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Original Research Article

Comparison of Biological Activities of *Synurus deltoides* (Aiton) Nakai Under Different Shading Conditions

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Abstract - Shade treatment of *Synurus deltoides* (Aiton) Nakai was carried out with 0, 35, and 55% shading net, and samples were marked as no shade, 35% shade, and 55% shade, respectively. We examined *in vitro* antioxidant and anti-inflammatory capacities using a 1,1-diphenyl-2-2-pricylhydrazyl (DPPH) radical scavenging assay, a reducing power assay, a total antioxidant assay, a metal chelating assay, a superoxide radical scavenging assay, and a nitric oxide inhibition assay. As a result, no shade and 35% shade possessed higher DPPH radical scavenging activity and reducing power ability than that of 55% shade. Notably, no shade had significantly higher total phenolic and flavonoid contents than those in the other samples. No shade exhibited significantly higher total antioxidant activity than that of 35% shade and 55% shade. However, the chelating ability of 55% shade was significantly greater than that of no shade and 35% shade; 55% shade also showed significantly higher anti-inflammatory capacity than that of no shade or 35% shade.

Key words - Anti-inflammatory, Antioxidant, Cytotoxicity, Shade treatment, Phenolic content

Introduction

Synurus deltoids is a perennial edible herbaceous plant that belongs to the Compositae family and is largely distributed in East Asia. *S. deltoids* has been used as a folk medicine to treat inflammatory edema, bleeding, vomiting, and urinary inflammation for a long time (Park *et al*., 2004). Some chemical and pharmacological researches about *S. deltoides* and related species have been reported. Anthocyanins and 20-hydroxyecdysone have been found in this plant, and the antioxidant activity of a hot water extracts of *S. deltoids* has been reported by Jung *et al*. (2008a). However, the effects of light treatment on the biological activity of *S. deltoids* have not been described. The intensity of photosynthetically active radiation irradiance of UV-exposed plants controls the activities of enzymes and the concentration of metabolites that prevent photo-oxidative damage (Guidi *et al*., 2011). Different light conditions may have a relatively minor influence on the biochemistry and biological activities of *S. deltoids* growing under natural conditions. The light condition in which plants grow is a very important factor for controlling their growth. Therefore, correlative measurements were carried out to compare the extraction yield and biological activities of *S. deltoids* under different light conditions. Additional studies to determine the proper environment are necessary.

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anions (O_2^-) has been considered to be a cause of oxidative damage to biological molecules in the human body (Song *et al*., 2006). For example, H_2O_2 lead to the cell death in many different cell types, but addition of natural antioxidants can provide protection (Jeong *et al*., 2011). Antioxidant provides protective against free radicals and ROS, and antioxidants are used to keep food quality increase the shelf life by regarding oxidative deterioration of lipids (Tyug *et al*., 2010). The major antioxidant compounds in plant are polyphenols. Phenolic compounds exhibit protection capacity against oxidative stress and several health beneficial effects such as antioxidant, anti-inflammatory, antihepatotoxic and antitumor activities (Luthria, 2008). Flavonoids as hydrophilic scavengers are a group of polyphenolic compounds. Flavonoids build up these activities as antioxidants and free radical scavengers and exhibit effect on antibacterial, antiviral, anti-

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inflammatory, antiallergic, and vasodilatory actions (Cook and Samman, 1996). Exogenous antioxidants are required to assist the body to decrease the antioxidant stress caused by excess free radicals (Sathuvan *et al.*, 2012). However, there is deep concern about the safety of some common antioxidants such as butylated hydroxyanisole (BHA) and butlylated hydroxytoluene (BHT) as they are toxic, carcinogenic and damage DNA and the liver (Aremu *et al*., 2011). For this reason, more attention has been focused on research for natural non-toxic antioxidants from plant sources (Milos *et al*., 2012). Because of the health-promoting potential of natural antioxidants, they should be incorporated into food products as more natural and healthy alternatives instead of some existing synthetic additives such as tocopherol, tertiary-butylhydroquinone, BHA, and BHT (Lue *et al*., 2010). Therefore, shade treatment of *S. deltoids* was carried out with 0, 35, and 55% shading net and *in vitro* antioxidant and anti-inflammatory capacities were examined to determine the effects of different shading conditions on the extraction yield and biological activities.

