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Optimization of flavonoid extraction from *Passiflora quadrangularis* leaves with sedative activity and evaluation of its stability under stress conditions

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ABSTRACT

Passiflora species have been widely used in folk medicine as tranquilizers, and previous pharmacological studies have reported sedative activity for *P. quadrangularis* L., Passifloraceae, leaf extracts. The aim of this work was to contribute to the standardization of *P. quadrangularis* leaf extract with sedative activity. For this purpose, the extraction of total flavonoids was optimized, evaluating variables such as drug-solvent ratio, extraction solvents and extraction time, using Response Surface Methodology. The stability of total and individual flavonoids on the optimized extract of *P. quadrangularis* leaves under stress conditions was also evaluated. Sedative activity was verified by the ethyl ether-induced hypnosis test in Swiss ICR mice. Based on the results, the highest concentration of total flavonoids was obtained at a drug-solvent ratio of 1:15 (w:v), extraction solvent EtOH:H_2O (1:1, v/v) and percolation time of 48 h. Regarding stability under stress conditions, it was found that the flavonoids from the optimized extract are photostable, and practically stable under neutral hydrolysis and oxidation, but labile by acid and basic hydrolysis, with the main degradation products being identified. Finally, it was demonstrated that the optimized extract improves the sedative effect when compared to previously evaluated extract in the ethyl ether-induced hypnosis test.

Introduction

Passiflora is the largest and most important genus of the family Passifloraceae. In South America, several species of *Passiflora* are widely distributed, being popularly known as 'maracujá', 'curuba', 'granadilla', among others (Ulmer and MacDougal, 2004). Several of them have commercial value for their edible fruits, as juices, and use in various food products.

In Colombia, *Passiflora quadrangularis* L. is popularly known as 'badea' and its fruit pulp is widely used for juice preparations, with production of more than 1000 tons of fruits per year (Agronet, 2018). In folk medicine, leaves of *P. quadrangularis* are used as sedatives and mild tranquilizers (Dhawan et al., 2004). In this aspect, Castro and co-workers (2007) have reported anxiolytic-like effect of hydroalcoholic extracts of leaves of *P. quadrangularis* in Wistar rats by the elevated plus maze, open field and hole-board tests. More recently, Gazola and co-workers have demonstrated the sedative activity of aqueous extract of leaves of *P. quadrangularis* by the ethyl-ether-induced sleeping model in ICR Swiss mice. The authors also suggest that the *C*-glycosylflavonoids present in the aqueous extract are responsible for this pharmacological activity (Gazola et al., 2018).

Since *P. quadrangularis* leaves are considered a by-product of its fruit harvest, this work aimed to optimize flavonoid extraction from *P. quadrangularis* leaves, using Response Surface Methodology (RSM), which has been successfully employed for the extraction of different metabolites in several plants (Liyana-Pathirana and Shahidia, 2005; Koocheki et al., 2009; Stroescu et al., 2013). It also evaluated the sedative activity and the stability of the optimized extract under stress conditions. This information contributes to knowledge of the bioactivity of *P. quadrangularis* leaves, proposing it as a potential active ingredient of herbal medicines and contributing to the value of this crop species as a source of high value-added compounds.

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Materials and methods

Chemicals

Ethanol, acetonitrile and sodium hydroxide were purchased from Merck®. Methanol and hydrochloric acid were obtained from J.T Baker® and Mallinkrodt® respectively. All solvents were of analytical reagent grade, with the exception of acetonitrile and methanol, which were of HPLC grade. Water was purified by a Milli-Q system. The standards vitexin, isovitexin, orientin and isoorientin were obtained from Sigma-Aldrich®.

Plant material

Passiflora quadrangularis L., Passifloraceae, leaves were collected in the city of Rivera, Huila, Colombia (Longitude: 75° 13′ 94.938; Latitude: 2° 45′ 41.899). A voucher specimen was deposited at the Colombian National Herbarium (COL 589241). The leaves were air-dried at 50 °C for 72 h, powdered, and stored at room temperature.

Experimental design for optimization of the extraction process

The optimum conditions for total flavonoid (TF) extraction were determined by using Response Surface Methodology (RSM) (SAS/STAT(R) 9.2 software). The central composite design (CCD) was used, and the treatments were established through a fractional factorial design (3^{3-1}). Percolation was chosen as the extraction method for the optimization of the extraction process, and the particle size of the dried plant material used to make the extractions was moderately coarse (WHO, 2011). Three factors at three levels were evaluated, to determine their influence on the final concentration of TF (drug:solvent ratio, at 1:10; 1:15 and 1:20, w/v; ethanol concentration, at 25, 50 and 75% and extraction times of 24, 48 and 72 h). The ethanol of the extracts was evaporated under reduced pressure at 40 °C. The remaining water was eliminated by freeze-drying and the dry extracts obtained were stored at 4 °C until HPLC quantification.

