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Antioxidant Activities of Powdered and Ultra-fine Powdered *Ulmus Davidiana* var. *Japonica*

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초미세 느릅 분말의 항산화 활성 연구

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ABSTRACT

The antioxidant activity was examined on ultra-fine powdered (UFP) *Ulmus davidiana* (*U. davidiana*) var. *japonica*. The average particle size of UFP or commercial powdered (CP) was 1–3 μm or 100 μm . The extraction was performed using either ethanol (EtOH) or hot-water. Contents of phenolic compound of CP and UFP *U. davidiana* extracts in EtOH was 40.38 and 65.61 mg/g, respectively. In DPPH, EtOH extract of UFP *U. davidiana* showed a significantly greater activity than hot-water extract at 40 and 200 $\mu\text{g/mL}$. At 200 $\mu\text{g/mL}$, the activity was over 90% in all groups. The reducing power of UFP *U. davidiana* var. *japonica* in EtOH extraction was 74.3%, which was significantly greater than in other samples ($p < 0.05$). In addition, reducing powder was significantly higher in UFP-EtOH than in other samples at all concentrations except for 0.32 $\mu\text{g/mL}$. The above results suggest that EtOH extraction of *U. davidiana* showed slightly higher DPPH radical scavenging activity, and ultra-fine powder of *U. davidiana* extracts may show higher antioxidative activities based on reducing power.

Key words: ultra-fine *Ulmus davidiana* var. *japonica*, antioxidant activity, phenolic compounds

I. Introduction

Ulmus davidiana (*U. davidiana*) is a traditionally

used in Korean medicine with anticancer, antiviral, antibacterial, and anti-inflammatory properties (Song et al., 2007). It is widely cultivated in Asia

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including Korea and its bark of the stem and roots are used extensively (Jung et al. 2008). Dried and grounded inner bark into powder is used in soups or cereal flours as a thickening agent in food manufacturing (Jung et al. 2008). Catechin and related compounds were detected as phytochemicals of *U. davidiana* stem bark (Jung et al. 2008).

Several oxygen species with high reactivity (ROS), such as superoxide anion ($O^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$), are nearly related to incidence of Alzheimer's disease, aging, cancer, infection, and cardiovascular disease (Freeman 1984; Squadrito & Pryor 1998). The interest has been raised in searching antioxidant phytochemicals, because those compounds may hinder the process of free radical reactions (Kinsella et al. 1993). These components are regarded as effective free radical scavengers and inhibitor of lipid oxidation due to their metal chelating properties (Miller 1997). The most effective agent appears to be herbs, seeds, and fruits in most of plants.

An antioxidant is defined that delays or inhibits the oxidation process with low concentration (Halliwell 1995). The antioxidants such as ascorbic acid, carotenoids, flavonoids and tannins, are considered to play an important role in inhibiting induced disease by free radicals (Halliwell et al. 1992). In addition, an inverse relationship was found between antioxidant-rich food consumption and disease incidence rate (Rice-Evans et al. 1997; Kim et al. 2014).

In recent, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) as synthetic antioxidants are widely used in processed foods and there is possibility showing side effects (Ahn & Park 2013). However, potent antioxidant activities without any side effect were found in plant as medicinal property in recent studies. By

using these medicinal components could be established as natural medicines for prevention and treatment of related diseases (Jung et al. 2008; Choudhary & Swarnkar 2011).

Several previous studies have indicated that water and various solvent extracts of *U. davidiana* exhibited a high antioxidant property (Guo & Wang 2007; Jung et al. 2008; Ahn & Park 2010; Park 2011). Also, the change of several aspects such as physicochemical and microbial properties of *U. davidiana* powder was investigated during storage (Ahn 2014). However, there was no information on antioxidant activity of ultra-fine *U. davidiana* particles, even though ultra-fine sizing of various plants increase the functional value (Seo et al. 2011; Ahn et al. 2013; Ahn et al. 2014). Therefore, the present study was designed to study the antioxidant properties of ultra-fine powdered *U. davidiana* stem barks.

