

Antioxidant Activities of Solvent Fractions from Root of *Ulmus davidiana*

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Abstract. The antioxidant potentials of various solvent fractions from root of *Ulmus davidiana* were evaluated using the following assays: DPPH radical scavenging, superoxide anion radical scavenging, ABTS radical scavenging, superoxide dismutase activity, and lipid peroxidation inhibition. The ethyl acetate fraction of *U. davidiana* showed significant effects in all the antioxidant assays, and contained high levels of total phenolics and flavonoids. Among the other solvent fractions, the *n*-butanol fraction exhibited significant activity, presenting the highest activities for superoxide anion and ABTS radical scavenging. These activities were superior to those of commercial synthetic and other natural antioxidants that were tested. Overall, the ethyl acetate and *n*-butanol fractions of *U. davidiana* were most effective.

Keywords: *Ulmus davidiana*, free radical, antioxidant activity, phenolics, flavonoids.

1. Introduction

Free radicals produced by radiation, chemical reactions and several redox reactions of various compounds may contribute to protein oxidation, DNA damage and lipid peroxidation in living tissues and cells [1]. Many studies have shown that natural antioxidants from various aromatic and medicinal plants are linked to reductions in chronic diseases caused by DNA damage, mutagenesis, and carcinogenesis [2]. It is recognized that naturally occurring substances in higher plants have antioxidant activities. Therefore, there is growing interest for research on alternative active antioxidant compounds, including plant extracts, which are relatively less damaging to human health and the environment [3].

Ulmus davidiana is a deciduous tree widely distributed in Korea. The root and stem bark are used in traditional oriental medicine to treat edema, mastitis, gastric cancer, and inflammation. *U. davidiana* is also known for maintaining or assisting blood circulation. *U. davidiana* water extract was developed based on the herb's known functions, as described in the literature of traditional Korean medicine [4]. Recently, we demonstrated that *U. davidiana* modulated the expression of inducible inflammatory cytokines in mice, and had therapeutic effects on collagen-induced arthritis when DBA/1 mice were immunized with bovine type II collagen. [5]. In addition, it was reported that *U. davidiana* has strong antioxidative activity on lipid peroxidation as well as an inhibitory effect on endogenous NO- induced apoptotic cell death. [6]. Although of *U. davidiana* is used to treat inflammatory diseases, the mechanism by which it functions is not well understood. There are some scientific reports on the biologically active compounds in *U. davidiana* as well as their biological actions. For example, catechin and the catechin glycoside, uldavioside, lignan xylosides, neolignan glycosides have been isolated from *U. davidiana*. Additionally, Guo and Wang [7] reported that *U. davidiana* extracts demonstrated strong antioxidant activities in assays for DPPH radical scavenging,

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hydroxyl radical scavenging, lipid peroxidation inhibition, and reducing power. In this study, the in vitro antioxidant activities of *U. davidiana* solvent fractions were investigated, with an objective to develop a natural antioxidant from the fractions. The antioxidant activities were evaluated using assays for DPPH radical scavenging, superoxide anion radical scavenging, ABTS radical scavenging, superoxide dismutase activity, and lipid peroxidation inhibition.

2. Materials and Methods

2.1. Extraction and Fraction Procedures

U. davidiana root (Jungsun, Kangwon-do, Korea) was purchased from an oriental herb market in Seoul, Korea. Dried *U. davidiana* (100 g) were extracted by stirring with 500 ml of 70% ethanol at 80 °C for 3 h two times, and then filtered through Whatman No. 2 filter paper. The solvent of the combined extracts was evaporated under reduced pressure using a rotary vacuum-evaporator at 50 °C and the remaining water was removed by freeze-dried. The ethanol extract of *U. davidiana* was suspended in distilled water and extracted successively with equal volumes of *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol. Each fraction was extracted three times, and the fractions from each step were removed by rotary evaporation and then freeze-dried.

2.2. Measurement of Total Phenolics and Flavaonoids

Total phenolic content was determined using Folin-Ciocalteu's phenol reagent [8]. The fractions (200 µl) were mixed with 400 µl of Folin-Ciocalteu's phenol reagent and 0.8 µl of 10% sodium carbonate. The mixtures were shaken thoroughly and allowed to stand for 1 h. Then, the absorbance at 750 nm was measured. The phenolic contents were determined using a standard curve obtained from various concentrations of gallic acid (GAE). Total flavonoid content was determined using the David deformed method [9]. The fractions (1.0 ml) were mixed with 10 ml of diethylen glycol and 0.1 ml of 1 N NaOH. The mixtures were shaken thoroughly and allowed to stand for 1 h at 37 °C. Then, the absorbance at 420 nm was measured. The flavonoid contents were determined using a standard curve obtained from various concentration of rutin (RE).

2.3. DPPH Radical Scavenging Assay

Each fraction in ethanol (4 ml) was mixed with 1 ml of ethanolic solution containing DPPH radical, resulting in a final concentration of 0.15 mM DPPH. The mixtures were shaken vigorously and left to stand in the dark for 30 min. Absorbance was measured at 517 nm against a blank.

