J Food Sci Nutr
Vol 9, p 289~294 (2004)

J Food Science and Nutrition

# Antioxidant Activity of Salad Vegetables Grown in Korea

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## Abstract

**The antioxidant activity of forty two kinds of salad vegetables grown in Korea was evaluated. Methanol extract of freeze-dried vegetable was assayed by radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and $Fe^{2+}$-catalyzed lipid peroxidation inhibition by TBA method. Total phenolics were determined colorimetrically using Folin-Ciocalteu reagent. The highest radical scavenging activity was expressed by perilla leaf, followed by dandelion leaf, red and green leafy lettuce, of which $IC_{50}$ was less than 0.10 mg/mL. Angelica leaf showed the highest inhibitory action for lipid peroxidation with 95%, and then dandelion leaf, water spinach, and perilla leaf inhibited over 80%. However, lettuce (Iceberg) and young Chinese cabbage exhibited the lowest antioxidant activity based on both assay methods. Highly positive correlations between antioxidative activities and total phenolics were observed ($p < 0.001$). The results suggested that salad vegetables, especially perilla leaf, leafy lettuce, dandelion or angelica, could be used for easily accessible sources of natural antioxidants.**

**Key words:** salad vegetables, antioxidant activities, total phenolics

## INTRODUCTION

A benefit of vegetable-rich diets has been partially attributed to the intake of phenolic compounds. Lots of phenolic antioxidants in vegetables are effective in preventing the oxidative damage that may be the causes of arteriosclerosis, brain disorders, cancer and immune system decline (1-3). Plant phenolic compounds have a significant capacity to scavenge free radicals and sequester transitional metal ions (4-6). Plant antioxidant components have repeatedly shown an affinity to quench reactive oxygen species by tea catechins (7), flavonoids of *Gingko biloba* (8), flaxseed lignan (9), and anthocyanins of fruits (10,11). Practically, complex mixtures of antioxidants in whole foods are responsible for their health benefits, and their advantage over single antioxidant may be due to a combination of additive and/or synergistic effects (12,13). Several analytical methods have been proposed for the determination of total antioxidant capacity of biological samples. Conventional methods for determining the antioxidant activity of plants are the measurements of phenolic content and radical scavenging activity. Color development using a Folin-Ciocalteu reagent (Folin-Ciocalteu assay) is the generally preferred method for measuring phenolics, because most plant-derived antioxidants contain large amounts of polyphenols. Free radical scavenging activity is used for measuring the antioxidant activity of edible plants with such activity varying according to radical species (14). Measurement of radical scavenging activity using discoloration of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH radical scavenging assay) has been widely used due to its stability, simplicity, and reproducibility (15). Also, $Fe^{2+}$-catalyzed lipid peroxidation inhibition by TBA method has been widely used, because food spoilage and *in vivo* membrane damage attributed to lipid oxidation (16,17). The antioxidant activity of several plant materials has been recently reported (3,16-20). In our study, the antioxidant activities of salad vegetables grown in Korea of which the antioxidant potential has not been well-investigated were measured by DPPH radical scavenging assay, TBA method and Folin-Ciocalteu assay.

## MATERIALS AND METHODS

### Materials

DPPH (1,1-diphenyl-2-picrylhydrazyl), ascorbic acid, $\alpha$-tocopherol, thiobarbituric acid (TBA), trichloroacetic acid (TCA), Folin-Ciocalteu's phenol reagent, caffeic acid, tannic acid and 1,1,3,3,-tetraethoxypropane were purchased from Sigma Chemical Company (MO, USA). Trolox from Acros Organics (NJ, USA), ethanol, acetic

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acid, methanol and hydrochloric acid from Merck (Darmstadt, Germany), sodium hydroxide, (NaOH) and ferric sulfate [$Fe(III)_2(SO_4)_3$] from Aldrich Chemical Company (MO, USA) were used.

**Salad vegetable samples**

Forty two kinds of salad vegetables, as listed in Table 1, were obtained from August of 2003 to May of 2004 at the organic farm in Gongju, Korea.

