Phytoecdysteroids of *Silene guntensis* and their *in vitro* Cytotoxic and Antioxidant Activity

Nilufar Z. Mamadalieva[a], Mahmoud Z. El-Readi[b], Abdulaziz A. Janibekov[a], Ahmad Tahrani[b], and Michael Wink[b,*]

[a] Institute of the Chemistry of Plant Substances AS RUz, Tashkent, Uzbekistan
[b] Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany.
Fax: +49 6221 54 4884. E-mail: wink@uni-hd.de

* Author for correspondence and reprint requests

Z. Naturforsch. **66c**, 215–224 (2011); received June 29, 2010/February 25, 2011

Phytoecdysteroids from aerial parts of *Silene guntensis* B. Fedtsch were investigated and three phytoecdysteroids were isolated: 2,3-diacetate-22-benzoate-20-hydroxyecdysone (**1**), 2-deoxy-20-hydroxyecdysone (**2**), and 20-hydroxyecdysone (**3**). Their chemical structures were elucidated by DEPT, COSY, ^{1}H and ^{13}C NMR spectroscopy. The isolated compounds **1**–**3** and crude extracts were evaluated for their antiproliferative and antioxidant activities. They exhibited substantial inhibition of cell growth against human cervix adenocarcinoma (HeLa), hepatocellular carcinoma (HepG-2), and breast adenocarcinoma (MCF-7) cells. The chloroform extract showed potent cytotoxic effects [IC_{50} values (26.58 ± 1.88) μg/mL, (20.99 ± 1.64) μg/mL, and (18.89 ± 2.36) μg/mL, respectively]. The new compound **1** was mildly cytotoxic compared to extracts [(127.97 ± 11.34), (106.76 ± 7.81), and (203.10 ± 19.56) μg/mL, respectively]. Water and *n*-butanol extracts exhibited good antioxidant activities [IC_{50} values of (68.90 ± 6.45) μg/mL and (69.12 ± 5.85) μg/mL, respectively].

Key words: Silene guntensis B. Fedtsch, Phytoecdysteroids, Antitumour and Antioxidant Activities

Introduction

Plants are an important source of medicinally potent secondary metabolites with a wide range of biological activities, including anticancer and antioxidant properties. Although many plants have been screened for active compounds, fundamental phytochemical investigations of previously neglected plants are still needed, especially in view of the interest in discovering new bioactive molecules with greater efficacy and fewer side effects than existing drugs.

Many species of the genus *Silene* (Caryophyllaceae) are abundant sources of ecdysteroids. The genus consists of more than 700 species (Mabberley, 2008) of which 84 grow in Central Asia (Bondarenko, 1971). Ecdysteroids have been recognized in more than 120 species and subspecies of *Silene* from the 155 species so far tested (Zibareva *et al.*, 2009). Ecdysteroids have anabolic, adaptogenic, tonic (Sláma *et al.*, 1996), carcinogenic (El-Mofty *et al.*, 1994), cardiotonic (Kurmukov and Yermishina, 1991), and antioxidant (Kuzmenko *et al.*, 1997) properties. Since plant ecdysteroids are similar in structure to the arthropod moulting hormone, ecdysone, they act as antifeedants and interfere with the growth of insects (Kubo *et al.*, 1983). Some species of *Silene* exert haemorheologic activity (Plotnikov *et al.*, 1998) and are strongly fungicidal (Vivek *et al.*, 2008). The steroid-containing fractions of some *Silene* species are immunosuppressive, potentially anti-inflammatory (Gasiorowski *et al.*, 1999), inhibit the proliferation of cancer cells by inducing apoptosis (Gaidi *et al.*, 2002), and show *in vivo* anticancer activity in mice (Zibareva, 2003).

The potential economic significance and biological value of ecdysteroids encouraged us to search for novel sources of phytoecdysteroids in the genus *Silene*. Thus, *Silene guntensis* B. Fedtsch from Uzbekistan, which had not been investigated previously (Vvedenskiy, 1953), is a promising target for phytochemical investigations and determination of its biological activity. We report here the isolation and structural elucidation of three phytoecdysteroids from extracts of this plant together with their cytotoxic and antioxidant activities. These compounds exhibit mild cytotoxic

activity. The chloroform extract exhibits the highest cytotoxic effects against HeLa, HepG-2, and MCF-7 cells. In addition, aqueous and *n*-butanol extracts exert substantial antioxidative activities.

Material and Methods

Plant material

Plants were collected in the Tashkent region of Uzbekistan in the flowering stage in the summer of 2008 and identified at the Department of Herbal Plants (Institute of the Chemistry of Plant Substances, Tashkent, Uzbekistan) by Dr. O. A. Nigmatullaev, with a voucher specimen (No. 2007168) being deposited at this department.

Plant extraction

The aerial parts of *S. guntensis* (3.75 kg) were extracted with methanol (5 × 20 L) at room temperature. The extract was filtered and the solvent evaporated to dryness using a vacuum evaporator (40 °C); 250 g methanol extract (6.65% of air-dried weight of the plant) were obtained. After evaporation the residue was repeatedly extracted with chloroform (8 × 1.5 L) for removing all hydrophobic compounds. The purified methanol extract was carefully extracted with *n*-butanol (5 × 1 L). After evaporating the solvent *in vacuo*, an *n*-butanol extract of 41.34 g (1.1%) was obtained.

