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Antioxidative Activities and Tyrosinase Inhibitory Effects of Korean Medicinal Plants

Seong-Il Heo, Mee-Jung Jung, Min-Kyeong Kim and Myeong-Hyeon Wang*

School of Biotechnology, Kangwon National University, Chuncheon 200-701, Korea

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To discover the sources with antioxidant and tyrosinase inhibitory activities in Korean traditional medicines, 10 extract of medicinal plants were screened for their potential to scavenge stable 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical, inhibit hydroxyl radical (·OH), total phenolic content, and inhibition of tyrosinase. The potency of DPPH radical scavenging activity was shown in the extract of *Ulmus davidiana* var. *japonica* Nakai that has a greater effect with IC_{50} values of 6.49 ± 5.43 μg/mL, than BHA (IC_{50} = 20.99 ± 0.74 μg/mL), L-ascorbic acid (IC_{50} = 20.59 ± 1.06 μg/mL), and α-tocopherol (IC_{50} = 25.55 ± 0.26 μg/mL) as a positive control. The ·OH scavenging activities were observed in the plants tested. *Acanthopanax senticosus, Cirsium setiders, U. davidiana* exhibited scavenging activity of more than 60% at 500 μg/mL. The scavenging activity (%) of BHA and a-tocopherol were 64.32 and 55.87% at 500 μg/mL, respectively. The total phenolic content was determined, in order to assess its effect on the extract's antioxidant activity. The total phenoic content of 33.37 ± 0.52 mg/g was conformed by methanolic extract of *U. davidiana*. The *U. davidiana* and *Morus bombycis* exhibited tyrosinase inhibitory activity with a 34.28 ± 1.32 and 75.57 ± 1.10%, respectively. In particular, *M. bombycis* has stronger tyrosinase inhibitory activity than albutin with 36.48 ± 3.56% as a positive control. This work showed that the inhibitory abilities of Korean medicinal plants, such as *U. davidiana* and *M. bombycis*, on DPPH free radical, inhibit hydroxyl radical (·OH), and inhibition of tyrosinase and total phenolic content, can be useful in the prevention and treatment of free radical-relate disease. Investigations into further isolation of inhibitory principles of *U. davidiana* and *M. bombycis* are now in progress.

Key words: *antioxidant activity, 1,1-diphenyl-2-picryhydrazyl, hydroxyl radical, total phenolic content, tyrosinase*

Two neutral free radicals, H·(hydrogen atom) and ·OH (hydroxyl radical), and charged free radicals can undergo reaction to produce other radicals or reactive species as followed; singlet oxygen ($^1O_2$), superoxide anion ($\cdot O_2^-$), peroxy radicals (ROO·), ·OH, hydrogen peroxide (H_2O_2), and organic hydroperoxides (ROOH). Many essential biochemical reactions, for example, prostaglandin synthesis, peroxidase action, and phagocytosis proceed via free radicals. On the other hand, they are implicated in disease and toxic reactions, for example, in the toxicity of bipyridyl herbicides, radiobiological effects, effects of cigarette smoke, ischemic heart disease, and cancer [Miquel, 1989].

Reactive oxygen species (ROS) such as hydroxyl radical, hydrogen peroxide, superoxide anion, and singlet oxygen have been known to be capable of chemically altering all majer classes of biomolecules including lipids, proteins, and nucleic acids, thus leading to change of their structures and functions [Simonian *et al.*, 1996; Waddington *et al.*, 2000; Kohen *et al.*, 2002]. That leads to lipid peroxidation, DNA and protein damages which result in various diseases, including inflammation, cancer, Parknson's disease, cardiovascular disease, multiple sclerosis, lupus, and aging [Halliwell, 1994; Waddington *et al.*, 2000; Kohen *et al.*, 2002]. ROS might be scavenged by antioxidants derived from natural source, mainly from plant kingdom [Halliwell *er al.*, 1995; Pietta, 2000].