**Preparation of vegetable extract**

Vegetables were washed, drained, weighed, and then dried. Dried samples were ground to a powder to pass through a 200 mesh sieve. Powdered samples (1 g) were immersed in absolute methanol (25 mL) and stored in the dark (15°C) for 3 days after which the methanol fraction was collected. The extraction was repeated three times and solvents were removed by a rotary evaporator. Chlorophyll was removed by extracting the residue with hexane. This chlorophyll-free residue was allowed to stand at room temperature under vacuum to obtain a solvent-free powder, and then stored at -24°C. After rotary evaporation, the residue was dissolved in methanol

**Table 1.** DPPH radical-scavenging activity and inhibition of lipid peroxidation of methanol extract of forty two salad vegetables

| English name | Scientific name | Water content (%) | Extraction (%) | Phenol content (μg/mL) | DPPH $IC_{50}$ | TBA[1] (%) |
|---|---|---|---|---|---|---|
| Agastache rugosa | *Isodon japonicus* | 81.5 | 26.7 | 4.3±0.1 | 3.2±0.8 | 80.4±11.9 |
| Angelica | *Angelica kiusiana* | 84.2 | 22.7 | 8.8±0.1 | 2.2±0.3 | 95.1±1.7 |
| Arugula | *Eruca sativa* | 90.4 | 15.8 | 6.7±0.4 | 7.4±0.7 | 28.3±13.1 |
| Beet leaf | *Beta vulgaris* var. | 91.1 | 15.7 | 4.3±0.2 | 5.2±0.9 | 35.4±3.4 |
| Cabbage, green | *Brassica oleracea* var. *capitata* | 91.7 | 12.4 | 1.6±0.2 | 17.6±8.2 | 14.8±2.4 |
| Cabbage, red | *Brassica oleracea* var. *capitata* | 91.9 | 12.1 | 5.7±0.1 | 11.5±3.2 | 54.1±7.2 |
| Celery | *Apium graveolens* spp. | 85.9 | 30.9 | 2.4±0.1 | 8.1±1.4 | 35.0±6.5 |
| Cherry tomato | *Lycopersicon esculentum* | 93.8 | 58.6 | 3.5±0.6 | 33.6±7.8 | 36.7±5.1 |
| Chicory, red | *Cichorium intybus* | 93.2 | 31.4 | 1.6±1.2 | 4.6±1.4 | 18.3±2.7 |
| Chicory, red | *Cichorium intybus* | 90.6 | 22.7 | 3.8±0.3 | 5.0±0.8 | 32.6±2.6 |
| Chicory, witloof | *Cichorium intybus* (green) | 93.2 | 21.8 | 4.4±0.2 | 6.7±0.9 | 64.5±1.5 |
| Chinese cabbage | *Brassica campestris* | 94.2 | 50.0 | 3.4±2.3 | 47.4±17.9 | 10.6±1.6 |
| Chinese radish, lobo | *Raphanus sativus* | 94.2 | 23.8 | 3.1±0.2 | 8.0±0.8 | 15.6±6.0 |
| Cos lettuce, green | *Lactuca sativa* var. | 94.2 | 22.0 | 4.2±0.1 | 10.4±1.1 | 70.9±5.2 |
| Cos lettuce, red | *Lactuca sativa* var. | 94.0 | 20.1 | 4.7±0.4 | 5.8±0.8 | 18.3±1.3 |
| Dandelion | *Taraxacum officinale wiggers* | 84.6 | 23.1 | 8.8±0.1 | 0.4±0.3 | 88.8±5.6 |
| Dill | *Anethum graveolens* | 97.5 | 22.3 | 3.5±0.4 | 5.9±0.8 | 35.5±1.1 |
