The Inhibitory Effects of 12 Medicinal Plants and Their Component Compounds on Lipid Peroxidation

Eun Ju Cho, Takako Yokozawa, Dong Young Rhyu, Hyun Young Kim and Naotoshi Shibahara
Institute of Natural Medicine, Toyama Medical and Pharmaceutical University
2630 Sugitani, Toyama 930-0194, Japan

Jong Cheol Park
Department of Oriental Medicine Resources, Sunchon National University
315 Maegok, Sunchon 540-742, Korea

Abstract: The antioxidative activities of 12 medicinal plants and the compounds isolated from them were investigated using the thiocyanate method to evaluate inhibitory effects on lipid peroxidation in the linoleic acid system. The peroxide levels gradually increased during incubation in the presence of linoleic acid over 3 days, and most of the plants inhibited lipid peroxidation. In particular, of the plants tested, Cudrania tricuspidata, Zanthoxylum piperitum, Houttuynia cordata and Ulmus parvifolia reduced lipid peroxidation more effectively as lipid peroxidation progressed, resulting in inhibition of about 80% relative to the control value by the 3rd day of incubation. In addition, the polyphenols isolated from the plants also showed marked and dose-dependent inhibitory effects on lipid peroxidation. The compounds with the strongest activities were 3,4-dihydroxybenzoic acid, quercetin, the quercetin glycosides quercetin-3-O-β-D-galactoside, quercetin-3-O-α-L-rhamnoside, quercetin-3-O-β-D-glucoside and quercetin-3-O-rutinoside, catechin, gallic acid, methyl gallate and rosamultin isolated from Zanthoxylum piperitum, Houttuynia cordata, Rosa rugosa and Cedrela sinensis. Moreover, quercetin glycosides showed stronger activity than quercetin, suggesting that glycosylation increases the antioxidative activity of quercetin. Our results indicate that the medicinal plants and their polyphenols show promise as therapeutic agents for various disorders involving free radical reactions.

Keywords: Medicinal Plant; Thiocyanate; Polyphenol; Lipid Peroxidation.

Correspondence to: Dr. Takako Yokozawa, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan. Fax: (+81) 76-434-4656, E-mail: yokozawa@ms.toyama-mpu.ac.jp
**Introduction**

Considerable attention has recently been focused on the inter-relationships of lipid peroxidation processes, free radical-related reactions and the development of a variety of pathological events. It is well established that lipid peroxidation is the deleterious result of free radical reactions, leading to disruption of biomembranes, and dysfunction of cells and tissues (Benzie, 1996). Therefore, lipid peroxidation is a crucial step in the pathogenesis of free radical-related disease states, including inflammatory injury, such as glomerulonephritis, vasculitis and rheumatoid arthritis, gastrointestinal diseases and cardiovascular, nervous system and other disorders (Halliwell, 1987).

Although natural antioxidant defense mechanisms exist to protect against oxidative damage, these mechanisms can be inefficient in situations of excessive oxidative stress, when dietary intake of antioxidant compounds becomes important. Some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole, are commonly used in processed foods, but these compounds have been reported to have some side effects (Ito *et al.*, 1983). Therefore, in recent years, there has been a global trend toward the use of natural phytochemicals present in herbs and functional foods as antioxidants. Actually, since ancient times, humans have derived many benefits from natural plants and compounds. It has been generally recognized that traditional Oriental medicines and the polyphenols isolated from them have potential therapeutic roles in the prevention and treatment of many human diseases related to excessive oxidative stress (Middleton *et al.*, 2000; Packer *et al.*, 1999). The various traditional herbs with medicinal functions have been developed, but there is no scientific evidence to support these functions. We have demonstrated that MeOH extracts and compounds isolated from several medicinal plants including *Rosa rugosa*, *Cudrania tricuspidata* and *Zanthoxylum piperitum* possess 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity (Cho *et al.*, 2003). Therefore, in order to learn more about the protective effects of these plants against free radical-induced damage, in this study, we used the thiocyanate method to investigate the inhibitory activities of medicinal plants and their components in the linoleic acid system.

