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Phenylethanoid Glycosides from *Digitalis viridiflora*

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Abstract: From the MeOH extract of the leaves of *Digitalis viridiflora* five phenylethanoid glycosides (**1**-**5**) including a new one were isolated. The structure of the new compound was established as 3,4-dihydroxy-β-phenylethoxy-*O*-β-quinovopyranosyl-(1→6)-4-*O*-caffeoyl-β-glucopyranoside (viridifloroside, **1**) based on extensive 1D- and 2D-NMR spectroscopy as well as MS. Compound **1** is the first example of a phenylethnoid glycoside bearing a quinovopyranose in its structure. The chemotaxonomic importance of the isolates was also discussed.

Keywords: *Digitalis viridiflora*; Plantaginaceae; phenylethanoid glycosides; viridifloroside; chemotaxonomy.
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1. Introduction

The genus *Digitalis* (Plantaginaceae), comprises around 36 species, is mainly distributed from Southwestern Europe to Asia. In the flora of Turkey nine species of *Digitalis* grow wild including *D. viridiflora* [1]. The genus is well known for its cardioactive glycosides content some of which (digoxin and digitoxin) are therapeutically used for the treatment of congestive heart failure. Previous phytochemical investigations on the genus *Digitalis* have shown that it contains diverse group of secondary metabolites. In addition to cardiac glycosides [2, 3], phenylethanoid glycosides [4, 5], steroidal saponins [6], pregnane glycosides, cleroindicins and flavonoids [3] have been reported from the genus *Digitalis*. Previous works on *D. viridiflora* revealed the presence of cardiac glycosides, flavonoids and anthraquinones as secondary metabolites [7, 8]. However, there is no chemical study regarding the phenylethanoid composition of this species. As a continuation of phytochemical studies on *Digitalis* species from flora of Turkey, this work led to the isolation of five phenylethanoid glycosides including a new one (**1**) from *D. viridiflora*.

2. Materials and Methods

2.1. Plant Source

Digitalis viridiflora Lindley was collected from Demirköy, Kırklareli, Turkey, in July 2012. A voucher specimen (YEF 12012) has been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, İstanbul, Turkey.

2.2 Extraction and Isolation

The air-dried and powdered leaves of *D. viridiflora* (185 g) were extracted twice with MeOH (2 L) at 45 °C for 4 h. The combined methanolic extracts were concentrated to a residue (45.1 g, yield

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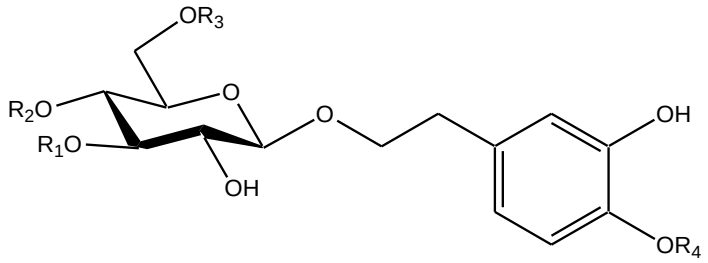
24.3%) under reduced pressure which was then suspended in H_2O (100 mL) and extracted with equal volumes of $CHCl_3$ (100 mL x 3). The H_2O layer was lyophilized (36.4 g) and an aliquot of the H_2O subextract (35 g) was subjected to a polyamide column (120 g) eluting with H_2O (200 mL) and a stepwise gradient of MeOH in H_2O (10–100%) to afford seven fractions, A–G. Fr. C (700 mg), which was rich in phenylethanoid glycosides, was separated by C_{18}-MPLC eluting with stepwise H_2O-MeOH gradient (10 to 80% MeOH) to obtain frs. $C_{1\text{-}3}$. Fr. C_2 (65 mg) was similary subjected to C_{18}-MPLC eluting with stepwise H_2O-MeOH gradient (10 to 30% MeOH) to give compounds lugrandoside (**2**, 16 mg) and maxoside (**4**, 12 mg). Fr. C_3 (40 mg) was rechromatographed on a SiO_2 column ($CHCl_3$/MeOH gradient, 100: 0 to 85:15) to give glucopyranosyl-(1→G_i-6)-martynoside (**5**, 4 mg). Fr. F (1.190 g) was applied to C_{18}-MPLC eluting with stepwise H_2O-MeOH gradient (15 to 60% MeOH) to yield isolugrandoside (**3**, 280 mg) and fr. F_3 (45 mg) which was then purified by SiO_2 CC ($CHCl_3$/MeOH gradient, 95:5 to 70:30) to yield viridifloroside (**1**, 12 mg).

