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Flavonol Glycosides with Antioxidant Activity from the Aerial Parts of *Epimedium koreanum* Nakai

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Abstract – The aerial parts of *Epimedium koreanum* Nakai have been used to stimulate hormone secretion in treating impotence. Ten flavonol glycosides, 3,4,5-trihydroxy-8-prenylflavone 7-*O*-[*β*-D-glucopyranosyl(1 → 2)-*β*-D-glucopyranoside] (**1**), hyperoside (**2**), icarisid II (**3**), 2"-*O*-Rhamnosylicarisid II (**4**), epimedin A (**5**), epimedin B (**6**), epimedin C (**7**), icariin (**8**), hexandraside E (**9**), and epimedoside A (**10**) were isolated from the an ethylacetate soluble extracts of the aerial parts of *Epimedium koreanum* Nakai through activity-monitord fractionation and isolation method. The structures of compounds **1** - **10** were elucidated by high resolution fast atom bombardment mass spectrometry and two dimentional nuclear magnetic resonance spectroscopy analysis. Compounds **1** and **4** showed potent antioxidant activity, with IC_{50} values of 19.7 and 11.5 μg/mL and 88.2 and 90.5 μM, respectively.
Keywords – *Epimedium koreanum*, Berberidaceae, prenylated flavonol glycoside, antioxidant, HR-FABMS, 2D-NMR

Introduction

With the nationwide growth of interest in health and the expanding health food market, research into the industrial uses of phytochemicals in plants has also increased. Development of high-quality varieties containing increased levels of bioactive compounds may increase the nutritional value of the harvest crops. The 'antioxidant' has been used in a broad sense, referring to agents capable of interfering with processes involved in oxidative and reductive processes. Mammals constantly form reactive oxygen species, by oxidative and reductive processes in the mitochondria, from oxygen derived from respiration or by the immune system exposed to foreign antigen, and externally by radiation or various chemicals (Hyun and Chung, 2006).

Epimedium koreanum Nakai (*E. koreanum*, Berberidaceae) is native to northwest Korea and is an important plant of Chinese herbal medicine (Kang *et al*., 1988). The underground parts of *E. koreanum* are used for treating asthmatic attacks and menstrual irregularities, whereas the aerial parts have been used as a tonic to stimulate hormone secretion, in treating impotence (Dou *et al*., 1992; Li *et al*., 1995; Li *et al*., 1996). Icariin, the main component of *E. koreanum*, possesses many biological effects, such as improving cardiovascular function, hormone regulation, immunological function modulation (Liang *et al*., 1997) and osteogenic differentiation enhancement (Chen *et al*., 2007).

As a part of our study on the identification of phytochemicals, we used bioactivity-monitored fractionation and isolation methods to isolate compounds from *E. koreanum*. This plants were chosen because the ethyl acetate (EtOAc)-soluble fraction of the aerial parts exhibited significant antioxidant activity based on scavenging activity of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals (IC_{50}: 18.5 μg/mL). Bioassay-monitored fractionation of the active EtOAc-soluble fraction led to the isolation of ten flavonol glycosides (**1** - **10**), which were then evaluated for their individual biological activities.

The structure of isolated compounds was elucidated on the basis of spectroscopic evidences, particularly the results of nuclear magnetic resonance (NMR) spectroscopy (^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, HMQC, and HMBC) and high-resolution fast atom bombardment mass (HR-FABMS) spectrometry.

