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FIRST PHYTOCHEMICAL INVESTIGATION OF SECONDARY METABOLITES OF *EUPHORBIA DAVIDII* SUBILS. AND ANTIPROLIFERATIVE ACTIVITY OF ITS EXTRACTS

SHORT COMMUNICATION

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The present work is the first phytochemical investigation of *Euphorbia davidii* Subils. After multistep separation process, three flavonoid glycosides were obtained from the ethyl acetate soluble fraction of the methanol extract of the whole plant. The structures of the isolated compounds were determined as kaempferol 3-*O*-rhamnoside, myricetin 3-*O*-rhamnoside, and quercetin 3-*O*-rhamnoside. Aqueous and organic extracts of the plant were screened *in vitro* for antiproliferative activity against HeLa (cervix epithelial adenocarcinoma), A431 (skin epidermoid carcinoma), A2780 (ovarian carcinoma) and MCF7 (breast epithelial adenocarcinoma) cells, using the MTT assay. *n*-Hexane and chloroform extracts demonstrated moderately dose-dependent cell growth inhibitory activity against all four cell lines.

Keywords: Euphorbia davidii – flavonoid glycoside – antiproliferative activity

Euphorbia species are reach in chemically and pharmacologically interesting secondary metabolites. Macrocyclic diterpenoids and their cyclisation products are the most important taxonomic markers in the Euphorbiaceae family in consequence of their extraordinary structural variability and limited distribution in the plant kingdom [11]. Besides specific Euphorbiaceae diterpenes, phenolic compounds (flavonoids, coumarins), other type diterpenoids and triterpenoids were isolated from different *Euphorbia* species [2, 3]. Many members of this family are used in folk medicine for the treatment of various diseases or have great economic importance like *Ricinus communis* L. and *Manihot esculenta* Crantz [9].

Euphorbia davidii Subils. is an invasive herbaceous annual weed, native in North America. Now, it has a scattered distribution in Europe (including Hungary) mostly with small populations restricted to railway areas and agricultural fields [1]. According to the latest molecular phylogeny and classification, *E. davidii* belongs to

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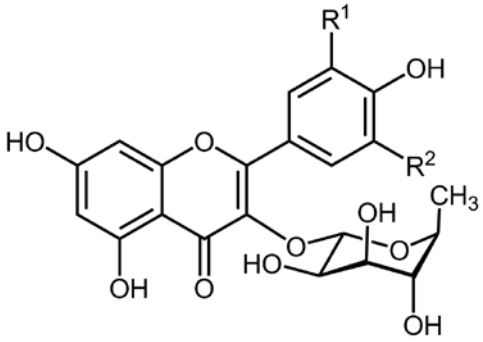
the subsection *Stormieae* within the *Euphorbia* section *Poinsettia* [12]. Neither the phytochemical investigation of *E. davidii* nor its biological activity have been reported previously.

Extensive phytochemical investigation was performed, in order to identify characteristic secondary metabolites of *E. davidii*. The whole plants were collected in Igar, Hungary, in September 2011. A herbarium sample (No. 836) has been deposited in the Department of Pharmacognosy, University of Szeged, Szeged, Hungary. The frozen fresh plant (15 kg) was percolated with 15 L methanol at room temperature. The crude extract was concentrated in vacuum and water was then added. The chlorophyll was removed by liquid-liquid extraction using $CHCl_3$ (5 × 1500 mL). The aqueous methanolic residue was extracted with ethyl acetate (5 × 1000 mL). The dried organic phase (29 g) was fractionated by vacuum liquid chromatography (silica gel 330 g; eluent: ethyl acetate/ethanol/water 64:4:0, 64:4:2, 64:4:3, 64:8:3, 64:8:4, 64:16:8, 64:32:16, 64:64:32; 1500 mL each) to yield 120 fractions. TLC monitoring of the fractions suggested, that fractions 4, 5 and 6 contain remarkable amounts of flavonoids. These fractions were first purified by preparative TLC (silica gel; ethyl acetate/formic acid/water 85:10:5), and, finally, by HPLC (LiChrospher RP-18, 10 μm, 250 × 10 mm; acetonitrile/water/formic acid 30:70:1, flow rate, 0.5 mL/min; 254 nm) to yield 1 (3.0 mg), 2 (3.0 mg) and 3 (3.0 mg).

