Turkish Journal of Chemistry
http://journals.tubitak.gov.tr/chem/
Research Article

Turk J Chem
(2017) 41: 345 – 353

doi:10.3906/kim-1607-68

The structure and cytotoxic activity of a new saponin: cephoside A from *Cephalaria elazigensis* var. *purpurea*

Peyker KAYCE[1], Nazlı BÖKE SARIKAHYA[1], Murat PEKMEZ[2], Nazlı ARDA[2], Süheyla KIRMIZIGÜL[1,*]
[1]Department of Chemistry, Faculty of Science, Ege University, İzmir, Turkey
[2]Department of Molecular Biology and Genetics, Faculty of Science, İstanbul University, İstanbul, Turkey

Received: 28.07.2016 • **Accepted/Published Online:** 07.11.2016 • **Final Version:** 16.06.2017

Abstract: Investigation of the aerial parts of *Cephalaria elazigensis* var. *purpurea* afforded one new oleanane-type saponin, namely cephoside A (**1**), and five known natural compounds (**2**–**6**). Compound **2**, named anemoclemoside A, which is an unusual triterpene glycoside, was identified in the family Dipsacaceae for the first time. Chemical structures of all compounds were determined on the basis of the HRESIMS and 1D and 2D NMR data. Cephoside A (**1**) and anemoclemoside A (**2**) were assessed for their cytotoxic activities against HeLa cells, having IC_{50} values of 495 and 135 μg/mL, respectively.

Key words: Cephoside A, Dipsacaceae, triterpene saponin, iridoid glycoside, cytotoxic activity, *Cephalaria*, anemoclemoside A

1. Introduction

Saponins are a diverse group of compounds widely distributed in the plant kingdom, and are characterized by their structure containing a triterpene or steroid aglycone and one or more sugar chains. This class of compounds has been accepted as the most important and characteristic chemical constituents in *Cephalaria* species. Great interest has been shown in their investigations, resulting in the discovery of triterpene saponins,[1–7] iridoids,[6,8] flavonoids,[9,10] alkaloids,[11] lignans, and their glycosidic derivatives,[12] and many of these compounds exhibit a wide range of pharmacological and biological properties.[13,14] For that reason, we decided to investigate *Cephalaria elazigensis* Gokturk & Sumbul var. *purpurea* Gokturk & Sumbul in detail. It is a perennial medicinal herb belonging to the family Dipsacaceae, widely distributed in southwestern Anatolia.[15] Previous pharmaceutical studies on the genus *Cephalaria* showed appealing pharmacological activities, e.g., anticancer,[7] antibacterial, molluscicidal,[16] antidiabetic, and antioxidative[17] properties.

As a part of continuous biochemical studies on the genus *Cephalaria*, our attention has been focused on *C. elazigensis* var. *purpurea*. A new triterpene saponin named cephoside A (Figure 1) and five known compounds were isolated from *C. elazigensis* var. *purpurea* and cytotoxic activity of compounds **1** and **2** against the HeLa cell line was exhibited by MTT assay for the first time. The chemical structure of the new compound was identified as 3-*O*-[α-L-2-*O*-methylarabinofuranosyl-(1$\rightarrow$2)-α-L-arabinopyranosyl] hederagenin (**1**). Additionally, compound **2** (anemoclemoside A),[18] which is an unusual triterpene glycoside, was identified in

*Correspondence: suheyla.kirmizigul@ege.edu.tr

the family Dipsacaceae for the first time. The structures of the other four known compounds were determined as hederagenin (**3**),[19] cyclopenta[c]pyran-4-carboxylic acid, octahydro-3,6-dihydroxy-7-methyl-methyl ester (**4**),[20] loganin (**5**),[21] and sweroside (**6**)[22] (Figure 1). Their structures were elucidated using chemical and spectroscopic methods, including 1D, 2D NMR, and HRESIMS techniques.

