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Bioactive Constituents from the *n*-Butanolic Fraction of *Aruncus dioicus* var. *kamtschaticus*

Quoc Hung Vo[1,2], Phi Hung Nguyen[1], Bing Tian Zhao[1], Yen Nguyen Thi[1], Duc Hung Nguyen[1], Won Il Kim[1], U Min Seo[1], Byung Sun Min[1], and Mi Hee Woo[1,*]

[1]*College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Republic of Korea*
[2] *Department of Pharmacy, Hue University of Medicine and Pharmacy, Hue City, Viet Nam*

Abstract – Six compounds were isolated from the *n*-BuOH fraction of the aerial parts of *Aruncus dioicus* var. *kamtschaticus* including: sambunigrin (**1**), prunasin (**2**), aruncide A (**3**), aruncide C (**4**), 1-*O*-caffeoyl-*β*-D-glucopyranose (**5**), and caffeic acid (**6**). Their structures were confirmed by comparing the spectral data with those reported in the literature. The isolated compounds (**1 - 6**) were then examined for their cytotoxic effects towards MCF-7, HL-60, and HeLa cancer cell lines, as well as their DPPH radical scavenging activity. The results indicated that compound **4** possessed the strongest inhibitory effect toward HeLa cell line with IC_{50} value of 5.38 ± 0.92 μM. Compound **3** possessed selective cytotoxic activity on HL-60 cells with IC_{50} value of 6.27 ± 0.17 μM, compound **5** was found as the best in inhibiting proliferation with IC_{50} value of 2.25 ± 0.09 μM, and the other compounds showed significant inhibition with IC_{50} values ranging from 6.10 to 11.27 μM. Compound **5** also displayed the strongest cytotoxic effect toward MCF-7 cell line (IC_{50} 4.32 ± 0.15 μM). Both **5** and **6** demonstrated strong radical scavenging activity (IC_{50} 6.87 ± 0.03 and 4.33 ± 0.22 μM, respectively). Compounds **1** and **5** were isolated for the first time from this plant.
Keywords – *Aruncus dioicus* var. *kamtschaticus*, Cyanogenic glycosides, Monoterpenes, Caffeoyl glucoside

Introduction

Increased public interest regarding the efficacy of natural products in the treatment of diseases has inspired pharmaceutical scientists to search for new avenues in drug discovery. Currently, the use of medicinal plants, as well as plant-derived molecules, remain a significant fraction of pharmaceuticals in the clinic.[1] Studies have found that natural products and related drugs are used to treat 87% of all categorized human diseases.[2]

Among existing diseases, cancer is a major cause of morbidity and mortality, with approximately 14 million new cases and 8 million cancer-related deaths reported in 2012; the disease affects populations in all countries and all regions. Particularly, breast cancer, which is the most common cancer diagnosis for women in 140 countries and the most frequent cause of cancer mortality in 101 countries, has a considerably higher incidence rate (43.3 per 100,000) than other types of cancer. Cervix is one of the five most common in incident sites of cancer in women which accounts for 7.9% of total.[3] Finally, leukemia ranks as the eleventh highest in terms of cancer incidence, and the tenth most common cause of cancer death.[4]

One of the causes of cancer is free radicals, primarily reactive oxygen species (ROS). Increased levels of ROS can damage the structure of biomolecules, alter their functions, and lead not only to cellular dysfunction, but also to cell death. The cumulative effect of increased ROS can increase oxidative stress at a systemic level, and it is manifested in the form of a variety of health problems such as cancer, age-related disease and cardiovascular diseases. Thus, natural antioxidants are known to minimize the adverse effects of free radicals in living system that brings benefits to human health.[5]

Therefore, in the interest of promoting drug discovery from natural sources, this research was conducted to identify bio-active compounds from *Aruncus dioicus* var. *kamtschaticus* (Rosaceae). This major leafy vegetable, which is cultivated in Ulleungdo - Rep. of Korea, has been proven to possess many bioactivities including antioxidant,[6] antidiabetic,[7] and anti-HIV[8] effects. The herb also has therapeutic value for treating ischemic,

