

Antioxidant activities of hot water extract of *Syneilesis palmata* root and aerial part

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우산나물 뿌리와 지상부 열수 추출물의 항산화 활성

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Abstract

This study was performed in order to investigate the antioxidant properties of hot water extract from the root and aerial part of the *Syneilesis palmata* in respect to its potential use as food, cosmetics material, or medicinal resource. The results showed that the *S. palmata* root hot water extract (RHW) possessed a higher content of total flavonoid compounds (4.58 mg/g) and total polyphenol compounds (59.11 mg/g). The SOD-like activities of the RHW and APHW were 23.74% and 21.61%, respectively, at a concentration 2,000 µg/mL. In the nitrite scavenging ability of a 2,000 µg/mL concentration, the RHW showed 63.06% (pH 1.2) and 47.16% (pH 3.0). The IC₅₀ values of the nitrite scavenging abilities were 99.93 µg/mL (ascorbic acid), 1,150.85 µg/mL (RHW), and 1,610.25 µg/mL (APHW). The IC₅₀ values of DPPH free radical scavenging abilities were 99.87 µg/mL (RHW) and 118.29 µg/mL (APHW). The inhibition values (IC₅₀) of xanthine oxidase were 139.62 µg/mL (RHW) and 111.11 µg/mL (APHW). In all of the experiments, the *S. palmata* root hot water extracts have higher activities than the aerial hot water extract, except for the xanthine oxidase inhibitory activity. These results suggest that the *S. palmata* is a potentially useful antioxidant source for the development of functional nutraceuticals, cosmetics and medicine.

Key words : *S. palmata*, nitrite scavenging ability, DPPH radical scavenging, xanthine oxidase inhibitory

Introduction

Free radical formation is associated with the normal natural metabolism of aerobic cells. The interaction of these species with molecules of a lipidic nature produces new radicals: reactive oxygen species (ROS) including free radicals such as O₂⁻, OH, ¹O₂ and H₂O₂ (1). Oxidative damage caused by ROS may be related to aging and disease, such as atherosclerosis, diabetes, cancer and coronary heart disease (2,3). Moreover, ROS end to work with reactive nitrogen species (RNS) to damage cells. RNS such as NO also have deleterious effects on the body due to their reactivity in cells, playing an important role in inflammation (4). Therefore,

regulating ROS and RNS is important to protect against degenerative disease including aging.

Recently there has been increasing interest in using oriental medicine and folk herbal resource for functional food, cosmetics and medicine. Plants are rich sources of antioxidants, such as vitamins, phenolic compounds and flavonoids (5), which prevents free radical damage, reducing risk of chronic diseases. Thus, the consumption of dietary physiological matters from these sources is beneficial in preventing many diseases (6).

Syneilesis palmata, which belongs to the family Compositae, has been used as a Korean traditional folk medicine in the treatment for arthritis, lumbago and bruise (7,8). Also *S. palmata* leaves used as food which is called "Usannamul" in Korea and "Tuershan" in China (9). Its chemical constituents include sesquiterpenes (10), pyrrolizidine

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alkaloids (11), monoterpene glycosides (12) and flavonoids (13). It contains abundant amounts of sugar, soluble proteins, minerals and amino acid (14), beneficial effects on antioxidation (15). Also, Lee et al. (16) was isolated sesquiterpenes and it had cytotoxicity against human cancer cell. Kwon et al. (17) have been reported that thrombin inhibition of methanol extract of *S. palmata* was higher than aspirin.

In this study, we evaluated the total flavonoid and polyphenol compounds contents of hot water extracts of *S. palmata* root and aerial part. Plus, we investigated the superoxide dismutase-like activity, nitrite scavenging activity and xanthine oxidase inhibitory as well as DPPH free radical scavenging activity of *S. palmata* root and aerial part hot water extract.

Materials and Methods

Materials

The *Syneilesis palmata* were collected at Mt. Palgong, Gyeongbuk Korea, in June to July 2009. The collecting sample, *S. palmata* was separated into root and aerial part. These were dried at 40°C for 48 hr until the moisture content was 10±1% using heated-air dryer (DR-0160, Hankwang, Siheung, Korea).

Preparation of extract

Each material (100 g) was extracted with 3L water using high-pressure extractor (DM-701, Daehan median, Seoul, Korea) at 105°C for 3 hr. After filtration, the filtrates were evaporated to dryness under vacuum and used throughout this study. The dried powders were used for preparing solutions of various concentrations. As a control, ascorbic acid was prepared at the same concentrations of *S. palmata* extract, and its physiological activities were also determined by the same experiments described below. The root and aerial part hot water extracts of *S. palmata* were named RHW, and APHW, respectively.