**Materials and Methods**

**Medicinal Plants**

The characteristics and origins of the medicinal plants used in this study and preparation of their MeOH extracts are described in previous reports (Hur *et al.*, 2001; Park and Ok, 1993; Park *et al.*, 1993, 2000a, b and c). MeOH extracts of the following 12 medicinal plants were prepared: *Eucommia ulmoides* (leaf), *Cudrania tricuspidata* (leaf), *Zanthoxylum piperitum* (leaf, stem, root and fruit), *Houttuynia cordata* (aerial part), *Angelica keiskei* (aerial part), *Cirsium japonicum* var. *ussuriense* (aerial part), *Ulmus parvifolia* (leaf), *Oenanthe javanica* (aerial part), *Armoracia rusticana* (aerial part), *Orostachys japonicus* (aerial part), *Rosa rugosa* (root) and *Cedrela sinensis* (rachis).
Isolation of Compounds

The MeOH extracts were partitioned with organic solvents of different polarities to yield CH$_2$Cl$_2$, EtOAc, $n$-BuOH and H$_2$O fractions, in sequence, as shown in Fig. 1. The EtOAc fraction of each plant was subjected to silica gel chromatography with CHCl$_3$-MeOH-H$_2$O (lower layers, by volume, 25:7:5, 7:3:1 or 65:35:10) as solvents to yield 3,4-dihydroxybenzoic acid, hesperidin, quercetin, quercetin-3-O-$\beta$-D-galactoside, quercetin-3-O-$\alpha$-L-rhamnoside and kaempferol-3-O-$\alpha$-L-rhamnoside from Zanthoxylum piperitum (Hur et al., 2001); kaempferol-3-O-$\beta$-D-xyloside, kaempferol-3-O-$\beta$-D-galactoside and kaempferol-3-O-$\beta$-D-xylosyl (1→2)-$\beta$-D-galactoside from Armoracia rusticana (Park et al., 2000a); quercetin, quercetin-3-O-$\beta$-D-galactoside, quercetin-3-O-$\alpha$-L-rhamnoside and kaempferol-3-O-$\alpha$-L-rhamnoside from Houttuynia cordata (Park et al., 2000b); kaji-ichigoside F 1, rosamultin, (+)-catechin, methyl gallate, gallic acid and quercetin-3-O-$\beta$-D-galactoside from Rosa rugosa (Park and Ok, 1993); and quercetin-3-O-$\beta$-D-glucoside, quercetin-3-O-$\alpha$-L-rhamnoside, quercetin-3-O-rutinose, (+)-catechin, methyl gallate and adenosine from Cedrela sinensis (Park et al., 1993), as described previously.

Plant
extracted with MeOH

MeOH extract
added with 10% MeOH
fractionated with CH$_2$Cl$_2$

Aqueous layer
CH$_2$Cl$_2$ fraction
fractionated with EtOAc

EtOAc fraction
chromatography
Aqueous layer
fractionated with $n$-BuOH

Compounds

$n$-BuOH fraction
H$_2$O fraction

Figure 1. Fractionation and isolation of compounds.
Assay of Lipid Peroxidation in a Linoleic Acid System by the Thiocyanate Method

Autoxidation of linoleic acid was carried out using the method of Mitsuda et al. (1966). Different amounts of samples dissolved in 100 µl EtOH (to produce concentrations of 100, 200 and 400 µg/ml) were added to reaction mixtures in screw-capped vials. Each reaction mixture consisted of 2.5 ml 20 mM linoleic acid in EtOH and 2.0 ml 200 µM phosphate buffer (pH 7.0). Each vial was incubated in an oven at 40°C and at regular intervals during incubation, a 100 µl aliquot of the reaction mixture was diluted with 4.0 ml 75% EtOH, followed by the addition of 100 µl 30% ammonium thiocyanate. Precisely 3 minutes after adding 100 µl 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance at 500 nm was measured. Identical solutions of 100 µl EtOH without samples were used as blank samples and EtOH solutions containing BHT were used as positive controls to compare the antioxidative activity of samples with the well-known antioxidant BHT.

Results

Inhibitory Effects of MeOH Extracts of 12 Medicinal Plants

Table 1 shows the effects of MeOH extracts of the 12 medicinal plants on linoleic acid-induced peroxidation. In the control samples, peroxidation gradually increased during incubation in the presence of linoleic acid over 3 days and most of the plants tested at the concentration of 50 µg/ml inhibited lipid peroxidation. Furthermore, the longer the incubation time and progression of lipid peroxidation, the stronger were the inhibitory effects of the plants.