General experimental procedures

Isolation of phytoecdysteroids was performed by column chromatography on silica gel KSK and L (63–100 μm; Chemapol, Praha, Czech Republic). Purity of phytoecdysteroids was verified using thin layer chromatography (TLC). TLC was conducted on plates precoated with Silufol F_{254} (Kavalier, Sazava, Czech Republic). Spots were visualized under UV light and by spraying with vanillin/H_2SO_4 reagent followed by heating to 120 °C for 10 min. The eluting solvent system for column chromatography used throughout the experiments was $CHCl_3/CH_3OH$ (15:1 and 9:1). Solvents were evaporated on a rotavapor. DEPT, COSY, ^1H and ^{13}C NMR spectra were recorded on a Varian UNITY (McKinley, Sparta, USA) plus 400 instrument in C_5D_5N (^1H NMR at 400 MHz; ^{13}C NMR at 100 MHz).

Liquid chromatography-mass spectrometry (LC-MS) analysis of phytoecdysteroids

HPLC conditions. The methanolic extract was injected in the HPLC system via a Rheodyne system with a 20-μL loop. Separation was achieved using an RP-$C18_e$ LichroCART 250–4, 5 μm column (Merck, Darmstadt, Germany). The concentration of sample was 20 μg/mL methanol. The mobile phase consisted of: A, HPLC-grade water with 0.5% formic acid; B, acetonitrile. A Merck-Hitachi L-6200A system (Darmstadt, Germany) was used with a gradient program at a flow rate of 1 mL/min as follows: 0% to 75% B in 45 min, then to 100% B in 5 min.

Mass spectrometry conditions. A Quattro II system from VG (Waters, Manchester, Great Britain) with an ESI interface was used in the positive ion mode under the following conditions: Drying and nebulizing gas, N_2; capillary temperature, 120 °C; capillary voltage, 3.50 kV; lens voltage, 0.5 kV; cone voltage, 30 V; full scan mode in the range *m/z* 300–900.

Isolation of phytoecdysteroids

The dry *n*-butanol extract (40 g) was subjected to a silica gel column (5.0 × 100.0 cm). The column was eluted with $CHCl_3/CH_3OH$ (9:1, 4 L), and fraction A was obtained containing a mix of compounds **1** and **2**. Continuous elution with $CHCl_3/CH_3OH$ (9:1, 4 L) gave 3.08 g (0.082% of air-dried plant material) of compound **3**, $C_{27}H_{44}O_7$, m.p. 241–242 °C (acetone), identified as 20-hydroxyecdysone by comparing with standard sample, TLC and spectral data (Saatov *et al.*, 1979).

Fraction A was applied to a silica gel column (3 × 60.0 cm) using $CHCl_3/CH_3OH$ (15:1, 3.5 L) to yield 8 mg of compound **1**, $C_{38}H_{54}O_{10}$, and 1.42 g (0.038%) of compound **2**, $C_{27}H_{44}O_6$, m.p. 252–254 °C (EtOH/H_2O). ^1H and ^{13}C NMR data were in agreement with the structure shown in Fig. 2, and compound **2** was identified as 2-deoxy-20-hydroxyecdysone by comparison of its spectral data with literature values (Chong *et al.*, 1970).

Preparation of samples for biological tests

The aerial parts of *S. guntensis* were dried at room temperature and milled to a coarse powder. The plant material (10 g) was extracted one time (24 h) with methanol (50 mL), chloroform (50 mL), and water (50 mL). Evaporation of the

solvents under reduced pressure provided 0.67 g methanol (6.65% of air-dried weight of the plant), 0.31 g chloroform (3.1%), and 0.32 g water (3.2%) extracts. The *n*-butanol extract was prepared as mentioned above (see section Plant extraction). All samples were stored frozen until further use.

Chemicals and reagents

Cell culture media, supplements, and dimethylsulfoxide (DMSO) were purchased from Roth (Karlsruhe, Germany) and Greiner Labortechnik (Frickenhausen, Germany). Other chemicals including 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), doxorubicin (≥ 98%), and quercetin (≥ 98%) were purchased from Sigma (Taufkirchen, Germany) and Gibco (Invitrogen, Karlsruhe, Germany). The purity of compounds **1**–**3** was > 95%, as determined by HPLC.

Cell cultures

HeLa (cervical cancer), HepG-2 (hepatic cancer), and MCF-7 (breast cancer) cell lines were maintained in DMEM complete medium (L-glutamine supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin) in addition to 10 mM non-essential amino acids in case of HeLa cell line. Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2. All experiments were performed with cells in the logarithmic growth phase.

Cytotoxicity and cell proliferation MTT assay

Sensitivity to drugs was determined in triplicate using the MTT cell viability assay (Mosmann, 1983). The extracts and phytoecdysteroids were dissolved in DMSO and diluted in the medium to final concentrations ranging from 0.977 to 500 μg/mL for extracts and from 0.977 to 500 μM for phytoecdysteroids **1**–**3**, in 96-well plates. Wells containing the solvent and wells without the solvent were included in the experiment. Cells ($2 \cdot 10^4$ cells/well of exponentially growing cells of each individual HeLa, HepG-2, and MCF-7 cell lines) were seeded in a 96-well plate (Greiner BioOne, Frickenhausen, Germany). The cells were cultivated for 24 h, then incubated with various concentrations of test samples at 37 °C for 24 h and then with 0.5 mg/mL MTT for 4 h. The formed formazan crystals were dissolved in 100 μL DMSO. The absorbance of the resulting solution was detected at 570 nm with a Tecan Safire II Reader (Männedorf, Switzerland). The cell viability rate (%) of three independent experiments was calculated by the following formula: cell viability rate (%) = (*OD* of treated cells/*OD* of control cells) · 100%, where *OD* is the optical density.