Aqueous extract from *P. quadrangularis* leaves was obtained by infusion according to previous works (Costa et al., 2013). Briefly, dried and milled leaves were directly extracted by infusion with boiled water (1:10 w/v, for 10 min). The aqueous extracts were subsequently filtered and freeze-dried.

Determination of the total flavonoids (TF) content

The TF of *P. quadrangularis* and its degradation products were quantified by an HPLC-DAD method adapted from previous investigations by our group (Costa et al., 2013). A Shimadzu Liquid Chromatography System equipped with a DGU-20 degasser, LC-6AD binary pumps, SPD M20-A DAD detector, CTO-20A column oven and SIL-20A HT autosampler was used for these analyses. The data were processed using Labsolution software®. The experiments were carried out on a reverse-phase Phenomenex Luna C18 column (250 mm × 4.6 mm i.d. 5 μm), maintained at 30 ± 1 °C. The mobile phase consisted of a liner gradient of phase A (water:acetonitrile:acetic acid, 90:10:1 v/v/v) and phase B (acetonitrile:water:acetic acid, 90:10:1 v/v/v) in two steps: 11% B (0–5 min), then 11–15% B (5–20 min). The flow rate was kept constant at 1 ml/min. The mobile phase was prepared daily and degassed by sonication before use. The chromatogram was monitored at 340 nm, and UV spectra of individual peaks were recorded in the range of 200–450 nm. The samples were prepared by dissolving the lyophilized crude extracts in methanol:water (1:1, v/v) and filtering through a 0.45 μm PVDA membrane (Millipore®) before injection. The concentration of the sample extracts was 1000 μg/ml. Vitexin was employed as standard and TF were quantified by the sum of all the chromatographic signals identified as flavonoids by their UV-DAD spectra, being expressed as mg-eq vitexin/g dry extract.

Validation of the analytical methodology was performed according to the guidelines of the International Conference on Harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use. (ICH, 2005), which establish the evaluation of parameters: linearity, selectivity, precision (repeatability and intermediate precision), accuracy, limit of quantitation (LOQ) and limit of detection (LOD).

Stability study under stress conditions

Stability studies under neutral, acid and alkali hydrolysis, oxidative and photolytic conditions were performed by adaptation to the methodology reported by Singh and Bakshi (2000). Briefly, 10 mg of the extract was subject to different hydrolytic, oxidative and photolytic conditions. The condition at which the TF content decreases by between 20% and 80% was used to classify the extract as extremely labile, very labile, labile, stable, very stable or practically stable. In order to better understand the extract stability, besides TF quantification, the variation on each *C*-glycosylflavonoid and degradation product was determined. The content of individual compounds was expressed as relative amount (%), according to Equation 1.

$$\text{Relative amount}\,(\%) = \frac{\text{Area of the peak of the individual flavonoid}}{\text{All flavonoid peaks total area}} \times 100$$

Evaluation of sedative activity

The sedative activity of the extract was evaluated in the ethyl ether-induced hypnosis test following the methodology reported by Gazola and co-workers (2018). Male adult Swiss ICR mice (age: 10–12 weeks and 30 g weight approximately) were used. The animals were supplied by the animal house of the Department of Pharmacy of the Universidad Nacional de Colombia, and were kept under constant temperature conditions (22 °C ± 1), 12 h light/dark cycles, with food and water *ad libitum*. The assays were carried out in accordance with the international and local ethical guidelines on the use and care of laboratory animals, and with approval of the local Research Ethics Committee (Act 02/2016 Faculty of Science).

The test was carried out with four groups of animals ($n = 10$ animals per group). Group I: vehicle (distilled water). Group II: Diazepam as positive control (1 mg/kg dissolved in distilled water). Group III: aqueous extract (60 mg/kg suspended in distilled water) and Group IV: the optimized hydroalcoholic extract (60 mg/kg suspended in distilled water).

After 12 h fasting, all treatments were given orally (0.1 ml/mg) 1 h prior to the start of the assay, except for the positive control, which was given 30 min earlier. After this time, each animal was placed in a glass chamber (previously saturated with ethyl ether for 5 min). Once the animal lost postural reflex, it was removed from the chamber and placed in the supine position. The time until the animal resumed the ventral position was recorded. For analysis of the data on pharmacological sedative activity, the software GraphPad Prism Version 7.0 was used, and one-way ANOVA was applied, followed by the Dunnett's test (95%).

