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Original Article

Comparative central effects of the aqueous leaf extract of two populations of *Passiflora edulis*

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ABSTRACT

Passiflora edulis Sims, Passifloraceae, has been used in Brazilian traditional folk medicine to the treatment of anxiety and insomnia. *P. edulis* is commonly known for its economic interests in Brazil. This species exhibits significant variability in the fruit rind color, then two subpopulations has been described (*P. edulis* fo. *flavicarpa* O. Deg. (PEF); *P. edulis* fo. *edulis* (PEE)). This study compared phytochemical profile and biological actions of aqueous leaf extract of PEE and PEF. HPLC analysis showed marked distinct chromatograms to the *P. edulis* varieties. However, in both extracts the major compounds observed were flavonoids C-glycosides. Behavioral studies showed that PEE (300 mg/kg, *p.o.*) and PEF (100 and 300 mg/kg, *p.o.*) reduced anxiety in the elevated plus maze test. PEE (300 and 1000 mg/kg, *p.o.*) and PEF (1000 mg/kg, *p.o.*) also induced antidepressant-like actions in the forced swimming test. PEE 1000 mg/kg significantly reduced distance moved, thus suggesting sedation. No alterations in sleeping time were observed with PEE and PEF extracts. In conclusion, despite the similarities between the biological actions observed for both *P. edulis* varieties, quite different phytochemical profile was herein reported. These data suggest that the anxiolytic and antidepressant actions are not due to a specific phytochemical component.
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Introduction

The genus *Passiflora*, Passifloraceae, comprises about 500 plant species, mostly distributed in tropical and subtropical regions of the world. Many species of this genus have long been used in traditional folk medicines worldwide to the treatment of anxiety, insomnia, epilepsy, spasm, and aches (for review see: Dhawan et al., 2004; Guzman-Gutiérrez et al., 2014). *Passiflora edulis* Sims is a variety commonly known for its tasty fruit but also used in Brazilian traditional folk medicine (Bernacci et al., 2008). As firstly noticed by Degener (1932), *P. edulis* species exhibits considerable morphological variability. This plant produces two types of fruit: the purple (*Passiflora edulis* fo. *edulis*) and yellow fruit (*Passiflora edulis* fo. *flavicarpa* O. Deg.) (Bernacci et al., 2008). These distinctions go further, the *P. edulis* fo. *edulis* species presents flowers with white petals and purple filaments on the lower half, and white on the top half and brownish-purple fruit, with a length of 6–7 cm. The flowers of *P. edulis* fo. *flavicarpa* exhibit highly purple corona at the base and larger yellow fruits (*i.e.*, 6–12 cm long) (Bernacci et al., 2008). Indeed, their genetic constitutions were found having low similarity (Aukar et al., 2002), and their volatile compounds were significantly different (Pontes et al., 2009). Despite these dissimilarities, *P. edulis* fo. *flavicarpa* is usually considered just a variety of the *P. edulis* species, thus the infraspecific taxonomy of *P. edulis* is contradictory and worth further discussion (for review see Bernacci et al., 2008).

In 2010, the official monograph of *P. edulis* species (no description of the variety) was included in the Brazilian Pharmacopoeia (2010) as well as the official monograph of *P. alata* Curtis. An important feature of *Passiflora* species is that, due to the folk medicinal uses, *P. edulis*, *P. alata* and *P. incarnata* were included in the National List of Medicinal Plants of Interest to Brazilian Public Health System (Ministério da Saúde do Brasil, 2009). This list includes 71 species of medicinal plants with potential to generate pharmaceutical products of interest to Brazilian public health (Ministério da Saúde do Brasil, 2009). Regarding the relevance of *Passiflora*

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species as food, Brazil is considered the top world producer of yellow fruits (*P. edulis* fo. *flavicarpa*). These observations reinforce the value of pharmacologically validating the traditional use of *Passiflora* species abundantly cultivated and medicinally used in Brazil.

In vivo studies have long been reporting the biological actions of *P. edulis* Sims in rodents (Petry et al., 2001; Coleta et al., 2006; Reginatto et al., 2006). However, no formal distinction between *P. edulis* varieties has been registered. An exception was found in a study published in 2011 by a Chinese group, which compared the chemical constitution and biological effects of the ethanol extract of two populations of *P. edulis* ('*edulis*' and '*flavicarpa*') cultivated in China (Li et al., 2011). In 2010, the same Chinese research group has reported the behavioral effects of ethanol extract of the aerial part of *P. edulis* fo. *flavicarpa* and its fractions in mice (Deng et al., 2010). Considering the importance of validating the traditional folk uses of *P. edulis* varieties, which has huge economic relevance in Brazil, the present study aimed to compare the phytochemical profile and the central effects of the aqueous leaf extract of the two populations of *P. edulis* ('*edulis*' and '*flavicarpa*') cultivated in South America.

