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Original Article

# Involvement of GABAergic pathway in the sedative activity of apigenin, the main flavonoid from *Passiflora quadrangularis* pericarp

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ABSTRACT

In the current study we showed that oral administration of an aqueous extract of *Passiflora quadrangularis* L., Passifloraceae, pericarp results in a significant prolongation of the sleep duration in mice evaluated in the ethyl ether-induced hypnosis test which indicates sedative effects. Apigenin, the main flavonoid of the extract, induced a similar sedative response when applied alone, at a dose equivalent to that found in the extract, suggesting that apigenin is mediating the sedative effects of *P. quadrangularis* extract. In addition, the sedative effect of apigenin was blocked by pretreatment with the benzodiazepine antagonist flumazenil (1 mg/kg), suggesting an interaction of apigenin with gamma-aminobutyric acid type A ($GABA_A$) receptors. However, apigenin at concentrations 0.1–50 μM failed to enhance GABA-induced currents through $GABA_A$ receptors ($\alpha_1\beta_2\gamma_{2S}$) expressed in *Xenopus* oocytes. Nevertheless, based on our results, we suggest that the *in vivo* sedative effect of the *P. quadrangularis* extract and its main flavonoid apigenin maybe be due to an enhancement of the GABAergic system.



## Introduction

The genus *Passiflora* has the most economical significance among all other genera in the family Passifloraceae. Its species are mainly distributed throughout Latin America. *Passiflora quadrangularis* L. is usually cultivated at altitudes below 2500 m (Killip, 1938). In Brazil, the occurrence of this species is described in the states of Amazonas and Maranhão (Cervi, 1997) and also in Colombia, in the regions of Chocó, Meta, Huila and Santander. Popularly known as "maracujá-açu" in Brazil (Cervi, 1997) and "badea" in Colombia (Hernández and Bernal, 2000), this species is widely consumed in Colombia as juice. The fruits are much bigger than the other fruits of *Passiflora* species showing pale yellowish green color when ripe (Vanderplank, 2000).

To date, only few pharmacological and chemical studies with pericarps of *Passiflora* species is available. In traditional medicine, preparations using leaves of some species are used as sedatives and mild tranquilizers (Lewis and Elvin-Lewis, 1977; Schindler, 1884). In particular, sedative, anxiolytic-like and anticonvulsant effects of leaves extracts from different species of *Passiflora* have been reported (for a review, see Dhawan et al., 2004).

Regarding the chemical composition, most of the studies is also related to leaves extracts and reported the presence of flavonoids (Petry et al., 1998; Ulubelen et al., 1982; Zucolotto et al., 2012) and saponins (Orsini et al., 1985; Reginatto et al., 2001). In this respect, due the lack in the literature about neuropharmacological and chemical data of pericarp from *Passiflora* species and considering that the sedative and anxiolytic-like activities of *P. edulis* var. *flavicarpa* pericarp were previously described (Sena et al., 2009), the aim of the present study was to evaluate the sedative activity of *P. quadrangularis* pericarp aqueous extracts, the correlation of the sedative activity to its flavonoids constituents besides to study the involvement of the gamma-aminobutyric acid system in the action of these compounds.

## Materials and methods

### *Plant material and extraction*

Fruits of *Passiflora quadrangularis* L., Passifloraceae, were collected in Colombia [Neiva, Huila (2°59′55″, −75°18′16″)], identified by Prof. Luis Carlos Jimenez (Instituto Nacional de Ciencias,

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Universidad Nacional de Colombia) and a voucher specimen was deposited in the Herbarium of the Universidad Nacional de Colombia (COL 572634).

Extract for the ethyl ether-induced hypnosis test as well as the HPLC-DAD analyses was obtained as follows. Fresh pericarp was directly extracted by infusion with hot water (90 °C; plant:solvent, 1:3, w/v) for 10 min. The aqueous extracts were subsequently filtered and freeze-dried.

*HPLC-DAD analyses*

The analyses were performed on a PerkinElmer Series 200 high-performance liquid chromatography (HPLC) system equipped with diode array detection (DAD), quaternary pump, online degasser and auto-sampler (injection volume was 20 μl). The separation was performed with a PerkinElmer Brownlee Analytical $C_{18}$ column (250 mm × 4.6 mm i.d.; 5 μm). The flow was maintained at a constant rate of 1.2 ml/min. The mobile phase used consisted of solvent A (acetonitrile) and solvent B (0.5% formic acid in water) in a linear gradient elution (0–20 min– 15% – 35% of A; 20–25 min – isocratic 35% A). The chromatograms were monitored at 340 nm and UV spectra were recorded in the 200–400 nm range. The data were processed with Chromera software® (Version 3.2.0.4847) (PerkinElmer®, Waltham, MA, USA).

