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Secondary Metabolites of *Phlomis viscosa* and Their Biological Activities

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Further phytochemical studies on the aerial parts of *Phlomis viscosa* (Lamiaceae) led to the isolation of 24 compounds: 3 iridoid glycosides, 10 phenylethanoid glycosides, a megastigmane glycoside and a hydroquinone glycoside, as well as 2 lignan glucosides and 7 neolignan glucosides, 1 of which is new (**17b**). Compound **17b** was obtained as a minor component of an inseparable mixture (2:1) of 2 neolignan glucosides (**17a/b**), and characterized as 3′,4-*O*-dimethylcedrusin 9-*O*-β-glucopyranoside. Full NMR data of the known 8-*O*-4′ neolignan glucoside, *erythro*-1-(4-*O*-β-glucopyranosyl-3-methoxyphenyl)-2-{2-methoxyl-4-[1-(*E*)-propene-3-ol]-phenoxyl}-propane-1,3-diol (**18**) are also reported. All isolated compounds were screened for cell growth inhibition versus 3 tumor cell lines (MCF7, NCI-H460, and SF-268) and several phenylethanoid glycosides were found to possess weak antitumoral activity. The phenylethanoid glycosides were also evaluated for their free radical (DPPH) scavenging, antibacterial and antifungal activities. The free radical (DPPH) scavenging activities of verbascoside (**4**), isoacteoside (**5**), forsythoside B (**10**), myricoside (**13**) and samioside (**14**) were found to be comparable to that of *dl*-α-tocopherol. Compounds **4**, **5**, **10** and **14** (MIC: 500 μg/mL) as well as Leucosceptoside A (**8**) and **13** (MIC:1000 μg/mL) showed very weak activity against Gram (+) bacteria.

Key Words: *Phlomis viscosa*, iridoids, phenylethanoid glycosides, lignan glucosides, 8-*O*-4′-oxylignan, neolignan glucosides, biological activity.

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Introduction

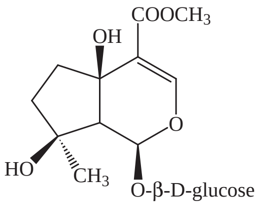
In a previous study, we reported a nortriterpene and 2 oleanan-type triterpene glycosides from *Phlomis viscosa*[1]. Further investigations into the chemistry of this plant resulted in the isolation of a variety of glycosides of iridoid, phenylethanoid, megastigmane, neolignan and lignan types (Figure). The present study reports the structural elucidation of the new compound, 3′,4-*O*-dimethylcedrusin 9-*O*-glucopyranoside (**17b**), as well as the complete NMR and other spectroscopic data for the known neolignan glucoside (**18**). All isolated compounds were tested for their cell growth inhibition activities. Phenylethanoid glycosides were also tested for their free radical scavenging and antimicrobial activities.

Experimental

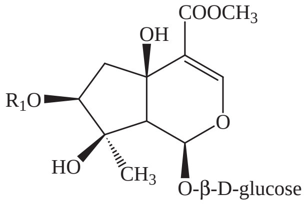
General Experimental Procedures: Optical rotations were measured on a Rudolph autopol IV Polarimeter using a sodium lamp operating at 589 nm. UV spectra were recorded on a Shimadzu UV-160A spectrophotometer. IR spectra (KBr) were measured on a Perkin Elmer 2000 FT-IR spectrometer. The 1D- and 2D-NMR spectra were obtained on a Bruker® AMX 300 instrument (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C) and DRX 500 FT spectrometer (500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C), at 295 K, for all 1D- and 2D-NMR experiments. A Bruker with XWIN NMR software package was used to acquire NMR data. Positive mode HR-MALDIMS were recorded on a Ionspec-Ultima-FTMS spectrometer with 2,5-dihydroxybenzoic acid (DHB) as matrix. TLC analyses were carried out on silica gel 60 F-254 precoated plates (Merck, Darmstadt); detection by 1% vanillin/H_2SO_4. For medium-pressure liquid chromatographic (MPLC) separations, a Büchi 681 pump, a Büchi 684 fraction collector, a Rheodyne injector, and Büchi glass columns (column dimensions 2.6 x 46 cm, and 1.8 x 35 cm) were used. Silica gel 60 (0.063-0.200 mm; Merck, Germany) was utilized for open column chromatography (CC) and vacuum liquid chromatography (VLC). LiChroprep C-18 (Merck) material was used for MPLC and VLC. Sephadex LH-20 (Fluka) was also used for further separations.

