Major Constituents and Cytotoxic Effects of *Ajuga chamaecistus* ssp. *tomentella*

Nargess Sadati^a, Kristina Jenett-Siems^b, Karsten Siems^c, Mohammad Reza Shams Ardekani^a, Abbas Hadjiakhoondi^a, Tahmineh Akbarzadeh^d, Seyed Nasser Ostad^e, and Mahnaz Khanavi^{a,*}

^a Department of Pharmacognosy and Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14155-6451, Iran.
Fax: +98 21 66954706. E-mail: khanavim@sina.tums.ac.ir

^b Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin, Berlin, Germany

^c Analyticon Discovery GmbH, Potsdam, Germany

^d Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14155-6451, Iran

^e Department of Toxicology and Pharmacology and Pharmaceutical Science Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14155-6451, Iran

* Author for correspondence and reprint requests

Z. Naturforsch. **67c**, 275–281 (2012); received May 29, 2011/January 8, 2012

The *n*-butanolic fraction of a methanolic extract (80%) from aerial parts of *Ajuga chamaecistus* ssp. *tomentella* was analysed using different chromatographic methods. Column (CC) and high-performance liquid chromatography (HPLC) were used for isolation and purification. ¹³C, ¹H NMR, H-H COSY, HSQC, HMBC, and ESI-MS were employed for identification of the compounds isolated from this fraction. The structures of the compounds were determined to be *cis*-melilotoside (**1**), *trans*-melilotoside (**2**), lavandulifolioside (**3**), 20-hydroxyecdysone (**4**), leonoside B (**5**), martynoside (**6**), ajugalactone (**7**), makisterone A (**8**), and 24-dehydroprecyasterone (**9**). This is the first report on the presence of *cis*- and *trans*-melilotoside in *Ajuga* species. Cytotoxic evaluation of the *n*-butanolic fraction, *cis*- and *trans*-melilotoside against cancer (T47D, HT-29, and Caco-2) and normal (NIH 3T3) cell lines by the mitochondrial tetrazolium test (MTT) showed no cytotoxic effects up to 400 µg/mL. The results of this study suggest that melilotoside, phenylethyl glycosides, and phytoecdysteroids are the main constituents of the *n*-butanolic fraction of *Ajuga chamaecistus* ssp. *tomentella*.

Key words: *Ajuga chamaecistus* ssp. *tomentella*, Melilotoside, Cytotoxic Effect

Introduction

More than one hundred species including fifty varieties and subspecies of *Ajuga* (Lamiaceae) are distributed throughout the world. The genus *Ajuga* with the common name bugle is found in China, Korea, Japan, and throughout Europe. Five species of this annual and perennial genus are found in Iran, of which *Ajuga chamaecistus* contains several exclusive subspecies, including *A. chamaecistus* ssp. *tomentella* (Mozaffarian, 2007). Some species belonging to this genus are used in traditional medicine of different countries of the world, including Iran, for treatment of joints pains, gout, and jaundice (Naghbi *et al.*, 2005). A broad range of biological effects has been reported from different species of *Ajuga* such as hypoglycemic (Hilaly and Lyoussi, 2002), treatment of

joint disease (Ono *et al.*, 2008), anti-inflammatory (Gautam *et al.*, 2011), and antimalarial (Kuria *et al.*, 2001). Many phytochemical studies on *Ajuga* species were performed which led to the isolation of phytoecdysteroids (Vanyolos *et al.*, 2009; Castro *et al.*, 2008), diterpenoids (Coll, 2002), iridoids (Manguro *et al.*, 2007), and phenylethyl glycosides (Akbay *et al.*, 2003). The aim of the present study was a phytochemical investigation of the *n*-butanolic fraction obtained from aerial parts of *Ajuga chamaecistus* ssp. *tomentella*, collected in Tehran (Iran), which has not been previously reported. Furthermore, we examined the cytotoxicity of the *n*-butanolic fraction and two major constituents, isolated from this fraction, against cancer and normal cell lines (T47D, Caco-2, HT-29, and NIH 3T3) by the MTT assay.

