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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-megastigmen-9-ene (**1**), (–)-loliolide (**2**), methyl-*p*-hydroxycinnamate (**3**), methyl caffeate (**4**), kaempferol (**5**), kaempferol-3-*O*-*β*-D-glucopyranoside (**6**), kaepmferol-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 cytotoxicty *in vitro* using the sulforhodamin 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 cytotoxic activities 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 a 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 F_{254} plates and RP-18 F_{254s} 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 $CHCl_3$: MeOH = 30 : 1 as the eluent to give seven fractions (TCM1-TCM7). The

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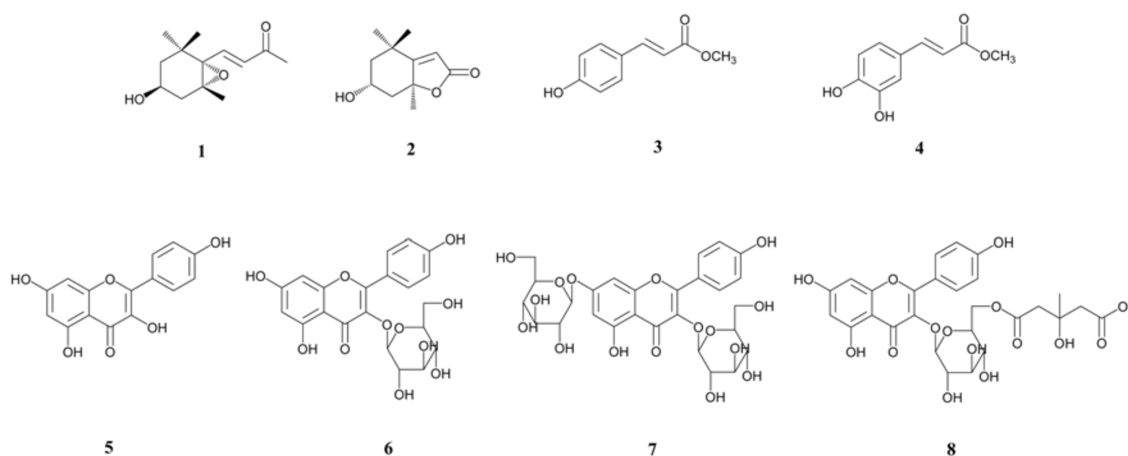

Fig. 1. The Structures of **1** - **8** from of *T. chinense* TURCZ.

fraction TCM3 (1.5 g) was subjected to a Sephadex LH-20 (MC : MeOH = 1 : 1) and purified with silica gel prep. HPLC (Econosil® 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; *n*-hexane : $CHCl_3$: EtOAc = 7 : 3 : 1) to yield compounds **3** (7 mg), **4** (10 mg) and **5** (10 mg). The fraction TCM7 (3.0 mg) was also subjected to a Sephadex LH-20 (MC : MeOH = 1 : 1) as the eluent and purified with silica gel prep. HPLC (Econosil® 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) (Skehan *et al.*, 1990).

5,6-Epoxy-3-hydroxy-7-megastigmen-9-one (1) – Colorless oil; $[\alpha]_D^{25}$: −55.7° (*c* 0.1, $CHCl_3$); EI-MS *m/z*: 194 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 7.16 (1H, d, *J* = 16.0 Hz, H-7), 6.18 (1H, d, *J* = 16.0 Hz, H-8), 3.77 (1H, m, H-3), 2.32 (1H, dd, *J* = 9.3, 5.2 Hz, H-4a), 2.30 (3H, s, H-10), 1.65 (1H, dd, *J* = 14.0, 9.3 Hz, H-4b), 1.57 (1H, dd, *J* = 14.0, 10.3 Hz, H-2b), 1.19 (3H, s, H-13), 1.17 (3H, s, H-11), 0.95 (3H, s, H-12); ^{13}C-NMR ($CDCl_3$, 125 MHz) : 199.0 (C-9), 144.2 (C-7), 132.7 (C-8), 69.7 (C-6), 67.6 (C-5), 63.2 (C-3), 46.5 (C-4), 40.2 (C-2), 34.9 (C-1), 28.6 (C-11), 26.3 (C-10), 23.9 (C-12), 18.9 (C-13).

(-)-Loliolide (2) – Colorless oil, ; $[\alpha]_D^{25}$: −98.7° (*c* 0.1, $CHCl_3$); FAB-MS *m/z*: 197 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500MHz) : δ 5.74 (1H, s, H-3), 4.20 (1H, m, H-6), 2.40 (1H, dt, *J* = 13.5, 2.3 Hz, H-5), 1.98 (1H, dt, *J* = 13.5, 2.3 Hz, H-7), 1.75 (3H, s, H-10), 1.74 (1H, dd, *J* = 13.0, 4.1 Hz, H-5), 1.53 (1H, dd, *J* = 14.0, 3.5 Hz, H-7), 1.46 (3H, s, H-8), 1.27 (3H, s, H-9); ^{13}C-NMR ($CDCl_3$, 125 MHz) : 184.5 (C-3a), 173.2 (C-2), 112.2 (C-3), 87.8 (C-7a), 66.1 (C-6), 46.8 (C-5), 45.3 (C-7), 36.0 (C-4), 29.8 (C-8), 26.3 (C-9), 25.8 (C-10).

