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Evaluation of the Antioxidant Contents of Korean Wild Leaf Vegetables*

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ABSTRACT

The purpose of this study was to evaluate the antioxidative potential of Korean wild leaf vegetables. Antixoidative activity of chamchwi (Aster scaber), nurucchwi (Pleurospermum kamtschaticum) sumssukbujengee (Aster glheni), moshidae (Adenophora remotiflora), spinach (Spinacia oleracea) was evaluated as a reference for commonly used synthetic antioxidant, BHA. We compared the content of β-carotene, vitamin C and vitamin E as antioxidant vitamins, and total phenolic compound including flavonoid as non-vitamin compounds in Korean wild leaf vegetables and spinach. Thiobarbituric acid (TBA) value of *Pleurospermum kamtschaticum*, *Aster scaber* and *Adenophora remotiflor* was only 18 – 20% of control. TBA value of *Aster glheni* was 40% of control. On the basis of moles, the high level of vitamin C is the major contributor to the total antioxidant vitamin contents of *Pleurospermum kamtschaticum* and *Adenophora remotiflor*. The amounts of flavonoid as well as total phenolic compound in *Pleurospermum kamtschaticum* and *Adenophora remotiflor* were also much higher than those of spinach. The amounts of flavonoid as well as total phenolic compound in *Aster glheni* were much higher than those of spinach while total amount of antioxidant vitamins was significantly lower than that of spinach. These results suggest that *Pleurospermum kamtschaticum* and *Adenophora remotiflor* could have antioxidative potency in food. Because of the higher content of antioxidant vitamins in *Pleurospermum kamtschaticum* and *Adenophora remotiflor*, these Korean wild leaf vegetables may have preventive effects on degenerative diseases, which have been associated with free radical mediated events.

KEY WORDS: antioxidant, wild leaf vegetables, vitamin E, vitamin C, β-carotene, flavonoid.

INTRODUCTION

There is increasing interest in the disease-preventing potential of naturally occurring substances in the diet and antioxidants have attracted a great attention for their preventive effects. The role of active oxygen and free radicals in tissue damage in various human diseases is becoming increasingly recognized.[1] Active oxygen and free radical are the byproducts of normal metabolism and attracts biological molecules, leading to cell or tissue injury. Living organisms have evolved antioxidant defenses to remove active oxygen and free radicals. However, our endogenous antioxidant defenses are inadequate to completely prevent oxidative damage.[2] Therefore, the finding sources of dietary antioxidants is especially important. Epidemiological evidence is accumulating to support the view that antioxidant vitamins like vitamin E, vitamin C and β-carotene have preventive effects on degenerating diseases.[3-6] Also, a dietary intake of the flavonoids and other plant phenolics may have the effect of lowering the risk of certain patho-physiologies that have been associated with free radical mediated events.[7-9]

When a plant is exposed to strong sunlight, it is forced to produce a much larger amount of harmful active oxygen in its photosynthetic system, which may cause it to have an efficient antioxidant in its chemical constituents for the protective system.[10][11] This may be the mechanism by which a plant produces active oxygen species during photosynthesis and normally, active oxygens produced in plant cells are under the control of the plant's own scavenging system. For the selection of plants, we focused on the wild leaf vegetables because they get relatively high amounts of sunlight compare to cultivated vegetables. Moreover, wild leaf vegetables also have been utilized as a stomachic, analgesic, antispasmodic and antitumorgenic agent in oriental folk medicine without scientific verification. Recently, physiological activity has been reported to have antiphlogistic, stomachic, analgesic, anti-tumor promoting effects in chamchwi (*Aster scaber*),[12-14] nurucchwi (*Pleurospermum kamtschaticum*),[15][16] Sumssukbujengee (*Aster glheni*) and Moshidae (*Adenophora remotiflor*).[14] Because these wild vegetables have been eaten in Korea for a long time, their toxicity or preference among Koreans does not need to be tested and the results of this study

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can be applied to humans directly. Thus, as a part of our continuing studies on the biological activity of natural products, we evaluated the antioxidant contents of Korean wild leaf vegetables.