*Author for correspondence
Mi Hee Woo, College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Republic of Korea
Tel: +82-53-8503620; E-mail: woomh@cu.ac.kr

degenerative brain diseases, as well as intoxification and tonsillitis.[9] As a part of our continuing study of *A. dioicus*, the constituents from the *n*-butanolic extract were identified and tested for their antiproliferation effects using MTT assay on three cancer cell lines including MCF-7 (Human breast cancer cells), HeLa (Human cervical epitheloid carcinoma cells) and HL-60 (Human promyelocytic leukemia cells). The isolates were also examined for antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

Experimental

General experimental procedures – IR spectra were measured on a Mattson Polaris FT/IR-300E spectrophotometer. The NMR spectra were recorded on Varian OXFORD-AS 400MHz instrument (PaloAlto, CA, USA). Low resolution EI-MS and FAB-MS data were collected on a Quattro II spectrometer. The optical density (OD) values of the solutions were measured on a plate reader Infinite® 200 PRO TECAN. Open column chromatography was performed using silica gel (Kieselgel 60, 70-230 mesh and 230 - 400 mesh, Merck) and reversed-phase silica gel (LiChroprep RP-18, 40-63 μm, Merck) as well as Sephadex LH-20 (25 - 100 μm, Sigma). Thin layer chromatography (TLC) was performed on Merck precoated TLC silica gel 60 F_{254} and/or TLC silica gel 60 RP-18 F_{254} S glass plates (0.25 mm), and spots were detected under UV light and by heating after spraying with 10% H_2SO_4. High-performance liquid chromatography (HPLC) was performed using a Waters 717 Autosampler with an UV 2487 detector and a Nova-Pak C18 Prep column (7.8 × 300 mm, 6 μm particle size, Waters, USA), HPLC solvents were from Burdick & Jackson, USA. All other chemicals and solvents were of analytical grade and used without further purification.

Plant material – The aerial parts of *A. dioicus* var. *kamtschaticus* were collected in July 2006 from Ulleungdo, Ulleung-gun, Gyeongbuk, Republic of Korea. This material was confirmed taxonomically by Professor Byung Sun Min, College of Pharmacy, Catholic University of Daegu, Korea. A voucher specimen (CUDP06001) has been deposited at the College of Pharmacy, Catholic University of Daegu, Korea.

Extraction and isolation – The freeze-dried aerial parts of *A. dioicus* var. *kamtschaticus* (6.9 kg) was extracted two times with 95% EtOH in H_2O (18 L/time) at room temperature for 36 hours. The resulting solution was concentrated under reduced pressure to yield a residue (3.03 kg). The EtOH extract was suspended in H_2O (6.0 L) and partitioned successively with *n*-hexanes (6 × 5.0 L, 308.0 g), CH_2Cl_2 (6 × 5.0 L, 41.7 g), EtOAc (6 × 7.0 L, 121.3 g), *n*-BuOH (6 × 7.0 L, 51.4 g) and H_2O-soluble fractions (600.0 g), respectively. The *n*-butanolic fraction (51.4 g) was subjected to open flash column chromatography (6.5 × 25 cm, silica gel 230 - 400 mesh) eluting with gradient of CH_2Cl_2-MeOH-H_2O (100 : 0 : 0 to 0 : 100 : 0.1). The eluates were combined into nineteen fractions (B1 to B19) according to their similar TLC patterns. The fraction B12 (5.3 g) was chromatographed on a silica gel column using a stepwise gradient of CH_2Cl_2-MeOH-H_2O mixture as a solvent system (5 : 1 : 0.1 to 3 : 1 : 0.1) to afford eight subfractions (B12.1 ~ B12.8). The subfraction B12.4 (308.0 mg) was applied for a reverse-phase column using MeOH-H_2O (2 : 5) as a mobile phase to yield compound **3** (17.7 mg) and three other subfractions (B12.4.1 ~ B12.4.3). B12.4.1 (70.0 mg) was chromatographed by HPLC on a RP-C18 column using 25% MeOH in H_2O as a mobile phase to yield compounds **2** (12.0 mg) and **4** (8.6 mg), respectively. The subfraction B12.7 (890.9 mg) was loaded on a reverse-phase RP-C18 column eluting with a stepwise gradient of MeOH-H_2O mixture (9 : 91 to 100 : 0) to give three subfractions (B12.7.1 ~ B12.7.3). B12.7.3 was further purified by an open column using Sephadex LH-20 and eluted by 99.8% MeOH to yield compounds **5** (140.0 mg) and **6** (21.5 mg), respectively. The fraction B19 (125.0 mg) was chromatographed by HPLC on a RP-C18 column using 20% MeOH in H_2O as a mobile phase to yield compound **1** (11.3 mg).

