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# Antioxidant Flavonoids from the Twigs of *Stewartia koreana*

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**Abstract** − In the course of screening for antioxidant compounds by measuring the radical scavenging effect on 1,1-diphenyl- 2-picrylhydrazyl (DPPH), a total extract of the twigs of *Stewartia koreana* (Theaceae) was found to show potent antioxidant activity. Subsequent activity-guided fractionation of the methanolic extract led to the isolation of six phenolic compounds, ampelopsin (1), catechin (2), proanthocyanidin-A2 (3), fraxin (4), (2R, 3R)-taxifolin-3-β-D-glucopyranoside (5), and (2S, 3S)-taxifolin-3-β-D-glucopyranoside (6), as active principles. Their structures were elucidated by spectroscopic studies. Compounds 1-6 were isolated for the first time from this plant. Among them, three compounds 1-3 showed the significant antioxidative effects on DPPH, and riboflavin originated superoxide quenching activity. In riboflavin-nitrobluetetrazolium (NBT)-light system, compound 1 showed better superoxide quenching activity than vitamin C.

**Keywords:** *Stewartia koreana*, Theaceae, Phenolic compounds, DPPH, Superoxide quenching activity

## INTRODUCTION

Reactive oxygen species produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects, such as DNA damage, carcinogenesis and cellular degeneration related to aging (Liu and Ng, 2000; Li *et al*., 2007; Dembinska-Kiec *et al*., 2008; Ma *et al*., 2008). In the past synthetic antioxidants such as 3,5-di-*t*-butyl-4-hydroxytoluene (BHT), 3-*t*-butyl-4-hydroxyanisole (BHA), troxol C were widely used because of their strong antioxidant effects. In recent years, however, the use of some synthetic antioxidants has been limited because of their possible toxic and carcinogenic effects (Branen, 1975; Moure *et al*., 2001). Thus, alternative natural antioxidants have attracted considerable interest because of their presumed safety and therapeutic effects (Branen, 1975).

In the course of screening for antioxidant compounds by measuring the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH), a total extract of the twigs of *Stewartia koreana* (Theaceae) was found to show potent antioxidant activity. Subsequent activity-guided fractionation of the methanolic extract led to the isolation of six phenolic compounds, as active constituents. *S. koreana* has not been considered a medicinal plant and phytochemical study of this plant has been not performed yet. But its extracts have various biological activities such as cyclooxygenase-2, inducible nitric oxide synthase gene expression, osteoclast differentiation, and bone resoption inhibitory activities (Kim *et al*., 2004; Lee *et al*., 2007; Park *et al*., 2007). Lee *et al*. reported that its extract stimulates proliferation and migration of human endothelial cells and induces neovasculization (Lee *et al*., 2010). This paper deals with the isolation and structural characterization of these compounds and their scavenging activity of the stable DPPH free radical and superoxide quenching activities.

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## MATERIALS AND METHODS

### General experimental procedures

$^1$H- and $^{13}$C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. TLC was carried out on Merck precoated silica gel $F_{254}$ plates and silica gel for column chromatography was Kiesel gel 60 (230-400 mesh, Merck). Spots were detected under UV and by spraying with 10% $H_2SO_4$ in ethanol followed by heating at 100-120°C for 3 min. And Sephadex LH-20 was used for column chromatography (Pharmacia, 25-100 μm). Preparative HPLC was carried out on a Jaigel GS310 column (Japan). All other chemicals and solvents were of analytical grade and used without further purification. Ascorbic acid and BHA were obtained from Sigma Chemical Co.

**Plant materials**

The twigs of *Stewartia koreana* were collected and air-dried in October 2006 at Wanju, Chonbuk, Korea. A voucher specimen was deposited in the herbarium of College of Pharmacy, Woosuk University (WSU-06-008).

