Pharmacological and Phytochemical Evaluation of *Adiantum cuneatum* Growing in Brazil

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This work describes the phytochemical analysis and analgesic activity of a non polar fraction obtained from *Adiantum cuneatum* grown in Brazil. The results showed that the hexane fraction as well as two pure compounds, identified as filicene (**1**) and filicenal (**2**), given intraperitoneally, exhibited potent analgesic activity when evaluated in two models of pain in mice, writhing test and formalin-induced pain. Compound **1** presented a calculated ID_{50} value of 19.5 μmol/kg body weight, when evaluated in writhing test, being about 7-fold more active than some reference drugs, like as acetyl salicylic acid and acetaminophen. It also inhibited both phases (neurogenic and inflammatory) of the formalin test at 10 mg/kg (24 μmol/kg). The chemical composition of the plant grown in Brazil is similar to that grown in other countries. The results confirm and justify the popular use of this plant for the treatment of dolorous processes.

Key words: Adiantum cuneatum, Analgesic Activity, Triterpenes

Introduction

Adiantum cuneatum Langsd. and Fisch. (Adiantaceae) is a common plant well-distributed in several countries of the South America, especially in Brazil. It is used as an ornamental plant, being also frequently employed in folk medicine as diuretic, expectorant, emollient, used for coughs, urinary disorders, alopecia and menstrual difficulties (De Feo, 1992). In Brazil, it is specially used to treat respiratory diseases and dolorous processes (Cirilo, 1993; Michalak, 1997). The chemical composition of this plant cultivated in Japan and other countries have been continuously studied, whose results have indicated the predominance of triterpenes (Shiojima *et al.*, 1996; 1997a; 1997b; 1997c).

However, little is known about its pharmacological action or regarding the plant growing in Brazil, where is known as "avenca". In a recent screening to determine Brazilian medicinal plant with analgesic potential, we have verified that the crude alcoholic extract from aerial parts of *A. cuneatum* caused antinociceptive effect in mice (unpubl. results). In this paper, we report the phytochemical analysis of this plant growing in Brazil as well as the analgesic activity of a fraction (hexane) and two pure compounds.

Material and Methods

Plant material

A. cuneatum was collected in March 2000 at a farm in Lageado Bonito, Caxambú do Sul, near to Chapecó city, west of Santa Catarina State, Brazil, and identified by Prof. Ademir Reis (Department of Botany, UFSC). The voucher specimen was deposited at Barbosa Rodrigues Herbarium (Itajaí) under number VC Filho 025.

Phytochemical analysis

The dried leaves (595 g) were powered and extracted twice with methanol (3 l) for five days at room temperature. The extract was evaporated under reduced pressure to obtain a greenish gummy residue (crude extract) (115.8 g). The

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crude extract was fractionated over silica gel with solvents of increasing polarities: *n*-hexane, dichloromethane, ethyl acetate and butanol, respectively. The hexanic fraction (2.2 g) was chromatographed on silica gel with hexane (100%) until ethyl acetate (100%) furnishing four compounds: filicene (**1**) (1.102 g = 0.19%), filicenal (**2**) (0.453 g = 0.08%), adiantol (**3**) (0.047 g = 0.008%) and isoadiantone (**4**) (0.025 g = 0.004%). All the compounds were identified on basis of spectroscopic evidence (IR, NMR ^{1}H and ^{13}C) which were identical to that reported in the literature (Shiojima *et al.*, 1993; Hveding-Bergseth *et al.*, 1983).

Animals

Swiss mice of both sexes (25–35 g) were housed in automatically controlled temperature conditions (23 ± 2° C and 12 h light-dark cycles). Food and water were freely available. The animals remained in the appropriate laboratory of UNIVALI until some hr before the experiments.

Pharmacological Assays: Abdominal constriction response caused by intraperitoneal injection of diluted acetic acid

Abdominal constriction was induced by intraperitoneal injection of acetic acid (0.6%), according to the procedures described previously (Collier *et al.*, 1968; Souza *et al.*, 1998) with minor modifications. Animals were pre-treated with the fraction or compounds intraperitoneally 30 min before the acid acetic injection. Control animals received a similar volume of 0.9% NaCl (10 ml/kg, i.p.). After the challenge, each mouse was placed in a separate glass funnel and the number of abdominal contractions of the abdominal muscles together with stretching, was 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 hexane fraction or compounds.

Formalin-induced pain

The procedure used was essentially similar to that described previously (Souza *et al.*, 1998; Hunskaar *et al.*, 1985; Hunskaar and Hole, 1987). 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 of PBS (phosphate buffered saline containing: NaCl 137 mM; KCl 2.7 mM and phosphate buffer 10 mM) was injected under the plantar surface of the left hindpaw. Animals were acclimatized to the laboratory for at least 24 h before the experiments. Two mice (control and treated) were observed simultaneously for 0 to 30 min following formalin injection. The initial nociceptive scores normally peaked after 5 min (first phase, representing the neurogenic pain), and after 15–30 min after formalin injection (second phase, representing the inflammatory pain) (Hunskaar and Hole, 1987). Animals were treated with saline 0.9% (10 ml/kg, i.p.), or compounds 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 timed with a chronometer and was considered indicative of pain.

Statistical analysis

The results are presented as mean ± s.e.m., and statistical significance between groups was analysed by means of the *t* test or analysis of variance followed by Dunnett's multiple comparison test, when appropriate. *P* values less than 0.05 were considered significant. When appropriate, the mean ID_{50} values (the dose of compound that reduced formalin- or acetic acid-induced pain by 50% relative to control values) were estimated by graphical interpolation from individual experiments.

