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Antioxidative Activity of the Korean Wild Leafy Vegetables: *Aster scaber* and *Ligularia fischeri*

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Abstract

The purpose of this study was to evaluate the antioxidative potential of Korean wild leafy vegetables *in vivo* as well as *in vitro*. Antioxidative activities of *Aster scaber* and *Ligularia fischeri* were evaluated against a reference of *Spinacia oleracea*. Forty rats were fed either control diet or respective vegetable diets for four weeks. The level of thiobarbituric acid reactive substances (TBARS) and the activities of catalase and superoxide dismutase (SOD) in liver were compared. The plasma concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) were also compared. Korean wild leafy vegetables were assayed for β-carotene, vitamin C and vitamin E and total phenolic compound including flavonoid and thiobarbituric acid (TBA) value using the linoleic acid model system. SOD activity in rats fed *Aster scaber* was significantly higher(as much as 289%) than those fed *Spinacia oleracea*. Compared to control animals, the *Aster scaber* fed animals had significantly lower TC and lower atherogenic index. Compared to *Spinacia oleracea*, *Aster scaber* and *Ligularia fischeri* had vitamin C concentration of 150% and 400%, flavonoid concentrations of 470% and 310%, and phenolic compound concentrations of 326% and 203%, respectively, but tended to have lower β-carotene and significantly lower vitamin E concentrations. TBA values were only 18% of the control value in *Aster scaber* and *Ligularia fischeri* and 41% of the control value in *Spinacia oleracea*. These results suggest that *Aster scaber* could have potent antioxidative activity *in vivo* as well as *in vitro* and potential value as a functional food to improve the plasma lipid profiles. Furthermore, phenolic and flavonoid compounds may be a major contributing factor to the antioxidative potential of Korean wild leafy vegetables.

Key words: antioxidant, antioxidative activity, *Aster scaber*, *Ligularia fischeri*

INTRODUCTION

The role of active oxygen species and free radicals in tissue damage in various human diseases is becoming increasingly recognized (1). To counteract these challenges, the human body has developed two primary defences: enzymatic and non-enzymatic antioxidant system. Detoxification enzymes may be controlled by the level of antioxidants that reduce free radical species and maintain the redox state of the cell (2). Among enzymatic antioxidant systems, catalase and superoxide dismutase (SOD) are important. Important non-enzymatic antioxidant systems include antioxidant such as vitamin E, vitamin C and β-carotene (3-6) as well as flavonoids and other plant phenolics (7-9), which may have similar effects.

There is increasing interest in the disease-preventing potential of naturally occurring substances in the diet. Antioxidants have attracted great attention for their preventive effects, espcially antioxidants for edible plants. These edible plants are also candidates for preventing aging and diseases. *Aster scaber* and *Ligularia fischeri* have been used as wild vegetables in Korea. These wild vegetables possess antiphlogistic, stomachic, analgesic, antimutagenic effects (10-12). Thus, as a part of our continuing studies of the biological activity of natural products, we evaluated the antioxidative activity of *Aster scaber* and *Ligularia fischeri in vivo* as well as *in vitro*.

MATERIALS AND METHODS

Animals and diets

A total of 40 male Sprague-Dawley rats (Samyang Exp. Animals Co.), weighing 120~140 g were divided into four groups of 10 rats each, with similar body weights; and fed either the control diet or diets containing *Aster scaber*, *Ligularia fischeri* and *Spinacia oleracea*. *Aster scaber*, *Ligularia fischeri* and *Spinacia oleracea* were purchased at a local market in Chuncheon, Korea. Vegetables were freeze-dried and powdered. The moisture content of dried vegetables was 10.70±1.0%. Animals received 10% vegetable diets or control diet for four weeks. The control diet was a vitamin-free casein-based semisyn-

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thetic diet which met AIN-93 recommendation (13). The nutritional composition of 10% vegetable diets was manipulated to achieve a similar composition to the control diet. Thus, all experimental diets contained 20% protein, 5% fat, 65% carbohydrates, 5% fibre and 3850 kcal/ kg by weight. Prior to initiating the respective vegetable diets, rats were given *ad libitum* access to the control diet for one week to adapt to the diet and feeding schedule and to bring all the rats to a similar metabolic status.

