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Antioxidant and Anti-Stress Activity of Shepherd's Purse

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ABSTRACT

In order to determine the antioxidant abilities of different parts of shepherd's purse (*Capsella brusa-pasteris*) of *Brassicaceae*, total polyphenol and flavonoid contents, ABTS and DPPH free radical scavenging activities were measured using three extraction solvents: hot water, 70% ethanol and 100% methanol. The proline content for each solvent extract was also determined to ascertain anti-stress activity along with SOD-like activity. The results showed that 70% ethanol is the best solvent for the extraction of polyphenol from the scape with flowers of shepherd's purse, whereas 100% methanol is the best solvent for the extraction of flavonoid from the leaf scape with leaves. The lowest contents of polyphenol and flavonoid compounds were obtained from hot water and 100% methanol extracts of the roots, respectively. The highest free radical scavenging activity determined by the ABTS and DPPH assays was observed in 70% ethanol extract of the scape with flowers while the lowest activity was seen in hot water extract of the roots. The highest SOD-like activity was observed in 70% ethanol extract of the scape with flowers among the fractions of samples of shepherd's purse and the lowest from the 100% methanol extract of the roots. Lastly, the highest proline content was observed from the 100% methanol extract of the scape with flowers and the lowest content from hot water extract of the leaf scape with leaves of shepherd's purse. The studies showed that all parts of shepherd's purse have high antioxidant activity and anti-stress activity, Overall, these results support that shepherd's purse can be used as a natural antioxidant.

Key words : Antioxidant, Free radical scavenging activity, Proline, SOD-like activity, Shepherd's purse

Introduction

Recently the life span of human being is increased along with the improvement of the standards of living in every country including Korea and people are very much interested in health problems. Accordingly, interests are steadily raised for the functional foods and natural products, containing biological activities which are related with antiaging and treating diseases [1,2].

Human diseases and aging are usually induced by transforming same parts of oxygen inhaled during the metabolism process into reactive oxygen which is a toxic substance. These reactive oxygen species (ROS) are super oxide, hydroxyl radical, hydrogen peroxide and singlet oxygen. Since these ROS are very unstable in molecular structures, they are formed by the accelerated oxidation in human body due to the unbalance of metabolic process or environmental pollution and overstress, etc. It is pointed out that the ROS easily invade cell-ingredients of high molecule weight, such as DNA, proteins and enzymes to incur the potential cell-damages such as DNA degeneration and cell senescence, etc. which are the causes of serious pathological troubles such as cardiac disorders, cerebrovascular disorders, arteriosclerosis and hypertension including cancers [3,4].

Recently it has been reported that adult diseases and senescence are caused by ROS. Accordingly studies on the antioxidants that can control or remove the effects of ROS are actively undertaken, and many research and development of antioxidants have been reported [5]. At present, synthetic antioxi-

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dants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are being used much because of prominent effectiveness and thrifty. Since cancers in human body can be induced by biological enzymes, transformation of fats, and toxicity, the development of natural antioxidants such as phenolics, flavonoids and carotenoids, etc., is urgently demanded for the sake of safety [6]. Therefore, remarkable efforts are made to find natural substances that can be obtained easily from our surroundings and new natural antioxidants [7].

Wild vegetables which are not artificially grown mean the edible plants without toxicities which are soft and tender and should be grown in the natural fields or mountains Thus, they surely have enough biological reactive substances in order to protect themselves from the various environments and enemies, while they grow themselves in the natural conditions. Along with the reports about the biological activities such as prevention of adult diseases and antibacterial, anticancer and antioxidant. etc., from the plants including the wild vegetable, teas and common vegetables, concerns are focused on the functional properties of daily used vegetables [8].

One of them is shepherd's purse of *Brassicaceae* distributed in Korea and all over the temperate regions of the world as annual herb or biennial plant. It is rich in biological activity substances such as α- and β-carotene, β- and γ-tocopherol, various kinds of vitamins, and sulfuraphane [9].

Also shepherd's purse contains plenty of protein compared to other wild vegetables and is an alkaline food that is rich in calcium and iron. Thus, young leaves of the shepherd's purse are used for salads and as condiment vegetable for soup in the western countries [10].

In this study, therefore, antioxidant activity, free radical scavenging activity, SOD-like activity and proline content were measured from shepherd's purse which is considered significant among the wild vegetables.

Materials and Methods

1. Materials

Shepherd's purse (*Capcella bursapastoris*) grown in Goryeong, Gyeongbuk was purchased from the local market in April 2014, and separated by the parts of roots, the scape with flowers and the scape with leaves for the experimental materials.