Material and Methods

General experimental procedures

^1H and ^{13}C NMR spectroscopy of compounds **1** and **2** were performed in CD_3OD on a Bruker Avance DPX 400 spectrometer (Karlsruhe, Germany) [400 MHz, tetramethylsilane (TMS) as internal standard]. ^1H and ^{13}C NMR spectra of compounds **3–9** were acquired in CD_3OD on a Jeol ECX-400 spectrometer (Peabody, MA, USA) (400 MHz, TMS as internal standard). ^1H - ^1H COSY, HMBC, and HSQC spectra were obtained on a Bruker DRX 500 MHz spectrometer. ESI-mass spectra were recorded on an Agilent 6210 ESI-TOP spectrometer (Santa Clara, CA, USA). Column chromatography (CC) was performed using Sephadex LH-20 (45 $\times$ 4.5 cm, lipophilic Sephadex, 25–100 μm ; Sigma, Dorset, UK) and RP-18 (30 $\times$ 4.5 cm, Lichroprep RP-18, 40–63 μm ; Merck, Darmstadt, Germany) columns. Analytical and preparative high-performance liquid chromatography (HPLC) separations were performed on a Shimadzu LC-10AD pumping system (Kyoto, Japan) with a Shimadzu variable wavelength detector (220 nm) equipped with a Knauer (Berlin, Germany) Eurospher 100 C-18 (7 μm , 250 $\times$ 4 mm) and Nucleosil 300-C18 (10 μm , 250 $\times$ 16 mm) column, respectively.

Plant material

Aerial parts of *Ajuga chamaecistus* Ging. ssp. *tomentella* (Boiss.) Rech. f. were collected from “Sorkhe Hesar”, east of Tehran, Iran, in June 2008 and verified by Prof. G. Amin. A voucher specimen (THE-6697) has been deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Extraction and isolation

The air-dried and ground plants of *A. chamaecistus* ssp. *tomentella* (1 kg) were extracted with 80% methanol (7 $\times$ 2.5 L) at room temperature and concentrated under reduced pressure to give a dark brown extract (180 g). The extract (150 g) was defatted through repeated extraction with *n*-hexane. The defatted extract was partitioned successively between 80% methanol, diethyl ether, and *n*-butanol. Twenty g of the *n*-butanolic fraction were loaded on a Sephadex LH-20 (150 g) column and eluted with aqueous methanol (60%)

to afford 4 fractions. Fraction 2 (10 g) was chromatographed on an RP-18 column, under medium pressure, and eluted with a gradient of water and methanol (80:20–50:50 v/v) to afford 5 fractions (B–F). Fraction B (8 g) was rechromatographed on RP-18 material with a gradient of aqueous methanol (5%, 20%, and 50%) to give 3 fractions (B_1 – B_3). B_1 (200 mg) was subjected to semipreparative reversed phase (RP)-HPLC using gradient elution with aqueous methanol (10%–35%; flow-rate, 1 mL/min; time per run, 30 min) to afford compound **1** (14 mg). Compound **2** (20 mg) was obtained by preparative RP-HPLC and a gradient of aqueous methanol (30%–35%) within 40 min from fraction B_2 (190 mg). Fraction B_3 (2.5 g) was chromatographed on an RP-18 column eluted with a gradient of water/methanol (80:20–50:50 v/v) to give 9 fractions ($B_{3.1}$ – $B_{3.9}$). Purification of $B_{3.2}$ (200 mg) using preparative RP-HPLC with a gradient of aqueous methanol (30%–60%; flow-rate, 7 mL/min) within 60 min afforded compound **3** (5 mg) and compound **4** (80 mg). Fraction $B_{3.7}$ (144 mg) was subjected to RP-HPLC with aqueous methanol (35%–60%) to give compounds **5** (13.5 mg) and **6** (17.6 mg) within 60 min. Fraction C (200 mg) was purified by RP-HPLC with a gradient of aqueous methanol (30%–60%) within 60 min to obtain compound **7** (8 mg) and compound **6** (11.5 mg). Fraction C (100 mg) was further purified by RP-HPLC using a gradient of water/acetonitrile (80:20–65:35) to afford compound **8** (1 mg) and compound **9** (1 mg). Direct sunlight was excluded during extraction and purification of the compounds.