Plant Material: *Phlomis viscosa* Poiret was collected from Osmaniye, Düziçi (Haruniye), above Çitli Village (Turkey) on July 1, 2001. The plant specimen was identified by Dr. Ali A. Dönmez. The voucher specimen (AAD 9493) has been deposited at the Herbarium of the Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey.

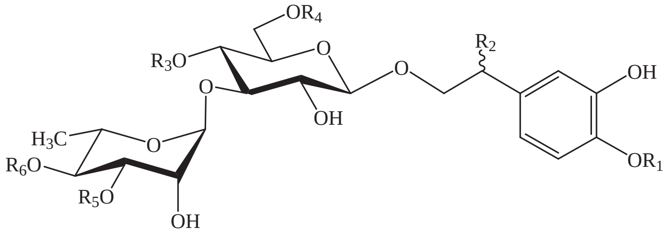
Extraction and Isolation: The powdered herb of *P. viscosa* (350 g) was extracted with EtOH (90°, 2 x 2.5 L, 5 h) and then filtered. The filtrates were combined and evaporated to dryness in vacuo to yield 65 g crude extract (yield 18.7%). The EtOH extract was suspended in H_2O (100 mL), and then extracted 3 times with $CHCl_3$ (100 mL x 3). The remaining water phase yielded 41.9 g crude extract upon concentration. An aliquot of the water extract (39.9 g) was mounted on a column packed with polyamide (200 g). Elution with increasing amounts of MeOH in H_2O (0-100%; H_2O 1250 mL, with increasing 20% MeOH, each 500 mL, and 1000 mL MeOH) yielded 42 fractions (each 100 mL), which were combined into 13 fractions (frs. A-M). Fr. A (4.5 g) was subjected to C_{18}-MPLC (column dimensions: 2.6 x 46 cm), eluted with H_2O (500 mL), MeOH-H_2O mixtures (5-50% MeOH, with increasing 10% MeOH, each 200 mL) and MeOH (300 mL) to yield 5 major fractions, A_1-A_5. Fraction A_2 yielded pure lamiide (**2**, 1794 mg). Fraction A_4 (120 mg) was rechromatographed over a SiO_2 (12 g) column (CH_2Cl_2-MeOH-H_2O, 90:10:1, 200 mL) to give **1** (23 mg) and **15** (3.5 mg). Fr. B (6.0 g) was rich in **2**. Fr. C (1.834 g) was chromatographed over C_{18}-MPLC (column



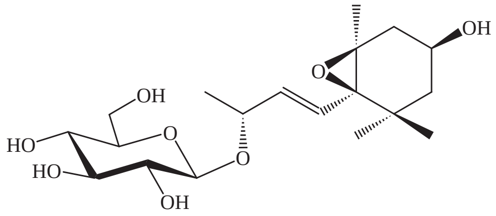
(**1**)



(**2**) $R_1 = H$
(**3**) $R_1 = p$-coumaroyl



Phenylethanoid Glycosides	R_1	R_2	R_3	R_4	R_5	R_6
4	H	H	*E*-caffeoyl	H	H	H
5	H	H	H	*E*-caffeoyl	H	H
6	H	H	H	H	H	H
7	Me	H	*E*-feruloyl	H	H	H
8	H	H	*E*-feruloyl	H	H	H
9	H	OH	*E*-caffeoyl	H	H	H
10	H	H	*E*-caffeoyl	$\beta-$ apiosyl	H	H
11	H	H	*E*-feruloyl	$\beta-$ apiosyl	H	H
12	Me	H	*E*-feruloyl	$\beta-$ apiosyl	H	H
13	H	H	*E*-caffeoyl	H	$\beta-$ apiosyl	H
14	H	H	*E*-caffeoyl	H	H	$\beta-$ apiosyl



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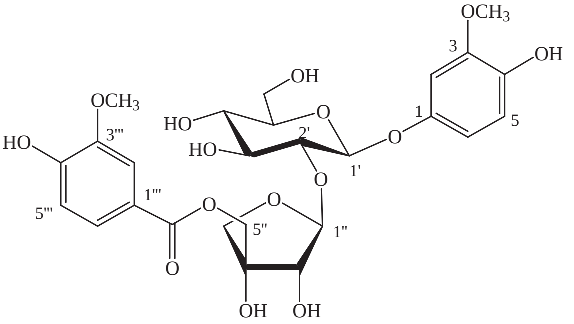

