Turkish Journal of Chemistry

Volume 35 | Number 5 Article 12

1-1-2011

Polyphenolic compounds from Geranium purpureum Vill. growing in Turkey

DİDEM ŞÖHRETOĞLU

MAHMUT KORAY SAKAR

OLOV STERNER

Turk J Chem
35 (2011) , 809 – 814.

doi:10.3906/kim-1103-52

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

Didem ŞÖHRETOĞLU[1,*], Mahmut Koray SAKAR[1], Olov STERNER[2]

[1] *Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Ankara, TURKEY*

e-mail: didems@hacettepe.edu.tr

[2] *Division of Organic Chemistry, Department of Chemistry, Lund University, P.O. Box 124, 22100 Lund-SWEDEN*

e-mails:didems@hacettepe.edu.tr, didemsohretoglu@yahoo.com

Received: 30.03.2011

Two new flavonol derivatives, Geranioside A (kaempferol 3-[3-(α-rhamnopyranosyl)-4-oxy-5-hydroxy-6-methyl-3,4-dihyropyran-2*H*-2-pyranoside]) (**1**) and Geranioside B (quercetin 3-[3-(α-rhamnopyranosyl)-4-oxy-5-hydroxy-6-methyl-3,4-dihyropyran-2*H*-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 kaemferol-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

*Corresponding author

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

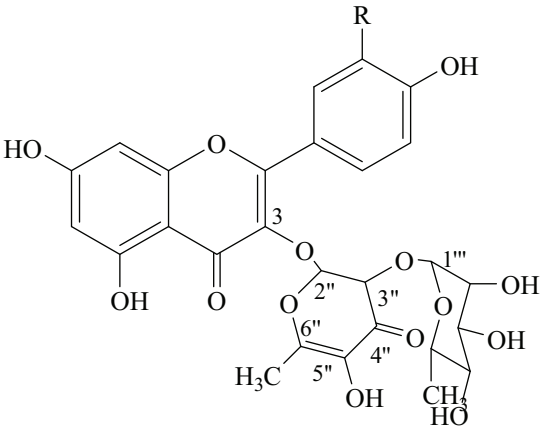
General experimental procedures

TLC: precoated silica gel 60 F_{254} (Merck) aluminum plates, elution with $CHCl_3$/MeOH/H_2O mixtures; visualization by spraying 10% H_2SO_4, followed by heating at 105 °C for 1-2 min. Preparative TLC (p-TLC) was carried out on Kieselgel GF_{254} 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 (^{1}H) and 100 MHz (^{13}C); δ in ppm rel to Me_4Si, 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 H_2O (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 H_2O, followed by increasing concentrations of MeOH in H_2O (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. B_{1-3}). Fr. B_2 (30) was then applied to p-TLC using AcOEt/MeOH/H_2O (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. C_1-C_5 and compound **9** (150 mg) in pure form. Fr. C_2 (40 mg) was further separated by p-TLC with $CHCl_3$/MeOH (80:20) solvent system to give compounds **6** (6.2 mg) and **7** (4 mg). Fr. C_4 was applied to silica gel CC and compound **5** (5 mg) obtained by using a $CHCl_3$/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. G_1-G_3). Fr. G_2 (104 mg) was further chromatographed by CC (SiO_2, $CHCl_3$/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 $CHCl_3$/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 (SiO_2, $CHCl_3$/MeOH 97:3, 95:5) and yielded compounds **3** (5.4 mg) and **4** (3.2 mg).

Geranioside A; **1**). Amorphous, yellow powder. $[\alpha]_D^{20} = -39.9$ ($c = 0.1$, MeOH). UV (MeOH): 267, 356. ^{1}H- and ^{13}C-NMR: see Table. ESI-TOF-MS: 597.1244 ($[M+Na]^+$, $C_{27}H_{26}O_{14}Na$; calc. 597.1220), IR: 3427, 2924, 2854, 1702, 1658, 1604, 1384.



	R
1	H
2	OH

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

Geranioside B; **2**) Amorphous, yellow powder. $[\alpha]_D^{20} = -67.0$ (c = 0.1, MeOH). UV (MeOH): 258, 359. ^{1}H- and ^{13}C-NMR: see Table. ESI-TOF-MS: 613.1190 ($[M+H]^+$, $C_{27}H_{26}O_{15}Na$; 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] kaemferol (**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 $C_{27}H_{26}O_{14}$ by the $[M+Na]^+$ ion peak at m/z597.1244 in the ESI-TOF-MS and indicated 15 degrees of unsaturation.

