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Screening for Antioxidant Activity of Plants and Marine Algae and Its Active Principles from *Prunus davidiana*

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Abstract—The antioxidant activity of methanol extracts of plants and marine algae was tested by using 1, 1-diphenyl-2-picrylhydrazyl(DPPH). Five plant extracts(*Prunus davidiana*, *Eriobotrya japonica*, *Artemisia iwayomogi*, *Stirodella tolyrrhiza* and *Ulmus davidiana*) and two algae (*Ecklonia stolonifera* and *Symphycoladia latiuscula*) were found to be the most effective in DPPH radical scavenging activity. The methanol extract obtained from the stems of *Prunus davidiana* was fractionated with several solvents. The ethylacetate soluble fraction exhibiting the strongest antioxidant activity was further purified by repeated silica gel and Sephadex LH-20 column chromatography. Antioxidant flavonoids and flavonoid glycosides were isolated and the most active ones was identified as (+)-catechin by MS, ^{1}H-NMR and ^{13}C-NMR. Its antioxidant activity was higher than that of vitamin C.

Keywords—antioxidant activity • natural antioxidants • plant extracts • algae extracts • flavonoid

Active oxygen species such as superoxide radicals, hydrogen peroxide and hydroxyl radicals has been recognized as the principal agent responsible for the deterioration of polyunsaturated fatty acids, or lipid containing foods when exposed to air[1].

Lipid peroxidation is strongly associated with aging and carcinogenesis[2~4]. Even though living systems are protected from active oxygen species by enzymatic inactivation systems, dietary antioxidants such as α-tocopherol and ascorbic acid may be effective in protection from peroxidative damage.

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants for food because of their excellent effects and low cost. Recently, it has been reported that synthetic antioxidants such as BHA and BHT are carcinogenic, so these antioxidants have been used less frequently although they have high antioxidant activity[5,6]. At present, natural antioxidants such as α-tocopherol and L-ascorbic acid are widely used because they are considered safer and have been linked with fewer adverse reactions. The antioxidant activities of α-tocopherol and L-

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ascorbic acid are, however, lower than those of synthetic antioxidants such as BHA and BHT. Hence, there is a pressing need to find safe, economic antioxidants with high antioxidant activity to replace these synthetic chemicals for the natural sources. Especially, the antioxidant compounds present in edible plants have recently been received as food additives because of a toxic side effect in synthetic antioxidants.

Although several measurements of antioxidant potency have been performed, a comparison of the extracts is often not possible because the extracts differ their property. It is reported that free radical scavengers that occur naturally and may be used as food additives or drugs (*e.g.* flavonoids) might also synergistically interact with vitamins C and E[7]. To evaluate the antioxidant activity, the present study was initiated to compare the radical scavenging effect of the plant extracts and algae on the stable free radical 1, 1-diphenyl-2-picrylhydrazyl(DPPH)[8], which shows a strong absorption band at 517 nm. This paper further extends the isolation of the antioxidant principles from the methanol extracts of *Prunus davidiana* stems used as a Korean folk medicine for treatment of neuritis and rheumatism.

Experimental Methods

Chemicals—DPPH, ascorbic acid and BHT (buthylated hydroxytoluene) were reagent grade, purchased from Sigma. All other reagents were of the highest grade commercially available.

Measurement of antioxidant activity—An 4 ml of MeOH solution of test extracts at various concentrations(2.5~120 μg/ml) was added to a solution of DPPH(1.5×10^{-4}M) in MeOH(1 ml), and the reaction mixture was shaken vigorously. After storage at room temperature for 30 min in air, remaining DPPH was determined by spectrophotometry at 517 nm, and the radical scavenging activity of each sample was expressed by the ratio of lowering of the absorption of DPPH(%), relative to the absorption(100%) of DPPH solution in the absence of test sample(control). The mean values were obtained from triplicate experiments.

Plants and algae—Plants and marine algae used in this experiment were identified by the authors. Voucher specimens are deposited in the Herbarium of College of Pharmacy, Pusan National University, Pusan, Korea.

Preparation of MeOH extract—Dried plants and marine algae were extracted with MeOH under refluex. The extracts were concentrated to dryness *in vacuo* at 40°C to produce the methanol extracts.

Fractionation and isolation of flavonoids from *Prunus davidiana*—The MeOH extract was partitioned with $CHCl_3$, EtOAc, BuOH and H_2O, sucessively. Flavonoids were isolated from the EtOAc fraction according to the procedure of Choi *et al.*[9,10] and identified by direct comparison with authentic samples(mp, IR, ^{1}H-NMR and ^{13}C-NMR).

Apparatus—Ultraviolet absorption spectrum was measured with a Shimadzu Double Beam spectrophotometer.

Results and Discussion

Antioxidant activity of plants and algae

A methanol solution of DPPH was found to be stable for over 60 min by spectrophotometry at 517 nm of an 80 μg/ml solution. The radical scavenging effects of some medicinal plant extracts and seaweeds were then measured by spectrophotometry of DPPH radical. The control intensity(absence of extracts) was taken as 100%, and the percentage intensity was calculated. The concentration for 50% inhibition is shown in Table I.

