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Article

Identification of Secondary Metabolites with Antioxidant and Antimicrobial Activities from *Artemisia iwayomogi* and *Chrysanthemum zawadskii*

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The present study describes the bioactive secondary metabolites of *Artemisia iwayomogy* and *Chrysanthemum morifolium*, a traditional medicinal plant widely distributed in Korea, through activity-monitored fractionation and isolation method. The aerial parts of plants were extracted with 80% ethyl alcohol, and an aqueous suspension of the extracts was partitioned successively with *n*-hexane, chloroform, ethylacetate, and *n*-butanol, leaving a residual water-soluble fraction. Secondary metabolites, compounds 1-12 were isolated from biologically active solvent-soluble fractions, and chemical structures were identified by high resolution mass spectroscopy and nuclear magnetic resonance spectroscopic analyses. All compounds were subjected to bioassay to evaluate their antioxidant and antimicrobial activities, as judged by scavenging stable 1,1-diphenyl-2-picrylhydrazyl free radicals and disk diffusion test with a minor modification of Institute of Clinical Laboratory Standards, respectively. Kaempferol (4), quercimeritrin (8), luteolin (10), and chlorogenic acid (12) were found to be antioxidants, and the concentrations at which resulted in 50% inhibition (IC_{50}) were 105.16±0.09, 101.72±0.76, 91.96±0.06, and 85.31±0.14 μM, respectively, and their activities compared favorably with those observed with the standards, ascorbic acid and BHA, which had IC_{50} values of 125.48±0.02 and 115.40±0.01 μM, respectively. Leucodin (5) was isolated from *A. iwayomogi* for the first time as moderate antioxidant and antimicrobial naturals.

Key words: antioxidant, antimicrobial, *Artemisia iwayomogi*, *Chrysanthemum morifolium*, Compositae, coumarin, flavonoid, phenolic acid, sesquiterpene lactone, HR-MS, NMR

Some secondary metabolites from natural plants exhibit biological activities, which are considered to be critical for maintaining the human health. The development of high-quality varieties containing increased levels of bioactive compounds may improve the medicinal value of plants [Hyun and Chung, 2006]. The food industry at present is facing a tremendous pressure from consumers for using chemical preservatives to prevent the growth of food-borne and spoiling microbes. Reduction or elimination of chemically synthesized additives from foods is a current demand worldwide. A new approach to prevent the proliferation of microorganism or protect food from oxidation is the use of essential oils as preservatives [Farah *et al*., 2008]. Plants and their extracts have played a great role in folk medicine, food flavoring, fragrance, and pharmaceutical industries [Kusmenoglu and Ozek, 1995]. *Artemisia iwayomogi* and *Chrysanthemum zawadskii* (Family Compositae) are perennial herbs found throughout the Asia region. *A. iwayomogi* is a popular herbal remedy in Korea that is used for its anti-inflammatory [Shin *et al*., 2006] and diuretic properties. Besides its anti-inflammatory activity, *A. iwayomogi* has been reported to be a modulator of the functional differentiation of bone marrow-derived dendritic cells [Lee *et al*., 2008] and is known to be involved in the suppression of thymocyte apoptosis [Hwang *et al*., 2005]. *Artemisia* oils also have inhibitory effects on bacterial and

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yeast growth [Lopes-Lutz *et al*., 2008; Chung *et al*., 2009]. *C. zawadskii*, commonly known as Gu-Jul-Cho in Korea, is used in traditional medicine to treat pneumonia, bronchitis, cough, common cold, pharyngitis, bladder-related disorders, gastroenteric disorders, and hypertension [Han *et al*., 2002]. Linarin and linarin acetate from *C. zawadskii* showed a moderate inhibition of cell growth [Singh *et al*., 2005]. In the current investigation, *A. iwayomogi* and *C. zawadskii* were selected for study, since the $CHCl_3$ and EtOAc soluble fractions showed inhibitory activities in 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and growth of microorganisms. Bioassay-monitoring of the active solvent fractions led to the isolation of twelve compounds, which were evaluated for their individual activity.

Materials and Methods

Chemicals. DPPH was obtained from Sigma Chemical Co. (St. Louis, MO). The compounds were dissolved in DMSO and stored at −4°C. All other chemicals were purchased from commercial sources and were of the highest purity available.

Plants. The aerial parts of *A. iwayomogi* and *C. zawadskii* were purchased from Kyung-Dong Oriental Market (Seoul, Korea). A voucher specimen was deposited at the College of Pharmacy at Duksung Women's University (Seoul, Korea).

Instrumental analysis. Uncorrected melting points (mp) were determined using a Mitamura-Riken melting point apparatus. A Hewlett Packard Model 5985B gas chromatography (GC)/mass spectrometry (MS) system was used for electron impact MS (EI-MS). FAB-MS spectra were obtained on a JEOL JMS 700-M Station (Tokyo, Japan). The ultraviolet (UV)/visible (Vis) and infrared (IR) spectra were recorded on a Hitachi 3100 UV/Vis and a JASCO Fourier transform (FT)-IR 5300 spectrophotometer, respectively. A Bruker AMX500 spectrometer (Billerica, MA) was used to record nuclear magnetic resonance (NMR) spectra (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) with tetramethylsilane (TMS) as an internal standard and $CDCl_3$-*d* (deuterium $CHCl_3$), CD_3OD-d_4 (deuterium MeOH), and DMSO-d_6 (deuterium dimethylsulfoxide) as NMR solvents. Thin-layer chromatography (TLC) was performed on 0.25-mm silica gels (Kiesel-60 F_{254} plates, Merck, Darmstadt, Germany). After development, the phenol and terpenoid were visualized and identified by spraying the plates with $FeCl_3$ and Liebermann-Bürchard reagents. Silica gel (Merck 60 A, 230-400 mesh, ASTM) and Sephadex LH-20 (25-100 mm; Pharmacia Fine Chemicals, Piscataway, NJ) were used for the open-column chromatographic separation.