<table>
<thead>
<tr>
<th>Material</th>
<th>Incubation Time (day)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.153 ± 0.005</td>
<td>1.459 ± 0.025</td>
<td>3.507 ± 0.040</td>
<td>8.515 ± 0.270</td>
</tr>
<tr>
<td>Eucommia ulmoides (leaf)</td>
<td>0.566 ± 0.006</td>
<td>0.997 ± 0.020</td>
<td>2.105 ± 0.060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cudrania tricuspidata (leaf)</td>
<td>0.557 ± 0.014</td>
<td>0.932 ± 0.005</td>
<td>1.905 ± 0.040</td>
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</tr>
<tr>
<td>Zanthoxylum piperitum (leaf)</td>
<td>0.576 ± 0.010</td>
<td>0.992 ± 0.015</td>
<td>1.695 ± 0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zanthoxylum piperitum (stem)</td>
<td>0.576 ± 0.006</td>
<td>1.042 ± 0.015</td>
<td>1.795 ± 0.060</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zanthoxylum piperitum (root)</td>
<td>0.587 ± 0.014</td>
<td>1.187 ± 0.130</td>
<td>2.135 ± 0.030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zanthoxylum piperitum (fruit)</td>
<td>0.564 ± 0.003</td>
<td>0.952 ± 0.025</td>
<td>1.925 ± 0.060</td>
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<tr>
<td>Houttuynia cordata (aerial part)</td>
<td>0.592 ± 0.002</td>
<td>1.037 ± 0.010</td>
<td>2.145 ± 0.060</td>
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<td></td>
</tr>
<tr>
<td>Angelica keiskei (aerial part)</td>
<td>0.574 ± 0.013</td>
<td>0.987 ± 0.000</td>
<td>2.425 ± 0.080</td>
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<tr>
<td>Cirsium japonicum var. ussuriense</td>
<td>0.521 ± 0.004</td>
<td>0.907 ± 0.020</td>
<td>1.645 ± 0.000</td>
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<tr>
<td>Ulmus parvifolia (leaf)</td>
<td>0.667 ± 0.006</td>
<td>1.212 ± 0.005</td>
<td>2.875 ± 0.050</td>
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</tr>
<tr>
<td>Oenanthe javanica (aerial part)</td>
<td>1.239 ± 0.006</td>
<td>2.342 ± 0.145</td>
<td>4.275 ± 0.070</td>
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<td></td>
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<tr>
<td>Armoracia rusticana (aerial part)</td>
<td>0.634 ± 0.015</td>
<td>1.302 ± 0.015</td>
<td>3.435 ± 0.110</td>
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<tr>
<td>Orostachys japonicus (aerial part)</td>
<td>0.617 ± 0.010</td>
<td>1.157 ± 0.010</td>
<td>2.515 ± 0.130</td>
<td></td>
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<tr>
<td>Rosa rugosa (root)</td>
<td>0.579 ± 0.005</td>
<td>1.253 ± 0.160</td>
<td>2.853 ± 0.200</td>
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</tbody>
</table>
medicinal plants. In particular, *Cudrania tricuspidata*, *Zanthoxylum piperitum*, *Houttuynia cordata* and *Ulmus parvifolia* inhibited lipid peroxidation strongly in an incubation time-dependent manner, resulting in inhibition, relative to the respective control values, of about 60%, 70% and 80% after incubation for 1, 2 and 3 days, respectively. *Eucommia ulmoides*, *Angelica keiskei*, *Cirsium japonicum var. ussuriense*, *Rosa rugosa* and *Cedrela sinensis* also reduced the lipid peroxidation level efficiently; they all reduced the lipid peroxidation level from the control value of 8.515 to below 3 by the 3rd day of incubation.

