Four New Triterpenes from *Anchusa azurea* var. *azurea*

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Four new triterpene glycosides, named oleanazuroside 1 (**1**), oleanazuroside 2 (**2**), ursolazuroside 1 (**3**), and ursolazuroside 2 (**4**), together with the seven known compounds **5**–**11**, were isolated from the MeOH extract of the aerial parts of *Anchusa azurea* MILLER var. *azurea*. Their structures were elucidated by means of spectroscopic evidence (UV, IR, MALDI-MS, and 1D- and 2D-NMR). The radical-scavenging activities against 2,2-diphenyl-1-picrylhydrazyl (DPPH) of the BuOH extract and of **8** and **10** were very strong (*Table 5*).

Introduction. – The genus *Anchusa* L. (Boraginaceae) is represented by 15 species in the flora of Turkey [1]. *Anchusa* species are used in folk medicine for wound healing and as a diuretic agent [2][3]. It was found that *A. strigosa* roots, which are used for the treatment of stomach ulcers in Jordan, prevented ulcer formation in an EtOH-induced gastric-ulcer model in rats [4]. Pyrrolizidine alkaloids, flavonoids, triterpene saponins, fatty acids, and phenolic acids were isolated from *Anchusa* species [5–17]. In our previous study on the aerial parts of *A. leptophylla*, flavonol glycosides and triterpene saponins were isolated [18]. In this study, we report the first phytochemical and biological work carried out on *A. azurea* MILLER var. *azurea*, which resulted in the isolation and characterization of four new saponins, oleanazuroside 1 (**1**), oleanazuroside 2 (**2**), ursolazuroside 1 (**3**), and ursolazuroside 2 (**4**), together with the seven known compounds **5**–**11** (*Fig.*). In addition, we tested the various extracts and isolated phenolic compounds for radical-scavenging activity by comparison with ascorbic acid as reference.

Results and Discussion. – The MeOH extract of the aerial parts of *A. azurea* var. *azurea* was fractionated with hexane and then BuOH. The BuOH-soluble fraction of the MeOH extract was chromatographed repeatedly on various columns and yielded the eleven compounds **1**–**11** (*Fig.*). Their structures were elucidated as oleanazuroside 1 (**1**), oleanazuroside 2 (**2**), ursolazuroside 1 (**3**), ursolazuroside 2 (**4**), (2α,3β,4α,19α)-2,3,19,23-tetrahydroxyurs-12-en-28-oic acid β-glucopyranosyl ester (=quercilicosid A; **5**) [12][19], kaempferol 3-(β-glucopyranoside) (=astragalin; **6**), quercetin 3-(β-glucopyranoside) (=isoquercitrin; **7**), quercetin 3-(α-rhamnopyranosyl-(1→6)-β-glucopyranoside) (=rutin; **8**), kaempferol 3-(α-rhamnopyranosyl-(1→6)-β-glucopyranoside) (**9**) [20–22], rosmarinic acid (**10**), and 3-(3,4-dihydroxyphenyl)lactic acid

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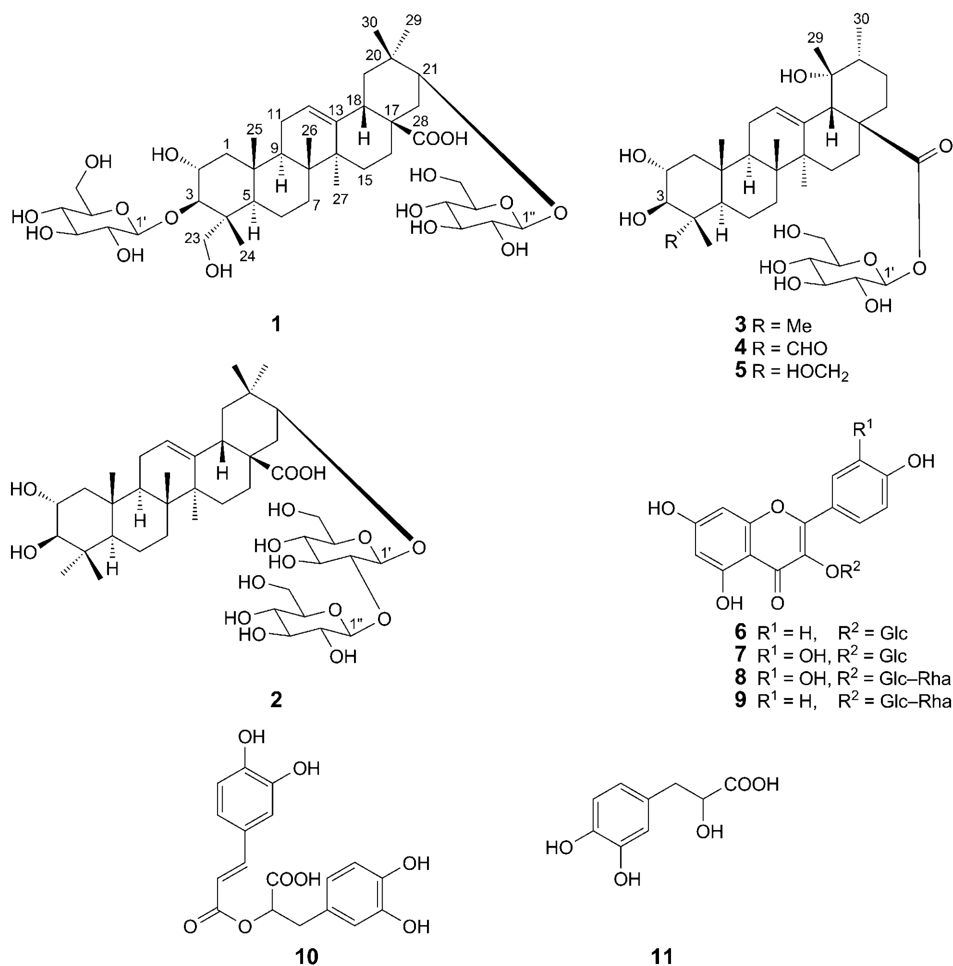