2. Chemicals and apparatus

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Fluka Biochemika AG (Buchs, Switzerland). 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), ascorbic acid, BHA, and other chemicals were purchased from Sigma (St Louis, MO, USA). All chemicals were of analytical grade. Refrigerator centrifuge (Kontron T-324), heating mantle (Wisd, Korea), shaking incubator (Hanbaek, Korea), rotary evaporator (Eyela, Japan), UV-VIS spectrophotometer (GeneQuant 100, UK), and ELISA microplate reader (Bio-Rad 680, USA) were used in our study.

3. Extracts preparation

Shepherd's purse was dried at 40˚C, ground into fine powder, and filtered using the sieve. For hot water extraction, 10 g of each powder sample was boiled in 500 mL of distilled water at 100˚C for 2 hr using a heating mantle, and then cooled and centrifuged at 8,000 rpm for 15 min. The pellets collected by centrifugation was suspended in 500 mL of distilled water, and centrifuged at 8,000 rpm for 15 min. For ethanol and methanol extractions, after 10 g of each powder samples were soaked in 500 mL of 70% ethanol and 100% methanol, respectively, extracted at 25˚C for 6 hr at 120 rpm, and then centrifuged at 8,000 rpm for 15 min.

The supernatant solutions collected by three times centrifugation at 8,000 rpm for 15 min, concentrated at 65˚C for hot water extraction and 50˚C for ethanol and methanol extractions until 10 mL remained using a rotary evaporator, respectively. Finally, freeze dried samples were stored at −70˚C until further analysis.

4. Total extraction yields

Total extraction yields of shepherd's purse were calculated as follows:

$$\text{Yields}\,(\%) = A/B \times 100$$

A: Amounts of freeze dried samples
B: Amounts of samples for extraction

5. Total polyphenol contents

The total phenolic content of the plant extracts and the standard antioxidant materials was determined according to the Folin-Ciocalteu method [11]. Folin-Ciocalteu's reagent was added to the extract. After 3 min, Na_2CO_3 was added and the

mixture was stored at 30˚C for 1 hr. The absorbance of the mixture was measured at 760 nm against water on a UV spectrophotometer. The results were calculated using the standard calibration curve of gallic acid and expressed as gallic acid equivalents (mg/g).

6. Total flavonoid contents

The determination of total flavonoid contents was performed according to Moreno et al. [12]. 62.5 μL of the different extract solutions diluted to 1 mg/mL were added to mixtures of 1.08 mL of 80% ethanol, 30 μL of 10% aluminum nitrate, and 30 μL of 1 M potassium acetate. After standing 40 min at room temperature, absorbance of the reaction mixture was measured at 415 nm. The contents of total flavonoid was expressed as mg of rutin hydrate equivalents per g of dry weight, calculated according to the standard calibration curve.

7. ABTS free radical scavenging activity

ABTS free radical scavenging activity was determined following ABTS cation decolorization assay described by Re et al. [13]. ABTS solution was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate keeping for 24 hr in darkness. Generated ABTS solution was diluted with phosphate buffer saline to give an absorbance of 0.7 ± 0.02 at 732 nm. 20 μL of each extract was added to 180 μL of dilution solution, respectively, and the absorbance reading was taken at 732 nm after 1 min. Ascorbic acid and BHA solutions were used as a positive control for comparing the antioxidant activity. Radical scavenging activity was calculated by measuring the absorbance of the sample and applying as follows:

$$\text{Radical scavenging activity}\,(\%) = [\,1 - (\text{As} - \text{Ac})] \times 100$$

Ac: Absorbance of control without sample
As: Absorbance of sample

8. DPPH free radical scavenging activity

DPPH free radical scavenging activity was determined by the method of Blois [14]. Each sample was prepared to contain 1 mg/mL, and the volume was adjusted to 1 mL. One mL of 0.05 mM DPPH was added to each sample. All samples were incubated in a dark place at room temperature for 30 min. Absorbance of DPPH was measured at 517 nm. Ascorbic acid and BHA were used as a positive control for comparison. DPPH free radical scavenging activity by samples and control was calculated as follows:

$$\text{Radical scavenging activity}\,(\%) = [\,1 - (\text{As} - \text{Ac})] \times 100$$

Ac: Absorbance of control without sample
As: Absorbance of sample

9. SOD-like activity

Superoxide dismutase (SOD)-like activity was assayed as described by Marklund and Marklund [15]. 200 μL of the extracts diluted to 1 mg/mL were added to mixtures of 2.6 mL of 50 mM Tris-HCl buffer (pH 8.5) contained 10 mM EDTA and 200 μL of 7.2 mm pyrogallol. After standing 10 min at 25˚C, reaction was stopped by addition of 100 μL of 1 N HCl. Ascorbic acid was used as a positive control. After measurement amount of pyrogallol oxidized in reaction mixture at 420 nm, these results were calculated as follows:

$$\text{SOD-like activity}\,(\%) = (1 - \text{B/A}) \times 100$$

A: Absorbance of Tris-HCl buffer + pyrogallol + HCl
B: Absorbance of Tris-HCl buffer + sample + pyrogallol + HCl

