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Screening of Antioxidant Activity of Domestic Trees*1

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ABSTRACT

This study was carried out to investigate the antioxidant activities of domestic trees grown in Korea. Based on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity method, the methanolic extracts of 23 species were screened in order to search for natural antioxidants. Among these species, *Acer ginnala, Cotinus coggygria, Acanthopanax koreanum, Thea sinensis* and *Pinus densiflora* showed stronger antioxidative activity comparing with reference compound, ascorbic acid.

Keywords : Antioxidative activity, DPPH free radical scavenging effect, domestic trees, ascorbic acid

1. INTRODUCTION

Free radicals which have the form of superoxide radical (O_2^-), hydroxyl radical($HO\cdot$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) appear to be an important factor in cellular degeneration involved in aging (Bohm, 1998). Accumulation of potentially harmful oxygen radicals increases with age in a number of species, and can cause cellular changes that could result in the loss of homeostatic control and organ function (Halliwell, 1991). Although many antioxidants are utilized, most antioxidants are synthetic butylated hydroxylanisole (BHA) and butylated hydroxyltoluene (BHT). Some researches about synthetic antioxidants have implicated the widely used synthetic inhibitors in promoting the natural food additives and their use has begun to be restricted due to their toxicity and are suspected to be carcinogenic (Branen, 1975). These results have reinforced interest in natural antioxidants. α-Tocopherol and L-ascorbic acid are effective natural antioxidants but has also limited usage because it is expensive for wide use. Therefore, the importance of search for natural antioxidants, especially of plant origin, has greatly increased in recent year. Jung *et al.* (1999) reported the antioxidant activity of *Eriobotrya japonica* leaf using method the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH). In their study, it was shown that the methanol extract of *E. japonica* and their fractions, and their components, flavonoids and chlorogenic acids, may be useful for the treatment of oxidative damage. Choi *et al.* (1993) screened for antioxidant activity of plants and marine algae and Na *et al.* (2001) studied antioxidative activity of MeOH extracts of 139 crude drugs such as *Nulumbo nucifera, Glycyrrhiza glabra* and *Angelica gigas.*

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In their study, they found that BuOH fraction from the extract of *N. nucifera* showed a potent activity. Choi, *et al.* (1992) also compared antioxidant activity of the crude extracts from 95 species of edible and medical plants. These studies mainly deal with plants which are used in traditional medicine. Because the studies of antioxidative activity for domestic tree are rare, we used woody plants for the surveying of antioxidants. Woods are harmless sources for obtaining natural antioxidants. From woods, many compounds can be extracted by solvents or water and have various biological activities.

In this paper, in search for antioxidants from Korean woody plants, we measured the radical scavenging effects of the extracts of 437 species of domestic tree on the stable free radical DPPH. Among these species, only the methanolic extracts of 23 species were compared the antioxidative activities in order to search for natural antioxidants.

2. MATERIALS and METHODS

2.1. Chemicals

DPPH and ascorbic acid were reagent grade and other reagents were of the highest grade commercially available.

2.2 Preparation of Extracts

Dried and powdered plants were extracted twice with 70% ethanol (EtOH) at room temperature. After the evaporation of the solvent under reduced pressure at 40°C, the crude extracts were obtained. The voucher specimens are deposited at the Korea Forest Research Institute, Suwon, Korea. (Lee *et al.*, 2002)

2.3 Antioxidative Activity Test

The antioxidant activity was measured by the DPPH method according to the procedure of Hatano *et al.* (1989). EtOH solutions (100 μl) of samples at various concentrations (20, 30, 60, 120, 240 and 500 ppm) were added to a solution of DPPH in EtOH (0.1 mM, 1.9 ml) and the reaction mixture were shaken vigorously for 10 sec. After storing mixtures for 30 min at 37°C, the remaining amounts of DPPH were determined by colorimetry (8452A Diode Array Spectrophotometer, Hewlett Packard Co.) at 515 nm. The mixture of 100 μl EtOH with a solution of 1.9 ml DPPH was used as control. Ascorbic acid was used as a positive compound.

3. RESULTS and DISCUSSION

Free radicals are chemical fragments that cause oxidation and antioxidants act as free radical scavengers. DPPH has been known as a free radical which is a purple compound having characteristic absorption at 515 nm in methanol. For this reason, DPPH (1,1-diphenyl-2-picrylhydrazyl) was used for measuring antioxidant activity. Change in absorbance value at 515 nm was investigated to evaluate antioxidation ability of sample. Decrease of the absorbance in test solution represents decrease of free radical type DPPH, that is DPPH reduction, by radical scavenger. In this study, the absorbance value at 515 nm was used to measure the DPPH concentration in testing solution. The purple color defaded when compound donating electron was added to DPPH solution. Fig. 1 showed the mechanisms of these reactions.

