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Original Research Article

Assessment on Antioxidant Potential and Enzyme Activity of Some Economic Resource Plants

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Abstract - The antioxidant potential and enzyme activities in *Salicornia herbacea, Corylopsis coreana, Erythronium japonicum, Phragmites communis*, *Momordica Charantia, Nelumbo nucifera*, *Salvia plebeia, Portulaca oleracea, Ficus carica, Citrus junos* and *Cornus officinalis* were determined. Their antioxidant activities were measured using DPPH radical scavenging and nitrite scavenging activity. Enzyme activities in investigated plants were evaluated as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). The DPPH scavenging rate from 100 to 2500 mgL^{-1} was the highest in the flower of *Corylopsis coreana*. However, it was not detected in most of the samples at concentration below 100 mgL^{-1}. The nitrite scavenging activity according to each kind of resource plants was significantly higher in the stem of *Corylopsis coreana* and leaf of *Nelumbo nucifera.*. The root extract of *Erythronium japonicum* had the highest SOD enzyme activity of 94.0% while leaf of *Salvia plebeia* showed the lowest SOD enzyme activity of 30.4%. The activity of CAT and APX showed higher values in the stem of *Corylopsis coreana,* root of *Erythronium japonicum* and root of *Phragmites communis* in comparison with other plants. The activity of POD showed significantly high values in stem of *Corylopsis coreana, Momordica Charantia* and pericarp of *Citrus junos* extracts. The antioxidant enzyme activities differ significantly in different plants. In conclusion, we showed that *Corylopsis coreana, Erythronium japonicum Cornus officinalis,* and *Momordica Charantia* had the potent biological activities. Therefore, these plant resources showing antioxidant activity could be good materials for development of source of functional healthy food.

Key words - Economic resource plant, DPPH radical scavenging activity, Nitrite scavenging activity, SOD, CAT, POD, APX

Introduction

Recently, plant and plant-derived products are treated a part of the healthcare system by applying the bioactive phytochemicals. Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Prakash *et al.*, 2001). Plant have antioxidant enzyme to keep the increase generation of ROS (reactive oxygen species) which includes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) (Zhou *et al.,* 2005). The production of activated oxygen species occurs when plants are subjected to stress conditions (Dionisio-Sese and Tobita, 1998). Higher

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levels of SOD, CAT, APX and POD activity may be correlated with a higher stress condition. Both enzymatic and non-enzymatic antioxidant systems are present in plants. Superoxide radicals are detoxified by SOD and hydrogen peroxide is destroyed by CAT and different kinds of peroxidases [e.g. guaiacol peroxidase (GPX)] (Kang and Saltveit, 2002). A major hydrogen peroxide-detoxifying system in plant is the ascorbate-glutathione cycle that includes APX and glutathione reductase (GR) (Asada, 1994). In plant cells chloroplasts, mitochondria and peroxisomes are important intracellular generators of ROS (Rich and Bonner, 1978). It is now widely accepted that reactive oxygen species (ROS) are responsible for various stress-induced damage to macromolecules and ultimately to cellular structure (Moftah and Michel, 1987; Kandpal *et al*., 1981), and needs to be scavenged for maintenance of normal growth. Ascorbate peroxidase, catalase and peroxidase, together with low-molecular mass scavengers such as ascorbate, glutathione and proline, act as the main defense against ROS produced in various parts of plant cells (Apel and Hirt. 2004). Catalase, which is located in peroxisomes, glyoxysomes and mitochondria, and is apparently absent in the chloroplast, dismutates mostly photorespiratory or respiratory H_2O_2 into water and molecular O_2 (Apel and Hirt, 2004), Peroxidases (POD) decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants. The induction of ROS-scavenging enzymes, such as SOD, POXs and CAT, is the most common mechanism for detoxifying ROS synthesized during stress responses (Wojtaszek, 1997; Mittler, 2002). In response to the increased production of oxygen radicals the capacity of the antioxidant defence system is increased but in most situations the response is moderate (Foyer *et al.,* 1994). Plant root cells differ in their capacity for nutrient absorption, storage, translocation and assimilation (Lazof *et al.,* 1992; Cruz *et al.,* 1995). Cell expansion and differentiation in higher plant tissues is accompanied by a quantitative rearrangement of protein that may be associated with changes in functional activities (Zeleneva *et al.,* 1982). Antioxidant enzymes play important roles in adaptation to stress conditions. In this study, the antioxidant activity and the antioxidative defence system enzymes SOD, CAT, APX and POD were measured in extracts of the stem, root, fruit and leaf of various economic resource plants containing medicinal plants.