Figure. *Compounds* **1**–**11** *isolated from* Anchusa azurea MILLER *var.* azurea

(**11**) [23] by extensive spectroscopic methods including 1D- (^{1}H, ^{13}C) and 2D-NMR (DQF-COSY, HSQC, and HMBC) experiments as well as positive-ion and reflectron-mode MALDI-MS analysis and by comparison of their physical and spectroscopic data with those reported for authentic samples.

The UV spectra of **1**–**5** showed only an end absorption at 208 nm, indicating the absence of any chromophore. The IR spectra of all compounds **1**–**5** showed OH group absorption (3400 cm^{-1}), a COOH group absorption (1697 cm^{-1}) for **1** and **2**, and an ester group absorption (1724 cm^{-1}) for **3**–**5**.

Compounds **1** and **2** were isolated as white amorphous powders. The molecular formulae of **1** and **2** were defined as $C_{42}H_{68}O_{16}$ and $C_{42}H_{70}O_{15}$, respectively, by positive-ion and reflectron-mode MALDI-MS (sodiated molecular ion $[M+Na]^+$ at *m/z* 851.4387 and 837.7763, resp.). The ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) of **1**,

which were assigned by various NMR experiments, showed signals assignable to six Me groups (6*s*, 3 H each, Me(24), Me(25), Me(26), Me(27), Me(29), and Me(30) at δ 0.68, 1.02, 0.80, 1.18, 1.21, and 0.96, resp.), the olefinic H−C(12) (br. *s* at δ 5.27), a CH_2 group (CH_2(23)), and three CH−O groups (br. *s*, H−C(2) at δ 3.66; br. *s*, H−C(3) at δ 3.54; *d*, H−C(21) at 3.56), besides two glucopyranosyl units (*d*, J = 7.3 Hz, H−C(1″) at δ 4.43 and *d*, J = 7.7 Hz, H−C(1′) at δ 4.64). These data also suggested the presence of an oleanolic acid derivative with one of the Me groups replaced by a CH_2OH function [24]. The coupling constants of the anomeric H-atoms and the ^{1}H- and ^{13}C-NMR spectra of **1** indicated the β-conformation for both glucose moieties. Two *dd* at δ 3.68 and 3.84 and δ 3.64 and 3.84 were due to the CH_2(6′) and CH_2(6″) groups, respectively, of the sugar units. Two C*H*−OH signals appeared at δ 3.66 (*ddd*, $J(2\beta,1\alpha)$ = 11.5, $J(2\beta,3\alpha)$ = 9.1 and $J(2\beta,1\beta)$ = 5.5 Hz) and 3.54 (*d*, J = 9.1 Hz) and were assigned to H−C(2) and H−C(3), respectively [25].

