Journal of Medicinal Plants Research Vol. 6(22), pp.3923-3930, 14 June, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.552
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Isolation and identification of phytochemical constituents from *Scrophularia takesimensis*

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Accepted 12 April, 2012

Phytochemical constituents were isolated from *Scrophularia takesimensis* (Scrophulariaceae) using repeated chromatography and prep-HPLC. Their structures were elucidated as stigmast-5-en-3-ol (1), α-spinasterol 3-*O*-β-D-glucopyranoside (2), 5-hydroxypyrrolidin-2-one (3), *trans*-cinnamic acid (4), 4-methoxycinnamic acid (5), 2-methoxycinnamic acid (6), and 5,7-dihydroxy-4′-methoxyflavone (7) by spectroscopic analysis. All compounds were isolated for the first time from *S. takesimensis* and compounds 2, 3, 6, and 7 were isolated for the first time from *Scrophularia* species. Among them, 5,7-dihydroxy-4′-methoxyflavone (7) exhibited strong AR inhibitory activity, with an IC_{50} value of 4.96 μM. The content of 5,7-dihydroxy-4′-methoxyflavone (7) was measured in aerial parts of *S. takesimensis*, *S. kakudensis* and *S. boreali-koreana* (54.3, 25.4, and 15.0 μg/g, respectively) by HPLC.

Key words: Aldose reductase inhibition, HPLC analysis, phytochemical constituent, *Scrophularia takesimensis*.

INTRODUCTION

The genus *Scrophularia* of the family Scrophulariaceae comprises about 300 species of herbaceous flowering plants. These plants are located throughout the Northern Hemisphere, with a high concentration in Asia and only a few species in Europe and North America (Chung and Shin, 1990). The dried roots of *Scrophularia* species have been used in Asian medicine as a treatment for fever, laryngitis, swelling, constipation, neuritis, and pharyngitis (Qian et al., 1992; Park et al., 2003). Five types of *Scrophularia* species, including *S. takesimensis*, *S. kakudensis*, *S. koraiensis*, *S. boreali-koreana*, and *S. buergeriana,* naturally grow in Korea. Among these, *S. buergeriana* has been cultivated and used as medicinal plant for diverse purposes while the other species are grown in the wild (Ahn, 2005). *S. takesimensis*, known as 'Seum Hyun Sam' in Korean, is a rare and endangered plant found just along the coast of Ulleung Island, a small volcanic island located in the East Sea approximately 150 km from the mainland of Korea (Ahn, 2005). *S. takesimensis* grows to a height of almost 3 feet with square stems and opposite leaves with saw-like edges that are generally 4 to 7.5 cm long. The flower blooms between June and July. There are five overlapping receptacles, approximately 1.5 mm long and 3 mm wide, and a violet corolla about 1 cm long with three divided ends.

Tiny fruits, about 8 to 9 mm long, are generally spherical with pointed ends (Ahn, 2005). Habitat loss, difficulties with seed germination in natural conditions, and overexploitation are major threats to the survival of this species (Kang et al., 2009). The Ministry of Environment in Korea classified it as a second-class endangered plant species (Park et al., 2010).

Until now, nothing has been reported on the phytochemical constituents and biological activities of *S. takesimensis* because it is a rare and endangered plant species. In our previous study, *Scrophularia* species (*S. takesimensis*, *S. kakudensis*, *S. boreali-koreana*, and *S. buergeriana*) were determined to exhibit rat lens aldose reductase (AR) inhibitory activity (Kim et al., 2010). The present study reports the isolation and characterization of two sterols, three phenypropanoids, an alkaloid, and a flavonoid from *S. takesimensis*. Additionally, we tested the effect of the flavonoid on rat lens AR inhibition and the content of the flavonoid in *Scrophularia* species.

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MATERIALS AND METHODS

Plant materials

The *S. takesimensis* specimen was collected on Ulleung Island and botanically authenticated by Prof. Y. H. Ahn, Chung-Ang University, Republic of Korea. A voucher specimen (No. LEE 2009-07) was deposited at the Herbarium of Department of Integrative Plant Science, Chung-Ang University, Republic of Korea. Additionally, the extracts of *S. kakudensis* (aerial part), *S. boreali-koreana* (aerial part), and *S. buergeriana* (root) were purchased from the Plant Extract Bank (PEB) of Korea Research Institute of Bioscience and Biotechnology (KRIBB) in Deajeon, Republic of Korea.

General instruments and reagents

Mass spectrometry (MS) was performed with a Jeol JMS-600W (Japan) mass spectrometer. ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AVANCE 300 and 500 NMR (Rheinstetten, Germany) spectrometer using TMS as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in hertz. Evaporation was conducted using an EYELA rotary evaporator system (Japan) under reflux *in vacuo*. Silica gel (No. 7734; Merck Co., Germany) was used for open column chromatography. Solvents such as *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), and *n*-butanol (BuOH) (SamChun Pure Chemical Co.; Pyeongtaek, Korea) were used as an elution solution in open column chromatography. Recycling preparative HPLC was performed using a JAI LC-9014 system equipped with an L-6050 pump and an UV-3702 UV/VIS detector. The auto collector was measured using an FC-339 fraction collector. Thin layer chromatography (TLC) was conducted using Kiesel gel 60 F254 (Art. 5715; Merck Co., Germany) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by spraying with 10% H_2SO_4 in MeOH. Fluorescence analysis was measured with a Hitachi U-3210 spectrophotometer. Solvents such as DL-glyceraldehyde, β-NADPH, sodium phosphate buffer, potassium phosphate buffer, and DMSO (Sigma-Aldrich Co.) were used for the rat lens AR assay. The HPLC system (Agilent, USA) consisted of a 1200 series quaternary pump, UV/visible detector, and fraction collector all controlled by a computer using Agilent 1200 infinity series LC ChemStation software. Separation was carried out on a Discovery® C18 (25 cm × 4.6 mm, 5μm) column (Sigma-Aldrich Co.). The column temperature was maintained at 25°C throughout the experiment. HPLC-grade solvents such as acetonitrile, chloroform, and distilled water (J. T. Baker®, USA) were used as elution solvents for HPLC.

