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Antioxidant compounds from the stem bark of *Sorbus commixta*

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Abstract – The MeOH extract of *Sorbus commixta* (Rosaceae) exhibited strong DPPH radical scavenging activity, and through activity-guided fractionation two antioxidant compounds were isolated and identified as catechin-7-*O*-β-D-xylopyranoside (**1**) and catechin-7-*O*- β-D-apiofuranoside (**2**) by physicochemical and spectrometric methods. To evaluate the antioxidant effect of these compounds, some *in vitro* tests, such as the DPPH radical scavenging activity test, the superoxide radical scavenging activity test and the lipid peroxidation inhibitory activity test, were performed. Compounds **1** and **2** showed stronger activities than both α-tocopherol and butylated hydroxy anisole (BHA) in each assay.

Key words – *Sorbus commixta*, Rosaceae, catechin-7-*O*-β-D-xylopyranoside, catechin-7-*O*- β-D-apiofuranoside, antioxidant effect

Introduction

Sorbus commixta Hedlund (Rosaceae) is a shrub growing in the base of mountainous regions and usually grows 6-8 m in height. The stem bark of *S. commixta* is used in traditional medicine as a tonic and for the treatment of cough, asthma and other bronchial disorders (Bae, 2000; Chiang, 1977).

In a search for effective antioxidants from plants, the MeOH extracts from approximately 200 Korean plants were investigated. Among them, the MeOH extract of the stem bark of *S. commixta* showed a potent activity in a DPPH radical scavenging assay. To date, there has been no systematic study on the *S. commixta* chemical constituents and reports on its biological activity are few. Therefore, this study was performed to evaluate the antioxidant compounds from the stem bark of *S. commixta*.

Experimental

Plant Material – The stem bark of *S. commixta* was collected in Mt. Sulak, Korea in June 1998 and identified by Prof. KiHwan Bae, College of Pharmacy, Chungnam National University. A voucher specimen (CNU 1081) was deposited in the herbarium of the College of Pharmacy, Chungnam National University.

Extraction, fractionation and isolation – The dried stem bark of *S. commixta* (2.2 kg) was extracted with MeOH by reflux. The MeOH extract (270 g) was suspended in water and then partitioned successively with hexane, EtOAc and BuOH. Among the solvent fractions, the BuOH fraction exhibited the strongest activity with an IC_{50} value of 18.2 ± 1.5 μg/ml in the DPPH assay. Accordingly, the BuOH fraction (70 g) was further subjected to column chromatography on a silica gel (9×40 cm, 70-230 mesh) eluting with $CHCl_3$-MeOH (gradient, 30:1→0:1). Eight fractions were obtained based on the monitoring of their TLC (silica gel) pattern. Among the fractions, the fraction 5 (Fr. 5, 7.2 g) had the most significant DPPH radical scavenging activity with an IC_{50} value of 8.0 ± 0.6 μg/ml. Silica gel column chromatography (5×30 cm, 230-400 mesh) of the Fr. 5 was then carried out using a mobile phase of $CHCl_3$-MeOH (7:3) to yield three subfractions (Fr. 5-1Fr. 5-3). The Fr. 5-2 (2.1 g) was purified by preparative MPLC on an ULTRA PACK ODS-18 column (YAMAZEN, 26×300 mm; mobile phase: MeOH-H_2O (30:70); flow rate: 12 ml/min; detection: 254 nm) to give compound **1** (180 mg, t_R 18 min). Compound **2** (120 mg, t_R 25 min) was isolated from Fr. 5-3 (1.6 g) by preparative MPLC on an ULTRA PACK ODS-18 column (YAMAZEN, 26×300 mm; mobile phase: MeOH-H_2O (30:70); flow rate: 12 ml/min; detection: 254 nm).