MATERIALS AND METHODS

1. Sample preparation

Chamchwi (*Aster scaber*) and nurucchwi (*Pleurospermum kamtschaticum*) were purchased at a local market in Chuncheon. Sumssukbujengee (*Aster glheni*) was purchased at a local market in Ulreung island. Moshidae (*Adenophora remotiflor*) and spinach (*Spinacia oleracea*) were purchased at a local market in Seoul, Korea. Vegetables were freeze-dried and stored at −20℃ until analyzed.

2. Analysis

Vitamin C was Measured by 2,4-dinitrophenylhydrazine colororimetric procedure.[17] Dried leaves of vegetables were extracted with 5% metaphosphoric acid under subdued light and filtered through Whatman No 1 filter paper. The filtrate was used for vitamin C analysis. β-carotene was measured by the HPLC method,[18][19] which was modified according to the following specification. Pump mobil phase (Benzene : Hexan/1 : 1.5/v : v) at a flow rate of 1.0 ml/min into the column (μBondpack ODS-2,4,6× 250 mm, 5 μm porus packing, C_{18}, Waters). Measure vitamin E using colorimetric method using α, α-dipyridyl.[20] For dried leaves of vegetables in standard taper round-bottom flask, add 30ml absolute ethanol and 1 ml 10% pyrogallol-ethanol solution. Attach reflux condenser and heat to boiling point in water bath. Raise condenser and add 3 ml concentrated KOH solution for dried leaves, replace condenser, and reflux 30 minutes. Stopper and cool rapidly under cold running water. Transfer solution to seperator, using 30 ml H_2O/ 10 g of dried vegetables. Extract unsaponifiable matter by rinsing saponification flask and shaking with each of two 30 ml portions of petroleum ether for dried vegetables. Combine petroleum ether extracts and wash with equal volumes H_2O until solution is neutral to phenolphtalein. Filter washed petroleum ether extract through anhydrous granular Na_2SO_4 into Erlenmyer, rinsing with petroleum ether. Concentrate petroleum ether solution under reduced pressure. Transfer this solution and dilute to 25 ml with ethanol. Use one aliquots of this ethanol solution for vitamin E analysis. Flavonoid measured using Kang *et al.* method.[21] Total phenol measured by colorimetric method modified by Chung *et al.*[22] Thiobarbituric acid (TBA) measured using linoleic acid model system modified by Kim *et al.*[23] Extraction and fractionation procedure[24] was as follows: Dried leaves (3 g) of vegetables were extracted three times with 80% methanol solution (100 ml) at 80℃ in waterbath for 20 minutes and concentrated *in vacuo* to evaporate methanol. The methanol extract was fractionated with chroloform and ethylacetate and was evaporated and freez-dried. Ethylacetate fraction was used to determine the antioxidative activity using TBA method.

For statistical analysis, all datum were first evaluated by analysis of variance. For those F values that were significant, the least significant difference test was peformed. A p value <0.001 was considered to be statistically significant.

RESULTS

Table 1 shows the content of antioxidant vitamins in Korean wild leaf vegetables. On a freeze dried weight basis, the content of β-carotene was 18593 ± 351 μg/100 g in *Pleurospermum kamtschaticum*, 25402 ± 128 μg/100 g in *Aster scaber*, 10183 ± 233 μg/100 g in *Adenophora remotiflor*, and 3938 ± 9.28 μg/100 g in *Aster glheni*. The content of vitamin C was 95.6 ± 5.21/100 g in *Pl-*

Table 1. Antioxidant vitamin contents as a percentage of RDA[1] in korean wild leaf vegetables[2][3]

Plant	Total antioxidant nutrition contents									
	Aster scaber		*Pleurospermum kamtschaticum*		*Aster glheni*		*Adenophora remotiflora*		*Spinacia oleracea L*	
	Contents	% RDA	Contents	% RDA	Contents	% RDA	Contents	% RDA	Contents	% RDA
β-carotene (μg/100 g)	25401 ± 128^b	605	18592 ± 351^c	443	3938 ± 9.28^e	94	10183 ± 232^d	242	39486 ± 350^a	940
Vitamin C (mg/100 g)	26.07 ± 2.30^c	37.2	95.59 ± 5.21^a	136	17.4 ± 2.30^d	24.9	60.8 ± 0.90^b	86.9	17.38 ± 0.87^d	24.8
Vitamin E (mg/100 g)	3.28 ± 0.45^c	32.8	8.51 ± 0.05^b	85.1	8.1 ± 0.55^b	81	12.5 ± 0.10^a	125	12.58 ± 0.46^a	125

