Scavenging Effect of Plant-Derived Materials on Free Radicals and Active Oxygen Species

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The *in vitro* antioxidative activities of the 33 plant-derived essential oils and 37 phytochemicals including 3 *Mentha arvensis* leaf-, 2 *Thymus vulgaris* leaf- and 2 *Syzygium aromaticum* flower-derived isolates were determined by the inhibition of linoleic acid autoxidation, the generation of superoxide anion and scavenging of DPPH radical. They were then compared to those of the widely used plant-derived antioxidants (pyrogallol and quercetin) and synthetic antioxidant BHT. At a concentration of 0.01%, potent antioxidative effect was observed in the essential oils from *Cinnamomum cassia* roots, *Mentha arvensis* leaves, *Ginkgo biloba* fruits, and *Syzygium aromaticum* flowers. Of the phytochemicals used, eugenol and isoeugenol at 0.01% showed potent antioxidative activity, and their activities were comparable to those of pyrogallol, quercetin, and BHT. The *Cinnamomum* root-, the *Mentha* leaf-, the *Ginkgo* fruit-, and the *Syzygium* flower-derived materials may be a good source for an alternative to the currently used antioxidants.

Key words : *antioxidant,* Cinnamomum cassia, Mentha arvensis, Ginkgo biloba, Syzygium aromaticum, *eugenol, isoeugenol.*

Activated oxygen species such as superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen may cause various disease states such as carcinogenesis, drug-associated toxicity, inflammation, atherogenesis, and aging in aerobic organisms, as well as food deterioration.[1-4] The naturally occurring antioxidants are known to be important for the prevention of spoilage process of foods as well as various diseases in animals. However, many of naturally occurring antioxidants have been limited in their practical usage because of their low effectiveness even though they are considered to be active in eliminating the reactive oxygens and controlling the toxic effects.[5] These economic, health, and environmental concerns have highlighted the need to develop new types of antioxidants.

Plants may be an alternative to currently used antioxidants because they are rich source of bioactive organic chemicals and biodegradable to nontoxic products. Therefore, much effort has been focused on plant-derived materials for potentially useful products as commercial antioxidants. Antioxidants are found in various plant products such as oils, fruits, teas, seeds, cereals, beans and nuts and include flavonoids, tannins, coumarins, curcuminoids, xanthons, phenolics and terpenoids.[4,6-8] Especially, essential oils are known to be a rich source of antioxidants which are mainly composed of monoterpenes, sesquiterpenes and diterpenes. For example, rosemary diterpenes have been found to inhibit the generation of superoxide anion in the xanthine/xanthine oxidase system and microsomal lipid peroxidation.[9] The main diterpenes of rosemary are rosemarinic acid, carnosic acid, and carnosol which have similar or higher antioxidative activity than that of the synthetic antioxidant butylated hydroxytoluene.[9] However, there are no much efforts to identify the relationship between the antioxidative activity and the terpenic skeleton.

In the laboratory studies described herein, we assessed the antioxidative activities of 17 plant-derived essential oils, 16 commercially available essential oils, and 37 phytochemicals to search plant-derived materials for potentially useful products as commercial antioxidants. Additionally, the relationship between the antioxidative activity and the essential monoterpenic skeleton is also discussed.

Materials and Methods

Chemicals. Thirty seven compounds which are found to exist in plant-derived essential oils were purchased from Wako Pure Chemical (Osaka, Japan). Pyrogallol, quercetin, BHT, DPPH, linoleic acid, NBT, and phenazine methosulphate were provided from Sigma Chemical (St. Louis, MO, USA). All other chemicals were of reagent grade.

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Abbreviations: BHT, butylated hydroxytoluene; DPPH, diphenyl-*p*-picrylhydrazyl; NBT, nitroblue tetrazolium.

Plants and essential oil preparation. A total of 17 plant species in 11 families and 16 plant-derived essential oils were purchased from a local market in Seoul (Korea) and Nature Co. (Sydney, Australia), respectively (Table 1). They have been used not only for flavoring foods but also for their antiseptic or medicinal properties. The plant species were dried in an oven at 60°C for 2 days and finely powdered using a blender. The essential oil of each dried plant (200 g) was obtained by steam distillation at 100°C for 5 hr, then the extracts were dehydrated with sodium sulphate. The yield of each essential oil extraction is shown in Table 1.