Sample collection and analysis

At the end of week 4, animals were anesthetized with ether and sacrificed by decapitation. Immediately following decapitation, blood was collected in heparinized tubes and centrifuged to separate the plasma. Organs were rapidly blotted dry and weighed. Plasma and tissues were stored at 70°C until analyzed. Plasma total cholesterol (TC) was analyzed with a commercial kit based on enzymatic method (Youngdong Pharmaceutical Co., Korea). High-density lipoprotein-cholesterol (HDL-C) was analyzed with a commercial kit based on the same analytical method as total cholesterol, after the precipitation of low-density lipoprotein, very-low-density lipoprotein and chylomicrons with polyethyleneglycol (International Reagent Co., Japan). Atherogenic index was calculated as (TC-HDL-C) / HDL-C. Triglyceride (TG) was analyzed with commercial kit based on the Trinder method (Youngdong Pharmaceutical Co., Korea).

Vitamin C was measured by a 2,4-dinitrophenylhydrazine colororimetric procedure (14), and β-carotene was measured by HPLC method (15,16). Vitamin E was measured by a colorimetric method using α, α-dipyridyl (16). Flavonoid was measured using the method of Kang et al. (17). Total phenol was measured by a colorimetric method modified by Chung et al. (18). Thiobarbituric acid (TBA) was measured using linoleic acid model system modified by Kim et al. (19). Liver thiobarbituric acid reactive substances (TBARS) was measured by a colorimetric method modified by Buckingham (20). A spectrophotometric method (21) was used for the determination of catalase activity. The activity of superoxide dismutase (SOD) was determined by the method of Flohe et al. (22).

For statistical analysis, all data were first evaluated by analysis of variance. When F values indicated significance of difference, the least significant difference test was performed. A value of $p<0.001$ was considered to be statistically significant.

RESULTS

As shown in Table 1, at week 4, there were no differences in body weight and FER between control animals and those fed either of the vegetable diets. Table 2 shows the effect of Korean wild leaf vegetable diet on organ weights. The mean liver weight of animals fed *Aster scaber* lower than that of control animals. Regardless of vegetable type, the spleen weights of vegetable-fed animals were significantly lower than those of control rats. Kidney and heart weights did not differ among the groups. Table 3 shows the effect of Korean wild leaf vegetable diet on antioxidative enzyme activities of liver. Neither TBARS nor catalase activity differed between anmals fed control or vegetable diet. However, SOD activity of *Aster scaber* animals was significantly higher than that of control animals. Moreover, the SOD activity of *Aster scaber* was significantly higher than that of *Spinacia oleracea,* reaching 289% of that of control animals. The effect of Korean wild leafy vegetable diets on plasma lipid composition is shown in Table 4. TG concentrations in the animals fed vegetable diets were not different from those of the control animals. Compared to control animals, TC and HDL-C levels of *Aster scaber* fed animals were significantly lower and they had lower atherogenic indices. Table 5 shows the content of antiox-

Table 1. The effect of Korean wild leafy vegetable diet on body weight gain (g) and feed efficiency ratio (FER)

	Control	*Spinacia oleracea*	*Aster scaber*	*Ligularia fischeri*
Body weight	279.9±7.0[1)a2)]	272.3±4.1[a]	276.6±7.2[a]	276.5±27.1[a]
FER	0.37±0.09[a]	0.35±0.01[a]	0.37±0.01[a]	0.36±0.01[a]

[1)]Values are mean±SEM, n = 10.
[2)]Within a given row, those values with different superscripts are significantly different ($p<0.05$).

