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ARTICLE

Influence of extraction conditions on antioxidant activities and catechin content from bark of *Ulmus pumila* L.

MyoungLae Cho[1] · Seok-Beom Ko[1] · Jae-Min Kim[1] · Ok-Hwan Lee[1] · Dae-Won Lee[2] · Jong-Yea Kim[1]

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Abstract This study investigated the influence of extraction conditions on antioxidant activities and catechin content from bark of *Ulmus pumila*. The *U. pumila* was extracted using various concentrations of ethanol (30, 50, 80, and 99 %) and different extraction times (1, 2, and 3 h) at 60 °C. The ethanol extracts were evaluated for their antioxidant activities [1,1-diphenyl-1-picrylhydrazyl, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activities and reducing power], total phenolic (TP), and proanthocyanidin (TA) contents. In addition, the antioxidant activities were correlated with TP and TA contents, and the catechin content was determined using high-performance liquid chromatography. The 99 % EtOH for 3-h extracts exhibited the highest antioxidant abilities with the highest phenolic and proanthocyanidin contents. TP content showed little correlation ($R^2 = 0.31$–0.61) with their antioxidant activities; however, a significant correlation ($R^2 = 0.65$–0.91) was observed with proanthocyanidin content. The catechin content of the 99 % EtOH for 3-h extracts was greater than that of other EtOH extracts. Therefore, these results indicate that the antioxidant capacity of EtOH extracts from bark of *U. pumila* may be due to catechin, and the optimal extraction condition was 99 % EtOH for 3 h.

Keywords Antioxidant activity · Catechin · Optimal extraction condition · Proanthocyanidin · *Ulmus pumila*

✉ Jong-Yea Kim
jongkim@kangwon.ac.kr

[1] Department of Food Science and Biotechnology, Kangwon National University, Chuncheon 24341, Republic of Korea

[2] Department of Jeongseon Yaccho, Jeongseon 26125, Republic of Korea

Introduction

Natural antioxidants from traditional plants have attracted attention in the last several decades because of their low side effects and strong bioactivities (Rates 2001; Chen et al. 2010; Ahn et al. 2013; Kim et al. 2015a). Reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and superoxide radicals, are natural byproducts of normal metabolism in living organisms (Aruoma and Cuppette 1997; Cavas and Yurdakoc 2005). However, excessive amounts of ROS produced by cellular metabolism may attack membrane lipids, proteins, and DNA. This can result in significant damage to these structures, which contributes to various diseases such as cancer, diabetes, cardiovascular disease, and degenerative processes associated with aging (Ames 1983; Stadtman 1992; Wiseman and Halliwell 1996; Mau et al. 2002). Therefore, commercial antioxidants have been in high demand, and most of them are synthesized, including butylated hydroxyanisole (BHA), butylated hydroxytoluene, and propyl gallate. However, these synthetic antioxidants were found to be toxic and carcinogenic in animal models (Ito et al. 1986; Safer 1999). Thus, the safety problems related to synthetic antioxidants have led to an increased interest in the development of safe and inexpensive supplements of natural origin. Many natural antioxidants have been found in various land plants such as cereals, vegetables, fruits, and herbal plants in which tocopherol, vitamin C, carotenoid, and flavonoid are good sources of antioxidants (Larson 1988). These plant polyphenols are known to possess strong antioxidant activity and have the ability to scavenge ROS and free radicals. Moreover, several studies have reported a positive correlation between the increased dietary intake of natural

antioxidants with a reduction in coronary heart disease, reduced cancer mortality, and longer life expectancy (Halliwell 2007; Rios et al. 2009).