Total flavonoid compound content

The total flavonoid compound contents were measured by the method of Nieva Moreno et al. (18) with slight modification. The root and aerial part hot water extracts of *S. palmata* were dissolved in 80% ethanol (10 mg/mL). An aliquot of 0.5 mL was mixed with 10% aluminum nitrate (0.1 mL), 1 M potassium acetate 0.1 mL and added 80%

ethanol (4.3 mL). The mixture was kept at 25°C for 40 min, and absorbance was measured at 415 nm. A calibration curve was obtained using various concentrations of quercetin (Sigma-Aldrich Co., St. Louis, MO, USA). The flavonoid compound content of the sample was expressed as mg of quercetin equivalents per gram of dried sample.

Total polyphenol compound content

Phenolic compound contents in the root and aerial part hot water extracts from *S. palmata* were based on procedures described by Folin-Denis method (19). Briefly, the extracts (0.2 mL) were mixed with 1.8 mL of distilled water and 0.2 mL of Folin-ciocalteu's phenol reagent (Junsei Chemical Co., Tokyo, Japan) and allowed to react for 3 min. Then, 0.4 mL of saturated Na₂CO₃ solution and 1.4 mL of distilled water were added and the mixture was mixed well. After incubation for 1 hr at room temperature, absorbance was measured at 725 nm. A calibration curve was obtained using various concentrations of tannic acid (Sigma-Aldrich Co.). The polyphenol compound content of the sample was expressed as mg of tannic acid equivalents per gram of dried sample.

Superoxide dismutase (SOD)-like activity

The SOD-like activity was measured by the modified method of Marklund and Marklund (20). The reaction mixture contained 0.2 mL of sample, 2.6 mL of tris-buffer (pH 8.5), and 0.2 mL of 7.2 mM pyrogallol, and incubated at 25°C for 10 min. The reaction was stopped with 0.1 mL of 1 N HCl, and the absorbance was measured at 420 nm. The SOD-like activity was expressed as follows: SOD-like activity (%) = {1 - (A₂ - A₁) / A₀} × 100, where A₀ was the absorbance of the control, A₁ was the absorbance of the sample without reagent, and A₂ was the absorbance of the sample with reagent. Ascorbic acid was used as a standard control.

Nitrite scavenging activity

According to the method described by Kato et al. (21) using Griess reagent. Sample 1 mL and 2 mL of 1 mM NaNO₂ made up to 10 mL solution with pH set at pH 1.2, 3.0, and 6.0. The reaction mixture was incubated at 37°C water bath for 1 hr. Subsequently, 1 mL of sample with 5 mL of 2% acetic acid and 0.4 mL of Griess reagent (mixed solution at 1: 1 ration with 1% of sulfanilic acid in 30% acetic acid and 1% of naphthylamine in 30% acetic acid) was mixed and kept at room temperature for 15 min. The absorbance was measured at 520 nm for residual nitrite determination.

The nitrite scavenging activity was expressed as follows: nitrite scavenging activity (%) = $\{1 - (A_2 - A_1)/A_0\} \times 100$, where A_0 was the absorbance of the control, A_1 was the absorbance of the sample without Griess reagent, and A_2 was the absorbance of the sample with Griess reagent. Also this activity was expressed as the inhibition concentration at 50% (IC_{50}). Ascorbic acid was used as a standard control.

DPPH free radical scavenging activity

Free radical scavenging activity was determined by using a stable free radical, 1,1-diphenyl-2-picryl-hydrazil (DPPH), according to a slightly modified method of Blois (22). Sample 2 mL was mixed with 1 mL of 2×10^{-4} DPPH solution and incubated at a room temperature for 30 min. The absorbance was measured at 517 nm. The DPPH free radical scavenging activity was expressed as follows: DPPH scavenging activity (%) = $\{1 - (A_2 - A_1)/A_0\} \times 100$, where A_0 is the DPPH without sample (control), A_1 is the sample without DPPH (blank), and A_2 is the sample with DPPH. Also this activity was expressed as the inhibition concentration at 50% (IC_{50}). Ascorbic acid was used as a standard control.