10. Proline contents

Proline was determined following the ninhydrin method described by Bates et al. [16]. 0.1 g of freeze-dried tissue was homogenized in 10 mL of 3% (w/v) aqueous sulphosalicylic acid and filtered. To 2 mL of the filtrate, 2 mL of acid ninhydrin was added, followed by the addition of 2 mL of glacial acetic acid and boiling for 1 hr. The mixture was extracted with 4 mL of toluene, and the free proline was quantified at 520 nm from the organic phase. The results were calculated using the standard calibration curve and expressed as proline equivalents (μg/g).

11. Statistical analysis

The experiments were performed three times exception the determination of total extraction yields. The results were expressed as the mean ± standard error (SE). Statistical significance of the mean mortality at each concentration was analyzed using one-way analysis of variance (ANOVA) and compared using Duncan's multiple range tests. Values of $p \leq 0.05$ were taken to be statistically significant.

Results

1. Total extraction yields

The sample powders of shepherd's purse were extracted three times each by the reflux cooling method in the hot water, the 70% ethanol and the 100% methanol solvents. After the extracts were vacuum-evaporated and freeze-dried, the yields were measured by the differences of the sample weights. The yields of the extracts from the roots of shepherd's purse were 30.98%, 17.06% and 11.94% for the hot water, the 70% ethanol and the 100% methanol, respectively (Table 1). The yields from the scape with flowers were 23.54%, 20.54% and 12.76 % for the hot water, the 70% ethanol and the 100% methanol, respectively (Table 2). And the yields from the leaf scape with leaves were 21.22%, 37.36% and 14.42%, respectively, for the hot water, the 70% ethanol and the 100% methanol (Table 3). The highest yield was observed from the leaf scape with leaves in the 70% ethanol. As a whole, the lowest yields were observed from the 100% methanol extract in all the parts, and in particular, the extracts from the roots showed the lowest yields over all trials. In general the 70% ethanol extract from the leaf scape with leaves showed the highest yields except the hot water extracts.

Table 1. Total extraction yields of Shepherd's purse roots.

Total extract yields (%)	
Hot water	30.98
70% Ethanol	17.06
100% Methanol	11.94

Table 2. Total extraction yields of Shepherd's purse scape with flowers.

Total extract yields (%)	
Hot water	23.54
70% Ethanol	20.54
100% Methanol	12.76

Table 3. Total extraction yields of Shepherd's purse scape with leaves.

Total extract yields (%)	
Hot water	21.22
70% Ethanol	37.36
100% Methanol	14.42

2. Total polyphenol contents

The freeze-dried samples from each part were diluted to the level of 1 mg/mL in distilled water for the measurement of the total polyphenol contents. The calibration curve was made using gallic acid, and the polyphenol contents per on gram (in mg/g) of the dried, sample was obtained through the calibration curve. The final total polyphenol contents were obtained by converting based on the gallic acid, and given in Fig. 1. In the case of the roots of shepherd's purse the total polyphenol contents from the hot water, the 70% ethanol and the 100% methanol extracts were 1.46 ± 0.003 mg/g, 1.55 ± 0.001 mg/g and 1.72 ± 0.002 mg/g, respectively. The highest content was observed from the 100% methanol, and in the case of the scape with flowers they were, respectively. 2.28 ± 0.003 mg/g, 2.90 ± 0.004 mg/g and 2.61 ± 0.003 mg/g. The 70% ethanol extract showed the highest content of polyphenol. The values for the leaf scape with leaves were 2.15 ± 0.002 mg/g, 2.43 ± 0.001 mg/g and 2.12 ± 0.003 mg/g, respectively. The highest content was obtained again from the 70% ethanol extract.

In common, the hot water, the 70% ethanol and the 100% methanol extracts of the scape with flowers showed higher contents than those of the roots and leaf scape with leaves in all solvents. In particular, the 70% ethanol extract of the scape with flowers showed as high as double amount of polyphenol as compared to the hot water of the roots.

