Natural Product Sciences
17(2) : 80-84 (2011)

Isolation of Compounds from Cimicifugae Rhizoma and their Cytotoxic Activity†

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Abstract – Five known compounds, cimigenol (**1**), 25-*O*-acetylcimigenol (**2**), cimigenol 3-*O*-β-D-xylopyranoside (**3**), ferulic acid methyl ester (**4**), and visnagin (**5**), were isolated from Cimicifugae Rhizoma (Ranunculaceae). The chemical structures of these compounds were determined on the basis of spectroscopic analyses including 2D NMR. Compounds **1** - **5** were evaluated for their cytotoxic activity against the HL-60, MCF-7, and A549 cancer cell lines in *in vitro*. Among them, compounds **4** and **5** showed moderate inhibitory activity against HL-60 cancer cell lines with IC_{50} values of 24.8 and 18.1 μM, respectively.
Keywords – Cimicifugae Rhizoma, Ranunculaceae, Visnagin, Cytotoxic activity

Introduction

The genus *Cimicifuga* is one of the smallest genera in the family Ranunculaceae. It comprises about 25 species distributed throughout East Asia, Europe, and North America. Cimicifugae Rhizoma originated from rhizomes of *Cimicifuga simplex, C. dahurica*, *C. racemosa*, *C. foetida* and *C. heracleifolia*, has been used as anti-inflammatory, analgesic, and antipyretic remedies in Chinese traditional medicine. More ever, it has been used in combination with other oriental medicinal herb as anti-inflammatory drugs (Liberman *et al.,* 1998). According to reports published, two main classes of compound have been isolated from Cimicifugae Rhizoma: 9,19-cycloartane, highly oxygenated triterpene glycosides (Bedir *et al*., 2000; Shao *et al*., 2000; Wende *et al*., 2001; Berger *et al*., 1988), and aromatic acids (Kruse et al., 1999). Also, cinnamic acid derivatives, chromones (Kondo *et al*., 1972), indolinones (Baba *et al*., 1981), fukilic acid esters, piscidic acid esters, and caffeic derivatives were isolated from rhizomes of *Cimicifuga* species. To date, 16 triterpene glycosides have been isolated from *C. racemosa*, more than 50 compounds of this type have been isolated from *C. simplex* (Shao *et al*., 2000), and more than 20 triterpenes or triterpene glycosides have been reported from *C. foetida* (Kadota *et al*., 1995; Li *et al*., 1996; Qiu *et al*., 2006) exhibiting cytotoxic, anticomplement and immunosuppressive activities. This study is part of an ongoing investigation into cytotoxic active compounds from herbal medicines. In our study, extraction and fractionation of Cimicifugae Rhizoma resulted in the isolation of five compounds (**1** - **5**). This paper describes the isolation, structural elucidation of isolated compounds and their cytotoxic activity against various cancer cell lines.

Experimental

General experimental procedures – Optical rotations were measured with a JASCO DIP 370 digital polarimeter. UV spectra were recorded on a JASCO V-530 spectrophotometer, and IR spectra were obtained on a JASCO FT/IR 300-E spectrometer. NMR experiments were conducted on a Varian Unity INOVA 400 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on 400 and 100 MHz, respectively, and tetramethylsilane was used as the internal standard. TLC was carried out on Merck silica gel F_{254}-precoated glass plates and RP-18 F_{254S} plates (0.25 mm). Column chromatography (CC): silica gel 60 (70 - 230 mesh and 230 - 400 mesh; Merck, Germany) or reversed-phase silica gel (LiChroprep® RP-18, 40 - 63 μm; 310 × 25 mm; Merck, Germany).

Plant material – The Cimicifugae Rhizoma was purchased from Gangwonyakcho, Pyeong Chang, Gangwon province, Korea and identified by Prof. Byung-Sun Min, College of Pharmacy, Catholic University of Daegu, Korea. A voucher specimen (CUD-1384) was deposited at the herbarium of the college of Pharmacy, Catholic University of Daegu, Korea.