cis-Melilotoside (**1**): ^1H NMR (400 MHz, CD_3OD): δ = 7.52 (1H, *d*, *J* = 7.46 Hz, H-6), 7.31 (1H, *d*, *J* = 12.52 Hz, H-7), 7.28 (1H, *dd*, *J* = 8.56 1.24, Hz, H-4), 7.18 (1H, *d*, *J* = 8.28 Hz, H-3), 6.95 (1H, *t*, *J* = 7.47 Hz, H-5), 5.95 (1H, *d*, *J* = 12.52 Hz, H-8), 4.93 (1H, *d*, *J* = 7.24 Hz, Glu-H-1'), 3.87 (1H, Ha-6'), 3.68 (1H, Hb-6'), 3.47 (1H, H-3'), 3.45 (1H, H-2'), 3.40 (2H, H-4', H-5'). – ^{13}C NMR (CD_3OD): δ = 138.42 (C-7), 130.48 (C-4), 130.27 (C-6), 121.64 (C-5), 120.02 (C-8), 115.27 (C-3); glucose: 101.43 (C-1'), 77.04 (C-5'), 76.95 (C-3'), 73.76 (C-2'), 70.00 (C-4'), 61.32 (C-6'). – ESI-TOF-MS (positive): *m/z* = 349.08 $[\text{M}+\text{Na}]^+$, 365.05 $[\text{M}]^+$.

trans-Melilotoside (**2**): ^1H NMR (400 MHz, CD_3OD): δ = 8.14 (1H, *d*, *J* = 16.20 Hz, H-7), 7.64 (1H, *d*, *J* = 7.68 Hz, H-6), 7.42 (1H, *m*, H-4), 7.26

(1H, *m*, H-3), 7.07 (1H, *t*, *J* = 7.48 Hz, H-5), 6.54 (1H, *d*, *J* = 16.2 Hz, H-8), 5.00 (1H, *d*, *J* = 7.68 Hz, Glu-H-1'). – ESI-TOF-MS (positive): *m/z* = 349.08 [M+Na]⁺, 365.05 [M]⁺.

Lavandulifolioside (**3**): ¹H NMR (400 MHz, CD₃OD): see Table I. – ESI-TOF-MS (positive): *m/z* = 779.2384 [M+Na]⁺.

20-Hydroxyecdysone (**4**): ¹H NMR (400 MHz, CD₃OD): δ = 5.79 (1H, *d*, *J* = 2.3 Hz, H-7), 3.95 (1H, *br s*, H_{eq}-3), 3.82 (1H, *m*, H_{ax}-2), 3.13 (1H, *br t*, H-9), 2.37 (1H, *dd*, H-5), 1.19 (6H, *s*, Me-26,27), 1.18 (3H, *s*, Me-21), 0.94 (3H, *s*, Me-19), 0.86 (3H, *s*, Me-18). – ESI-TOF-MS (positive): *m/z* = 503.298 [M+Na]⁺, 983.607 [M₂+Na]⁺.

Leonoside B (**5**): ¹H NMR (400 MHz, CD₃OD): see Table I. – ESI-TOF-MS (positive): *m/z* = 807.27 [M+Na]⁺, 823.24 [M+K]⁺.

Martynoside (**6**): ¹H NMR (400 MHz, CD₃OD): see Table I. – ESI-TOF-MS (positive): *m/z* = 675.23 [M+Na]⁺, 691.20 [M+K]⁺.

Ajugalactone (**7**): This is the first report on NMR data of ajugalactone achieved in CD₃OD. ¹H NMR (400 MHz, CD₃OD): δ = 5.95 (1H, *J* = 2.56 Hz, H-7), 4.19 (1H, *dd*, *J* = 12.96, 4.16 Hz, H-22), 3.95 (1H, *br s*, H-3), 3.79 (1H, *m*, H-2), 3.55 (1H, *br t*, *J* = 10.9 Hz, H-9), 2.92 (1H, *br t*, *J* = 10.2 Hz, H-17), 2.79 (1H, *q*, H_a-11), 2.38 – 2.32 (2H, *m*, H-23, 28), 2.10 – 2.04 (1H, *m*, H_a-16), 1.76 – 1.72 (1H, *m*, H_b-16), 2.23 – 2.17 (1H, *m*, H_a-15), 1.85 (3H, *br s*, Me-18), 1.76 – 1.72 (1H, *m*, H_b-15), 1.64 (1H, *d*, *J* = 4.4 Hz, H_a-1), 1.47 (1H, *br s*, H_b-1), 1.25 (3H, *s*, Me-26), 1.21 (3H, *s*, Me-18), 1.12 (3H, *t*, *J* = 7.64 Hz, Me-29), 1.07 (3H, *s*, Me-19). – ¹³C NMR (500 MHz, CD₃OD): δ = 202.1 (C-6), 123.7 (C-7), 162.2 (C-8), 210.2 (C-12), 89.4

Table I. ¹H NMR spectral data of **3**, **5**, and **6**^a.