Sambunigrin (1): White powder; IR (KBr) cm^{-1}: 3421, 1083, 745, 701; MS (FAB) *m/z* 296 $[M + H]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 7.61 - 7.59 (2H, m, H-4, 8), 7.46–7.44 (3H, m, H-5, 6, 7), 6.06 (1H, s, H-2), 4.69 (1H, d, J = 7.6 Hz, H-1'), 3.95 (1H, dd, J = 2.0, 12.0 Hz, H-6'a), 3.70 (1H, dd, J = 6.4, 12.0 Hz, H-6'b), 3.43 - 3.27 (4H, m, H-2', 3', 4', 5'); ^{13}C NMR (CD_3OD, 100 MHz) δ: 135.4 (C-3), 130.9 (C-6), 130.0 (C-4, 8), 128.9 (C-5, 7), 118.6 (C-1), 102.2 (C-1'), 78.7 (C-5'), 78.0 (C-3'), 74.9 (C-2'), 71.7 (C-4'), 68.6 (C-2), 62.9 (C-6').

Prunasin (2): White powder; IR (KBr) cm^{-1}: 3300, 1067, 745, 700; MS (FAB) *m/z* 296 $[M + H]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 7.59 - 7.57 (2H, m, H-4, 8), 7.46 - 7.45 (3H, m, H-5, 6, 7), 5.91 (1H, s, H-2), 4.24 (1H, d, J = 7.2 Hz, H-1'), 3.91 (1H, dd, J = 2.0, 11.6 Hz, H-6'a), 3.70 (1H, dd, J = 5.2, 11.6 Hz, H-6'b), 3.44 - 3.26 (4H, m, H-2', 3', 4', 5'); ^{13}C NMR (CD_3OD, 100 MHz) δ: 135.0 (C-3), 131.2 (C-6), 130.3 (C-4, 8), 129.1 (C-5, 7), 118.6 (C-1), 102.2 (C-1'), 78.5 (C-5'), 78.0 (C-3'), 74.9 (C-2'), 71.6 (C-4'), 68.5 (C-2), 62.9 (C-6').

Aruncide A (3): Colorless syrup; IR (KBr) cm^{-1}: 3400, 2930, 1744; MS (FAB) *m/z* 367 $[M + Na]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 6.42 (1H, dd, J = 2.4, 3.2 Hz, H-4), 5.28 (1H, m, H-6), 5.26 (1H, m, H-7), 4.89 (1H, m, H-11a), 4.83 (1H, m, H-11b), 4.33 (1H, d, J = 7.6 Hz, H-1'), 3.87 (1H, dd, J = 2.0, 11.6 Hz, H-6'a), 3.69 (1H, dd, J = 4.8, 12.0 Hz, H-6'b), 3.39 - 3.19 (4H, m, H-2', 3', 4', 5'), 3.14 (1H, m, H-5a), 2.65 (1H, d, J = 1.2 Hz, H-5b), 1.79 (3H, d, J = 1.2 Hz, H_3-10), 1.77 (3H, d, J = 1.2 Hz, H_3-9); ^{13}C NMR (CD_3OD, 100 MHz) δ: 171.9 (C-2), 141.1 (C-8), 140.5 (C-4), 127.9 (C-3), 124.6 (C-7), 104.4 (C-1'), 78.2 (C-5'), 76.9 (C-3'), 76.9 (C-6), 75.2 (C-2'), 71.6 (C-4'), 67.0 (C-11), 62.8 (C-6'), 36.7 (C-5), 26.0 (C-10), 18.5 (C-9).