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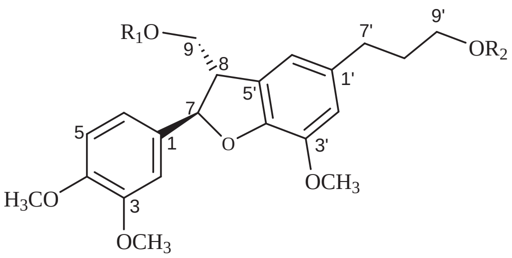
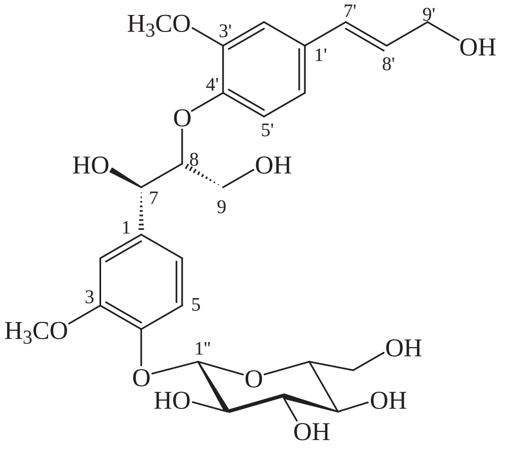
Figure. Secondary metabolites of *P. viscosa*.



17a R$_1$ = H, R$_2$ = β-Glc
17b R$_1$ = β-Glc, R$_2$ = H



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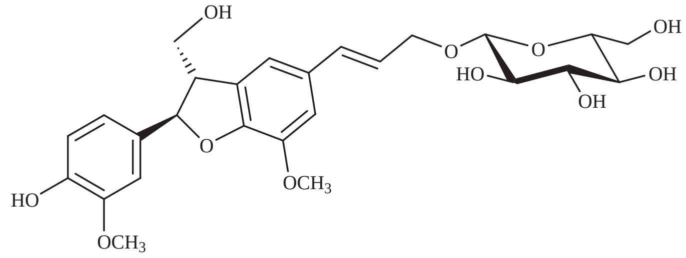

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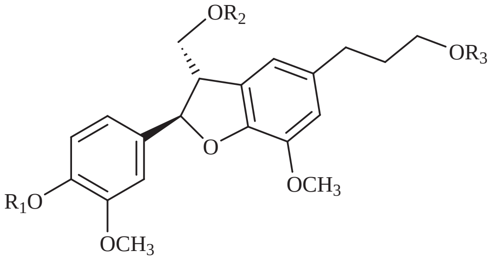
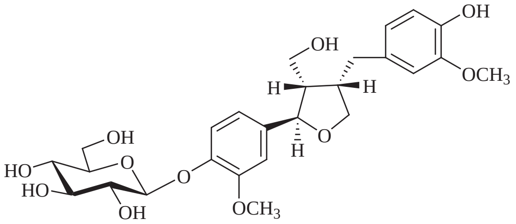


20 R$_1$ = β-Glc R$_2$ = R$_3$ = H
21a R$_2$ = β-Glc, R$_1$ = R$_3$ = H
21b R$_3$ = β-Glc, R$_1$ = R$_2$ = H



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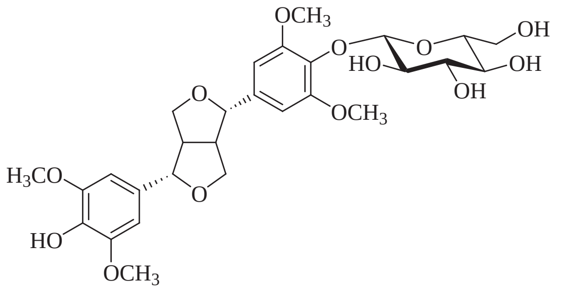

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Figure. Contunied.

