Antitumor activity of *Trichosanthes kirilowii*

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The activity-directed fractionation upon the MeOH extract of the root of *Trichosanthes kirilowii* led to the isolation of eight cucurbitane triterpenes namely cucurbitacin B (I), isocucurbitacin B (II), cucurbitacin D (III), isocucurbitacin D (IV), 3-epi-isocucurbitacin B (V), dihydrocucurbitacin B (VI), dihydroisocucurbitacin B (VII) and dihydrocucurbitacin E (VIII), as active principles. All isolates were shown to exhibit significant cytotoxicity against cultured human tumor cells, including A-549, SK-OV-3, SK-MEL-2, XF-498 and HCT 15, with an exceptionally high potency.

**Key words**: *Trichosanthes kirilowii*, Cucurbitacin B, Cucurbitacin D, Isocucurbitacin B, Isocucurbitacin D, 3-epi-isocucurbitacin B, Dihydrocucurbitacin B, Dihydroisocucurbitacin B, Dihydrocucurbitacin E, Antitumor

**INTRODUCTION**

The plant *Trichosanthes kirilowii* (Cucurbitaceae), called "Kwalu" in Korean, is one of the important herb drugs in northeast Asian Countries since ancient times. It has been widely used as a remedy for the regulation of the water balance and for the pyretolysis or as an anti-inflammatory agent. Earlier investigation upon the chemical constituents of *Trichosanthes kirilowii* was mainly dealt with the isolation of fatty acid or its methyl ester, amino acids such as citrulline and arginine, some sterols including the campesterol, sitosterol and stigmasterol, etc. (Kanaoka et al., 1982), glycans named trichosan A, B, C, D and E (Hikino et al., 1989), and a plant protein called trichosanthin which was regarded as a promising abortifacient or antitumor agent (Feng et al., 1986).

In the course of continuing search for the tumour inhibitors of plant origin, the methanolic extract of the root or the seed of *Trichosanthes kirilowii* was found to exhibit a significant inhibitory activity with an exceptionally high potency upon the growth of some human tumor cells carried out in cell culture. And it was also found that such a cytotoxic activity of *Trichosanthes kirilowii* was mainly concentrated in the CH$_2$Cl$_2$ soluble fraction, whereas the water soluble fraction, presumably believed to contain the trichosanthin which was reported to be a prominent antitumor agent, was shown to exhibit negligibly poor activity. These results suggested that this plant could contain another active compounds, totally different from the plant protein trichosanthin and this suggestion prompted us to reinvestigate the active constituents of *Trichosanthes kirilowii* guided by the bioassay. Present paper dealt with the isolation of active principles I-VIII, from the root of *Trichosanthes kirilowii* by the way of the activity-directed fractionation on the basis of the inhibitory activity upon the growth of tumor cells, *in vitro*.

**MATERIALS AND METHODS**

$^1$H-NMR spectra were run at 300 MHz and $^{13}$C-NMR at 75 MHz and recorded by Bruker AM-300. Low resolution MS (70 eV) were taken with a direct inlet and recorded by JMS-DX303 mass spectrometer (JEOL). Human tumor cells used in the experiment, i.e., A549 (non small cell lung), SK-OV-3 (ovary), SK-MEL-2 (skin), XF498 (central nerve system) and HCT-15 (colon) were obtained from the National Cancer Institute (NCI) in the USA, which were currently used in the NCI's *in vitro* anti-cancer drug screening. Stock cell cultures were grown in T-25 (falcon) flasks containing 10 ml of RPMI-1640 medium with glutamine, sodium bicarbonate and 5% fetal calf serum, which were dissociated with 0.25% trypsin and 30 mM 1,2-cyclohexane-
diaminotetraacetic acid (CDTA) in PBS in case of transfer or dispense before experiment.

Test for the cytotoxicity in vitro

All experimental procedures were followed up the NCI's protocol based on the SRB method (Skehan et al., 1990). Detailed experimental procedures were described on the previous paper (Ryu et al., 1992).