### Inhibitory Effects of the Compounds Isolated from MeOH Extracts of Medicinal Plants

The inhibitory effects of the compounds isolated from the medicinal plants on lipid peroxidation are presented in Table 2. Although the compounds were tested at 10-fold lower concentrations than the MeOH extracts of the plants themselves, they showed significant and dose-dependent inhibition of lipid peroxidation. Moreover, their inhibitory activities were stronger as the peroxide level was higher. The compounds that were the strongest inhibitors of lipid peroxidation were 3,4-dihydroxybenzoic acid, quercetin, quercetin glycosides, catechin, gallic acid, methyl gallate and rosamultin isolated from *Zanthoxylum piperitum*, *Houttuynia cordata*, *Rosa rugosa* and *Cedrela sinensis*. Interestingly, the quercetin glycosides quercetin-3-O-β-D-galactoside, quercetin-3-O-α-L-rhamnoside, quercetin-3-O-β-D-glucoside and quercetin-3-O-rutinose showed stronger activity than quercetin. On the 3rd day of incubation, the addition of quercetin at a final concentration of 10 µg/ml reduced the lipid peroxidation level by 62%, while the quercetin glycosides did so by more than 70%. Kaempferol and its glycosides, however, showed relatively low activity.

### Discussion

In the present study, we evaluated the antioxidant activities of 12 medicinal plants by the thiocyanate method, which measures the amount of peroxides formed in emulsion during incubation in the presence of linoleic acid, the target of lipid peroxidation. The addition of MeOH extracts of the medicinal plants to the linoleic acid emulsion reduced peroxide formation. In particular, *Cudrania tricuspidata*, *Zanthoxylum piperitum*, *Houttuynia cordata*, *Rosa rugosa* and *Cedrela sinensis* showed strong inhibitory activity. In our previous study, extracts of these plants also exerted high DPPH radical-scavenging activity (Cho et al., 2003). Since ancient times, herbs were the basis for nearly all medicinal therapy until synthetic drugs were developed and these plants have been used widely for several pathological conditions. *Cudrania tricuspidata* has been used for various diseases: lumbago, pollution, hemoptysis, avascularization, bruising, eczema, mumps and pulmonary tuberculosis. In addition, it has been reported to be effective in the treatment of various cancerous lesions of the stomach, colon, rectum, esophagus and liver. Several compounds have been reported to be its components, namely isoprenylated xanthones such as cudraxanthone, isoprenylated flavones such as cudraflavone, cycloartocarpesin, populnin and quercimetrin, β-sitosterol glucoside, arthocarpesin, norarthocarpentin and 5-O-methyl genistein (Park et al., 1992).
Table 2. Effect of the Compounds Isolated from MeOH Extracts of Medicinal Plants on Peroxidation Generated by Linoleic Acid

<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration (µg/ml)</th>
<th>Incubation Time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>0.139 ± 0.003</td>
<td>1.431 ± 0.002</td>
</tr>
<tr>
<td>Zanthoxylum piperitum (leaf)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extract</td>
<td>10</td>
<td>0.788 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.705 ± 0.002</td>
</tr>
<tr>
<td>3,4-Dihydroxybenzoic acid</td>
<td>5</td>
<td>0.812 ± 0.017</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.795 ± 0.006</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>5</td>
<td>1.868 ± 0.033</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.321 ± 0.025</td>
</tr>
<tr>
<td>Quercetin</td>
<td>5</td>
<td>0.849 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.713 ± 0.006</td>
</tr>
<tr>
<td>Quercetin-3-O-β-D-galactoside</td>
<td>5</td>
<td>0.859 ± 0.014</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.799 ± 0.019</td>
</tr>
<tr>
<td>Quercetin-3-O-α-L-rhamnosed</td>
<td>5</td>
<td>0.760 ± 0.009</td>
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<td>10</td>
<td>0.531 ± 0.011</td>
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<tr>
<td>Kaempferol-3-O-α-L-rhamnoside</td>
<td>5</td>
<td>1.682 ± 0.023</td>
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<td></td>
<td>10</td>
<td>1.562 ± 0.028</td>
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<tr>
<td>Armoracia rusticana (aerial part)</td>
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<tr>
<td>Extract</td>
<td>10</td>
<td>1.869 ± 0.066</td>
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<tr>
<td></td>
<td>50</td>
<td>0.844 ± 0.047</td>
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<tr>
<td>Kaempferol-3-O-β-D-xylene</td>
<td>5</td>
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<td>1.838 ± 0.037</td>
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<tr>
<td>Kaempferol-3-O-β-D-galactoside</td>
<td>5</td>
<td>1.466 ± 0.022</td>
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<td>1.008 ± 0.036</td>
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<td>Kaempferol-3-O-β-xylonyl (1→2)-β-D-galactoside</td>
<td>5</td>
<td>1.778 ± 0.020</td>
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<td>1.433 ± 0.033</td>
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<td>Houttuynia cordata (aerial part)</td>
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<tr>
<td>Extract</td>
<td>10</td>
<td>0.777 ± 0.019</td>
</tr>
<tr>
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<td>0.480 ± 0.003</td>
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<td>1.562 ± 0.028</td>
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### Table 2. (continued)

