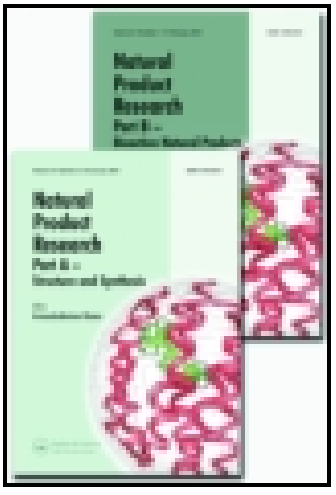

Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

Structural elucidations and spectral assigments of two novel triterpene glycosides from Cephalaria paphlagonica

S. Çapanlar [a] & S. Kırmızıgül [a]
[a] Chemistry Department, Faculty of Science , Ege University , 35100 Bornova, Izmir, Turkey
Published online: 27 Aug 2010.

To cite this article: S. Çapanlar & S. Kırmızıgül (2010) Structural elucidations and spectral assigments of two novel triterpene glycosides from Cephalaria paphlagonica , Natural Product Research: Formerly Natural Product Letters, 24:14, 1337-1346, DOI: 10.1080/14786410903381335

To link to this article: http://dx.doi.org/10.1080/14786410903381335

Natural Product Research
Vol. 24, No. 14, 10 September 2010, 1337–1346

Taylor & Francis
Taylor & Francis Group

Structural elucidations and spectral assigments of two novel triterpene glycosides from *Cephalaria paphlagonica*

S. Çapanlar and S. Kırmızıgül*

Chemistry Department, Faculty of Science, Ege University, 35100 Bornova, Izmir, Turkey

(*Received 19 December 2008; final version received 20 February 2009*)

Two novel triterpene glycosides, paphlagonoside A (**1**) and B (**2**), were characterised as 28-O-[β-D-glucopyranosyl(1 → 2)-α-L-arabinopyranosyl (1 → 4)-β-D-glucopyranosyl]-hederagenin (**1**) and 28-O-[β-D-xylopyranosyl (1 → 4)-α-L-rhamnopyranosyl(1 → 3)-β-D-xylopyranosyl (1 → 6)-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl]-hederagenin (**2**) from *Cephalaria paphlagonica* (Dipsacaceae). In addition to these, a common natural product (hederagenin) (**3**) was also isolated. The structures of all compounds were identified by spectroscopic and chemical methods. The antimicrobial and antioxidant activities of the plant extracts were examined by MIC and DPPH activity methods, respectively.

Keywords: *Cephalaria paphlagonica*; Dipsacaceae; paphlagonoside; triterpene glycoside; antimicrobial and antioxidant activity

1. Introduction

There are 93 *Cephalaria* species belonging to the Dipsacaceae family worldwide, of which 23 are endemic to and widely distributed in Turkey (Bobrov, 1972). According to the literature, alkaloids and different types of glycosidic compounds have been reported from this species (Aliev, Movsumov, & Bagirov, 1975; Aliev, Movsumov, & Serkerov, 1975, Aliev & Serkerov, 1976; Godevac et al., 2004, 2006a, 2006b; Movsumov & Aliev, 1975; Movsumov, Aliev, Kondratenko, & Abubakirov, 1975; Mustafaeva et al., 2008; Tabatadze et al., 2007). In connection with this, elucidation of some triterpene glycosides and antimicrobial results of *Cephalaria transsylvanica* were reported previously by us (Kırmızıgül & Anıl, 1994, 2002; Kırmızıgül, Anıl, & Rose, 1995, 1996; Kirmizıgül, Anıl, Uçar, & Akdemir, 1996; Kırmızıgül & Rose, 1997).

The present article describes the isolation and elucidation of two new triterpene glycosides, named paphlagonosides A (**1**) and B (**2**) from the aerial parts of *C. paphlagonica* Bobrov. In addition to these, hederagenin (**3**) was also isolated from this plant. Their structures were established on the basis of 1D- and 2D-NMR, ESI-MS, FT-IR and UV experiments. The antimicrobial and antioxidant activities of the plant extracts were examined by MIC and 2,2′-diphenyl-1-picrylhydrazyl (DPPH) activity methods, respectively.