Materials and Methods

Plant materials and shading treatments

S. deltoids was sown in 105 cell tray pots in March 2012 and grown in a grass house. They were planted in a 30 × 20 cm field located at the Gangwon Agricultural Research and Extension Services, Specialty Crops Research Institute, Wild Vegetable Branch in Pyeongchang, Gangwon, Korea. The shading treatments were carried out in the last 10 days of May using 35% and 55% shading net sold commercially. Light density was measured 10 times at about 11 o'clock using a portable light density measuring instrument (Almemo 2590, FL A603-PS5, Ahlborn, Holzkirchen, Germany) on sunny days from July 26 to September 10. The average shading effect under the 35% shading net was 39% and the average shading effect under the 55% shading net was 72%. The yields and hardness of the leaves were measured on 15 plants during the first 10 days of August (3 months after planting). The hardness of the leaves was measured using a hardness measuring instrument (Rheo-meter Compac-100 II/SUN). The temperatures under the shading treatments were measured with an automatic temperature measuring instrument (Onset, Hobo, Contoocook, NH, USA) at 1 meter from the ground from May to September. The data were analyzed by SPSS program version 19.0 (SPSS, Inc., Chicago, IL, USA) and were evaluated with Duncan's multiple range test. A $p < 0.05$ was considered significant.

Chemicals

1,1-diphenyl-2-2-pricylhydrazyl (DPPH), 2-deoxy-D-ribose, α-tocopherol, ethylenediaminetetraacetic acid (EDTA), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), trichloroacetic acid (TCA), Folin-Ciocalteu reagent, BHT, nitro blue tetrazolium (NBT), ferric chloride, phenazine methosulfate (PMS), dinucleotide-reduced (NADH), 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT), and lipopolysaccharide (LPS) (*E. coli* 0111:B4) were purchased from Sigma (St, Louis, MO, USA). RPMI medium 1640 and fetal bovine serum (FBS) were acquired from Gibco BRL (Grand Island, NY, USA). All culture supplies were obtained from BD-Falcon (BD, Franklin Lakes, NJ).

Preparation of the extract

S. deltoides was dried in the shade at room temperature and powdered. One hundred g of the powder was immersed in 2 L of methanol and extracted with an ultrasonic cleaner for 6 h at the boiling point. The extract was filtered through filter paper (100 mm; Whatman, Maidstone, UK) and evaporated using a vacuum rotary evaporator (CCA-1110; Eyela, Tokyo, Japan) to produce a crude extract. The dried extract was weighed and kept in a refrigerator for further analysis. The extracts of samples without treatment and treated with 35% and 55% shading net were marked as control, 35% treatment, and 55% treatment, respectively.

Determination of total phenolic and flavonoid contents

Total phenolic content was determined using Folin-Ciocalteu reagent according to the method of Jung *et al*. (2008b) with a slight modification. Samples of 1 mg/mL and tannic acid of five different concentrations (20, 40, 80, 160, and 320 μg/mL) were prepared. One hundred μL of sample or tannic acid was mixed with 500 μL of 10% Folin-Ciocalteu reagent. Then, 400 μL of sodium carbonate solution (7.5%) was added to the mixture. The reactants were mixed adequately. After 30 min

at room temperature, the absorbance was measured at 750 nm. Total phenolic content was expressed as mg tannic acid equivalents/g (Tan mg/g extract).

