate fraction and compounds **3**, **4** + **5** and **12** inhibited both the first and the second phase of the formalin-induced pain. The inhibition rates were 30.9, 34.7, 58.9 and 40.7% against the first phase and 83.8, 83.0, 97.8 and 46.4% against the second phase. As can be observed, the antinociceptive action was more pronounced

Table 1: Antinociceptive effects of constituents isolated from *I. pes-caprae* and some reference drugs against acetic acid-induced abdominal constrictions in mice

Treatment (10 mg/kg, i.p.)	Number of constrictions	Inhibition (%)
Control	35.3 ± 4.2	–
Ethyl acetate fraction	6.7 ± 3.2	81.0
Glochidone	8.7 ± 1.4	75.5
α,β-Amyrin acetate	15.9 ± 1.6	54.4
Betulinic acid	4.2 ± 1.6	88.1
Isoquercitrin	23.1 ± 3.0	34.5
Aspirin	22.9 ± 2.0	35.0
Paracetamol	21.9 ± 1.0	38.0

Each group represents the mean ± s.e.m. of 6–8 experiments.

Table 2: Effects of indomethacin and compounds isolated from *I. pes-caprae* given intraperitoneally in the formalin test in mice

Treatment (10 mg/kg, i.p.)	1st phase[1]	Inhibition (%)	2nd phase[2]	Inhibition (%)
Control	78.2 ± 6.2	–	204.3 ± 6.8	–
Ethyl acetate fraction	54.0 ± 7.8	30.9	33.0 ± 4.6	83.8
Glochidone	46.6 ± 4.0	40.4	34.7 ± 3.8	83.0
α,β-Amyrin acetate	32.1 ± 5.0	58.9	4.5 ± 2.3	97.8
Betulinic acid	73.2 ± 3.3	6.5	102.2 ± 4.2	50.0
Isoquercitrin	40.7 ± 3.8	47.9	109.5 ± 5.2	46.4
Indomethacin	70.4 ± 1.8	10.0	61.3 ± 3.4	70.0

Each group represents the mean ± s.e.m. of 6–8 experiments; [1] 0–5 min licking (s); [2] 15–30 min licking (s).

in the late phase of the formalin-induced licking response. Indomethacin, a peripheral acting drug, and betulinic acid (**11**) inhibited only the second phase of the formalin test, with inhibitions of 70.0 and 50.0%, respectively.

Although all compounds reported here have been chemically identified earlier, it is interesting to mention that some of them have exhibited different types of biological activity. In this context, xanthoxyline (**8**), which was previously isolated in our laboratories from *Sebastiania schottiana* Muell. Arg. in a yield of 0.25%, resulted as the main antispasmodic component of this plant [16, 20]. Sericic acid (**10**), isolated from *Vochysia divergens* Pohl, possesses antibacterial and antifungal action against pathogenic microorganisms [21, 22] besides antinociceptive properties [15]. Quercetin (**13**) and isoquercitrin (**12**) are common flavonoids present in many plants and foods and several pharmacological or biological properties have been reported [14, 23–25], α-amyrin (**6**) and β-amyrin (**7**) have shown antiinflammatory [26–28] and analgesic [13] effects and their acetates demonstrated to have antiinflammatory and antiarthritic properties [29, 30]. Phytosterols (**1**, **2**) are well-known constituents widely distributed in higher plants and several pharmacological properties have been attributed to these compounds, such as analgesic [12] and antiinflammatory [31]. Recent studies have reported the therapeutic efficacy of these sterols for the treatment of patients with benign prostatic hyperplasy (BHP) [32, 33]. Betulinic acid (**11**), a well-known lupane-type triterpene, has received special attention by some researchers because of its promising pharmacological and/or biological activities. Although this compound has been extracted from many plants, such properties have just recently been reported. Among them, there are antitumoral [34], spasmogenic [35], antiinflammatory [27, 28], anti-HIV [36], antimalarial [37], and other properties [28, 37].

To our best knowledge, this is the first report about the antinociceptive action of glochidone (**3**), α-amyrin acetate (**4**) and β-amyrin acetate (**5**), betulinic acid (**11**) and isoquercitrin (**12**).

It is important to mention that we have sometimes observed the existence of plants with a pharmacological profile similar to *I. pes-caprae*, which produces many compounds with analgesic activity [38–41]. Such a fact suggests that their mechanism of action is distinct or suggests the possibility of the existence of some special effects that deserves further investigation. In summary, our results clearly demonstrate that the plant *I. pes-caprae* produces several active compounds, which support, at least in part, its use in folk medicine as a remedy against different diseases, in particular those associated with dolorous pathologies. Presently, investigations are in progress to confirm these effects by other routes of administration as well as to characterize the mechanism of the antinociceptive action of these compounds.