Materials and methods

Chemical and reagents

All solvents used were HPLC grade and Milli Q water, filtered in 0.45 μm membranes (MILEX®) and degassed by ultrasound bath. The reference standards used were vitexin-2″-*O*-rhaminoside (Fluka, 98.2%), isoorientin (Sigma, >98%), orientin (Sigma, >97%), isovitexin (Fluka, >95%), vitexin (Fluka, >95%), and luteolin-7-*O*-glucoside (Fluka, >98%). The compound 6,8-di-*C*-glycosylchrysin was isolated from *Lychnophora ericoides* leaves and was provided by Dr. Norberto Peporine Lopes. Spinosin and swertisin were isolated from *Wilbrandea ebracteata* roots (Santos et al., 1996). Vicenin-2 was isolated from *P. edulis* fo. *flavicarpa* leaves and identified by NMR spectral data (^{1}H and 2D-NMR techniques-COSY, HSQC and HMBC) and the purity was confirmed by HPLC/DAD (Zucolotto et al., 2009). The reference standards were analyzed at 50 μg/ml concentration. The solutions were filtered in 0.45 μm membranes (MILEX®) and a 20 μl aliquot of the filtrate was injected for HPLC analysis.

Plant material

The leaves of *Passiflora edulis* fo. *flavicarpa* O. Deg. were collected in Antônio Carlos, Santa Catarina, Brazil and identified by the botanist Prof. Dr. Daniel Falkenberg (Department of Botany of the Federal University of Santa Catarina, Florianópolis, SC, Brazil). A voucher specimen was deposited in the Herbarium at the same university (FLOR 33886). The leaves of *P. edulis* Sims fo. *edulis* were collected in Santa Sofia, Boyacá, Colombia and identified by the botanist Luis Carlos Jimenez (Instituto Nacional de Ciencias, Universidad Nacional de Colombia). A voucher specimen was deposited in the herbarium at the same university (COL 530661).

Preparation of extracts

The leaves of *P. edulis* fo. *edulis* and *P. edulis* fo. *flavicarpa* were air-dried at 40 °C, powdered and extracted using hot water (90 °C) by infusion (plant: solvent, 1:10, w/v) for 10 min. After that, the extracts were filtered and freeze-dried. For HPLC analysis, each extract was previously filtered through a 0.45 μm membrane (MILEX®). The extracts were solubilized in methanol:water (1:1, v/v) at 7.5 mg/ml and 20 μl was used for HPLC analysis.

Phytochemical analysis

HPLC analysis was performed in a Varian® chromatograph pump ProStar 240, ProStar 410 auto injector, coupled to ProStar 335 DAD detector and Phenomenex-Luna 5 μm C18 (2) 100A (250 mm × 4.6 mm) (column). The mobile phase was (A) acetonitrile and (B) acetic acid 0.3%, using the following gradient 0–10 min 10:90% (A) in (B), 10–40 min 20:80% (A) in (B), and 40–90 min 20:80% (A) in (B) at a flow rate of 1 ml/min and UV 345 nm. Peaks found in the chromatograms were identified by comparing the retention time (*RT*) and UV spectra of the reference standards and by co-injection of reference standards plus extracts to compare the increase of peak area.

Animals

Male Swiss mice (weighing 25–40 g) from our breeding colony were used. Animals were housed in plastic cages (41 cm × 34 cm × 16 cm), 12 per cage, under a 12-h light/dark cycle (lights on 6 a.m.) at room temperature of 23 ± 1 °C, with water and food ad libitum. All experimental procedures were conducted between 2 and 5 p.m. and were performed in accordance with the Brazilian law n° 11.794/2008 for experimental use of animals. This study was approved by local Ethic Committee for Animal Use (License N° 032/2010).

Drugs and treatments

Diazepam (Cristália Prod. Quím. Farmacêuticos Ltda., São Paulo, Brazil), an anxiolytic drug, was solubilized in tween 80 (0.5%) and saline, and it was injected intraperitoneally (*i.p.*) at 1 mg/kg (10 ml/kg), 30 min before testing. Nortriptyline (Novartis Biociências S.A., São Paulo, Brazil), an antidepressant drug, was solubilized in saline and *i.p.* administered at 30 mg/kg (10 ml/kg), 30 min prior the forced swimming test. Saline was used as control.

Aqueous extracts of *Passiflora* were solubilized in tap water and administered orally (*p.o.*), by using a syringe coupled to an oral cannula (0.1 cm × 4 cm), in a volume of 10 ml/kg, 60 min before testing. Tap water was used as control. Thiopental sodium 50 mg/kg (Cristália Prod. Quím. Farmacêuticos Ltda., São Paulo, Brazil), a barbituric acid, was injected *i.p.* 60 min prior the *Passiflora* extracts.

