PHYTOTHERAPY RESEARCH
Phytother. Res. **20**, 1067–1073 (2006)
Published online 28 September 2006 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.1997

Neuropharmacological Evaluation of the Putative Anxiolytic Effects of *Passiflora edulis* Sims, its Sub-fractions and Flavonoid Constituents

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***Passiflora edulis* Sims together with several other plants of the genus *Passiflora* have been reported to possess anxiolytic properties. It has been suggested recently that flavonoids may be partly responsible for the neuropharmacological activity of these plants but there are still few data reporting the relation between the constituents of these plants and their activity. This work evaluated the anxiolytic/sedative activity of an aqueous extract of *Passiflora edulis* Sims and bioguided its fractionation using the elevated plus-maze model of anxiety and other complementary pharmacological tests. The aqueous extract presented an anxiolytic-like activity without any significant effect upon the motor activity whilst the total flavonoid fraction (TFF) presented an anxiolytic-like activity but compromised motor activity. Through fractionation of TFF it was possible to isolate and characterize luteolin-7-O-[2-rhamnosylglucoside] which showed an anxiolytic-like activity without compromising motor activity. Copyright © 2006 John Wiley & Sons, Ltd.**

Keywords: Passiflora edulis Sims; flavonoid glycosides; anxiety; elevated plus-maze; luteolin-7-O-[2-rhamnosylglucoside].

INTRODUCTION

Passiflora incarnata L. is the one Passifloraceae most commonly used in Europe for its anxiolytic/sedative properties and is referred to as the medicinal species in different official publications (ESCOP, German Commission E Monographs). Despite this fact there have been several reports over the years on the anxiolytic/sedative activity of various other species of the genus *Passiflora*, namely *P. edulis* Sims (Petry *et al.*, 2001), *P. coerulea* L. (Wolfman *et al.*, 1994), *P. alata* Curtis (Oga *et al.*, 1984). These studies carried out with different animal models generally show insufficient characterization of the extracts and/or botanical materials used and this has brought some controversy on which of the *Passiflora* genus species do possess any neuropharmacological activity and which are the active constituents involved in it (Dhawan *et al.*, 2004).

Passiflora edulis Sims is the edible variety most commonly known for its tasty fruit but also reported to be used in Brazilian traditional folk medicine (Maluf *et al.*, 1991; Vale and Leite, 1983) and to be active in animal models of anxiety (Petry *et al.*, 2001). This plant is widely available in Portugal as a garden plant or a crop and previous work by some members of our group showed that *P. edulis* Sims aqueous extracts had significant affinity for 5-HT_3 rat brain receptors thus suggesting an eventual anxiolytic activity (Cotrim *et al.*, 1995). As it has been suggested that flavonoids may play a role in the neuropharmacological activity of several plants (Medina *et al.*, 1997; Paladini *et al.*, 1999) including *P. incarnata* L. (Zanoli *et al.*, 2000) and *P. coerulea* L. (Wolfman *et al.*, 1994) we wanted to establish if the aqueous extract of *Passiflora edulis* Sims growing in Portugal and the flavonoids it contains had any activity in classical animal models of anxiety using properly characterized extracts and also to isolate and identify those flavonoid constituents possibly involved in this activity.

Two non-conditioned tests were chosen that are well accepted for determining the anxiolytic-like activity of these preparations in mice: the elevated plus-maze (EPM) (Lister, 1987; Rodgers and Cole, 1994; Rodgers and Dalvi, 1997) and the marble-burying test (MBT) (Broekkamp *et al.*, 1986). To evaluate any eventual effects on motor activity the wire test (Boissier *et al.*, 1961) and the chimney test (Boissier *et al.*, 1960) were chosen.

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Contract/grant sponsor: Praxis XXI; contract/grant number: BD/16264/98.

Received 13 February 2006
Revised 20 July 2006
Accepted 3 August 2006

METHODS

Plant material. Leaves from plants growing wild in Beira Litoral, Portugal, were used. The leaves were collected during the flowering season, dried in a flow of hot air (25 °C), ground and stored below –10 °C. A properly identified voucher specimen of the starting plant material, *Passiflora edulis* Sims, was deposited in the Botanical Institute, University of Coimbra and referenced by the author's name.

Flavonoids extraction and fractionation. A 6% (w/v) infusion was prepared by adding boiling water to the powder sample. The filtered infusion was stirred, for 1 h with Amberlite XAD 2 (BDH Laboratory Reagents) and the phenolic compounds fixed in this resin were eluted with MeOH and 75% MeOH. Both eluates were mixed and concentrated to dry residue, which was re-dissolved in 75% MeOH and chromatographed on Sephadex LH-20 (Sigma, 25–100 μm). All fractions collected were monitored by TLC (silica gel), EtOAc/HCOOH/H_2O, 9:1:1, detection under UV_{354nm} before and after the action of ammonia vapour and after spraying with the reagent NEU/PEG (Markham, 1989). Fractions exhibiting the typical flavonoid profile were pooled and concentrated to dryness resulting in the total flavonoid fraction (TFF).

