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Antioxidant Activities of Different Parts of *Synurus deltoids* Nakai Extracts *in Vitro*

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Abstract The antioxidant activity of hot water extracts of various parts, the leaf, stem, and root of *Synurus deltoides* was evaluated by various antioxidant assays, including total phenolic content, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, hydroxyl radical (·OH) scavenging, superoxide dismutase (SOD), and xanthine oxidase (XOI) activities. The various antioxidant activities were compared with the standard antioxidants such as L-ascorbic acid, α-tocopherol, and butylated hydroxyanisole (BHA). Among the different plant parts, stem has been found to possess the highest activity in all tested model systems, the activity decreased in the order stems>roots>leaves. These results indicate that stem extract could be used as potential source of natural antioxidant.

Keywords: *Synurus deltoids*, antioxidant activity, scavenging effect, total phenolic content

Introduction

High levels of free radicals or reactive oxygen species create oxidative stress, which leads to a variety of biochemical and physiological lesions and results in metabolic impairment and cell death (1,2). Epidemiological evidence indicates that the consumption of foodstuffs containing antioxidant phytochemicals is advantageous for health (3-5), since they can protect human body from free radicals and retard the progress of many chronic diseases (2). A number of synthetic antioxidant, such as butylated hydroxyanisole (BHA), butylated hydroxyltoluene (BHT), and *tert*-butylhydroquinone, are commonly used in processed food. Yet, these antioxidants suffer from the drawback that they are volatile and easily decompose at high temperatures. Additionally, it is still unclear whether chronic consumption can lead to health risks (6). For this reason, the development natural antioxidant from plant species, especially edible plant, are in progress.

Synurus deltoids (Ait.) Nakai (*S. deltoids*), one of the family Compositae, is an edible plant and distributed in East Asia including Korea, China, and Japan (7). In Korea, 2 species (*Synurus excelsus* Kitamura and *S. deltoides*) and 1 variety (*Synurus palmatopinnatifidus* Kitamura var. *palmatopinnatifidus*) of the *Synurus* genus are distributed widely (8). It has been used as a folk medicine for treating edema, bleeding, vomiting, and urinary inflammation (7-9). Some researchers reported the isolation of anthocyanins and 20-hydroxyecdysone (10,11). However, the possibility of antioxidant activity of *S. deltoids* has not been investigated. This work investigates the possible antioxidative activity of different part of *S. deltoids* to use as a natural preservative in food or functional food.

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Materials and Methods

Preparation of *S. deltoids* extracts The *S. deltoides* was collected in September, 2007 at Chuncheon. The leaves, roots, and stems (5 g each) of *S. deltoides* were separately extracted with water (25 mL) at 80°C for 3 hr. The extracts were filtered through filter paper (Whatmen 70-mm) and evaporated using a vacuum rotary evaporator (CCA-1110; Eyela, Tokyo, Japan). Finally, the samples were dried by freezing in a high vacuum (FD-5N; Eyela) for 2 days to obtain the crude extracts. Dried samples were weighed and kept at 4°C for further analysis.

Chemicals L-Ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-deoxy-D-ribose, 2 N folin-ciocalteu's phenol reagent, iron (II) sulfate heptahydrate, tannic acid (TA), α-tocopherol, trichloroacetic acid (TCA), BHA, and ethylene diamine tetraacetic acid (EDTA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 4,6-Dihydroxy-2-mercaptopyrimidine was purchased from Alfa Aesar (Ward Hill, MA, USA). Hydrogen peroxide (H_2O_2) and sodium carbonate were purchased from Junsei Chemical Co., Ltd. (Osaka, Japan). Iron (III) chloride hexahydrate was purchased from Kanto Chemical (Osaka, Japan).

Total phenolic contents The concentration of phenolics in the extracts was estimated according to the method of Jayaprakasha *et al.* (12). The results were expressed as TA equivalent. The leaves, roots, and stems of *S. deltoides* extracts (2 mg) and TA (2 mg) were dissolved in a 1 mL of mixture of methanol:water (6:4, v/v). The hot water extracts (100 μg) of *S. deltoides* and different concentrations (10-100 μg) of TA in 0.1 mL were mixed with 0.5 mL of 10-fold diluted Folin-Ciocalteu reagent and 0.4 mL of 7.5% sodium carbonate solution. After standing for 30 min at ambient temperature, the absorbance was measured at 750 nm using multiplate spectrophotometer (ELx800TM; BioTek, Winooski, VT, USA). The estimation of phenolics in the fresh different parts of *S. deltoides* was calculated using a standard graph of TA.