2.4. Superoxide Anion radical Scavenging Assay

The superoxide anion radical scavenging assay of the fractions was measured according to the method of Robak and Gryglewski [10]. One milliliter aliquots (prepared in 0.1 M Tris HCl buffer, pH 7.4) of 150 µM nitroblue tetrazolium, 60 µM phenazine methosulphate, and 468 µM nicotinamide adenine dinucleotide were added to 1 ml of sample containing the various fractions.

2.5. ABTS Radical Scavenging Assay

The total antioxidant activity of the fractions was measured by the ABTS radical cation decolorization assay [11]. ABTS was dissolved in water to a 7 mM concentration, and ABTS radical cations (ABTS.⁺) were produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. After the mixture had remained in the dark at room temperature for 16 h to allow the completion of radical generation, it was diluted with ethanol (99.5 %) so that its absorbance was adjusted to 0.70 ± 0.02 at 734 nm. To determine the scavenging activity, 0.9 ml of ABTS reagent was mixed with 0.1 ml of sample and the absorbance was measured at 734 nm after 6 min of reaction time at room temperature, using ethanol as a control. The antioxidant activities of the fractions were compared to the Trolox equivalent antioxidant capacity in 1.0 g of sample.

2.6. Superoxide Dismutase Activity

Superoxide dismutase activity was determined according to the method of Kim et al. [12]. Each fraction (1-10 mg/ml) in water (0.2 ml) was mixed with 3 ml of 50 mM Tris-HCl buffer (pH 8.5) and 0.2 ml of 7.2 mM pyrogallo, and incubated at 25 °C for 10 min. The reaction was stopped by adding 1 ml of 1 N HCl. The absorbance was determined at 420 nm against a blank.

2.7. Determination of Lipid Peroxidation in the Liver Homogenates

Mitochondria were isolated from the livers of male Sprague-Dawley rats (300±20 g b.w). Rats (n=5) were killed by decapitation and their livers were removed and washed with isolation medium (ice cold 0.25 M sucrose containing 10 mM Tris-HCl, pH 7.4). Ten percent liver homogenates were then prepared by differential centrifugation. During the experiment, the mitochondrial fractions were appropriately diluted with phosphate buffer (pH 7.4). Lipid peroxidation was determined by TBARS after exposing the mitochondrial samples to H₂O₂, in both the presence and absence of fractions. The reaction was initiated by adding H₂O₂ to reaction mixture containing 0.5 ml of mitochondrial sample (10 mg/protein/ml), in a total volume of 1.5 ml. After incubation at 37 °C for 1 h, the tubes were shaken vigorously for 2 min. The reaction was stopped with TCA and the color was developed with TBA (0.375% w/v). The tubes were placed in a boiling water bath for 30 min. The samples were then allowed to reach room temperature and 3 ml of n-butanol was added. After vigorous shaking, absorbance was read at 532 nm [13].

3. Results and Discussions

3.1. Total Phenolic and Flavonoid Contents

Total phenolic contents of the *U. davidiana* fractions, which varied significantly, are presented in Table 1. The ethyl acetate fraction had the highest phenolic content (213.60 mg/g), as determined by the Folin-Denis method. The content of the ethyl acetate fraction was followed by that of the other fractions as follows: n-butanol > water > dichloromethane > n-hexane. Total flavonoid contents of the *U. davidiana* fractions were determined using the David deformed method in Table 1. The flavonoid contents of the n-hexane, dichloromethane, ethyl acetate, n-butanol, and water fractions were 36.94, 44.05, 107.84, 84.48, and 82.42 mg/g, respectively.

Table 1. Comparative total phenolic and flavonoid contents of *U. davidiana* fractions

Samples	GAE (mg/g) ¹⁾	RE (mg/g) ¹⁾
n-Hexane	105.27 ± 8.74 ^a	36.94 ± 2.62 ^a
Dichloromethane	120.97 ± 2.21 ^b	44.05 ± 1.24 ^b
Ethyl acetate	213.60 ± 7.77 ^d	107.84 ± 3.91 ^d
n-Butanol	187.93 ± 7.53 ^c	84.48 ± 1.43 ^c
Water	139.98 ± 75.99 ^c	82.42 ± 3.49 ^c

¹⁾ The total phenolic and flavonoid contents are expressed as gallic acid equivalents (GAE) and rutin equivalents (RE), mg/g of dry weight for the samples, respectively. ^{a-d} Values within a column followed by different letters are significantly different (p < 0.001).

3.2. DPPH Radical Scavenging Activity

The IC₅₀ values of the DPPH radical scavenging activity are shown in Table 2. The ethyl acetate fraction was found to have the highest DPPH radical scavenging activity, with an IC₅₀ of 2.59 µg/ml. The IC₅₀ values of the n-hexane, dichloromethane, n-butanol, and water fractions were 7.37, 74.12, 7.55, and 33.34 µg/ml, respectively, which were significantly lower (p < 0.001) than that of L-ascorbic acid (IC₅₀ 2.44 µg/ml).