The TFF was chromatographed on Whatman 3 mm paper, EtOAc/HCOOH/H_2O, 44:16:20. The three distinct absorption bands observed under UV_{354nm} were cut out and eluted with 50%–100% MeOH to yield three flavonoid subfractions: SF I, SF II and SF III. SF I was subsequently chromatographed on Whatman® 3 mm paper, HOAc 15% and the two distinct UV_{354nm} absorption bands (compounds A and B) were cut out and eluted with MeOH. The residue of these eluates was dissolved in water and purified with Isolute® C18 pre-packed columns.

Analytical procedures. The composition of the infusion, TFF, SF I, SF II, SF III, A and B was analysed by HPLC using a Gilson® apparatus, a reverse phase column (250 × 4 mm; Spherisorb® S5 ODS 2) and a Nucleosil® C18 pre-column (particle size 5 μm; 30 × 4 mm). Gradient 1: linear gradient of MeOH/5% HCOOH (35% to 80% in 60 min) with a flow rate of 1 mL/min. Gradient 2: water (pH = 2.2 with H_3PO_4) (A)/acetonitrile (B) (0–12 min: 100%–91% of A; 12–20 min: 91%–87% of A; 20–40 min: 87%–67% of A; 40–42 min: 67% of A; 42–52 min: 67%–57% of A; 52–60 min: 57% of A) with a flow of 0.8 mL/min. Peak detection and absorption spectra were obtained with a Gilson® '170 diode array detector' (Campos *et al.*, 1997). The infusion and the TFF were screened for the presence of tannins and alkaloids. For tannins TLC (silica gel) was used with $CHCl_3$/EtOAc/HCOOH, 5:4:1, detection with 5% phosphomolybdic acid in MeOH after heating at 80 °C during 10 min. For alkaloid screening the procedure recommended by French Pharmacopoeia X was followed. The flavonoid contents of the different fractions were determined according to the *Passiflora incarnata* L. monograph in French Pharmacopoeia X.

NMR spectra of the two isolated compounds and their hydrolysates (dissolved in 600 μL DMSO-d_6) were recorded in a Varian Unity-500 spectrometer using either a 5 mm reverse detection (^{1}H-NMR, HMBC and COSY) or a 5 mm broadband probe (^{13}C-NMR).

Acid hydrolysis of compounds A and B was performed (Markham, 1989) and the genin was analysed by HPLC and co-chromatographed with standard luteolin and orientin, purchased from Extrasynthése (Genay, France).

Pharmacological assays. Adult male Swiss mice (25–35 g) were used. Animals were housed in a local animal house in groups of ten animals per plastic cage, with a controlled dark/light 12 h cycle (lights on at 07:00 h) and food was supplied *ad libitum*. Each animal was used just once. All experiments were conducted in accordance with international standards of animal welfare recommended by the Brazilian Society of Neuroscience and Behavior (Act 1992) and the experimental protocols were approved by the University Committee for Animal Care in Research (#23080.001156/2001-50/UFSC). The minimum number of animals and the duration of observation required to obtain consistent data were used.

The animals were orally administered, using an intragastric cannula (by gavage), with the different test solutions, diazepam or distilled water (vehicle) 60 min before testing. To ascertain the best time-course for observation, the infusion and TFF were previously tested with 30, 60, 90 and 120 min delays before the testing. On the test day, mice were placed in the laboratory 1 h prior to the experiment, which was always conducted during the same period of the day (09.00–13.00 h). The lyophilized infusion was restored with water immediately before testing and doses were evaluated in the range between 14.4 and 230 mg/kg. TFF, SF (I, II, III), A and B were also dissolved in distilled water and tested in doses corresponding to its percentage in the extract from which they were obtained. Complementary doses ranging from 1 to 100 mg/kg were also tested. Diazepam was used in a dose of 1 mg/kg and extemporaneous solutions were prepared by slowly diluting a commercial diazepam formulation (Valium® Roche/5 mg) in distilled water immersed in an ultrasound bath.

Elevated plus-maze test (EPM). The elevated plus-maze was slightly modified from that used by Lister (1987). Briefly, it consisted of two open arms (30 × 5 × 0.25 cm) and two enclosed arms (30 × 5 × 15 cm), extending from a central platform (5 × 5 cm) and arranged so that two pairs of identical arms were opposite to each other. The apparatus was raised to a height of 50 cm above floor level. The maze floor was constructed from black Plexiglas and the walls from clear Plexiglas. At the beginning of the test, each mouse was placed on the central platform facing an enclosed arm. After the test (5 min), the maze was carefully cleaned with wet tissue paper (10% ethanol solution). Mouse behaviour was recorded under red light illumination (15 W) by using a video-camera located 100 cm above the maze. Tapes were analysed 'blind' for the treatment used. The conventional spatiotemporal measures were the number of entries (all four paws on open or enclosed arms and expressed as the percentage of total entries), the time spent on open arms (expressed as the percentage of time spent on closed plus open arms), number of entries on enclosed arms and the time on the central

DOI: 10.1002/ptr

platform. Ethologically derived measures were grooming, rearing, stretched attend postures (SAP), head-dipping (HD) and defaecation as an emotionally related parameter (Rodgers and Dalvi, 1997; Espejo, 1997). A selective increase in the spatiotemporal parameters of exploration of the open arms of the maze reveals an anxiolytic-like effect (Pellow *et al.*, 1985).

