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Antioxidative Activities of Methanol Extracts of Tropical and Oriental Medicinal Plants

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Introduction

In biological membranes, a change of the unsaturated fatty acids by the oxidation reaction may be caused by reactive oxygen species such as superoxide ion radical, hydrogen peroxide, hydroxyl radical, singlet oxygen, and the nitric oxide radical. The oxidation leads to change the membrane fluidity and membrane structure, and stimulate to malfunction of membrane.[1,2] Active oxygen species have also been implicated in several different diseases including cardiovascular diseases, chronic gut inflammation and cancer.[3-5] Antioxidant defenses normally protect against such oxidative damages. The generation mechanism of reactive oxygen species may be collapsed by non-enzymatic inactivation involving antioxidants and free radical scavengers and by enzymatic inactivation such as superoxie dismutase, which catalyzes the conversion of superoxide to hydrogen peroxide and oxygen.[6] Some naturally occurring antioxidants including α-tocopherol, ascorbic acid, and carotenoids have been used in food industry and preventive medicine. However, even though α-tocopherol and other naturally occurring antioxidants are considered to be active in eliminating the reactive oxygens and controlling the toxic effects, they have been limited of their usage as antioxidants because of low effectiveness.[7] A number of artificial antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been developed and used in food industry today. However, the safety of these chemicals has received increasing attention because of their toxicity to human beings.[8,9] Therefore, there is a need to find an alternative for the currently used antioxidants. In the study described herein, we assessed the antioxidant activity of thirty-seven plant species to develop new safer types of antioxidant agents.

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Materials and Methods

Plants and sample preparation

Thirteen different kinds of oriental medicinal plants we purchased from local stores in Seoul, Korea. *Rosmarinu officinalis* and *Salvia officinalis* were purchased from loc stores in California, USA. Each sample (100 g) was extracte twice with 600 m*l* methanol at room temperature and filtere (Whatman filter paper No. 1). The filtrate was concentrated i vacuo at 40°C using a rotary vacuum evaporator. Twent one plant extracts were kindly gifted by Dr. Brandao, Unive sidade Federal de minas Gerais, Brazil. Butylated hydroxy anisole (BHA), Butylated hydroxytoluene (BHT), dipheny p-picrylhydrazyl (DPPH), Linoleic acid, nitroblue tetrazoliu (NBT), and phenazine methosulphate were purchased fro Sigma Co. (St. Louis, MO).

Assay of autooxidation

The Oxidation of linoleic acid was measured by th modified method described in Haraguchi *et al.*.[10] Differe amounts of samples dissolved in 30 ml EtOH were adde to a reaction mixture in a screw cap vial. Each reactio mixture consisted of 0.57 m*l* of 2.51% linoleic acid i EtOH and 2.25 m*l* of 40 mM phosphate buffer (pH 7.0 The vial was placed in an oven at 37°C. After 3 day incubation, 0.1 m*l* aliquot of the mixture was diluted 4.6 m*l* of 75% EtOH, which was followed by adding 0.1 ml o 30% ammonium thiocyanate. At precisely 3 min after th addition of 0.1 m*l* of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance a

00 nm was measured.

Radical scavenging activity on DPPH

DPPH scavenging activity was measured by the method f Blois.[11] The reaction mixture contained 1 m*l* of 100 mM cetate buffer (pH 5.5), 1 m*l* of EtOH and 0.5 m*l* of 2.5 M ethanolic solution of DPPH. After allowing the mixture stand at room temperature for 20 min, the absorbance of ie remaining DPPH was determined colorimetrically at 517 m. The scavenging activity was measured as the decrease absorbance of the DPPH expressed as a percentage of ie absorbance of a control DPPH solution.

Assay of superoxide anion

The non-enzymatic generation of superoxide anion was easured by the method of Robak *et al.*.[12] The reaction ixture was composed of 10 μM phenazine methosulfate, 78 μM NADH, 25 μM NBT and 0.1 M phosphate buffer (pH 7.4). After 3 min of incubation at room temperature, the absorbance at 560 nm was measured. The levels of antioxidant activities of plant were arbitrarily divided into five categories by calculating the ratio of the O.D. value of the sample containing plant extract (S) to to of the control (C) after certain incubation time (++++: S/C < 0.2, +++: 0.2 < S/C < 0.4, ++: 0.4 < S/C < 0.6, +: 0.6 < S/C < 0.8, -: 0.8 < S/C).

