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Flavonoid constituents of *Sideritis caesarea*

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Abstract: The acetone extract of the aerial parts of *Sideritis caesarea* Duman, Aytaç & Başer (Lamiaceae) afforded the flavonoids penduletin (**1**) and apigenin (**2**) and 6 glycosylated flavonoids, 4'-*O*-methyl-isoscutellarein-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-6"-*O*-acetyl-β-D-glucopyranoside (**3**), 4'-*O*-methylhypolaetin-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-6"-*O*-acetyl-β-D-glucopyranoside (**4**), isoscutellarein-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-6"-*O*-acetyl-β-D-glucopyranoside (**5**), isoscutellarein-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-β-D-glucopyranoside (**6**), 4'-*O*-methylhypolaetin-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-β-D-glucopyranoside (**7**), and hypolaetin-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-β-D-glucopyranoside (**8**). The compounds were identified by the use of 1D- and 2D-NMR and UV spectroscopic techniques and by comparisons with the reported data. The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the acetone, methanol, and water extracts of the plant and of the flavones penduletin and apigenin were evaluated at 200 μg/mL. The water extract exhibited better activity against the enzyme AChE as compared to both the acetone and the methanol extracts. Penduletin (**1**) showed significant activity against BChE (66.58%) while apigenin (**2**) showed weak activity against both enzymes.

Key words: Penduletin, methoxyflavones, flavone glycosides, *Sideritis caesarea*, anticholinesterase activity, Lamiaceae

1. Introduction

The genus *Sideritis* (Lamiaceae) is distributed mainly in the Mediterranean (including North Africa, the Iberian Peninsula, the Mediterranean countries, and the Middle East) and Macaronesian regions. *Sideritis* comprises more than 150 species. In Turkey 46 species, 12 subspecies, and 2 varieties grow, among which 36 species, 10 subspecies, and 2 varieties are endemic.[1–3] *Sideritis* species are widely used as herbal teas and they have served as folk medicine. Traditional uses and the pharmacological activities of the constituents of *Sideritis* species have been covered in a review by Gonzalez-Burgoz et al.[4] *Sideritis caesarea* Duman, Aytaç & Başer is an endemic species to Turkey. In previous studies the aqueous and methanol extracts of *Sideritis caesarea* Duman, Aytaç & Başer have been found to show gastroprotective effects on ethanol-induced ulcerogenesis[5] and antimicrobial and antioxidant activities.[6]

The genus *Sideritis* is characterized by its essential oil constituents,[7] diterpenoids,[8–11] and flavonoids.[12,13] In a former study we reported 5 diterpenoids, all with the *ent*-kaurane skeleton, from the acetone extract of *Sideritis caesarea*.[11] The present study is the first report on the isolation and structure elucidation of its

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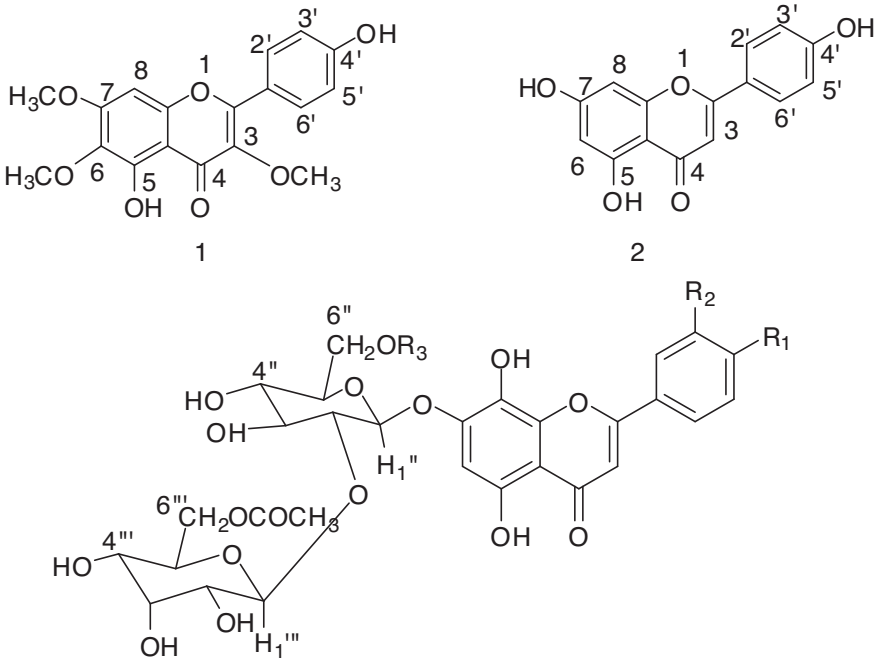
flavonoid constituents. Although the flavone apigenin (**2**) and glycosylated flavonoids (**3**–**8**) have been isolated from other *Sideritis* species, the trimethoxylated flavone penduletin (5,4'-dihydroxy-3,6,7-trimethoxyflavone) (**1**) was isolated for the first time from a *Sideritis* species (Figure 1).



