Acta Farm. Bonaerense **21** (1): 5-8 (2002)
Recibido el 27 de agosto de 2001
Aceptado el 11 de octubre de 2001

Pharmacochemical Study of Aqueous Extracts of *Passiflora alata* Dryander and *Passiflora edulis* Sims

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SUMMARY. Pharmacological properties and chemical characterization of standard aqueous extracts from *P. alata* and *P. edulis* leaves were studied. The phytochemical results showed that aqueous extract of *P. edulis* leaves presented twice the flavonoid content than *P. alata.* Chromatographic comparison of *P. alata* and *P. edulis* extracts showed different flavonoid and saponin compositions. The pharmacological results of *P. alata* at doses of 100 and 150 mg/kg and *P. edulis* at doses of 50, 100 and 150 mg/kg showed anxiolytic effect according to the elevated plus-maze model via i.p.

RESUMEN. "Estudio Farmacoquímico de Extractos Acuosos de *Passiflora alata* Dryander y *Passiflora edulis* Sims". Se realizaron estudios farmacológicos y químicos de extractos acuosos estandarizados de hojas de *P. alata* y *P. edulis.* Los resultados fitoquímicos mostraron que el extracto acuoso de hojas de *P. edulis* contiene el doble de flavonoides que el extracto de *P. alata*. La comparación cromatográfica de los extractos de *P. alata* y *P. edulis* demostró que la composición en flavonoides y saponinas es distinta. Los resultados farmacológicos mostraron que los extractos de *P. alata* en dosis de 100 y 150 mg/kg y *P. edulis* en dosis de 50, 100 y 150 mg/kg resultaron ansiolíticos de acuerdo con el modelo del laberinto en cruz elevada por vía i.p.

INTRODUCTION

The aerial parts of *Passiflora* species have been traditionally used in Europe and America to treat anxiety, insomnia and nervousness [1]. Although several compounds like maltol [2], alkaloids [3] and flavonoids [4,5] have been proposed as responsible for their pharmacological effects, the active principles have not yet been identified. Furthermore, there are few pharmacological and phytochemical data available concerning the most used species in Brazilian herbal medicines, *Passiflora alata* [6-8] and *Passiflora edulis* [1,3,9-11].

In a previous paper [12] we showed that hydroethanol extracts of *P. alata* and *P. edulis* leaves possessed anxiolytic activity. Herewith we report the anxiolytic activity of standard aqueous extracts of *P. alata* and *P. edulis* leaves together with a phytochemical characterization of these extracts. Finally, we compared the anxiolytic properties of *P. alata* and *P. edulis* leaves extracts in relation to their chemical compositions.

MATERIALS AND METHODS

Plant material

Leaves of *P. alata* Dryander and *P. edulis* Sims were collected in the cities of São Leopoldo and Presidente Lucena, respectively, State of Rio Grande do Sul, Brazil. These specimens

KEY WORDS: Anxiolytic activity, Elevated plus-maze test, Isoorientin, Isovitexin, Orientin, *Passiflora alata, Passiflora edulis*, Passifloraceae, Vitexin.
PALABRAS CLAVE: Actividad ansiolítica, Isoorientina, Isovitexina, Modelo del laberinto en cruz elevada, Orientina, *Passiflora alata, Passiflora edulis, Passifloraceae, Vitexina.*

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were identified by Marcos Sobral and are on deposit in the herbarium of Departamento de Botânica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil (ICN 8344 *P. alata;* ICN 114356 *P. edulis).*

Preparation of aqueous plant extracts

P. alata and *P. edulis* leaves were air-dried at 40 °C for 7 days. 50 g of dry and powdered leaves were extracted, separately, using 500 ml water (plant:solvent, 1:10, w/v) under reflux during one hour. The extracts were filtered and freeze-dried. These extracts were characterized by thin-layer chromatography (TLC) profiles of flavonoids and saponins, the total flavonoids content and flavonoids analysis by HPLC.

Instrumentation

Ultraviolet (UV) spectra were recorded using a HP 5820 spectrophotometer. HPLC analyses were performed on a liquid chromatograph (Waters, model 600E) with a Rheodyne injection valve fitted with a 20 µL injection loop, a variable ultraviolet detector (Waters, model 486), and an integrator (Waters 746). Peak identification was performed using a photodiode-array detector (Waters PDA 996).

