



Revista Brasileira de Farmacognosia

BRAZILIAN JOURNAL OF PHARMACOGNOSY

www.journals.elsevier.com/revista-brasileira-de-farmacognosia



Short communication

First phytochemical studies of japecanga (*Smilax fluminensis*) leaves: flavonoids analysis

Edith E.A. Petrica^{a,b}, Adilson P. Sinhorin^{a,b,*}, Valéria D.G. Sinhorin^{a,b}, Gerado M.V. Júnior^{a,b}

^aLaboratórios Integrados de Pesquisa em Ciências Químicas, Instituto de Ciências Naturais, Humanas e Sociais, Universidade Federal de Mato Grosso, Sinop, MT

^bPrograma de Pós-graduação em Química, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil

ARTICLE INFO

Article history:

Received 5 June 2014

Accepted 21 July 2014

Keywords:

Smilax fluminensis

Quercetin

Chromatography

NMR

A B S T R A C T

This is the first chemical study of the antiradical potential of *Smilax fluminensis* Steud., Smilacaceae, leaves crude extract and fractions and the elucidation of two structurally isolated flavonoids. Quercetin-3-O- α -L-rhamnopyranoside (1-6)-O- β -D-glucopyranoside and quercetin-3-O- β -L-galactopyranoside were elucidated by spectrometric methods (¹H and ¹³C NMR and mass).

© 2014 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. All rights reserved.

Introduction

The genus *Smilax* is comprised of 300 to 350 species, found in the tropics and subtropics worldwide, they are bramble woody vines with a paired tendrils for climbing (Andreato, 2006). Several species of *Smilax* have been traditionally used as food or pharmaceutical materials in many countries, like the leaves of *S. excelsa*, which are widely used in some parts of Turkey in the daily diet (Ozsoy et al., 2008). Roots and young shoots of *S. aspera* are used as an ingredient to elaborate soft drinks and as a substitute of asparagus (Mariani et al., 2008). The roots and rhizomes of several species are used as folk medicine for their antibacterial, antifungal, anti-inflammatory and hepatoprotective activities as well as a syphilis treatment (Xu et al., 2005; Mandal et al., 2008). Phenolic compounds as gallic acid, protocatechuic acid, caffeic acid, gentisic acid, *trans*-o-coumaric acid and some flavonoids (Ozsoy et al., 2008; Yang et al., 2008; Zhang et al., 2009; Wungsintaweekul et al., 2011) were isolated from some species

of the genus *Smilax*. So far, the chemical constituents of *Smilax fluminensis* Steud., Smilacaceae, have not been investigated. Therefore, the aim of this study was to elucidate the structure of flavonoids by ¹H and ¹³C NMR and ESI-MS.

Results and discussion

The isolated compounds were analyzed using HPLC and ¹H and ¹³C NMR. The obtained compound 1 was a dark yellow solid; the molecular formula was established as MS (ESI): *m/z* [M⁺] calculated for C₂₇H₃₀O₁₆: 610.15; found *m/z* 609, 12 (M-H)⁻. MP: 201-203°C. The chromatogram peak for compound 1 was detected at 254 nm with 32 min RT.

The compound 2 was a dark yellow solid; the molecular formula was established as C₂₁H₂₀O₁₂, MW 464 g.mol⁻¹. MP: 231-233°C. The chromatogram peak of compound 2 was detected at 254 nm with 25.8 min RT.

* Corresponding author.

E-mail: sinhorin@ufmt.br (A.P. Sinhorin).

Compound 1: Quercetin-3-O- α -L-rhamnopyranoside(1-6)-O- β -D-glucopyranoside

^1H NMR (300 MHz CD_3OD): 6.18/6.19 (1H, d, H-6), 6.39/6.38 (1H, d, H-8), 7.72/7.52 (1H, d/d, H-2'), 6.90/6.83 (1H, d, H-5'), 7.62/7.53 (1H, d/d, H-6'), 4.21/5.34 (1H, d/d, H-1''), 3.08 (1H, m, H-2''), 3.10 (1H, m, H-3''), 2.93 (1H, m, H-4''), 2.98 (1H, m, H-5''), 3.53 (2H, m, H-6''), 4.24/4.38 (1H, s, H-1'''), 3.07 (1H, m, H-2'''), 3.34 (1H, m, H-3'''), 2.93 (1H, m, H-4'''), 3.12 (1H, m, H-5'''), 0.94/0.98 (3H, s, H-6''').