GC-MS conditions. For the determination of the components from essential oils, a Shimadzu QP-5000 GC/MS system was used. The mass data were analyzed by a Shimadzu CLASS 5000 system. The column conditions were as follows: glass capillary column, CBP5 0.25 mm i.d.×25 m; helium carrier gas flow rate, 0.25 ml/min; split ratio, 50 : 1; temperature program, isotherm 3 min at 100°C, 15°C/min gradient to 250°C, 10°C/min gradient to 280°C, isotherm 20 min; injection port temperature, 250°C; detector temperature, 250°C. The ionizing voltage was 70 eV and the ion source temperature 250°C.

Autoxidation assay. The oxidation of linoleic acid was measured by the method of Haraguchi *et al.*[10] with slight modification. Each sample dissolved in 30 μl ethanol were added to a reaction mixture in a screw cap vial. Each reaction mixture consisted of 0.57 ml of 2.51% linoleic acid in ethanol and 2.25 ml of 40 mM phosphate buffer (pH 7.0). The vial was placed in an oven at 37°C. After 3 day-incubation, 0.1 ml aliquot of the mixture was diluted with 4.65 ml of 75% ethanol, which was followed by adding 0.1 ml of 30% ammonium thiocyanate. At 3 min after the addition of 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm.

Scavenging effects on DPPH radical. DPPH radical scavenging activity was measured by the method of Blois.[11] The reaction mixture consisted of 1 ml of 100 mM acetate buffer (pH 5.5), 1 ml of ethanol and 0.5 ml of 2.5 mM ethanol solution of DPPH. After allowing the mixture to stand at room temperature for 20 min, the absorbance of the remaining DPPH was determined at 517 nm. The scavenging activity was evaluated as the decrease in absorbance.

Table 1. List of plants tested and yield of essential oils extracted by steam distillation.

Plant species	Familiy	Tissue sampled	Yield (%)	Remarks[a]
Adenophora triphylla	Campanulaceae	Leaf	0.25	ST
Adenophora remotiflora	Campanulaceae	Leaf	0.33	ST
Artemisia princeps	Compositae	Leaf	1.08	ST
Chrysanthemum zawadskii	Compositae	Leaf	0.02	ST
Cirsium japonicum	Compositae	Leaf	0.04	ST
Taraxacum platycarpum	Compositae	Leaf	0.05	ST
Capsella bursa-pastoris	Curciferae	Leaf	0.05	ST
Caragana sinica	Leguminosae	Leaf	0.12	ST
Cinnamomum cassia	Lauraceae	Root	1.45	ST
Ginkgo biloba	Ginkgoaceae	Fruit	0.84	ST
Leonurus sibiricus	Labiatae	Leaf	0.34	ST
Mentha arvensis	Labiatae	Leaf	0.75	ST
Perilla frutescens	Labiatae	Leaf	0.14	ST
Liriope platyphylla	Liliaceae	Leaf	0.07	ST
Lonicera japonica	Caprifoliaceae	Leaf	0.04	ST
Panax ginseng	Araliaceae	Leaf	0.05	ST
Pinus koraiensis	Pinaceae	Leaf	0.05	ST
Cananga odorata (Ylang Ylang)	Annonaceae	Leaf		CAO
Boswellia carteri (Frankincense)	Burseraceae	Leaf		CAO
Commiphora myrrh (Myrr)	Burseraceae	Leaf		CAO
Juniperus communis (Juniper Berry)	Cupressaceae	Fruit		CAO
Cymbopogon nardus (Citronella)	Cyperaceae	Leaf		CAO
Pogostemon patchouli (Patchouli)	Labiatae	Leaf		CAO
Thymus vulgaris (Thyme)	Labiatae	Leaf		CAO
Lavandula officinalis (Lavender)	Lamiaceae	Leaf		CAO
Rosmarinus officinalis (Rosemary)	Lamiaceae	Leaf		CAO
Syzygium aromaticum (Clove)	Myrtaceae	Flower		CAO
Eucalyptus globulus (Eucalyptus)	Myrtaceae	Stem		CAO
Melaleuca alternifolia (Tea tree)	Myrtaceae	Leaf		CAO
Citrus paradisi (Grapefruit)	Rutaceae	Peel		CAO
Citrus aurantium (Petitgrain)	Rutaceae	Leaf		CAO
Foeniculum vulgare (Fennel seed)	Umbelliferae	Seed		CAO
Pimpinella anisum (Anise seed)	Umbelliferae	Seed		CAO

[a]ST, steam distillate; and CAO, commercially available oil.

Scavenging effects on superoxide anion. The non-enzymatic generation of superoxide anion was measured by the method of Robak *et al.*[12] The reaction mixture consisted of 10 μM phenazine methosulfate, 78 μM NADH, 25 μM NBT and 0.1 M phosphate buffer (pH 7.4). After 3-min incubation at room temperature, the absorbance was measured at 560 nm.