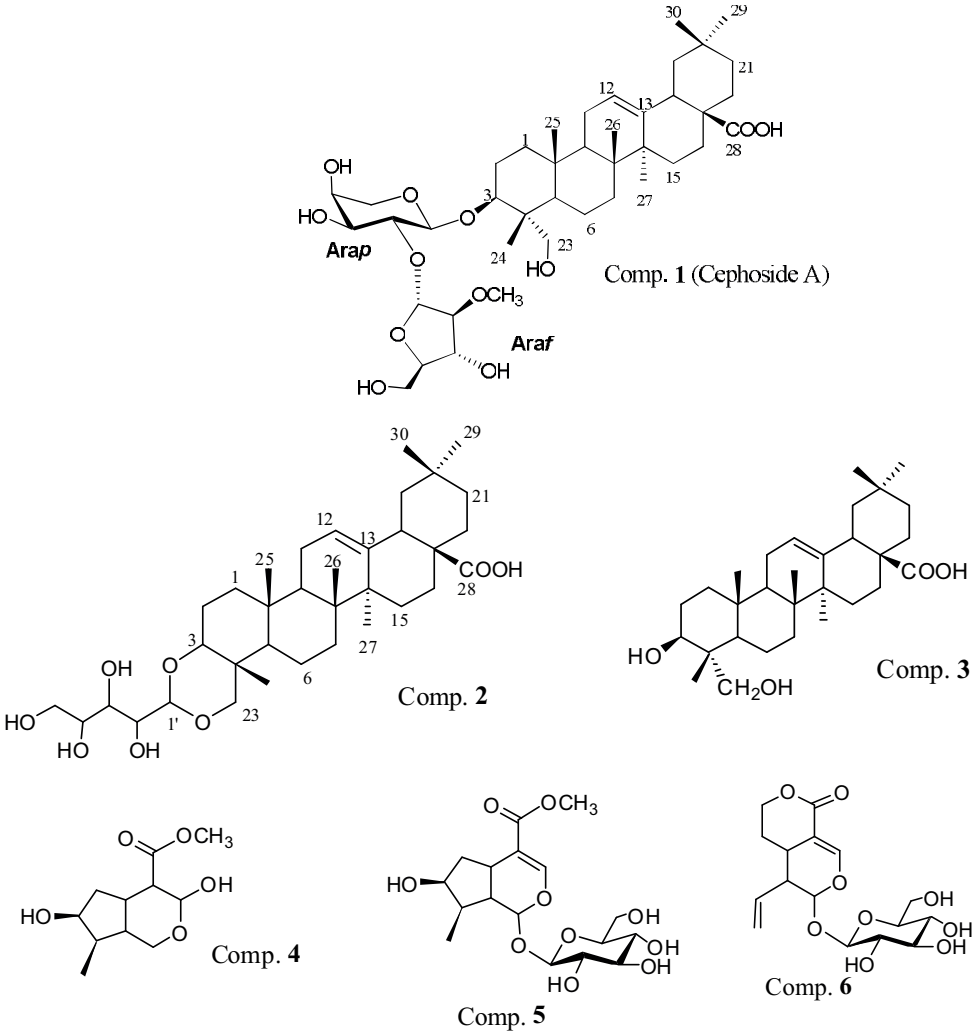

Figure 1. The structures of cephoside A (**1**) and compounds **2**–**6**.

2. Results and discussion

The *n*-butanol extract (51.3 g) of the aerial parts of *C. elazigensis* var. *purpurea* (1.5 kg) was subjected to reversed-phase (RP) C18 VLC apparatus, silica gel and RP open column chromatography applications, and MPLC experiments to afford one new (**1**) and five known compounds (**2**–**6**).

Cephoside A (**1**) was isolated as a white amorphous powder. The positive-ion HRESIMS of **1** exhibited an ion peak at m/z 773.3865 $[M + Na]^+$ (calcd. 773.3871) compatible with the molecular formula $C_{41}H_{66}O_{12}$. The FTIR spectrum of **1** exhibited the characteristic absorptions for hydroxy (3386 cm^{-1}), carbonyl (1694 cm^{-1}), olefin (1594 cm^{-1}), and aliphatic C–H (2942 cm^{-1}) functionalities. The ^{13}C NMR spectrum gave 41 signals, of which 11 were assigned to the sugar moieties and 30 signals to a triterpene moiety, including six