The ^{1}H-NMR spectrum of **1** (see Table) showed the typical signals arising from the kaempferol moiety, an AA′BB′ system for the ring B at δ(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 ^{1}H-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 ^{1}H-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. ^{1}H- and ^{13}C-NMR data[a] of **1** and **2**. In CH_3OH-d_4; in ppm, J in Hz. Arbitrary atom numbering.

C/H	(δ_H)[b] ppm, J Hz		(δ_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-dihyropyran-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-dihyropyran-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-dihyropyran-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-dihyropyran-2*H*-2-pyranoside], and named geranioside 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 $[M+\mathrm{Na}]^+$ peak at m/z 613.1190, corresponding to the molecular formula $C_{27}H_{26}O_{15}$. The $[M+\mathrm{Na}]^+$ ion of **2** was 16 mass units bigger than that of **1**, which indicated an additional hydroxyl group in **2**.

The ^{1}H- 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 ^{1}H-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 ^{1}H-NMR spectra of **2** indicated the presence of 5-hydroxy-6-methyl-2,3-dihyropyran-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-dihyropyran-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-dihyropyran-4-one and C-3 of quercetin, and between H-1‴ of rhamnose and C-3″ of 5-hydroxy-6-methyl-2,3-dihyropyran-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-dihyropyran-2*H*-2-pyranoside], and named Geranioside B.

This is the first time that a 5-hydroxy-6-methyl-2,3-dihyropyran-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.

Acknowledgments

The authors thank Prof. Dr. Mecit Vural, Gazi University, Faculty of Science, Department of Botany, for the authentication of the plant specimen. The authors are also grateful to Dr. Keriman Özadalı and Prof. Dr. Erhan Palaska, Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, for recording the IR spectra. A part of this study was supported by 2214-Fellowship, BİDEB, TÜBİTAK.

References

1. Aedo, C.; Garmendia, F. M.; Pando, F. *Anales Jard. Bot. Madrid* **1998**, *56*, 211-252.
2. Baytop, T. *Therapy with Medicinal Plants in Turkey: Past and Present*, Nobel Tıp Kitabevi, İstanbul, 1999; Bremness, L. *Herbs*, Dorling Kindersley, London, 1995.
3. Maldonado, P. D.; Rivero-Cruz, I.; Mata, R.; Pedraza-Chaverri, J. *J. Agr. Food Chem.* **2005,** *53*, 1996-2005.
4. Calzada, F.; Cervantes-Martinez, J. A.; Yepez-Mulia, L. *J Ethnopharmacol* **2005**, *98*, 191-193.
5. Kızılarslan, Ç. Master Thesis, University of İstanbul at İstanbul, 2008.
6. Proestos, C.; Boziaris, I. S.; Nychas, G. J. E.; Komaitis, M. *Food Chem.* **2006**, *95*, 664-671.
7. Şöhretoğlu, D.; Atasayar Sabuncuoğlu, S.; Sakar, M. K.; Özgüneş, H.; Sterner, O. *Turk. J Chem.* **2009**, *33*, 685-692.
8. Şöhretoğlu, D.; Sakar, M. K.; Sterner, O. *Helv. Chim. Acta* **2009**, *92*, 520-524.
9. Şöhretoğlu, D.; Atasayar Sabuncuoğlu, S.; Sakar, M. K.; Özgüneş, H.; Sterner, O. *Nat. Prod. Commun.* **2010**, *5*, 899-902.
10. Harborne, J. B.; Mabry, T. J. *The Flavonoids: Advances in Research*, Chapman and Hall Ltd., London, 1982.
11. Kobakhidze, K. B.; Alaniya, M. D. *Chem. Nat. Compd.* **2001**, *39*, 262-264.
12. Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307-3330.
13. Çalış, İ.; Yürüker, A.; Rüeegger, H.; Wright, A. *Helv. Chim. Acta* **1993**, *76*, 416-424.
14. Ivancheva, S.; Petrova, A. *Biochem. Syst. Ecol.* **2000**, *28*, 255-260.
15. Miliauskas, G.; Van Beek, T. A.; Venskutonis, P. R.; Linssen, J. P. H.; Waar, P. *Eur. Food Res. Technol.* **2004**, *218*, 253-261.