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Phenylethanoid Glycosides from *Scutellaria galericulata*

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From the aerial parts of *Scutellaria galericulata* L., four phenylethanoid glycosides, 2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside (**1**), calceolarioside B (**2**), osmanthuside E (**3**) and martynoside (**4**), were isolated. The structure elucidations of the isolated compounds were performed by spectroscopic (UV, IR, ESI-MS, 1D- and 2D-NMR) methods. Compounds **1**-**4** demonstrated scavenging properties toward the 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical in TLC autographic assays.

Key Words: *Scutellaria galericulata*, Lamiaceae, phenylethanoid glycosides, radical scavenging activity.

Introduction

The genus *Scutellaria* L. (Lamiaceae) is represented by fifteen species in the flora of Turkey[1]. *S. orientalis* subsp. *pinnatifida* has been used as an antidiarrheaic, hemostatic, tonic and for wound healing in Anatolian folk medicine[2]. Previous investigations of Turkish *Scutellaria* species by our research group led to the isolation and characterization of iridoid glucosides from *S. albida* subsp. *colchica*[3], phenylethanoid glycosides from *S. albida* subsp. *colchica*[4], *S. orientalis* subsp. *pinnatifida*[5] and *S. salviifolia*[6]. *Scutellaria galericulata* is a rhizomatous perennial herb, growing in marshy ground, and on the edges of lakes and rivers at elevations of 1-2000 m in Northern Turkey and Inner Anatolia[1]. Several neo-clerodane diterpenoids with insect anti-feedant activity have been reported from *S. galericulata*[7−9]. In this paper, we describe the isolation and structure determination of four phenylethanoid glycosides, 2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside (**1**), calceolarioside B (**2**), osmanthuside E (**3**) and martynoside (**4**) from the title plant.

Experimental

General experimental procedures: UV spectra (λ_{max}) were recorded on a Hitachi HP 8452 A spectrophotometer, in MeOH. IR spectra (υ_{max}, in KBr pellets) were recorded on a Perkin-Elmer 2000 FTIR

spectrophotometer. NMR measurements in CD_3OD (room temperature) were performed on Varian spectrometers, operating at 400 or 500 MHz. ESIMS spectra were recorded in the positive and negative ion modes on a Finnigan LCQDECA ion trap mass spectrometer. For open-column chromatography (CC), silica gel 60 (0.063-0.200 mm, Merck), Sephadex LH-20 (Fluka), and polyamide (Polyamid-MN-Polyamid SC-6, Machery-Nagel, Düren) were used. MPLC separations were performed on a Labomatic glass column (18x352 mm, i.d.) packed with LiChroprep RP-18 (Merck), using a Lewa M5 peristaltic pump. TLC was carried out on precoated silica gel $60F_{254}$ aluminum sheets (Merck). Compounds were detected by UV fluorescence and spraying with vanillin-H_2SO_4 reagent followed by heating at 105^{o}C for 1-2 min. For radical-scavenging TLC autographic assay, 1,1-diphenyl-1-picrylhydrazyl (DPPH, Fluka) was used as spray reagent.

Plant material: *Scutellaria galericulata* L. (Lamiaceae) was collected by Abant Lake, Bolu, Turkey, in August 1998. Voucher specimens have been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University [HUEF 96-007].