	H	3	5	6
Aglycone	H-C(2)	6.68 (<i>d</i> , <i>J</i> = 2.08)	6.72 (<i>d</i> , <i>J</i> = 2.08)	6.71 (<i>d</i> , <i>J</i> = 2.08)
	H-C(5)	6.66 (<i>d</i> , <i>J</i> = 7.92)	6.81 (<i>d</i> , <i>J</i> = 4.64)	6.81 (<i>d</i> , <i>J</i> = 4.4)
	H-C(6)	6.56 (<i>dd</i> , <i>J</i> = 8.12, 2.08)	6.68 (<i>dd</i> , <i>J</i> = 8.12, 2.08)	6.68 (<i>d</i> , <i>J</i> = 8.12, 2.12)
	CH ₂ (α)	4.04, 3.77 – 3.23	4.08, 3.73 (<i>m</i>)	4.07, 3.77 – 3.35 (<i>m</i>)
	CH ₂ (β)	2.79 (<i>br t</i> , <i>J</i> = 6.96)	2.82 (<i>br t</i> , <i>J</i> = 7.44)	2.82 (<i>br t</i> , <i>J</i> = 7.4)
	CH ₃ O	–	3.86 (<i>s</i>)	3.86 (<i>s</i>)
	CH ₃ O	–	3.79 (<i>s</i>)	3.79 (<i>s</i>)
β-Glucose	H-C(1')	4.36 (<i>d</i> , <i>J</i> = 7.92)	4.37 (<i>d</i> , <i>J</i> = 8.12)	4.37 (<i>d</i> , <i>J</i> = 8.12)
	H-C(2')	3.77 – 3.23	3.70 – 3.47	3.77 – 3.35
	H-C(3')	3.77 – 3.23	3.70 – 3.47	3.77 – 3.35
	H-C(4')	4.92 (<i>t</i>)	4.94 (<i>t</i>)	4.92 (<i>t</i>)
	H-C(5')	3.77 – 3.23	3.70 – 3.47	3.77 – 3.35
	H-C(6' _A)	3.77 – 3.23	3.70 – 3.47	3.77 – 3.35
	H-C(6' _B)	3.85 (<i>dd</i> , <i>J</i> = 12.96, 2.4)	3.85	3.77 – 3.35
α-Rhamnose	H-C(1'')	5.46 (<i>br s</i>)	5.47 (<i>d</i> , <i>J</i> = 1.4)	5.18 (<i>d</i> , <i>J</i> = 1.6)
	H-C(2'')	3.92 (<i>dd</i> , <i>J</i> = 3.24, 1.36)	3.93 (<i>br s</i>)	3.92 (<i>br s</i>)
	H-C(3'')	3.77 – 3.23	3.70 – 3.47	3.77 – 3.35
	H-C(4'')	3.77 – 3.23	3.70 – 3.47	3.77 – 3.35
	H-C(5'')	3.77 – 3.23	3.70 – 3.47	3.77 – 3.35
	CH ₃ (6'')	1.05 (<i>d</i> , <i>J</i> = 6.28)	1.05 (<i>d</i> , <i>J</i> = 6.28)	1.08 (<i>d</i> , <i>J</i> = 6.24)
	H-C(1''')	4.29 (<i>d</i> , <i>J</i> = 7.2)	4.30 (<i>d</i> , <i>J</i> = 7.44)	–
α-Arabinose	H-C(2''')	3.77 – 3.23	3.70 – 3.47	–
	H-C(3''')	3.77 – 3.23	3.70 – 3.47	–
	H-C(4''')	3.77 – 3.23	3.75 (<i>br s</i>)	–
	H-C(5''')	3.77 – 3.23	3.70 – 3.47	–
	H-C(2''''')	7.03 (<i>d</i> , <i>J</i> = 1.84)	7.18 (<i>d</i> , <i>J</i> = 1.8)	7.18 (<i>d</i> , <i>J</i> = 1.84)
	H-C(5''''')	6.76 (<i>d</i> , <i>J</i> = 8.12)	6.79 (<i>d</i> , <i>J</i> = 4.64)	6.79 (<i>d</i> , <i>J</i> = 4.64)
	H-C(6''''')	6.95 (<i>dd</i> , <i>J</i> = 8.36, 2.08)	7.07 (<i>dd</i> , <i>J</i> = 8.36, 1.88)	7.07 (<i>dd</i> , <i>J</i> = 8.36, 1.88)
Caffeic acid	H-C(α')	6.27 (<i>d</i> , <i>J</i> = 15.8)	6.37 (<i>d</i> , <i>J</i> = 16)	6.37 (<i>d</i> , <i>J</i> = 16)
	H-C(β')	7.59 (<i>d</i> , <i>J</i> = 15.88)	7.66 (<i>d</i> , <i>J</i> = 16)	7.66 (<i>d</i> , <i>J</i> = 15.76)