II. Materials and Methods

1. Materials

Commercial bark of stem powder of *Ulmus davidiana* (*U. davidiana*) var. *japonica*, which was dried under room temperature was purchased. Ultra-fine *U. davidiana* powder (UFP) was manufactured by the dry milling in Apexcel Co. (Pohang, Korea) and stored at room temperature. The average particle size of UFP was in the range of 1-3 μm and commercial powder (CP) was about 100 μm . All chemicals and reagents were purchased from Sigma Chemicals Co. (St. Louis, MO, USA).

2. Preparation of extracts

For ethanol (EtOH) extraction, three hundred grams of either CP or UFP were dissolved in 1,000

mL of EtOH after 24 hr soaking. Then, soluble collected EtOH fraction was concentrated to about 20 mL volume using a vacuum evaporator and kept in a refrigerator until use. For hot-water extraction, 300 g of either CP or UFP *U. davidiana* var. *japonica* were boiled in 1,000 mL for 2 hr, filtered and concentrated with freeze-dryer.

3. DPPH radical scavenging activity

The antioxidant activity was determined with its ability in stabilizing the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl). The mixture containing 40 μ L of extracts (4 mg/mL dissolved in dimethyl sulfoxide (DMSO)) and 760 μ L of 300 μ M DPPH solution was heated at 37°C for 30 min and the absorbance was determined at 515 nm. The DPPH ability was calculated and all determination was triplicated.

$$\begin{aligned} &\text{DPPH radical scavenging activity (\%)} \\ &= [(A_0 - A_1)/A_0] \times 100, \end{aligned}$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

4. ABTS radical scavenging activity

ABTS (2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenger ability was measured with modification (Re et al, 1999). Seven mM of ABTS reagent was mixed with 2.45 mM potassium persulfate and stood for 12-16 hr in dark. After ABTS radical was produced, it was diluted upto 0.70 absorbance value at 734 nm. The 40 μ L of sample (4 mg/mL dissolved in DMSO) and 760 μ L ABTS radical solution were mixed and incubated at room temperature for 10 min. At 734 nm, the absorbance was measured and the ABTS ability was calculated. All measurements were in

triplicate.

$$\begin{aligned} &\text{ABTS radical scavenging activity (\%)} \\ &= [(A_0 - A_1)/A_0] \times 100, \end{aligned}$$

where A_0 was the absorbance of the control and A_1 was the absorbance of sample.

5. Hydroxyl radical scavenging activity

Hydroxyl radical scavenger ability was measured with a few modification (Smirnov & Cumbes 1989). The mixture contained 250 μ L of FeSO_4 (1.5 mM), 175 μ L of hydrogen peroxide (6 mM) and 300 μ L of sodium salicylate (20 mM) and reacted for 30 min at 37°C. The absorbance was determined at 562 nm and activity was calculated. All determination was triplicated.

6. Reducing power

The reducing power was examined by the method of Oyaizu (1986). Different concentrations of 2.5 mL of each samples were reacted with 2.5 mL (200 mM) of sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. After 20 min incubation at 50°C, 2.5 mL of 10% trichloroacetic acid (w/v) were added. Then, the mixture was centrifuged at 650 rpm for 10 min, and the upper layer (5 mL) was collected. Five mL of deionized water and 1 mL of 0.1% of ferric chloride were added and the absorbance was measured at 700 nm.

7. Phenolic compound

One mg of the sample was mixed with 2 mL of Folin-Denis reagent and 2 mL of 35% sodium carbonate, mixed vigorously and filled up to 10 mL with distilled water. After 30 min incubation at ambient temperature, the absorbance was measured

at 765 nm, The contents of phenolic compound were determined through a standard curve prepared at different concentrations of tannic acid.

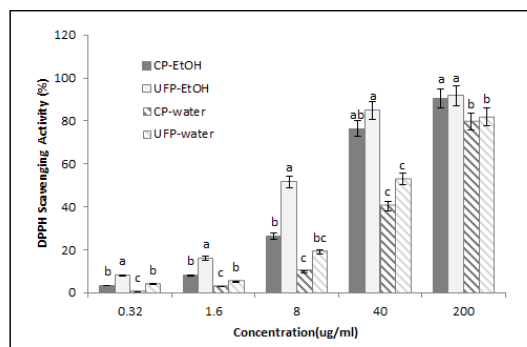
8. Statistical analysis

Statistical analysis system (version 9.0, SAS Institute Inc, Cary, NC, USA) was used. ANOVA was used to test significant differences among the samples, and means were performed by Duncan's multiple range test ($p < 0.05$).

