Arch Pharm Res Vol 27, No 4, 390-395, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Flavonol Glycosides from the Aerial Parts of *Aceriphyllum rossii* and Their Antioxidant Activities

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(Received October 7, 2003)

The methanol extract obtained from the aerial parts of *Aceriphyllum rossii* (Saxifragaceae) was fractionated into ethyl acetate (EtOAc), *n*-BuOH and H_2O layers through solvent fractionation. Repeated silica gel column chromatography of EtOAc and *n*-BuOH layers afforded six flavonol glycosides. They were identified as kaempferol 3-*O*-β-D-glucopyranoside (astragalin, **1**), quercetin 3-*O*-β-D-glucopyranoside (isoquercitrin, **2**), kaempferol 3-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (**3**), quercetin 3-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (rutin, **4**), kaempferol 3-*O*-[α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside] (**5**) and quercetin 3-*O*-[α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside] (**6**) on the basis of several spectral data. The antioxidant activity of the six compounds was investigated using two free radicals such as the ABTS free radical and superoxide anion radical. Compound **1** exhibited the highest antioxidant activity in the ABTS {2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)} radical scavenging method. 100 mg/L of compound **1** was equivalent to 72.1±1.4 mg/L of vitamin C, and those of compounds **3** and **5** were equivalent to 62.7±0.5 mg/L and 54.3±1.3 mg/L of vitamin C, respectively. And in the superoxide anion radical scavenging method, compound **5** exhibited the highest activity with an IC_{50} value of 17.6 ± 0.3 μM. In addition, some physical and spectral data of the flavonoids were confirmed.

Key words: *Aceriphyllum rossii*, Saxifragaceae, Antioxidant activity, ABTS free radical, Superoxide anion radical, Flavonol glycoside

INTRODUCTION

Aceriphyllum rossii (Saxifragaceae), a perennial herb indigenous to Korea, grows in scant amounts of soil on damp rocks along valleys in mid-northern parts of Korea. The young leaflets and stems are commonly ingested in the Korean diet. Until authors isolated triterpenoids with the inhibitory effect of ACAT (Acyl-CoA: Cholesterol acyltransferase) from *A. rossii* (Han *et al.*, 2002), any constitutional or pharmacological study of the plant had never been reported. In this study, the isolation and structure elucidation of the six flavonol glycosides from *A. rossii* was carried out. In addition, the antioxidant activity of the flavonoids was evaluated using two free radicals (ABTS and superoxide radicals).

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MATERIALS AND METHODS

Plant materials

The *Aceriphyllum rossii* was collected from Kapyung-Kun, Korea in July, 2002. A voucher specimen (KHU020712) was preserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Suwon, Korea.

Instrumentation

Melting points were determined on a Fisher-John apparatus and uncorrected. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. FAB-MS were recorded on a VG AutoSpec E mass spectrometer. IR

(KBr) spectra were run on a Perkin Elmer Spectrum One FT-IR spectrometer. UV spectra were recorded on a Shimadzu UV-1601 spectrophotometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer. HPLC analysis was performed on a Hewlett Packard HPLC series 1100.

Chemicals

ABTS as diammonium salt, NBT (nitrotetrazolium blue chloride), EDTA (ethylenediaminetetraacetic acid), L-ascorbic acid, xanthine, xanthine oxidase were obtained from Sigma Chemical Co. AAPH {2,2-azobis(2-amidinopropane) dihydrochloride} was obtained from Wako Chemicals.