Table 1. *^{1}H-NMR Data* (CD_3OD, 400 MHz) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

	1	**2**		**1**	**2**
CH_2(1)	3.31[a])	3.30[a])	Me(25)	1.02 (*s*)	0.80 (*s*)
H−C(2)	3.66[a])	3.68[a])	Me(26)	0.80 (*s*)	0.80 (*s*)
H−C(3)	3.54[a])	2.90[a])	Me(27)	1.18 (*s*)	1.07 (*s*)
H−C(5)	0.88[a])	0.83[a])	Me(29)	1.21 (*s*)	1.00 (*s*)
CH_2(6)	0.92[a])	1.04[a])	Me(30)	0.96 (*s*)	0.98 (*s*)
CH_2(7)	[a])	1.28[a])	H−C(1′)	4.64 (*d*, J = 7.7)	4.43 (*d*, J = 7.3)
H−C(9)	1.69[a])	1.72[a])	H−C(2′)	3.19–3.24 (*m*)	3.52–3.55 (*m*)
CH_2(11)	1.98[a])	1.94[a])	H−C(3′)	3.33–3.38 (*m*)	3.56–3.59 (*m*)
H−C(12)	5.27 (br. *s*)	5.26 (br. *s*)	H−C(4′)	3.28–3.33 (*m*)	3.22–3.26 (*m*)
CH_2(15)	1.09[a])	1.18[a])	H−C(5′)	3.30–3.38 (*m*)	3.25–3.29 (*m*)
CH_2(16)	1.96[a])	1.98[a])	CH_2(6′)	3.68 (*dd*, J = 12, 5.5),	3.69 (*dd*, J = 12.0, 5.1),
H−C(18)	2.88 (*dd*,	2.87 (*dd*,		3.84 (*dd*, J = 12, 2.2)	3.84 (*dd*, J = 12.0, 2.9)
	J = 10.2, 3.6)	J = 10.1, 3.6)	H−C(1″)	4.43 (*d*, J = 7.3)	4.64 (*d*, J = 7.7)
CH_2(19)	1.37[a])	1.82[a]), 1.94[a])	H−C(2″)	3.20–3.24 (*m*)	3.17–3.19 (*m*)
H−C(21)	3.56[a])	3.56[a])	H−C(3″)	3.50–3.59 (*m*)	3.30–3.36 (*m*)
CH_2(22)	1.72[a])	1.70[a]), 1.90[a])	H−C(4″)	3.20–3.24 (*m*)	3.27–3.32 (*m*)
CH_2(23)	[a])	0.96 (*s*)	H−C(5″)	3.20–3.29 (*m*)	3.30–3.39 (*m*)
or Me(23)			CH_2(6″)	3.64 (*dd*, J = 12, 5.1)	3.65 (*dd*, J = 12, 5.0),
Me(24)	0.68 (*s*)	1.15 (*s*)		3.84 (*dd*, J = 12, 2.2)	3.82 (*dd*, J = 12, 2.0)

[a]) Signal pattern unclear due to overlapping.

The ^{13}C-NMR and DEPT-90 spectra of **1** showed resonances for all 42 C-atoms revealing 6 Me, 11 CH_2, 17 CH, and 8 quaternary C-atoms. They also showed one COOH signal at δ 179.5, two olefinic C-atoms at δ 122.6 and 143.2 for C(12) and C(13), eleven CH−O groups at δ 68.4–83.6 for C(2), C(3), C(21), C(2′)–C(5′), and C(2″)–C(5″), three CH_2−O groups at δ 61.5, 61.8, and 65.0 for C(6′), C(6″), and C(23), and two anomeric C-atoms at δ 103.5 and 103. 8 for C(1′) and C(1″), respectively. Direct one-bond ^{1}H,^{13}C-connectivities of each protonated C-atom were deduced with the help of HSQC data. The structure of **1** was characterized by an HMBC experiment, in which long-range correlations were observed between the

Table 2. ^{13}*C-NMR Data* (CD_3OD, 100 MHz) *of Compounds* **1** *and* **2**[a]). δ in ppm.

	1	**2**		**1**	**2**
CH_2(1)	47.8	47.8	CH_2(22)	39.1	39.2
CH(2)	68.4	68.3	CH_2(23) or Me(23)	65.0	28.1
CH(3)	80.3	83.2	Me(24)	12.6	15.9
C(4)	42.9	38	Me(25)	16.3	16.2
CH(5)	46.9	55.5	Me(26)	16.5	16.5
CH_2(6)	17.9	18.4	Me(27)	25.1	25.1
CH_2(7)	32.2	32.7	C(28)	179.5	[b])
C(8)	39.3	39.3	Me(29)	29.9	28.3
CH(9)	47.1	47.1	Me(30)	17.2	17.2
C(10)	37.8	36.4	CH(1′)	103.5	103.8
CH_2(11)	23.9	23.4	CH(2′)	75.2	80.3
CH(12)	122.6	122.5	CH(3′)	76.9	77.2
C(13)	143.2	143.3	CH(4′)	70.3	70.6
C(14)	41.8	41.7	CH(5′)	76.6	77.1
CH_2(15)	28.3	27.7	CH_2(6′)	61.5	61.8
CH_2(16)	23.4	24	CH(1″)	103.8	103.5
C(17)	48.3	48.1	CH(2″)	76.3	75.2
CH(18)	40.8	40.9	CH(3″)	77.2	76.6
CH_2(19)	46.7	46.9	CH(4″)	70.6	70.3
C(20)	36.4	36.4	CH(5″)	77.1	76.3
CH(21)	83.6	83.7	CH_2(6″)	61.8	61.5