Antioxidant activity

The antioxidant and radical scavenging activities of the isolated compounds and extracts were evaluated according to Brand-Williams *et al.* (1995) using diphenylpicryl hydrazyl (DPPH). Equal volumes of sample solutions containing 0.02–10 mg/mL of the test samples and 0.2 mM methanolic solution of DPPH were pipetted into 96-well plates. The absorbance was measured against a blank at 517 nm using a Tecan Safire II Reader after incubation in the dark for 30 min at room temperature and compared with a DPPH control after background subtraction. Quercetin was used as a positive control (Ashour *et al.*, 2009). The percent inhibition was calculated from three different experiments using the following equation: $RSA\ (\%) = [(A_{517\text{control}} - A_{517\text{sample}})/A_{517\text{control}}] \cdot 100\%$, where *RSA* is the radical scavenging activity, A_{517} is the absorption at 517 nm, and control is the non-reduced DPPH.

Statistical analysis

IC_{50} values were calculated using a four-parameter logistic curve (SigmaPlot® 11.0), and all data were statistically evaluated using Student's t-test or the Kruskal-Wallis test (GraphPad Prism® 5.01; GraphPad Software, Inc., San Diego, USA) followed by Dunn's post-hoc multiple comparison test when the significance value was $P < 0.05$ using the same significance level. The criterion for statistical significance was taken as $P < 0.05$.

Results

LC-MS analysis (Fig. 1) revealed the presence of compounds **2** and **3** in the methanol extract of *S. guntensis* with retention times of 18.95 and 15.52 min, respectively. In addition, trihydroxyecdysone (Fig. 1, b), 22-dehydro-12-hydroxy-sengosterone (Fig. 1, c), and trihydroxyergosta-7,22-dien-6-one (Fig. 1, f) could be tentatively identified by LC-MS, whereas compound **1** could not be detected because of its small amount present (0.0002% of original extract).

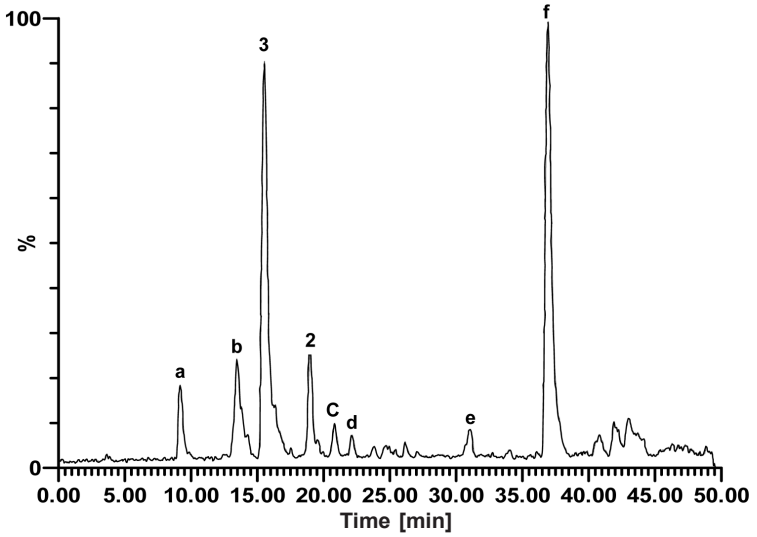
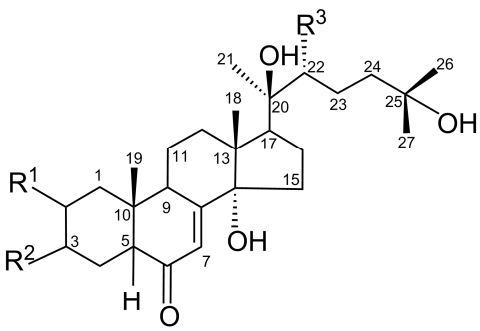


Fig. 1. Representative LC-MS chromatogram of *S. guntensis*: 3, 20-hydroxyecdysone [MW 480; (M+H$^+$) 481]; 2, 2-deoxy-20-hydroxyecdysone [MW 464; (M+H$^+$) 465]; tentative identification of: b, trihydroxyecdysone [MW 512; (M+H$^+$) 513]; c, 22-dehydro-12-hydroxy-sengosterone [MW 550; (M+H$^+$) 551]; and f, trihydroxyergosta-7,22-dien-6-one [MW 444; (M+H$^+$) 445]; a, d, and e, are unknown compounds with molecular ions (M+H$^+$) *m/z* 301, 431, and 353, respectively.

The DEPT data of compound **1** showed 38 carbon atoms, the ecdysterol skeleton accounting for five CH_3, eight CH_2, and seven CH groups, as well as seven quaternary carbon atoms. The olefinic resonance at δ_H 6.346 ppm (H-C-7) and δ_C 203.42 (C-6), 165.822 (C-8), 83.68 (C-14), and 34.131 ppm (C-9) indicated the presence of an α,β-unsaturated C=O group at C-6 one of the diagnostical features of ecdysteroids (Table I). The downfield resonances at δ_C 68.843, 68.843, 83.68, 80.891, and 68.843 ppm indicated further oxygenations and were assigned to C-2, C-3, C-14, C-22, and C-25, respectively, on the basis of COSY data. These characteristic features indicated the presence of an ecdysteroid skeleton similar to 20-hydroxyecdysone (**3**), and detailed NMR spectroscopic analysis showed the skeleton of **1** to be different from **3** at positions 2, 3, and 22.