*Corresponding author. Email: suheyla.kirmizigul@ege.edu.tr

ISSN 1478–6419 print/ISSN 1029–2349 online

DOI: 10.1080/14786410903381335
http://www.informaworld.com

2. Results and discussion

It is known that glycosides represent a large group of secondary metabolites which are generally found in wild plants, vegetables and flowers. They have great importance in the field of phytochemistry, pharmacology, biochemistry and nutriology (Alam et al., 2008; Hostettmann & Marston, 1995; Rai & Mares, 2003). In the context of our investigation, the chemical constituents of *Cephalaria* plants belonging to the Dipsacaceae have been discussed for many years. Here, two novel glycosides have been derived from the most active BuOH phase of *C. paphlagonica* Bobrov. (Dipsacaceae), according to the antimicrobial and antioxidant activity results of the extracts.

Paphlagonoside A (**1**) was isolated by open silica gel CC. It was obtained as a light brown amorphous powder and its molecular formula was assigned as $C_{47}H_{76}O_{18}$, as indicated by the $[M+Na]^+$ ion peak at m/z 952.1 by an ESI-MS ion trap instrument in positive mode. In the UV spectrum, a main maximum at 207.0 nm was an n–π^* absorption of the CH_3COOH group. In the FT-IR spectrum, the peaks at 3367.79, 2943.30, 1736.63, 1630.32 and 1069.95 cm^{-1} indicated the groups –OH, –C–H, –C=O, –C=C– and C–O–C, respectively.

The ^{1}H-NMR spectrum of compound **1** exhibited six methyl signals at 0.55 (3H, s), 0.65 (3H, s), 0.84 (9H, s) and 1.05 (3H, s) ppm, belonging to H-24, H-25, H-26, H-29, H-30 and H-27 protons. The C-12 proton was observed at 5.14 ppm as a broad singlet. H-3 proton gave a signal at δ 3.41 (1H, m). From these data the aglycone was found to be hederagenin. In addition to these, the three anomeric proton signals were determined at δ 5.20 (d, $J=8.4$ Hz), δ 4.19 (bs) and δ 4.18 (d, $J=7.6$ Hz), belonging to glucose, arabinose and glucose, respectively.

Comparison of ^{13}C-NMR and DEPT spectra gave eight quaternary carbon atoms. Two specific quaternary carbon atoms in this molecule were seen at C-28 and C-13, and their signals were assigned at 175.98 and 144.17 ppm, confirming the esteric carbonyl carbon and olefinic carbon of aglycone, respectively. In addition to these signals, we also observed a C-3 signal at 80.69 and C-12 signal at 122.37 ppm. From this information, it is clear that the aglycone is a hederagenin type of triterpene. According to the micro-hydrolysis technique, compound **1** has two glucose and one arabinose unit. The anomeric carbon signals of these sugars were detected at 94.74, 103.65 and 105.40 as glucose, arabinose and glucose, respectively.

The linking point of the sugar units was deduced by HMBC correlations which were observed between the carboxylic carbon at 175.98 ppm and the anomeric proton signal at δ 5.20 (1H, d, $J=8.4$ Hz, Glc H-1). These findings confirmed that the sugar moiety was linked to the aglycone through the carboxyl terminus. When we looked at the linking points of sugars with each other, we clearly observed the correlations between C-1″ and H-4′ and C-1‴ and H-2″. However there was no correlation between the C-3 carbon and the anomeric proton in the HMBC spectrum. The other important correlation was seen between C-23 and H-24. All these findings proved that compound **1** is a monodesmosidic acyl glycoside.

On the basis of these results, the structure of paphlagonoside A (**1**) was assigned as 28-O-[β-D-glucopyranosyl(1 → 2)-α-L-arabinopyranosyl(1 → 4)-β-D-glucopyranosyl]-hederagenin, a new monodesmosidic acyl glycoside (Table 1, Figure 1).