^a The spectra were measured in CD₃OD (400 MHz). Chemical shifts in ppm relative to the internal standard TMS; *J* in Hz.

(C-14), 83.3 (C-22), 168.2 (C-27), 154.8 (C-24), 121.5 (C-25). – ESI-TOF-MS (positive): m/z = 539.26 $[M+Na]^+$, 555.23 $[M+K]^+$, 1055.53 $[M_2+Na]^+$.

Makisterone A (8): 1H NMR (400 MHz, CD_3OD): δ = 5.79 (1H, *d*, J = 2.5 Hz, H-7), 1.17 (3H, *s*, Me-21), 1.14 (3H, *s*, Me-26), 1.11 (3H, *s*, Me-27), 0.94 (3H, *s*, Me-19), 0.92 (3H, *d*, J = 6.72 Hz, Me-28), 0.87 (3H, *s*, Me-18). – ESI-TOF-MS (positive): m/z = 517.32 $[M+Na]^+$.

24-Dehydroprecyasterone (9): 1H NMR (400 MHz, CD_3OD): δ = 5.80 (1H, *d*, J = 2.3 Hz, H-7), 1.83 (3H, *d*, J = 2.0 Hz, Me-26), 1.31 (3H, *s*, Me-21), 1.28 (3H, *d*, J = 6.4 Hz, Me-19), 0.94 (3H, *s*, Me-19), 0.87 (3H, *s*, Me-18). – ESI-TOF-MS (positive): m/z = 541.28 $[M+Na]^+$.

Cell culture

The colon carcinoma (HT-29), colorectal adenocarcinoma (Caco-2), and breast ductal carcinoma (T47D) cell lines, respectively, were maintained as exponentially growing cultures in RPMI 1640 cell culture medium (PAA, Pasching, Austria) supplemented with 10% fetal bovine serum (FBS; PAA) for HT-29 cells and 15% FBS for Caco-2 and T47D cells. The Swiss mouse embryo fibroblast (NIH 3T3) cell line was kept in Dulbecco's modified Eagle's medium (DMEM; PAA) supplemented with 10% FBS. One hundred IU/mL penicillin and 100 μ g/mL streptomycin (Roche, Penzberg, Germany) were added to the media. All cell lines were cultured at 37 °C in air/ CO_2 (95:5 v/v) atmosphere.

Determination of cell viability by the MTT assay

Cytotoxic activities of the *n*-butanolic fraction, *cis*- and *trans*-melilotoside from *Ajuga chamaecistus* ssp. *tomentella* against breast ductal carcinoma (T47D), colon carcinoma (HT-29), colorectal adenocarcinoma (Caco-2), and Swiss mouse embryo fibroblast (NIH 3T3) cell lines were performed according to our previous study (Khanavi *et al.*, 2010) by the mitochondrial tetrazolium test (MTT).

Results and Discussion

Isolated compounds **1–9** from the *n*-butanolic fraction of the total methanolic extract of aerial parts of *Ajuga chamaecistus* ssp. *tomentella* were identified by comparison of their NMR (1H , ^{13}C

NMR, HMBC, HSQC and 1H - 1H COSY) and ESI-mass spectral data with those reported in the literature.

Compounds **1** and **2** (Fig. 1) were identified as *cis*-melilotoside and *trans*-melilotoside, respectively. This is the first report on the occurrence of *cis*- and *trans*-melilotosides in the genus *Ajuga*. Melilotoside is a coumaric acid derivative which was reported for the first time from *Melilotus altissima* and *M. arvensis* (Takaishi, 1968). *cis*-Melilotoside has been reported from several plants (Ferreira and Rodriguezde Oliveira, 2010; Yang *et al.*, 2007a, b), also there is an older report on the occurrence of both *cis*- and *trans*-forms of melilotoside in *Melilotus albus* depending on growing conditions (Kahnt, 1962). Antiprotozoal activity of melilotoside isolated from *Teloxys graveolens*, a medicinal plant for treatment of dysentery and diarrhea, has been shown (Calzada *et al.*, 2003).

