Food Sci. Biotechnol. Vol. 16, No. 1, pp. 55 ~ 61 (2007)

Food Science and Biotechnology

Antioxidant and Antidiabetic Activities of *Ulmus davidiana* Extracts

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Abstract The antioxidant activities of water, ethanol, methanol, and chloroform extracts of *Ulmus davidiana* were evaluated using various antioxidant assays: DPPH (1,1-diphenyl-2-pricrylhydrazyl) free radical scavenging; hydroxyl radical scavenging; lipid peroxidation scavenging; and reducing power assays. All extracts, except the chloroform extract, demonstrated strong antioxidant activity in all assays. The chloroform extract had the highest hydroxyl radical scavenging activity, and its activity was equivalent to α-tocopherol at a concentration of 0.5 mg/mL. Additionally, the antidiabetic activity of their extracts was also evaluated using a rat intestinal α-glucosidase inhibition assay. Among all extracts investigated, the methanol extract had the highest α-glucosidase inhibitory activity, although its activity was less than acarbose at 0.5 μg/mL. This result suggested that *U. davidiana* extracts may have antidiabetic activity. Total phenolic compounds and flavonoids were also measured. Phenolic compounds such as tannic acid, *p*-coumatric acid, and kaempferol were detected by high-performance liquid chromatography (HPLC). These results suggest that *U. davidiana* extracts may be useful as a potential source of antioxidant and antidiabetic materials.

Keywords: *Ulmus davidiana*, antioxidant activity, lipid peroxidation, α-glucosidase, phenolic content

Introduction

Free radicals, from both endogenous and exogenous sources, are implicated in the etiology of several degenerative diseases, such as coronary artery diseases, stroke, rheumatoid arthritis, diabetes, and cancer (1). Serious attention is now being paid to the cytotoxicity of reactive oxygen as the cause of various pathological conditions. Lipid peroxides produced from unsaturated fatty acids via radicals cause histotoxicity and promote the formation of additional free radicals in the manner of a chain reaction (2, 3).

An antioxidant is defined as any substance that, when present at low concentrations compared with that of an oxidizable substrate, delays, or prevents oxidation of that substrate (4). Some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are commonly used in processed foods. There is thought to be an inverse relationship between dietary intake of antioxidant-rich foods and the incidence of human disease (5). The antioxidants in some plants, including ascorbic acid, carotenoids, flavonoids, and hydrolysable tannins, are considered to play an important role in preventing diseases induced by free radicals (6).

Polyphenols belong to a heterogeneous class of compounds with a variety of antioxidant actions. In searching for sources of natural antioxidants, the antioxidant and radical scavenging activities of medicinal plants and fruits have been extensively studied in the last few decades (7, 8). Among the various natural antioxidants, phenolic compounds are reported to quench oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical (9, 10). Phenolic compounds in plants have been shown to neutralize free radicals in various model systems (11, 12).

Scavenging of the stable 1,1-diphenyl-2-pricrylhydrazyl (DPPH) radical is a widely used method to evaluate the free radical scavenging ability of various samples (13). Antioxidant molecules quench the DPPH free radical (by providing hydrogen atoms or donating electrons, conceivably via a free-radical attack on the DPPH molecule) and convert it to a colourless/bleached product (i.e., 2,2-diphenyl-1-hydrazine, or a substituted analogue of hydrazine), resulting in a decrease in absorbance at 517 nm. Results are expressed as the percentage reduction in A_{517}, and the IC_{50} is used to define the antioxidant activity of each extract.

Ulmus davidiana is a deciduous tree and widely distributed in Korea. Its bark and roots are used in oriental traditional medicine to treat edema, mastitis, gastric cancer, and inflammation. Some scientific reports on biologically active compounds of *U. davidiana* and their biological actions have already been reported so far. For example, catechin and catechin glycoside named uldavioside A were isolated from *U. davidiana* in 1989 (14). Additionally, 4 ligan xylosides and 2 neoligan glycosides were isolated from its stem and root barks (15). Recently, it was reported that *U. davidiana* Panch (UDP), a kind of Ulmaceae, has a strong anti-oxidative activity on lipid peroxidation and an inhibitory effect on an endogenous NO-induced apoptotic cell death (16).