Xanthine oxidase inhibitory activity

Xanthine oxidase inhibitory (XIO) activity was measured by the method of Stirpe and Corte (23) with some modification. The assay mixture consisted of 0.1 mL of each extract and 0.6 mL of 2 mM xanthine solution dissolved into 0.6 mL of 0.1 M potassium phosphate buffer (pH 7.5), and 0.1 mL of enzyme solution 0.2 units/mL xanthine oxidase (XO) in phosphate buffer, pH 7.5). After incubation at 37°C for 5 min, the reaction was stopped by the addition of 1 mL of 1 N HCl, and the absorbance was measured at 292 nm using a spectrophotometer. XIO activity was expressed as follows: xanthine oxidase inhibition (%) = $[(A-B) - (C-D)/(A-B)] \times 100$, where A is the activity of the enzyme without sample, B the control of A without sample and enzyme, C and D are the activities of the sample with and without XO, respectively. Also this activity was expressed as the inhibition concentration at 50% (IC_{50}). Ascorbic acid was used as a standard control.

Statistical analysis

Data are expressed as means \pm SD of three replicated determinations and were analyzed by SPSS version 19.0 for windows (SPSS Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA), and Duncan's multiple range tests were used to compare differences among mean values. A

p value <0.05 denoted the presence of statistically significant difference.

Results and Discussion

Extract yields, total flavonoid and polyphenol compound contents

S. palmata were divided into two different parts: root and aerial part and extract yield, total flavonoid and polyphenol compound contents in two parts were determined. As shown in Table 1, the extract yield of root (RHW) was 33.42% and aerial part (APHW) was 24.39%. The total flavonoid compound contents of RHW and APHW were 4.58 mg/g and 2.79 mg/g, respectively. The total polyphenol compound contents of RHW were 59.11 mg/g and 48.01 mg/g in APHW. The extract yield, flavonoid and polyphenol content of root had a higher than aerial part. Especially total flavonoid of root was more 1.6 times than aerial part. This is agreement with Lee et al. (15) reported that the roots water and ethanol extracts were consistently higher than the level of the aerial part extracts. The phenolic compounds have been shown to possess strong antioxidant activity, and flavonoids are one of the most diverse and widespread group of natural phenolic compounds (24). It had been reported that the antioxidant activity of plant materials is well correlated with the content of phenolic compounds (25-27). Thus polyphenol compound content including flavonoid compound content could be used as an important indicator of antioxidant capacity (28,29). This suggests that the water extract of *T. nucifera* needles, which contained a higher level of polyphenol and flavonoids than the ethanol extract of *T. nucifera*, might have high antioxidant properties. The higher content of total flavonoid and polyphenol compounds in the RHW might account for the better results found in their SOD-like activity, nitrite scavenging and DPPH radical scavenging activity.

Table 1. The extract yields, total flavonoid and polyphenol compounds of hot water extracts from root and aerial part of *S. palmata*

| | RHW ¹⁾ | APHW ²⁾ |
|----------------------------------|-------------------------------|-------------------------------|
| Extraction yield (%) | 33.42 | 24.39 |
| Total flavonoid compound (mg/g) | 4.58 \pm 0.45 ³⁾ | 2.79 \pm 0.21 ³⁾ |
| Total polyphenol compound (mg/g) | 59.11 \pm 2.52 | 48.01 \pm 0.85 |

¹⁾RHW is hot water extract from root of *S. palmata*.

²⁾APHW is hot water extract from aerial part of *S. palmata*.

³⁾Values represent the mean \pm SD.

Superoxide dismutase (SOD)-like activity

SOD is a primary enzyme in enzymatic antioxidant defense. O_2^- can be transformed into hydrogen peroxide by superoxide dismutase (SOD), a defense enzyme protecting cells from cellular damage caused by reactive oxygen species (30). SOD regulated the concentration of superoxide anionic radical, and receives much attention because of its protective effect against oxygen toxicity. However, SOD is inactivated by either digestive enzyme or gastric juice when it is orally administered, since its enzyme properties with molecular weight exceed 30 kDa, and it cannot be absorbed into the gastrointestinal tract (31). An alternative means of achieving this goal is to find low-molecular weight compounds that mimic sod behavior and can act as healthier alternatives. Numerous natural materials having SOD-like activity have been under investigation, because the active oxygen formed in the organism is supposed to cause oxidative hindrance. Therefore, SOD-like activity is regarded as a preventive parameter of oxidative damages (32). As shown Table 2, all extracts had dosage-dependant SOD-like activity, RHW and APHW showed the 23.74% and 21.61% at the 2,000 $\mu\text{g/mL}$. RHW had significantly higher SOD-like activity compared to the APHW at all concentrations, but there was no significant difference at concentration of 2,000 $\mu\text{g/mL}$ ($p < 0.05$). On the other hand, ascorbic acid showed the excellent SOD-like activity greater than 90% at concentrations of 100 $\mu\text{g/mL}$. A previous study, Lee et al. (15), reported that the root extract of *S. palmata* had the higher SOD-like activity than aerial part extract, which agrees with the present results. The hot water extract of the root and aerial part showed higher SOD-like activities compared to the Lee et al. (15), water and ethanol extract of root and aerial part from *S. palmata*.