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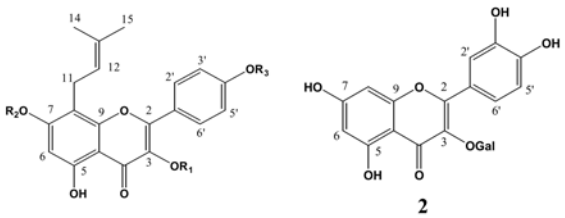
Experimental

Instrument and reagents – Melting points were measured by using an Electro thermal apparatus and are not corrected. Positive and Negative FABMS, and HR-FABMS spectra were obtained on a JEOL JMS 700-Mstation, Japan. Ultra-violet (UV) spectra were recorded on a Gilford System 2600 spectrophotometer as methyl alcohol (MeOH) solutions. NMR spectra were recorded in deuteriorated dimethylsulfoxide (DMSO-d_6) on a Varian unity Inova 500 spectrometer at 11.7T (500 MHz for ^{1}H and 125 MHz for ^{13}C) and Bruker Avance DRX 300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C), respectively. Chemical shifts were referenced to solvent peaks, DMSO-d_6 spectra: ^{1}H-NMR at 2.49 ppm, ^{13}C NMR at 39.5 ppm. Two-dimensional (2D) NMR spectroscophic techniques were used for ^{1}H-^{1}H correlation spectroscopy (COSY) and for experiments on heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC). Thin-layer chromatographic (TLC) analysis was performed on 0.25 mm silica gel Kiesel gel 60 F_{254} plates (Merck, Darmstadt, Germany). After samples developed, compounds were visualized by spraying plates with $FeCl_3$ and 5% anisaldehyde-sulfuric acid reagents for the identification of phenols and glycosides, respectively. Silica gel (Merck 60 A, 230 - 400 mesh, ASTM), RP-18 (Merck, 40 - 63 μM) and Sephadex LH-20 (25 - 100 μm; Pharmacia Fine Chemicals, Piscataway, NJ) were used for open column and flash column chromatographic separations.

Plant material – The aerial parts of *E. koreanum* were collected in Kang Won Province of Korea in summer of 2005. A voucher specimen has been deposited at the College of life Sciences and Biotechnology, Korea University. The samples were kept in a refrigerator until use for experiments.

Extraction and isolation – The dried aerial part of *E. koreanum* (8.0 kg) was extracted 5 times with 20% MeOH for 3 hours at hot water bath. The combined MeOH extracts (2.8 kg) were partitioned between n-hexane and water, with the more polar layer then partitioned with chloroform ($CHCl_3$), EtOAc, and *n*-butanol (*n*-BuOH). The EtOAc-soluble fraction of *E. koreanum* showed a strong antioxidant activity (IC_{50}: 18.5 μg/mL), and was subjected to a series of activity-monitored column chromatographic separation steps to yield pure compounds. The dried EtOAc-soluble fraction (120.3 g) was chromatographed over a silica flash column using a step-wise gradient EtOAc-MeOH (50 : 1, v/v) to MeOH to give eight sub-fractions. Sub-fraction 6 was further chromatographed on a silica gel open column using $CHCl_3$-MeOH (100 : 1, v/v) to yield compound **3** (350.8 mg). Sub-fraction 7 was further purified by recrystallization with highly purified MeOH to give the pure compound **7** (12.5 mg). Sub-fraction 7 was further chromatographed on a silica gel open column to afford compound **8** (570 mg). Sub-fraction 8 was rechromatographed on a silica gel eluted with $CHCl_3$-MeOH-H_2O (25 : 4 : 1 and 15 : 4 : 1, v/v) to give seven fractions, 8 - 1~8 - 7. Combined sub-fractions 8 - 5, 8 - 6 and 8 - 7 were subjected to RP-18 silica gel column chromatography using MeOH-H_2O (1 : 10 to 100 : 0, v/v), followed by Sephadex LH-20 column chromatography eluted with MeOH to yield compounds **1** (32.2 mg), **2** (55.4 mg), **4** (15.6 mg), **5** (7.2 mg), **6** (58.1 mg), **9** (10.3 mg) and **10** (11.4 mg), respectively. Complete identification of isolated compounds made use of varieties of physical and chemical techniques, HR-FABMS, Positive and Negative FABMS spectrometry, UV/Visible spectrophotometry, ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, HMQC and HMBC spectroscopy (Fig. 1).



