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N-trans-feruloyltyramine and flavonol glycosides from the leaves of *Solanum sordidum*

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Abstract: Chemical investigation of the leaves of *Solanum sordidum* Sendtn., Solanaceae, resulted in the isolation and identification of sitosterol, stigmasterol, 3 β -*O*- β -glycopyranosyl stigmasterol, 3 β -*O*- β -glycopyranosyl sitosterol, kaempferol-3-*O*- α -rhamnopyranosyl-(1-6)- α -glycopyranoside, rutin, and *N-trans-feruloyltyramine*. The structures of these compounds were established by analysis of 1D and 2D NMR spectrometric data and comparison with data in the literature. The evaluation of antioxidant activity showed an IC₅₀ of 159.5 ppm for the chloroformic fraction and IC₅₀ of 77.5 ppm for the hydromethanolic fraction.

Introduction

Solanum is the most representative genus of Solanaceae and comprises about 1700 species distributed all over the world. Plants in the genus are distributed in the tropical and subtropical regions and an estimated 1000-1100 species of the genus are found in South America regions (Willis, 1980). Due to the large number of species in the genus, the family was named Solanaceae.

Many species of the genus are known for its economic importance, such as tomato (*S. lycopersicum*), egg-plant (*S. melongena*) and potato (*S. tuberosum*), and some are used in folk and traditional medicine, like *S. paniculatum* ("jurubeba"), used for treatment of anemia and liver and digestion problems, and *S. americanum*, ("maria-pretinha"), used in the treatment of gastralgia, bladder spasm, joint pains, and as an effective vermifuge (Lorenzi & Matos, 2002).

Phytochemical studies of the *Solanum* species report the occurrence of flavonoids, free flavones and their glycosides (Silva et al., 2003), in addition to steroid alkaloids with spirosolane and solanidane skeletons, such as solasodine and solanine, as well as non-steroids constituted of amines and amides (Evans & Somanabandhu, 1980; Costa, 1994).

The present work describes the isolation of two flavonoids and an alkamide from *Solanum sordidum* Sendtn., Solanaceae, popularly named "falsa-jurubeba".

Material and Methods

The ¹H and ¹³C NMR spectra were obtained in a Varian spectrometer model Mercury Plus at 300 MHz for ¹H, and 75.5 MHz for ¹³C, using tetramethylsilane (TMS) or the solvent itself as internal reference. The chemical shifts (δ) were recorded in ppm and the deuterated solvents CDCl₃, CD₃OD, C₅D₅N, and D₂O were used. Glass chromatography columns (CC) of various sizes with silica gel (0.063-0.200 mm) stationary phase from Merk and silica gel 60 (0.04-0.063 mm) from Fluka were used.

In the analytic and preparative thin layer chromatography (TLC), glass plates with silica gel 60 GF254 from Merck were visualized with ultraviolet radiation $\lambda=254$ and 366 nm, followed by nebulization with either an H₂SO₄/MeOH (1:1) or H₂SO₄ anisaldehyde/acetic acid (1:0.5:50) solution and heating.

Plant material

Solanum sordidum Sendtn., Solanaceae, was collected on the island of Porto Rico, in the municipality of Taquaruçu, Mato Grosso do Sul State. A voucher of the vegetable material was deposited in the herbarium of the State University of Maringá under HUM 2482.

Extraction and isolation of the chemical constituents of S. sordidum

Pulverized dried leaves from *S. sordidum* (785 g) were extensively extracted using MeOH at room temperature. The combined extracts were concentrated *in vacuo* to give 166.2 g of methanolic extract. Part of the extract (81.4 g) was chromatographed on a silica gel column eluted with CHCl₃/MeOH with increasing polarity gradient. The fraction of CHCl₃/MeOH 99.5:0.5 of this treatment yielded a mixture of stigmasterol and sitosterol. The fraction CHCl₃/MeOH 95:05 was recrystallized in methanol, giving a mixture of the same glycosylated steroids. The second part of the extract (84.8 g) was dissolved in water and partitioned in butanol. Next, the butanolic fraction was concentrated, diluted in MeOH/H₂O 80:20 and partitioned with hexane. The resulting hydromethanolic fraction was concentrated, diluted in MeOH/H₂O 60:40, and extracted with CHCl₃. The chloroform fraction was submitted to column chromatography in eluted neutral alumina with CHCl₃/MeOH with increasing polarity gradient, leading to the isolation of alkamide **3**. The remaining aqueous MeOH fraction was chromatographed on silica gel CC with CHCl₃/MeOH, with increasing polarity gradient, and yielded the flavonol **1** after recrystallization in MeOH, and the flavonol **2** after silica gel preparative TLC with CHCl₃/MeOH 7:3.