Extraction and isolation – The rhizomes (10 kg) were extracted three times with MeOH (3 × 15 L) at room

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temperature for seven days. The MeOH solution was combined and concentrated to yield a residue (985 g) that was suspended in water (2 L) and extracted with *n*-hexane (3 L × 3 times), EtOAc (3 L × 3 times), and *n*-BuOH (3 L × 3 times), successively, to afford *n*-hexane (186.5 g), EtOAc (260.3 g), and *n*-BuOH-soluble fractions (528.2 g), respectively. The EtOAc-soluble fraction (260.3 g) was chromatographed on a silica gel column eluting with a gradient of $CHCl_3$-MeOH (50 : 1 → 0 : 1) to give eight fractions (Fr.1 to Fr.8) according to their TLC profiles. Rechromatography of fraction 2 (9.4 g) on a silica gel column eluting with a gradient of $CHCl_3$-MeOH (30 : 1 → 5 : 1) afforded three subfractions (Fr.2-1 to Fr.2-3). Rechromatography of subfraction 2-2 (5.4 g) on a silica gel column eluting with a gradient of $CHCl_3$-acetone (10 : 1 → 0 : 1) afforded nine subfractions (Fr.2-2-1 to Fr.2-2-9). Subfraction Fr.2-2-3 (450.0 mg) was purified by silica gel column eluting with $CHCl_3$-MeOH (30 : 1 → 10 : 1) to afford compound **2** (54.2 mg). Subfraction Fr.2-2-4 (542.0 mg) was subjected to a silica gel column eluting with a gradient of hexane-acetone (5 : 1 → 1 : 1) to afford **1** (78.8 mg). Further purification of subfraction Fr.2-2-5 (462.0 mg) using silica gel column with a gradient of $CHCl_3$-MeOH (10 : 1 → 2 : 1) resulted in the isolation of **3** (15.0 mg). Subfraction Fr.2-2-7 (420.0 mg) was chromatographed over a reverse phase C_{18} column chromatography eluting with a gradient of MeOH-H_2O (from 3 : 1 to 10 : 1) resulted in the isolation of **4** (5.0 mg) and **5** (5.3 mg), respectively.

Cimigenol (**1**) – white powder; mp. 216 - 217 °C; $[\alpha]_D^{25}$ +12.8 (*c* 0.07, MeOH); UV λ_{max} (MeOH): 265.5 nm; IR (KBr): 3280 cm^{-1}; ESI-MS *m/z* 511.1 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_5Na$); ^{1}H-NMR (400 MHz, C_5D_5N): δ 3.30 (1H, dd, *J* = 5.2, 11.6 Hz, H-3), 3.92 (1H, d, *J* = 8.8 Hz, H-15), 2.55 (1H, d, *J* = 8.8 Hz, 15-OH), 1.40 (1H, d, *J* = 11.2 Hz, H-17), 0.38 (1H, d, *J* = 4.4 Hz, H-19a), 0.64 (1H, d, *J* = 4.4 Hz, H-19b), 4.47 (1H, br d, *J* = 9.2 Hz, H-23), 3.45 (1H, s, H-24), 1.10 (3H, s, H-18), 0.90 (3H, d, *J* = 6.8 Hz, H-21), 1.19 (3H, s, H-26), 1.20 (3H, s, H-27), 0.97 (3H, s, H-28), 0.98 (3H, s, H-29), 0.82 (3H, s, H-30); ^{13}C-NMR (100 MHz, C_5D_5N): δ 32.3 (C-1), 30.5 (C-2), 78.9 (C-3), 40.7 (C-4), 47.1 (C-5), 21.1 (C-6), 26.3 (C-7), 48.3 (C-8), 20.0 (C-9), 26.7 (C-10), 26.3 (C-11), 33.8 (C-12), 42.0 (C-13), 47.1 (C-14), 80.0 (C-15), 111.7 (C-16), 59.2 (C-17), 31.0 (C-19), 23.8 (C-20), 37.7 (C-22), 71.8 (C-23), 89.0 (C-24), 71.5 (C-25), 19.2 (C-18), 19.4 (C-21), 26.3 (C-26), 26.7 (C-27), 11.2 (C-28), 25.6 (C-29), 14.2 (C-30).