**Extraction and isolation**

The shade dried plant material (1 kg) was extracted three times with MeOH at 50°C and filtered. The extracts were combined and evaporated *in vacuo* at 50°C. The resultant methanolic extract (38 g) was subjected to successive solvent partitioning to give *n*-hexane (3.9 g), methylene chloride (3.7 g), ethyl acetate (9.8 g), *n*-BuOH (10.8 g) and $H_2O$ soluble fractions. Each fraction was tested for the radical scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl). Among these fractions, the ethyl acetate soluble fraction showed the most significant free radical scavenging effect on DPPH (Table I). The ethyl acetate soluble extract was chromatographed on silica gel column ($CHCl_3$-EtOAc-MeOH, 7:2:1) as an eluent to give nine fractions (E1-E9). Fraction E4 (360 mg) was chromatographed on silica gel column chromatography ($CHCl_3$-MeOH-$H_2O$, 90:20:1) as an eluent to give three subfractions (E41-E43). Subfraction E42 (138 mg) was further chromatographed on a JAI-GS310 cloumn (MeOH) and purified by Sephadex LH-20 (MeOH) to give compound 1 (83 mg). Fraction E5 (733 mg) was chromatographed on a JAI-GS310 cloumn (MeOH) and purified by Sephadex LH-20 (MeOH) to give compound 2 (239 mg). Fraction E7 (1,880 mg) was chromatographed on Sephadex LH-20 (MeOH) to give five subfractions (E71-E75). E75 (118.4 mg) was chromatographed over silica gel ($CHCl_3$-MeOH-Water, 40:10:1) and purified on a JAI-GS310 cloumn (MeOH) to give compound 3 (12 mg) (Scheme 2). Fraction E8 (1,312 mg) was chromatographed on Sephadex LH-20 (MeOH) to give four subfractions (E81-E84). Subfraction E81 (308.2 mg) was chromatographed on a JAI-GS310 cloumn (MeOH) to give compound 4 (4 mg). Subfraction E83 (501 mg) was chromatographed on a JAI-GS310 cloumn (MeOH) to give compound 5 (43 mg). Subfraction E83 (501 mg) was applied on a JAI-GS310 cloumn (MeOH) and purified by Sephadex LH-20 (MeOH) to give compound 6 (25 mg).

**Table I.** Scavenging effects of methanol extract and its subsequent fractions from the twigs of *Stewartia koreana* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

| Fractions | $IC_{50}$ (μg/ml)[a] |
|---|---|
| MeOH extract | 52.8 |
| *n*-Hexane fraction | 787.5 |
| Methylene chloride fraction | 69.1 |
| EtOAc fraction | 14.9 |
| *n*-BuOH fraction | 42.1 |
| Ascorbic acid | 4.6 |
| 3-*t*-Butyl-4-hydroxyanisole | 6.4 |

[a]The values indicate 50% decrease of DPPH radical and are the means of triplicate data.

**Ampelopsin (1)**

White powder (MeOH), $^1$H-NMR (400 MHz, $CD_3OD$) δ: 6.50 (2 H, s, H-2', 6'), 5.87 (1 H, d, *J*=2.0 Hz, H-8), 5.83 (1 H, d, *J* = 2.0 Hz, H-6), 4.79 (1 H, d, *J*=11.6 Hz, H-2), 4.43 (1 H, d, *J*=11.6 Hz, H-3). $^{13}$C-NMR (100 MHz, $CD_3OD$): Table II.

**Catechin (2)**

White powder (MeOH), $^1$H-NMR (400 MHz, $CD_3OD$) δ: 6.79 (1 H, d, *J*=2.0 Hz, H-2'), 6.72 (1 H, d, *J*=8.4 Hz, H-5'), 6.67 (1 H, dd, *J*=8.4, 2.0 Hz, H-6'), 5.89 (1 H, d, *J*=2.4 Hz, H-8), 5.82 (1 H, d, *J*=2.4 Hz, H-6), 4.53 (1 H, d, *J*=7.2 Hz, H-2), 3.94 (1 H, ddd, *J*=8.0, 7.2, 5.6 Hz, H-3), 2.80 (1 H, dd, *J*=16.4, 5.6 Hz, H-4a), 2.46 (1H, ddd, *J*=16.4, 8.0, 2.0 Hz, H-4b). $^{13}$C-NMR (100 MHz, $CD_3OD$): Table II.