[a]) Assignments were based on COSY, HSQC, and HMBC experiments. [b]) Signal pattern unclear due to overlapping.

following H-atom and C-atom pairs: H−C(1′) (δ 4.64 (d, J = 7.7 Hz))/C(3) (δ 80.3) and H−C(1″) (δ 4.43 (d, J = 7.3 Hz))/C(21) (δ 83.6).

The 1D- and 2D-NMR data of **2** were in agreement with those of **1** (*Tables 1* and *2*). The only difference between **2** and **1** was the lack of one CH_2 group and the presence of an additional Me group in **2** (Me(23) at δ(C) 28.1 and δ(H) 0.96), besides long-range correlations between these Me H-atoms and C(3), C(4), and C(5). Furthermore, the position of the sugar residues was evident from the HMBC spectra where the anomeric H−C(1′) (δ 4.43) and H−C(1″) (δ 4.64) exhibited connectivities with C(21) (δ 83.7) and C(2′) (δ 80.3), respectively.

Assigment of all the ^{1}H- and ^{13}C-NMR data of **1** and **2** was accomplished by further comparisons with those of oleanolic acid (=(3β)-3-hydroxyolean-12-en-28-oic acid) and its glycosides [26][27], hederagenin (=(3β,4α)-3,23-dihydroxyolean-12-en-28-oic acid), and its glycosides [28][29], caccigenin (=(2α,3β,4α,21β)-2,3,21,23-tetrahydroxy-olean-12-en-28-oic acid) and its glycosides [8][9][19][30], and arjunolic acid (=(2α,3β,4α)-2,3,23-trihydroxyolean-12-en-28-oic acid) and arjunglucoside II (=(2α,3β,4α)-2,3,23-trihydroxyolean-12-en-28-oic acid β-D-glucopyranosyl ester) [31]. The type of aglycone of compounds **1** and **2** was determined as caccigenin. Consequently, the structures of compounds **1** and **2**, which are novel natural products, were concluded to be (2α,3β,4α,21β)-3,21-bis(β-glucopyranosyloxy)-2,23-dihydroxy-olean-12-en-28-oic acid and (2α,3β,21β)-21-[(β-glucopyranosyl-(1 $\rightarrow$ 2)-β-glucopyra-

nosyl)oxy]-2,3-dihydroxyolean-12-en-28-oic acid, respectively. Their trivial names are proposed as oleanazuroside 1 (**1**) and oleanazuroside 2 (**2**).

Saponins **3**–**5** were obtained as amorphous colorless compounds. Their ^{13}C-NMR spectra revealed 36 C-atom signals of which 6 were assigned to a hexose unit and the remaining 30 signals to a triterpenoid skeleton. The aglycones appeared to have ursane-type skeletons according to their NMR spectra (*Tables 3* and *4*) [26][32]. In the ^{13}C-NMR spectrum, the signal of C(28) at δ 177.3, consistent with the IR absorption at 1724 cm^{-1}, indicated the presence of an ester group. Assignments for all H- and C-atom resonances (*Tables 3* and *4*) were achieved by COSY, HSQC, and HMBC experiments. The ^{1}H-NMR spectra of **3**–**5** exhibited resonances for the anomeric H-atom of the sugar moiety at δ *ca.* 5.31 (*d*, J = 8.0 Hz, 1 H) which was assigned to the anomeric H-atom of β-glucose. In addition, the shifts observed for the C-atoms of the β-glucose unit, the values of the anomeric C-atom C(1′) (δ *ca.* 94.5) were in agreement with a site of glycosylation at the C(28)OOH group. Furthermore, HMBCs between the anomeric H-atoms H–C(1′) (δ *ca.* 5.31) and C(28) (δ 177.3) were found. The ^{1}H-NMR of **3** showed two CH–O groups at δ 4.10 and 2.85, assigned to H–C(2) and H–C(3), respectively.