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<tr>
<th>Material</th>
<th>Concentration (µg/ml)</th>
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<th>1</th>
<th>2</th>
<th>3</th>
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<td><em>Rosa rugosa</em> (root)</td>
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<td>50</td>
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<td>1.342 ± 0.050</td>
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<td>Kaji-ichigoside F₁</td>
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<td>1.263 ± 0.013</td>
<td>2.297 ± 0.015</td>
<td>6.867 ± 0.207</td>
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<td>2.545 ± 0.038</td>
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<td>(+)-Catechin</td>
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<td>0.882 ± 0.013</td>
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<td>10</td>
<td>0.699 ± 0.013</td>
<td>1.218 ± 0.015</td>
<td>3.231 ± 0.031</td>
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<td>Rosamultin</td>
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<td>0.909 ± 0.008</td>
<td>1.758 ± 0.035</td>
<td>3.951 ± 0.031</td>
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<td>1.675 ± 0.069</td>
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<td>Procyanidin B3</td>
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<td>0.991 ± 0.004</td>
<td>2.145 ± 0.000</td>
<td>5.043 ± 0.332</td>
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<td>0.843 ± 0.003</td>
<td>1.708 ± 0.015</td>
<td>4.110 ± 0.021</td>
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<td><em>Rosa rugosa</em> (stem)</td>
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<td>Gallic acid</td>
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<td>3.841 ± 0.000</td>
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<td>0.893 ± 0.013</td>
<td>1.618 ± 0.045</td>
<td>3.737 ± 0.021</td>
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<td>Quercetin-3-O-β-D-galactoside</td>
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<td>0.799 ± 0.019</td>
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<td>2.686 ± 0.035</td>
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<td>0.790 ± 0.015</td>
<td>1.117 ± 0.038</td>
<td>2.417 ± 0.019</td>
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<tr>
<td><em>Cedrela sinensis</em> (rachis)</td>
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<tr>
<td>Extract</td>
<td>10</td>
<td>0.804 ± 0.005</td>
<td>1.767 ± 0.074</td>
<td>5.833 ± 0.137</td>
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<tr>
<td></td>
<td>50</td>
<td>0.616 ± 0.005</td>
<td>1.214 ± 0.157</td>
<td>2.989 ± 0.211</td>
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<tr>
<td>Quercetin-3-O-β-D-glucoside</td>
<td>5</td>
<td>0.855 ± 0.003</td>
<td>1.978 ± 0.098</td>
<td>4.861 ± 0.075</td>
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<tr>
<td></td>
<td>10</td>
<td>0.718 ± 0.011</td>
<td>1.342 ± 0.020</td>
<td>2.894 ± 0.116</td>
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<tr>
<td>Quercetin-3-O-α-L-rhamnoside</td>
<td>5</td>
<td>0.760 ± 0.009</td>
<td>0.992 ± 0.038</td>
<td>2.255 ± 0.035</td>
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<td></td>
<td>10</td>
<td>0.531 ± 0.011</td>
<td>0.986 ± 0.018</td>
<td>2.110 ± 0.035</td>
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<tr>
<td>Quercetin-3-O-rutinose</td>
<td>5</td>
<td>0.751 ± 0.022</td>
<td>1.747 ± 0.024</td>
<td>4.838 ± 0.095</td>
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<td></td>
<td>10</td>
<td>0.721 ± 0.013</td>
<td>1.263 ± 0.048</td>
<td>2.999 ± 0.075</td>
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<tr>
<td>(+)-Catechin</td>
<td>5</td>
<td>0.882 ± 0.013</td>
<td>1.719 ± 0.005</td>
<td>4.817 ± 0.116</td>
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<td>10</td>
<td>0.837 ± 0.005</td>
<td>1.512 ± 0.035</td>
<td>3.698 ± 0.095</td>
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<td>Methyl gallate</td>
<td>5</td>
<td>0.729 ± 0.011</td>
<td>1.327 ± 0.015</td>
<td>3.368 ± 0.021</td>
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<td>10</td>
<td>0.699 ± 0.013</td>
<td>1.218 ± 0.015</td>
<td>3.231 ± 0.031</td>
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<tr>
<td>Adenosine</td>
<td>5</td>
<td>1.340 ± 0.024</td>
<td>3.276 ± 0.035</td>
<td>7.269 ± 0.116</td>
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<tr>
<td></td>
<td>10</td>
<td>1.305 ± 0.005</td>
<td>3.051 ± 0.074</td>
<td>6.573 ± 0.286</td>
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<tr>
<td>BHT</td>
<td>5</td>
<td>0.459 ± 0.009</td>
<td>0.918 ± 0.008</td>
<td>1.892 ± 0.000</td>
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<tr>
<td></td>
<td>10</td>
<td>0.452 ± 0.004</td>
<td>0.888 ± 0.008</td>
<td>1.851 ± 0.000</td>
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In view of its traditional uses and active components, it is also considered to be effective for the treatment of free radical-related disorders and our results provide scientific evidence in support of a protective effect against free radical reactions.