Extraction and isolation

The dried and powdered aerial parts of *S. takesimensis* (613.5 g) were extracted with MeOH (8 L × 5) under reflux at 65-75°C. The filtrate was concentrated until dry *in vacuo* to produce MeOH extract (333.2 g), suspended in H_2O, and then partitioned using *n*-hexane, $CHCl_3$, EtOAc, and BuOH, in that order. A portion of the *n*-hexane fraction (6.2 g) was chromatographed on a silica gel column (6 × 80 cm, No. 7734) using a stepwise gradient of the *n*-hexane-EtOAc solvent system to yield 19 sub-fractions. Sub-fraction 3 (2.6 g) was recrystallized ($CHCl_3$-MeOH) to yield 1 (50.3 mg). A portion of the $CHCl_3$ fraction (3.8 g) was applied to a silica gel column (6 × 80 cm, No. 7734) eluted with a step-wise gradient using *n*-hexane-EtOAc and EtOAc-MeOH to yield 22 sub-fractions. Sub-fraction 7 (0.3 g) was subjected to recycling prep-HPLC eluted with $CHCl_3$-MeOH (8:2, flow rate: 3 mL/min) to yield sub-fractions 7a-7e. Recrystallization of sub-fraction 7e (0.2 g) from MeOH yielded 7 (8.9 mg). Sub-fraction 8 (0.9 g) was further fractionated on the silica gel column (4.5 × 80 cm) using $CHCl_3$-MeOH as an eluent to yield 7 sub-fractions (8a-8g). Sub-fraction 8g (0.2 g) was purified by recrystallization with MeOH to yield 2 (72.6 mg) (Figure 1A).

The sliced and dried roots of *S. takesimensis* (678.8 g) were extracted and partitioned using the same method described previously. A portion of the $CHCl_3$ fraction (8.9 g) was chromatographed on a silica gel column (6 × 80 cm, No. 7734) using the stepwise gradient of the *n*-hexane-EtOAc and EtOAc-MeOH solvent system (up to increasing polarity) to yield 18 sub-fractions. Sub-fraction 5 (2.7 g) was subjected to recycling prep-HPLC eluted with $CHCl_3$-MeOH (8:2, flow rate: 3 mL/min) to yield sub-fractions 5a-5e. Among them, sub-fraction 5e (0.7 g) was recrystallized with MeOH to yield 4 (4.1 mg). Sub-fraction 6 (0.8 g) was subjected to recycling prep-HPLC to yield sub-fractions 6a-6e using the same method described previously. Compounds 5 (5.9 mg) and 6 (6.8 mg) were recrystallized with MeOH from sub-fractions 6d (0.3 g) and 6e (0.4 g), respectively. Sub-fraction 11 (1.6 g) was re-chromatographed in a silica gel column (4.5 × 80 cm) using $CHCl_3$-MeOH as the eluent to yield 10 sub-fractions (11a-11j). Among these, recrystallization of sub-fraction 11j (0.2 g) from MeOH yielded 3 (8.6 mg) (Figure 1B).

Compound 1: white powder; EI-MS *m/z*: 414 $[M]^+$ (100), 396 (50), 381 (24), 302 (36), 273 (50), 257 (44), 215 (31); ^{1}H- and ^{13}C-NMR (300 MHz, $CDCl_3$): see Table 1.

Compound 2: white powder; EI-MS *m/z*: 574 $[M]^+$ (17), 412 $[M+H_2O\text{-Glc}]^+$ (100), 271 (97), 255 (22); ^{1}H- and ^{13}C-NMR (500 MHz, pyridine): see Table 1.

Compound 3: colorless crystal; EI-MS *m/z*: 101 $[M]^+$ (22), 84 (100); ^{1}H-NMR (500 MHz, DMSO): δ 1.81 (1H, m, H-4eq), 2.18 (1H, m, H-4ax), 2.18-2.28 (2H, m, H-3ax and 3eq), 5.04 (1H, d, *J* = 5.5 Hz, H-5), 8.59 (1H, s, NH); ^{13}C-NMR (125 MHz, DMSO): δ 28.0 (C-3), 28.1 (C-4), 82.6 (C-5), 177.4 (C=O).

Compound 4: white powder; EI-MS *m/z*: 148 $[M]^+$ (89), 131 (18), 103 (30), 77 (15); ^{1}H-NMR (300 MHz, $CDCl_3$): see Table 2.

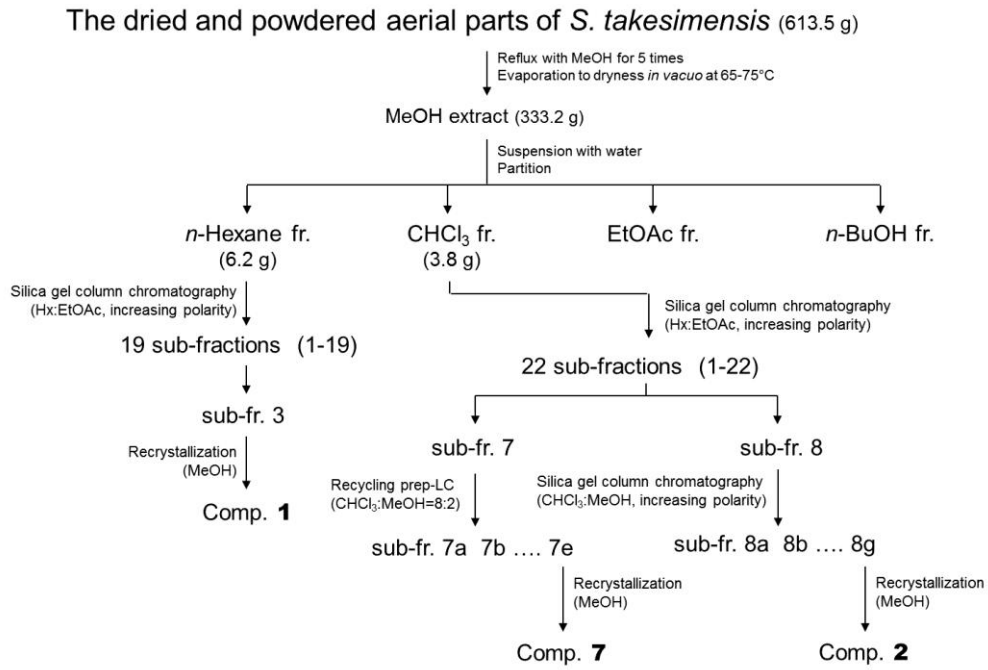
Compound 5: white powder; EI-MS *m/z*: 178 $[M]^+$ (100), 161 (39), 147 (4), 133 (17), 77 (6); ^{1}H-NMR (300 MHz, $CDCl_3$): see Table 2.