Antioxidant activity

The antioxidant activity was evaluated using chloroform and hydromethanolic fractions of the vegetable species *S. sordidum*, by monitoring the consumption of the free radical DPPH by measuring the decrease in absorbance of the samples in different concentrations. Absorbance measurements were performed in an UV-Vis spectrophotometer at wavelength 517 nm. Catechin and 3 mL of HPLC-grade methanol were used as controls. Solutions of the chloroform fraction were diluted in HPLC-grade methanol at the concentrations of 25, 50, 75, 100, 125, 150, 175, and 200 ppm, and the hydromethanolic fraction was diluted in HPLC-grade methanol at concentrations of 25, 50, 75, 90, and 100 ppm. The tests were performed in triplicate for all concentrations for obtaining the IC₅₀. Absorbance was measured 30 min after the addition of DPPH to the sample at intervals of 3 min for each concentration.

Results and Discussion

The phytochemical study of the leaves of *Solanum sordidum* Sendtn., Solanaceae, led to the isolation of flavonoids rutin (**1**) and 3-*O*-rhamnopyranosyl-(1→6)-β-glycopyranosyl kaempferol (**2**), of the alkaloid *N-trans-feruloyltyramine* (**3**), of mixture of steroid saponins 3β-

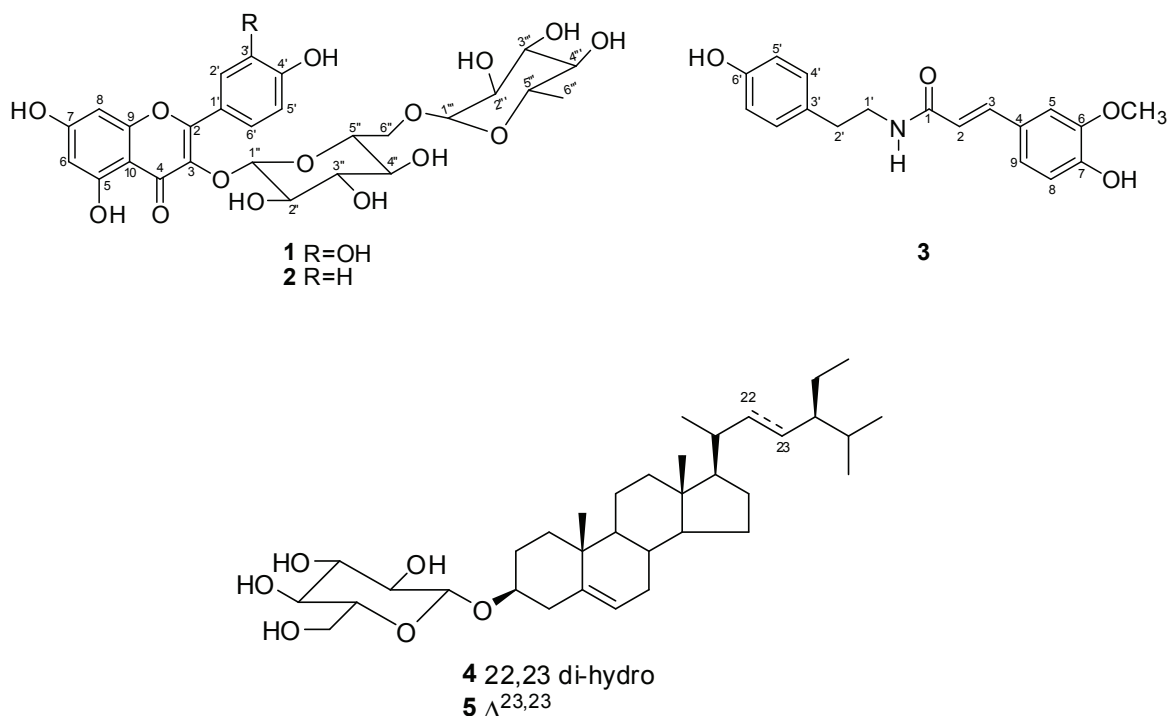


Table 1. ^1H (δ and J Hz, CD_3OD) of ^1H (300 MHz) and ^{13}C (75.5 MHz) NMR data of substance **3** and literature values for *N-trans-feruloyltyramine*.