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Compound 1 – Pale yellow powder, mp 165-167, $[\alpha]_D$ 37.9° (c 1.6, MeOH), FAB-MS *m/z*: 423 $[M+H]^+$, IR ν_{max} (KBr) cm^{-1}: 3400, 2900, 1610, 1590, 1510, 1450, 1280, 1160, 1100, 1040, UV (MeOH) λ_{max} (log ε): 280 nm (3.60), ^{1}H-NMR (300 MHz, CD_3OD) δ: 4.59 (1H, d, *J*=7.4 Hz, H-2), 4.01 (1H, ddd, *J*=8.0, 7.4, 5.2 Hz, H-3), 2.53 (1H, dd, *J*=16.3, 8.0 Hz, H-4*α*), 2.85 (1H, dd, *J*=16.3, 5.2 Hz, H-4*β*), 6.17 (1H, d, *J*=2.3 Hz, H-6), 6.12 (1H, d, *J*=2.3 Hz, H-8), 6.83 (1H, d, *J*=1.8 Hz, H-2), 6.76 (1H, d, *J*=8.1 Hz, H-5), 6.71 (1H, dd, *J*=8.1, 1.8 Hz, H-6), 4.76 (1H, d, *J*=7.3 Hz, xyl-1), ^{13}C-NMR (75 MHz, CD_3OD) δ: 82.8 (C-2), 68.5 (C-3), 28.4 (C-4), 157.5 (C-5), 97.4 (C-6), 158.4 (C-7), 96.9 (C-8), 156.8 (C-9), 103.7 (C-10), 132.0 (C-1), 115.2 (C-2), 146.2 (C-3), 146.2 (C-4), 116.1 (C-5), 120.0 (C-6), 102.7 (xyl-1), 74.6 (xyl-2), 77.7 (xyl-3), 71.0 (xyl-4), 66.8 (xyl-5).

Compound 2 – Pale yellow powder, mp 171-174, $[\alpha]_D$ 33.3° (c 0.48, MeOH), FAB-MS *m/z* : 423 $[M+H]^+$, IR ν_{max} (KBr) cm^{-1}: 3400, 2900, 1610, 1590, 1510, 1450, 1280, 1160, 1100, 1040, UV (MeOH) λ_{max} (log ε): 280 nm (3.60), ^{1}H-NMR (300 MHz, CD_3OD) δ: 4.62 (1H, d, *J*=7.3 Hz, H-2), 4.05 (1H, ddd, *J*=7.9, 7.3, 5.3 Hz, H-3), 2.55 (1H, dd, *J*=16.3, 7.9 Hz, H-4*α*), 2.88 (1H, dd, *J*=16.3, 5.3 Hz, H-4*β*), 6.17 (1H, d, *J*=2.3 Hz, H-6), 6.12 (1H, d, *J*=2.3 Hz, H-8), 6.85 (1H, d, *J*=1.8 Hz, H-2), 6.78 (1H, d, *J*=8.1 Hz, H-5), 6.73 (1H, dd, *J*=8.1, 1.8 Hz, H-6), 5.51 (1H, d, *J*=2.8 Hz, api-1), 4.16 (1H, d, *J*=2.8 Hz, api-2), 3.87, 4.08 (each 1H, d, *J*=9.8 Hz, api-4), 3.64 (2H, br., api-5), ^{13}C-NMR (75 MHz, CD_3OD) δ: 82.6 (C-2), 68.4 (C-3), 28.2 (C-4), 157.3 (C-5), 97.2 (C-6), 157.9 (C-7), 96.8 (C-8), 156.6 (C-9), 103.2 (C-10), 131.9 (C-1), 115.1 (C-2), 146.0 (C-3), 146.0 (C-4), 116.1 (C-5), 119.9 (C-6), 108.4 (api-1), 78.2 (api-2), 80.2 (api-3), 75.3 (api-4), 64.9 (api-5).

DPPH radical scavenging activity – DPPH radical scavenging activity was measured as by the method described by Taco *et al.* (1994) with some modifications. Ten μl of each sample, dissolved in DMSO, was prepared in 96 well plate and then 190 μl of 200 μM ethanolic DPPH solution was added. The mixture was incubated at room temperature for 30 min. The absorbance of reaction mixture was measured at 517 nm. DPPH radical scavenging activity (%) was expressed as follows:

$$\text{DPPH radical scavenging activity (\%)} = (A_{control} - A_{sample})/A_{control} \times 100$$

Where, $A_{control}$ is the absorbance of control group and A_{sample} is the absorbance of test group.

Superoxide (O_2^-) radical scavenging activity – Superoxide was generated by xanthine/xanthine oxidase and measured by the NBT reduction method (Nishikimi *et al.*, 1972; Cheng *et al.*, 1998). 50 μl of 4 mM xanthine, 50 μl of 225 μM NBT, 50 μl of 50 mM phosphate buffer (pH 7.8, 1 mM EDTA) and 10 μl of test compounds were prepared in 96 well plate, and then 40 μl of xanthine oxidase were added to the mixture. Depending upon the passage of time, the absorbance of each reaction mixture was measured at 550 nm. Superoxide radical scavenging activity (%) was expressed by the degree of NBT reduction decrease of test group in comparison with that in the control group after 3 minutes.

$$\text{Superoxide radical scavenging activity (\%)} = (A_{control} - A_{sample}) / (A_{control} - A_{blank}) \times 100$$

Where, $A_{control}$ is the absorbance of control group where the sample was not treated, A_{sample} is the absorbance of test group where the sample was treated and A_{blank} is the absorbance of blank to which the sample and the NBT solution were not added. BHA and α-tocopherol were used as a positive control.

