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Antioxidant and Anti-inflammatory Activity of Stem Bark Extracts from *Ulmus davidiana* var. *japonica*[*1]

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ABSTRACT

Ulmus davidiana var. *japonica* is a deciduous tree used in traditional medicine. In this study, we examined the antioxidant and anti-inflammatory activity of extracts and compounds isolated from *U. davidiana* var. *japonica* stem barks for development of cosmetic phyto-materials. Phytochemical examination of the stem bark led to the isolation and characterization of three known compounds, (+)-catechin (**1**), (+)-catechin-7-O-β-D-apiofuranoside (**2**), and procyanidin B3 ((+)-catechin-(4α→8)-(+)-catechin) (**3**). Their bioactivities including antioxidant (DPPH ree radical scavenging assay) activity, anti-inflammatory (nitric oxide inhibition assay) were evaluated. Most of the crude extracts and isolates indicated significant antioxidant potential compared with BHT and α-tocopherol as controls. Futhermore, all compounds showed higher inhibitory activities for NO production in Raw 264.7 cells than the L-NMMA using the positive control. Eespecially, (+)-catechin (**1**) and (+)-catechin-7-O-β-D-apiofuranoside (**2**) which could inhibit more than 90% of the NO production at a concentration of 100 μg/㎖, implying excellent anti-inflammatory activity.

Keywords : antioxidant, anti-inflammatory, (+)-catechin derivatives, *Ulmus davidiana* var. *japonica*

1. INTRODUCTION

Ulmus davidiana var. *japonica* Nakai (Ulmaceae) is a deciduous tree that is widely distributed in Korea and Japan. Its stem and root bark have been used as a traditional oriental medicine for the treatment of edema, mastitis, gastric cancer, inflammation and rheumatoid arthritis (Lee, 1996). Research on the phytochemical composition of *U. davidiana* var. *japonica* has reported isolation of flavonoids from the root bark (Son *et al*., 1989), glycoproteins, terpenoids, lignan and neolignan glycosides from the stem and bark (Lee *et al*., 2005; Lee *et al*., 2001).

Pharmacological studies have shown that the extract of this species has anti-inflammatory

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(Kim *et al*., 2007), anti-cancer (Lee *et al*., 2005), anti-angiogenic (Jung *et al*., 2007), neuroprotective activity (Lee and Kim, 2001), helps with gastroenteric disorder (Choi *et al*., 2006), and enhances the immunocompetent properties (Lee *et al*., 2006).

Historically, cosmetics have focused on developing products based on synthetic chemical ingredients. Long term usage may result in issues with skin health. Current consumer trends towards healthier lifestyles have resulted in a shift in preference from synthetic to natural based products. This has lead to a need for natural additives and ingredients for use as cosmetic ingredients.

The aim of the present study was to investigate the potential of extracts and several isolated compounds from the stem bark of *U. davidiana* var. *japonica* to be used as cosmetic ingredients by examining their bioactivity.

2. MATERIALS AND METHODS

2.1. Reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), dimethyl sulfoxide (DMSO), iso-propanol and α-tocopherol were purchased from Sigma Chemicals (St. Louis, MO, USA). Lipopolysaccharide (LPS), N^G-monomethyl-L-arginin (NMMA), Griess reagent (equal volumes of 1% sulfanilamide in 5% phosphoric acid and 0.1% naphtylethylenediamine-HCl), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), Dulbecco's modified Eagle medium (DMEM), 10% fetal bovine serum (FBS), penicillin (100 U/㎖), streptomycin (100 μg/㎖) and phosphate buffered saline (PBS) were purchased from Gibco Laboratories (Gaithersburg, MD, USA). All other chemicals and reagents were analytical grade.

2.2. Plant Materaials

The stem bark of *U. davidiana* var. *japonica* was collected from the experimental forest at the Forest Research Institute, Gangwon Province in January of 2005. Collected bark (WSE0513) was processed at the Herbarium of Department of Forest Biomaterials Engineering, Kangwon National University. Samples were air-dried at room temperature for 3 weeks, and then ground to fine powder for further use.

2.3. General Experimental Procedures

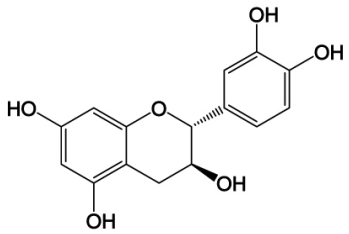
NMR spectra were obtained by a Bruker Avance DPX 400 MHz NMR spectrometer using CD_3OD. FAB-MS was recorded with a Micromass Autospec M363 Spectrometer at the Central Laboratory, Kangwon National University.

