Antimicrobially Active Hederagenin Glycosides from *Cephalaria elmaliensis*

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Key words
- *Cephalaria elmaliensis*
- Dipsacaceae
- triterpene glycoside
- elmalienoside A–C
- antimicrobial activity

Abstract

A phytochemical investigation of the aerial parts of *Cephalaria elmaliensis* resulted in the isolation of ten hederagenin-type triterpene saponins (**1**–**10**) including three new ones, elmalienoside A (**1**), elmalienoside B (**2**), elmalienoside C (**3**), and two known flavonoid glycosides (**11**–**12**). Their structures were identified by extensive spectroscopic techniques (1D and 2D NMR, HR ESIMS) and chemical evidence. The antimicrobial activity of the extracts and the pure compounds was evaluated by the MIC method. According to the results, all pure compounds including the new ones were found to be very active against both gram-positive and gram-negative bacteria.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

Triterpene glycosides are constituents of many plant drugs and folk medicines, especially from the Orient. Consequently, great interest has been shown in their characterization and in the investigation of their pharmacological and biological properties. The list of biological activities associated with triterpene glycosides is very long. Certain attributes of saponins, such as their fungicidal and piscicidal effects, have been known for many years, while new activities are continually being discovered [1–3]. In light of all of this knowledge, we decided to investigate the glycosidic compounds in *Cephalaria elmaliensis* (Dipsacaceae). Previous phytochemical investigations on different *Cephalaria* species have reported a number of triterpene saponins, iridoids, flavonoids, alkaloids, lignans, and their glycosidic compounds [4–15]. Triterpene saponins are the major examples of these types of substances [4–11]. Furthermore, many *Cephalaria* species have been used in traditional medicine for many years due to their antimicrobial, antifungal, antioxidant, alleviative, anti-infectant, hypothermic, relaxant, insecticidal, and cytotoxic activities [7–13, 16]. *Cephalaria elmaliensis* (Hub.-Mor. & Matthews) is an endemic species which is widespread mainly in the southwestern Anatolia region [17].

Preliminary TLC analysis of the MeOH extract of *C. elmaliensis* suggested that it contains numerous triterpene glycosides and a glycoside-enriched *n*-BuOH fraction. Since this extract showed antimicrobial activity, we decided to make a phytochemical examination of its BuOH fraction. As a result, three new hederagenin type triterpene glycosides (**1**–**3**), named elmalienoside A–C, together with nine known natural compounds, macranthoidin A (**4**) [18], dipsacoside B (**5**) [19], 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester (**6**) [20], 3-*O*-*β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester (**7**) [21], *α*-hederin (**8**) [22], sapindoside B (**9**) [23], macranthoside A (**10**) [24], tiliroside (**11**) [25], and luteolin 7-*O*-*β*-D-glycoside (**12**) [26], were isolated from the aerial parts of *C. elmaliensis*. The structures of these compounds were identified by extensive spectroscopic analysis, including 1D, 2D NMR, HR ESIMS data, and chemical evidence. The antimicrobial activity of the extracts and the pure isolated compounds was evaluated by the MIC method.

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Materials and Methods

General

Optical rotations and FTIR spectra were recorded on a Rudolph Research Analytical Autopol I automatic polarimeter and on an ATI Mattson Genesis Series Fourier transform infrared spectrophotometer, respectively. High resolution electrospray ionization mass spectra (HR ESIMS) and the 1D and 2D NMR spectra were recorded on a Bruker LC micro-Q-TOF instrument and on a Varian AS 400 MHz spectrometer in DMSO-d_6 with TMS as an internal standard, respectively. Medium pressure liquid chromatography (MPLC) was carried out using a Buchi system (Buchi C-605 pumps, coupled to a UV detector) with Buchi glass columns (15/460, 36/460). 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 F_{254} (Merck) and RP-18 $F_{254\,s}$ (Merck) precoated aluminum sheets. They were detected after developing with suitable solvent systems spraying with 20% H_2SO_4 solution followed by heating at 120 °C for 3 minutes.

Plant material

Cephalaria elmaliensis (Hub.-Mor. & Matthews) was collected from Antalya-Elmalı, Çiğlikara, at about 1750 m altitude in July 2007. It was identified by H. Sümbül and R. S. Göktürk (Department of Biology, Faculty of Art and Science, Akdeniz University). A voucher specimen has been deposited (No: R. S. Göktürk 6097) at the Herbarium Research and Application Centre of the Akdeniz University.