DPPH will oxidize and be decolorized by ascorbic acid, Tocopherol and polyhydroxy aromatic compounds. Because it measures the most common natural antioxidants, the DPPH method a convenient one for antioxidant assay of biological materials (Blois, 1958). Additionally, because of the ease and convenience of this reaction it now has widespread use in the free

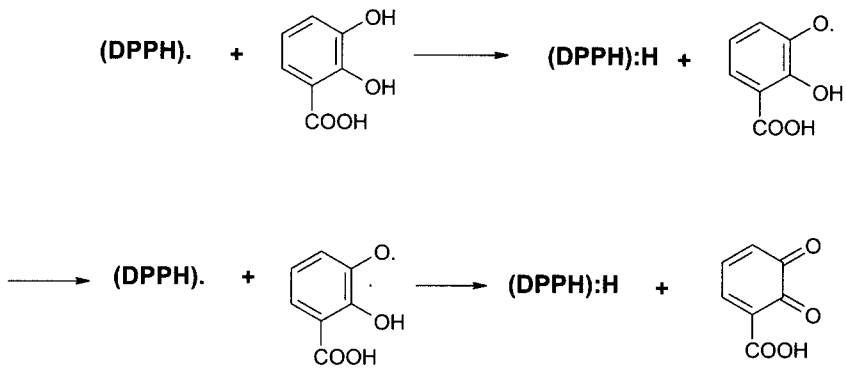

Fig. 1. The mechanisms of the reactions between the DPPH radical and oxidizable groups.

Table 1. Free radical scavenging activity of several hard wood extracts

Species	Family	Parts	IC_{50} (μg/ml)[1)]
Acer geiseum	Aceraceae	Whole Plant	34
Acer ginnala	Aceraceae	Whole Plant	31
Acer psedo-siebolianum	Aceraceae	Whole Plant	49
Cotinus coggygria	Alangiaceae	Whole Plant	29
Acanthopanax koreanum	Araliaceae	Stem	31
Berberis koreana	Berberidaceae	Whole Plant	43
Viburnum erosum var.*taguetii*	Caprifoliaceae	Whole Plant	46
Cercidiphyllum japonicum	Cercidiphyllaceae	Whole Plant	36
Cornus walteri	Cornaceae	Whole Plant	48
Mallotus japonicus	Euphorbiaceae	Whole Plant	58
Erythrina crista galli	Fabaceae	Whole Plant	51
Gymnocladus dioicus	Fabaceae	Whole Plant	39
Castanopsis cuspidata var.*thunbergii*	Fagaceae	Whole Plant	40
Quercus dentata	Fagaceae	Leaf	38
Corylopsis willmotiae cv. Spring Purple	Hamamelidaceae	Whole Plant	40
Distylum racemosum	Hamamelidaceae	Whole Plant	35
Loropetalum chinense	Hamamelidaceae	Whole Plant	56
Osmanthus heterophyllus var. *rotundifolius*	Oleaceae	Whole Plant	38
Prunus leveilleana	Rosaceae	Whole Plant	50
Thea sinensis	Theaceae	Leaf	9
		Stem	17
Ascorbic Acid			34

[1)] Amounts required for 50% reduction of DPPH after 30 min.

radical-scavenging activity assessment. The radical scavenging activities of domestic tree were measured by spectrophotometry of DPPH radical. Free radical scavenging activity of several hard wood extracts expressed by IC_{50}, the concentration for 50% inhibition, was shown in Table 1. In Table 1, the crude extracts from *Acer ginnala* (Amur Maple), *Cotinus coggygria,*

Table 2. Free radical scavenging activity of several soft wood extracts

Species	Family	Parts	IC_{50} (μg/ml)[1)]
Thuijopsis dolabrata cv. *Aurea*	Cupressaceae	Whole Plant	34
		Leaf	236
Pinus densiflora	Pinaceae	Bark	16
		Stem	19
Torreya nucifera	Taxaceae	Stem	38
Ascorbic Acid			34

[1)] Amounts required for 50% reduction of DPPH after 30 min.

Acanthopanax koreanum and *Thea sinensis* (Thea Bohea) exhibited higher scavenging activity on DPPH than that of reference compound, ascorbic acid. Although the antioxidative activities of the extracts from *Acer geiseum, Cercidiphyllum japonicum* (Katsura Tree), *Gymnocladus dioicus, Distylum racemosum Quercus dentata* (Daimyo Oak) and *Osmanthus heterophyllus* var. *rotundifolius* have similar or lower than that of ascorbic acid and these species also have potential possibility for using antioxidants. From the ethyl acetate soluble fraction of *Distylum racemosum* leaf, Park *et al.* (2003) isolated antioxidants such as kaempferol, quercetin, methyl gallate.

Free radical scavenging activity of several soft wood extracts expressed by IC_{50} was shown in Table 2. As shown in Table 2, the results showed that the crude extracts of the bark (16 μg/ml) and stem (19 μg/ml) of *Pinus densiflora,* Japanese red pine, have better antioxidant activities than that of reference compound, ascorbic acid (34 μg/ml). According to the study of Cha *et al.* (1997), the extracts from *P. densiflora* and *Euphorbia humifusa* showed strong antioxidative active. In their study, they suggested that antioxidative activities of EtOAc and BuOH extracts were similar or even higher than that of natural (tocopherol) or synthetic antioxidants (BHA) and concluded that major fraction for the antioxidative activity of *P. densiflora* was the EtOAc and BuOH extract compartments. Since, the study of antioxidant activity was mainly deals with common plants which are used in traditional medicine, the further study of antioxidant compounds from domestic trees which screened in this study.

4. CONCLUSIONS

In search for natural antioxidants from Korean woody plants, we measured the radical scavenging effects of the extracts of 437 species of domestic trees. Among these species, only the ethanol extracts of 23 species were compared the antioxidative activities in order to search for natural antioxidants. Form the results of this study, we can concluded that *Acer ginnala, Cotinus coggygria, Acanthopanax koreanum, Thea sinensis* and *Pinus densiflora* showed stronger antioxidative activity than ascorbic acid which was used reference compound and have potential possibility for using antioxidants.

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