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Article

Antioxidant and Antidiabetic Activities of *Eucommia ulmoides* Bark

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Eucommia ulmoides **bark extracts by cold water, boiling water, 100% EtOH, 70% EtOH, 100% MeOH, 70% MeOH and $CHCl_3$ were assayed for their medicinal effects. The antioxidant activity of the extracts ranged from IC_{50} 125.2 to IC_{50} 872.7 μg/m*l* in the 1,1-diphenyl-2-picrylhydrazyl (DDPH) free radical-scavenging assay, and cold water extracts had the highest antioxidant activity. $CHCl_3$ extracts had the highest inhibitory effect on angiotensin I-converting enzyme (ACE) giving inhibition of up to 56.4% at a concentration of 1 mg/m*l*. Extracts in 100% EtOH had the greatest inhibitory effect on á-amylase activity (IC_{50} = 174.6 μg/m*l*), and 70% MeOH extracts had the greatest inhibitory effect on α-glucosidase activity (IC_{50} = 14.0 μg/m*l*). Taken together, these results provided the *in vitro* evidence on the ACE, amylase and glucosidase inhibitory actions of *E. ulmoides* bark that form the pharmacological basis for its antihypertensive and antidiabetic action.**

Key words: *Eucommia ulmoides, DPPH, antioxidant activity, ACE, α-amylase, α-glucosidase*

Hypertension is one of the most serious chronic cardiovascular diseases, carrying a high risk of arteriosclerosis, stroke, myocardial infarction and end-stage renal disease.[1] Angiotensin I-converting enzyme (ACE) is involved in blood pressure regulation and electrolyte homeostasis. ACE inhibitors can be used to treat hypertension by inhibiting the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor.[2] Diabetes mellitus is a serious disease associated with many complications, and the incidence of this disease is increasing rapidly.[3,4] Diabetes may be caused by disorders of carbohydrate uptake.[5] Amylase inhibitors slow starch digestion by inhibiting amylase activity in the intestinal lumen, thereby improving postprandial carbohydrate tolerance in patients with diabetes mellitus,[6] and. inhibitors of α-glucosidase limit the absorption of dietary carbohydrates and suppress postprandial hyperglycemia.[3] Therefore, both amylase and glucosidase inhibitors can be used to treat diabetes.

There is growing interest in the use of medicinal plants. To date, ACE, amylase and glucosidase inhibitors have been found in many plants.[3,7,8] *Eucommia ulmoides* Oliv., also referred to as Tu-chung or Du-zhong, the only species of the Eucommiaceae family, has long been used as a traditional medicine to strengthen internal organs, bones and muscles,[9] as well as to treat hypertension.[10] In the present study, extracts of *E. ulmoides* made in different solvents were assayed for their ACE, amylase and glucosidase inhibitory activities, to assess their potential use as antihypertensives and antidiabetics. In addition the antioxidant activity of the extracts was measured with the DPPH free radical-scavenging assay.

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Abbreviations: *E. ulmoides, Eucommia ulmoides*; DDPH, 1,1-diphenyl-2-picrylhydrazyl; ACE, angiotensin I-converting enzyme; HHL, hippuryl-histydil-leucine

Materials and Methods

Plant material and extracts. *E. ulmoides* bark was air dried at room temp. in the shade, and each 30 g of pulverized sample was soaked in 600 m*l* of cold water (room temperature), boiling water, 100% EtOH, 70% EtOH, 100% MeOH, 70% MeOH, or $CHCl_3$. The extract in boiling water was heated in boiling water for 10 h with continuous stirring, and the extracts made in the other solvents were treated for 48 h at room themperature. They were then filtered and the solvents evaporated with an Eyela evaporator at 42°C. For further analytic experiments, the extracts were dissolved in dimethyl sulfoxide (DMSO) as stock solution at 100 mg/m*l*, and diluted to the desired concentrations in distilled water.

DPPH free radical-scavenging assay. The antioxidant activities of the extracts were assessed on the basis of the radical scavenging effect of the stable DPPH free radical, and the assay was carried out as described by Kilani *et al.* with some modifications.[11] Aliquots (0.5 m*l*) of different concentrations (0.05-1 mg/m*l*) of extract were mixed with 0.5 m*l* freshly prepared DPPH in MeOH (final concentration 65 μM). The absorbance of the mixtures was measured photometrically at 517 nm after they were incubated for 30 min in the dark at room temperature. Distilled water was used as control, and ascorbic acid as reference compound. Each dilution was assayed in triplicate and the three readings were averaged. The ability to scavenge the DPPH radical was calculated according to the equation: scavenging effect (%) = $[1 - A_{sample}/A_{control}] \times 100$. The IC_{50} is the concentration of an extract

required to decrease the absorbance at 517 nm by 50% relative to the control. It is used to define the antioxidant capability of each extract.