*Zanthoxylum piperitum*, which is used as a spice and traditional medicine (Epple et al., 2001), is well known as an antidote, antiphlogistic and a cure for gastrointestinal disorders. Moreover, *Zanthoxyli Fructus*, the pericarp of *Zanthoxylum piperitum*, has been recognized to act on the gastrointestinal tract, so it has been used as an aid to digestion since ancient times. Although used traditionally for several disorders, scientific research into its effects on pathological conditions has rarely been carried out. This study showed that *Zanthoxylum piperitum* itself and its components inhibit lipid peroxidation in vitro, suggesting therapeutic potential of this medicinal plant for disorders involving free radical-induced damage.

In addition, *Houttuynia cordata* Thunb. is described as a pungent tasting herb with good properties and it is used for treating hypertension and edema, and as a detoxicant, diuretic, anti-inflammatory, anti-pyretic and anti-purulent agent (Probstle and Bauer, 1992). Furthermore, Hayashi et al. (1995) reported that it has direct inhibitory activity against herpes simplex virus type I, influenza virus and human immunodeficiency virus type 1 without being cytotoxic to the host. In particular, quercetin, a flavonoid present in *Houttuynia cordata*, has been reported to have inhibitory effects on several viruses (Mucsi and Pragai, 1985). This study provides evidence that *Houttuynia cordata* and its components, quercetin and its glycosides, inhibit lipid peroxidation in vitro. Taken together with these reports, our findings indicate that *Houttuynia cordata* and its flavonoids could be potential therapies for several pathological events, including disorders involving free radicals.

Rosaceae, including *Rosa rugosa*, have been used as folk medicines for several disorders, such as diabetes mellitus, mastitis, asthma, dyspepsia, gastroenteritis and menoxenia, although scientific confirmation of their benefits is still awaited. The root of *Rosa rugosa*, a perennial shrub, has been used as an astringent and stomachic, and it is known as a Korean folk remedy for treating mastitis and diabetes mellitus (Song et al., 1977). In fact, the hypoglycemic effects of Rosaceae in rats with alloxan- or streptozotocin-induced diabetes are well established (Lemus et al., 1999). Furthermore, *Rosa rugosa* has been reported to have a hypolipidemic effect through the inhibition of microsomal HMG-CoA reductase activity, resulting in the suppression of cholesterol synthesis (Lee et al., 1991), and the juice of *Rosa rugosa* fruit strongly inhibited the proliferation of cancer cell lines and induced differentiation of HL-60 leukemia cells (Yoshizawa et al., 2000). Our previous study demonstrated that Rosaceae possess radical-scavenging activity and suggested that they may be useful therapeutic agents for radical-related pathological damage through scavenging free radicals (Cho et al., 2003). In addition, the results of the present study suggest that *Rosa rugosa* and its compounds could be expected to exert therapeutic properties by virtue of inhibition of lipid peroxidation.