Extraction and solvent fractionation. The dried and ground aerial parts of *A. iwayomogi* and *C. zawadskii* were extracted three times with 80% EtOH for 3 h in an 80°C water bath. The combined dark-brown EtOH extracts were partitioned between *n*-hexane and water, and the more polar layer (aqueous layer) was successively partitioned with chloroform ($CHCl_3$), ethylacetate (EtOAc), and *n*-butyl alcohol (BuOH) (Table 1). The solvent-soluble fractions obtained from the chromatographic separation were tested for their biological activities.

Isolation of *Artemisia iwayomogi*. The dried EtOAc-soluble fraction (7.0 g) was separated on a silica gel column (4.5×50 cm) using a $CHCl_3$:MeOH gradient (95:5-25:5) and yielded six subfractions. Subfraction 4 (1.2 g) was further separated on a silica-gel vacuum column by elution with $CHCl_3$:MeOH (12:1-7:1) to produce a yellow solid material. This material was further purified to nine sub-subfractions using a Sephadex LH-20 column. Sub-subfractions 7 (192.8 mg) and 11 (240.5 mg) were recrystallized with highly purified MeOH to yield pure forms of compounds **1** (16.7 mg), **2** (18.3 mg), **3** (20.8 mg), **4** (32.1 mg), and **5** (27.4 mg).

Isolation of *Chrysanthemum zawadskii*. The dried $CHCl_3$-soluble fraction (5.0 g) was separated on a silica gel open column (3.0×70 cm) using an *n*-hexane-EtOAc gradient (5:1-1:1) and yielded 12 subfractions. Subfraction 7 (1.4 g) was eluted with *n*-hexane-EtOAc (5:1), vacuum-concentrated, and further purified by recrystallization with highly purified EtOAc to produce compound **6** (32.7 mg). The dried EtOAc-soluble fraction (4.0 g) was separated on a silica gel open column (4.0×60 cm) using a $CHCl_3$:MeOH:H_2O gradient (95:5:0-80:10:2.0) and yielded 24 subfractions. Subfractions 7-11 (312.2 mg) were eluted with $CHCl_3$:MeOH:H_2O (92:8:0.5) and separated on a vacuum-liquid column using a $CHCl_3$:MeOH gradient (95:5-88:12) to yield compounds **7** (28.1 mg), **8** (32.5 mg), **9** (16.4 mg), and **11** (36.2 mg). Subfractions

Table 1. Total yields (g) of EtOH extract and solvent-soluble fractions of plants

Plants	EtOH extract	Solvent-soluble fractions			
		n-Hexane	$CHCl_3$	EtOAc	*n*-BuOH
Artemisia iwayomogi (1.2 kg)	83.47	22.31	14.71	8.29	19.50
Chrysanthemum zawadskii (1.2 kg)	173.71	23.63	30.86	13.91	16.93

19-21 (755.2 mg) were further separated on a Sephadex LH-20 column with a $CHCl_3$:MeOH:H_2O gradient (85:12:1.0), followed by a separation with highly purified MeOH to produce pure compounds **10** (36.2 mg) and **12** (8.6 mg).

Assay for DPPH free radical scavenging activity. This assay is based on the scavenging activity of stable DPPH free radicals [Chung and Shin, 2007]. Reaction mixtures containing 5 mL of test sample dissolved in DMSO and 95 mL of 300 mM DPPH in ethanol solution (final DPPH concentration) were incubated at 37°C for 30 min in 96-well micro filter plates, and the absorbance was measured at 515 nm. The percent inhibitions of the samples were determined by comparison to that of the DMSO-treated control group. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. To monitor the reaction conditions and confirm the usefulness of the assay, commercially available antioxidants, including ascorbic acid and 2(3)-*tert*-butyl-4-hydroxyanisole (BHA), were also evaluated for their free-radical scavenging activity.

Selection of strains and assay of solvent-soluble fractions. The bacterial strains to be tested were obtained from the Culture Collection of Antimicrobial Resistant Microbes (CCARM) and the Korean Culture Center of Microorganisms (KCCM) [Byun *et al*., 2006]. The solvent-soluble fractions of 80% ethanolic extracts of *A. iwayomogi* and *C. zawadskii* were screened for their susceptibility to pathogens using the disk diffusion method. The microorganisms tested for pure compounds **1-12** were *Streptococcus pyrogenes* CCARM0206, *Staphylococcus aureus* CCARM0201, *Escherichia coli* CCARM0230, *Salmonella typhimurium* CCARM0240, and *Pseudomonas aeruginosa* CCARM0219. The bacteria were inoculated on Muller-Hilton agar plates (MHA; BBL, Sparks, MD), according to the methods of the Performance Standards for antimicrobial Susceptibility Testing (M100-S14) of CLSI (Clinical Laboratory Standards Institute, 2006). The pure compounds **1-12** were diluted in DMSO to make a final concentration of 100 μM. Each bacterial species was suspended in saline to make a Macfarland concentration of 0.5. Using a sterile cotton swab an aliquot of the diluent was inoculated on culture media, a 5-μL aliquot containing 500 μg of sample was laid onto medium inoculated with each bacterium. After overnight incubation at 37°C, the antibacterial activity was evaluated by measuring the diameter (mm) of the inhibition zones (DIZ) with a ruler. All tests were performed in triplicates. As a control, 5 μL of DMSO was streaked onto the plates.