3.	$R_1 = OCH_3$	$R_2 = H$	$R_3 = CH_3CO$
4.	$R_1 = OCH_3$	$R_2 = OH$	$R_3 = CH_3CO$
5.	$R_1 = OH$	$R_2 = H$	$R_3 = CH_3CO$
6.	$R_1 = OH$	$R_2 = H$	$R_3 = H$
7.	$R_1 = OCH_3$	$R_2 = OH$	$R_3 = H$
8.	$R_1 = OH$	$R_2 = OH$	$R_3 = H$

Figure 1. Structures of compounds **1**–**8**.

Sideritis extracts and constituents exhibit various biological activities[4] including antiinflammatory,[14] antirheumatoid,[4] gastroprotective,[5] and antioxidant[15,16] activities, as well as anticholinesterase activity.[9,17] Oxidative stress is a factor contributing to the progress of neurodegeneration. Acetylcholinesterase (AChE) enzyme and butyrylcholinesterase (BChE) enzyme inhibitors have the potential to alleviate neurodegenerative diseases and dementia. Compounds such as flavonoids, which show antioxidant activity, have been shown to also possess AChE and BChE inhibitory activities. Since the extracts of *S. caesarea* have been investigated previously for antioxidant activity,[6] in the present study AChE and BChE inhibitory activities of the plant extracts, as well as the flavones penduletin and apigenin, were analyzed.

2. Results and discussion

From the aerial parts of *Sideritis caesarea*, which is an endemic species to Turkey, 2 flavones and 6 flavone glycosides consisting of 3 isoscutellarein and 3 hypolaetin glycosides were isolated. The flavonoids were identified by UV and 1D- and 2D-NMR techniques and by comparison with the reported data as penduletin

(**1**),[18] apigenin (**2**),[19] 4'-*O*-methyl-isoscutellarein-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-6"-*O*-acetyl-β-D-glucopyranoside (**3**),[20] 4'-*O*-methylhypolaetin-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-6"-*O*-acetyl-β-D-glucopyranoside (**4**),[21] isoscutellarein-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-6"-*O*-acetyl-β-D- glucopyranoside (**5**),[13,21] isoscutellarein-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-β-D-glucopyranoside (**6**),[13,20,21] 4'-*O*-methylhypolaetin-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-β-D-glucopyranoside (**7**),[21] and hypolaetin-7-*O*-[6"'-*O*-acetyl-β-D-allopyranosyl-(1$\rightarrow$2)]-β-D-glucopyranoside (**8**)[20,21] (Figure 1; Tables 1–3).

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data in $CDCl_3$ for **1**.

C/H	δ_H (*J*,Hz)	DEPT	δ_C
2		C	155.82
3		C	138.95
4		C	179.19
5		C	152.57
6		C	132.49
7		C	158.29
8	6.50, s	CH	90.54
9		C	153.02
10		C	106.80
1'		C	121.37
2'	8.03, d *(7.6)*	CH	130.65
3'	6.96, d *(7.6)*	CH	115.86
4'		C	159.00
5'	6.96, d *(7.6)*	CH	115.86
6'	8.03, d *(7.6)*	CH	130.65
3-OMe	3.86, s	CH_3	61.12
6-OMe	3.92, s	CH_3	60.39
7-OMe	3.95, s	CH_3	56.54
5-OH	12.60, s	-	-