Total flavonoid content was determined in 500 μL of a 1 mg/mL sample mixed with 500 μL of 2% $AlCl_3$ solution. The mixed solution was kept at room temperature for 1 h, and absorbance was recorded at 405 nm. Total flavonoid content was expressed as mg quercetin equivalents/g (Que mg/g extract).

DPPH radical-scavenging activity

The activity of the DPPH scavenging free radical was assessed using the method of Hu *et al*. (2009a) with a slight modification. Five-hundred μL of a positive control or sample at concentrations of 1-16 μg/mL was mixed with 500 μL of 0.1 mM DPPH methanol solution. The mixture was kept in the dark for 30 min at room temperature after shaking vigorously. Absorbance was measured at 515 nm, and DPPH radical scavenging activity was calculated. BHA and α-tocopherol were used as positive controls.

Reducing power assay

The reducing power activity of the extract was measured according to the method of Hu *et al*. (2009b) with some modifications. Samples was prepared at three concentrations (100, 300, and 500 μg/mL) and 200 μL of 0.2 M sodium phosphate buffer (pH 6.6) was added to each sample. Then, 200 μL of 0.1 % potassium ferricyanide was mixed and the mixture was incubated at 50℃ for 30 min. After adding 200 μL of TCA solution (10%), the reactants were centrifuged at 574 × g for 10 min at room temperature. Then, 500 μL of the supernatant was vacuumed out and added to a new tube with 500 μL of distilled water and 100 μL of 0.1% ferric chloride. The absorbance was determined at 700 nm. Ascorbic acid was used as the positive control.

Metal chelating activity

Metal chelating activity of the extracts was measured according to Liu *et al*. (2011) with a slight modification. Samples (200 μL) at four different concentrations (0.5, 1, 2, and 4 mg/mL) were mixed with 20 μL of 2 mM $FeCl_2$ in 740 μL of methanol. Then, 40 μL of 5 mM ferrozine was added to initiate the reaction. The mixture was kept at room temperature for 10 min, and absorbance of the solution was determined at 562 nm. BHA and EDTA were used as positive controls.

Superoxide radical scavenging assay

Superoxide radical scavenging activity was determined by the PMS-NADH generating system approach described by Singh and Rajini (2004) with minor modifications. One hundred μL of various concentrations (25, 50, 100, and 200 μg/mL) of each sample solution and 1 mL of 0.1 M phosphate buffer (pH 7.4) were mixed. Then, 100 μL of 150 μM NBT, 100 μL of 468 μM NADH, and 20 μL of 60 μM PMS were added. The mixture was incubated at room temperature for 8 min, and absorbance was measured at 560 nm. Ascorbic acid and gallic acid were used as positive controls.

Nitrite scavenging ability

The nitrite scavenging assay was carried out according to the method of Yin *et al*. (2007) with minor modifications. Two hundred μL of each sample was mixed with 200 μL of 1 mM nitrite sodium. Then, 1.6 mL of 0.1 M HCl buffer (pH 1.2 adjusted with NaOH) or 0.2 M citrate buffer at pH 4.2 or 6.0 was added. After a 1 h pre-incubation at 37℃, 200 μL of solution was removed and mixed with 400 μL of 2% acetic acid. Then, Griess reagent [0.1% aqueous solution of naphthyl-ethylenediamine dihydrochloride, 40 μL; 1% sulfanilamide (in 5% phosphoric acid), 40 μL] was added. The reactants were mixed by vigorous shaking and kept at room temperature for 15 min. Absorbance was measured at 515 nm. BHA was used as the positive control.

Total antioxidant activity

Total antioxidant activity was measured according to the method of Li *et al*. (2010) with some modifications and expressed as BHA and ascorbic acid equivalents. BHA and ascorbic acid were prepared at five concentrations of 20-100 μg/mL. Two hundred μL of each sample (1 mg/mL), BHA, or ascorbic acid was mixed with 200 μL of 0.6 M sulfuric acid in a 1.5 mL tube. Then, 200 μL of 28 mM sodium phosphate was added to the tubes and 200 μL of 4 mM ammonium molybdate solutions was added to the mixture. All tubes were shaken vigorously and incubated for 90 min at 95℃. Absorbance was measured at 695 nm against a blank.