Table 2. SOD-like activity of hot water extracts from root and aerial part of *S. palmata*

| Concentration ($\mu\text{g/mL}$) | Extract | | Control |
|------------------------------------|-------------------------------|--------------------------------|-------------------------------|
| | RHW | APHW | Ascorbic acid |
| 100 | 3.44 \pm 0.56 ^{bi} | 2.76 \pm 0.60 ^b | 90.59 \pm 0.78 ^a |
| 300 | 7.13 \pm 0.77 ^b | 5.40 \pm 1.39 ^{bc} | 93.86 \pm 0.23 ^a |
| 500 | 10.58 \pm 0.56 ^b | 8.05 \pm 0.80 ^c | 96.86 \pm 0.39 ^a |
| 1,000 | 14.76 \pm 0.37 ^b | 12.99 \pm 1.63 ^{bc} | 98.56 \pm 0.23 ^a |
| 2,000 | 23.74 \pm 0.56 ^b | 21.61 \pm 1.21 ^{bc} | 99.08 \pm 0.23 ^a |

¹⁾Values represent the mean \pm SD and mean with different superscripts within the row are significantly different at $p < 0.05$ by Duncan's multiple range test.

Nitrite scavenging activity

Nitrosamines are known to be potent carcinogens when

human consume them in the diet, or when they are produced from endogenous biosynthesis in the body (33). Nitrosamines are formed by the reaction of 2nd and 3rd grade amines in protein-rich food, medicines with nitrosating agents under acidic conditions in the stomach (34). Therefore, effective nitrite scavenging in an acidic condition is very helpful in inhibiting the formation of carcinogenic nitrosamines.

Nitrite scavenging activities of the prepared RHW and APHW of *S. palmata* were assessed at pH 1.2, 3.0 and 6.0 (Table 3). The nitrite scavenging activity of the sample was increased as the pH decreased in a dose-dependent manner. The estimated maximum nitrite scavenging activity was 63.06% under the pH 1.2 condition of the concentration 2.0 mg/mL from RHW, APHW was 56.31% at the same condition. The IC_{50} values of RHW and APHW were 1,150.85 $\mu\text{g/mL}$ and 1,610.25 $\mu\text{g/mL}$, and ascorbic acid was 99.93 $\mu\text{g/mL}$, respectively. In the pH 3.0, RHW and APHW were 47.16% and 39.26%. Ascorbic acid had the highest scavenging ability of all the samples under the same conditions. The RHW had a higher nitrite scavenging activity than that of APHW at pH 1.2 and 3.0. At the pH 6.0 condition, RHW value were not significantly different with APHW ($p < 0.05$). Thus, RHW is expected that nitrosation inhibition

Table 3. Nitrite scavenging ability of hot water extracts from root and aerial part of *S. palmata*

| Concentration ($\mu\text{g/mL}$) | Extract | | Control | |
|------------------------------------|-----------|----------------------------------|-----------------------------------|-------------------------------|
| | RHW | APHW | Ascorbic acid | |
| pH 1.2 | 100 | 5.86 \pm 0.57 ^{bi} | 2.95 \pm 0.00 ^c | 52.38 \pm 0.21 ^a |
| | 300 | 16.03 \pm 0.42 ^b | 14.28 \pm 0.20 ^c | 91.34 \pm 0.51 ^a |
| | 500 | 26.91 \pm 1.07 ^b | 22.53 \pm 0.56 ^c | 93.67 \pm 0.36 ^a |
| | 1,000 | 47.68 \pm 0.14 ^b | 40.12 \pm 0.31 ^c | 97.40 \pm 0.28 ^a |
| | 2,000 | 63.06 \pm 0.63 ^b | 56.31 \pm 0.62 ^c | 98.06 \pm 0.08 ^a |
| | IC_{50} | 1,150.85 \pm 7.33 ^b | 1,610.25 \pm 15.27 ^c | 99.93 \pm 0.16 ^a |
| pH 3.0 | 100 | 3.89 \pm 0.45 ^b | 3.33 \pm 0.28 ^c | 30.52 \pm 0.69 ^a |
| | 300 | 13.97 \pm 0.49 ^b | 12.57 \pm 0.15 ^c | 52.58 \pm 0.94 ^a |
| | 500 | 20.72 \pm 0.77 ^b | 18.70 \pm 0.38 ^c | 65.77 \pm 0.65 ^a |
| | 1,000 | 35.07 \pm 0.85 ^b | 30.07 \pm 0.48 ^c | 81.03 \pm 0.45 ^a |
| | 2,000 | 47.16 \pm 0.92 ^b | 39.26 \pm 0.40 ^c | 85.26 \pm 0.43 ^a |
| pH 6.0 | 100 | 1.82 \pm 0.65 ^b | 2.82 \pm 0.87 ^b | 5.24 \pm 1.01 ^a |
| | 300 | 3.39 \pm 0.38 ^b | 3.99 \pm 0.37 ^b | 13.87 \pm 0.63 ^a |
| | 500 | 5.51 \pm 0.88 ^b | 5.84 \pm 0.54 ^b | 29.85 \pm 1.44 ^a |
| | 1,000 | 8.09 \pm 0.51 ^b | 8.50 \pm 0.49 ^b | 50.19 \pm 0.51 ^a |
| | 2,000 | 11.18 \pm 0.77 ^b | 10.60 \pm 0.36 ^b | 59.41 \pm 0.58 ^a |