Table 3. *^{1}H-NMR Data* (CD_3OD, 400 MHz) *for Compounds* **3** *and* **4**. δ in ppm, J in Hz.

	3	**4**		**3**	**4**
CH_2(1)	3.40[a]), 3.20[a])	3.40[a]), 3.20[a])	CH_2(22)	1.75[a])	1.75[a])
H–C(2)	4.10[a])	4.10[a])	Me(23) or H–C(23)	1.33 (*s*)	9.8 (*s*)
H–C(3)	2.85 (*d*, J = 9.5, 3.1)	2.85 (*d*, J = 9.5, 3.1)	Me(24)	0.79 (*s*)	0.78 (*s*)
H–C(5)	1.09[a])	[a])	Me(25)	0.96 (*s*)	0.93 (*s*)
CH_2(6)	1.81[a])	1.81[a])	Me(26)	1.94 (*s*)	1.94 (*s*)
CH_2(7)	1.79[a])	1.79[a])	Me(27)	1.19 (*s*)	1.18 (*s*)
H–C(9)	1.70[a])	1.70[a])	Me(29)	1.37 (*s*)	1.35 (*s*)
CH_2(11)	2.01[a])	2.01[a])	Me(30)	0.92 (*d*, J = 6.5)	[a])
H–C(12)	5.30 (br. *s*)	5.31 (br. *s*)	H–C(1′)	5.31 (*d*, J = 8)	5.30 (*d*, J = 8)
CH_2(15)	1.78[a])	1.78[a])	H–C(2′)	3.28–3.32 (*m*)	[a])
CH_2(16)	1.21[a])	1.21[a])	H–C(3′)	3.30–3.33 (*m*)	[a])
H–C(18)	2.51 (*s*)	2.51 (*s*)	H–C(4′)	3.32–3.37 (*m*)	[a])
H–C(20)	1.33[a])	1.33[a])	H–C(5′)	3.37–3.43 (*m*)	[a])
CH_2(21)	1.21[a])	1.21[a])	CH_2(6′)	3.65 (*dd*, J = 12, 4.3), 3.80 (*dd*, J = 12, 2.0)	3.67 (*dd*, J = 12, 4.5), 3.77 (*dd*, J = 12, 2.0)

[a]) Signal pattern unclear due to overlapping.

Comparison of the NMR spectra of **3** with that of quercilicoside A (=(2α,3β,4α,19α)-2,3,19,23-tetrahydroxyurs-12-en-28-oic acid β-D-glucopyranosyl ester; **5**), the major triterpene isolated from *Quercus laurifolia* previously [33], showed that most of the C-atom resonances (*Table 4*) were almost superimposable; the only meaningful difference was observed for the C(23) position which was attributed to the presence of a Me(23) group in **3** instead of a CH_2(23)OH group in quercilicoside A (**5**). The ^{1}H- and ^{13}C-NMR spectra of **4** were very similar to those of **3**, except for the replacement of a Me signal by an CHO group. This was consistent with a signal at δ

Table 4. *^{13}C-NMR Data for Compounds* **3** *and* **4** (CD_3OD, 100 MHz)[a]). δ in ppm.

	3	**4**		**3**	**4**
CH_2(1)	47.1	46.3	C(19)	72.4	72.4
CH(2)	68.2	68.0	CH(20)	41.7	41.7
CH(3)	83.3	81.5	CH_2(21)	28.4	28.4
C(4)	40.0	54.2	CH_2(22)	37.0	37.1
CH(5)	56.5	56.9	Me(23) or CH(23)	23.3	207.4
CH_2(6)	20.3	20.2	Me(24)	16.3	15.4
CH_2(7)	33.2	32.5	Me(25)	14.4	16.3
C(8)	40.0	39.9	Me(26)	15.4	18.8
CH(9)	46.7	46.2	Me(27)	25.9	24.0
C(10)	38.4	38.1	C(28)	177.3	177.3
CH_2(11)	23.7	23.4	Me(29)	23.9	26.0
CH(12)	128.4	128.2	Me(30)	15.4	16.4
C(13)	138.5	138.5	CH(1′)	94.6	94.5
C(14)	41.6	41.5	CH(2′)	72.7	72.6
CH_2(15)	25.3	29.1	CH(3′)	77.3	77.4
CH_2(16)	26.0	25.3	CH(4′)	69.9	69.9
C(17)	48.4	[b])	CH(5′)	77.1	77.0
CH(18)	53.8	53.7	CH_2(6′)	61.2	61.2

[a]) Assignments were based on COSY, HSQC, and HMBC experiments. [b]) Signal pattern unclear due to overlapping.