C (DEPT)	3		<i>N-trans-feruloyltyramine</i>	
	δ_{C}	δ_{H} (multiplicity; J Hz)	δ_{C}	δ_{H} (multiplicity; J Hz)
1 (C)	169.4		169.5	
2 (CH)	118.8	6.40 (<i>d</i> ; 15.8)	119.0	6.49 (<i>d</i> ; 15.6)
3 (CH)	142.2	7.43 (<i>d</i> ; 15.8)	142.5	7.44 (<i>d</i> ; 15.6)
4 (C)	128.4		128.5	
5 (CH)	111.6	7.12 (<i>d</i> ; 2.1)	112.0	7.16 (<i>d</i> ; 2.0)
6 (C)	149.5		149.7	
7 (C)	150.0		150.5	
8 (CH)	116.6	6.79 (<i>d</i> ; 8.3)	117.0	6.83 (<i>d</i> ; 8.0)
9 (CH)	123.4	7.01 (<i>dd</i> ; 8.3 and 2.1)	123.5	7.03 (<i>dd</i> ; 8.0 and 2.0)
1' (CH ₂)	42.5	3.46 (<i>t</i> ; 7.2)	43.0	3.47 (<i>m</i>)
2' (CH ₂)	35.8	2.75 (<i>t</i> ; 7.2)	36.3	2.74 (<i>t</i> ; 7.7)
3' (C)	131.5		131.5	
4'/8' (CH)	130.9	7.05 (<i>d</i> ; 8.3)	131.0	7.06 (<i>d</i> ; 8.6)
5'/7' (CH)	116.4	6.71 (<i>d</i> ; 8.3)	116.5	6.76 (<i>d</i> ; 8.6)
6' (C)	157.1		157.5	
OCH ₃	56.4	3.88 (<i>s</i>)	56.5	3.87 (<i>s</i>)

O-glycopyranosyl-24 α -ethylcolesta-5-enol (**4**) and 3 β -*O*- β -D-glycopyranosyl-24 α -ethylcolesta-5,22*E*-dien-3 β -ol (**5**) (Alam et al., 1996) and of the steroids sitosterol and stigmasterol (Goulart et al., 1993).

Substances **1** and **2** were characterized as being glycosylated flavonoids containing the same sugar unit at position 3 and different levels of oxidation of the C ring of the genins, which had substitution patterns of quercetin and kaempferol. These substances were identified as being rutin and 3-*O*-rhamnopyranosyl-(1 \rightarrow 6)- β -glycopyranosyl kaempferol by comparison of the ^1H and ^{13}C NMR data to those in the literature (Niassy et al., 2004).

Substance **3** was isolated from the chloroform fraction as colorless crystals. In the ^1H NMR spectrum of **3** (Table 1), two duplets at δ 7.43 and 6.40 characteristic of hydrogens of α,β -unsaturated carbonyl system with constant coupling ($J=15.8$ Hz) can be observed, indicated by the *trans* configuration, also three-substituted benzene ring signals at δ 7.12 (*d*, $J=2.1$ Hz), 7.01 (*dd*, $J=8.3$, 2.1 Hz) and 6.79 (*d*, $J=8.3$ Hz) and the methoxyl group signal at δ 3.88, evidencing the presence of a *trans* feruloyl group. The two duplets at δ 7.05 ($J=8.3$) and 6.71 ($J=8.3$) attributed to hydrogens of para substituted benzene ring and the triplets at δ 2.75 and 3.46 of two methylenes are indicative of a four-hydroxyphenyl ethyl system. The carbons of the α,β -unsaturated carboxamide system were observed in the ^{13}C NMR spectrum (Table 1) at δ 169.4, 142.2, and 118.8, the methylenes of 4-hydroxyphenylethyl at δ 35.8, 42.5, and the methoxyl at δ 56.4. The correlations

of the signals at δ 2.75 and 3.46 and at δ 6.40 and 7.43 observed in the COSY confirmed the presence of the saturated and vinyl systems of the hydroxyphenyl ethyl and *trans*-feruloyl groups. The analysis of one- and two-dimensional NMR data combined with the data in the literature (Muñoz et al., 1996; King & Calhoun, 2005) allowed the characterization of substance **3** as being *N-trans-feruloyltyramine*.

The oxidation patterns of the oxidation of the flavonoid rings 3-*O*- α -rhamnopyranosyl-(1 \rightarrow 6)- β -glycopyranosyl kaempferol and of rutin isolated from *S. sordidum* agree with the patterns of the flavonoids found in other species of the genus, as described by Silva et al. (2003) and Steinharter et al. (1986), who reported glycosylated flavonols quercetin and kaempferol as the most frequent. *N-trans-feruloyltyramine* has been reported in plants and cell cultures of *Solanum* species. This alkamide has also been associated with the cell walls of some Solanaceae species and seems to play an important role in the defense of the plant (Keller et al., 1996; Muhlenbeck et al., 1996; Negrel et al., 1996; Turnock et al., 2001; Syu et al., 2001). The isolation of these substances corroborates the classification of this species in the *Solanum* genus.