1	R_1 =H	R_2 = Glc —(2)— Glc	R_3 = H
3	R_1 =Rha	R_2 = H	R_3 = CH_3
4	R_1 =Glc	R_2 = Glc	R_3 = H
5	R_1 =Rha	R_2 = Glc	R_3 = CH_3
6	R_1 =Rha	R_2 = Glc	R_3 = H
7	R_1 =Rha —(2)— Rha	R_2 = H	R_3 = CH_3
8	R_1 =Rha —(2)— Glc	R_2 = Glc	R_3 = CH_3
9	R_1 =Rha —(2)— Xyl	R_2= Glc	R_3 = CH_3
10	R_1= Rha —(2)— Rha	R_2= Glc	R_3 = CH_3

Fig. 1. Chemical Structure of Compounds **1** - **10**.

3,4,5-trihydroxy-8-prenylflavone 7-*O*-[*β*-D-glucopyranosyl (1 → 2)-*β*-D-glucopyranoside] (1) : Pale yellow powder (MeOH); mp: 200 - 204 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ : 8.03 (d, J = 9.0 Hz, H-2',6'), 6.93 (d, J = 9.0 Hz, H-3',5'), 6.58 (s, H-6), 5.18 (d, J = 8.0 Hz, H-1"), 4.56 (d, J = 7.5 Hz, H-1'"), 1.72 (s, H-15), 1.62 (s, H-14); ^{13}C-NMR (125 MHz, DMSO-d_6) δ : 176.4 (C-4), 158.5 (C-5), 156.9 (C-7), 152.7 (C-9), 147.5 (C-2), 135.8 (C-3), 130.8 (C-13), 122.9 (C-12), 121.8 (C-1'), 115.5 (C-3',5'), 108.1 (C-8), 104.4 (C-10), 104.1 (C-1'"), 98.2 (C-1"), 97.1 (C-6), 81.2 (C-2"), 77.0 (C-3",5"), 76.3 (C-3'",C-5'"), 74.7 (C-2'"), 69.5 (C-4'"), 69.3 (C-4"), 60.6 (C-6'"), 60.5 (C-6"), 25.5 (C-14), 21.4 (C-11), 18.0 (C-15); Negative FABMS

m/z 677 $[M-H]^-$, 515 [M - H - Glucose]$^-$, 353 [M - H - 2Glucose]$^-$; HR-FABMS *m/z* 701.2058 ($[M+Na]^+$) (calculated $C_{32}H_{38}O_{16}Na$, 701.2064).

Hyperoside (2) : Pale yellow plates (MeOH); mp: 205 - 206 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ : 12.63 (1H, *s,* OH-5), 7.65 (2H, dd, *J* = 2.2, 8.5 Hz, H-6'), 7.53 (1H, d, *J* = 2.2 Hz, H-2'), 6.81 (1H, d, *J* = 8.5 Hz, H-5'), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.37 (1H, d, *J* = 7.7 Hz, anomeric proton); ^{13}C-NMR (75 MHz, DMSO-d_6) δ : 177.3 (C-4), 164.0 (C-7), 161.1 (C-5), 156.1 (C-2,9), 148.3 (C-4'), 144.7 (C-3'), 133.3 (C-3), 121.8 (C-6'), 120.9 (C-1'), 115.8 (C-5'), 115.0 (C-2'), 103.8 (C-10), 101.6 (C-1"), 98.5 (C-6), 93.4 (C-8), 75.7 (C-5"), 73.0 (C-3"), 71.0 (C-2"), 67.8 (C-4"), 60.0 (C-6"'); Positive FABMS *m/z* 465 ($[M+H]^+$).