Statistical analyses. The results are expressed as the mean±SD (n=3) of triplicate experiments.

Results and Discussion

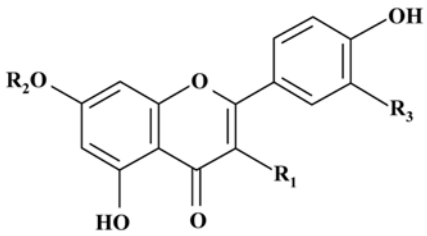
The dried and ground plants were extracted with 80% EtOH and successively fractionated with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH to identify antioxidant solvent-soluble fractions (Table 2). Of the fractions, the EtOAc-soluble fraction of *A. iwayomogi*, and the $CHCl_3$ and EtOAc-soluble fractions of *C. zawadskii* showed strong and moderate antioxidant activities in DPPH free radical scavenge, respectively. Each solvent-soluble fraction was column-chromatographed for the identification of pure compounds.

Compounds **1-5** were isolated from the EtOAc-soluble fraction of *A. iwayomogi* and had significant antioxidant activity with an IC_{50} value of 19.2±0.7 μg/mL based on DPPH free-radical scavenging activity. The $CHCl_3$ and EtOAc-soluble fractions of *C. zawadskii* respectively showed moderate antioxidant activity with IC_{50} values of 56.1±1.8 and 32.7±1.7 μg/mL. The $CHCl_3$ and EtOAc-soluble fractions were further chromatographed with silica gel and Sephadex LH-20 to isolate compounds **6-7**, and **8-12**, respectively. Chemical structures of the pure compounds **1-12** were characterized based on the published data [Glasl *et al*, 2002; Taskova *et al*., 2003; Cheng *et al*, 2008; Zheng *et al*., 2008], physicochemical methods, co-TLC, mp, EI-MS, UV, IR, and NMR (1D- and 2D-) spectral data.

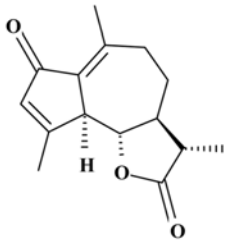
Apigenin (1). Yellow amorphous powder; $FeCl_3$ color reaction: positive; mp: 122-125°C; UV λ_{max} (MeOH) nm: 269 (4.50), 347 (4.32); IR ν_{max} (KBr) cm^{-1}: 3350 (OH), 1650 (C=O), 1610, 1510, 1450 (aromatic C=C); EI-MS *m/z*: 270 $[M]^+$, 252 $[M-H_2O]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.73 (2H, d, *J*=8.0, H-2',6'), 6.91 (2H, d, *J*= 8.0, H-3',5'), 6.70 (1H, s, H-3), 6.41 (1H, d, *J*=1.2, H-6), 6.20 (1H, d, *J*=1.9, H-8); ^{13}C-NMR (125 MHz, CD_3OD): δ 159.1 (C-2), 136.1 (C-3), 179.4 (C-4), 163.1 (C-5), 99.7 (C-6), 165.7 (C-7), 94.7 (C-8), 158.4 (C-9), 105.8 (C-10),

Table 2. Antioxidant activity of solvent-soluble fractions of plants

Plants	DPPH free radical scavenging activities (IC_{50}: mg/mL)			
	n-Hexane	$CHCl_3$	EtOAc	*n*-BuOH
Artemisia iwayomogi	110.3±1.2	83.2±1.4	19.2±0.7	46.2±0.4
Chrysanthemum zawadskii	>200	56.1±1.8	32.7±1.7	82.1±0.2



1 : R_1=H; R_2=H; R_3=H
2 : R_1=H; R_2=Glc; R_3=H
3 : R_1=OH; R_2=H; R_3=OCH_3
4 : R_1=H; R_2=OH; R_3=H
7 : R_1=Rha; R_2=H; R_3=H
8 : R_1=OH; R_2=Clc; R_3=OH
9 : R_1=O-Glc-O-Rha; R_2=H; R_3=OH
10 : R_1=H; R_2=H; R_3=OH
11 : R_1=Rha; R_2=H; R_3=OH



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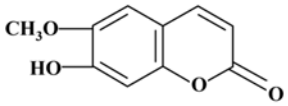

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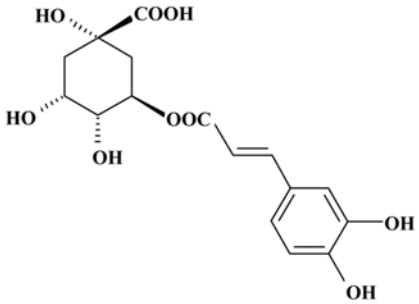

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Fig. 1. Chemical structures of compounds 1-12.

122.5 (C-1'), 131.9 (C-2',6'), 110.1 (C-3), 116.4 (C-3',5'), 161.4 (C-4').