dimensions: 2.6 x 46 cm) employing a MeOH-H_2O gradient (10-30% with increasing 5%; each 200 mL) to yield **6** (39 mg). Fr. D (926 mg) was similarly subjected to C_{18}-MPLC (column dimensions: 3 x 24 cm) employing MeOH-H_2O gradient (25-80% with increasing 2.5-5%; each 100 mL) to yield 8 major fractions, D_1-D_8. Repeated chromatography of fr. D_2 (62 mg) on a SiO_2 (35 g) column (EtOAc-MeOH-H_2O, 100:10:5, 200 mL) yielded **18** (9 mg). Fr. D_7 (67 mg) was applied to a (15 g) column packed with SiO_2 using CH_2Cl_2-MeOH-H_2O (90:10:1, 300 mL, and 80:20:2, 200 mL) to obtain **23** (8 mg). Fr. E (214 mg) was subjected to a SiO_2 CC (35 g) employing a EtOAc-MeOH-H_2O mixture (100:10:5, 600 mL) to afford **3** (28 mg) and **20** (18 mg). Fr. F (848 mg) was likewise subjected to a SiO_2 (100 g) CC utilizing CH_2Cl_2-MeOH-H_2O mixtures (80:20:2 and 80:20:1, both 300 mL) to yield 4 fractions, F_{1-4}. Fr. F_3 (390 mg) was subjected to C_{18}-MPLC (column dimensions: 3 x 24 cm) using stepwise gradients of MeOH in H_2O (25-50% with increasing 5%; each 200 mL) to yield **16** (8.5 mg), **22** (8 mg), a mixture **21a** and **b** (17 mg), **3** (153 mg) and a mixture of **17a** and **b** (18 mg). Fr. G (424 mg) was also applied to a SiO_2 (40 g) CC employing CH_2Cl_2-MeOH-H_2O mixtures (90:10:0.5 to 61:32:7, totally 1200 mL) to afford **19** (11 mg), **12** (20 mg) and **11** (34 mg). Purification of fr. H (435 mg) by SiO_2 (40 g) CC using EtOAc-MeOH-H_2O (100:10:5, 500 mL) furnished **10** (103 mg). Fr. I (763 mg) was subjected to C_{18}-MPLC (column dimensions: 3 x 24 cm) employing a MeOH-H_2O gradient (20-40% with increasing 2.5%; each 200 mL) to yield **9** (48 mg), a crude fraction of **13** (237 mg), **10** (106 mg) and norviscoside (16 mg) [1]. The fraction containing myricoside (**13**) was applied to a SiO_2 (35 g) column using EtOAc-MeOH-H_2O (100:15:5 to 100: 15:10, totally 300 mL) to obtain **10** (7 mg) and **13** (66 mg). Fr. J (2737 mg) was likewise subjected to C_{18}-MPLC (column dimensions: 2.6 x 46 cm) using stepwise gradients of MeOH in H_2O (20-100% with increasing 5%; each 200 mL) to yield **4** (1380 mg) and **8** (63 mg), in addition to crude **7** (143 mg) and 2 triterpenic glycosides[1]. The fraction containing compound **7** was further purified over a SiO_2 (12 g) column eluting with CH_2Cl_2-MeOH-H_2O mixtures (90:10:1, 80:20:2 and 70:30:3, each 100 mL) to afford pure **7** (29 mg). Fr. K (3800 mg) was subjected to C_{18}-MPLC (column dimensions: 2.6 x 46 cm) using stepwise gradients of MeOH in H_2O (25-100% with increasing 5%; each 200 mL) to yield **4** (1634 mg) and **5** (557 mg) in addition to fractions rich in flavonoid glycosides (fr. K_3; PV-37, PV-38), and triterpenes (fr. K_3 and fr. K_4). Fr. L (2145 mg) was rich in chlorogenic acid.

***3′,4-O-dimethylcedrusin 9′-O-β-glucopyranoside and 3′,4-O-dimethylcedrusin 9-O-β-glucopyranoside* (17a/17b):** ESI-MS: m/z 559 $[M + Na]^+$ ($C_{27}H_{36}O_{11}$). ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 75 MHz): see Table 1.

Erythro-1-(4-O-β-glucopyranosyl-3-methoxyphenyl)-2-{2-methoxyl-4-[1-(E)-propene-3-ol]-phenoxyl}-propane-1,3-diol (**18**): Amorphous powder; $[\alpha]_D^{25}$: - 12.0° (c 0.1, MeOH). CD (EtOH, c 0.13 gl^{-1}; 2.24 x 10^{-4} M): $[\theta]_{214}$ –2500, $[\theta]_{226}$ –4600,$[\theta]_{235}$ –1700, $[\theta]_{250}$ –100. IR (KBr): ν_{max} 3395 (OH), 1502, 1450 (arom.), 1066, 1024 cm^{-1} (C-O-C). UV (MeOH): λ_{max} 215, 268, 299 sh, 324 nm. HR-MALDI-MS: m/z 561.1950 $[M + Na]^+$ (calcd. for $C_{27}H_{34}O_{13}Na$, 561.1943). ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 75 MHz): see Table 2.

Cytotoxicity Assay: Compounds were dissolved in DMSO to yield a final test solution with 0.25% DMSO. For both the 3-cell prescreen[2] and 60-cell screen[3], compounds were dissolved in DMSO at 40 mM to achieve a final concentration of 100 μM in the assay. Five doses were titrated from this high concentration in the 60-cell tests. The endpoint was read at 48 h in both cases; for the prescreen, the endpoint was determined by Alamar blue, while in the 60-cell assay the endpoint was checked using sulforhodamine B staining.