Results and Discussion

Antioxidative activities of methanol extracts of plants against the oxidation of linoleic acid. Among the plant extracts tested, seven species shown strong antioxidative activities (++++) on the oxidation of linoleic acid (Table 1). These results clearly indicated that some of the tested plants were rich in natural antioxidants at a low concentration. The activities of

able 1. Antioxidative activities of extracts of thirty-seven plants and several antioxidants

Plant species	Inhibition of autooxidation (0.01%)	DPPH scavenging activity (0.01%)	Superoxide anion scavenging activity (0.01%)
canthopanax sessilifolinus	+++	-	-
mpelozizyphus amazonicus (L)	++++	+++	++
mpelozizyphus amazonicus (Rt)	+++	++	+++
ngelica polymorpha	+++	++	++
ralia eleta	-	-	-
rringeria actisma	++++	+++	+++
accahris trimera	+++	+++	+
astanea crenata	+++	+++	+++
optis chinensis	+++	-	-
albergia myriantha	+++	+++	++
ioscorea rotununda	+++	+++	++
olichus kilimandscharicus	++	+++	+
uonymus sieboliana	+++	++	-
uterpe oleracea	++++	+++	+++
raxinus densata	+++	+++	++
nidia subcordata	+++	+++	-
pomoea stans	++++	+++	+++
udigofera anil	++	+++	-
acaranda mimosifolia	+++	+++	++++
alopanax pictus	+++	-	-
agerstroemia speciosa	+++	-	+
eptadenia madagascariensis	++	+++	-
lorus alba	+++	+	+
lyristica fragrans	+++	+++	+++
lea uropaea	+++	++++	++
anax ginseng	+++	+++	++
icrasma quassioides	+++	-	-
seudospondias microcarpa	+++	++++	+
sychotria vellosiana	+++	+++	+
osmarinus officinalis	++++	+++	+++
umex obtusifolius	+++	+++	-
alvia officinalis	++++	+++	++
clerocarya bierra	+++	++++	++
olanum aculeatissimum	+++	+++	++
orbus commixta	+++	++	-
yzygium guineense	++++	++++	++
Ulmus paraifolia	++	+++	+++
BHA	++++	++++	++
BHT	++++	++++	++
Pyrogallol	++	++++	+++
Catechin	+++	++++	+++
Catechol	+++	++++	+++
Quercetin	+++	+++	+++

Table 2. Antioxidative activities from extracts of four species with various solvents

Plant Extracting solvent	Inhibition of autooxidation (0.01%)	DPPH scavenging activity (0.01%)	Superoxide anion scavenging activity (0.01%)
Arringeria actisma			
Methanol	++++	+++	+++
Acetone	++	+++	++
Ethyl acetate	-	++	-
Hexane	-	-	+
Euterpe oleracea			
Methanol	++++	+++	+++
Acetone	++	+	++
Ethyl acetate	-	-	++
Hexane	-	+	-
Ipomoea stans			
Methanol	++++	+++	+++
Acetone	++	+	-
Ethyl acetate	++++	+++	+++
Hexane	-	-	-
Jacaranda mimosifolia			
Methanol	+++	+++	++++
Acetone	-	+	-
Ethyl acetate	-	+	-
Hexane	-	-	-

extracts of *Euterpe oleracea* seed and *Ipomoea stans* stem bark were similar to those of the synthetic antioxidants, BHT and BHA, while their antioxidative activities were slightly stronger than those of naturally occurring compounds, pyrogallol, catechin, catechol and quercetin. The antioxidative activities of two extracts have not been reported in previous literature. The oriental plants may be a source of alternative for the currently used antioxidants, as Kim *et al.*[13] reported 44 species among 180 different herbal plants endemic in Korea that showed very strong antioxidative activities against the oxidation of linoleic acid. Furthermore, they reported that methanol extracts of *Psolarea corylifolia* and *Sorphora angustifolia* greatly decreased the epoxide formation of the lard during storage. However, the thirteen oriental medicinal plants had moderate or weak antioxidative activities.

DPPH radical scavenging activities of the extracts of plants. Only the extract of *Syzygium guineense* had higher radical scavenging activity than pyrogallol at the concentration used. This is a traditional medicinal plant for remedies of bacterial infection in Africa. Another three extracts of *Pseudospondias microcarpa, Olea uropaea* and *Sclerocarya bierra* showed similar radical scavenging activities to the activity of pyrogallol. Hence, the antioxidative activities of the four extracts may be attributed to their hydrogen-donating ability. These results indicate that the four extracts are free-radical inhibitors, possibly as primary antioxidants that react with free radicals.[14] Nevertheless, the four plants have not been reported in the literature regarding with DPPH scavenging activity. The thirteen oriental medicinal plants did not show strong DDPH radical scavenging activities.