Methyl-*p*-hydroxycinnamate (3) – White powder, mp: 135 °C; FAB-MS *m/z*: 179 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz) : δ 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, $-OCH_3$); ^{13}C-NMR (CD_3OD, 125 MHz) : δ 168.6 (C-9), 160.1 (C-4), 145.4 (C-7), 129.9 (C-2, 6), 126.5 (C-1), 115.7 (C-3, 5), 114.7 (C-8), 50.8 ($-OCH_3$).

Methyl caffeate (4) – Colorless oil, FAB-MS *m/z*: 194 $[M]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) : δ 7.58 (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_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 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), 114.7 (C-5, 8), 55.3 (-OCH3).

Kaempferol (5) – Yellow powder, mp: 265 °C; FAB-MS *m/z*: 287 $[M + H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz) : δ 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); ^{13}C-NMR (CD_3OD, 125 MHz) : δ 176.2 (C-4), 164.4 (C-7), 161.3 (C-5), 159.4 (C-4'), 157.1 (C-9),

146.9 (C-2), 135.9 (C-3), 129.5 (C-2', 6'), 122.6 (C-1'), 115.4 (C-3', 5'), 103.4 (C-10), 98.1 (C-6), 93.3 (C-8).

Kaempferol-3-*O*-*β*-D-glucopyranoside (6) – Yellow amorphous powder, mp: 186 °C; FAB-MS *m/z*: 449 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz) : δ 12.6 (1H, br s, -OH), 8.04 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.89 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.43 (1H, d, *J* = 2.0 Hz, H-6), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 5.46 (1H, d, *J* = 7.0 Hz, H-1''); ^{13}C-NMR (DMSO-d_6, 125 MHz) : δ 178.2 (C-4), 164.9 (C-7), 161.9 (C-5), 160.7 (C-4'), 157.1 (C-9), 156.9 (C-2), 133.9 (C-3), 131.6 (C-2',6'), 121.6 (C-1'), 115.8 (C-3',5'), 104.7 (C-10), 101.6 (C-1''), 99.4 (C-6), 94.4 (C-8), 78.1 (C-3''), 77.2 (C-5''), 74.9 (C-2''), 70.6 (C-4''), 61.6 (C-6'').

Kaepmferol-3,7-di-*O*-*β*-D-glucopyranoside (7) – Yellow amorphous powder, mp: 165 °C; FAB-MS *m/z*: 611 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz) : δ 8.06 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.89 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.79 (1H, d, *J* = 2.3 Hz, H-8), 6.44 (1H, d, *J* = 2.3 Hz, H-6), 5.47 (1H, d, *J* = 7.5 Hz, H-1''), 5.08 (1H, d, *J* = 7.5 Hz, H-1'''); ^{13}C-NMR (DMSO-d_6, 125 MHz) : δ 178.4 (C-4), 163.5 (C-7), 161.6 (C-5), 160.8 (C-4'), 157.5 (C-2), 156.7 (C-9), 134.2 (C-3), 131.7 (C-2', 6'), 121.5 (C-1'), 115.9 (C-3'), 106.4 (C-10), 101.4 (C-1''), 100.5 (C-1'''), 100.0 (C-6), 95.2 (C-8) 78.2 (C-5'') 77.8 (C-5''') 77.2 (C-3''), 77.1 (C-3'''), 74.9 (C-2'') 73.8 (C-2'''), 70.6 (C-4''), 70.3 (C-4'''), 61.5 (C-6''), 61.3 (C-6''').

Kaepmferol-3-*O*-*β*-D-glucopyranoside-6''-(3-hydroxy-3-methylglutarate) (8) – Yellow amorphous powder, mp: 215 °C; FAB-MS *m/z*: 593 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6, 500 MHz) : δ 7.98 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.87 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.42 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.38 (1H, d, *J* = 7.5 Hz, H-1''), 4.15 (1H, dd, *J* = 11.7, 1.7 Hz, H-6''), 3.93 (1H, dd, *J* = 11.7, 7.0 Hz, H-6''), 2.50 - 2.42 (2H, ABq, *J* = 14.0 Hz, H-2''' or -4'''), 2.34 - 2.31 (2H, ABq, *J* = 14.0 Hz, H-H-2''' or -4'''), 1.10 (3H, s, H-6'''); ^{13}C-NMR (DMSO-d_6, 125 MHz) : δ 178.1 (C-4), 173.0 (C-5'''), 170.8 (C-1'''), 164.9 (C-7), 161.9 (C-5), 160.7 (C-4'), 157.2 (C-2), 157.1 (C-9), 133.7 (C-3), 131.5 (C-2'), 121.5 (C-1'), 115.8 (C-3'), 104.6 (C-10), 101.7 (C-1''), 99.4 (C-6), 94.4 (C-8), 76.8 (C-3''), 74.7 (C-2''), 74.7 (C-5''), 70.7 (C-4''), 69.7 (C-3'''), 63.8 (C-6''), 45.9 (C-4'''), 45.7 (C-2'''), 27.8 (C-6''').