Table 1. ^{13}C- and ^{1}H-NMR spectroscopic data of compounds **17a** and **17b** (CD_3OD, δ_C: 75 MHz, δ_H: 500 MHz).

		17a			**17b**		
C/H	DEPT	δ_C	δ_H	HMBC^a	δ_C	δ_H	HMBC^a
1	C	136.91	-	H-2, H-2, H-8	136.91	-	H-7
2	CH	110.61	6.98 d (1.8)	H-7	110.79	7.03 d (1.8)	H-7
3	C	150.53	-	H-2, H-5, 3-OMe	150.40	-	3-OMe
4	C	150.22	-	4-O-Me	150.20	-	4-O-Me
5	CH	112.79	6.91 d (8.3)		112.75	6.90 d (8.3)	
6	CH	119.41	6.74 dd (8.3, 1.8)	H-7	119.41	6.97 dd (8.3, 1.8)	H-7
7	CH	88.70	5.52 d (6.2)	H-2, H-6	88.70	5.64 d (6.0)	
8	CH	55.53	3.46 dt (6.2, 7.3)	H_2-9, H-7, H-6′	53.38	3.63 m	H-7
9	CH_2	65.02	3.85^b 3.76 dd (11.1, 7.3)		72.50	3.75^b	H-1″
3-OMe	CH_3	56.46	3.79 s		56.46	3.80 s	
4-OMe	CH_3	56.41	3.81 s		56.41	3.82 s	
1′	C	137.10	-	H-7′	137.02	-	H_2-7′
2′	CH	114.17	6.76 br s	H-6′, H_2-7′	114.12	6.73 br s	H_2-7′
3′	C	145.20	-	H-2′, 3′-OMe	145.50	-	H-2′, 3-OMe
4′	C	147.46	-	H-2′, H-6′	145.46	-	
5′	C	129.65	-	H-7, H-8, H_2-9	129.40	-	H-7
6′	CH	118.07	6.75 br s	H-2′, H_2-7′	118.16	6.78 br s	
7′	CH_2	32.90	2.68 t (7.4)	H-2′, H-6′, H_2-9′	32.90	2.62 t (7.4)	
8′	CH_2	32.89	1.91 tt (7.4, 6.5)	H_2-9′	35.83	1.81 tt (7.4, 6.5)	H_2-7′, H_2-9′
9′	CH_2	68.89	3.92 dt (10.2, 6.5) 3.51 dt (10.2, 6.5)	H-1″, H_2-7′	62.20	3.56 t (6.5)	
3′-OMe	CH_3	56.75		56.75			
1″	CH	104.56	4.25 d (7.8)	H-2″	104.60	4.36 d (7.8)	
2″	CH	75.17	3.20 dd (7.8, 9.0)		75.17	3.22 dd (7.8, 9.0)	
3″	CH	78.12	3.36 t (9.0)		78.26	3.36 t (9.0)	
4″	CH	71.64	3.30 t		71.64	3.30 t	
5″	CH	77.91	3.26 ddd (9.0, 5.0, 2.0)		78.07	3.26 ddd (9.0, 5.0, 2.0)	
6″	CH_2	62.75	3.88^b 3.66 dd (12.5, 6.0)		62.21	3.88^b 3.66 (12.5, 6.0)	

[a] From C to H.

[b] Overlapped.

Table 2. ^{13}C- and ^{1}H-NMR spectroscopic data of **18** (CD_3OD, δ_C: 75 MHz, δ_H: 500 MHz).

			18	
C/H	DEPT	δ_C	δ_H	HMBC^a
1	C	137.4	-	H-2, H-6, H-7, H-8
2	CH	112.4	7.13 d (2.1)	H-6, H-7
3	C	150.5	-	H-2, H-5, 3-OMe
4	C	147.4	-	H-1″, H-2, H-5, H-6
5	CH	117.6	7.11 d (8.6)	
6	CH	120.6	6.96 dd (8.6, 2.1)	H-2, H-7
7	CH	73.6	4.94 d (5.1)	H-2, H-6, H-8
8	CH	86.5	4.33 m	H_2-9, H-7
9	CH_2	62.2	3.77 dd (12.0, 6.4) 3.49 dd (12.0, 6.0)	H-7, H-8
3-OMe	CH_3	56.5	3.82 s	
1′	C	133.1	-	H-2′, H-6′, H-7′, H-8′
2′	CH	111.2	7.04 d (2.0)	H-6′
3′	C	151.7	-	3′-OMe
4′	C	149.1	-	H-8, H-2′, H-5′, H-6′
5′	CH	118.5	6.96 d (8.0)	
6′	CH	120.7	6.90 dd (2.0, 8.0)	H-2′, H-7′
7′	CH	131.4	6.52 d (15.8)	H-2′, H-6′, H-8′, H_2-9′
8′	CH	128.6	6.26 dt (15.8, 6.5)	H_2-9′
9′	CH_2	63.8	4.20 d (6.5)	H-7′, H-8′
3′-OMe	CH_3	56.6	3.84 s	
1″	CH	102.9	4.81 d (7.8)	H-2″
2″	CH	74.9	3.47^b	
3″	CH	78.2	3.40^b	
4″	CH	71.3	3.41^b	
5″	CH	77.8	3.48^b	
6″	CH_2	62.5	3.88^b, 3.69 dd (12.0, 5.0)	