Evaluation. Each experiment was performed in triplicate. The antioxidative activity of plant-derived materials was arbitrarily divided into five categories by calculating the ratio of the O.D. value of each sample used (S) to the O.D. value of the control (C) after certain incubation time: very strong, ++++, S/C < 0.2; strong, +++, 0.2 < S/C < 0.4; moderate, ++, 0.4 < S/C < 0.6; weak, +, 0.6 < S/C < 0.8; and little or no activity, -, 0.8 < S/C.

Results

Scavenging effects of plant-derived oils on free radicals and active oxygen species. The *in vitro* antioxidative activities of the 17 plant-derived steam distillates and 16 commercially available oils were determined by linoleic acid autoxidation (Table 2). At a concentration of 0.01%, very strong activity (++++) was obtained in the essential oils from *Cinnamomum cassia* (Lauraceae) roots, *Mentha arvensis* (Labiatae) leaves, and *Syzygium aromaticum* (Myrtaceae) flowers, whereas essential oils from *Rosmarinus officinalis* (Lamiaceae) leaves and *Thymus vulgaris* (Labiatae) leaves exhibited strong activity (+++). Moderate activity (++) was observed in the 9 essential oils.

The scavenging effects of the 33 plant-derived essential oils on DPPH radical are shown in Table 2. At 0.01%, essential oils of *C. cassia* roots and *S. aromaticum* flowers showed strong scavenging activity, whereas moderate activity was produced from the essential oils of *Ginkgo biloba* (Ginkgoaceae) fruits, *Chrysanthemum zawadskii* (Compositae) leaves and *Foeniculum vulgare* (Umbelliferae) leaves. The other essential oils exhibited weak or little scavenging activity.

The superoxide-scavenging abilities of plant-derived essential oils used are given in Table 2. At a concentration of 0.01%, the essential oils of *C. cassia* roots, *G. biloba* fruits and *S. aromaticum* flowers revealed moderate superoxide-scavenging activity, whereas weak or little activity was obtained in the other oils.

Scavenging effects of various compounds on free radicals and active oxygen species. The three main monoterpenes (menthol, menthone and carvacrol), carvacrol and thymol, and eugenol and isoeugenol were isolated from the essential oils of the *Mentha* leaves, the *Cinnamomum* roots, and the *Syzygium* flowers, respectively.

The antioxidative activities of 37 phytochemicals used were examined by measuring the inhibition of linoleic acid autoxidation and compared to those of the commonly used plant-derived antioxidants (pyrogallol and quercetin) and synthetic antioxidant BHT (Table 3). At 0.01%, isoeugenol showed very strong activity, whereas strong activity was obtained in carvacrol, eugenol and thymol. Their activities were comparable to those of quercetin and BHT.

Table 2. Antioxidative activities of essential oils, 0.01%.[a]

Sample	Inhibition of Autoxidation	DPPH Scavenging Activity	Superoxide Anion Scavenging Activity
A. triphylla	+	+	-
A. remotiflora	-	-	-
A. princeps	+	+	+
C. bursa-pastoris	++	+	+
C. sinica	+	-	-
C. zawadskii	++	++	+
C. cassia	++++	+++	++
C. japonicum	+	-	-
G. biloba	++	++	++
L. sibiricus	++	+	+
L. platyphylla	+	-	+
L. japonica	++	+	-
M. arvensis	++++	+	-
P. ginseng	++	+	+
P. frutescens	+	-	-
P. koraiensis	+	-	-
T. platycarpum	++	+	+
P. anisum	-	+	+
C. nardus	-	-	-
S. aromaticum	++++	+++	++
E. globulus	++	-	+
F. vulgare	+	++	-
B. carteri	-	-	-
C. paradisi	+	-	-
J. communis	+	+	-
L. officinalis	+	+	-
C. myrrh	+	-	+
P. patchouli	-	-	-
C. aurantium	-	-	-
R. officinalis	+++	+	+
M. alternifolia	++	-	+
T. vulgaris	+++	+	-
C. odorata	+	-	-

[a]O.D. value of sample/O.D. value of control: very strong, ++++, S/C<0.2; strong, +++, 0.2<S/C<0.4; moderate, ++, 0.4<S/C<0.6; weak, +, 0.6<S/C<0.8; and little or no activity, -, 0.8<S/C.

Table 3 shows the scavenging activity of the compounds used on DPPH radical when tested at 0.01%. Eugenol and isoeugenol showed very strong activity, and their activity was comparable to that of pyrogallol. Weak or little activity was observed from the other phytochemicals.