Compound 6: white powder; EI-MS *m/z*: 178 $[M]^+$ (31) 147 (100), 131 (37), 103 (63), 77 (33); ^{1}H-NMR (300 MHz, $CDCl_3$): see Table 2.

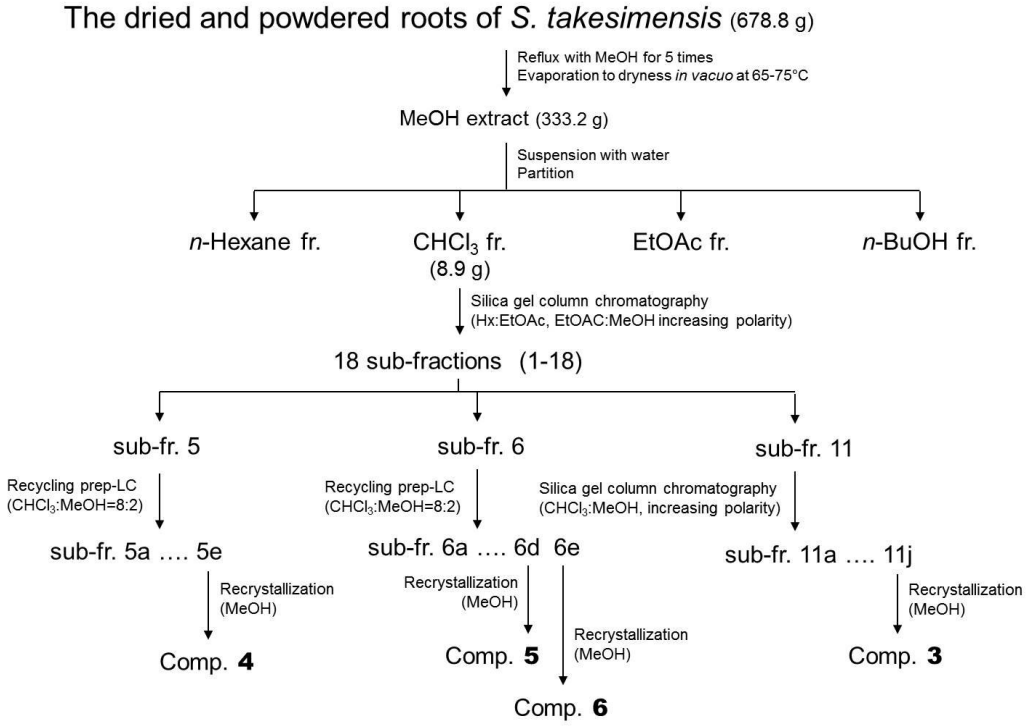
Compound 7: yellow powder; EI-MS *m/z*: 284 $[M]^+$ (100), 241 (7.0), 152 (5.9), 132 (14.4); ^{1}H-NMR (500 MHz, DMSO): δ 6.87 (1H, s, H-3), 6.51 (1H, d, *J* = 2.3 Hz, H-8), 6.20 (1H, d, *J* = 2.3 Hz, H-6), 8.04 (2H, d, *J* = 10.7 Hz, H-2′,6′), 7.11 (2H, d, *J* = 10.7 Hz, H-3′,5′), 3.86 (3H, s, OMe), 12.9 (1H, s, 5-OH); ^{13}C-NMR (125 MHz, DMSO): δ 164.3 (C-2), 103.6 (C-3), 181.8 (C-4), 161.4 (C-5), 98.9 (C-6), 162.3 (C-7), 94.1 (C-8), 157.1 (C-9), 103.8 (C-10), 122.8 (C-1′), 128.3 (C-2′, 6′), 114.6 (C-3′, 5′), 163.3 (C-4′), 55.6 (OMe).

Measurement of AR activity

Rat lenses were removed from Sprague-Dawley rats (weighing 250 - 280 g, 7-weeks) and preserved by freezing it until use. These were homogenized and centrifuged at 10,000 rpm (4°C, 20 min) and the supernatant was used as an enzyme source. AR activity was spectrophotometrically determined by measuring the decrease in absorption of NADPH at 340 nm for a 4 min period at room temperature with DL-glyceraldehydes as a substrate (Sato and Kador, 1990). The assay mixture contained 0.1 M potassium phosphate buffer (pH 7.0), 0.1 M sodium phosphate buffer (pH 6.2), 1.6 mM NADPH, and test sample (in DMSO) with 0.025 M DL-



(**A**)



(**B**)

Figure 1. Isolation scheme of the aerial part (A) and root (B) of *S. takesimensis.*

glyceraldehyde as the substrate in the quartz cell. A flavonoid (1.0 mg) isolated from the aerial parts of *S. takesimensis* was dissolved in DMSO (1 mL). IC_{50} values, the concentration of inhibitors giving 50% inhibition of enzyme activity, were calculated from the least-

Table 1. ^{1}H- and ^{13}C-NMR spectral data for compounds 1 and 2.