Marble-burying test (MBT). This test consisted of a Plexiglas cage of 23 × 17 × 14 cm with a smooth lid punctured by small ventilation holes. The floor was covered with a 5 cm layer of sawdust and 25 glass marbles were placed in contact with each other in the centre of the maze. The mouse was placed in the cage for 30 min after which it was removed and the burying response quantified by counting the number of marbles that were more than two thirds covered with sawdust. A diminution of the burying reflex reveals a positive anxiolytic-like effect (Broekkamp *et al.*, 1986).

Motor performance evaluation. Muscle relaxant effects were evaluated using the horizontal-wire test which consisted of a stretched copper wire placed 20 cm above the ground (Boissier *et al.*, 1961). The animal is suspended in the wire by its fore paws and the time taken for the animal to reach the wire with the hind paws or tail is counted. Animals who failed to do it within 5 s were considered to have failed the test and this was considered to be synonymous with muscle relaxation (Vogel and Vogel, 1997).

Motor coordination was assessed using the chimney test which consisted of a simple glass tube in which the experimental mouse entered and when it reached the other end, the tube was placed in a vertical position. The normal reaction of the animal was to climb the tube backwards. Animal performance was evaluated by the time taken to reach the upper edge of the glass tube considering motor impairment the inability of the mouse to climb backwards up the tube within 30 s (Boissier *et al.*, 1960).

Statistical analysis. Statistical analysis of the results obtained was done with GraphPad Instat® software, using ordinary ANOVA followed by post-hoc Tukey's test for comparisons between treatments and Fisher's exact test for the Horizontal-wire and chimney tests. Differences between the experimental groups were considered statistically significant when $p < 0.05$.

RESULTS

Flavonoid extraction and analytical procedures

Infusions of *Passiflora edulis* Sims leaves were found to contain 2.45 ± 0.1% (w/w) of flavonoids expressed in rutin. The TLC control of the fractionation process on Sephadex LH-20 revealed the presence of a majority of compounds with strong UV_{354nm} absorption, yellow/greenish fluorescence after exposure to ammonia vapour and yellow/orange fluorescence after revelation with NEU/PEG thus suggesting a flavone type structure (Markham, 1989). This fractionation procedure yielded 1.4 ± 0.3% (w/w) of TFF which presented a flavonoid content of 60 ± 0.2% (w/w). Tannin screening was positive for the infusion but negative for TFF. Alkaloids were absent from the infusion as well as from TFF.

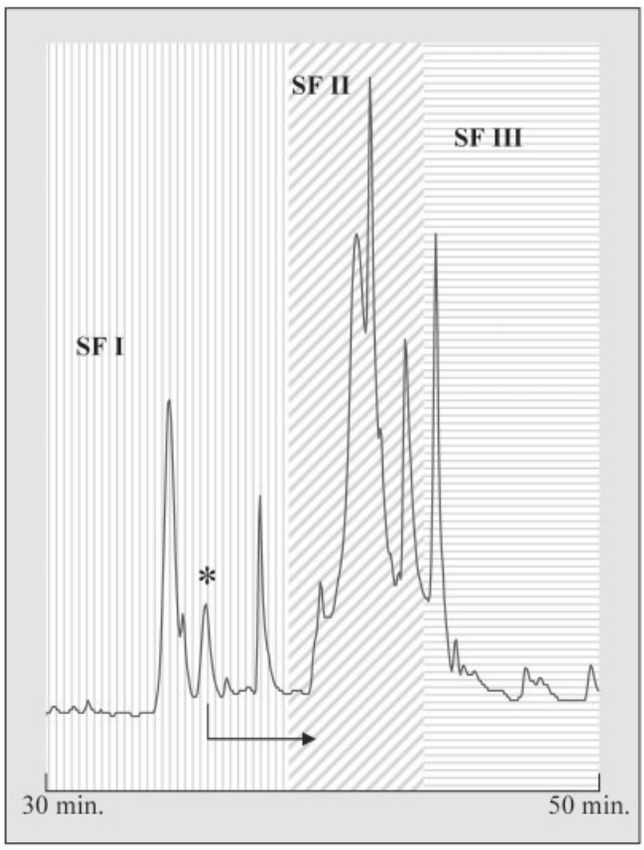

Figure 1. Schematic representation of a total flavonoid fraction (TFF) HPLC chromatogram (Gradient 2; detection: 340 nm), signalling each sub-fraction (SF I, II and III) constituents obtained after preparative paper chromatography (PC) of TFF.