The ^{1}H NMR spectrum of ecdysteroid **1** showed an olefinic proton at δ_H 6.346 ppm (1H, H-7), and seven methyl signals at δ_H 1.065 (3H, s, CH_3-18), 0.942 (3H, s, CH_3-19), 1.643 (3H, s, CH_3-21), 1.216 (3H, s, CH_3-26), 1.211 (3H, s, CH_3-27), 1.926 (3H, s, 2-CH_3CO), and 1.895 ppm (3H, s, 3-CH_3CO). Also, examination of the ^{1}H NMR data of ecdysteroid **1** showed (in comparison to the data for 20-hydroxyecdysone) a high-frequency shift ($\Delta\sigma$ 1.55 and 1.56 ppm) of the H-2 and H-3 signals (5.656 and 5.678 ppm) and there were two new 1H methyl singlet signals at δ_H 1.926 and 1.895 ppm. These are in agreement with an acetylation of the 2- and 3-hydroxy groups. This was confirmed by ^{13}C NMR data from the correlation of H-2 and H-3 → CO-Me (δ_C 169.83 ppm).

The ^{1}H NMR spectrum of ecdysteroid **1** also contained a signal for five aromatic protons at



1 R^1 = OAc, R^2 = OAc, R^3 = OBz
2 R^1 = H, R^2 = OH, R^3 = OH
3 R^1 = OH, R^2 = OH, R^3 = OH

Fig. 2. Chemical structures of the phytoecdysteroids **1**–**3** isolated from *S. guntensis*.

Table I. DEPT, ^{1}H and ^{13}C NMR data of phytoecdysteroids **1**–**3** recorded at 400 and 100 MHz, respectively, in C_5D_5N; δ in ppm; numbering as in Fig. 2.

DEPT	Position	**1**		**2**		**3**	
		δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
CH_2	1	33.949		27.14		37.63	1.91, 2.14 m
CH	2	68.843	5.656	26.43	2.06 m	67.82	4.10 m
CH	3	68.843	5.678	63.73	4.00 br s	67.72	4.11 m
CH_2	4	31.597		28.73	4.00 br s	32.11	1.70, 2.01 m
CH	5	51.216	2.504	51.40		51.06	2.92
C	6	203.42		212.5		203.18	
CH	7	121.1	6.346	121.16	6.13	121.32	6.25
C	8	165.822		166.08		165.78	
CH	9	34.131	3.48	36.10	3.75	34.01	2.88 m
C	10	38.193		36.65		38.32	
CH_2	11	21.179		21.21		21.14	1.70, 1.88 m
CH_2	12	31.597		31.30		31.67	2.46, 1.96
C	13	47.817		48.56		47.77	
C	14	83.68		86.24		83.85	
CH_2	15	31.32		31.91		31.42	2.14, 1.89
CH_2	16	25.889		21.21		21.35	2.45, 2.04
CH	17	50.162	2.92	49.80	2.91 m	49.76	2.90
CH_3	18	17.466	1.065 s	17.57	1.25 s	17.55	1.08 s
CH_3	19	22.089	0.942 s	24.05	0.94 s	24.13	0.94 s
C	20	76.137		76.53		76.53	
CH_3	21	20.757	1.643 s	21.36	1.48 s	20.79	1.48 s
CH	22	80.891	5.503	77.22	3.77 m	77.22	3.76 m
CH_2	23	29.005		26.76		27.13	1.85, 2.15
CH_2	24	41.403		42.30		42.29	2.32, 1.82
C	25	68.843		69.23		69.25	
CH_3	26	29.762	1.216 s	29.67	1.25 s	26.65	1.25 s
CH_3	27	29.296	1.211 s	29.78	1.26 s	29.77	1.25 s
2-CH_3CO		23.662, 169.834	1.926 s				
3-CH_3CO		23.662, 169.834	1.895 s				
Bz:			8.134				
1′		131.372	(2′,6′-H)				
2′,6′		129.661	7.08				
3′,5′		128.373	(3′,5′-H)				
4′		132.588	7.235				
COO		166.623	(4′-H)				

8.134 (2′,6′-H), 7.08 (3′,5′-H), and 7.235 ppm (4′-H), confirming the presence of one benzoate group. The chemical shifts of the benzoate moiety in the ^{13}C NMR spectrum (δ_C 131.372, 129.661, 128.373, 132.588, and 166.623 ppm) also confirmed the presence of a benzoate group. Comparison of the ^{1}H and ^{13}C NMR spectra of ecdysteroids **1** and **3** revealed a significant difference for the position of the proton of C-22. C-22, which appeared at 77.22 ppm in ecdysteroid **3**, was shifted downfield to δ_C 80.891 ppm in ecdysteroid **1**, and results indicated that the benzoate group is bound to the C-22 hydroxy group. Thus, from the above data, the structure of the new ecdysteroid **1** was established as 2,3-diacetate-22-benzoate-20-hydroxyecdysone (Fig. 2).

The cytotoxicity of the three isolated phytoecdysteroids, of doxorubicin (as positive control), and of the individual extracts was tested against HeLa, HepG-2, and MCF-7 cell lines (Fig. 3). The IC_{50} values are shown in Table II. 2,3-Diacetate-22-benzoate-20-hydroxyecdysone (**1**) was most active against HeLa and HepG-2 cells [IC_{50} values (127.97 ± 11.34) and (106.76 ± 7.81) μM, respectively], while 2-deoxy-20-hydroxyecdysone (**2**) was most active against MCF-7 cells [IC_{50} (126.54 ± 12.09) μM]. The chloroform extract potently inhibited cell growth in all cells tested [IC_{50}