Isolation of flavonol glycosides from the aerial parts of *Aceriphyllum rossii*

The dried aerial parts of *A. rossii* (2.8 kg) were extracted with 80% aqueous MeOH (15 L×2) for 12 h at room temperature. The filtrate was concentrated *in vacuo* at 40°C to render the MeOH extracts. The extracts were suspended in water (1500 mL) and extracted with EtOAc (1.5 L×2) and *n*-BuOH (1 L×2), successively, to yield the EtOAc (75 g) and *n*-BuOH (126 g) extracts. The EtOAc extract (70 g) was applied to the silica gel column (7×18 cm) chromatography and eluted with EtOAc : *n*-BuOH : H_2O (20 : 2 : 0.5) monitored with thin layer chromatography (TLC) to produce eight fractions (ARE-1~ARE-8). ARE-4 (1.3 g) was further purified by the silica gel column chromatography (5× 15 cm) using $CHCl_3$: MeOH (4 : 1) for elution to yield compounds **1** (43 mg) and **2** (46 mg). ARE-7 (1.2 g) was subjected to the silica gel column (5×15 cm), eluted with $CHCl_3$: MeOH (3 : 1) to obtain compound **3** (43 mg). The *n*-BuOH extract (120 g) was chromatographed on the silica gel column (10×20 cm) with $CHCl_3$: MeOH : H_2O (7 : 4 : 1 → 6 : 4 : 1), collecting eleven fractions (ARB-1~ARB-11). ARB-5 (2.1 g) was applied to the silica gel column chromatography (5×16 cm), eluted with EtOAc : *n*-BuOH : H_2O (14 : 1 : 0.5) to ultimately produce compound **4** (38 mg). ARB-7 (4.1 g) was applied to the silica gel column chromatography (5×21 cm), eluted with EtOAc : *n*-BuOH : H_2O (7 : 2 : 0.5) to ultimately produce compound **5** (32 mg). ARB-9 (3 g) was subjected to the silica gel column chromatography (5×18 cm), eluted with EtOAc : *n*-BuOH : H_2O (4 : 4 : 1) to obtain compound **6** (36 mg).

Kaempferol 3-*O*-β-D-glucopyranoside (astragalin, 1)

Yellow powder (MeOH-H_2O); m.p. 178-180°C; $[\alpha]_D$ = +16.0° (*c*=1.1, MeOH); pos. FAB/MS *m/z*: 471, 449, 287; IR (KBr, ν) 3348, 2925, 2360, 1655, 1608, 1500 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) 8.03 (2H, d, *J*=8.8 Hz, H-2', 6'), 6.86 (2H, d, *J*=8.8 Hz, H-3',5'), 6.35 (1H, d, *J*=1.4 Hz, H-8), 6.16 (1H, d, *J*=1.4 Hz, H-6), 5.22 (1H, d, *J*=7.6 Hz, H-1"), 3.21~3.48 (4H, m, H-2", 3", 4", 5"), 3.69 (1H, dd, *J*=12.0, 2.2 Hz, H-6a"), 3.53 (1H, dd, *J*=12.0, 5.4 Hz, H-6b"), ^{13}C-NMR (100 MHz, CD_3OD) 179.2 (C-4), 165.7 (C-7), 162.7 (C-5), 161.3 (C-4'), 158.8 (C-9), 158.2 (C-2), 135.3(C-3), 132.2 (C-2', 6'), 122.6 (C-1'), 115.9 (C-3', 5'), 105.6 (C-10'), 104.0 (C-1"), 99.8 (C-6), 94.7 (C-8), 78.2 (C-3"), 77.9 (C-5"), 75.6 (C-2"), 71.2 (C-4"), 62.5 (C-6").

Quercetin 3-*O*-β-D-glucopyranoside (isoquercitrin, 2)