Qualitative and quantitative analyses were performed with the extract in a concentration of 5000 μg/ml. For qualitative analyses the reference standards co-eluted with the extract were apigenin (≥95% – Sigma–Aldrich®, USA), isoorientin (≥98% – Extrasynthèse®, France), vitexin-2″-*O*-rhamnoside (≥98% – Sigma–Aldrich®, USA), vitexin-2″-*O*-xyloside (Costa et al., 2013), isovitexin (≥98% – Fluka®, USA) and swertisin (Santos et al., 1996).

The quantitative analyses were developed using the external standard method, being apigenin used as a reference substance to create a calibration curve with six points in a 0.5–40 μg/ml concentration range. All of the standard solutions were analyzed in triplicate, and the average peak areas were measured. The validation of the analytical procedures was performed in accordance with ICH guidelines (2005). In particular, the following parameters were evaluated: specificity, linearity, accuracy, precision (both repeatability and intermediate precision), the limit of quantification (LOQ) and the limit of detection (LOD).

*Animals*

Male adult Swiss mice (3–4 months) weighing 35–50 g were used for behavioral evaluations. Animals were maintained on a 12-h light-dark cycle (lights on at 7 a.m.) at a constant room temperature (23 ± 2 °C). Mice were housed in groups of twenty per plastic cage (30 cm × 37 cm × 16 cm) with food and water provided *ad libitum* except during the experiments. All animals were allowed to adapt to the laboratory conditions for at least one week prior to the behavioral assessments of the study. On the day of the experiment, animals were housed in the experimental room for at least 1 h prior to the start of the testing procedures. Each animal was used only once. Experiments were conducted in accordance with national and international standards of animal welfare (Brazilian Law #11,794, 10/08/2008; NIH publication #85-23, revised in 1996) and approved by the local Committee for Animal Care in Research (#23080.044085/2009-37/CEUA/UFSC). All efforts were made to minimize the number of animals used and their suffering.

*Drugs and treatments*

Mice were orally treated (*p.o.*) through an intragastric cannula, in a constant volume of 0.1 ml/10 g of animal weight, with *P. quadrangularis* aqueous extract or apigenin, both dissolved in distilled water. In the ethyl ether-induced hypnosis test, the extract was tested at the doses of 100, 300 and 600 mg/kg and apigenin was tested at the doses of 0.1, 0.3, 0.6 and 1.0 mg/kg. An equal volume of distilled water (vehicle, *p.o.*) treated mice of the control groups. Diazepam (DZP – Hoffman-La Roche®, Switzerland) was used as positive drug control, dissolved in water with 10% of propylene glycol, and administered *p.o.* at a dose of 1 mg/kg in the sedative test (Sena et al., 2009). Animals were individually tested 1 h after their treatments with the extract or apigenin and 30 min after their treatment with DZP.

In order to assess the involvement of the GABAergic system, a group of animals were pretreated (*i.p.*) with physiological saline solution (Quimibrás Indústrias Químicas S.A., Brazil) or flumazenil (Flumazen®, União Química Lab., Brazil – 1 mg/kg – Carvalho et al., 2011) 15 min prior the oral treatment with the test compound apigenin (0.6 mg/kg), the reference drug DZP (1 mg/kg) or distilled water (control group).

*Ethyl ether-induced hypnosis test*

Animals were placed in an ethyl ether-saturated glass cage (6 ml, 13 min of saturation, 30 cm × 20 cm glass cage) after treatments. A stopwatch was used to record the duration of hypnosis in seconds (s). The hypnosis time was measured by the loss of the righting reflex, and the recovery of this reflex was considered as the end-point of the hypnosis (Vieira, 2001).

*Voltage clamp analysis*

Preparation of stages V–VI oocytes from *Xenopus laevis* and synthesis of capped off run-off poly(A+) cRNA transcripts from linearized cDNA templates (pCMV vector) were performed as previously described (Khom et al., 2006). Briefly, female *Xenopus Laevis* frogs (Nasco, Fort Atkinson, USA) were anaesthetized by exposing them for 15 min to a 0.2% solution of MS-222 (methane sulfonate salt of 3-aminobenzoic acid ethyl ester; Sigma, Vienna, Austria) before surgically removing parts of the ovaries. Follicle membranes from isolated oocytes were digested with 2 mg/ml collagenase solution (Type 1A). Selected stages V–VI oocytes were injected with about 10–50 nl of DEPC-treated water (diethyl pyrocarbonate) containing the cRNAs at a concentration of approximately 300–3000 pg/nl. The amount of cRNA was determined by means of a NanoDrop ND-1000 (Kisker Biotech, Steinfurt, Germany). To ensure expression of the gamma-subunit, cRNAs for expression of $\alpha_1\beta_2\gamma_{2s}$ receptors were mixed in a ratio of 1:1:10, respectively. After injection, oocytes were stored at 18 ± 8 °C for 24–48 h in ND96 solution containing penicillin G (10,000 IU/100 ml) and streptomycin (10 mg/100 ml). Electrophysiological experiments on $GABA_A$ receptors were performed using the two-microelectrode-voltage-clamp method at a holding potential of −70 mV, making use of a TURBO TEC 01 C amplifier (npi electronic, Tamm, Germany) and an Axon Digidata 1322A interface (Molecular Devices, Sunnyvale, CA). Data acquisition was done using pCLAMP v.9.2. The bath solution contained 90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2.6H_2O$, 1 mM $CaCl_2$ and 5 mM HEPES (pH 7.4). Microelectrodes were filled with 3 M KCl.