tertiary methyl groups at δ_C 13.4 (C-24), 16.0 (C-25), 17.4 (C-26), 26.1 (C-27), 33.4 (C-29), and 23.9 (C-30); a hydroxyl methyl carbon at δ_C 63.2 (C-23); an oxygen-bearing methine carbon at δ_C 80.4 (C-3); an olefinic carbon at δ_C 122.3 (C-12) and 144.3 (C-13); and a carboxylic acid carbonyl carbon at δ_C 180.0 (C-28). The δvalues of C-3 and C-28 suggested that compound **1** is a mono-desmosidic glycoside with saccharide units attached at the C-3 position. The ^{1}H NMR spectrum of **1** showed six singlets assignable to the aglycone methyls between δ_H 0.56 and 1.07, an olefinic proton signal at δ_H 5.09 (1H, brs), and methine protons at δ_H 3.07 and 3.40 (2H, m). These analyses, together with the literature data,[23] clearly indicate that compound **1** is a hederagenin-type triterpene saponin. Additionally, the signals of two anomeric protons were observed at δ_H 4.18 (1H, brs) and 4.52 (1H, d, J = 2.0 Hz) in the ^{1}H NMR spectrum, which gave correlations in the HSQC spectrum with two anomeric carbons at δ_C 105.0 and 110.0, respectively, suggesting the presence of two sugar units (Table). The chemical shifts of the signal multiplicities, the absolute values of the coupling constants, and their magnitude in the ^{1}H NMR spectrum, as well as the ^{13}C NMR data, indicated that both sugar units have an alpha configuration. This was also confirmed by the COSY, NOESY, and HSQC spectra. The linkage sites and the sequences of the two saccharides to each other and to the aglycone were deduced from an HMBC experiment by specific correlations between H-1′ of Ara$p$(δ_H 4.18, brs) and C-3 (δ_c 80.4) of the aglycone and between H-1″ of Ara$f$ (δ_H 4.52, d, J = 2.0 Hz) and C-2′ of Ara$p$ (δ_c 73.0).[24] In addition, there is one methoxy signal that resonated at δ_c 55.0 in ^{13}C NMR and at δ_H 3.22 (3H, s) in the ^{1}H NMR spectra, which gave the exact correlation with arabinofuranose in the HMBC spectrum. The exact location of the methoxy group was identified by HMBC spectrum including the correlations between H-1″ of Ara$f$ (δ_H 4.52) and methoxy carbon (δ_c 55.0), and C-1″ of Ara$f$(δ_C 110.0) and methoxy protons (δ_H 3.22) (Figure 2). Thus, the structure of **1** was elucidated as 3-O-[α-L-2-O-methylarabinofuranosyl-(1→2)-α-L-arabinopyranosyl] hederagenin (**1**), namely cephoside A.

The cytotoxicity of **1** and **2** was tested against HeLa human cervical carcinoma cells by MTT assay. The results revealed that **1** (cephoside A) and **2** (anemoclemoside A) could inhibit the viability of HeLa cells in a concentration-dependent manner (Figure 3) by IC_{50} of 495 and 135 μg/mL, respectively. These concentrations correspond to 660 μM for cephoside A and 220 μM for anemoclemoside A. Thus, it seems both compounds are inactive, at least for HeLa cells.

While having the same aglycone as cephoside A, kalopanaxsaponin A, which has an O-linked arabinose attached to C-3 and a terminal rhamnose isolated from *Anemone taipaiensis*, exhibited cytotoxic activity against HeLa cells with an IC_{50} value of 18.16 μM.[25,26] Furthermore, this compound was found to be more active against lung carcinoma (A549), glioblastoma (U87MG), promyleocytic leukemia (HL-60), and hepatocellular liver carcinoma (HepG2) cells (IC_{50} values of 15.49, 10.25, 8.68, and 6.42 μM, respectively).[26] The IC_{50} values of many saponins, isolated from *Pulsatilla chinensis* having similar aglycone as cephoside A, have been found as 7.1, >10, 7.8, and 3.8 μg/mL, against HL-60 human promyelocytic leukemia cells.[27] Thus, the occurrence of rhamnose on the sugar chain, especially in the terminal position, in active monodesmosidic oleanane-type saponins indicates that rhamnose is an effective sugar for cytotoxicity.[28]

On the other hand, the hydroxyl group at C-23 has been suggested to have a negative effect on cytotoxic activity, probably due to the electron donating effect of two unbound outer shell electrons of the –OH group toward C-3 of the aglycone.[28,29] Yokosuka et al. also concluded that the hydroxyl group at C-23 diminished the cytotoxicity, as prosapogenin CP6 from *Anemone hypehensis* var. *japonica* exhibited cytotoxic activity but lower than its derivative prosapogenin CP4, which lacks –OH at C-23.[30]

Table. ^{13}C NMR and ^{1}H NMR data of compounds **1** and **2**[a–e].