Extraction and Isolation: Air-dried and powdered over-ground parts of *S. galericulata* (450 g) were extracted twice with MeOH (2x1500 ml) at 40^{o}C. The combined MeOH extracts were evaporated to dryness *in vacuo*. The crude extract (45 g) was suspended in water (500 ml) and the water insoluble material was removed by filtration. The filtrate was extracted with *n*-BuOH (3x150 ml) and concentrated (20 g *n*-BuOH extract). An aliquot of the *n*-BuOH extract (10 g) was chromatographed over polyamide (100 g) eluting with H_2O (350 ml), followed by increasing concentrations of MeOH in H_2O (25%, 50%, 75% and 100%, each 250 ml) to yield five main frs: Frs A (1.62 g), B (0.75 g), C (0.93 g), D (2.99 g) and E (1.60 g). 310 mg of fraction C was chromatographed over CC, using silica gel (30 g) as stationary phase and eluting with $CHCl_3$-MeOH-H_2O (80:20:0.5, 80:20:1, 80:20:2 and 70:30:3, each 100 ml) to give impure **4**, which was purified over Sephadex LH-20 (MeOH) to yield **4** (15.3 mg) in pure form. 1.06 g of fraction D was subjected to silica gel (100 g) column chromatography, employing $CHCl_3$-MeOH-H_2O mixtures (80:20:2, 70:30:3 and 60:40:4, each 500 ml) to afford fractions D_1-D_4. Si gel (30 g) chromatography of fraction D_1 (243 mg) eluting with $CHCl_3$-MeOH-H_2O mixtures (90:10:0.5, 90:10:1, 80:20:1 and 80:20:2, each 150 ml) gave impure **1** and **3**. Compounds **1** and **3** were further purified by CC on Sephadex (MeOH) with the following yields: **1** (13.7 mg) and **3** (5.3 mg). Fr. D_2 (127 mg) was subjected to RP-18 MPLC using *i*PrOH-H_2O gradients (5% to 20% *i*-PrOH, each 100 ml) to yield **2** (15 mg).

2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside (1): Amorphous powder, $C_{23}H_{26}O_{10}$; UV (MeOH) λ_{max} 324, 285sh, 249, 222 nm; IR (KBr) υ_{max} 3400 (OH), 1690 (α,β-unsaturated ester), 1635 (olefinic C=C), 1600, 1515 (arom. ring) cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz): phenylethyl moiety: δ 7.02 (2H, *d*,J = 8.4 Hz, H-2, H-6), 6.64 (2H, *d*, J = 8.4 Hz, H-3, H-5), 3.94 (1H, *m*, H-α_b), 3.71 (1H, *m*, H-α_a), 2.82 (2H, *t*, J = 7.9 Hz, H-β); glucose moiety: δ 4.32 (1H, *d*, J = 7.3 Hz, H-1′), 3.20 (1H, *dd*, J = 7.3/8.4 Hz, H-2′), 3.36 (2H, overlapping H-3′, H-4′), 3.51 (1H, *m*, H-5′), 4.49 (1H, *dd*, J = 12.0/2.0 Hz, H-6′$_b$), 4.34 (1H, *dd*, J = 12.0/6.4 Hz, H-6′$_a$); caffeoyl moiety: δ 7.03 (1H, *d*, J = 2.0 Hz, H-2″), 6.76 (1H, *d*, J = 8.4 Hz, H-5″), 6.88 (1H, *dd*, J = 8.4/2.0 Hz, H-6″), 6.28 (1H, *d*, J = 15.9 Hz, H-α'), 7.56 (1H, *d*, J = 15.9 Hz, H-β'); ^{13}C NMR (CD_3OD, 125 MHz): Table; positive ion ESIMS: m/z 485 [M+Na]$^+$, 947 [2M+Na]$^+$; negative ion ESIMS: m/z 461 [M-H]$^-$.

Calceolarioside B (2): Amorphous powder, $C_{23}H_{26}O_{11}$; UV (MeOH) λ_{max} 329, 290, 249sh, 216 nm; IR (KBr) υ_{max} 3350 (OH), 2940, 1690 (α,β-unsaturated ester), 1650 (olefinic C=C), 1600, 1515 (arom.

ring) cm^{-1}; ^1H NMR (CD_3OD, 500 MHz): phenylethyl moiety: δ 6.65 (1H, *br s*, H-2), 6.61 (1H, *d*, J = 8.0 Hz, H-5), 6.52 (1H, *br d*, J = 8.0 Hz, H-6), 3.93 (1H, *m*, H-α_b), 3.69 (1H, *m*, H-α_a), 2.76 (2H, *t*, J = 7.5 Hz, H-β); glucose moiety: δ 4.30 (1H, *d*, J = 7.3 Hz, H-1′), 3.19 (1H, *dd*, J = 7.3/8.5 Hz, H-2′), 3.35 (2H, overlapping, H-3′, H-4′), 3.50 (1H, *m*, H-5′), 4.47 (1H, *br d*, J = 12.0 Hz, H-6′$_b$), 4.32 (1H, *dd*, J = 12.0/6.5 Hz, H-6′$_a$); caffeoyl moiety: δ 7.01 (1H, *br s*, H-2″), 6.76 (1H, *d*, J = 8.2 Hz, H-5″), 6.86 (1H, *br d*, J = 8.2 Hz, H-6″), 6.26 (1H, *d*, J = 15.9 Hz, H-α'), 7.54 (1H, *d*, J = 15.9 Hz, H-β'); ^{13}C NMR (CD_3OD, 125 MHz): Table; positive ion ESIMS: m/z 501 $[M+Na]^+$, 979 $[2M+Na]^+$; negative ion ESIMS: m/z 477 $[M-H]^-$.