[a] From C to H.

[b] Overlapped.

Measurement of DPPH Radical-Scavenging Activity: MeOH solutions of the compounds at various concentrations were added to 1.5 x 10^{-5} M DPPH (2,2-diphenyl-1-picrylhydrazil) in MeOH. Absorbance at 520 nm was determined after 30 min of incubation at room temperature. The radical scavenging activity was determined by comparing the absorbance with that of a blank (100%) containing only DPPH and solvent. *dl*-α-tocopherol was used as standard, and samples were prepared using the same dilution procedures[4].

Antimicrobial Activity Studies: Minimum inhibitory concentrations (MICs) were determined by broth microdilution following the procedures recommended by the National Committee for Clinical Laboratory Standards[5]. Two Gram-positive and two Gram-negative bacteria were used (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853). Antifungal activities of the compounds were tested towards 3 yeast-like fungi: *Candida albicans* ATCC 90028, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019. Mueller-Hinton broth (Difco Laboratories, Detroit, MI, USA) was used for testing the bacterial strains. For *Candida* species, RPMI-1640 medium with L-glutamin, buffered with 3-(N-Morpholino) Propanesulfonic acid (MOPS) (ICN, FLOW; Aurora, OH, USA) was used. The inoculum densities were 5 x 10^5 colony forming units per milliliter (cfu/mL) and 0.5–2.5 x 10^3 cfu/mL for bacteria and fungi, respectively. The compounds tested were dissolved in distilled water, except for 4 (**7**, **8**, **11**, **12**) of them, which were first solubilized in a minimal amount of dimethylsulfoxide and the rest of the volume was adjusted with distilled water for the required concentration. The final 2-fold concentrations were prepared from 1000 to 0.98 μg/mL in microtiter plates. Ampicillin and fluconazole were used as references. Two-fold dilutions were used for both of them from 64 to 0.0625 μg/mL. Microtiter plates were incubated for 18-24 h at 35 °C for testing the bacterial strains. For yeast-like fungi, MICs were determined after incubation for 48 h at 35 °C. Minimum inhibitory concentrations were defined as the lowest concentration of the antimicrobial agents that inhibited visible growth of the microorganism.

Results and Discussion

Compounds **17a** and **17b** were obtained as an inseparable (2:1) mixture. The positive ion ESIMS of this mixture exhibited a single pseudomolecular ion $[M + Na]^+$ at m/z 559 and $[M + K]^+$ at m/z 575 compatible with the molecular formula $C_{27}H_{36}O_{11}$ for both. Although most of the NMR signals appeared double, indicating a close structural similarity between **17a** and **17b**, DQF-COSY, HSQC and HMBC experiments enabled distinct assignments for the individual components of the mixture. ^{1}H- and ^{13}C-NMR data (Table 1) assigned for the major compound of the mixture (**17a)** were identical with those reported for (-)-3′,4-*O*-dimethylcedrusin 9′-*O*-*β*-glucopyranoside (= 4-*O*-methyldihydrodehydrodiconiferyl alcohol 9′-*O*-*β*-glucopyranoside), previously reported from *Phlomis chimerae*[6]. The NMR resonances observed for H-7, H-8, H_2-9, H-7′, H-8′ and H_2-9′ and C-8, C-9, C-8′ and C-9′ atoms of **17b** (Table 1) indicated that the only difference between **17a** and **17b** stems from the site of glycosidations. The slight (+ 0.11) downfield shifting of the anomeric proton of glucose (H-1″, δ_H 4.36 d, J = 7.8 Hz), as well as a prominent long range correlation between this proton (H-1″) and the oxymethylene carbon (C-9, δ_C 72.50) of the benzofuran moiety, suggested that the glucose unit was attached at C-9, and not at C-9′. Based on these results, the structure of the minor constituent of the mixture (**17b**) was determined as 3′,4-*O*-dimethylcedrusin 9-*O*-*β*-glucopyranoside. A survey showed **17b** was a new compound to the literature.