Preparative PC of TFF yielded three sub-fractions: SF I [17.3% (w/w)], SF II [51.8% (w/w)] and SF III [11.4% (w/w)] and their HPLC profiles were established (Fig. 1). The different chromatograms (HPLC, PC) of SF I have shown that it was composed of two compounds: A [retention time (R_t) = 37.4 min (gradient 2) and R_t = 9.4 min (gradient 1)] and B (R_t = 34.5 min (gradient 2) and R_t = 10.8 min (gradient 1). PC of SF I with HOAc 15% allowed the isolation of compound A [20.3% (w/w); retention factor (R_f) = 0.29] and compound B [72.1% (w/w); R_f = 0.58]. After acid hydrolysis, A presented the same R_t (gradient 1 = 28.1 min; gradient 2 = 49.5 min), R_f (PC/HOAc 70%: 0.56) and UV spectrum (absorption maxima: 245sh, 254, 267, 291sh, 349 nm) as standard luteolin. After acid hydrolysis B presented the same R_t (gradient 1: 9.4 min; gradient 2: 34.9 min) and R_f (PC/HOAc 15%: 0.15) as pure standard orientin and its UV absorption maximums were also superimposable (255, 266, 292sh, 348 nm).

NMR analysis of compounds A and B

Compound A – ^{1}H-NMR (500 MHz, DMSO-d6): δ (ppm) = 7.45 (1H, d, J = 2.2 Hz, H-6′), 7.40 (1H, s, H-2′), 6.90 (1H, d, J = 8.4 Hz, H-5′), 6.77 (2H, s, H-3, H-8), 6.37 (1H, d, H-6), 5.24 (1H, d, J = 7.3 Hz, H-1Glc), 5.13 (1H, s, H-1 Rha). After hydrolysis: 7.42

DOI: 10.1002/ptr

Table 1. Time course of the behavioural effects tested in the EPM

	30 min Plus-maze		60 min Plus-maze		90 min Plus-maze		120 min Plus-maze	
	%OE	%OT	%OE	%OT	%OE	%OT	%OE	%OT
Vehicle	21.3 ± 5.8	19.4 ± 8.6	18.3 ± 4.5	16.3 ± 6.6	14.5 ± 10.5	13.6 ± 11.1	20.8 ± 6.7	14.2 ± 10.2
Diazepam	25.5 ± 13.4	30.3 ± 16.5	49.9 ± 14.7[b]	54.8 ± 12.8[b]	44.8 ± 17.6[a]	47.8 ± 19.8[a]	33.9 ± 16.8	43.2 ± 15.0[a]
TFF	22.5 ± 16.4	14.6 ± 9.3	43.4 ± 16.5[a]	40.1 ± 12.1[b]	39.4 ± 12.2[a]	37.3 ± 8.4[b]	29.6 ± 12.4	34.3 ± 14.4
Infusion	30.3 ± 9.5	23.4 ± 7.5	36.9 ± 9.3[a]	35.3 ± 10.1[a]	29.8 ± 4.3[a]	25.1 ± 5.2	30.1 ± 7.4	14.5 ± 12.3

Behavioural effects 30, 60, 90 and 120 min after administration of vehicle, diazepam, TFF and infusion.
%OE, % number of entries in the open areas of the plus-maze vs total number of entries; %OT, % time spent in the open areas of the plus-maze. Data presented as mean ± SEM. Statistical analysis, [a] $p < 0.05$, [b] $p < 0.001$ with ANOVA and Tukey's test.

(1H, s, H-2′), 7.39 (1H, d, $J = 2.3$ Hz, H-6′), 6.88 (1H, d, $J = 8.4$ Hz, H-5′), 6.67 (1H, s, H-3), 6.45 (1H, s, H-6), 6.19 (1H, s, H-8). ^{13}C-NMR (500 MHz, DMSO-d_6): δ (ppm) = 94.4 [Glucose (G)1], 73.3 (G2), 72.3 (G3), 77.3 (G4), 72.4 (G5), 60.3 (G6), 99.3 [Rhamnose (R)1], 77.2 (R2), 77.0 (R3), 76.4 (R4), 71.9 (R5), 18.1 (R6).

Compound B – ^{1}H-NMR (500 MHz, DMSO-d_6): δ (ppm) = 13.14 (1H, s, 5-OH), 7.55 (1H, d, $J = 8.5$ Hz, H-6′), 7.49 (1H, s, H-2′), 6.87 (1H, d, $J = 8.4$ Hz, H-5′), 6.66 (1H, s, H-3), 6.26 (1H, s, H-6), 4.98 (1H, s, H-1 Rha), 4.75 (1H, d, $J = 9.9$ Hz, H-1 Glc). After hydrolysis: δ (ppm) 7.54 (1H, d, $J = 8.4$, H-6′), 7.48 (1H, s, H-2′), 6.87 (1H, d, $J = 8.4$ Hz, H-5′), 6.65 (1H, s, H-3), 6.27 (1H, s, H-6), 4.67 (1H, d, $J = 9.0$, H-1 Glc). ^{13}C-NMR (500 MHz, DMSO-d_6): δ (ppm) = 71.5 (G1), 75.1 (G2), 80.0 (G3), 70.4 (G4), 82.0 (G5), 61.5 (G6), 100.4 (R1), 70.9 (R2), 70.5 (R3), 71.7 (R4), 68.3 (R5), 17.8 (R6).