DPPH radical scavenging assay The DPPH radical scavenging effect was evaluated according to Blois (13) with a slight modification. The different hot water extract samples (0-1 mg/mL) in 0.4 mL of methanol were added to a 0.4 mL DPPH methanol solution (0.2 mM). After mixing gently and standing at room temperature for 30 min, the optical density was measured at 515 nm using a multiplate spectrophotometer (ELx800TM; BioTek). The antioxidant activity of each sample was expressed in terms of the IC_{50} [concentration (μg/mL) to decrease the initial absorbance of DPPH by 50%], which was calculated from the log-dose inhibition curve.

Hydroxyl radical (·OH) scavenging assay ·OH Scavenging activity was carried out using the 2-deoxyribose oxidation assay according to Guo and Wang (14). The solution (0.2 mL) of $FeSO_4 \cdot 7H_2O$ (10 mM) and EDTA (10 mM) was prepared in a screw-capped test tube, and 0.2 mL of a 2-deoxyribose solution (10 mM), the samples (extracts) solution and a sodium phosphate buffer (pH 7.4, 0.1 M) were added to give a total volume of 1.8 mL. Finally, 200 μL of H_2O_2 solution (10 mM) was added to this reaction mixture and incubated at 37°C for 4 hr. After incubation, 1 mL each of a TCA solution (2.8%) and thiobarbituric acid solution (1.0%) were added to the reaction mixture. The sample was boiled at 100°C for 10 min, cooled in ice and its absorbance was measured with multiplate spectrophotometer (ELx800TM; BioTek) at 515 nm. The capability to scavenge hydroxyl radical was calculated by the following equation:

Scavenging effect (%)
=[1-(Abs. sample at 515 nm/Abs. control at 515 nm)]×100

Assay of superoxide dismutse (SOD)-like activity The activity of SOD was assayed as described by Beauchamp and Fridovich (15). The reaction mixture contained 1.17×10^{-6} M riboflavin, 0.1 M methionine, 2×10^{-5} M KCN, and 5.6×10^{-5} M nitroblue tetrazolium salt dissolved in 3 mL of 0.05 M sodium phosphate buffer (pH 7.8). Three mL of the reaction medium was added to 1 mL of enzyme extract. The mixtures were illuminated in glass test tubes by 2 sets of Philips 40 W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30°C for 1 hr. Identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD-like activity is expressed in U/mg protein (U=change in 0.1 absorbance/hr/mg protein).

Xanthine oxidase (XOI) inhibition assay XOI activity was estimated by the formation of uric acid from xanthine-XOI system (16). Test samples were dissolved in DMSO, and then diluted with 0.1 M phosphate buffer (pH 7.5) to various concentrations (1, 10, and 100 mg/mL). After 1 mL of xanthine (2 mM) and 0.1 mL of XOI (0.25 units) were added, samples were vigorously mixed and then incubated at 37°C. After 5 min, 1 mL of 20% TCA solution was added to the mixture, which was then centrifugation for 15 min at 3,500×g. Superoxide formation was counted by spectrophotometric measurement of uric acid production at 292 nm.

Table 1. Effect of yields on different parts and total phenolic content of hot water extracts from *S. deltoides*

Samples	Yield (%)	Total phenolic content (g TA[1])/100 g)
Leaves	10.6	3.44±1.1
Stems	5.9	3.35±1.2
Roots	11.9	3.11±1.0

[1]Tannic acid (TA) was used as a standard for measuring the total phenolic content; fresh plants weight.

Statistical analysis All experimental data were expressed as mean±standard derivation (SD). Data analyses were performed using the SPSS 7.5 (Window Version 7.5 Software Inc., New York, NY, USA) and $p<0.05$ was considered as significant.