The objectives of the present study were to investigate the *in vitro* antioxidant activities of the bark extracts of *U. davidiana* and to develop a natural antioxidant from the bark. We evaluated the antioxidant activities of various extracts *in vitro* using DPPH free radical scavenging, hydroxyl radical scavenging, lipid peroxidation scavenging, and reducing power assays. We also investigated the possibility that these extracts may also possess antidiabetic activity by studying the effects of these various plant extracts using the α-glucosidase inhibition assay.

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Received August 8, 2006; accepted November 3, 2006

Material and Methods

Plant material and preparation of extracts *U. davidiana* bark was air dried under shade at room temperature and powdered. Ten g of sample was extracted separately with 200 mL water, ethanol, 70% ethanol, methanol, 70% methanol and chloroform for 72 hr. The extracts were filtered and evaporated in a rotary evaporator at 50°C to obtain the final extract.

DPPH radical scavenging activity The free radical scavenging activities of the extracts, based on the scavenging activity of DPPH free radical, were determined by the modified method of Kilani *et al.* (17). Aliquots (0.5 mL) of the extracts at different concentrations (5-20 μg/mL) were mixed with freshly prepared DPPH in methanol (final concentration 250 μM) and the absorbance at A_{517} was determined after incuba-tion for 30 min in the dark at room temperature. Distilled water was used as a control, and ascorbic acid as a reference compound. The percentage of inhibitory activity was calculated as:

$$[1\text{-}A_{sample}/A_{control}]\times 100\%.$$

$FeCl_2$-ascorbic acid-stimulated lipid peroxidation in liver homogenates A young broiler chick (Ross, 2.1 kg body weight) was used in this assay. A chick liver was harvested immediately after decapitation. Two g of liver tissue was sliced and homogenized with 10 mL of 150 mM KCl-Tris-HCl buffer (pH 7.2). The reaction mixtures were composed of 0.25 mL of liver homogenate, 0.1 mL of Tris-HCl buffer (pH 7.2), 0.05 mL of 0.1 mM ascorbic acid, 0.05 mL of 4 mM $FeCl_2$, and 0.05 mL of extracts. Five tenth mL of HCl (0.1 N), 0.2 mL of sodium dodecyl sulfate (SDS) (9.8%), 0.9 mL of distilled water and 2 mL of thiobarbituric acid (TBA, 0.6%) were added to this mixture in capped tubes. After incubation at 37°C for 1 hr, these tubes were shaken vigorously for 2 min. The tubes were placed in a boiling water bath (100°C) for 30 min and cooled down to room temperature. The supernatant was removed by adding 5 mL of *n*-butanol and centrifuged (25 min, 80×g). The absorbance of the supernatant was then measured at A_{532}.

Reducing power assay One mL of *U. davidiana* extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium hexacyano-ferrate (III) ($K_3Fe(CN)_6$). The mixture was then placed in a 50°C water bath for 30 min. Two and half mL of 10% trichloroacetic acid was added and the mixture was centrifuged (10 min, 800×g). Two and half mL distilled water and 0.5 mL of 0.1% ferric chloride were added to its supernatant. The absorbance of the samples was then measured at A_{700} with a UV-visible spectrophotometer (Mecasys Co., Daejeon, Korea).

Hydroxyl radical scavenging The Fenton reaction mixture consisted of 200 μL of $FeSO_4{\cdot}7H_2O$ (10 mM), ethylenediamine tetraacetate (EDTA, 10 mM) and 2-deoxyribose (10 mM). Then, 200 μL of *U. davidiana* extract and 1 mL of 0.1 M phosphate buffer (pH 7.4) were added to make up a total volume of 1.8 mL. Thereafter, 200 μL of 10 mM H_2O_2 was added and the reaction mixture was incubated at 37°C for 4 hr. After incubation, 1 mL of 2.8% tricarboxylic acid (TCA) and 1 mL of 1% TBA were added and the mixture was placed in a boiling water bath for 10 min. After cooling, it was centrifuged (5 min, 800×g) and the sample absorbance was measured at A_{532}.