Apigenin-7-*O*-β-D-glucopyranoside (2). Pale yellowish powder (MeOH); $FeCl_3$ and aniline hydrogen phthalate color reaction: positive; mp 200-204°C; UV λ_{max} (MeOH) nm: 270 (4.54), 368 (4.36); IR ν_{max} (KBr) cm^{-1}: 3380 (OH), 1661 (α,β-unsaturated C=O), 1607, 1499 (aromatic C=C), 1240 (aromatic C-O), 1062, 1015 (glycosidic C-O); FAB-MS *m/z*: 432 $[M]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.27 (1H, s, 5-OH), 7.73 (2H, d, *J*=8.0, H-2',6'), 6.91 (2H, d, *J*=8.0, H-3',5'), 6.43 (1H, d, *J*=1.9, H-8), 6.22 (1H, d, *J*=1.9, H-6), 5.36 (1H, d, *J*=7.6, anomeric proton); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 181.4 (C-4), 164.4 (C-2), 162.4 (C-7), 161.0 (C-5), 160.1 (C-3), 156.6 (C-9), 150.2 (C-4'), 145.2 (C-3'), 121.2 (C-1'), 119.1 (C-6'), 116.1 (C-5'), 113.2 (C-2'), 105.1 (C-10), 103.1 (C-3), 99.1 (C-8), 97.7 (Glc-1"), 77.0 (Glc-5"), 76.7 (Glc-2"), 76.1 (Glc-3"), 70.1 (Glc-4"), 60.4 (Glc-6").

Isorhamnetin (3). Yellow amorphous powder; $FeCl_3$ color reaction: positive; mp: 132-133°C; UV λ_{max} (MeOH) nm: 269, 347; IR ν_{max} (KBr) cm^{-1}: 3320 (OH), 1647 (C=O), 1610, 1605, 1450 (aromatic C=C); EI-MS *m/z*: 316 $[M]^+$, 298 $[M-H_2O]^+$, 280 $[M-H_2O-CO]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.73 (2H, d, *J*=8.0, H-2',6'), 6.82 (1H, dd, *J*=8.0, 1.2, H-5'), 6.70 (1H, s, H-3), 6.32 (1H, d, *J*=1.2, H-8), 6.15 (1H, d, *J*=1.2, H-6), 3.10 (3H, q, OCH_3); ^{13}C-NMR (125 MHz, CD_3OD): δ 159.1 (C-2), 136.1 (C-3), 179.4 (C-4), 163.1 (C-5), 99.7 (C-6), 165.7 (C-7), 94.7 (C-8), 158.4 (C-9), 105.8 (C-10), 122.5 (C-1'), 131.9 (C-2',6'), 116.4 (C-5'), 160.1 (C-3'), 161.4 (C-4').

Kaempferol (4). Yellow amorphous powder; $FeCl_3$ color reaction: positive; mp: 202-210°C; UV λ_{max} (MeOH) nm: 269, 347; IR ν_{max} (KBr) cm^{-1}: 3350 (OH), 1650 (C=O), 1610, 1510, 1450 (aromatic C=C); EI-MS *m/z*: 287 $[M+H]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.73 (2H, d, *J*=8.0, H-2',6'), 6.91 (2H, d, *J*=8.0, H-3',5'), 6.71 (1H, s, H-3), 6.32 (1H, d, *J*=1.2, H-8), 6.15 (1H, d, *J*=1.2, H-6); ^{13}C-NMR (125 MHz, CD_3OD): δ 159.1 (C-2), 136.1 (C-3), 179.4 (C-4), 163.1 (C-5), 99.7 (C-6), 165.7 (C-7), 94.7 (C-8), 158.4 (C-9), 105.8 (C-10).

Leucodin (5). White amorphous powder; Liebermann-Bürchard reagents: positive; mp: 204-206°C; UV λ_{max} (MeOH) nm: 205; IR ν_{max} (KBr) cm^{-1}: 3420 (OH), 1740 (γ-lactone); EI-MS *m/z*: 246 $[M]^+$, 218 $[M-CO]^+$, 203 $[M-CO-CH_3]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 1.29 (br d, *J*=7.0, H-15), 1.30 (d, *J*=7.1, H-13), 1.47 (br d, *J*=7.0, H-14), 1.91 (dq, *J*=2.0, 8.1, H-5), 2.06 (ddd, *J*=2.0, 8.0, 10.2, H-7), 2.32 (dd, *J*=4.5, 13.1, H-9), 2.37 (d, *J*=2.0, H-3), 3.24 (dq, *J*=7.0, H-11), 4.20 (dt, *J*=4.5, H-8), 4.63 (t, *J*=10.1, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 13.2 (C-14), 13.2 (C-13), 18.4 (C-15), 35.5 (C-10), 36.8 (C-11), 42.8 (C-1), 45.1 (C-9), 52.0 (C-5), 56.1 (C-7), 63.2 (C-8), 78.0 (C-3), 82.1 (C-6), 142.4 (C-4), 178.0 (C-12), 198.1 (C-2).

Scopoletin (6). Colorless needle; mp: 202-204°C; UV λ_{max} (MeOH) nm: 220, 248; IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 1470 (C=C); EI-MS *m/z*: 292 $[M]^+$, 149 $[M-CH_3-CO]^+$, 121 $[M-CH_3-2CO]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 3.81 (d, *J*=8.0, OCH_3), 6.20 (d, *J*=9.5, H-3), 6.77 (s, H-8), 7.20 (s, H-5), 7.89 (d, *J*=9.5, H-4), 10.35 (s, H-7); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 56.08 (OCH_3),

102.84 (C-8), 109.64 (C-5), 110.63 (C-10), 111.75 (C-3), 144.59 (C-4), 145.34 (C-6), 149.57 1(C-9), 51.20 (C-7), 160.82 (C-2).