**Proanthocyanidin-A2 (3)**

Brown powder (MeOH), $^1$H-NMR (400 MHz, $CD_3OD$), (T unit) δ: 6.06 (1 H, d, *J*=2.4 Hz, H-8), 5.95 (1 H, d, *J*=2.4 Hz, H-6), 4.22 (1 H, d, *J*=3.2 Hz, H-4), 4.06 (1 H, d, *J*=3.2 Hz, H-3), (B unit) δ: 6.08 (1 H, s, H-6), 4.72 (1 H, d, *J*=8.0 Hz, H-2), 4.13 (1 H, m, H-3), 2.93 (1 H, dd, *J*=16.4, 5.2 Hz,

**Table II.** $^{13}$C-NMR spectral data of compounds 1, 2, 5 and 6

| C | 1 | 2 | 5 | 6 |
|---|---|---|---|---|
| 2 | 85.1 | 82.8 | 83.5 | 83.4 |
| 3 | 73.6 | 68.7 | 77.1 | 77.8 |
| 4 | 198.2 | 28.4 | 195.8 | 196.1 |
| 5 | 165.2 | 157.5 | 165.4 | 165.5 |
| 6 | 96.3 | 96.3 | 96.4 | 96.4 |
| 7 | 168.6 | 156.8 | 169.1 | 169.1 |
| 8 | 97.3 | 95.5 | 96.4 | 96.4 |
| 9 | 164.3 | 157.7 | 164.1 | 164.0 |
| 10 | 101.4 | 100.8 | 102.5 | 102.3 |
| 1' | 129.1 | 132.2 | 129.0 | 129.0 |
| 2' | 108.1 | 115.2 | 115.9 | 116.0 |
| 3' | 146.8 | 146.2 | 147.3[a] | 147.0[a] |
| 4' | 134.8 | 146.2 | 146.3[a] | 146.0[a] |
| 5' | 146.8 | 116.1 | 116.2 | 116.3 |
| 6' | 108.1 | 120.0 | 121.1 | 121.1 |
| 1" | | | 102.5 | 104.6 |
| 2" | | | 74.6 | 75.4 |
| 3" | | | 78.1 | 77.9 |
| 4" | | | 71.2 | 71.4 |
| 5" | | | 77.6 | 77.9 |
| 6" | | | 62.6 | 62.8 |

Recorded at 400 MHz for $^1$H and 100 MHz for $^{13}$C (in $CD_3OD$). [a]This values may be changeable.

H-4a), 2.56 (1 H, dd, *J*=16.4, 8.4 Hz, H-4b), (T and B unit) δ: 7.12 and 6.91 (each 1 H, d, *J*=2.0, H-2'), 7.01 and 6.80 (each 1 H, dd, *J*=8.4, 2.4 Hz, H-6'), 6.79 (2 H, d, *J*=8.4, H-5'). $^{13}$C-NMR (100 MHz, $CD_3OD$): (T unit) δ: 104.0 (C-10), 100.3 (C-2), 98.2 (C-6), 96.6 (C-8), 67.8 (C-3), 29.0 (C-4), (B unit) δ: 106.8 (C-8), 103.2 (C-10), 96.6 (C-6), 84.5 (C-2), 68.1 (C-3), 29.2 (C-4), (T and B unit) δ: 158.2, 156.8, 156.2, 154.2, 152.2, 151.4 (C-5, 7, 9), 132.3, 130.6 (C-1'), 120.7, 119.8 (C-6'), 116.3, 115.8, 115.7, 115.6 (C-2', 5').