The trimethoxylated flavone penduletin (**1**) has been isolated from a *Sideritis* species for the first time in this study. The substitution pattern was indicated by the UV spectrum and verified by other spectroscopic techniques. The presence of hydroxyl groups at C-5 and C-4' and oxygenation at C-3 were observed from the UV data. Oxygenation at position C-3, but no 3-OH group, was indicated by λ_{max} at 333 nm in MeOH. A 56-nm bathochromic shift from λ_{max} 333 nm to λ_{max} 389 nm on addition of NaOMe showed the presence of 4'-OH. A shift from λ_{max} 333 nm to λ_{max} 356 nm was due to complex formation with $AlCl_3$ by 5-OH and the carbonyl function at C-4. No hypsochromic shift was observed by the addition of HCl to the $AlCl_3$ solution, verifying the absence of *ortho*-dihydroxyl groups.

The ^{1}H NMR spectrum of compound **1** in $CDCl_3$ (Table 1) indicated the presence of 3 methoxyl groups at δ_H 3.95 (s, 3H), 3.92 (s, 3H), and 3.86 (s, 3H). The 2 pairs of *ortho*-coupled (J = 7.6 Hz) doublets at δ_H 6.96 (H-3'/5') and at δ_H 8.03 (H-2'/6') showed that ring B is monosubstituted at C-4'. The peak at δ_H 12.60 belongs to 5-OH. There is a 1-proton singlet at δ_H 6.50. There are 3 possible positions for this singlet. These are C-3, C-6, or C-8. The ^{13}C NMR spectrum displays 16 carbon signals. Three methoxyl, 3 methine (C-8, C-3'/5', and C-2'/6'), 1 carbonyl (C-4), and 9 quaternary carbon signals (1', 4', 2, 3, 5, 6, 7, 9,

Table 2. Proton chemical shifts (δ_H), multiplicities, and coupling constants (J, Hz) for **3–8** in CD_3OD.