GABA and apigenin were applied by means of a fast perfusion system (Screening Tool, npi electronic, Tamm, Germany) (Baburin et al., 2006) to study the GABA-induced chloride current ($I_{GABA}$) modulation. To elicit $I_{GABA}$, the chamber was perfused with 120 μl of GABA containing solution at a volume rate between 300 and 1000 ml/s. The $I_{GABA}$ rise time ranged from 100 to 250 ms (Khom et al., 2006). To exclude voltage-clamp errors, oocytes with maximal current amplitudes >3 mA were discarded.

Potentiation of the $I_{GABA}$ in percent was defined according to the formula: $I_{GABA}\ (\%) = [I_{(GABA\ +\ apigenin)}/I_{GABA} - 1] \times 100$, where $I_{(GABA+apigenin)}$ is the current response in the presence of different

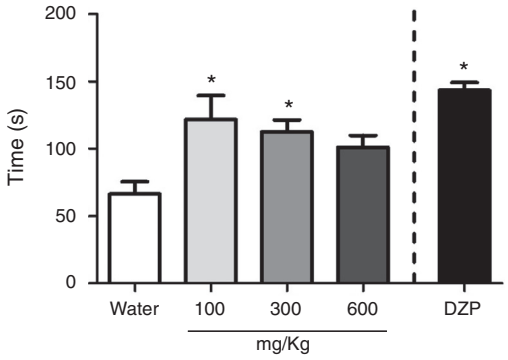


**Fig. 1.** Effects of the oral treatment with the aqueous extract from the pericarp of *P. quadrangularis* (100, 300 and 600 mg/kg) on the duration of the hypnosis induced by ethyl ether in mice. Diazepam (DZP) (1 mg/kg, *p.o.*) was used as control drug. $n = 7–8$ animals/group. Data are expressed as mean ± S.E.M. $^{*}p \leq 0.05$ compared with the control group. Comparisons were accomplished with one-way ANOVA followed by Dunnett's test or Student's *t* test (DZP group × control group).

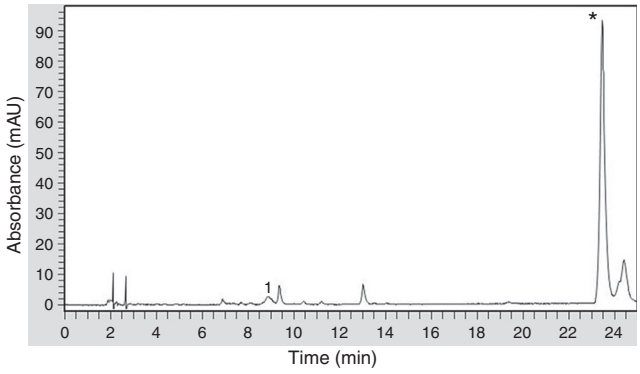


**Fig. 2.** Chromatogram of the aqueous extract from *P. quadrangularis* pericarp (5000 μg/ml) at 340 nm. Co-elution with standards showed the presence of vitexin-2″-*O*-xyloside (1) and apigenin (*). See subsection HPLC-DAD analysis in Materials and Methods section for the description of the chromatographic conditions.

concentrations of apigenin, and $I_{GABA}$ is the control GABA-induced chloride current.

### *Statistical analysis*

Results of the *in vivo* experiments are expressed as means ± S.E.M. Data of the experiments without flumazenil pre-treatment were analyzed by Student's *t* test (positive control groups X control groups) or one-way analysis of variance (ANOVA) followed by Dunnett's test. In flumazenil pretreatment protocol data were analyzed by two-way ANOVA followed by Newman–Keuls' test. All of the statistical analyses were performed using GraphPad Prism® 5.0 (La Jolla, CA, USA) and/or Statistica Statsoft® 7.0 (Tulsa, OK, USA). Differences were considered to be statistically significant when $p \leq 0.05$.

For *in vitro* experiments, Origin Software® (OriginLab Corporation) was used. Responses were graphed as mean ± S.E.M. from at least three oocytes out of ≥2 different batches. Statistical significance was calculated using one-way ANOVA followed by Tukey's test with a confidence interval of $p \leq 0.05$.