No.	1		2	
	δ_H	δ_C	δ_H	δ_C
1		37.2		37.8
2		29.9		29.4
3	3.58 (m)	71.6	4.01 (m)	77.6
4	2.27 (m)	39.9		35.2
5		138.5		40.7
6	5.35 (m)	129.4		30.5
7		32.0		118.4
8		31.9		139.2
9		51.4		50.1
10		36.6		35.0
11	2.00 (m)	21.3		22.1
12		40.7		40.1
13		42.7		44.0
14		56.2		55.8
15		24.1		22.3
16		29.0		26.2
17		54.6		56.6
18	0.70 (s)	12.4	0.61 (s)	12.7
19	1.00 (s)	19.2	0.75 (s)	13.5
20		35.7		41.6
21	0.99 (d, 6.3)	19.0	1.09 (d, 6.5)	21.9
22		35.0	5.23 (m)	140.1
23		25.6	5.08 (m)	130.2
24		45.0		51.9
25		29.8		32.7
26	0.89 (d, 6.3)	19.4	0.79 (d, 6.6)	19.7
27	0.81 (d, 6.6)	19.3	0.89 (d, 6.4)	21.8
28		23.0		23.8
29	0.80 (t, 6.0)	12.5	0.87 (t, 7.8)	13.0
1′		-	5.06 (d, 7.6)	102.8
2′		-		75.9
3′		-		78.9
4′		-		72.3
5′		-		79.1
6′		-		63.5

Chemical shifts are reported in parts per million (δ) and coupling constants (J) are expressed in hertz.

squares regression line of the logarithmic concentrations plotted against the residual activity. Quercetin, a typical AR inhibitor, was used as a positive control by dissolving in DMSO to a final concentration of 1.0 mg/mL.

Chromatographic conditions for HPLC

For analysis of the flavonoid isolated from *Schrophularia* spp., each 3.0 mg sample (*S. takesimensis*, *S. kakudensis*, *S. boreali-koreana*, and *S. buergeriana*) was dissolved in 1.0 mL 50% $CHCl_3$ in methanol. For injection, soluble samples were passed through a Whatman 0.45 µm PVDF syringe filter (Cat No. 6779 1304, USA). The resulting solvent was used for HPLC analysis. For identification and quantification of the flavonoid *via* HPLC, the mobile phase was an isocratic elution (acetonitrile: 1% acetic acid in distilled water = 26: 74) for 55 min. The flow rate was 0.5 mL/min. The detection wavelength was 330 nm and sample injection volume was 10 µL. All injections were performed in triplicate.

Calibration

Stock solutions of the flavonoid (0.5 mg/mL) were repeatedly blended with 50% $CHCl_3$ in MeOH, and then loaded onto an HPLC column for preparation of the calibration functions. The calibration function of the flavonoid was calculated with peak area (Y), concentration (X, mg/mL), and mean values (n = 12) ± standard deviation.

RESULTS AND DISCUSSION

A chromatographic separation of the MeOH extracts from the aerial and root portions of *S. takesimensis* led to the isolation of compounds 1-7 (Figure 2). Their structures were identified through spectral analysis.

Compounds 1 and 2 were identified as phytosterols. Compound 1 was obtained as a white powder that had a molecular ion peak at *m/z* 414 $[M]^+$ in the EI-MS along with a molecular formula of $C_{29}H_{50}O$. The ^{1}H-NMR spectrum of 1 exhibited a sterol skeleton and is summarized in Table 1. Two angular methyl singlets of 18- and 19-Me at δ 0.70 and 1.00, three doublets of 21-, 26-, and 27-Me at δ 0.99, 0.88 and 0.81, and one triplet of 29-Me at δ 0.80 were observed. A single broad doublet signal at δ 5.35 corresponding to the H-6 olefinic proton was observed. The ^{13}C-NMR spectrum of 1 had 29 resonances, and C-5 and -6 signals were noted at δ 138.5 and 129.4, respectively. Accordingly, the structure of 1 was identified as a phytosterol, formally named stigmast-5-en-3-ol (β-sitosterol) by spectral analysis (Chang et al., 1981). β-Sitosterol, the most common plant sterol, exhibits anti-inflammatory, anti-tumor, and anti-microbial activities (Park et al., 2001; Yuk et al., 2007). β-Sitosterol also inhibits the growth of several specific types of tumor cells *in vitro* and decreases the size and extent of tumor metastases *in vivo* (Awad et al., 2007).

Compound 2 had a molecular formula of $C_{35}H_{58}O_6$, according to EI-MS spectral and NMR data. The presence of a molecular ion at *m/z* 574 $[M]^+$ and a fragment at *m/z* 412 $[M+H_2O\text{-Glc}]^+$ in the EI-MS spectrum suggested the presence of a sugar and a C-29 aglycone in the molecule. The ^{1}H-NMR spectrum of 2 indicated the presence of six methyl proton signals at δ 0.61, 0.75, 0.79, 0.87, 0.89, and 1.09 and an anomeric proton signal at δ 5.06. The ^{13}C-NMR spectroscopic data of 2 were similar to those of 1 with the exception of the sugar (δ 102.8, 75.9, 78.9, 72.3, 79.1, and 63.5) and the additional double bond (δ 140.1 and 130.2) peaks at the C-22 position (Table 1). Additionally, double bond signals at δ 118.4 and 139.2 indicated C-6 and -7 positions, unlike the

Table 2. ^{1}H-NMR spectral data for compounds 4, 5, and 6.

No	4	5	6
2	7.68 (m)	6.37 (d, 8.9)	-
3	7.40-7.42 (m)	7.63 (d, 8.9)	7.41 (m)
4	7.40-7.42 (m)	-	7.42 (m)
5	7.40-7.42 (m)	7.63 (d, 8.9)	7.43 (m)
6	7.68 (m)	6.37 (d, 8.9)	7.68 (m)
7	7.57 (d, 16.1)	7.53 (d, 16.1)	7.58 (d, 16.1)
8	6.52 (d, 16.1)	6.97 (d, 16.1)	6.97 (d, 16.1)
OMe	-	3.78 (s)	3.80 (s)

Chemical shifts are reported in parts per million (δ) and coupling constants (J) are expressed in hertz.

double bond position in 1. These results indicated the presence of α-spinasterol 3-*O*-β-D-glucopyranoside (Ali et al., 1975; Choi and Kim, 2002). α-Spinasterol 3-*O*-β-D-glucopyranoside has been reported to affect heme oxygenase-1 (HO-1) expression, inhibit matrix metalloproteinase-1 expression, and increase HO-1 expression in HepG2 cells (Cui et al., 2011; Lee et al., 2011b).