Icarisid II (3) : Yellow plates (MeOH); mp: 204 - 206 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ : 12.55 (1H, s, OH-5), 7.90 (2H, d, *J* = 8.9 Hz, H-2',6'), 7.13 (2H, d, *J* = 8.9 Hz, H-3',5'), 6.63 (1H, s, H-6), 3.42 (3H, s, OCH_3), 1.69 (3H, s, H-14), 1.60 (3H, s, H-15); ^{13}C-NMR (125 MHz, DMSO-d_6) δ : 178.6 (C-4), 161.7 (C-7), 160.8 (C-5), 159.4 (C-4'), 157.6 (C-9), 153.3 (C-2), 134.9 (C-3), 131.4 (C-13), 130.9 (C-2',6'), 122.5 (C-12), 122.4 (C-1'), 114.4 (C-3',5'), 108.6 (C-8), 105.9 (C-10), 102.3 (C-1"), 98.4 (C-6), 71.0 (C-3"), 70.6 (C-2"), 70.4 (C-5"), 70.0 (C-4"), 55.8 (OCH_3), 25.8 (C-14), 21.7 (C-11), 18.2 (C-6"), 17.7 (C-15); Positive FABMS *m/z* 861 ($[M+Na]^+$).

2"-*O*-Rhamnosylicarisid II (4) : Pale yellow plates (MeOH); mp: 212 - 214 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ : 12.60 (1H, s, OH-5), 7.88 (2H, d, *J* = 9.0 Hz, H-2',6'), 7.06 (2H, d, *J* = 9.0 Hz, H-3',5'), 6.62 (1H, s, H-6), 5.14 (1H, br t, *J* = 6.5 Hz, H-12), 4.99 (1H, br s, anomeric proton), 4.84 (1H, br s, anomeric proton), 3.84 (3H, s, OCH_3), 1.68 (3H, s, H-14), 1.59 (3H, s, H-15), 1.08 (3H, d, *J* = 6.0 Hz, H-6"), 0.79 (3H, d, *J* = 5.5 Hz, H-6"'); ^{13}C-NMR (125 MHz, DMSO-d_6) δ : 178.2 (C-4), 161.4 (C-5), 160.4 (C-7), 159.0 (C-4'), 157.1 (C-2), 152.9 (C-9), 131.0 (C-13), 130.4 (C-2',6'), 122.1 (C-12,1'), 114.1 (C-3',5'), 108.2 (C-10), 105.4 (C-8), 100.4 (C-1'"), 100.0 (C-1"), 98.0 (C-6), 77.2 (C-2"), 76.5 (C-5"), 73.3 (C-2"',4"'), 70.3 (C-5"'), 69.6 (C-4",3"'), 60.5 (C-3"), 55.4 (OCH_3), 25.5 (C-14), 22.9 (C-6"), 21.4 (C-11), 17.8 (C-15), 17.4 (C-6"'); Positive FABMS *m/z* 661 ($[M+H]^+$).

Epimedin A (5) : Pale yellow plates (MeOH); mp: 197 - 199 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ : 12.55 (1H, s, OH-5), 7.90 (2H, d, *J* = 8.9 Hz, H-2',6'), 7.13 (2H, d, *J* = 8.9 Hz, H-3',5'), 6.63 (1H, s, H-6), 3.42 (3H, s, OCH_3), 1.69 (3H, s, H-14), 1.60 (3H, s, H-15), 0.85 (3H, d, *J* = 6.2 Hz, H-6"); ^{13}C-NMR (125 MHz, DMSO-d_6) δ : 178.6 (C-4), 161.7 (C-7), 160.8 (C-5), 159.4 (C-4'), 157.6 (C-9), 153.3 (C-2), 134.9 (C-3), 131.4 (C-13), 130.9 (C-2',6'), 122.5 (C-12), 122.4 (C-1'), 114.4 (C-3',5'), 108.6 (C-8), 105.9 (C-10), 102.3 (C-1"), 100.8 (C-1"'), 98.4 (C-6), 77.5 (C-3"'), 76.9 (C-5"'), 73.7 (C-2"'), 71.4 (C-4"'), 71.0 (C-3"), 70.6 (C-2"), 70.4 (C-5"), 70.0 (C-4"), 60.9 (C-6"'), 55.8 (OCH_3), 25.8 (C-14), 21.7 (C-11), 18.2 (C-6"), 17.7 (C-15); Positive FABMS *m/z* 861 ($[M+Na]^+$).

