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Two Cyanidin compound from the Fruits of *Acanthopanax divaricatus* var. *albeofructus*

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Abstract – *Acanthopanax divaricatus* var. *albeofructus* is one of the indigenous medicinal plant and the fruits of *Acanthopanax spp*. used as a remedial for "wipe out evil wind". Two anthocyanin were isolated from the fruits of *Acanthopanax divaricatus* var. *albeofructus.* Their structures were elucidated as cyanidin 3-lathyroside (**1**) and cyanidin 3-galactoside (**2**) by chemicophysical and spectroscopic analysis. And also, four chemical, syringin, chlorogenic acid, caffeic acid and acanthoside D were identified. Both anthocyanide were isolated for the first time from *Acanthopanax* species. cyanidin 3-lathyroside is one of the rare anthocyanin in natural resources.
Key words – *Acanthopanax divaricatus* var. *albeofructus* Fruits, cyanidin 3-lathyroside, cyanidin 3-galactoside.

Introduction

Acanthopanax species are herbaceous genus of the family Araliaceae. They are distributed in Korea, China and Japan. Among then sixteen species of *Acanthopanax* growing in the Korean peninsular, *Acanthopanax divaricatus* var. *albeofructus* is known to be one of the most available species in Korea.

Above 160 chemical constituent of *Acanthopanax* species were isolated and have been shown to have various levels of bioactive effects. (Shin and Lee, 2002). And also some of them have non-specific adaptogenic activity (Davydov and Krikorian, 2000).

Acanthopanax divaricatus var. *albeofructus* is closely related with morphological and chemotaxonomic character of *Acanthopanax divaricatus*.

Some 3,4-seco-lupane triterpened have been reported from *Acanthopanax divaricatus.* (Matsumoto *et al*., 1987; Shirasuna *et al*., 1997), an anthocyan (delphinidin 3-xylosylgalactoside) (Ishikura, 1975) and two lignans, pimalic acid, *d*-sesamin, three steroids, six fatty acid (Yook *et al*., 1996). Farmasol, farcarin, *l*-sesamin, farcaridiol (Miyakoshi *et al*., 1995).

The constituents of *Acanthopanax divaricatus* var. *albeofructus* are revealed as follow, chiisanogenin, *l*-sesamin, savinin, chiisanoside, 24-hydroxy chiisanogenin, 22 α-hydroxychiisanoside (Oh *et al*., 2000a and b). Also, anti-oxidantive triterpenoids and lignans have been isolated from the bark of *Acanthopanax divaricatus* var. *albeofructus* (Kim and Yang, 2004). The constituent of *Acanthopanax divaricatus* forma *flavi-flos*, chiisanogenin, chiisanoside, isochiisanoside and 11-deoxyisochiisanoside were identified (Nam *et al*., 2006).

The ripe berries of *Acanthopanax* species, *Acanthopanacis* Fructus, has been introduced to "wipe out evil wind" from the bodies of pathological patient (Li Shi Chen, 1518~1593).

The constituent of *Acanthopanacis* Fructus, *d*-sesamine, scoparone, protochatechuic acid, ursolic acid, hyperin, hydroxymethylfurfural from *A. sessilifrorum* (Lee *et al*., 2002), carbohydrate (72.33%) potasium (5951. 3 mg/100 g) xylose, mannose, galactose and glucose were measured and estimated antioxidantive, antimicrobial activity of *Acanthopanax senticosus* HARMS (Kim, 2006). Savinin, secotriterpenoid and syringaresinol diglucoside from *Acanthopanacis* Fruits (Shin *et al*., 1992).

The current study revealed that the water extract of *Acanthopanax divaricatus* var. *albeofructus* (ADA) possessed anti-oxidantive activity (Lyu *et al*., 2006). The methanol extract of ADA exhibited anti-lipid peroxidative activity (Zu and Yang, 2004) and reported the Antimitogenic and cytotoxicity effects of *Eleutherococcus senticosus* Maxim (Jun *et al*., 2003). The colored berries of *Acanthopanax divaricatus* var. *albeofructus* (ADAF) were suggested that it containing much of a anthocyanides. The anthocyanide in Araliaceus plant drugs are reported previously, Cyanidim 3-xylosyl-galactoside from *Aralia elata* (Sakamura and Kawano, 1970), Cyanidin-3-

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[O-β-D-xylopyranosyl(1 → 2)-β-D-galactopyranoside] from *Aralia elata* and *Aralia cordata* (Kawano and Sakamura, 1972), Delphinidin 3-xylosylgalactoside from *Acanthopanax divaricatus* (Ishikura, 1975), Cyanidin 3-O-lathyloside from *Fatsia japonica* (Terahara *et al*., 1992).