## Results and discussion

In the current study, we evaluated the sedative activity of the aqueous extract obtained from pericarps of *P. quadrangularis* by means of ethyl ether-induced hypnosis test. This test allows the assessment of sedative effect without introducing possible pharmacokinetic biases caused by hepatic metabolism of the sleep inductor, such as in the case of pentobarbital-induced hypnosis test (Duarte et al., 2007; Carvalho et al., 2011).

### *The ethyl ether-induced hypnosis test of Passiflora extract*

As depicted in Fig. 1, the pericarp extract of *P. quadrangularis* increased the hypnosis duration (100 and 300 mg/kg – [$F_{3.27} = 3.859$, $p = 0.0203$]), an effect similar to DZP. The sedative activity of *P. quadrangularis* pericarp extract was not previously reported. In the literature was found just one neuropharmacological study with this species describing the anxiolytic-like effect of the hydroethanolic extract from leaves in rats evaluated in the elevated plus-maze test (Castro et al., 2007). At higher doses (600 mg/kg), however, the extract did not show a sedative activity. The reason for this finding should be due to the fact that at higher doses all chemical compounds present in this extract could be present at higher amount and the lack of response may be attributed to the competition between several compounds of the extract acting in different receptors, even excitatory ones.

### *The qualitative analysis of the flavonoid constituents in the extract*

Regarding the qualitative analysis, most studies are indicating flavonoids as the main chemical constituents responsible for the CNS activities of *Passiflora* species (Petry et al., 2001; Li et al., 2011; Sena et al., 2009). Therefore, the presence of flavonoid constituents was investigated in *P. quadrangularis* pericarp extract by co-elution with apigenin, isoorientin, vitexin-2″-*O*-rhamnoside, vitexin-2″-*O*-xyloside, isovitexin and swertisin standards. Previous studies of our research group identified the flavonoid vitexin-2″-*O*-xyloside as the major compound in the leaves extract of this plant species (Costa et al., 2013). Nevertheless, the qualitative HPLC-DAD analysis of the pericarp extract in co-elution with reference standards showed that the main flavonoid is not a glycoside, but an aglycone identified as apigenin (Fig. 2). The presence of other minor flavonoids was visible in the chromatogram but their contents were very low and could not be isolated. The co-elution procedures employed allowed the identification of one of them as the same glycoside found in the leaves, *i.e.* vitexin-2″-*O*-xyloside, and excluded the presence of isoorientin, vitexin-2″-*O*-rahmnoside, isovitexin and swertisin (Fig. 2). Therefore, as apigenin is present in a much higher amount, quantitative analysis was focused in this main flavonoid.

### *The quantitative analysis of apigenin in the extract*

In order to quantify the content of apigenin in the extract, a methodology was developed and validated. The parameters specificity, linearity, accuracy, precision, limit of quantification (LOQ) and limit of detection (LOD) were evaluated (Tables 1 and 2). The quantification demonstrated a good linear relationship between peak area and concentration ($r^2 > 0.999$). LOQ and LOD were defined by the relative standard deviation (RSD > 5%) and by a signal:noise ratio of 3:1, respectively. The RSD values for repeatability and intermediate precision ranged from 0.5 to 1.9%. The recovery of the spiked samples ranged from 99 to 103%, showing that the analytical approach used in this study is repeatable and accurate.

Since the procedures of the quantitative analysis were accurate allowing the correct quantification of apigenin in the extract, they were employed and this flavonoid was found as 2.449 ± 0.004 mg/g of *P. quadrangularis* pericarp extract.

**Table 1**
Validation data for apigenin quantification by HPLC-DAD.

| Flavonoid | Linearity range (μg/ml) | Calibration equation[a] | Correlation factor ($r^2$) | LOD[b] (μg/ml) | LOQ[b] (μg/ml) |
|---|---|---|---|---|---|
| Apigenin | 0.5–40 | $y = 112834x - 23037$ | 0.9996 | 0.0625 | 0.25 |

[a] Six data points ($n = 3$).
[b] LOQ, limit of quantification; LOD, limit of detection.

**Table 2**
Apigenin quantification in *Passiflora* species as evaluated by HPLC-DAD.

| Flavonoid | Repeatability[a] | | Intermediate precision[a] | | Recovery[a,b] | |
|---|---|---|---|---|---|---|
| | Concentration (μg/ml) | RSD (%) | Concentration (μg/ml) | RSD (%) | *P. quadrangularis* (5000 μg/ml) | |
| | | | | | Mean (%) | RSD (%) |
| Apigenin | 1 | 1.9 | 10 | 0.5 | 103.7 | 0.1 |
| | 10 | 0.7 | | | | |
| | 40 | 1.1 | | | | |

RSD, relative standard deviation.
[a] Limits: RSD: <5%.
[b] Spiked samples with apigenin standard solution at 5 μg/ml.

