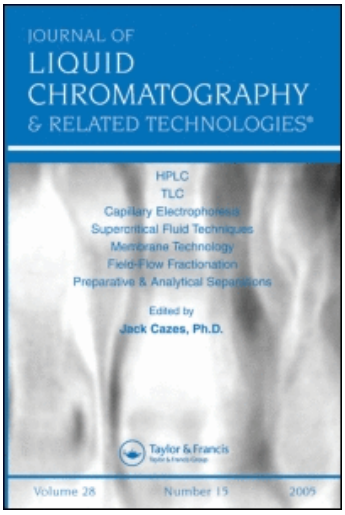


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# TLC Fingerprint of Flavonoids and Saponins from *Passiflora* Species

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# TLC Fingerprint of Flavonoids and Saponins from *Passiflora* Species

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**Abstract:** Several *Passiflora* species, known in Brazil as *maracujás*, are used as flavour and as juice in food industries, whereas passionflower extract has an ancient tradition in folk medicine as a sedative. Due to few phytochemical and pharmacological studies, there are no quality criteria for these *Passiflora* species as raw material. In this work, we present unique fingerprints of fourteen samples of *Passiflora* species relating to the presence of flavonoids and saponins. These chemical characterisations can provide, for example, authentication of samples, detection of adulterations, and differentiation between closely related species.

**Keywords:** *Passiflora*, Flavonoid, Saponin, TLC, Control quality

## INTRODUCTION

The aerial parts of *Passiflora* species (Passifloraceae) specially *Passiflora alata* Dryander, *Passiflora edulis* Sims, and *Passiflora incarnata* Linneaus

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have been traditionally used in Europe and America to treat anxiety and nervousness.[1,2] Although their anxiolytic activities have been extensively studied,[3–9] the active substances of *Passiflora* spp. are not yet identified.

*Passiflora alata* is the only species present in the Brazilian Pharmacopoeia[10] and its aerial parts are included in registered pharmaceutical preparations in Brazil.[11] There are few investigations on its chemical components[12,13] and pharmacological properties.[6,9,14]

Considering that at this moment the active component is unknown and in view of the large pharmaceutical utilisation of *Passiflora* species, the development of chromatographic identification based on chemical constituents may contribute significantly to the standardisation of the crude drug.

Relating to the chemical composition, flavonoids such as apigenin, vitexin, and homorientin were found in *Passiflora* species, while saponins are present in *P. alata* and *P. edulis*.[13,15,16] Flavonoids and saponins, due to their overall prevalence, structural diversity, chemical stability, and methods available to qualitative and quantitative analysis, are good chemical markers to provide authentication of samples, and to detect adulterations and differentiation between closely related species.

Concerning analytical procedures, the literature describes quantitative methods for routine evaluation of flavonoids in *Passiflora*.[17–22] As for saponins, as far as we know, only our research group presented a quantitative HPLC method.[23]

We present herein chemical characterisation using different thin-layer chromatographic systems to differentiate native *Passiflora* species from the States of Rio Grande do Sul and Santa Catarina, Brazil, as to the presence of flavonoids and saponins.



## EXPERIMENTAL

### Plant Material

Aerial parts of *Passiflora actinia* Hooker (ACT), *P. alata* Dryander (ALA), *P. amethystina* Mikan (AME), *P. caerulea* L. (CAE), *P. capsularis* L. (CAP), *P. edulis* Sims var. *flavicarpa* (EDU), *P. elegans* Masters (ELE), *P. foetida* L. (FOE), *P. misera* H.B.K. (MIS), *P. organensis* Gardner (ORG), *P. suberosa* L. (SUB), *P. tenuifila* Killip (TEN), *P. tricuspis* Masters (TRI), *P. warmingii* Masters (WAR) were collected in native areas in the States of Rio Grande do Sul and Santa Catarina, Brazil, in February 2000. Herbarium specimens are on deposit in the Herbarium of the Departamento de Botânica of the Universidade Federal do Rio Grande do Sul. Each plant material was air-dried and powdered separately.