Tyrosinase (EC 1.14.18.1), which is known as polyphenol oxidase (PPO) [Mayer, 1987; Whitaker, 1981], is a multifunctional, copper-containing oxidase that catalyzes three distinct reactions of melanin synthesis, hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), oxidation of DOPA to dopaquinone and the conversion of 5,6-dihydroxyindole to melanochrome. Tyrosinase is

*Corresponding author
Phone: +82-33-250-6486; Fax: +82-33-241-6480
E-mail: mhwang@kangwon.ac.kr

found in microorganisms, animals and plants, and is known to cause the browning of some fruits, vegetables, and crustaceans, which significantly decreases their nutritional and market values. The enzymatic oxidation of L-tyrosine to melanin is of considerable importance because melanin has many functions, and alterations in melanin synthesis occur in many disease states so that tyrosinase is a key enzyme in the insect molting process. Tyrosinase inhibitors might ultimately provide clues for controlling insect pests [Andersen, 1979]. Tyrosinase inhibitors have become increasingly important in cosmetic and medical products related to hyperpigmentation [Kim *et al.*, 2002; Pérez-Bernal *et al.*, 2000]. Tyrosinase might be central to dopamine neurotoxicity as well as contributing to the neurogeneration that is associated with Parkinsons disease [Xu *et al.*, 1997].

The researchers have investigated powerful antioxidants and cold creams from natural sources, edible or medicinal plants replace synthetic compounds, which may be carcinogenic and harmful to the lungs and liver [Ames *et al.*, 1990; Branen, 1975]. There are few reports on DPPH radical and ·OH scavenging activities, and tyrosinase inhibitory activities of plants [Jung *et al.*, 2006; Thuong *et al.*, 2006; Na *et al.*, 2003; Kim *et al.*, 2006; Choi *et al.*, 2001]. In this paper, the medicinal plants, traditional uses in ethnomedicine and the results obtained from antioxidant and tyrosinase inhibition screening are presented and discussed.

Materials and Methods

Materials. The plant samples were purchased around in Kangwon areas. Each of the plants dried at room temperature in the shade, and then was extracted with MeOH at room temperature for two days. Two hundred gram of each samples were extracted with 1 L MeOH and repeated twice. The extracts were filtered and evaporated using a vacuum rotary evaporator at 50°C.

DPPH radical scavenging activity. The antioxidant activity was determined on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picryhydrzyl (DPPH) free radical by a described method with a slight modification [Amarowicz *et al.*, 2000]. An aliquot of 100 μL of 0.2 mM DPPH solution in methanol and 100 μL extract at various concentrations were mixed. The mixture was shacked vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 515 nm with an ELISA reader (ELx800TM, BioTek, USA). The free radical scavenging activity was expressed as followed:

$$\text{DPPH scavenging activity (\%)} = \{(Ac-As)/(Ac-Ab)\}\times 100$$

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank. The IC_{50} values were defined as the concentration that could scavenge 50% DPPH free radical. L-ascorbic acid, á-tocopherol and 2(3)-*t*-Butyl-4-hydroxyanisole (BHA) were used as positive control.

Hydroxyl radical scavenging assay. Hydroxyl radical scavenging activity was determined according to the method with modification [Chung *et al.*, 1997]. 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 sample and 1 mL of 0.1 M phosphate buffer (pH 7.4) were mixed together and made the total volume of 1.8 mL. Thereafter, 200 μL 10 mM H_2O_2 was added and the reaction mixture incubated at 37°C for 4 hr. After incubation, 1 mL of 2.8% tricaboxylic acid (TCA) and 1 mL of 1% TBA were mixed and placed in a boiling water bath for 10 min. The mixture was centrifuged (5 min, 800 × g) and the absorbance was measured at 532 nm with a UV-vis spectrophotometer.

Determination of total polyphenolic content. Total phenolic content was determined by method [Yen *et al.*, 1993]. 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 765 nm was determined after incubation at room temperature for 30 min using a UV-vis spectrometer. A tannic acid standard curve was obtained for the calculation of phenolic content.

Tyrosinase inhibitory activity. Tyrosinase inhibitory activity was measured according to the method [Kubo *et al.*, 1999] with modification. Forty micro liter of samples dissolved in methanol and 80 μL of 0.1 M phosphate buffer (pH 6.8) and 40 μL mushroom tyrosinase (125 U/mL) that incubated at 25°C for 5 min. After incubation, the mixed solution was added 40 μL of 2.5 mM 3,4-dihydroxypenylalanine (L-DOPA). The amount of dopachrome formed was determined by measuring the optical density (OD) at 490 nm by an ELISA reader (ELx800TM, BioTek, USA), and L-ascorbic acid was used ad standard agent. The percentage of inhibition of tyrosinase was calculated as followed:

$$\text{Inhibition (\%)} = \{(Ac - As)/(Ac - Ab)\} \times 100$$

Where Ac was the absorbance of the control, As was the absorbance of the sample and Ab was the absorbance of the blank.