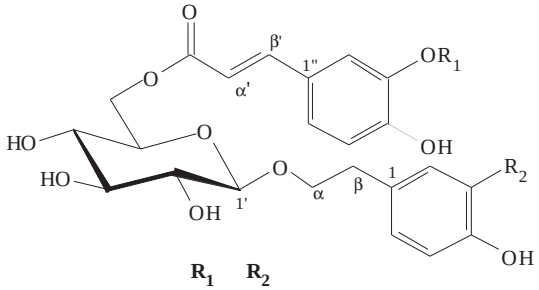
Osmanthuside E (3): Amorphous powder, $C_{24}H_{28}O_{11}$; UV (MeOH) λ_{max} 320, 285, 226, 210 nm; IR (KBr) υ_{max} 3400 (OH), 1690 (α,β-unsaturated ester), 1630 (olefinic C=C), 1605, 1520 (arom. ring) cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): phenylethyl moiety: δ 6.66 (1H, *d*, J = 2.0 Hz, H-2), 6.61 (1H, *d*, J = 8.0 Hz, H-5), 6.52 (1H, *dd*, J = 8.0/2.0 Hz, H-6), 3.95 (1H, *m*, H-α_b), 3.73 (1H, *m*, H-α_a), 2.78 (2H, *t*, J = 7.6 Hz, H-β); glucose moiety: δ 4.31 (1H, *d*, J = 7.3 Hz, H-1′), 3.23 (1H, *dd*, J = 7.3/8.5 Hz, H-2′), 3.36 (2H, overlapped, H-3′, H-4′), 3.52 (1H, *m*, H-5′), 4.51 (1H, *dd*, J = 12.0/2.4 Hz, H-6′$_b$), 4.32 (1H, *dd*, J = 12.0/6.4 Hz, H-6′$_a$); feruloyl moiety: δ 7.14 (1H, *d*, J = 2.0 Hz, H-2″), 6.80 (1H, *d*, J = 8.4 Hz, H-5″), 7.01 (1H, *dd*, J = 8.4/2.0 Hz, H-6″), 6.37 (1H, *d*, J = 15.9 Hz, H-α'), 7.61 (1H, *d*, J = 15.9 Hz, H-β'), 3.84 (3H, *s*, OMe); ^{13}C NMR (CD_3OD, 100 MHz): Table; negative ion ESIMS: m/z 491 $[M-H]^-$.