3. Total flavonoid contents

Calibration curve was prepared by the use of rutin hydrate as the standard material. The total flavonoid contents per a gram of dried sample were read through the calibration curve and the total flavonoid content for each part of the plant were

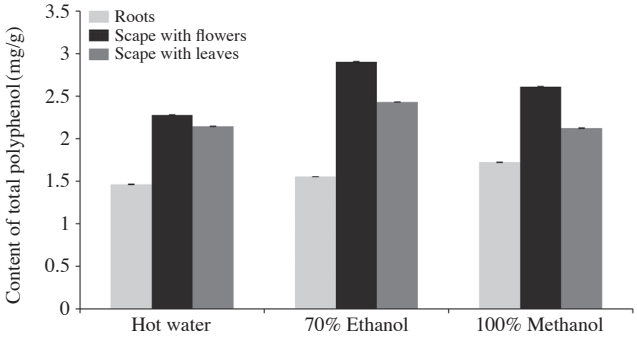

Fig. 1. Total polyphenol contents in shepherd's purse by solvents. The bars represent the mean ± SE. Values of $p \leq 0.05$ were taken to be statistically significant.

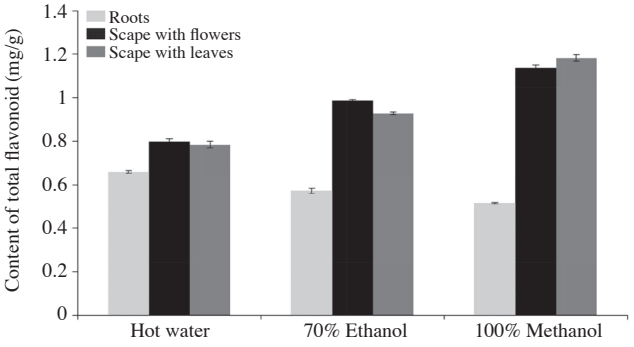

Fig. 2. Total flavonoid contents in shepherd's purse by solvents. The bars represent the mean ± SE. Values of $p \leq 0.05$ were taken to be statistically significant.

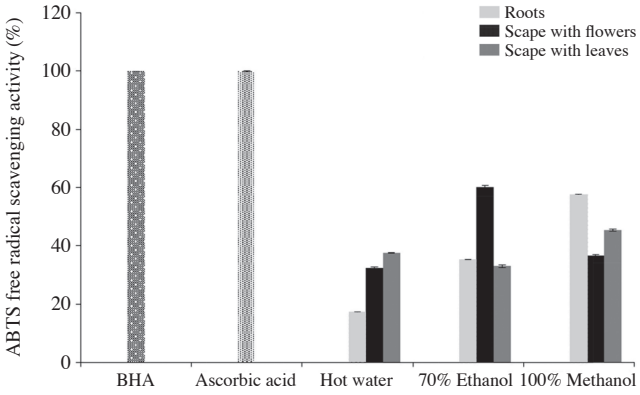

Fig. 3. ABTS free radical scavenging activity in shepherd's purse by solvents. BHA and ascorbic acid prepared in the same concentration as the test extracts were used as a positive control. The bars represent the mean ± SE. Values of $p \leq 0.05$ were taken to be statistically significant.

obtained in mg/g by converting based on the rutin hydrate, and given in Fig. 2. The total flavonoid contents of the roots from the hot water, the 70% ethanol and the 100% methanol extracts were respectively, 0.66 ± 0.007 mg/g, 0.57 ± 0.012 mg/g and 0.54 ± 0.003 mg/g. The hot water showed the highest content of the total flavonoid. The values from the scape with flowers were 0.80 ± 0.045 mg/g, 0.99 ± 0.003 mg/g and 1.14 ± 0.015 mg/g, respectively, showing that the 100% methanol gave the highest content. The leaf scape with leaves for the solvents showed, respectively, 0.78 ± 0.015 mg/g, 0.93 ± 0.007 mg/g and 1.18 ± 0.015 mg/g. The highest contents was obtained from the 100% methanol extract as in the case of the scape with flowers.

Consequently the highest total flavonoid content was observed from the 100% methanol extract of leaf scape with leaves, and excepting the 100% methanol extract, the 70% ethanol extract of the scape with flowers showed high contents. In general, the roots showed low total flavonoid contents, and among these the 100% methanol extracts of roots showed the lowest content.

4. ABTS free radical scavenging activity

ABTS free radical scavenging activity was measured, by the comparative measurement, using synthetic antioxidants, BHA and ascorbic acid. The results were given in Fig. 3. Free radical scavenging activities of BHA and ascorbic acid were 99.85 ± 0.130% and 99.90 ± 0.043% respectively, showing very high free radical scavenging activities. The activities from the roots for the hot water, the 70% ethanol and the 100% methanol extracts were 17.29 ± 0.302%, 35.22 ± 0.690% and 57.55 ± 0.075%, respectively. In the case of the scape with flowers they were 32.39 ± 0.262%, 60.19 ± 0.457% and 36.52 ± 0.425% respectively, where the 70% ethanol extract showed the highest scavenging activity.