Compound 3 was obtained as a colorless crystal, with the molecular formula $C_4H_7NO_2$, determined by the EI-MS at *m/z* 101 $[M]^+$. The ^{1}H-NMR spectrum of 3 revealed the presence of an amide NH signal at δ 8.59 (1H, s, NH), a methine signal at δ 5.04 (1H, d, *J* = 5.5 Hz, H-5), and two methylene signals at δ 2.18-2.28 (2H, m, H-3ax and -3eq), 1.81 (1H, m, H-4eq), and 2.18 (1H, m, H-4ax). The ^{13}C-NMR spectrum of 3 indicated the presence of a quaternary carbon, a methine, and two methylenes. The peak corresponding to the quaternary carbon was noted at δ 177.4 and was attributed to an amide carbonyl carbon and a methine, while the peak at δ 82.6 was assigned to a hemi-aminoacetal carbon. As a result, the structure of 3 was identified as an alkaloid, 5-hydroxypyrrolidin-2-one, whose spectral data were consistent with the literature (Chen et al., 2008; Dai et al., 2008). 5-Hydroxypyrrolidin-2-one was previously isolated as an antibacterial agent from the leaves of *Hyptis verticillata* (Kuhnt et al., 1995; Michaela et al., 1995; Ruth et al., 1999; Lee et al., 2011a).

Compounds 4-6 were identified as phenylpropanoids. Compound 4 had a molecular formula of $C_9H_8O_2$ according to a molecular ion at *m/z* 148 in its EI-MS spectrum. Five aromatic (δ 7.68 and 7.40-7.42) and two olefinic (δ 7.57 and 6.52, AB system, *J* = 16.1 Hz) protons in the ^{1}H-NMR spectrum were attributed to a *trans*-cinnamoyl moiety (Table 2). Compound 4 was therefore identified as *trans*-cinnamic acid (3-phenyl-2-propenoic acid) (Gao et al., 2012; Jing et al., 2011; Kuk et al., 1997). A previous study found *trans*-cinnamic acid to be the most potent inhibitor of FtsZ assembly (Rastogi et al., 2008). *trans*-Cinnamic acids were also found to effectively inhibit cataracts in the eye's natural lens(Chethan et al., 2008). Compounds 5 and 6 exhibited the same molecular ion at *m/z* 178 in the EI-MS spectra, despite slight differences in NMR spectra (Table 2). The ^{1}H-NMR spectroscopic data of 5 were similar to those of 4, with the exception of aromatic protons and an additional methoxy group in the *para* position (δ 3.78). Two protons from the aromatic ring [A_2B_2 type, δ 6.37 (d, *J* = 8.9 Hz, H-2,6) and 7.63, (d, *J* = 8.9 Hz, H-3,5)] and two *trans* olefinic protons [AB type, δ 7.53 (d, *J* = 16.1 Hz, H-7) and 6.97 (d, *J* = 16.1 Hz, H-8)] produced signals on the spectrum. The ^{1}H-NMR spectroscopic data of 6 resembled that of 5 except for proton peaks in the aromatic ring. The aromatic proton peaks of 6 at δ 7.41 (1H, m, H-3), 7.42 (1H, m, H-4), 7.43 (1H, m, H-5), and 7.68 (1H, m, H-6) suggested that a methoxy group must be located in the *ortho* position. The structures of 5 and 6 were identified as 4-methoxycinnamic acid (*E*-*p*-methoxycinnamic acid) (Kim and Kim, 2000) and 2-methoxycinnamic acid (Wondrazek and Heinze, 2008), respectively. 4-Methoxycinnamic acid, a major component in the root of *S. buergeriana*, appears to have neuroprotective properties, most likely as a result of partial glycine antagonism and anti-amnesic activity (Kim et al., 2002, 2003). 2-Methoxycinnamic acid has been shown to facilitate the inhibition of tyrosinase activity and exhibit lipase-catalyzed esterification activity (Lee, 2002; Vosmann et al., 2006).

Compound 7 had an $[M]^+$ peak at *m/z* 284 in the EI-MS, which was consistent with the molecular formula $C_{16}H_{12}O_5$. This result was further substantiated by analysis of its ^{1}H- and ^{13}C-NMR spectra. The ^{1}H-NMR spectrum of 7 demonstrated two typical *para*-coupled doublets (A_2B_2 type, *J* = 10.7 Hz) at δ 8.04 and 7.11. Each doublet signal was integrated for two protons, which were assigned to coupled H-2′,6′ and -3′,5′, respectively. The downfield shift of H-3′,5′ protons at δ 7.11 compared to the apigenin of 5,7,4′-trihydroxyflavone at δ 6.98 indicated that the 4′-hydroxyl group of 7 was substituted (Marin et al., 2001). A sharp three-proton resonance for the one methoxyl group at δ 3.86 was also observed. The presence of two singlet signals at δ 6.87 (H-3) and 12.9 (5-OH) was observed. Additionally, the proton resonances at δ 6.51 (1H, d, *J* = 2.3 Hz, H-8) and 6.20 (1H, d, *J* = 2.3 Hz, H-6) were aromatic protons, which suggested the *meta*-coupling pattern of the A-ring in the skeleton. The ^{13}C-NMR-spectrum indicated 16 carbon resonances. One of the characteristic carbonyl carbon signals for the C-ring was observed at δ 181.8 and one methoxy carbon signal for the B-ring was observed at δ 55.6. Compound 7 was therefore identified as 5,7-dihydroxy-4′-methoxyflavone (acacetin) (Agrawal, 1989). Acacetin has been described as an inhibitor of eukaryotic topoisomerase I and also shows bacteriostatic activity (Boege et al., 1996; Fawe et al., 1998).

