Cytotoxic and Anti-oxidant Constituents from the Aerial Parts of *Aruncus dioicus* var. *kamtschaticus*

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**Abstract** – Ten compounds (1-10), palmitic acid (1), 10-nonacosanol (2), pentacosan-1-ol (3), phytol (4), β-sitosterol (5), β-sitosterol-3-O-β-D-glucopyranoside (6), 2,4-dihydroxycinnamic acid (7), hyperoside (8), uridine (9) and adenosine (10), were isolated from the *n*-hexane and EtOAc-soluble fractions of the aerial parts of *A. dioicus* var. *kamtschaticus* (Rosaceae). The structures of these compounds were elucidated on the basis of spectroscopic evidence. All compounds (1-10) were isolated for the first time from this plant. Cytotoxicity of 1-10 against Jurkat T (T-lymphocytic leukemia cells), HeLa (Human cervical epitheloid carcinoma cells), MCF-7 (Human breast cancer cells), and HL-60 (Human promyelocytic leukemia cells) cell lines was measured. Compound 6 showed good cytotoxicity against HL-60 cell line with IC₅₀ value of 8.13 µg/mL. In addition, compounds 7 and 8 exhibited antioxidant activity with IC₅₀ values of 16.30 and 12.42 µg/mL, respectively.

**Keywords** – *Aruncus dioicus* var. *kamtschaticus*, Rosaceae, Cytotoxicity.

**Introduction**

Ulleungdo (Island) which is located at the east longitude 130° 54' and the north latitude 37° 29' of the East Sea, is 130 km away from the mainland of Korea, and has special local products such as edible wild vegetables and marine products (Park et al., 1997). The major wild leafy vegetable produced in Ulleungdo is *Aruncus dioicus* var. *kamtschaticus* (Rosaceae) (Ulleung-Gun Agriculture Technology Center, 2002). Biological studies have revealed that this plant possesses numerous effects such as anti-oxidant (Kwon et al., 2006; Kim et al., 2002), anti-diabetic (Shin et al., 2008), anti-AIDS (Min et al., 1998) and prevention and treatment of ischemic and degenerative brain diseases (Lee et al., 2006). The dried sprout of this plant has been used for food, and the aerial parts of this plant also have been used to treat detoxification and tonsillitis (Kim et al., 1998).

This study was conducted to identify bio-active compounds from this plant. The aerial parts of *A. dioicus* var. *kamtschaticus* were extracted with 95% ethanol (EtOH), and its extract was concentrated and fractionated into five parts; *n*-hexane, methylene chloride (CH₂Cl₂), ethyl acetate (EtOAc), n-butanol (n-BuOH) and water (H₂O) fractions. The EtOH extract and fractions were examined on DPPH radical scavenging activity. Among the samples tested, *n*-hexane and EtOAc fractions showed radical scavenging activity.

**Experimental**

**Instruments and chemicals** – Melting points were determined on a Yanaco micro melting point apparatus and were uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. IR spectra were measured on a Mattson Polaris FT/IR-300E spectrophotometer. UV spectra were measured on a Thermo 9423AQA2200E UV spectrophotometer. NMR spectroscopy was taken on a Varian Unity INOV A-400 spectrometer. Low- and high-resolution EI-MS and FAB-MS data were collected on a Quattro II spectrometer. 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). TLC tests were performed on Merck precoated silica gel 60 F₂₅₄ (EM 5717) and/or RP-18 F₂₅₄g glass plates (0.25 mm),
and visualized by spraying with 10% H$_2$SO$_4$ and subsequent heating. 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 in KyeongBuk, Republic of Korea. These materials were confirmed taxonomically by Professor Byung Sun Min, College of Pharmacy, Catholic University of Daegu, Korea. A voucher specimen (CUDP 200601) has been deposited at the College of Pharmacy, Catholic University of Daegu, Korea.

**Extraction and isolation** – The aerial parts of *A. dioicus* var. *kamtschaticus* (6.90 kg) were extracted with H$_2$O (600.0 g), respectively. Fraction H13 (4.7 g) was chromatographed on a reverse-phase column (4.0 × 57 cm) using H$_2$O-Cl$_2$ mixture as a solvent and eluted with a stepwise gradient (100% H$_2$O to 0% Cl$_2$) gradient. Fractions (H1 to H37) were collected and pooled according to their similar TLC patterns. Fraction H1 (92.1 mg) was chromatographed on a normal column (3.5 × 15 cm) using n-hexane-CH$_2$Cl$_2$ mixture as a solvent and eluted with a stepwise gradient (100 : 1 to 20 : 1) to yield compound 1 (30.1 mg). Fraction H3 (100.1 mg) was chromatographed on a normal column (3.5 × 15 cm) using n-hexane-CH$_2$Cl$_2$ mixture as a solvent and eluted with a stepwise gradient (80 : 1 to 2 : 1) to yield compound 2 (30.0 mg). Fraction H6 (230.2 mg), respectively. Fraction H12 (7.6 g) was chromatographed on a normal column (5.0 × 15 cm) using n-hexane-CH$_2$Cl$_2$ mixture as a solvent and eluted with a stepwise gradient (5 : 1 to 2 : 1) to yield compound 5 (3.05 g). Fraction H13 (4.7 g) was chromatographed on a normal column (5.0 × 15 cm) using n-hexane-CH$_2$Cl$_2$ mixture as a solvent and eluted with a stepwise gradient (3 : 1 to 1 : 1) to yield compound 6 (4.3 g).