TLC was performed on 25 DC-Plastikfolien Celulose F (0.25 mm, Merck) and developed with *t*-BuOH-HOAc-water (3:1:1, v/v/v) or HOAc-water (3:47, v/v). Visualization was done by illuminating ultraviolet light (254 and 365 nm) or by spraying 1% $FeCl_3$ (in EtOH) and vanillin-HCl-EtOH (60:0.15:6, w/v/v) followed by heating.

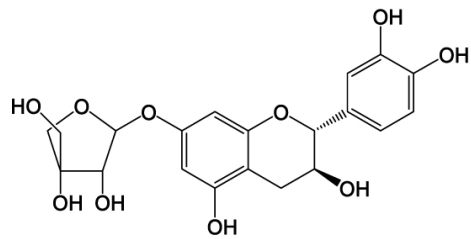
2.4. Isolation and Identification

Ground bark samples (2.8 kg) were extracted with 95% EtOH at room temperature. Extracts were combined, filtered and evaporated under reduced pressure. The residue was sequentially partitioned with *n*-hexane, CH_2Cl_2, EtOAc, and H_2O using a separatory funnel. Each fraction was concentrated and freeze dried to give 28.05 g, 3.69 g, 25.94 g and 177.17 g, respectively.

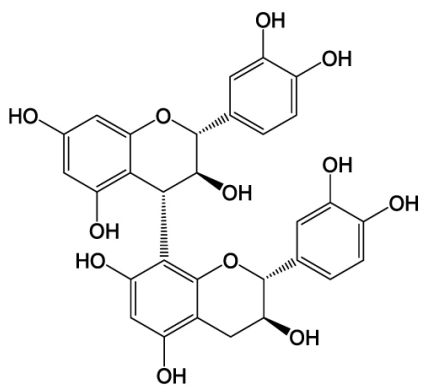
A portion of the resulting EtOAc soluble (20 g) powder was applied to a Sephadex LH-20 (Pharmacia, Sweden) column eluting with MeOH-H_2O (3:1, v/v) to give 7 fractions (E1-E7). The H_2O soluble fraction (37 g) was subjected to Sephad-



(+)-catechin (**1**)



(+)-catechin-7-O-β-D-apiofuranoside (**2**)



procyanidin B3 ((+)-catechin-(4α→8)-(+)-catechin) (**3**)

Fig. 1. Structures of the compounds isolated from *U. davidiana* var. *japonica* stem bark.

ex LH-20 column chromatography using MeOH-H_2O (1:1, v/v) to give 6 fractions (W1-W6).

Fraction E5 was reapplied to a Sephadex LH-20 column with MeOH-H_2O (1:1 and 1:2, v/v) to give 1.87 g of compound (**1**). Fraction E3, E5 and W5 were reapplied to the column and eluted with MeOH-H_2O (1:1, v/v) to yield 13.94 g of compound (**2**). Fraction E6 and E7 were again reapplied and eluted with MeOH -H_2O (1:2, v/v) to yield 59 mg of compound (**3**).

2.5. Antioxidant Activity (DPPH Radical Scavenging Assay)

The antioxidant activity of individual samples was determined on the basis of their radical scavenging activity in the presence of DPPH free radical using the method introduced by M. S. Blois (Blois, 1958) with slight modification. Samples of different concentrations (20～160 μg /㎖) were added to a solution of DPPH (0.15 mM) in 4 ㎖ MeOH. After mixing gently and standing at room temperature for 30 min, the optical density was measured at 517 nm with a UV-visible spectrophotometer (Libra S32, Biochrom LTD). The results were calculated by taking the mean of all triplicate values. IC_{50} values were obtained through extrapolation from concentration of sample necessary to scavenge 50% of the DPPH free radicals. BHT and α-tocopherol were used as controls.

Table 1. IC_{50} values of antioxidant activity of the crude fractions and compounds **1-3**

	Samples	IC_{50} (μg/mℓ)
Controls	BHT	30.0
	α-Tocopherol	26.0
Fractions	Crude extract	15.5
	n-Hexane fraction	19.5
	CH_2Cl_2 fraction	23.1
	EtOAc fraction	11.9
	H_2O fraction	12.2
Compounds	(+)-Catechin (**1**)	11.6
	(+)-Catechin-7-O-β-D-apiofuranoside (**2**)	14.3
	Procyanidin B3 (**3**)	15.7