Yellow powder (MeOH-H_2O); m.p. 230-232°C; $[\alpha]_D$ = -12.5° (*c*=0.9, MeOH); pos. FAB/MS *m/z*: 487, 465, 325, 303; IR (KBr, ν) 3388, 2945, 2350, 1656, 1608, 1505 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) 7.71 (1H, d, *J*=2.4 Hz, H-2'), 7.56 (1H, dd, *J*=2.4, 8.6 Hz, H-6'), 6.85 (1H, d, *J*=8.6 Hz, H-5'), 6.35 (1H, d, *J*=2.0 Hz, H-8), 6.17 (1H, d, *J*=2.0 Hz, H-6), 5.23 (1H, d, *J*=7.6 Hz, H-1"), 3.22~3.51 (4H, m, H-2", 3", 4", 5"), 3.71 (1H, dd, *J*=12.0, 2.4 Hz, H-6a"), 3.58 (1H, dd, *J*=12.0, 5.2 Hz, H-6b"), ^{13}C-NMR (100 MHz, CD_3OD) 179.2 (C-4), 165.7 (C-7), 162.7 (C-5), 158.8 (C-9), 158.2 (C-2), 149.6(C-4), 145.6 (C-3'), 135.4 (C-3), 123.1 (C-1'), 122.8 (C-6'), 117.4 (C-5), 115.8 (C-2'), 105.5 (C-10), 104.2 (C-1"), 99.8 (C-6), 94.6 (C-8), 78.2(C-3"), 77.9 (C-5"), 75.6 (C-2"), 71.1 (C-4"), 62.4 (C-6").

Kaempferol 3-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (3)

Yellow powder (MeOH-H_2O); m.p. 168-170°C; $[\alpha]_D$ = -14.9° (*c*=1.3, MeOH); pos. FAB/MS *m/z*: 617, 595, 449, 287; IR (KBr, ν) 3364, 2935, 2362, 1654, 1605, 1510 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) 8.05 (2H, d, *J*=8.8 Hz, H-2', 6'), 6.88 (2H, d, *J*=8.8 Hz, H-3', 5'), 6.37 (1H, s, H-8), 6.19 (1H, s, H-6), 5.11 (1H, d, *J*=7.2 Hz, H-1"), 4.51 (1H, s, H-1"), 3.25~3.81 (10H, m, H-2", 2"', 3", 3"', 4", 4"', 5", 5"', 6"), 1.12 (3H, d, *J*=6.0 Hz, H-6"'), ^{13}C-NMR (100 MHz, CD_3OD) 179.0 (C-4), 165.7 (C-7), 162.7(C-5), 161.2(C-4), 159.2(C-9), 158.2(C-2), 135.4(C-3), 132.2 (C-2', 6'), 122.5 (C-1'), 116.0 (C-3', 5'), 105.5 (C-10'), 104.6 (C-1"), 102.2 (C-1"'), 99.9 (C-6), 94.8 (C-8), 78.0 (C-5"), 77.0 (C-5"), 75.6 (C-2"), 73.8 (C-4"), 72.2 (C-3"'), 71.9 (C-2"'), 71.3 (C-4"'), 69.6 (C-5"'), 68.5 (C-6"), 18.0 (C-6"').

Quercetin 3-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (rutin, 4)

Yellow powder (MeOH-H_2O); m.p. 210-212°C; $[\alpha]_D$ = -8.8° (*c*=1.4, MeOH); pos. FAB/MS *m/z*: 633, 611, 465, 303; IR (KBr, ν) 3410, 2935, 1658, 1560, 1502 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) 7.66 (1H, d, *J*=2.0 Hz, H-2'), 7.62 (1H, dd, *J*=8.4, 2.0 Hz, H-6'), 6.86 (1H, d, *J*=8.4 Hz, H-5'), 6.37 (1H, d, *J*=2.2 Hz, H-8), 6.19 (1H, d, *J*=2.2 Hz, H-6), 5.08 (1H, d, *J*=7.6 Hz, H-1"), 4.52 (1H, d, *J*=1.2 Hz, H-1"'), 3.25~3.81 (10H, m, H-2", 2"', 3", 3"', 4", 4"', 5", 5"', 6"), 1.12 (3H, d, *J*=6.0 Hz, H-6"'), ^{13}C-NMR (100 MHz,

CD_3OD) 179.5 (C-4), 165.7 (C-7), 162.6 (C-5), 159.1 (C-9), 158.2 (C-2), 149.6 (C-4'), 145.6 (C-3'), 135.5(C-3), 123.4 (C-1'), 122.9 (C-6'), 117.6 (C-5'), 115.9(C-2'), 105.5 (C-10), 104.7 (C-1"), 102.2 (C-1'"), 99.8 (C-6), 94.8 (C-8), 78.0 (C-5"), 77.0 (C-5"), 75.6 (C-2"), 73.8 (C-4"), 72.1 (C-3'"), 72.0 (C-2'"), 71.2 (C-4'"), 69.6 (C-5'"), 68.5 (C-6"), 17.9 (C-6'").

