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Free radical scavenging and total phenolic contents from methanolic extracts of *Ulmus davidiana*

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Abstract

A methanolic (MeOH) extract of *Ulmus davidiana* was analyzed for antioxidant activity using model systems, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, hydroxyl radical (·OH) scavenging, reducing power, and total phenolic content. The MeOH extract exhibited strong antioxidant activity in the tested model systems. Among fractions using several solvents, the ethyl acetate (EtOAc)-soluble fraction, which exhibited strong antioxidant activity, was further purified by silica–gel and Sephadex LH-20 column chromatography. The (−)-Catechin (1) and (−)-catechin-7-*O*-β-D-apiofuranoside (2) were isolated as the active principles. Compounds 1 and 2 exhibited strong antioxidant activity on DPPH radicals, with IC_{50} values of 6.37 ± 0.02 μM and 6.41 ± 0.03 μM, respectively, and strong activity on ·OH radicals at 10 μg/ml, with 53.65 ± 0.01% and 52.56 ± 0.01% inhibition. *U. davidiana* extracts may be exploited as biopreservatives in food applications as well as for health supplements of functional food, to alleviate oxidative stress.

Keywords: *Ulmus davidiana*; (−)-Catechin; (−)-Catechin-7-*O*-β-D-apiofuranoside; 1,1-Diphenyl-2-picryl hydrazyl; Antioxidant activity

1. Introduction

Reactive oxygen species (ROS), such as superoxide anion ($O^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH), are closely involved in human diseases such as Alzheimer's disease, aging, cancer, inflammation, rheumatoid arthritis, and atherosclerosis (Freeman, 1984; Squadrito & Pryor, 1998). There has been an increased interest in identifying antioxidant phytochemicals, because these molecules can inhibit the propagation of free radical reactions, protect the human body from diseases (Kinsella, Frankel, German, & Kanner, 1993), and retard lipid oxidative rancidity in foods (Duthie, 1993). The most effective agents appear to be flavonoids and other phenolic compounds of many plant raw materials, particularly from herbs, seeds, and fruits. Because of their metal-chelating and radical-scavenging properties, phenolic compounds are considered effective free radical scavengers and inhibitors of lipid peroxidation (Bors & Saran, 1987; Miller, 1997).

Ulmus davidiana Planch *var*. *japonica* Nakai (Ulmaceae) is hardy to zone 5 and is not frost tender. It is in flower in May, and the seeds ripen from May to June. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Wind. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil.

Traditionally, root and stem barks of *U. davidiana* are frequently used to brew a tea in Asia. Dried inner bark, ground into powder and used as a thickening in soups or added to cereal flours when making bread (Kunkel, 1984). *U. davidiana* reputed to be effective against gastric cancer, gastroenteric disorders, granulating, eruption, edema, rheumatoid arthritis, hemorrhoids, and mastitis (Lee & Kim, 2001; Son, Park, & Zee, 1989). Investigations of the phytochemical components of *U. davidiana* stem bark have resulted in the isolation of (+)-catechin, catechin

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rhamnoside, and catechin apiofuranoside (Kim, Lee, Choi, Park, & Eom, 2003; Son et al., 1989), triterpene esters (Lee & Kim, 2001), sesquiterpene *O*-naphthaquinones (Kim, Kim, Koshino, Jung, & Yoo, 1996), and lignan and neolignan glycosides (Lee, Sung, Lee, Cho, & Kim, 2001). The bioactive ingredients from *U. davidiana* have been reported to have medicinal activities, such as neuroprotective effects (Lee & Kim, 2001), antitumor activity (Lee, Cho, & Yoon, 2004), and nitric oxide inhibition (Jun et al., 1998). In a previous study, we reported the antioxidant and antidiabetic activities of *U. davidiana* extracts (Guo & Wang, 2007). In this study, we reports the evaluation of the antioxidant activity of the extracts and compounds that have been isolated from *U. davidiana*, examining their reducing power, their total phenolic content, and their potential to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and to inhibit the generation of hydroxyl radicals (•OH).