Cell line and cell culture

The RAW 264.7 cell line was purchased from the Korean Cell Line Bank (Seoul, Korea). RAW 264.7 cells were maintained in RPMI 1640 supplemented with 10% FBS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. The cells were incubated at 37℃ in a humidified atmosphere of 95% air and 5% CO_2.

Cell viability assay

The cytotoxicity of samples on RAW 264.7 cells was investigated. Cells were seeded into 96-well plates at a density of 1×10^5 cells/well for 16 h and then exposed to medium in the presence of different sample concentrations for 24 h. After removing the supernatant from each well, 10 μL of MTT solution (5 mg/mL in phosphate-buffered saline) and 90 μL of FBS-free medium were added to each well followed by a 4 h incubation at 37℃. The dark blue formazan crystals formed inside the intact mitochondria were solubilized with 100 μL of MTT stop solution (containing 10% sodium dodecyl sulfate and 0.01 M HCl). The amount of MTT formazan was quantified by measuring absorbance at 550 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader (ELx800TM, Bio-Tek, Winooski, VT, USA). The optical density of formazan formed in the control cells was considered 100% viability. Cell viability was expressed as a percentage of the control culture value.

Quantification of NO production in LPS-induced RAW 264.7 cells

RAW 264.7 cells were plated in 96-well cell plate and stimulated with LPS (2 μg/mL) in the presence or absence of various concentration of samples for 24 h. Aliquots of 100 μL of cell culture medium were mixed with Griess reagent [0.1% aqueous solution of naphthyl-ethylenediamine dihydrochloride, 50 μL; 1% sulfanilamide (in 5% phosphoric acid), 50 μL] at room temperature. The absorbance was determined at 550 nm using an ELISA plate reader (ELx800TM).

Statistical analyses

All tests were carried out independently in triplicate. Data are expressed as mean ± standard derivation. One-way analysis of variance was used to determine the significant differences between the groups followed by Dunnett's *t*-test for multiple comparisons. Values of $*p < 0.05$ were considered significant. All analyses were performed using SPSS for Windows XP, version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Total phenolic and total flavonoid contents

The effect of light treatment on *S. deltoides* yield was studied after extraction with aqueous methanol. As shown in Table 1, the control sample, 35% shade-treated sample, and 55% shade-treated sample produced yields of 14.14, 13.51, and 14.52%, respectively. Compared the three extracts, control showed the highest phenolic content (292.99 ± 2.86 Tan mg/g extract) and the highest flavonoid content (22.63 ± 1.64 Que mg/g extract). 55% shade showed the lowest content in both phenolic (236.41 ± 2.21Tan mg/g extract) and flavonoid (2.60 ± 1.73 Que mg/g extract).

DPPH radical scavenging activity

The value of IC_{50} of no shade was the lowest in three treatment, it means that the no shade possess the strongest DPPH scavenging ability (Table 1). The DPPH scavenging ability of 35% shade was between the no shade and 55% shade. The IC_{50} value of 55% shade was significantly larger

Table 1. Effect of shade treatment on yield and DPPH free radical-scavenging activity of *Synurus deltoides*

Extracts	Yield (%)[z]	DPPH free radical scavenging activity (IC_{50})[y]
No shade	14.14	17.65 ± 0.87[c]
35% shade	13.51	18.42 ± 0.38[c]
55% shade	14.52	21.49 ± 0.49[d]
Positive control		
BHA	-	3.09 ± 0.07[a]
-Tocpherol	-	13.34 ± 0.04[b]

[z]Extraction yield (%) is expressed as: (sample extract weight / sample weight) × 100.
[y]IC_{50}: The effective concentration at which DPPH radicals were scavenged by 50%. L-Ascorbic acid was used as a positive control.
Values are means of three determinations ± standard deviation (n = 3). Different letters of upper index in the same column are significantly different by Duncan's multiple range test (p < 0.05).

than that of no shade or 35% shade. Results showed that the control was better to be a DPPH radical scavenger.