Aruncide C (4): Colorless syrup; IR (KBr) cm^{-1}: 3386, 1760, 1130; MS (FAB) *m/z* 383 $[M+Na]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 5.30 (1H, d, J = 9.2 Hz, H-8), 4.83 (1H, d, J = 6.0 Hz, H-4), 4.41 (1H, dd, J = 10.8, 11.2 Hz, H-12a), 4.33 (1H, d, J = 7.6 Hz, H-1'), 4.29 (1H, dd, J = 7.6, 8.8 Hz, H-5), 3.94 (1H, dt, J = 2.4, 2.8, 10.4 Hz, H-7), 3.88 (1H, dd, J = 2.8, 11.6 Hz, H-12b), 3.87 (1H, dd, J = 2.0, 11.6 Hz, H-6'a), 3.69 (1H, dd, J = 4.8, 12.0 Hz, H-6'b), 2.74 (1H, dd, J = 2.4, 9.2 Hz, H-3), 1.82 (3H, s, H_3-11), 1.80 (3H, s, H_3-10), 3.53 - 3.00 (4H, m, H-2', 3', 4', 5'); ^{13}C NMR (CD_3OD, 100 MHz) δ: 175.0 (C-2), 142.8 (C-9), 122.3 (C-8), 99.6 (C-1'), 82.5 (C-4), 81.3 (C-5'), 79.9 (C-3'), 75.2 (C-7), 75.0 (C-2'), 73.3 (C-5), 71.9 (C-4'), 68.5 (C-12), 62.6 (C-6'), 50.8 (C-3), 26.2 (C-11), 18.8 (C-10).

1-*O*-Caffeoyl-β-D-glucopyranose (5): Dark-colored syrup; IR (KBr) cm^{-1}: 3400, 1690, 1635, 1605, 1515; MS (FAB) *m/z* 341 $[M - H]^-$, ^1H NMR (CD_3OD, 400 MHz) δ: 7.66 (1H, d, J = 15.6 Hz, H-7), 7.08 (1H, brs, H-2), 6.96 (1H, d, J = 6.4 Hz, H-6), 6.80 (1H, d, J = 7.6 Hz, H-5), 6.31 (1H, d, J = 16.0 Hz, H-8), 5.62 (1H, d, J = 6.0 Hz, H-1'), 3.81 (1H, d, J = 12.0 Hz, H-6'a), 3.74 (1H, d, J = 11.6 Hz, H-6'b), 3.57 - 3.36 (4H, m, H-2', 3', 4', 5'); ^{13}C NMR (CD_3OD, 100 MHz) δ: 167.9 (C-9), 149.8 (C-4), 148.5 (C-7), 146.7 (C-3), 127.6 (C-1), 123.4 (C-6), 116.6 (C-5), 115.4 (C-2), 114.3 (C-8), 95.7 (C-1'), 78.6 (C-5'), 77.9 (C-3'), 74.0 (C-2'), 71.0 (C-4'), 62.3 (C-6').

Caffeic acid (6): Yellow powder; IR (KBr) cm^{-1}: 3380, 1690, 1627, 1600, 1511; MS (EI) *m/z* 180 $[M]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 7.48 (1H, d, J = 15.6, H-7), 6.99 (1H, d, J = 2.0, H-2), 6.89 (1H, dd, J = 2.0, 8.0 Hz, H-6), 6.73 (1H, d, J = 8.0, H-5), 6.18 (1H, d, J = 15.6, H-8); ^{13}C NMR (CD_3OD, 100 MHz) δ: 171.3 (C-9), 149.7 (C-4), 147.1 (C-7), 146.9 (C-3), 128.0 (C-1), 123.0 (C-6), 116.6 (C-5), 115.8 (C-2), 115.2 (C-8).