2. Materials and methods

2.1. Plant material

The stem bark of *Ulmus davidiana* Planch *var. japonica* Nakai (Ulmaceae) were purchased in April 2006, from the Herbal Medicine Co-operative Association Chuncheon Province, Kangwon-do, Korea.

2.2. Chemicals

L-Ascorbic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH•), 2-deoxy-D-ribose, ferrous chloride, 2N folin-ciocalteu's phenol reagent, iron (II) sulfate heptahydrate, tannic acid, α-tocopherol, trichloroacetic acid (TCA), 4, 6-dihydroxy-2-mercaptopyrimidine, 2-thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), and ethylenediaminetetraacetic acid (EDTA) disodium salt were purchased from Sigma Chemical Company (St. Louis, MO, USA). Hydrogen peroxide, gallic acid, and sodium carbonate were purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). Iron (III) chloride hexahydrate was purchased from Kanto Chemical Co., Ltd. (Osaka, Japan). All other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Preparation of plant extracts

The stem bark (1.5 kg) of *U. davidiana* was refluxed with MeOH for 3 h (10 L × 3 times). The total filtrate was concentrated and dried in vacuo at 40 °C to render the MeOH extract (228.12 g). The extract was then suspended in distilled water and sequentially partitioned with CH_2Cl_2 (16.26 g), EtOAc (75.00 g), *n*-BuOH (74.47 g), and H_2O (62.30 g). Each extract was tested for its antioxidant activity in the tested model systems, and the EtOAc fraction exhibited strong activity. Therefore, the EtOAc (25.35 g) fraction was column chromatographed on a Si gel column using CH_2Cl_2:MeOH = 10:1 – MeOH (gradient) to yield 6 (1–6, 7–12, 13–17, 18–24, 25–41, 42–60) subfractions. Fraction 2 (270 mg) was further column chromatographed on a Sephadex LH-20 column using MeOH to yielded compound 1 (320 mg). Fraction 3 (860 mg) was column chromatographed on a Sephadex LH-20 column with MeOH, which yielded compound 2 (170 mg). Optical rotation was obtained using a Perkin–Elmer 341 Polarimeter. UV spectra were recorded on a Varian Cary UV–visible spectrophotometer and FAB-MS data were obtained with Autospec. M363 series (Micromass, Euroscience, Manchester, UK) mass spectrometer. ^{1}H and ^{13}C NMR spectra were measured using a Bruker DPX 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) spectrometer. The chemical shifts were referenced to the respective residual solvent peaks (δ_H 2.50 and δ_C 39.5 for DMSO-d_6). The distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond connectivity (HMBC) spectra were recorded pulsed field gradients. Column chromatography was carried out using Si gel (BW-820MH (S), Fuji Silysia Chemical Ltd., Aichi, Japan), Sephadex LH-20 (25–100 μm, GE Healthcare Bio-Sciences, Uppsala, Sweden).

The thin layer chromatography (TLC) was performed on a precoated Merck Kieselgel 60 F_{254} plate (0.25 μm), with a mobile phase composed of CH_2Cl_2–MeOH–H_2O (5:1:0.1, v/v). 50% H_2SO_4 was used as spray reagent.

(–)-Catechin (1): yellowish amorphous powder; mp. 175–176 °C; $[\alpha]_D^{20}$-5.45° (c 0.011, MeOH); UV λ_{max}^{MeOH} nm (log ε): 280 (3.95); FAB-MS: *m*/*z* 290 $[M]^+$; ^{1}H NMR (DMSO-d_6): δ 9.18 (s, OH), 8.94 (s, OH) 8.87 (s, OH), 8.82 (s, OH), 6.72 (1H, d, *J* = 1.9 Hz, H-2′), 6.69 (1H, d, *J* = 8.1 Hz, H-5′), 6.59 (1H, dd, *J* = 1.9, 8.1 Hz, H-6′), 5.89 (1H, d, *J* = 2.3 Hz, H-6), 5.69 (1H, d, *J* = 2.3 Hz, H-8), 4.48 (1H, d, *J* = 7.4 Hz, H-2), 3,30 (1H, ddd, *J* = 5.2, 7.4, 12.9 Hz, H-3), 2.65 (1H, dd, *J* = 5.3, 16.0 Hz, H-4α), 2.35 (1H, dd, *J* = 8.0, 16.0 Hz, H-4β); ^{13}C NMR (DMSO-d_6): δ 156.8 (C-7), 156.5 (C-9), 155.7 (C-5), 145.2 (C-3′, 4′), 130.9 (C-1′), 118.8 (C-6′), 115.4 (C-5′), 114.9 (C-2′), 99.4 (C-10), 95.4 (C-6), 94.2 (C-8), 81.3 (C-2), 66.7 (C-3), 28.2 (C-4).