Table 2. The effect of Korean wild leafy vegetable diet on organ weights (g)

	Control	*Spinacia oleracea*	*Aster scaber*	*Ligularia fischeri*
Liver	8.81±0.34[1)a2)]	8.37±0.19[ab]	7.87±0.23[b]	8.49±0.31[ab]
Heart	0.89±0.03[a]	0.88±0.01[a]	0.93±0.03[a]	0.89±0.03[a]
Kidney	1.06±0.03[a]	1.04±0.03[a]	1.03±0.03[a]	0.99±0.04[a]
Spleen	0.82±0.05[a]	0.72±0.03[b]	0.77±0.05[b]	0.77±0.03[b]

[1)]Values are mean±SEM, n = 10.
[2)]Within a given row, those values with different superscripts are significantly different ($p<0.05$).

Table 3. The effect of Korean wild leafy vegetable diet on antioxidative enzyme activities of liver

	Control	*Spinacia oleracea*	*Aster scaber*	*Ligularia fischeri*
TBARS[3)]	7.729±0.825[1)a2)]	7.412±0.988[a]	7.262±3.749[a]	7.163±1.388[a]
SOD[4)]	17.27±0.38[a]	23.24±1.39[b]	49.98±1.90[c]	20.42±1.07[ab]
Catalase[5)]	5857±81[ab]	5963±74[ab]	5539±164[b]	6064±76[a]

[1)]Values are mean±SEM, n = 10.
[2)]Within a given row, those values with different superscripts are significantly different ($p<0.05$).
[3)]TBARS: thiobarbituric acid reactive substance, nmol/g liver.
[4)]SOD: superoxide dismutase; SOD activities are expressed as units per minute per mg protein (One unit inhibits the rate of reduction of cytochrome C by 50% in a coupled system with xanthine and xanthine oxidase at pH 7.8 and 25°C in a 3 ml reaction volume).
[5)]Catalase activities are expressed as nmol formaldehyde utilyzed per mg protein.

Table 4. The effect of Korean wild leafy vegetable diet on plasma lipid composition

	Control	*Spinacia oleracea*	*Aster scaber*	*Ligularia fischeri*
Triglyceride (mg/dL)	67.72±4.76[1)a2)]	76.27±7.73[ab]	72.09±7.09[ab]	90.64±7.05[b]
Total cholesterol (mg/dL)	54.76±4.01[ab]	58.48±3.43[a]	32.74±2.47[c]	46.46±2.55[b]
HDL-cholesterol (mg/dL)	17.13±2.08[a]	16.13±2.09[a]	11.83±0.45[b]	9.59±0.89[b]
Atherogenic index[3)]	2.47±0.41[a]	2.47±0.40[a]	1.80±0.27[a]	3.49±0.22[b]

[1)]Values are mean±SEM, n = 10.
[2)]Within a given row, those values with different superscripts are significantly different ($p<0.05$).
[3)]Atherogenic index was calculated as (TC - HDL-C) / HDL-C.

idants in Korean wild leafy vegetables. On a freeze-dried weight basis, β-carotene concentrations in *Aster scaber* and *Ligularia fischeri* tended to be lower than that of *Spinacia oleracea.* The vitamin E content of *Aster scaber* and *Ligularia fischeri* was significantly lower than that of *Spinacia oleracea.* The vitamin C content of *Aster scaber* and *Ligularia fischeri* was 150% and 400% of *Spinacia oleracea*'s, respectively. Flavonoid content of *Aster scaber* and *Ligularia fischeri* was 470% and 310% of *Spinacia oleracea*, respectively. Total phenolic compounds in *Aster scaber* and *Ligularia fischeri* was 326% and 203% higher than in *Spinacia oleracea*, respectively.

As shown in Table 6, all the vegetable diets tested had higher antioxidant activity than the control diet. TBA values of *Aster scaber* and *Ligularia fischeri* were only 18% of the control value. TBA value of *Spinacia oleracea* was 41% of control value.

DISCUSSION

The value of spinach was used as a reference to compare the relative antioxidative potency of wild vegetables because spinach is a cultivated vegetable that is commonly accepted to be an excellent source of antioxidant nutrients.