Cytotoxic activity assay – The cancer cell lines were maintained in RPMI 1640, which included L-glutamine with 10% FBS and 2% penicillin-streptomycin. Cells were cultured at 37 °C in a 5% CO_2 incubator. Cytotoxic activity was measured using a modified 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) assay with some modifications.[10] Viable cells were seeded in the growth medium (180 μL) into 96-well microplate (1×10^4 cells per well) and incubated at 37 °C in a 5% CO_2 incubator. The test sample was dissolved in a minimum volume of dimethyl sulfoxide (DMSO) and adjusted to final sample concentrations ranging from 1.0 to 100.0 μg/mL by diluting with the growth medium. Each sample was prepared in triplicate. The final DMSO concentration was adjusted to < 0.1%. After standing for 24 h, 20 μL of the test sample was added to each well. The same volume of DMSO was added to the control wells. On removing medium after 45 h of the test sample treatment, MTT (5 mg/mL, 20 μL) was also added to the each well. After 3 h incubation, the plates were removed, and the resulting formazan crystals were dissolved in DMSO (150 μL). A plate reader was used to measure the OD values of the solutions at 570 nm. The IC_{50} value was defined as the concentration of sample that reduced absorbance by 50% relative to the vehicle-treated control. All cell lines were purchased from the Korean Cell Line Bank (Seoul, Korea).

DPPH radical scavenging activity assay – The DPPH radical scavenging activity was measured using the method described by Tagashira and Ohtake with some modifications.[11] 100 μL of each sample dissolved in MeOH was prepared in the 96-well microplate, and then 100 μL of methanolic DPPH solution (0.2 mM) was added. After mixing and standing in the dark for 30 min at room temperature, the absorbance of the reaction mixture was measured at 515 nm. L-ascorbic acid (Sigma-Aldrich; purity: > 99%) was used as the positive control.

Result and Discussion

Six compounds (**1 - 6**) were isolated from the aerial parts of *A. dioicus* var. *kamtschaticus* using various chromatographic methods including: silica gel, Lichroprep RP-18, Sephadex LH-20, and preparative HPLC. Those were identified as: sambunigrin (**1**), prunasin (**2**), aruncide A (**3**), aruncide C (**4**), 1-*O*-caffeoyl-β-D-glucopyranose (**5**) and caffeic acid (**6**), respectively, by spectroscopic methods, as well as by comparing their data with literature-based values (Fig. 1). To the best of our knowledge, compounds **1** and **5** were isolated for the first time from this plant.

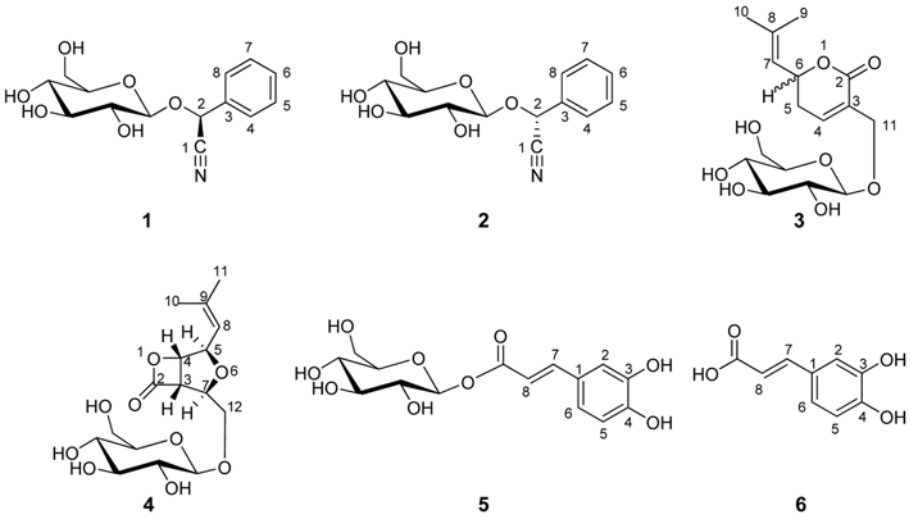

Fig. 1. Chemical structures of compounds **1 - 6** isolated from the aerial parts of *A. dioicus* var. *kamtschaticus.*