Epimedin B (6) : Pale yellow plates (MeOH); mp: 206 - 208 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ : 12.61 (1H, s, OH-5), 7.90 (2H, d, *J* = 8.9 Hz, H-2',6'), 7.15 (2H, d, *J* = 8.9 Hz, H-3',5'), 6.64 (1H, s, H-6), 4.16 (1H, d, *J* = 7.0 Hz, anomeric proton), 3.86 (3H, s, OCH_3), 1.70 (3H, s, H-14), 1.62 (3H, s, H-15), 0.88 (3H, d, *J* = 5.7 Hz, H-6"); ^{13}C-NMR (75 MHz, DMSO-d_6) δ : 178.6 (C-4), 161.8 (C-7), 160.8 (C-5), 159.4 (C-4'), 157.5 (C-2), 153.3 (C-9), 135.0 (C-3), 131.5 (C-2',6'), 130.8 (C-13), 122.4 (C-12), 122.3 (C-1'), 114.5 (C-3',5'), 108.6 (C-8), 106.7 (C-10), 105.8 (C-1"'), 101.3 (C-1"), 100.8 (C-1""), 98.4 (C-6), 80.9 (C-2"), 77.5 (C-5""), 76.9 (C-3""), 76.5 (C-3"'), 74.0 (C-2""), 73.6 (C-2"'), 71.9 (C-4"), 70.7 (C-3"), 70.5 (C-4"'), 69.9 (C-5"), 69.6 (C-4""), 66.1 (C-5"'), 60.9 (C-6""), 55.8 (OCH_3), 25.8 (C-14), 21.7 (C-11), 18.2 (C-6"), 17.7 (C-15); Positive FABMS *m/z* 809 ($[M+H]^+$).

Epimedin C (7) : Pale yellow plates (MeOH); mp: 175 - 178 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ : 7.83 (2H, d, *J* = 8.8 Hz, H-2',6'), 7.04 (2H, d, *J* = 8.8 Hz, H-3',5'), 6.64 (1H, s, H-6), 5.52 (1H, s, anomeric proton), 5.06 (1H, d, *J* = 6.5 Hz, anomeric proton), 3.87 (3H, s, OCH_3), 1.71 (3H, s, H-14), 1.63 (3H, s, H-15), 1.20 (3H, d, *J* = 6.0 Hz, H-6"), 0.90 (3H, br s, H-6"'); ^{13}C-NMR (75 MHz, DMSO-d_6) δ : 179.9 (C-4), 163.4 (C-7), 162.0 (C-5), 160.9 (C-4'), 159.1 (C-2), 154.9 (C-9), 136.5 (C-3), 132.6 (C-13), 131.8 (C-2',6'), 123.7 (C-1'), 123.5 (C-12), 115.2 (C-3',5'), 110.5 (C-8), 107.5 (C-10), 103.7 (C-1"'), 102.3 (C-1""), 100.8 (C-1"), 99.3 (C-6), 78.2 (C-5""), 78.2 (C-3""), 74.9 (C-2"), 73.9 (C-2""), 73.4 (C--4"), 72.2 (C-4"'), 72.0 (C-3"'), 71.9 (C-3",2"'), 71.8 (C-5"'), 71.1 (C-5"), 70.3 (C-4""), 62.3 (C-6""), 56.0 (OCH_3), 25.9 (C-14), 22.7 (C-11), 18.3 (C-15), 17.9 (C-6"), 17.8 (C-6"'); Positive FABMS *m/z* 823 ($[M+H]^+$).