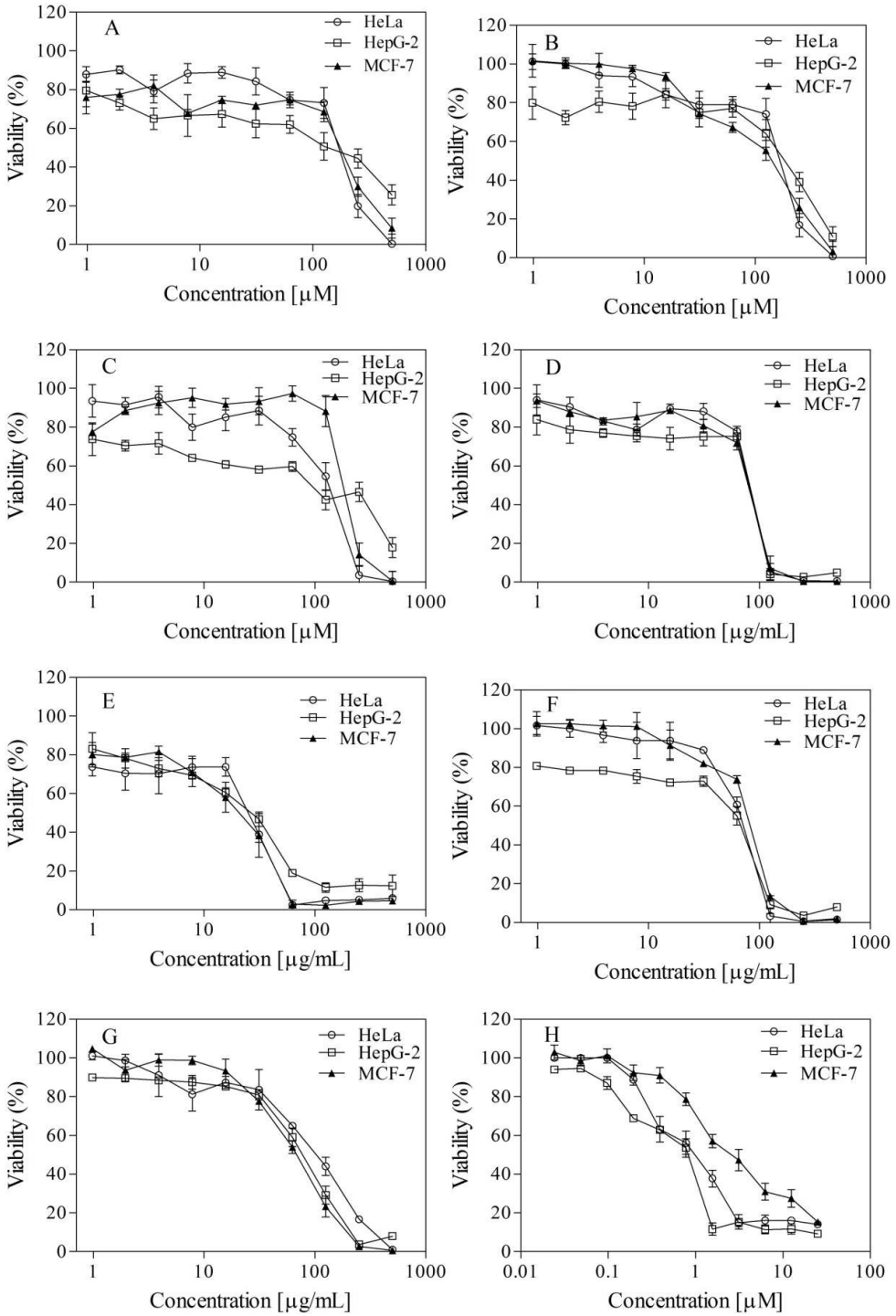


Fig. 3. Cytotoxicity of phytoecdysteroids and extracts from *S. guntensis*: dose-response curves of (A) 20-hydroxyecdysone (**3**), (B) 2-deoxy-20-hydroxyecdysone (**2**), (C) 2,3-diacetate-22-benzoate-20-hydroxyecdysone (**1**), (D) BuOH, (E) $CHCl_3$, (F) MeOH, (G) H_2O extracts, and (H) doxorubicin (positive control) in HeLa, HepG-2, and MCF-7 cell lines. The data shown are means ± SD obtained from three independent experiments.

Table II. Antiproliferative activities of phytoecdysteroids **1**–**3** and extracts of *S. guntensis* in HeLa, HepG-2, and MCF-7 cell lines. The data are presented as IC_{50} values (means ± SD).

Sample	IC_{50}		
	HeLa	HepG-2	MCF-7
2,3-Diacetate-22-benzoate-20-hydroxyecdysone (**1**) [μM]	127.97 ± 11.34	106.76 ± 7.81	203.10 ± 19.56
2-Deoxy-20-hydroxyecdysone (**2**) [μM]	174.88 ± 19.10	195.61 ± 17.26	126.54 ± 12.09
20-Hydroxyecdysone (**3**) [μM]	175.02 ± 16.34	130.26 ± 12.87	168.33 ± 25.30
Chloroform extract [μg/mL]	26.58 ± 1.88	20.99 ± 1.64	18.89 ± 2.36
Methanol extract [μg/mL]	65.81 ± 2.78	64.59 ± 3.98	83.86 ± 8.04
n-Butanol extract [μg/mL]	84.08 ± 3.30	83.21 ± 7.12	80.83 ± 0.88
Water extract [μg/mL]	106.70 ± 0.84	80.47 ± 7.92	70.97 ± 5.00
Doxorubicin [μM] (positive control)	1.84 ± 0.19	0.67 ± 0.07	0.48 ± 0.04
Doxorubicin [μg/mL]	1.07 ± 0.11	0.39 ± 0.04	0.28 ± 0.02

(26.58 ± 1.88) μg/mL on HeLa, (20.99 ± 1.64) μg/mL on HepG-2, and (18.89 ± 2.36) μg/mL on MCF-7 cells]. As compared to doxorubicin (IC_{50} between 0.28 to 1.07 μg/mL) the extracts and isolated compounds showed moderate cytotoxicity. In general, the IC_{50} values of extracts were much lower in all tested cell lines as compared to the pure isolated compounds (Fig. 4) indicating that further cytotoxic secondary metabolites must be present.