ACE inhibitory assay. Angiotensin I-converting enzyme (ACE) in the form of the rabbit lung acetone powder, and hippuryl-histydil-leucine (HHL), were purchased from Sigma Chemical Co. ACE inhibitory activity was measured by the method of Cheung and Cushman with some modifications.[12] Reaction mixtures (125 μ*l*) contained 50 μ*l* of ACE solution (60 mU/m*l*) in 150 mM sodium borate buffer (pH 8.3), and 25 μl of different concentrations of extract added to 50 μl HHL solution (12.5 mM) in 0.4 M NaCl buffer (pH 8.3). Reactions were carried out at 37°C for 30 min, and terminated by adding 50 μl of 2.5 N HCl. Distilled water was used as control. Reactions terminated before incubation at 37°C served as blanks. The hippuric acid liberated was extracted with 1 m*l* of ethyl acetate. After centrifugation at 13000 × g, the ethyl acetate was evaporated at 95°C in a drying oven. The hippuric acid was dissolved in 500 μl of 0.4 M NaCl and the absorbance of the solution was measured at 228 nm. Inhibition was calculated as: % inhibition = $\{[(A_{control} - A_{control\ blank}) - (A_{sample} - A_{sample\ blank})]/(A_{control} - A_{control\ blank})\} \times 100$.

Assays of α-amylase and α-glucosidase inhibition. A modification of the method of Kim *et al.* was used to measure α-amylase inhibition.[13] Total of 200 μ*l* reaction mixtures contained 2 units á-amylase from porcine pancreas (Sigma), 0.02 M phosphate buffer (pH 6.9) and various concentrations (10-2000 μg/m*l*) of extract. Reactions were started by adding 250 μ*l* of 2% starch. Two hundred microliters of DNS solution (28.5 mM 3,5-dinitrosalicylic acid, 0.65 N NaOH, 0.5 M glycerol) was added to terminate the reactions after incubation for 7 min at 37°C. The reaction mixtures were heated for 5 min at 100°C and diluted to a final volume of 5 m*l* with distilled water in an ice bath. Distilled water was used as control. Blank reaction mixtures contained no α-amylase, and acarbose was used as reference compound. The amount of reducing sugar liberated was determined by measuring absorbance at 540 nm, and the inhibitory effect of a sample was calculated as: inhibitory effect (%) = $\{[(A_{control} - A_{control\ blank}) - (A_{sample} - A_{sample\ blank})]/(A_{control} - A_{control\ blank})\} \times 100$. The IC_{50} value was defined as the concentration of an extract that inhibited 50% of α-amylase activity.

Inhibition of α-glucosidase was assayed according to Kim *et al.* with some modifications.[13] Reaction mixtures contained 0.015 units of α-glucosidase from *Bacillus strearothermophilus* (Sigma) as a control, and various concentrations (1-1000 μg/m*l*) of extract. After 10 min pre-incubation at 37°C, 100 μ*l* of 3 mM glucopyranoside (pNPG) was added and 10 min later the reactions were terminated by adding 750 μ*l* of 0.1 Na_2CO_3. Liberated glucose was measured photometrically at 400 nm.

Results and Discussion

Assays of DPPH free radical scavenging. Free radicals are involved in a number of diseases due to the oxidative damage to DNA, lipids, and proteins and which can result in failure of cellular functions.[14,15] DPPH has been widely used to measure free radical-scavenging activity. The IC_{50} values of the *Ecommia* extracts ranged from 125.2 to 872.7 μg/m*l* as shown in Fig. 1. The cold water extract had the highest activity, and the chloroform extract the least. Thus, *E. ulmoides* bark appears to contain compounds that could scavenge DPPH free radicals, suggesting that extracts of *E. ulmoides* may treat some diseases associated with excess free radicals. Leaves of *E. ulmoides* were found to have effective DPPH free radical-scavenging activity.[16,17] Chen *et al.* carried out identification of antioxidants from *E. ulmoides* by DPPH assay.[18] Some studies showed that boiling water extracts of *E. ulmoides* bark and leaves have been shown to inhibit oxidation of deoxyribose by Fe^{3+}-EDTA/H_2O_2/ascorbic acid and the oxidative modification of human low-density lipoprotein by Cu^{2+}.[19,20] The present study showed the antioxidant activity of *E. ulmoides* and we hope that our results will provide some useful information for pharmaceutical study.

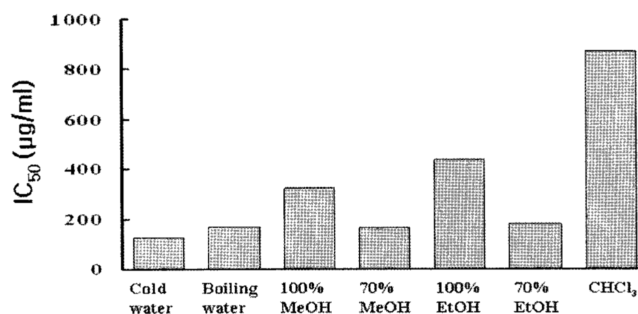

Fig. 1. DPPH free radical-scavenging activity of *E. ulmoides* bark extracts made in different solvents. Values are means of three determinations. (Ascorbic acid with an IC_{50} of 2.7 μg/m*l* is also included as a control).