Kaempferol-3-*O*-α-L-rhamnopyranoside (7). Yellow amorphous powder; $FeCl_3$ and aniline hydrogen phthalate color reaction: positive; mp: 172-175°C; UV λ_{max} (MeOH) nm: 269, 347; IR ν_{max} (KBr) cm^{-1}: 3350 (OH), 1650 (C=O), 1610, 1510, 1450 (aromatic C=C), 1365, 1170 (glycosidic C-O); FAB-MS *m/z*: 455 $[M+Na]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.73 (2H, d, *J*=8.0, H-2',6'), 6.91 (2H, d, *J*=8.0, H-3',5'), 6.32 (1H, d, *J*=1.2, H-8), 6.15 (1H, d, *J*=1.2, H-6), 5.36 (1H, d, *J*=1.6, Rha-1), 0.92 (3H, d, *J*=5.5, Rha-6); ^{13}C-NMR (125 MHz, CD_3OD): δ 159.1 (C-2), 136.1 (C-3), 179.4 (C-4), 163.1 (C-5), 99.7 (C-6), 165.7 (C-7), 94.7 (C-8), 158.4 (C-9), 105.8 (C-10), 122.5 (C-1'), 131.9 (C-2',6'), 116.4 (C-3',5'), 161.4 (C-4'), 103.4 (Rha-1"), 71.9 (Rha-2"), 72.0 (Rha-3"), 73.1 (Rha-4"), 71.8 (Rha-5"), 17.6 (Rha-6").

Quercimeritrin (8). Pale yellowish powder (MeOH); $FeCl_3$ and aniline hydrogen phthalate color reaction: positive; mp: 200-204°C; UV λ_{max} (MeOH) nm: 282 (4.54), 360 (4.36); IR μ_{max} (KBr) cm^{-1}: 3380 (OH), 1661 (α,β-unsaturated C=O), 1607, 1499 (aromatic C=C), 1240 (aromatic C-O), 1062, 1015 (glycosidic C-O); FAB-MS *m/z*: 465 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 8.27 (1H, s, 5-OH), 7.66 (1H, dd, *J*=2.1, 8.4, H-6'), 7.56 (1H, d, *J*=2.1, H-2'), 6.82 (1H, d, *J*=8.4), 6.43 (1H, d, *J*=1.9, H-8), 6.22 (1H, d, *J*=1.9, H-6), 5.36 (1H, d, *J*=7.6, anomeric proton); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 181.4 (C-4), 164.4 (C-2), 162.4 (C-7), 161.0 (C-5), 160.1 (C-3), 156.6 (C-9), 150.2 (C-4'), 145.2 (C-3'), 121.2 (C-1'), 119.1 (C-6'), 116.1 (C-5'), 113.2 (C-2'), 105.1 (C-10), 103.1 (C-3), 99.1 (C-8), 97.7 (Glc-1"), 77.0 (Glc-5"), 76.7 (Glc-2"), 76.1 (Glc-3"), 70.1 (Glc-4"), 60.4 (Glc-6").

Rutin (9). Pale yellowish powder (MeOH); $FeCl_3$ and aniline hydrogen phthalate color reaction: positive; mp: 200-204°C; UV λ_{max} (MeOH) nm: 271 (4.50), 375 (4.26); IR ν_{max} (KBr) cm^{-1}: 3390 (OH), 1658 (α,β-unsaturated C=O), 1600, 1509 (aromatic C=C), 1238 (aromatic C-O), 1060, 1011 (glycosidic C-O); FAB-MS *m/z*: 611 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.67 (1H, d, *J*=2.1, H-2'), 7.54 (1H, dd, *J*=2.1, 8.4, H-6'), 6.88 (1H, d, *J*=8.4, H-5'), 6.40 (1H, d, *J*=1.8, H-8), 6.18 (1H, d, *J*=1.8, H-6), 5.31 (1H, d, *J*=7.6, anomeric proton), 0.92 (3H, d, *J*=5.5, Rha-6); ^{13}C-NMR (125 MHZ, DMSO-d_6): δ 177.24 (C-4), 163.97 (C-7), 161.09 (C-9), 156.50 (C-5), 156.30 (C-2), 148.29 (C-4'), 144.63 (C-3'), 133.16 (C-3), 121.48 (C-6'), 121.05 (C-1'), 116.15 (C-5'), 115.11 (C-2'), 103.83 (C-10), 101.04 (C-Glc"), 100.63 (C-Rha'"), 98.58 (C-6), 93.49 (C-8), 76.30 (Rha-5"), 75.77 (Glc-5"), 73.95 (Glc-2"), 71.70 (Rha-4'"), 70.43 (Rha-3'"), 70.25 (Rha-2'"), 69.86 (Glc-4"), 68.14 (Rha-5'"), 66.89 (Glc-6"), 17.63 (Rha-6'").

Luteolin (10). Pale yellow plate from MeOH; $FeCl_3$ color reaction: positive; mp: 320°C; UV λ_{max} (MeOH) nm: 254 (4.32), 267 (4.29), 297 (sh, 4.25), 360 (4.21); IR ν_{max} (KBr) cm^{-1}: 3330 (OH), 1648 (C=O), 1625, 1610, 1445 (aromatic C=C); EI-MS *m/z*: 286 $[M]^+$, 258 $[M\text{-}CO]^+$, 229 $[M\text{-}CO\text{-}CHO]^+$ (24.5), 153 $[A_1+H]^+$ (49.3), 152 $[A_1]^+$ (10.1), 134 $[B_1]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 11.6 (1H, s, 5-OH), 7.43 (1H, dd, *J*=1.8, H-2'), 7.41 (1H, dd, *J*=8.4, 1.8, H-6'), 6.92 (1H, d, *J*=8.4, H-5'), 6.67 (1H, s, H-3), 6.58 (1H, d, J=1.8, H-8), 6.22 (1H, d, J=1.8, H-6) ^{13}C-NMR (125 MHz, DMSO-d_6): δ 177.1 (C-4), 167.0 (C-7), 161.5 (C-5), 157.6 (C-2), 150.2 (C-4'), 145.1 (C-3'), 133.8 (C-3), 122.5 (C-6'), 116.8 (C-5'), 116.1 (C-2'), 158.0 (C-9), 122.8 (C-1'), 106.6 (C-10), 102.1 (C-3), 99.0 (C-6), 94.7 (C-8).