Kaempferol 3-*O*-α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (5)

Yellow powder (MeOH-H_2O); m.p. 171-172°C; $[\alpha]_D$ = -73° (*c*=0.4, MeOH); pos. FAB/MS *m/z*: 763, 741, 595, 449, 287; IR (KBr, ν) 3420, 2930, 1660, 1565 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) 8.01 (2H, d, *J*=8.8 Hz, H-2', 6'), 6.89 (2H, d, *J*=8.8 Hz, H-3', 5'), 6.37 (1H, d, *J*=2.0 Hz, H-8), 6.17 (1H, d, *J*=2.0 Hz, H-6), 5.59 (1H, d, *J*=8.4 Hz, H-1"), 5.11 (1H, d, *J*=1.2 Hz, H-1'"), 4.51 (1H, d, *J*=1.2 Hz, H-1""), 3.21~4.08 (14H, m, H-2", 2'", 2"", 3", 3'", 3"", 4", 4'", 4"", 5", 5'", 5"", 6"), 1.07 (3H, d, *J*=6.0 Hz, H-6'"), 0.98 (3H, d, *J*=6.0 Hz, H-6""), ^{13}C-NMR (100 MHz, CD_3OD) 179.0 (C-4), 165.4 (C-7), 162.9 (C-5), 161.1 (C-4'), 158.8 (C-9), 158.2 (C-2), 134.2 (C-3), 132.0 (C-2', 6'), 123.4 (C-1'), 116.0 (C-3', 5'), 105.8 (C-10'), 102.4 (C-1"), 102.2 (C-1'"), 100.3 (C-1""), 99.7 (C-6), 94.7 (C-8), 79.7 (C-4'"), 78.8 (C-3"), 76.9 (C-5"), 73.9 (C-2"), 73.7 (C-4""), 72.3 (C-3""), 72.2 (C-2'"), 72.2 (C-3'"), 72.0 (C-2'"), 71.8 (C-4"), 69.8 (C-5'"), 69.7 (C-5""), 68.2 (C-6"), 17.9 (C-6'"), 17.6 (C-6'").

Quercetin 3-*O*-α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (6)

Yellow powder (MeOH-H_2O); m.p. 204-205°C; $[\alpha]_D$ = -2.3° (*c*=0.4, MeOH); pos. FAB/MS *m/z*: 779, 757, 611, 465, 303; IR (KBr, ν) 3415, 2940, 1660, 1520 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) 7.60 (1H, d, *J*=2.4 Hz, H-2'), 7.59 (1H, dd, *J*=8.8, 2.4 Hz, H-6'), 6.86 (1H, d, *J*=8.8 Hz, H-5'), 6.36 (1H, d, *J*=2.0 Hz, H-8), 6.17 (1H, d, *J*=2.0 Hz, H-6), 5.58 (1H, d, *J*=8.0 Hz, H-1"), 5.22 (1H, d, *J*=1.2 Hz, H-1'"), 4.50 (1H, d, *J*=1.2 Hz, H-1""), 3.21~4.08 (14H, m, H-2", 2'", 2"" 3", 3'", 3"", 4", 4'", 4"", 5", 5'", 5"", 6"), 1.06 (3H, d, *J*=6.0 Hz, H-6'"), 1.00 (3H, d, *J*=6.0 Hz, H-6""), ^{13}C-NMR (100 MHz, CD_3OD) 179.0 (C-4), 165.4 (C-7), 162.9 (C-5), 158.7 (C-9), 158.2 (C-2), 149.4 (C-4'), 145.7 (C-3'), 134.3 (C-3), 123.4 (C-1'), 123.2 (C-6'), 117.3 (C-5'), 115.9 (C-2'), 105.8 (C-10), 102.5 (C-1"), 102.1 (C-1'"), 100.4 (C-1""), 99.6 (C-6), 94.6 (C-8), 79.9 (C-4'"), 78.8 (C-3"), 77.0 (C-5"), 74.0 (C-2"), 73.8 (C-4""), 72.3 (C-3""), 72.2 (C-2'"), 72.2 (C-3'"), 72.1 (C-2'"), 71.8 (C-4"), 69.9 (C-5'"), 69.7 (C-5""), 68.3 (C-6"), 17.9 (C-6'"), 17.5 (C-6'").