*Apigenin in the ethyl ether-induced hypnosis test*

Apigenin was evaluated in the ethyl ether-induced hypnosis test at doses of 0.1, 0.3, 0.6 and 1.0 mg/kg. These doses were selected considering the approximated quantity of apigenin present in the active doses of the extract, in view of the quantification result for this compound. Thus, for the doses 100 and 300 mg/kg of *P. quadrangularis* pericarp extract, the corresponding quantities of apigenin are 0.24 and 0.73 mg/kg, respectively.

Among all the administered doses of apigenin, 0.6 mg/kg demonstrated a sedative effect ($F_{4,35} = 2.657$, $p = 0.0490$), resulting in a significant increase in the sleeping duration (Fig. 3).

The results obtained suggest a positive relationship between apigenin levels and the sedative effect of the extract. This assumption is supported by other authors who demonstrate that apigenin at different doses possesses sedative effect. For example, Avallone et al. (2000) described this effect when apigenin (*i.p.*) was evaluated by spontaneous locomotor behavior in rats. Moreover, by telemetry tests, Chow et al. (2011) demonstrated that apigenin (*p.o.*) promoted sedative effect in C57BL/6J mice.

On the other side, when apigenin was administered in the doses of 0.1 and 0.3 mg/kg, *i.e.*, the approximated amount of apigenin in 100 mg/kg of *P. quadrangularis* pericarp extract, this flavonoid did not show any sedative activity (Fig. 3), showing a U inverted dose-effect curve. Some findings indicate that, as occurs in our results, the hormesis model could predicts responses in the low doses zone very well in biological tests (Calabrese, 2009). This fact indicates that the effect observed for pericarp extract is not due only to the content of apigenin. Actually, this compound can be the main responsible, but probably there is also a significant contribution of other compounds.

*Studying a potential involvement of the GABAergic system in the sedative activity of apigenin*

Losi et al. (2004) reported an interaction of apigenin with the GABAergic and glutamatergic neurotransmission in cultured cortical neurons. These neurotransmitter systems play a key role in controlling the excitability of the brain and are thus targeted by many psychoactive substances as well as clinically used drugs (Mihic and Harris, 2011). In addition, Lolli et al. (2007) showed that methanol and hydromethanol extracts from *P. actinia* exerts anxiolytic-like and sedative effects in mice after oral administration. Authors also show that previous administration of flumazenil in the elevated plus maze test indicate that the anxiolytic-like activity is related to the influence of the GABAergic activation (Lolli et al., 2007) pointing that this pathway can be modulate by *Passiflora* extracts.

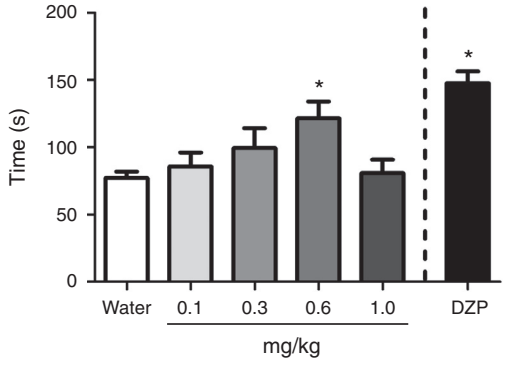


**Fig. 3.** Effects of the oral treatment with the flavonoid apigenin (0.1, 0.3, 0.6, and 1.0 mg/kg) on the duration of ethyl ether-induced hypnosis in mice. Diazepam (DZP) (1 mg/kg, *p.o.*) was used as a standard drug. $n = 8$ animals/group. Data are expressed in terms of means ± S.E.M. $^{*}p \leq 0.05$ compared with the control group. Comparisons were accomplished with one-way ANOVA followed by Dunnett's test or Student's *t* test (DZP group × control group).

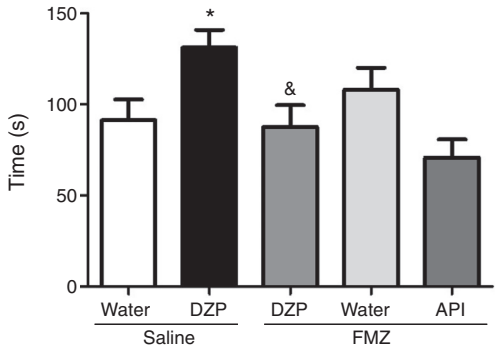


**Fig. 4.** Effects of the oral treatment of apigenin (API – 0.6 mg/kg), diazepam (DZP – 1.0 mg/kg) and water in animals pre-treated (*i.p.*) with flumazenil (FMZ – 1.0 mg/kg) or saline on the duration of hypnosis induced by ethyl ether in mice. DZP was used as control drug. $n = 7$–8 animals/group. Data are expressed as mean ± S.E.M. $^{*,\&}p \leq 0.05$ compared with: saline/water and saline/DZP groups, respectively (two-way ANOVA test followed by Newman–Keuls' test).