Pharmacological studies

When testing the infusion and TFF to determine the most suitable time-course for the observation of the behavioural effects (Table 1), positive results were found (i.e. statistical significant differences with relation to groups administered only with vehicle-distilled water) were observed after 60 min of the oral administration. After 90 min, the results were less significant and after 120 min there was a general increase in the dispersion of the results. Therefore, all other results presented in this paper were obtained 60 min after oral administration of all test solutions.

For the lyophilized infusion doses were tested between 5 and 230 mg/kg. Only the highest dose (230 mg/kg) produced a statistical significant increase in both the percentage of time spent and the number of entries into the open arms of the EPM in relation to the group administered with vehicle (Fig. 2). Also, in the MBT there was a significant diminution of the object-burying reflex only at the highest dose (230 mg/kg; Fig. 3). In the range of doses tested for the infusion there were no significant changes in the parameters evaluated in the wire and chimney tests (Fig. 4).

For TFF doses between 1 and 100 mg/kg were tested. For the dose corresponding to the active dose of the infusion (3.2 mg/kg) there were no significant changes in any of the tests. However, with 100 mg/kg there was an increase in the percentage of entries and the time spent in the open arms of the EPM (Fig. 2). Results of the horizontal wire test showed an increase in the percentage of animals considered to be under muscle

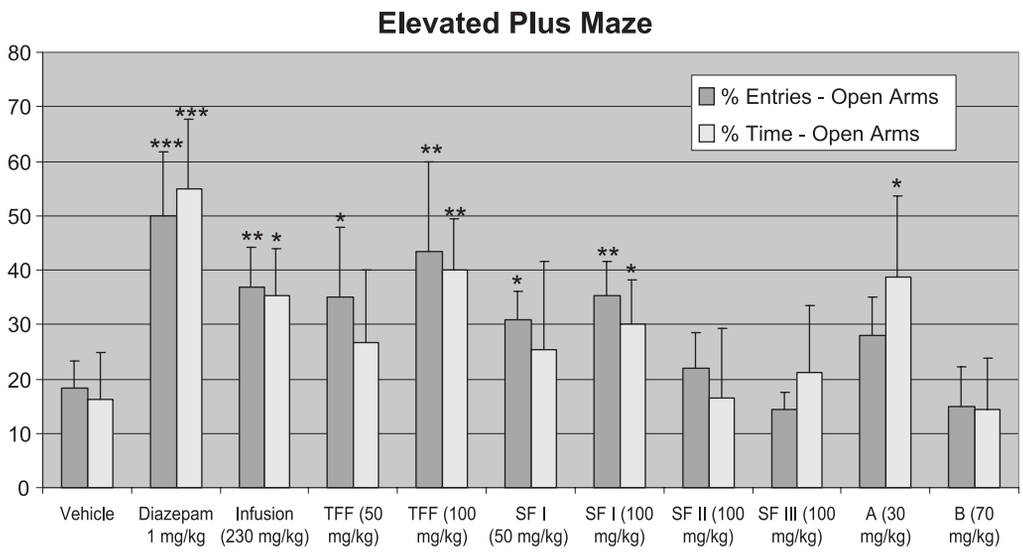

Figure 2. Analysis of the behaviour of mice in the elevated plus maze test of anxiety. Results expressed as average (± SEM) percentage of entries in the open areas of the maze (closed bars) and average percentage of time in the open areas of the maze (hatched bars). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ are significantly different from control (ANOVA; Tukey's test). The number of mice per group ranged between 9 and 16.

DOI: 10.1002/ptr

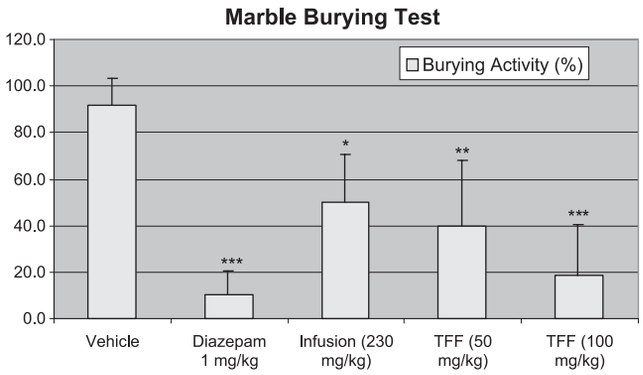

Figure 3. Behavior of mice in the marble burying test. Results expressed as average percentage (± SEM) of buried marbles. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ are significantly different from control (ANOVA; Tukey's test). The number of mice per group ranged between 8 and 11.

relaxant effects and an increase in the percentage of animals presenting motor uncoordination in the chimney test (Fig. 4). With both 50 and 100 mg/kg a reduction of the marble-burying reflex was observed (Fig. 3).