Table 2. IC₅₀ values for radical scavenging and antioxidant activities of *U. davidiana* fractions

Antioxidant activities	Fractions	IC ₅₀ (µg/ml)
DPPH radical scavenging	n-Hexane	7.37 ± 0.08 ^b
	Dichloromethane	74.12 ± 5.30 ^d

	Ethyl acetate	2.59 ± 0.01 ^a
	<i>n</i> -Butanol	7.55 ± 0.22 ^b
	Water	33.34 ± 1.11 ^c
	L-ascorbic acid	2.44 ± 0.04 ^a
	<i>n</i> -Hexane	1966.72 ± 114.77 ^d
	Dichloromethane	1580.71 ± 60.43 ^c
Superoxide anion radical scavenging	Ethyl acetate	90.65 ± 3.58 ^a
	<i>n</i> -Butanol	80.67 ± 0.70 ^a
	Water	198.55 ± 3.21 ^b
	Tannic acid ^e	49.45 ± 1.10 ^a
	<i>n</i> -Hexane	3761.48 ± 256.25 ^e
	Dichloromethane	9497.47 ± 228.07 ^d
	Ethyl acetate	9315.40 ± 203.04 ^d
Superoxide dismutase activity	<i>n</i> -Butanol	11869.50 ± 152.97 ^e
	Water	23794.17 ± 677.50 ^f
	BHT ^e	1666.65 ± 33.80 ^b
	L-ascorbic acid	1134.10 ± 17.38 ^a
	<i>n</i> -Hexane	857.81 ± 88.55 ^c
	Dichloromethane	782.50 ± 48.00 ^c
Inhibitory effect on lipid peroxidation in a rat liver	Ethyl acetate	481.31 ± 13.14 ^a
	<i>n</i> -Butanol	524.56 ± 24.34 ^a
	Water	813.32 ± 47.41 ^c
	α-Tocopherol	691.23 ± 13.52 ^b

^{a-f} Values within a column followed by different letters are significant different ($p < 0.001$).

3.3 Superoxide Anion Radical Scavenging Assay

The superoxide anion radical scavenging activities of each of the fractions increased markedly as the concentration increased (Table 2). However, although the *n*-butanol fraction had the highest superoxide anion scavenging activity ($IC_{50} = 80.67 \mu\text{g/ml}$), its activity was less than that of tannic acid ($IC_{50} = 49.45 \mu\text{g/ml}$). Finally, the ethyl acetate, water, dichloromethane, and *n*-hexane fractions were found to have IC_{50} values of 90.65, 198.55, 1580.71, and 1966.72 $\mu\text{g/ml}$, respectively.

3.4. ABTS Radical Scavenging Activity

The antioxidant activities of the *U. davidiana* fractions based on the Trolox equivalent antioxidant capacity (TEAC) were as follows: the TEAC of the *n*-butanol fraction was highest, at 43079.98 mmol kg^{-1} , whereas those of the *n*-hexane, dichloromethane, ethyl acetate, and water fractions were 5148.58, 172.64, 4060.80, and 1687.55 mmol kg^{-1} , respectively (Table 3).

Table 3. ABTS radical scavenging activity of *U. davidiana* fractions using TEAC value

Fractions	TEAC (mmol kg^{-1}) ^f
<i>n</i> -Hexane	5148.58 ± 361.57 ^b
Dichloromethane	172.64 ± 0.78 ^a
Ethyl acetate	4060.80 ± 651.31 ^b

<i>n</i> -Butanol	43079.98 ± 2710.59 ^c
Water	1687.55 ± 8.57 ^a

¹⁾ TEAC: millimolar concentration of Trolox solution having an ABTS radical scavenging activity equivalent to solutions of *U. davidiana* fractions. ^{a-c} Values within a column followed by different letters are significantly different ($p < 0.001$).

3.5. Superoxide Dismutase Activity

As shown in Table 2, the *U. davidiana* fractions reacted directly with and quenched superoxide to different degrees, with increasing activities at higher concentrations. The IC₅₀ values of the *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol, and water fractions were 3761.48 µg/ml, 9497.47 µg/ml, 9315.40 µg/ml, 11869.50 µg/ml, and 23794.17 µg/ml, respectively.

3.6. Determination of Lipid Peroxidation in the Liver Homogenates

The TBARS formation inhibitory effect of the organic fractions of ethyl acetate and *n*-butanol were significantly higher than that of α -tocopherol, and the inhibition effect was dose dependent (Table 2). The IC₅₀ values of the *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol and water fractions, as well as α -tocopherol, were 857.81, 782.50, 481.31, 524.56, 813.32, and 691.23 µg/ml.

4. Conclusion

In summary, the antioxidant activities of *U. davidiana* fractions were evaluated with various antioxidant assays. All fractions showed strong antioxidant activities. Overall, the ethyl acetate and *n*-butanol fractions showed higher scavenging activities than the other *U. davidiana* fractions. The results suggest that the ethyl acetate and *n*-butanol fractions of *U. davidiana* could offer health benefits and be applied in foods.

5. References

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