Total antioxidant activity

Total antioxidant activity of the different samples was expressed as BHA and ascorbic acid equivalents (Table 2). No shade revealed the highest values [(107.13 ± 1.15) BHA equivalent mg/g and (71.80 ± 0.80) ascorbic acid equivalent mg/g], followed by 35% shade [(102.50 ± 0.61) BHA equivalent mg/g and (68.58 ± 0.43) ascorbic acid equivalent mg/g], and 55% shade [(90.38 ± 0.86) BHA equivalent mg/g and (60.16 ± 0.60) ascorbic acid equivalent mg/g].

Table 2. Effect of shade treatment on total antioxidant activity of *Synurus deltoides*

Extracts	BHA equivalent (mg/g)	Ascorbic acid equivalent (mg/g)
No shade	107.13 ± 1.15[c]	71.80 ± 0.80[c]
35% shade	102.50 ± 0.61[b]	68.58 ± 0.43[b]
55% shade	90.38 ± 0.86[a]	60.16 ± 0.60[a]

BHA and ascorbic acid were used as standards for measuring the total antioxidant activity.
Values are means of three determinations ± standard deviation (n = 3). Different letters of upper index in the same column are significantly different.

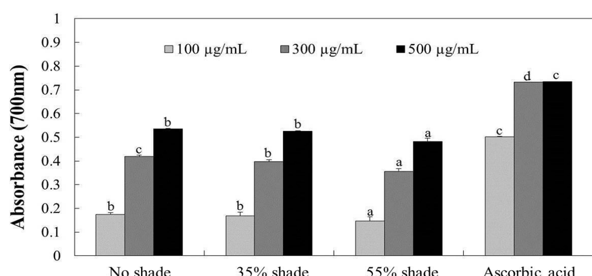

Fig. 1. Effect of shade treatment on total reducing power activity of *Synurus deltoides*. Each value is expressed as the mean ± SD (n = 3). Values at the same concentration are significantly different by Duncan's multiple range test ($p < 0.05$).

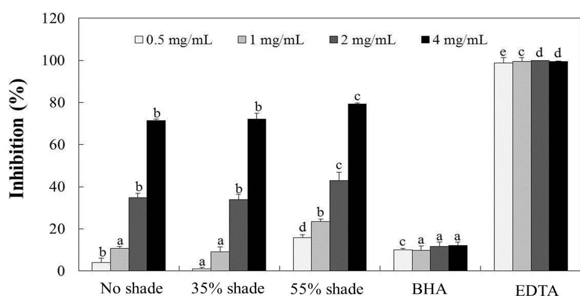

Fig. 2. Effect of shade treatment on metal chelating activity of *Synurus deltoides*. Each value is expressed as the mean ± SD (n = 3). Values at the same concentration are significantly different by Duncan's multiple range test ($p < 0.05$).

Reducing power activity

The results shown in Fig. 1 revealed that no shade and 35% shade had significantly stronger reducing power compared with that of 55% shade. No shade and 35% shade exhibited the same reducing power at concentrations of 100 and 500 µg/mL. However, at a concentration of 300 µg/mL, no shade exhibited higher reducing power than that of 35% shade. All samples showed significantly lower reducing power compared with that of ascorbic acid.

Metal chelating activity

As shown in Fig. 2, metal chelating activity of the samples increased with increasing concentration. The chelating ability of 55% shade was significantly greater than that of no shade and 35% shade and no significant difference in scavenging capacity was observed between no shade and 35% shade at concentrations of 1, 2, or 4 mg/mL. The metal chelating activity of the samples was stronger than that of BHA when concentrations were 2 or 4 mg/mL. At a concentration of 4 mg/mL, the percentage inhibition of samples reached its maximum, which was significantly lower compared with that of EDTA.