However, there has been no report on the constituents of ADAF. In the present study, we isolated two cyanidins; cyanidin-3-lathyroside(1) and cyanidin-3-galactoside(2) (2) and syringin, chlorogenic acid, caffeic acid and acanthoside D. were identified by HPLC.

Experimental

General experimental procedures – $^{1}H/^{13}C$-NMR and HMBC spectra were recorded on Bruka AMX-600. The chemical shifts were shown in δ (ppm) relative to TMS. HPLC-ESI-POS-MS was performed on Agilent 1100 HPLC coupled to API-3000 Tandem Mass system. Chromatographic separation was conducted using a Phenomenex Luna C18 column (3 μm, 150 × 2 mm). The mobile phase was 10% acetonitrile containing 0.1% formic acid. Injection volume was 10 μl and flow rate was 150 μl/min. Preparative HPLC was performed on a Waters 600F pump with a COSMOSIL 5C 18-AR-II column (250 × 20 mm) eluting with H_2O : CH_3CN : HCOOH = 85.5 : 9.5 : 5 (flow rate 20 ml/min) and detected at 532 nm on Younglin UV 730D detector. TLC analysis for anthocyanidins was performed on microcrystalline cellulose plate (MERK) with HCOOH : HCl : H_2O 4 : 1 : 5 (solvent A) and detected with UV lamp. After acid hydrolysis of sugar associated with anthocyanins, the sugars were developed with n-BuOH : CH_3COOH : H_2O 4 : 1 : 5 (solvent B) and detected with aniline hydrogenen phthalate.

Plant material – The ripe berries of ADA were collected from Native Korea Acanthopanaxes Farm, Sushin-Myeon, Cheonan, Korea in November 2008 and certificated by Dr. Han. A voucher specimen was deposited in the author's laboratory (ADA200811).

Extraction and isolation – The freeze dried fruit were extracted with 30% CH_3OH containing 1% HCl under sonication at 4 ºC for 1 h three times and filtered. The dark red extracts were applied on Sephadex LH-20 resin column (700 × 3.0 cm) eluting with 0 - 30% CH_3OH containing 1% HCl and resulted 3 subfractions (I - III). Each fraction II and III was subjected to preparative RP-HPLC eluting with H_2O : CH_3CN : HCOOH = 85.5 : 9.5 : 5 and each peak was observed at 7.56 min (**1**) and 8.63 min (**2**), respectively. After these were then dissolved in a small amount of trifluoroacetic acid (TFA) and precipitated with diethylether, each TFA salt of **1** (30 mg) and **2** (2.4 mg) yielded, respectively.

Acid hydrolysis – Acid hydrolysis were perfomed by the usual method. Sugars associated with anthocyanins were identified using TLC analysis, carried out on microcrystalline cellulose plate (MERK) with BAW developing solvent (n-BuOH : CH_3COOH : H_2O 4 : 1 : 5). The sugar spots of (1) were detected as galactose (Rf 0.29) and xylose (Rf 0.39) and that of (2) was detected as galactose (Rf 0.30) by spraying aniline hydrogenen phthalate.

HPLC analysis for minor compounds of ADAF – Quantitative analysis of minor compounds with HPLC, quantitative HPLC was performed, on a waters 510 pump with a COSMOSIL 5C 18-AR-II column (250 × 4.6 mm), wave length 220 nm, developed with CH_3CN : H_2O = 15 : 85, detector UV 730D.

The material of pericarp and fruit meat of *Acanthopanax divaricatus* var. *albeofructus* fruits were extracted with 50% MeOH.

Contents of minor compounds, from pericarp : syringin 45.25, chlorogenic acid 183.00, caffeic acid 5.20, acanthoside D 6.72ppm and from fruit meat : syringin 19.03, chlorogenic acid 82.98, caffeic acid 4.15, acanthoside D 2.89ppm respectivery.