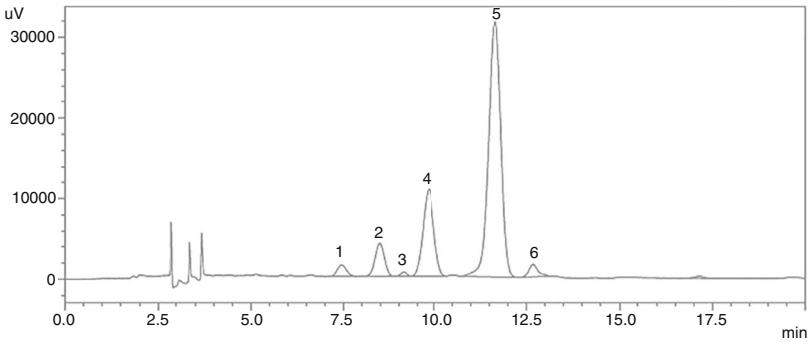

Fig. 1. Chromatographic profile of hydroalcoholic extract of leaves from *Passiflora quadangularis*. (1). orientin-2″-*O*-glucoside, (2) orientin-2″–*O*-xyloside, (3) orientin, (4) vitexin-2″–*O*-glucoside, (5) vitexin-2″–*O*-xyloside, (6) vitexin. For chromatographic conditions, see section 'Material and methods'.

Table 1
Calibration, sensitivity, precision and accuracy data for vitexin quantification in leaf extracts of *Passiflora quadrangularis*.

Flavonoid	Linearity range (μg/ml)	Calibration equation[a]	Correlation factor (r^2)	LOD[b] (μg/ml)	LOQ[b] (μg/ml)	Repetability[c]		Intermediate precision[c]		Recovery[d]			
						Concentration (μg/ml)	RSD (%)	Concentration (μg/ml)	RSD (%)	Vitexin 1 μg/ml		Vitexin 120 μg/ml	
Vitexin	1.0–120	$y = 16{,}856x + 5351.1$	0.9989	0.19	0.62	1	2.3	1	3.6	Mean (%)	RSD (%)	Mean (%)	RSD (%)
						2	1.9	2	2.5	99.2	2.1	100.6	1.8
						20	0.5	20	1.0				
						40	2.8	40	4.0				
						100	1.6	100	2.9				
						120	2.1	120	1.9				

[a] Eleven data points ($n = 3$).
[b] LOD, limit of detection; LOQ, limit of quantification.
[c] Limits: RSD: <5%.
[d] Recovery was determined by injection of sample (*P. quadrangularis* leaf extract, 1000 μg/ml) spiked with standard solution, in triplicate.

Results and discussion

Analytical methodology

Preliminary investigations by our research group have already reported an HPLC method to qualitative evaluation of flavonoids in *P. quadrangularis* leaf extract (Costa et al., 2013). Nevertheless, this method was not developed for the quantification of TF, and also resulted in poor resolution of the compounds analyzed when the extract was submitted to the degradation process.

The HPLC method developed in the present work for TF quantification in *P. quadrangularis* leaf extract proved to be linear in

Table 2
Passiflora quadrangularis leaf extract (matrix) influences the total flavonoid quantification.

	Standard plot[a]	Standard + extract plot
n	33	33
Slope (b)	16,856.2	17,219.7
Intercept (a)	5351.1	23,205.1
s_2xy	553,726,032.2	1,315,815,977.0
s_2b	9796.5	23,279.5
s_2a	32,525,337.2	77,289,771.3

[a] Standard: Vitexin.

Table 3
Flavonoid content in hydroethanolic leaf extracts of *Passiflora quadrangularis*.