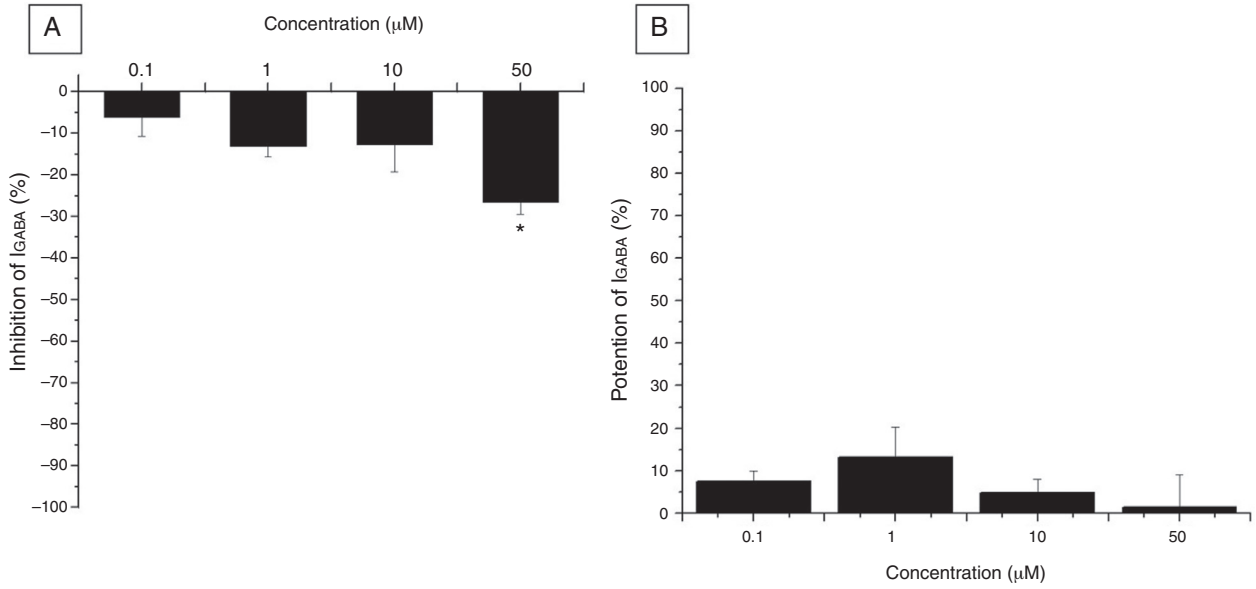


**Fig. 5.** Modulation of $I_{GABA}$ through $GABA_A$ receptors ($\alpha_1\beta_2\gamma_{2s}$) at GABA concentrations eliciting 3–7% of the maximal GABA response ($EC_{3-7}$) by apigenin (A) and luteolin (B) at the indicated concentrations. Each bar represents the mean ± S.E.M. from at least 3 different oocytes of two different oocyte batches. Statistical significance ($p < 0.05$, one-way ANOVA followed by Tukey's test) is indicated by (*).

As depicted in Fig. 3, 0.6 mg/kg of apigenin increased sedation in mice in the ethyl ether-induced hypnosis test. This sedative effect was blocked by application of flumazenil at a dose of 1.0 mg/kg (Fig. 4). Since flumazenil is a benzodiazepine antagonist, this result indicates that apigenin might interact with the benzodiazepine-binding site of $GABA_A$ receptors to promote its sedative effect.

We have therefore expressed $GABA_A$ receptors composed of $\alpha_1$, $\beta_2$ and $\gamma_{2S}$ subunits in *Xenopus laevis* oocytes and studied a potential modulation of GABA-induced currents ($I_{GABA}$) by apigenin and luteolin making use of the 2-microelectrode voltage-clamp technique and a fast perfusion system.

However, as illustrated in Fig. 5A, apigenin failed to enhance $I_{GABA}$ through this receptor subtype at all tested concentrations (0.1–50 μM). Actually, as not expected, it inhibited $I_{GABA}$ at the higher concentration, a result that has further investigated.

Taking into account that the sedative effect of apigenin was observed after oral application it is tempting to speculate that not only apigenin, but any of the metabolites formed after gastrointestinal and/or hepatic metabolism might *in vivo* modulate $GABA_A$ receptors. Gradolatto et al. (2004) reported that at least seven compounds are originated from hepatic metabolism of apigenin with luteolin being identified as the main metabolite. Therefore, we have also analyzed the modulation of $I_{GABA}$ through $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors expressed in *Xenopus* oocytes by luteolin. As illustrated in Fig. 5B, luteolin also did not elicited a significant enhancement of $I_{GABA}$. Thus, this result could not explain the sedative effect of apigenin observed after oral treatment of mice, but it shows the importance of better understand the metabolism of *in vivo* administered compounds.