The *Ulmus* species are naturally growing in countries in North-East Asia such as Korea, Japan, and China, and have been used in traditional medicine to treat cancer, inflammation, and rheumatoid arthritis (Wang et al. 2004; Kim et al. 2005). It was reported that the 80 % EtOH extracts from the root bark of *U. pumila* showed strong antioxidant abilities, such as 1,1-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activities and reducing power (RP; Kim et al. 2015a). In addition, the major antioxidant compounds from *U. davidiana* were identified as (−)-catechin and (−)-catechin-7-*O*-β-D-apiofuranoside (Jung et al. 2008). Moreover, the triterpenes isolated from methanol extracts of *U. pumila* exhibited anti-obesity activities (Ghosh et al. 2012), and the 80 % EtOH extracts of *U. pumila* facilitated the inhibition of HepG2 cell growth (Jeong and Kim 2012). It has been demonstrated that the solvents and conditions used to prepare the *Angelica gigas* extracts affected antioxidant and ACE inhibitory activities (Noh et al. 2014). Furthermore, the yields and antioxidant activities of the *Acanthopanax senticosus* extracts are strongly dependent on the extraction solvent and conditions (Lee et al. 2011). In addition, the EtOH/water mixture proved to be a more efficient extraction condition than other mono-component solvent systems (Yilmaz and Toledo 2006). Therefore, it is necessary to investigate the optimal extraction conditions for preparing EtOH *U. pumila* extracts. Thus, the first objective of this study was to investigate the effect of solvents on the extraction from the bark of *U. pumila* using a binary solvent of EtOH and water at various concentrations (30, 50, 80, and 99 % EtOH) at 60 °C for 1, 2, and 3 h. Furthermore, the second objective of the study was to determine antioxidant abilities using DPPH radical scavenging activity, ABTS radical scavenging activity, RP, and oxygen radical absorbing capacity (ORAC). In addition, the antioxidant activities were correlated with antioxidant compounds, including total phenolic (TP) and proanthocyanidin (TA) contents, as well as catechin content, which were identified using high-performance liquid chromatography (HPLC).

Materials and methods

Sample collection and materials

The bark of *U. pumila* L. was obtained from a local market in Jeongseon, Gangwon province, Republic of Korea. The sample was air-dried at 50 °C for 24 h, and milled using a blender, passed through a sieve (<1.0 mm), and then stored at −20 °C until use. Gallic acid, caffeic acid, catechin, chlorogenic acid, gallic acid, vanillic acid, Trolox, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), ABTS, DPPH, trichloroacetic acid (TCA), vanillin, potassium ferricyanide, potassium persulfate, ferric chloride ($FeCl_3$), Folin–Ciocalteu reagent, ascorbic acid (VC), and BHA were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and other reagents used in this work were of analytical grade.

EtOH extracts from the bark of *U. pumila*

The milled samples (5 g) were prepared with 500 mL of aqueous ethanol (30, 50, 80, and 99 %) and were extracted using a water bath at 60 °C for 1, 2, and 3 h. The precipitate was isolated by filtering the solution with a Whatman filter paper no. 40 (0.8 μm pore size; GE Healthcare Life Sciences, Buckinghamshire, UK), and the EtOH extracts were obtained by concentrating the supernatant using a rotary evaporator and a vacuum drier at 50 °C and then lyophilized. The EtOH extracts were stored in the dark at −20 °C before analysis.

Determination of total phenolic and proanthocyanidin contents

TP content was determined using the Folin–Ciocalteu assay (Cho et al. 2011). The sample solution (500 μL) was mixed with the Folin–Ciocalteu reagent (250 μL, 1.0 M) and 12.5 % (w/v) sodium carbonate (1.25 mL). After 40 min at RT, absorbance was measured at 750 nm against the control (500 μL of distilled water plus 1.5 mL of reagent mixture). Gallic acid (0.01–0.2 mg/mL) was used to produce a calibration curve, and the results were expressed as mg of gallic acid equivalent (GAE)/g sample.