**Fraxin (4)**

Yellowish powder (MeOH), $^1$H-NMR (400 MHz, $CD_3OD$) δ: 7.87 (1 H, d, *J*=9.6 Hz, H-3), 6.97 (1 H, s, H-5), 6.23 (1 H, d, *J*=9.6 Hz, H-4), 4.97 (1 H, d, *J*=8.0 Hz, H-1'), 3.89 (3 H, s, $OCH_3$). $^{13}$C-NMR (100 MHz, $CD_3OD$) δ: 163.7 (C-2), 147.7 (C-7), 146.6 (C-4, 6), 144.4 (C-8), 133.3 (C-9), 112.8 (C-3), 112.0 (C-10), 106.2 (C-1'), 78.5 (C-3'), 77.9 (C-5'), 75.5 (C-2'), 71.0 (C-4'), 62.3 (C-6'), 57.0 (C-$OCH_3$).

**(2R,3R)-Taxifolin-3-β-$_D$-glucopyranoside (5)**

White powder (MeOH), $^1$H-NMR (400 MHz, $CD_3OD$) δ: 6.95 (1 H, d, *J*=2.0 Hz, H-2'), 6.83 (1 H, dd, *J*=8.0, 2.0 Hz, H-6'), 6.77 (1 H, d, *J*=8.0 Hz, H-5'), 5.88 (2 H, d, *J*=2.0 Hz, H-6, 8), 5.21 (1 H, d, *J*=9.6 Hz, H-2), 4.91 (1 H, d, *J*=9.6 Hz, H-3), 3.85 (1 H, d, *J*=7.6 Hz, H-1"). $^{13}$C-NMR (100 MHz, $CD_3OD$): Table II.

**(2S,3S)-Taxifolin-3-β-$_D$-glucopyranoside (6)**

White powder (MeOH), $^1$H-NMR (400 MHz, $CD_3OD$) δ: 6.95 (1 H, d, *J*=2.0 Hz, H-2'), 6.79 (1 H, dd, *J*=8.0, 2.0 Hz, H-6'), 6.74 (1 H, d, *J*=8.0 Hz, H-5'), 5.89 (2 H, d, *J*=2.0 Hz, H-6, 8), 5.23 (1 H, d, *J*=8.8 Hz, H-2), 4.88 (1 H, d, *J*=8.0 Hz, H-1"), 4.67 (1 H, d, *J*=8.8 Hz, H-3). $^{13}$C-NMR (100 MHz, $CD_3OD$): Table II.

**DPPH radical scavenging effect**

Ethanol solutions of test samples at various concentrations (0.1-100 μg/ml) were added to a solution of DPPH in methanol (0.2 mM) in 96 well plates. After storing these mixtures for 30 minutes at room temperature, the remaining amounts of DPPH were determined by colorimetry at 520 nm on a microplate reader (Yoshida *et al*., 1989). And the radical scavenging activity of each compound was expressed by the ratio of the lowering of the DPPH solution in the absence of compounds. The mean values were obtained from triplicate experiments.

**Superoxide quenching activity**

Superoxide quenching activities of test samples were measured photochemically, using an assay system consisting of methionine, riboflavin, and nitrobluetetrazolium (NBT) (Ginnopolitis and Ries, 1977; Choi *et al*., 2001). The reaction mixture was composed of 0.13 μM riboflavin, 13 mM methionine, 75 μM NBT, 0.1 mM EDTA, PBS buffer (pH 7.4), and various concentrations of test samples. The sample was randomly placed in a light storage box and replaced randomly every 5 min for 15 min. The temperature within the light storage box was 20 ± 1°C during the light illumination. The light intensity at the sample level was 5,500 lux. During the light illumination, NBT was reduced to blue formazan formation was measured by the absorbance at 560 nm. The inhibition of blue formazan formation was taken as superoxide quenching activity.

## RESULTS AND DISCUSSION

After screening of various plant extracts for their scavenging activity on DPPH radical, a methanolic extract of the twigs of *S. koreana* was found to be potent at a con-