Extraction and Isolation

The root of Trichosanthes kirilowii, which was commercially available, was purchased at market and 6 kg of the crude material was extracted with MeOH by reflux for 4 hours. The resultant MeOH extract was subjected to evaporation and suspended in water, followed by the successive solvent partition with CH2Cl2 and EtOAc, and finally gave 38 g of CH2Cl2 soluble fraction, 13 g of EtOAc soluble fraction and 110 g of water soluble fraction. The CH2Cl2 soluble fraction was adsorbed in 1 kg of neutral alumina (Al2O3, activity 1, Merck), and was eluted with 5 L of CH2Cl2 and then washed with 5 L of MeOH. The eluate and wash was pooled up and evaporated to dryness to give 7 g of Fr.N. The alumina gel was further eluted with 25% NH3/MeOH 5 L and the eluate was collected and evaporated to give 25 g of Fr.A. Each fraction, i.e., Fr.N, Fr.A, Fr.EtOAc and Fr.H2O was examined for the cytotoxicity in vitro, and it was found that the total activity of the crude MeOH extract was almost completely recovered in the Fr.N. Therefore, the Fr.N was subjected to the SiO2 column chromatography and the repeated preparative TLC using various solvent system, such as CH2Cl2/MeOH gradient system, 3% and the repeated preparative TLC using various solvent system, such as CH2Cl2/MeOH gradient system, 3%

Compound I (cucurbitacin B). yield 0.0005%, colorless needle in dil. MeOH, mp. 180-183°C, [α]D +82 (c, 0.2; MeOH), UV(λmax; 229 nm (MeOH), MS: m/z (rel. int.); 498(M+-AcOH, 15), 455(12), 385(12), 370(12), 111(34), 96(100). 1H-NMR (CDCl3, δ): 7.03 (1H, d, J= 15.6 Hz, 24-H), 6.44 (1H, d, J=15.6 Hz, 23-H), 5.91 (1H, m, 6-H), 4.32 (1H, m, 16-H), 4.25 (1H, brs, 3-H), 3.08 (1H, d, J=14.5 Hz, 12α-H), 2.48 (1H, d, J=7.0 Hz, 17-H), 1.98 (3H, s, -OAc), 1.54, 1.52, 1.40, 1.32, 1.30, 1.25, 1.04 and 0.95 (each 3H, s, -CH3), 13C-NMR: Table I.

Compound II (isocucurbitacin B). yield 0.0013%, colorless needle in dil. MeOH, mp. 220-222°C, [α]D +35 (c, 0.2; CHCl3), UV(λmax; 229 nm (MeOH), MS: m/z (rel. int.); 498(M+-AcOH, 15), 455(12), 385(12), 370(12), 111(34), 96(100). 1H-NMR (CDCl3, δ): 7.03 (1H, d, J= 15.6 Hz, 24-H), 6.44 (1H, d, J=15.6 Hz, 23-H), 5.91 (1H, m, 6-H), 4.32 (1H, m, 16-H), 4.25 (1H, brs, 3-H), 3.08 (1H, d, J=14.5 Hz, 12α-H), 2.70 (1H, m, 10-H), 2.58 (1H, d, J=14.5 Hz, 12β-H), 2.42 (1H, d, J=7.0 Hz, 17-H), 1.98 (3H, s, -OAc), 1.52, 1.50, 1.42, 1.32, 1.25, 1.15, 0.98 and 0.82. (each 3H, s, -CH3), 13C-NMR: Table I.