3. Experimental

3.1. Plant material

Aerial parts of *I. pes-caprae* were collected from a population growing on the dunes of Jurerê beach, Florianopolis city, Brazil, in March 1997. The plant was authenticated by Dr. Ademir Reis (Department of Botany, UFSC, Florianópolis) and a voucher was deposited at Barbosa Rodrigues Herbarium (Itajaí) under number VC Filho 009.

3.2. Isolation and identification of active principles

Fresh aerial parts of *I. pes-caprae* (6.5 kg) were cut in small pieces and macerated with methanol at room temperature for approximately 10 days. The solvent was evaporated under reduced pressure to the desired volume and the methanolic extract was then successively partitioned with hexane, dichloromethane, ethyl acetate and butanol, respectively, according to a previously described methodology [11]. Evaporation of the solvents afforded the respective fractions. The ethyl acetate fraction (8.0 g) presented a considerable antinociceptive activity and the best yield, being therefore chosen for phytochemical analysis. Thus, a part of this fraction (7.5 g) was chromatographed on a silica gel column eluted with a $CHCl_3$/MeOH gradient. The fractions that showed a similar chromatographic profile by TLC were combined in order of their respectives polarities and successively rechromatographed in CC to afford the following compounds: stigmasterol (**1**) (12 mg), β-sitosterol (**2**) (49 mg), glochidone (**3**) (58 mg), α-amyrin acetate (**4**) (19.5 mg), β-amyrin acetate (**5**) (19.5 mg), α-amyrin (**6**) (24.5 mg), β-amyrin (**7**) (12.0 mg), 2-hydroxy-4,6-dimethoxy acetophenone (**8**) (16.6 mg), 2,4-dihydroxy-6-methoxy acetophenone (**9**) (41.1 mg), sericic acid (**10**) (21.6 mg), betulinic acid (**11**) (4.0 mg), isoquercitrin (**12**) (171.4 mg), quercetin (**13**) (35.6 mg) and salicylic acid (**14**) (86.3 mg). All compounds were identified by spectroscopic data (IR, ^{1}H- and ^{13}C NMR) and direct comparison with authentic samples. Compounds **1**, **2** and **4**–**7** were analysed by high resolution gas chromatography (HRGC) by co-injection with authentic samples. Compounds **1**–**8** also were detected in the fractions of hexane and dichloromethane, but additional studies are required to determine their concentrations.

3.3. Pharmacological analysis

3.3.1. Abdominal constriction response caused by intraperitoneal injection of dilute acetic acid

The abdominal constriction induced by intraperitoneal injection of acetic acid (0.6%), was carried out according to the procedures described previously [43] with minor modifications. Animals were pretreated with the compounds intraperitoneally ($10\ mg \cdot kg^{-1}$) 30 min before the acid acetic injection. Control animals received a similar volume of 0.9% NaCl ($10\ ml \cdot kg^{-1}$, 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 abdominal constrictions of the abdominal muscles together with a stretching, were cumulatively counted over a period of 20 min. Antinociceptive activity was expressed as the reduction of the number of abdominal contractions between control animals and mice pretreated with the compounds studied.

3.3.2. Formalin-induced pain

The procedure used was essentially similar to that previously described [18, 19]. Animals from the same strain were slightly anaesthetized with ether, except when used to analyse the first phase of formalin-induced pain, and 20 µl of 2.5% (0.92% formaldehyde) made up PBS (phosphate buffered solution, containing: NaCl 137 mM; KCl 2.7 mM and phosphate buffer 10 mM) was injected s.c. under the plantar surface of the left hindpaw with a Hamilton syringe. Animals were acclimatized to the laboratory for at least 24 h before experiments. Two mice (control and treated) were simultaneously observed from 0 up to 30 min following formalin injection. The initial nociceptive scores normally peaked after 5 min (first phase, representing the neurogenic pain), and 15–30 min after formalin injection

(second phase, representing the inflammatory pain) [19]. Animals were treated with saline 0.9% (10 ml/kg, i.p.) or with compounds isolated from *I. pes-caprae* (10 mg · kg^{-1}, i.p.) 60 min before formalin injection. After intraplantar irritant application, the animals were immediately placed into a glass cylinder (20 cm diameter). The time spent by animals licking or biting the injected paw was observed and was considered indicative of pain.

3.4. Statistical analysis

The results are presented as mean ± s.e.m., and statistical significance between the groups was analysed by means of the t-test or analysis of variance followed by the Dunnett's multiple comparison test, when appropriate. P values less than 0.05 were considered as indicative of significance.

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