Experiment	Factor			TF	Relative amount of flavonoids in the extract (%)					
	D-S.R	E.C	T.E		Orientin-2″-*O*-glucoside	Orientin-2″-*O*-xyloside	Orientin	Vitexin-2″-*O*-glucoside	Vitexin-2″-*O*-xyloside	Vitexin
1	1:10	25	24	61.88 ± 2.31^{d}	3.8 ± 0.01	10.6 ± 0.35	0.4 ± 0.01	21.1 ± 0.06	63.1 ± 0.71	1.2 ± 0.08
2	1:10	50	72	68.01 ± 3.01^{bc}	2.0 ± 0.12	6.9 ± 0.14	0.5 ± 0.08	22.3 ± 0.08	67.1 ± 0.30	1.1 ± 0.05
3	1:10	75	48	61.70 ± 0.74^{d}	2.0 ± 0.02	7.0 ± 0.05	0.6 ± 0.02	22.5 ± 0.08	66.7 ± 0.02	1.2 ± 0.05
4	1:15	50	48	71.05 ± 0.81^{b}	2.6 ± 0.16	8.3 ± 0.12	0.6 ± 0.01	21.8 ± 0.08	65.7 ± 0.16	1.0 ± 0.19
5	1:15	25	72	61.85 ± 2.14^{d}	3.7 ± 0.03	11.2 ± 0.05	0.5 ± 0.01	21.2 ± 0.03	62.3 ± 0.18	1.1 ± 0.18
6	1:20	25	48	64.44 ± 2.02^{cd}	2.7 ± 0.67	7.8 ± 0.12	0.5 ± 0.04	22.0 ± 0.07	65.9 ± 0.61	1.2 ± 0.07
7	1:15	75	24	68.89 ± 0.58^{bc}	2.0 ± 0.18	6.9 ± 0.09	0.5 ± 0.03	22.3 ± 0.26	66.8 ± 0.76	1.5 ± 1.01
8	1:20	50	24	65.16 ± 0.29^{cd}	2.0 ± 0.15	6.8 ± 0.14	0.5 ± 0.09	22.3 ± 0.06	67.2 ± 0.40	1.3 ± 0.04
9	1:20	75	72	75.82 ± 0.16^{a}	2.0 ± 0.10	6.7 ± 0.19	0.6 ± 0.02	22.4 ± 0.08	67.2 ± 0.50	1.2 ± 0.01
Aqueous extract				55.12 ± 0.63^{f}	3.0 ± 0.04	11.4 ± 0.3	N.D.	22.7 ± 0.01	61.1 ± 0.22	1.0 ± 0.13

D-S.R, drug-solvent radio; E.C, ethanol extraction (%); T.E, time extraction (hours); TF, total flavonoid content expressed as mg-eq vitexin/g dry extract; N.D, not detectable. Data represent the mean ± SD (standard deviation) ($n = 3$). Different letters indicate significant differences between mean values ($p < 0.05$).

the range of 1–120 μg/ml, with a correlation coefficient (r^2) of 0.9989. The obtained profile is presented in Fig. 1 and the results obtained for calibration, sensitivity, and precision and accuracy of the method are shown in Table 1.

A possible effect of the matrix in the quantification was evaluated, and an additive change was detected in the evaluated range by matrix addition, indicating that there is no matrix influence on TF quantification, according to the student *t* test (*p*-value = 0.05) (Table 2).

Optimization of extract

Content of TF and the relative content of the individual flavonoids (orientin 2″-*O*-glucoside **(1)**, orientin 2″-*O*-xyloside **(2)**, orientin **(3)**, vitexin 2″-*O*-glucoside **(4)**, vitexin 2″-*O*-xyloside **(5)** and vitexin **(6)**) in the extracts obtained are summarized in Table 3. The results show that in all the hydroethanolic extracts, TF is higher than in the aqueous extract according to previous reports (Do et al., 2014; Lee at al., 2014).

It is noted that orientin **(3)**, not detected previously in aqueous extract (Costa et al., 2013, 2016), was detected in all the hydroalcoholic extracts. This result may be due to differences in the solvents employed and the extraction method, since percolation is a more exhaustive method, unlike other methods, such as decoction and infusion (Chanda and Kaneria, 2012).

There were significant differences in the TF content of the different extracts prepared according to the experimental statistical design. Nevertheless, in all of them, vitexin 2″-*O*-xyloside **(5)** remained as the major flavonoid. This result is consistent with previous reports in which this flavonoid has been proposed as a chemical marker for differentiation between *P. quadrangularis* and *P. alata* (Costa et al., 2013).

Regards orientin derivatives, the content of these flavonoids changes slightly according to the extraction conditions. In treatments 1 and 5 (ethanol 25% solvent extraction), a slight increase in the content of orientin-2″-*O*-glucoside **(1)** and orientin-2″-*O*-xyloside **(2)** was observed, as well as a significant decrease in the content of the flavonoids derived from the vitexin. This fact may be related to the greater affinity of the solvent used (higher proportion