Compound **1** was obtained as white powder with a molecular weight of *m/z* 296 $[M + H]^+$, based on FAB-MS data. The IR spectrum of compound **1** showed absorptions characteristic of mono-substituted ring (745, 701 cm^{-1}), ethereal stretching band (1083 cm^{-1}), and hydroxyl (3421 cm^{-1}). The ^{1}H-NMR spectrum of compound **1** displayed the presence of a phenyl group with multiplet signals at δ_H 7.61 - 7.59 and 7.46 - 7.44. The anomeric proton was detected as a doublet at δ_H 4.69, whose coupling constants (J = 7.6 Hz) indicated the β-configuration for glycosidic linkage. The methine proton was observed as a singlet at δ_H 6.06. The ^{13}C-NMR spectrum of compound **1** showed the presence of a mono-substituted ring (δ_C 135.4, 130.9, 130.0, 128.9), a nitrile (δ_C 118.9), a methine carbon (δ_C 68.6) and a hexose sugar (δ_C 102.2, 78.7, 78.0, 74.9, 71.7, 62.9). Detailed comparison of the ^{1}H and ^{13}C NMR spectra of compound **1** with those published in the literature, led structural identification of compound **1** as sambunigrin.[12] The ^{1}H and ^{13}C NMR spectra of compound **2** revealed a similar structure with compound **1**, but different in chiral center at C-2, which was determined as prunasin. Sambunigrin (**1**) and prunasin (**2**) are two stereoisomeric compounds that can be clearly distinguished by a notable chemical shift difference in the anomeric protons (4.69 and 4.24 ppm, respectively), and the slightly shift difference in the cyanogenic methine protons at δ_H 6.06 and 5.91 ppm, respectively.[13]

Compound **3** was obtained as a colorless syrup. The molecular weight of compound **3** was obtained from FAB-MS with *m/z* 367 $[M + Na]^+$. The IR spectrum of **3** with absorption bands at 3400 cm^{-1} and 1744 cm^{-1} indicated the presence of hydroxy and α,β-unsaturated lactone functional groups. The ^{1}H NMR spectrum demonstrated an α,β-unsaturated δ-lactone moiety with four protons at δ_H 6.42 (1H, dd, J = 2.4, 3.2 Hz, H-4), 3.14 (1H, m, H-5a), 2.65 (1H, d, J = 1.2 Hz, H-5b) and 5.28 (1H, m, H-6); there were corresponding ^{13}C NMR signals at δ_C 140.5 (C-4), 36.7 (C-5), 76.9 (C-6) and 171.9 (C-2). The presence of an isobutenyl side-chain was identified due to the ^{1}H and ^{13}C NMR spectra {δ_H 5.26 (1H, m, H-7), δ_C 124.6 (C-7), 141.1 (C-8)}, {δ_H 1.77 (3H, d, J = 1.2 Hz, H_3-9), δ_C 18.5 (C-9)}, {δ_H 1.79 (3H, d, J = 1.2 Hz, H_3-10), δ_C 26.0 (C-10)}. The signals of β-D-glucose were detected at δ_H 4.33 (1H, d, J = 7.6 Hz, H-anomeric), and δ_C 104.4, 78.2, 76.9, 75.2, 71.6, 62.8. Base on the above evidence and comparison with the literature data, compound **3** was identified as aruncide A.[14]

Compound **4** was obtained as a colorless syrup. The molecular weight of **4** was suggested by a $[M + Na]^+$ peak at *m/z* 383 in the FAB-MS. The IR spectrum of **4** demonstrated absorption bands due to hydroxyl (3386 cm^{-1}), four-membered lactone (1760 cm^{-1}), and ether (1130 cm^{-1}) groups. The ^{1}H NMR spectrum revealed the presence of a four-membered lactone with two signals at δ_H 2.74 (1H, dd, J = 2.4, 9.2 Hz, H-3) and δ_H 4.83 (1H, d, J = 6.0 Hz, H-4), together with their corresponding ^{13}C NMR signals at δ_C 175.0 (C-2), 50.8 (C-3) and 82.5 (C-4). The signals of an isobutenyl groups were observed at {δ_H 5.30 (1H, d, J = 9.2 Hz, H-8), δ_C 122.3 (C-8), δ_C 142.8 (C-9)}, {δ_H 1.80 (3H, s, H_3-10), δ_C 18.8 (C-10)}, {δ_H 1.82 (3H, s, H_3-11), δ_C 26.2 (C-11)}. The ^{1}H and ^{13}C NMR spectra of compound **4** were presented signals of a glucoside of hydroxymethyl {δ_H 4.41 (1H, dd, J = 10.8, 11.2 Hz, H-12a), δ_H 3.88 (1H, dd, J = 2.8, 11.6 Hz, H-