207.4 for CH(23)=O. The positive-ion and reflectron-mode MALDI-MS quasi-molecular ions $[M+H]^+$ of **3** and **4** gave peaks at *m/z* 651.4016 for $C_{36}H_{59}O_{10}^+$ and 665.3812 for $C_{36}H_{57}O_{11}^+$, respectively. Thus, compounds **3** and **4** were established as (2*α*,3*β*,19*α*)-2,3,19-trihydroxyurs-12-en-28-oic acid *β*-glucopyranosyl ester and (2*α*,3*β*,4*α*,19*α*)-2,3,19-trihydroxy-23-oxours-12-en-28-oic acid *β*-glucopyranosyl ester for which the trivial names ursolazuroside 1 and ursolazuroside 2 are proposed, respectively.

DPPH-Radical-scavenging activity of the extracts prepared from *A. azurea* aerial parts and phenolic compounds which were isolated from their BuOH extract are shown in *Table 5* (DPPH = 2,2-diphenyl-1-picrylhydrazyl). The scavenging activities of the BuOH extract and of **8** and **10** were very strong. Recent research suggests a role for antioxidants in wound healing. *A. azurea* is rich in antioxidant phenolic compounds like flavonoids and phenolic acids. Additionally, oleanolic acid has shown a good wound-healing activity [34]. In summary, these isolated compounds may play a significant role in the ethnobotanical usage of *A. azurea.*

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Table 5. *DPPH-Radical-Scavenging Activities of the Extracts and of Compounds* **6–10**

	IC_{50} [a]) [µg/ml]		IC_{50} [a]) [µM]
Hexane extract	–	**6**	–
BuOH extract	24.42	**7**	65.93
MeOH extract	–	**8**	25.92
H_2O extract	88.65	**9**	–
		10	24.38
		Ascorbic acid [b])	12.01

[a]) IC_{50} values were calculated from regression lines obtained with six different concentrations in triplicate. [b]) Positive control.

Experimental Part

General. DPPH (2,2-diphenyl-1-picrylhydrazyl = 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazinyl) was used for the radical-scavenging-activity test. TLC: precoated silica gel *60 F_{254}* aluminium sheets (*Merck*); detection by UV fluorescence and spraying with 1% vanillin/H_2SO_4 reagent, followed by heating at 105° for 1–2 min. Column chromatography (CC): silica gel *60* (0.063–0.200 mm; *Merck*) and *Sephadex LH-20* (*Fluka*). Vacuum liquid chromatography (VLC): reversed-phase *Lichroprep RP-18* (25–40 µm; *Merck*). Prep. HPLC: *Büchi* (3 × 45 cm) glass columns packed with *LiChroprep C_{18}* (40–63 µm; *Merck*); *Dionex-P680* pump. Optical rotation: *Rudolph-Research-Analytical-Autopol-IV* automatic polarimeter; in MeOH. UV Spectra: *Biotek-µQuant-MQX200* microplate spectrophotometer; in MeOH; λ_{max} (log ε) in nm. IR Spectra: *Mattson-1000*-FT-IR spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Varian-Mercury-plus* spectrometer; at 400 (^1H) and 100 MHz (^{13}C) in CD_3OD; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR- (pos.) and MALDI-TOF-MS (reflectron pos., mass resolution 14700): *Applied Biosystems Voyager DE*™, PRO; in *m/z* (rel. %).

Plant Material. Anchusa azurea MILLER var. *azurea* was collected from Ankara-Beytepe, Turkey on 07/15/2003. A voucher specimen was deposited with the Herbarium of Hacettepe University, Faculty of Pharmacy, Ankara, Turkey, under the number HUEF 03012.

Extraction and Isolation. Air-dried and powdered aerial parts (600 g) were extracted with 3 × 3 l of MeOH at 45° for 4 h. The filtered, combined, and concentrated MeOH extract (78 g) was dissolved in dist. H_2O (150 ml) and partitioned with hexane and BuOH (7 g). The BuOH-soluble fraction of the MeOH extract was subjected to CC (*Sephadex LH-20*): *Fractions A–E*. *Fr. A* (3.6 g) was subjected to reversed-phase prep. HPLC (30–100% MeOH/H_2O) and repeatedly to normal-phase CC (SiO_2, $CHCl_3$/MeOH/H_2O 90 : 10 : 1, 80 : 20 : 2, 70 : 30 : 3, and 60 : 40 : 4), and CC (*Sephadex LH-20*, MeOH): **1** (96 mg), **2** (15 mg), **3** (28 mg), **4** (29 mg), **5** (16 mg), and **10** (200 mg). *Fr. C* (1.2 g) was purified by repeated-reversed-phase VLC (0–100% MeOH/H_2O), normal-phase CC (SiO_2, AcOEt/MeOH : H_2O 100 : 7.5 : 2.5 and 100 : 10 : 5), and CC (*Sephadex LH-20*): **6** (46 mg), **7** (20 mg), **8** (14 mg), **9** (70 mg), and **11** (5 mg).