Extraction and isolation

The air-dried and powdered aerial parts of the plant (1.35 kg) were extracted with MeOH (3 × 2 L) overnight at room temperature, and the combined extracts were concentrated under vacuum at ~ 40 °C. The MeOH residue was extracted with *n*-BuOH-H_2O, 1 : 1 (3 × 300 mL) solvent system, and then the *n*-BuOH fraction was defatted with hexane (10 × 50 mL) to remove chlorophyll and oily substances. The repurified *n*-BuOH extract which was found to be the most biologically active fraction, was taken under investigation. A part of the *n*-BuOH fraction (42.5 g) was chromatographed on a RP silica gel via vacuum liquid chromatography (VLC) eluted with an MeOH-H_2O solvent system (0 : 100 → 100 : 0% with increasing polarity by an MeOH 10% gradient) to give 11 fractions. The combined fractions 7 and 8 were loaded into a silica gel column (1200 g, 115 × 6.0 cm) with solvent systems $CHCl_3$-MeOH-H_2O (80 : 20 : 2; 70 : 30 : 3; 61 : 32 : 7) and MeOH (3 L each) to give 30 fractions. The 24th of these fractions (1.2 g) was purified on a silica gel column (110 g, 85 × 2.0 cm) eluted with $CHCl_3$-MeOH-H_2O (80 : 20 : 2) to give the first novel compound, **1** (111 mg), and the known compound **4** (109 mg). Compound **2** (1.3 g) was obtained by silica gel column (185 g, 135 × 2.0 cm) chromatography eluted with the $CHCl_3$-MeOH-H_2O (90 : 10 : 1) solvent system from the combined fractions 15 and 17 as a second novel natural product. Fraction 18 (2 g) was purified by silica gel CC (185 g, 135 × 2.0 cm) using solvent system $CHCl_3$-MeOH-H_2O (80 : 20 : 2) to afford the last new saponin, compound **3** (210 mg), and a known compound, **5** (349 mg). Fraction 10 (538 mg) was re-chromatographed on a silica gel column (55 g, 120 × 1.35 cm) using solvent system $CHCl_3$-MeOH-H_2O (90 : 10 : 1) to yield compound **6** (195 mg). The combined fractions 12 and 13 (435 mg) were further separated by a silica gel column (40 g, 100 × 1.2 cm) chromatography eluting with the $CHCl_3$-MeOH-H_2O (90 : 10 : 1) solvent system to give compound **7** (83 mg). The 9th of the VLC fractions was subjected to MPLC [silica gel, 36 × 460 mm Buchi glass column, solvent systems $CHCl_3$-MeOH-H_2O 90 : 10 : 1; 80 : 20 : 2; 70 : 30 : 3; 61 : 32 : 7 (900 mL each), flow rate: 30 mL/min, max. pressure: 30 bar], and 14 fractions were obtained. Compound **8** (200 mg) was purified by silica gel column (35 g, 45 × 1.8 cm) chromatography with the solvent system $CHCl_3$-MeOH-H_2O (90 : 10 : 1) from the 6th of these fractions. Fraction 7 was loaded into a silica gel column (40 g, 100 × 1.2 cm) with the solvent system $CHCl_3$-MeOH-H_2O (90 : 10 : 1) to give compounds **9** (69 mg) and **10** (89 mg). Compound **11** (42.3 mg) was obtained from the 2nd-4th combined fractions of the thirty fractions obtained earlier by MPLC (silica gel, 15 × 460 mm Buchi glass column, solvent system $CHCl_3$-

Table 1 [1]H-NMR data for compounds **1, 2**, and **3**[a–c].