III. Results and Discussion

1. Phenolic contents

The total phenolic contents of either CP or UFP *U. davidiana* var. *japonica* were shown in Table 1. The phenolic content of CP and UFP extraction samples in EtOH were 40,38 and 65,61 mg tannic acid equivalent/g, respectively. However, a lower content of phenolic compound was found in hot-water such as 39,68 mg tannic acid equivalent/g in



CP-EtOH; EtOH extract of commercial powder, UFP-EtOH; EtOH extract of ultra-fine powder, CP-water; hot-water extract of commercial powder, UFP-water; hot-water extract of ultra-fine powder. Values are the means \pm SD ($n=3$). Bars with different letters indicate significantly different values ($p < 0.05$).

Fig. 1. DPPH radical scavenging activities of commercial or ultra-fine *Ulmus davidiana* powder at different concentrations

CP and 45,62 mg tannic acid equivalent/g in UFP *U. davidiana* var. *japonica*.

Earlier study showed that phenolic compounds have been shown to possess a high antioxidant property (Guo & Wang 2007). Other previous study have also shown a close relationship between total phenolic content and an antioxidant capacity in some berry crops (Kalt et al, 1999).

Polyphenols belong to a several kinds of materials with a high antioxidant actions. Those compounds are generally known to stabilize oxygen-derived free radicals by contributing a hydrogen atom to the free radical (Guo & Wang 2007). In searching for sources of natural antioxidants, the radical scavenging activities of medicinal plants and fruits have been studied in the last few decades (Guo & Wang 2007).

2. DPPH radical scavenging activity

The antioxidant activity of DPPH is thought to be due to their hydrogen-donating ability. In this study, scavenging activity of EtOH or hot-water extract on DPPH was compared between CP and UFP *U. davidiana* var. *japonica* and shown in Fig. 1.

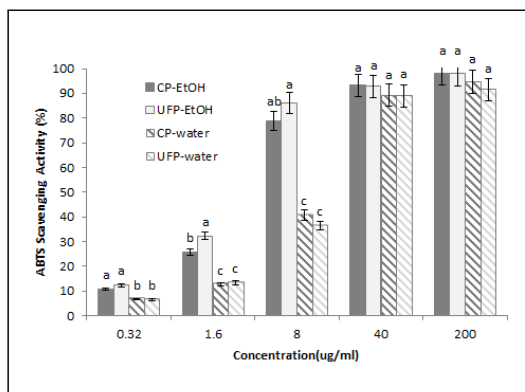
DPPH radical scavenging activity increased with increasing extract concentrations (Fig. 1). At every concentrations, UFP-EtOH showed the highest activity among other samples. Also, the DPPH

Table 1. Total phenolic contents in extracts of *Ulmus davidiana* stem bark

	mg tannic acid equivalent/g	
	Ethanol	Hot-water
Commercial powder	40,38 \pm 1,55 ^a	39,68 \pm 1,31 ^a
Ultra-fine powder	65,61 \pm 3,01 ^c	45,62 \pm 3,70 ^b

Values are the means \pm SD ($n=3$).

Different letters within the same column indicate significantly different values ($p < 0.05$).



CP-EtOH; EtOH extract of commercial powder, UFP-EtOH; EtOH extract of ultra-fine powder, CP-water; hot-water extract of commercial powder, UFP-water; hot-water extract of ultra-fine powder. Values are the means \pm SD (n=3). Bars with different letters indicate significantly different values ($p < 0.05$).

Fig. 2. ABTS radical scavenging activities of commercial or ultra-fine *Ulmus davidiana* powder at different concentrations.

scavenging activity was significantly higher at lower concentrations (0.32, 1.6, and 8 $\mu\text{g}/\text{mL}$) (Fig. 1). The activity reached up to 81.8 and 76.7% at 200 $\mu\text{g}/\text{mL}$ concentration in UFP-water and CP-water, which were significantly lower activities compared with those in EtOH extracts (90.4 and 91.8%). DPPH radical scavenging activity of water extract was decreased dramatically at 40 $\mu\text{g}/\text{mL}$ concentration.