Results and Discussion

Effect of yields on different parts of *S. deltoids* The yields of the extracts were recorded as % of crude hot water extract/5 g of fresh plant material as indicated in Table 1. The hot water extract yields obtained from different parts of *S. deltoids* were as follows: followed by roots (11.9%, 0.595 g roots hot water extract/5 g fresh roots), leaves (10.6%, 0.53 g leaves hot water extract/5 g fresh leaves), and stems (5.9%, 0.295 g stems hot water extract/5 g fresh stems)

Total phenolic contents The total phenolic contents in different parts of *S. deltoids* extracts were determine and presented in Table 1. The phenolic contents were calculated by using TA. The total phenolic contents of leaves, stems, and roots were 3.44±1.1, 3.35±1.2, and 3.11±1.0 g TA equivalent/100 g, respectively. It was noted that water extract of leaf had significant higher total phenol contents than did other parts. Phenols and polyphenolic compounds are sidely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (17,18). In this study, the total phenolic compounds of the different part extracts of *S. deltoids* were found to be in the ranges of 3.11-3.44 g TA/100 g, and they may cause the antioxidative activities of the *S. deltoids* extracts.

Scavenging effect on DPPH radical The stable DPPH radical is a widely used method to evaluate the free radical scavenging activity of various samples (19-22). The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. Table 2 shows the DPPH radical scavenging activities of the different parts from *S. deltoids* extracts. The half-inhibition concentrations (IC_{50}) for DPPH radical scavenging activity of leaves, stems, and roots were 83.6±3.7, 64.9±2.4, and 76.6±1.8 mg/mL, respectively. Based on the IC_{50} results, stem had the highest DPPH scavenging activity, while leaves showed the least activity. α-Tocopherol, a positive control, had an IC_{50} value of 28.3±2.4 mg/mL. In other words, to reach a similar extent of DPPH scavenging effect, the concentrations required for *S. deltoids* extracts were significantly higher than that required for α-tocopherol. Although the DPPH

Table 2. DPPH radical scavenging activity of hot water extracts from *S. deltoides*

Sample	DPPH radical activity IC_{50}[1] (μg/mL)
Leaves	83.6±3.7
Stems	64.9±2.4
Roots	76.6±1.8
Positive control	
α-Tocopherol	28.3±2.4

[1]The effective concentration at which DPPH radicals were scavenged by 50%; significant difference compared to the control (α-tocopherol) at $p<0.05$.

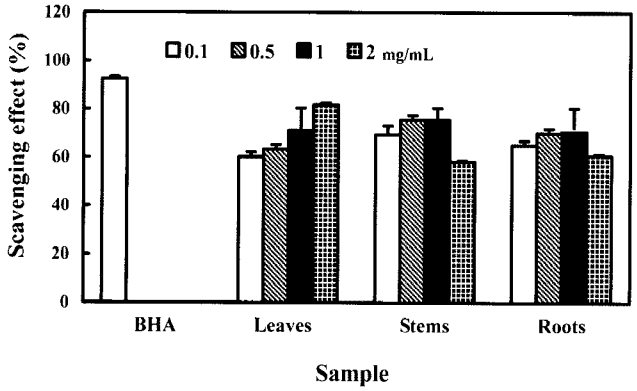

Fig. 1. Hydroxyl radical (·OH) scavenging activities in different part of *S. deltoides* hot water extract. Data were presented as the mean±SD (n=3). The concentrations of different solvent extracts were 0.1, 0.5, 1, and 2 mg/mL, respectiviely. BHA at the concentration of 0.1 mg/mL was used as a positive control.

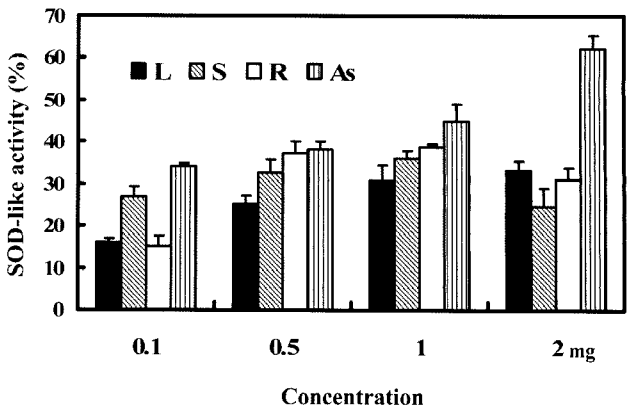

Fig. 2. Superoxide dismutase (SOD) activities in different part of *S. deltoides* hot water extract. The leaves (L), stems (S), and roots (R) are used test samples. L-Ascorbic acid (As) is a positive control.