Materials and Methods

Experimental materials

In this experiment, 11 kinds of plant materials (*Salicornia herbacea, Corylopsis coreana, Erythronium japonicum, Phragmites communis*, *Momordica Charantia, Nelumbo nucifera*, *Salvia plebeia, Portulaca oleracea, Ficus carica, Citrus junos* and *Cornus officinalis*) of economic resource plants were used. These plants were chosen because of the possibility to obtain various physiological functionalities. Each sample was freeze-dried and then ground. Each plant powder was stored at -20℃ for further experiments.

DPPH radical scavenging assay

100 μL of various concentrations (100, 250, 500, 1000 and 2500 mg L^{-1}) of extracts of the investigated plants were added to 900 μL of 100% methanol containing 100 μM DPPH, and the reaction mixture was shaken vigorously. After storage at room temperature for 30 min in darkness, the absorbance of DPPH was determined by spectrophotometer at 517 nm. The DPPH radical-scavenging activity was calculated according to the following equation: Scavenging effect on DPPH radical (%) = [(A－B)/A]×100, Where A is the absorbance at 517 nm without pigment compositions and B is the change in absorbance at 517 nm with pigment compositions incubation (Brand-Williams *et al.,* 1995).

Nitrite scavenging assay

The nitrite scavenging activity (NSA) was determined according to a method using Griess reagent (Kato *et al.,* 1987). First, 40 μL of each sample was mixed with 20 μL of 1 mM nitrite sodium. Then the mixture was added to 140 μL of 0.2 M citrate buffer (pH 3.0, 4.2, or 6.0). The final volume of each sample wad adjusted to 200 μL. After the mixtures had been incubated for 1 h at 37℃, and added to 1000 μL of 2% acetic acid and 80 μL of Griess reagent (1% sulfanilic acid and 1% naphthylamine in a methanol solution containing 30% acetic acid). After vigorous mixing with a vortex, the mixture was placed at room temperature for 15 min, and absorbance was measured at 520 nm. The nitrite scavenging activity was determined based on the following formula: NSA (%) = ((1-A-C)/B)*100. Where A is the absorbance of

the mixture sample during a reation with 1 mM $NaNO_2$ after a 1 h reaction, B is the absorbance of a mixture of distilled water and 1 mM $NaNO_2$ after a 1 h reaction and C is the absorbance of the sample.

Enzyme assay

– SOD activity

The superoxide dismutase (SOD) activity was measured using SOD assay Kit-WST purchased from Sigma-Aldrich. This assay is based on the colorimetric assay for the measurement of total antioxidant capacity of crude aqueous fractions. The 60 μL of sample solution (sample and blank2) or double-distilled water (blank1 and blank3) was mixed with 600 μL of WST working solution. For Blank2 and Blank3, 60 μL of dilution buffer was added. Then, 60 μL of enzyme working solution was added to each sample and blank1. The plate was incubated at 37℃ for 20 min, and the OD was determined at 450 nm using a spectrophotometer. SOD activity (inhibition rate percent) was calculated using the following equation: SOD activity=$\{[(A_{blank1}-A_{blank3})-(A_{sample}-A_{blank2})]/(A_{blank\ 1}-A_{blank\ 3})\} \times 100$.

– CAT activity

Catalase (CAT) activity was assayed by the method of Mishra *et al.* (1993). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 11 mM H_2O_2, and the crude enzyme extract. The reaction was initiated by addition of H_2O_2 to the mixture, and enzyme activity was determined by monitoring the decline in absorbance at 240 nm (ε=36 M^{-1} cm^{-1}), because of H_2O_2 consumption.