Results and Discussion

The column chromatographic separation of the MeOH extract of *T. chinense* TURCZ. led to the isolation of two norsesquiterpenes (**1** - **2**) two phenylpropanes (**3** - **4**) and four flavonoids (**5** - **8**). Compounds **5**, **6** and **7** were identified to be kaempferol (Choi *et al*., 2004) (**5**), kaempferol-3-*O*-*β*-D-glucopyranoside (Lee *et al*., 2004) (**6**) and kaempferol-3,7-di-*O*-*β*-D-glucopyranoside (Do *et al*., 1992) (**7**), by the comparison of ^{1}H-, ^{13}C-NMR and MS data with those reported in the literatures. Compounds **1**-**4**, **7** and **8** were isolated for the first time from this source. The following describes the structural elucidation of compounds **1**, **2** and **8**, which were not so often isolated from natural sources.

Compound **1** was obtained as colorless oil. The MS spectrum of **1** showed a molecular ion peak at m/z 224 $[M]^+$. From the MS, ^{1}H- and ^{13}C-NMR spectral data, the molecular formula of **1** was deduced to be $C_{13}H_{22}O_3$. The ^{1}H-NMR spectrum showed olefinic proton signals at δ 7.16 (1H, d, *J* = 16.0 Hz) and 6.18 (1H, d, *J* = 16.0 Hz), an oxygenated proton signal at δ 3.77 (1H, m), and four methyl groups at δ 2.30 (3H, s), 1.19 (3H, s), 1.17 (3H, s), and 0.95 (3H, s). The ^{13}C-NMR spectrum exhibited 13 carbon signals, consisting of a carbonyl carbon signal at δ 199.0, two olefinic carbon signals at δ 144.2 and 132.7, and three oxygenated carbon signals at δ 69.7, 67.5, and 63.2. These spectral data suggested that **1** was an ionane-type norsesquiterpene (Kim *et al.*, 2008). Based on further comparison the NMR and physical data with those in the literature (Kim *et al.*, 2008), the structure of compound **1** was determined to be 5,6-epoxy-3-hydroxy-7-megastigmen-9-one.

Compound **2** was obtained as colorless oil. The MS spectrum of **2** showed a molecular ion peak at m/z 197$[M]^+$. The ^{1}H-NMR spectrum showed three methyl group signals at δ 1.75 (3H, s), 1.46 (3H, s) and 1.27 (3H, s), two methylene group signals at δ 2.40 (1H, dt, *J* = 13.5, 2.3 Hz), 1.98 (1H, dt, *J* = 13.5, 2.3 Hz), 1.74 (1H, dd, *J* = 13.0, 4.1 Hz), 1.53 (1H, dd, *J* = 14.0, 3.5 Hz), oxygenated proton at δ 4.20 (1H, m), and one olefinic proton signal at δ 5.74 (1H, s). The ^{13}C-NMR spectrum exhibited 11 carbon signals, consisting of a *α*,*β*-unsaturated-*γ*-lactone group signals at δ 184.5, 173.2 and 112.2, oxygenated carbon signals at δ 87.8 and 66.1, three methyl carbon signals at δ 29.8, 26.3, 25.8. Based on the NMR spectral evidences, and a further comparison with published data (Park *et al.*, 2004), the structure of compound **2** was determined to be (–)-loliolide.

Compound **8** was obtained as yellow amorphous powder. The MS spectrum of **8** showed a molecular ion peak at m/z 593 $[M+H]^+$. ^{1}H- and ^{13}C-NMR spectral data of **8** were similar to those of **6** (kaempferol-3-*O*-*β*-D-glucopyranoside) (Lee *et al.,* 2004), except for the additional presence of six carbon substituent in the ^{13}C-NMR spectrum of **8**, which was composed of a methyl group (δ 27.8), two methylene carbons (δ 45.9, and 45.7),

a hydroxylated methine carbon (δ 69.7), one ester carbonyl carbon (δ 170.2) and a carboxyl carbon (δ 175.2). These data implied that **8** could be an esterified kaepmferol-3-*O*-*β*-D-glucopyranoside with a substituent group, 3-hydroxy-3-methylglutaric acid (Berhow *et al.,* 1994; Jung *et al.*, 1993). Based on the NMR spectral evidences, and a further comparison with published data (Berhow *et al.*, 1994; Jung *et al.*, 1993), the structure of compound **8** was determined to be kaempferol-3-*O*-*β*-D-glucopyranoside-6''-(3-hydroxy-3-methylglutarate). The isolated compounds were tested *in vitro* for cytotoxicity against four tumor cells using the SRB assay. Compound **1** exhibited moderate cytotoxicities against A549, SK-OV-3, SK-MEL-2 and HCT15 with ED_{50} values of 20.19, 21.83, 21.56 and 26.07 μM, respectively. The other compounds showed little activities against tested cell lines ($ED_{50} > 100$ μM).

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