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# Antioxidative Activities of 60 Plant Extracts

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**Abstract**–The methanol extracts from 60 plant extracts were prepared and evaluated for their antioxidative activity. *Arctium lappa, Diospyros kaki, Eugenia caryophyllata, Melia azedarach* and *Forsythia suspensa* showed the inhibitory activity against lipid peroxidation. *Caesalpinia sappan, Crataegus pinnatifida, Eugenia caryophyllata, Gleditsia japonica, Osmunda japonica, Rhus javanica* and *Sanguisorba officinalis* showed the highest inhibitory activity against DPPH radical formation. In particular, *Eugenia caryophyllata* demonstrated strong inhibitory activity on the lipid peroxidation and free radicals. The results suggested that selected plant extracts have a potential as natural antioxidant.

**Keywords** □ antioxidative activity, inhibition, lipid peroxidation, free radical scavenging, plant extract

## INTRODUCTION

Since reactive oxygen species generated by ultraviolet, environmental pollutants and metabolic processes can cause a wide variety of pathological conditions such as DNA damage, carcinogenesis and cellular degeneration (Simic *et al.*, 1994; Jovanovic *et al.*, 2000; Yaar *et al.*, 2002), an antioxidant use may give significant protection against aging process. The free radical theory of aging further supports the scientific rationale for using antioxidant to delay aging, particularly, skin aging (Beckman *et al.*, 1998). Actually, several antioxidants including ascorbic acid, dl-α-tocopherol and butyl hydroxy toluene (BHT) have been widely used in the cosmetic products (Dreher *et al.*, 2001; Lupo. *et al.*, 2001; Podda *et al.*, 2001). But, there is still a need for new natural antioxidants.

Many previous investigations of plant screening showed the antioxidant activity of various plant materials (Wang *et al.*, 1991; Fukuda *et al.*, 1995; Masaki, 1995; Liu *et al.*, 2000, Ng et *al.*, 2000 ; Ogata *et al.*, 2000; Chen *et al.*, 2001; Schinella *et al.*, 2002). Especially, several recent publications suggested that plant antioxidants have a potential as anti-aging agent in cosmetic use (Afaq *et al.*, 2002a; Afaq *et al.*, 2002b; Pinnell, 2003). Thus, as our continuous efforts to develop useful natural antioxidants for cosmetic use (Kim *et al.*, 1997), the methanol extracts from 60 plants were prepared and evaluated for their inhibitory activity of lipid peroxidation and scavenging activity of free radical in this investigation. And the results suggested that selected plant extracts have a potential as anti-aging agent in cosmetics.

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## MATERIALS AND METHODS

### Preparation of Plant Extracts

Sixty plant materials were obtained from the oriental medicine market in Chunchon, South Korea, and each specimen was deposited in the herbarium of College of Pharmacy, Kangwon National University. Each powdered plant materials (100 g) was soaked in 300 mL of 80% methanol aquous solution at room temperature for 7 days. After filtration, the methanolic filtrate was evaporated to dryness under vacuum. These extracts without further purification were used to study their inhibitory effect of lipid peroxidation and scavenging activity of free radical.

### Inhibition of Lipid Peroxidation

Inhibition of lipid peroxidation was measured according to the previous reported procedure of Ohkawa *et al.*, 1979. Each test sample (0.1 mL) and ethyl linoleate (10 μM, Sigma-Aldrich) were added to incubation medium (4.89 mL) containing 2% sodium dodecyl sulfate, 1 μM ferrous chloride and 0.5 mM hydrogen peroxide. Butyl hydroxy toluene (BHT, Sigma-Aldrich) was used as a reference compound. The incubation medium was kept at 55°C for 16 hrs. Each reaction mixture (0.3 mL)

was transferred into a test tube followed by addition of 4% BHT (50 μL) to prevent further oxidation. The extent of lipid peroxidation was determined by measuring the quantity of thiobarbituric acid reactive substance (TBARS). The reaction mixture was added 1 mL of 0.67% TBA. The samples were vortexed and incubated at 95°C for 30 min. After cooling, 4 mL of 15% methanolic butanol solution was added and mixed. The reaction mixture was centrifuged (2,500 rpm) for 10 min and absorbance of the supernatant was measured at 532 nm.