Oleanazuroside 1 (= *(2α,3β,4α,21β)-3,21-Bis(β-glucopyranosyloxy)-2,23-dihydroxyolean-12-en-28-oic Acid*; **1**): Amorphous colorless powder. $[\alpha]_D^{20} = +3.2$ ($c = 0.5$, MeOH). IR: 3379, 2921, 1697, 1634, 1078. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. MALDI-MS (pos. and reflectron mode): 851.4387 ($[M + Na]^+$, $C_{42}H_{68}NaO_{16}^+$; calc. 851.4405).

Oleanazuroside 2 (= *(2α,3β,21β)-21-[(2-O-β-Glucopyranosyl-β-glucopyranosyl)oxy]-2,3-dihydroxyolean-12-en-28-oic Acid*; **2**): Amorphous colorless powder. $[\alpha]_D^{20} = -0.8$ ($c = 0.5$, MeOH). IR: 3374, 2945, 1699, 1634, 1077. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. MALDI-MS (pos. and reflectron mode): 837.7763 ($[M + Na]^+$, $C_{42}H_{70}NaO_{15}^+$; calc. 837.4607).

Ursolazuroside 1 (= *(2α,3β,19α)-2,3,19-Trihydroxyurs-12-en-28-oic Acid β-Glucopyranosyl Ester*; **3**): Amorphous colorless powder. $[\alpha]_D^{20} = -30$ ($c = 0.06$, MeOH). IR: 3391, 2931, 1724, 1462, 1070. ^1H- and ^{13}C-NMR: *Tables 3* and *4*. MALDI-MS (pos. and reflectron-mode): 651.4016 ($[M + H]^+$, $C_{36}H_{59}O_{10}^+$; calc. 651.4108).

Ursolazuroside 2 (=*(2α,3β,4α,19α)-2,3,19-Trihydroxy-23-oxours-12-en-28-oic Acid β-Glucopyranosyl Ester*; **4**): Amorphous colorless powder. $[\alpha]_D^{20} = -10$ ($c = 0.2$, MeOH). IR: 3400, 2935, 1735, 1070. ^{1}H- and ^{13}C-NMR: *Tables 3* and *4*. MALDI-MS (pos. and reflectron mode): 665.3812 ($[M+H]^+$, $C_{36}H_{57}O_{11}^+$; calc. 665.3901).

DPPH-Radical-Scavenging Activity. The DPPH assay was carried out by the method described in [35]. The radical-scavenging effect of the extracts and the isolated compounds **6**–**10** was assessed spectroscopically by the decoloration of the MeOH soln. of DPPH; ascorbic acid was used as standard. Each MeOH soln. (230 μl) of the tested compounds at various concentrations (100, 50, 25, 10, 5, and 1 μg/ml for extracts and 200, 100, 50, 25, 10, and 1 μM for substances) was added to the DPPH soln. (50 μl; 0.022% in MeOH). The mixture was allowed to react for 30 min at r.t. The absorbance of the soln. was read at 517 nm with a spectrophotometer The radical-scavenging activity was determined by comparing the absorbance with that of a blank (100%) containing only DPPH and solvent. The percentage of radical-scavenging activity (*RSA* [%]) was calculated as follows: $RSA\ [\%] = [(A_c - A_t)/A_c] \times 100\%$, where A_c is the average absorbance of the control, and A_t is the absorbance of the test compounds. All the analyses were performed in triplicates. IC_{50} Values are given in *Table 5*.

REFERENCES

[1] D. F. Chamberlain, 'Anchusa L.', in 'Flora of Turkey and East Aegean Islands', Ed. P. H. Davis, University Press, Edinburgh, 1978, Vol. 6, p. 388.

[2] E. Yeşilada, G. Honda, E. Sezik, M. Tabata, F. Tetsuro, T. Tanaka, Y. Takeda, Y. Takaishi, *J. Ethnopharmacol.* **1995**, *46*, 133.

[3] G. Honda, E. Yeşilada, M. Tabata, E. Sezik, F. Tetsuro, Y. Takeda, Y. Takaishi, T. Tanaka, *J. Ethnopharmacol.* **1996**, *53*, 75.