Structures of 1–3 were established by NMR spectroscopy (Bruker Avance DRX 500 spectrometer, δ in ppm rel. to Me_4Si as internal standard, J in Hz). The ^{1}H- and ^{13}C-NMR data of isolated compounds were identical with those of kaempferol 3-*O*-rhamnoside [10], quercetin-*O*-rhamnoside [4, 5], and myricetin 3-*O*-rhamnoside [6] (Fig. 1).



	R^1	R^2
[1]	H	H
[2]	OH	H
[3]	OH	OH

Fig. 1. Chemical structure of flavonoid glycosides isolated from *E. davidii*. [1] kaempferol-3-*O*-rhamnoside; [2] quercetin-3-*O*-rhamnoside; [3] myricetin 3-*O*-rhamnoside

For pharmacological investigations 50 g of fresh plant material was extracted with 500 mL methanol with the use of an ultrasonic bath. After filtration, the extract was evaporated to dryness under reduced pressure. The residue was dissolved in 50 mL 50% aqueous methanol and was subjected to solvent–solvent partition between *n*-hexane (3×50 mL) and chloroform (3 × 50 mL). The *n*-hexane, chloroform and the residue phases were evaporated to dryness. After extraction with methanol, the dried plant material was extracted with 400 mL of boiling water for 15 min. The filtered extract was freeze-dried. All five extracts were screened *in vitro* for antiproliferative activity against HeLa (cervix epithelial adenocarcinoma), A431 (skin epidermoid carcinoma), A2780 (ovarian carcinoma) and MCF7 (breast epithelial adenocarcinoma) cells by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [8]. All *in vitro* experiments were carried out on two microplates with at least five parallel wells. Cisplatin was used as reference compound. Stock solutions of the tested extracts (10 mg/mL) were prepared with DMSO. The cell lines were treated with extracts at concentrations of 10 or 30 mg/mL. The higher concentration of DMSO (0.3%) did not exert substantial action on the viability of the utilized cell lines [7]. Antiproliferative effects of extracts are presented in Table 1.

Table 1
The antiproliferative effects of the prepared extracts against the utilized cancer cell lines

Extract	Concentration (μg/mL)	Inhibition of cell growth (%) ± SEM			
		HeLa	MCF7	A2780	A431
n-Hexane	10	14.60 ± 1.76	33.07 ± 1.95	19.39 ± 1.37	18.11 ± 0.84
	30	22.44 ± 2.66	45.88 ± 1.58	26.72 ± 0.91	25.65 ± 2.99
Chloroform	10	–*	30.81 ± 2.16	21.83 ± 0.66	–
	30	22.28 ± 2.74	52.63 ± 0.88	47.10 ± 0.68	21.64 ± 0.37
Ethyl acetate	10	–	–	–	–
	30	–	–	–	–
50% methanol	10	–	–	–	–
	30	–	15.84 ± 2.27	–	–
Water	10	–	–	–	–
	30	–	27.89 ± 1.92	15.20 ± 0.80	–

*Extracts eliciting less than 10% inhibition of cancer cell proliferation were regarded as ineffective and the values are not presented.

In the course of our previous chemical screenings, 33 Euphorbiaceae species were tested for their diterpene content using the extraction and chromatographic methods published in ref. [11]. This investigation suggested, that diterpenes are not detectable in the extract of *E. davidii* [11]. Extensive literature searches on all species belonging to the section *Poinsettia* led to the conclusion that plants of this section are poorly investigated, and characteristic metabolites of this section are flavonoids and triterpenoids. Isolation of three flavonoid gylcosides from *E. davidii* confirms this establishment. In our pharmacological investigation flavonoid containing ethyl acetate extract did not inhibit the proliferation of any cancer cell lines. However, *n*-hexane and

chloroform extracts demonstrated dose-dependent cell growth inhibitory activity against all cell lines. Bioassay guided phytochemical investigation of these lipophilic extracts would be a proper way of finding antiproliferative agents of *E. davidii*.

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