Other possible explanation for our *in vivo* findings might be that apigenin can be acting as a second order modulator. Previous studies showed that apigenin did not modulate $I_{GABA}$ when applied alone to the receptor, but it showed strong effects when co-applied with another $GABA_A$ receptor modulator such as diazepam (Campbell et al., 2004).

In addition, the influence of other $GABA_A$ receptor subtypes in the sedative activity of apigenin could not be discarded. In this sense, it was previously showed that $\alpha_5$ subunit of $GABA_A$ receptors exerts influence of the sedative activity (Savić et al., 2008).

In conclusion, this study provides evidences that the aqueous extract from the pericarp of *P. quadrangularis* induces sedation in mice. In addition, apigenin, the main flavonoid found in the extract, also induced sedative effect, suggesting that this flavonoid plays an important role in the sedative effect of the extract. Furthermore, a potential involvement of $GABA_A$ receptors in mediating the sedative effect of apigenin was demonstrated *in vivo* but not *in vitro* and the molecular mechanism remains to be elucidated.

## Conflict of interest

All authors have none to declare.

## Authors' contributions

ACG (PhD student) contributed in running all the laboratory work, analysis of the data and drafted the paper. GMC contributed in phytochemical analyses. LC and FAR contributed in collection of the plant, confection of voucher specimen, supervision of laboratory work and critical reading of the manuscript. FHR supervised the laboratory work and contributed to critical reading of the manuscript. TCML and EPS designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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## References


Avallone, R., Zanoli, P., Puia, G., Kleinschnitz, M., Schreier, P., Baraldi, M., 2000. Pharmacological profile of apigenin a flavonoid isolated from *Matricaria chamomilla*. Biochem. Pharmacol. 59, 1387–1394.

Baburin, I., Beyl, S., Hering, S., 2006. Automated fast perfusion of *Xenopus* oocytes for drug screening. Pflugers. Arch. 453, 117–123.

Calabrese, E.J., 2009. Getting the dose–response wrong: why hormesis became marginalized and the threshold model accepted. Arch. Toxicol. 83, 227–247.

Campbell, E.L., Chebib, M., Johnston, G.A.R., 2004. The dietary flavonoids apigenin and (−)-epigallocatechin gallate enhance the positive modulation by diazepam of the activation by GABA of recombinant $GABA_A$ receptors. Biochem. Pharmacol. 68, 1631–1638.

Carvalho, R.S.M., Duarte, F.S., de Lima, T.C.M., 2011. Involvement of GABAergic non-benzodiazepine sites in the anxiolytic-like and sedative effects of the flavonoid baicalein in mice. Behav. Brain Res. 221, 75–82.

Castro, P.C.F., Hoshino, A., Silva, J.C., Mendes, F.R., 2007. Possible anxiolytic effect of two extracts of *Passiflora quadrangularis* L in experimental models. Phytother. Res. 21, 481–484.

Cervi, A.C., 1997. Passifloraceae do Brasil: Estudo do gênero *Passiflora* L. subgênero *Passiflora*. Fontqueria 45, 1–92.

Chow, N.K., Fretz, M., Hamburger, M., Butterweck, V., 2011. Telemetry as a tool to measure sedative effects of valerian root extract and its single constituents in mice. Planta Med. 77, 795–803.

Costa, G.M., Gazola, A.C., Madóglio, F.A., Zucolotto, S.M., Reginatto, F.H., Castellanos, L., Ramos, F.A., Duque, C., Schenkel, E.P., 2013. Vitexin derivatives as chemical markers in the differentiation of the closely related species *Passiflora alata* Curtis. and *Passiflora quadrangularis* Linn. J. Liq. Chromatogr. Relat. Technol. 36, 1697–1907.

Dhawan, K., Dhawan, S., Sharma, A., 2004. Passiflora: a review update. J. Ethnopharmacol. 94, 1–23.

Duarte, F.S., Duzzioni, M., Mendes, B.G., Pizzolatti, M.G., De Lima, T.C.M., 2007. Participation of dihydrostyryl-2-pyrones and styryl-2-pyrones in the central effects of *Polygala sabulosa* (Polygalaceae) a folk medicine topical anesthetic. Pharmacol. Biochem. Behav. 86, 150–161.

Gradolatto, A., Canivenc-Lavier, M.C., Basly, J.P., Siess, M.H., Teyssier, C., 2004. Metabolism of apigenin by rat liver phase I and phase II enzymes and by isolated perfused rat liver. Drug Metab. Dispos. 32, 58–65.

Hernández, A., Bernal, R., 2000. Lista de especies de Passifloraceae de Colombia. Biota Colombiana 1, 320–335.

ICH, 2005. Validation of Analytical Procedures: Text and Methodology – Q2(R1). International Conference on Harmonization, London.

Khom, S., Baburin, I., Timin, E.N., Hohaus, A., Sieghart, W., Hering, S., 2006. Pharmacological properties of $GABA_A$ receptors containing gamma1 subunits. Mol. Pharmacol. 69, 640–649.