2.6. Anti-inflammatory Activity (NO assay)

Nitric oxide (NO) inhibition activity was measured in the culture medium by Griess reaction (Green *et al*., 1982). Raw 264.7 (mouse macrophage) cells were incubated at 37°C in DMEM medium supplemented with 10% FBS, penicillin (100 U/mℓ) and streptomycin (100 μg /mℓ) in a humidified atmosphere of 5% v/v CO_2 with lipopolysaccharide (LPS) 0.1 μg/mℓ. Cells were seeded in the growth medium (1 mℓ) into 96-well plates (1×10^6 cells per each well) and incubated for 24 hr. The test samples were dissolved in DMSO and adjusted to sample concentrations 20, 50 and 100 μg/mℓ by diluting with the growth medium. 50 μM of positive control L-NMMA was treated with same method. After 24 hr of incubation at 37°C, 50 μl of cell culture medium was mixed with 50 μl of Griess reagent and incubated for 10 min, and then the absorbance at 540 nm was measured in ELISA reader (Sunrise-basic Tecan, Austria).

3. RESULTS and DISCUSSION

The 95% v/v EtOH extract of the bark of *U. davidiana* var. *japonica* was successively partitioned with *n*-hexane, CH_2Cl_2, EtOAc, and H_2O. A portion of both EtOAc and H_2O fractions were further fractionated by successive column chromatography (Sephadex LH-20), yielding compounds (**1-3**).

The three known natural products, (+)-catechin (**1**), (+)-catechin-7-O-β-D-apiofuranoside (**2**) and procyanidin B3 (**3**) were identified by comparing their physical and spectral data with literature values (Park *et al*., 1996; Brandon *et al*., 1982). The major constituent of the bark extract was (+)-catechin-7-O-β-D-apiofuranoside.

Antioxidant ability plays an important role in the defense mechanism against harmful effects from oxygen free radicals in the cell. The antioxidant activity of each crude fraction and isolate was evaluated with the DPPH assay. The purple color rapidly bleached when DPPH encountered any radical scavengers (Choi *et al*., 2005). As shown in Table 1, all of the fractions and isolates indicated strong antioxidant activity

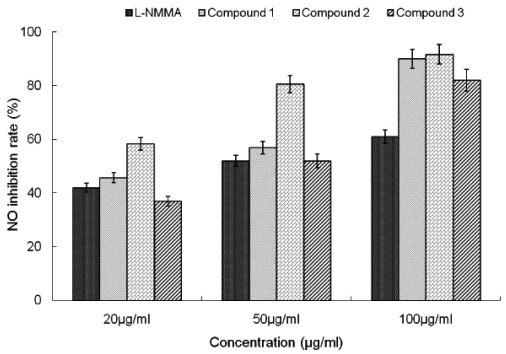


Fig. 2. NO inhibition effects of the compounds **1-3** at 20, 50 and 100 μg/㎖ on NO production in LPS-stimulated Raw 264.7 cells. Experiments were performed in triplicate.

compared with BHT and α-tocopherol, which were used as controls. Compounds **1-3** showed high antioxidant potentials with IC_{50} values of 11.6, 14.3, and 15.7 μg/㎖, respectively. EtOAc and H_2O soluble fractions also showed high activities suggesting a synergistic effect of the isolated compounds.

The LPS-stimulated Raw 264.7 cell assay was used to evaluate the NO inhibition activity of the isolates. As shown in Fig. 2, pretreatment of Raw 264.7 cells with samples showed concentration-dependent inhibition of NO production. All compounds showed higher inhibitory activities for NO production than the L-NMMA using the positive control. (+)-Catechin (**1**) and (+)-catechin-7-O-β-D-apiofuranoside (**2**) which could inhibit more than 90% of the NO production at a concentration of 100 μg/㎖, implying excellent anti-inflammatory activity. This observation is consistent with previous report that the sesquiterpenes from this plant inhibited LPS-induced NO production in murine microglial BV2 cells (Kim *et al*., 2007) and supports its traditional role in treating inflammatory diseases.

4. CONCLUSIONS

Phytochemical constituents were isolated from the stem bark of *U. davidiana* var. *japonica* through open column chromatography. Their structures were identified as (+)-catechin (**1**), (+)-catechin 7-O-β-D-apiofuranoside (**2**), and procyanidin B3 (**3**) on the basis of spectroscopic analysis. Antioxidant activity fo each crude fraction and isolated compounds showed higher than that of the controls. In anti-inflammatory test, (+)-catechin (**1**) and (+)-catechin-7-O-β-D-apiofuranoside (**2**) which could inhibit more than 90% of the NO production at a concentration of 100 μg/㎖, implying excellent anti-inflammatory activity. Accordingly, these results demonstrate that *U. davidiana* var. *japonica* contaning catechin derivatives have promising potential as new additives for the development of natural cosmetics. Investigation of further growth inhibition of cancer cells (human melanoma and human fibrosarcoma cell) are now in progress.

ACKNOWLEDGEMENTS

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