1			**2**	
Position	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR
1	38.4	0.84, 1.48, m	38.6	1.00, 1.54, m
2	25.6	1.52, 1.68, m	23.5	1.41, 1.56, m
3	80.4	3.46, s	85.0	3.21, m
4	42.8	-	36.7	-
5	46.6	1.16, m	50.9	0.80, m
6	17.7	1.18, 1.40, m	17.7	1.12, 1.32, m
7	32.4	1.16, 1.42, m	32.4	1.20, 1.36, m
8	42.1	-	45.9	-
9	47.6	1.49, m	47.4	1.52, m
10	36.7	-	37.2	-
11	23.4	1.46, 1.80, m	23.3	1.44, 1.77, m
12	122.3	5.09, brs	121.1	5.10, brs
13	144.3	-	145.1	-
14	41.8	-	41.8	-
15	27.7	0.94, 1.66, m	27.8	0.91, 1.71, m
16	23.2	1.42, 1.84, m	23.2	1.56, 1.80, m
17	45.9	-	47.3	-
18	41.4	nd	41.5	2.76, m
19	46.3	1.02, 1.58, m	46.6	1.00, 1.56, m
20	30.9	-	30.9	-
21	33.9	1.10, 1.28, m	34.1	1.10, 1.28, m
22	32.7	1.16, 1.42, m	32.8	1.38, 1.58, m
23	63.2	3.07, 3.40, m	77.5	3.18, 3.70, m
24	13.4	0.56, s	13.5	0.95, s
25	16.0	0.87, s	16.6	0.88, s
26	17.4	0.70, s	17.4	0.70, s
27	26.1	1.07, s	26.2	1.08, s
28	180.0	-	179.9	-
29	33.4	0.86, s	33.4	0.88, s
30	23.9	0.86, s	23.9	0.85, s
Sugars				
Ara*p*				
1′	105.0	4.18, brs	102.7	4.50, d, (6.4)
2′	73.0	3.30, m	70.0	3.43, m
3′	71.5	3.30, m	70.1	3.59, m
4′	68.3	3.59, m	71.2	3.41, m
5′	65.0	3.30, 3.64	64.0	3.34, 3.55, m
Ara*f*				
1″	110.0	4.52, d, (2.0)		
2″	81.8	3.74, m		
3″	77.2	3.62, m		
4″	84.0	3.67, m		
5″	61.8	3.40, 3.54, m		
–OMe	55.0	3.22, s		

[a] ^{13}C NMR data (δ) were measured in DMSO-d_6 at 100 MHz.

[b] ^{1}H NMR data (δ) were measured in DMSO-d_6 at 400 MHz.

[c] Coupling constants (J) in Hz are given in parentheses.

[d] The assignments are based on COSY, HSQC, and HMBC experiments.

[e] nd: not determined

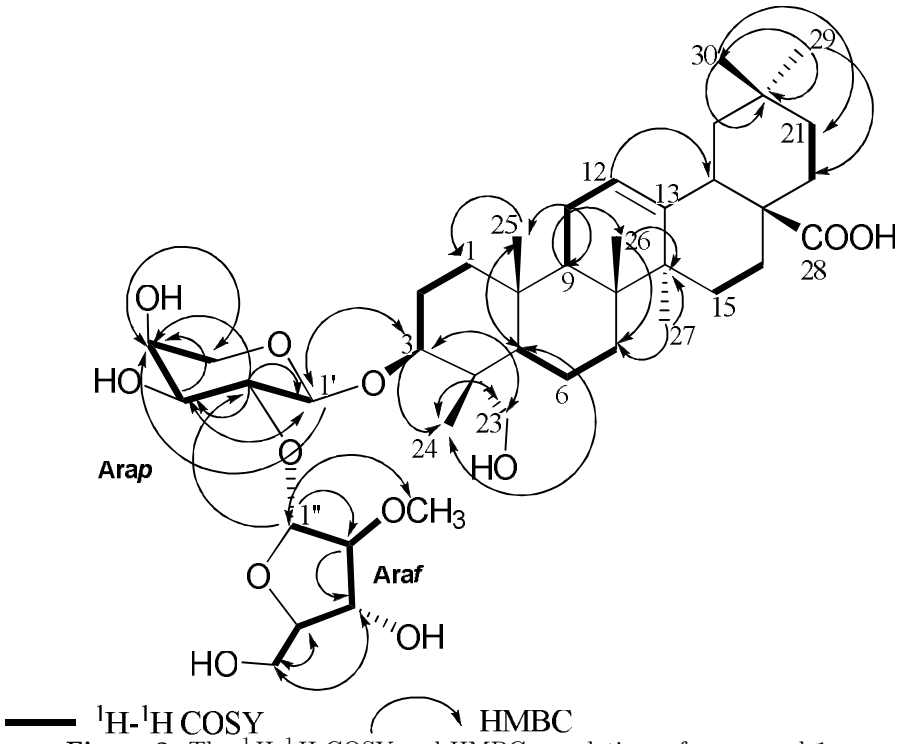

Figure 2. The ^{1}H-^{1}H COSY and HMBC correlations of compound **1**.