25-*O*-acetylcimigenol (**2**) – white powder; mp. 193 - 194 °C; $[\alpha]_D^{25}$ +28.7 (*c* 0.08, MeOH); UV λ_{max} (MeOH): 270 nm; IR (KBr): 3300, 1739, 1230, 1021 cm^{-1}; ESI-MS *m/z* 553.0 $[M + Na]^+$ (calcd for $C_{32}H_{50}O_6Na$); ^{1}H-NMR (400 MHz, C_5D_5N): δ 3.29 (1H, m, H-3), 3.91 (1H, d, *J* = 8.4 Hz, H-15), 2.66 (1H, d, *J* = 8.4 Hz, 15-OH), 4.38 (1H, d, *J* = 8.8 Hz, H-23), 3.89 (1H, s, H-24), 1.09 (3H, s, H-18), 0.89 (3H, d, *J* = 6.4 Hz, H-21), 1.41 (3H, s, H-26), 1.46 (3H, s, H-27), 0.95 (3H, s, H-28), 0.97 (3H, s, H-29), 0.81 (3H, s, H-30), 1.99 (3H, s, $COC\underline{H}_3$); ^{13}C-NMR (100 MHz, C_5D_5N): δ 32.2 (C-1), 30.5 (C-2), 78.9 (C-3), 40.7 (C-4), 47.1 (C-5), 21.1 (C-6), 26.2 (C-7), 48.3 (C-8), 20.0 (C-9), 26.7 (C-10), 26.3 (C-11), 33.8 (C-12), 42.0 (C-13), 47.1 (C-14), 79.8 (C-15), 112.1 (C-16), 59.0 (C-17), 31.0 (C-19), 23.8 (C-20), 37.9 (C-22), 71.9 (C-23), 86.5 (C-24), 82.6 (C-25), 19.2 (C-18), 19.4 (C-21), 21.9 (C-26), 23.2 (C-27), 11.2 (C-28), 25.5 (C-29), 14.2 (C-30), 22.6 (25-OCO$\underline{C}H_3$), 170.5 (25-O$\underline{C}$OCH$_3$).

Cimigenol 3-*O*-β-D-xylopyranoside (**3**) – white amorphous powder; mp. 266 - 269 °C; $[\alpha]_D^{25}$ +15.3 (*c* 0.13, MeOH); UV λ_{max} (MeOH): 284 nm; IR (KBr): 3400 cm^{-1}; ESI-MS *m/z* 643.0 $[M + Na]^+$ (calcd for $C_{35}H_{56}O_9Na$); ^{1}H-NMR (400 MHz, C_5D_5N): δ 3.54 (1H, dd, *J* = 3.6, 11.2 Hz, H-3), 4.04 (1H, d, *J* = 8.4 Hz, H-15), 4.61 (1H, d, *J* = 9.2 Hz, H-23), 3.66 (1H, s, H-24), 1.27 (3H, s, H-18), 0.86 (3H, d, *J* = 6.4 Hz, H-21), 1.33 (3H, s, H-26), 1.20 (3H, s, H-27), 1.16 (3H, s, H-28), 1.27 (3H, s, H-29), 1.07 (3H, s, H-30), 4.88 (1H, d, *J* = 6.4 Hz, H-1'), 4.13 (1H, d, *J* = 6.8 Hz, H-2'), 3.74 (1H, t, *J* = 10.4 Hz, H-3'), 4.4.25 (1H, m, H-4'), 4.20 (1H, m, H-5'a), 4.37 (1H, dd, *J* = 6.4, 11.2 Hz, H-5'b); ^{13}C-NMR (100 MHz, C_5D_5N): δ 32.9 (C-1), 30.6 (C-2), 89.1 (C-3), 38.5 (C-4), 47.7 (C-5), 21.5 (C-6), 26.2 (C-7), 48.0 (C-8), 20.0 (C-9), 26.8 (C-10), 26.7 (C-11), 34.5 (C-12), 41.8 (C-13), 49.1 (C-14), 80.6 (C-15), 112.4 (C-16), 59.8 (C-17), 31.4 (C-19), 24.4 (C-20), 37.7 (C-22), 71.7 (C-23), 88.7 (C-24), 72.0 (C-25), 20.0 (C-18), 19.8 (C-21), 26.5 (C-26), 26.7 (C-27), 12.3 (C-28), 24.5 (C-29), 15.9 (C-30), 108.0 (C-1¢), 76.7 (C-2'), 79.0 (C-3'), 72.1 (C-4'), 67.5 (C-5').