(−)-Catechin-7-*O*-β-D-apiofuranoside (2): yellowish amorphous powder; mp 171–174 °C; $[\alpha]_D^{20}$-9.09° (c 0.011, MeOH); UV λ_{max}^{MeOH} nm (log ε): 280 (3.74); FAB-MS: *m*/*z* 445 $[M + Na]^+$, 423 $[M + H]^+$; ^{1}H NMR (DMSO-d_6): δ 9.44 (s, OH), 8.87 (s, OH), 8.82 (s, OH), 6.72 (1H, d, *J* = 1.7 Hz, H-2′), 6.69 (1H, d, *J* = 8.1 Hz, H-5′), 6.59 (1H, dd, *J* = 1.7, 8.1 Hz, H-6′), 6.09 (1H, d, *J* = 2.2 Hz, H-8), 5.90 (1H, d, *J* = 2.2 Hz, H-6), 5.33 (1H, d, *J* = 3.9 Hz, H-1′), 4.55 (1H, d, *J* = 7.2 Hz, H-2), 4.03 (1H, dd, *J* = 3.8, 6.7 Hz, H-2″), 4.00, 3.68 (each 1H, d, *J* = 9.4 Hz, H-4″, 3,87 (1H, ddd, *J* = 5.2, 7.4, 12.9 Hz, H-3), 3.40, 3.33 (each, 1H, dd, *J* = 5.6, 11.2 Hz, H-5″), 2.65 (1H, dd, *J* = 5.2, 16.2 Hz, H-4α), 2.40 (1H, dd, *J* = 7.8, 16.2 Hz, H-4β); ^{13}C NMR (DMSO-d_6): δ 156.7 (C-7), 156.6 (C-5), 155.6 (C-9), 145.2 (C-3′, 4′), 130.8 (C-1′), 118.6 (C-6′), 115.5 (C-5′), 114.7 (C-2′), 107.3 (C-1″), 102.1

(C-10), 96.0 (C-8), 95.2 (C-6), 81.4 (C-2), 78.8 (C-3″), 76.3 (C-2″), 74.3 (C-4″), 66.4 (C-3), 62.6 (C-5″), 27.9 (C-4).

2.4. DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

The DPPH radical scavenging effect was evaluated according to Blois (1958) with a slight modification. One hundred sixty microliter of the test extracts and compounds in MeOH with different concentrations (1, 5, 10, 50, and 100 μg/ml) were added to a 40 μl DPPH methanol solution (1.5×10^{-4} M). After mixing gently and standing at room temperature for 30 min, the optical density was measured at 515 nm using a multiplate spectrophotometer (EL × 800TM, Bio-Tek, Vermont, USA). The antioxidant activity of each sample was expressed in terms of the IC_{50} (μg/ml of μM required to inhibit DPPH radical formation by 50%), which was calculated from the log-dose inhibition curve.

2.5. Hydroxyl radical scavenging assay (·OH assay)

Hydroxyl radical scavenging activity was carried out using the 2-deoxyribose oxidation assay according to Chung and Osawa (1998). The solution (0.2 ml) of $FeSO_4 \cdot 7H_2O$ (10 mM) and ethylenediaminetetraacetic acid (EDTA) (10 mM) was prepared in a screw-capped test tube, and 0.2 ml of a 2-deoxyribose solution (10 mM), the samples (extracts and compounds) solution and a sodium phosphate buffer (pH 7.4, 0.1 M) were added to give a total volume of 1.8 ml. Finally, 200 μl of H_2O_2 solution (10 mM) were added to this reaction mixture and incubated at 37 °C for 4 h. After incubation, 1 ml each of a trichloroacetic acid solution (2.8%) and thiobarbituric acid solution (1.0%) were added to the reaction mixture. The sample was boiled at 100 °C for 10 min, cooled in ice and its absorbance was measured with multiplate spectrophotometer (EL × 800TM, Bio-Tek, Vermont, USA) at 515 nm. The capability to scavenge hydroxyl radical was calculated by the following equation: scavenging effect (%) = [1 − (absorbance of sample at 515 nm/absorbance of control at 515 nm)] × 100%.