Cyanidin-3-lathyloside TFA (1) – Faint dark red powder; Rf 0.62 (**1**), UV (0.01 % HCl - MeOH) λ_{max} nm: 530 and a bathochromic shift of 13 nm by the addition of $AlCl_3$ solution inducating the presence of a free O-dihydroxyl group in the B-ring. IR ν_{max} (KBr) cm^{-1} : 3356, 1680, 1646, 1335, 1198, 1068, 724; HPLC-ESI-POS-MS *m/z*: 581 ; ^{1}H-NMR (600 MHz, CD_3OD) δ: 8.93 (1H, s, H-4), 8.27 (1H, dd, *J* = 1.8, 8.8 Hz, H-6'), 8.02 (1H, d, *J* = 1.8 Hz, H-2'), 6.99 (1H, d, *J* = 8.8 Hz, H-5'), 6.88 (1H, m, Hz, H-8), 6.63 (1H, d, *J* = 1.8 Hz, H-6), 5.42 (1H, d, *J* = 7.7, H-1''), 4.72 (1H, d, *J* = 7.7 Hz, H-1'''), 4.23 (1H, t, *J* = 8.4, H-2''), 3.97 (1H, m, H-4''), 3.92 (1H, dd, *J* = 2.5, 9.5, H-3''), 3.84 (1H, m, H-5''), 3.82(1H, m, H-6''a), 3.65 (1H, dd, *J* = 5.1, 11.4 Hz, H-5'''a), 3.60 (1H, dd, *J* = 5.9, 11.0 Hz, H-6'''b), 3.38 (1H, m, H-4'''), 3.28 (1H, t, J = 9.0 Hz, H-3'''), 3.17 (1H, t, *J* = 8.4 Hz, H-2'''), 3.04 (1H, t, *J* = 10.8, H-5'''b); ^{13}C-NMR (125 MHz, CD_3OD) δ: see Table 1

Cyanidin 3-galactoside TFA (2) – Red powder; Rf 0.32 (**2**). UV (0.01% HCl - MeOH) λ_{max} nm: 530 and bathochromic schifted 13nm. IR ν_{max} (KBr) cm^{-1} : 3356, 1680, 1646, 1335, 1198, 1068, 724; HPLC-ESI-POS-MS *m/z*: 449 'H-NMR (600MHz, CD_3OD) δ : 8.93 (1H, s, H-4), 8.27 (1H, d, *J* = 1.8, 8.8 Hz, H-6'), 8.02 (1H, d, *J* = 1.8 Hz, H-2'), 6.99 (1H, d, *J* = 8.8 Hz, H-5'), 6.88 (1H, *J* = 1.8

Table 1. ^{13}C-NMR data of **1** and **2** (inCD_3OD)

	Carbon	1[a]	2[a]	Aglycon*
Cyanidin	2	164.0	164.0	162.4
	3	145.5	145.6	146.5
	4	136.2	136.6	134.0
	5	159.3	159.2	157.9
	6	103.4	103.5	103.0
	7	170.3	170.3	168.8
	8	98.2	95.2	94.7
	9	157.6	157.6	156.9
	10	113.3	113.2	113.5
	1	121.4	121.3	121.9
	2	118.7	118.5	117.9
	3	147.5	147.4	147.9
	4	155.9	155.8	155.1
	5	117.5	117.3	117.2
	6	128.8	128.7	127.1
Galactopyranosyl	1	106.1	106.0	
	2	80.0	80.0	
	3	75.2	75.3	
	4	70.1	70.5	
	5	78.0	78.0	
	6	62.4	62.3	
Xylopyranosyl	1	102.1		
	2	75.9		
	3	77.8		
	4	71.1		
	5	67.3		

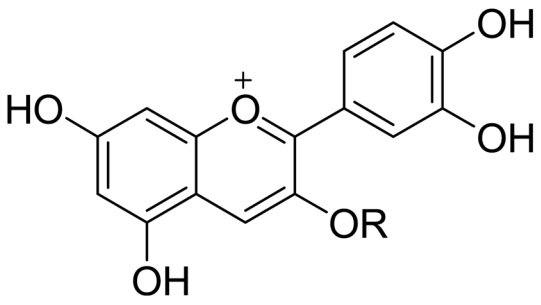
[a] Recorded at 150 MHz

Hz, H-8), 6.63 (1H, d, J = 1.8 Hz, H-6), 5.46 (1H, d, J = 7.7 Hz, H-1"), 4.23 (1H, t, J = 7.7 Hz, H-2"), 3.97 (1H, m H-4"), 3.92 (1H, m, J = 2.9, Hz, H-3"), 3.84 (1H, m, H-5"), 3.78 (2H, m, H-6" a and b); ^{13}C-NMR (125 MHz, CD_3OD) δ: see Table 1