1) Recommended dietary allowance (RDA) was based on that of 20 – 29years men (2000, 7th)
2) Data represents means ± standard deviation, based on the freeze dried vegetable weight
3) Within a given row, those values with different alphabets are significantly different ($p < 0.001$)

Table 2. The contents of antioxidant vitamins in Korean wild leaf vegetables on the basis of moles[1)2)]

	β-carotene	Vitamin C	Vitamin E
Aster scaber	47.3 ± 0.24[b]	148 ± 13.1[c]	7.60 ± 1.04[c]
Pleurospermum kamtschaticum	34.6 ± 0.65[c]	542 ± 29.6[a]	19.8 ± 0.12[b]
Aster glheni	7.34 ± 0.02[e]	98.7 ± 13.0[d]	18.7 ± 1.28[b]
Adenophora remotiflora	18.7 ± 0.02[d]	345 ± 4.94[b]	29.0 ± 0.02[a]
Spinacia oleracea	73.5 ± 0.65[a]	98.7 ± 4.94[d]	29.2 ± 1.06[a]

1) Data represents means ± standard deviation, based on the freeze dried vegetable weight
2) Within a given column, those values with different alphabets are significantly different ($p < 0.001$)

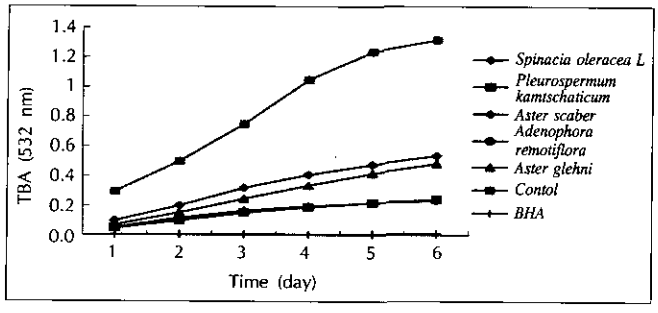

Fig. 1. Change of thiobarbituric acid (TBA) value of linoleic acid substrates containing BHA and plant extract during storage at 40℃. Two hundred ppm of each sample was used for the measurement

eurospermum kamtschaticum, 26.1 ± 2.30 mg/100 g in *Aster scaber*, 60.8 ± 0.90 mg/100 g in *Adenophora remotiflor*, and 17.4 ± 2.30 mg/100 g in *Aster glheni*. The content of vitamin E was 8.52 ± 0.05 mg/100 g in *Pleurospermum kamtschaticum*, 3.28 ± 0.45 mg/100 g in *Aster scaber*, 12.5 ± 0.10 mg/100g in *Adenophora remotiflor*, and 8.1 ± 0.55 mg/100g in *Aster glheni*. Table 2 showes the amount of antioxidant vitamins on the basis of moles. On a freeze dried weight basis, the content of β-carotene was 34.6 ± 0.65 μmol/100 g in *Pleurospermum kamtschaticum*, 47.3 ± 0.24 μmol/100 g in *Aster sc 0aber*, 18.7 ± 0.02 μmol/100 g in *Adenophora remotiflor*, and 7.34 ± 0.02 μmol/100 g in *Aster glheni*. The content of vitamin C was 542 ± 29.6 μmol/100 g in *Pleurospermum kamtschaticum*, 148 ± 13.1 μmol/100 g in *Aster scaber*, 345 ± 4.94 μmol/100 g in *Adenophora remotiflor*, and 98.7 ± 13.0 μmol/100 g in *Aster glheni*. The content of vitamin E was 19.8 ± 0.12 μmol/100 g in *Pleurospermum kamtschaticum*, 7.60 ± 1.04 μmol/100 g in *Aster scaber*, 29.0 ± 0.02 μmol/100 g in *Adenophora remotiflor*, and 18.7 ± 1.28 μmol/100 g in *Aster glheni*. As shown in Fig. 1, all the vegetables tested had marked antioxidative activity when compared with the control. TBA value of *Pleurospermum kamtschaticum*, *Aster scaber* and *Adenophora remotiflor* was 80% lower than that of control. TBA value of *Aster glheni* and spinach was 40% lower than that of control. Table 3 shows the content of non-vitamin antioxidant compounds. The flavonoid content of wild vegetables was 749 – 5155 mg/100 g of freeze dried weight which was 100 – 650% more than that of spinach. The flavonoid content of *Pleurospermum kamtschaticum* was the highest one. The total phenoic compound content of wild vegetables was 8870 – 29850 mg/100 g of freeze dried weight which was 100 – 330% more than that of spinach. The total phenolic content of *Adenophora remotiflor* was the highest one.