Compound **2** was isolated from the same open column as another fraction. It was isolated as an off-white amorphous powder. The molecular formula was obtained

Table 1. ^{1}H and ^{13}C-NMR data of compounds **1** and **2** in DMSO (δ ppm).

	Compounds			
	1		**2**	
Position	δ ^{13}C	^{1}H (J Hz)	δ ^{13}C	^{1}H (J Hz)
1	33.94	1.11 m	33.74	
2	25.80	1.68 m	26.07	
3	80.69	3.41 m	80.14	3.44 m
4	42.95	–	46.73	–
5	46.25	1.92 m	46.26	1.05 m; 1.56 m
6	17.80	1.20 m	17.77	
7	36.66	–	39.59	–
8	46.81	1.20 m	46.89	1.15 m
9	49.20	–	42.95	–
10	41.40	2.72 m	41.38	2.70 d ($J=9$ Hz)
11	23.66	1.58 m	23.41	
12	122.37	5.14 bs	122.36	5.12 bs
13	144.17	–	144.15	–
14	42.00	–	41.99	–
15	27.95	1.30 m	27.89	
16	23.20		23.20	0.94 m
17	46.65	–	36.63	–
18	47.82	1.45 m	47.42	1.47 m
19	38.73	1.38 m	39.45	0.90 m; 1.47 m
20	30.95	–	30.94	–
21	32.52		32.47	1.28 m
22	32.33	1.12 m	32.27	1.28 m
23	63.81	3.06 m; 3.38 m	63.05	3.08 m; 3.31 m
24	13.70	0.55 s	13.79	0.55 s
25	17.42	0.65 s	16.30	0.84 s
26	16.35	0.84 s	17.39	0.65 s
27	26.22	1.05 s	26.21	1.08 s
28	175.98	–	175.99	–
29	33.46	0.84 s	33.46	0.84 s
30	23.66	0.84 s	24.04	0.84 s
28-O-Sugar moieties				
1′	94.74	5.20 d ($J=8.4$ Hz)	94.80	5.20 d ($J=8$ Hz)
2′	70.11	3.09 m	74.39	
3′	73.40	3.27 m	76.98	
4′	76.95	3.31 m	74.11	3.52 m
5′	77.23	3.28 m	77.02	
6′	65.70	3.29 m; 3.64 m	61.98	3.44 m; 3.59 m
1″	105.40	4.19 bs	103.82	4.30 d ($J=6.8$ Hz)
2″	74.14	2.95 m	74.02	
3″	72.92	3.10 m	74.96	
4″	69.92	3.29 m	70.58	
5″	68.50	3.59 m; 3.90 m	76.89	3.09 m
6″	103.65	4.18 d ($J=7.6$ Hz)	66.40	3.02 m; 3.68 m
1‴	71.82	3.29 m	106.37	4.20 d ($J=7.6$ Hz)
2‴	77.02	3.14 m	72.98	
3‴	70.58	2.99 m	81.76	3.61 m
4‴	77.37	3.03 m	68.78	

(*Continued*)

Table 1. Continued.

	Compounds			
	1		**2**	
Position	δ ^{13}C	^{1}H (*J* Hz)	δ ^{13}C	^{1}H (*J* Hz)
5‴	61.58	3.41 m; 3.61 m	68.85	3.53m
1⁗			100.21	5.12 bs
2⁗			68.02	
3⁗			69.77	
4⁗			79.78	3.43 m
5⁗			68.52	3.91 d ($J = 10.4$ Hz)
6⁗			18.38	1.05 d ($J = 6$ Hz)
1⁗′			101.78	4.40 d ($J = 7.6$ Hz)
2⁗′			71.83	
3⁗′			79.78	
4⁗′			71.20	3.12 m
5⁗′			65.60	3.28m; 3.64 m

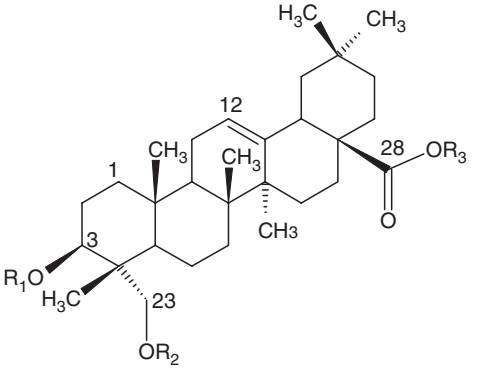

Figure 1. Structures of compounds **1**–**3**.