Behavioral tests

Elevated plus-maze (EPM) test

The elevated plus maze consists of two open arms (15 cm × 5 cm) and two enclosed arms (15 cm × 5 cm × 5 cm), connected to a central platform (5 cm × 5 cm) and elevated 40 cm above the floor. This test was performed as described by Lister (1987). After treatments, each mouse was individually placed in the center of the EPM, with the head turned to the open arms, and they were allowed to freely explore the apparatus for 5 min. Experiments were performed in a dimly light and quiet room. After each test, the maze was cleaned with 10% ethanol solution. The parameters registered were the number of entries into and the time spent in the open and enclosed arms. The ratios 'time spent in the open arms/time spent in all arms' and 'frequency of entries into open arms/total entries into all arms' were calculated to yield the percentages of time spent in and frequency of entries into open arms, respectively. These parameters can be related to the 'anxiety' level experienced by the animal (Rodgers and Dalvi, 1997). Additionally, the number of entries into enclosed arms was used and an index of general activity.

Open field test

Spontaneous locomotor activity of mice was measured using the open field test. The apparatus, made of wood covered with impermeable formica, had a black floor of 40 cm × 40 cm and black walls of 40 cm high. The test room had a controlled illumination (dimly-light condition). Each mouse was placed in the center of the open field and the distance traveled every 5 min were registered during 30 min through automatic observation (Anymaze, Stoelting Co., Wood Dale, IL, USA). After the behavioral evaluation of each mouse, the arena was cleaned with 10% ethanol solution.

Forced swimming test

This test was performed according to Porsolt et al. (1977). Mouse was individually forced to swim in a transparent glass cylinder (24 cm in height, 18 cm in diameter) filled with 18 cm of water at 23 ± 1 °C. The time spent immobility (in s) was measured during the last 4 min of a single 6-min test session. Mice were considered immobile when they made no further attempts to escape except the movements necessary to keep their heads above the water.

Thiopental-induced sleeping time test

Thiopental was used to induce sleep as previously described by Vogel and Vogel (1997). Thiopental 50 mg/kg was administered *i.p.* 60 min after administration of *Passiflora* extracts. The interval time between injection of thiopental and time that animal loss righting reflex was recorded (in s) as latency time. The interval, in seconds, between loss and recovery of righting reflex was registered as sleeping duration, and it was used as index of hypnotic effect.

Statistical analysis

The data herein presented were reported as mean ± SEM. All data were analyzed using GraphPad InStat 3.06 (GraphPad Software Inc., San Diego, USA). All results were initially submitted to Levene's test for homogeneity of variance and to Kolmogorov–Sminorv's test for normality. Comparisons between treated and control groups were performed using one-way ANOVA followed by Dunnett's test or Student's *t*-test, as detailed in the figure legends. Differences were considered significant when $p < 0.05$.

Results and discussion

Phytochemical analysis

The aqueous extracts from *P. edulis* fo. *edulis* and *P. edulis* fo. *flavicarpa* were analyzed by HPLC/PAD. The chemical profile showed in chromatograms is markedly different to the *P. edulis* varieties. *P. edulis* fo. *edulis* presents peaks between 30 and 80 min, the major peaks were observed in *RT* = 32.4 and 58 min (Fig. 1A). In contrast, *P. edulis* fo. *flavicarpa* shows peaks between 20 and 50 min and major peaks with *RT* = 29.3 and 36 min (Fig. 1B). According to the maxima absorption found in the UV spectra, the main peaks observed in the chromatograms of both extracts seem to be flavonoids.