The superoxide-scavenging effects of test compounds were examined (Table 3). Eugenol and isoeugenol at 0.01% revealed strong activity (+++), and their activities were comparable to those of quercetin and BHT. The other phytochemicals exhibited weak or little scavenging activity.

Discussion

In the laboratory study with plant-derived essential oils, the essential oils of *C. cassia* roots, *G. biloba* fruits,

Table 3. Antioxidant activities of various compounds contained in essential oils, 0.01%.[a]

Compound	Inhibition of Autoxidation	DPPH Scavenging Activity	Superoxide Anion Scavenging Activity
Allylbenzene	+	-	+
Anisaldehyde	+	+	-
Anethole	++	-	-
Anisole	++	-	-
Benzaldehyde	+	-	-
Borneol	++	+	+
4-*t*-Butylaniline	-	-	-
Camphene	++	-	-
Camphor	++	-	-
Carvacrol	+++	+	+
Carvone	++	-	+
Cinnamyl alcohol	+	-	-
Cinnamaldehyde	+	-	-
1,8-Cineole	++	-	+
Citral	+	-	-
Citronellal	++	-	+
Citronellol	+	-	-
p-Cymene	+	-	-
Eugenol	+++	++++	++
Geraniol	-	-	-
Isoeugenol	++++	++++	+++
d-Limonene	-	-	+
Linalool	+	-	+
Linalylacetate	+	-	-
Menthol	-	-	-
Menthone	-	+	-
Myternal	-	-	-
Myternol	+	-	-
Perilla aldehyde	+	-	-
Perilla alcohol	+	-	-
α-Pinene	++	-	+
β-Pinene	++	-	+
α-Terpinene	-	-	-
Terpineol	+	-	-
Terpine-4-ol	+	-	-
Thymol	+++	+	+
Thujone	+	-	+
Pyrogallol	++	++++	++++
Quercetin	+++	+++	+++
BHT	+++	+++	+++

[a]For explanation, see Table 2.

C. zawadskii and *S. aromaticum* flowers at 0.01% revealed strong antioxidative activities, although the effects varied with test model (linoleic acid autoxidation, the generation of superoxide anion, and scavenging of DPPH radical). The steam distillates of plants belonging to the families Lauraceae, Ginkgoaceae, Compositae and Myrtaceae showed potent antioxidant activity. The strong antioxidative activities of these plant-derived essential oils confirm their superiority and usefulness as a potent antioxidant, although the antioxidative effect of plant-derived materials has been extensively studied.[8] It has been well acknowledged that antioxidative activity has positively correlated with antimutagenicity. Barclay and Perdue[13] suggested that the most promising botanical anti-tumor agents are in the families Annonaceae, Apocynaceae, Celestraceae, Cephalotaxaceae, Euphorbiaceae, Liliaece, Menispermaceae, Podocarpaceae, Rutaceae, Simanubaceae, Taxaceae, and Thymelaeaceae.

It has been well acknowledged that many of plant-derived extracts and phytochemicals are potential alternatives to synthetic antioxidants.[8] Various compounds including phenolics, terpenoids and alkaloids exist in plants and jointly or independently contribute to antioxidative activities. Many of them appear to have no secondary hazards to animals, but there are few studies in this regard. The relationship between antioxidative activity, antimutagenicity, and oxidative DNA damage has been reported. For example, antioxidative activity of tea extracts has been observed to correlate with the antimutagenicity.[14] Halliwell and Gutteridge[15] already report-ed that the oxidative DNA damage, mediated by active oxygen radical, induces carcinogenesis. It has been also reported that green tea solution reduced hepatic lipid peroxide levels and effectively blocked oxidative DNA damage in liver as well as hepatotoxicity of rats treated with 2-nitropropane as a hepatocarcinogen.[16] In our study, carvacrol and thymol exhibited strong antioxidative effect on linoleic acid autoxidation, but weak antioxidative effect on the generation of superoxide anion and scavenging of DPPH radical. On the contrary, eugenol and isoeugenol exhibited highly strong antioxidative activity on three test models, and their activities were comparable to those of the reference compounds (pyrogallol, quercetin, and BHT). These results indicate that the antioxidative activity of phenols might be generally ascribed to their hydroxyl groups, but it is not the only factor in determining the potency of their activities because the other phenolic monoterpene cinnamyl alcohol does not exhibit an antioxidative activity toward the autoxidation of linoleic acid, the generation of superoxide anion and scavenging of DPPH.

In conclusion, the antioxidative activity of some plant-derived essential oils described may be an indication of at least one of the pharmacological actions of these plant species. Based upon our limited data and some earlier findings, the plant essential oils and their components described seem to be a promising compounds in the prevention of several diseases by their antioxidative function.

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