With SF I, II and III there were no positive results in any of the tests when a dose corresponding to the active dose of TFF (100 mg/kg) was used. But when higher doses were tested, SF I significantly increased the time spent (100 mg/kg) and the number of entries (50 and 100 mg/kg) in the open arms of the plus-maze (Fig. 2), without any effect on motor activity parameters (Fig. 4). SF II (10–100 mg/kg) and SF III (1–100 mg/kg) induced no significant changes in any of the tests used.

With compounds A (0.3–30 mg/kg) and B (0.7–100 mg/kg), isolated from SF I, only A (30 mg/kg) significantly increased the percentage of time spent in the open arms of the EPM (Fig. 2) and none of them induced any effects on the motor activity tests (Fig. 4).

Analysis of the ethological parameters in the EPM was performed only for SF I, II, III and compounds A and B (Table 2). The only significant differences were found for SF I and compound A. SF I decreased the number of total stretch approach postures (SAP) with 50 and 100 mg/kg, whereas it also increased the total number of head-dippings. Compound A (30 mg/kg) significantly increased the number of head-dips.

DISCUSSION

The elevated plus-maze is now widely accepted as an animal model of anxiety (Reibaud and Böhme, 1993). The ratio of the number of entries into the open arms to the total number of arm entries and the ratio of the time spent in the open arms to the time spent in both types of arms are used as markers of the natural aversion of rodents for the open arms and these two parameters have been shown to be increased by clinically effective anxiolytics and to be decreased by anxiogenic drugs (Lister, 1987; Pellow *et al.*, 1985). The defensive burying is a behaviour that can be elicited in rodents in response to aversive stimuli and inhibited by diazepam or chlordiazepoxide (Treit *et al.*, 1981). Glass marbles provide an effective unconditioned stimulus

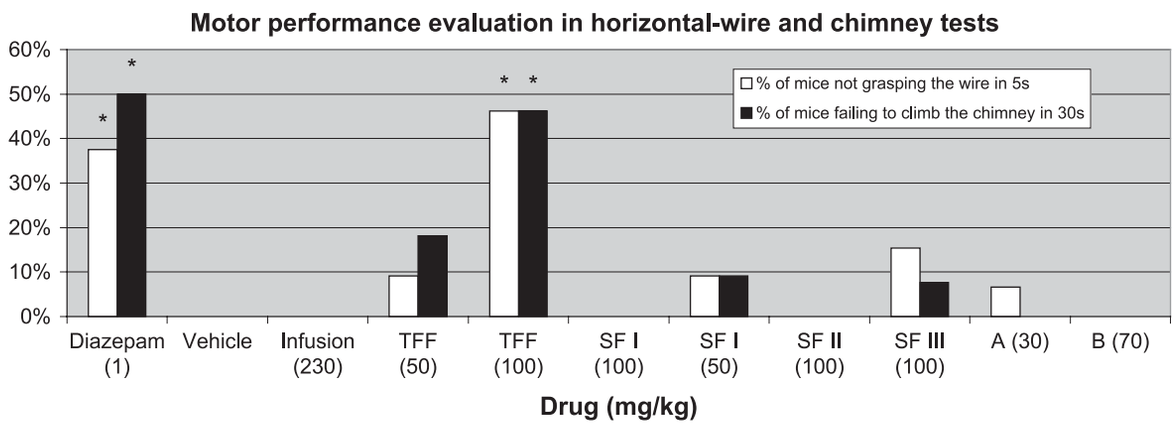

Figure 4. Performance of mice in the horizontal-wire and in the chimney test: mice who failed to grasp the wire with their hind paws in 5 s were considered to be under a myorelaxant effect and those who failed to climb the chimney backwards in 30 s were considered to be motor uncoordinated. * $p < 0.001$ significantly different from control, Fisher's exact test. The number of mice per group ranged between 11 and 16.

Table 2. Analysis of ethological parameters in the EPM

	Protected head-dips (%)	Total stretched attend postures
Vehicle ($n = 13$)	72.1/6.7	16.2/3.8
Diazepam (1 mg/kg) ($n = 11$)	50.1/11.2[b]	6.8/2.1[b]
SF I (100 mg/kg) ($n = 10$)	60.3/5.4[a]	6.4/2.5[b]
SF I (50 mg/kg) ($n = 9$)	68.2/7.3	7.3/5.7[a]
A (30 mg/kg) ($n = 9$)	58.6/6.5[a]	17.3/8.0
B (70 mg/kg) ($n = 10$)	70.1/9.2	18.4/7.1

Data presented as mean/SEM. Statistical analysis [a] $p < 0.05$; [b] $p < 0.001$ with ANOVA and Tukey's test.