Results and Discussion

It is well known that ROS are considered to be implicated in many diseases and aging [Halliwell *et al.*, 1984; Vishwanath, 1995; Barja, 2002; Sohal *et al.*, 2002]. Accordingly, antioxidants are expected to play a role in the prevention and treatment of various diseases caused by ROS.

DPPH free radical scavenging activity. The free radical scavenging activity of plant extracts was evaluated, and the results are shown in Table 1. The *U. davidiana*, with IC_{50} value of 6.49 ± 5.43 μg/mL, had a greater effect than BHA (IC_{50} = 20.99 ± 0.74 μg/mL), L-ascorbic acid (IC_{50} = 20.59 ± 1.06 μg/mL), and α-tocopherol (IC_{50}= 25.55 ± 0.26 μg/mL) as positive control.

Hydroxyl radical scavenging activity. The hydroxyl radical is so reactive that it can damage biomolecules by direct oxidation such as the hydroxylation of aromatic amino acids and the oxidation of thiols, and induce lipid peroxidation. Hydroxyl radicals generated by the Fenton system (Fe^{3+}/H_2O_2/EDTA) attack deoxyribose to form malondialdehyde (MDA)-like thiobabituric acid and trichloroacetic acid reactive substances [Chung *et al.*, 1998]. As shown in Table 2, *Acanthopanax senticosus, Cirsium setiders, U. davidiana* exhibited 60% inhibitory activity compare to that of the BHA and α-tocopherol were 64.32 and 55.87% at 500 μg/mL, respectively.

Total phenolic contents. It has been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds [Velioglu *et al.*, 1998]. BHT (butylated hydroxytoluene) and tannin, are known to be effective antioxidants. Table 3 shows the phenols concentration in the medicinal plant extracts, expressed as mg of tannin equivalents (TA) per gram dry weight (g DW). The content of total phenols in methanolic extract from *U. davidiana* was 33.37 ± 0.52 mg/g.

Tyrosinase enzyme inhibitory activity. The tyrosinase

Table 1. DPPH radical scavenging activities of various plant extracts

Sample names	Part[a]	IC_{50} (μg/mL)[b]
Acanthopanax senticosus	FT	424.95 ± 15.32
Cirsium setiders	LF	229.82 ± 10.32
Cirsium setiders	RT	258.01 ± 13.24
Ulmus davidiana	SB	6.49 ± 5.43
Eucommia ulmoides	SB	1106.16 ± 104.62
Aster ciliosus	AG	367.33 ± 52.92
Aster ciliosus	BG	578.47 ± 25.27
Sonchus asper	AG	253.76 ± 2.34
Morus bombycis	RT	252.89 ± 4.54
Picrasma quassioides	ST	432.98 ± 13.06
Positive controls		
BHA		20.99 ± 0.74
L-Ascorbic acid		20.59 ± 1.06
α-Tocopherol		25.55 ± 0.28

[a]Sample used: FT (fruit), LF (leaves), SB (stem bark), AG (above ground part), BG (bellow ground part), and ST (stem).
[b]Values represent the mean ± SD (n = 3).

Table 2. Hydroxyl radical (·OH) scavenging effects of various plant extracts

MeOH extracts[a]	Part[b]	HO scavenging activity (%)[c]
Acanthopanax senticosus	FT	84.51 ± 4.21
Cirsium setiders	LF	64.32 ± 3.89
Cirsium setiders	RT	53.99 ± 4.10
Ulmus davidiana	SB	72.30 ± 3.56
Eucommia ulmoides	SB	48.36 ± 2.87
Aster ciliosus	AG	44.13 ± 3.96
Aster ciliosus	BG	40.38 ± 2.84
Sonchus asper	AG	54.46 ± 5.24
Morus bombycis	RT	32.51 ± 3.63
Picrasma quassioides	ST	51.64 ± 3.91
Positive controls		
α-Tocopherol		55.87 ± 1.17
BHA		64.32 ± 2.11

[a]Sample concentration was 500 μg/mL and α-tocopherol and BHA ant the concentration of 50 μg/mL were used as controls.
[b]Sample used: FT (fruit), LF (leaves), SB (stem bark), AG (above ground part), BG (bellow ground part), and ST (stem).
[c]Values represent the mean ± SD (n = 3).

