Phytochemical Constituents of *Thesium chinense* TURCZ and Their Cytotoxic Activities *In Vitro*

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**Abstract** – Column chromatographic separation of the MeOH extract from the aerial parts of *Thesium chinense* TURCZ led to the isolation of two norsesquiterpenes (1-2), two phenylpropanes (3-4) and four flavonoids (5-8). Their structures were determined by spectroscopic means to be 5,6-epoxy-3-hydroxy-7-megastigm-9-ene (1), (−)-loliiolide (2), methyl-p-hydroxycinnamate (3), methyl caffeate (4), kaempferol (5), kaempferol-3-O-β-D-glucopyranoside (6), kaempferol-3,7-di-O-β-D-glucopyranoside (7) and kaempferol-3-O-β-D-glucopyranoside-6''-(3-hydroxy-3-methylglutarate) (8). Compounds 1-4, 7 and 8 were first isolated from this source. The isolated compounds were evaluated for their cytotoxicity *in vitro* using the sulforhodamine B bioassay (SRB).

**Keywords** – *Thesium chinense*, Norsesquiterpene, Phenylpropane, Flavonoid, Cytotoxicity.

**Introduction**

*Thesium chinense* TURCZ has been used as a Korean traditional medicine in the treatment for inflammatory, mastitis, bronchial trouble and tuberculosis (Ahn, 1998). Alkaloids (Wang et al., 2006) and flavonoids (Lu et al., 2004) were reported from *T. chinense*. As parts of our continuing search for biological active compounds from natural sources, we investigated the constituents of *T. chinense*. The column chromatographic separation of the MeOH extract (500 g) resulted in the isolation of two norsesquiterpenes (1-2), two phenylpropanes (3-4) and four flavonoids (5-8). Compounds 1-4, 7 and 8 were first isolated from this plant source. The isolated compounds were tested for their cytotoxicity against four human cancer cell lines *in vitro* using a SRB bioassay.

**Experimental**

**General** – Melting points were determined on Gallenkamp melting point apparatus and uncorrected. Optical rotations were measured on a JASCO P-1020 Polarimeter. NMR spectra were recorded on Varian UNITY INOVA 500 NMR spectrometer. FAB-MS data were obtained on an Agilent 1100 mass spectrometer. Preparative HPLC used a Wellchrom K1001 A pump with Knauer Dual Detector and Apollo Silica 5u column (250 × 22 mm) or Econosil® RP-18 10u column (250 × 22 mm). Silica gel 60 (Merck, 70~230 mesh and 230~400 mesh) was used for column chromatography. TLC used Merck precoated Silica gel F₂₅₄ plates and RP-18 F₂₅₄ plates. Packing material of molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). Low pressure liquid chromatography was carried out over Merck LiChroprep Lobar®-A Si 60 (240 × 10 mm) or LiChroprep Lobar®-A RP-18 (240 × 10 mm) column with FMI QSY-0 pump (ISCO).

**Plant materials** – *T. chinense* TURCZ. (1.8 kg) was collected at Yeongcheon, Gyeongbuk Province, Korea in August, 2008. A voucher specimen (SKKU-2008-2) was deposited at the College of Pharmacy in Sungkyunkwan University, Korea.

**Extraction and Isolation** – The half dried and chopped aerial parts of *T. chinense* TURCZ. (1.8 kg) were extracted with 80% MeOH at room temperature and evaporated under reduced pressure to give residue (500 g), which was dissolved in water (800 ml) and partitioned with solvent to give methylene chloride fraction (MC) (16 g).

The MC fraction (16 g) was chromatographed over a silica gel column with C HCl₃ : MeOH = 30 : 1 as the eluent to give seven fractions (TCM1-TCM7). The
fraction TCM3 (1.5 g) was subjected to a Sephadex LH-20 (MC : MeOH = 1 : 1) and purified with silica gel prep. HPLC (Econosil\textsuperscript{®} RP-18 10 µ column, 250 × 22 mm; 47% MeOH) to yield compounds 1 (32 mg) and 2 (3 mg). The fraction TCM4 (2.4 g) was also subjected to a Sephadex LH-20 (MC : MeOH = 1 : 1) and purified with silica gel prep. HPLC (Apollo Silica 5 µ column, 250 × 22 mm; 50% MeOH) to yield compounds 3 (7 mg), 4 (10 mg) and 5 (10 mg). The fraction TCM5 (3.0 g) was also subjected to a Sephadex LH-20 (MC : MeOH = 1 : 1) as the eluent and purified with silica gel prep. HPLC (Econosil\textsuperscript{®} RP-18 10 µ column, 250 × 22 mm; 50 and 70% MeOH) to yield compounds 6 (55 mg), 7 (32 mg) and 8 (230 mg).