Martynoside (4): Amorphous powder, $C_{31}H_{40}O_{15}$; UV (MeOH) λ_{max} 330, 287, 220 nm; IR (KBr) υ_{max} 3400 (OH), 1700 (α,β-unsaturated ester), 1625 (olefinic C=C), 1605, 1515 (arom. ring) cm^{-1}; ^1H NMR (CD_3OD, 500 MHz), phenylethyl moiety: δ 6.73 (1H, *d*, J = 2.0 Hz, H-2), 6.78 (1H, *d*, J = 8.0 Hz, H-5), 6.69 (1H, *dd*, J = 8.0/2.0 Hz, H-6), 3.95 (1H, *m*, H-α_b), 3.73 (1H, *m*, H-α_a, overlapped with OMe signal), 2.78 (2H, *t*, J = 7.3 Hz, H-β), 3.75 (3H, *s*, OMe); glucose moiety: δ 4.33 (1H, *d*, J = 7.3 Hz, H-1′), 3.32 (1H, *dd*, J = 7.3/9.0 Hz, H-2′), 3.84 (1H, *t*, J = 9.0 Hz, H-3′), 4.86 (1H, *t*, J = 9.4 Hz, H-4′), 3.56 (1H, *m*, H-5′), 3.96 (1H, *br d*, J = 12.0 Hz, H-6′$_b$), 3.55 (signal pattern unclear due to overlapping, H-6′$_a$); rhamnose moiety: δ 5.15 (1H, *d*, J = 1.8 Hz, H-1″), 3.85 (1H, *dd*, 1.8/3.2 H-2″), 3.80 (1H, overlapped with OMe signal, H-3″), 3.29 (1H, *t*, J = 7.3 Hz, H-4″), 3.53 (signal pattern unclear due to overlapping, H-5″), 1.05 (1H, *d*, J = 6.2 Hz, H-6″); feruloyl moiety: δ 7.18 (1H, *br s*, H-2‴), 6.76 (1H, *d*, J = 8.4 Hz, H-5‴), 7.03 (1H, *dd*, J = 8.4/2.0 Hz, H-6‴), 6.35 (1H, *d*, J = 15.9 Hz, H-α'), 7.61 (1H, *d*, J = 15.9 Hz, H-β'), 3.83 (3H, *s*, OMe); ^{13}C NMR (CD_3OD, 125 MHz), Table.

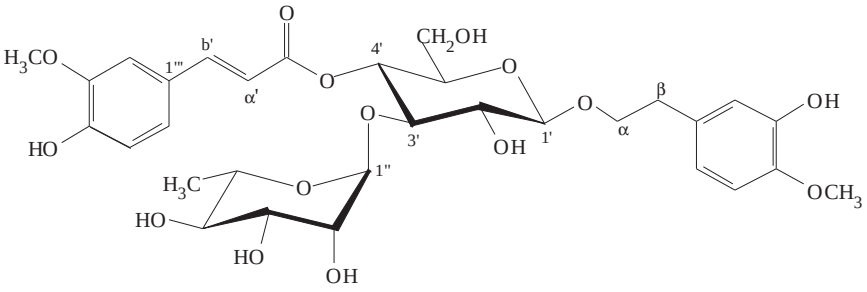
Reduction of DPPH Radical. Methanolic solutions (0.1%) of compounds **1-4** were chromatographed on a Si gel TLC plate using $CHCl_3$-MeOH-H_2O (61:32:7). After drying, TLC plates were sprayed with 0.2% DPPH (Fluka) solution in MeOH. Compounds showing a yellow-on-purple spot were regarded as antioxidant.

Results and Discussion

Phenylethanoid glycosides **1-4** (Figure) were isolated from the methanolic extract of the aerial parts of *S. galericulata* by a combination of MPLC and open column chromatographic methods. All compounds were obtained as amorphous colorless powders, whose UV spectra indicated their polyphenolic nature. Their IR spectra showed absorption bands typical for hydroxyl groups, α,β-unsaturated esters, olefinic double bonds and aromatic rings.