Ferulic acid methyl ester (**4**) – white solid; mp. 62 - 65 °C; UV λ_{max} (MeOH): 293, 323 nm; IR (KBr): 3383, 2950, 2844, 2645, 2356, 1599, 1169, 816, 567 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.29 (1H, d, *J* = 16.0 Hz, H-2), 7.60 (1H, d, *J* = 16.0 Hz, H-1) 7.14 (1H, s, H-5), 6.85 (1H, d, *J* = 8.4 Hz, H-5'), 7.03 (1H, d, *J* = 8.4 Hz, H-6'), 3.93 (3H, s, 3'-OCH$_3$), 3.79 (3H, s, 3-OCH$_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 168.0 (C-3), 115.8 (C-2), 145.0 (C-1), 128.1 (C-1'), 110.8 (C-2'), 146.1 (C-3'), 148.9 (C-4'), 117.3 (C-5'), 122.0 (C-6'), 56.1 (3'-O$\underline{C}$H$_3$), 51.8 (3-O$\underline{C}$H$_3$).

Visnagin (**5**) – pale yellow needles; mp. 141 - 143 °C; UV λ_{max} (MeOH): 243, 321 nm; IR (KBr): 1650, 1618, 1589 cm^{-1}; ESI-MS *m/z* 231.0 $[M + H]^+$ (calcd for

$C_{13}H_{11}O_4$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.60 (1H, d, J = 4.4 Hz, H-2), 7.04 (1H, d, J = 4.4 Hz, H-3) 6.05 (1H, s, H-6), 7.24 (1H, s, H-8), 2.34 (3H, s, 7-CH_3), 4.17 (3H, s, 4-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 145.3 (C-2), 105.5 (C-3), 117.0 (C-3a), 153.7 (C-4), 112.3 (C-4a), 178.9 (C-5), 110.8 (C-6), 164.4 (C-7), 156.1 (C-7a), 95.3 (C-8), 158.1 (C-8a), 61.8 (4-O<u>C</u>H_3), 20.1 (7-<u>C</u>H_3).

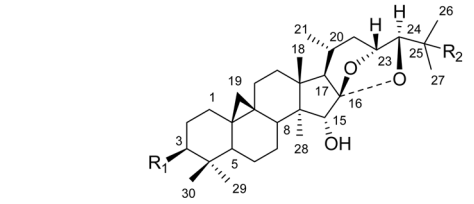
Determination of Sugar Component – Compound **3** (5 mg) was dissolved in 5% HCl (50 mL) and heated at 90 °C for 2 hours. The mixture was evaporated under vacuum to 20 mL and then extracted with EtOAc (25 mL × 3 time). After addition of H_2O, the acidic solution was evaporated again and then dried *in vacuo* to furnish a monosaccharide residue. From the residue, xylose was detected by co-TLC [solvent system: $CHCl_3$: MeOH : H_2O (8 : 5 : 1 v/v/v)] with authentic sample. The R_f value for the above sugar was 0.46, respectively.

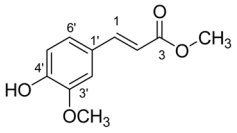
Cytotoxicity Assay – The cancer cell lines were maintained in RPMI 1640, which included L-glutamine with 10% FBS and 2% penicillin-streptomycin. Cells were cultured at 37 °C in a 5% CO_2 incubator. Cytotoxic activity was measured using a modified MTT assay (Kim Van *et al.,* 2009). Viable cells were seeded in the growth medium (100 μL) into 96-well microtiter plates (1×10^4 cells per well) and incubated at 37 °C 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.

Results and discussion

Cimicifugae Rhizoma was extracted with methanol. The MeOH extract was partitioned into *n*-hexane-, EtOAc-, and n-BuOH-fractions, successfully. Chromatographic purification of the EtOAc-soluble fraction led to the isolation of five compounds (**1** - **5**) (Fig. 1).