– APX activity

Ascorbate peroxidase (APX) activity was determined by monitoring the decline of absorbance at 290 nm as ascorbate (ε=2.8 mM^{-1} cm^{-1}) was oxidized, by the method of Chen and Asada (1989). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.5), 0.5 mM ascorbate, and 0.2 mM H_2O_2.

– POD activity

Peroxidase (POD) activity was determined specifically with guaiacol at 470 nm (ε=26.6mM^{-1} cm^{-1}), following the method of Egley *et al.* (1983). The reaction mixture contained 40 mM potassium phosphate buffer (pH 6.9), 1.5 mM guaiacol, and 6.5 mM H_2O_2 in 1 ml with crude enzyme extract. Control assays in which the enzyme extracts or substrates were replaced by buffer were performed.

Data analysis

The statistical analysis was performed using the procedures of the Statistical Analysis System. ANOVA procedure followed by Duncan test was used to determine the significant difference ($p < 0.05$) between treatment means.

Results and Discussion

DPPH radical scavenging activity

The measurement results of free radical scavenging activity are shown in Table 1 of the investigated samples. The investigation of the antioxidant activity of natural substances is based on the measuring of the electron donor capacity of DPPH with the ability to inhibit the oxidation by donating electrons in free radicals causing this lipid peroxidation. Active oxygen caused by in vivo metabolism removed by the body's antioxidant system, but excessive free radicals induced stress, causing the lipid peroxidation by combining with unsaturated fatty acids in the cell membrane, and brought intracellular structural and functional damage. Looking at the results, the antioxidant capacity of the economic resource plants, showed relatively high scavenging activity in *Corylopsis coreana*, *Salvia plebeia* and *Cornus officinalis,* but it was not detected in most of the samples at concentration below 100 mg/L. Overall, the DPPH radical scavenging activity showed that the increase was proportional to the concentration. Cells are oxidized and damaged by the free radical, depending on the growth of cells. It has been reported that phenolic compounds have antioxidant capacity to inhibit the oxidation by donating electrons to the free radical due to strong reduction (Sanchez *et al.,* 2007; Saija *et al.,* 1998). The content of phenolic compounds increases the radical scavenging activity, which also is reported to be increased (Boo *et al.,* 2011; Chon *et al.,* 2012; Oki *et al,.* 2002). In our study, the DPPH radical scavenging activity appeared to concentration-dependent, and depending on the kind of plants, there were

significant differences in the results. The effective source of the resource plants could be employed in all medicinal preparation to combat myriad diseases associated with oxidative stress, including cancer and related disorders.

Nitrite scavenging activity

Nitrite ions in the acidic environment of the stomach induce mutagenic and cell-damaging reactions (Kato and Puck, 1971). Exposure to excess nitrite from the diet is implicated as a potential etiological factor in the development of stomach and colorectal cancers (Lee *et al.,* 2006). Nitrite reacts with second and third grade amines to form nitrosamine in protein-rich foods, medicines, and residual pesticides. It is also present in large quantities in meat and both leafy and root vegetables. Nitrosamine is converted to diazoalkane (alkane nucleic acid), proteins, and intracellular components, which can increase the risk for cancer (Choi *et al.,* 2008). In order to investigate the nitrite scavenging activity in the several resource plants, various acidic conditions were tested. The results are shown in Table 2. The maximum nitrite scavenging activity was obtained at pH of 1.2. The nitrite scavenging activities were affected by changes in pH. The nitrite scavenging activity according to each kind of resource plants was significantly higher in the stem of *Corylopsis coreana* and leaf of *Nelumbo nucifera.*. The results of the nitrite scavenging activity varied depending on the kind of plant from 62.1 to 96.4% at pH of 1.2. However, the nitrite scavenging activity at pH of 6.0 was not almost detected. These results were consistent with other findings that had the highest the nitrite scavenging at pH of 1.2 in fermented pine extract (Hong *et al.,* 2004) and extracts from different parts of citron (Shin *et al.,* 2005). The fact that the nitrite scavenging activity was high at pH 1.2 suggests that nitrosamine production can be inhibited in vivo (Choi *et al.,* 2008).