Both CP-EtOH and UFP-EtOH showed a strong antioxidant activity (IC_{50} at 12.56 and 6.75 $\mu\text{g}/\text{mL}$), whereas IC_{50} which reflects 50% depletion of DPPH free radicals in CP-water and UFP-water were 70.05 and 31.64 $\mu\text{g}/\text{mL}$ (data not shown). These data were similar to those of other study, which evaluated the free radical scavenging activity of *U. davidiana* extracts with various solvents (Guo & Wang 2007). A high positive correlation has been found between total polyphenolic contents and DPPH radical scavenging

ability (Guo & Wang 2007).

Therefore, the present study indicated that EtOH extract showed the highest antioxidative activity compared with other samples, therefore, EtOH extract may act as free radical inhibitors or scavengers.

3. ABTS radical scavenging activity

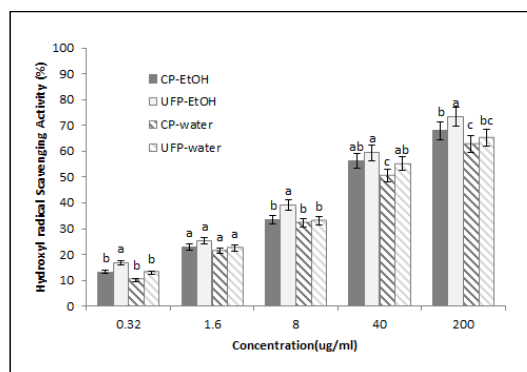
Scavenging activity of EtOH or hot-water extracts on ABTS was compared between CP and UFP *U. davidiana* var. *japonica* and shown in Fig. 2. There was no difference in samples at 40 and 200 $\mu\text{g}/\text{mL}$ concentrations ($p < 0.05$). At 200 $\mu\text{g}/\text{mL}$, scavenging activities were found in the range of 91.6 to 98.2%, and over 89% of scavenging activity was shown in all samples at 40 $\mu\text{g}/\text{mL}$ concentration. The IC_{50} values was 3.76 and 2.96 $\mu\text{g}/\text{mL}$ of CP-EtOH and UFP-EtOH (data not shown) and UFP and CP in hot-water extraction showed the similar scavenging activity with IC_{50} (8.62 and 9.48 $\mu\text{g}/\text{mL}$, respectively). However, extracts of hot-water showed the significantly lower activity than that of EtOH at 8 $\mu\text{g}/\text{mL}$ and lower concentrations ($p < 0.05$). These results indicated that both UFP and CP extracts of *U. davidiana* var. *japonica* showed a strong antioxidative activity on ABTS at high concentration, regardless of EtOH and hot-water extraction.

4. Hydroxyl radical scavenging activity

In hydroxyl radical scavenging assay, both activities of CP and UFP in EtOH extraction increased moderately up to 73.5 and 67.9% at 200 $\mu\text{g}/\text{mL}$ (Fig. 3). There was no dramatic difference between samples. Hot-water extracts showed slightly lower activities at 40 and 200 $\mu\text{g}/\text{mL}$ concentration than EtOH extracts. Also, not much difference was found between CP and UFP in

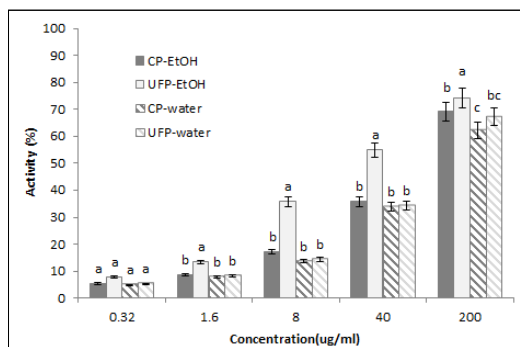
hydroxyl scavenging activity in both EtOH and hot-water extraction.