H	**3**	**4**	**5**	**6**	**7**	**8**
Aglycone						
H-3	6.65 s	6.64 s	6.65 s	6.64 s	6.64 s	6.60 s
H-6	6.75 s	6.71 s	6.72 s	6.79 s	6.78 s	6.78 s
H-2′	7.98 d *(8.59)*	7.47 d *(2.34)*	7.93 d *(8.97)*	7.94 d *(8.97)*	7.48 d *(2.34)*	7.46 d *(1.95)*
H-3′	7.06 d *(8.98)*	-	6.95 d *(8.97)*	6.94 d *(8.97)*	-	-
H-5′	7.06 d *(8.98)*	7.09 d *(8.97)*	6.95 d *(8.97)*	6.94 d *(8.97)*	7.10 d *(8.58)*	6.93 d *(8.97)*
H-6′	7.98 d *(8.98)*	7.59 dd *(8.97; 2.34)*	7.93 d *(8.97)*	7.94 d *(8.97)*	7.59 dd *(8.58; 2.34)*	7.48 dd *(8.97; 1.95)*
4′-OCH_3	3.85 s	3.86 s	-	-	3.95 s	-
Glucopyranose						
H-1″	4.95 d (*7.80*)	4.92 d (*7.60*)	4.92 d (*7.41*)	4.94 d *(7.80)*	4.94 d (*7.80*)	4.94 d (*7.80*)
H-2″	3.70 m	3.73 dd *(7.61; 9.60)*	3.74 dd *(7.80; 8.97)*	3.73 dd *(7.41; 8.97)*	3.73 dd *(7.60; 9.20)*	3.73 dd *(7.41; 9.36)*
H-3″	3.74 m	3.69 dd *(9.20; 9.60)*	3.68 dd *(8.58; 9.75)*	3.67 dd *(8.58; 8.97)*	3.67 m	3.63 m
H-4″	3.40 dd *(8.92; 9.80)*	3.44 dd *(9.20; 10.00)*	3.45 dd *(8.97; 10.14)*	3.48 m	3.49 m	3.45 m
H-5″	3.68 ddd *(2.00; 5.22; 9.80)*	3.67 t *(9.20)*	3.71 ddd *(2.20; 4.90; 10.00)*	3.49 m	3.49 m	3.47 m
H-6a″	4.48 dd *(1.95; 1.70)*	4.47 dd *(2.00; 2.00)*	4.48 dd *(2.20; 12.09)*	3.94 brd *(11.31)*	3.93 brd *(11.60)*	3.96 brd *(10.50)*
H-6b″	4.32 dd *(5.27;11.71)*	4.30 dd *(4.80; 2.00)*	4.31 dd *(4.68; 2.09)*	3.77 dd *(4.68; 1.80)*	3.77 dd *(5.20; 1.60)*	3.75 dd *(4.80; 0.50)*
Acetyl	2.14	2.14	2.14	-	-	-
Allopyranose						
H-1‴	5.05 d *(8.00)*	5.06 d *(8.40)*	5.06 d *(8.10)*	5.07 d *(7.80)*	5.07 d *(7.80)*	5.08 d *(8.20)*
H-2‴	3.46 dd *(3.60; 8.00)*	3.46 dd *(3.20; 8.80)*	3.46 dd *(3.12; 8.00)*	3.46 dd *(2.73; 7.80)*	3.46 dd *(2.73; 7.80)*	3.46 brd *(8.20)*
H-3‴	4.12 t *(3.60)*	4.12 t *(2.80)*	4.12 t *(3.12)*	4.12 t *(2.73)*	4.12 t *(2.73)*	4.12 t *(2.73)*
H-4‴	3.64 dd *(3.40; 9.80)*	3.64 dd *(3.20; 8.10)*	3.63 dd *(3.12; 10.00)*	3.64 dd *(2.73; 9.75)*	3.64 dd *(2.73; 10.14)*	3.67 dd *(2.40; 8.80)*
H-5‴	4.05 ddd *(2.34; 5.20; 9.90)*	4.04 ddd *(2.34; 4.40; 9.60)*	4.04 ddd *(2.34; 5.10; 10.00)*	4.04 ddd *(2.34; 5.46; 9.75)*	4.03 ddd *(2.30; 5.10; 9.90)*	4.03 ddd *(1.50; 5.00; 9.00)*
H-6a‴	4.33 dd *(2.20; 12.11)*	4.33 dd *(2.20; 12.10)*	4.34 dd *(2.34; 12.09)*	4.33 dd *(2.34; 12.09)*	4.33 dd *(2.20; 12.09)*	4.34 dd *(2.00; 12.10)*
H-6b‴	4.24 dd *(5.08; 12.11)*	4.24 dd *(4.80; 12.10)*	4.24 dd *(5.07; 12.09)*	4.24 dd *(5.46; 12.09)*	4.24 dd *(5.10; 12.09)*	4.24 dd *(5.46; 12.10)*
Acetyl	1.98	2.00 s	1.98	1.97 s	2.00 s	2.00 s

Table 2. Continued.