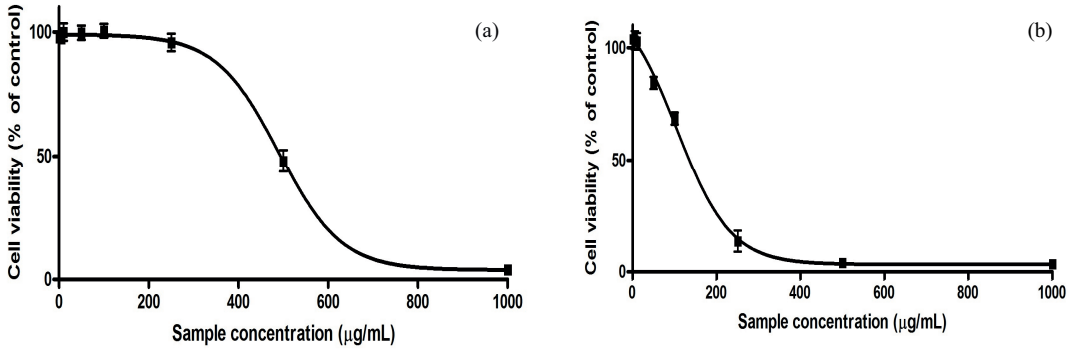

Figure 3. (a) The effect of cephoside A (**1**) ($P < 0.0001$, $R^2 = 0.980$); (b)Anemoclemoside A (**2**) ($P < 0.0001$, $R^2 = 0.983$) on HeLa cell proliferation.

Cephoside A lacks a rhamnose unit and carries a hydroxyl group at C-23. Thus it is to be expected that cephoside A has no activity. Although the cytotoxic activity of anemoclemoside A (IC_{50} = 135 μg/mL or 220 μM) was higher than that of cephoside A (IC_{50} = 495 μg/mL or 660 μM), probably due to free hydroxyl groups of acyclic sugar moiety, this compound was also regarded as inactive on HeLa cells.

3. Conclusion

One new and five known natural compounds have been isolated from *C. elazigensis* var. *purpurea.* While the aglycones of two triterpenic glycosides were hederagenin, the other two glycosides include iridoidal aglycones. The last two compounds are detected as hederagenin and iridoid aglycones.

The cytotoxicity of cephoside A (**1**) (Figure 3a) and anemoclemoside A (**2**) (Figure 3b) was examined by MTT assay for the first time. The cytotoxic activities of both compounds were outside the range for them to be assumed as active compounds (IC_{50} values were <250 μg/mL for **1** and <100 μg/mL for **2**). However, further studies are needed to check their effects in combination with each other and/or current anticancer agents as it is well known that natural compounds may enhance cytotoxic activity synergistically.[31] Moreover, their efficiency and selectivity on different cancer cell lines and normal cells might be tested to understand selectivity, as the cell-specific action of saponins is observed in some cases,[25–27,29,30,32,33] and the treatment period might be extended to 72 h for exhibiting whether it increases activity or not.

4. Experimental

4.1. General

Optical rotations of pure compounds were measured at 23 °C using a Rudolph Research Analytical Autopol I automatic polarimeter fitted with a sodium lamp with 1 mL of cells. IR spectra were obtained on ATI Mattson 1000 Genesis Series FTIR instrument using KBr discs. 1D and 2D NMR measurements were obtained on a Varian AS 400 MHz in DMSO-d_6. All chemical shifts (δ) were given in ppm units with reference to tetramethylsilane (TMS) as an internal standard, and the coupling constants (J) were recorded in Hz. HRESIMS analyses were carried out using a Bruker LC micro-Q-TOF mass spectrometer. Medium pressure liquid chromatography (MPLC) applications were run using a Buchi system (Buchi C-605 pumps, coupled to a UV detector) with a Buchi glass column (26/920). Lichroprep RP-18 (25–40 μm; Merck) and silica gel 60 (0.063–0.200 mm; Merck) were used both for column chromatography and MPLC studies. Thin-layer chromatography (TLC) was performed on F254 (Merck) and RP-18 F254s (Merck) precoated aluminum sheets. Spots were visualized under UV light and/or by spraying with H_2SO_4:H_2O (1:5, v/v) followed by heating at 120 °C. L-Arabinose, L-rhamnose, D-xylose, D-mannose, D-galactose, and D-glucose were used as standard sugar moieties for the sugar analysis of compound **1**.