Proanthocyanidin content was determined using the vanillin–hydrochloric acid method (Mitsunaga et al. 1998). The samples (0.5 mg) were dissolved with methanol (5 mL), and 4 % (w/v) vanillin solution (3 mL) was then added. After vigorous stirring, concentrated hydrochloric acid (1.5 mL) was added to the mixture and incubated for 15 min at RT. Then, the absorbance was measured at 490 nm using a microplate reader. Catechin (0.06–1.0 mg/mL) was used to produce a calibration curve, and the results were expressed as mg of catechin equivalent (CE)/g sample.

DPPH radical scavenging activity assay

The DPPH radical scavenging assay was performed according to the method of Cho et al. (2011). The samples (100 μL) were mixed with 0.2 mM DPPH (100 μL) followed by incubation for 30 min at RT. Absorbance at 515 nm was measured using a microplate reader

(Spectramax GEMINI EM, Molecular Devices, Sunnyvale, CA, USA). The blank was similarly prepared, except the distilled water was used instead of the sample. The DPPH radical scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging activity } (\%) = [(A_c - A_s)/A_c] \times 100,$$

where A_c is the absorbance of the control (100 μL of ethanol with 100 μL of the DPPH solution) and A_s is the absorbance of the sample. VC and BHA were used as positive controls.

ABTS radical scavenging activity assay

The ABTS radical scavenging assay was modified according to the method of Re et al. (1999). ABTS stock solution was dissolved in distilled water to 7 mM ABTS with 2.45 mM potassium persulfate, and the following mixture remained in the dark at RT for 12–16 h. The ABTS solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. An aliquot of each sample (50 μL) was mixed with 1.5 mL of ABTS solution (150 μL) followed by incubation at RT for 20 min. Absorbance at 734 nm was measured using a microplate reader. The ABTS radical scavenging effect was calculated using the following equation:

$$\text{ABTS scavenging activity } (\%) = [(A_c - A_s)/A_c] \times 100,$$

where A_c is the absorbance of the control (50 μL of ethanol with 150 μL of the ABTS solution) and A_s is the absorbance of the sample. VC and BHA were used as positive controls.

Reducing power

The RP of the samples was determined according to the method of Cho et al. (2011). An aliquot of each sample (500 μL) was mixed with a 200 mM sodium phosphate buffer (pH 6.6, 500 μL) and 1 % potassium ferricyanide (500 μL), followed by incubation in a water bath at 50 °C for 20 min. Following incubation, 10 % (w/v) TCA (500 μL) was added to the reaction mixture and centrifuged at 12,000 rpm for 10 min. The supernatant (1.0 mL) was mixed with distilled water (1.0 mL) and 0.1 % ferric chloride (200 μL), and reacted for 10 min. The absorbance at 700 nm was measured using a microplate reader. VC with a 50 μg/mL concentration was used as a positive control.

Oxygen radical absorbance capacity (ORAC)

The ORAC assay was performed using a modified method of Ou et al. (2001) using AAPH as a source of peroxyl radicals and fluorescein as an oxidizable probe. The sample was diluted with a phosphate buffer (75 mM, pH 7.4), and 25 μL of either the Trolox (0–10 μM) or the sample was transferred to a black-walled 96-well microplate containing 150 μL of 40 nM fluorescein and 25 μL of 18 mM AAPH. The plate was immediately carried to the fluorescence microplate reader, and the fluorescence (485 nm Ex/520 nm Em) was monitored continuously every 3 min for 90 min at 37 °C. The ORAC value results were calculated using the Trolox calibration curve and the area under the fluorescence decay curve. The ORAC value was expressed as Trolox equivalents in micromoles per milliliter. The area under the curve (AUC) was calculated for each sample by integrating the relative fluorescence curve. The net AUC of the sample was calculated using the following equation, and the ORAC values were expressed as μM TE/mg sample.

$$\text{Area under the curve (AUC)} = 1 + f_1/f_0 + f_2/f_0 + f_3/f_0 + f_4/f_0 + \cdots + f_{31}/f_0.$$