*Viridifloroside (**1**):* Yellowish powder; UV (MeOH) λ_{max} nm: 222, 250 (sh), 290 (sh), 331. IR (KBr) ν_{max} cm^{-1}: 3314, 1697, 1606, 1523, 1278. ^{1}H- and ^{13}C- NMR: Table 1. LC-ESI-MS *m/z*: 623.2 $[M - H]^-$, $C_{29}H_{35}O_{15}^-$. HR-ESI-MS *m/z*: 647.1964 $[M + Na]^+$ (calcd for $C_{29}H_{36}O_{15}Na^+$, 647.1952).



1 R_1 = H, R_2 = (*E*)-Caff, R_3 = β-Quin, R_4 = H
2 R_1 = H, R_2 = (*E*)-Caff, R_3 = β-Glc, R_4 = H
3 R_1 = (*E*)-Caff, R_2 = H, R_3 = β-Glc, R_4 = H
4 R_1 = β-Glc, R_2 = (*E*)-Caff, R_3 = β-Glc, R_4 = H
5 R_1 = α-Rha, R_2 = (*E*)-Fer, R_3 = β-Glc, R_4 = CH_3

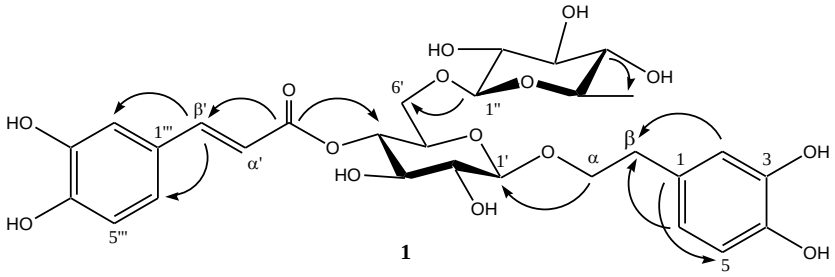
Figure 1. Structures of phenylethanoid glycosides isolated from *D. viridiflora*.

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was isolated as a yellowish amorphous powder. Its IR absorption bands revealed the presence of hydroxyl (3314 cm^{-1}), α,β-unsaturated ester carbonyl (1697 cm^{-1}), conj. C=C (1606 cm^{-1}) and aromatic ring (1523 cm^{-1}) functionalities. The molecular formula was determined to be $C_{29}H_{36}O_{15}$ by HR-ESI-MS (*m/z*: 647.1964 $[M + Na]^+$) as well as NMR data (Table 1). The ^{1}H NMR spectrum displayed the characteristic signals arising from a 2-(3,4-dihydroxyphenyl)ethyl (δ_H 6.71, 6.67 and 6.57 as an *ABX* system and benzylic methylene and adjacent nonequivalent oxymethylene signals at δ_H 2.80 and 4.04, 3.73), and a *trans*-caffeoyl (δ_H 7.05, 6.95, 6.77 as an *ABX* system and *trans* coupled *AX* system at δ_H 7.59 and 6.29, each J = 15.8 Hz) moieties. Moreover, the signals at δ_H 4.35 (*d*, J = 7.8 Hz, H-1′) and 4.27 (*d*, J = 7.6 Hz, H-1″) in the ^{1}H NMR were indicative of its diglycosidic nature which was supported by the corresponding anomeric carbon resonances assigned by HSQC spectrum at δ_C 104.5 and 104.6. The core sugar portion of the phenylethnoid structure was determined as β-glucopyranosyl by the help of COSY and HSQC spectra. The remaining six carbon resonances (104.6, 77.7, 77.1, 75.5, 73.4 and 18.2 ppm) in the ^{13}C NMR spectrum of **1** revealed that the second sugar unit was a methyl pentose. The β-configuration of this terminal sugar was deduced from the large coupling constant of the anomeric proton signal (J = 7.6 Hz). When its ^{13}C NMR data were compared with the