## Reference Substances

We used vitexin (**1**) purchased from Rotichrom®; whereas 3-O-$\beta$-D-glicopiranosil-1 $\rightarrow$ 2-$\beta$-D-glicopiranosil-oleanolic acid (**2**) and quadranguloside (**3**) (9,19-cyclolanost-24*Z*-en-3$\alpha$,21-26-trihydroxy-3,26-di-O-gentiobiose) were isolated from *P. alata* leaves.[13]

## Thin-Layer Chromatographic Analysis

Extracts from *Passiflora* species were prepared, separately, using EtOH 40° GL under reflux (1:10, plant:solvent, w/v) for one hour (1 h). Filtered extracts were evaporated under vacuum to eliminate the alcoholic content and freeze-dried. Sample solutions (10 mg/mL) were prepared ultrasonically using methanol as solvent. Each sample of 15 μL was applied to TLC plates as a band at the starting line, with the exception of *P. warmingii* (20 μL).

Analytical TLC aluminium sheets coated with Si gel $GF_{254}$ (Merck, 20 × 20 cm plates) were used. Flavonoids were analysed using AcOEt: acetone:AcOH:$H_2O$ (60:20:10:10, v/v) and saponins were analysed using $CHCl_3$:EtOH:AcOH (60:40:5, v/v) as the mobile phase. Each mobile phase was allowed to migrate 15 cm from the starting line. Two identical TLC plates were obtained for the first mobile phase: one plate was sprayed using anisaldehyde-$H_2SO_4$ then heating (100°C) and the other one was submitted to methanol solutions of diphenylboryloxyethylamine (0.5%), followed by PEG 400 (5%, w/v) (Natural Product Reagent A, NA)[24] as colour reagents. Saponin plates were sprayed using anisaldehyde-$H_2SO_4$ then heating (100°C) as colour reagent. Spots were observed under visible light and long-wave UV light after being sprayed by colour reagents. Figures 1, 2, and 3 are photographed chromatographs in long-wave UV light.



## RESULTS AND DISCUSSION

TLC analysis is a valuable method in the identification and quality assurance of vegetable drugs. The chromatographic conditions used herein allowed good resolution and Rf-values to different compounds present at the *Passiflora* extracts.

Figure 1 presents the flavonoid pattern of *Passiflora* species using silica gel plates $GF_{254}$, AcOEt:acetone:AcOH:$H_2O$ (60:20:10:10) as the mobile phase and NA as the colour reagent. It is possible to verify that some extracts have important characteristics. So, we can differentiate the *P. suberosa* extract through a blue spot not detected elsewhere, whereas *P. alata* presents only two major spots (one yellow and another one orange, just bellow). *P. amethystina* and *P. misera* extracts have pale spots. Two couples of extracts are very similar using these chromatographic con-

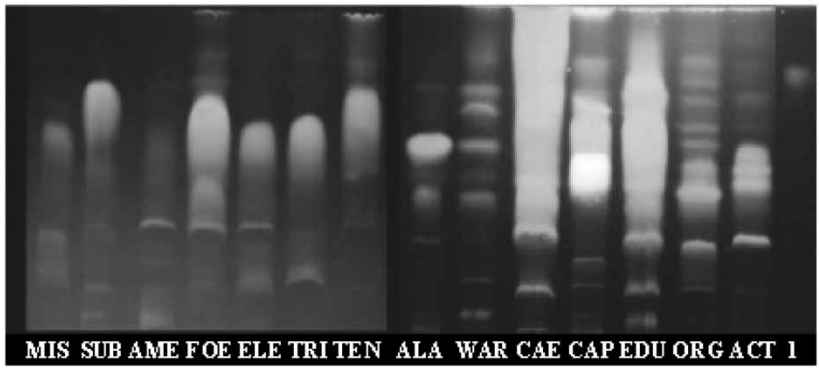


***Figure 1.*** Pattern of hidroethanolic extracts from *Passiflora* species. Chromatographic system: Si gel $GF_{254}$, AcOEt:acetone:AcOH:$H_2O$ (60:20:10:10, v/v), Natural Reagent A/$UV_{366}$. To sample codes see materials and methods. Reference: vitexin (**1**).



ditions: *P. organensis*/*P. actinia*, and *P. caerulea*/*P. edulis*. Relating *P. alata* and *P. edulis*, species used mainly for industrial purposes, it was possible to verify different chromatographic profiles for flavonoids.

The utilisation of anisaldehyde-$H_2SO_4$ as colour reagent for flavonoids was also a good alternative to achieve chemical differentiation among *Passiflora* species (Figure 2). Considering the couple of extracts cited above, it is possible to differentiate *P. organensis*/*P. actinia* extracts by the presence of blue spots in the first one, while *P. caerulea*/*P. edulis* extracts also present different chromatographic profiles.