Preparation of rat brain homogenate: The rat brain homogenate was prepared as described method with some modifications (Nguyen *et al.*, 1998). Sprague-Dawley rat brain was removed and washed with ice-cold saline. The brain was homogenized in 9 volume of ice-cold phosphate buffer (pH 7.4) using a glass homogenizer and then centrifuged at 1000 rpm for 10 min. The supernatant was stored at –70°C until the lipid peroxidation experiment.

Lipid peroxidation inhibitory activity: The lipid peroxidation inhibitory activity in rat brain homogenate was evaluated by the thiobarbituric acid (TBA) method with some modifications (Nguyen *et al.*, 1998). The reaction mixture was composed of 10 μl of sample (DMSO), 740 μl of 50 mM-phosphate buffer (pH 7.4), 50 μl of rat brain homogenate (10 mg protein/ml) and 200 μl of the free radical generating system: 0.1 mM $FeSO_4 7H_2O$ + 1 mM ascorbic acid. The reaction mixture was incubated at 37°C for 30 min and the reaction was terminated by adding 250 μl of 20% TCA and 250 μl of 1% TBA (in 50 mM NaOH). After boiling at 95°C for 5 min, the mixture was centrifuged at 10,000 rpm for 10 min. The absorbance of supernatant was measured at 532 nm. Lipid peroxidation inhibitory activity (%) was expressed as follows:

$$\text{Lipid peroxidation inhibitory activity (\%)} = (A_{control} - A_{sample})/(A_{control} - A_{blank}) \times 100$$

Where, $A_{control}$ is the absorbance of control group, A_{sample} is the absorbance of test group and A_{blank} is the absorbance of blank to which the sample and the free radical generating system (Fe^{2+}/ascorbate) were not added.

Results and Discussion

Compound 1 had a molecular weight of 422, as identified by positive ion FAB-MS ($[M+H]^+$ at *m/z* 423). The characteristic ^{1}H-NMR signals at δ 4.59 (1H, d, *J* = 7.4 Hz), 4.01 (1H, ddd, *J* = 8.0, 7.4, 5.2 Hz), 2.53 (1H, dd, *J* = 16.3, 8.0 Hz) and 2.85 (1H, dd, *J* = 16.3, 5.2 Hz) were indicative of H-2, H-3, H-4α and H-4β, respectively, of the C ring of a catechin moiety. In addition, the ^{1}H-NMR spectrum indicated five aromatic protons including an AB spin system at δ 6.17 (1H, d, *J* = 2.3 Hz) and 6.12 (1H, d, *J* = 2.3 Hz) with *meta* coupling (H-6 and H-8) and an ABX system attributable to a 3′,4′ disubstituted B ring. A doublet at δ 4.76 (1H, d, *J* = 7.3 Hz) and complicated signals at δ 3.27-3.98 were indicative of one sugar. The presence of five signals in the ^{13}C-NMR spectrum at δ 102.7 (C-1″), 74.6 (C-2″), 77.7 (C-3″), 71.0 (C-4″) and 66.8 (C-5″), respectively, was indicative of a D-xylopyranoside (Hiroyuki *et al.*, 1995). The coupling constant (*J* = 7.3 Hz) supported a β configuration for the anomeric proton. The linkage of this sugar at C-7 was established by a COLOC correlation. Thus, the structure of **1** was determined to be catechin-7-*O*-β-D-xylopyranoside (Fig. 1). This was confirmed by a physicochemical and spectral data comparison with the published data (Hiroyuki *et al.*, 1995; Raymond *et al.*, 1973). Compound **2** also had a molecular weight of 422, as identified by positive ion FAB-MS ($[M+H]^+$ at *m/z* 423). The ^{1}H and ^{13}C-NMR spectra of compound **2** were similar to those of **1**. The most apparent difference was in the sugar moiety. The ^{1}H-NMR spectrum showed unique signals at δ 5.51 (1H, d, *J* = 2.8 Hz), 4.16 (1H, d, *J* = 2.8 Hz), 3.87, 4.08 (each 1H, d, *J* = 9.8 Hz), and 3.64 (2H, br.). ^{13}C-NMR signals were observed at δ 108.4 (C-1″), 78.2 (C-2″), 80.2 (C-3″), 75.3 (C-4″), 64.9 (C-5″), which were indicative of a D-apiofuranoside (Shengjun *et al.*, 2000). The linkage of this sugar at C-7 was established by a COLOC correlation. Thus, the structure of **2** was determined to be catechin-7-*O*-β- D-apiofuranoside (Fig. 1), which was also verified by a comparison with the published physicochemical and spectral data (Christian *et al.*, 1982). These two compounds were isolated from *Sorbus* spp. for the first time.