Rat intestinal α-glucosidase inhibitory activity α-Glucosidase inhibitory activity was assayed by the modified method of Kim *et al.* (18). α-Glucosidase (0.075 unit) was mixed with 50 μL of *U. davidiana* extract in phosphate buffer at various concentrations (100-500 μg/mL). After pre-incubation at 37°C for 15 min, 100 μL of 3 mM *p*-nitrophenyl glucopyranoside (*p*NPG) was added to the mixtures as the substrate. The reactions were incubated at 37°C for 10 min and stopped by adding 750 μL of 0.1 M Na_2CO_3. α-Glucosidase activity was determined by measuring the concentration of *p*-nitrophenol released from the *p*NPG at A_{405}.

Measurement of total phenolic compounds and flavonoids One mg of the various extracts at different concentrations was mixed with 2 mL of Folin–Denis reagent and 2 mL of 35% sodium carbonate. The mixtures were shaken thoroughly and made up to 10 mL with distilled water. The absorbance at A_{765} was determined after incubation at room temperature for 30 min. Phenolic content was determined from a standard curve determined at differents concentrations of tannic acid.

Flavonoid content was determined as follows: 1 mL of plant extract in methanol (10 mg/mL) was mixed with 1 mL aluminium trichloride in ethanol (20 mg/mL), and the absorption at A_{415} was measured after stood up for 40 min. Blank was the mixture of 1 mL of plant extract and 1 mL ethanol. The absorption of a standard rutin solution (0.5 mg/mL) in ethanol was measured under the same conditions. The rutin equivalents (RE) of the flavonoids in plant extracts were calculated from the formula:

$$X = (A{\cdot}m_0)/(A_0{\cdot}m)$$

X: the flavonoid content in mg/mg plant extract
A: the absorption of the plant extract
A_0: the absorption of the standard rutin solution
m: the weight of plant extract in mg
m_0: the weight of rutin in the solution in mg

HPLC analysis of *U. davidiana* phenolic compounds High-performance liquid chromatography (HPLC) was used to separate and identify individual phenolic compounds in *U. davidiana* samples. Different solvent extracts were dissolved in 1 mL of acidified water (3% formic acid), and then passed through a 0.20 μm membrane filter (SCA Co., Germany), and 20 μL was analyzed by HPLC. The samples were determined using a Waters Corp. HPLC system coupled with a photodiode array detector (990 Series; Waters, Millipore, MA, USA) and equipped with two pumps (600E system controller). Samples were injected at ambient temperature (20°C) into a reversed-phase NOVA-PAK C18 column (250×4.6 mm^2). The mobile phase consisted of 2.5% aqueous formic acid (A)

and HPLC grade acetonitrile (B). The mobile phase was acidified water containing 2.5% formic acid (A) and acetonitrile (B) in a linear gradient from 5 to 20% B in the first 15 min, followed by a linear gradient from 20 to 30% B for 5 min, then an isocratic mixture for 5 min, and then another isocratic mixture for 10 min before returning to the initial conditions. The flow rate was 1.0 mL/min, and the wavelengths of detection were set at 320 and 350 nm. Scanning between 240 and 550 nm was performed, and data were collected by the Waters 990 3D chromatography data system. Retention times and spectra were compared to those of authentication standards.