¹⁾Values represent the mean \pm SD and mean with different superscripts within the row are significantly different at $p < 0.05$ by Duncan's multiple range test. IC_{50} values were determined by linear regression analysis.

might be feasible under stomach conditions (pH 1.2). This finding is similar to that reported by Lee et al. (15) who found that the nitrite scavenging activity in a root extract was higher than aerial parts and low pH. Phenolic compounds including flavonoid compounds and ascorbic acid are reported to have high nitrite scavenging effects, and have higher activities under conditions of low environmental pH (35). These results showed that when compared to the APHW, the higher antioxidant activity of the RHW is in good accordance with its greater amount of phenolic compound.

DPPH free radical scavenging activity

DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol, but becomes pale yellow when it is neutralized by antioxidants (36). The use of DPPH free radicals is a common method to evaluate antioxidant activities in a relatively short time compared to other methods. Removal of free radicals plays an important role in preventing lifestyle diseases and aging of our body (37). Hence, the DPPH radical scavenging method is used for measuring electron donating ability, and DPPH free radical scavenging has been widely used to evaluate the antioxidant activity of various natural plants.

Table 4 showed concentration-dependent DPPH radical scavenging activity of RHW and APHW. As a reference, ascorbic acid showed an excellent scavenging ability of greater than 84% at a concentration of 50 $\mu\text{g/mL}$. The scavenging ability of RHW, APHW and ascorbic acid were 94.41%, 93.98% and 96.85% at 2,000 $\mu\text{g/mL}$, respectively. RHW was higher than the activities of APHW below the concentrations of 1,000 $\mu\text{g/mL}$. However, no significant difference was shown between the RHW and APHW at

concentrations of 2,000 $\mu\text{g/mL}$ ($p < 0.05$). The IC_{50} of the RHW (99.87 $\mu\text{g/mL}$) was significantly higher than that of the APHW (118.29 $\mu\text{g/mL}$), and the control (ascorbic acid) was 48.79 $\mu\text{g/mL}$. Lee et al. (15) reported that the DPPH free radical scavenging activity of water extract from *S. palmata* root and aerial part showed 91.39% and 60.70% at 1,000 $\mu\text{g/mL}$, respectively. APHW result were higher free radical scavenging activity than the Lee et al. (15) report, but RHW were similar to that reported by Lee et al. (15). Previous studies report that free radical scavenging activity have shown positive correlations between phenolic compound content (34,35), and antioxidant activity for RHW were higher total polyphenol compound contents than APHW. This suggests that phenolic compounds constituents in the RHW may contribute to the highest antioxidant activities in the DPPH free radical activity.

Xanthine oxidase inhibition

Xanthine oxidase (XO) is the enzyme responsible for the formation of uric acid from the purines, hypoxanthine and xanthine, and its accumulation in blood and bone results in gout and accumulation in the kidney (38). Gout is induced by the deposition of uric acid in the joints, resulting in painful inflammation, with XO inhibition resulting in a remission in gout (39). XO also functions as an important biological source of oxygen-derived free radicals, which contribute to oxidative damage to living tissues that are involved in a variety of pathological processes, including inflammation, atherosclerosis, cancer, and aging (40). XO inhibitors may potentially prove useful for the treatment of gout or other XO-induced diseases (41). Allopurinol is the only clinically used XO inhibitor, which also suffers from many side effects such as hepatitis, hypersensitivity syndrome, Stevens Johnson syndrome, nephropathy allergic reaction and renal toxicity (42,43). Therefore, there is an urgent need to search for new XO inhibitors. Ascorbic acid had the highest XO inhibition activity at concentrations between 50~500 $\mu\text{g/mL}$, also APHW were significantly higher than that of the RHW at 50~500 $\mu\text{g/mL}$ ($p < 0.05$). But XO activity of all extract were over 95% and there was no significant difference at concentration of 1,000 $\mu\text{g/mL}$ (Table 5). The IC_{50} value of APHW (111.11 $\mu\text{g/mL}$) was higher than those of RE (139.62 $\mu\text{g/mL}$), ascorbic acid was 52.17 $\mu\text{g/mL}$. These results suggest that *S. palmata* may potentially be useful for treating gout, inflammation, and other XO induced diseases.