DOI: 10.1002/ptr

which provokes burying (Poling *et al.*, 1981) and this model has been used for the screening of anxiolytic-like drugs with the diminution of the burying activity indicating an anxiolytic-like effect (Broekkamp *et al.*, 1986). Therefore the results obtained with *P. edulis* Sims infusions in the EPM and MBT are consistent with an anxiolytic-like effect. As there were no significant changes on the motor activity parameters measured by the wire and the chimney tests it was concluded that this anxiolytic-like effect was devoid of any effects on motor activity.

TFF also revealed an anxiolytic-like activity in both EPM and MBT parameters but unlike the infusion and despite the number of entries into the enclosed arms of the EPM [parameter directly related with motor activity (Rodgers and Cole, 1994)] remaining unchanged, the results obtained in the wire and in the chimney tests indicate muscle relaxant effects with motor impairment. As TFF is mainly composed of flavonoids and tested negatively for other possible active substances, such as tannins or alkaloids (Takahashi *et al.*, 1986; Aoagy *et al.*, 1974), it was concluded that the flavonoids extracted from *P. edulis* Sims could be involved in the neuropharmacological activity. However, as the doses (50 and 100 mg/kg) necessary to induce positive effects were much higher than the TFF content in the active dose of the infusion and the pharmacological profile was also different a possible direct or indirect involvement of other compounds in the activity of the infusion cannot be ruled out.

Ethological derived parameters such as protected head-dipping or stretched attend postures are risk assessment measures inversely related with an anxiolytic effect and are generally more sensitive to drug action than the traditional indices of anxiety in this test (Rodgers and Cole, 1994). Hence it can be concluded that the SF I (50, 100 mg/kg) increase in the conventional spatiotemporal parameters of open arms exploration and decrease of head-dipping and stretched attend postures is consistent with an anxiolytic-like effect. However, SF I did not have any effect on MBT which can be described as a model of novelty-induced or proximal threat anxiety, whereas EPM is a model of approach-avoidance conflict. It is known that the same compound may act at different dose ranges in distinct models (Olivier *et al.*, 2000), however, the differences obtained spanned all the dose ranges tested and this aspect should be further investigated.

The positive results of SF I obtained in the EPM were observed for doses much higher than the corresponding dose of the active extract from which it was obtained thus suggesting that other compounds in TFF could also be involved in its neuropharmacological activity. The same doses did not elicit any effect on the motor activity measured in the EPM and by the chimney and wire tests, hence indicating a specific anxiolytic-like effect without sedation or muscle relaxation.

When both isolated components of SF I were tested it was verified that only compound A (30 mg/kg) induced changes in both classical and ethological derived parameters of the EPM, consistent with an anxiolytic-like effect which in this case correlated very well with its content in the active dose of SF I (100 mg/kg).

^{1}H-NMR analysis of SF I isolated constituents, revealed that both compound A and B, were substituted flavones. Through the assignment of ^{13}C-NMR signals and from HMBC and COSY correlations, the type and relative position of the sugar residues in the flavone nucleus were established. The results were consistent with the structure of luteolin-7-O-[2-rhamnosylglucoside] (compound A) and 8-C-glucosylluteolin-2″-rhamnoside (compound B). This leads to the conclusion that if the C-linked rhamnoglucosyl appears in position 8 of the flavone nucleus this can negatively affect the neuropharmacological activity.

Recently Dhawan *et al.* (2004) questioned the correct identification of the *Passiflora* species used in previous studies and attributed the discrepancy in the literature to this fact. These authors also reported on a comparison of *P. incarnata* L. with *P. edulis* Sims in the elevated plus-maze test of anxiety concluding that *P. edulis* Sims was devoid of pharmacological activity (Dhawan *et al.*, 2001). Although it is agreed that in some cases there is a strong possibility of mistaken identity between *P. incarnata* L. and *P. edulis* Sims due to their morphological resemblance, it is not believed that this assumption can be used to simply rule out all the anxiolytic-like activity reports of other Passifloraceae. Instead it is believed that the apparent discrepancy between our results and those of Dhawan is surely a consequence of the differences in the chemical composition of the plant extracts used, maybe originating in the processing and extraction procedures of the botanical materials or even in the different *P. edulis* Sims chemotypes used by each group.

Although we did not identify all the active constituents in *P. edulis* Sims and our results cannot be used to justify the medicinal use of this plant, this study clearly demonstrated that this plant's aqueous extracts and flavonoids did have anxiolytic-like activity and that at least one of these flavonoids, luteolin-7-O-[2-rhamnosylglucoside], can elicit the same kind of neuropharmacological activity.

Acknowledgement

M. Coleta was a recipient of a PhD Scholarship from Praxis XXI (BD/16264/98).

REFERENCES

Aoagy N, Kimura R, Murata T. 1974. Studies on *Passiflora incarnata* dry extract. I. Isolation of maltol and pharmacological action of maltol and ethyl maltol. *Chem Pharm Bull* **22**: 1006–1013.