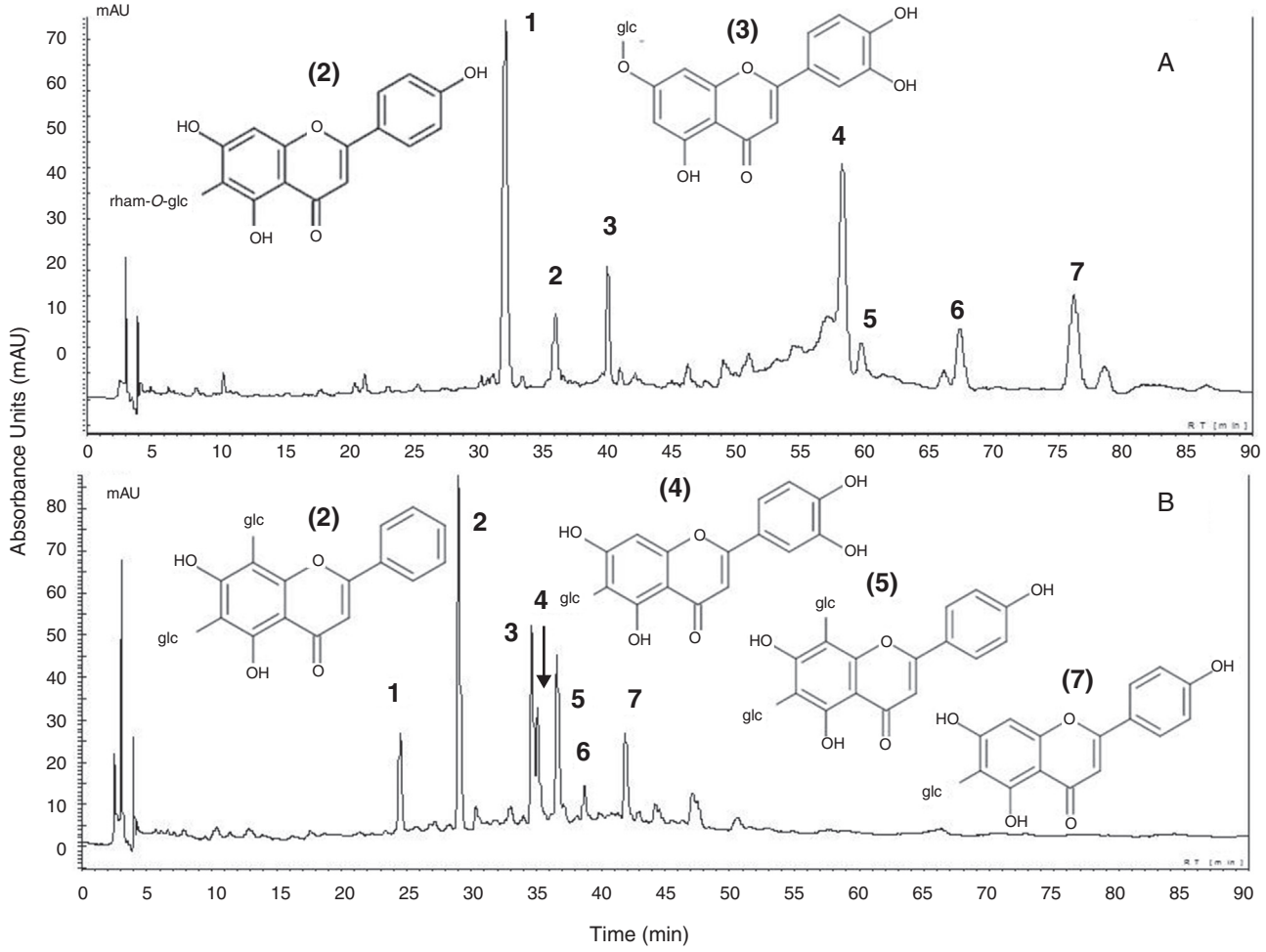

Fig. 1. HPLC chromatograms of aqueous extracts of *Passiflora edulis* fo. *edulis* (A) and *Passiflora edulis* fo. *flavicarpa* (B) leaves 7.5 mg/ml. UV = 345 nm. (A) 1, 4, 5 and 7 = luteolin derivatives, 2 = vitexin-2″-*O*-rhamnoside, 3 = luteolina-7-*O*-glucoside, 6 = apigenin derivative. (B) 1 and 6 = luteolin derivatives, 2 = vicenin-2,3 = apigenin derivative, 4 = isoorientin, 5 = 6,8-di-*C*-glycosylchrysin, 7 = isovitexin. Chromatographic conditions: see in "Materials and methods".

Table 1
Flavonoids identified in *Passiflora edulis* fo. *edulis* and *P. edulis* fo. *flavicarpa*.

Flavonoid	Isoorientin	Orientin	Vitexin	Isovitexin	Vitexin-2″-*O*-rhamnoside	Luteolin-7-*O*-glucoside	Vicenin-2	Spinosin	6,8-di-*C*-glycosyl chrysin	Swertisin
P. edulis fo. *flavicarpa*	+	–	–	++	–	–	++	–	++	–
P. edulis fo. *edulis*	–	–	–	–	+	+	–	–	–	–

Notes: +, Compound identified; ++ major compound; – compound not identified.

Co-injection of extracts plus reference standards showed an increase in the peak area of some flavonoids as summarized in Table 1.

Regarding the *P. edulis* fo. *edulis* extract, only two minority peaks were identified: vitexin-2″-*O*-rhamnoside (peak 2, *RT* = 36 min) and luteolin-7-*O*-glucoside (peak 3, *RT* = 42 min) (Fig. 1A). In general, peaks showed the typical UV absorption of luteolin derivatives: peak 1 (λ_{max} = 261,349 nm), 4 (λ_{max} = 264,348 nm), 5 (λ_{max} = 264,348 nm), and 7 (λ_{max} = 297,348 nm); except for peak 6 (λ_{max} = 267,342 nm) that presented UV absorption similar to flavonoid apigenin (Fig. 1A) (Mabry et al., 1970). As regards *P. edulis* fo. *flavicarpa* extract, vicenin-2 (peak 2, *RT* = 28.2 min), isoorientin (peak 4, *RT* = 30.2 min), 6,8-di-*C*-glycosylchrysin (peak 5, *RT* = 35.5 min) and isovitexin (peak 7, *RT* = 38.5 min) were identified as the major peaks (Fig. 1B). The peaks 1 (λ_{max} = 266,347 nm) and 6 (λ_{max} = 266,345 nm) displayed an UV absorption similar to luteolin derivatives, while peak 3 (λ_{max} = 268,339 nm) was characterized as an apigenin derivative (Fig. 1B) (Mabry et al., 1970).