Icariin (8) : Pale yellow plates (MeOH); mp: 207 - 210 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ : 12.57 (1H, s, OH-5), 7.88 (2H, d, *J* = 8.7 Hz, H-2',6'), 7.11 (2H, d, *J* = 8.7 Hz, H-3',5'), 6.64 (1H, s, H-6), 5.14 (1H, d, *J* = 7.0 Hz, anomeric proton), 3.85 (3H, s, OCH_3), 1.69 (3H, s, H-14), 1.60 (3H, s, H-15), 0.78 (3H, d, *J* = 5.7 Hz, H-6"); ^{13}C-NMR (75 MHz, DMSO-d_6) δ : 178.6 (C-4), 161.7 (C-7), 160.8 (C-5), 159.4 (C-4'), 157.6 (C-9), 153.3 (C-2), 134.9 (C-3), 131.4 (C-13), 130.9 (C-2',6'), 122.5 (C-12), 122.4 (C-1'), 114.4 (C-3',5'), 108.6 (C-8), 105.9 (C-10), 102.3

(C-1"), 100.8 (C-1'''), 98.4 (C-6), 77.5 (C-3'''), 76.9 (C-5'''), 73.7 (C-2'''), 71.4 (C-4'''), 71.0 (C-3"), 70.6 (C-2"), 70.4 (C-5"), 70.0 (C-4"), 60.9 (C-6'''), 55.8 (OCH_3), 25.8 (C-14), 21.7 (C-11), 18.2 (C-6"), 17.7 (C-15); Positive FABMS *m/z* 677 ($[M+H]^+$).

Hexandraside E (9) : Pale yellow plates (MeOH); mp: 212 - 213 °C; ^{1}H-NMR (500 MHz, DMSO-d_6) δ : 12.60 (1H, s, OH-5), 8.07 (2H, d, *J* = 8.8 Hz, H-2',6'), 6.89 (2H, d, *J* = 8.8 Hz, H-3',5'), 6.62 (1H, s, H-6), 5.48 (1H, d, *J* = 7.5 Hz, anomeric proton), 5.01 (1H, d, *J* = 7.0 Hz, anomeric proton), 1.72 (3H, s, H-14), 1.62 (3H, s, H-15); ^{13}C-NMR (125 MHz, DMSO-d_6) δ : 177.8 (C-4), 160.2 (C-7), 160.0 (C-4'), 158.9 (C-5), 156.5 (C-2), 152.7 (C-9), 133.1 (C-3), 131.0 (C-13), 130.8 (C-2',6'), 122.1 (C-12), 120.8 (C-1'), 115.0 (C-3',5'), 108.0 (C-8), 105.2 (C-10), 100.7 (C-1"), 100.3 (C-1'''), 97.9 (C-6), 77.3 (C-5"), 77.0 (C-5'''), 76.4 (C-3"), 76.3 (C-3'''), 74.1 (C-2"), 73.2 (C-2'''), 69.7 (C-4"), 69.5 (C-4'''), 60.6 (C-6"), 60.5 (C-6'''), 25.3 (C-14), 21.3 (C-11), 17.7 (C-15); Positive FABMS *m/z* 703 ($[M+H+Na]^+$).

Epimedoside A (10) : Pale yellow plates (MeOH); mp: 204 - 206 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ : 12.85 (1H, s, OH-5), 7.79 (2H, d, *J* = 8.7 Hz, H-2',6'), 6.93 (2H, d, *J* = 8.7 Hz, H-3',5'), 6.63 (1H, s, H-6), 5.30 (1H, br s, anomeric proton), 5.14 (1H, br t, *J* = 7.0 Hz, H-12), 5.00 (1H, br d, *J* = 7.0 Hz, anomeric proton), 1.69 (3H, s, H-14), 1.61 (3H, s, H-15), 0.79 (3H, d, *J* = 5.4 Hz, H-6"); ^{13}C-NMR (75 MHz, DMSO-d_6) δ : 178.1 (C-4), 160.3 (C-7), 160.1 (C-4'), 158.9 (C-5), 157.6 (C-2), 152.8 (C-9), 134.1 (C-3), 131.0 (C-13), 130.5 (C-2',6'), 122.1 (C-12), 120.4 (C-1'), 115.3 (C-3', 5'), 108.1 (C-8), 105.4 (C-10), 101.7 (C-1'''), 100.4 (C-1"), 97.9 (C-6), 77.0 (C-5'''), 76.5 (C-3'''), 73.9 (C-2'''), 71.0 (C-5"), 70.6 (C-4"), 70.2 (C-2"), 70.0 (C-4'''), 69.5 (C-3"), 60.5 (C-6'''), 25.3 (C-15), 21.3 (C-11), 17.7 (C-14), 17.3 (C-6"); Positive FABMS *m/z* 663 ($[M+H]^+$).