4.2. Plant material

C. elazigensis Gokturk & Sumbul var. *purpurea* Gokturk & Sumbul was collected from Kırıkkale–Kırşehir, at about 1255 m altitude, in July 2007. This species was identified by Prof Dr H Sumbul and Prof Dr RS Göktürk (Department of Biology, Faculty of Arts and Science, Akdeniz University). A voucher specimen (R. S. Gokturk 6090) was deposited at the Herbarium Research and Application Center of Akdeniz University.

4.3. Extraction and isolation

The dried and ground aerial parts (1.5 kg) of *C. elazigensis* var. *purpurea* were extracted with MeOH at room temperature (4 × 3 L). Evaporation of the solvent in a vacuum provided a dark residue (51.3 g). This residue was suspended in n-BuOH:H_2O water mixture (1:1) (350 mL × 3) and n-BuOH and water fractions were obtained. Then, for removing apolar and oily parts, the n-BuOH fraction was extracted with n-hexane (9 × 50 mL). The n-BuOH residue (51.3 g) of *C. elazigensis* var. *purpurea* was subjected to a VLC apparatus using Lichroprep RP-18 as an adsorbent by MeOH:H_2O solvent system with a gradient from 0% to 100% MeOH to give 7 fractions. The combined fractions 6 and 7 (8.7 g) of RP-VLC were exposed to MPLC over silica gel using a suitable column (Buchi, 26 mm × 920 mm) and program (max. pressure: 40 bar, flow rate: 23 mL/min, $CHCl_3$:MeOH:H_2O solvent system, 90:10:0.5–61:32:7). Twelve subfractions were derived after

MPLC application. Compounds **1** (30.5 mg), **2** (45.2 mg), and **3** (80.5 mg) were purified by an open silica gel column chromatography with the solvent systems $CHCl_3$:MeOH:H_2O (90:10:1–61:32:7) from subfraction 8 of MPLC. Fraction 1 of RP-VLC was exposed to MPLC over silica gel using a suitable column (Buchi, 26 mm × 920 mm) and program (max. pressure: 40 bar, flow rate: 30 mL/min, $CHCl_3$:MeOH:H_2O solvent systems, 90:10:1; 80:20:2; 70:30:3; 61:32:7) and 17 subfractions were derived. Compound **4** (53.7 mg) was purified by open RP column chromatography with the solvent system MeOH:H_2O (1:4) from the 3rd subfraction of this last MPLC application. Finally, compounds **5** (49.0 mg) and **6** (32.2 mg) were purified by the same methods from subfractions 12 and 14 of the MPLC application, respectively.

4.4. Cephoside A (1)

Cephoside A handled as a white amorphous solid (30.5 mg); $[\alpha]_D^{23}$–4.81 (c0.5, MeOH); IR (KBr) νmax 3386, 2942, 1694, 1594, 1458, 1055 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz) see Table; Positive-ion ESIMS m/z(rel. %): 773 ($[M + Na]^+$, 6), 741 ($[M + Na]^+ - OCH_3$, 5), 705 ($[M - CO_2]^+$, 100), 627 ($[M + Na]^+ -$ Ara$f$, 46), 547 (63), 491 (17), 478 (20), 463 (28), 439 (36), 431 (15), 417 (10); positive-ion HRESIMS m/z773.3865, $[M + Na]^+$ (calcd. for $C_{41}H_{66}O_{12}Na$, 773.3871).

4.5. Sugar analysis of pure compound

The sugar analysis of compound **1** was performed using microhydrolysis on a TLC plate. Pure compound was applied on a TLC layer (silica gel HF 254) and treated with concentrated HCl vapor in a closed vessel saturated with the acidic vapor for 40 min at 60 °C. After the vessel was cooled and the excess HCl was removed from the plate, the sugar references were applied on a TLC layer. TLC was eluted using a $CHCl_3$:MeOH:H_2O:gAcOH/16:9:2:2 solvent system. For detecting the compounds, it was sprayed with α-naphthol-H_2SO_4 solution and then heated at 120 °C for 5 min. Hexoses, 6-deoxy sugars and pentoses gave purple, orange, and blue spots on the TLC plate, respectively.[34]