### Free Radical Scavenging Activity

Free radical scavenging activity was routinely determined according to the previous reported procedure of Fugita *et al.*, 1988. The sample solution (2 mL) was added to 2 mL of 60 μM 1,1-diphenyl-2-picryl (DPPH) ethanolic solution and kept at room temperature for 30 min. The absorbance was measured at 520 nm. Gallic acid was used as a reference compound.

## RESULTS AND DISCUSSION

The inhibitory activities of 60 plant extracts against lipid peroxidation and free radical formation were evaluated at 10 μg/mL. As demonstrated in Table 1, *Arctium lappa, Diospyros kaki, Eugenia caryophyllata, Melia azedarach* and *Forsythia suspensa* showed more than 30% inhibition against lipid peroxidation. *Caesalpinia sappan, Crataegus pinnatifida, Eugenia*

**Table I.** Lipid peroxidation inhibitory and free radical scavenging activities of selected medicinal plants.

| Plant Name | Part Used | % Inhibition (10 μg/mL) | |
|---|---|---|---|
| | | lipid peroxidation | free radical |
| *Acantopanax sessilifolium* | cortex | - | 3 |
| *Achyrantes japonica* | radix | - | - |
| *Adenophora triphylla* | radix | - | 3 |
| *Alpinia katsumadaii* | fructus | 19 | 25 |
| *Alpinia oxyphylla* | fructus | 24 | - |
| *Angelica gigas* | radix | 14 | - |
| *Arctium lappa* | fructus | 66 | 13 |
| *Aristolochia contorta* | fructus | 9 | 3 |
| *Artemisia capillaris* | herba | 26 | 11 |
| *Bletilla striata* | rhizoma | 19 | 2 |
| *Caesalpinia sappan* | lignum | 7 | 37 |
| *Carthamus tinctorius* | flos | - | 2 |
| *Celosia argentia* | semen | 7 | 3 |
| *Cimicifuga heracleifolia* | rhizoma | 21 | 8 |
| *Cinnamomum cassia* | cortex | 15 | 7 |
| *Cinnamomum cassia* | ramulus | 16 | 27 |
| *Clematis mandshurica* | radix | - | - |
| *Coix lachryma-jobi* | semen | 15 | - |
| *Crataegus pinnatifida* | fructus | - | 30 |
| *Croton tiglium* | semen | 20 | - |
| *Dendrobium nobile* | herba | 24 | 14 |
| *Dioscorea japonica* | radix | 9 | 1 |
| *Diospyros kaki* | calyx | 35 | 15 |
| *Dolichos lablab* | semen | 5 | 6 |
| *Ephedra sinica* | herba | 12 | 30 |
| *Epimedium koreanum* | herba | 10 | - |
| *Eriobotrya japonica* | herba | 15 | 17 |
| *Eucommia ulmroides* | folium | 4 | - |
| *Eugenia caryophyllata* | flos | 88 | 38 |
| *Euphoria longana* | fructus | 26 | 3 |
| *Forsythia suspensa* | fructus | 30 | 14 |
| *Gentiana scabra* | radix | 17 | 4 |
| *Gleditsia japonica* | ramulus | 22 | 34 |
| *Ledobourilla seseloides* | radix | - | 6 |
| *Lithospermum erythrorhizon* | radix | - | 2 |
| *Lonicera japonica* | flos | - | 5 |
| *Magnolia kobus* | flos | 5 | 3 |
| *Magnolia officinalis* | cortex | 50 | 16 |
| *Melia azedarach* | corex | 28 | 5 |
| *Mucuna birdwoodiana* | caulis | - | 39 |
| *Nelumbo nucifera* | semen | 46 | 2 |
| *Osmunda japonica* | rhizoma | 1 | 5 |
| *Paeonia albiflora* | radix(red) | - | 16 |
| *Panax ginseng* | radix | 11 | - |
| *Peucedanum praeruptorum* | rhizoma | 10 | - |
| *Phlomis umbrosa* | radix | 29 | 3 |
| *Polygonum multiflorum* | radix(red) | 1 | - |
| *Polygonum multiflorum* | radix(white) | 13 | 6 |
| *Pulsatilla koreana* | radix | - | - |
| *Prunella vulgaris* | herba | 18 | 9 |
| *Rhapanus sativa* | semen | - | 1 |
| *Rhemania glutinosa* | radix | - | 2 |
| *Rhus javanica* | galla rhois | 15 | 58 |
| *Rosa laevigata* | fructus | - | 5 |
| *Salvia miltiorhiza* | radix | 10 | 5 |
| *Sanguisorba officinalis* | radix | 26 | 41 |
| *Sesamum indicum* | semen | 11 | - |
| *Sinomenium acutum* | rhizoma | 11 | - |
| *Sophora subprostrata* | radix | 1 | 2 |
| *Zizypus jujuba* | fructus | 6 | - |

Values represent arithmetic mean of three separate experiments. % Inhibition = (A-B)/A × 100, where A was the absorbance of positive control, and B was the absorbance of test sample.