Compounds **3**, **5**, and **6** (Fig. 1) were characterized as phenylethanoid glycosides, lavandulifolioside (**3**), leonoside B (**5**), and martynoside (**6**), by comparison of their spectral data with literature values (Basaran *et al.*, 1988; Calis *et al.*, 1992; Sasaki *et al.*, 1978). This group of phenolic compounds has interesting biological properties such as antimicrobial, antibacterial, cytotoxic, antioxidant, enzyme inhibitory, and immunomodulatory. Lavandulifolioside, a trisaccharide phenylethyl glycoside, showed inhibition of peroxy lipid formation (Jimenez and Riguera, 1994), a negative chronotropic effect, and decrease of blood pressure (Milkowska-Leyck *et al.*, 2002). Several pharmacological activities of martynoside have been reported, including antioxidant (Miao *et al.*, 2003) and estrogenic/antiestrogenic properties in breast cancer cells (Papoutsi *et al.*, 2006).

The isolated compounds **4** and **7–9** (Fig. 1) were identified as ecdysteroids, 20-hydroxyecdysone (**4**), ajugalactone (**7**), makisterone A (**8**), and 24-dehydroprecyasterone (**9**) by comparison of their spectral data with data in the literature (Wessner *et al.*, 1992; Imai *et al.*, 1968, 1970). The ecdysteroids are a large class of polyhydroxy-steroids isolated from both the animal and plant kingdom. Most of the *Ajuga* species have been used in traditional medicine all over the world. Several studies have shown that ecdysteroids isolated from *Ajuga* species are responsible for their biological activities. This group of natural products produces a wide range of pharmacological

Fig. 1. Molecular structures of *cis*-melilotoside (1), *trans*-melilotoside (2), lavandulfolioside (3), 20-hydroxyecdysone (4), leonoside B (5), martynoside (6), ajugalactone (7), makisterone A (8), and 24-dehydroprecyasterone (9), isolated from the *n*-butanolic fraction of *Ajuga chamaecistus* ssp. *tomentella*.

activities in mammals, including adaptogenic and anabolic, and shows increasing protein synthesis in muscles (Gorelick-Feldman *et al.*, 2010), anti-diabetic and hypoglycemic (Hamden *et al.*, 2008; Kutepova *et al.*, 2001), hepatoprotective, immunoprotective, wound-healing (Dinan, 2009), antioxidant, and free radical scavenging effects (Cai *et al.*, 2002), and perhaps even antitumour effects (Akabay *et al.*, 2002). 20-Hydroxyecdysone and cyasterone, in addition to ajugalactone, seem to be the most common compounds in *Ajuga* species (Ramazanov, 2005).

In the cytotoxic evaluation of the *n*-butanolic fraction, *cis*- (1) and *trans*-melilotosides (2) did not show cytotoxic effects up to 400 µg/mL against cancer (T47D, HT-29, and Caco-2) and normal (NIH 3T3) cell lines in the MTT assay.

Previous to this study, we isolated three major compounds (20-hydroxyecdysone, cyasterone, and

8-acetylharpagide) from the diethyl ether fraction of *Ajuga chamaecistus* ssp. *tomentella*, which were inactive in the cytotoxicity evaluation (Sadati *et al.*, 2012).

In conclusion, our study showed that *cis*- and *trans*-melilotosides, phenylethyl glycosides, and phytoecdysteroids can be considered major constituents of the *n*-butanolic fraction of *Ajuga chamaecistus* ssp. *tomentella*. According to these results, it can be stated that the *n*-butanolic fraction and two major compounds isolated from this fraction are not cytotoxic against cancer and normal cell lines.

Acknowledgements

This study was part of a PhD thesis funded and supported by Tehran University of Medical Sciences (TUMS) (grant No. 11302).