2.6. Reducing power assay

The reducing power of methanolic extract and its various soluble fractions of *U. davidiana* were determined according to the method of Elmastas, Isildak, Turkekul, and Temur (2007). Various concentrations of sample extract (10–500 μg/ml) in 0.1 ml of methyl alcohol were mixed with sodium phosphate buffer (0.25 ml 0.2 M, pH 6.8) and 0.25 ml of 1% potassium ferricyanide $[K_3Fe(CN)_6]$. The mixture was incubated at 50 °C for 20 min, then 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifugation for 10 min at 1220*g* (Centrifuge 5415 D, Eppendrof, Hamburg, Germany). The upper layer of solution (0.25 ml) was mixed with distilled water (0.25 ml) and $FeCl_3$ (50 μl, 0.1%), and the absorbance was measured with multiplate spectrophotometer (EL × 800TM, Bio-Tek, Vermont, USA) at 750 nm. A higher absorbance indicates a higher reductive capability.

2.7. Determination of total phenolics

The concentration of phenolics in the extracts was determined according to the method described by Jayaprakasha, Negi, Jena, and Rao (2007) with slight modification. The results were expressed as tannic acid and gallic acid equivalents. The *U. davidiana* extract and its fractions (2 mg), tannic acid (2 mg), and gallic acid (2 mg) were dissolved in a 1 ml of mixture of methanol:water (6:4 v/v). The MeOH extract and its various soluble fractions (100 μg) of *U. davidiana* and different concentrations (10–100 μg) of tannic acid and gallic acid in 0.1 ml were mixed with 0.5 ml of ten-fold diluted Folin–Ciocalteu reagent and 0.4 ml of 7.5% sodium carbonate solution. After standing for 30 min at ambient temperature, the absorbance was measured at 750 nm using multiplate spectrophotometer (EL × 800TM, Bio-Tek, Vermont, USA). The estimation of phenolics in the MeOH extract and fractions were calculated using standard graph (tannic acid and gallic acid).

2.8. Statistical analysis

The data is expressed as a mean ± standard error of three experiments.

3. Results and discussion

3.1. Bioactivity assay of U. davidiana MeOH extract and its soluble fractions

3.1.1. DPPH radical scavenging activity of U. davidiana MeOH extract and its soluble fractions

DPPH is a stable free radical that has widely been used as a substrate to evaluate the antioxidative activity of various samples (Blois, 1958; Jung et al., 2003). The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. In this study,

Table 1
Antioxidant activities of the *Ulmus davidiana* extract on DPPH and ·OH

Samples	DPPH (μg/ml)[a]	·OH[b]
MeOH	2.81 ± 0.01	57.12 ± 0.01
CH_2Cl_2	16.11 ± 0.00	57.72 ± 0.01
EtOAc	2.17 ± 0.01	58.56 ± 0.01
n-BuOH	1.95 ± 0.01	58.50 ± 0.01
H_2O	4.55 ± 0.02	56.92 ± 0.00
L-Ascorbic acid	1.86 ± 0.02	56.65 ± 0.02

Results are mean ± SD ($n = 3$).
[a] DPPH is the free radical scavenging activity (IC_{50}).
[b] ·OH is the inhibition percent of hydroxyl radical generation in 10 mM H_2O_2 and 10 mM $FeSO_4$ at the test concentration of 10 μg/ml. Data are Means ± SD of triplicates.

