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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 IC_{50} value of 8.13 μg/mL. In addition, compounds **7** and **8** exhibited antioxidant activity with IC_{50} 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 (CH_2Cl_2), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH) and water (H_2O) 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 INOVA-400 spectrometer (USA), and chemical shifts are expressed as δ values using TMS as an internal standard. 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 F_{254} (EM 5717) and/or RP-18 F_{254s} glass plates (0.25 mm),

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and visualized by spraying with 10% H_2SO_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 95% EtOH (18 L × 2) at room temperature for 36 hours. The EtOH extract was concentrated under reduced pressure to make an EtOH extract (2.03 kg). The concentrated EtOH extract was suspended in H_2O (6.0 L) and partitioned successively with *n*-hexane (6 × 5 L, 308.0 g), CH_2Cl_2 (6 × 5 L, 41.7 g), EtOAc (6 × 5 L, 121.3 g), *n*-BuOH (6 × 5 L, 353.8 g), and H_2O (600.0 g), respectively. The EtOH extract, *n*-hexane, CH_2Cl_2, EtOAc, *n*-BuOH, and H_2O-soluble fractions were assayed for DPPH radical scavenging activity. The *n*-hexane fraction (150.0 g) was subjected to open flash column chromatography over silica gel (6.5 × 25 cm) eluted with *n*-hexane-CH_2Cl_2 (100 : 0 to 0 : 100) and CH_2Cl_2-MeOH-H_2O (100 : 0 : 0.1 to 0 : 100 : 0.1) 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_2Cl_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_2Cl_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) was chromatographed on a normal column (3.5 × 15 cm) using *n*-hexane-CH_2Cl_2 mixture as a solvent and eluted with a stepwise gradient (20 : 1 to 5 : 1) to yield compounds **3** (32.1 mg) and **4** (42.3 mg), respectively. Fraction H12 (7.6 g) was chromatographed on a normal column (5.0 × 15 cm) using *n*-hexane-CH_2Cl_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_2Cl_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_2Cl_2-MeOH-H_2O 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_2O mixture as a solvent and eluted with a stepwise gradient (100% H_2O 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_2O mixture as a solvent and eluted with a stepwise gradient (100% H_2O 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; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 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, $CDCl_3$) *δ*: 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; ^1H-NMR ($CDCl_3$, 400 MHz) *δ*: 0.88 (6H, t, *J* = 6.8 Hz, H-1, 29), 3.58 (1H, m, H-20); ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ*: 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; ^1H-NMR ($CDCl_3$, 400 MHz) *δ*: 5.51 (1H, t, *J* = 7.2 Hz, H-2), 4.15 (2H, d, *J* = 6.8 Hz, H-1), 1.99 (2H, t, *J* = 8.0 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) *δ*: 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; ^1H NMR ($CDCl_3$, 400 MHz): *δ* 4.15 (2H, d, *J* = 6.8 Hz, H-1), 5.41 (2H, t, *J* = 6.8 Hz, H-2), 1.99 (1H, t, *J* = 8.0 Hz, H-4), 0.86 (3H, d, *J* = 6.4 Hz, H-16), 0.85 (3H, d, *J* = 6.4 Hz, H-17), 0.87 (3H, d, *J* = 6.4 Hz, H-18), 0.88 (3H, d, *J* = 6.4 Hz, H-19), 1.67 (3H, s, H-20): ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 59.6 (C-1), 123.3 (C-2), 140.4 (C-3), 40.1 (C-4), 25.3 (C-5), 36.8 (C-6), 33.0 (C-7), 37.6 (C-8), 24.7 (C-9), 37.5 (C-10), 32.9 (C-11), 37.5 (C-12), 25.0 (C-13), 39.8 (C-14), 28.2 (C-15), 22.8 (C-16), 22.9 (C-17), 20.0 (C-18), 19.9 (C-19), 16.4 (C-20).

***β*-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; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 5.55 (1H, d, *J* = 1.2 Hz, H-6), 3.53~3.46 (1H, m, H-3); ^{13}C

NMR (100 MHz, $CDCl_3$) δ: 37.4 (C-1), 31.8 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 32.1 (C-7), 32.1 (C-8), 50.3 (C-9), 36.7 (C-10), 21.2 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.2 (C-17), 12.2 (C-18), 19.6 (C-19), 36.3 (C-20), 18.9 (C-21), 34.1 (C-22), 26.2 (C-23), 46.0 (C-24), 29.3 (C-25), 20.0 (C-26), 19.2 (C-27), 23.2 (C-28), 12.0 (C-29).