- Akabay P., Gertsch J., Calis I., Heilmann J., Zerbe O., and Sticker O. (2002), Novel antileukemic sterol glycosides from *Ajuga salicifolia*. *Helv. Chim. Acta* **85**, 1930–1942.
- Akabay P., Calis I., Heimann J., and Sticher O. (2003), Ionon, iridoid and phenylethanoid glycosides from *Ajuga salicifolia*. *Z. Naturforsch.* **58c**, 177–180.
- Basaran A. A., Calis I., Anklin C., Nishibe S., and Sticher O. (1988), Lavandulifolioside: A new phenylpropanoid glycoside from *Stachys lavandulifolia*. *Helv. Chim. Acta* **71**, 1483–1489.
- Cai Y.-J., Dai J.-Q., Fang J.-G., Ma L.-P., Hou L.-F., Yang L., and Liu Z.-L. (2002), Antioxidative and free radical scavenging effects of ecdysteroids from *Serratula stragulate*. *Can. J. Physiol. Pharmacol.* **80**, 1187–1194.
- Calis I., Ersoz T., Tasdemir D., and Ruedi P. (1992), Two phenylpropanoid glycosides from *Leonurus glaucescens*. *Phytochemistry* **31**, 357–359.
- Calzada F., Velazquez C., Cedillo-Rivera R., and Esquivel B. (2003), Antiprotozoal activity of the constituents of *Teloxys graveolens*. *Phytother. Res.* **17**, 731–732.
- Castro A., Coll J., Tandron Y. A., Pant A. K., and Mathela C. (2008), Phytoecdysteroids from *Ajuga macrosperma* var. *breviflora* roots. *J. Nat. Prod.* **71**, 1294–1296.
- Coll J. (2002), NMR shift data of neo-clerodane diterpenes from the genus *Ajuga*. *Phytochem. Anal.* **13**, 372–380.
- Dinan L. (2009), Phytoecdysteroids: what use are they? *Arch. Insect Biochem. Physiol.* **72**, 126–141.
- Ferreira F. P. and Rodrigues de Oliveira D. C. (2010), New constituents from *Mikania laevigata* Schult Bip. ex Baker. *Tetrahedron Lett.* **51**, 6856–6859.
- Gautam R., Jachak S. M., and Saklani A. (2011), Anti-inflammatory effect of *Ajuga bracteosa* Wall ex Benth. mediated through cyclooxygenase (COX) inhibition. *J. Ethnopharmacol.* **133**, 928–930.
- Gorelick-Feldman J., Cohick W., and Raskin I. (2010), Ecdysteroids elicit a rapid Ca²⁺ flux leading to Akt activation and increased protein synthesis in skeletal muscle cells. *Steroids* **75**, 632–637.
- Hamden K., Ayadi K., Jamoussi K., Masmoudi H., and Eifeki A. (2008), Therapeutic effect of phytoecdysteroids rich extract from *Ajuga iva* on alloxan induced diabetic rats liver, kidney and pancreas. *BioFactors* **33**, 165–175.
- Hilaly J. and Lyoussi B. (2002), Hypoglycemic effect of lyophilized aqueous extract of *Ajuga iva* in normal and streptozotocin diabetic rats. *J. Ethnopharmacol.* **80**, 109–113.
- Imai S., Hori M., Fujioka S., Murata E., Goto M., and Nakanishi K. (1968), Isolation of four new phytoecdysones, makisterone A, B, C, D and the structure of makisterone A, a C₂₈ steroid. *Tetrahedron Lett.*, 3883–3886.
- Imai S., Murata E., Fujioka S., Matsuoka T., Koreeda M., and Nakanishi K. (1970), Ajugalactone, an insect moulting inhibitor as tested by the *chio* dipping method. *J. Am. Chem. Soc.* **92**, 7512–7513.
- Jimenez C. and Riguera R. (1994), Phenylethanoid glycosides in plants: structure and biological activity. *Nat. Prod. Rep.* **11**, 591–606.
- Kahnt G. (1962), Isolierung des *trans*- und *cis*-o-Oxyzimtsäureglucosids aus Steinkleeblättern (*Melilotus albus*) und Umwandlung der *trans*-Form in die isomere Verbindung *in vivo* und *in vitro* durch Sonnenlicht. *Naturwissenschaften* **49**, 207–208.

