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Evaluation of the Antioxidant Potential of Korean Indigenous Plant Extracts by Free Radical Scavenging Activity

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Abstract – Since reactive oxygen radicals play an important role in carcinogenesis and other human diseases including neurodegenerative states, antioxidants present in natural products have received considerable attention for alleviation of these disease states. Therefore, in order to identify antioxidants in plant extracts, fifty-seven methanolic extracts derived from indigenous Korean plants were primarily assessed for potential to scavenge stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. As a result, nine plant extracts were found to exhibit the DPPH free radical scavenging activity in the criteria of IC_{50} <40 μg/ml. In particular, the extracts of *Melioma oldhami* (IC_{50} = 0.1 μg/ml), *Myrica rubra* (IC_{50} = 16.2 μg/ml), *Sympolocos paniculata* (IC_{50} = 23.0 μg/ml), *Carpinus laxiflora* (IC_{50} = 25.1 μg/ml), and *Cleyera japonica* (IC_{50} = 26.2 μg/ml) showed a potent radical scavenging activity. Further study for the identification of active compounds from these lead extracts might be warranted.

Keywords – DPPH free radical scavenging activity, *Melioma oldhami*, *Myrica rubra*, *Sympolocos paniculata*, *Carpinus laxiflora*, *Cleyera japonica*

Introduction

Free radicals are produced in normal or pathological cell metabolism, from xenobiotics, or through ionizing radiation. Especially, oxygen free radicals (OFR) play an important role in mediating OFR-related effects (Freeman and Crapo, 1982). It is generally believed that membrane lipid peroxidation and nucleic acid damage are induced and thus several disease states are enhanced by reactive oxygen species (ROS) (Halliwell *et al.*, 1992). In recent years, natural product antioxidants have been shown to alleviate these acute or chronic ROS-mediated diseases. Thus, in the course of searching for antioxidants from natural products, we evaluated the in vitro free radical scavenging activity of indigenous Korean plant extracts.

Experimental

Chemicals – 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St. Louis, MO).

Extracts of plant materials tested – Methanolic plant extracts used for this study was purchased from Plant Extracts Bank of Plant Diversity Research Center (Daejon, Korea).

Evaluation of DPPH free radical scavenging activity – Reaction mixtures containing test samples (5 μl, dissolvd in DMSO) and 316 μM DPPH ethanolic solution (95 μl, final DPPH concentration is 300 μM) in 96-well microtiter plates were incubated at 37C for 30 min and absorbance was determined at 515 nm. Percent inhibition by sample treatment was determined by comparison with a DMSO-treated control group. IC_{50} values denote the concentration of sample which is required to scavenge 50% DPPH free radicals (Lee *et al.*, 1998).

Results and Discussion

Oxygen radicals or reactive oxygen species (ROS) such as superoxide anion ($O_2\cdot^-$), hydrogen peroxide (H_2O_2), hydroxy radicals (·OH), and singlet oxygen ($^1O_2$) are continuously generated in cells exposed to an aerobic environment, and have been associated with a diverse of diseases such as carcinogenesis, atherosclerosis, arthritis, and neurodegenerative disorders (Halliwell *et al.*, 1992; Ames *et al.*, 1993; Guyton and Kensler, 1993; Cerutti,

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Table 1. DPPH free radical scavenging potential of plant extracts

