Chemistry of Natural Compounds, Vol. 46, No. 2, 2010

BIOACTIVE CHEMICAL CONSTITUENTS FROM *Smilax excelsa*

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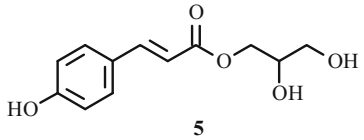
UDC 547.572

Plants from the genus *Smilax* are used to treat syphilis, acute bacillary dysentery, acute and chronic nephritis, eczema, dermatitis, cystitis, and mercury and silver poisoning [1, 2]. Several species from this genus have demonstrated antimutagenic [3], antioxidant [4], anti-inflammatory, and anti-nociceptive activities [5]. A survey of the literature showed that several *Smilax* species contain phenylpropanoid glycosides [3], anthocyanins [6], flavanoid glucosides [2], and steroidal saponins [7, 8].

We investigated the constituents of the rhizomes of *Smilax excelsa*, a climbing shrub growing in Bulgaria. In this paper we report the isolation and structural identification of *trans*-resveratrol (**1**) [9], naringenin (**2**) [10], 5-*O*-caffeoylshikimic acid (**3**) [11], 1-*O*-*trans*-feruloylglycerol (**4**) [12], 1-*O*-*trans*-*p*-coumaroylglycerol (**5**) [12], and 1,2-*O*-di-*trans*-feruloylglycerol (**6**) [12]. The antimicrobial and cytotoxic activities of the methanol, chloroform, *n*-butanol, and water-methanol extracts were tested.



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Compounds **1–3** are new for *Smilax excelsa* L., and compounds **4–6** are new for the genus *Smilax*. According to the erroneous numbering of the ring of shikimic acid in the literature, the same compound is identified as the 3- or 5-caffeoyl derivative [11, 13]. Our 1D and 2D NMR data unambiguously proved the structure, in agreement with IUPAC nomenclature.

All the extracts exhibited good cytotoxic activity (Table 1). Among them chloroform and butanol extracts demonstrated the highest activity. In our previous investigations we found that the last two extracts showed significant antioxidant activity [4]. The extracts showed no antimicrobial activity.

General Procedure. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 250 (^{1}H 250.13 MHz/^{13}C 62.9 MHz) spectrometer in solvent methanol-d_4 and TMS as internal standard. Bruker standard pulse programs were used.

Plant Material. The rhizomes of *Smilax excelsa* L. (Smilaceae) were collected in February 2004 near Golden Sands, Varna, Bulgaria. A voucher specimen SOM Co-3313 is deposited at the Institute of Botany, Bulgarian Academy of Sciences, Sofia.

Extraction and Isolation. The air dried milled rhizomes of *Smilax excelsa* (2.00 kg) were extracted three times with methanol at room temperature, yielding, after concentration, 213.2 mg methanol extract that was resuspended in a mixture of methanol–water. This suspension was successively extracted with chloroform (3 × 500 mL) and *n*-butanol (3 × 500 mL) to give the corresponding chloroform (6.0 g) and *n*-butanol (27.5 g) extracts. The *n*-butanol fraction was subjected to liquid vacuum chromatography eluting with $CHCl_3$–MeOH–H_2O (6:1:0.1 to 1:1:0.1) to give 18 fractions (F1-F18). Fraction F4 (0.46 g) was purified by silica gel CC eluting with $CHCl_3$–MeOH (30:1 to 10:1) to give seven subfractions (M1–M7). From M1 (18.6 mg) by preparative normal phase silica TLC ($CHCl_3$–MeOH–H_2O 12:1:0.1) naringenin (**2**, 9.1 mg) was obtained. From M4 (27.7 mg) using preparative TLC ($CHCl_3$–MeOH-H_2O 8:1:0.1) 1-*O*-*trans*-feruloylglycerol (**4**, 5.6 mg) was obtained.

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0009-3130/10/4602-0295 ©2010 Springer Science+Business Media, Inc.

TABLE 1. Cytotoxic Activity of Extracts from *Smilax excelsa* L.