Compound **1** was isolated as white powder, mp 216 - 217 °C and showed $[\alpha]_D^{25}$ +12.8° (*c* 0.07, MeOH). The *electrospray* ionization *mass spectrometry* (ESI-MS)





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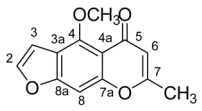

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Fig. 1. Chemical structures of isolated compounds **1 - 5**.

showed a molecular ion peak at *m/z* 511.1 $[M + Na]^+$, regarding to the molecular formula of **1** as $C_{30}H_{48}O_5$. The infrared (IR) spectrum of **1** showed absorption band at 3280 (OH). The ^{1}H-NMR spectrum of **1** showed signals due to a cyclopropane methylene (δ_H 0.38 and 0.64, each d, J = 4.4 Hz), four methine protons on carbon substituted by oxygen (δ_H 3.30, 3.45, 3.92 and 4.47), and a secondary methyl group (δ_H 0.90, d, J = 6.8 Hz), along with six *tert*-methyl groups (Fig. 1). These data can consider that **1** is highly oxygenated cyclotriterpenoid (Kimura *et al.,* 1983). The relative stereochemistry of **1** was determined on the basis of the chemical shift and coupling constants of each proton. The configuration at the C-23 and C-24 position was deduced to be *R* and *S* on the basis of the ^{1}H- and ^{13}C-NMR spectra [(δ_H 4.47, br d, J = 9.2 Hz, H-23/δ_C 71.8, C-23) and (δ_H 3.45, s, H-24/δ_C 89.0, C-24)], which is comparable with that of 25-*O*-acetyl-7,8-didehydrocimigenol [(δ_H 4.39, br d, J = 10.0 Hz, H-23/δ_C 71.88, C-23) and (δ_H 3.92, s, H-24)/δ_C 86.26, C-24] (Jian *et al.,* 1993). Furthermore, the signal at δ_H [2.55 (1H, d, J = 8.8 Hz)/δ_C 80.0], was assigned for 15-OH by comparison with that of 25-*O*-acetyl-7,8-didehydrocimigenol [(δ_H 2.73, d, J = 8.4 Hz, 15-OH)/δ_C 76.84, C-15]. Based on a comparison of the ^{1}H- and ^{13}C-NMR spectral data with those reported in the literature for a cycloartane-type triterpene isolated from *C. racemosa*, we speculated that the structure of **1** was assigned as cimigenol (Takemoto *et al.,* 1967).

Compound **2** was isolated as white powder with optical rotation as +28.7° (*c* 0.08, MeOH). In the ESI-MS, it showed a molecular ion peak at *m/z* 553.0 $[M + Na]^+$, which determined molecular formula of **2** as $C_{32}H_{50}O_6$. The infrared (IR) spectrum of **2** showed absorption band

at 3300 (OH) and 1739 (C = O). The ^{1}H-NMR spectrum resembled that of **1** except for the signals attributable to an acetyl group {[δ_H 1.99 (3H, s), δ_C 22.6], [δ_C 170.5]} in. When the ^{13}C-NMR spectrum of **2** was compared with those of **1**, the chemical shifts due to the 24-, 25-, 26-, 27-carbon signals (δ_C 86.5, 82.6, 21.9, 23.2) of **2** were distinctly different form those of **1** (δ_C 89.0, 71.5, 26.3, 26.7), and the acetyl group was suggested to be attached at the 25-position, replacing of hydroxyl group. Base on the above analysis, **2** was determined to be 25-*O*-acetylcimigenol (Takemoto *et al.,* 1969).

Compound **3** was isolated as white amorphous powder with positive optical rotation as +15.3° (*c* 0.13, MeOH). Its molecular formula was determined to be $C_{35}H_{56}O_9$ based on the ESI-MS (*m/z* 643.0 $[M + Na]^+$). The infrared (IR) spectrum of **3** showed absorption band at 3400 (OH). The ^{1}H- and ^{13}C-NMR spectra of **3** showed much closed similarity to those of **1**. However, additional signals due to a sugar were observed at [δ_C 108.0, 76.7, 79.0, 72.1, 67.5]. In the HMBC spectrum, the carbon signal due to C-3 (δ_C 89.1) showed long-range correlation with a signal due to an anomeric proton (δ_H 4.88), which suggested that xylose was substituted at the 3-position. To identify the sugar moiety, acid hydrolysis of **3** yielded D-xylose that was confirmed by co-TLC with authentic sample and in combination with NMR data interpretation. On the basis of these finding, the structure of **3** was assigned as cimigenol 3-*O*-β-D-xylopyranoside (Sakurai *et al.,* 1972).