Table 5. Antioxidant contents in Korean wild leafy vegetables

	Spinacia oleracea	*Aster scaber*	*Ligularia fischeri*
β-carotene (μg/100 g)	39486±350[1)a2)]	25402±128[b]	3098±398[c]
Vitamin C (mg/100 g)	17.38±0.87[a]	26.07±2.30[b]	69.79±1.75[c]
Vitamin E (mg/100 g)	12.58±0.46[a]	3.28±0.45[b]	2.31±1.02[b]
Flavonoid (mg/100 g)	749±97[a]	3535±214[c]	2321±239[b]
Total phenolic compounds (mg/100 g)	8870±420[a]	28880±240[c]	18006±372[b]

[1)]Values are mean±SEM, n = 10.
[2)]Within a given row, those values with different superscripts are significantly different ($p<0.05$).

Table 6. Change of thiobarbituric acid value (O.D.) of linoleic acid substrates during storage at 40°C

	Control	BHA	*Spinacia oleracea*	*Aster scaber*	*Ligularia fischeri*
1 hour	0.294±0.026[1)d2)]	0.003±0.001[a]	0.096±0.028[c]	0.050±0.003[b]	0.049±0.006[b]
2 hour	0.501±0.104[d]	0.005±0.001[a]	0.200±0.038[c]	0.115±0.026[b]	0.098±0.029[b]
3 hour	0.750±0.118[d]	0.006±0.001[a]	0.320±0.044[c]	0.165±0.027[b]	0.154±0.036[b]
4 hour	1.050±0.160[d]	0.006±0.001[a]	0.405±0.087[c]	0.198±0.060[b]	0.190±0.053[b]
5 hour	1.204±0.151[d]	0.009±0.001[a]	0.477±0.091[c]	0.213±0.023[b]	0.221±0.054[b]
6 hour	1.323±0.171[d]	0.011±0.003[a]	0.537±0.071[c]	0.235±0.030[b]	0.242±0.046[b]

[1)]Values are mean±SEM, n = 10.
[2)]Within a given row, those values with different superscripts are significantly different ($p<0.05$).

Because there were no differences in body weight and FER between animals on control or vegetable diets, regardless of vegetable type, the biochemical indices were not affected by differences in body weight and FER due to differences in palatability or intake of the diets.

The potent antioxidative activities of the vegetables were observed *in vivo* and *in vitro*. Although Jeong et al. (23) reported antioxidative activity, in terms of the value of TBARS, this study found on differences in TBARS between animals fed control or vegetable diets. However, the SOD activities of *Aster scaber* fed animals were significantly higher than those of control animals. Moreover, the SOD activity in animals fed *Aster scaber* was significantly higher than that of *Spinacia oleracea* fed animals, reaching 289% of the activity in control animals. Furthermore, the TBA value of *Aster scaber* was 80% of the value of BHT, a strong synthetic antioxidant, while the value of spinach was only 40% of BHT. Thus, the higher antioxidative activity of *Aster scaber* may have an effect on blood lipid composition, which has been associated with free radical mediated events. This is consistent with our observation that both the TC level and the atherogenic index were significantly lower in the *Aster scaber* fed animals. The strong antioxidative potency of the wild vegetables may be due to either antioxidant vitamin, non-antioxidant vitamin, phenoic compounds including flavonoid, or the combined effect of all of the above. Flavonoids inhibit both lipid peroxidation and the formation of lipid peroxides (24-27). The hypocholesterolemic effect has also been reported in other polyphenol-containing foods (28-30). Numerous studies have demonstrated a multitude of interactions among the antioxidants (31-33). Vitamin A, C, and E have additive antioxidative effects when used together (31). Dietary flavonoids are an important category of antioxidants that have a sparing effect on vitamin E and β-carotene (34). The concentrations of flavonoid and total phenolic compounds were much higher in *Aster scaber and Ligularia fischeri* than in spinach, whereas the vitamin E and β-carotene concentrations were lower. This suggest that the flavonoid and other dietary phenolic compounds, which are known to have potent free-radical scavenging properties (35,36), are the major contributors to the antioxidative potential of *Aster scaber and Ligularia fischeri*.

Despite the many uncertainties regarding mode of action, these results demonstrate that *Aster scaber* has potent antioxidative activity, both *in vivo* and *in vitro*, and potential value as a functional food to improve the plasma lipid profile.

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