other two common methyl pentoses, β-quinovopyranosyl [9-11] and β-fucopyranosyl [10,12] the second sugar was unambiguously characterized as β-quinovopyranosyl due to the superimposition of its ^{13}C NMR findings with those of β-quinovopyranosyl. The glycosidation point of this terminal sugar was evidenced to be at C-6′(OH) of β-glucopyranosyl unit due to the appearance of C-6′ shifted downfield at 69.5 ppm.



1

Figure 2. Key HMBC for **1** (C→H)

This assumption was further confirmed by a three-bond correlation between the anomeric carbon of β-quinovopyranose and hydroxymethylene protons (H_2-6′) of β-glucopyranose in the HMBC spectrum (figure 2). The remaining depicted interfragmental connectivities were also determined by the cross-peaks in the HMBC spectrum, C=O (168.7 ppm)/H-4′ (4.87 ppm); C-α (72.5 ppm)/H-1′ (4.35 ppm). Thus, compound **1** was identified as 3,4-dihydroxy-*β*-phenylethoxy-*O*-*β*-quinovopyranosyl-(1→6)-4-*O*-caffeoyl-β-glucopyranoside and named as viridifloroside.

The known compounds were characterized as lugrandoside [5], isolugrandoside [13], maxoside [14] and glucopyranosyl-(1→G_i-6)-martynoside [15] by compairing of their NMR data with those published in the literatures.

3.2 Chemotaxonomic significance

Five phenylethanoid glycosides (**1-5**) were isolated from the leaves of *D. viridiflora* for the first time. Phenylethanoid glycosides are considered as significant chemotaxonomic marker due to their limited distribution. They are usually encountered in the Plantaginaceae, Lamiaceae, Scrophulariaceae and Oleaceae families [16]. The genus *Digitalis* has recently been categorized under the family "new" Plantaginaceae" based on the molecular phylogenetic studies [17]. Moreover, close relationships were found between *Digitalis* and the genera *Plantago*, *Globularia*, *Isoplexis* and *Wulfenia* within the expanded Plantaginaceae. There also exist several phytochemical studies to reveal the secondary metabolite profile of these genera and to support the molecular data. Accordingly, the common secondary metabolite class to all these genera is phenylethanoid glycosides [3, 18-20]. Taken into consideration of the phenylethanoid glycosides isolated from *Digitalis* species up to now, it can be concluded that the genus *Digitalis* seems to be more close to *Plantago* within Plantaginaceae, as two compounds namely, plantainoside D and plantamajoside (= purpureaside A) that were isolated from the genus *Digitalis* [21], were also obtained from *Plantago* species [19, 22]. On the other hand, lugrandoside (**2**) and isolugrandoside (**3**) isolated in this study along with the other phenylethanoid glycosides (calceolariosides A and B) [23] and ferruginoside A [5] characterized in some *Digitalis* species were also reported from the genus *Fraxinus* (Oleaceae) [24, 25]. Thus, the presence of these compounds in both *Digitalis* and *Fraxinus* may imply a close relationship between these two genera though they belong to different families. Concerning viridifloroside (**1**), it is being reported as a new and unique phenylethanoid glycoside which contains a rare methyl pentose, quinovose moiety in its structure. This compound is the first example of a phenylethanoid glycoside that possesses a quinovose unit. Isolugrandoside (**3**) is also being reported for the first time from a *Digitalis* species with this work. So, compounds **1** and **3** could be useful chemotaxonomic marker for *D. viridiflora* within the genus *Digitalis*.