Analysis of catechin content

The catechin content of HPLC analysis was a modified method of Li et al. (2006). The samples were analyzed using a Sunfire^{TM} C_{18} column (250 × 4.6 mm, 5 μm particle size) (Waters Co., Milford, MA, USA) at 40 °C. A sample volume of 10 μL was injected into the column and eluted with a constant flow rate of 1.0 mL/min. The gradient elution was applied, with acetonitrile (A) and water (0.5 % phosphoric acid) (B) as solvents (0–26 min: 8 % A and 92 % B to 15 % A and 85 % B). Catechin was identified by comparing the retention times and PDA spectra (Waters 996 photodiode array detector, Waters Co.). The catechin content quantification was performed using the external standard method with five-point calibration curves generated via linear regression (correlation coefficients >0.999).

Statistical analysis

All data are presented as the mean ± SD. The data were analyzed using the one-way ANOVA procedure of SAS 9.3 (SAS Institute, Inc., Cary, NC, USA). Differences were analyzed using Duncan's multiple range tests at a $p < 0.05$. Correlations were calculated using Pearson's correlation coefficient (R) by the IBM SPSS Statistics software package (IBM SPSS Statistics 21, IBM, NY, USA).

Results and discussion

Yields and total phenolic (TP) and proanthocyanidin (TA) contents

The yields of EtOH extracts from the bark of *U. pumila* using different concentrations of EtOH and different

Table 1 The yield of various EtOH extracts from the bark of *U. pumila*

EtOH (%)	Time (h)	Yield (%)
99	3	8.46 ± 0.07^{d}
	2	7.90 ± 0.54^{d}
	1	8.40 ± 0.63^{c}
80	3	9.45 ± 0.33^{bc}
	2	10.14 ± 0.03^{bc}
	1	9.81 ± 0.05^{bc}
50	3	11.08 ± 0.11^{a}
	2	11.15 ± 0.05^{a}
	1	10.44 ± 0.02^{ab}
30	3	10.54 ± 0.11^{ab}
	2	10.37 ± 0.02^{ab}
	1	9.89 ± 0.10^{bc}

Values with different superscript within a same column are significantly different ($p < 0.05$) by Duncan's test

Table 2 Total phenolic and proanthocyanidin contents from the bark of *U. pumila*

EtOH (%)	Time (h)	TP (mg GAE/g)	TA (μg CE/g)
99	3	430.63 ± 1.53^{a}	601.50 ± 55.77^{a}
	2	418.57 ± 1.65^{b}	531.96 ± 4.08^{b}
	1	366.27 ± 0.96^{e}	496.12 ± 5.07^{c}
80	3	392.58 ± 1.67^{d}	341.27 ± 4.07^{d}
	2	391.67 ± 1.24^{d}	325.49 ± 2.96^{de}
	1	369.76 ± 0.55^{e}	299.51 ± 4.40^{ef}
50	3	399.88 ± 3.46^{c}	269.75 ± 11.95^{fg}
	2	348.17 ± 6.85^{f}	246.10 ± 5.85^{gh}
	1	339.48 ± 0.18^{g}	216.17 ± 6.47^{h}
30	3	404.25 ± 2.48^{c}	239.82 ± 6.90^{gh}
	2	341.55 ± 1.92^{g}	228.71 ± 0.67^{h}
	1	328.49 ± 3.11^{h}	219.93 ± 6.50^{h}

Values with different superscripts within a same column are significantly different ($p < 0.05$) by Duncan's test

GAE gallic acid equivalent, *CE* catechin equivalent

extraction times are shown in Table 1. Among the various extracts, the 50 % EtOH extracts showed the highest yield (20.44–11.15 %), followed by the 30 % EtOH (9.89–10.54 %), 80 % EtOH (9.45–10.14 %), and 99 % EtOH (7.90–8.46 %) extracts. These results were comparable to those of the 70 % EtOH extracts from the root bark of *U. pumila* (12.0 %) (Jeong and Kim 2012). However, the 50 % EtOH extract yields from *U. davidiana* after 1 month of extraction at RT were considerably lower than those of the 80 % methanol extracts (41.4 %) (Jung et al. 2007). Therefore, the current study indicates that the yields of EtOH extracts from *Ulmus* species may be significantly influenced by solvent conditions and extraction times (Cho et al. 2010; Lee et al. 2011).