Boissier JR, Dremont C, Robins R, Pagny J. 1961. Tentative de pharmacology prévisionnelle dans le domaine des neuroleptiques: actions sedative centrale et adrénolytique de la N(diméthoxy-3,4 phénéthyl)N′ (chloro-2-phényl)piperazine. *Arch Int Pharmacodyn Ther* **133**: 29–32.

Boissier JR, Tardy J, Diverres JC. 1960. Une nouvelle méthode simple pour explorer l'action 'tranquillisante': le test de la cheminée. *Med Exp* **3**: 81–84.

Broekkamp CL, Rijk W, Joly-Gelouin D. 1986. Major tranquilizers can be distinguished from minor tranquilizers on the basis of effects on marble burying and swim-induced grooming in mice. *Eur J Pharmacol* **126**: 223–229.

Campos M, Markham KR, Mitchell KA, da Cunha AP. 1997. An approach to the characterization of bee pollens via

their flavonoid/phenolic profiles. *Phytochem Anal* **8**: 181–185.
Cotrim MD, Martins MA, Figueiredo IV *et al.* 1995. Effects of *Passiflora edulis* aqueous extracts on 5-HT3 receptors in guinea-pig ileum and rat brain. *Pharmacol Res Suppl*. EPHAR (Proceedings of the Federation of European Pharmacological Societies): 266.
Dhawan K, Dhawan S, Sharma A. 2004. Passiflora: a review update. *J Ethnopharmacol* **94**: 1–23.
Dhawan K, Kumar S, Sharma A. 2001. Comparative biological activity study on *Passiflora incarnata* and *P. edulis*. *Fitoterapia* **72**: 698–702.
Espejo EF. 1997. Structure of the mouse behaviour on the elevated plus-maze test of anxiety. *Behav Brain Res* **86**: 105–112.
Lister RG. 1987. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* **92**: 180–185.
Maluf E, Barros MT, Frochtengarten ML, Benti R, Leite JR. 1991. Assessment of the hypnotic/sedative effects and toxicity of *Passiflora edulis* aqueous extract in rodents and humans. *Phytother Res* **5**: 262–266.
Markham KR. 1989. Plant phenolics. In *Methods in Plant Biochemistry*, Vol. 1, Dey PM, Harborne JB (eds). Academic Press: London, 202–203.
Medina JH, Viola H, Wolfman C *et al.* 1997. Overview – Flavonoids: a new family of benzodiazepine receptor ligands. *Neurochemistry* **22**: 419–425.
Oga S, Freitas PCD, Silva AG, Hanada S. 1984. Pharmacological trials of crude extract of *Passiflora alata. Planta Med* **50**: 303–306.
Olivier B, van Wijngaarden I, Soudijn W. 2000. 5 HT-3 receptor antagonists and anxiety, a pre-clinical and clinical review. *Eur Neuropsychopharmacol* **10**: 77–95.
Paladini AC, Marder M, Viola H, Wolfman C, Wasowski C, Medina JH. 1999. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. *J Pharm Pharmacol* **51**: 519–526.
Pellow S, Chopin P, File SE, Briley M. 1985. Validation of open closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Meth* **14**: 149–167.
Petry RD, Reginatto F, de-Paris F *et al.* 2001. Comparative pharmacological study of hydroethanol extracts of *Passiflora alata* and *Passiflora edulis* leaves. *Phytother Res* **15**: 162–164.
Poling A, Cleary J, Monaghan M. 1981. Burying by rats in response to aversive and nonaversive stimuli. *J Exp Anal Behav* **35**: 31.
Reibaud M, Böhme GA. 1993. Evaluation of putative anxiolytics in the elevated plus-maze test. *Methods Neurosci* **14**: 230–239.
Rodgers RJ, Cole JC. 1994. The elevated plus-maze: pharmacology, methodology and ethology. In *Ethology and Psychopharmacology*, Cooper SJ, Hendrie CA (eds). John Wiley & Sons: New York, 9–44.
Rodgers RJ, Dalvi A. 1997. Anxiety, defense and the elevated plus-maze. *Neurosci Biobehav Rev* **21**: 801–810.
Takahashi RN, Lima TCM, Morato GS. 1986. Pharmacological actions of tannic acid; II. Evaluation of CNS activity in animals. *Planta Med* **4**: 272–275.
Treit D, Pinel JPJ, Fibiger HC. 1981. Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. *Pharmacol Biochem Behav* **15**: 619.
Vale NB, Leite JR. 1983. Efeitos psicofarmacológicos de preparações de *Passiflora edulis* (maracujá). *Ciênc Cult* **35**: 11–24.
Vogel HG, Vogel WH. 1997. *Drug Discovery and Evaluation, Pharmacological Assays.* Springer: Berlin, 211–212.
Wolfman C, Viola H, Paladini AC, Dajas F, Medina JH. 1994. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea*. *Pharmacol Biochem Behav* **47**: 1–4.
Zanoli P, Avallone R, Baraldi M. 2000. Behavioural characterisation of the flavonoids apigenin and chrysin. *Fitoterapia* **71**(Suppl 71): S117–S123.

DOI: 10.1002/ptr