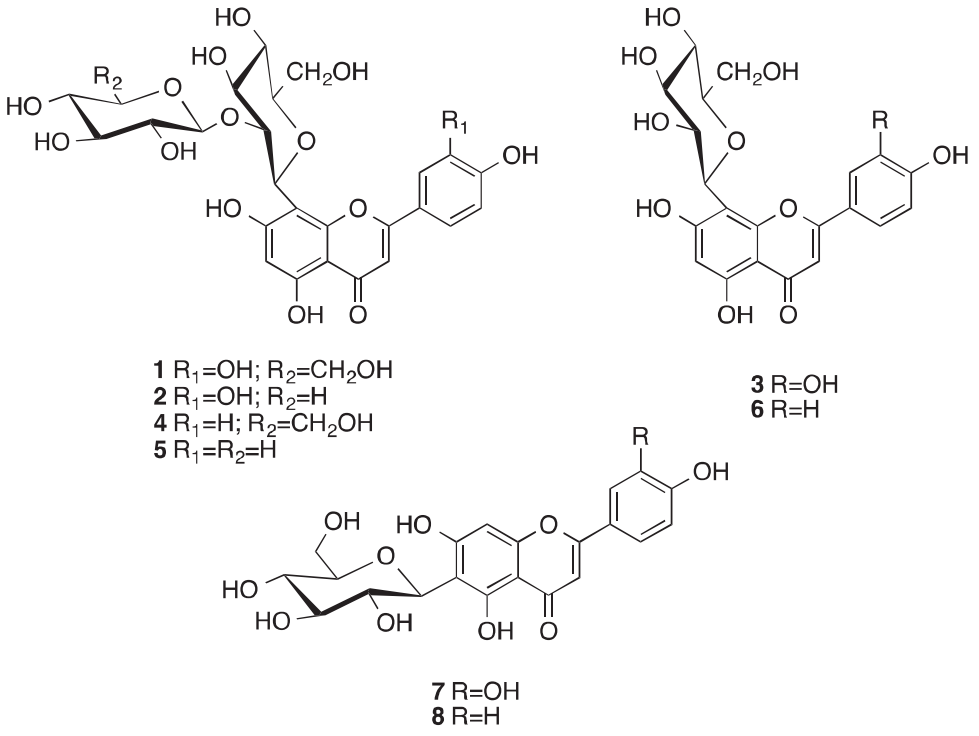

Table 4
ANOVA including covariates for total flavonoids in leaf extracts of *Passiflora quadrangularis*.

Regression	DF	Type I sum of squares	*R* – square	*F* value	Pr > *F*
Lineal	3	301,083,609	0.5246	39.4	0.0001
Quadratic	2	37,429,771	0.0652	7.35	0.005
Cross product	3	192,106,256	0.3347	25.14	0.0001
Total model	8	530,619,637	0.9246	26.04	0.0001

Residual	DF	SS	MS
Total error	17	43.298027	2.546943

Parameter	DF	SS	Pr > [*t*]
Drug–solvent ratio	1	2.034603	0.00160
Ethanol concentration	1	0.298813	0.12800
Time extraction	1	0.091251	0.61740
Drug–solvent ratio × ethanol concentration	1	0.005940	0.00010
Drug–solvent ratio × time extraction	1	0.011120	0.06040
Ethanol concentration × time extraction	1	0.002116	0.00320

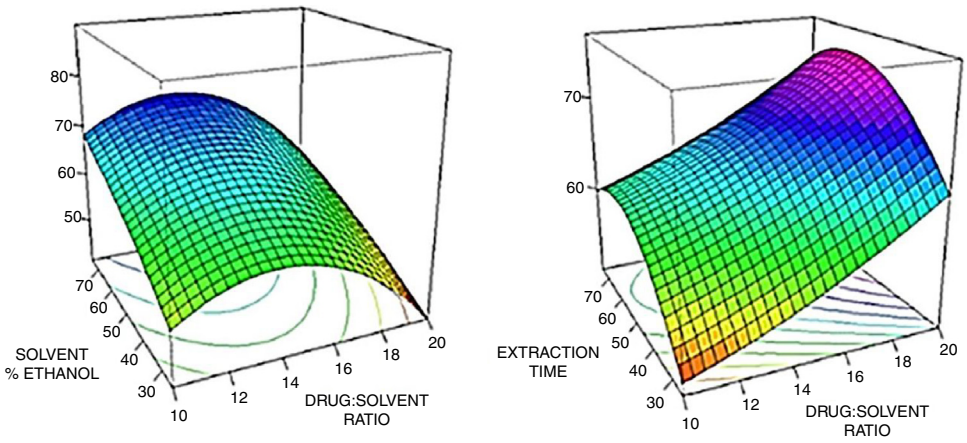

Fig. 2. Response surface estimated for the extraction of total flavonoids from *Passiflora quadrangularis* leaves.

of water in the mixture) for orientin derivatives, as its nucleus (luteolin) has a higher number of phenolic hydroxyls groups than vitexin derivatives (nucleus apigenin). Similar findings have been previously reported for other species (Wang et al., 2014).