^{13}C NMR (75 MHz CD_3OD): 157.7/156.7 (C), 157.7/156.7(C), 176.6/177.4 (C), 161.3/162.3 (C), 100.2/98.2 (CH), 166.7/164.1 (C), 92.5/93.7 (CH), 155.9/156.5 (C), 104.2/104.0 (C), 122.1/121.3 (C), 116.3/116.4 (CH), 145.8/144.8 (C), 149.5/148.4 (C), 116.1/115.2 (CH), 121.7/121.7 (CH), 104.3/102.0 (CH), 74.4/74.9 (CH), 77.2/77.4 (CH), 69.7/70.5 (CH), 76.0/76.0 (CH), 67.9/69.3 (CH_2), 102.3/101.6 (CH), 71.3/70.7 (CH), 69.1/70.5 (CH), 71.7/71.9 (CH), 68.6/68.4 (CH), 17.2/17.3 (CH_3).

Compound 2: Quercetin-3-O- β -D-galactopyranoside

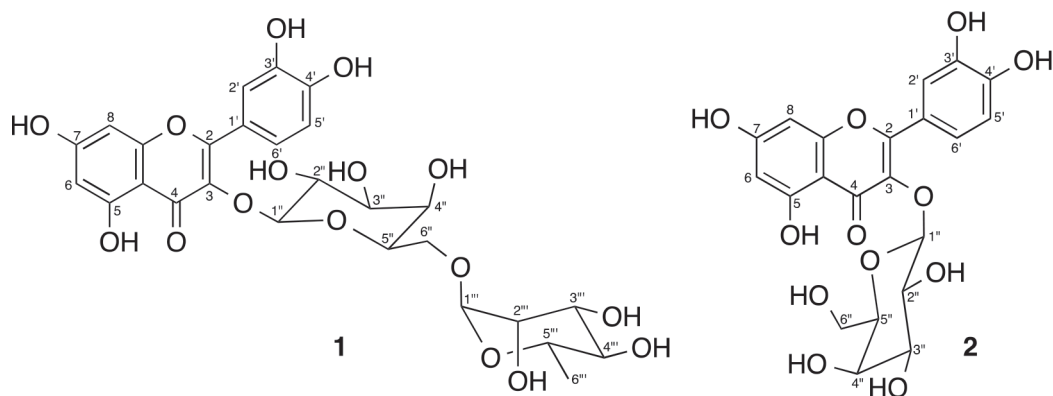
^1H NMR (300 MHz CD_3OD): 6.21/6.20 (1H, d, H-6), 6.41/6.41 (1H, d, H-8), 7.54/7.54 (1H, d, H-2'), 6.82 (1H, d, H-5'), 7.67/7.66 (1H, d/d, H-6'), 5.37/5.37 (1H, d, H-1''), 3.57/3.57 (1H, m, H-2''), 3.55/3.38 (1H, d, H-3''), 3.65/3.66 (1H, m, H-4''), 3.10/3.34 (1H, m, H-5''), 3.60/3.46 (2H, m, H-6'').

^{13}C NMR (75 MHz CD_3OD): 157.0/156.9 (C), 134.0/134.0 (C), 178.0/178.0 (C), 161.0/161.7 (C), 98.9/99.3 (CH), 164.9/164.9 (C), 93.7/94.1 (CH), 157.3/156.8 (C), 104.7/104.3 (C), 121.6/121.6 (C), 116.8/116.5 (CH), 144.7/145.4 (C), 148.8/149.0 (C), 115.0/115.3 (CH), 121.9/122.5 (CH), 102.4/102.4 (CH), 71.1/71.7 (CH), 73.9/73.7 (CH), 68.5/68.5 (CH), 76.9/76.3 (CH), 60.4/60.4 (CH_2).

The ^1H NMR spectrum of compounds 1 and 2 elicited a signal typical of an A ring between δH 6.18-6.21 ppm (1H, H-6) and δH 6.39-6.41 ppm (1H, H-8). Furthermore, two typical B ring signals, were observed at δH 7.72-7.54 ppm (1H, H-2'), δH 6.82-6.90 ppm (1H, H-5') and at δH 7.62-7.67 ppm (1H, H-6'). These groups suggest two polyphenolic compounds from quercetin derivatives. The sugar moieties of compound 1 identified as α -L-rhamnopyranoside(1-6)-O- β -D-glucopyranoside with chemical shifts for (H-1'') at δH 4.21/5.34 ppm (1H, d/d, $J = 1.0/6.0$ Hz, H-1''), at δH 4.24/3.38 (1H, s, H-1'''), at δH 0.94/0.98 ppm (3H, s, H-6''') and (H-sugar) at δH 2.93-3.53 ppm (m). The sugar moieties of compound 2 identified β -D-galactopyranosyl with chemical shifts for (H-1'') at δH 5.37/5.37 ppm (1H, d, $J = 9.0$ Hz, H-1'') and H-sugar at δH 3.55-3.66 ppm (m).

