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Anthocyanins from *Vaccinium arctostaphylos* Berries

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Abstract

The systematic investigation of the berries of *Vaccinium arctostaphylos* L. (Ericaceae), a native medicinal plant growing in Iran, permitted the identification of three major anthocyanins. The compounds were isolated from the acidified extract of the berries using the repeated paper chromatography with different mobile phases and were characterized using chromatographic, chemical, and spectroscopic methods as delphinidin 3-*O*-β-glucoside, petunidin 3-*O*-β-glucoside, and malvidin 3-*O*-β-glucoside.

Keywords: Anthocyanin, Ericaceae, *Vaccinium arctostaphylos*.

Introduction

The genus *Vaccinium* (Ericaceae) comprises nearly 200 species, most of them found in the Northern Hemisphere (Morazzoni & Bombardelli, 1996). This genus is represented with only one taxon (*Vaccinium arctostaphylos* L.) in Iran (Wendelbo, 1965). *V. arctostaphylos* is a compact shrub about 1.5–2.5 m in height with many ascending branches and is found in the northern forests of the country between 1600–1800 m above sea level and known by the local names "Qaraqat" and/or "Cyah-gileh" (Ghahreman, 2001). The plant is therapeutically important in Iranian traditional medicine, and the decoction from the berries has been used as an antidiabetic and antihypertensive agent for a long time (Amin, 1991).

In regard to phytochemical profiles of *V. arctostaphylos*, previous studies have shown the presence of flavonol glycosides (Mzhavanadze, 1971a) and coumarins (Mzhavanadze et al., 1971b) in leaves and the presence phenolic acids and their derivatives in leaves (Mzhavanadze et al., 1971b) and unripe fruits (Mzhavanadze et al., 1972). In addition, the essential oil composition of the flowering aerial parts has been determined (Nickavar et al., 2002).

In past decades, the anthocyanin content of the genus *Vaccinium*, especially of *V. myrtillus*, has received attention due to its relevant pharmacological properties (Francis et al., 1966; Ballinger et al., 1981, 1982; Baj et al., 1983; Andersen, 1985; Fuleki, 1986; Andersen, 1987a, 1987b, 1989; Gao & Mazza, 1994; Morazzoni & Bombardelli, 1996; Cabrita & Andersen, 1999) but, to the best of our knowledge, there is no previous report on the anthocyanin content of *V. arctostaphylos*.

The aim of this study was to determine the major anthocyanins in the berries of *V. arctostaphylos*. This investigation will be useful to identify the bioactive compounds, which may be responsible for the therapeutic properties of the plant berries.

Materials and Methods

Plant material

The ripe berries of *V. arctostaphylos* were collected from the forest region of Asalem in the north of Iran in August 2000. Voucher specimens were deposited in the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran (no. 6520 THE).

Extraction and isolation of compounds

The pigments were extracted by maceration of 400 g of the berries with methanol : glacial acetic acid : water (70 : 2 : 28). The extract was filtrated, concentrated, and then partitioned

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against ethyl acetate (18 g). The pigments were isolated from the final aqeous extract by repeated descending paper chromatography (PC) on Whatman No. 3 MM paper with *n*-butanol : glacial acetic acid : water (4 : 1 : 5, upper phase) and 15% aqueous acetic acid as developing solvents. The bands were separately eluted with methanol : glacial acetic acid : water (90 : 5 : 5), concentrated and monitored by PC on Whatman No. 1 paper (Glassgen et al., 1992).

Identification of compounds

The structures of isolated compounds were determined on the basis of chromatographic, chemical, and spectral studies and confirmed by comparison with literature (Francis et al., 1966; Cabrita & Andersen, 1999).

UV-Vis spectra were recorded on Shimadzu UV-160A spectrophotometer in 0.01 N HCl-CH_3OH; ^{1}H NMR spectra were taken on a Bruker-Spectrospin 500 spectrometer in CD_3OD-CF_3COOD (5 : 1), and chemical shifts were recorded in δ-values; positive fast atom bombardment (FAB) mass spectra were obtained in glycerol-HCl as the matrix by Finigan MAT TSQ 700 mass spectrometer.

The nature of the sugar moiety in isolated compounds was determined through acid hydrolysis, according to the standard methods and direct comparison of each sugar moiety with authentic carbohydrates by co-chromatography on Merck cellulose TLC plates (Francis et al., 1966; Skaltsa et al., 1999).

Results and Discussion

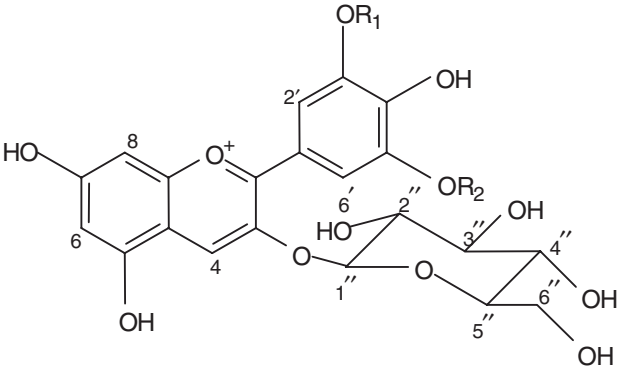
V. arctostaphylos berries were extracted by the acidified hydroalcoholic solvent. The extract was chromatographed successively on PC with different mobile phases. Finally, three major pigments, **1** (13 mg), **2** (10 mg), and **3** (16 mg), were obtained. The structures of the isolated anthocyanins are shown in Figure 1. R_f data observed in standard PC conditions used for this group of pigments are presented in Table 1. The spectroscopic data for the compounds are in agreement with the reported values for delphinidin 3-*O*-β-glucoside, petunidin 3-*O*-β-glucoside, and malvidin 3-*O*-β-glucoside (Francis et al., 1966; Cabrita & Andersen, 1999).