In the leaf scape with leaves, the values were 37.47 ± 0.114 %, 32.99 ± 0.486% and 45.39 ± 0.376%, respectively. As a whole the 70% ethanol extract of the scape with flowers and the 100% methanol extract of the roots showed the highest scavenging activity. The lowest scavenging activity was observed from the hot water extract of roots. And each part of shepherd's purse showed for low scavenging activity as compared to BHA and ascorbic acid. It, however, could be proved that the shepherd's purse plant has significant ABTS free radical scavenging activity.

5. DPPH free radical scavenging activity

DPPH free radical scavenging activity was measured like ABTS free radical scavenging activity, using BHA and ascorbic acid as dual control plots, and the results are given in Fig. 4. The control plots, BHA and ascorbic acid, showed very high free radical scavenging activities, respectively, being 77.01 ± 0.142% and 24.17 ± 0.110%. The scavenging activities for the hot water, the 70% ethanol and the 100% methanol extracts of roots were, respectively, 87.19 ± 0.767%, 105.02 ± 0.293% and 98.57 ± 0.333%. The 70% ethanol extract showed the highest scavenging activity. The scavenging activity of the scape with flowers were, respectively, 103.26 ± 0.338%, 122.01 ± 0.347% and 118.16 ± 0.528%. The 70% ethanol extract showed the highest value. And those of the leaf scape with leaves were 101.27 ± 0.254%, 104.39 ± 0.287% and 118.96 ± 1.198% from the hot water, the 70% ethanol and the 100% methanol extract, respectively. The highest scavenging activity was obtained

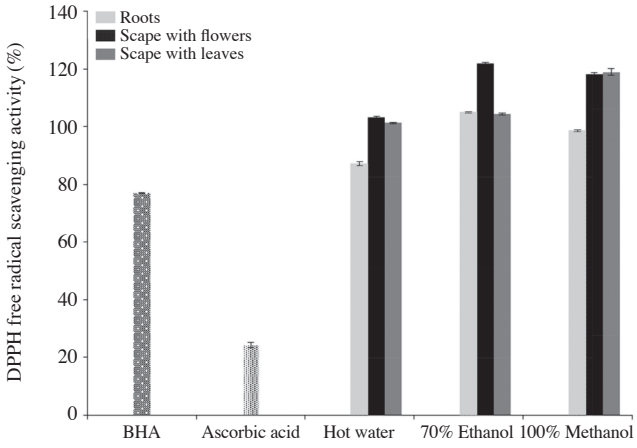

Fig. 4. DPPH free radical scavenging activity in shepherd's purse by solvents. BHA and ascorbic acid prepared in the same concentration as the test extracts were used as a positive control. The bars represent the mean ± SE. Values of $p \leq 0.05$ were taken to be statistically significant.

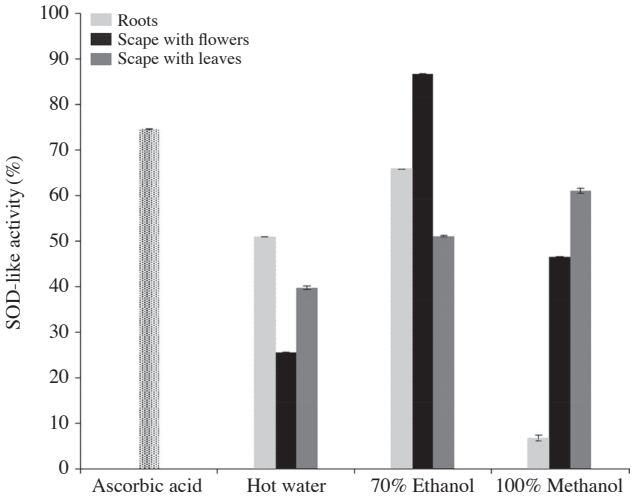

Fig. 5. SOD like-activities ability of shepherd's purse by solvents. Ascorbic acid prepared in the same concentration as the test extracts was used as a positive control. The bars represent the mean ± SE. Values of $p \leq 0.05$ were taken to be statistically significant..

from the 100% methanol extract.

Consequently, the hot water, the 70% ethanol and the 100% methanol extracts of the roots. The scape with flowers and the leaf scape with leaves showed much higher scavenging activities than the dual control plots, BHA and ascorbic acid. In particular, the 70% ethanol extract of the scape with flowers showed the highest scavenging activity, which is about 5 times higher than ascorbic acid.

