

Polyphenolic compounds from *Geranium purpureum* Vill. growing in Turkey

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Two new flavonol derivatives, Geranioside A (kaempferol 3-[3-(α -rhamnopyranosyl)-4-oxy-5-hydroxy-6-methyl-3,4-dihydropyran-2H-2-pyranoside] (1) and Geranioside B (quercetin 3-[3-(α -rhamnopyranosyl)-4-oxy-5-hydroxy-6-methyl-3,4-dihydropyran-2H-2-pyranoside] (2), along with 5 known flavonols (quercetin (3), kaempferol (4), quercetin 4'-O- β -glucopyranoside (5), quercetin 3-O- β -glucopyranoside (6), and kaempferol-3-O- β -glucopyranoside (7)), a gallic acid derivative (methyl gallate (8)), and a hydrolysable tannin (pusilagin (9)), were isolated from the aerial parts of *Geranium purpureum*. The structures of the isolated compounds were elucidated on the basis of UV, IR, and 1D- and 2D-NMR experiments as well as TOF-MS.

Key Words: *Geranium purpureum*, Geraniaceae, flavonoids, Geraniosides A and B

Introduction

The genus *Geranium* L., with its around 400 species, is distributed throughout the temperate and tropical regions of the world.¹ Some species of the genus *Geranium* (cranesbill) are utilized as an antidiabetic, hemostatic, antihemorrhoidal, and antidiarrheal, and as a remedy for tonsillitis, cough, whooping cough, urticaria, dysentery, pain, fevers, and gastrointestinal ailments in some folkloric medicines.²⁻⁴ The aerial parts of *Geranium purpureum* Vill. are consumed as food in Turkey.⁵ In a previous work performed on *G. purpureum*, the

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antioxidant and antimicrobial activities of the methanolic extract and the presence of several phenolic acids and flavonoids were reported.⁶ As part of our continuing phytochemical investigations on *Geranium* species growing wild in Turkey,⁷⁻⁹ we herein report on the isolation and the structure elucidation of 2 new flavonoids (**1** and **2**) in addition to 7 known metabolites (**3-9**) from the aerial parts of the title plant.

Experimental

General experimental procedures

TLC: precoated silica gel 60 F₂₅₄ (Merck) aluminum plates, elution with CHCl₃/MeOH/H₂O mixtures; visualization by spraying 10% H₂SO₄, followed by heating at 105 °C for 1-2 min. Preparative TLC (p-TLC) was carried out on Kieselgel GF₂₅₄ glass plates (Analtech- Uniplate 81300) Column chromatography (CC): silica gel 60 (0.063-0.200 mm; Merck, Darmstadt) and Sephadex LH-20 (Sigma). Optical rotations: Rudolph Autopol-IV Automatic polarimeter. UV Spectra: Bio-Tek Instruments, M-Quant Biomolecular spectrophotometer; λ_{max} in nm. NMR Spectra: Bruker DRX 400 spectrometer; at 400 MHz (¹H) and 100 MHz (¹³C); δ in ppm rel to Me₄Si, J in Hz. IR Spectra: PerkinElmer, FT-IR System Spectrum BX.

Plant material: *Geranium purpureum* Vill was collected from Amasya, around Borabay Lake, in north Anatolia, Turkey, in June 2004. A voucher specimen has been deposited at the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 04161).

Extraction and isolation: The air-dried and powdered aerial parts of *G. purpureum* (960 g) were extracted with MeOH (4 × 3 L, 5 h) at 35 °C, and then filtered. The combined MeOH extracts were evaporated to dryness under reduced pressure. The crude extract was dissolved in H₂O (150 mL), and extracted with petroleum ether (40-60 °C) (PE) (4 × 150 mL), AcOEt (6 × 150 mL), and *n*-BuOH (4 × 150 mL), successively. The AcOEt soluble fraction (24 g) was chromatographed over Sephadex LH-20 (3.2 × 55 cm), eluting with H₂O, followed by increasing concentrations of MeOH in H₂O (0% → 100% MeOH) to yield 11 main fractions (Frs. A-K) according to the TLC chromatogram. Fr. B (600 mg eluted with 3% MeOH) was subjected to CC (Sephadex LH-20, MeOH) to give 3 subfractions (Fr. B₁₋₃). Fr. B₂ (30) was then applied to p-TLC using AcOEt/MeOH/H₂O (100:10:2) solvent system to give compound **8** (5.5 mg). Fr. C (740 mg, 5% → 10%) was rechromatographed over Sephadex LH-20 CC with MeOH to give Frs. C₁-C₅ and compound **9** (150 mg) in pure form. Fr. C₂ (40 mg) was further separated by p-TLC with CHCl₃/MeOH (80:20) solvent system to give compounds **6** (6.2 mg) and **7** (4 mg). Fr. C₄ was applied to silica gel CC and compound **5** (5 mg) obtained by using a CHCl₃/MeOH (88:12) mixture. Fr. G (900 mg, eluted with 45% MeOH) was subjected to CC (Sephadex LH-20, MeOH) to afford 3 fractions (Frs. G₁-G₃). Fr. G₂ (104 mg) was further chromatographed by CC (SiO₂, CHCl₃/MeOH 98:2, 85:15, 50:50) and 3 fractions were obtained (Frs. G_{2A}-G_{2C}). Fr. G_{2B} (40 mg) was applied to p-TLC using CHCl₃/MeOH (80:20) to give **1** (12.0 mg) and **2** (5.5 mg). Fr. J (100 mg, eluted with MeOH) was subjected to CC (SiO₂, CHCl₃/MeOH 97:3, 95:5) and yielded compounds **3** (5.4 mg) and **4** (3.2 mg).