A large body of literature demonstrates that the development of diabetic complications can be reduced or prevented by the use of natural therapies, particularly with those plants having high phenolic compounds and

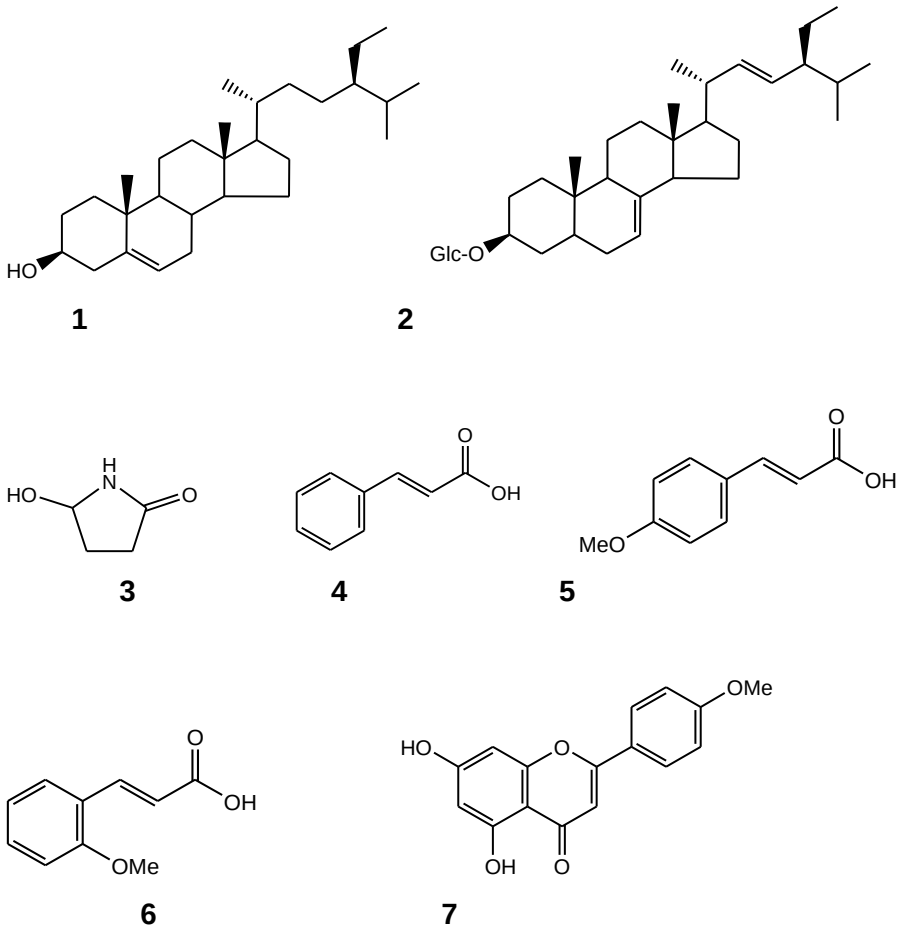


Figure 2. Structures of compounds 1-7.

Table 3. IC_{50} of 5,7-dihydroxy-4′-methoxyflavone (7) on rat lens AR inhibition.

Compound	Concentration (µg/ml)	AR inhibition [a] (%)	IC_{50} [b] (µM)
7	10	76.11	4.96
	5	69.55	
	1	43.81	
*Quercetin	1	61.10	1.56
	0.5	56.66	
	0.1	18.33	

Each sample concentration was 1 mg/ml in DMSO. [a] Inhibition rate was calculated as percentage with respect to the control value. [b] IC_{50} value was calculated from the least-squares regression equations in the plot of the logarithm of three graded concentrations *vs.* % inhibition. *Quercetin was used as a positive control.

flavonoid contents and substantial *in vivo* AR inhibiting effects (Jung et al., 2008, 2009). Several flavonoids such as luteolin, hyperin, quercetin, and quercitrin have been reported to have inhibitory activity against AR (Andrew et al., 2008; Mok et al., 2011; Lee et al., 2003). Among compounds 1-7, 5,7-dihydroxy-4′-methoxyflavone (7) was tested for AR inhibitory activity and the results are shown in Table 3. The IC_{50} value of 5,7-dihydroxy-4′-methoxyflavone (7) was calculated as 4.96 µM.

The optimum mobile phase for the analysis of acacetinwas determined by performing HPLC flow with isocratic solvent (26% ACN) for 55 min. From the wavelengths monitored, a peak was observed at 330 nm. The retention time of standard components such as 5, 7-

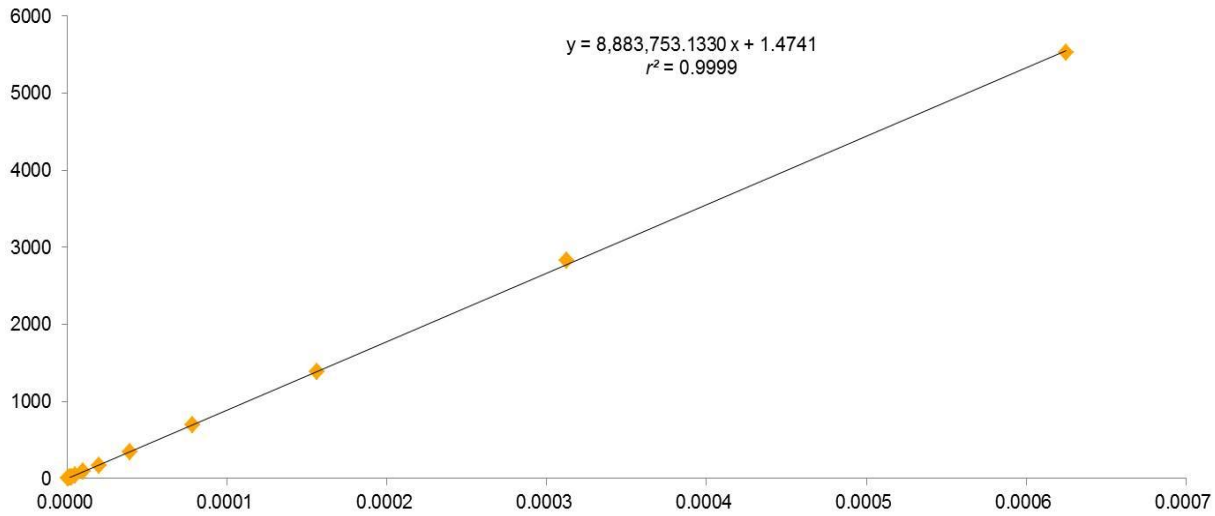


Figure 3. Calibration curve of 5,7-dihydroxy-4′-methoxyflavone (7).