6. SOD-like activity

For SOD-like activity the effect of inhibiting the autoxidation of pyrogallol was measured by the use of the principle that brown substances are generated when the pyrogallol is automatically oxidized by superoxide under the neutral or alkaline stales. Ascorbic acid was used as a control plot and the SOD-like activities for the different parts of the shepherd's purse are given in Fig. 5. Ascorbic acid, the control plot, showed high activity being 74.60 ± 0.009%. In the case of roots, the hot water, the 70% ethanol and the 100% methanol extracts showed, respectively, 50.80 ± 1.022%, 65.79 ± 0.502% and 6.70 ± 1.021%. The 70% ethanol extract showed the highest activity. In the scape with flowers, the activities were, respectively, 25.53 ± 0.427%, 86.67 ± 0.219% and 46.49 ± 0.592 %. The 70% ethanol extract showed the highest activity as in the roots. In the leaf scape with leaves the activities were, respectively, 39.66 ± 0.041%, 51.06 ± 0.030% and 61.01 ± 0.673%, and the 100% methanol extract showed the highest activity.

In the SOD-like activity of the different parts of the shepherd's purse plants, the 70% ethanol extracts showed the highest SOD-like activity which is higher than that of ascorbic acid, the control plot, whereas, the 100% methanol extract of roots showed the lowest SOD-like activity. The 70% ethanol extract of the scape with flowers showed the highest SOD-like activity which is 19 times of that from the 100% methanol extract of the roots.

7. Proline contents

The samples of the different parts of the shepherd's purse were diluted to the level of 250 μg/g and the calibration curves were prepared. The proline contents per mg of samples were measured in μg/g by converting on the basis of the standard material proline through the calibration curve, and the results are given in Fig. 6. The proline contents of roots from the hot water, the 70% ethanol and the 100% methanol extracts were 1.77 ± 0.004 μg/g, 1.45 ± 0.002 μg/g and 1.37 ± 0.003 μg/g, respectively. In the case of the scape with flowers they were 1.43 ± 0.005 μg/g, 1.77 ± 0.002 μg/g and 2.03 ± 0.003 μg/g, respectively, and the values of the leaf scape with leaves were 1.17 ± 0.005 μg/g, 1.21 ± 0.002 μg/g and 1.28 ± 0.003 μg/g respectively.

The highest proline content was observed from the 100% methanol extract of the scape with flowers, and the values were in order of the 100% methanol, the 70% ethanol and the hot water. In the case of leaf scape with leaves, the proline contents were in general lower than those of the scape with flow-

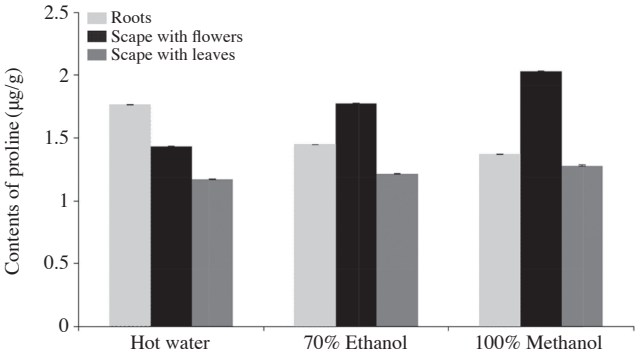

Fig. 6. Proline contents of shepherd's purse by solvents. The bars represent the mean ± SE. Values of $p \leq 0.05$ were taken to be statistically significant.

ers in the same order. In the case of roots, however, the proline contents were in the order of the hot water, the 70% ethanol and the 100% methanol extracts.

Discussion

In this study an attempt has been made to ascertain the efficacy of the shepherd's purse on antioxidation and to evaluate the economic effects, disease prevention and retarding senescence etc, for practical use of the plant which can easily be found in our surroundings. The results obtained from the hot water, the 70% ethanol and the 100% methanol extracts of the roots, the scape with flowers and leaf scape with leaves were examined for the possibility of an antioxidant.

The yields of the different parts of the shepherds' purse by solvents were, in general, high from the hot water extract, but the 70% ethanol extract of the leaf scape with leaves showed the highest yields. The high yield, however, just means that the plant contains a large amount of the substance, and not be concluded that the plant has high effect of antioxidation.

Polyphenol compounds are widely distributed in the plant kingdom, and most of the polyphenol compounds including hydroxycinnamic acid are known to prevent oxidation by eliminating the radicals in human body. The antioxidant potential of phenols is believed to be conferred on them by their hydroxyl group (-OH), which is bonded directly to and aromatic hydrocarbon (phenyl) ring. This makes them donate electrons easily to electron-seeking free radicals, thus down-regulating their menace in living cell [17].

The 70% ethanol extracts of the scape with flowers showed the highest total polyphenol among the parts of shepherd's purse, and the total polyphenol contents for the parts of the plant by solvents were seen in order of the scape with flowers > leaf scape with leaves > roots. As a whole, the hot water extracts showed relatively low polyphenol contents.

Ivanova et al. [18]. Reported that the total polyphenol contents by parts of the plant were higher than that of the whole plant, and Thaipong et al. [19]. reported that the leaves of *Ilex paraguariensis* showed a high total polyphenol content being 202.60 ± 5.16 mg/g.