The antioxidant and radical scavenging activities of the isolated compounds, extracts, and quercetin (as positive control) are summarized in Table III. Maximum scavenging activity of DPPH* was observed with the water extract (IC_{50} 68.90 μg/mL), followed by the activity of the *n*-butanol, methanol, and chloroform extracts with IC_{50} values of 69.12, 122.48, and 148.28 μg/mL, respectively (Fig. 5). The activity of 20-hydroxyecdysone (**3**), 2-deoxy-20-hydroxyecdysone (**2**), and 2,3-diacetate-22-benzoate-20-hydroxy-

Table III. Antioxidant activity of pure isolated phytoecdysteroids **1**–**3** and extracts of *S. guntensis* using the DPPH* radical scavenging assay. The data are represented as IC_{50} values (means ± SD).

Sample	IC_{50} [μg/mL]
2,3-Diacetate-22-benzoate-20-hydroxyecdysone (**1**)	291.38 ± 25.31
2-Deoxy-20-hydroxyecdysone (**2**)	157.29 ± 16.72
20-Hydroxyecdysone (**3**)	144.75 ± 11.53
Chloroform extract	148.28 ± 13.82
Methanol extract	122.48 ± 11.09
n-Butanol extract	69.12 ± 5.85
Water extract	68.90 ± 6.45
Quercetin (positive control)	3.37 ± 0.77

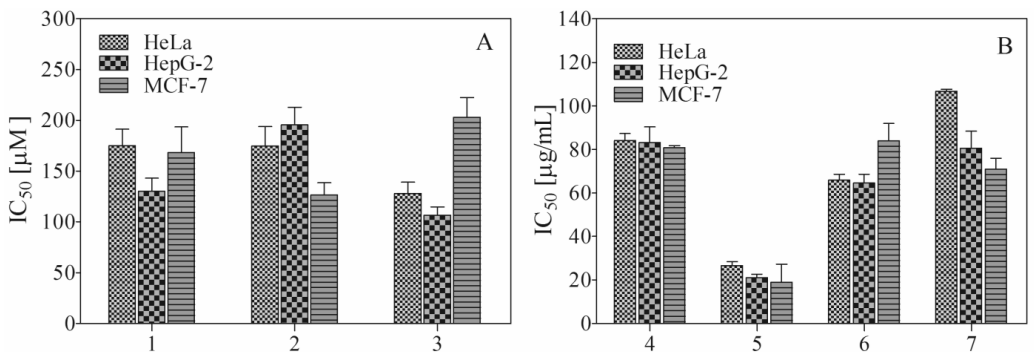


Fig. 4. Antiproliferative activities of (A) isolated phytoecdysteroids 2,3-diacetate-22-benzoate-20-hydroxyecdysone (1), 2-deoxy-20-hydroxyecdysone (2), and 20-hydroxyecdysone (3), and (B) BuOH (4), $CHCl_3$ (5), MeOH (6), and H_2O (7) extracts from *S. guntensis* in human tumour cell lines. The data are presented as IC_{50} values (means ± SD).

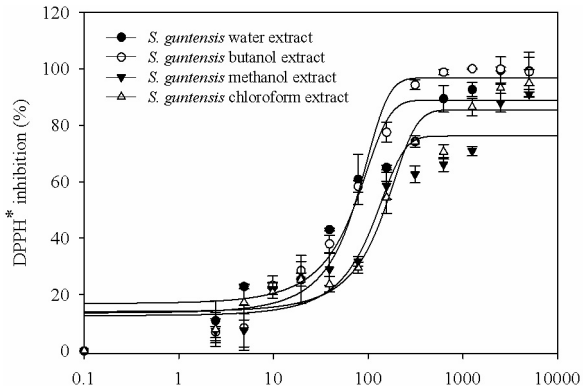


Fig. 5. Dose-response curve for DPPH* scavenging activity of H_2O, BuOH, MeOH, and $CHCl_3$ extracts of *S. guntensis*.

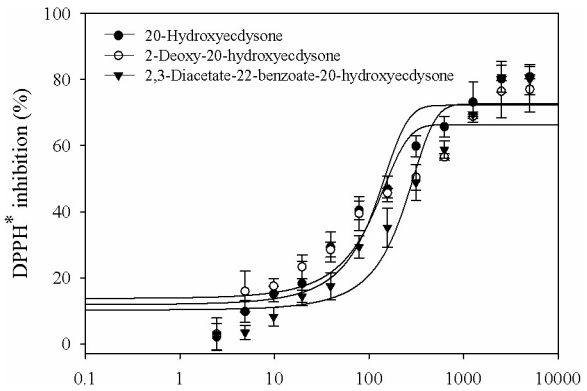


Fig. 6. Dose-response curve for DPPH* scavenging activity of 20-hydroxyecdysone (**3**), 2-deoxy-20-hydroxyecdysone (**2**), and 2,3-diacetate-22-benzoate-20-hydroxyecdysone (**1**).