The TP and TA contents of different EtOH extracts from the bark of *U. pumila* are shown in Table 2. However, flavonoid compounds were not detected in the present study. The TP content of various EtOH extracts ranged from 328.49 to 430.63 mg GAE/g sample, which was slightly higher than that of the root bark of *U. pumila* (218.4–363.5 mg GAE/g sample) (Kim et al. 2015a). However, the TP content was significantly higher than in other herbal plants such as *A. senticosus* (23.50–56.08 mg GAE/g sample) and *A. koreanum* (35.45–73.34 mg GAE/g sample) (Kim et al. 2015b). Among the samples, the extraction time influenced the TP content to a larger extent than EtOH concentration. Thus, the 3-h extraction samples were observed to have the highest TP content for each EtOH concentration. It was reported by Spigno et al. (2007) that the TP content of the sample (1.62 %) extracted for 7 h was higher than of the samples extracted for 1, 3, and 5 h (0.97–1.53 %). On the other hand, the TA content of different EtOH extracts from bark of *U. pumila* was 219.93–601.50 μg CE/g sample (Table 2) and was observed to have similar trends to those of the 3-h EtOH extracts, which had a significantly higher TA content than other EtOH extracts. In other herbal plants, the TA content from different plant parts of *A. senticosus* and *A. koreanum* were observed to have a higher TA content (2.88–33.01 mg CE/g sample) than *U. pumila* (Kim et al. 2015b). Pinelo et al. (2005) reported that a 1:1 (v/v) ratio of EtOH and water was the optimal condition for extracting polyphenol from a grape pomace. However, in the current study, we observed that 99 % EtOH is a more optimal condition for extracting phenolic and proanthocyanidin compounds. Especially, the TA contents of all 99 % EtOH extracts were significantly higher than other samples. Therefore, these results imply that the differences in the TP and TA contents in herbal plants may be species specific and depend on different extraction conditions (Cho et al. 2011).

Antioxidant activities

The antioxidant abilities of various EtOH extracts from the bark of *U. pumila* were determined using the DPPH and ABTS radical scavenging activities, RP, and ORAC assays. The IC_{50} values of the DPPH radical scavenging activity of EtOH extracts from the bark of *U. pumila* are shown in Table 3. The various concentrations of EtOH extracts exhibited time-dependent DPPH radical scavenging activities from 1 to 3 h. Among the samples, the 99 % EtOH extracts (IC_{50} = 16.17–21.08 μg/mL) showed higher DPPH radical scavenging than other samples (IC_{50} = 27.82–39.30 μg/mL). In addition, the 99 % EtOH for 3-h extracts exhibited a significantly higher DPPH radical

Table 3 IC_{50} values of the DPPH and ABTS radical scavenging activities from the bark of *U. pumila*

EtOH (%)	Time (h)	DPPH (μg/mL)	ABTS (μg/mL)
99	3	16.78 ± 0.06^{j}	21.27 ± 0.22^{e}
	2	18.11 ± 0.78^{i}	21.40 ± 0.28^{e}
	1	21.08 ± 0.39^{h}	23.52 ± 0.31^{d}
80	3	29.25 ± 1.50^{f}	25.92 ± 0.48^{c}
	2	29.48 ± 0.26^{f}	27.89 ± 1.63^{b}
	1	32.17 ± 0.60^{cd}	31.12 ± 0.16^{a}
50	3	27.82 ± 0.53^{g}	24.83 ± 0.50^{cd}
	2	30.99 ± 0.43^{e}	30.65 ± 0.81^{a}
	1	39.30 ± 0.21^{a}	31.51 ± 2.19^{a}
30	3	31.65 ± 0.13^{de}	25.95 ± 1.59^{c}
	2	33.04 ± 0.47^{c}	30.38 ± 0.38^{a}
	1	36.69 ± 0.21^{b}	30.18 ± 0.29^{a}
Vitamin C		10.89 ± 0.37^{k}	23.62 ± 1.53^{d}
BHA		16.52 ± 0.30^{j}	12.95 ± 0.58^{f}