we investigated a *U. davidiana* MeOH extract and its solvent-partitioned fractions, including CH_2Cl_2-, EtOtAc-, *n*-BuOH-, and H_2O-soluble fractions, for general antioxidant effects, as indicated by their potential to scavenge stable DPPH radicals. As summarized in Table 1, the scavenging activity of the extracts on DPPH increased in the order of *n*-BuOH > EtOAc > MeOH > H_2O > CH_2Cl_2, with IC_{50} values of 1.95 ± 0.01, 2.17 ± 0.01, 2.81 ± 0.01, 4.55 ± 0.02, and 16.11 ± 0.00 μg/ml, respectively, indicating that the *n*-BuOH and EtOAc fractions of the MeOH extract have significant free radical scavenging abilities. These values are comparable to that of L-ascorbic acid (IC_{50} 1.86 ± 0.02 μg/ml). Although the DPPH radical-scavenging abilities of the MeOH extract and its fractions were significantly less than that of L-ascorbic acid, it was evident that the EtOAc and *n*-BuOH fractions have hydrogen-donating ability and could serve as free radical inhibitors or scavengers, possibly acting as primary antioxidants.

3.1.2. Hydroxyl radicals scavenging activity of U. davidiana MeOH extract and its soluble fraction

Both the MeOH extract of stem bark of *U. davidiana* and its soluble fractions had consistently more scavenging ability on hydroxyl radicals (·OH) than L-ascorbic acid (Table 1). The CH_2Cl_2-, EtOAc-, *n*-BuOH-, and H_2O-soluble fractions exhibited strong activity on ·OH at concentrations of 50 μg/ml, showing $57.72 \pm 0.01\%$, $58.56 \pm 0.01\%$, $58.50 \pm 0.01\%$, and $56.92 \pm 0.00\%$, inhibition, respectively. These activities are comparable to that ($56.65 \pm 0.02\%$) of L-ascorbic acid at 50 μg/ml, which was used as a positive control.

3.1.3. Reducing power of U. davidiana MeOH extract and its soluble fraction

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (Meir, Kanner, Akiri, & Philosoph-Hadas, 1995). In this assay, the yellow color of the test solution changes to green depending on the reducing power of test specimen. Fig. 1 presents the reductive capabilities of the methanolic extract of *U. davidiana* and its soluble fractions. The reducing powers of the extract and all of the fractions increased with increasing concentration. The reducing power of the *U. davidiana* MeOH extract, its soluble fractions, and standard compounds followed the order EtOAc > *n*-BuOH > MeOH > BHT > α-tocopherol > H_2O > CH_2Cl_2. The EtOAc and *n*-BuOH fractions exhibited strong reducing powers of 0.26 and 0.22 at 100 μg/ml, respectively. In comparison, the reducing power of α-tocopherol at 100 μg/ml was 0.19.

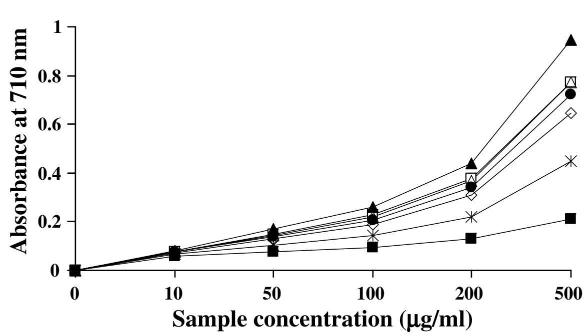


Fig. 1. Reducing powers of the *Ulmus davidiana* extract at different concentrations (□: methanol extract; ■: dichloromethane fraction; ▲: ethyl acetate fraction; △: *n*-buthanol fraction; ∗: water fraction; ●: 2,6-Di-*tert*-butyl-4-methylphenol; ○: α-tocopherol).

3.1.4. Total phenolic contents in U. davidiana MeOH extract and its soluble fraction

The total phenolic contents in *U. davidiana* extracts and its soluble fractions were determined and are presented in Fig. 2. The phenolic contents were calculated using tannic acid and gallic acid. Analysis of the phenolic content in all of the extracts using the Folin–Ciocalteu method revealed that the EtOAc fraction contained the maximum phenolic content (72.64 μg/ml) in terms of tannic acid equivalents, followed by the *n*-BuOH fraction (68.54 μg/ml), the MeOH extract (66.53 μg/ml), the H_2O fraction (23.54 μg/ml), and the CH_2Cl_2 fraction (21.25 μg/ml).