4.6. Cytotoxic activity test

The MTT assay[35] was used to test the cytotoxic activities of compounds **1** and **2** with a minor modification as reported earlier.[36] The assay is based on the reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to a colored formazan product by mitochondrial dehydrogenase, which is active only in living cells. Human HeLa cervical carcinoma cells were cultured in Eagle's minimum essential medium (MEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, and antibiotic–antimycotic mixture [penicillin (100 U/mL), streptomycin (100 μg/mL), and amphotericin B (0.25 μg/mL)]. The cells were maintained in 96-well plates and each well contained 200-μL cell suspensions at a density of 1×10^5 cells/mL. After reaching confluence (1 day later), the cells were treated with increasing concentrations (1 μg/mL–1000 μg/mL) of the samples diluted with MEM. Cells treated with MEM instead of sample were considered as the control group. After growth of the cells for 48 h at 37 °C in a humidified 5% CO_2 atmosphere, the adherent cells were washed with phosphate buffered saline (PBS); then 10 μL of MTT stock solution (5 mg/mL) and 90 μL of PBS buffer were added to each well and the plates were further incubated at 37 °C for 4 h. At the end of this period, supernatants were discarded and DMSO (150 μL) was added to each well to solubilize the water-insoluble purple formazan crystals. The absorbance was measured at 570 nm in a microplate reader

(μQuant, Bio-Tek Instruments, Inc., Winooski, VT, USA). The cell viability was calculated using the following equation:

$$\text{Cell viability}(\%) = (A_{\text{sample}}/A_{\text{control}}) \times 100$$

The half maximal inhibitory concentration (IC_{50}) of the extracts on HeLa cells was calculated from a graph of cell viability versus the sample concentrations.

Statistical comparisons were conducted using the one-way analysis of variance (ANOVA) module of GraphPad Prism 5. Differences in mean values were considered significant when $P < 0.05$.

Acknowledgments

The authors would like to thank the Research Grant Office of Ege University, İzmir, Turkey (2013/Fen/035), and the Scientific Research Projects Coordination Unit of İstanbul University(29681) for financial support, and Peyker Kayce is grateful to TÜBİTAK BIDEB 2211-A for the research fellowship. We would like to thank Prof Dr H Sümbül and Prof Dr RS Göktürk for collection and identification of the plant material and TÜBİTAK-UME, Gebze, Kocaeli, Turkey, for HRESIMS analysis, and EBILTEM Ege University, Bornova, İzmir, Turkey, for NMR analysis.

References

1. Kayce, P.; Sarıkahya, N. B.; Kırmızıgül, S. *Phytochem. Lett.* **2014**, *10*, 324-329.
2. Sarıkahya, N. B. *Phytochem. Lett.* **2014**, *8*, 149-155.
3. Sarıkahya, N. B.; Kırmızıgül, S. *Planta Med.* **2012**, *78*, 828-833.
4. Sarıkahya, N.B.; Kırmızıgül, S. *Turk. J. Chem.* **2012**, *36*, 323-334.
5. Godevac, D.; Menkovic, N.; Vujisic, L.; Tesevic, V.; Vajs, V.; Milosavljevic, S. *Nat. Prod. Res.* **2010**, *24*, 1307-1312.
6. Godevac, D.; Mandic, B.; Vajs, V.; Tesevic, V.; Menkovic, N.; Janackovic, P.; Milosavljevic, S. *Biochem. Syst. Ecol.* **2006**, *34*, 890-893.
7. Tabatadze, N.; Elias, R.; Faure, R.; Gerkens, P.; Pauw-Gillet, M. C.; Kemertelidze, E.; Chea, A.; Ollivier, E. *Chem. Pharm. Bull.* **2007**, *55*, 102-105.
8. Mustafaeva, K.; Elias, R.; Balansard, G.; Suleimanov, T.; Mayu-Lede, V.; Kerimov, Y. *Chem. Nat. Comp.* **2008**, *44*, 132-133.
9. Movsumov, I. S.; Garaev, E. A.; Isaev, M. I. *Chem. Nat. Compd.* **2009**, *45*, 422-423.
10. Godevac, D.; Vajs, V.; Menkovic, N.; Tesevic, V.; Janackovic, P.; Milosavljevic, S. *J. Serb. Chem. Soc.* **2004**, *69*, 883-886.
11. Aliev, A. M.; Movsumov, I. S.; Bagirov, E. *Khim. Prir. Soedin.* **1975**, *5*, 667.
12. Pasi, S.; Aligiannis, N.; Skaltsounis, A. L.; Chinou, I. B. *Nat. Prod. Lett.* **2002**, *16*, 365-370.
13. Kırmızıgül, S. In: *Plant-derived Antimycotics: Current Trends and Future Prospects*; Rai, M.; Mares, D. Eds. Food Products Press: New York, NY, USA, 2003, pp. 459-495.
14. Podolak, I.; Galanty, A.; Sobolewska, D. *Phytochem. Rev.* **2010**, *9*, 425-474.
15. Göktürk, R. S.; Sümbül, H.; Açık, L. *Israel J. Pl. Sci.* **2003**, *51*, 59-65.
16. Pasi, S.; Aligiannis, N.; Pratsinis, H.; Skaltsounis, A. L.; Chinou, I. B. *Planta Med.* **2009**, *75*, 163-167.
17. Mbhele, N.; Balogun, F. O.; Kazeem, I.; Ashafa, T. *Bangladesh J. Pharma.* **2015**, *10*, 214-221.