H	**3**	**4**	**5**	**6**	**7**	**8**
Aglycone						
H-3	6.65 s	6.64 s	6.65 s	6.64 s	6.64 s	6.60 s
H-6	6.75 s	6.71 s	6.72 s	6.79 s	6.78 s	6.78 s
H-2′	7.98 d *(8.59)*	7.47 d *(2.34)*	7.93 d *(8.97)*	7.94 d *(8.97)*	7.48 d *(2.34)*	7.46 d *(1.95)*
H-3′	7.06 d *(8.98)*	-	6.95 d *(8.97)*	6.94 d *(8.97)*	-	-
H-5′	7.06 d *(8.98)*	7.09 d *(8.97)*	6.95 d *(8.97)*	6.94 d *(8.97)*	7.10 d *(8.58)*	6.93 d *(8.97)*
H-6′	7.98 d *(8.98)*	7.59 dd *(8.97; 2.34)*	7.93 d *(8.97)*	7.94 d *(8.97)*	7.59 dd *(8.58; 2.34)*	7.48 dd *(8.97; 1.95)*
4′-OCH_3	3.85 s	3.86 s	-	-	3.95 s	-
Glucopyranose						
H-1″	4.95 d (*7.80*)	4.92 d (*7.60*)	4.92 d (*7.41*)	4.94 d *(7.80)*	4.94 d (*7.80*)	4.94 d (*7.80*)
H-2″	3.70 m	3.73 dd *(7.61; 9.60)*	3.74 dd *(7.80; 8.97)*	3.73 dd *(7.41; 8.97)*	3.73 dd *(7.60; 9.20)*	3.73 dd *(7.41; 9.36)*
H-3″	3.74 m	3.69 dd *(9.20; 9.60)*	3.68 dd *(8.58; 9.75)*	3.67 dd *(8.58; 8.97)*	3.67 m	3.63 m
H-4″	3.40 dd *(8.92; 9.80)*	3.44 dd *(9.20; 10.00)*	3.45 dd *(8.97; 10.14)*	3.48 m	3.49 m	3.45 m
H-5″	3.68 ddd *(2.00; 5.22; 9.80)*	3.67 t *(9.20)*	3.71 ddd *(2.20; 4.90; 10.00)*	3.49 m	3.49 m	3.47 m
H-6a″	4.48 dd *(1.95; 1.70)*	4.47 dd *(2.00; 2.00)*	4.48 dd *(2.20; 12.09)*	3.94 brd *(11.31)*	3.93 brd *(11.60)*	3.96 brd *(10.50)*
H-6b″	4.32 dd *(5.27;11.71)*	4.30 dd *(4.80; 2.00)*	4.31 dd *(4.68; 2.09)*	3.77 dd *(4.68; 1.80)*	3.77 dd *(5.20; 1.60)*	3.75 dd *(4.80; 0.50)*
Acetyl	2.14	2.14	2.14	-	-	-
Allopyranose						
H-1‴	5.05 d *(8.00)*	5.06 d *(8.40)*	5.06 d *(8.10)*	5.07 d *(7.80)*	5.07 d *(7.80)*	5.08 d *(8.20)*
H-2‴	3.46 dd *(3.60; 8.00)*	3.46 dd *(3.20; 8.80)*	3.46 dd *(3.12; 8.00)*	3.46 dd *(2.73; 7.80)*	3.46 dd *(2.73; 7.80)*	3.46 brd *(8.20)*
H-3‴	4.12 t *(3.60)*	4.12 t *(2.80)*	4.12 t *(3.12)*	4.12 t *(2.73)*	4.12 t *(2.73)*	4.12 t *(2.73)*
H-4‴	3.64 dd *(3.40; 9.80)*	3.64 dd *(3.20; 8.10)*	3.63 dd *(3.12; 10.00)*	3.64 dd *(2.73; 9.75)*	3.64 dd *(2.73; 10.14)*	3.67 dd *(2.40; 8.80)*
H-5‴	4.05 ddd *(2.34; 5.20; 9.90)*	4.04 ddd *(2.34; 4.40; 9.60)*	4.04 ddd *(2.34; 5.10; 10.00)*	4.04 ddd *(2.34; 5.46; 9.75)*	4.03 ddd *(2.30; 5.10; 9.90)*	4.03 ddd *(1.50; 5.00; 9.00)*
H-6a‴	4.33 dd *(2.20; 12.11)*	4.33 dd *(2.20; 12.10)*	4.34 dd *(2.34; 12.09)*	4.33 dd *(2.34; 12.09)*	4.33 dd *(2.20; 12.09)*	4.34 dd *(2.00; 12.10)*
H-6b‴	4.24 dd *(5.08; 12.11)*	4.24 dd *(4.80; 12.10)*	4.24 dd *(5.07; 12.09)*	4.24 dd *(5.46; 12.09)*	4.24 dd *(5.10; 12.09)*	4.24 dd *(5.46; 12.10)*
Acetyl	1.98	2.00 s	1.98	1.97 s	2.00 s	2.00 s

10) have been assigned by HSQC and HMBC correlations (Table 1). The methine carbon resonance at δ_C 90.54 is characteristic only of a nonoxygenated C-8 in flavones.[22] Instead, the methine proton of a nonoxygenated C-6 appears at δ 94–98. The HMBC experiment (Figure 2), exhibiting 3-bond away correlations from H-8 to C-10 (δ 106.8) and from H-8 to C-6 (δ 132.49), verified that the δ_H 6.50 singlet belongs to H-8. In the case of free C-3, C-3 would be observed at about 100–105 ppm in the ^{13}C NMR spectrum. However, there is no methine signal observed in this range. An HMBC correlation between the singlet signal at δ_{H} 3.86 and the carbon signal at δ_C 138.95 verified the presence of a methoxyl group at C-3. The location of the remaining 2 methoxyl groups was also assigned by 3-bond away HMBC correlations: from δ 3.92 (OCH_3) to 132.49 ppm (C-6) and from δ 3.95 (OCH_3) to 158.29 ppm (C-7) (Table 1).