| Endive | *Cichorium endivia* var. | 92.6 | 23.2 | 4.2±0.1 | 4.8±0.6 | 74.4±3.8 |
| Grumoro | *Cichorium intybus* | 90.0 | 23.9 | 5.1±0.5 | 4.9±0.6 | 25.3±3.4 |
| Kale | *Brassica oleracea* var. *acephala* | 89.8 | 30.3 | 3.4±0.2 | 10.9±0.1 | 19.3±5.8 |
| Kale, green | *Brassica oleracea* var. *acephala* | 91.0 | 16.3 | 5.5±0.1 | 4.5±0.6 | 84.3±7.1 |
| Kale, red | *Brassica oleracea* var. *acephala* | 90.4 | 25.6 | 3.3±0.1 | 7.0±0.7 | 20.3±5.9 |
| Kale, Scotch | *Brassica olerecea* var. *acephala* | 90.8 | 20.2 | 4.6±0.2 | 5.3±0.1 | 77.0±1.4 |
| Laciniata | *Brassica juncea* var. *laciniata* | 91.2 | 24.3 | 3.6±0.3 | 8.1±0.5 | 4.8±2.8 |
| Leaf broccoli | *Brassica oleracea* var. *botrytis* | 88.8 | 17.3 | 4.8±0.1 | 9.5±1.5 | 36.4±8.3 |
| Leaf lettuce, green | *Lactuca sativa* | 93.4 | 25.5 | 5.7±0.1 | 0.8±0.1 | 72.1±3.4 |
| Leaf lettuce, red | *Lactuca sativa* | 92.0 | 20.3 | 4.9±0.1 | 0.6±0.1 | 72.9±1.8 |
| Lettuce, iceberg | *Lacluce sativa* var. *capitata* | 96.2 | 40.3 | 1.6±0.2 | 52.1±2.3 | 6.4±2.7 |
| Lettuce, Romaine | *Lactuca sativa* | 94.1 | 19.2 | 3.7±0.3 | 3.5±0.2 | 11.4±2.9 |
| Lollo Rosso | *Lactuca sativa* | 94.2 | 16.8 | 4.7±0.1 | 6.5±1.8 | 54.0±4.8 |
| Mustard leaf, green | *Brassica juncea* | 90.8 | 21.8 | 8.1±0.4 | 5.0±2.0 | 82.3±10.7 |
| Mustard leaf, red | *Brassica juncea* | 93.5 | 20.5 | 4.6±0.3 | 6.1±1.9 | 33.2±8.6 |
| Oak leaf lettuce | *Lactuca sativa* var. | 92.5 | 22.0 | 4.2±0.1 | 8.0±1.2 | 56.5±5.5 |
| Parsley | *Petroselinum crispum* | 88.5 | 24.7 | 1.8±0.2 | 67.1±18.1 | 28.4±4.3 |
| Perila leaf | *Perilla frutescens* var. *japonica* | 93.7 | 22.0 | 4.6±0.3 | 0.3±0.4 | 84.7±6.6 |
| Radish leaf, red | *Rhaphanus sativus* | 92.6 | 22.0 | 3.3±0.1 | 7.1±1.5 | 47.7±9.2 |
| Short-frui | *Cryptotaenia japonica* | 87.2 | 17.2 | 4.4±0.1 | 7.1±1.1 | 74.6±1.5 |
| Sugar loaf | *Cichorium intybus* | 93.0 | 24.8 | 5.9±0.3 | 6.2±0.8 | 48.9±4.8 |
| Tah tasai Chinese cabbage | *Brassica campestris* var. *narinosa* | 92.1 | 22.3 | 3.6±0.1 | 8.3±1.5 | 29.3±5.9 |
| Teragon | *Tetragonia tetragonoides* | 92.5 | 14.0 | 5.7±0.1 | 4.3±0.8 | 67.7±5.9 |
| Water spinach | *Ipomoea aquatica* | 89.4 | 14.9 | 7.3±0.3 | 3.7±1.4 | 87.0±3.1 |
| Witloof | *Cichorium intybus* | 89.7 | 20.6 | 6.4±0.1 | 5.1±0.4 | 3.0±2.0 |
| Trolox | | | | | 0.066±0.006 | 94.2±1.4 |
| Ascorbic acid | | | | | 0.048±0.002 | 93.1±1.6 |

[1]Thiobarbituric acid.

and the solvent fraction was assayed for antioxidant activity.