	R_1	R_2
1	H	H
2	H	OH
3	CH_3	OH



4

Figure Phenylethanoid glycosides isolated from *Scutellaria galericulata*

The positive ion ESIMS of compound **1** showed pseudomolecular ion peaks $[M+Na]^+$at m/z 485 and $[2M+Na]^+$at m/z 947, while the negative ion ESIMS exhibited the ion $[M-H]^-$at m/z 461, compatible with the molecular formula $C_{23}H_{26}O_{10}$, and in good agreement with the observation of fourteen methine, three methylene and six quaternary carbon resonances in its ^{13}C NMR spectrum (Table). The ^1H NMR spectrum of **1** exhibited characteristic signals arising from a (*E*)-caffeic acid and *p*-substituted phenylethyl alcohol moieties: protons of aromatic rings (an ABX system and an AA′BB′ system at δ_H 6.60-7.05 region), two *trans*-olefinic protons (AB system, δ_H 7.56 and 6.28, both *d*, J_{AB} = 15.9 Hz), benzylic β-methylene protons (δ_H 2.82, 2H, *m*) and two non-equivalent protons (δ_H 3.94 and 3.71, each 1H, *m*). The complete assignments of all proton and carbon resonances were based on the gCOSY, gHSQC and gHMBC experiments. A doublet proton resonance at δ_H 4.32 (*d*, J = 7.3 Hz) was readily assigned to the anomeric proton of a β-D-glucopyranose unit, indicating the monoglycosidic structure in **1**. The corresponding carbon resonance at δ_C 104.57 (*d*, C-1′) confirmed this assumption. The caffeoyl moiety was assumed to be positioned at the C-6′ atom of the glucose, on the basis of the strong deshielding of the H_2-6′ signals of the glucose unit (δ_H 4.49 *dd*, J = 12.0/2.0 Hz and 4.34 *dd*, J = 12.0/6.4 Hz). A gHMBC experiment revealed the determination of the relevant interfragmental connectivities. A gHMBC correlation observed between H_2-6′ protons of the glucose unit and the carbonyl carbon resonance (δ_C 169.13) of the acyl moiety showed the attachment of the acyl group at the C-6′ position of glucose. Additional gHMBC cross-peaks, observed from anomeric proton of glucose (δ_H 4.31, H-1′) to C-α (δ_C 72.42) and from H-β (δ_H 2.80) to C-1 (δ_C 130.64) of the phenylethyl alcohol aglycon confirmed the proposed structure of **1**. On the basis of its NMR data, the structure of **1** was established as 2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside. A literature survey indicated that this compound was previously isolated from *Prunus grayana* (Rosaceae)[10].

Table. ^{13}C NMR (CD_3OD, 125 MHz*) data for **1-4**

	1	**2**	**3**	**4**
C Atom	δ_c	δ_c	δ_c	δ_c
Aglycon				
1	130.64	131.41	131.37	132.94
2	130.92	117.09	117.07	112.88
3	116.17	146.15	146.16	147.58
4	156.79	144.67	144.67	147.43
5	116.17	116.56	116.49	117.09
6	130.92	121.28	121.25	121.26
α	72.42	72.40	72.49	72.12
β	36.51	36.71	36.77	36.57
OMe	-	-	-	56.50
Glucose				
$1'$	104.57	104.56	104.60	104.25
$2'$	75.08	75.10	75.08	76.23
$3'$	77.98	77.95	77.96	81.51
$4'$	71.81	71.76	71.81	70.65
$5'$	75.46	75.48	75.48	76.08
$6'$	64.65	64.65	64.71	62.41
Rhamnose				
$1''$				103.00
$2''$				72.37
$3''$				72.09
$4''$				73.81
$5''$				70.42
$6''$				18.43
Acyl moiety				
$1''$ ($'''$)	127.68	127.71	127.66	127.68
$2''$ ($'''$)	115.06	115.07	111.58	111.80
$3''$ ($'''$)	149.70	149.66	149.39	149.42
$4''$ ($'''$)	146.85	146.81	150.68	150.88
$5''$ ($'''$)	116.53	116.38	116.32	116.52
$6''$ ($'''$)	123.12	123.17	124.28	124.38
α'	114.88	114.88	115.24	115.14
β'	147.22	147.26	147.12	147.90
C=O	169.13	169.18	168.27	168.27
OMe	-	-	56.44	56.45

*100 MHz for **3**

Compound **2** was isolated as an amorphous powder, with the molecular formula of $C_{23}H_{26}O_{11}$, as determined by ESIMS, ^{1}H NMR and ^{13}C NMR (Table) data. The positive ion ESIMS of **2** exhibited the pseudomolecular ions $[M+Na]^+$ at m/z 501 and $[2M+Na]^+$ at m/z 979 and the negative ion ESIMS showed an ion $[M-H]^-$at m/z 477. These data suggested that compound **2** was 16 mass units higher than that of **1**, indicating the presence of an additional oxygen function in **2**. The proton and carbon chemical shifts due to the acyl and sugar moieties were in good agreement with those of **1**, suggesting the similar substructures and glucosidation pattern. However, in the ^{1}H NMR spectrum of compound **2**, the proton resonances at δ_H 6.50-6.65 region, observed as an ABX system, indicated the presence of a 3,4-dihydroxyphenylethanol moiety in **2** instead of a 4-hydroxyphenethyl alcohol in **1**. All structural assignments, substantiated by the

results obtained from the 2D shift-correlated gCOSY, gHSQC and gHMBC spectra, identified **2** as 2-(3,4-dihydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside. This compound was previously isolated from *Prunus grayana* (Rosaceae)[10], and named calceolarioside B, which was further identified from *Calceolaria hypericina* (Scrophulariaceae)[11], *Scutellaria prostrata* (Lamiaceae)[12], *Chirita sinensis* (Gesneriaceae)[13] and *Digitalis purpurea* (Scrophulariaceae)[14].