The antioxidant activity was evaluated by the DPPH radical scavenging method. The activity was determined through the capacity of the fractions to bleach diluted solutions of the DPPH radical. IC₅₀, which corresponds to 50% of the inhibition of the DPPH radical, was calculated from the plot of percent inhibition versus

the concentration of the extract or fraction in $\mu\text{g/mL}$. The antioxidant used as a reference was catechin, which had an IC₅₀ of 8.25 ppm. The results obtained show that the chloroform fraction had moderate antioxidant activity, with an IC₅₀ of 159.5 ppm. The hydromethanolic fraction had significant antioxidant activity, with an IC₅₀ of 77.5 ppm, as shown in Figure 1. The flavonoids present in this species may be responsible for the significant antioxidant activity of the hydromethanolic fraction, as the literature cites them as being the most potent antioxidants (Furusawa et al., 2005; Alves et al., 2007; Rosa et al., 2010).

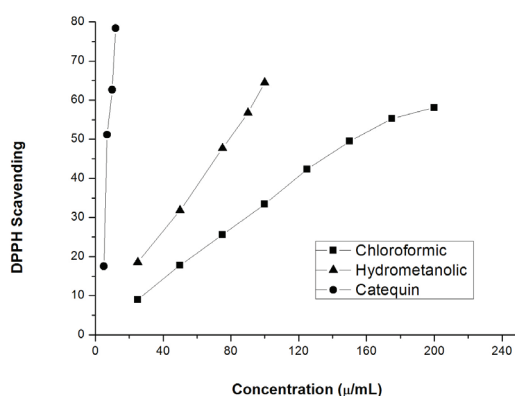


Figure 1. Scavenging activity of the DPPH radical on the chloroformic and hydromethanolic fractions of the leaves of *Solanum sordidum*.

Spectroscopic data of substances 1 and 2

Rutin (1): ^1H NMR (300 MHz, CD_3OD) δ ppm (multiplicity, J): 6.20 (d , $J=2.1$ Hz, 1H, H-6), 6.38 (d , $J=2.1$ Hz, 1H, H-8), 7.67 (d , $J=2.05$ Hz, 1H, H-2'), 6.88 (d , $J=8.4$ Hz, 1H, H-5'), 7.63 (dd , $J=8.5$ and 2.2 Hz, 1H, H-6'), 5.10 (d , $J=7.5$ Hz, 1H, H-1''), 4.52 (d , $J=1.5$ Hz, 1H, H-1'''), 1.12 (d , $J=6.3$ Hz, 3H, H-6'''), 3.30- 3.85 (m); NMR ^{13}C (75.5 MHz, CD_3OD) δ ppm: 158.8 (C-2), 135.9 (C-3), 179.7 (C-4), 163.3 (C-5), 100.2 (C-6), 166.3 (C-7), 95.1 (C-8), 159.7 (C-9), 105.8 (C-10), 123.4 (C-1'), 118.0 (C-2'), 146.1 (C-3'), 150.1 (C-4'), 116.3 (C-5'), 123.8 (C-6'), 105.0 (C-1''), 75.9 (C-2''), 78.3 (C-3''), 71.5 (C-4''), 77.4 (C-5''), 68.7 (C-6''), 102.6 (C-1'''), 72.3 (C-2'''), 72.4 (C-3'''), 74.1 (C-4'''), 69.9 (C-5'''), 18.0 (C-6''').

3-O-rhamnopyranosyl-(1 \rightarrow 6)- β -glycopyranosyl kaempferol (2): ^1H NMR (300 MHz, CD_3OD) δ ppm (multiplicity, J): 6.21 (d , $J=1.5$ Hz, 1H, H-6), 6.40 (d , $J=1.5$ Hz, 1H, H-8), 8.06 (d , $J=8.7$ Hz, 2H, H-2' and 6'), 6.89 (d , $J=8.7$ Hz, 2H, H-3' and 5'), 5.10 (d , $J=7.5$ Hz, 1H, H-1''), 4.50 (d , $J=1.2$ Hz, 1H, H-1'''), 1.12 (d , $J=6$, 0 Hz, 3H, H-6''') 3.25- 3.78 (m); ^{13}C NMR (75.5 MHz, CD_3OD) δ ppm: 159.6 (C-2), 136.6 (C-3), 180.6 (C-4), 167.3 (C-5), 101.2 (C-6), 164.0 (C-7), 96.1 (C-8), 160.7

(C-9), 106.7 (C-10), 123.8 (C-1'), 133.5 (C-2' and 6'), 117.3 (C-3' and 5'), 162.7 (C-4'), 105.7 (C-1''), 74.9 (C-2''), 79.1 (C-3''), 72.4 (C-4''), 78.2 (C-5''), 69.6 (C-6''), 103.5 (C-1'''), 73.1 (C-2'''), 73.3 (C-3'''), 76.7 (C-4'''), 70.7 (C-5'''), 18.8 (C-6''').

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