**Preparation of bovine brain homogenate**

The bovine brain was obtained from slaughter in Daejeon. The brain was washed in cooled 0.15 M NaCl, kept on ice, and subsequently blotted on filter paper. The brain tissue was then homogenized for 2 min in a glass-teflon homogenizer with an equal volumes of cold 10 mM phosphate buffer. The homogenates were used to analyse the contents of thiobarbituric acid- reacting substances (TBARS) content.

**Biological lipid peroxidation assay**

$Fe^{+2}$-mediated lipid peroxidation in bovine brain was induced with 0.2 mM $Fe^{+2}$ and 25 μM ascorbic acid *in vitro* as described by Lee (21). The extent of lipid peroxidation was assayed as TBARS contents according to the method described by Bidlack and Tappel (22). Brain homogenates containing $Fe^{+2}$ and ascorbic acid, with or without plant extract, were placed in a shaking water bath at 37°C for 30 min; equal volumes of 15% trichloroacetic acid (TCA) and 0.75% thiobarbituric acid (TBA) were then added. The reaction mixtures were heated in boiling water for 15 min, kept in ice for 5 min, and then centrifuged for 10 min at 3,000 rpm to separate corpuscolate particles. The absorbance of the supernatant was read at 533 nm by a spectrometer (Model 80-2088-64, Pharmacia Biotech. Co., Cambridge, England). Calibration was performed using a malondialdehyde standard prepared by hydrolysis of 1,1,3,3,-tetraethoxypropane (23). Extract concentrations at which lipid peroxidation was inhibited by 50% inhibition ($IC_{50}$) were derived by interpolation of a log concentration against inhibition plot using eight concentrations of the extract, spanning the 50% inhibition point. All experiments were run in triplicate. The antioxidative activity was expressed as the percentage decrease of TBARS relative to the control using the following equation.

$$\text{Antioxidant activity (\%)} = 1 - \frac{[A(\text{brain homogenate+ascorbate+}Fe^{2+}\text{+sample}^{*}) - A(\text{brain homogenate+sample})]}{A(\text{brain homogenate+ascorbate+}Fe^{2+}) - A(\text{brain homogenate})} \times 100$$

*Sample: Vegetable extracts.

A(brain homogenate): Absorbance from the incubation containing brain homogenate only.

A(brain homogenate + sample): Absorbance from the incubation containing brain homogenate and sample.

A(brain homogenate + ascorbate + $Fe^{2+}$): Absorbance for the incubation containing brain homogenate, ascorbate and $Fe^{2+}$.

A(brain homogenate + ascorbate + $Fe^{2+}$ + sample): Absorbance from the incubation containing brain homogenate, ascorbate, $Fe^{2+}$ and sample.

**DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical-scavenging assay**

The free radical-scavenging activity of vegetables was measured using the method described by Shimada et al. (24) with some modification. Samples (0 ~ 1 mg/mL) in 4 mL methanol were added to a solution of DPPH (10 mM, 1 mL) in methanol. The mixture was shaken and left to stand at room temperature for 10 min; the absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The percentage of scavenging effect (%) was calculated as follow: Scavenging effect % (capacity to scavenge the DPPH radical) = (1-absorbance of sample/absorbance of control) × 100. α-Tocopherol, ascorbic acid and trolox were used as positive controls.

**Determination of total phenolics**

Total phenolics of vegetabe were determined by Folin-Ciocalteu colorimetric method (25). One milliliter of vegetable extract was mixed with 0.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22°C for 5 min; 1.0 mL of sodium bicarbonate (60 g/L) solution was added to the mixture. After 30 min at 22°C, absorbance was measured at 760 nm. Results were expressed as milligrams of tannic acid equivalent per gram of the sample.

**Statistics**

All results were obtained from average of three independent experiments. Data were expressed as mean ± SD. The statistical analysis system software (26) was used to perform statistical computations. Pearson's correlation between total phenolics content and antioxidant activity was performed.

