CHEMICAL COMPONENTS OF *Silene viridiflora* AND THEIR BIOLOGICAL PROPERTIES

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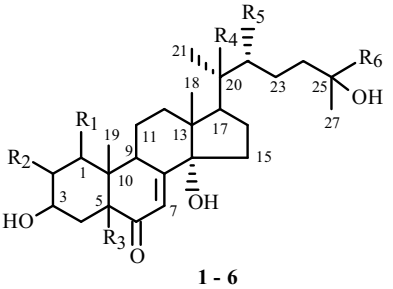
We have previously reported that 2-deoxyecdysone, 2-deoxy-20-hydroxyecdysone, polipodin B, 20-hydroxyecdysone, 26-hydroxypolipodin B, integristerone A, 2,22-diacetate- and 3,22-diacetate-20,26-dihydroxyecdysone, sileneoside A, and sileneoside D were isolated from the aerial part of *Silene viridiflora* L. (Caryophyllaceae) introduced into the experimental plot of the ICPS AS RU [1, 2]. In continuation of research on the chemical composition of this plant, we isolated fractions containing phytoecdysteroid **1** by chromatography of the BuOH extract over silica gel using $CHCl_3$:CH_3OH (9:1). Fractions were rechromatographed using $CHCl_3$:CH_3OH (15:1) to afford **1**. The PMR and mass spectra allowed **1** to be identified as taxisterone, $C_{27}H_{44}O_6$. Mass spectrum (CI, NH_3, *m*/*z*): 465 $[M + H]^+$, 447, 429, 411, 364, 346, 330, 304, 244, 202, 162, 145, 124, 118.

PMR spectrum (500 MHz, CD_3OD, δ, ppm, 0 = TMS): 1.77 (1H, H_a-1), 1.41 (1H, H_e-1), 3.80 (1H, dd, H_a-2), 3.94 (1H, H_e-3), 1.74 (1H, H_a-4), 1.69 (1H, H_e-4), 2.33 (1H, dd, H-5), 5.79 (1H, dd, H-7), 3.12 (1H, t, H-9), 1.78 (1H, H_a-11), 1.66 (1H, H_e-11), 2.10 (1H, dd, H_a-12), 1.88 (1H, H_e-12), 1.93 (1H, H_a-15), 1.60 (1H, H_b-15), 1.70 (1H, H_a-16), 1.90 (1H, H_b-16), 2.35 (1H, dd, H-17), 0.87 (3H, s, CH_3-18), 0.96 (3H, s, CH_3-19), 1.28 (3H, s, CH_3-21), 1.39-1.50 (1H, H-22), 1.39-1.50 (1H, H_a-23), 1.39-1.50 (1H, H_b-23), 1.39-1.50 (1H, H_a-24), 1.39-1.50 (1H, H_b-24), 1.17 (3H, s, CH_3-26), 1.17 (3H, s, CH_3-27).

Taxisterone (**1**) was isolated earlier from *Silene italica* ssp. *nemoralis* [3] and *S. nutans* [4]. This phytoecdysteroid was isolated from *S. viridiflora* for the first time.

We also studied the biological activity of certain pure phytoecdysteroids and the MeOH extract of *S. viridiflora* that was obtained by treatment of raw material with MeOH at room temperature and evaporation of the solvent to dryness. It is known that total phytoecdysteroids obtained from the aerial parts of *S. viridiflora* are recommended as an effective actoprotector for use in sports medicine, reduced functioning, and poor restoration after serious illnesses and heavy physical exertion [5, 6]. Results of an *in vivo* study of the biological activity showed that the extract of *S. viridiflora* had antitumor activity [7]. It reliably increased by 1.9 times the amount of leucocytes in peripheral blood in mice that received combined treatment and enhanced the antimetastatic activity of cyclophosphan by decreasing the amount of metastases in one mouse by 2.4 times. These data prompted further study of the extract and ecdysteroids in cytotoxic *in vitro* tests. The major ecdysteroids integristerone A (**2**), 2-deoxyecdysone (**3**), 2-deoxy-20-hydroxyecdysone (**4**), 20-hydroxyecdysone (**5**), and 26-hydroxypolipodin B (**6**) were studied.



1 - 6

1: $R_1 = R_3 = R_5 = H$, $R_2 = R_4 = OH$, $R_6 = CH_3$
2: $R_1 = R_2 = R_4 = R_5 = OH$, $R_3 = H$, $R_6 = CH_3$
3: $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = OH$, $R_6 = CH_3$
4: $R_1 = R_2 = R_3 = H$, $R_4 = R_5 = OH$, $R_6 = CH_3$
5: $R_1 = R_3 = H$, $R_2 = R_4 = R_5 = OH$, $R_6 = CH_3$
6: $R_1 = H$, $R_2 = R_3 = R_4 = R_5 = OH$, $R_6 = CH_2OH$

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TABLE 1. Activity of Methanol Extract and Phytoecdysteroids from *S. viridiflora* on Proliferation of P3X Cells after 24-h Cultivation (MTT Test)

Sample	Sample concentration, μg/mL	P3X cell vitality, %
MeOH extract	20	60.0
	40	125.0
2	0.4	96.3
	4	75.4
3	0.4	102.8
	4	128.9
4	0.4	84.5
	4	73.3
5	0.4	109.5
	4	135.0
6	0.4	79.5
	4	104.2
Control		100

The antitumor activity of the tested samples was estimated for murine myeloma cells P3X. For this, myeloma cells P3X were cultivated at 37°C in 96-well planchettes (2 × 105 cell/well) in DMEM culture medium (Dulbecco's modified Eagle's medium) (pH 7.4) containing fetal calf serum (5%), horse serum (10%), and penicillin/streptomycin (50 U/mL) in an atmosphere with 90% humidity and 5% CO_2. After this, extract (20 and 40 μg/mL per dry compound) and pure phytoecdysteroids (0.4 and 4 μg/mL) were added to the P3X cells and placed into an incubator for 24 h at 37°C. The control was P3X cells (2 × 105 cell/well) without active compound. The data were recalculated in percent of the control. All experiments were carried out in triplicate.

The cytotoxic activity was estimated using the MTT test [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] [8]. Medium in each well was replaced by fresh medium (100 μL DMEM) containing MTT (0.5 mg/mL) 4 h before the cultivation was finished. The plates were placed for 3 h in an incubator. When the test was finished, the resulting crystals of formazan were dissolved in DMSO. Absorption was determined in an apparatus for reading planchettes. Cytotoxic activity of extracts and compounds were expressed in percent reduction of color in control samples at 595 nm.

The *in vitro* studies showed that the MeOH extract and pure phytoecdysteroids of *S. viridiflora* suppressed growth of cells to different degrees. The MeOH extract had the highest antitumor activity.

The MeOH extract (20 μg/mL) in MTT tests exhibited high (60%) cytotoxicity for P3X cells. However, the extract activity decreased at 40 μg/mL to 125% (Table 1). Phytoecdysteroids 2-deoxyecdysone (**3**), 20-hydroxyecdysone (**5**), and 26-hydroxypolipodin B (**6**) were inactive toward P3X cells whereas **2** and **4** at 4 μg/mL were more active (75.4 and 73.3%, respectively) than the other phytoecdysteroids. This was apparently due to differences in the structures of the compounds.

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