Table 1. DPPH radical scavenging activities according to each kind of several economic resource plants

Plants	DPPH radical scavenging activity, % of control				
	Concentration (mg/L)				
	100	250	500	1000	2500
Salicornia herbacea	ND	1.5±0.08^{g}	5.4±0.18^{f}	13.6±0.49^{f}	27.6±0.42^{f}
Corylopsis coreana(Stem)	2.5±0.10^{c}	7.3±0.24^{d}	17.9±0.17^{c}	42.7±0.07^{b}	64.4±0.66d
Corylopsis coreana(Flower)	6.2±0.32^{b}	14.2±0.73^{b}	21.9±1.09^{b}	36.2±0.25^{c}	76.2±0.17^{b}
Erythronium japonicum(Leaf)	ND	1.7±0.35^{g}	7.1±0.22^{e}	9.7±0.63^{h}	18.6±0.36^{g}
Erythronium japonicum(Root)	ND	ND	ND	2.2±0.43^{k}	9.5±0.29^{k}
Erythronium japonicum(Flower)	ND	0.1±0.34^{h}	1.0±0.29^{gh}	4.5±0.35^{ij}	17.7±0.03^{gh}
Phragmites communis(Root)	ND	ND	1.2±0.16^{gh}	3.6±0.65^{jk}	7.8±0.32^{i}
Nelumbo nucifera(Leaf)	1.9±0.29^{c}	5.6±0.18^{e}	10.8±0.26^{d}	15.7±0.83^{e}	46.2±0.77^{e}
Salvia plebeia	7.8±0.79^{a}	12.8±0.54^{c}	17.7±0.27^{c}	29.7±0.26^{d}	72.7±1.29^{c}
Momordica Charantia	ND	2.1±0.09^{g}	5.6±0.07^{ef}	12.4±0.30^{fg}	26.6±0.11^{f}
Portulaca oleracea	ND	ND	1.6±0.09^{gh}	5.4±0.59^{ij}	12.6±0.26^{j}
Ficus carica(Leaf)	ND	0.4±0.08^{h}	2.4±0.21^{g}	6.3±0.44^{i}	8.6±0.57^{kl}
Citrus junos(Pericarp)	0.4±0.15^{d}	4.1±0.09^{f}	9.3±0.03^{d}	10.7±0.31^{gh}	16.3±0.06^{hi}
Citrus junos(Leaf)	ND	0.4±0.24^{h}	1.6±0.06^{gh}	5.8±1.57^{i}	15.3±0.05^{i}
Cornus officinalis	7.2±1.50^{ab}	17.3±0.83^{a}	37.6±1.75^{a}	70.7±0.95^{a}	89.9±0.52^{a}

[z]Data represent the mean values±SE of three independent experiments. Means with the same letter in column are not significantly different at $p<0.05$ level by Duncan's multiple range test.

N.D. : Not detected.

Table 2. Nitrite scavenging activities according to each kind of several economic resource plants

Plants	Nitrite scavenging activity (%)		
	pH 1.2	pH 4.2	pH 6.0
Salicornia herbacea	83.8±1.25^{g}	31.2±2.09^{b}	ND
Corylopsis coreana(Stem)	96.1±0.10^{a}	34.5±0.75^{a}	0.9±0.06^{e}
Corylopsis coreana(Flower)	94.9±0.21^{ab}	29.5±0.34^{b}	ND
Erythronium japonicum(Leaf)	89.7±0.10^{d}	25.3±1.27^{cd}	ND
Erythronium japonicum(Root)	62.1±0.27^{i}	13.5±0.76^{f}	ND
Erythronium japonicum(Flower)	90.1±0.56^{d}	22.4±0.95^{d}	ND
Phragmites communis(Root)	64.5±0.91^{h}	18.2±0.63^{e}	ND
Nelumbo nucifera(Leaf)	96.4±0.30^{a}	25.7±0.92^{c}	ND
Salvia plebeia	86.8±0.63^{f}	31.2±1.27^{b}	2.6±0.05^{d}
Momordica Charantia	93.5±0.27^{bc}	22.4±0.54^{cd}	ND
Portulaca oleracea	87.9±0.82^{ef}	30.2±0.62^{b}	6.9±1.11^{b}
Ficus carica(Leaf)	92.1±1.13^{c}	24.9±0.41^{cd}	ND
Citrus junos(Pericarp)	88.9±0.50^{de}	18.8±0.82^{e}	3.8±0.23^{c}
Citrus junos(Leaf)	95.3±0.40^{a}	29.0±1.25^{b}	ND
Cornus officinalis	95.8±0.00^{a}	36.4±2.07^{a}	12.6±0.29^{a}