Briefly, the chromatograms of *P. edulis* fo. *flavicarpa* were quite different from those of *P. edulis* fo. *edulis*. Six major peaks (between 55 and 80 min) observed in the *P. edulis* fo. *edulis* had not been detected in *P. edulis* fo. *flavicarpa*. These observations reinforce the view that these two forms of *P. edulis* are different from each other. Zucolotto and colleagues (2011) have previously demonstrated the presence of *C*-glycosyl flavonoids in South American *Passiflora* species, including the *P. edulis* fo. *edulis* and *P. edulis* fo. *flavicarpa*. Concerning the *P. edulis* fo. *flavicarpa*, in this study, vicenin-2, isoorientin, isovitexin, orientin, vitexin, spinosin and 6,8-di-*C*-glycosylchrysin were identified, while to the *P. edulis* fo. *edulis* extract no reference standards used in this work were identified (see Table 1). Previously, Li et al. (2011) showed the presence of the following flavonoids in *P. edulis* fo. *flavicarpa* ethanol extract: lucenin-2, vicenin-2, isoorientin, isovitexin, luteolin-6-*C*-chinovoside, and luteolin-6-*C*-fucoside. In agreement with our data, Li et al. (2011) also demonstrated that none of these flavonoids were detected in *P. edulis* fo. *edulis*. These observations reinforce the view that these two varieties of *P. edulis* are different from each other. However, distinct local and collection times of the herein studied *P. edulis* varieties should be taken into account to explain differences in the phytochemical profile.

Behavioral tests

Elevated plus-maze test

Before starting the behavioral evaluation of *Passiflora* extracts, the effects of the standard anxiolytic drug, diazepam, was assessed in our experimental conditions. Mice *i.p.* injected with diazepam at 1 mg/kg displayed a significant increase in the percentage of time spent in (vehicle: 12.3 ± 7.0%; diazepam: 42.0 ± 4.5%*; *$p < 0.05$, Student's *t*-test) and entries into (vehicle: 19.3 ± 5.0%; diazepam: 51.0 ± 4.5* %; *$p < 0.05$, Student's *t*-test) open arms. As showed in Fig. 2, *P. edulis* fo. *edulis* 300 mg/kg significantly increased the percentage of entries into open arms (Fig. 2A; $F(3,42) = 3.096$; $p < 0.05$, ANOVA, Dunnett's test). Similarly, *P. edulis* fo. *flavicarpa* 100 and 300 mg/kg evoked a significant increase in the percentage of entries into open arms (Fig. 2D; $F(4,51) = 4.206$; $p < 0.05$, ANOVA, Dunnett's test). No significant effects in the percentage of time spent into open arms and frequency of entries into closed arms were observed in mice treated with the extracts of these two populations of Passiflora (Fig. 2B, C, E, F; $p > 0.05$).

Open-field test

Considering the cumulative distance traveled, a trend to reduce this behavioral parameter was observed in *P. edulis* fo. *edulis* (1000 mg/kg)-treated mice (Fig. 3A; $F(2,25) = 2.965$; #$p = 0.07$, ANOVA, Dunnett's test). The administration of *P. edulis* fo. *edulis* at 1000 mg/kg significantly reduced the distance traveled at the first 5 min of observation compared to control (Fig. 3B; $F(2,25) = 3.233$; *$p < 0.05$, ANOVA, Dunnett's test). *P. edulis* fo. *flavicarpa* did not