Superoxide radical scavenging activity

All three samples showed an obvious scavenging effect on the superoxide radical, and no significant differences were observed in the scavenging capacity at 25, 50, or 100 µg/mL (Fig. 3). However, at a concentration of 200 µg/mL, the percentage inhibitory activity of the samples against the superoxide radical decreased and the decline of 35% shade and 55% shade was significantly steeper than that of no shade. When the concentration was 100 µg/mL, the samples exhibited the strongest superoxide radical scavenging ability which was stronger than that of ascorbic acid but lower than that of gallic acid.

Nitric oxide (NO) inhibition activity

The scavenging activity of the samples against nitrite by

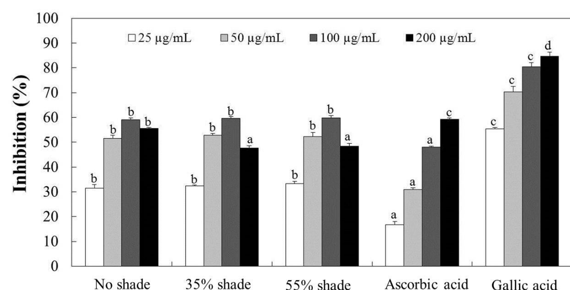

Fig. 3. Effect of shade treatment on superoxide radical scavenging activity of *Synurus deltoides*. Each value is expressed as the mean ± SD (n = 3). Values at the same concentration are significantly different by Duncan's multiple range test ($p < 0.05$).

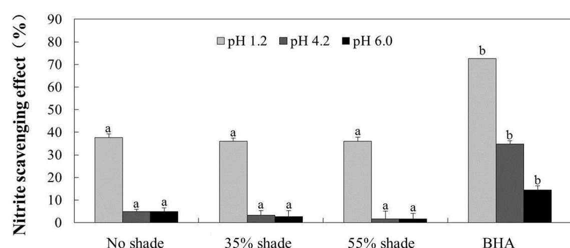

Fig. 4. Effect of shade treatment on nitrite scavenging activity of *Synurus deltoides* on the different acidic conditions. Each value is expressed as the mean ± SD (n = 3). Values in the same column are significantly different by Duncan's multiple range test (*p < 0.05*).

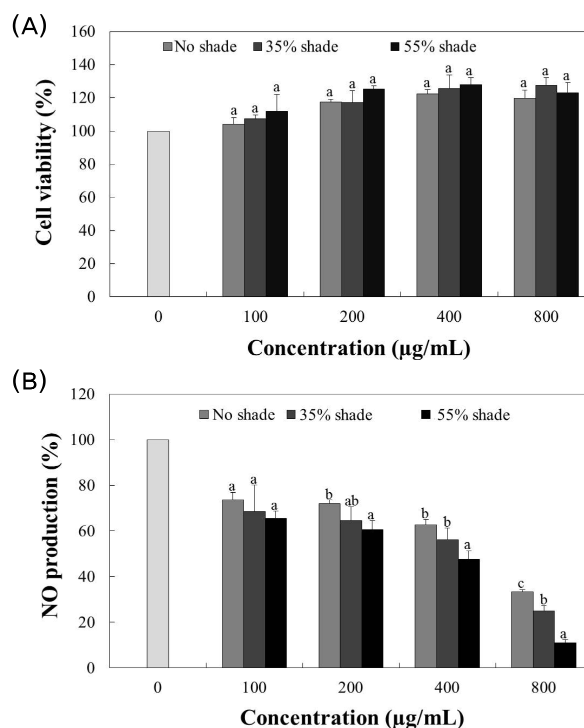

Fig. 5. Effect of shade treatment on cell viability (A) and NO production (B) of *Synurus deltoides* in LPS induced RAW 264.7 cells. Each value is expressed as the mean ± SD (n = 3). Values at the same concentration are significantly different by Duncan's multiple range test (*p < 0.05*).

sodium nitrite was investigated, and the results are presented in Fig. 4. The percentage inhibition of the samples and BHA at pH 1.2 was significantly higher than that at pH 4.2 or 6.0. In addition, the scavenging activity was not significantly different among the three samples under the same pH conditions. BHA exhibited stronger nitrite scavenging activity than all samples at each pH.