Results and Discussion

The dried leaves of *A. cuneatum* were extracted with methanol, and the concentrated extract (crude extract) was filtered on silica gel with solvents of the different polarities (*n*-hexane, dichloromethane, ethyl acetate and butanol). Phytochemical preliminary analysis by TLC revealed that, similar to previous studies with this plant, it contains mainly triterpenes. Further fractionation of the most active fraction (hexane, Table I), resulted in isolation of four known triterpenes, such as filicene (**1**), filicenal (**2**), adiantol (**3**) and isoadiantone (**4**). They were identified on basis of

Table I. Analgesic activity of *n*-hexane fraction, filicene (**1**) and filicenal (**2**) from *A. cuneatum*, acetyl salicylic acid and acetaminophen against acetic acid-induced abdominal constriction in mice.

Treatment	ID_{50} [mg/kg]	ID_{50} [μmol/kg]	Maximum inhibition (%)*
n-Hexane fraction	19.7 (14.6–25.8)	–	73 ± 4
Filicene (1)	8.0 (7.1–9.0)	19.5 (17.3–22.0)	76 ± 6
Filicenal (2)	~ 10	~ 25	58 ± 6
ASA	24.0 (13.0–44.0)	133 (73.0–247.0)	35 ± 2
ACE	19.0 (16.0–24.0)	125 (140–250)	38 ± 1

Each group represents the mean ± s. e. m. of 6–8 experiments. Fraction and compounds were given intraperitoneally. ASA = acetyl salicylic acid; ACE = acetaminophen. * Maximum inhibition at 10 mg/kg.

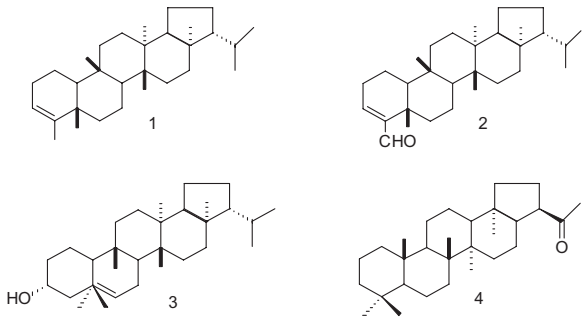

Fig. 1. Molecular structures of triterpenes isolated from *A. cuneatum* leaves (**1** = filicene, **2** = filicenal, **3** = adiantol, **4** = isoadiantone).

spectroscopic evidence (NMR ^{1}H and ^{13}C, IR) and comparison with data reported in literature (Shiojima *et al.*, 1993; Hveding-Bergseth *et al.*, 1983). Other terpenes were detected, but they were not isolated because of the low concentrations. Our experimental results with respect to the triterpenes were similar to those reported for the plant cultivated in Japan (Shiojima *et al.*, 1997a).

The results shown in Table I indicate that the hexane fraction from *A. cuneatum* exhibits considerable analgesic profile in mice, dose-dependently inhibiting acetic acid-induced writhing responses. It presented a calculated ID_{50} value (and 95% of confidence limit) of 19.7 (14.6–25.8) mg/kg, given intraperitoneally, with maximum inhibition of 73%. The hexane fraction was equipotent to some reference drugs like acetyl salicylic acid and acetaminophen, included in this study with the purpose of comparison (Table I). Because the limited quantity of the isolated compounds, only filicene (**1**) and filicenal (**2**) were tested as analgesic in mice. Compound **1** was about 2.5-fold more active than the respective hexanic fraction, with ID_{50} (and 95% confidence limit) of 8.0 (7.1–9.0) mg/kg (i. p.) and maximum inhibition of 76%. At μmol/kg level, it was about 7-fold more potent than the standard drugs. Compound **2** was less active, causing inhibition of 58 ± 6% at 10 mg/kg. In order to confirm the analgesic effects of compounds **1** and **2**, they were analysed on formalin-induced pain (10 mg/kg, i. p.), a test wich defines two distinct periods of response, first phase (neurogenic) and second phase (inflammatory). Filicene (**1**) inhibited both phases of pain, with inhibition of 51 and 60% for first and second phase, respectively, whereas filicenal (**2**) inhibited only the inflammatory phase, with 79% of inhibition (Table II). Both compounds presented similar activity of standard drugs in relation to second phase of formalin test.

In summary, our results demonstrate that *A. cuneatum* cultivated in Brazil produces active compounds, especially triterpenes, which act as analgesic in different models of pain in mice. The potent

Table II. Analgesic activity of filicene (**1**) and filicenal (**2**) from *A. cuneatum*, acetyl salicylic acid and indomethacin against formalin induced-pain in mice.

Compound	Inhibition (%) First phase[1]	Second phase[2]
Filicene (1)	51.0 ± 9	60.0 ± 8
Filicenal (2)	26.1 ± 11.5	79.0 ± 10.4
ASA	Inactive	39.0 ± 4
IND	Inactive	33.0 ± 5

Each group represents the mean ± s.e.m. of 6–8 experiments. [1] 0–5 min licking (s); [2] 15–30 min licking (s). Compounds were given intraperitoneally in a dose of 10 mg/kg. ASA = acetyl salicylic acid; IND = indomethacin.

analgesic effect of filicene (**1**) and filicenal (**2**) encourages further investigations on structural modifications to obtain new analgesic compounds. The mechanism by which the plant or active principles exert analgesic activity still remains undetermined, but our studies are currently in progress to confirm the pharmacological effects described here in other models and different routes of administration. Finally, our results confirm and justify the popular use of this plant in folk medicine to treat dolorous processes.

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