^{z}Data represent the mean values±SE of three independent experiments. Means with the same letter in column are not significantly different at $p<0.05$ level by Duncan's multiple range test.
N.D. : Not detected.

Enzyme activity

The comparative results of the antioxidant enzyme activity in the 11 kinds of economic resource plants are shown in figure 1～4. The root extract of *Erythronium japonicum* had the highest SOD enzyme activity of 94.0% while leaf of *Salvia plebeia* showed the lowest SOD enzyme activity of 30.4%. The activity of CAT showed higher values in the root of *Erythronium japonicum* and in the stem of *Corylopsis coreana,* and the APX activity showed higher values in the root of *Erythronium japonicum,* root of *Phragmites communis* and pericarp of *Citrus junos* in comparison with other plants. The activity of POD showed significantly high values in stem of *Corylopsis coreana, Momordica Charantia* and pericarp of *Citrus junos* extracts. Significant roles of POD have been suggested in plant development processes (Gaspar *et al.,* 1985), which was involved in scavenging of H_2O_2 produced in chloroplasts (Asish and Anath, 2005). The antioxidant enzyme activities differ significantly in different plants. The SOD is one of the enzymes, in vivo, to catalyze the reaction that converts the harmful reduced oxygen formed in cell due to rancidity into hydrogen peroxide; is generated in most aerobic or anaerobic biological organisms; is switched to water and oxygen by the CAT and APX, and loses then its toxicity. Typically, the APX plays the most important scavenger role in the cytoplasm and chloroplasts of plants, and ascorbic acid is used as a reduction substrate (Wheeler *et al.,* 1998). APX activity, which is important component of the antioxidant system, plays a key role in eliminating H_2O_2 molecules and in the modulation of its steady-state levels in various plant subcellular compartments (Najami *et al.,* 2008). The CAT is also an antioxidant enzyme that protects cells by dispatching of in vivo harmful oxygen and is a typical enzyme that acts to decompose and scavenge the H_2O_2 together with APX. The antioxidant enzymes, indicating a high activity to remove harmful free radicals, have the effect of prevention and inhibition of various diseases and aging, and in various economic resource plants, we can also expect to see these benefits for the next variety of natural foods and

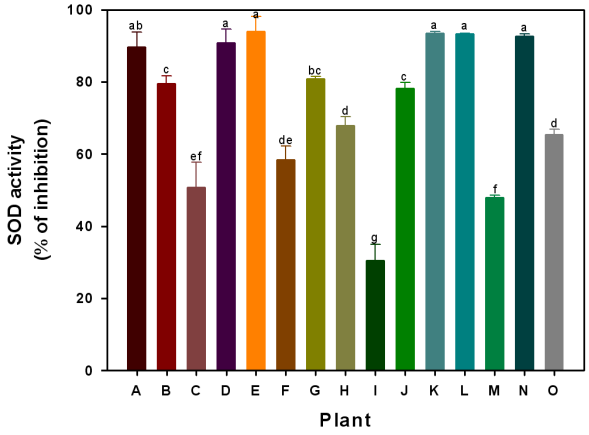

Fig. 1. SOD activities according to each kind of several economic plants. Means with the same letter in a column are not significantly different at p<0.05 level by Duncan's multiple range test. The bars represent the standard error. A: *Salicornia herbacea*, B: *Corylopsis coreana* (Stem), C: *Corylopsis coreana* (Flower), D: *Erythronium japonicum* (Leaf), E: *Erythronium japonicum* (Root), F: *Erythronium japonicum* (Flower), G: *Phragmites communis* (Root), H: *Nelumbo nucifera* (Leaf), I: *Salvia plebeia*, J: *Momordica Charantia*, K: *Portulaca oleracea*, L: *Ficus carica* (Leaf), M: *Citrus junos* (Pericarp), N: *Citrus junos* (Leaf), O: *Cornus officinalis*.