Table 4. The content of 5,7-dihydroxy-4′-methoxyflavone (7) in *Scrophularia* species.

Species	7 (μg/g)
Scrophularia takesimensis	54.26 ± 1.42
S. kakudensis	25.36 ± 0.01
S. boreali-koreana	14.96 ± 0.01
S. buergeriana	14.96 ± 0.57

Data are given as the mean ± S.D. (n = 3).

dihydroxy-4′-methoxyflavone (7) was 29.68 min. We constructed a calibration curve for 5,7-dihydroxy-4′-methoxyflavone (7) as shown in Figure 3. The calibration equation for 5,7-dihydroxy-4′-methoxyflavone (7) was Y = 8,883,753.1330X + 1.4741 (r^2 = 0.9999). The content of 5,7-dihydroxy-4′-methoxyflavone (7) was measured in *S. takesimensis* (54.3 μg/g), *S. kakudensis* (25.4 μg/g), *S. boreali-koreana* (15.0 μg/g), and *S. buergeriana* (15.0 μg/g) (Table 4). Consequently, the content of 5,7-dihydroxy-4′-methoxyflavone (7) in the aerial parts of *S. takesimensis* had the highest.

In conclusion, phytochemical constituents, including stigmast-5-en-3-ol (1), α-spinasterol 3-*O*-β-D-glucopyranoside (2), 5-hydroxypyrrolidin-2-one (3), *trans*-cinnamic acid (4), 4-methoxycinnamic acid (5), 2-methoxycinnamic acid (6), and 5,7-dihydroxy-4′-methoxyflavone (7), were isolated from *S. takesimensis*. We believe all compounds were isolated for the first time from *S. takesimensis* and compounds 2, 3, 6, and 7 were isolated for the first time from *Scrophularia* species. To the best of our knowledge, our study is the first to comparatively analyze the content of 5,7-dihydroxy-4′-methoxyflavone (7) in *Scrophularia* species. As a result, *S. takesimensis* should be considered in various applications, such as functional foods and pharmaceuticals.

REFERENCES

Agrawal PK (1989). Carbon-13 NMR of Flavonoids. Elsevier Press, Amsterdam, Netherlands, p.132.

Ahn YH (2005). Ecological characteristics and distribution of native *Scrophularia takesimensis* in Ulleung Island. J. Environ. Sci., 14: 1087-1092.

Ali E, Giri VS, Pakrashi SC (1975). α-Spinasterol glucoside and other constituents of *Maesa chisia*. Phytochemistry, 14: 1133-1134.

Andrew GM, Duchowicz PR, Fernandez FM, Castro EA, Bennardi DO, Autino JC, Romanelli GP (2008). QSAR prediction of inhibition of aldose reductase for flavonoids. Bioorg. Med. Chem., 16: 7470-7476.

Awad AB, Chinnam M, Fink CS, Bradford PG (2007). β-Sitosterol activates Fas signaling in human breast cancer cells. Phytomed., 14: 747-754.

Boege F, Straub T, Kehr A, Boesenberg C, Christiansen K, Andersen A, Jakob F, Köhrle J (1996). Selected novel flavones inhibit the DNA binding or the DNA religation step of eukaryotic topoisomerase I. J. Biol. Chem., 271: 2262-2270.

Chang IM, Yun HS, Yamasaki K (1981). Revision of ^{13}C-NMR assignments of β-sitosterol and β-sitosteryl-3-*O*-β-D-glucopyranoside isolated from *Plantago asiatica* Seed. Kor. J. Pharmacogn., 12: 12-14.

Chen Y, Zhao Y, Hu Y, Wang L, Ding Z, Liu Y, Wang J (2008). Isolation of 5-hydroxypyrrolidin-2-one and other constituents from the young fronds of *Pteridium aquilinum*. J. Nat. Med., 62: 358-359.