Table 1. ^{13}C- (100 MHz) and ^{1}H- (400 MHz) NMR data[a] for viridifloroside **(1)** in CD_3OD.

Position	δ_C (ppm)	δ_H (ppm)
Aglycone		
1	131.6	-
2	117.3	6.71 (d, *J* = 2.0)
3	146.2	-
4	144.8	-
5	116.5	6.67 (d, *J* = 8.0)
6	121.5	6.57 (dd, *J* = 8.0, 2.0)
α	72.5	4.04 (m)
		3.73 (m)
β	36.8	
Glucose		
1′	104.5	4.35 (d, *J* = 7.8)
2′	75.3	3.28 (dd, *J* = 7.8, 9.5)
3′	75.9	3.60†
4′	72.6	4.87 (t, *J* = 9.5)
5′	74.9	3.71 (m)
6′	69.5	3.85 (dd, *J* = 11.5, 1.7)
		3.61†
Quinovose		
1″	104.6	4.27 (d, *J* = 7.6)
2″	75.5	3.17 (dd, *J* = 7.6, 9.0)
3″	77.7	3.27 (t, *J* = 9.1)
4‴	77.1	2.96 (t, *J* = 9.1)
5″	73.4	3.23 (m)
6″	18.2	1.23 (d, *J* = 6.1)
Caffeoyl		
1‴	127.8	-
2‴	115.3	7.05 (d, *J* = 2.0)
3‴	147.0	-
4‴	149.9	-
5‴	116.6	6.77 (d, *J* = 8.3)
6‴	123.2	6.95 (dd, *J* = 8.3, 2.0)
α′	114.8	6.29 (d, *J* = 15.8)
β′	147.9	7.59 (d, *J* = 15.8)
C=O	168.7	-

[a]Assignments are based on COSY, HSQC and HMBC experiments. † Overlapped

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] P. H. Davis (1978). *Digitalis* L. in "Flora of Turkey and the East Aegean Islands, Vol. 6, P. H. Davis (ed.).University Press: Edinburgh, p. 680.

[2] I. Calis, P. Akbay, A. Kuruuzum, F. N. Yalcin, P. Sahin and G. F. Pauli (1999). Phenylethanoid and cardioactive glycosides from *Digitalis ferruginea*, *Pharmazie* **54**, 926-930.

[3] H. Kirmizibekmez, M. Masullo, M. Festa, A. Capasso and S. Piacente (2014). Steroidal glycosides with antiproliferative activities from *Digitalis trojana*. *Phytother. Res.* **28**, 534-538.

[4] H. Kirmizibekmez, D. Tasdemir, T. Ersoz,, C. M. Ireland and I. Calis (2002). A new pregnane glycoside and a furostanol glycoside from *Digitalis cariensis*, *Pharmazie* **57**, 716-720.