3.2. Identification of active compounds (1 and 2) and its antioxidant activity

The active EtOAc-soluble fraction was subjected to further chemical analysis and, after successive column chromatography, two active flavonoids (1 and 2) were isolated (Fig. 3). Compound 1, $[\alpha]_D^{20}$-5.45° (MeOH), was obtained as yellowish amorphous powder. The molecular formula of 1 was determined as $C_{15}H_{14}O_6$ based on the NMR and FAB-MS [M^+, m/z 290]. The characteristic ^1H NMR signals at δ 4.48 (1H, d, $J = 7.4$ Hz), 3.30 (1H, ddd, $J = 5.2$, 7.4, 12.9 Hz), 2.65 (1H, dd, $J = 5.3$, 16.0 Hz), and 2.35 (1H, dd, $J = 8.0$, 16.0 Hz) were indicative of H-2, H-3, H-4α, and H-4β, respectively, on the C-ring of a catechin moiety. In addition, the ^1H NMR spectrum indicated five aromatic protons including an AB spin system at δ 5.89 (1H, d, $J = 2.3$ Hz) and 5.69 (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. Thus, the structure of 1 was determined to be (−)-catechin (1). This was confirmed by a physicochemical and spectral data comparison with the published data

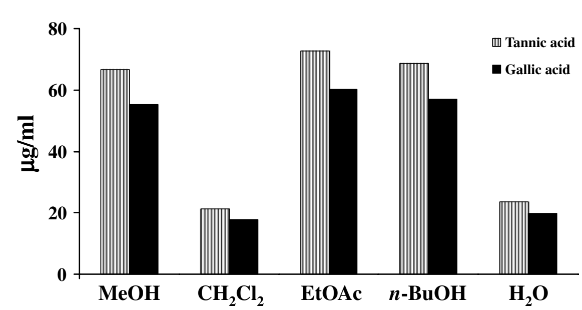
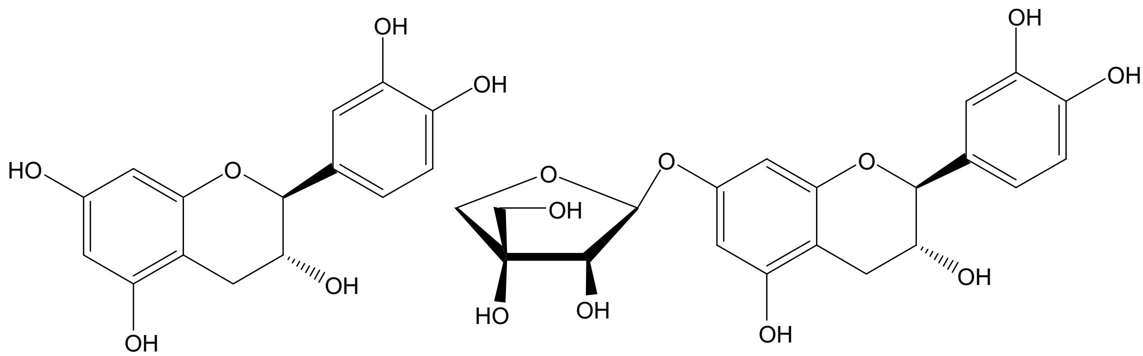


Fig. 2. Total phenolic content in the MeOH extract and its soluble fractions of *Ulmus davidiana*.



(1) (2)

Fig. 3. Isolated compounds 1 and 2. (1), (−)-Catechin; (2), (−)-Catechin-7-*O*-β-D-apiofuranoside.