Quantification of NO production in LPS-induced RAW 264.7 cells

We measured the cytotoxicity of *S. deltoides* extracts on RAW 264.7 cells. As shown in Fig. 5A, the cell viability rate was not reduced after treated cells with no shade, 35% shade, or 55% shade. In order to investigate the NO inhibition ability, LPS was used to stimulate RAW 264.7 cells. NO production was measured by Griess reaction through the cell response to inflammatory. Our results in Fig. 5B demonstrated that all samples had the same potency on NO inhibition at 100 μg/mL. NO production decreased as sample concentration increased. 55% shade showed significantly higher inhibition of NO production than that of no shade or 35% shade at 400 and 800 μg/mL.

Discussion

Phenolic compounds are secondary metabolites synthesized which contain hydroxylated derivatives of benzoic acid and cinnamic acids. Phenolics play an important role in plant as antioxidants and protective agents (Naczk and Shahidi, 2006). These compounds possess the effect not only against invading bacteria and other types of environmental stress (Ndhlala *et al*., 2007), but also on protect DNA from oxidative damaged, antioxidative, antimicrobial and antiinflammatory (Kim *et al*., 2003). Flavonoids are phytochemicals including flavanones, flavones, flavonols, isoflavonoids, anthocyanins, and flavans (Peterson and Dwyer, 1998). The beneficial effect of flavonoids have been reported as antioxidant, antifungal, anticancerous

Table 3. Effect of shade treatment on total phenolic and flavonoid contents of *Synurus deltoides*

Extracts	Phenolic content (Tan mg/g extract)[z]	Flavonoid content (Que mg/g extract)[y]
No shade	292.99 ± 2.86[c]	22.63 ± 1.64[c]
35% shade	269.58 ± 2.44[b]	14.37 ± 3.83[b]
55% shade	236.41 ± 2.21[a]	2.60 ± 1.73[a]

[z]Tannic acid (Tan) was used as a standard for measuring the total phenolic content. [y]Quercetin (Que) was used as a standard for measuring the total flavonoid content.

Values are means of three determinations±standard deviation (n = 3). Different letters of upper index in the same column are significantly different by Duncan's multiple range test ($p < 0.05$).