Geranoside A; **1**). Amorphous, yellow powder. $[\alpha]_D^{20} = -39.9$ ($c = 0.1$, MeOH). UV (MeOH): 267, 356. ¹H- and ¹³C-NMR: see Table. ESI-TOF-MS: 597.1244 ($[M+Na]^+$, C₂₇H₂₆O₁₄Na; calc. 597.1220), IR: 3427, 2924, 2854, 1702, 1658, 1604, 1384.

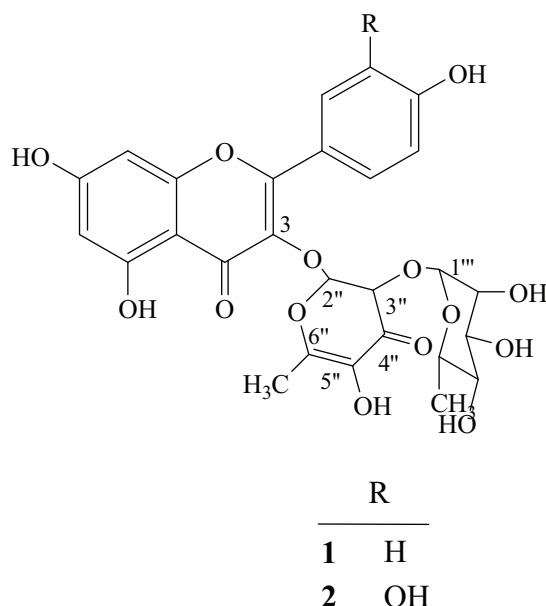


Figure. Compounds **1** and **2**.

Geranoside B; **2**) Amorphous, yellow powder. $[\alpha]_D^{20} = -67.0$ ($c = 0.1$, MeOH). UV (MeOH): 258, 359. ^1H - and ^{13}C -NMR: see Table. ESI-TOF-MS: 613.1190 ($[M+H]^+$, $\text{C}_{27}\text{H}_{26}\text{O}_{15}\text{Na}$; calc. 613.1169), IR: 3208, 2937, 2864, 1698, 1655, 1603, 1362, 1292.

Results and discussion

The air-dried aerial parts of *G. purpureum* were extracted with MeOH. The EtOAc soluble part of the crude MeOH extract was separated by a combination of various chromatographic methods to obtain 2 new flavonol glycosides (**1-2**) (see Figure) in addition to 7 known metabolites. The known compounds were identified as quercetin (**3**),¹⁰ kaempferol (**4**),¹⁰ quercetin 4'-*O*- β -glucopyranoside (**5**),¹⁰ quercetin 3-*O*- β -glucopyranoside (**6**),¹⁰ kaempferol 3-*O*- β -glucopyranoside (**7**),¹⁰ methyl gallate (**8**),⁷ and pusilagin (**9**)¹¹ by comparison of their spectroscopic data (UV, 1D- and 2D-NMR, and MS) with previously published data.

Compound **1** was isolated as a yellow amorphous powder. The UV absorption maxima (267 and 356 nm) were indicative of a flavonol skeleton. The molecular formula was determined as $\text{C}_{27}\text{H}_{26}\text{O}_{14}$ by the $[M+\text{Na}]^+$ ion peak at m/z 597.1244 in the ESI-TOF-MS and indicated 15 degrees of unsaturation.