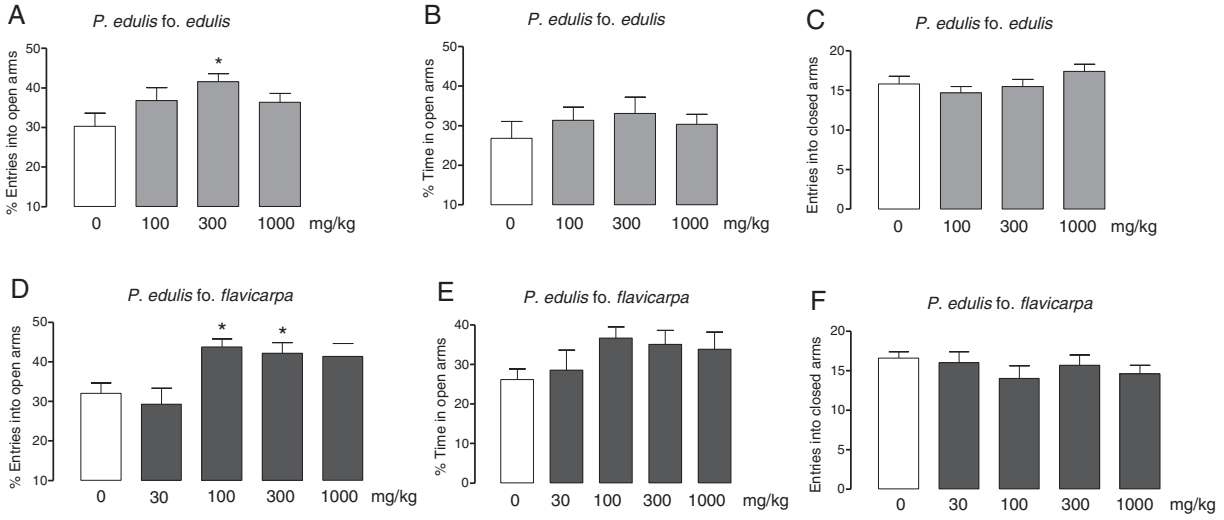

Fig. 2. Effects of the acute administration of *P. edulis* fo. *edulis* (100, 300 and 1000 mg/kg, *p.o.*) and *P. edulis* fo. *flavicarpa* (30, 100, 300 and 1000 mg/kg, *p.o.*) on the percentage of entries into (A, D) and time spent in open arms (B, E), besides on the frequency of entries into enclosed arms (C, F) in the elevated plus maze test. Results are represented as mean ± SEM of 10–15 animals per group. *$p < 0.05$, ANOVA followed by Dunnett's test.