Inhibition of the non-enzymatic generation of superoxide anion by the extracts of plants. Among thirty-seven plant extracts, eight extracts showed strong antioxidative activities (+++) on the generation of superoxide anion radicals. Interestingly, two synthetic antioxidants, BHA and BHT, did not show strong antioxidative activities on the generation of superoxide anion radicals in this system. The activities of the two oriental medicinal plants, *Ulmus paraifolia* and *Castanea crenata,* were higher than that of BHT and BHA, and were similar to those naturally occurring antioxidants. The extract of *Jacaranda mimosifolia* was shown to have a strong antioxidative activity on the generation of superoxide anion.

Comparison of antioxidative activities from extracts of four species with various solvents. Because of their potent antioxidative activities on the oxidation of linoleic acid, DPPH radical scavenging activity and superoxide anion scavenging activity, the activity of each solvent extract from the extracts of *Arringeria actisma, Euterpe oleracea, Ipomoea stans* and *Jacaranda mimosifolia* was evaluated (Table 2). These results indicate that the antioxidative activities increases with increasing polarity of solvent. Apparently, methanol is the most effective extraction of antioxidants from the four plant species followed by acetone. This result is similar to the data by Economou *et al.*[15] that show the methanol is a widely used and effective solvent for extraction of antioxidants. Therefore, this result demonstrates that the antioxidative activity is greatly dependent on the kinds of solvents used for the extraction.

In conclusion, the strong antioxidative activity of tropical

plants described confirms their superiority and usefulness on an antioxidant. Additionally, natural product-derived materials are found to be effective instead of the synthetic antioxidants. Further research for the isolation and characterization of the antioxidative components from the four tropical plants are in progress.

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References

1. Huang, M. T., T. Osawa., C. T. Ho and R. T. rosen (1994) Food phytochemicals for cancer prevention I. **50**. pp 57-73. American Chemical Society. Washington.
2. Kwon. M. T., Y. S. Joun and Y. O. Song (1994) The degree of lipid oxidation of rat liver fed peroxidized lipid its effects on antioxidative system. *J. Korean Soc. Food Nutr.* **23**(6), 899-970.
3. Yagi, K. (1987) Lipid peroxides and human disease. *Chem. Phys. Lipids* **45**, 337-341.
4. Huang, H. T., C. T. Ho and C. Y. Lee (1992) Phenolic compounds in food and their effects on health II. **122**. pp 54-70. American Chemical Society. Washington.
5. Halliwell, B., and O. I. Arouma (1992) *Molecular Biology of Free Radical Scavenger System*, Scandalios, Ed., Vol. 9, pp 47-67. Cold Spring Harbor Laboratory Press, New York.
6. Witting, L. A. (1980) *Free Radicals in Biology*, Pryored, W. A., Ed., Vol. 4, pp 295-319. Academic Press, New York.
7. Fang, X., and S. Wada (1993) Enhancing the antioxidant effect of α-tocopherol with rosemary in inhibiting catalyzed oxidation caused by Fe^{2+} and hemoprotein. *Food Res. Int.* **26**, 405-411.
8. Branen, A. L. (1975) Toxicology and biochemistry of butylated hydroxy anisole and butylatedhydroxytoluene. *J. Am. Oil Chem. Soc.* **52**, 59-63.
9. Nakami, M. (1990) Antioxidants/Antimutagens in food. *Crit. Rev. Food Sci. Nutr.* **29**, 273-300.
10. Haraguchi, H., H. Ishikawa, Y. Sanchez, T. Ogura, Y. Kubo, and I. Kubo (1997) Antioxidant constituents in *Heterotheca inuloides. Bioorg. Med. Chem.* **5**, 865-871.
11. Blois, M. S. (1958) Antioxidant determinations by the use of a stable free radical. *Nature* **181**, 1199-1200
12. Robak, J., and R. J. Gryglewski (1988) Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol.* **37**, 837-841.
13. Kim, S. Y., S. K. Kim, M. J. Oh, and M. Y. Jung (1994) Antioxidant actives of selected oriental herb extracts. *J. Am. Oil Chem. Soc.* **71**, 633-640.
14. Shimada, K., K. Fujikawa, K. Tahara and T. nakamura (1992) Antioxidative properties of xanthone on the antioxidation soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.* **40**, 945-948.
15. Economou, K. D., V. Oreopoulou, C. D. Thomopoulos (1991) Antioxidant activity of some plant extracts of the family labiate. *J. Am. Oil Chem. Soc.* **68**, 109-113.

열대 및 국내산 약용식물 조추출물의 항산화 효과

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