Compound **18** was obtained as an amorphous powder ($[\alpha]_D = -12°$). Its molecular formula ($C_{26}H_{34}O_{12}$) was determined by positive-ion HR-MALDIMS ($[M + Na]^+ m/z$ 561.1950, calcd. for $C_{26}H_{34}O_{12}Na$, 561.1943) and ^{13}C NMR data (Table 2). The ^{1}H-NMR spectrum displayed 2 sets of signals due to 1,3,4-trisubstituted aromatic rings, 2 oxymethines, 2 oxymethylenes and 2 *trans*-oriented olefinic protons together with an anomeric proton and two methoxyl signals. The ^{13}C NMR spectrum exhibited 26 signals, 6 of which were ascribed to a hexose unit. The complete assignments of all proton and carbon resonances were based on the DQF-COSY, HSQC, HSQC-TOCSY and HMBC experiments. The anomeric proton resonance appeared at δ_H 4.81 (d, J = 7.8 Hz, H-1″) and the corresponding carbon resonance (δ_C 102.9) along with the other sugar signals indicated the presence of a *β*-glucopyranose unit in **18**. The doublet signal at δ_H 4.94 (J = 5.1 Hz, H-7) was coupled to the multiplet at δ_H 4.33 (H-8), which, in turn, was coupled to 2 double doublets at δ_H 3.77 (J = 12.0 and 6.4 Hz, H-9a) and δ_H 3.49 (J = 12.0 and 6.0 Hz, H-9b), thus suggesting a chain of 3 carbons, all bearing oxygen (C-7, C-8 and C-9, respectively). The 2 *trans*-oriented olefinic protons (δ_H 6.26

dt, J = 15.8, 6.5 Hz, 6.52 d, J = 15.8 Hz) and the second oxymethylene protons (δ_H 4.20 d, J = 6.5 Hz) were observed in the same spin system, and were assigned to an (*E*)-coniferyl alcohol side chain. The HMBC spectrum (Table 2) revealed the connectivities between the molecular fragments exhibiting long-range correlations from the quaternary carbon resonance at δ_C 147.4, assigned as C-4, to the anomeric proton of the glucose unit, and the protons assigned to H-2, H-5 and H-6 of one of the 1,3,4-trisubstituted aromatic rings. The long-range correlations from C-4′ (δ_C 149.1) to H-2′, H-5′ and H-6′ of the second aromatic ring, as well as to H-8, suggested the presence of an 8-*O*-4′-neolignan structure. Furthermore, HMBC correlations from C-1 to H-2, H-6, H-7 and H-8, as well as from C-1′ to H-2′, H-6′, H-7′ and H-8′, established the remaining connectivities. Finally, the long-range correlations from C-3 to 3-OMe and C-3′ to 3′-OMe allowed the locations of 2 methoxyl groups on the aromatic rings. These results showed that **18** has the same constitution as reported for an 8-*O*-4′ neolignan glucoside isolated from *Arum italicum* (established as its peracetylated derivative)[7]. Moreover, the small coupling constant ($J_{7,8}$ = 5.1 Hz) of the benzylic proton (H-7), and the chemical shifts of H-7 and H-8, as well as those of C-7 (δ_C 73.6) and C-8 (δ_C 86.5), agreed with an *erythro*-configuration[8]. Based on the above evidence, and due to the almost superimposable CD-spectrum with that of the derivative[7] (see Experimental), the structure of **18** was considered to be (7*S*,8*R*)-8-*O*-4′ neolignan 4-*O*-β-glucopyranoside {= *erythro*-1-(4-*O*-β-glucopyranosyl-3-methoxyphenyl)-2-{2-methoxyl-4-[1-(*E*)-propene-3-ol]-phenoxyl}-propane-1,3-diol}.