The most active DPPH radical scavenging EtOAc fraction (121.3 g) was chromatographed over a silica gel column (15 × 35 cm) and eluted with a gradient of CH$_3$Cl-MeOH-H$_2$O to afford 20 fractions (E1-E20). Fraction E4 (3.5 g) was chromatographed on a reverse-phase column (4.0 × 57 cm) using MeOH-H$_2$O mixture as a solvent and eluted with a stepwise gradient (100% H$_2$O to 70% MeOH) to yield compounds 7 (23.0 mg) and 8 (280.2 mg), respectively. Fraction E6 (8.1 g) was chromatographed on a reverse-phase column (4.0 × 57 cm) using MeOH-H$_2$O mixture as a solvent and eluted with a stepwise gradient (100% H$_2$O to 80% MeOH) to yield compounds 9 (12.3 mg) and 10 (13.7 mg), respectively.

**Palmitic acid (1)** – White powder; EI-MS *m/z* 256 [M]$^+$; m.p. 66.5–67.5 °C; IR (KBr) cm$^{-1}$ 3317, 1738, 1061; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 2.36 (2H, t, $J$ = 6.8, H-2), 1.63 (2H, m, H-3), 1.31-1.26 (24H, m) 0.89 (3H, t, $J$ = 6.4, H-16); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$: 179.9 (C-1), 34.2 (C-2), 24.9 (C-3), 29.9-29.3 (C-4-13), 32.1 (C-14), 22.9 (C-15), 14.3 (C-16).

**10-Nonacosanol (2)** – White powder; EI-MS *m/z* 424 [M]$^+$; m.p. 80.0–82.7 °C; IR (KBr) cm$^{-1}$ 3289, 2912, 2848, 1065; $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$: 0.88 (6H, t, $J$ = 6.8 Hz, H-1, 29), 3.58 (1H, m, H-20); $^{13}$C-NMR (CDCl$_3$, 100 MHz) $\delta$: 14.3 (C-1, 29), 72.3 (C-20), 22.9 (C-2, 28), 25.9 (C-3, 27), 37.7 (C-19, 21), 32.1 (C-18, 22).

**Pentacosan-1-ol (3)** – White powder; EI-MS *m/z* 368 [M]$^+$; m.p. 66.8–68.2 °C; IR (KBr) cm$^{-1}$ 3273, 1061; $^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$: 5.51 (1H, t, $J$ = 6.2 Hz, H-2), 1.67 (3H, s, H-20), 1.03–1.56 (20H, m, H-5–15), 0.86–0.84 (H-16–19); $^{13}$C-NMR (CDCl$_3$, 100 MHz) $\delta$: 59.6 (C-1), 123.3 (C-2), 140.4 (C-3), 40.0 (C-4), 25.3 (C-5), 36.8 (C-6), 32.9 (C-7), 37.6 (C-8), 24.6 (C-9), 37.5 (C-10), 32.9 (C-11), 37.5 (C-12), 25.1 (C-13), 39.5 (C-14), 28.1 (C-15), 22.8 (C-16), 22.9 (C-17), 19.9 (C-18), 19.9 (C-19), 16.3 (C-20).

**Phytol (4)** – White powder; EI-MS *m/z* 296 [M]$^+$; IR (KBr) cm$^{-1}$ 3743, 2924, 1670, 1078; $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$: 4.15 (2H, d, $J$ = 6.8 Hz, H-1), 5.41 (2H, t, $J$ = 6.8 Hz, H-2), 1.99 (2H, t, $J$ = 8.0 Hz, H-4), 1.67 (3H, s, H-20), 1.03–1.56 (20H, m, H-5–15), 0.86–0.84 (H-16–19); $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$: 59.6 (C1), 123.3 (C2), 140.4 (C3), 40.0 (C4), 25.3 (C5), 36.8 (C6), 32.9 (C7), 37.6 (C8), 24.6 (C9), 37.5 (C10), 32.9 (C11), 37.5 (C12), 25.1 (C13), 39.5 (C14), 28.1 (C15), 22.8 (C16), 22.9 (C17), 19.9 (C18), 19.9 (C19), 16.3 (C20).

**$\beta$Sitosterol (5)** – White powder; EI-MS *m/z* 414 [M]$^+$; m.p. 135.6–139.1 °C; IR (KBr) cm$^{-1}$ 3420, 2935, 2864, 1457, 1375, 1052; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$: 5.55 (1H, d, $J$ = 1.2 Hz, H-6), 3.53–3.46 (1H, m, H-3); $^{13}$C