Position	1	2	3
3	3.46, m	3.48, m	3.48, m
5	1.18, m	1.15, m	1.15, m
9	1.48, m	1.47, m	1.47, m
12	5.14, brs	5.13, brs	5.13, brs
23	3.10, 3.34, m	3.10, 3.23, m	nd, 3.38, m
24	0.55, s	0.54, s	0.54, s
25	0.85, s	0.84, s	0.84, s
26	0.66, s	0.65, s	0.64, s
27	1.07, s	1.05, s	1.06, s
29	0.85, s	0.84, s	0.84, s
30	0.85, s	0.84, s	0.84, s
	Ara at C-3	**Ara at C-3**	**Ara at C-3**
1	4.30, d (6.0)	4.31, d (5.6)	4.32, d (6.0)
2	3.47, m	3.50, m	3.50, m
3	3.48, m	3.48, m	3.47, m
4	3.54, m	3.56, m	3.57, m
5	3.35, 3.66, m	3.28, 3.66, m	3.31, 3.69, m
	Rha	**Rha**	**Rha**
1	5.10, brs	5.04, brs	5.13, brs
2	3.91, m	3.68, m	3.78, m
3	3.63, m	3.44, m	3.60, m
4	3.37, m	3.15, m	3.16, m
5	3.80, m	3.66, m	3.80, m
6	1.07, d (6.4)	1.05, d (6.0)	1.05, d (6.0)
	Glc		**Xyl**
1	4.29, d (7.2)		4.29, d (7.2)
2	3.04, m		3.06, m
3	3.31, m		3.07, m
4	3.11, m		3.21, m
5	3.08, m		3.00, 3.68, m
6	3.42, 3.62, m		
	Glc I at C-28	**Glc at C-28**	**Glc at C-28**
1	5.20, d (8.0)	5.20, d (8.0)	5.20, d (6.8)
2	3.10, m	3.10, m	3.10, m
3	3.11, m	3.30, m	3.21, m
4	3.22, m	3.20, m	3.29, m
5	3.22, m	3.21, m	3.10, m
6	3.45, 3.91, m	3.66, 3.91, m	3.56, 3.90, m
	Gal	**Gal**	**Gal**
1	4.44, d (7.2)	4.44, d (7.6)	4.44, d (7.2)
2	3.80, m	3.80, m	3.80, m
3	3.10, m	3.10, m	3.11, m
4	3.26, m	3.26, m	3.26, m
5	3.42, m	3.41, m	3.43, m
6	3.40, 3.60, m	3.39, 3.59, m	3.43, 3.60, m

[a] [1]H-NMR data (δ) were measured in DMSO-d_6 at 400 MHz. [b] Coupling constants (J) in Hz are given in parentheses. [c] nd: Not detected

Table 2 ^{13}C-NMR data for compounds **1, 2**, and **3**[a–c].

	1	2	3		1	2	3
Position	**Aglycon**			**Position**	**Sugars**		
					Ara at C-3	**Ara at C-3**	**Ara at C-3**
1	39.0	39.0	39.0	1	103.9	103.6	103.8
2	26.1	26.0	26.0	2	74.6	74.9	74.1
3	80.1	80.0	80.1	3	73.9	73.5	73.9
4	43.0	42.9	43.0	4	68.8	68.5	68.9
5	46.9	46.9	46.9	5	65.6	65.0	65.7
6	17.8	17.8	17.8		**Rha**	**Rha**	**Rha**
7	32.5	32.5	32.0	1	100.5	100.6	100.2
8	39.7	39.6	nd	2	70.0	71.0	70.7
9	47.8	47.8	47.9	3	82.3	71.2	81.6
10	36.6	36.6	36.6	4	71.5	72.7	71.6
11	23.7	23.6	23.6	5	68.6	68.8	68.5
12	122.4	122.4	122.4	6	18.5	18.4	18.4
13	144.2	144.2	144.2		**Glc**		**Xyl**
14	42.0	42.0	42.0	1	105.4		106.3
15	27.9	27.9	27.9	2	74.6		74.4
16	23.2	23.2	23.2	3	77.0		77.4
17	46.7	46.6	46.6	4	70.5		69.8
18	41.4	41.4	41.4	5	77.4		66.4
19	46.3	46.2	46.3	6	61.6		
20	31.0	30.9	30.9		**Glc I at C-28**	**Glc at C-28**	**Glc at C-28**
21	33.9	33.9	34.0	1	94.8	94.8	94.8
22	32.3	32.3	32.3	2	71.3	71.3	71.3
23	63.1	63.2	63.2	3	77.0	77.0	76.9
24	13.8	13.7	13.8	4	69.8	69.8	70.1
25	16.3	16.3	16.3	5	77.1	77.1	77.0
26	17.4	17.4	17.4	6	68.8	68.8	68.7
27	26.2	26.2	26.2		**Gal**	**Gal**	**Gal**
28	176.0	176.0	176.0	1	101.8	101.8	101.7
29	33.5	33.5	33.5	2	71.9	71.9	71.8
30	24.1	24.1	24.0	3	73.0	73.0	72.9
				4	68.1	68.1	68.0
				5	75.1	75.0	74.9
				6	62.0	62.0	62.0