Values with different superscripts within a same column are significantly different ($p < 0.05$) by Duncan's test

scavenging activity than the 1- and 2-h extraction samples. This result was comparable to that of the positive control, BHA ($IC_{50} = 16.52$ μg/mL). However, VC ($IC_{50} = 10.89$ μg/mL) was observed to have the strongest DPPH radical scavenging effect among in the current study. In another study, the root bark extracts of *U. pumila* was observed to have a 55.7 % DPPH radical scavenging activity at a concentration of 100 μg/mL, which was significantly lower than the scavenging activity noted this study (Kim et al. 2015a). However, the 70 % EtOH extracts from *U. davidiana* exhibited relatively strong DPPH radical scavenging capacity (61.3 %) at a concentration of 10 μg/mL (Guo and Wang 2007). The IC_{50} values of the ABTS radical scavenging activities of various EtOH extracts from *U. pumila* ranged from 21.27 to 31.51 μg/mL (Table 3). Specifically, the 99 % EtOH extracts exhibited strong ABTS radical scavenging ability, which was similar to the DPPH radical scavenging activity. The ABTS radical scavenging capacity of the 99 % EtOH extracts was close to that of the positive control, VC ($IC_{50} = 23.62$ μg/mL). The 3-h extracts of each sample showed higher ABTS radical scavenging activities than the 1- and 2-h extracts. The IC_{50} values of ABTS radical scavenging activity from the EtOH extracts of *A. senticosus* (245–348 μg/mL) and black raspberry (213–288 μg/mL) were significantly lower than those of the EtOH extracts from the bark of *U. pumila* (Lee et al. 2009, 2011). In general, the binary solvent (EtOH with water) was exhibited stronger radical activity than the mono EtOH solvent (Pinelo et al. 2005). However, the 99 % EtOH extracts had more efficient radical scavenging activities than other binary solvents in this study. These results suggest the optimal extraction of radical scavenging compounds depends on their agricultural materials. The RP of various EtOH extracts from the bark of *U. pumila* was determined by measuring the amount of reductones in samples (Cho et al. 2010) and was expressed as the percentage of activity exhibited by 0.05 mg/mL VC. As shown in Fig. 1(A), the 99 % EtOH for 3-h extracts exhibited considerably higher RP than the other extracts. In addition, the 3-h extraction of each EtOH sample showed a higher RP than the 1- and 2-h extracts. Figure 1(B) demonstrates that the EtOH extracts from the bark of *U. pumila* exhibited time-dependent ORAC values from 1 to 3 h. Among the various samples, the 99 % 3-h extracts had the most efficient ORAC values,