[5] I. Calis, D. Tasdemir, O. Sticher and S. Nishibe (1999). Phenylethanoid glycosides from *Digitalis ferruginea* subsp. *ferruginea* (= *D. aurea*), *Chem. Pharm. Bull*. **47**, 1305-1307.
[6] A. Perrone, A. Capasso, M. Festa, E. Kemertelidze, C. Pizza, A. Skhirtladze and S. Piacente (2012). Antiproliferative steroidal glycosides from *Digitalis ciliata*, *Fitoterapia* **83**, 554-562.
[7] S. Ninov, Y. Buchvarov and C. Akhtardzhiev (1979). Glucoside composition of the leaves of *Digitalis viridiflora* Lindl. Communication II, *Farmatsiya* **29**, 28-30.
[8] S. Imre (1972). Flavone and anthraquinone pigments of *Digitalis* species. III. Anthraquinone and flavone compounds from the leaves of *Digitalis viridifloria*, *Planta Med*. **21**, 274-278.
[9] X.-T. Zhang, L.-H. Zhang, W.-C. Ye, X.-L. Zhang, Z.-Q. Yin, S.-X. Zhao and X.-S. Yao (2006). Four new cycloartane glycosides from *Thalictrum fortunei*, *Chem. Pharm. Bull*. **54**, 107-110.
[10] I. H. Hwang, D. W. Kim, S. J. Kim, B. S. Min, S. H. Lee, J. K. Son, C.-H. Kim, H. W. Chang and M. Na, (2011). Asterosaponins isolated from the starfish *Asterias amurensis*, *Chem. Pharm. Bull*. **59**, 78-83.
[11] A. Itoh, T. Tanahashi, N. Nagakura and T. Nishi (2003). Two triterpenoid saponins from *Neonauclea sessilifolia*, *Chem. Pharm. Bull.* **51**, 1335-1337.
[12] S. O. Lee, S. Z. Choi, S. U. Choi, K. C., Lee, Y. W. Chin, J. Kim, Y. C. Kim and K. R. Lee (2005). Labdane diterpenes from *Aster spathulifolius* and their cytotoxic effects on human cancer cell lines, *J. Nat. Prod.* **68**, 1471-1474.
[13] T. Iossifova, B. Vogler, I. Klaiber, I. Kostova and W. Kraus (1999). Caffeic acid esters of phenylethanoid glycosides from *Fraxinus ornus* bark, *Phytochemistry* **50**, 297-301.
[14] D. Brieger, S. Liedtke, R. Weber, M. Kirschke and J. J. Lichıus (1995). A new ester glucoside derived from *Digitalis lanata* leaves maxoside. *Pharmazie* **50**, 707-708.
[15] H. Otsuka (1993). Phenylethanoids from *Linaria japonica*, *Phytochemistry* **32**, 979-981.
[16] G. Fu, H. Pang and Y. H. Wong (2008). Naturally occurring phenylethanoid glycosides: potential leads for new therapeutics, *Curr. Med. Chem.* **15**, 2592-2613.
[17] D. C. Albach, H. M. Meudt, and B. Oxelman (2005). Piecing together the "new" Plantaginaceae, *Am. J. Bot.* **92**, 297-315.
[18] H. Kirmizibekmez, I. Calis, S. Piacente and C. Pizza (2004). Iridoid and phenylethyl glycosides from *Globularia sintenisii*, *Hel. Chim. Acta*. **87**, 1172-1179.
[19] R. M. Taskova, C. H. Gotfredsen and S. R. Jensen (2005). Chemotaxonomic markers in Digitalideae (Plantaginaceae), *Phytochemistry* **66**, 1440-1447.
[20] R. M. Taskova, C. H. Gotfredsen and S. R. Jensen (2006). Chemotaxonomy of Veroniceae and its allies in the Plantaginaceae, *Phytochemistry* **67**, 286-301.
[21] J. W. Oh, J. Y. Lee, S. H. Han, Y. H. Moon, Y. G. Kim, E. R. Woo and K. W. Kang (2005). Effects of phenylethanoid glycosides from *Digitalis purpurea* L. on the expression of inducible nitric oxide synthase, *J. Pharm. Pharmacol.* **57**, 903-910.
[22] F. Geng, L. Yang, G. X. Chou and Z. T. Wang (2010). Bioguided isolation of angiotensin-converting enzyme inhibitors from the seeds of *Plantago asiatica* L. *Phytother. Res*. **24**, 1088-1094.
[23] B.-N. Zhou, B. D. Bahler, G. A. Hofmann, M. R. Mattern, R. K. Johnson and D. G. I. Kingston (1998). Phenylethanoid glycosides from *Digitalis purpurea* and *Penstemon linarioides* withPKCα-inhibitory activity. *J. Nat. Prod*. **61**, 1410-1412.
[24] I. Kostova and T. Iossifova (2007). Chemical components of *Fraxinus* species, *Fitoterapia* **78**, 85-106.
[25] Y.-J. Chen, H.-G. Zhang and X. Li, (2009). Phenylethanoid glycosides from the bark of *Fraxinus mandschurica*, *Chem. Nat. Compd*. **45**, 330-332.

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