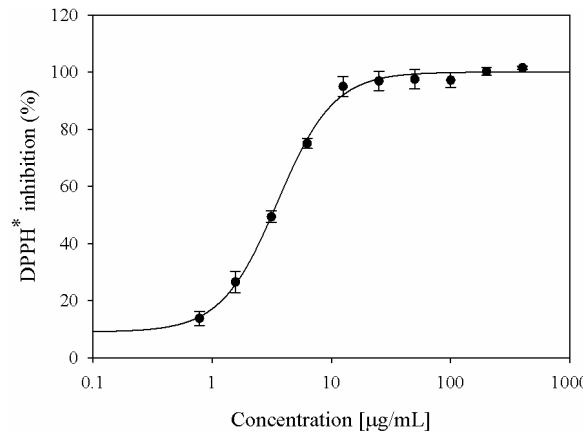


Fig. 7. Dose-response curve for DPPH* scavenging activity of quercetin (positive control), IC_{50} was 3.32 μg/mL.

ecdysone (**1**) were 144.75, 157.29, and 291.38 μg/mL, respectively (Fig. 6). The activity of quercetin was 3.37 μg/mL (Fig. 7).

Discussion

Phytoecdysteroids **1**–**3** (Fig. 2) were isolated from the aerial parts of *S. guntensis* for the first time. Compound **1** (2,3-diacetate-22-benzoate-20-hydroxyecdysone) could be described as a novel natural compound; it had previously been prepared by chemical synthesis (Saatov *et al.*, 1986). Phytoecdysteroids **2** (2-deoxy-20-hydroxyecdysone) and **3** (20-hydroxyecdysone) had been reported before (Saatov *et al.*, 1979; Chong *et al.*, 1970). The presence of acetate and benzoate derivatives of phytoecdysteroids is typical for *Silene*. A large number of acetate derivatives of phytoecdysteroids have been described, and more than 30 acetates have been isolated from different species of *Coronaria*, *Lychnis*, *Melandrium, Silene* (Caryophyllaceae), *Cyanothis* (Commelinaceae), *Rhaponticum* and *Serratula* (Asteraceae), *Ajuga* and *Vitex* (Lamiaceae) (Lafont *et al.*, 2002). About ten benzoate derivatives of ecdysteroids have already been described from *Silene* and *Rhaponticum* (Saatov *et al.*, 1993). In *Silene* they occur as 2-deoxy- and 20-hydroxyecdysone benzoates (mainly 22-benzoates). The simultaneous presence of acetate and benzoate groups in the ecdysteroid molecule is reported here for the first time in a plant, and this demonstrates the combinatorial character of ecdysteroid biosynthesis.

The isolated phytoecdysteroids displayed mild antiproliferative activity against HeLa, HepG-2, and MCF-7 cell lines, while the extracts were more active. Particularly the chloroform extract of *S. guntensis* exhibited a remarkable cytotoxic activity against all tested cell lines (Figs. 3 and 4), and this may be due to lipophilic compounds other than ecdysteroids that easily penetrate the cell membrane, affect membrane fluidity, or interact with membrane proteins (Wink, 2008). Flavonoids, triterpene saponins, ecdysteroids, and polysaccharides are common constituents in the genus *Silene*. In particular the saponins may be responsible for the cytotoxic effects of the extracts. The saponins jenisseensosides C and D from *Silene fortunei* stimulated the proliferation of Jurkat tumour cells at low concentrations; at high concentrations they were cytotoxic and apparently induced apoptosis (Gaidi *et al.*, 2002). The extract

of *Silene viridiflora* exerted antitumour activity *in vivo* (Zibareva, 2003).

The phytoecdysteroids cyasterone, polypodine B, and decumbesterone A showed potent antitumour activities in a mouse-skin model *in vivo* in a two-stage carcinogenesis trial, using 7,12-dimethylbenz[*a*]anthracene as initiator and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as promoter (Takasaki *et al.*, 1999). However, Lagova and Valueva (1981) reported that 20-hydroxyecdysone was mainly ineffective in preventing tumour growth in mice, but it stimulated the growth of mammary gland carcinomas. Because ecdysteroids structurally resemble sex hormones, they may bind to steroid hormone receptors in mammals and stimulate the growth of hormone-dependent tumours.

Many natural products, especially polyphenols, have antioxidant properties. The antioxidative properties of natural compounds or plant extracts can result from metal chelating, free radical scavenging (hydrogen-donating capability or free radical quenching), or all these properties together. The extracts of *S. guntensis* were able to reduce DPPH* in a concentration-dependent manner. The maximum scavenging activity was produced with the water extract with an IC_{50} value of 68.90 μg/mL followed by the activity of the *n*-butanol, methanol, and chloroform extracts with IC_{50} values of 69.12, 122.48, and 148.28 μg/mL, respectively. We assume that water and *n*-butanol extracts of *S. guntensis* contain flavonoids, known for their strong antioxidant activity. The isolated ecdysteroids were less active, as expected from their chemical structures. These observations are contradictory to what was found in other studies (Osinskaia *et al.*, 1992). Miliauskas and coworkers (2005) reported mild radical scavenging activity for 20-hydroxyecdysone. However, they suggested that the antioxidant effect might be attributed to some co-eluting phenolic compounds other than 20-hydroxyecdysone. The latter explanation seems to be more convincing, since the structure of ecdysteroid molecules is unlikely to exert an antioxidant effect, as compared to the common antioxidative flavonoids (Lu and Yeap, 2001).

Acknowledgements

Financial support by DAAD for a research grant to N. Z. M. is gratefully acknowledged. The authors express their special thanks to Kh. Bobokulov for recording the ^{1}H and ^{13}C NMR spectra. M. Z. E. R. thanks the Egyptian Ministry of Higher Education and Scientific Research for providing financial support. Theodor C. H. Cole improved the English and made valuable suggestions.