18. Li, X. C.; Yang, C. R.; Liu,Y. Q.; Kasai, R.; Ohtani, K.; Yamasaki, K.; Miyahara, T. K.; Shingu, K. *Phytochemistry* **1995**, *39*, 1175-1179.
19. Braca, A.; Autore, G., De Simone, F.; Marzocco, S.; Morelli, I.; Venturella, F.; De Tommasi, N. *Planta Med.* **2004**, *70*, 960-966.
20. Kocsis, A.; Szabo, L. F.; Podanyi, B. *J. Nat. Prod.* **1993**, *56*, 1486-1499.
21. Dunstan, W. R.; Short, F. W. *Pharm. J.* **1983**, *14*, 1025-1028.
22. Inouye, H.; Ueda, S.; Nakamura, Y. *Chem. Pharm. Bull.* **1970**, *18*, 1856-1865.
23. Sharma, A.; Sati, S. C.; Sati, O. P.; Sati, M. D.; Kothiyal, S. K. *H.P.C. J. Chem.* **2013**, *2013*, 1-5.
24. Qi, J.; Ojika, M.; Sakagami, Y. *Biorg. Med. Chem.* **2002**, *10*, 1961-1966.
25. Jung, H. J.; Lee, C. O.; Lee, K. T.; Choi, J.; Park, *H. J. Biol. Pharm. Bull.* **2004**, *27*, 744-747.
26. Wang, X. Y.; Zhang, W.; Gao, K.; Lu, Y. Y.; Tang, H. F.; Sun, X. L. *Fitoterapia* **2013**, *89*, 224-230.
27. Mimaki, Y.; Kuroda, M.; Asano, T.; Sashida, Y. *J. Nat. Prod.* **1999**, *62*, 1279-1283.
28. Bang, S. C.; Lee, J. H.; Song, G. Y.; Kim, D. H.; Yoon, M. Y.; Ahn, B. Z. *Chem. Pharm. Bull.* **2005**, *53*, 1451-1454.
29. Park, H. J.; Kwon, S. H.; Lee, J. H.; Lee, K. H.; Miyamoto, K. I.; Lee, K. T. *Planta Med.* **2001**, *67*, 118-121.
30. Yokosuka, A.; Sano, T.; Hashimoto, K.; Sakagami, H.; Yoshihiro, M. *Chem. Pharm. Bull.* **2009**, *57*, 1425-1430.
31. Sadeghi-Aliabadi, H.; Minaiyan, M.; Dabestan, A. *Res. Pharm. Sci.* **2010**, *5*, 127-133.
32. Thu, V. K.; Thang, N. V.; Nhiem, N. X.; Tai, B. H.; Nam, N. H.; Kiem, P. V.; Minh, C. V.; Anh, H. L. T.; Kim, N.; Park, S.; et al. *Phytochemistry* **2015**, *116*, 213-220.
33. Wang, J.; Zhao, X. Z.; Qi, Q.; Tao, L.; Zhao, Q.; Mu, R.; Gu, H. Y.; Wang, M.; Feng, X.; Guo, Q. L. *Food Chem. Toxicol.* **2009**, *47*, 1716-1721.
34. Su, Y.; Guo, D.; Guo, H.; Liu, J.; Zheng, J.; Koike, K.; Nikaido, T. *J. Nat. Prod.* **2001**, *64*, 32-36.
35. Mossman, T. *J. Immunol. Methods* **1983**, *65*, 55-63.
36. Onay-Uçar, E.; Erol, O.; Kandemir, B.; Mertoğlu, E.; Karagöz, A.; Arda, N. *eCAM.* **2012**, *2012*, 1-7.