As mentioned earlier SAS/STAT(R) 9.2 software was used to analyze and calculate the factors on the response variables and estimated response surface analysis with covariates through the RSREG Procedure, which uses the method of least squares to fit quadratic response surface regression models. Response surface models are a kind of general linear model in which attention focuses on the characteristics of the fit response function and in particular, where optimum estimated response values occur (Muhammad et al., 2016). Statistical analysis suggests a lineal model. The model F – value of 39.40 (Table 4) implies that the model was highly significant ($p < 0.001$). The drug-solvents ratio was the only significant factor of the model. Furthermore, ethanol concentration × time extraction interaction was found to be statistically significant ($p < 0.05$) while drug-solvent × ethanol concentration interaction was highly significant ($p < 0.001$). According to the statistical analyses, the model is described by equation 2.

$$[\mathrm{TF}] = 17.158713 + 7.638003f1 + 0.039862f1 * f2 + 0.007243f2 * f3$$

where TF, Total flavonoids content; f_1, drug–solvent ratio; f_2, ethanol concentration; and f_3,time extraction.

Through the RSREG procedure, it was also possible to elaborate the response surface plots in order to evaluate the behavior among the evaluated factors (Fig. 2). According to the surface diagrams obtained in the MSR analysis for the optimization of the TF content of the leaves of *P. quadrangularis*, the stationary point was reached when factor 1 (drug-solvent ratio) was 15.192; factor 2 (Extraction solvent) 50.962 and factor 3 (extraction time) 48.923. Thus, optimum conditions for TF extraction from *P. quadrangularis*, by percolation, were defined as: 1:15 drug:solvent ratio, 48 h of percolation and ethanol 50% (v/v) as extraction solvent. This result is similar to other studies that suggest the use of ethanol between 50 and 70% as solvent for optimized extraction of flavonoids from *Passiflora* leaves (Noriega et al., 2012; Gomes et al., 2017).

The content of TF in the hydroalcoholic optimized extract was 17% higher than the TF in aqueous extract (65.29 ± 2.08 mg-eq vitexin/g dry extract and 55.10 ± 1.12 mg-eq vitexin/g dry extract, respectively) as well as the yield (2.1% for the aqueous and 38% for the hydroalcoholic extract).

Sedative activity

According to the statistical analysis of the data, it was found that diazepam and the evaluated extracts of *P. quadrangularis* leaves (aqueous and optimized hydroalcoholic percolation) increased the mice sleep time, presenting highly significant differences with respect to the vehicle. This result is in accordance with the sedative activity previously reported for this species (Gazola et al., 2018). In addition, it was found that the optimized extract presents a higher increase in sleep time than the aqueous extract, suggesting that the increase flavonoid content contributes positively to the sedative activity (Fig. 3).

Stability

Photolysis

The extract was subjected to the strongest photolytic conditions, and after 30 days exposed to 6 × 106 lux/h, the TF just present 5.2% of degradation, classifying the extract as photostable. This result is in accordance with the literature that describes the flavonoid as a photoprotector (Saewan and Jimtaisong, 2013; Julkunen-Tiitto et al., 2015;).

Oxidation

Flavonoids on the extract were degraded 32.2% after 24 h of exposure to hydrogen peroxide (30%), without a change in the

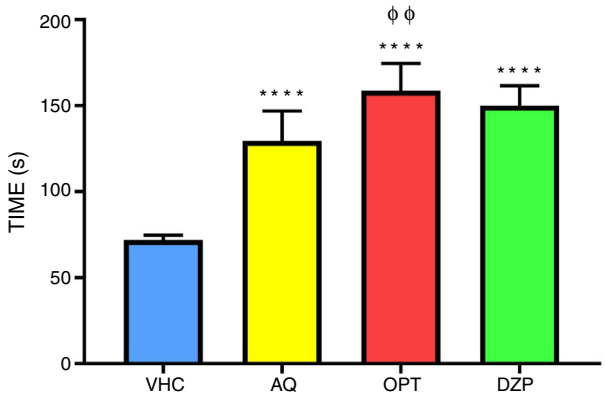

Fig. 3. Effect of the leaf extracts of *Passiflora quadrangularis* on sleep induced by ethyl ether in mice. DZP: Diazepam 1 mg/kg *p.o.* (positive control); VHC: Vehicle (distilled water); AQ: aqueous extract 60 mg/kg (*p.o*); OPT: optimized hydroalcoholic percolation 60 mg/kg (*p.o*). Each group: $n = 10$ animals. Data are expressed as the mean ± S.D and were submitted to analysis of variance (ANOVA), followed by a Dunnett's test with a value of $p < 0.05$. The significance values are given by $^{****}p < 0.0001$ with respect to the vehicle group, ϕϕ $p < 0.01$ with respect to the aqueous extract.