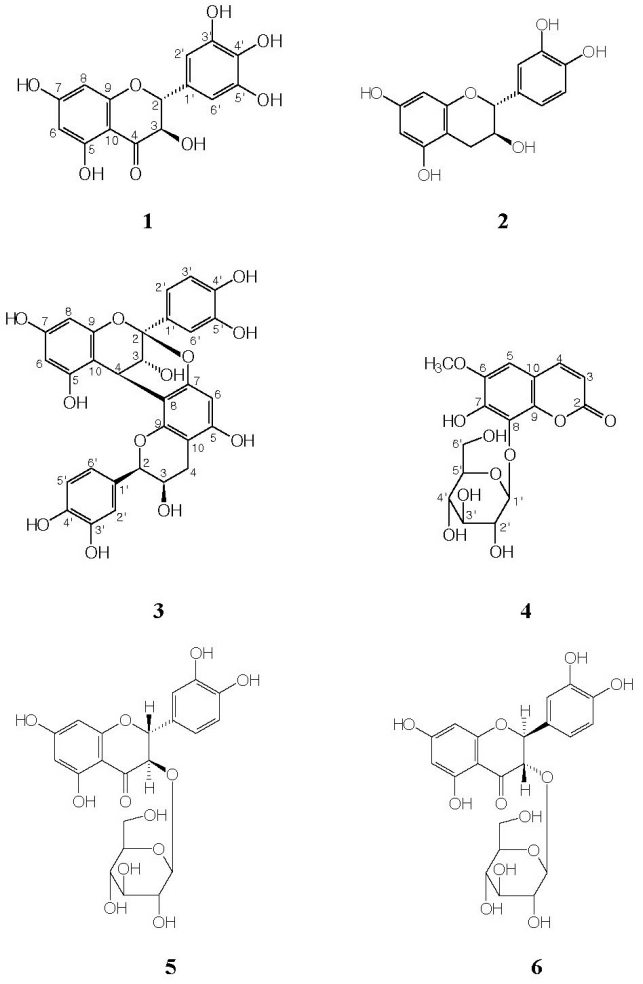


**Fig. 1.** Structures of compounds 1-6 isolated from *Stewarita koreana*.

centration of 52.8 μg/ml ($IC_{50}$) (Table I). Activity-guided fractionation of ethyl acetate soluble fraction of *S. koreana* led to the isolation of six compounds (Fig. 1).

Compound 1, white powder, showed positive results toward $FeCl_3$ reagent. The $^1$H-NMR spectrum of 1 showed two *meta* coupling peaks at δ 5.87 (1 H, d, *J*=2.0 Hz, H-8) and 5.83 (1 H, d, *J*=2.0 Hz, H-6), and a peak at δ 6.50 (2 H, s, H-2', 6') in the aromatic region. And a pair of doublet coupling peak at δ 4.79 (1 H, d, *J*=11.6 Hz, H-2), 4.43 (1 H, d, *J*=11.6 Hz, H-3). The $^{13}$C-NMR spectrum of 1 showed a carbonyl signal at 198.2, and two oxygenated carbon signals at 85.1 and 73.6. From these results, compound 1 was deduced to be one of pentahydroxy flavanonol compounds. On the basis of the above evidence, together with a direct comparison of the above data with those published in the literature, the structure of compound 1 was determined to be 5,7,3',4',5'-pentahydroxyflavanonol (ampelopsin) (Yeom *et al*., 2003).

Compound 2, white powder, showed positive results toward $FeCl_3$ reagent. The $^1$H-NMR spectrum of 2 showed two meta coupling peaks at δ 5.89 (1 H, d, *J*=2.4 Hz, H-8), 5.82 (1 H, d, *J*=2.4 Hz, H-6) of A ring, and two germinal coupling signals δ 2.80 (1 H, dd, *J*=16.4, 5.6 Hz, H-4a), 2.46 (1 H, ddd, *J*=16.4, 8.0, 2.0 Hz, H-4b). And in the $^1$H-NMR spectrum of 2, the proton signals of δ 6.79 (1 H, d, *J*=2.0 Hz, H-2'), 6.72 (1 H, d, *J*=8.4 Hz, H-5') and 6.67 (1 H, dd, *J*=8.4, 2.0 Hz, H-6') showed ABX splitting pattern of B ring. In the $^{13}$C-NMR spectrum of 2, fifteen carbon signals including two oxygenated carbon signals at δ 82.8 and 68.7, and a aliphatic signal at δ 28.4 were observed. From these results, compound 2 was deduced to be catechin. On the basis of the above evidence, together with a direct comparison of the above data with those published in the literature, the structure of compound 2 was determined to be catechin (Yeo *et al*, 1992).