Table 3. ^{13}C NMR chemical shifts (δ_C) for **3**–**7** in CD_3OD.

C	**3**	**4**	**5**	**6**	**7**
Aglycone					
2	165.9	164.3	165.2	165.5	165.3
3	102.1	103.1	101.2	103.0	103.2
4	183.8	183.2	183.0	183.3	183.3
5	151.1	151.4	150.2	150.8	151.7
6	100.8	101.0	100.3	102.6	100.6
7	152.0	152.6	152.6	152.9	152.9
8	130.92	128.0	128.3	128.4	128.5
9	nd*	144.1	143.8	144.1	142.6
10	106.8	105.2	106.4	106.6	106.6
1'	123.3	122.1	121.7	122.0	123.8
2'	128.5	111.3	128.4	128.6	113.0
3'	114.5	147.0	115.6	115.8	147.0
4'	163.7	150.4	161.5	161.7	150.9
5'	114.5	112.7	115.6	115.8	111.5
6'	128.5	119.0	128.4	128.6	119.3
4'-OMe	54.9	55.1	-	-	55.3
Glucopyranose					
1"	102.5	101.1	102.3	100.6	101.8
2"	83.8	82.3	82.5	82.8	82.5
3"	76.2	76.1	76.0	76.4	76.4
4"	70.3	70.0	71.1	69.6	69.6
5"	74.6	73.1	74.3	77.3	77.3
6"	63.8	63.5	63.3	61.1	61.1
CO**C**H_3	19.9	20.0	19.5	-	-
COCH_3	172.3**	172.2	171.7	-	-
Allopyranose					
1"'	103.1	103.0	102.9	101.7	102.8
2"'	72.2	72.0	72.4	72.2	72.0
3"'	71.9	71.6	72.0	71.4	71.2
4"'	67.4	67.1	67.0	67.3	67.0
5"'	71.4	71.2	71.6	71.9	71.6
6"'	63.9	63.6	63.6	63.8	63.6
CO **C**H_3	19.9	20.1	19.4	19.6	19.5
CO CH_3	172.5**	172.4	171.4	171.8	171.6

*Not detected.

**Interchangeable peaks.

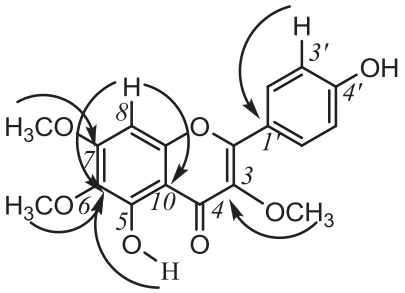


Figure 2. HMBC correlations for **1**.

In previous studies on Turkish *Sideritis* species, only the trimethoxylated flavone xanthomicrol (5,4'-dihydroxy-6,7,8-trimethoxyflavone) was obtained from *Sideritis stricta*.[13] It differs from compound **1** with the 3 methoxyl groups located on ring A.

The anticholinesterase activity of the crude acetone, methanol, and water extracts of the aerial parts of *S. caesarea* and of compounds **1** and **2** were determined. The water extract exhibited better activity against AChE while the acetone extract exhibited better activity against BChE. Penduletin showed significant activity against BChE with an inhibition value of 66.58%. However, apigenin's inhibition on the enzymes was insignificant (Table 4).

Table 4. Anticholinesterase activity results at 200 μg/mL[a] (inhibition %).

Sample	AChE	BChE
Acetone extract	24.35 ± 2.02	37.88 ± 1.13
Methanol extract	23.16 ± 1.14	9.50 ± 1.41
Water extract	58.10 ± 1.49	30.38 ± 1.84
Penduletin	21.03 ± 0.99	66.58 ± 1.42
Apigenin	24.21 ± 0.28	12.33 ± 1.02
Galantamine[b]	74.12 ± 0.15	74.48 ± 0.63

[a]Values expressed are means ± standard deviations of 3 parallel measurements ($P < 0.05$).
[b]Standard drug.