12b), δ_C 68.5 (C-12)} at C-7. The signals of two protons at δ_H 4.29 (1H, dd, J = 7.6, 8.8 Hz, H-5), δ_H 3.94 (1H, dt, J = 2.4, 2.8, 10.4 Hz, H-7) and no ethoxyl group proved that a tetrahydrofuran ring was formed. The signals of β-D-glucose were identified at δ_H 4.33 (1H, d, J = 7.6 Hz, H-1'), and δ_C 99.6, 81.3, 79.9, 75.0, 71.9, 62.6. Finally, compound **4** was determined to be aruncide C by comparing with reported data.[14]

Compound **5** was isolated as a dark-colored syrup. Its ^{1}H NMR spectrum displayed the presence of an aromatic ABX-spin system with a *meta*-coupled aromatic signal at δ_H 7.08 (1H, brs, H-2), *ortho*-coupled aromatic resonance at δ_H 6.80 (1H, d, J = 7.6 Hz, H-5), and *ortho–meta*-coupled aromatic signal δ_H 6.96 (1H, d, J = 6.4 Hz, H-6) for a 3,4-dihydroxyphenyl unit. In addition, the signals of two olefinic protons at δ_H 7.66 (1H, d, J = 15.6 Hz, H-7), and δ_H 6.31 (1H, d, J = 16.0 Hz, H-8) were consistent with a *trans*-configured double bond of a caffeoyl moiety. The signals of β-D-glucose were observed at δ_H 5.62 (1H, d, J = 6.0 Hz, H-anomeric), and δ_C 95.7, 78.6, 77.9, 74.0, 71.0, 62.3. The ^{1}H and ^{13}C NMR spectra of compound **6** were similar to compound **5** without the signals of β-D-glucose moiety. Compounds **5** and **6** were finally identified as 1-*O*-caffeoyl-β-D-glucopyranose and caffeic acid, respectively, by comparison of the spectroscopic data with those in literature.[15]

The six isolated compounds were tested for their cytotoxic activity against HeLa, HL-60, and MCF-7 cancer cell lines. Their results were shown in Table 1. Compounds **1** and **2** possessed strong inhibitory activity against HL-60 cell line with IC_{50} values of 6.43 ± 1.46 and 6.10 ± 0.71 μM, respectively, as well as considerable inhibition towards HeLa and MCF-7 cancer cells with IC_{50} values ranging from 55.64 to 69.52 μM. Compound **3** demonstrated selective cytotoxic effect on HL-60 cell line with IC_{50} value of 6.27 ± 0.17 μM. Compound **4** showed high ability on inhibition towards HeLa and HL-60 cells with IC_{50} values of 5.38 ± 0.92 and 7.22 ± 0.94 μM, respectively. Especially, compound **5** exhibited potent cytotoxic effect toward HL-60 (IC_{50} 2.25 ± 0.09 μM) and significant cytotoxicity on MCF-7 cells (IC_{50} 4.32 ± 0.15 μM). In addition, the results displayed good inhibition of compound **6** towards all three cell lines with IC_{50} values ranging from 11.27 to 26.81 μM.

The results of the DPPH radical scavenging assay indicated that compounds **5** and **6** had strong antioxidant activity with IC_{50} values of 6.87 ± 0.03 and 4.33 ± 0.22 μM, respectively (Table 1), compared with the reference standard, L-ascorbic acid (IC_{50} 3.46 ± 0.11 μM). These are in accord with previous publications in which 1-*O*-caffeoyl-β-D-glucopyranose showed high activity with IC_{50} values ranging from 4.7 to 46.4 μM.[16]

Compounds **1** and **2** belong to cyanogenic glycosides group occurring widely in plants.[12] Sambunigrin (**1**) has been reported in a number of plants of various families such as Caricaceae, Caprifoliaceae, and Fabaceae.[17] Prunasin (**2**) has been demonstrated in at least six families including: Polypodiaceae, Myrtaceae, Rosaceae, Saxifragaceae, Scrophulariaceae, and Myoporaceae. These two compounds had weak or no inhibitory activity against