Acidic hydrolysis of compounds 5 and 6

Compounds **5** and **6**, 5 mgs each, were dissolved in 1.2 N HCl/MeOH and heated for 2 h. After concentration to remove the MeOH, the reaction mixture was partitioned in H_2O and EtOAc. The aqueous fraction was developed on TLC with the solvent as $CHCl_3$-MeOH-H_2O (6:4:1). The HPLC analysis on the EtOAc fraction was carried out using the following information. Column : Waters Symmetry 5 μm C_{18} (250×4.5 mm); mobile phase : pH 2.6, 50 mM $NH_4H_2PO_4$ (A), pH 1.5, 80:20 (v/v) acetonitrile/50 mM $NH_4H_2PO_4$ (B), pH 2.6, 200 mM H_3PO_4 (C), gradient : 100% A at zero, 92% A/8% B at 4 min, 14% B/86% C at 10 min, 16.5% B/83.5% C at 22.5 min, 25% B/75% C at 27.5 min, 80% B/20% C at 50 min, 100% A at 55 min, 100% A at 60 min, flow rate : 0.7 mL/min. r.t. : kaempferol 42'12", quercetin 39'26".

VCEAC (vitamin C equivalent antioxidant capacity) assay using ABTS radical

ABTS radical anion was used according to the method of Kim *et al.* (2002). In short, 1.0 mM AAPH, a radical initiator, was mixed with 2.5 mM of ABTS in a phosphate-buffered saline (pH 7.4: 100 mM potassium phosphate buffer containing 150 mM NaCl). The mixed solution was heated in a water bath at 68°C for 13 min. The resulting blue-green $ABTS^{\cdot-}$ solution was adjusted to the absorbance of 0.650±0.020 at 734 nm with an additional phosphate-buffered saline. Twenty μL of the sample was added to 980 μL of the ABTS radical solution. The mixture was incubated at 37°C under restricted lighting for 10 min. The reduction of absorbance at 734 nm was measured after 10 min. The ABTS radical scavenging activity of pure chemical compounds at the level of 100 mg/L was expressed as mg/L vitamin C equivalents antioxidant capacity (VCEAC) at 10 min.

Superoxide radical anion scavenging activity assay

For the assay, the method of Kweon *et al.* (2001) was used with minor modification. Tetrazolium blue solution, the mixture of 0.1 mM xanthine and 0.1 mM NBT, were mixed in 50 mM potassium phosphate buffer (pH 7.4), which included 0.05 mM EDTA (PBE). An aliquot (0.9 mL) of the tetrazolium blue solution was added to 0.1 mL of the flavonoid sample properly diluted in 50% aqueous methanol in 9 mL of test tube. The reaction was initiated by the addition of 1 mL of xanthine oxidase solution (0.05 unit/mL) in PBE. The resulting mixture was incubated for 20 min at 37°C. By adding 2 mL of 2.0 N HCl to the reaction mixtures, the reaction was terminated. The coloration of NBT was measured at 560 nm against a blank that was similarly prepared. To calculate the IC_{50} values, a graphical method was employed from the dose-response curves. The IC_{50} value is defined as the concentration of sample required to achieve NBT reduction by a 50% decrease.

Statistics

The data were analyzed by a two tailed Students *t*-test.