Results and Discussion

DPPH radical scavenging activity The DPPH method was used to evaluate the free radical scavenging activity of *U. davidiana* extracts (19). All extracts except the chloroform extract demonstrated strong antioxidant activity (IC_{50} from 6.64 to 10.41 μg/mL, Table 1). DPPH is a stable free radical that accepts an electron or hydrogen radical and becomes a stable diamagnetic molecule (20). It has been found that compounds such as cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines (*p*-nitrophenol diamine, *p*-aminophenol, etc.), reduce and bleach DPPH by their hydrogen donating ability (21, 22). Previous studies have also shown a linear relationship between total phenolic or anthocyanin content and antioxidant capacity in some berry crops (23).

Table 1. DPPH radical scavenging activity of various extracts from *U. davidiana*

Solvent extract	Concentration (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)
Water extract	5	39.7	6.95
	10	64.5	
	20	90.9	
EtOH extract	5	35.6	8.74
	10	56.4	
	20	88.8	
70% EtOH extract	5	33.8	8.35
	10	61.3	
	20	93.2	
MeOH ext.	5	42.4	6.64
	10	67.1	
	20	91.8	
70% MeOH extract	5	35.1	10.41
	10	47.8	
	20	82	
$CHCl_3$ extract	10	15.1	>100
	50	26.5	
	100	41.1	
Vit. C	0.5	5.4	3.42
	1	15.7	
	5	71.8	
Vit. E	1	7.3	7.59
	5	35.4	
	15	96.5	

$FeCl_2$-ascorbic acid stimulated lipid peroxidation in rat liver homogenates Chicken liver homogenate was incubated with ascorbic acid/Fe^{2+} ($FeCl_2$-AA) to cause non-enzymatic lipid peroxidation (24). Different extracts were incubated with the chicken liver homogenate in the presence of $FeCl_2$-AA, and the lipid peroxide concentration was determined by the absorbance of the MDA–TBA adduct (a complex of malondialdehyde with thiobarbituric acid) at A_{532} (25). When incubated at 37°C for 1 hr, each of the extracts inhibited the formation of TBA-RS at a concentration of 0.5 mg/mL ($p<0.01$; Table 2).

Lipid peroxidation is a typical free radical oxidation and proceeds via a cyclic chain reaction. It is thought to be an important cause of cellular damage, and is strongly associated with aging, carcinogenesis and other diseases (26). In addition, lipid peroxidation also plays an important role in the deterioration of foods during storage (27). *U.*

Table 2. The inhibitory effects of 6 extracts of *U. davidiana* on $FeCl_2$-Ascorbic acid induced lipid peroxidation in a rat liver homogenate *in vitro*[1)]

Groups	Concentrations (mg/mL)	MDA (mmol/mg protein)	Inhibition (%)
Control	-	7.27±0.27	-
$FeCl_2$-AA+water extract	0.5	5.06±0.60	27.2
$FeCl_2$-AA+EtOH extract	0.5	6.74±0.80	32.1
$FeCl_2$-AA+70% EtOH extract	0.5	5.45±0.23	33.5
$FeCl_2$-AA+MeOH extract	0.5	6.37±0.49	33.6
$FeCl_2$-AA+70% MeOH extract	0.5	5.55±0.55	37.8
$FeCl_2$-AA+$CHCl_3$ extract	0.5	5.55±0.33	38.4
$FeCl_2$-AA+Vit. E	0.5	5.76±0.97	35.3

[1)]Vit. E at the concentration of 0.5 mg/mL was used as control. Values represent the mean±SD (n=3).

davidiana extracts may protect against lipid peroxidation by radical scavenging and reduction (28).

Reducing activity The reducing activity of the *U. davidiana* extracts increased with increasing sample concentration (Fig. 1). All the extracts except the chloroform extract had greater reducing activity than vitamin E (control) at the same concentration. Reducing activity is generally associated with the presence of reductones (29), which have been shown to exert an antioxidant effect by donating a hydrogen atom and breaking the free radical chain (30). Reductones are reported to react with certain peroxide precursors, which preventing peroxide formation. In our study, most of solvent extracts had higher reducing activity, which suggests that the reducing activity of the tested extracts contributes significantly to their antioxidant effect and there should be some kinds of reductones in *U. davidiana*.