***β*-Sitosterol-3-*O*-*β*-D-glucose (6)** – White powder; EI-MS *m/z* 576 $[M]^+$, 414 $[M\text{-glucose}]^+$, 399 $[M\text{-glucose-}CH_3]^+$, 396 $[M\text{-glucose-}H_2O]^+$, 381 $[M\text{-glucose-}CH_3\text{-}H_2O]^+$, 329 $[M\text{-glucose-}C_6H_{13}]^+$, 303 $[M\text{-glucose-}C_7H_{11}O]^+$; m.p. 293.5~296.3 ºC; IR (KBr) cm^{-1} 3420, 1456, 1375, 1052, 1021; ^1H NMR (400 MHz, pyridine-d_5) δ: 5.34(1H, s, H-6), 4.40 (1H, d, $J = 8.0$, H-1), 0.65 (3H, s, H-28), 0.67 (3H, s, H-29), 0.88 (3H, s, H-18), 0.93 (3H, s, H-26), 0.90 (3H, s, H-19), 1.13(3H, s, H-21); ^{13}C NMR (100 MHz, pyridine-d_5) δ: 38.0 (C-1), 30.8 (C-2), 78.6 (C-3), 40.5 (C-4), 141.4 (C-5), 122.4 (C-6), 32.6 (C-7), 32.7 (C-8), 50.9 (C-9), 37.4 (C-10), 21.8 (C-11), 39.9 (C-12), 43.0 (C-13), 57.3 (C-14), 25.0 (C-15), 29.1 (C-16), 56.8 (C-17), 12.5 (C-18), 19.9 (C-19), 36.9 (C-20), 19.5 (C-21), 34.7 (C-22), 26.9 (C-23), 46.6 (C-24), 30.0 (C-25), 19.7 (C-26), 20.5 (C-27), 23.9 (C-28), 12.7 (C-29), 103.1 (C-1'), 75.9 (C-2'), 79.0 (C-3'), 72.2 (C-4'), 79.1 (C-5'), 63.4 (C-6').

2,4-Dihydroxycinnamic acid (7) – Yellow powder; EI-MS *m/z* 180 $[M]^+$; m.p. 192.3~194.2 ºC; IR (KBr) cm^{-1} 3432, 1721, 1642; ^1H NMR (CD_3OD, 400 MHz) δ: 7.02 (1H, d, $J = 2.0$ Hz, H-3), 6.92 (1H, dd, $J = 8.0$, 2.0 Hz, H-5), 6.77 (1H, dd, $J = 8.0$ Hz, H-6), 6.20 (1H, d, $J = 15.6$ Hz, H-7), 7.51 (1H, d, $J = 15.6$ Hz, H-8); ^{13}C NMR (CD_3OD, 100 MHz) δ: 127.9 (C-1), 149.6 (C-2), 115.3 (C-3), 147.2 (C-4), 123.0 (C-5), 115.7 (C-6), 116.7 (C-7), 171.3 (C-8).

Hyperoside (8) – Yellow powder; FAB-MS *m/z* 465 $[M + H]^+$; m.p. 232.1~233.5 ºC; IR (KBr) cm^{-1} 3444, 1654, 1608, 1508, 1054; ^1H NMR (400 MHz, CD_3OD) δ: 6.18 (1H, d, $J = 2.0$, H-6), 6.38 (1H, d, $J = 2.0$, H-8), 8.04 (1H, d, $J = 2.0$, H-2'), 6.84 (1H, d, $J = 8.4$, H-5'), 7.57 (1H, dd, $J = 8.4$, 2.0, H-6'), 5.15 (1H, d, $J = 7.6$, H-1"); ^{13}C NMR (100 MHz, CD_3OD) δ: 158.9 (C-2), 135.9 (C-3), 179.7 (C-4), 163.2 (C-5), 100.1 (C-6), 166.4 (C-7), 94.9 (C-8), 158.6 (C-9), 105.5 (C-10), 123.0 (C-1'), 116.2 (C-2'), 146.0 (C-3'), 150.1 (C-4'), 117.9 (C-5'), 123.0 (C-6'), 105.5 (C-1"), 77.3 (C-2"), 75.2 (C-3"), 73.3 (C-4"), 70.2 (C-5), 62.1 (C-6").