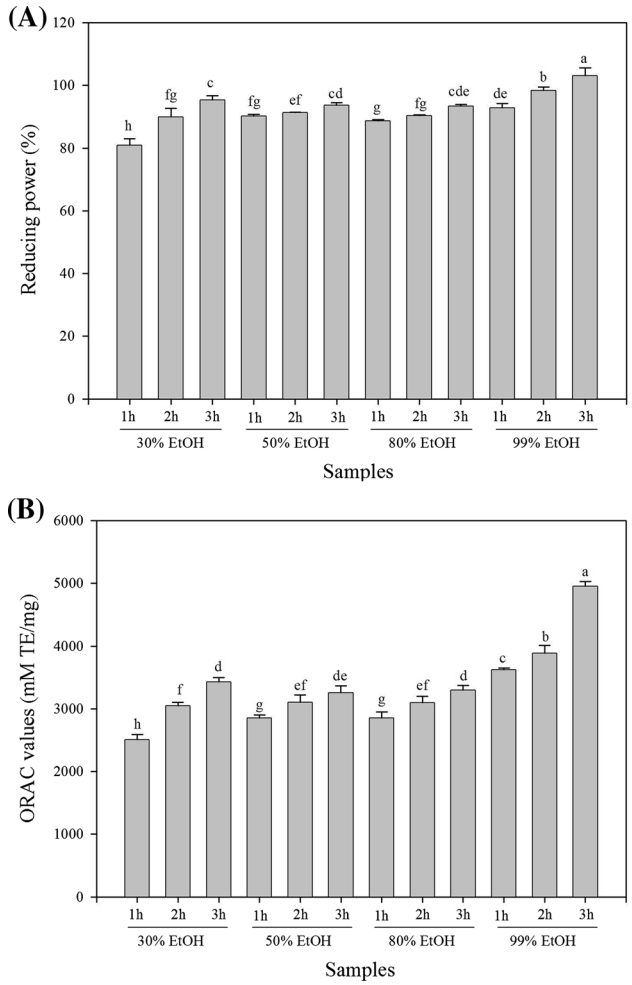

Fig. 1 Reducing power (**A**) and of oxygen radical absorbing capacity (**B**) of various EtOH extracts from the bark of *U. pumila*. All values are presented as the mean ± SD. Each sample was used at a concentration of 100 μg/mL, and the reducing power was expressed as a percentage of activity exhibited by 50 μg/mL of VC. The *bars* with *different letters* indicate statistically significant differences among groups at $p < 0.05$ using a one-way ANOVA

with a value of higher than 5000 μM TE/g sample. Moreover, all 99 % EtOH extracts were observed to have significantly higher ORAC values than the other samples. In a previous study, the ORAC value of the 80 % EtOH for 3-h extracts from the root bark of *U. pumila* was observed to be 3158 μM TE/g sample (Kim et al. 2015a), which suggests that the ORAC values of *U. pumila* were different for different plant parts such as the leaves, root bark, or stem bark. These results are in good agreement with the above results for the DPPH and ABTS radical scavenging activities and the RP, which suggests the 99 % EtOH 3-h extracts was the strongest antioxidants. It has been reported that the antioxidant abilities of EtOH extracts may be correlated with their antioxidant compounds such as TP, flavonoid, and TA contents (Kim et al. 2015b). Therefore, the antioxidant activities of various EtOH extracts from the bark of *U. pumila* were correlated with TP and TA contents.

Relationship between antioxidant activities and antioxidant compounds

The relationship between antioxidant activities and antioxidant compounds was evaluated using a regression analysis (correlation coefficient = R). In Table 4, a significant correlation was obtained between TA and DPPH ($R = 0.91$). In addition, TA was strongly correlated with ABTS ($R = 0.65$), RP ($R = 0.78$) and ORAC ($R = 0.75$). However, weak correlations were observed between TP and ABTS ($R = 0.31$), and TP and ORAC ($R = 0.55$). Among the antioxidant activities, DPPH was significantly correlated with RP and ORAC ($R = 0.84$), and RP was correlated well with ORAC ($R = 0.84$). Kim et al. (2015b) observed that the antioxidant activity of various extracts from *A. senticosus* and *A. koreanum* was directly correlated between proanthocyanidin content and ABTS ($R = 0.88$) and ORAC ($R = 0.77$). In addition, the TP content of the EtOH extracts from marine plants had a weak correlation with their antioxidant abilities (Cho et al. 2010). The antioxidant activity of plant extracts were usually correlated with their TP, flavonoid, and proanthocyanidin contents. However, some studies have reported that the strong antioxidant abilities of the plant extracts are due to specific bioactive compounds rather than TP content (Shon et al. 2008; Cho et al. 2011; Kim et al. 2012). These results suggest that the strong antioxidant activities of various EtOH extracts from the bark of *U. pumila* may be due to specific proanthocyanidin compounds. Therefore, we determined the catechin content from each EtOH concentration for 3-h extracts from the bark of *U. pumila*.