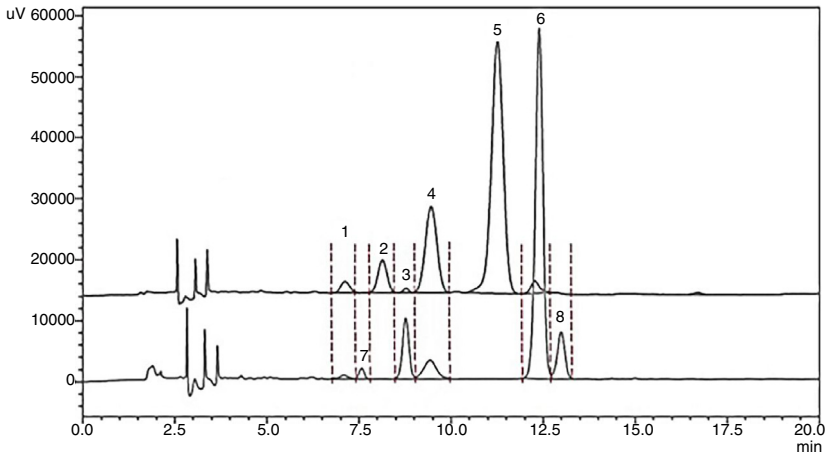

Fig. 4. Chromatographic profile of the optimized extract of *Passiflora quadrangularis* leaves before and after acidic hydrolysis. Up line: $t = 0$ h. Down line: $t = 8$ h from the evaluation of stability under stress conditions with HCl 0.1 N. (1) orientin-2″-*O*-glucoside, (2) orientin-2″-*O*-xyloside, (3) orientin, (4) vitexin-2″-*O*-glucoside, (5) vitexin-2″-*O*-xyloside, (6) vitexin, (7) isoorientin and (8) isovitexin.

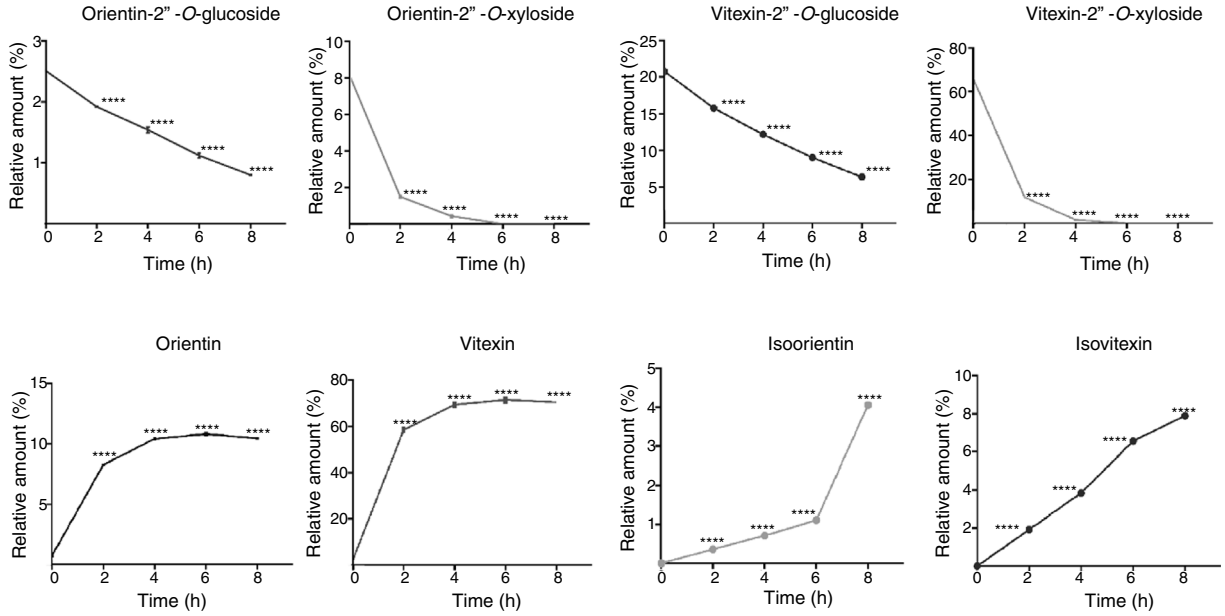

Fig. 5. Relative content of flavonoids in optimized extracts of leaves from *P. quadrangularis* during acidic hydrolysis (Reflux with HCl 0.1N). The data are expressed as the mean ± S.D and were submitted to analysis of variance (ANOVA), followed by Dunnett's test, *** $p < 0.001$.

flavonoid chromatographic profile. This fact allows the extract to be classified as practically stable under oxidative stress according to previous reports that indicated that *C*-glycosylflavonoids with sugar moiety at carbon 8 are more stable to oxidation degradation that *O*-glycosylated flavonoids (Umeo, 1988).