P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

When the methanol extract of *A. rossii* was developed on the silica gel TLC, the spots showed, not only the UV absorbance at 254 or 365 nm, but also a yellow colorization by spraying 10% H_2SO_4 solution and then heating the TLC plate, indicating the presence of flavonoids in the extracts. The methanol extract obtained from the aerial parts of the *A. rossii* was fractionated into the EtOAc layer, *n*-BuOH layer and H_2O layer through solvent fractionation. The repeated silica gel column chromatography of EtOAc and *n*-BuOH fractions supplied six flavonol glycosides, compounds **1-6**.

Structural identifications of these compounds were carried out by interpretation of several spectral data and in comparison with the data described in the literature. Compounds **1-6** were readily identified as kaempferol 3-*O*-β-D-glucopyranoside, astragalin (**1**) (Liu *et al.*, 1997) and quercetin 3-*O*-β-D-glucopyranoside, isoquercitrin (**2**) (Choi *et al.*, 1998), kaempferol 3-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (**3**) (Ho *et al.*, 2002), quercetin 3-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (**4**) (Choi *et al.*, 1998), kaempferol 3-*O*-α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (**5**) (Webby & Markham, 1990) and quercetin 3-*O*-α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside] (**6**) (Beck & Haberlein, 1999).

Flavonoids are generally known as important antioxidants (Cao *et al.*, 1997; Duthie *et al.*, 1997; Rice-Evans *et al.*, 1997; Arora *et al.*, 1998; Ohshima *et al.*, 1998; Nuutila *et al.*, 2003). ABTS and superoxide anion radical scavenging methods have been used to evaluate the antioxidant activity of various chemicals (Re *et al.*, 1999; Kweon *et al.*, 2001). In this study, the general antioxidant effects for the potential to scavenge stable ABTS free radicals and superoxide anion radicals were investigated. The effect of number of hydroxyl groups and sugars in the flavonoid compounds on the antioxidant activity was found.

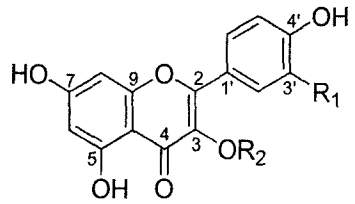
The flavonol glycosides **1-4** are widespread in the plants whereas compounds **5** and **6**, which have linear trisaccharides moieties, have been rarely found. In the ^{1}H-NMR spectrum of compound **5**, signals due to kaempferol moiety [δ_H 8.01 (2H, d, *J*=8.8 Hz), 6.89 (2H, d, *J*=8.8 Hz) (H-2,6 or 3,5 of *para*-disubstituted benzene), 6.37 (1H, d, *J*=2.0 Hz), 6.17 (1H, d, *J*=2.0 Hz) (H-4 or H-6 of 1,2,3,5-tetrasubstituted benzene)], to two 6-deoxyhexoses [δ_H 5.11 (1H, d, *J*=1.2 Hz, anomer-H), 4.51 (1H, d, *J*=1.2 Hz, anomer-H), 1.07 (3H, d, *J*=6.0 Hz, H-6 of deoxyhexose), 0.98 (1H, d, *J*=6.0 Hz, H-6 of deoxyhexose) and to one hexose [δ_H 5.59 (1H, d, *J*=8.4 Hz, anomer-H) were observed. The acidic hydrolysis of compound **5** gave not only a kaempferol but also rhamnose and glucose with a ratio of 2:1. Therefore compound **5** was identified as a kaempferol triglycoside with two rhamnoses and one glucose. Glycosidic linkage of compound **5** was determined from the ^{13}C-NMR data. The chemical shifts of one glucose and one rhamnose were very similar to those of compounds **3** or **4**, which have rutinose, α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside, as the sugar moiety, with the exception of rhamnosyl C-4. The carbon signal was observed at δ_C 79.7 owing to the glycosidation effect, which is usually observed at δ_C 73.7 in rutinose. Hence, the terminal rhamnose was shown to be linked at C-4 of the second rhamnosyl moiety, and consequently, compound **5** was identified as kaempferol 3-*O*-α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside. The NMR data of compound **6** were very similar to those of compound **5** with the exception of B-ring of aglycone that showed the characteristics of 1,2,4-trisubstituted benzene. Therefore, compound **6** was identified as quercetin 3-*O*-α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside. Some physical data such as the melting point or $[\alpha]_D$ values of compounds **3**, **5** and **6** were not found in the literature. This paper has supplied all of the physical and spectral data including the exact assignments of NMR signals, which were carried out by the 2-D NMR experiments, gHMBC (gradient heteronuclear multiple bonding connectivity), gHSQC (gradient heteronuclear single quantum correlation) etc..