[a] ^{13}C-NMR data (δ) were measured in DMSO-d_6 at 100 MHz. [b] The assignments are based on DEPT, COSY, NOESY, HMQC, and HMBC experiments. [c] nd: Not determined

MeOH 9 : 1, flow rate: 10 mL/min, max. pressure: 50 bar). The last compound **12** (40 mg) was purified from the 3rd fraction of VLC firstly by MPLC [silica gel, 36 × 460 mm Buchi glass column, solvent system $CHCl_3$-MeOH-H_2O 90 : 10 : 1; 80 : 20 : 2; 70 : 30 : 3; 61 : 32 : 7 (1 L each), flow rate: 30 mL/min, max. pressure: 30 bar], and secondly by MeOH-acetone (1 : 3) precipitation procedures.

Elmalienoside A (**1**): white, amorphous powder (111 mg); $[\alpha]_D^{25}$–7.4 (*c* 2.4, MeOH); IR (KBr) ν_{max} 3368, 2941, 1741, 1591, 1388, 1261, 1055 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see **Tables 1** and **2**, respectively; positive-ion HR ESIMS *m/z* 1259.6054 [M + Na]$^+$ (calcd. for $C_{59}H_{96}O_{27}Na$, 1259.6031).

Elmalienoside B (**2**): white, amorphous powder (1.3 g); $[\alpha]_D^{25}$–8.6 (*c* 3.1, MeOH); IR (KBr) ν_{max} 3399, 2944, 1752, 1640, 1458, 1389, 1261, 1059 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see **Tables 1** and **2**, respectively; positive-ion HR ESIMS *m/z* 1097.5487 [M + Na]$^+$ (calcd. for $C_{53}H_{86}O_{22}Na$, 1097.5503).

Elmalienoside C (**3**): white, amorphous powder (210 mg); $[\alpha]_D^{25}$–11.0 (*c* 2.0, MeOH); IR (KBr) ν_{max} 3368, 2942, 1736, 1595, 1459, 1388, 1261, 1055 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see **Tables 1** and **2**, respectively; positive-ion HR ESIMS *m/z* 1229.5948 [M + Na]$^+$ (calcd. for $C_{58}H_{94}O_{26}Na$, 1229.5926).

Alkaline hydrolysis

Pure compounds **1**–**3** (20 mg each) were refluxed with 5% KOH in water solution (5 mL) at 80 °C for 2 h. The reaction mixtures were neutralized with 5% HCl in water solution and then concentrated to dryness. The residues were extracted with *n*-BuOH-H_2O (1 : 1), and all of the organic layers were evaporated to dryness to afford prosapogenins [7]. Finally, they were analyzed by ^{1}H NMR and HR ESIMS methods. Alkaline hydrolysis of **1, 2**, and **3** afforded three known triterpene glycosides which were also isolated in this study, namely, macranthoside A (**10**) [24], α-hederin (**8**) [22], and sapindoside B (**9**) [23], respectively.

Acid hydrolysis

The identification of the monosaccharide units of the glycosides was performed by the microhydrolysis technique on a TLC plate and GC-MS analysis with authentic samples [7, 27, 28]. Each compound (5 mg) was hydrolyzed with 1 N HCl (2 mL) for 6 h at 90 °C. After extraction with $CHCl_3$ (3 × 5 mL), the aqueous layer was evaporated to dryness and then analyzed by TLC over silica gel ($CHCl_3$-MeOH-H_2O-gAcOH/16 : 9 : 2 : 2) by comparison with au-

thentic monosaccharide samples. Furthermore, the residue of monosaccharides was dissolved in anhydrous pyridine (1 mL); then 1 mL of HMDS-TMCS (hexamethyldisilazane–trimethylchlorosilane, 1 : 1) was added, and the mixture was stirred at 70 °C for 1 h. The mixture was concentrated under an N_2 stream and dissolved in *n*-hexane for GC-MS analyses. A mixture which contained standard monosaccharide units was silylated by the same procedure, too. L-Arabinose, L-rhamnose, D-xylose, D-galactose, and D-glucose were detected by co-injection of the hydrolysate with standard silylated monosaccharide samples for GC-MS analyses. The retention times of the standard monosaccharides L-arabinose, L-rhamnose, D-xylose, D-galactose, and D-glucose were found to be 14.77, 15.25, 18.37, 29.22, and 30.70 min, respectively. The monosaccharide moieties of the samples were determined as L-arabinose, L-rhamnose, D-galactose, and D-glucose giving peaks at 14.74, 15.18, 29.20, and 30.66 min for **1**, 14.76, 15.15, 29.22, and 30.64 min for **2**, 14.75, 15.16, 29.20, and 30.68 min for **3**, respectively. D-xylose was found only for **3**, giving a peak at 18.26 min.