The ^{13}C NMR spectrum (75 MHz in CD_3OD) for compound 1 signaled 27 carbons, with a methyl group at carbon at 67.9 ppm confirming the presence of a glycoside chain (Niassy et al., 2004; Moura et al., 2011). A carbonyl signal was detected at 176.6 ppm, and signals at 102.3 and 104.3 ppm were determined to be the anomeric carbons of rhamnose (C1'') and glucose (C1'''), respectively. A spectrum band at 67.9 ppm was suggested to be a methylene carbon of glucose (C6''). The binding region rhamnosyl unit was assigned to (C6''), due to the signal at 6.2 ppm relative to the unsubstituted monomer. The methyl group of the rhamnose signals at 17.2 ppm (C6'''). So the heteroside chain was assigned the structure α -L-rhamnopyranoside-(1''-6'')-O- β -D-glucopyranoside. The aromatic carbons signals assigned were (C8) 92.5 ppm, (C6) 100.2 ppm, (C5') 116.1 ppm, (C2'') 116.3 ppm and (C6') 121.7 ppm. The signal on the carbonyl was assigned to (C4) and non-hydrogenated carbons were assigned based on data retrieved from literature (Pizzolatti et al., 2003; Braca et al., 2004; Niassy et al., 2004; Silva et al., 2005; Cha and Lee, 2007; Maisuthisakul et al., 2007; Peres et al., 2009; Moura et al., 2011) (C3) 132.4 ppm, (C5) 161.3 ppm, (C7) 166.7 ppm, (C9) 155.9 ppm and (C10) 104.2 ppm, (C1') 122.1 ppm, (C3') 145.8 ppm and (C4') 149.5 ppm (Pizzolatti et al., 2003; Yang et al., 2008). Compound 1 was identified by HPLC and confirmed by ^1H and ^{13}C NMR, and mass spectrometry, which showed m/z 609.12 $[\text{M}-\text{H}]^-$. Fragmentation produced a fragment representing m/z 301.21 $[\text{M}-147-163-\text{H}]^-$, indicating the loss of rhamnose (m/z 147) and glucose (m/z 163), respectively, (calculated for $\text{C}_{27}\text{H}_{30}\text{O}_{16}$).

The ^{13}C NMR spectrum (75 MHz in CD_3OD) of compound 2 displayed 21 carbon signals, and a methyl carbon at 60.4 ppm confirming the presence of a glycoside chain (Pizzolatti et al., 2003; Braca et al., 2004; Silva et al., 2005; Moura et al., 2011). A carbonyl was identified at 178.0 ppm, and signal at 102.4 ppm were assigned to the anomeric carbons of rhamnose (C1''). The methylenic carbon of glucose (C6'') signal was determined at 60.4 ppm. Thus the glycoside chain was assigned the structure β -D-galactopyranosyl. The aromatic carbons were determined at (C8) 93.7 ppm, (C6) 98.9 ppm, (C5') 115.0 ppm (C2'') 116.8 ppm and (C6') 121.9 ppm. The signal of the carbonyl, assigned to C4, and non-hydrogenated carbons were assigned by comparison with data from literature (Pizzolatti et al., 2003; Braca et al., 2004; Mariani et al., 2008; Moura et al., 2011) (C3) 134.0 ppm, (C5) 161.0 ppm, (C7) 164.9 ppm, (C9) 157.3 ppm and (C10) 104.7 ppm, (C1') 121.6 ppm, (C3') 144.7 ppm and (C4') 148.8 ppm (Niassy



et al., 2004, Moura et al., 2011). Compound 2 was identified by HPLC and confirmed by ^1H NMR and ^{13}C NMR.

Conclusion

This is the first phytochemical study of *S. fluminensis* leaves and the flavonoids described in the literature. Considering the results obtained from the fractions of the studied extract, we isolated and elucidated the structure of quercetin-3-O- α -L-ramnopyranoside (1-6)-O- β -D-glucopyranoside and quercetin-3-O- β -D-galactopyranoside.

Authors' contributions

EEAP (PPGQ student) carried out laboratory work as part of her final year research project. GMVJ obtained the NMR and MS data and contributed to compound identification. APS and VDGS supervised this project, provided intellectual input and prepared the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

All authors declare that there are no conflicts of interest and affirm that this paper consist of original and unpublished work.