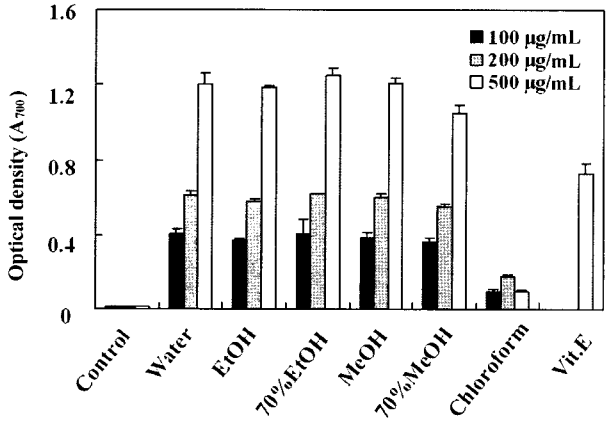

Fig. 1. Effects of different solvents used to produce extracts from *U. davidiana* on reducing power. Data were presented as the mean±SD (n=3). The concentrations of different solvent extracts were 100, 200, and 500 μg/mL, respectively. Vitamin E at the concentration of 500 μg/mL was used as a control.

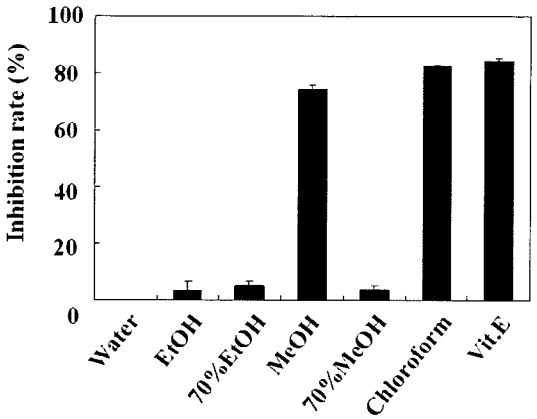

Fig. 2. Effects of different solvents used to produce extracts from U. davidiana on hydroxyl radical (OH•) scavenging assay. Data were presented as the mean±SD (n=3). The concentration of different extracts was 500 μg/mL. Vitamin E at the concentration of 500 μg/mL was used as a control.

Hydroxyl radical scavenging activity The methanol and chloroform extracts of *U. davidiana* showed considerable hydroxyl radical scavenging activity (Fig. 2). The methanol extract at 0.5 mg/mL scavenged 74.36% of the available free radicals, whereas chloroform extracts and vitamin E (control) scavenged 82.39 and 84% at a concentration of 0.5 mg/mL, respectively. Studies of effective hydroxyl radical scavengers indicate that hydroxyl radical scavenging activity is related to the levels of phenolic compounds present in the scavenger. Phenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triple oxygen, or decomposing peroxides (31, 32). We hypothesize that the significant hydroxyl activity demonstrated by *U. davidiana* extracts is likely due to the presence of phenolic compounds in the prepared extracts, and to investigate this possibility, we attempted to measure the phenolic content of our extracts as well as identify the compounds themselves (see below).

Phenolic and flavonoid content of *U. davidiana* extracts As phenolic compounds have been shown to possess strong antioxidant activity (33), we decided to measure the phenolic and flavonoid centent of the *U. davidiana* extracts as it probably contributes to the antiioxidant activity of *U. davidiana*.

The total phenolic content of *U. davidiana* extracts was measured using the Folin-Denis method. The phenolic content of the water, ethanol, 70% ethanol, methanol, 70% methanol, and chloroform extracts were 15.34, 12.43, 14.02, 15.16, 10.83, and 2.21 mg/g, respectively (Fig. 3). A strong positive correlation has been reported between total polyphenolic content and DPPH radical scavenging activity (34, 35), which had been strongly demonstrated in our study. In our result, the DPPH radical scavenging activity and the phenolic content of the six extracts were, in descending order: MeOH > water > 70% EtOH > EtOH > 70% MeOH > $CHCl_3$.