Results and Discussion

The 30% CH_3OH (containing 1 % HCl) extract of the dark red berries of ADAF was subjected to Sephadex and reversed phase preparative HPLC yielded two anthocyanidins as a red powder of the TFA salts (**1** and **2**) (Fig. 1).

Compound (**1**) was obtained as a red amorphous powder. The 1H-NMR spectrum showed an aromatic ABX-spin system at δ 8.27 (1H, dd, J = 1.8, 8.8 Hz, H-6'), δ 8.02 (1H, d, J = 1.8 Hz, H-2') and δ 6.99 (1H, d, J = 8.8 Hz, H-5'), and two meta-coupled protons at δ 6.88 (1H, d, J = 1.8, H-8) and δ 6.63 (1H, d, J = 1.8, H-6). In



	R
1	Xyl-(1→2)-Gal
2	Gal

Fig. 1. Anthocyanidins from the ADA.

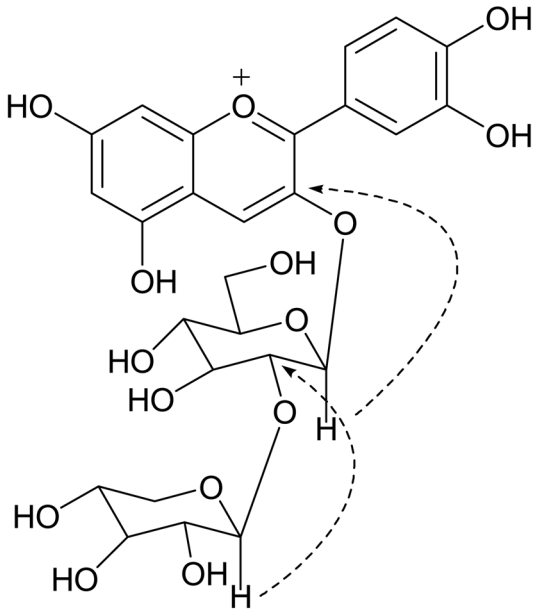


Fig. 2. Key correlations on the HMBC of **1**.

particular, a downfield shifted aromatic proton at δ 8.93 (1H, s, H-4) suggested a cyanidin moiety. Furthermore, the two anomeric protons with large coupling constants at δ 5.42 (1H, d, J = 7.7, H-1") and δ 4.72 (1H, d, J = 7.7, H-1'"). On ^{1}H-NMR spectrum indicated the existence of β-linked two sugar moieties. The R_f values, 0.29 and 0.39, on the TLC after acid hydrolysis of (1) indicated that these two sugars were galactose and xylose, and the correlations between H-1" and C-3, and between H-1'" and C-2" were observed on the HMBC (Fig. 2). Based on above spectral data and the comparison with reported literatures (Sakamura and Kawano, 1970; Kawano and Sakamura, 1972; Terahara *et al*., 1992), compound (1)

was identified as cyanidin-O-3-lathyroside.

Compound **(2)** was obtained as a red amorphous powder. All the signals on $^{1}H/^{13}C$-NMR spectra were almost same to (1) However, only one anomeric protons δ 5.46 (1H, d, J = 7.7 Hz, H-1'') observed on the ^{1}H-NMR spectrum and one hexose moiety was observed at δ 106.0, 80.0, 78.0, 75.3, 70.5 and 62.3 on the ^{13}C-NMR spectrum. And, acid hydrolysis of **(2)** exhibited that this hexose was galactose due to the Rf value, 0.39 on the TLC. Thus, (2) was identified as cyanidin-O-3-β-D-galactopyranoside (Jan and Jean 1988). Cyanidin 3-galactoside is the major constituent of *Aronia melanocarpa* which induced the polish paradox. (use in medieval underfunction of cardio-vascular metabolic system)

We firstly isolated **(1)** from the ADAF which is rare anthocyanidins in nature.

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