The antioxidant activities of the isolated compounds **1-6** are shown in Table I. Compound **1** exhibited the highest antioxidant activity in the ABTS radical scavenging method. 100 mg/L of compound **1** was equivalent to 72.1 ±1.4 mg/L of vitamin C, and those of compounds **3** and **5**

Table I. ABTS (Vitamin C equivalent antioxidant capacity/VCEAC) and superoxide anion scavenging activity (IC_{50}) of the flavonol glycosides from the aerial parts of *Aceriphyllum rossii*

Flavonol glycosides	ABTS scavenging activity		Superoxide anion scavenging activity (IC_{50}: μM)
	A734nm	VCEAC (mg/L)**	
Control	0.644 ± 0.001		
Compound 1	0.402 ± 0.008*	72.1 ± 1.4	39.1 ± 0.7
Compound 2	0.506 ± 0.007*	40.5 ± 0.5	42.2 ± 0.4
Compound 3	0.433 ± 0.009*	62.7 ± 0.5	29.6 ± 0.4
Compound 4	0.499 ± 0.010*	42.7 ± 0.9	27.2 ± 0.5
Compound 5	0.461 ± 0.012*	54.3 ± 1.3	17.6 ± 0.3
Compound 6	0.506 ± 0.008*	40.4 ± 0.7	29.6 ± 0.5
Vitamin C	0.312 ± 0.010*	100	57.2 ± 0.6

The data are presented as mean ± standard deviations of five replications. *Significantly different from the control (P<0.01). **The value denotes VCEAC corresponding to the level of 100 mg/L of each compound.



	R_1	R_2
1	H	β-D-Glc
2	OH	β-D-Glc
3	H	α-L-Rhm(1→6)-β-D-Glc
4	OH	α-L-Rhm(1→6)-β-D-Glc
5	H	α-L-Rhm(1→4)-α-L-Rhm(1→6)-β-D-Glc
6	OH	α-L-Rhm(1→4)-α-L-Rhm(1→6)-β-D-Glc

Fig. 1. Structures of flavonol glycosides from the aerial parts of *Aceriphyllum rossii*.

were equivalent to 62.7±0.5 mg/L and 54.3±1.3 mg/L of vitamin C, respectively. The increase in the number of sugars linked to kaempferol led to the decrease in activity, which might be a result of steric hindrance by sugar substituents (Cholbi *et. al.*, 1991). Kaempferol glycosides, with 4-hydroxy moiety in the B-ring, appeared to be a better antioxidant than quercetin glycosides, with 3,4-dihydroxy (Rice-Evans *et al.*, 1996).

In the superoxide anion radical scavenging method, all the compounds showed higher activity than vitamin C. In particular, compound **5** exhibited the highest activity with the IC_{50} value of 17.6±0.3 μM. The increase in number of sugars linked to quercetin led to the increase in activity, which might be due to the increase of the polarity. This superoxide anion radical scavenging method showed a reverse result to the ABTS radical scavenging method.

Antioxidants have been reported to play a major role in ameliorating peroxidative damage, induced by free radicals and xenobiotics, in membranes and tissues (Carini *et al.*, 1990). Therefore, they could be used as preventive and/or therapeutic agents for carcinogenesis, the aging process and cardiovascular diseases such as ischemia-reperfusion injury and hypercholesterolemic atherosclerosis (Gey, 1993).

ACKNOWLEDGEMENTS

This study was supported by the BioGreen 21 Program from Rural Development Administration, Republic of Korea, and by a grant from the Korea Science & Technology Foundation through Plant Metabolism Research Center, Kyung Hee University.

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