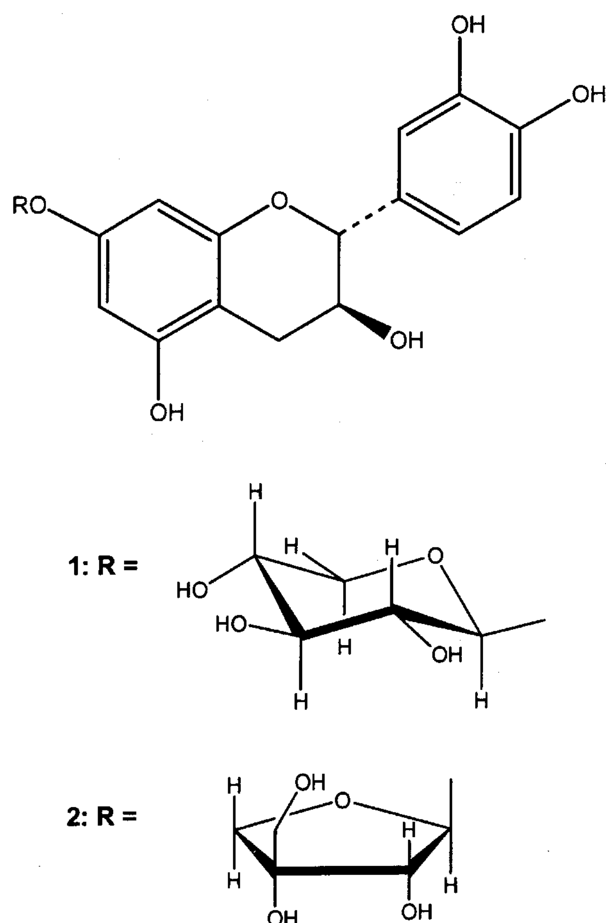

Fig. 1. Structures of catechin-7-*O*-β-D-xylopyranoside (**1**) and catechin-7-*O*-β-D-apiofuranoside (**2**).

In order to evaluate antioxidant effect of both compounds **1** and **2**, *in vitro* activity tests were performed. In the DPPH radical scavenging assay, **1** and **2** showed a significant scavenging activity with IC_{50} values of 3.6 ± 0.2 μM and 4.0 ± 0.3 μM, respectively. The activity of **1** and **2** was higher than either α-tocopherol or BHA, which had IC_{50} values of 6.7 ± 0.3 μM and 4.8 ± 0.2 μM, respectively (Table 1). In the superoxide radical scavenging assay, both **1** and **2** showed potent activity with IC_{50} values of 8.5 ± 0.9 μM and 9.2 ± 1.0 μM, respectively (Table 1), while α-tocopherol did not show an activity in this assay system.

Table 1. Antioxidative activities of compound 1 and 2 isolated from the bark of *S. commixta*

Compounds	DPPH radical scavenging activity IC_{50}[a] (μM)	Superoxide radical scavenging activity IC_{50} (μM)	Lipid peroxidation inhibitory activity IC_{50} (μM)
catechin-7-*O*-β-D-xylopyranoside (**1**)	3.6	8.5	9.0
catechin-7-*O*-β-D-apiofuranoside (**2**)	4.0	9.2	9.2
α-tocopherol	6.7	>100.0	15.6
BHA	4.8	24.6	10.8

[a] IC_{50} values were calculated from regression lines using five different concentrations in triplicate experiments.

The IC_{50} value of α-tocopherol was not reached even at concentrations as high as 100.0 μM, and the IC_{50} value of BHA was 24.6 ± 3.2 μM. In the lipid peroxidation inhibitory assay, **1** and **2** also exhibited potent inhibitory activity with IC_{50} values of 9.0 ± 0.8 μM and 9.2 ± 0.9 μM, respectively (Table 1). These compounds showed stronger activity than either α-tocopherol (IC_{50} 15.6 ± 1.9 μM) or BHA (IC_{50} 10.8 ± 0.7 μM), which were used as positive controls.

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