Quercitrin (11). Yellow crystal from MeOH; $FeCl_3$ and aniline hydrogen phthalate color reaction: positive; mp: 238-240°C; UV λ_{max} (MeOH) nm: 257 (4.33), 267 (sh, 4.26), 359 (4.20); IR ν_{max} (KBr) cm^{-1}: 3410 (OH), 1655 (α,β-unsaturated C=O), 1607, 1510 (aromatic C=C), 1365, 1210, 1087 (glycosidic C-O); FAB-MS *m/z*: 449 $[M+1]^+$, 285 $[M+1\text{-}Rha]^+$, 267 $[M+1\text{-}Rha\text{-}H_2O]^+$, 249 $[M+1\text{-}Rha\text{-}2H_2O]^+$, 221 $[M+1\text{-}Rha\text{-}2H_2O\text{-}CO]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.0 (1H, s, 5-OH), 7.25 (1H, d, *J*=2.0, H-2'), 7.21 (1H, dd, *J*=8.4, 2.0, H-6'), 6.86 (1H, d, J=8.4, H-5'), 6.28 (1H, d, *J*=2.0, H-8), 6.22 (1H, d, *J*=2.0, H-6), 5.26 (1H, d, *J*=2.0, anomeric proton), 0.80 (3H, d, *J*=6.0 Hz, CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 179.0 (C-4), 167.1 (C-7), 162.5 (C-5), 157.9 (C-2), 149.2 (C-4'), 145.8 (C-3'), 135.9 (C-3), 122.7 (C-6'), 116.8 (C-5'), 116.1 (C-2'), 158.8 (C-9), 122.8 (C-1'), 105.6 (C-10), 103.1 (Rha-1"), 99.6 (C-6), 94.6 (C-8), 73.1 (Rha-4"), 71.9 (Rha-3"), 12.7 (CH_3).

Chlorogenic acid (12). White powder from MeOH; mp: 156-158°C; UV λ_{max} (MeOH) nm: 270 (2.20), 258 (4.18); IR ν_{max} (KBr) cm^{-1}: 3360 (OH), 1720, 1709, 1640, 1529 (aromatic C=C); EI-MS *m/z*: 354 $[M]^+$, 318 $[M\text{-}2H_2O]^+$, 270 $[M\text{-}2H_2O\text{-}COOH]^+$, 180 $[M\text{-}C_7H_{10}O_5]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.42 (1H, s, H-1'), 7.03 (1H, d, *J*=1.8, H-2'), 7.01 (1H, dd, *J*=8.2, 1.8, H-6'), 6.78 (1H, d, *J*=8.2, H-5'), 6.19 (1H, s, CH-), 3.76 (1H, s, H-3), 3.25 (1H, s, H-4), 1.90 (2H, d, *J*=13.2, H-2), 1.87 (2H, d, *J*=13.7, H-6), 1.79 (2H, d, *J*=13.2, H-2), 1.72 (2H, d, *J*=13.7, H-6); ^{13}C-NMR (125 MHz DMSO-d_6): δ 175.4 (COOH), 150.1 (C-4'), 144.9 (C-3'), 122.3 (C-6'), 121.8 (C-1'), 116.7 (C-2'), 115.3 (C-5'), 74.5 (C-3), 69.1 (C-4), 68.5 (C-5), 66.5 (C-1), 41.0 (C-2), 40.5 (CH-), 37.8 (CH-), 37.4 (C-6).

Antioxidant activity. Compounds **1-12** were assayed for biological activity. The DPPH radical is a stable organic free-radical with an absorption band of 515-528

Table 3. Antioxidant activities of pure compounds 1-12

Compounds	DPPH free radical scavenging activities (IC_{50}: μM/mL)
1	147.04±0.31
2	225.03±0.12
3	128.16±0.17
4	105.16±0.09
5	174.18±0.15
6	129.45±0.26
7	178.01±0.08
8	101.72±0.76
9	134.59±0.11
10	91.96±0.06
11	145.31±0.18
12	85.31±0.14
Ascorbic acid[a]	125.48±0.02
BHA[a]	115.40±0.01