Ashour M. L., El-Readi M., Youns M., Mulyaningsih S., Sporer F., Efferth T., and Wink M. (2009), Chemical composition and biological activity of the essential oil obtained from *Bupleurum marginatum* (Apiaceae). J. Pharm. Pharmacol. **61**, 1079–1087.

Bondarenko O. N. (1971), Identification guide of plants of Central Asia. Tashkent. Fan Publ. **2**, 253.

Brand-Williams W., Cuvelier M., and Berset C. (1995), Use of a free-radical method to evaluate antioxidant activity. Lebensm.-Wiss. Technol. **28**, 25–30.

Chong Y. K., Galbraith M. N., and Horn D. H. S. (1970), Isolation of deoxycrustecdysone, deoxyecdysone and α-ecdysone from the fern *Blechnum minus*. J. Chem. Soc. Chem. Commun., 1217–1220.

El-Mofty M. M., Sakre S. A., Rizk A. M., and Moussa E. A. (1994), Induction of breast and lung neoplastic lesions in mice by alpha-ecdysone. Oncol. Rep. **1**, 435–438.

Gaidi G., Miyamoto T., Laurens V., and Lacaille-Dubois M. A. (2002), New acylated triterpene saponins from *Silene fortunei* that modulate lymphocyte proliferation. J. Nat. Prod. **65**, 1568–1572.

Gasiorowski K., Brokos B., Glensk M., and Schöpke T. (1999), Immunomodulatory activity of the saponin-rich fraction from roots of *Silene vulgaris* Garcke: initial study. Pharmazie **54**, 864–866.

Kubo I., Klocke J. A., and Asano S. (1983), Effects of ingested phytoecdysteroids on the growth and development of two lepidopterous larvae. J. Insect Physiol. **29**, 307–316.

Kurmukov A. G. and Yermishina O. A. (1991), Effect of ecdysterone on experimental arrhythmias, changes in hypodynamics and contractility of the myocardium produced by a coronary artery occlusion. Farmakol. Toksikol. (Moscow) **54**, 27–29.

Kuzmenko A. I., Morozova R. P., Nikolenko I. A., Korniets G. V., and Kholodova Y. D. (1997), Effect of vitamin D3 and ecdysterone on free radical lipid peroxidation. Biochemistry (Moscow) **62**, 609–612.

Lafont R., Harmatha J., Marion-Poll F., Dinan L., and Wilson I. D. (2002), Ecdybase – The Ecdysone Handbook, 3rd ed. (continuously updated). Available at: http://ecdybase.org/ (accessed April 21, 2010).

Lagova N. D. and Valueva I. M. (1981), Effect of ecdysterone isolated from *Rhaponticum carthamoides* on the growth of experimental tumours. Eksp. Onkol. (Kiev) **3**, 69–71.

Lu Y. and Yeap F. L. (2001), Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food Chem. **75**, 197–202.

Mabberley D. (2008), The Plant-Book, 3rd ed. Cambridge University Press, Cambridge, New York.

Miliauskas G., van Beek T. A., de Waard P., Venskutonis R. P., and Sudhölter E. J. R. (2005), Identification of radical scavenging compounds in *Rhaponticum carthamoides* by means of LC-DAD-SPE-NMR. J. Nat. Prod. **68**, 168–172.

Mosmann T. (1983), Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods **65**, 55–63.

Osinskaia L. F., Saad L. M., and Kholodova I. (1992), Antiradical properties and antioxidant activity of ecdysterone. Ukr. Biokhim. Zh. **64**, 114–117.

Plotnikov M. B., Zibareva L. N., Koltunov A. A., Aliev O. I., Yakimova T. V., and Maslov M. Yu. (1998), The hemorheologic properties of the extracts from some plants containing ecdysteroids. Rastit. Resyr. (St. Peterburg) **34**, 91–97.

Saatov Z., Usmanov B. Z., and Abubakirov N. K. (1979), Phytoecdysone of *Silene praemixta*. Chem. Nat. Compd. **15**, 700–701.

Saatov Z., Gorovits M. B., Melibaev S., and Abubakirov N. K. (1986), Phytoecdysteroids of genus *Silene*. Ecdysterone-22-benzoate from *Silene scabrifolia*. Khim. Prir. Soedin., 77–79.

Saatov Z., Gorovits M. B., and Abubakirov N. K. (1993), Phytoecdysteroids from plants of genus *Silene*. Chem. Nat. Compd. **29**, 551–557.

Sláma K., Koudela K., Tenora J., and Mathova A. (1996), Insect hormones in vertebrates: anabolic effects of 20-hydroxyecdysone in Japanese quails. Experientia **52**, 702–706.

Takasaki M., Tokuda H., Nishino H., and Konoshima T. (1999), Cancer chemopreventive agents (antitumour promoters) from *Ajuga decumbens*. J. Nat. Prod. **62**, 972–975.

Vivek L., Bajpai K., Shukla S., and Kang S. Ch. (2008), Chemical composition and antifungal activity of essential oil and various extracts of *Silene armeria*. Bioresour. Technol. **99**, 8903–8908.

Vvedenskiy A. I. (1953), Flora of Uzbekistan. Tashkent, AS RUz Publ. **2**, 391.

Wink M. (2008), Evolutionary advantage and molecular modes of action of multi-component mixtures used in phytomedicine. Curr. Drug Metab. **9**, 996–1009.

Zibareva L. (2003), Phytoecdysteroids of the family Caryophyllaceae. Doctoral Thesis, Tomsk University, p. 31.

Zibareva L., Yeriomina V. I., Munkhjargal N., Girault J. P., Dinan L., and Lafont R. (2009), The phytoecdysteroid profiles of 7 species of *Silene* (Caryophyllaceae). Arch. Insect Biochem. Physiol. **72**, 234–248.