Uridine (9) – White powder; EI-MS *m/z* 245 $[M]^+$; m.p. 163.4~165.9 ºC; IR (KBr) cm^{-1} 3418, 2954, 1702, 1552; ^1H NMR (CD_3OD, 400 MHz) δ: 5.71 (1H, d, $J = 8.0$ Hz, H-5), 8.03 (1H, d, $J = 8.0$ Hz, H-6), 5.92 (1H, d, $J = 4.8$ Hz, H-1'); ^{13}C NMR (CD_3OD, 100 MHz) δ: 152.8 (C-2), 166.3 (C-4), 102.8 (C-5), 142.9 (C-6), 90.9 (C-1'), 86.5 (C-2'), 75.9 (C-3'), 71.5 (C-4'), 62.4 (C-5').

Adenosine (10) – White powder; FAB-MS *m/z* 310 $[M + H]^+$; m.p. 234.1~235.2 ºC; ^1H NMR (CD_3OD, 400 MHz) δ: 8.42 (1H, s, H-2), 8.63 (H, s, H-8), 6.74 (1H, d, $J = 6.4$ Hz, H-1'); ^{13}C NMR (CD_3OD, 100 MHz) δ: 151.8 (C-2), 148.4 (C-4), 119.9 (C-5), 156.2 (C-6), 139.1 (C-8), 89.3 (C-1'), 86.3 (C-2'), 74.0 (C-3'), 70.9 (C-4'), 61.5 (C-5').

DPPH radical-scavenging activity – The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity was measured using the method described by Tagashira et al. (1998). Briefly, 10 μL of each sample dissolved in EtOH was prepared in the 96-well microplate, and then 200 μL of 100 μM methanolic DPPH solution was added. After mixing and standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 517 nm. L-Ascorbic acid (Sigma-Aldrich; purity: > 99%) was used as the positive control for DPPH radical-scavenging activity.

Cytotoxicity – 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 ºC in a 5% CO_2 incubator. Cytotoxic activity was measured using a modified MTT assay (Kim Van *et al.*, 2009). Viable cells were seeded in the growth medium (100 μL) into 96-well microtiter plates (1×10^4 cells per well) and incubated at 37 ºC in a 5% CO_2 incubator. The test sample was dissolved in DMSO and adjusted to final sample concentrations ranging from 5.0 to 150 μM 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, 10 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 48 h of the test sample treatment, MTT (5 mg/mL, 10 μL) was also added to the each well. After 4 h incubation, the plates were removed, and the resulting formazan crystals were dissolved in DMSO (150 μL). The OD was measured 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).

Results and Discussion

Ten compounds (**1 - 10**) were isolated from the *n*-hexane and EtOAc fractions of the aerial parts of *A. dioicus* var. *kamtschaticus* by repetitive column chroma-

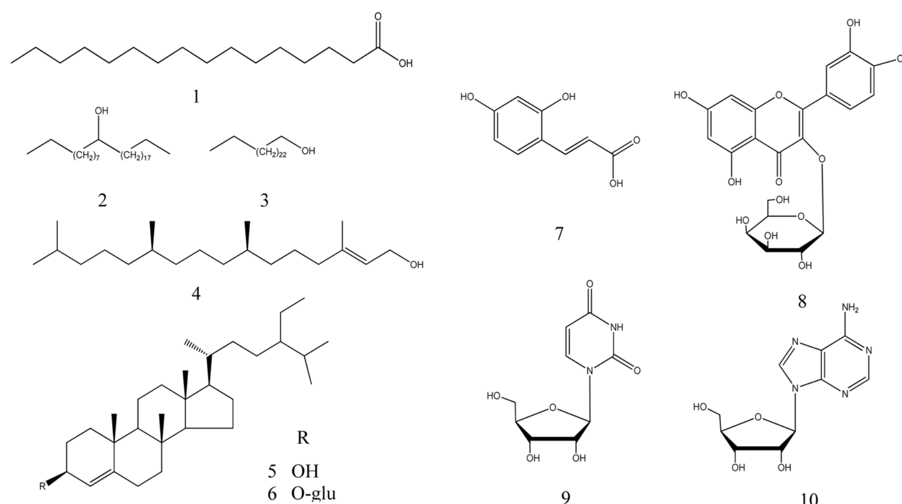

Fig. 1. Structures of compounds **1** - **10** isolated from the aerial parts of *A. dioicus* var. *kamtschaticus*.