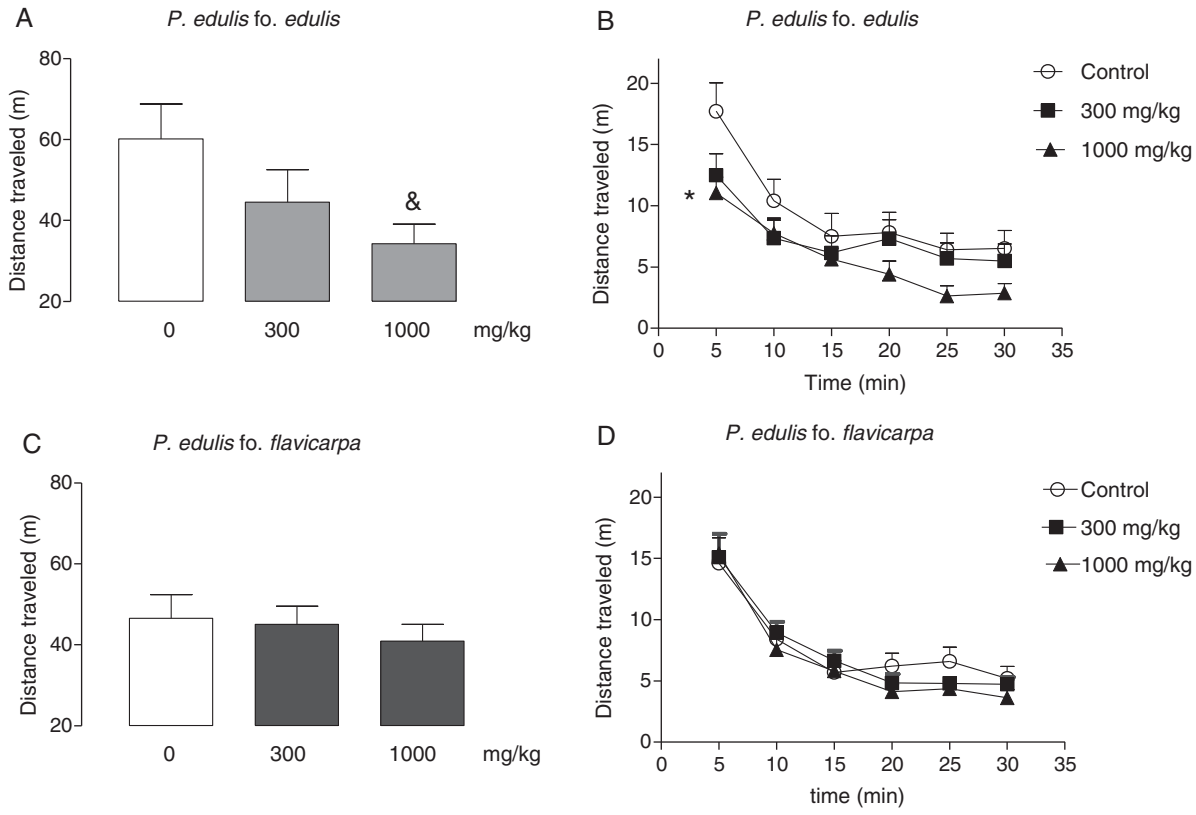

Fig. 3. Effects of the acute administration of *P. edulis* fo. *edulis* (300 and 1000 mg/kg, *p.o.*) and *P. edulis* fo. *flavicarpa* (300 and 1000 mg/kg, *p.o.*) on the cumulative distance traveled during 30 min (A, C) and on the accumulated distance traveled over consecutive 5 min time (B, D) in the open field test. Results are expressed as mean ± SEM of 8–10 animals per group. $^{*}p < 0.05$ vs. control; $^{\&}p = 0.07$ vs. control, ANOVA followed by Dunnett's test.

affect the distance traveled by mice during the 30 min of observation (Fig. 3C and D; $p > 0.05$).

Several studies investigated the effects of *P. edulis* in anxiety. However, experimental results have brought some controversy. Petry et al. (2001) showed that *P. edulis* Sims reduced anxiety in rodents, as well as Coleta et al. (2006) and Barbosa et al. (2008). However, Dhawan et al. (2001) showed that *P. edulis* Sims was devoid of anxiolytic activity. These differences could be due to the fact that distinct subpopulations of *P. edulis* were used. In favor of this view, Li et al. (2011) compared the anxiety effects ethanol leaf extracts of two varieties of *Passiflora edulis* ('*edulis*' and '*flavicarpa*'). They showed that *P. edulis* fo. *flavicarpa* displayed anxiolytic-like activity only at the higher dose tested (*i.e.*, 400 mg/kg). However, *P. edulis* fo. *edulis* was inactive at lower doses, while at 400 mg/kg evoked sedation. Based on these observations, Li et al. (2011) suggested that the biological actions of these subpopulations of *P. edulis* are distinct. In contrast with Li et al., we observed that both varieties of *P. edulis* reduces anxiety behaviors; being *P. edulis* fo. *flavicarpa* more potent than the variety '*edulis*'. Indeed, the hypolocomotor/sedative effects of *P. edulis* fo. e*dulis*, firstly reported by Li et al. (2011), was also herein observed. Differences observed between the present study and Li et al. (2011) can be explained by the distinctions in technical extract preparations (*i.e.*, aqueous and ethanol, respectively), besides the differences in local and collection times of the used *P. edulis* varieties.

Forced swimming test

Student's *t*-test revealed a significant effect for the acute administration of nortriptyline in mice in the forced swimming test. Nortriptyline reduced the time that animals spent immobile in the water compared to controls (saline: 100.16 ± 15.11 s; nortriptyline: 49.28 ± 7.31 s*; $^{*}p < 0.05$, Student's *t*-test). As illustrated in Fig. 4, the administration of the aqueous extract of *P. edulis* fo. *edulis* 300 mg/kg (Fig. 4A; $F(3,81) = 5.028$; $p < 0.05$, ANOVA, Dunnett's test) and *P. edulis* fo. *flavicarpa* 1000 mg/kg (Fig. 4B; $F(2,56) = 3.489$; $p < 0.05$, ANOVA, Dunnett's test) significantly reduced immobility time of mice in the forced swimming test.

Very little literature information supports the antidepressant actions of *Passiflora* species. A study suggested that the antidepressive effects of *Hypericum perforatum* are potentiated when it is combined with *P. incarnata* (Fiebich et al., 2011). Recently, for the first time it was showed that a *Passiflora* extract induces antidepressant-like effects in mice. An ethanol extract of leaves and stems of *P. edulis* (the variety of *P. edulis* was not detailed), 400–500 mg/kg, given orally during seven days, reduced immobility time in the tail suspension and forced swimming tests (Wang et al., 2013). In the same study, cyclopassiflosides IX and XI, compounds observed in large amount in the ethanol extract, were isolated, and these cycloartane triterpenoids were suggested to be mediating these effects (Wang et al., 2013). In our study, both extracts evoked antidepressant actions. However, *P. edulis* fo. *edulis* seemed to be more potent compared to the variety '*flavicarpa*'. These findings are promising and they support an innovative biological action induced by *P. edulis* varieties.

Thiopental-induced sleeping time test

At the doses tested, the treatment with the aqueous extract of *P. edulis* fo. *edulis and P. edulis* fo. *flavicarpa* did not significantly affect neither the latency to sleep nor the sleeping time duration induced by thiopental (Table 2).