Hidrolysis

For hydrolysis under neutral conditions, the extract was classified as practically stable, since the degradation of the flavonoids in the extract was only 20.1%, the chromatographic profile did not show changes, and the relative amount of the individual flavonoids did not vary significantly.

Alkaline hydrolysis

In alkali-induced degradation, the flavonoids in the extract were degraded to 28.2%. This result classifies the extract as labile under conditions of alkaline stress. The relative amounts of all flavonoids (di and monoglycosides) decreased. This finding is in accordance with previous reports that suggest that flavonoids under conditions of alkaline hydrolysis can undergo an opening in the C ring, producing two fragments composed of ring A and ring B (Markham, 1982).

Acidic hydrolysis

Although the TF in the extract had a degradation just of 7.8% under acid hydrolysis, an important change was detected in the chromatographic profile of the sample after 8 h of reflux with HCl 0.1 N. An increase was observed in the content of vitexin **(6)** and orientin **(3)**, due to the rupture of the bond between the first and the second sugar moieties, converting the di-*C*-*O*-glycosylflavonoids to mono-*C*-glycosylflavonoids. Also, two new chromatographic signals suggest the presence of additional degradation products, identified by co-injection with standards as isoorientin **(7)** and isovitexin **(8)** (Fig. 4). The presence of these compounds could be explained by an arrangement on the benzopyrone nucleus known as the Wessely–Moser rearrangement, as the sugar bond attached directly to the nucleus (C–C link) cannot be hydrolyzed in these conditions. This arrangement involves the opening of the heterocyclic ring, subsequent rotation of the ring around the single bond between the carbonyl and ring A, and finally, the restoration of the

heterocycle; giving the isomeric form (Day and Harborne, 1989; Vázquez, 1998; Krishnaswamy, 1999).

Our results are in disagreement with those reported Gomez and co-workers (Gomes et al., 2017), who only found isovitexin **(8)** (rutin, orientin, isoorientin and vitexin were not detected) in an ethanolic extract of leaves of *P. quadrangularis* obtained by accelerated solvent extraction (ASE) at 80 °C. The presence of isovitexina **(8)** in this case could be due to the plant material source or, as observed in our results, a possible degradation associated with the high temperature of that extraction process.

The relative amount of the individual flavonoids present in the extract was also affected in the acidic hydrolysis. In this test, the flavonoids orientin-2″-*O*-glucoside **(1)** and vitexin-2″-*O*-glucoside **(4)** was decreasing, orientin-2″-*O*-xyloside **(2)** and vitexin-2″-*O*-xyloside **(5)** were completely degraded while the relative abundance of its monoglycosides were on the rise (Fig. 5). The degradation of the flavonoids orientin-2″-*O*-xyloside **(2)** and vitexin-2″-*O*-xyloside **(5)** was given at a higher rate than the flavonoids, whose second sugar unit was glucose (Fig. 5). This behavior is due to the fact that under acidic hydrolysis conditions, pentoses are hydrolyzed faster than hexoses (Gámez et al., 2006). For all these reasons, the sample was classified as labile under stress conditions with acid, although the percentage of degradation of total flavonoid content was less than 20%

Conclusions

According to the response surface statistical model employed, the optimal extraction conditions to achieve the highest flavonoid content in the extract of *Passiflora quadrangularis* were 1:15 drug-solvent ratio, with an ethanol 50% (v/v) and a percolation time of 48 h; the optimized extract shows higher sedative activity than the aqueous extract previously evaluated. In addition, this extract was classified as photostable, practically stable at oxidation and hydrolysis, and labile to acidic and alkaline hydrolysis. In addition, the flavonoids isoorientin **(7)** and isovitexina **(8)** were identified as degradation products of acidic hydrolysis of the optimized extract. This information contributes by generating added value to *P. quadrangularis* crops through the characterization of a leaf extract as potential active ingredient of herbal medicinal drugs.

Authors' contributions

SME and HIM (Msc students) contributed in collecting plant sample, running the laboratory work, analysis of the data and drafted the paper. GMC and DMA designed the study, supervised the laboratory work and contributed to the drafting and critical reading of the manuscript. All authors have read the final manuscript and approved the submission.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

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