In conclusion, the screening of antioxidant, content of total flavonoid and polyphenol compound and XO activities was

Table 4. DPPH free radical scavenging ability of hot water extracts from root and aerial part of *S. palmata*

| Concentration ($\mu\text{g/mL}$) | Extract | | Control |
|---------------------------------------|--------------------------------|--------------------------------|-------------------------------|
| | RHW | APHW | Ascorbic acid |
| 50 | 15.95 \pm 1.74 ^{bl} | 11.83 \pm 1.22 ^c | 84.32 \pm 0.63 ^a |
| 100 | 50.09 \pm 0.96 ^b | 46.86 \pm 1.42 ^c | 86.40 \pm 0.37 ^a |
| 300 | 67.58 \pm 1.20 ^b | 64.03 \pm 1.44 ^c | 89.46 \pm 0.80 ^a |
| 500 | 81.59 \pm 1.53 ^b | 72.01 \pm 1.01 ^c | 91.43 \pm 0.62 ^a |
| 1,000 | 90.32 \pm 0.69 ^b | 86.88 \pm 0.71 ^c | 95.07 \pm 0.24 ^a |
| 2,000 | 94.41 \pm 0.42 ^b | 93.98 \pm 0.51 ^b | 96.85 \pm 0.18 ^a |
| IC_{50} | 99.87 \pm 0.84 ^b | 118.29 \pm 4.92 ^c | 48.79 \pm 0.13 ^a |

¹⁾Values represent the mean \pm SD and mean with different superscripts within the row are significantly different at $p < 0.05$ by Duncan's multiple range test. IC_{50} values were determined by linear regression analysis.

Table 5. Xanthine oxidase inhibitory activity of hot water extracts from root and aerial part of *S. palmata*

| Concentration ($\mu\text{g/mL}$) | Extract | | Control |
|---------------------------------------|----------------------------------|--------------------------------|-------------------------------|
| | RHW | APHW | Ascorbic acid |
| 50 | 17.36 \pm 1.20 ^{bc1)} | 19.64 \pm 1.79 ^b | 70.90 \pm 2.43 ^a |
| 100 | 35.42 \pm 0.00 ^c | 48.21 \pm 1.79 ^b | 88.89 \pm 2.75 ^a |
| 300 | 72.22 \pm 1.20 ^c | 78.57 \pm 1.79 ^b | 90.48 \pm 0.00 ^a |
| 500 | 84.72 \pm 1.20 ^{bc} | 86.90 \pm 2.06 ^b | 94.71 \pm 0.92 ^a |
| 1,000 | 97.92 \pm 2.08 ^a | 95.83 \pm 1.03 ^a | 96.30 \pm 0.92 ^a |
| IC ₅₀ | 139.62 \pm 3.82 ^c | 111.11 \pm 5.56 ^b | 52.17 \pm 0.21 ^a |

¹⁾Values represent the mean \pm SD and mean with different superscripts within the row are significantly different at $p < 0.05$ by Duncan's multiple range test. IC₅₀ values were determined by linear regression analysis.

performed on hot water extracts of *S. palmata* root and aerial part. Total flavonoid and polyphenol compound contents of root hot water extract (RHW) were higher than aerial part hot water extract (APHW) and the antioxidant activity of RHW were higher than APHW except XO activity at concentrations between 50~500 $\mu\text{g/mL}$. XO activity of all extract were over 95% and there was no significant difference at concentration of 1,000 $\mu\text{g/mL}$. This result shows that high content of phenolic compounds with the lowest IC₅₀ value from DPPH free radical activity and nitrite scavenging ability. Thus this study suggests that the hot water extract of *S. palmata* root could be used as an effective antioxidant source for functional nutraceuticals, cosmetic and medicine. Further, more detailed work should be followed to isolate and identify the active ingredients with strong antioxidation ability in *S. palmata*.