As shown in Table I, some medicinal plant

Table I. Effects of several plants on DPPH

Plants	Part	RC_{50}[a] (μg)
Prunus davidiana	stem	21.4
Cassia tora	seed	299.6
Spinacia oleracea	whole plant	159.7
Elaegnus crispa	leaf	261.9
Ixeris sonchifolia	root	439.6
Ixeris sonchifolia	leaf	139.4
Phragmites communis	rhizome	480<
Allium monanthum	whole plant	168.8
Boehmeria frutescens	leaf	480<
Ficus erecta	leaf	480<
Eriobotrya japonica	leaf	37.2
Catalpa ovata	stembark	131.8
Artemisia iwayomogi	whole plant	11.8
Artemisia asiatica	whole plant	125.8
Impatiens balsamina	seed	480<
Ulmus davidiana	stem	11.8
Wistaria brachybotrys	lump	134.2
Stirodella tolyrrhiza	whole plant	85.0
Ailanthus altissima	bark	480<
Solanum lyratum	whole plant	172.7
Melia toosendan	fruits	480<
Ascorbic acid		8.1
BHT		9.5

[a] Amount required for 50% reduction of DPPH after 30min.

Table II. Effects of marine algae on DPPH

Seaweeds	RC_{50}[a] (μg)
CHLOROPHYTA	
Enteromorpha erinita	480<
Ulva pertusa	480<
PHAEOPHYTA	
Hizikia fusiformis	335.4
Undaria pinnatifida	480<
Sargassum ringgoldianum	347.8
Sargassum miyabei	480<
Sargassum thunbergii	480<
Ecklonia stolonifera	68.8
RHODOPHYTA	
Gelidium amansii	480<
Carpopeltis cornea	480<
Chondrus cripus	480<
Pachymeniopsis elliptica	480<
Symphycoladia latiuscula	54.2
Grateloupia filicina	480<
Rhodymenia intricata	480<
Acrosorium flabellatum	480<
Gracilaria verrucosa	480<
Gigartina tenella	480<
Ascorbic acid	8.1
BHT	9.5

[a] Amount required for 50% reduction of DPPH after 30 min.

extracts such as *Prunus davidiana*, *Eriobotrya japonica*, *Artemisia iwayomogi*, *Stirodella tolyrrhiza* and *Ulmus davidiana* examined in this study exhibited somewhat high scavenging effects on DPPH. *Artemisia iwayomogi* and *Ulmus davidiana* showed the strongest effects. These crude MeOH extracts showed activities almost equivalent to those of BHT. This result suggests that these plants contained a strong antioxidant(s). Further comprehensive chemical investigation will be needed to elucidate the exact mechanism of radical scavenging effect and to isolate the active principles responsible.

From the results obtained in Table II, *Symphycoladia latiuscula* and *Ecklonia stolonifera* were found to be potent scavenging effects on DPPH, although this was less marked than the effect of plant extracts. The previous authors reported that the chloroform-soluble fraction extracted from *Undaria pinnatifida* showed excellent antioxygenic activity in their screening test for antioxygenic principles in marine algae[11,12]. These results are contradictory to the our result that the MeOH extract of *U. pinnatifida* did not exhibit the antioxidative activity. The difference in the antioxidant activity might be ascribed to the difference in the method of extraction or concentration of the extract, and measurement of antioxidant potency.

Antioxidant activity of *Prunus davidiana*

The present study was also carried out to investigate the active principles from the methanol extract of *Prunus davidiana*. The methanol extract of *P. davidiana* was partitioned between $CHCl_3$, EtOAc, BuOH and water, successively and the EtOAc-soluble fraction showed strong radical scavenging effect against DPPH(Table **III**). This fraction was further purified to obtain active compounds, (+)-catechin along with several flavonoids. Table **IV** summarized the radical scavenging results of flavonoids on DPPH. Table **IV** shows that (+)-catechin was more active than ascorbic acid or BHT. Previous workers reported that (+)-catechin has been shown to have a powerful free radical scavenging capacity[13] and to inhibit lipid peroxidation in different experimental methods[14~16]. The antioxidative mechanism of (+)-catechin was demonstrated as cytoprotective through the drastic inhibition of Fe^{2+}-induced linoleate peroxidation[17]. Among flavonoids isolated, kaempferol and its glucoside(populnin) revealed especially potent scavenging effect.

Antioxidant activities of various flavonoids are well known. As to flavonoids, the relationship between the position of the hydroxy groups and the antioxidant activity has been discussed[18].

Table III. Effects of methanol extract and their fractions from *Prunus davidiana* on DPPH

Fractions	RC_{50}[a] (μg)
MeOH extract	23.2
$CHCl_3$ fraction	117.1
EtOAc fraction	12.6
BuOH fraction	12.6
H_2O fraction	480<

[a] Amount required for 50% reduction of DPPH after 30 min.

Table IV. Effects of flavonoids isolated from *P. davidiana* on DPPH

Compounds	RC_{50}[a] (μg)
Catechin	4.9
Prunin	480<
Hesperetin	480<
Hesperetin 5-O-glucoside	69.8
Kaempferol	38.9
Dihydrokaempferol	480<
Populnin	30.6
Naringin	480<
Naringenin	480<
Quercetin	4.9
Rutin	8.3
Persiconin	480<
Persicogenin	140.1
BHT	9.5
Ascorbic acid	8.1

[a] Amount required for 50% reduction of DPPH after 30 min.

Quercetin is an effective flavonols in the same ways as morin, kaempferol, and luteolin[7]. Kaempferol and its glucoside(populnin) isolated from the MeOH extract of *P. davidiana* also may be usable as an antioxidant agent. The findings of the present study indicate that the methanolic extract of *Prunus davidiana* stems and its components [(+)-catechin and flavonoids] may be useful for antioxidant.

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