Delphinidin 3-*O*-β-glucoside (1)

UV-Vis λ_{max} (in CH_3OH-HCl): 539.5, 273, 219; + $AlCl_3$ (3 drops of 5% $AlCl_3$ in CH_3OH): 557.5, 273, 219 nm; $E_{440}/E_{vis,max}$ = 29%.

^{1}H-NMR (500 MHz, in CD_3OD-CF_3COOD): δ (aglycone moiety) 8.93 (1H, *s*, H-4), 7.74 (2H, *s*, H-2′ and H-6′), 6.84 (1H, *d*, *J* = 1.5 Hz, H-8), 6.64 (1H, *d*, *J* = 1.5 Hz, H-6); δ (sugar moiety) 5.32 (1H, *d*, *J* = 7.6 Hz, H-1″), 3.96–3.51 (5H, *m*, H-2″-H-6″) ppm.

FAB-MS *m/z*: 465 for $M^+ = C_{21}H_{21}O_{12}^+$.



Delphinidin 3-*O*-β-glucoside (1) : $R_1 = R_2 = H$

Petunidin 3-*O*-β-glucoside (2) : $R_1 = CH_3$; $R_2 = H$

Malvidin 3-*O*-β-glucoside (3) : $R_1 = R_2 = CH_3$

Figure 1. Structures of the anthocyanins isolated from *Vaccinium arctostaphylos*.

Table 1. R_f data of the anthocyanins isolated from *Vaccinium arctostaphylos*.

	PC ($R_f \times 100$)	
Anthocyanin	BAW[a]	15% HOAc[b]
Delphinidin 3-*O*-β-glucoside (**1**)	22	32
Petunidin 3-*O*-β-glucoside (**2**)	26	37
Malvidin 3-*O*-β-glucoside (**3**)	32	49

Solvent systems: [a] BAW, *n*-butanol : glacial acetic acid : water (4 : 1 : 5, upper phase); [b] 15% HOAc, water : glacial acetic acid (85 : 15). PC, paper chromatography.

Petunidin 3-*O*-β-glucoside (2)

UV-Vis λ_{max} (in CH_3OH-HCl): 536, 275, 218.5; + $AlCl_3$ (3 drops of 5% $AlCl_3$ in CH_3OH): 550, 275, 218.5 nm; $E_{440}/E_{vis,max}$ = 28%.

^{1}H-NMR (500 MHz, in CD_3OD-CF_3COOD): δ (aglycone moiety) 8.98 (1H, *s*, H-4), 7.94 (1H, *d*, *J* = 1.7 Hz, H-2′), 7.77 (1H, *d*, *J* = 1.7 Hz, H-6′), 6.88 (1H, *d*, *J* = 1.6 Hz, H-8), 6.64 (1H, *d*, *J* = 1.6 Hz, H-6), 3.97 (3H, *s*, OCH_3-3′); δ (sugar moiety) 5.23 (1H, *d*, *J* = 7.6 Hz, H-1″), 3.84–3.26 (5H, *m*, H-2″-H-6″) ppm.

FAB-MS *m/z*: 479 for $M^+ = C_{22}H_{23}O_{12}^+$.

Malvidin 3-*O*-β-glucoside (3)

UV-Vis λ_{max} (in CH_3OH-HCl): 539, 277.5, 215.5; + $AlCl_3$ (3 drops of 5% $AlCl_3$ in CH_3OH): 539, 277.5, 215.5 nm; $E_{440}/E_{vis,max}$ = 23%.

^{1}H-NMR (500 MHz, in CD_3OD-CF_3COOD): δ (aglycone moiety) 9.03 (1H, *s*, H-4), 7.97 (2H, *s*, H-2′ and H-6′), 6.94 (1H, *d*, *J* = 1.5 Hz, H-8), 6.66 (1H, *d*, *J* = 1.5 Hz, H-6), 3.99 (6H, *s*, OCH_3-3′ and OCH_3-5′); δ (sugar moiety) 5.25 (1H, *d*, *J* = 7.6 Hz, H-1″), 3.84–3.30 (5H, *m*, H-2″, H-6″) ppm.

FAB-MS *m/z*: 493 for $M^+ = C_{23}H_{25}O_{12}^+$.

The previous studies on the berries of the genus *Vaccinium* revealed the presence of 3-*O*-monoglycosides in which the aglycones delphinidin, cyanidin, petunidin, malvidin, and peonodin are combined with the sugars galactose, arabinose, and glucose. The current study, the first to report the anthocyanin content in *V. arctostaphylos*, also shows a similar chemical profile. These anthocyanins are likely the active compounds responsible for some therapeutic effects that have been supposed for the berries of *V. arctostaphylos* in Iranian traditional medicine (Amin, 1991). On the other hand, these compounds seem to have potential as taxonomic markers in the genus *Vaccinium* (Cabrita & Andersen, 1999).

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