Compound III (cucurbitacin D). yield 0.0003%, colorless needle in dil. MeOH, mp. 150-155°C, [α]D +50 (c, 0.1; MeOH), UV(λmax); 229 nm (MeOH), MS: m/z (rel. int.); 498(M+-H2O, 25), 480(6), 385(12), 370(12), 111(40), 96(100). 1H-NMR (CDCl3, δ): 7.07 (1H, d, J= 15.2 Hz, 24-H), 6.58 (1H, d, J=15.2 Hz, 23-H), 5.72 (1H, m, 6-H), 4.35 (1H, dd, J=12.9, 5.9 Hz, 2-H), 4.28 (1H, m, 16-H), 3.24 (1H, d, J=14.5 Hz, 12α-H), 2.65 (1H, m, 10-H), 2.64 (1H, d, J=14.5 Hz, 12β-H), 2.48 (1H, d, J=7.0 Hz, 17-H), 1.30 and 1.28 (each 6H, s, -CH3), 1.33, 1.23, 1.01, 0.92 (each 3H, s, -CH3), 13C-NMR: Table I.

Compound IV (isocucurbitacin D). yield 0.0003%, colorless needle in dil. MeOH, mp. 185-190°C, [α]D +35 (c, 0.1; CHCl3), UV(λmax); 229 nm (MeOH), MS: m/z (rel. int.); 498 (M+-H2O, 20), 480(6), 385(12), 370 (12), 111(50), 96(100). 1H-NMR (CDCl3, δ): 7.08 (1H, d, J=15.2 Hz, 24-H), 6.59 (1H, d, J=15.2 Hz, 23-H), 5.91 (1H, m, 6-H), 4.32 (1H, m, 16-H), 4.27 (1H, brs, 3-H), 3.12 (1H, d, J=14.5 Hz, 12α-H), 2.70 (1H, m, 10-H), 2.62 (1H, d, J=14.5 Hz, 12β-H), 2.48 (1H, d, J=7.0 Hz, 17-H), 1.33 (6H, s, -CH3), 1.35, 1.29, 1.22, 1.17, 0.94 and 0.87 (each 3H, s, -CH3), 13C-NMR: Table I.

Compound V (3-epi-isocucurbitacin B). yield 0.0003%, white amorphous powder, [α]D +25 (c, 0.2; CHCl3), UV(λmax); 229 nm (MeOH), MS: m/z (rel. int.); 498 (M+-AcOH, 10), 480(5), 455(12), 385(12), 370 (12), 111(50), 96(100). 1H-NMR (CDCl3, δ): 7.02 (1H, d, J=15.6 Hz, 24-H), 6.43 (1H, d, J=15.6 Hz, 23-H), 5.81 (1H, m, 6-H), 4.32 (1H, m, 16-H), 4.09 (1H, brs, 3-H), 3.08 (1H, d, J=14.5 Hz, 12α-H), 2.94 (1H, m, 10-H), 2.62 (1H, d, J=14.5 Hz, 12β-H), 2.45(1H, d, J=7.0 Hz, 17-H), 1.98(3H, s, -OAc), 1.53, 1.51, 1.41, 1.36, 1.32, 1.05, 0.94 and 0.83(3H, s, -CH3), 13C-NMR: Table I.

Compound VI (23,24-dihydrocucurbitacin B). yield 0.0015%, white amorphous powder, [α]D +50 (c, 0.2; CHCl3), UV(λmax); end absorption (MeOH), MS: m/z (rel. int.); 500 (M+-AcOH, 38), 482(20), 402(75), 385 (75), 368(45), 113(98), 96(52), 43(100). 1H-NMR (CDCl3, δ): 5.74 (1H, m, 6-H), 4.38 (1H, dd, J=12.8, 5.9 Hz, 2-H), 4.32 (1H, m, 16-H), 3.20 (1H, d, J=14.5 Hz, 12α-H), 2.72 (1H, m, 10-H), 2.65 (1H, d, J=14.5 Hz, 12β-H), 2.48 (1H, d, J=7.0 Hz, 17-H), 1.90 (3H, s, -OAc), 1.40, 1.38, 1.36, 1.33, 1.31, 1.24, 1.02 and 0.91 (each 3H, s, -CH3), 13C-NMR: Table I.