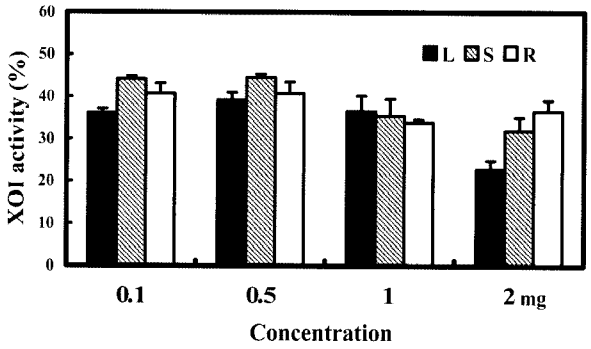

Fig. 3. Xanthine oxidase (XOI) activities in differ different part of *S. deltoides* hot water extract. The leaves (L), stems (S), and roots (R) are used test samples.

radical scavenging abilities of the extracts were significantly less than that of α-tocopherol, it was evident that the extracts did show the hydrogen-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

·OH activity Figure 1 shows the ·OH radical scavenging activity of the different parts from *S. deltoids* extracts. The leaves, stems, and roots extract exhibited strong activity on ·OH at concentration of 0.1 mg/mL, showing 60.1±2.0, 69.3±3.8, and 65.0±1.8% inhibition, respectively. Moreover, all extracts exhibited inhibitory effects higher than 60% at 0.1, 0.5, 1.0, and 2.0 mg/mL. These activities are comparable to that (92.5±0.9%) of BHA at 0.1 mg/mL, which was used as a positive control.

·OH is the most reactive free radical and can be formed from hydrogen peroxidate and superoxide anion, in the presence of metal ions. ·OH react with protein, lipid, polypeptides, and DNA (23). The hydroxyl radicals scavenging ability may be related with the inhibition of lipid peroxidation observed in the present study.

SOD-like scavenging activity SOD is a major scavenger of superoxide anion radical that catalyses the dismutations of superoxide anion radical with great efficiency resulting in the production of H_2O_2 and O_2 (24). Figure 2 shows the dose-response effects of SOD-like activities of the different part extracts from *S. deltoids*. All concentrations except the concentration of 2 mg/mL demonstrated strong SOD-like activity. The scavenging effect was in the order: roots (37.3%) >stems (32.8)>leaves (25. 3%) at a concentration of 0.5 mg/mL. A comparison with the commercial antioxidant showed that the concentration needed to obtain 38.2% SOD-like activity for L-ascorbic acid was 0.5 mg/mL, which was about nearly equal to the scavenging effect of roots. SOD activity increased under drought stressed higher plants like wheat, rice (25,26).

XOI activity The XOI activity in different parts of *S. deltoids* extracts were determined (Fig. 3). Leaves and stems exhibited an inhibitory effect on xanthine oxidase activity in concentration of 0.1-0.5 mg/mL. At concentration 0.1-0.5 mg/mL, the XOI inhibitory activity varies from 36.1 to 39.2 for leaves and 44.1 to 44.5 for stems. XOI is a flavoprotein, which catalyses the oxidation of hypoxanthine to xanthine and generates superoxid and uric acid (27). Studies have shown that XOI inhibitors may be useful for the treatment of hepatic disease and gout, that is caused by the generation of uric acid and superoxide anion radical (28). XOI-derived superoxide anion has been linked to post-ischaemic tissue injury and edema (29,30).

This result indicates that the leaves, stems, and roots hot water extracts of *S. deltoids* may be useful for treating oxidative damage. A further investigation into the antioxidant

activity of these natural components in view of preventing various radical-mediated injuries in pathological situation *in vivo* is currently underway.

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