Antimicrobial assay

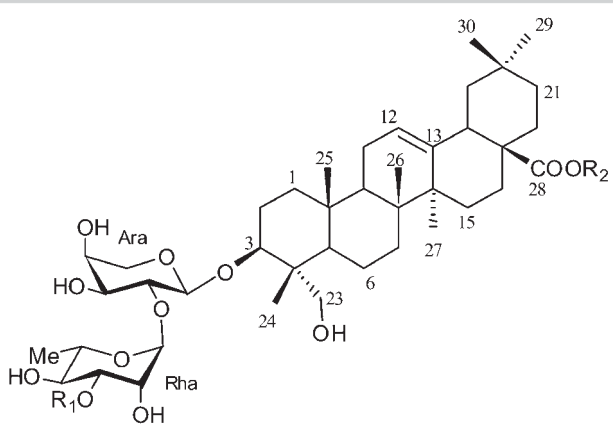
In vitro antibacterial activity tests of all compounds were evaluated using minimum inhibitory concentration (MIC) measurements against eight bacterial strains [four gram-negative: *Escherichia coli* (ATCC 23999), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (CCM 5445), and *Klebsiella pneumoniae* (CCM 2318); and four gram-positive: *Staphylococcus aureus* (ATCC 6538-P), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus cereus* (ATCC 7064), and *Enterococcus faecalis* (ATCC 29212)]. The bacterial strains were inoculated on Mueller-Hinton broth (Difco) and incubated for 24 h at 37 ± 0.1 °C. The inocula from 24-h broth cultures were adjusted to 0.5 McFarland standards. The dilution series of the compounds were prepared in test tubes then transferred to the broth in 96-well microtiter plates. Final concentrations were from 256 to 0.5 µg/mL in the medium. The last well containing 100 µL of nutrient broth without compounds and 10 µL of the inocula on each strip was used as a negative control. All plates were covered with a sterile plate sealer and incubated at 37 °C for 24 h. The MIC is defined as the lowest concentration that appeared clear against a black background. Samples from clear wells were subcultured by plotting onto Mueller Hinton agar. Gentamycin (Sigma) was used as the positive control. Dilutions were prepared from 128 to 0.25 µg/mL concentrations in microtiter plates [29].

Supporting information

The 1D, 2D NMR, and HR ESIMS spectra of the novel compounds **1**–**3** (elmalienoside A–C) from *C. elmaliensis* are available as Supporting Information.

Results and Discussion

Twelve compounds were isolated from the *n*-BuOH fraction of *Cephalaria elmaliensis*. Seven compounds (**4**–**10**) (**Fig. 1**) were determined to be known hederagenin-type triterpene glycosides which can be classified as monodesmosidic and bisdesmosidic ones. The bisdesmosidic glycosides were macranthoidin A (**4**) [18], dipsacoside B (**5**) [19], 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester (**6**) [20], and 3-*O*-*β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester (**7**) [21]. The monodesmosidic ones were elucidated as α-hederin (**8**) [22], sapindoside B (**9**) [23], and macranthoside A (**10**) [24]. The other two known compounds, **11** and **12**, were determined as tiliroside [25] and luteolin-7-*O*-*β*-D-glycoside [26], respectively, by comparison of their physical and spectroscopic data with those reported in the literature. Tiliroside (**11**) and compounds **6, 7**, and **10** were isolated for the first time from the *Cephalaria* genus. All known compounds were identified by comparison of the results of the 1D, 2D NMR, and HRESIMS techniques and chemical methods with reference data. Compound **1** (elmalienoside A) (**Fig. 1**) was isolated as an amorphous solid and had a molecular formula $C_{59}H_{96}O_{27}$, determined by HR ESIMS (1259.6054 $[M + Na]^+$) and confirmed by ^1H and ^{13}C NMR data (see **Tables 1** and **2**). The IR spectrum of **1** suggested a glycoside (3368, 1055 cm^{-1}) and indicated the presence of a carbonyl group (1741 cm^{-1}) in the molecule. The ^1H NMR spectrum of **1** showed characteristic singlets due to six quaternary methyl groups at δ_H 1.07, 0.85 (× 3), 0.66, and 0.55, a hy-