Extract	Death, % (μg/mL)				LC_{50}[a] ± SD[b] (μg/mL)
	1000	100	10	1	
MeOH	100	76	33	10	15.84±3.75
$CHCl_3$	100	71	48	43	10.08±2.24
BuOH	100	81	48	43	4.36±2.06
H_2O–MeOH	100	57	24	10	32.62±2.65
CAPE	100	100	83	63	0.45±0.05

[a]Lethal concentration for 50% of *Artemia salina* nauplii.

[b]Mean of three measurements (10 nauplii per concentration plus control in one measurement; dead nauplii were counted).

trans-Resveratrol (**1**, 4.8 mg) was purified by preparative TLC ($CHCl_3$–MeOH–H_2O 10:1:0.1) from M5 (96.1 mg). From M7 (140.5 mg) using preparative TLC ($CHCl_3$–MeOH–H_2O 12:1:0.1) 1-*O*-*trans*-*p*-coumaroylglycerol (**5**, 12.0 mg) and 1,2-*O*-di-*trans*-feruloylglycerol (**6**, 6.4 mg) were obtained. Fraction F9 (0.8 g) was subjected to RP8 LPLC eluting with MeOH–H_2O (3:7 to 4:1, v/v) to give 10 subfractions (F9.1–F9.10). Fraction F9.4 (43.2 mg) was purified by silica gel CC eluting with $CHCl_3$–MeOH–H_2O (5:1:0.1 to 1:1:0.1) to give five subfractions (S1-S5). From S4 (14.8 mg) by preparative TLC ($CHCl_3$–MeOH–H_2O 13:7:2) 5-*O*-caffeoylshikimic acid (**3**, 9.4 mg) was obtained.

Tested Material. Total methanol extract (yield 10.7%). This extract was subjected to solvent-solvent partition to give the chloroform ($CHCl_3$), *n*-butanol (BuOH), and H_2O–MeOH extracts (yields: 2.8, 14.6 and 79.2, respectively).

Cytotoxic Assay. Cytotoxcicity was tested by the *Artemia salina* lethality test (Brine shrimp assay) using caffeic acid phenethyl ester (CAPE) as active reference substance [14]. Antibacterial and antifungal activities were tested by the modified disk diffusion method [15].

Antibacterial Assay. This was performed on gram (–) strain *Escherichia coli* WF^+, Gram (+) strain *Staphylococcus aureus* 209, and the fungus *Candida albicans* 562 (antimicrobial).

5-*O*-Caffeoylshikimic acid (3). ^{1}H NMR (250.13 MHz, CD_3OD, δ, ppm, J/Hz): 7.55 (1H, d, J = 15.9, H-7′), 7.04 (1H, d, J = 2.0, H-2′), 6.93 (1H, dd, J = 2.0, 8.2, H-6′), 6.77 (1H, J = 8.2, H-5′), 6.69 (1H, m, H-2), 6.28 (1H, d, J = 15.9, H-8′), 5.23 (1H, q, J = 6.4, H-5), 4.36 (1H, t, J = 4.1, H-3), 3.85 (1H, dd, J = 4.2, 8.3, H-4), 2.90 (1H, m, H-6a), 2.30 (1H, m, H-6b).

^{13}C NMR (62.8 MHz, CD_3OD): 172.7 (C-7), 168.9 (C-9′), 149.7 (C-4′), 147.1 (C-7′), 146.9 (C-3′) 135.1 (C-2), 134.2 (C-1) 127.8 (C-1′) 123.0 (C-6′), 116.5 (C-5′), 115.3 (C-8′), 115.1 (C-2′), 71.7 (C-5), 70.7 (C-4), 67.6 (C-3), 30.7 (C-6).

ACKNOWLEDGMENT

The authors are grateful to Dr. H. Najdenski, Institute of Microbiology, Bulgarian Academy of Sciences for performing the antimicrobial tests. Partial support of this work by the National Council for Scientific Research in Bulgaria (contracts X-1312 and X-1601) is gratefully acknowledged.

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