Compound 3, brown powder, showed positive results toward $FeCl_3$ reagent. NMR spectra of 3 were similar to those of 2, suggesting the same skeleton. The main differences were the appearance two fold of signals in NMR spectra. In the $^1$H-NMR spectrum, six signals showing the two typical ABX coupling system were observed at δ 7.12-6.79 of B and B' rings. And two *meta* coupling peaks at δ 6.06 (1 H, d, *J*=2.4 Hz, H-8) and 5.95 (1 H, d, *J*=2.4 Hz, H-6), and a singlet peak δ 6.08 (1 H, s, H-6) were observed. In the $^{13}$C-NMR spectrum of 3, thirty carbon signals including three oxygenated carbon signals at δ 84.5, 68.1 and 67.8, and two aliphatic signal at δ 29.2 and 29.0 were observed. From these results, compound 3 was deduced to be a dimer of catechin derivatives. On the basis of the above evidence, together with a direct comparison of the above data with those published in the literature, the structure of compound 3 was determined to be epicatechin-(2β→7, 4β→8)-epicatechin (proanthocyanidin A-2) (Jacques and Haslam, 1974).

Compound 4, yellowish powder, showed positive results toward FeCl3 reagent. The 1H-NMR spectrum of 4 showed two ortho coupling peaks of typical coumarin signals at d 7.87 (1 H, d, J=9.6 Hz, H-3), and 6.23 (1 H, d, J=9.6 Hz, H-4), and a singlet peak d 6.97 (1 H, s, H-5) in the aromatic region. A methoxy signal at d 3.89, and anomeric proton signal of glucose were observed d 4.97 (1 H, d, J=8.0 Hz, H-1'). The 13C-NMR spectrum of 4 showed a carbonyl signal at d 163.7, a anomeric carbon signal at d 106.2, and a methoxy signal at d 57.0. From these results, compound 4 was deduced to be a coumarin glycoside. On the basis of the above evidence, together with a direct comparison of the above data with those published in the literature, the structure of 4 was determined to be fraxin (Shi et al, 2007). Compound 5, white powder, showed positive results toward FeCl3 reagent. In the 1H-NMR spectrum of 5, the proton signals of d 6.95 (1 H, d, J=2.0 Hz, H-2'), 6.83 (1 H, dd, J=8.0, 2.0 Hz, H-6'), and 6.77 (1 H, d, J=8.0 Hz, H-5') showed ABX splitting pattern. The 1H-NMR spectrum of 5 showed two meta coupling peaks at d 5.88 (2 H, d, J=2.0 Hz, H-6, 8) in the aromatic region. And a pair of doublet coupling peak at d 5.21 (1 H, d, J=9.6 Hz, H-2) and 4.91 (1 H, d, J=9.6 Hz, H-3). An anomeric proton signal of glucose were observed d 3.85 (1 H, d, J=7.6 Hz, H-1''). The 13C-NMR spectrum of 5 showed a carbonyl signal at 195.8, and two oxygenated carbon signals at 83.5 (C-2) and 77.1 (C-3). From these results, compound 5 was deduced to be one of tetrahydroxy flavanonol compounds. On the basis of the above evidence, together with a direct comparison of the above data with those published in the literature, the structure of compound 5 was determined to be 5,7,3',4'- tetrahydroxyflavanonol-3-b -D-glucopyranoside [(2R,3R)-taxifolin-3-O-b -D-glucopyranoside)] (Xia 1995; Sakushima *et al*., 2002).