Studies of effective hydroxyl radical scavengers indicate that the activity is closely related to the phenolic contents (Guo & Wang 2007). Phenolic compounds are widely contained in both edible and inedible plants, and those have been known to show various functional effects (Guo & Wang 2007). These antioxidant activity is mostly due to their redox properties, which can be an aspects in absorbing free radicals (Siriwardhana et al, 2003). Therefore, a slightly higher activity found in EtOH extracts of UFP is likely due to higher contents of phenolic compound in samples.



CP-EtOH; EtOH extract of commercial powder, UFP-EtOH; EtOH extract of ultra-fine powder, CP-water; hot-water extract of commercial powder, UFP-water; hot-water extract of ultra-fine powder. Values are the means \pm SD (n=3). Bars with different letters indicate significantly different values ($p < 0.05$).

Fig. 3. Hydroxyl radical scavenging activities of commercial or ultra-fine *Ulmus davidiana* powder at different concentrations



CP-EtOH; EtOH extract of commercial powder, UFP-EtOH; EtOH extract of ultra-fine powder, CP-water; hot-water extract of commercial powder, UFP-water; hot-water extract of ultra-fine powder. Values are means \pm SD (n=3). Bars with different letters indicate significantly different values ($p < 0.05$).

Fig. 4. Reducing power of commercial *Ulmus davidiana* powder at different concentrations

5. Reducing power

The reducing power may be a significant indicator of its potential antioxidant ability (Jung et al, 2008). Fig. 4 presents the reductive capabilities of both CP and UFP *U. davidiana* var. *japonica* on EtOH and hot-water. The significantly high reducing power was shown in UFP-EtOH group, compared with others at every concentrations except for 0.32 $\mu\text{g/mL}$ ($p < 0.05$).

Reducing power is closely related to the reductones, which have been known as an antioxidant agent by giving a hydrogen atom (Duh 1998). In our study, EtOH extracts of UFP showed a higher reducing activity, which suggests that there may be some reductones contained in UFP *U. davidiana* var. *japonica*.

In present, BHA and BHT, the synthetic antioxidants, are commonly used to reduce lipid peroxidation and to prolong the shelf-life of food products. Recent studies have been focused on natural compounds as a natural antioxidants derived from medicinal plants. In consequence, it

is widely accepted that some of synthetic antioxidants need to be replaced because of their potential health risk (Li et al, 2008).

Several previous studies have indicated that *U. davidiana* extracts of various solvents showed a strong antioxidant activity (Guo & Wang 2007; Jung et al, 2008; Ahn & Park 2010; Park 2011). However, no study has been done to evaluate the effect of ultra-fine size for extraction process. The results of the various inhibitor assays revealed that EtOH extract of *U. davidiana* var. *japonica* could be considered as an effective additive in food and pharmaceutical agent.

IV. Summary and Conclusion

The present study was designed to compare the antioxidant activities between commercially powdered (CP) and ultra-fine powdered (UFP) *Ulmus davidiana* (*U. davidiana*) var. *japonica*. The extraction was performed using either ethanol (EtOH) or hot-water. The phenolic content of CP and UFP *U. davidiana* extracts in EtOH were 40.38 and 65.61 mg tannic acid equivalent/g. In DPPH, EtOH extracts showed a significantly higher activity than hot-water extracts at all concentrations ($p < 0.05$). The reducing power of UFP *U. davidiana* var. *japonica* in EtOH extraction exhibited 74.3%, which was the significantly higher value than other samples ($p < 0.05$). In addition, reducing powder was significantly higher in UFP-EtOH than those of others at every concentrations except for 0.32 μ g/mL ($p < 0.05$).

In present, synthetic antioxidants are commonly used in food manufacturing for prevention of oil oxidation and extension of food shelf-life, while some synthetic antioxidants may possess potential health risks. The present data indicated that

ultra-fine powder of *U. davidiana* extracts show higher antioxidative activities such DPPH radical scavenging activity and reducing power, therefore, could be used as potential source of antioxidant materials. In the health view point, *U. davidiana* var. *japonica* powder could be considered as an effective additive in food and pharmaceutical agent.

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