## RESULTS AND DISCUSSION

Forty two kinds of salad vegetables including commonly-consumed traditional vegetables and newly-introduced Western vegetables in Korea, were evaluated for antioxidant activity. Here, the antioxidative activities of the vegetable extracts were examined by measuring DPPH free radical-scavenging activity and the ability to inhibit $Fe^{+2}$-mediated lipid peroxidation in bovine brain tissue. Table 1 shows the water content and extraction yield of each vegetable.

**DPPH free radical scavenging activity of salad vegetables**

The peroxyl radical is a key step in lipid peroxidation and scavenging of non-lipid radicals is of great im-

portance for protection against early events in oxidative damage *in vivo* system (27). Generally, DPPH has been used a generator of peroxy radicals, which has been generated *in vitro* system. In this regard, the extracts from salad vegetables were subjected to DPPH radical-scavenging assay. As represented in Fig. 1, the DPPH radical-scavenging activities of salad vegetable extract as well as standard antioxidant, trolox, linearly increased in a dose-dependent manner with a correlation coefficient of each plot of over 0.99. Based on these plots, the DPPH radical-scavenging activity was expressed as $IC_{50}$ (Concentration for 50% free radical inhibition) value for each salad vegetable as shown in Table 1. $IC_{50}$ values of trolox and ascorbic acid were 0.007 mg/mL and 0.048 mg/mL, respectively. Among vegetable extracts, the highest radical-scavenging activity was expressed by the extract of perilla leaf and dandelion leaf ($IC_{50}$, 0.04 mg/mL), followed by red leafy lettuce ($IC_{50}$, 0.07 mg/mL), green leafy lettuce ($IC_{50}$, 0.08 mg/mL), and angelica leaf ($IC_{50}$, 0.22 mg/mL). Meanwhile, lettuce (Iceberg) and young Chinese cabbage showed the lowest antioxidant activity ($IC_{50}$, $>10$ mg/mL). These results reveal that perilla leaf and leafy lettuce may contain potent antioxidant compounds, which may act as primary antioxidants that react with peroxy radicals. Therefore, the use of perilla leaf, red and green leafy lettuce, which have been consumed traditionally as salad vegetables, would be beneficial to prevent oxidative damage caused by the intake of broiled meat. In addition to these Korean native vegetables, newly introduced Western vegetables, such as dandelion and angelica leaf, romain ($IC_{50}$, 3.5 mg/mL), water spinach ($IC_{50}$, 3.7 mg/mL), which possessed high radical-scavenging activities, are also supposed to be effective in preventing free radical generation.

**Inhibition of lipid peroxidation of salad vegetables**

Metal ions such as $Fe^{2+}$ or $Cu^{2+}$ have been known to catalyze the oxidation of lipid in the presence of reducing agents such as ascorbic acid. Virtually, all cellular components such as lipids, proteins, nucleic acids, and carbohydrates are all known to undergo metal-catalyzed oxidative modification (28). The process of lipid peroxidation is initiated by the abstraction of a hydrogen atom in an unsaturated fatty acyl chain and propagated as a chain reaction during the lipid peroxidation of membranes (27). Therefore, the inhibition of lipid peroxidation in the initial stage is of great importance in the prevention of disease processes involving free radicals. Hence, we examined the inhibitory effect of vegetable extract on lipid peroxidation of brain homogenate caused by $H_2O_2/Fe^{2+}$/ascorbic acid system. When forty two salad vegetable extracts were tested for the antioxidant action against $Fe^{2+}$-catalyzed peroxidation of brain lipid, it was found that all salad vegetable extracts expressed an antioxidant action in a dose-dependent manner. Angelica leaf exhibited the highest inhibition with over 90%, and then dandelion leaf, water spinach, perilla leaf, kale and mustard leaf showed over 80% inhibition. However, lettuce (Iceberg), young Chinese cabbage and laciniata showed the lowest inhibition with below 10%. When compared to that of DPPH radical-scavenging activity, inhibition of lipid peroxidation in leafy lettuce which showed remarkably high in DPPH radical-scavenging activity, showed moderately high activity with 72%. Although discrepancies in some vegetables were observed between two methods, generally, vegetable extract with higher antioxidant action against DPPH oxidation and $H_2O_2/Fe^{2+}$/ascorbate system, contained higher content of total phenolics. These results suggest that there was some correlationship between DPPH method