Flavonoids are one of the most diverse and widespread groups of natural compounds. Flavones, isoflavones, flavonones, anthocyanins, and catechins are likely to be the most important natural phenolics (36). These compounds possess a broad spectrum of chemical and biological activities including radical scavenging and strong antioxidant capacity. The flavonoid content of different solvent extracts from *U. davidiana* is comparatively high compared to other plants species which had been studied (37). The higher content of flavonoid in *U. davidiana* extracts might account for the better results found in their reducing power and radical scavenging effect on DPPH radicals. The flavonoid content of the extracts was shown in Table 4.

HPLC identification of phenolic compounds HPLC analysis of *U. davidiana* extracts showed that certain phenolic compounds were present in different solvent extracts (Fig. 3). Compounds such as tannic acid, *p*-coumatric acid, and kaempferol were detected. Tannic acid

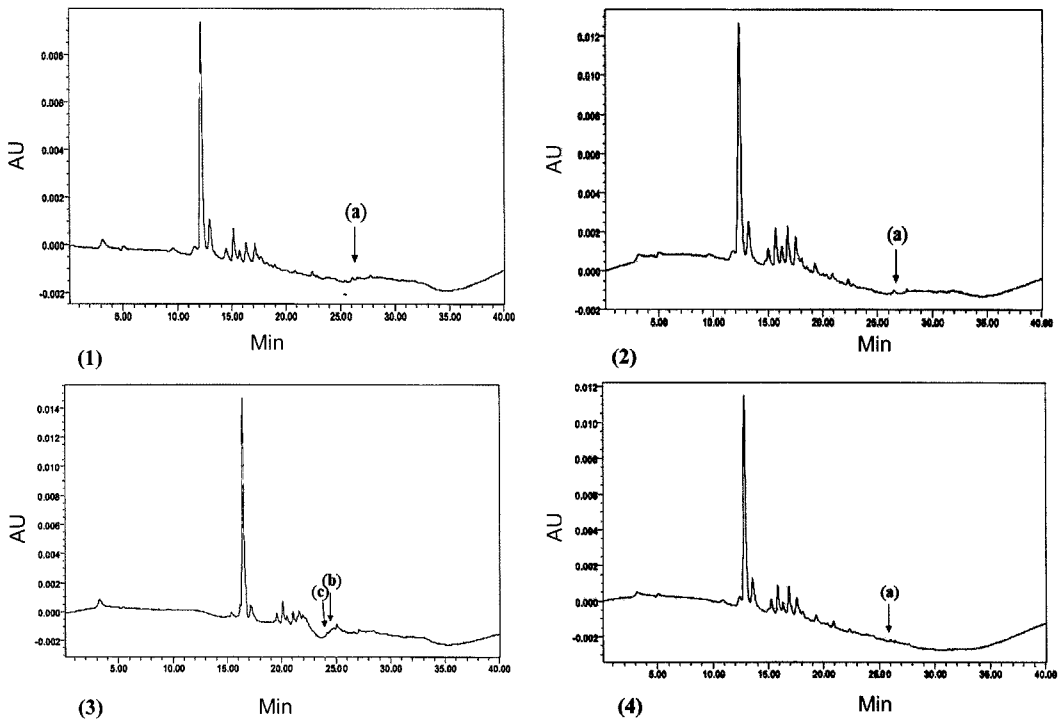

Fig. 3. HPLC phenolic profile of different solvent extracts from *U. davidiana*. Detection at A_{320}: (1) EtOH solvent extract; (2) 70% EtOH solvent extract; (3) MeOH solvent extract; (4) 70% MeOH solvent extract. Peaks: (a) kaempferol; (b) tannic acid; (c) *p*-coumatric acid.

was detected in MeOH solvent extract at a concentration of 13.46 mg/g. Kaempferol was detected in all ethanol and methanol extracts where its concentration was 11.32, 7.81, and 17.0 mg/g in EtOH, 70% EtOH, and 70% MeOH solvent extracts, respectively. *p*-Coumatric acid occurred only in small amounts in MeOH solvent extract at a concentration of 0.73 mg/g.