요 약

본 연구는 식품, 화장품 및 의약품 소재로 활용 가능성에 대해 알아보하고자 우산나물 뿌리와 지상부에 대한 항산화 활성을 측정하였다. 우산나물 뿌리 열수 추출물(RHW)은 4.58 mg/g의 플라보노이드와 59.11 mg/g의 폴리페놀 화합물을 함유하였으며, 지상부 열수 추출물(APHW)은 각각 2.79 mg/g과 48.01 mg/g을 함유하였다. SOD 유사활성능은 RHW에서 23.74%, APHW는 21.61%를 나타내었다. 아질산염 소거능은 2,000 $\mu\text{g/mL}$ 에서 RHW는 pH 1.2에서 63.06%였으며, pH 3.0에서는 47.16%로 아질산염을 50% 소거하는 IC₅₀은 ascorbic acid 99.93 $\mu\text{g/mL}$, RHW 1,150.85 $\mu\text{g/mL}$, 그리고 APHW에서는 1,610.25 $\mu\text{g/mL}$ 이었다. DPPH free radical 소거능에 대한 IC₅₀은 RHW 99.87 $\mu\text{g/mL}$, APHW 118.29 $\mu\text{g/mL}$ 를 나타내었다. Xanthine oxidase에 대한 IC₅₀

은 RHW에서 139.62 $\mu\text{g/mL}$ 였으며, APHW는 111.11 $\mu\text{g/mL}$ 으로 xanthine oxidase 저해능은 우산나물 지상부의 열수 추출물인 APHW가 뿌리 열수 추출물인 RHW보다 좀더 낮은 농도에서 50%의 활성을 나타내었으나, 플라보노이드와 폴리페놀 화합물 함량, SOD 유사활성, 아질산염 소거 및 DPPH free radical 소거능은 RHW가 APHW보다 높은 활성을 나타내었다. 이상의 결과 우산나물은 기능성 식품과 화장품 그리고 의약품 개발을 위한 항산화 소재로 활용할 수 있을 것으로 판단된다.

References

1. Torel J, Cillard J, Cillard P (1986) Antioxidant activity of flavonoids and reactivity with peroxy radical. *Phytochem*, 2, 383-385
2. Halliwell B, Gutteridge JM (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J*, 219, 1-14
3. Lipworth L, Martinez ME, Angell J, Hsien CC, Trichopoulos D (1997) Olive oil and human cancer; an assessment of evidence. *Prev Med*, 26, 181-190
4. Bashan N, Kovsan J, Kachko I, Ovadia H, Rudoch A (2009) Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. *Physiol Rev*, 89, 27-71
5. Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evan C, Roberfroid M (1998) Functional food science and defence against reactive oxidative species. *Brit J Nutr*, 80, 77-112
6. Hu FB (2000) Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol*, 13, 3-9
7. Lee YN (1998) *Flora of Korea* 3th edition. Kyohaksa, Seoul, Korea p 814
8. An DK (1998) *Illustrated book of Korean medicinal herbs*. Kyohaksa, Seoul, Korea p 347
9. Kim H, Song MJ, Potter D (2006) Medicinal efficacy of plants utilized as temple food in traditional Korean Buddhism. *J Ethnopharm*, 104, 32-46
10. Kuroda C, Murae T, Toda M, Nagano H, Takahashi T (1978) New 14-oxofuranoeremophilanes and related sesquiterpenes from *Syneilesis palmata* (Thunb.) Maxim. *Chem Lett*, 1313-1316
11. Manabu H, Tsutomu F (1974) Syneilesine, a new pyrrolizine alkaloid from *Syneilesis palmata*. *Tetrahedron Lett*, 41, 3657-3660
12. Bolhmann F, Grenz M (1977) Terpeneglucoside aus