Passiflora species has long been used in traditional folk medicine due to its sedative effects. More than 30 years ago, the aqueous extract of *P. edulis* have been reported to prolong barbiturate- and

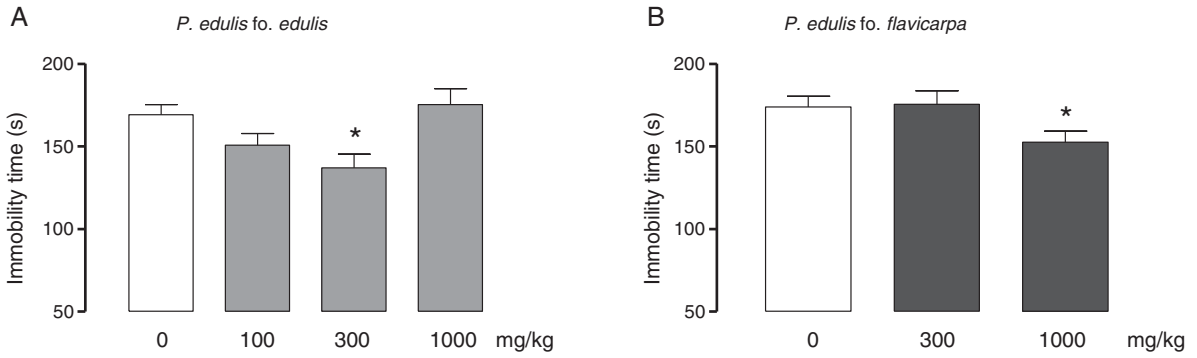

Fig. 4. Effects of the acute administration of *P. edulis* fo. *edulis* (100, 300 and 1000 mg/kg, *p.o.*) (A), and *P. edulis* fo. *flavicarpa* (300 and 1000 mg/kg, *p.o.*) (B) on the immobility time of mice in the forced swimming test. Results are represented mean ± SEM of 16–18 animals per group. $^{*}p < 0.05$, ANOVA followed by Dunnett's test.

Table 2
Effects of acute administration of *Passiflora edulis* fo. *edulis* (PEE; 300 and 1000 mg/kg, *p.o.*) and *P. edulis* fo. *flavicarpa* (PEF; 300 and 1000 mg/kg, *p.o.*) on the latency to sleep and sleeping time duration induced by thiopental.

Extract	Dose	Latency to sleep (s)	Sleeping time duration (s)
Vehicle	–	155.2 ± 8.0	403.5 ± 66.9
PEE	300 mg/kg	163.3 ± 17.3	599.8 ± 167.2
PEE	1000 mg/kg	146.2 ± 27.8	584.2 ± 87.3
Vehicle	–	223.1 ± 17.0	647.7 ± 187.1
PEF	300 mg/kg	174.7 ± 15.3	513.2 ± 117.1
PEF	1000 mg/kg	181.3 ± 14.0	686.0 ± 267.4

Results are expressed as mean ± SEM of 10–12 animals per group.

morphine-induced sleep time in mice, and this extract also partially blocked the amphetamine-induced stimulated effects (Do et al., 1983). Few studies showed that *P. edulis* aqueous extracts reduce spontaneous locomotor activity and prolong sleep in mice (Maluf et al., 1991; Meier, 1995). Sena et al. (2009) showed that the aqueous extract from the pericarp of *P. edulis* fo. *flavicarpa* potentiated the hypnotic effects of ethyl ether in mice. In our study, the treatment with the aqueous extract of *P. edulis* fo. *edulis and P. edulis* fo. *flavicarpa* did not significantly affect thiopental-induced sleeping time. As commented above, a potential sedative effect was only detected for the aqueous extract of *P. edulis* fo. *edulis* in mice at 1000 mg/kg. Possibly, the low sedative effect of our extracts could be due to the part of plant used in our study (which was not stated in those old reports), methods of extract preparation or differences between bioassays; additionally, aspects related to seasonal variation and geographical origin of plants should be taken into account.

Conclusion

The present findings support that both extracts share anxiolytic- and antidepressant-like activities. By contrast, quite distinct phytochemical profile was reported for the aqueous extract of *P. edulis* varieties. In both extracts the major compounds observed were flavonoids *C*-glycosides, suggesting that these biological actions are not due to a specific glycoside. A possible explanation to the biological similarities between *P. edulis* fo. *edulis* and *P. edulis* fo. *flavicarpa* could be on the fact that flavonoids C-glycosides or other similar product of the metabolism of *P. edulis* varieties are promoting these shared biological actions. Finally, both varieties of *P. edulis* could be used as a remedy for anxiety and depression.

Authors' contributions

SMZ and ECG defined the experimental design of this study. ASFSJA, LLSA, TCS and GMC contributed by performing the biological and phytochemical assays. FHR, FAR, LC and EPS contributed to prepare the extracts. ASFSJA, SMZ and ECG drafted the paper, while EPS, VPSR contributed to critical reading of the manuscript. All authors read and approved the final manuscript submission.

Conflicts of interest

The authors declare no conflicts of interest.

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