Chethan S, Dharmesh SM, Malleshi NG (2008). Inhibition of aldose reductase from cataracted eye lenses by finger millet (*Eleusine coracana*) polyphenols. Bioorg. Med. Chem., 16: 10085-10090.
Choi BJ, Kim CW (2002). Studies on the constituents of *Impatiens nolitangere* L. Kor. J. Pharmacogn., 33: 263-266.
Chung BS, Shin MG (1990). Dictionary of Korean Folk Medicine. Young Lim Sa Press, Seoul, Korea, p.910.
Cui EJ, Park JH, Park HJ, Chung IS, Kim JY, Yeon SW, Baek NI (2011). Isolation of sterols from cowpea (*Vigna sinensis*) seeds and their promotion activity on HO-1. Kor. Soc. Appl. Biol. Chem., 54: 362-366.
Dai YH, Cui Z, Li JL, Wang D (2008). A new thiaziedione from the fruits of *Xanthium sibricum*. J. Asian Nat. Prod. Res., 10: 303-305.
Fawe A, Abou-Zaid M, Menzies JG, Bélanger RR (1998). Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. Phytopathology, 88: 396-401.
Gao L, Xu X, Nan H, Yang J, Sun G, Wu H, Zhong M (2012). Isolation of cinnamic acid derivatives from the root of *Rheum tanguticum* Maxim.ex Balf. and its significance. J. Med. Plant Res., 6: 929-931.
Jing J, Chan C, Xu L, Jin D, Cao X, Mok DKW, Parekh HS, Chen S (2011). Development of an in-line HPLC fingerprint ion-trap mass spectrometric method for identification and quality control of *Radix scrophulariae*. J. Pharmaceut. Biomed. Anal., 56: 830- 835.
Jung HA, Jung YJ, Yoon NY, Jeong DM, Bae HJ, Kim DW, Na DH, Choi JS (2008). Inhibitory effects of *Nelumbo nucifera* leaves on rat lens aldose reductase, advanced glycation endproducts formation, and oxidative stress. Food Chem. Toxicol. 46: 3818-3826.
Jung HA, Kim YS, Choi JS (2009). Quantitative HPLC analysis of two key flavonoids and inhibitory activities against aldose reductase from different parts of the Korean thistle, *Cirsium maackii*. Food Chem. Toxicol., 47: 2790-2797.
Kang JH, Choi KO, Ahn SY, Kim DS, Chon YS, Yun JG (2009). Improvement of seed germination in *Scrophularia takesimensis*, Korean native plant. Kor. J. Hort. Sci. Technol., 27: 535-539.
Kim HM, Han S, Lee YS, Chung JM, Lee S (2010). Aldose reductase inhibitory activity of *Scrophularia* species. Nat. Prod. Sci., 16: 54-57.
Kim SR, Kang SY, Lee KY, Kim SH, Markelonins GJ, Oh TH, Kim YC (2003). Anti-amnestic activity of *E-p*-methoxycinnamic acid from *Scrophularia buergeriana*. Cogn. Brain Res., 17: 454-461.
Kim SR, Kim YC (2000). Neuroprotective phenylpropanoid esters of rhamnose isolated from roots of *Scrophularia buergeriana*. Phytochemistry, 54: 503-509.
Kim SR, Sung SH, Jang YP, Park MJ, Markelonins GJ, Oh TH, Kim YC (2002). *E-p*-Methoxycinnamic acid protects cultured neuronal cells against neurotoxicity included by glutamate. Br. J. Pharmacol., 135: 1281-1291.
Kuhnt M, Pröbstle A, Rimpler H, Bauer R, Heinrich M (1995). Biological and pharmacological activities and further constituents of *Hyptis verticillata*. Planta Med., 61: 227-232.
Kuk JH, Ma SJ, Park KH (1997). Isolation and characterization of cinnamic acid with antimicrobial activity from needle of *Pinus densiflora*. Kor. Soc. Food Sci. Technol., 29: 823-826.
Lee HS (2002). Tyrosinase inhibitors of *Pulsatilla cernua* root-derived materials. J. Agric. Food Chem., 50: 1400-1403.
Lee S, Han S, Kim HM, Lee JM, Mok SY, Lee S (2011a). Isolation and identification of phytochemical constituents from *Taraxacum coreanum.* Kor. Soc. Appl. Biol. Chem., 54: 73-78.
Lee S, Jung SH, Lee YS, Shin KH (2003). Hyperin, an aldose reductase inhibitor from *Acanthopanax senticosus* leaves. Nat. Prod. Sci., 9: 4-6.
Lee TH, Lee SM, Lee DY, Son Y, Chung DK, Baek NI, Kim J (2011b). A glycosidic spinasterol from *Koreana stewartia* promotes procollagen production and inhibits matrix metalloproteinase-1 expression in UVB-irradiated human dermal fibroblasts. Biol. Pharm. Bull., 34: 768-773.
Marin PD, Grayer RJ, Veitch NC, Kite GC, Harborne JB (2001). Acacetin glycosides as taxonomic markers in *Calamintha* and *Micromeria*. Phytochem., 58: 943-947.
Michaela K, Andrea P, Horst R, Ruldolf B, Michael H (1995). Biological and pharmacological activities and further constituents of *Hyptis verticillata*. Planta Med., 61: 227-232.
Mok SY, Lee S, Kim HM, Lee JM, Lee DG, Ahn YH, Park CG, Cho EJ, Lee S (2011). Inhibition of rat lense aldose reductase by flavonoids from dandelions. Nat. Prod. Sci., 17: 130-134.
Park EH, Kahng JH, Lee SH, Shin KH (2001). An anti-inflammatory principle from cactus. Fitoterapia 72: 288-290.
Park J, Kim M, Park KR (2010). Genetic variation in endangered *Scrophularia takesimensis* (Scrophulariaceae) from Ulleung Island. Bot. Stud. 51: 371-376.
Park SU, Chae YA, Facchini PJ (2003). Genetic transformation of the figwort, *Scrophularia buergeriana* Miq., an oriental medicinal plant. Plant Cell Rep., 21: 1194-1198.
Qian J, Hunkler D, Rimpler H (1992). Iridoid-related aglycone and its glycosides from *Scrophularia ningpoensis*. Phytochem., 31: 905-911.
Rastogi N, Domadia P, Shetty S, Dasgupta D (2008). Screening of natural phenolic compounds for potential to inhibit bacterial cell division protein FtsZ. Indian. J. Exp. Biol., 46: 783-787.
Ruth S, Manfred SZ, Alois H, Theodor K (1999). A complex of 5-hydroxypyrrolidin-2-one and pyrimidine-2,4-dione isolated from *Jatropha curcas*. Phytochem., 50: 337-338.
Vosmann K, Weitkamp P, Weber N (2006). Solvent-free lipase-catalyzed preparation of long-chain alkyl phenylpropanoates and phenylpropyl alkanoates. J. Agric. Food Chem., 54: 2969-2976.
Wondrazek H, Heinze T (2008). Efficient synthesis and characterization of new photoactive dextran esters showing nanosphere formation. Macromol. Biosci., 8: 606-614.
Yuk JE, Woo JS, Yun CY, Lee JS, Kim JH, Song GY, Yang EJ, Hur IK, Kim IS (2007). Effects of lactose-β-sitosterol and β-sitosterol on ovalbumin-induced lung inflammation in actively sensitized mice. Int. Immunopharmacol., 7: 1517-1527.