Compounds	R_1	R_2	R_3
1	H	H	Glc(1→2)Ara (1→4) Glc-
2	H	H	Xyl(1→4)Rha(1→3)Xyl(1→6)Glc (1→4)Glc-
3	H	H	H

as $C_{58}H_{94}O_{26}$ and the molecular ion peak was observed at *m*/*z* 1230 $[M + Na]^+$ by an ESI-MS ion trap instrument in positive mode. The UV spectrum showed a specific maximum at 208.0 nm, which was an n–π^* absorption of the CH_3COOH group. The IR spectrum signals of –OH, –C=C– and C–O–C were observed at 3350.72, 1629.64 and 1055.58 cm^{-1}, respectively.

In the ^{1}H-NMR spectrum, six methyl signals of the aglycone were exhibited at 0.55 (3H, s), 0.65 (3H, s), 0.84 (9H, s) and 1.08 (3H, s) ppm. Two other main signals,

H-12 and H-3, were obtained at δ 5.12 (1H, bs) and δ 3.44 (1H, m). The anomeric proton signals of the sugar moieties were determined at 5.20 (d, $J = 8.0$ Hz), 4.30 (d, $J = 6.8$ Hz), 4.20 (d, $J = 7.6$ Hz), 5.12 (bs) and 4.40 (d, $J = 7.6$ Hz) ppm, belonging to glucose, glucose, xylose, rhamnose and xylose, respectively.

Characteristic quaternary carbon signals of C-13 and C-28 were determined at 144.15 and 175.99 ppm. These data show that the aglycone of the glycoside is hederagenin. In the ^{13}C-NMR spectrum, the anomeric carbon atoms were determined at 94.8 (C-1′), 103.82(C-1″), 106.37(C-1‴), 100.21(C-1⁗) and 101.78(C-1⁗′) ppm for glucose, glucose, xylose, rhamnose and xylose, respectively. These results were confirmed by HMQC spectrum. In the HMBC spectrum, the C-3 carbon atom was assigned at 80.14 ppm and there is no correlation concerning the connection of carbohydrates from this point; in other words, this point is not branched. Besides that, the other specific correlations between C-3 and H-24 and C-23 and H-24 also showed that sugar moieties were not connected to C-3, but the same HMBC spectrum revealed that the correlations between C-28 and H-1′ exhibited the sugar moieties that were linked to the aglycone from the carbonyl carbon. This means that this compound is a monodesmosidic acyl glycoside. The connection points of the sugars with each other were also determined by the HMBC spectrum as C-1″ and 3.52 (H-4′); C-1‴ and 3.02, 3.68 (H-6″); C-3‴ and 4.20 (H-1‴); C-3‴ and 5.12 (H-1⁗); C-1⁗′ and 3.43 (H-4⁗); in addition to these, C-5⁗′ and 5.12 (H-1⁗); C-5⁗′ and 4.40 (H-1⁗′); C-4⁗′ and 1.05 ppm (H-6⁗′) correlations were observed in sugar moieties.

Thus, paphlagonoside B (**2**), which is named 28-O-[β-D-xylopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 3)-β-D-xylopyranosyl(1 → 6)-β-D-glucopyranosyl(1 → 4)-β-D-glucopyra-nosyl]-hederagenin, is assigned as another new monodesmosidic acyl glycoside (Table 1, Figure 1).

The structure of the last compound (**3**) was determined by spectroscopic and co-chromatographic experiments as hederagenin. Thus, the structures of the two acyl triterpenic glycosides which have been observed rarely in natural product chemistry will contribute markedly to this genus, in addition to providing a considerable improvement in the chemotaxonomic markers of this family.