***Figure 2.*** Pattern of hidroethanolic extracts from *Passiflora* species. Chromatographic system: Si gel $GF_{254}$, AcOEt:acetone:AcOH:$H_2O$ (60:20:10:10, v/v), anisaldehyde-$H_2SO_4$ then heating (100°C)/$UV_{366}$. To sample codes see materials and methods. Reference: vitexin (**1**).

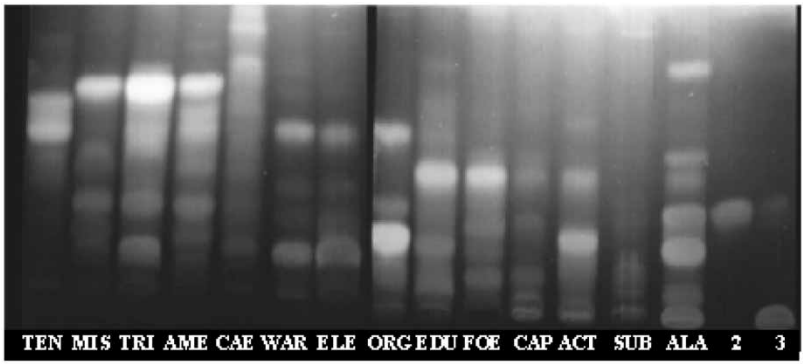


***Figure 3.*** Pattern of hidroethanolic extracts from *Passiflora* species. Chromatographic system: Si gel $GF_{254}$, $CHCl_3$:EtOH:AcOH (60:40:5, v/v), anisaldehyde-$H_2SO_4$ then heating (100°C)/$UV_{366}$. To sample codes see materials and methods. Reference: 3-O-$\beta$-D-glicopiranosil-1 → 2-$\beta$-D-glicopiranosil-oleanolic acid (**2**) and quadranguloside (**3**).



Figure 3 presents the pattern of *Passiflora* species using silica gel plates $GF_{254}$, $CHCl_3$:EtOH:AcOH (60:40:5) as the mobile phase and anisaldehyde-$H_2SO_4$ then heating (100°C) as colour reagent. Under visible light, only *P. alata* extracts and reference substances **3** and **4** presented violet spots. Under $UV_{366}$, some extracts have similar chromatographic profiles as *P. actinia, P. capsularis, P. edulis*, and *P. foetida.* It is also difficult to differentiate the extracts from P. *amethystina, P. misera*, and *P. tricuspis.* It is important to emphasise that only *P. alata* and reference substances **3** and **4** present orange spots under UV light, the characteristic colour of triterpenoids.

The *P. alata* extract presents saponins as main metabolites, whereas flavonoids are the major metabolites to other studied species. The *P. alata* extract presented two spots with characteristic flavonoid colour, however, their Rf-values were not the same as any flavonoid used as a reference substance.

Accordingly to these results, saponins can be used as quality markers in the identification of *P. alata* and detection of adulterations in raw material. Moreover, using altogether the three chromatographic conditions presented herein, it is possible to differentiate all studied species through their chromatographic profiles, either as flavonoids or saponins.