DPPH free radical scavenging activity – This assay is based on the scavenging activity of stable DPPH free radicals. Reaction mixtures containing 5 mL of test samples dissolved in DMSO and 95 mL of 300 mM DPPH in ethanolic solution (final DPPH concentration) were incubated at 37 °C for 30 minutes in 96-well micro filter plates. Absorbance was then measured at 515 nm. Percent inhibition in sample treatments was determined by comparison with a DMSO-treated control group. IC_{50} values denote the concentration of sample required to scavenge 50% of DPPH free radicals. On the basis of reaction conditions, and in order to confirm the usefulness of the assay, commercial antioxidants including ascorbic acid and 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) were also evaluated for their free radical scavenging activity (Chung *et al*., 1997).

Results and Discussion

The combined MeOH extracts of the aerial parts of *E. koreanum* were partitioned via successive extractions with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. The EtOAc-soluble fraction of *E. koreanum*, showed a strong antioxidant activity (IC_{50}: 18.5 μg/mL), and was subjected to a series of activity-guided chromatographic separation using a step-wise gradient EtOAc-MeOH to generate eight sub-fractions. Sub-fractions 6 - 8 were then further chromatographed on a silica gel open column using $CHCl_3$-MeOH, $CHCl_3$-MeOH-H_2O, respectively. The resulting subfraction with antioxidant activity was rechromatographed on a RP-18 and Sephadex LH-20 column using MeOH-H_2O and MeOH in order to isolate the compounds **1 - 10**.

Compound **1**, was obtained as a pale yellow amorphous powder. FABMS in the negative mode exhibited a quasi-molecular ion peak at *m/z* 677 $[M-H]^-$ as well as other fragment ions caused by elimination of two glucose moieties, respectively.

The positive HR-FABMS data indicated molecular formula $C_{32}H_{38}O_{16}Na$ for compound **1**, with a molecular ion at *m/z* 701.2058. The structure of the aglycone moiety was deduced from the ^{1}H-NMR spectrum. The presence of two-proton doublets at δ 6.93 ppm (*J* = 9.0 Hz) and δ 8.03 (*J* = 9.0 Hz), and a one-proton singlet at δ 6.58 ppm suggested that the aglycone could be based on kaempferol with a substituent carbon linked at C-8 (Li *et al*., 1996b). The characteristic signals based on an isopentenyl group as the substituent were observed at δ 1.62 and δ 1.72 ppm in each three-proton singlet. The chemical shifts based on anomeric protons of the glucosyl residue at δ 5.18 (*J* = 8.0 Hz) and δ 4.56 ppm (*J* = 7.5 Hz) were also observed. In the ^{13}C-NMR spectrum, the chemical shift based on inner glucose at δ 81.2 ppm was observed, suggesting that the terminal glucose was connected at C-2", since its chemical shift appeared further downfield (commonly at δ 71.7 ppm). Each correlation between proton and carbon peaks of compound **1** is shown in HMQC spectral data. In the HMBC spectrum, cross were observed between an inner anomeric proton at δ 5.18 ppm of a glucosyl moiety and a carbon at δ 156.9 ppm assigned to C-7, a terminal anomeric proton at δ 4.56 ppm in ^{1}H-NMR, also caused a cross peak with a carbon of C-2" at δ 81.2 ppm. These results indicated a *β*-D-glucopyranosyl site was connected with two hydroxyl groups, one at C-7 and other at C-2"

Table 1. Antioxidant activity (IC_{50}) of compounds **1 - 10** in DPPH assay

Compounds	μg/mL	μM
1	19.7	88.2
2	37.2	73.2
3	26.1	102.7
4	11.5	90.5
5	30.4	70.1
6	37.1	107.9
7	72.9	142.0
8	67.1	172.8
9	55.7	157.3
10	58.9	136.3
Ascorbic acid[1)]	21.9	113.1
BHA[1)]	20.0	104.8

[1)] Control compounds.