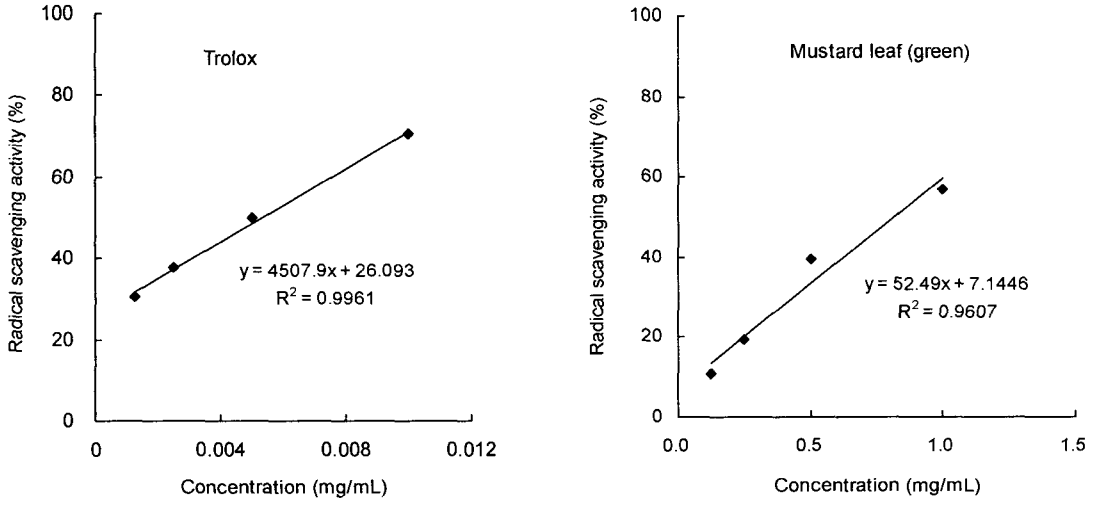


**Fig. 1.** Dose-dependent curve for DPPH radical scavenging activity of trolox as a standard (left) and mustard leaf (right).

and /$Fe^{2+}$/ascorate system. Although these antioxidative activities of vegetable extracts may be partially due to phenolics, antioxidative activities of salad vegetables could be the synergistic effect of more than two compounds present in the vegetable extract; it has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that create an effective defense system against free radical attack (13). Based on these results, it is suggested that salad vegetables would contribute to reduce the level of peroxy radicals produced during oxidative damage (29). Virtually all cellular components appear to be sensitive to oxidative damage. Lipids, proteins, nucleic acids, and carbohydrates are all known to undergo oxidative modification (28). Further studies on the identification and purification of components responsible for the antioxidative activities in different fractions of salad vegetable extract are now in progress.

**Total phenolics**

Phenolic compounds are very important plant constituents because they have a scavenging capability due to the existence of hydroxyl groups (30). In this respect, total phenolics content was determined according to the Folin-Ciocalteu method, varied from 1.6 to 8.8 μg/mL extract. (Table 1). Especially, vegetables such as dandelion, angelica leaf, mustard leaf, water spinach, kale, leafy lettuce and perilla leaf contained high amount of phenolic compounds. Noteworthy, the antioxidant activity of salad vegetables showed to be in a proportion to the total amount of phenolic compounds in vegetable extract. Generally, vegetables, which had higher value of phenolic compound content, demonstrated greater antioxidant action in DPPH free radical-scavenging activity and inhibition of lipid peroxidation as demonstrated with angelica leaf, dandelion and lettuce. However, despite its relatively moderate content of phenolic compounds,