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## REFERENCES

1. Ross, M.S.F.; Anderson, A. Selection of plants for phytopharmacological study based on modernal herbal practice. Int. J. Crude Drug Res. **1986**, *24*, 1–6.
2. *The Review of Natural Products*®, Third Edition; DeMarderosian, A., Beutler, J.A., Eds.; Facts and Comparisons®: St. Louis, 2002; 547–550.
3. Speroni, E.; Billi, R.; Mercati, V.; Boncompagni, E.; Toja, E. Sedative effects of crude extract of *Passiflora incarnata* after oral administration. Phytother. Res. **1996**, *10*, S92–S94.
4. Speroni, E.; Billi, R.; Perellino, N.C.; Minghetti, A. Role of chrysin in the sedative effects of *Passiflora incarnata* L. Phytother. Res. **1996**, *10*, S98–S100.
5. Soulimani, R.; Younos, C.; Jarmouni, S.; Bousta, D.; Misslin, R.; Mortier, F. Behavioural effects of *Passiflora incarnata* L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. J. Ethnopharmacol. **1997**, *57*, 11–20.
6. Petry, R.D.; Reginatto, F.H.; De-Paris, F.; Gosmann, G.; Salgueiro, J.B.; Quevedo, J.; Kapczinski, F.; González-Ortega, G.; Schenkel, E.P. Comparative pharmacological study of hydroethanol extracts of *Passiflora alata* and *Passiflora edulis* leaves. Phytother. Res. **2001**, *15*, 162–164.
7. Dhawan, K.; Kumar, S.; Sharma, A. Comparative biological activity study on *Passiflora incarnata* and *P. edulis*. Fitoterapia **2001**, *72*, 698–702.
8. Dhawan, K.; Kumar, S.; Sharma, A. Anti-anxiety studies on extracts of *Passiflora incarnata* Linneaus. J. Ethnopharmacol. **2001**, *78*, 165–170.
9. De-Paris, F.; Petry, R.D.; Reginatto, F.H.; Gosmann, G.; Quevedo, J.; Salgueiro, J.B.; Kapczinski, F.; González-Ortega, G.; Schenkel, E.P. Pharmacochemical study of aqueous extracts of *Passiflora alata* Dryander and *Passiflora edulis* Sims. Acta Farm. Bonaerense **2002**, *21*, 05–08.
10. *Farmacopéia Brasileira*, 3rd Ed.; Andrei: São Paulo, 1977.
11. Anvisa, Agência Nacional de Vigilância Sanitária. Ministério da Saúde: Brazil. www.anvisa.gov.br/bancodedados (accessed Feb. 2004).
12. Ulubelen, A.; Oksuz, S.; Mabry, T.J.; Dellamonica, G.; Chopin, J. C-glycosylflavonoids from *Passiflora pittieri, P. alata, P. ambigua and Adenia manii*. J. Nat. Prod. **1982**, *45*, 783.
13. Reginatto, F.H.; Kauffmann, C.; Schripsema, J.; Guillaume, D.; Gosmann, G.; Schenkel, E.P. Steroidal and triterpenoidal glucosides from *Passiflora alata*. J. Braz. Chem. Soc. **2001**, *12*, 32–36.
14. Oga, S.; de Freitas, P.C.; Gomes da Silva, A.C.; Hanada, S. Pharmacological trials of crude extract of *Passiflora alata*. Planta Med. **1984**, 303–306.
15. Yoshikawa, K.; Katsuta, S.; Mizumori, J.; Arihara, S. Four cycloartane triterpenoids and six related saponins from Passiflora edulis. J. Nat. Prod. **2000**, *63*, 1229–1234.
16. Yoshikawa, K.; Katsuta, S.; Mizumori, J.; Arihara, S. New cycloartane triterpenoids from *Passiflora edulis*. J. Nat. Prod. **2000**, *63*, 1377–1380.
17. Schmidt, P.C.; González-Ortega, G. Passionsblumenkraut: Bestimmung des Gesamtflavonoidgehaltes von Passiflorae herba. Dtsch. Apoth. Ztg. **1993**, *133*, 4457–4466.

18. Rehwald, A.; Meier, B.; Sticher, O. Qualitative and quantitative reversed-phase high-performance liquid chromatography of flavonoids in *Passiflora incarnata* L. Pharm. Acta Helv. B, *69*, 153–158.
19. Bokstaller, S.; Schmidt, P.C. A comparative study on the content of passionflower flavonoids and sesquiterpenes from valerian roots extracts in pharmaceutical preparations by HPLC. Pharmazie **1997**, *52*, 552–557.
20. Petry, R.D.; Souza, K.C.B.de; Bassani, V.L.; Petrovick, P.R.; González-Ortega, G. Doseamento do teor de flavonóides totais em extratos hidroalcoólicos de *Passiflora alata* Dryander (maracujá). Rev. Bras. Farm. **1998**, *79*, 7–10.
21. Grice, I.D.; Ferreira, L.A.; Griffiths, L.R. Identification and simultaneous analysis of harmane, harmine, harmol, isovitexin, and vitexin in *Passiflora incarnata* extracts with a novel HPLC method. J. Liq. Chrom. & Rel. Technol. **2001**, *24*, 2513–2523.
22. Abourashed, E.A.; Vanderplank, J.R.; Khan, I.A. High-speed extraction and HPLC fingerprinting of medicinal plants– I. Application to *Passiflora* flavonoids. Pharm. Biol. **2002**, *40*, 81–91.
23. Reginatto, F.H.; Gosmann, G.; Schripsema, J.; Schenkel, E.P. HPLC/UV assay of quadranguloside, the major saponin from *Passiflora alata* Leaves. Phytochem. Anal. **2004**, *15*, 195–197.
24. Wagner, H.; Bladt, S.; Zgainski, E.M. *Plant Drug Analysis*; Springer: Berlin, 1984, 320.

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