3. Experimental

3.1. *General*

The 1D and 2D NMR spectra of the compounds were recorded on a Varian AS 400 spectrometer in DMSO using TMS as an internal standard. ESI-MS measurements were performed by a Bruker HCT Ultra ESI-MS ion trap instrument. IR spectra were recorded on an ATI Mattson Genesis Series FT-IR in KBr. UV spectra were run on a Shimadzu UV-160S double beam spectrophotometer using 1.0 cm quartz cells. Optical rotations were measured in DMSO with a Rudolp Research Analitical Autopol I automatic polarimeter. Thin layer chromatography (TLC) was carried out using silica gel 60 F_{254} (Merck 5554) and reverse-phase silica gel RP-18 F_{254S} (Merck 15685) pre-coated aluminium sheets. Open column chromatography (CC) was carried out using silica gel 60 (0.063–0.200 mm) (Merck 7734) as a sorbent. Vacuum liquid chromatography (VLC) and medium pressure liquid chromatography (MPLC) were performed over reverse-phase silica gel LiChroprep RP-18

(25–40 μm) (Merck 9303). TLC analysis of the compounds and sugar moieties was performed on pre-coated silica gel plates. All chromatographic studies were run using hexane, BuOH : H_2O (1 : 1), CH_2Cl_2 : MeOH : H_2O (80 : 20 : 2–61 : 32 : 7 + 10% MeOH) and $CHCl_3$: MeOH (9 : 1) solvent and solvent systems. The spots were detected by spraying the plates with 20% H_2SO_4 solutions in water followed by heating at about 110°C.

For antioxidant activity studies, 2,2′-diphenyl-1-picrylhydrazyl, EtOH and 3-ter-but-4-hydroxyanisole (BHA) were used as reactives for the DPPH radical activity method. Ascorbic acid was used as the standard.

Klebsiella pneumonie (CCM 2318), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus cereus* (ATCC 7064), *Escherichia coli* (ATCC 23999), *Salmonella typhimurium* (CCM 5445), *Pseudomonas aureginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 6538-P) were used as test microorganisms on the basis of MIC values for *C. paphlagonica* in the antimicrobial activity analysis. Gentamycin was used as a positive control for bacteria.

3.2. *Plant material*

Cephalaria paphlagonica was collected from Çankırı, Turkey: Ilgaz rocky places between Başaraz-Kazancı villages about 1200–1300 m height in July 2007. A voucher specimen was collected and identified by Prof. Dr Hüseyin Sümbül and Asst. Prof. R. Süleyman Göktürk. It was deposited at the Herbarium of the Research and Application Center of Akdeniz University, Antalya, Turkey, with the number R.S. Göktürk 6100.

3.3. *Extraction and isolation*

Dried and powdered aerial parts of *C. paphlagonica* (1.76 kg) were extracted with 3 × 7000 mL of MeOH at room temperature. Each extraction was treated with solvent overnight. The MeOH extracts were combined and the solvent was removed by rotary evaporator under reduced pressure at ~40°C. The residue was extracted with MeOH : H_2O (1 : 1). The activity results showed that the MeOH : H_2O extract was more active than the other extracts for this plant. Therefore, this phase was fractionated with a 1 : 1 / BuOH : H_2O (250 : 250 mL) solvent system to obtain, in particular, the glycosidic components successively.

The MeOH extract (100 g) was also extracted with with a 1 : 1 / BuOH : H_2O (400 : 400 mL) solvent system. The BuOH and H_2O extracts were then separated from each other. The water extract was treated with a 1 : 1 / BuOH : H_2O (400 : 400 mL) solvent system. All BuOH extracts were combined and extracted with hexane (14 × 25 mL) to remove apolar parts of the plant. At the end of the extractions, two main points played an important role in deciding the isolation processes. The first was the biological activity results of the MeOH : H_2O extract, which showed higher antimicrobial and antioxidant activity than the other fractions (Tables 2 and 3). The second was the TLC findings of the BuOH phase, which showed a greater number of intense spots, indicating the presence of more glycosides in that fraction than the others. These two results may indicate that the

Table 2. Antimicrobial activity (MIC) results of the extracts and gentamicin ($\mu g\,mL^{-1}$).