*Cedrela sinensis* has been used for food in Korea and as an Oriental medicine for treating enteritis, dysentery and itching (Park et al., 1993). Its active components were reported to be kaempferol, methyl gallate, quercetin, afzelin, quercitrin, isoquercitrin and rutin (Park et al., 1993). We confirmed that *Cedrela sinensis* and its polyphenols, such as quercetin, quercetin glycosides, catechin and methyl gallate, possess antioxidative activity, suggesting their potential for the treatment and/or prevention of free radical-related disorders.
Although Armoracia rusticana showed relatively low antioxidative activity compared with the other medicinal plants, it contains polyphenols that are known to be active components of various medicinal plants. Therefore, we isolated its polyphenol and investigated its antioxidative activity. Armoracia rusticana belongs to the Cruciferae and has been used widely as a spice and an Oriental medicine for arthritis, rheumatism and vascular disorders. In addition, it not only improves circulation of the blood and the appetite, but it is also used as an antidote and an antiseptic. Moreover, Hur et al. (1998) reported that kaempferol glycoside isolated from the aerial parts of Armoracia rusticana P. reduced lipid peroxide formation in the bromobenzene-treated rat liver. However, this study indicated that kaempferol and its glycosides had relatively low lipid peroxide inhibitory activity compared with other polyphenols, such as quercetin, catechin and gallic acid.

It has been reported that there is an inverse relationship between the antioxidative status and incidence of human diseases (Rice-Evans et al., 1997). In addition, antioxidant compounds, which are responsible for such antioxidative activity, could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders (Middleton et al., 2000; Packer et al., 1999). Therefore, research to identify antioxidative compounds is an important issue. Although it remains unclear which of the components of medicinal plants are the active compounds, polyphenols have received increasing attention recently because of some interesting new findings regarding their biological activities. From the pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as scavenging free radicals and inhibition of lipid peroxidation, are the most crucial. Even though a variety of herbs are known to be sources of phenolic compounds, studies isolating polyphenols and evaluating their antioxidative effects have rarely been carried out. Thus, in the present study, polyphenol compounds were isolated from the medicinal plants that contained high levels of polyphenols and their antioxidative activities were evaluated.

In this investigation, 3,4-dihydroxybenzoic acid, quercetin, quercetin glycosides, catechin, methyl gallate and rosamultin showed efficient antioxidative potential by inhibiting lipid peroxidation. These observations are in good agreement with our previous findings and other studies that demonstrated their reactive oxygen species- and DPPH radical-scavenging activities (Cho et al., 2003; Yokozawa et al., 1998). Hong et al. (1995) also showed that polyphenols protected lipids from peroxidation.

Polyphenols can exert their antioxidant activity by various mechanisms, for example, by scavenging radicals, binding metal ions, and inhibiting enzymatic systems responsible for free radical generation (Cotelle et al., 1996). Thus, based on the data from our present study, we propose that the inhibitory effects of polyphenols on lipid peroxidation result from scavenging radicals and/or inhibition of a wide range of enzymes involved in oxidation systems. Of the polyphenols studied, numerous in vitro studies have demonstrated that quercetin and its related flavonoids inhibit oxidative modification by scavenging reactive oxygen species, chelating transition metal ions responsible for the generation of reactive oxygen species, or inhibiting lipoxygenase (DeWhalley et al., 1990; Yamamoto et al., 1999). Our findings that quercetin and its glycosides inhibited lipid peroxidation also indicate that they have antioxidative activity.
Furthermore, the radical-scavenging activities of flavonoids may be influenced greatly by glycosylation. This study revealed that the quercetin glycosides quercetin-3-O-β-D-galactoside, quercetin-3-O-α-L-rhamnoside, quercetin-3-O-β-D-glucoside and quercetin-3-O-rutinose inhibited lipid peroxidation more efficiently than quercetin. As demonstrated in our previous study, the glycoside forms of quercetin showed similar or greater radical-scavenging activity than quercetin (Cho et al., 2003). We confirmed that glycosylation of quercetin increases its antioxidative activity, although whether glycosylation enhances the antioxidative properties of flavonoids remains to be elucidated. Our present study suggests that the 12 medicinal plants and their component compounds have the potential to be treatments for oxidative stress-related disorders.

References