Compounds	R_1	R_2
1	Glc-	Gal (1→6) Glc I-
2	H-	Gal (1→6) Glc-
3	Xyl-	Gal (1→6) Glc-
4	Glc-	Glc II (1→6) Glc I-
5	H-	Glc I (1→6) Glc-
6	H-	Glc-
7	Glc-	Glc I-
8	H-	H-
9	Xyl-	H-
10	Glc-	H-

Fig. 1 Chemical structures of elmalienoside A–C (**1**–**3**) and the seven known triterpene glycosides (**4**–**10**).

Table 3 MIC results (μg/mL) of compounds **1**–**12** from *C. elmaliensis*.

	Compounds												
Microorganisms	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Gentamycin**
S. aureus	32	32	64	64	32	32	32	32	32	32	8	32	1.0
S. epidermidis	16	16	32	32	16	32	16	16	32	32	16	16	1.0
S. typhimurium	16	16	32	32	32	16	16	16	16	16	16	16	1.0
E. coli	16	16	32	32	16	16	16	16	16	16	16	16	1.0
B. cereus	8	8	32	16	16	16	8	16	16	8	8	8	4.0
K. pneumoniae	32	32	32	64	32	32	32	16	32	32	16	16	4.0
E. faecalis	2	2	4	4	2	4	1	2	1	8	2	4	16.0
P. aeruginosa	16	16	32	32	16	16	16	16	16	16	16	16	2.0

droxymethyl group at δ_H 3.46 (m), and an olefinic proton at δ_H 5.14 (brs). The ^{13}C NMR spectrum also revealed the signals for six quaternary carbons at δ_C 33.5, 26.2, 24.1, 17.4, 16.3, and 13.8; an oxygen-bearing methine carbon at δ_C 80.1, a hydroxymethyl group at δ_C 63.1, a set of olefinic carbons at δ_C 144.2 and 122.4, and one carbonyl carbon at δ_C 176.0 confirmed that **1** has a hederagenin aglycone [30]. The C-3 oxymethine carbon and C-28 carbonyl carbon were observed at δ_C 80.1 and 176.0, respectively, which suggested that **1** is a 3,28-bisdesmoside of hederagenin. In addition to these signals, the ^1H and ^{13}C NMR spectra of **1** contained five clear signals for anomeric protons and carbons at δ_H 5.20 (d, J = 8.0 Hz), 5.10 (brs), 4.44 (d, J = 7.2 Hz), 4.30 (d, J = 6.0 Hz), 4.29 (d, J = 7.2 Hz), and δ_C 105.4, 103.9, 101.8, 100.5, 94.8. All proton signals for the sugar moieties were associated with COSY, NOESY, and HMQC spectra. The acid hydrolysis of compound **1** gave L-arabinose, L-rhamnose, D-glucose, D-galactose, and hederagenin whereas alkaline hydrolysis gave compound **10** (macranthoside A). The identity of each monosaccharide was confirmed by TLC and GC-MS analyses comparing them with authentic samples [27,28]. These results were also confirmed by HMBC data. In the HMBC spectrum, the H-3 proton of the aglycone at δ_H 3.46 and the H-1 proton of glucose I at δ_H 5.20 showed long-range correlations with C-1 of the arabinose moiety at δ_C 103.9 and C-28 of the aglycone at δ_C 176.0, respectively. On the other hand, long-range correlations between the H-1 proton of D-glucose at δ_H 4.29 and the C-3 carbon of L-rhamnose at δ_C 82.3, the H-1 proton of L-rhamnose at δ_H 5.10 and the C-2 carbon of L-arabinose at δ_C 74.6, and the H-1 proton of D-galactose at δ_H 4.44 and the C-6 carbon of D-glucose I at δ_C 68.8 showed the linkage points of the sugar molecules to each other. Accordingly, the structure of **1** was formulated as 3-*O*-*β*-D-glucopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-galactopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester, namely elmalienoside A.