Acknowledgment

The authors would like to thank Professor Virgínia Claudia da Silva (UFMT-ICET) for her important contributions to this work; we also thank Professor Lourdes Campaner dos Santos for equipment accessibility (Mass Spectra acquisition, Department of Chemistry, UNESP-Araraquara) and for CNPq for financial support (Process N^o 558225/2009-8).

REFERENCES

- Andreato, R.H.P., 2006. Smilacaceae na Reserva Biológica de Poço das Antas, Silva Jardim, Rio de Janeiro, Brasil. *Rodriguésia* 57, 647-657.
- Braca, A., Prieto, J.M., Tommasi, N. de, Tomé, F., Morelli, I., 2004. Furostanol saponins and quercetin glycosides from the leaves of *Helleborus viridis* L. *Phytochemistry* 65, 2921-2928.
- Cha, B.C., Lee, E.H., 2007. Antioxidant activities of flavonoids the leaves of *Smilax china*. *Korean J. Pharmacogn.* 38, 31-36.
- Maisuthisakul, P., Suttajit, M., Pongsawatmanit, R., 2007. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. *Food Chem.* 100, 1409-1418.
- Mandal, S.C., Jana, G.K., Das, S., Sahu, R., Venkidesh, R., Dewanjee, S., 2008. Hepatoprotective and antioxidant activities of *Smilax chinensis* L. root. *Pharmacologyonline* 2, 529-535.
- Mariani, C., Braca, A., Vitalini, S., Tommasi, N. de, Visioli, F., Fico, G., 2008. Flavonoid characterization and *in vitro* antioxidant activity of *Aconitum anthora* L. (Ranunculaceae). *Phytochemistry* 69, 1220-1226.
- Moura, A.C.S., Vilegas, W., Santos, L.C., 2011. Identificação de alguns constituintes químicos de *Indigofera hirsuta* L. (Fabaceae) por CLAE-IES-EM (TOF) e avaliação da atividade antirradicalar. *Quim. Nova* 34, 1136-1140.
- Niassy, B., Um, B.H., Lobstein, A., Weniger, B., Koné, M., Anton, R., 2004. Flavonoides from *Tephrosia deflexa* et *Tephrosia albifoliolis*. *Comptes Rendus Chimie.* 7, 993-996.
- Ozsoy, N., Can, A., Yanardag, R., Akev, N., 2008. Antioxidant activity of *Smilax excelsa* L. leaf extracts. *Food Chem.* 110, 571-583.
- Peres, M.T.L.P., Simionatto, E., Hess, S.C., Bonani, V.F.L., Candido, A.C.S., Castelli, C., Poppi, N.R., Honda, N.K., Cardoso, C.A.L., Faccenda, O., 2009. Estudos químicos e biológicos de *Microgramma vacciniifolia* (Langsd. & Fisch.) Copel (Polypodiaceae). *Quim. Nova* 32, 897-901.
- Pizzolatti, M.G., Cunha, J.R.A., Szpoganicz, B., Sousa, E., 2003. Flavonoides glicosilados das folhas e flores de *Bauhinia forficata* (Leguminosae). *Quim. Nova* 26, 466-469.
- Silva, D.A., Costa, D.A., Silva, D.F., Souza, M.F.V., Agra, M.F., Medeiros, I.A., Barbosa-Filho, J.M., Braz-Filho, R., 2005. Flavonoides glicosilados de *Herissantia tiubae* (K. Schum) Brizicky (Malvaceae) e testes farmacológicos preliminares do canferol 3,7-di-O- α -L-ramnopiranosídeo. *Rev. Bras. Farmacogn.* 15, 23-29.
- Wungsintaweekul, B., Umehara, K., Miyase, T., Noguchi, H., 2011. Estrogenic and anti-estrogenic compounds from the Thai medicinal plant, *Smilax corbularia* (Smilacaceae). *Phytochemistry* 72, 495-502.
- Xu, J., Li, X., Zhang, P., Li, Z.L., Wang, Y., 2005. Antiinflammatory constituents from the roots of *Smilax bockii*. *Warb. Arch. Pharm. Res.* 28, 395-399.
- Yang, C., Tang, Q.J., Zhang, L.Z., Wenying, L., 2008. Preparative isolation and purification of phenolic acids from *Smilax china* by high-speed counter-current chromatography. *Sep. Purif. Technol.* 61, 474-478.
- Zhang, Q.F., Zhang, Z.R., Cheung, H.Y., 2009. Antioxidant activity of rhizoma *Smmilacis glabrae* extracts and its key constituent-astilbin. *Food Chem.* 115, 297-303.