**Evaluation of cytotoxicity in vitro** – A sulforhodamin B bioassay (SRB) was used to determine the cytotoxicity of the compounds. The cytotoxic activity of each compound against four cultured human tumor cells was examined in vitro at the Korea Research Institute of Chemical Technology. The tumor cell lines were A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma) and HCT15 (colon cancer cells) (Skohan \textit{et al}.), 1990).

**5,6-Epoxy-3-hydroxy-7-megastigmen-9-one (1)** – Colorless oil; \(\alpha\)\textsubscript{D} \(\approx \) \(-98.7^\circ\) (0.1, CHCl\textsubscript{3}); FAB-MS \(m/z\) 194 [M]+; \(\text{\textsuperscript{1}H-NMR}\) (CDCl\textsubscript{3}, 500 MHz) : \(\delta\) 7.60 (1H, d, \(J = 15.8\) Hz, H-7), 7.44 (2H, d, \(J = 8.8\) Hz, H-2, 6), 6.80 (1H, d, \(J = 8.8\) Hz, H-3, 5), 6.30 (1H, d, \(J = 15.8\) Hz, H-8), 3.75 (3H, s, OCH\textsubscript{3})\textsuperscript{13}; \(\text{\textsuperscript{13}C-NMR}\) (CDCl\textsubscript{3}, 125 MHz) : \(\delta\) 169.8 (C-9), 160.1 (C-4), 145.4 (C-7), 129.9 (C-2, 6), 126.3 (C-1), 115.7 (C-3, 5), 114.7 (C-8), 50.8 (OCH\textsubscript{3}).

**Methyl-\(\beta\)-hydroxycinnamate (3)** – White powder, mp: 135 °C; FAB-MS \(m/z\) 179 [M+H]+; \(\text{\textsuperscript{1}H-NMR}\) (CD\textsubscript{3}OD, 500 MHz) : \(\delta\) 7.60 (1H, d, \(J = 15.8\) Hz, H-7), 7.17 (1H, d, \(J = 1.7\) Hz, H-2), 7.05 (1H, dd, \(J = 8.2, 1.7\) Hz, H-6), 6.80 (1H, d, \(J = 8.2\) Hz, H-5), 6.32 (1H, br d, \(J = 15.3\) Hz, H-8), 4.83 (3H, s, OCH\textsubscript{3})\textsuperscript{13}; \(\text{\textsuperscript{13}C-NMR}\) (CDCl\textsubscript{3}, 125 MHz) : \(\delta\) 169.8 (C-9), 149.3 (C-4), 148.2 (C-3), 145.7 (C-7), 126.7 (C-1), 122.8 (C-6), 115.3 (C-2, 5), 114.7 (C-5, 8), 55.3 (OCH\textsubscript{3}).

**Methyl caffeate (4)** – Colorless oil, FAB-MS \(m/z\) 194 [M]+; \(\text{\textsuperscript{1}H-NMR}\) (CDCl\textsubscript{3}, 500 MHz) : \(\delta\) 7.60 (1H, d, \(J = 15.8\) Hz, H-7), 7.17 (1H, d, \(J = 1.7\) Hz, H-2), 7.05 (1H, dd, \(J = 8.2, 1.7\) Hz, H-6), 6.80 (1H, d, \(J = 8.2\) Hz, H-5), 6.32 (1H, br d, \(J = 15.3\) Hz, H-8), 4.83 (3H, s, OCH\textsubscript{3})\textsuperscript{13}; \(\text{\textsuperscript{13}C-NMR}\) (CDCl\textsubscript{3}, 125 MHz) : \(\delta\) 169.8 (C-9), 149.3 (C-4), 148.2 (C-3), 145.7 (C-7), 126.7 (C-1), 122.8 (C-6), 115.3 (C-2, 5), 114.7 (C-5, 8), 55.3 (OCH\textsubscript{3}).

**Kaempferol (5)** – Yellow powder, mp: 265 °C; FAB-MS \(m/z\) 287 [M+H]+; \(\text{\textsuperscript{1}H-NMR}\) (CD\textsubscript{3}OD, 500 MHz) : \(\delta\) 8.08 (2H, d, \(J = 8.5\) Hz, H-2, 6), 6.90 (2H, d, \(J = 8.5\) Hz, H-3', 5'), 6.40 (1H, d, \(J = 2.0\) Hz, H-8), 6.20 (1H, d, \(J = 2.0\) Hz, H-6); \(\text{\textsuperscript{13}C-NMR}\) (CDCl\textsubscript{3}, 125 MHz) : 176.2 (C-4), 164.4 (C-7), 161.3 (C-5), 159.4 (C-4'), 157.1 (C-9), 148.2 (C-3), 145.7 (C-7), 126.7 (C-1), 122.8 (C-6), 115.3 (C-2, 5), 114.7 (C-5, 8), 55.3 (OCH\textsubscript{3}).

Fig. 1. The Structures of 1 - 8 from of \textit{T. chinense} TURCZ.