The ^1H -NMR spectrum of **1** (see Table) showed the typical signals arising from the kaempferol moiety, an AA'BB' system for the ring B at $\delta(\text{H})$ 7.81 and 6.91 H-(2'/6') and H-(3'/5'), respectively, (both d , $J = 8.8$ Hz) and a *meta*-coupled signal for the ring A at δ_H 6.37 H-8 and 6.19 H-6 (both d , $J = 2.0$).¹⁰ The ^1H -NMR spectrum also displayed characteristic resonances for an α -rhamnopyranosyl unit, at δ_H 5.07 (d , $J = 1.6$ Hz, H-1''') and 1.38 (d , $J = 6.0$ Hz, H-6''').¹² Moreover, 2 ortho-coupled protons at δ_H 5.89 and 4.45 (both d , $J = 2.0$ Hz) and a methyl group at δ_H 1.43 (s) were observed in the ^1H -NMR spectrum of **1**. The ^{13}C -NMR spectrum (Table) displayed 27 signals, 15 of which could be attributed to the kaempferol unit, while 6 of which were ascribed to the rhamnopyranosyl moiety. The remaining 6 resonances were attributed

Table. ¹H- and ¹³C-NMR data^a of **1** and **2**. In CH₃OH-*d*₄; in ppm, *J* in Hz. Arbitrary atom numbering.

C/H	$(\delta_H)^b$ ppm, <i>J</i> Hz		$(\delta_C)^c$ ppm		HMBC (H→C)	
	1	2	1	2		
2	-	-	159.1	159.7		
3	-	-	135.5	136.1		
4	-	-	178.7	179.0		
5	-	-	163.2	163.8		
6	6.19 d 2.0	6.15 d 2.0	100.0	102.3	5, 7, 8, 10	5, 10
7	-	-	166.1	166.1		
8	6.37 d 2.0	6.32 d 2.0	95.0	96.8	6, 7, 8, 10	10
9	-	-	158.5	159.4		
10	-	-	105.7	105.3		
1'	-	-	122.0	123.2		
2'	7.81 d 8.8	7.39 d 2.0	132.3	117.3	2, 4'	2, 4', 6'
3'	6.91 d 8.8	-	116.2	147.4	1', 4'	
4'	-	-	161.7	151.2		
5'	6.91 d 8.8	6.87 d 8.4	116.2	117.1	1', 4'	3', 6'
6'	7.81 d 8.8	7.35 dd 2.0/8.4	132.6	124.2	2, 4'	2, 4', 5'
2''	5.89 d 2.0	5.85 d 2.0	102.2	103.2	3, 3'', 4'', 6''	3, 4'', 6''
3''	4.45 d 2.0	4.47 d 2.0	74.3	75.2	2'', 4'', 5''	4'', 6'', 1'''
4''	-	-	184.6	185.5		
5''	-	-	133.6	134.5		
6''	-	-	156.1	157.3		
7''	1.43 s	1.50 s	14.8	15.8	5'', 6''	5'', 6''
Rhamnose						
1'''	5.07 d 1.6	5.07 d 1.6	101.4	102.2	3'', 2''', 3'''	2'''
2'''	3.81 d 1.6/3.6	3.80 dd 1.6/3.2	71.9	72.9	3''', 4'''	
3'''	3.62 dd 3.6/9.6	3.62 dd 3.2/9.6	72.0	72.8	2''', 4'''	
4'''	3.44 t 9.6	3.43 t 9.6	73.7	74.6	3''', 5'''	
5'''	3.73 dd 6.0/9.6	3.72 dd 5.8/9.6	70.9	71.7	3''', 4''', 6'''	
6'''	1.38 d 6.0	1.36 d 5.8	18.0	18.8	4''', 5'''	4''', 5'''

^a All δ (H) and δ (C) assignments are based on 2D NMR (DQF-COSY, HMQC, HMBC). ^b Recorded at 400 MHz.^c Recorded at 100 MHz.