Microorganisms	MeOH	Hexane	MeOH : H_2O	BuOH	H_2O	Gentamycine
S. aureus ATCC 6538/P	1000	1250	>2000	82.5	>2000	1.0
S. epidermidis ATCC 12228	750	>2000	>2000	82.5	>2000	1.0
S. typhimurium CCM 5445	1250	>2000	>2000	82.5	>2000	1.0
E. coli ATCC 23999	750	1000	>2000	82.5	>2000	1.0
B. cereus ATCC 7064	750	>2000	>2000	82.5	>2000	4.0
K. pneumonie CCM 2318	1250	1500	>2000	82.5	>2000	4.0
E. faecalis ATCC 29212	750	>2000	>2000	82.5	>2000	16.0
P. aeroginosa ATCC 27853	1250	>2000	>2000	82.5	>2000	2.0

Table 3. DPPH activity results of the extracts and vitamin C (IC_{50}, $\mu g\,mL^{-1}$).

Samples	MeOH	Hexane	MeOH : H_2O	BuOH	H_2O
C. paphlagonica	385	2284	194	410	462
Vitamin C	–	–	–	–	11

most active compounds are present in the BuOH fraction. Therefore, isolation studies were started with the BuOH (35.73 g) extract. This extract was chromatographed by VLC using RP silica gel (230 g) column, eluting from 100% H_2O to 100% MeOH, increasing the MeOH by 10%, and 10 fractions were obtained. Fraction 8 (9.89 g) was subjected to open silica gel column and eluted with a gradient of 80 : 20 : 2–61 : 32 : 7 / CH_2Cl_2 : MeOH : H_2O solvent systems, giving paphlagonoside A (**1**) (507.40 mg) and paphlagonoside B (**2**) (291.00 mg) (Table 1).

3.4. *Acid hydrolysis of 1 and 2*

The sugar moieties of the glycosides were identified by the micro-hydrolysis method on TLC under hydrochloric acid vapour. Pure compounds were applied on a TLC layer (silica gel 60 F_{254}) and treated with concentrated HCl vapour in a closed vessel for 45 min at 60°C. After the excess HCl was removed from the plate, the sugar references (glucose, galactose, arabinose, xylose, rhamnose, mannose and fucose) were applied to the TLC layer. The TLC was developed by a $CHCl_3$: MeOH : H_2O : gAcH /16 : 9 : 2 : 2 solvent system. Detection of the spots was realised by heating the plates at 110°C after spraying with α-naftol-H_2SO_4 (2%) solution (Su et al., 2001).

3.5. *Biological activities*

Two different activity experiments were undertaken on the studied extracts of the plant material. These activity methods are given below in detail.

3.5.1. *Antioxidant activity*

Antioxidant activity studies were prepared using the DPPH radical method for *C. paphlagonica*. This method is representative of the methods employing model

radicals in the evaluation of radical scavengers; such methods have gained high popularity over the last decade because of their rapidity and sensitivity. The important reagent DPPH, which has the molecular formula $C_{18}H_{12}N_5O_6$, was provided from Sigma (80% D-9132) and DPPH was dissolved in EtOH (200 mL) to obtain a 10^{-4} M solution. L-Ascorbic acid (Vitamin C) (Sigma) and 3-ter-but-4-hydroxyanisole (BHA) (Fluka, 20021) were used as standards at a concentration of 0.01% (w/v). The mass ratios of the solutions were prepared as extract (DPPH = 5.5 : 1) and standard (DPPH 0.5 : 1). For the control, 1 : 1/MeOH : DPPH solution (A_B) was used. Extract : EtOH/1 : 1 solution was used to prevent absorbance mistakes during measurement of the coloured extracts. The samples were incubated for 15 min in the dark at 30°C and from each sample 200 µL was added to a 96-well plate (PS Microplate 96 well, Greiner). A decrease in absorbance at 517 nm was measured against ethanol using a spectrophotometer (μ Quant Universal Microplate Spectrophotometer, KCJunior). Ethanol was used as a blank in spectrophotometric assay; a blank sample containing the same amount of ethanol and DPPH was prepared and measured daily. They were stored in a flask covered with aluminium foil and kept in the dark at 4°C between consecutive measurements (Koleva, van Beek, Linssen, Groot, & Evstatieva, 2002). All values were performed in triplicate. The radical scavenging activities of the tested samples are expressed as percentage inhibition of DPPH and they were calculated according to the following formula (Yen & Duh, 1994):