Table 1. MTT cytotoxic and DPPH radical scavenging activities of **1** - **10**

Compound	Cytotoxic activity IC_{50} (μg/mL)				Antioxidant activity
	Jurkat T[c)]	HeLa[d)]	MCF-7[e)]	HL-60[f)]	IC_{50} (μg/mL)
1	> 100	37.38 ± 3.21	88.81 ± 4.41	61.97 ± 2.75	> 100
2	> 100	> 100	> 100	> 100	> 100
3	> 100	> 100	> 100	> 100	> 100
4	> 100	> 100	> 100	88.23 ± 2.12	> 100
5	> 100	48.61 ± 3.45	> 100	52.68 ± 4.23	> 100
6	> 100	31.83 ± 0.86	> 100	8.13 ± 1.10	> 100
7	78.84 ± 2.35	> 100	> 100	> 100	16.30 ± 0.21
8	> 100	77.45 ± 3.42	> 100	> 100	12.42 ± 0.38
9	> 100	> 100	> 100	> 100	> 100
10	75.76 ± 2.14	54.51 ± 2.58	> 100	> 100	> 100
Adriamycin[a)]	9.62 ± 0.20	2.80 ± 0.04	5.32 ± 0.02	0.68 ± 0.03	NT[g)]
L-ascorbic acid[b)]	NT	NT	NT	NT	2.88 ± 0.02

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

tography using silica gel and Lichroprep RP-18. Compounds **1** - **10** were identified as palmitic acid (**1**) (Crane *et al*., 2005), 10-nonacosanol (**2**) (Wen *et al*., 2006), pentacosan-1-ol (**3**) (Grella *et al*., 1990), phytol (**4**) (Brown *et al*., 1994), *β*-sitosterol (**5**) (Iribarren *et al*., 1983), *β*-sitosterol-3-O-*β*-D-glucopyranoside (**6**) (Cheng *et al*., 1992), 2,4-dihydroxycinnamic acid (**7**) (Lin *et al*., 1999), hyperoside (**8**) (Schittgo *et al*., 1999), uridine (**9**) (Luyten *et al*., 1998), and adenosine (**10**) (Zhou *et al*., 2005), respectively, by spectroscopic methods as well as by comparison of their data with literature values. To the best of our knowledge, all compounds (**1** - **10**) were isolated for the first time from this plant (Fig. 1).

Ten compounds isolated from *A. dioicus* var. *kamtschaticus* were tested for their cytotoxic activity against the Jurkat T, HeLa, MCF-7 and HL-60 cell lines. Compounds **7** and **10** showed cytotoxic activity with IC_{50} values of 78.84 and 75.76 μg/mL against Jurkat T cell

line, compounds **1**, **5**, **6**, **8** and **10** with IC_{50} values of 37.38, 48.61, 31.83, 44.45 and 54.51 μg/mL against HeLa cell line, compound **1** with IC_{50} value of 88.81 against MCF-7 cell line, and compounds **1**, **4**, **5** and **6** with IC_{50} values of 61.97, 88.26, 52.68 and 8.13 μg/mL against HL-60 cell line, respectively (Table 1). There is no report on cytotoxicity of **1 - 10** against Jurkat T, HeLa, MCF-7 and HL-60 cell lines. Especially, compound **6** (*β*-sitosterol-3-O-*β*-D-glucopyranoside) showed good cytotoxicity against HL-60 cell line.

The radical scavenging activity of compounds **1 - 10** was evaluated in the DPPH radical scavenging assay. Compounds **7** and **8** exhibited antioxidant activity with IC_{50} values of 16.30 and 12.42 μg/mL, respectively, compared with the reference standard, L-ascorbic acid (IC_{50} of 2.88 μg/mL) (Table 1). This observation is consistent with previous report that compounds **7** and **8** showed potent antioxidant activity (Terpinc *et al*., 2010; Soberón *et al*., 2010).

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