to a carbonyl (δ_C 184.6, C=O), an oxymethine (δ_C 74.3), and a hemiacetal carbon (δ_C 102.2), as well as 2 olefinic carbons (δ_C 156.1 and 133.6) and a methyl (δ_C 14.8) carbon. These carbon signals together with the corresponding proton resonances secured by COSY, HSQC, and HMBC experiments revealed the presence of a 6-membered ring with 3 degrees of unsaturation, 5-hydroxy-6-methyl-2,3-dihydropyran-4-one. Interpretation

of the HMBC spectrum permitted the determination of all the interfragmental connectivities of these units. Thus, cross-peaks were observed between H-2'' (δ_H 5.89) of 5-hydroxy-6-methyl-2,3-dihydropyran-4-one unit and C-3 (δ_C 135.5) of the kaempferol moiety, and between H-1''' (δ_H 5.07) of rhamnose and C-3''' (δ_C 74.3) of 5-hydroxy-6-methyl-2,3-dihydropyran-4-one unit. Correlations between H-2''/H-3'' and H-3''/H-1''' were observed in the NOESY spectrum. NOE correlations between H-2''/H-3'' indicated a *cis* relationship of these protons. Thus, compound **1** was established as kaempferol 3-[3-(α -rhamnopyranosyl)-4-oxy-5-hydroxy-6-methyl-3,4-dihydropyran-2*H*-2-pyranoside], and named geranoside A.

Compound **2** was isolated as a yellow amorphous powder. The UV spectrum of **2** in MeOH showed maxima at 258 and 359 nm, suggesting a flavonol derivative. ESI-TOF-MS showed the $[M+Na]^+$ peak at m/z 613.1190, corresponding to the molecular formula $C_{27}H_{26}O_{15}$. The $[M+Na]^+$ ion of **2** was 16 mass units bigger than that of **1**, which indicated an additional hydroxyl group in **2**.

The 1H - and ^{13}C -NMR spectra of compound **2** were similar to those of **1**, except for the signals derived from the B ring of the flavonol aglycone. The 1H -NMR spectrum of **2** showed a double doublet at δ_H 7.35 (*dd*, $J=2.0, 8.4$) and 2 doublets at δ_H 7.39 (*d*, $J=2.0$) and 6.87 (*d*, $J=8.4$), which were observed as an ABX system revealing the presence of a 3',4'-dihydroxy functional structure of a flavonoid B ring. These findings suggested the presence of a quercetin skeleton instead of a kaempferol skeleton in **2**.¹⁰ The anomeric proton appeared at δ_H 5.07 (*d*, $J=1.6$) and the anomeric C-atom resonance shown at δ_C 102.2 together with the proton and carbon resonances at δ_H 1.36 and δ_C 18.8 revealed an α -rhamnopyranose unit. Furthermore, 2 *ortho*-coupled protons at δ_H 5.86 and 4.47 (each *d*, $J=2.0$ Hz) and a methyl group (δ_H 1.50, *s*) in the 1H -NMR spectra of **2** indicated the presence of 5-hydroxy-6-methyl-2,3-dihydropyran-4-one ring in **2** as in **1**. The ^{13}C -NMR spectrum of **2** displayed 27 resonances, 15 of which could be ascribed to the aglycone, and 6 of which were attributed to a rhamnose unit. All the remaining ^{13}C signals (185.5, 157.3, 134.5, 103.2, and 15.8) established by HMQC and HMBC experiments were assignable to a hydroxy-6-methyl-2,3-dihydropyran-4-one ring. The complete assignments of **2** were made by 2D-NMR experiments (DQF-COSY, HMQC, and HMBC). HMBC correlations observed between H-2'' of 5-hydroxy-6-methyl-2,3-dihydropyran-4-one and C-3 of quercetin, and between H-1''' of rhamnose and C-3'' of 5-hydroxy-6-methyl-2,3-dihydropyran-4-one confirmed that the attachment of sugars was the same as in **1**. Thus, the structure of **2** was identified as quercetin 3-[3-(α -rhamnopyranosyl)-4-oxy-5-hydroxy-6-methyl-3,4-dihydropyran-2*H*-2-pyranoside], and named Geranoside B.

This is the first time that a 5-hydroxy-6-methyl-2,3-dihydropyran-4-one ring has been reported from nature. This ring was supposed to be an oxide derivative of a rhamnose unit. Taking into account its structure's similarity to a rhamnopyranose moiety, this could be derived from a rhamnopyranose unit. Reports on the presence of oxidative sugars in nature are rare. For example, a monoterpene glycoside (Anatolioside D) from *Viburnum orientale* contains an oxidative derivative of glucose in its structure.¹³

Flavonoids are widely distributed and recognized as taxonomic markers in the genus *Geranium*, and some kaempferol and quercetin glycosides were found in *Geranium* species. It has been reported that both kaempferol and quercetin 3-glycosides are the most common flavonoid glycosides of *Geranium* species.¹⁴ However, a much rarer compound, quercetin 4'-*O*- β -glucopyranoside, which was previously isolated from *G. macrorrhizum*,¹⁵ is reported for the second time from the genus *Geranium* in the present study.

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