- Syneilesis aconitifolia*. Phytochem, 16, 1057-1059
13. Bolhmann F, Zdero C (1978) Neue furaneremophilane und andere sesquiterpene aus vertretern gattung Europs. Phytochem, 17, 1135-1153
 14. Lee YS, Seo SJ, Kim NW (2009) Analysis of the general components of *Syneilesis palmata* Maxim. Korean J Food Preserv, 16, 412-418
 15. Lee YS, Ahn DS, Joo EY, Kim NW (2009) Antioxidative activities of *Syneilesis palmata* extract. J Korean Soc Food Sci Nutr, 38, 1471-1477
 16. Lee KH, Choi SU, Lee KR (2005) Sesquiterpenes from *Syneilesis palmata* and their cytotoxicity against human cancer cell lines in vitro. Arch Pharm Res, 28, 280-284
 17. Kwon CS, Kwon YS, Kim YS, Kwon GS, Jin UG, Ryu GC, Sohn HY (2004) Inhibitory activities of edible and medicinal herbs against human thrombin. J Life Sci, 14, 509-513
 18. Nieva Moreno MI, Isla MI, Sampietro AR, Vattuone MA (2000) Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. J Ethnopharmacol, 71, 109-114
 19. AOAC (2005) Official method of analysis. 18th ed. Association of official analytical Chemists, Washington, DC, USA, 45, p 21-22
 20. Marklund S, Marklund G (1975) Involvement of superoxide amino radical in the oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem, 47, 468-474
 21. Kato H, Lee IE, Chuyen NV, Kim SB, Hayase F (1987) Inhibition of nitrosamine formation by nondialyzable melanoidins. Agric Biol Chem, 51, 1333-1338
 22. Blois MS (1958) Antioxidant determination by the use of a stable free radical. Nature, 181, 1199-1200
 23. Stirpe F, Corte ED (1969) The regulation of rat liver xanthine oxidase. J Biol Chem, 244, 3855-3861
 24. Shahidi F, Wanasundara PK (1992) Phenolic antioxidant. Crit Rev Food Sci, 32, 67-103
 25. Velioglu YS, Mazza G, Gao L, Oomh BC (1998) Antioxidant activity and total phenolics in selected fruits, vegetables. and grain products. J Agri Food Chem, 46, 413-417
 26. Cai Y, Luo Q, Su M, Corke H (2004) Antioxidant activity and phenolic compounds of 112 Chinese medicinal plants associated with anticancer. Life Sci, 74, 2157-2184.
 27. Matkowski A (2008) Plant in vitro culture for the production of antioxidants. Rev Biotech Adv, 26, 548-560
 28. Vinson JA, Pinch J, Bose P (2001) Determination of quantify and quality of polyphenol antioxidants in foods and beverages. Method Enzymol, 335, 103-114
 29. Duan X, Wu G, Jiang Y (2007) Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Molecules, 12, 759-771
 30. Conner EM, Grisham MB (1996) Inflammation, free radicals, and antioxidants. Nutrition, 12, 274-280
 31. Zielinski H, Frias J, Piskula MK, Kozłowska H, Vidal-Valverde C (2006) The effect of germination process on the superoxide dismutase-like activity and thiamine, riboflavin and mineral contents of rapeseeds. Food Chem, 99, 516-521
 32. Fridovich I (1997) Superoxide anion radical (O_2^-), superoxide dismutases, and related matters. J Biol Chem, 272, 18515-18517
 33. Shin SR, Hong JY, Nam HS, Yoon KY, Kim KS (2006) Anti-oxidative effects of extracts of Korean herbal materials. Korean J Soc Food Sci Nutr, 35, 187-191
 34. Bartsch H, Montesano R (1984) Relevance of nitrosamines to human cancer. Carcinogenesis, 5, 1381-1393
 35. Mirivish SS, Wallcave L, Eagen M, Shubik P (1972) Ascorbate nitrite reaction: Possible means of the formation of carcinogenic N-nitroso compounds. Science, 177, 65-67
 36. Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI, Nishioka I (1998) Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl radical. Biochem Pharmacol, 56, 213-222
 37. Kang YH, Park YK, Lee GD (1996) The nitrite scavenging and electron donating ability of phenolic compounds. Korean J Food Sci Technol, 28, 232-239
 38. Stroch J, Ferber E (1988) Amplifies chemiluminescence of lucigenin for determination of superoxide anion production by NADPH oxidase and xanthine oxidase. Analytical Biochem, 169, 262-266
 39. Chiang HC, Lo YJ, Lu FJ (1994) Xanthine oxidase inhibitors from the leaves of *Alsophila spinulosa* (Hook) Tryon. J Enzyme Inhib, 8, 61-71
 40. Borges F, Fernandes E, Roleira F (2002) Progress towards the discovery of xanthine oxidase inhibitors. Cur Med Chem, 9, 195-217
 41. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Poel BV, Pieters L, Viletinck AJ, Berghe DV (1998) Structure activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and super oxide scavengers. J Nat Prod, 61, 71-76

42. Adkins WK, Taylor AE (1990) Role of xanthine oxidase and neutrophils in ischemia-reperfusion injury in rabbit lung. *J Appl Physiol*, 69, 2012-2019
43. Hande KR, Noone RM, Stone WJ (1984) Severe allopurinol toxicity: description and guidelines for prevention in patients with renal insufficiency. *Am J Med*, 76, 47-52
44. Urban T, Maquarre E, Housset C, Chouaid C, Devin E, Lebeau B (1995) Allopurinol hypersensitivity. A possible cause of hepatitis and mucocutaneous eruptions in a patient undergoing antitubercular treatment. *Rev Mal Respir*, 12, 314-316

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