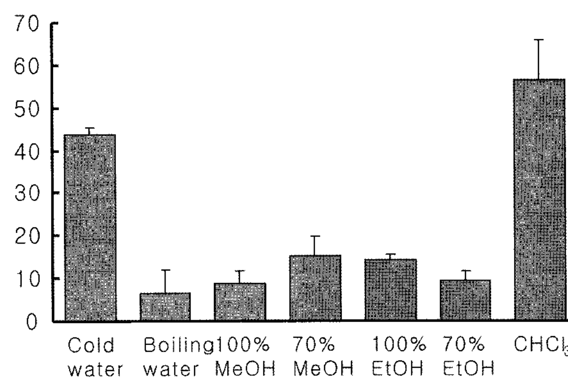

Fig. 2. ACE inhibitory activity of *E. ulmoides* bark extracts at final concentrations of 1 mg/m*l*. Values are means of three determinations.

Assays of ACE inhibition. Extracts in each of the solvents had ACE inhibitory activity. The inhibitory effects of concentrations of 1 mg/m*l* of each extract are shown in Fig. 2. The $CHCl_3$ extract had the highest inhibitory effect. The cold water extract was more inhibitory than the boiling water extract, suggesting that one or more ACE inhibitory

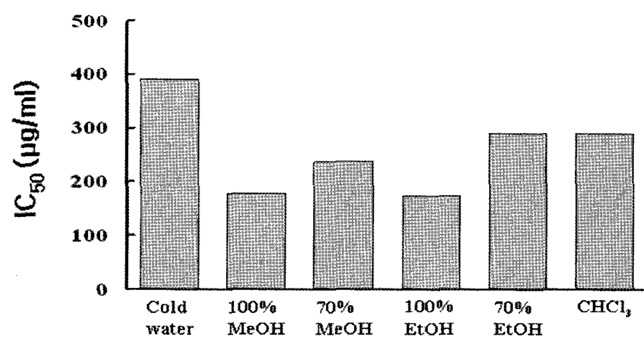

Fig. 3. The α-amylase inhibitory activity of *E. ulmoides* bark extracts. Values are means of three determinations. (The extract made in boiling water has an IC_{50} of >2.0 mg/m*l*, and acarbose has an IC_{50} of 0.1 μg/m*l*).

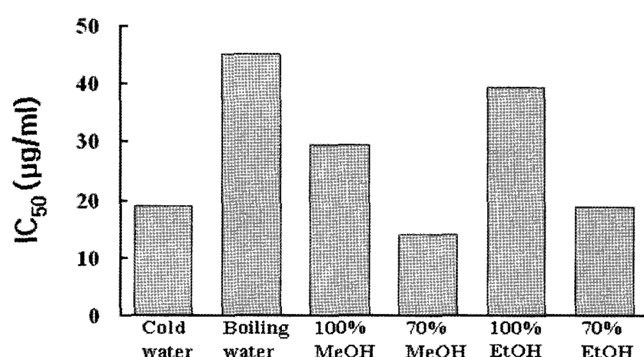

Fig. 4. The α-glucosidase inhibitory activity of *E. ulmoides* bark extracts. Values are means of three determinations. (The extract in $CHCl_3$ has an IC_{50} of more than 1 mg/m*l*, and acarbose has an IC_{50} of 0.015 μg/m*l*).

compound is/are not stable on boiling water. Several compounds from *E. ulmoides* have been reported to be involved in its hypotensive effect.[9]

Assays of α-amylase and α-glucosidase inhibition. All the extracts were capable of inhibiting amylase activity except for the boiling water extract which had an IC_{50} of more than 2.0 mg/m*l* (Fig. 3). The other IC50 values ranged from 174.6 to 390.2 μg/m*l*. The 100% MeOH and EtOH extracts had the highest inhibitory activity, with IC_{50} of 179.3 and 174.6 μg/m*l*, respectively. The $CHCl_3$ extract had weak inhibitory activity in the glucosidase inhibitory assay, with an IC_{50} of more than 1 mg/m*l*, while the 70% MeOH extract had the highest activity, with an IC_{50} of 14.0 μg/m*l* (Fig. 4). *E. ulmoides* extracts showed α-glucosidase inhibition using methanol solution.[21] The present findings suggest that extracts of *E. ulmoides* bark are potential candidates for treating diabetes by inhibiting amylase and glucosidase activities. They also indicate that heat treatment reduces the amylase and glucosidase inhibitory activities of aqueous extracts.

Our study gives the in vitro evidence that *E. ulmoides* bark possesses antioxidant, antihypertensive and antidiabetic actions, and takes on significant differences in physiological activities from different solvent extracts. Comparison of the cold water and boiling water extracts suggests that heat treatment of aqueous extracts reduces their physiological activities. We hope our results will contribute to the pharmaceutical development of *E. ulmoides*.

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