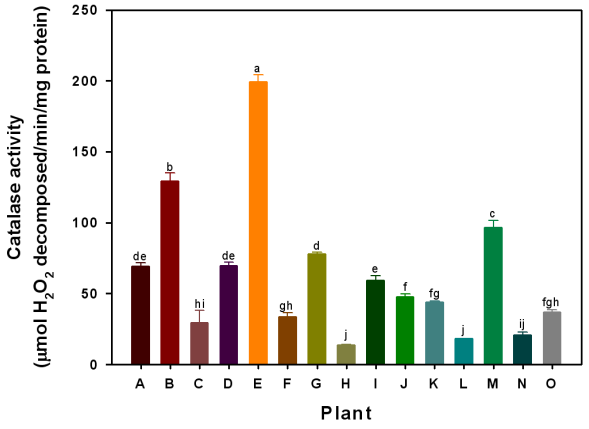

Fig. 2. CAT activities according to each kind of several economic plants. Means with the same letter in a column are not significantly different at p<0.05 level by Duncan's multiple range test. The bars represent the standard error. A: *Salicornia herbacea*, B: *Corylopsis coreana* (Stem), C: *Corylopsis coreana* (Flower), D: *Erythronium japonicum* (Leaf), E: *Erythronium japonicum* (Root), F: *Erythronium japonicum* (Flower), G: *Phragmites communis* (Root), H: *Nelumbo nucifera* (Leaf), I: *Salvia plebeia*, J: *Momordica Charantia*, K: *Portulaca oleracea*, L: *Ficus carica* (Leaf), M: *Citrus junos* (Pericarp), N: *Citrus junos* (Leaf), O: *Cornus officinalis*.

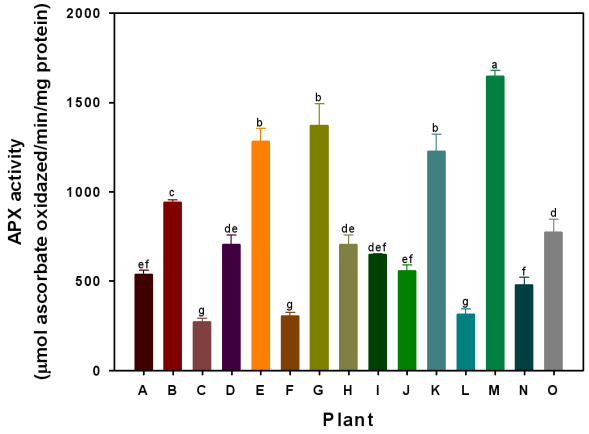

Fig. 3. APX activities according to each kind of several economic plants. Means with the same letter in a column are not significantly different at p<0.05 level by Duncan's multiple range test. The bars represent the standard error. A: *Salicornia herbacea*, B: *Corylopsis coreana* (Stem), C: *Corylopsis coreana* (Flower), D: *Erythronium japonicum* (Leaf), E: *Erythronium japonicum* (Root), F: *Erythronium japonicum* (Flower), G: *Phragmites communis* (Root), H: *Nelumbo nucifera* (Leaf), I: *Salvia plebeia*, J: *Momordica Charantia*, K: *Portulaca oleracea*, L: *Ficus carica* (Leaf), M: *Citrus junos* (Pericarp), N: *Citrus junos* (Leaf), O: *Cornus officinalis*.