$$\text{Percentage inhibition} = 1 - [(A_B - A_A)/A_B] \times 100$$

where A_B and A_A are the absorbance values of the test and of the blank samples, respectively (Table 3).

3.5.2. *Antimicrobial activity*

Antimicrobial activity was examined by the minimum inhibition concentration (MIC) values. In this technique, the MIC values of the extracts were developed by National Committee of Clinical Laboratory Standards (NCCLS, 2003) and Atlas, Parks and Brown (1995). *In vitro* antimicrobial activity studies were carried out against different bacteria, which were obtained from the Microbiology Department Culture Collection of the Faculty of Science, Ege University. The bacteria strains were inoculated on Mueller–Hinton broth (Difco) and incubated for 24 h at 37 ± 0.1°C. The inocula of the bacterial strains were prepared from 24 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. This test was carried out using a series of test tubes containing the concentrations from 0.5 to 40 mg mL^{-1} for *C. paphlagonica* extracts. The 96-well plates were prepared by dispensing into each well 100 µL of broth containing the inoculum (1×10^5 CFU mL^{-1}). The extract prepared at a concentration of 40 mg mL^{-1} was added into the first well. Then, its serial dilutions were transferred into consecutive wells. The last well, containing Mueller–Hinton broth without compound and the inoculum on each strip, was used as negative control. The plate was covered with a sterile plate sealer. The plates were incubated at 37°C for 24 h. Gentamycin (Sigma) and clotrimazole (Sigma) were used as positive controls for bacteria strains. All assays were performed in duplicate (Table 2).

Acknowledgements

The authors are grateful to Prof. Dr H. Sümbül and Asst. Prof. R.S. Göktürk for collection and identification of the plant, and Prof. Dr N. Arda and Assoc. Prof. Dr Ü.K. Yavaşoğlu for discussing the antioxidant and antimicrobial activity results. We thank our Faculty for NMR spectra and the Biology Department of our Faculty for ESI-MS analysis, respectively. We are also thankful to TUBITAK (107T028) for financial support.

References

Alam, M.S., Kaur, G., Ali, A., Hamid, H., Ali, M., & Athar, M. (2008). Two new bioactive oleanane triterpene glycosides from *Terminalia arjuna*. *Natural Product Research, 22*(14), 1279–1288.

Aliev, A.M., & Movsumov, I.S. (1976). Triterpenoid glycosides from *Cephalaria kotschyi*. *Khimiia Prirodnykh Soedineniĭ, 2*, 264–265.

Aliev, A.M., Movsumov, I.S., & Bagirov, E. (1975). Alkaloids from certain *Cephalaria* species. *Khimiia Prirodnykh Soedineniĭ, 5*, 667.

Aliev, A.M., Movsumov, I.S., & Serkerov, S.V. (1975). Triterpene acid from the roots of *Cephalaria kotschyi* and *Cephalaria nachiczevanica*. *Khimiia Prirodnykh Soedineniĭ, 11*, 518.

Atlas, R.M., Parks, L.C, & Brown, A.E. (1995). *Laboratory manual of experimental microbiology*. St. Louis, MI: Mosby-Year Book, Inc.

Bobrov, E.G. (1972). *C. paphlagonica*. In P.H. Davis (Ed.), *Flora of Turkey and the East Aegean Islands* (Vol. 4, pp. 585–597). Edinburgh, Scotland: Edinburgh University Press.