Since flavonoid is one of phenolic compounds, the total flavonoid content of each sample showed the same tendency as in the phenolic compound, providing similar significance. The result that the rate of flavonoid is relatively high was coincided with the fact that higher amount of phenolic compounds was obtained from the ethanol solvent rather than other solvents, implying that the ethanol extraction is a better means than the hot water extraction for the antioxidant phenolic compounds [20,21].

The level of polyphenol and flavonoid in the scape with flowers are higher than that in other parts of the plant. In 100% methanol extracts for the total flavonoid contents, except the case that the leaf scape with leaves showed higher values than the scape with flowers, the scape with flowers showed higher values than the roots in other solvents. The 100% methanol extract from the roots showed the least value, whereas the leaf scape with leaves showed the highest value, resulting in double of the roots.

Kim et al. [22] studied the polyphenol and flavonoid contents of the autogenous plants and herb medicine resources and found the similar fashion in the rates of the total flavonoid content to the total phenol contents in each sample. These results are similar to that in the present study.

Oboh and Akindahunsi [23] also studied the total phenol contents and antioxidation activities for the green leaves vegetables dried under the natural conditions. They reported that the increase of reducing property and free radical scavenging activity were related with the increase of the total phenol contents. Both BHA and ascorbic acid used as control plots showed very high and similar ABTS free radical scavenging activities. The highest scavenging activity has been observed from the 70% ethanol extract of the scape with flowers and the next high activity from the 100% methanol extract of the roots. Although the scavenging activities of the samples were for lower than those of the control plots, significant ABTS free radical scavenging activities were convinced. In the case of roots of shepherd's purse, the scavenging activity by solvents

was in order of the 100% methanol > the 70% ethanol > the hot water and the 100% methanol and the 70% ethanol extracts showed respectively, 3 and 2 times higher scavenging activities than the hot water extract.

The highest scavenging activity from the 70% ethanol extract of the scape with flowers was about 3.5 times of the lowest scavenging activity from the hot water extract of the roots, implying that the scavenging activities depend upon the solvents used.

Ghasemzadeh et al. [24] reported that Chinese cabbage, one of *Brassicaceae* like the shepherd's purse, showed DPPH scavenging activity being 58.05±0.81%, which is much lower than those observed from the shepherd's purse in the present study. In DPPH free radical scavenging activity, as in ABTS free radical activity, BHA and ascorbic acid were used as dual control plots whose scavenging activities were lower than those in the case of ABTS free radical scavenging activities.

DPPH free radical scavenging activity for all the parts of shepherd's purse plant and all solvents were higher than those of the control plots. The 70% ethanol extract of the scape with flowers showed the highest content being 122.008±0.347% which is about 1.5 times of BHA and about 5 times of ascorbic acid. Even the lowest activity from the hot water extract of roots was higher than BHA and about 3 times higher than ascorbic acid. Grosso et al. [25] reported that a high DPPH scavenging activity of 1041.49 mg/mL from the methanol extract of the whole plant of shepherd's purse, and hence high scavenging activity is obtainable from the whole plant rather than the parts of the plant.

ROS which are noxious to cells are transferred to H_2O_2 by the SOD-like activity, one of antioxidant enzymes and this is known that the H_2O_2 is dissolved into harmless oxygen and hydrogen molecular by peroxidase or catalase to protect the living matters from the damages by oxygen [26], and that this is closely related with the restraint of senescence not to mention antioxidation [27].

The SOD-like activities were observed from the extracts of all solvents for each part of shepherd's purse, and the 70% ethanol extract of the scape with flowers showed higher activity than the control plot, ascorbic acid, On the other hand the hot water extract of the scape with flowers showed low activity as compared to other solvents. The activities from the leaf scape with leaves were increased in the order of the hot water, the 70% ethanol and the 100% methanol extracts.

The salt tolerances are different according to the plant species [28], and when plants are placed under the states of salt stresses or water shortage, glutamic acid is transferred to free proline which plays the role of osmosis regulator, and is used as an index of salt tolerance [29].

The 100% methanol extract of the scape with flowers showed the highest proline content for anti-stress, and the hot water extract showed large differences in proline, whereas the 70% ethanol extracts showed smallest differences among the parts of the plant. Thus, it could be confirmed that proline is contained in all the parts of shepherd's purse plant and is highly effective in salts resistance, water stress and anti-stress [30].

Consequently, it could be ascertained in this study that each part of shepherd's purse plant contains high contents of antioxidant materials, and could be concluded that the scape with flowers containing large quantities of flavonoid, phenol acid, and proline, etc. are worthy to be used as the natural antioxidant.