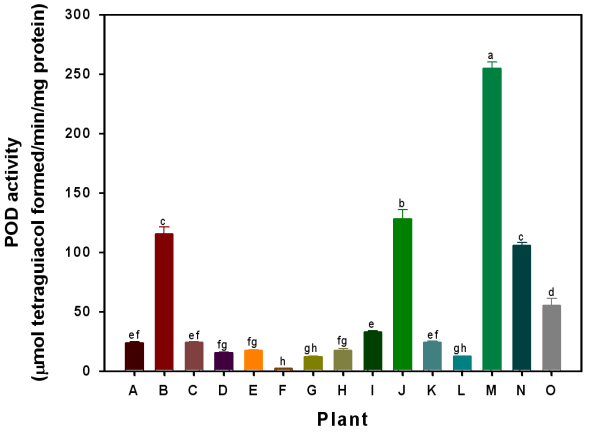

Fig. 4. POD activities according to each kind of several economic plants. Means with the same letter in a column are not significantly different at p<0.05 level by Duncan's multiple range test. The bars represent the standard error. A: *Salicornia herbacea*, B: *Corylopsis coreana* (Stem), C: *Corylopsis coreana* (Flower), D: *Erythronium japonicum* (Leaf), E: *Erythronium japonicum* (Root), F: *Erythronium japonicum* (Flower), G: *Phragmites communis* (Root), H: *Nelumbo nucifera* (Leaf), I: *Salvia plebeia*, J: *Momordica Charantia*, K: *Portulaca oleracea*, L: *Ficus carica* (Leaf), M: *Citrus junos* (Pericarp), N: *Citrus junos* (Leaf), O: *Cornus officinalis*.

cosmetics where the need to apply functional substances may also be required. Therefore, the results of this experiment are believed to be meaningful. That is, with this study, in a variety of resource plants, we can expect to take advantage of their higher value as valuable materials of healthy functional foods, as they showed higher antioxidant enzyme activity. Therefore, in the future, developing the healthy functional food and natural antioxidants can be possible.

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Literature Cited

Apel, K. and H. Hirt. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55:373-99.

Asada, K. 1994. Production and action of active oxygen species in photosynthetic tissues. *In* C.H. Foyer, P.M. Mullineaux, (eds.), Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants. CRC Press, London, pp.77-105.

Asish, K.P. and B.D. Anath. 2005. Salt tolerance and salinity effects on plants. A review Ecotoxicol. Environ. Saf. 60: 324-349.

Brand-Williams, W., M.E. Cuvelier and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. Tech. 28:25-30.

Boo, H.O., S.J. Hwang, C.S. Bae, S.H. Park and W.S. Song. 2011. Antioxidant activity according to each kind of natural plant pigments. Korean J. Plant Res. 24(1):134-141.

Chen, G.X. and K. Asada. 1989. Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol. 30:987-998.

Choi, D.B., K.A. Cho, M.S. Na, H.S. Choi, Y.O. Kim, D.H. Lim, S.J. Cho and H. Cho. 2008. Effect of bamboo oil on antioxidative activity and nitrite scavenging activity. J. Ind. Eng. Chem. 14:765-770.

Chon, S.U., C.H. Bae and S.C. Lee. 2012. Antioxidant and cytotoxic potentials of methanol extracts from *Taraxacum officinale* F. H. Wigg. at different plant parts. Korean J. Plant Res. 25(2):232-239.

Cruz, C., S.H. Lips and M.A. Martins-Loução. 1995. Uptake regions of inorganic nitrogen in roots of carob seedlings. Physiol. Plant 95:167-175.

Dionisio-Sese, M.L. and S. Tobita. 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135:1-9.

Egley, G.H., R.N. Paul, K.C. Vaughn and S.O. Duke. 1983. Role of peroxidase in the development of water-impermeable seed coats in Sida spinos L. Planta 157:224-232.

Foyer, C.H., P. Descourvières and K.J. Kunert. 1994. Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant Cell Env. 17:507-523.

Gaspar, T., C. Penel, F.J. Castillo and H. Greppin. 1985. A two step control of basic and acid peroxidases and its significance for growth and development. Plant Physiol. 64:418-423.

Hong, T.G., Y.R. Lee, M.H. Yim and C.N. Hyun. 2004. Physiological functionality and nitrite scavenging ability of fermentation extracts from pine needles. Korean J. Food Preserv. 11:94-99.