*caryophyllata, Gleditsia japonica, Osmunda japonica, Rhus javanica* and *Sanguisorba officinalis* showed more than 30% inhibition against DPPH free radical formation. Especially *Eugenia caryophyllata* showed potent inhibition against lipid peroxidation and free radical formation.

To determine $IC_{50}$ values of several plant extract showing high antioxidative activities, the concentration-dependent inhibition was studied. *Eugenia caryophyllata* and *Crataegus pinnatifida* exhibited the highest potency in inhibiting of lipid peroxidation and free generation, respectively. $IC_{50}$ values of *Eugenia caryophyllata* was found to be 17.2 μg/mL against lipid peroxidation. $IC_{50}$ values of *Crataegus pinnatifida* was found to be 66.9 μg/mL against DPPH free radical generation, respectively. Using the same procedure, $IC_{50}$ values of other plant extracts were determined and summarized in Table 2.

Although inhibitory potency of the selected plant extracts against lipid peroxidation were less than that of reference material, BHT, *Arctium lappa, Eugenia caryophyllata, Forsythia suspensa, Magnolia officinalis* and *Melia azedarach* showed higher inhibition when compared with gallic acid. In a similar way, *Crataegus pinnatifida* and *Osmunda japonica* possessed comparable radical scavenging activity with that of BHT, while gallic acid showed much higher radical scavenging activity.

Natural antioxidants are usually classified into preventive antioxidants and chain breaking ones (Halliwell *et al.*, 1990). *Eugenia caryophyllata* potently inhibited both lipid peroxidation and DPPH radical generation (Table 1 and 2). Therefore, it is suggested that the inhibition of lipid peroxidation is attributable to free radical scavenging as preventive antioxidant. Although active as lipid peroxidation inhibitors, *Arctium lappa, Forsythia suspensa* and *Magnolia officinalis* do not seem to be relevant as DPPH scavengers suggesting that they could act as chain-breaking antioxidant.

**Table II.** $IC_{50}$ of medicinal plants selected.

| Plant Name | Part Used | $IC_{50}$(μg/mL) | |
|---|---|---|---|
| | | lipid peroxidation | free radical |
| *Arctium lappa* | semen | 37.6 | |
| *Eugenia caryophyllata* | flos | 17.2 | |
| *Forsythia suspensa* | fructus | 21.0 | |
| *Melia azedarach* | cortex | >100.0 | |
| *Magnolia officinalis* | cortex | 88.3 | |
| *Crataegus pinnatifida* | fructus | | 66.9 |
| *Gleditsia sinensis* | ramulus | | >100.0 |
| *Osmunda japonica* | rhizoma | | 90.4 |
| BHT | | <5.0 | 37.2 |
| Gallic acid | | >100.0 | <5.0 |

The $IC_{50}$ was determined using % inhibition of four different concentrations between 5 μg/mL and 100 μg/mL.
% Inhibition = (A-B)/A × 100, where A was the absorbance of positive control, and B was the absorbance of test sample.

Various palant materials were previously reported to show antioxidative effect. *Arctium lappa, Crataegus pinnatifida, Eugenia caryophyllata, Forsythia suspensa, Magnolia officinalis, Melia azedarach* and *Sanguisorba officinalis* were evaluated for their antioxidative activity and showed similar results as ours (Chen *et al.*, 2001; Wang *et al.*, 1991; Fukuda *et al.*, 1995; Masaki, 1995; Liu *et al.*, 2000, Ogata *et al.*, 2000; Schinella *et al.*, 2002).

In addition, our result of *Eugenia caryophyllata* is well correlated with previous investigation describing the extract of *Eugenia caryophyllata* and its one of major component, eugenol possessed potent antioxidative activity (Ogata *et al.*, 2000; Fujisawa *et al.*, 2002). For further identifying another active principles from *Eugenia caryophyllata* is now being under investigation.

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