Compound 6, white powder, showed positive results toward FeCl3 reagent. NMR spectra of 6 were very similar of 5. The main differences were the chemical shifts of the position of C-2 and C-3, and the anomeric carbon. In the 1H-NMR spectrum of 6, the proton signals of d 6.95 (1 H, d, J=2.0 Hz, H-2'), 6.74 (1 H, dd, J=8.0, 2.0 Hz, H-6') and 6.77 (1 H, d, J=8.0 Hz, H-5') showed ABX splitting pattern. The 1H-NMR spectrum of 6 showed two meta coupling peaks at d 5.89 (2 H, d, J=2.0 Hz, H-6, 8) in the aromatic region. And a pair of doublet coupling peak at d 5.23 (1 H, d, J=8.8 Hz, H-2), 4.67 (1 H, d, J=8.8 Hz, H-3). An anomeric proton signal of glucose were observed d 4.88 (1 H, d,

**Table III.** Scavenging effects of compounds 1-6 from the twigs of *Stewartia koreana* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

| Fractions | $IC_{50}$ (μg/ml)[a] |
|---|---|
| Ampelopsin (1) | 4.6 |
| Catechin (2) | 5.4 |
| Proanthocyanidin-A2 (3) | 3.9 |
| Fraxin (4) | 40.5 |
| (2R, 3R)-Taxifolin-3-β-D-glucopyranoside (5) | 7.1 |
| (2S, 3S)-Taxifolin-3-β-D-glucopyranoside (6) | 9.6 |
| Ascorbic acid | 2.9 |
| 3-*t*-Butyl-4-hydroxyanisole | 6.9 |

[a]The values indicate 50% decrease of DPPH radical and are the means of triplicate data.

**Table IV.** Superoxide quenching activities of compounds 1-6 from the twigs of *Stewartia koreana*

| Fractions | $IC_{50}$ (μg/ml)[a] |
|---|---|
| Ampelopsin (1) | 2.6 |
| Catechin (2) | 7.9 |
| Proanthocyanidin-A2 (3) | 6.8 |
| Fraxin (4) | 48.7 |
| (2R, 3R)-Taxifolin-3-β-D-glucopyranoside (5) | 20.1 |
| (2S, 3S)-Taxifolin-3-β-D-glucopyranoside (6) | 18.5 |
| Ascorbic acid | 6.4 |
| 3-*t*-Butyl-4-hydroxyanisole | 49.9 |

[a]The values indicate 50% superoxide quenching activities and are the means of triplicate data.

J=8.0 Hz, H-1"). The 13C-NMR spectrum of 6 showed a carbonyl signal at 196.1 and two oxygenated carbon signals at 83.4 (C-2) and 77.8 (C-3). From these results, compound 6 was deduced to be isomer of 5. On the basis of the above evidence, together with a direct comparison of the above data with those published in the literature, the structure of compound 6 was determined to be [(2S,3S)-taxifolin-3-O-b -D-glucopyranoside)] (Xia 1995; Sakushima *et al*., 2002). To our best knowledge, this is the first report on the isolation of compounds 1-6 from this plant.

s The DPPH radical scavenging effect of the methanolic extract and its solvent partitioned fractions from *S. koreana* are shown in Table I. The radical scavenging effects of six compounds isolated from ethyl acetate soluble fraction of *S. koreana* were also shown in Table III. Among six isolated compounds, compound 3 and 1 exhibited higher scavenging activity on DPPH with $IC_{50}$ values of 3.9 and 4.6 μg/ml, respectively. The inhibition of blue formazan formation was taken as superoxide quenching activity. Superoxide quenching activities of six compounds isolated from ethyl acetate soluble fraction of *S. koreana* were shown in Table IV. Among six isolated compounds, compound 1 exhibited higher activity with $IC_{50}$ value of 2.6 μg/ml than vitamin C, used as a positive control ($IC_{50}$ value, 6.4 μg/ml) (Table IV). It was reported that compound 1, ampelopsin has protective effect on oxidant stress-induced apoptosis induced by $H_2O_2$ in MT-4 cells, antitumor effects, inhibitory effects on angiogenesis, and hepatoprotective activity (Murakami *et al*., 2004; Zeng *et al*., 2004; Luo *et al*., 2006; Ye *et al*., 2008). The results from free radical scavenging systems revealed that the ethyl acetate soluble fraction of *S. koreana*, and isolated compounds 1-3 may be useful for the treatment of various oxidative damage.

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