Kandpal, R.P., C.S. Vaidyanathan, M. Udaykumar, K.S. Krishnasastry and N. Appaji-Rao. 1981. Alternation in the activities of the enzyme of proline metabolism in ragi (*Eleusine coracane*) leaves during water stress. J. Biosci. 3:361-369.

Kang, H.M. and M.E. Saltveit. 2002. Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. Physiol. Plant. 115: 571-576.

Kato, F.T. and T.T. Puck. 1971. Mutagenesis by carcinogenic nitroso compounds. J. Cell Physiol. 78:139-144.

Kato, H., I.E. Lee, N.V. Chuyen, S.B. Kim and F. Hayase. 1987. Inhibition of nitrosamine formation by nondialyzable melanoidins. Agric. Biol. Chem. 51:1333-1338.

Lazof, D.B., T.W. Rufty and M.G. Redinbaugh. 1992. Localization of nitrate absorption and translocation within morphological regions of the corn root. Plant Physiol. 100: 1251-1258.

Lee, S.C., S.Y. Kim, S.M. Jeong and J.H. Park. 2006. Effect of far-infrared irradiation on catechins and nitrite scavenging activity of green tea. J. Agric. Food Chem. 54:399-403.

Mishra, N.P., R.K. Mishra and G.S. Singhal. 1993. Changes in the activities of anti-oxidant enzymes during exposure of intact wheat leaves to strong visible light at different temperatures in the presence of protein synthesis inhibitors. Plant Physiol. 102:903-910.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410.

Moftah, A.H. and B.E. Michel. 1987. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. Plant Physiol. 83:238-240.

Najami, N., J. Tibor, W. Barriah, G. Kayam, T. Moshe, M. Guy and M. Volokita. 2008. Ascorbate peroxidase gene family in tomato: its identification and characterization. Mol. Genet. Genom. 279:171-182.

Oki, T, M. Masuda, S. Furuta, Y. Nishibia, N. Terahara and I. Suda. 2002. Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. Food Chem. Tox. 67:1752-1756.

Prakash, A., F. Rigelhof and E. Miller. 2001. Antioxidant activity. Analytical Progress (Medallion laboratories). pp. 1-4.

Rich, P.R. and W.D. Jr. Bonner. 1978. The sites of superoxide anion generation in higher plant mitochondria. Arch. Biochem. Biophys. 188:206-213.

Saija, A.D., A. Trombetta, R.L. Tomaino, P. Cascio, N. Princi, F.B. Uccella and F. Castelli. 1998. 'In vitro' evaluation of the antioxidant activity and biomembrane interaction of the plant phenols oleu-ropein and hydroxytyrosol. Int. J. Pharmacol. 166:123-133.

Sanchez, C.S., A.M.T. Gozalez, M.C. Garcia-Parrilla, J.J.Q. Granados, H.L.G. Serrana and M.C.L. Martinez. 2007. Different radical scavenging tests in virgin olive oil and their relation to the total phenol content. Anal. Chemical Acta. 593:103-107.

Shin, J.H., J.Y. Lee, J.C. Ju, S.J. Lee, H.S. Cho and N.J. Sung. 2005. Chemical Properties and Nitrite Scavenging Ability of Citron (*Citrus junos*). J. Korean Soc. Food Sci. Nutr. 34:496-502.

Wheeler, G.L., M.A. Jones and N. Smirnoff. 1998. The biosynthetic pathway of vitamin C in higher plants. Nature 393:363-369.

Wojtaszek, P. 1997. Oxidative burst: an early plant response to pathogen infection. Biochem. J. 322:681-692.

Zeleneva, L.V., E.V. Savost'yanova and É.E. Khavkin. 1982. Enzyme distribution of the cortex and the stele in cytodifferentiating maize seedlings. Biochem. Physiol. Pflanzen. 177: 97-106.

Zhou, B.Y., Z.F. Guo and Z.L. Liu. 2005. Effects of abscisic acid on antioxidant systems of *Stylosanthes guianensis* (Aublet) Sw. under chilling stress. Crop Sci. 45:599-605.

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