Table 3. The contents of other non-vitamin antioxidant in korean wild leaf

	Flavonoid	Total phenolic
Aster scaber	3535 ± 214.8[b]	28880 ± 240[a]
Pleurospermum kamtschaticum	5155 ± 418.4[a]	19920 ± 120[c]
Aster glehni	3787 ± 85.4[b]	24540 ± 780[b]
Adenophora remotiflora	3123 ± 55.1[c]	29850 ± 1910[a]
Spinacia oleracea	749 ± 97.4[d]	8870 ± 420[d]

1) Data represents means ± standard deviation, based on the freeze dried vegetable weight
2) Within a given column, those values with different alphabets are significantly different ($p < 0.001$)

DISCUSSION

The value of spinach was used as reference value to compare the relative antioxidative potency of wild vegetables because spinach is commonly accepted to be an excellent source of antioxidant nutrient among the cultivated vegetables. The potent antioxidative activities of the vegetables were observed in a linoleic acid auto-oxidation model system. On the basis of TBA value, the potent antioxidant activity of vegetables tested was strong and the antioxidant activity of *Aster scaber*, *Pleurospermum kamtschaticum*, and *Adenophora remotiflor* reached to 80% that of BHT, a strong synthetic antioxidant, while the antioxidative activity of spinach or *Aster glheni* reached to 40% that of BHT. Although the antioxidant activity of vegetables was not as strong as much as that of BHA, which is a synthetic antioxidant, these wild vegetables still can be a good source of antioxidants. There is increasing interest in the protective biological effects of natural oxidants, especially from edible plants. These edible plants are also candidates for preventing aging and diseases. β-carotene content in 100 g of all the freeze dried wild vegetables tested except *Aster glheni* were 200 – 600% of recommended dietary allowance (RDA). The strong antioxidative

potency of the wild vegetables might be from either antioxidant vitamin or non-antioxidant vitamin compounds, phenolic compounds including flavonoid or both. Much evidence demonstrated a multitude of interactions among the antioxidants.[25-27] The vitamin A, C, and E showed additive antioxidative effects when used together.[25] The antioxidant vitamins content of *Pleurospermum kamtschaticum* and *Adenophora remotiflor* was significantly higher than that of spinach on a molecular basis. The high level of vitamin C is the major contributor to the total antioxidant vitamin contents of *Pleurospermum kamtschaticum* and *Adenophora remotiflor*.

Dietary flavonoids represent an important source of antioxidants and showed the sparing effect on vitamin E and β-carotene.[28] Thus phenolic and flavonoid compounds could be the major contributing factor in the antioxidative potential of Korean wild leaf vegetables. Because the amounts of flavonoid as well as total phenolic compound in *Pleurospermum kamtschaticum* and *Adenophora remotiflor* were much higher than those of spinach and total antioxidative vitamin was also significantly higher than that of spinach, the antioxidative potency of *Pleurospermum kamtschaticum* and *Adenophora remotiflor* might be from antioxidant vitamin and non-vitamin antioxidant compounds, phenoic compounds including flavonoid. Because the amounts of non-vitamin antioxidant compound in *Aster glheni* were much higher than those of spinach, while total antioxidative vitamin content was significantly lower that of that of spinach, the antioxidative potency of *Aster glheni* might be from the non-vitamin antioxidant compounds and phenoic compounds, including flavonoid. Because many antioxidant compound other than the constituent observed in this paper could not be examined, an assay-guided isolation of these compounds is necessary for further antioxidant investigation of Korean wild leaf vegetables.

Thus, despite the many uncertainties regarding mode of action, these results suggest that *Pleurospermum kamtschaticum* and *Adenophora remotiflor* could have antioxidative potency in food. Because of the higher content of antioxidant vitamins in *Pleurospermum kamtschaticum* and *Adenophora remotiflor*, these Korean wild leaf vegetables may have preventive effects on degeneratives diseases, which have been associated with free radical mediated events, and potential value for functional food.

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