Plant name and Authority	Family	Part used[a]	DPPH[b]
Abelia tyaihyoni Nakai	Caprifoliaceae	ST	> 100
Actaea asiatica Hara	Ranunculaceae	WP	> 100
Actinodaphne lancifolia (S. et Z.) Meissn	Lauraceae	TW	47.2
Asperula odorata L.	Rubiaceae	WP	> 100
Caesalpinia japonica S. et Z.	Leguminosae	LS	78.1
Calystegia soldanella Roem. et Schult.	Convolvulaceae	WP	> 100
Capsella bursa-pastoris (L.) Medicus	Cruciferae	HR	> 100
Cardamine amaraeformis Nakai	Cruciferae	WP	> 100
Cardamine flexuosa With	Cruciferae	HR	> 100
Carpinus laxiflora Bl.	Betulaceae	SB	25.1
Cayratia japonica (Thunb.) Gagnep.	Vitaceae	FR	> 100
Celtis choseniana Nakai	Ulmaceae	FR	> 100
Cinnamomum japonicum Sieb.	Lauraceae	SB	38.9
Citrus tachibana (Makino) C. Tanaka	Rutaceae	SB	> 100
Cleyera japonica Thunb.	Theaceae	SB	26.2
Cornus walteri Wagner.	Cornaceae	ST	> 100
Crataegus pinnatifida Bunge	Rosaceae	ST	52.7
Daphniphyllum glaucescens Blume	Euphorbiaceae	ST	> 100
Dystaenia takeshimana (Nak.) Kitagawa	Umbelliferae	HR	> 100
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Erysimum aurantiacum Max.	Cruciferae	LS	> 100
Heloniopsis orientalis (Thunb.) C. Tanaka	Liliaceae	WP	> 100
Hovenia dulcis Thunb.	Rhamnaceae	ST	95.5
Ilex macropoda Miq.	Aquifoliaceae	ST	61.5
Ixeris dentate var. albiflora Nak.	Compositae	RT	> 100
Kirengeshoma koreana Nakai	Saxifragraceae	RT	> 100
Kirengeshoma koreana Nakai	Saxifragraceae	WP	39.5
Lamium album var. barbatum (S. et Z.) Fr. et Sav.	Labiatae	WP	> 100
Lepidium ruderale L.	Cruciferae	WP	> 100
Ligularia fischeri (Ledeb.) Turcz.	Compositae	WP	> 100
Ligustrum japonicum Thunb.	Oleacae	TW	> 100
Lindera obtusiloba Bl.	Lauraceae	LS	64.9
Lonicera maackii Max.	Caprifoliaceae	ST	> 100
Lonicera vidalii Fr. et Sav.	Caprifoliaceae	LF	> 100
Maackia fauriei (Lev.) Takeda	Leguminosae	SB	> 100
Meliosma oldhamii Max.	Sabiaceae	SB	0.1
Mitchella undulata S. et Z.	Rubiaceae	WP	> 100
Myrica rubra S. et Z.	Myricaceae	SB	16.2
Neolitsea aciculate (Bl.) Koidz	Lauraceae	LF	58.3
Osmanthus insularis Koidz.	Oleacae	SB	> 100
Phlomis umbrosa Turcz	Labiatae	WP	> 100
Sambucus sieboldiana Bl.	Caprifoliaceae	SB	> 100
Sorbus alnifolia (S. et Z.) K. Koch	Rosaceae	ST	56.1
Staphylea bumalda Dc.	Staphyleaceae	FR	> 100
Stewartia koreana Nakai	Theaceae	ST	> 100
Symplocos paniculata Miq.	Symplocaceae	LF	23.0
Syringa velutina var. kamibayashi T. Lee.	Oleacae	LS	> 100
Tetragonia tetragonoides O. Kuntze	Aizoaceae	WP	> 100
Thuja koraiensis Nakai	Cupressaceae	LF	65.9
Tiarella polyphylla D. Don.	Saxifragraceae	RT	59.8
Vaccinium bracteatum Thunb.	Ericaceae	SB	54.3
Vaccinium oldhami Miq.	Ericaceae	ST	33.3
Viburnum awabuki K. Koch	Caprifoliaceae	LF	71.0
Viburnum erosum Thunb.	Caprifoliaceae	FR	37.6
Vicia angustifolia var. segetilis K. Koch	Leguminosae	WP	> 100
Wasabia koreana Nakai	Cruciferae	RT	> 100
Wasabia koreana Nakai	Cruciferae	WP	> 100
Ascorbic acid			23.0
Butylated hydroxyanisole (BHA)			20.6
Butylated hydroxytoluene (BHT)			100.0

[a]Part used: FT (Fruit), HR (Herba), LF (Leaf), LS (Leaf+stem), RT (Root), SB (Stem bark), ST (Stem), TW (Twig), WP (Whole plant).
[b]DPPH: DPPH free radical scavenging activity (IC_{50}: μg/ml).

1994; Feig *et al.*, 1994). Therefore, antioxidants have received considerable attention to alleviate these diseases. In the previous study the antioxidant potential of some medicinal plants was evaluated using xanthine oxidase inhibition assay or DPPH free radical scavenging activity assay by our group and others (Nam and Lee, 1999; Na *et al.*, 2001). In the present study we further extended to evaluate the antioxidant potential of additional indigenous Korean plant extracts. Accordingly, the potential antioxidant activity of plant extracts has been assessed based on scavenging DPPH free radicals. Among tested fifty-seven methanolic extracts of plant materials, nine extracts were found to be active as shown in Table 1 as judged in the criteria of IC_{50} <40 μg/ml. In particular, the extracts of *Melioma oldhami* (IC_{50} = 0.1 μg/ml), *Myrica rubra* (IC_{50} = 16.2 μg/ml), *Sympolocos paniculata* (IC_{50} = 23.0 μg/ml), *Carpinus laxiflora* (IC_{50} = 25.1 μg/ml), and *Cleyera japonica* (IC_{50} = 26.2 μg/ml) showed a potent radical scavenging activity. Further study for the identification of active compounds from these lead extracts might be warranted. In addition, since the indigenous Korean plant *Melioma oldhami* showed a strong DPPH free radical scavenging activity (IC_{50} = 0.1 μg/ml) and has not been thoroughly studied for phytochemical investigation, the monitoring of antioxidant principles for this stem bark extract of the plant might be particularly valuable.

In conclusion, for the discovery of novel antioxidants from natural products, we primarily approached and evaluated the DPPH free radical scavenging activity for indigenous Korean plant extracts. Several plant extracts exhibited potential free radical scavenging activity. Therefore, the information from this study will be useful to the isolation of active compounds with antioxidant potential.

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