The negative ion ESIMS of compound **3** exhibited a pseudomolecular ion $[M\text{-}H]^-$ at m/z 491, suggesting the molecular formula to be $C_{24}H_{28}O_{11}$. The ^{1}H NMR spectrum of **3** was very similar to that of **2**, except for the aromatic region. The major difference between these two compounds was the existence of an aromatic methoxyl resonance (δ_H 3.84, 3H, *s*) in the ^{1}H NMR spectrum of **3**. The assignments of the chemical shifts for the methoxyl group and the related aromatic protons (δ_H 7.14, 6.80 and 7.01, ABX system; 6.37 and 7.61, AB system) were established by comparison with those of alyssonoside[15], a phenylethanoid glycoside with ferulic acid as the acyl moiety. Therefore, the acyl group in **3** was assigned as (*E*)-ferulic acid. On the basis of the above mentioned evidence, **3** was determined as 2-(3,4-dihydroxyphenyl)ethyl-(6-*O*-feruloyl)-β-D-glucopyranoside. Compound **3** was previously isolated from *Prunus grayana* (Rosaceae)[16], and *Osmanthus asiaticus* (Oleaceae)[17].

The ^{1}H NMR spectrum of **4** exhibited characteristic signals belonging to a (*E*)-ferulic acid and 3-hydroxy,4-methoxyphenylethanol moieties. Additionally, two anomeric proton signals were observed at δ_H 4.28 (*d*, J = 7.3 Hz) and 5.19 (*d*, J = 1.8 Hz), which were consistent with the β-glucopyranose and α-rhamnopyranose units, respectively. The corresponding anomeric carbon resonances at δ_C 104.25 and 103.00, respectively, also confirmed the disaccharidic structure. The ^{13}C NMR spectrum of **4** showed 31 carbon resonances, 12 of which could be assigned to the sugar moieties. The feruloyl group was determined to be positioned at C-4′ of the glucose, on the basis of the strong deshielding of H-4′ signal (δ_H 4.86, *t*, J = 9.4 Hz) due to acylation. On the other hand, HMBC correlation from H-1″ (δ_H 5.19) of rhamnose to C-3′ (δ_C 81.51) of glucose indicated the interglycosidic linkage to be C-3′$_{Glc}$ →C-1″$_{Rha}$. Therefore, the structure of **4** was identified as 2-(3-hydroxy-4-methoxyphenyl)ethyl-*O* − α-L-rhamnopyranosyl-(1→3)-4-*O*-feruloyl-β-D-glucopyranoside (= martynoside)[18,19]. Martynoside (**4**) was previously isolated in the genus *Scutellaria* from *Scutellaria albida* subsp. *colchica*[4], *S. orientalis* subsp. *pinnatifida*[5] *S. salviifolia*[6], *S. prostrata*[12] and *S. planipes*[20].

Conclusion

Of the phenylethanoid glycosides described above, 2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside (**1**), calceolarioside B (**2**) and osmanthuside E (**3**) are all phenylethanoid monosaccharides. Although several papers have been published on the isolation and characterization of the phenylethanoid glycosides from *Scutellaria* species[4–6,12,20], calceolarioside B (**2**), isolated from *S. prostrata*[12], is the only monosaccharidic phenylethanoid glycoside that has been reported from the genus *Scutellaria* so far. Therefore, this is the second instance of the characterization of phenylethanoid monosaccharides from the genus *Scutellaria*. Furthermore, this is the first report of the isolation of **1** and **3** from *Scutellaria* species.

Compounds **1-4** were found to have significant antioxidant properties, based on the experiments with 1,1-diphenyl-1-picrylhydrazyl (DPPH), which indicated their ability to efficiently scavenge free radicals[21,22].

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