Table 4 Correlation analysis (R^2) between the antioxidant compounds content and antioxidant activities (DPPH, ABTS, reducing power, and ORAC) from the bark of *U. pumila*

Traits	TP	TA	DPPH	ABTS	RP	ORAC
TP	1.00	0.43	0.56*	0.58*	0.61*	0.55*
TA	0.43	1.00	0.91*	0.57*	0.78*	0.75*
DPPH	0.56*	0.91*	1.00	0.74	0.84**	0.84*
ABTS	0.31*	0.65*	0.74	1.00	0.64*	0.63*
RP	0.61*	0.78*	0.84**	0.64*	1.00	0.84*
ORAC	0.55*	0.75*	0.84*	0.63*	0.84*	1.00

TP total phenolic contents, *TA* total proanthocyanidin contents, *RP* reducing power

* $p < 0.001$, ** $p < 0.01$

Analysis of catechin content

In the present study, we determined various phenolic compounds such as, gallic acid, catechin, chlorogenic acid, vanillic acid, and caffeic acid. However, the catechin was only found in EtOH extracts from the bark of *U. pumila*, and other compounds were not detected in the sample (data not shown). Therefore, the catechin content was analyzed using the HPLC comparison method with a standard compound. Catechin is one of the proanthocyanidin compounds and is the major bioactive compound in the *Ulmus* species (Kwon et al. 2011). As shown in Table 5, the catechin content of various EtOH extracts dramatically increased (from 7.84 to 14.90 mg/g sample) with increasing EtOH concentrations. The highest catechin content (14.90 mg/g sample) was observed for the 99 % EtOH for 3-h extracts. Catechin, which was isolated from *U. davidiana*, exhibited strong DPPH radical scavenging activity with IC_{50} values of 6.37 μM (Jung et al. 2008). In addition, the strong metal chelating activity of catechin isolated from *U. davidiana* has been reported by Jung et al. (2010). These results agree with the above-mentioned results, which indicated that the 99 % EtOH extracts showed the highest proanthocyanidin content and the strongest antioxidant abilities compared with other extracts. This result suggests that the catechin is the major antioxidant in EtOH extracts from the bark of *U. pumila*.

In conclusion, the optimal conditions for preparing bark extracts from *U. pumila* were investigated, and TP and TA

Table 5 Catechin content in different concentrations of the EtOH for 3-h extracts from the bark of *U. pumila*

Extraction condition	(+)-Catechin (mg/g sample)
30 % EtOH	7.84 ± 0.04^{cd}
50 % EtOH	8.47 ± 0.07^{c}
80 % EtOH	10.60 ± 1.02^{b}
99 % EtOH	14.90 ± 0.95^{a}

Values with different superscripts within a same column are significantly different ($p < 0.05$) by Duncan's test

contents were determined. In addition, antioxidant abilities were measured using DPPH and ABTS radical scavenging activities, RP, and ORAC. Moreover, the antioxidant compounds were correlated with antioxidant activities, and the catechin content was analyzed using HPLC. Among various EtOH extracts, the 99 % EtOH for 3-h extracts was observed to have the strongest antioxidant abilities with the highest TP and TA content. The antioxidant compound, especially proanthocyanidin content, was significantly correlated with DPPH radical scavenging activity ($R = 0.91$). Furthermore, a good correlation was observed among TA content with ABTS radical scavenging activity, RP and ORAC. Moreover, the highest catechin content was observed in the 99 % EtOH for 3-h extracts. Therefore, the strong antioxidant activities of EtOH extracts from the bark of *U. pumila* may be attributed to the specific proanthocyanidin compound, catechin. The current study suggests that the bark extracts of *U. pumila* may have natural antioxidant benefits, which are useful as nutraceuticals, supplements, and cosmeceuticals.

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