[4] A. M. Disi, S. O. Tamimi, G. M. Abuereish, *J. Ethnopharmacol.* **1998**, *60*, 189.

[5] G. Romussi, S. Cafaggi, L. Mosti, *Pharmazie* **1979**, *34*, 751.

[6] G. Romussi, G. Ciarallo, G. Falsone, C. Schneider, *Liebigs Ann. Chem.* **1979**, *12*, 2028.

[7] G. Romussi, S. Cafaggi, G. Bignardi, *Pharmazie* **1980**, *35*, 498.

[8] G. Romussi, G. Falsone, A. E. G. Crea, E. Finner, *Arch. Pharm.* **1983**, *316*, 499.

[9] G. Romussi, G. Falsone, D. Wendisch, B. Parodi, *Liebigs Ann. Chem.* **1984**, 1869.

[10] G. Romussi, G. Falsone, D. Wendisch, *Arch. Pharm.* **1986**, *319*, 549.

[11] G. Romussi, S. Cafaggi, C. Pizza, *Arch. Pharm.* **1988**, *321*, 753.

[12] G. Romussi, G. Falsone, *Pharmazie* **1983**, *38*, 787.

[13] G. Romussi, G. Falsone, *Arch. Pharm.* **1985**, *318*, 219.

[14] H. Hendriks, A. P. Bruins, H. J. Huizing, *Biomed. Environ. Mass Spectrom.* **1988**, *17*, 129.

[15] A. El-Shazly, M. El-Domiaty, L. Witte, M. Wink, *Biochem. Syst. Ecol.* **1998**, *26*, 619.

[16] N. Erdemoglu, S. Kusmenoglu, M. Vural, *Eur. J. Lipid Sci. Technol.* **2004**, *106*, 160.

[17] J. L. Guil-Guerrero, F. Maroto, F. Garcia, A. Gimenez, *J. Am. Oil Chem. Soc.* **2001**, *78*, 677.

[18] L. Ö. Demirezer, A. Zeeck, *H. U. J. Fac. Pharm.* **2000**, *20*, 7.

[19] G. Romussi, G. Bignardi, F. Sancassan, G. Falsone, *Liebigs Ann. Chem.* **1983**, 1448.

[20] T. J. Mabry, K. R. Markham, M. B. Thomas, 'The Systematic Identification of Flavonoids', Springer-Verlag, New York, 1970.

[21] K. R. Markham, H. Geiger, 'The Flavonoids: Advances in Research', Ed. J. B. Harborne and Mabry, Chapman & Hall Ltd., New York, 1982, Chapt. 2, p. 19.

[22] K. R. Markham, V. M. Chari, 'The Flavonoids: Advances in Research', Ed. J. B. Harborne and Mabry, Chapman & Hall Ltd., New York, 1982, Chapt. 10, p. 441.

[23] C. J. Kelley, R. C. Harruff, M. Carmack, *J. Org. Chem.* **1976**, *41*, 449.

[24] S. B. Mahato, A. P. Kundu, *Phytochemistry* **1994**, *35*, 1515.

[25] H. Kojima, H. Ogura, *Phytochemistry* **1989**, *28*, 1703.

[26] W. Seebacher, N. Simic, R. Weis, R. Saf, O. Kunert, *Magn. Reson. Chem.* **2003**, *41*, 636.

[27] Y. Zang, D. L. DeWitt, S. Murugesan, M. G. Nair, *Life Sci.* **2005**, *77*, 3222.

[28] K. Hostettmann, *Helv. Chim. Acta.* **1980**, *63*, 606.

[29] H. B. Wang, R. Mayer, G. Rücker, *Phytochemistry* **1993**, *33*, 1469.
[30] F. N. Ngounou, Atta-ur-Rahman, M. I. Choudhary, S. Malik, S. Zareen, R. Ali, D. Lontsi, B. L. Sondengam, *Phytochemistry* **1999**, *52*, 917.
[31] G. Romussi, N. De Tommasi, *Pharmazie* **1992**, *47*, 877.
[32] B. Baykal, T. Panayır, D. Tasdemir, O. Sticher, I. Çalıs, *Phytochemistry* **1998**, *48*, 867.
[33] G. Romussi, G. Caviglioli, C. Pizza, N. De Tommasi, *Arch. Pharm.* **1993**, *326*, 525.
[34] G. Moura-Letts, L. F. Villegas, A. Marcalo, A. J. Vaisberg, G. B. Hammond, *J. Nat. Prod.* **2006**, *69*, 978.
[35] A. Cavin, K. Hostettmann, W. Dyatmyko, O. Potterat, *Planta Med.* **1997**, *64*, 393.

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