In addition to these compounds, 3 known iridoid glycosides [ipolamiide (**1**)[9], lamiide (**2**)[9] and lamiidoside (**3**)[10]], 10 known phenylethanoid glycosides [verbascoside (**4**)[11], isoacteoside (**5**)[12], decaffeoylverbascoside (**6**)[12], martynoside (**7**)[13], leucosceptoside A (**8**)[14], β-hydroxyverbascoside (**9**)[15], forsythoside B (**10**)[16], alyssonoside (**11**)[17], leucosceptoside B (**12**)[15] and myricoside (**13**)[18]] a known megastigmane glycoside [phlomuroside (**15**)[19]], a known hydroquinone glycoside [seguinoside K[20]], 4 known neolignan glucosides [dehydrodiconiferylalcohol 9′-*O*-β-glucopyranoside (**19**)[21], dihydrodehydrodiconiferylalcohol 4-*O*-β-glucopyranoside (**20**)[22], a mixture of dihydrodehydrodiconiferylalcohol 9-*O*-β-glucopyranoside and dihydrodehydrodiconiferylalcohol 9′-*O*-β-glucopyranoside (**21a/21b**)[22]] and 2 lignan glucosides [lariciresinol 4-*O*-β-glucopyranoside (**22**)[23], and syringaresinol 4′-*O*-β-glucopyranoside (**23**)[24]] were also isolated and identified by comparison of their spectroscopic (NMR and MS) data and optical rotation values with those published in the literature.

Samioside (**14**) has been isolated from *P. samia* as reported in a previous study[25]. Due to its structural similarity to compounds **4-13**, samioside was also evaluated for its biological activities.

All compounds including samioside (**14**) were tested for cell growth inhibition versus 3 tumor cell lines (MCF7, NCI-H460, and SF-268) in the US National Cancer Institute cancer prescreen at a single dose of 100 μM[3]. None of the iridoid glycosides were active in the prescreen. The other 6 phenylethanoid glycosides (**4**, **5**, **10**, **12**, **13**, **14**) tested reduced the growth of at least 1 cell line to <32% of the control, and were tested further against the full 60-cell line panel[3]. The phenylpropanoid glycosides acteoside (**4**), isoacteoside (**5**), forsythoside B (**10**), alyssonoside (**11**), myricoside (**13**) and samioside (**14**) were tested against the full panel; however, the concentrations required to inhibit cell growth at the GI-50 level were greater than 47 μM in all cases, with minimal differential inhibition among the cell lines. Thus, these compounds were not investigated further as inhibitors of cancer cell growth.

The DPPH radical scavenging activities of phenylethanoid glycosides are summarized in Table 3. The results confirmed that the respective activities of compounds **4**, **5**, **10**, **13** and **14** were comparable to that of *dl*-α-tocopherol, while the other compounds showed moderate activities. These results suggested that the antioxidative effect of these compounds might be potentiated by an increase in the number of free phenolic hydroxyl groups in the molecule.

Table 3. Free radical (DPPH) scavenging activity of compounds **4-14**.

Compounds	10 (μM)	25 (μM)	50 (μM)	100 (μM)	200 (μM)	IC$_{50}$ (μM)
(**4**) Verbascoside	40.83	45.39	54.96	92.36	97.53	75.5
(**5**) Isoacteoside	16.33	25.93	57.82	93.89	94.52	225.4
(**6**) Decaffeoylacteoside	-	-	10.7	14.2	46.0	355.3
(**7**) Martynoside	-	-	7.6	15.7	26.4	87.0
(**8**) Leucosceptoside A	4.2	16.4	26.7	58.0	80.7	125.4
(**9**) β-hydroxyacteoside	-	11.8	51.7	46.8	66.0	52.1
(**10**) Forsythoside B	7.6	38.8	44.3	89.6	92.2	137.1
(**11**) Alyssonoside	-	5.0	12.2	27.6	79.7	131.2
(**12**) Leucosceptoside B	15.0	16.8	24.2	38.2	72.5	61.3
(**13**) Myricoside	17.4	24.8	42.8	75.6	81.4	46.8
(**14**) Samioside	18.5	34.0	59.4	86.6	94.7	115.0
dl-α-tocopherol	2.3	13.1	25.4	32.6	92.00	75.5

Phenylethanoid glycosides (**4-14**) were tested for their antibacterial and antifungal activities. Compounds **4**, **5**, **10** and **14** (MIC: 500 μg/mL) as well as **8** and **13** (MIC:1000 μg/mL) showed very weak activity against Gram (+) bacteria. All compounds were inactive towards Gram (-) bacteria and *C. albicans* (MICs $>$ 1000 μg/mL). Isoverbascoside (**5**) exhibited slight antifungal potential against 2 yeast-like fungi (MIC: 1000 μg/mL), while samioside (**14**) weakly inhibited the growth of *C. krusei* (MIC: 1000 μg/mL).

As part of our continuing project to identify the secondary metabolites of *Phlomis* species growing in Turkey, we have studied all 33 species of this genus. Among them, *P. viscosa* had the richest chemical diversity, especially for its lignan and phenylethanoid glycoside content.

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