In conclusion, as part of our studies of the *Sideritis* species of Turkey, we have investigated the flavonoid constituents of *Sideritis caesarea*. The flavones apigenin and penduletin, as well as 6 glycosylated derivatives of the 8-hydroxyflavones isoscutellarein and hypolaetin, were isolated and identified. This is the first report on the isolation of penduletin from a *Sideritis* species. The investigated anticholinesterase activity of the crude extracts of *Sideritis caesarea* and of its flavones was found to be weak-moderate, except for penduletin, which exhibited high activity against BChE.

3. Experimental

3.1. General

UV spectra were recorded on UV-VIS Varian Techtron model 635-D at İstanbul University, Faculty of Pharmacy, Department of General Chemistry. NMR spectra were recorded on a Varian-400 spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, with $CDCl_3$ and CD_3OD as solvents. Kieselgel 60 (0.063–0.200 mm, Merck) and Sephadex LH-20 (Pharmacia) were used for column chromatography and precoated Kieselgel 60 F_{254}

(Merck) aluminum sheets were used for preparative thin layer chromatography (TLC). The isolated compounds were detected by UV fluorescence and by spraying with a solution of $Ce(SO_4)_2$ reagent (2 g of $Ce(SO_4)_2$ in 100 mL of 6 M H_2SO_4), followed by heating at 100–110 °C for 1 to 2 min. The anticholinesterase activity measurements were done on a 96-well microplate reader, Spectra-Max 340PC[384], Molecular Devices (USA). The measurements and calculations were evaluated by using Softmax PRO v5.2 software.

3.2. Plant material

The whole plant *S. caesarea* Duman, Aytaç & Başer (Lamiaceae) was collected from the Binboğa Mountains above Yeşilköy, Pınarbaşı-Göksun, Kayseri, on 21 June 2006. It was authenticated by Dirmenci (3000) and Arabacı at Balıkesir University, Turkey. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, İstanbul University (ISTE 98910), and also in the Special Collection of Dr Tuncay Dirmenci at Balıkesir University.

3.3. Extraction and isolation

First, 795 g of the powdered whole plant was extracted successively with petroleum ether, acetone, methanol, and water. The acetone extract (21.37 g) was fractionated on a silica gel column with petroleum ether, dichloromethane, acetone, and methanol gradients. The isolated compounds were purified on Sephadex LH-20 columns eluted with MeOH with repeated preparative TLC applications. The acetone extract yielded compounds **1**–**8**. Penduletin (**1**) was isolated from fractions 39–42 (50 mL each) eluted with dichloromethane-acetone, 8:2. The dichloromethane-acetone fractions also yielded diterpenoids.[11] Fractions 119–126 eluted with acetone-methanol (9:1) yielded **2**, **3**, **4**, and **5**. Fractions 127–131 eluted with acetone-methanol (8:2) yielded **3**, **6**, **7**, and **8**, all after being subjected to further fractionation on silica gel and subsequently on Sephadex LH-20 columns (MeOH eluent) with repeated preparative TLC.

3.4. Penduletin (1)

Amorphous yellow powder. UV λ_{max} nm (log ε), MeOH: 275 (2.10), 333 (1.96); NaOMe: 247 (sh), 277 (1.88), 300 (sh), 389 (2.10); $AlCl_3$: 280 (1.71), 300 (sh), 356 (1.03); $AlCl_3$+HCl: 282 (1.08), 300 (sh), 354 (1.01); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz): Table 1.

3.5. Anticholinesterase activity

AChE and BChE inhibitory activities were measured by a slight modification of the spectrophotometric method developed by Ellman et al.[23] Acetylthiocholine iodide and butyrylthiocholine chloride were used as substrates of the reaction and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) was used for the measurement of the anticholinesterase activity. First, 160 μL of 100 mM sodium phosphate buffer (pH 8.0), 10 μL of test compound solution, and 10 μL of AChE or BChE solution were mixed and incubated for 15 min at 25 °C and 10 μL of DTNB was added. The reaction was then initiated by the addition of 10 μL of acetylthiocholine iodide or butyrylthiocholine chloride. The hydrolysis of these substrates was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, at a wavelength of 412 nm. Methanol was used as a solvent for the test compounds and for the control.

3.5.1. Statistical analysis

All data on all anticholinesterase activity tests are the average of triplicate analyses. The data were recorded as mean ± standard deviation. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Student's t-test. $P < 0.05$ was regarded as significant.

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