Godevac, D., Mandic, B., Vajs, V., Menkovic, N., Macura, S., & Milosavljevic, S. (2006a). Complete assignments of ^{1}H and ^{13}C NMR spectra of leucanthoside A, a new triterpenoid saponin from *Cephalaria leucantha* L. *Magnetic Resonance in Chemistry, 44*, 731–735.

Godevac, D., Mandic, B., Vajs, V., Tesevic, V., Menkovic, N., Janackovic, P., et al. (2006b). Triterpenoid saponins and iridoid glycosides from the aerial parts of *Cephalaria pastricensis*. *Biochemical Systematics and Ecology, 34*, 890–893.

Godevac, D., Vajs, V., Menkovic, N., Tesevic, V., Janackovic, P., & Milosavljevic, S. (2004). Flavonoids from flowers of *Cephalaria pastricensis* and their antiradical activity. *Journal of Serbian Chemical Society, 69*(11), 883–886.

Hostettmann, K., & Marston, A. (1995). *Chemistry and pharmacology of natural products*. New York: Cambridge University Press.

Kırmızıgül, S., & Anıl, H. (1994). Transsylvanoside A, an acidic triterpene glycoside from *Cephalaria transsylvanica*. *Phytochemistry, 35*, 1075–1076.

Kırmızıgül, S., & Anıl, H. (2002). New triterpenic saponins from *Cephalaria transsylvanica*. *Turkish Journal of Chemistry, 26*, 947–954.

Kırmızıgül, S., Anıl, H., & Rose, M.E. (1995). Triterpenoid glycoside from *Cephalaria transsylvanica*. *Phytochemistry, 39*, 1171–1174.

Kırmızıgül, S., Anıl, H., & Rose, M.E. (1996). Triterpenoid saponins from *Cephalaria transsylvanica*. *Journal of Natural Products, 59*, 415–418.

Kırmızıgül, S., Anıl, H., Uçar, F., & Akdemir, K. (1996). Antimicrobial and antifungal activities of three new triterpenoid glycosides. *Phytotherapy Research, 10*, 274–276.

Kırmızıgül, S., & Rose, M.E. (1997). Hederagenin glycosides from the flowers of *Cephalaria transsylvanica*. *Planta Medica, 63*, 51–54.

Koleva, I.I., van Beek, T.A., Linssen, J.P.H., Groot, A., & Evstatieva, L.N. (2002). Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis, 13*(8), 17.

Movsumov, I.S., & Aliev, A.M. (1975). Flavonoids of some *Cephalaria* species. *Khimiia Prirodnykh Soedineniĭ, 11*(6), 804–805.

Movsumov, I.S., Aliev, A.M., Kondratenko, E.S., & Abubakirov, N.K. (1975). Triterpene glycosides of *Cephalaria kotschyi* and *Cephalaria nachiczevanica*. *Khimiia Prirodnykh Soedineniĭ, 11*, 519.

Mustafaeva, K., Elias, R., Balansard, G., Suleimanov, T., Mayu-Lede, V., & Kerimov, Y. (2008). Iridoid glycosides from *Cephalaria kotschyi* roots. *Chemistry of Natural Compounds, 44*(1), 132–133.

National Committee for Clinical Laboratory Standards. 2003. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard*. NCCLS Document M7-A6 (8th ed.). Wayne, Pennsylvania, USA: NCCLS.

Rai, M., & Mares, D. (2003). *Plant-derived antimycotics: Current trends and future prospects*. New York: Food Products Press.

Su, Y., Guo, D., Guo, H., Liu, J., Zheng, J., Koike, K., et al. (2001). Four new triterpenoid saponins from *Conyza blinii*. *Journal of Natural Products, 64*, 32–36.

Tabatadze, N., Elias, R., Faure, R., Gerkens, P., Pauw-Gillet, M.C., Kemertelidze, E., et al. (2007). Cytotoxic triterpenoid saponins from the roots of *Cephalaria gigante*. *Chemical & Pharmaceutical Bulletin, 55*(1), 102–105.

Yen, G.-C., & Duh, P.-D. (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *Journal of Agricultural and Food Chemistry, 42*, 629–632.