and antiinflammatory activity (López-Posadas *et al*., 2008). Therefore, it is necessary to determine the phenolic and flavonoid compounds when measuring biological activity. The control treatment had significantly higher total phenolic and flavonoid contents than that of the other extracts (Table 3). 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) is a stable free radical and in its structure there is an odd electron (Jin and Chen, 1998). In order to evaluate antioxidant effective, DPPH radical scavenging capacity assay has always been used of natural extracts, but this assay is sensitive to light, oxygen, and impurities (Mot *et al*., 2011). Result of this assay may be interfered by light, oxygen, and impurities, which may lead to increase the experimental error. Therefore, the reaction process of DPPH assay was kept in dark and the level of DPPH scavenging was determined quickly by absorbance at 517 nm. No shade and 35% shade possessed higher DPPH free radical scavenging activity than that of 55% shade (Table 1). Total antioxidant activity was measured according to the molybdenum blue assay. The formation of isopoly-molybdenum blue results in an intense blue color and has been used as a sensitive test for reducing reagents. High absorbance values indicate that a sample possesses higher antioxidant activity (Puoci *et al*., 2011). No shade exhibited significantly higher total antioxidant activity that may have been attributed to its chemical composition and high phenolic and flavonoid contents. The reducing power assay has been became a useful test with inexpensive reagents and equipment. The reaction process of this assay was with an appropriate speedy through a wide concentration range (Berker *et al*., 2007). In food and science, ferrous reducing power assay has been widely used to estimate the antioxidant activity (Kuda and Yano, 2009). Although the reducing power of no shade and 35% shade was significantly different at 300 μg/mL (Fig. 1), the numerical values were approximate and they were not significantly different at 100 or 500 μg/mL. Therefore, the significant difference may have been caused by a deviation. Iron biological characters include enzyme co-factor, oxygen transport and redox reactions (Kontoghiorghes, 1995). Iron chelation plays an important role in the regulation of transfusion-dependent anaemias. For iron-overloaded patients, iron-chelating therapy is always been used (Cappellini, 2005). Therefore, the metal chelating activity of the three samples was measured, and the 55% shade sample revealed the strongest chelating ability among the samples at any concentration (Fig. 2). Superoxide anion (O2-) production is stimulated in neutrophils and treats with activators of protein kinase (Kitaoka *et al*., 2005). Superoxide scavenging activities were measured by the PMS-NADH system (Samak *et al*., 2009). Therefore, it is necessary to identify some antioxidants with strong superoxide radical scavenging ability. There was a fact that nitrate ingestion may cause death of gastric cancer (Kako *et al*., 1992). Nitric oxide (NO), peroxynitrite (ONOO_) and nitrogen dioxide (NO2) as reactive nitrogen intermediates are one of the important reason introduce inflammatory process (Yen *et al*., 2001). In this study, the NO inhibition ability of the samples at pH 1.2 was higher than that at pH 4.2 or 6.0, which indicated that pH 1.2 was more advantageous for samples to scavenge nitrite.

Inflammation is generally recognized to be a cause of numerous diseases such as cancer, diabetes, atherosclerosis, sepsis, and obesity (O'Neill *et al*., 2009). Inflammatory processes are mediated by multiple molecular mechanisms. During the inflammatory process, a number of different inflammatory mediators, including NO, prostaglandin E2, and tumor necrosis factor-α generated by macrophages upon stimulation with LPS (a primary component of the Gram-negative bacteria cell wall) are representative toxins and play an important role in immune-pathology of acute or chronic inflammatory diseases (Castro *et al*., 2006). In this study, none of the samples showed a significant effect on inhibiting NO at 100 μg/mL. However, a significant difference was observed at a higher concentration of 800 μg/mL. The 55% shade sample

displayed significantly greater NO inhibitory capacity than that of the 35% shade sample, which showed significantly greater NO inhibition capacity than the no shade sample at 800 μg/mL. These results suggest that the effect of light treatment on the anti-inflammatory activity of *S. deltoids* was remarkable and that reducing the light intensity during their growth was more beneficial to exert anti-inflammatory effects.

In conclusion, we demonstrated the antioxidant and anti-inflammatory activities of *S. deltoids*, which were treated with different light intensities during growth. Samples with different light treatments exhibited different biological activities. The effects of the light condition on total antioxidant activity and the accumulation of phenolic and flavonoid contents in *S. deltoids* were clear. The total phenolic and flavonoid contents of the 55% shade sample were significantly lower than that of the 35% shade sample whose phenolic and flavonoid contents were lower than that of the no shade sample. In the total antioxidant activity assay, samples showed similar results for total phenolic and flavonoid contents. Only the 55% shade sample exhibited a significant difference in total reducing power activity and metal chelating activity with the no shade sample at all concentrations. The superoxide radical scavenging activities of the 35% and 55% shade samples were significantly different from the no shade sample only at a concentration of 200 μg/mL. In addition, no significant difference in nitrite scavenging activity or cytotoxicity was observed among the three samples. The 55% shade sample exhibited greater anti-inflammatory activity.

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