- Khanavi M., Nabavi M., Sadati N., Shams Ardekani M. R., Sohrabipour J., Nabavi S. M. B., Ghaeli P., and Ostad S. N. (2010), Cytotoxic activity of some marine brown algae against cancer cell lines. *Biol. Res.* **43**, 31–37.
- Kuria K. A. M., Coster S., Muriuki G., Masengo W., Kibwage I., Hoogmartens J., and Laekeman G. M. (2001), Antimalarial activity of *Ajuga remota* Benth (Labiateae) and *Caesalpinia volkensii* Harms (Caesalpinaceae): *in vitro* confirmation of ethnopharmacological use. *J. Ethnopharmacol.* **74**, 141–148.
- Kutepova T. A., Syrov V. N., Khushbaktova Z. A., and Saatov Z. (2001), Hypoglycemic activity of the total ecdysteroid extract from *Ajuga turkestanica*. *Pharm. Chem. J.* (Translation of Khimiko-Farmatsevticheskii Zhurnal) **35**, 608–609.
- Manguro L. O. A., Ogur J. A., Okora D. M., Wagal S. O., and Lemen P. (2007), Further flavonol and iridoid glycosides from *Ajuga remota*. *J. Asian Nat. Prod. Res.* **9**, 617–629.
- Miao J., Wang W., Yao S., Navaratnam S., and Parsons B. J. (2003), Antioxidative properties of martynoside: pulse radiolysis and laser photolysis study. *Free Radical Res.* **37**, 829–833.
- Milkowska-Leyck K., Filipek B., and Strezelecka H. (2002), Pharmacological effects of lavandulifolioside from *Leonurus cardiac*. *J. Ethnopharmacol.* **80**, 85–90.
- Mozaffarian V. (2007), A Dictionary of Iranian Plant Names. Farhang moaser, Tehran, pp. 21–22.
- Naghibi F., Mosaddegh M., Mohammadi Motamed S., and Ghorbani A. (2005), Labiateae family in folk medicine in Iran: from ethnobotany to pharmacology. *Iran. J. Pharm. Res.* **2**, 63–79.
- Ono Y., Fukaya Y., Imai S., and Yamakuni T. (2008), Beneficial effects of *Ajuga decumbens* on osteoporosis and arthritis. *Biol. Pharm. Bull.* **31**, 1199–1204.
- Papoutsi Z., Kassi E., Mitakou S., Aliogiannis N., Tsiapara A., Chrousos G. P., and Moutsatsou P. (2006), Acteoside and martynoside exhibit estrogenic/antiestrogenic properties. *J. Steroid Biochem. Mol. Biol.* **98**, 63–71.
- Ramazanov N. Sh. (2005), Phytoecdysteroids and other biologically active compounds from plants of genus *Ajuga*. *Chem. Nat. Compd.* **41**, 361–369.
- Sadati N., Ostad S. N., Karimian Z., Shams Ardekani M. R., Akbarzadeh T., Hadjiakhoondi A., and Khanavi M. (2012), Phytochemical study and *in vitro* cytotoxic effect of *Ajuga chamaecistus* ssp. *tomentella*. *Asian J. Chem.* **24**, 2871–2874.
- Sasaki H., Taguchi H., Endo T., Yosioka I., Higashiyama K., and Otomasu H. (1978), The glycosides of *Martynia louisiana* Mill. A new phenylpropanoid glycoside, martynoside. *Chem. Pharm. Bull.* **26**, 2111–2121.
- Takaishi K. (1968), Studies on the coumarin components in the leaves of cherry-tree. *Yakugaku Zasshi* **88**, 1467–1471.
- Wessner H., Champion B., Girault J.-P., Saidi B., and Lafont R. (1992), Ecdysteroids from *Ajuga iva*. *Phytochemistry* **31**, 3785–3788.
- Vanyolos A., Simon A., Toth G., Polgar L., Kele Z., Ilku A., Matyus P., and Bathori M. (2009), C-29 Ecdysteroids from *Ajuga reptans*. *J. Nat. Prod.* **72**, 929–932.
- Yang L., Han H., Nakamura N., Hattori M., Wang Z., and Xu L. (2007a), Bio-guided isolation of antioxidants from the stems of *Dendrobium auranticum* var. *denneanum*. *Phytother. Res.* **21**, 696–698.
- Yang L., Nakamura N., Hattori M., Wang Z., Bligh S. W. A., and Xu L. (2007b), High-performance liquid chromatography-diode array detection/electrospray ionization mass spectrometry for simultaneous analysis of *cis*-, *trans*- and dihydro-2-glucosyloxycinnamic acid derivatives from *Dendrobium* medicinal plants. *Rapid Commun. Mass Spectrom.* **21**, 1833–1840.