Previous work on elm-derived phenolics is limited. It was reported that the flavonols quercetin and kaempferol, as well as caffeic acid in acid-hydrolyzed leaf extracts of *Ulmus procera* Salisb were identified (38). Equal amounts of quercetin and kaempferol were identified in *Ulmus parvifolia* Jacq, but only quercetin in *Ulmus campestris* was detected (39). In our study, 7 phenolic compounds were chosen to be standard compounds, including quercetin dehydrate, kaempferol, *p*-coumatric acid, tannic acid, caffeic acid, (-)-epicathchin, and gallic acid. However, only tannic acid, *p*-coumatric acid, and kaempferol were tested in some solvent extracts from *U. davidiana*.

α-Glucosidase inhibition To determine if *U. davidiana* extracts possess antidiabetic properties, we studied the effect of these extracts in α-glucosidase inhibition assays. All *U. davidiana* extracts, except the water extract, demonstrated α-glucosidase inhibitory activity (Table 3) that increased steadily with increasing sample concentration. In particular, the 70% MeOH extract at a concentration of 500 μg/mL inhibited α-glucosidase activity by 89 %. This result was only slightly lower than that of acarbose, an α-glucosidase inhibitor, at 0.5 μg/mL.

One therapeutic approach to decreasing postprandial hyperglycemia is to retard absorption of glucose by

Table 3. Effects of different *U. davidiana* extracts on rat intestinal α-glucosidase inhibition assay[1)]

	Concentration (μg/mL)	Inhibition (%)
Water extract	100	-
	200	-
	500	-
EtOH extract	100	40.41±1.92
	200	63.48±5.09
	500	80.78±8.38
70% EtOH extract	100	-
	200	-
	500	59.08±9.08
MeOH extract	100	58.49±4.23
	200	73.18±6.46
	500	78.04±1.12
70% MeOH extract	100	67.28±0.00
	200	60.01±1.82
	500	89.09 ±3.15
$CHCl_3$ extract	100	-
	200	58.16±1.87
	500	88.11±1.10
	0.5	99.15±1.07

[1)]Acarbose at the concentration of 0.5 μg/mL was used as control. Values represent the mean±SD (n=3).

Table 4. Total phenolic compounds and flavonoid content of *U. davidiana* extracts[1)]

Solvent extract	Yield (%)	Phenolic content (mg/g)	Flavonoid content (mg/g)
Water	5.9	15.34±0.99	18.49±0.29
EtOH	13	12.43±0.45	20.26±0.31
70% EtOH	10.2	14.02±0.59	19.06±0.30
MeOH	13.2	15.16±0.43	19.14±0.32
70% MeOH	19.6	10.83±0.55	19.67±0.24
$CHCl_3$	0.02	2.21±0.07	6.76±0.20

[1)]Values represent the mean±SD (n=3).

inhibiting carbohydrate hydrolysing enzymes, for example α-glucosidase, in the digestive organs (40). Inhibitors of intestinal α-glucosidases are used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM) and represent a huge proportion of the antidiabetic drug market (41). Nowadays, much attention has been paid to looking for new α-glucosidase inhibitors. Most of the *U. davidiana* extracts possessed α-glucosidase inhibitory activity, meaning that they contain effective α-glucosidase inhibitors that may have potential antidiabetic activity.

The results of the various inhibitor assays (including DPPH free radical scavenging, hydroxyl radical scavenging, lipid peroxidation scavenging and reducing power assays) revealed that the various extracts of *U. davidiana* had significant antioxidant activity. The extracts had different levels of antioxidant activity in different assays. *U. davidiana* could be considered as a functional food ingredient and pharmaceutical agent. However, further study of the individual compounds present in *U. davidiana* extracts and further *in vivo* experiments of their bioactivity are needed to understand their mechanisms of action.

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