(Na et al., 2002; Son et al., 1989). Compound 2, $[\alpha]_D^{20}$-9.09° (MeOH), had a molecular weight of 422, as identified by FAB-MS ($[M+H]^+$ at *m*/*z* 423). The ^1H and ^{13}C NMR spectra of compound 2 were similar to those of 1. The most apparent difference was in the sugar moiety. The ^1H NMR spectrum showed unique signals at δ 5.33 (1H, d, $J = 3.9$ Hz, H-1″), 4.03 (1H, dd, $J = 3.8$, 6.7 Hz, H-2″), 4.00, 3.68 (each 1H, d, $J = 9.4$ Hz, H-4″), 3.40, 3.33 (each, 1H, dd, $J = 5.6$, 11.2 Hz, H-5″). ^{13}C NMR signals were observed at δ 107.3 (C-1″), 78.8 (C-3″), 76.3 (C-2″), 74.3 (C-4″), 62.6 (C-5″), which were indicative of a D-apiofuranoside (Na et al., 2002). The linkage of this sugar at C-7 was established by an HMBC correlation. Thus, the structure of 2 was determined to be (−)-catechin-7-*O*-β-D-apiofuranoside (2), which was also verified by a comparison with the published physicochemical and spectral data (Hori, Satake, Saiki, Murakami, & Chen, 1988; Na et al., 2002; Park, Goo, & Na, 1996; Son et al., 1989). These two compounds were isolated from *U. davidiana* for the first time. The antioxidant activities of the two isolated compounds, 1 and 2, are shown (Table 2). Compounds 1 and 2 exhibited strong antioxidant activity on the DPPH radical with IC_{50} values of 6.37 ± 0.02 μM and 6.41 ± 0.03 μM, respectively. Their IC_{50} values were lower than the IC_{50} of 6.78 ± 0.00 μM for L-ascorbic acid. On ·OH, compounds 1 and 2 exhibited strong activity at a concentration of 10 μg/ml, with 53.65 ± 0.01% and 52.56 ± 0.01% inhibition, respectively. These values are comparable to that (50.55 ± 0.01%) of L-ascorbic acid at a concentration of 10 μg/ml, which was used as a positive control. Compounds 1 and 2 exhibited good activity in all of the tested model systems. The results suggest that the 3′,4′-ortho functional group on the B ring is the most important feature for the antioxidant activity. Flavonoids, hydroxycinnamates, and related phenolic acids have been reported to function as potent antioxidants by virtue of their hydrogen-donating properties (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). Apparently, the better ability of EtOAc fraction than other fractions might be due to more hydrogen-donating components extracted by EtOAc solvent.

Table 2
Antioxidant activities of the compounds (1 and 2) derived from *Ulmus davidiana* on DPPH and ·OH

Compounds	DPPH (μM)[a]	·OH[b]
(−)-Catechin (1)	6.37 ± 0.02	53.65 ± 0.01
(−)-Catechin-7-*O*-β-D-apiofuranoside(2)	6.41 ± 0.03	52.56 ± 0.01
L-Ascorbic acid	6.78 ± 0.00	50.55 ± 0.01

Results are mean + SD ($n = 3$).
[a] DPPH is the free radical scavenging activity (IC_{50}).
[b] ·OH is the inhibition percent of hydroxyl radical generation in 10 mM H_2O_2 and 10 mM $FeSO_4$ at the test concentration of 10 μg/ml. Data are Means + SD of triplicates.

Catechins can exist as two geometrical isomers, trans-catechins and *cis*-epicatechins, depending on the stereochemical configuration of the 3′,4′-dihydroxyphenyl and hydroxyl groups at the 2- and 3-positions of the C ring (Friedman et al., 2007). Each of the isomers exists as two optical isomers: (+)-catechin and (−)-catechin and (+)-epicatechin and (−)-epicatechin, respectively. (−)-Catechin can be modified by esterification with gallic acid to form (−)-catechin-3-gallate and epicatechin-3-gallate. Theaflavins are formed by the enzyme-catalyzed oxidative dimerization of catechins (Sang et al., 2004; Schwimmer, 1981; Shahidi & Naczk, 2004). Catechin was a well-know flavonoid, which has been reported to possess excellent inhibitory effect on DPPH and Reducing power (Abreu, Braham, Jannet, Mighri, & Matthew, 2007).

The results suggest that the methanol extract of *U. davidiana* and its various fractions, as well as its components, may be an alternative to more toxic synthetic antioxidants as additive in food, pharmaceutical and cosmetic preparations. A further investigation into using the antioxidant activities of these natural compounds to prevent various radical-mediated injuries in pathological situations in vivo is currently underway.

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