Elmalienoside B (**2**) (**Fig. 1**) exhibited in the HR ESIMS the $[M + Na]^+$ peak at *m/z* 1097.5487 consistent with the molecular formula $C_{53}H_{86}O_{22}$. The NMR data (see **Tables 1** and **2**) of the aglycone of **2** were similar to those of compound **1**. Acid hydrolysis of **2** with conc. HCl gave hederagenin, L-arabinose, L-rhamnose, D-glucose, and D-galactose moieties whereas alkaline hydrolysis of **2** with 5% KOH in water yielded a known compound **8**. The ^1H NMR spectrum of **2** displayed signals for four anomeric protons at δ_H 5.20 (d, J = 8.0 Hz, Glc), 5.04 (brs, Rha), 4.44 (d, J = 7.6 Hz, Gal), and 4.31 (d, J = 5.6 Hz, Ara), which gave correlations in the HMQC spectrum, with four anomeric carbon signals at δ_C 94.8, 100.6, 101.8, and 103.6, respectively. The correlations which were observed in the HMBC spectrum between the anomeric proton signals and aglycone carbons at δ_H 4.31 (Ara) and δ_C 80.0 (C-3 of aglycone), δ_H 5.20 (Glc) and δ_C 176.0 (C-28 of aglycone) confirmed the linkage points of the sugar moieties to the aglycone. A correlation between δ_H 5.04 and δ_C 74.9 revealed the (1 → 2) linkage between rhamnose and arabinose. Moreover, a characteristic correlation between δ_H 4.44 and δ_C 68.8 revealed the (1 → 6) linkage between galactose and glucose. On the basis of the above results, the structure of **2** was elucidated as 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-galactopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester which was named elmalienoside B.

Compound **3** was shown to have the molecular formula $C_{58}H_{94}O_{26}$ on the basis of the HR ESIMS data at *m/z* $[M + Na]^+$ = 1229.5948. Comparison of the ^1H and ^{13}C NMR data (see **Tables 1** and **2**) of **3** with those of **1** showed considerable structural similarity except for the presence of one xylose moiety instead of glucose. The sugar part of **3** was found to consist of five monosaccharide residues, identified as L-arabinose (δ_C 103.8/δ_H 4.32, d, J = 6.0 Hz), L-rhamnose (δ_C 100.2/δ_H 5.13, brs), D-xylose (δ_C 106.3/δ_H 4.29, d, J = 7.2 Hz), D-glucose (δ_C 94.8/δ_H 5.20, d, J = 6.8 Hz), and D-galactose (δ_C 101.7/δ_H 4.44, d, J = 7.2 Hz) from its NMR spectroscopic data. In the HMBC spectrum, long-range correlations between δ_H 4.32 (H-1 of Ara) and δ_C 80.1 (C-3 of aglycone), between δ_H 5.13 (H-1 of Rha) and δ_C 74.1 (C-2 of Ara), between δ_H 4.29 (H-1 of Xyl) and δ_C 81.6 (C-3 of Rha), between δ_H 5.20 (H-1 of Glc) and δ_C 176.0 (C-28 of aglycone), and between δ_H 4.44 (H-1 of Gal) and δ_C 68.7 (C-6 of Glc) showed the linkage points of the monosaccharides to each other and to the aglycone. The identity of each monosaccaride was determined by acid hydrolysis using TLC and GC-MS techniques comparing with authentic sugar samples, in addition to the COSY, NOESY spectral data and literature reports [27,28]. The alkaline hydrolysis of compound **3** gave the known compound **9** (sapindoside B). Consequently, compound **3** (elmalienoside C) was determined to be 3-*O*-*β*-D-xylopyranosyl-(1 → 3)-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-galactopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester.

Antibacterial activity tests of all extracts and pure compounds were evaluated using the MIC method (**Table 3**). All identified compounds (1–12) were obtained from the most active fraction of *C. elmaliensis*. According to the results, all pure compounds, including the new ones, were found to be very active against both gram-positive and gram-negative bacteria. Moreover, all compounds of *C. elmaliensis* showed very strong antibacterial activity against *E. faecalis*, their MIC values being even much lower than those of the standard antibiotic gentamycin. These results are similar to those of other reports in the literature concerning the genus *Cephalaria* [9,10].

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Conflict of Interest

There is no conflict of interest between the authors.

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