Compound **4** was isolated as white solid. The IR spectrum of compound **4** showed one peak at 3383 cm^{-1} suggested the presence of OH functional group. The ^{1}H-NMR spectra of **4** showed three aromatic protons at [δ_H: 7.14 (1H, s, H-2'), 6.85 (1H, d, J = 8.4 Hz, H-5'), 7.03 (1H, d, J = 8.0 Hz, H-6')], two olefinic protons [δ_H: 7.60 (1H, d, J = 16.0 Hz, H-1), 6.29 (1H, d, J = 16.0 Hz, H-2)] and two methoxy signals [δ_H: 3.93 (3H, s, 3'-OCH$_3$), 3.79 (3H, s, 3-OCH$_3$)]. The ^{13}C-NMR spectrum of **4** showed eleven carbon signals including six carbon signals of benzene rings [δ_C: 128.1 (C-1'), 110.8 (C-2'), 146.1 (C-3'), 148.9 (C-4'), 117.3 (C-5'), 122.0 (C-6')], two olefinic carbons [δ_C: 145.0 (C-1), 115.8 (C-2)], two methoxy carbon groups [δ_C: 51.8 (3-O<u>C</u>H$_3$), 56.1 (3'-O<u>C</u>H$_3$)] and one carbonyl group [δ_C: 168.0 (C-3)]. Thus, the structure of compound **4** was determined as (*E*)-methyl-3-(4'-hydroxy-3'-methoxyphenyl)acrylate by comparison its physicochemical and spectral data with those of literature (Voisin-Chiret *et al.,* 2007).

Compound **5** was isolated as pale yellow needles with with the molecular formula $C_{13}H_{10}O_4$, as determined by the ESI-MS ($[M + H]^+$ *m/z* 231.0). The IR spectrum of compound **5** suggested the presence of C = O functional group at 1650 (ester C = O). The ^{1}H-NMR spectra of **5** showed one aromatic proton at [δ_H: 7.24 (1H, s, H-8], three olefinic protons [δ_H: 7.60 (1H, d, J = 4.4 Hz, H-2), 7.04 (1H, d, J = 4.4 Hz, H-3), 6.05 (1H, s, H-6)], one methoxy signal [δ_H: 4.17 (3H, s, 4-OCH$_3$), and one methyl signal [δ_H: 2.34 (3H, s, 7-CH$_3$)]. The ^{13}C-NMR spectrum of **5** showed thirteen carbon signals including six carbon signals of benzene rings [δ_C: 117.0 (C-3a), 153.7 (C-4), 112.3 (C-4a), 156.1 (C-7), 95.3 (C-8), 158.1 (C-8a)], three olefinic carbons [δ_C: 145.3 (C-2), 105.5 (C-3), 110.8 (C-6)], one methoxy carbon group [δ_C: 61.8 (4-O<u>C</u>H$_3$)], one methyl carbon signal [δ_C: 20.1 (7-<u>C</u>H$_3$)], and one carbonyl group [δ_C: 178.9 (C-5)]. Thus, the structure of compound **5** was determined as 4-methoxy-7-methyl-5*H*-furo[3,2-*g*]chromen-5-one, and named as visnagin by comparison its physicochemical and spectral data with those of literature (Ito *et al.,* 1976).

Table 1. Cytotoxic activity of isolated compounds against cancer cell lines

Compounds	IC_{50} (μM)		
	A549	HL-60	MCF-7
1	> 100	> 100	> 100
2	> 100	84.3	> 100
3	> 100	> 100	> 100
4	> 100	24.8	> 100
5	> 100	18.1	93.1
Adriamycin[a]	0.63	0.70	0.65

[a] Used as positive control.

Compounds **1 - 5** were evaluated for their *in vitro* cytotoxic activity against MCF-7, HL-60, and A549 cancer cell lines using MTT assay method with slight modification (Kim Van *et al.,* 2009). As the results in Table 1, compounds **2, 4**, and **5** showed inhibitory activity against HL-60 cancer cell lines with IC_{50} values of 84.3, 24.8 and 18.1 μM, respectively. In the case of MCF-7 and A549, these isolates displayed very weak cytotoxic activities against with IC_{50} values over than 100 μM.

Acknowledgements

This work was supported by a grant (09112 HerbalMedicine811) from the National Center for Standardization of Herbal Medicine funded by the Food Drug and Administration, Republic of Korea (2010) and supported by the National Research Foundation of Korea Grant funded by the Korean Government (MEST) (KRF-2010-0027720).

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Received November 29, 2010
Revised April 20, 2011
Accepted April 30, 2011