[a]Control compounds

nm. However, the radical looses this absorption feature when it accepts an electron or free-radical species, resulting in a visually noticeable discoloration from purple to yellow. Because the DPPH radical can accommodate many samples in a short time period and is sensitive enough to detect active ingredients at low concentrations, it has been extensively used to screen the antiradical activities of compounds or plant extracts [Zou *et al*., 2004]. Except for compounds **5**, **6** and **12**, each of the compounds had a characteristic flavonoid skeleton as shown by the $FeCl_3$ color reaction, UV, and ^{1}H-NMR and ^{13}C-NMR spectral data [Chung *et al*., 1999]. Compounds **10** and **12** respectively showed potent antioxidant activity with IC_{50} values of 91.96±0.06 and 85.31±0.14 μM, 1.36- to 1.47-fold more efficacy as compared to the ascorbic acid, which had an IC_{50} value of 125.48±0.02 μM (Table 3). Compound **10** had four non-*ortho*-hydroxyl groups in its structure, suggesting that radical-scavenging activity could be enhanced by increasing hydroxyl substitution and that these four hydroxyl groups may confer strong antioxidant activity as compared to compounds **8** and **11**, possibly because there were C-3-OH and/or C-7-OH glycosylation of flavonoid skeleton. Heilmann *et al*. [1995] reported that all flavonoids possessing C-3,4-dihydroxyl substitution have excellent inhibitory activity against the DPPH radical. We may hypothesize that glycosylation could reduce the number of free hydroxyl groups or destroy the *ortho*-hydroxyl structure, and the sugar linkage may hinder access of free radical scavengers to the center of the DPPH radical. These results confirmed that the DPPH free radical scavenging activity of flavonoids is greatly influenced by glycosylation and is related to the type and configuration of the glycons [Cho *et al*., 2003]. Our results suggest that the compounds **4**, **8**, **10** and **12** isolated from *A. iwayomogi* and *C. zawadskii* exhibited effective radical scavenging activity and may be promising agents for scavenging free radicals. The biological activity of flavonoids is a comprehensive effect of multiple factors, and observing the antioxidant activity *in vitro* from a structural perspective is only preliminary. Although the structure-dependent correlation between chemical structure and antioxidative activity is not applicable to all flavonoids, it exists in certain flavonoids. Compound **5**, which showed positive reaction with Liebermann-Bürchard reagent, exhibited a molecular ion peak at *m/z* 246, in accord with the formula $C_{15}H_{18}O_3$, as well as fragment ions at *m/z* 218 $[M\text{-}CO]^+$ and *m/z* 203 $[M\text{-}CO\text{-}CH_3]^+$. The IR spectrum showed a characteristic absorption band for a saturated γ-lactone at 1740 cm^{-1}. The ^{13}C-NMR spectrum displayed fifteen carbon resonances. The lactone carbonyl resonances were located at δ 82.1 and 178.0. Judging from the DEPT spectrum, it is clear that the remaining carbon resonances are due to three methyl, two carbonyl, one methylene and six methines. The splitting pattern of the oxymethine proton signal at δ 4.63 (1H, t, *J*=10.1) correlated with the ^{13}C resonance at δ 82.1, and was assigned to the lactone proton at C-6. This indicated the *trans*-diaxial disposition of the protons at C-5 (α), C-6 (β), C-7 (α), suggesting a quaianolide-type sesquiterpene lactone [Chung and Woo, 1994]. On the basis of spectral data and literature [Glasl *et al*., 2002], compound **5** was elucidated as Leucodin for the first time from *A. iwayomogi*.

Antimicrobial activity. The results of screening of antimicrobial fractions from *A. iwayomogi* and *C. zawadskii* were demonstrated as published paper [Byun *et al*., 2006]. Except for the aqueous layer, all tested fractions of *A. iwayomogi* and *C. zawadskii* exhibited prominent activity against both of *S. pyrogenes* strains with 6-10 mm growth inhibition zones, whereas *S. aureus* revealing a significant deviation in sensitivity between strains *S. aureus* 285 and *S. aureus* 503 showed highest sensitivity of 9-14 mm of inhibition when treated with $CHCl_3$ or EtOAc fraction [Byun *et al*., 2006]. Compounds **1-12** were tested on inhibition zone against *S. pyogenes* CCARM0206, *S. aureus* CCARM0201, *E. coli* CCARM0230, *S. typhimurium* CCARM0240, and *P. aeruginosa* CCARM0219. Among all tested compounds, compounds **4-5** and **11-12** exhibited inhibitory growth zone, except on *E. coli* CCARM0230. Compounds **4** and **5** showed 8 and 12 mm of inhibition zones against *S. pyogenes*, respectively. Compounds **5**, **10**, and **11** also showed 13, 12, and 17 mm of inhibition zones against *S.*

Table 4. Diameters of growth inhibition zones of pure compounds

Strain Name	Antibacterial activity (DIZ[a], mm)				
	C[b]	**4**	**5**	**10**	**11**
Streptococcus pyogenes CCARM0206	0	8	12	0	0
Staphylococcus aureus CCARM0201	0	0	13	12	17
Escherichia coli CCARM0230	0	0	0	0	0
Salmonella typhimurium CCARM0240	0	12	0	10	10
Pseudomonas aeruginosa CCARM0219	0	0	0	0	0

[a]DIZ: Diameter of inhibition zone
[b]DMSO: Negative control

aureus (Table 4). Compound **5**, isolated from *A. iwayomogi* for the first time, showed inhibitory growth against *S. pyogenes* and *S. aureus*. Although its activity is much weaker than synthetics, it is an attractive promising compound in terms of safety [Eggimann *et al*., 2003]. Tshikalange *et al*. [2005] reported that compound **10** showed antimicrobial activity at the highest non-toxic concentrations of 500 μg/mL, whereas compound **12** showed a strong inhibitory effect against *Serratia marcescens* and *Enterobacter cloacae* [Almeida *et al*., 2006]. The interesting finding from our experiments was that *A. iwayomogi* and *C. zawadskii* are rich in secondary metabolites with antioxidant and antimicrobial activities. In conclusion, the solvent-soluble fractions and pure compounds with biological activities could be used as functional materials for the development of safe natural sources.

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References

Almeida AA, Farah A, Silva DA, Nunan EA, and Glória MB (2006). Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria. *J Agric Food Chem* **54**, 8738-8743.