Table 1. Cytotoxic and DPPH radical scavenging activities of compounds **1 - 6** from the aerial parts of *A. dioicus* var. *kamtschaticus*

Compound	Cytotoxic activity IC_{50} (μM)			Antioxidant activity
	HeLa[c)]	HL-60[d)]	MCF-7[e)]	IC_{50} (μM)
1	69.52 ± 1.83	6.43 ± 1.46	61.50 ± 2.98	> 20
2	65.77 ± 3.16	6.10 ± 0.71	55.64 ± 1.35	> 20
3	> 100	6.27 ± 0.17	> 100	> 20
4	5.38 ± 0.92	7.22 ± 0.94	> 100	> 20
5	> 100	2.25 ± 0.09	4.32 ± 0.15	6.87 ± 0.03
6	26.81 ± 1.67	11.27 ± 3.72	12.93 ± 0.50	4.33 ± 0.22
adriamycin[a)]	3.09 ± 0.42	0.22 ± 0.11	2.83 ± 0.02	NT[f)]
L-ascorbic acid[b)]	NT	NT	NT	3.46 ± 0.11[g)]

[a)] Positive control for MTT assay.
[b)] Positive control for DPPH assay.
[c)] Human cervical epitheloid carcinoma cells.
[d)] Human promyelocytic leukemia cells.
[e)] Human breast cancer cells.
[f)] Not tested.
[g)] Results are expressed as IC_{50} values (μM) ± standard deviation of three experiments performed in triplicate.

hACAT1/2 (human AcylCoA: cholesterol acyltransferase) and LDL-oxidation (low-density lipoprotein).[18] Sambunigrin was also reported to have no inhibitory effects on 15-LO (15-lipoxygenase) and XO (xanthine oxidase).[19] Prunasin, however, significantly inhibited the Epstein-Barr virus early antigen activation induced by tumor promoter, an *in vitro* assay for assessing anti-tumor promoting activity.[20] In the present study, these cyanogenic glycosides showed inhibitory effect towards HeLa, HL-60, and MCF-7 cancer cells. Nevertheless, all cyanogenic glycosides have potential toxicity to animals and humans because of the generation of HCN via hydrolysis.[21] Therefore, further research should be conducted to determine their content in *A. dioicus* var *kamtschaticus,* as well as determine a safe dosage when using this plant as a traditional medicine.

An ideal anticancer drug must be selective and cytotoxic to cancer cells. It should be noted that of over 600,000 compounds screened, less than 40 agents were routinely used in the clinic.[22] Most molecularly targeted agents do not proceed to advanced stages in human clinical trials due to either efficacy or toxicity concerns.[23] In our previous report, compounds **3** and **4** demonstrated no cytotoxic effect toward Jurkat T cells.[14] Our present study reveals that aruncide A (**3**) possessed selective cytotoxic effect toward HL-60 cell line, while it had no or a weak effect towards HeLa and MCF-7 cancer cell lines. Hence, it can be considered a potential agent in anticancer drug development. Additional investigation, however, should be performed to clarify the mechanism of this activity in detail.

Park *et al*.[24] reported that compound **5** exhibited good scavenging effect on not only DPPH radical but also NBT superoxide with IC_{50} values of 31.69 ± 1.58 and 70.94 ± 0.91 μM, respectively. Nevertheless, the result of *in vitro* anti-inflammatory assay showed that compound **5** had no inhibitory effect on nitric oxide production in LPS-stimulated RAW264.7 cells. Caffeic acid (**6**) is widely distributed in vegetables, fruits, coffee and tea.[25] Besides strong antioxidant activity, caffeic acid has been proven to possess various bioactivities such as increasing collagen production, prevention of premature aging, antimicrobial activity,[26] *in vitro* hepatoprotective effect from ethanol-induced oxidative stress,[27] protecting human blood lymphocytes from UV-induced oxidative insult,[28] and anti-HBV activity[29]. The present bioactive results of compounds **5** and **6,** together with those in published literature have proved the potential use of caffeic acid and its derivatives in pharmaceutical preparations. Therefore, further studies should be conducted to investigate their applicability in human clinical trials.

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