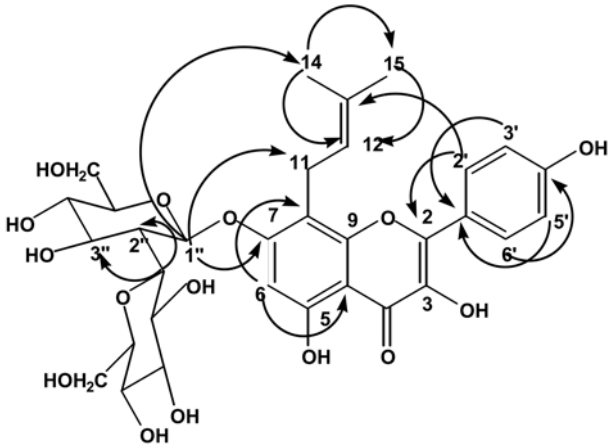


Fig. 2. Selective HMBC Correlations of Compound **1**.

(Fig. 2). For these reason, compound **1** was identified as 3,4,5-trihydroxy-8-prenylflavone 7-*O*-[*β*-D-glucopyranosyl (1 → 2)-*β*-D-glucopyranoside].

Compounds **2 - 10** were obtained as pale yellow amorphous powder. All compounds were showed positive reaction in $FeCl_3$ and 5% anisaldehyde-sulfuric acid color applications. The ^{1}H-NMR and ^{13}C-NMR spectral data of compounds **2 - 10** indicated the presence of prenylated flavonol and glycoside moieties. Complete determination of chemical structure was accomplished by HR-FABMS, ^{1}H-NMR, ^{13}C-NMR, COSY, HMQC, HMBC spectroscopic methods and published literature (**1**: Zhao *et al*., 2007; **2**: Kang *et al*., 1988; **3**: Fukai and Nomura, 1988; **4**: Li *et al*., 1996b; **5**: Mizuno *et al*., 1988; **6**: Mizuno *et al*., 1988; **7**: Muzuno *et al*., 1988; **8**: Kang *et al*., 1988; **9**: Mizuno *et al*., 1992; **10**: Mizuno *et al*., 1991).

Compounds **1 - 10** showed characteristic flavonoid skeleton in $FeCl_3$ color reaction, ^{1}H-NMR and ^{13}C-NMR spectral data. The IC_{50} of compounds **1** and **4** were 19.7 and 11.4 μg/mL and 88.2 and 90.5 μM, respectively, and their activity compared favorably with the activity observed with a number of standards, ascorbic acid and BHA in this same assay. Compounds **7 - 10** were moderately active in this assay, possibly because there was a more degree of glycosidation in their phenol rings than in those of compounds **1 - 6** (Table 1).

The flavonoids in plants comprise the largest single class of secondary metabolites. Flavonoids have often enormous physiological activities, hence their wide use in diet as a source of health foods (Chung *et al*., 1997). It is well known that flavonoids have antioxidant properties and free radical scavenging capabilities. Recently, researchers mentioned the position of the geranyl or prenyl groups on the flavonoid skeleton plays an important role in exhibiting antioxidant activity (Kumazawa *et al*., 2007). They are isoprenoid units, which are usually incorporated into glycoside moieties. The present study suggests that flavonol glycosides of *E. koreanum* showed strong antioxidant activity in DPPH free radical scavenging assay. The further research on the identification of mechanism should be conducted *in vivo* experimental system.

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