Evaluation of flavonoids

Flavonoids content was determined by the UV absorption of the $AlCl_3$-flavonoid complex, expressing total flavonoids content as apigenin, according to previous work [12-15].

Chromatographic analysis

TLC analyses were performed as previously described [12]. Flavonoid qualitative analysis by HPLC was carried out using a column Nova Pack® RPC18 (3.9 x 150 mm i.d., 4 µm), acetonitrile:phosphoric acid 0.05% (20:80, m/m) as mobile phase at a flow rate of 0.8 ml/min at 21 ± 2 °C. Detection was performed at 340 nm and 0.05 AUFS. Sample solutions were injected in triplicate. Flavonoids vitexin, isovitexin, orientin, isoorientin (Roth®, Germany) and chrysin (Sigma® USA) were used as reference substances. Peak identification and purity checking of test samples and flavonoid standards were conducted comparing the UV spectra between 200-400 nm using a photodiode-array detector.

Animals

Adult female Wistar rats (age 2-3 months; weight 180-250 g) from our breeding colony were used. The animals were housed in plastic cages, five to a cage, under 12 h light/dark cycle (lights on 7:00 a.m.) at constant temperature of 23° ± 1 °C, with water and food *ad libitum*.

Drugs and dosage

Saline, vehicle (propyleneglycol and saline), diazepam (1 mg/kg) dissolved in vehicle and freeze-dried aqueous extracts of *P. alata* and *P. edulis* (25, 50, 100 and 150 mg/kg) dissolved in saline were used. Saline, drug and extracts were injected (at injection volume of 1 ml/kg) intraperitoneally 30 min before testing.

Elevated Plus-Maze test

The elevated plus-maze test used as anxiety animal model is described in detail elsewhere [12,16,17].

Statistical analysis

Data from elevated plus-maze behaviour are expressed as mean ± SEM and were examined by one-way ANOVA followed by a Student-Newman-Keuls multiple range test.

RESULTS

Chemical results

The UV spectra of aqueous extracts from *P. alata* and *P. edulis* leaves were similar presenting λ_{max} at 278, 300, 400 nm for *P. alata* and 278, 300, 392 nm for *P. edulis.* However, evaluation of the content of flavonoids showed that the *P. edulis* extract has twice the flavonoid amount than *P. alata*. The total flavonoid content in aqueous extracts related to the dried plant material was 1.90% (w/w) for *P. alata* and, 4.04% (w/w) for *P. edulis.*

The flavonoid composition of the *P. alata* extract was simpler than that of *P. edulis.* In the *P. alata* extract three spots were detected with characteristic flavonoid colour by TLC. However, their chromatographic characteristics did not correspond to any of the flavonoids used as reference. Chrysin was not detected in either extract and saponin compounds were only observed in the *P. alata* extract. Using HPLC, it was possible to detect five substances in the P. alata extract but their retention time (rt) and HPLC-PDA analysis were different from those of the reference substances used. In contrast, *P. edulis* extract showed to contain vitexin (rt 9.1 min), isovitexin (rt 8.1 min), orientin (rt 13.7 min) and isoorientin (rt 15.7 min) by HPLC-PDA analysis.

Pharmacological results

The test results are shown in Table 1. All tested groups were compared with saline and