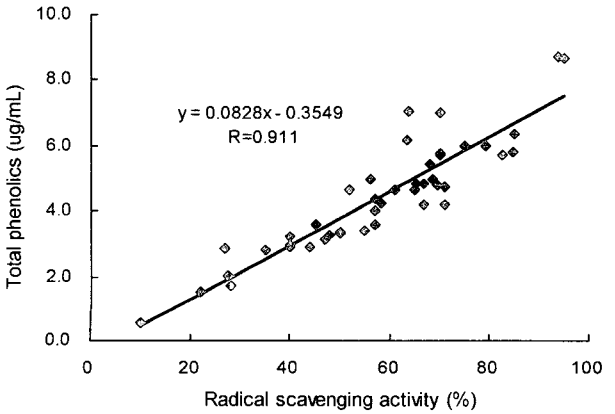


**Fig. 2.** Relationship between total phenolics and DPPH radical scavenging activity of salad vegetables.

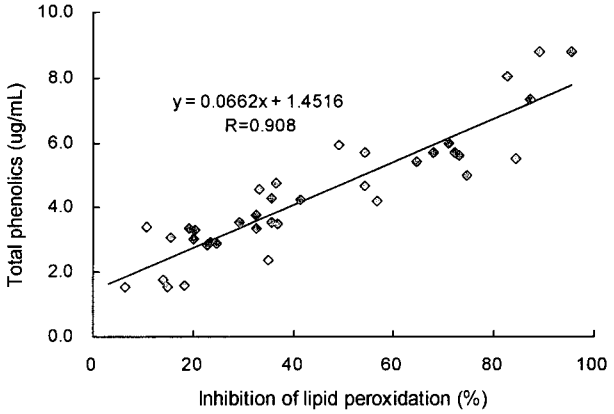


**Fig. 3.** Relationship between total phenolics and inhibition of lipid peroxidation (TBA assay) of salad vegetables.

leafy lettuce expressed a higher DPPH radical-scavenging effect, compared to the other vegetables. These strong antioxidant activity in these vegetables may be derived from the phenolic compounds such as caffeic acid, luteolin, quercetin, protocatechulic acid and *p*-hydroxybenzoate, 3,5-dicaffeoylquinic acid or rosmarinic acid (31,32).

**Relationship between total phenolics and antioxidant activity**

The relationship between total phenolics and antioxidant activity of salad vegetables is shown in Fig. 2. The results indicate that when all salad vegetables were included in the statistical analysis, there was a significant positive relationship between total phenolics and DPPH radical-scavenging activity ($R=0.911$, $p<0.001$). However, the relationship between phenolics and antioxidant activity for the anthocyanin-rich vegetables was not highly significant. The lack of a significant correlation between total phenolics and DPPH radical-scavenging activity of the anthocyanin-containing plant materials, such as purple leafy lettuce, purple cos lettuce, purple kale, purple chicory, purple mustard leaf or purple cabbage, reflects that the compounds other than phendics may be responsible for antioxidant action. Statistically significant relationships were also observed between total phenolics and inhibition of lipid peroxidation of salad vegetables as shown in Fig. 3. There was a significant positive relationship between total phenolics and lipid peroxidation inhibition ($R=0.908$, $p<0.001$). The correlation between the antioxidant activities and total phenolics of plants was derived from the effective hydrogen donors in phenolic compounds (33). Total antioxidative activity of wine was reported to be correlated well with the phenolics contents (34). Further work is in progress in our laboratory to elucidate the identity of compounds responsible for the antioxidant activity.

Based on our results, it can be concluded that salad

vegetables, especially perilla leaf, leafy lettuce, dandelion and angelica leaf showing great free radical-scavenging activity and lipid peroxidation inhibition activity, could be used for easily accessible natural antioxidant sources.

## ACKNOWLEDGEMENT

This work was supported by ARPC under the Ministry of Agricultural and Forestry.

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(Received October 26, 2004; Accepted December 3, 2004)