Table 3. Total phenol contents of various plant extracts

MeOH extracts	Part[a]	Total phenolic (mg TA/g)[b]
Acanthopanax senticosus	FT	7.28 ± 1.11
Cirsium setiders	LF	11.75 ± 0.68
Cirsium setiders	RT	14.70 ± 0.30
Ulmus davidiana	SB	33.37 ± 0.52
Eucommia ulmoides	SB	5.70 ± 0.59
Aster ciliosus	AG	11.51 ± 0.86
Aster ciliosus	BG	6.47 ± 1.46
Sonchus asper	AG	11.90 ± 0.46
Morus bombycis	RT	12.66 ± 0.52
Picrasma quassioides	ST	10.32 ± 0.22

[a]Sample used: FT (fruit), LF (leaves), SB (stem bark), AG (above ground part), BG (bellow ground part), and ST (stem).
[b]Values represent the mean ± SD (n = 3).

Table 4. Tyrosinase inhibitory activities of various plant extracts

MeOH extracts	Part[a,b]	Tyrosinase inhibitory activity (%)[c]
Acanthopanax senticosus	FT	16.27 ± 3.73
Cirsium setiders	LF	16.48 ± 3.56
Cirsium setiders	RT	10.58 ± 1.64
Ulmus davidiana	SB	34.28 ± 1.32
Eucommia ulmoides	SB	10.07 ± 4.83
Aster ciliosus	AG	7.80 ± 2.08
Aster ciliosus	BG	6.27 ± 6.54
Sonchus asper	AG	13.93 ± 1.49
Morus bombycis	RT	75.57 ± 1.10
Picrasma quassioides	ST	5.25 ± 1.94
Positive controls		
L-Ascorbic acid		91.49 ± 5.38
α-Tocopherol		36.27 ± 3.73
Albutin		36.48 ± 3.56

[a]Sample used: FT (fruit), LF (leaves), SB (stem bark), AG (above ground part), BG (bellow ground part), and ST (stem).
[b]Sample concentration was 1000 μg/mL and L-ascorbic acid, α-tocopherol and albutin at the concentration of 1000 μg/mL were used as controls.
[b]Values represent the mean ± SD (n = 3).

inhibitory activity of medicinal plants, as shown in Table 4, was determined using L-tyrosine as a substrate. *U. davidiana and Morus bombycis* exhibited tyrosinase inhibitory activity with a 34.28 ± 1.32 and 75.57 ± 1.10%, respectively. *M. bombycis* was a stronger tyrosinase inhibitor than albutin with 36.48 ± 3.56% as a positive control. *Ulmus davidiana* var. Japonica Nakai (*Ulmaceae*) has been used for treatment of edema, theumatoid arthritis, and cancer in Korea [Lee, 2001]. This plant is a producer of antibacterial flavonoids, such as catechin, catechin rhamnoside, and chtechin apiofuranoside, used for antioxidant [Kim *et al.*, 2003]. *Cirsium setidens* Nakai (*Compositae*) a perennial herb, is distributed mainly in Kangwon province, Korea, and *Cirsium* species have been used to treat edema, bleeding and hemoptysis. Flavonoids, aplotaxane and furan derivatives were reported form Cirsium species [Lee *et al.*, 2002]. *Morus bombycis* Koidzumi (MK) is widely distributed in Asia and is used in traditional medicine on account of its apparent anti-inflammatory, antibiotic, and antioxidant effects, and its lowering of blood hyperlipemia [Jin *et al.*, 2005]. *U. davidiana, C. setidens* and *M. bombycis* exhibited potent scavenging activities both on the DPPH, ·OH and tyrosinase.

Free or non-free radicals including ROS and RNS cause a variety of diseases such as inflammation, cardiovascular diseases, cancer, Alzheimers disease, rheumatoid arthritis, and atherosclerosis [Beckman *et al.*, 1996; Podrez *et al.*, 1999]. These diseases have been reported to be ameliorated by radical scavenger [Aruoma, 1999; Hermann *et al.*, 1999]. Therefore, the medicinal plants tested with radical scavenging and tyrosinase inhibitory activities can be useful in the prevention and treatment of free radical and tyrosinase related disease. With further study, we may be able to find potential beneficial effects, active components and action mechanism of the medicinal plants to prevent and treat the free radical and tyrosinase related disease.

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