Byun Y-H, Shim Y, Lim S, Choi S-H, Park N-H, Moon S-R, Koo In-H, Lee K-U, Lee M-Y, Hong H-J, Chung H-S, Lee Y-H, and Shin S-W (2006) Development of natural drugs against antibiotics-resistant bacteria from *Artemisia* and *Chrysanthemum* species in Korea and study on its active mechanism (1). *Duksung Bull Pharm Sci* **17**, 13-21.

Cheng N, Abraham L, Masakuni T, Isao H, and Hajime T (2008) Antioxidant flavonoid glycosides from the leaves of *Ficus pumila* L. *Food Chem* **109**, 415-420.

Cho EJ, Yokozawa T, Rhyu DY, Kim SC, Shibahara N, and Park JC (2003). Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1,1-diphenyl-2-picrylhydrazyl radical. *Phytomedicine* **10**, 544-551.

Chung EY, Byun YH, Shin EJ, Chung HS, Lee YH, and Shin S (2009). Antibacterial effects of Vulgarone B from *Artemisia iwayomogi* alone and in combination with Oxacillin. *Arch Pharm Res* **32**, 1711-1719.

Chung HS, Chang LC, Lee SK, Shamon LA, van Breeman RG, Mehta RG, Farnsworth NR, Pezzuto JM, and Kinghorn AD (1999) Flavonoid constituents of *Chorizanthe diffusa* with potential cancer chemopreventive activity. *J Agric Food Chem* **47**, 36-41.

Chung HS and Shin JC (2007) Characterization of antioxidant alkaloids and phenolic acids from anthocyanin-pigmented rice (*Oryza sativa* cv. *Heugjinjubyeo*). *Food Chem* **104**, 1670-1677.

Chung HS and Woo WS (1994) Dentalactone, A sesquiterpene from *Ixeris dentata. Phytochem* **35**, 1583-1584.

Clinical and Laboratory Standards Institute. 2006. M100-S16, Performance standards for antimicrobial susceptibility testing; 16th informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Eggimann P, Garbino J, and Pittet D (2003) Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. *The LANCET Infectious Diseases* **3**, 685-702.

Farah D, Tran DX, Masaaki Y, and Shinkichi (2008) Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. Radiata. *Food Control* **19**, 346-352.

Glasl S, Mucaji P, Werner I, Presser A, and Jurenitsch J (2002) Sesquiterpenes and flavonoid aglycones from Hungarian taxon of the *Achillea millefolium* group. *Z Naturforsch C* **57**, 976-982.

Han S, Sung KH, Yim D, Lee S, Lee CK, Ha NJ, and Kim K (2002) The effect of linarin on LPS-induced cytokine production and nitric oxide inhibition in murine macrophages cell line RAW264.7. *Arch Pharm Res* **25**, 170-177.

Heilmann J, Merfort I, and Weiss M (1995) Radical scavenger activity of different 3',4'-dihyroxyflavonols and 1,5-dicafeoylquinic acid studied by inhibition of chemi-luminescence. *Planta Med* **61**, 435-438.

Hwang JS, Ji HJ, Koo KA, Lee NH, Yeo HK, Cheong SW, Park JH, Oh GS, Yoon CS, and Youn HJ (2005) AIP1, a

water-soluble fraction from *Artemisia iwayomogi*, suppresses thymocyte apoptosis in vitro and down-regulates the expression of Fas gene. *Biol Pharm Bull* **28**, 921-924.

Hyun JW and Chung HS (2006) In *Globalisation of Herbal Health*, Govil JN and Singh VK (eds.), *Recent Progress in Medicinal Plants* **12**, 193-202, Studium Press, Texas, TX, USA.

Kusmenoglu S, Baser KHC, and Ozek T (1995) Constituents of the essential oils from the hulls of *Pistacia vera* L. *J Essential Oil Res* **7**, 441-442.

Lee JA, Sung HN, Jeon CH, Gill BC, Oh GS, Youn HJ, and Park JH (2008) AIP1, a carbohydrate fraction from *Artemisia iwayomogi*, modulates the functional differentiation of bone marrow-derived dendritic cells. *Int Immunopharmacol* **8**, 534-541.

Lopes-Lutz D, Alviano DS, Alviano CS, and Kolodzeiejczyk PP (2008) Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. *Phytochem* **69**, 1732-1738.

Shin TY, Park JS, and Kim SH (2006) *Artemisia iwayomogi* inhibits immediate-type allergic reaction and inflammatory cytokine secretion. *Immunopharmacol Immunotoxicol* **28**, 421-430.

Singh RP, Agrawal P, Yim D, Agarwal C, and Agarwal R (2005) Acacetin inhibits cell growth and cell cycle progression, and induces apoptosis in human prostate cancer cells: structure-activity relationship with linarin and linarin acetate. *Carcinogenesis* **26**, 845-854.

Taskova R, Mitova M, Mikhova B, and Duddeck H (2003) Bioactive phenolics from *Carthamus lanatus* L. *Z Naturforsch* **58**, 704-707.

Tshikalange TE, Meyer JJ, and Hussein AA (2005) Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. *J Ethnopharmacol* **96**, 515-519.

Zheng ZP, Cheng KW, Chao J, Wu J, and Wang M (2008) Tyrosinase inhibitors from paper mulberry (*Broussonetia papyrifera*). *Food Chem* **106**, 529-535.

Zou Y, Lu Y, and Wei D (2004) Antioxidant activity of a flavonoid rich extract of *Hypericum perforatum* L. *in vitro*. *J Agric Food Chem* **52**, 5032-5039.