Killip, E.P., 1938. The American Species of Passifloraceae. v.XIX. Part II. Botanical Series, Field Museum of Natural History, Chicago.

Lewis, W.H., Elvin-Lewis, M.P.F., 1977. Medical Botany: Plants Affecting Man's Health. John Wiley & Sons, New York.

Li, H., Zhou, P., Yang, Q., Shen, Y., Deng, J., Li, L., Zhao, D., 2011. Comparative studies on anxiolytic activities and flavonoid compositions of *Passiflora edulis 'edulis'* and *Passiflora edulis 'flavicarpa'*. J. Ethnopharmacol. 133, 1085–1090.

Lolli, L.F., Sato, C.M., Romanini, C.V., Villas-Boas, L.D.B., Santos, C.A.M., Oliveira, R.M.W., 2007. Possible involvement of $GABA_A$-benzodiazepine receptor in the anxiolytic-like effect induced by *Passiflora actinia* extracts in mice. J. Ethnopharmacol. 111, 308–314.

Losi, G., Puia, G., Garzon, G., Vuono, M.C., Baraldi, M., 2004. Apigenin modulates GABAergic and glutamatergic transmission in cultured cortical neurons. Eur. J. Pharmacol. 502, 41–46.

Mihic, S.J., Harris, R.A., 2011. Hypnotics and sedatives. In: Brunton, L.L., Chabner, B.A., Knollmann, B. (Eds.), Goodman & Gilman's: The Pharmacological Basis of Therapeutics. McGraw Hill Medical, New York, pp. 457–480.

Orsini, F., Pelizzoni, F., Verotta, L., 1985. Quadranguloside, a cycloartane triterpene glycoside from *Passiflora quadrangularis*. Phytochemistry 25, 191–193.

Petry, R.D., De Souza, K.C.B., Basani, V.L., Petrovick, P.R., González-Ortega, G., 1998. Doseamento do teor de flavonoides totais em extratos hidroalcoolicos de *Passiflora alata* Dryander (maracujá). Rev. Bras. Farm. 79, 7–10.

Petry, R.D., Reginatto, F., De-Paris, F., Gosmann, G., Salgueiro, J.B., Quevedo, J., Kapczinski, F., Ortega, G.G., Schenkel, E.P., 2001. Comparative pharmacological study of hydroethanol extracts of *Passiflora alata* and *Passiflora edulis* leaves. Phytother. Res. 15, 162–164.

Reginatto, F.H., Kauffmann, C., Schripsema, J., Guillaume, D., Gosmann, G., Schenkel, E.P., 2001. Steroidal and triterpenoidal glycosides from *Passiflora alata*. J. Braz. Chem. Soc. 12, 32–36.

Santos, R.I., Santos, M.A., Schenkel, E.P., 1996. Analysis of the plant drug *Wilbrandia ebracteata* (Cogn) Cogn. Int. J. Pharmacogn. 34, 300–302.

Savić, M.M., Huang, S., Furtmüller, R., Clayton, T., Huck, S., Obradović, D.I., Ugrešić, N.D., Sieghart, W., Bokonjić, D.R., Cook, J.M., 2008. Are $GABA_A$ receptors containing $\alpha5$ subunits contributing to the sedative properties of benzodiazepine site agonists? Neuropsychopharmacology 33, 332–339.

Schindler, S.S., 1884. Catalogue of Brazilian Medicinal Plants: Giving A Detailed Account of Their Therapeutic Properties, Uses and Doses. Typ. E Lith de Moreira, Maximino & Co, Rio de Janeiro.

Sena, L.M., Zucolotto, S.M., Reginatto, F.H., Schenkel, E.P., de Lima, T.C.M., 2009. Neuropharmacological activity of the pericarp of *Passiflora edulis flavicarpa* Degener: putative involvement of *C*-glycosylflavonoids. Exp. Biol. Med. 234, 967–975.

Ulubelen, A., Topcu, G., Mabry, T.J., Dellamonica, G., Chopin, J., 1982. C-glycosylflavonoids from *Passiflora foetida* var hispida and *P. foetida* var. *hibicifolia*. J. Nat. Prod. 45, 103.

Vanderplank, J., 2000. Passion flowers. The MIT Press, Cambridge.

Vieira, R.A., (MSc thesis) 2001. Avaliação da possível atividade central da *Stachytarpheta cayenensis* (gervão-roxo). Programa de Pós-graduação em Farmacologia, Universidade Federal de Santa Catarina, Florianópolis, 121 pp.

Zucolotto, S.M., Fagundes, C., Reginatto, F.H., Ramos, F.A., Castellanos, L., Duque, C., Schenkel, E.P., 2012. Analysis of *C*-glycosyl flavonoids from South American *Passiflora* species by HPLC-DAD and HPLC-MS. Phytochem. Anal. 23, 232–239.