Groups	Behaviour				
	Open Arms		Closed Arms		Open and Closed Arms
	Number of Entries	Time Spent (s)	Number of Entries	Time Spent (s)	Total of Entries
Saline	3.55 (± 0.59)	60.18 (± 11.56)	5.73 (± 0.69)	207.64 (± 13.33)	9.27 (± 1.23)
Control	4.5 (± 0.79)	88.5 (± 17.16)	7.08 (± 1.0)	179.25 (± 18.99)	11.58(± 1.68)
Diazepam	7.25(± 0.65)*	176.42(± 19.2)*	2.92(± 0.47)*	88.58(± 17.43) *	10.17 (± 0.87)
P. alata 25	4.78 (± 0.43)	105.89(± 18.74)	6.33(± 0.73)	153.0(± 22.31)	11.11(± 2.03)
P. alata 50	6.0 (± 0.82)	118.89(± 19.83)	5.67 (± 0.67)	150.78 (± 22.17) *	11.67(± 0.90)
P. alata 100	6.38 (± 0.63)	161.13(± 26.44)*	3.5 (± 0.50)	113.38 (± 26.9) *	9.88 (± 0.67)
P. alata 150	7.6 (± 0.70)	146.0(± 24.16)*	4.4 (± 0.48)	116.7 (± 21.69) *	12.0 (± 0.56)
P. edulis 25	4.8 (± 0.70)	96.2 (± 18.08)	5.4 (± 0.52)	159.3 (± 19.76) *	10.2(± 0.95)
P. edulis 50	6.33(± 0.69)*	140.33(± 14.22)*	5.78 (± 0.70)	122.67(± 18.64) *	12.11(± 0.82)
P. edulis 100	7.33 (± 0.53)*	171.56(± 16.12)*	4.78 (± 0.78)	91.78 (± 14.46) *	12.11 (± 0.75)
P. edulis 150	7.0 (± 0.77)	158.1 (± 23.01)*	5.6 (± 0.92)	98.8 (± 18.83) *	12.6 (± 0.99)

Table 1. Effect of pre-treatment with diazepam and aqueous extracts from *Passiflora alata* (25, 50, 100 and 150 mg/kg) and *Passiflora edulis* leaves (25, 50, 100 and 150 mg/kg) on elevated plus-maze behaviour via i.p. Data are expressed as mean ± S.E.M. *N*= 9-10. * as compared to saline and control groups ($p < 0.05$).

vehicle injected animals. There were no differences between these groups.

Diazepam (1 mg/kg) was used as a standard anxiolytic drug. Diazepam-injected animals showed an increase in the number of entries and in the time spent in open arms ($p < 0.05$) and impairment in the number of entries and in time spent in the closed arms ($p < 0.05$).

Animals treated with *P. alata* (100 and 150 mg/kg) and *P. edulis* (50, 100 and 150 mg/kg) showed an increase in the time spent in open arms ($p < 0.05$) and impairment in the time spent within the closed arms ($p < 0.05$). *P. alata* (150 mg/kg) and *P. edulis* (50, 100 and 150 mg/kg) showed also an increase in the number of entries in open arms ($p < 0.05$).

Lower doses of *P. alata* (25 and 50 mg/kg) and *P. edulis* (25 mg/kg) did not show behavioural effects. There were not differences in the total number of entries (open + closed arms) among these groups.

DISCUSSION

The elevated plus-maze test is a pre-clinical test commonly used for searching new anxiolytic agents [16,17]. An increase in the time spent in the open arms have generally been used as indices for anxiolytic agents. *P. alata* at doses of 100 and 150 mg/kg and *P. edulis* at doses of 50, 100 and 150 mg/kg showed anxiolytic effect according to the elevated plus-maze model.

The phytochemical results showed that aqueous extract of *P. edulis* leaves presented twice the flavonoid content than *P. alata*. Chromatographic comparison of *P. alata* and *P. edulis* extracts showed different flavonoid and saponin compositions between them. Notwithstanding these observations, similar pharmacological properties were observed to both species.

By comparing these results using aqueous extracts with previous ones [12] using ethanolic extracts, it can be observed that the solvent (ethanol 40 °GL or water) did not change qualitatively the chemical composition regarding flavonoids and saponins content and, moreover, aqueous and hydroethanol extracts have identical anxiolytic effect.

The results presented here are consistent with others [18-20] that found anxiolytic effects for hydroethanol and aqueous extracts from aerial parts of *Passiflora incarnata* and could not demonstrate that these actions were related to flavonoids, harman alkaloids or maltol. In the literature, flavonoids have been reported as anxiolytic compounds [21]. On the other hand, it was reported that pure vitexin and isovitexin showed

no activity [18], isoorientin and orientin possessed very mild anxiolytic effects [22] and some alkaloids and maltol [2] presented in *Passiflora* species are not active in the central nervous system.

Further studies are in course to investigate which compounds in *Passiflora* extracts are responsible for their diazepam-like activities.

Acknowledgements. We are grateful to Marcos Sobral (Universidade Federal do Rio Grande do Sul) for locating, collecting and identifying the plant materials. This work was supported by CAPES, CNPq, PRONEX, FAPERGS and FIPE-HCPA (Brazil).

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