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References

1. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 1993;90:7915-7922.
2. Goleberg I. Functional foods: designer foods, pharmafoods, nutraceuticals. New York USA: Chapman Hall Press 1994;350-550.
3. Helen W. Dietary influences on membrane function: importance in protection against oxidative damage and disease. Nutr Biochem 1996;7:2-15.
4. Papa S, Skulachv VP. Reactive oxygen species, mitochondria, apoptosis and aging. Mol Cell Biochem 1997;174:305-319.
5. Coleman MD, Fernandes S, Khanderia L. A preliminary evaluation of a novel method to monitor a triple antioxidant combination vitamins E, C and α-lipoic acid) in diabetic volunteers using in vitro methaemoglobin formation. Environ Toxicol Pharmacol. 2003;14:69-75.
6. Williams GM, Wang CX, Iatropoulos MJ. Toxicity studies of butylated hydroxyanisole and butylated hydroxytoluene. II. Chronic feeding studies. Food Chem Toxicol 1990;28:799-806.
7. Hahm TS, King DL, Min DB. Food antioxidants. Food Biotech 1993;2:1-8.
8. Kim EY, Baik IH, Kim JH, Kim SR, Rhyu MR. Screening of the antioxidant activity of some medicinal plants. Korean J

Food Sci Technol. 2004;36:333-338.
9. Kurilich AC, Tsau GJ, Brown A, Howard L, Klein BP, Jeffery EH, et al. Carotene, tocopherol, and ascorbate contents in subspecies of *Brassicaoleracea*. J Agric Fd Chem 1999;47:1576-1581.
10. Peterson LA. A field guide to edible plants of eastern and central North America. Boston USA: Houghton Miffin Co 1997;26.
11. Gamez-Meza N, Noriega-Rodriguez J, Medina-Juarez L, Ortega-Garcia J, Cazarez-Casanova R, Angulo-Guerrero O. Antioxidant activity in soybean oil of extracts from Thompson grape bagasse. JAOCS 1999;76:1445-1447.
12. Moreno MIN, Isla MI, Sampietro AR, Vattuone MA. Comparison of the free radical scavenging activity of propolis from several regions of Argentina. J Ethnopharm 2000;71:109-114.
13. Re R, Pellegrini, Proteggente A, Pannala A, Yang M, Rice Evans C. Antioxidant activity applying an improved ABTS radical cation delcolorization assay. Free Radic Biol Med 1999;26:1231-1237.
14. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1198-1200.
15. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Europe J Biochem 1974;47:469-474.
16. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil 1973;39:205-207.
17. Uyoh EA, Chukwurah PN, David IA, Bassey AC, Evaluation of antioxidant capacity of two *Ocimum* species consumed locally as spices in Nigeria as a justification for increased domestication. American J Plant Sci 2013;4:222-230.
18. Ivanova D, Gerova D, Chervenkov T, Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J Ethnopharmacology 2005;96:145-150.
19. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Hawkins Byrne D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Compositi Analysis 2006;19:669-675.
20. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr 2004;134:3479S-3485S.
21. Manach C, Williamson G, Morand C, Scalbert A, Remesy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. American J Clin Nutr 2005;81:230S-242S.
22. Kim EJ, Choi JY, Yu M, Kim MY, Lee S, Lee BH. Total polyphenols, total flavonoid contents, and antioxidant activity of Korean natural and medicinal plants. Korean J Food Sci Technol 2012;44:337-342.
23. Oboh G, Akindahunsi AA. Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. Nutr Health 2004;18:29-36.
24. Ghasemzadeh A, Azarifar M, Soroodi O, Jaafar HZ. Flavonoid compounds and their antioxidant activity in extract of some tropical plants. J Medic Plants Research 2012;6:2639-2643.
25. Grosso C, Vinholes J, Silva LR, Pinho PGD, Goncalves RF, Valentao P, et al. Chemical composition and biological screening of *Capsella bursa-pastoris*. Revista Brasileira de Farmacognosia 2011;21:635-643.
26. Klug D, Rabani J, Fridovich I. A direct demonstration of the catalytic action of superoxide dismutase through the use of pulse radiolysis. J Biol Chem 1972;247:4839-4842.
27. Gupta AS, Webb RP, Holaday AS, Allen RD. Overexpression of superoxide dismutase protects plants from oxidative stress (induction of ascorbate peroxidase in superoxide dismutase-overexpressing plants). Plant Physiology 1993;103:1067-1073.
28. Parida AK, Das AB. Salt tolerance and salinity effects on plants. Ecotoxicol. Environ Saf 2005;60:324-349.
29. Choung JI, Kim KY, Choi DH, Oh MK, Lee DJ. Growth responses and proline accumulation by salt treatment in rice cultivars. Korean J Soc Inter Agric 2003;15:288-293.
30. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Experiment Botany 2007;59:206-216.