

## 꿀풀 에탄올 추출물 및 분획물의 항산화성

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### Antioxidant Activity of Different Solvent Fractions from *Prunella vulgaris* var. *lilacina*

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**ABSTRACT:** The potential antioxidant activities of different fractions from *Prunella vulgaris* var. *lilacina* were assayed *in vitro*. Among several fractions, *n*-BuOH fraction showed the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging ( $IC_{50} = 0.50 \mu\text{g/mL}$ ). The results of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity and ferric reducing antioxidant power (FRAP) assay showed the concentration dependency and *n*-BuOH fraction appeared a better result than the other fractions at the same concentration in this study. Moreover the total phenol and flavonoid contents of *n*-BuOH fraction contained the highest level. Additionally, correlation analysis indicated a high correlation between the antiradical activity and the total phenolic and flavonoid contents ( $p < 0.001$ ). It suggests that *n*-BuOH fraction obtained from the 70% EtOH crude extract of *Prunella vulgaris* var. *lilacina* has wide potential for use as a source of antioxidant material.

**Key Words :** ABTS, DPPH, Flavonoid Contents, Phenolic Contents, *Prunella vulgaris* var. *lilacina*

## INTRODUCTION

There is considerable epidemiological evidence indicating an association between diets rich in fruits and vegetables and a decreased risk of cardiovascular diseases and certain forms of cancer originated by the presence of free radicals in the body (Gaté *et al.*, 1999; Yeum *et al.*, 2003). Therefore, there is consumer interesting in there being included in foods as additives and within the manufacture of medicines (Willcox *et al.*, 2004). Some of the most widely used antioxidants are vitamin C, vitamin E and carotenoids. These play an important role in reducing oxidative stresses that are caused by different factors but are mainly the by-product of physiological stress within the human body (Nordberg and Arnér, 2001). Some of these reactive oxygen species (ROS) can include super-oxide anion radicals, hydroxyl radicals and hydrogen peroxide. The imbalance of ROS and attempted elimination by the body can be the cause of

several chronic diseases, inflammations and neurological disorders (Halliwell, 1996). Antioxidants protect the body by scavenging free radicals and reducing the production of hydrogen peroxide within the body (Shahidi and Wanasundara, 1992).

Traditionally, herb and plant based medicines have played a large role in the history of human health. However, there is still a lot of research to evaluate their properties and mechanisms. Some have suggested that the antioxidant properties of some herbs are behind their use as medicines. The phenol compounds of some herbs have acted as reducing agents, hydrogen donators and free radicals eliminators (Lee *et al.*, 2011; Seo *et al.*, 2009; Shahidi and Wanasundara, 1992).

*Prunella vulgaris* var. *lilacina* is one of the most widely used medical plants in north-eastern Asian countries and have been used for traditional herbal medicine to treat fever, inflammation, dropsy, gonorrhoea and cancer (Park *et*

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*al.*, 2010). Although several studies have reported that it has many biological activities (Ryu *et al.*, 2000; Psoťová *et al.*, 2003), little is known to regarding its antioxidant properties which is related to these beneficial properties.

The purpose of this study was to evaluate the several fractions (*n*-Hexane, CHCl<sub>3</sub>, *n*-BuOH, and H<sub>2</sub>O) from 70% EtOH crude extract of *Prunella vulgaris* var. *lilacina* as new potential source of natural antioxidants. So, this study examined total phenolic and flavonoid contents of various fractions from 70% EtOH crude extract of *Prunella vulgaris* var. *lilacina* and evaluated their potential antioxidative activities by DPPH and ABTS radical scavenging activities. Additionally, the correlation between the total phenolic and flavonoid contents in the tested fractions and their antioxidant activities was investigated.

## MATERIALS AND METHODS

### 1. Sample Preparation

The *Prunella vulgaris* var. *lilacina* was collected from agricultural fields in Yeongju, Gyeongbuk, Korea in Jul, 2007. Washed and chopped fresh *Prunella vulgaris* var. *lilacina* was dried in a freeze dryer (at -70°C). The dried leaves and stem of *Prunella vulgaris* var. *lilacina* were extracted three times with 70% EtOH. The 70% EtOH extract powder (10 g) was suspended in 500 ml distilled water and extracted with 500 ml of the following solvents stepwise: *n*-hexane, CHCl<sub>3</sub> and *n*-BuOH. Each fraction was filtered through Advantec No. 6 filter paper (Advantec, Toyo Roshi Kaish, Ltd., Tokyo, Japan). Filtered extracts concentrated under reduced pressure by rotary evaporator (EYELA N-1000 Rikakikai Co., LTD., Tokyo, Japan) at 40°C and lyophilized by lyophilizer (Bondiro-PVTFD20R, Ilshine Lab Co. Ltd., Korea), and stored at -20°C until used. The concentration extract powder (4.16 g) was further partitioned sequentially to give *n*-Hexane (0.56 g), CHCl<sub>3</sub> (0.57 g), *n*-BuOH (1.41 g), H<sub>2</sub>O (1.57 g) fractions in powder (Fig. 1).

### 2. Total phenol determination

Total phenols were determined by using the Folin-Ciocalteu reagent according to the method of Ainsworth and Gillespie (Ainsworth and Gillespie, 2007). Briefly, a 50 µL of various fractions was assayed with 250 µL Folin reagent and 500 µL of sodium carbonate (20%, w/v). The

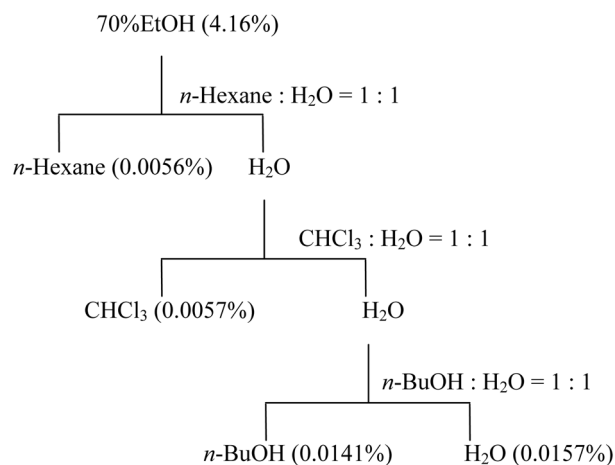


Fig. 1. Systemic solvent fractions of *Prunella vulgaris* var. *lilacina*.

mixture was vortexed and diluted with water to a final volume of 5 mL. After incubation for 30 min at room temperature, the absorbance was read at 765 nm.

### 3. Total flavonoid determination

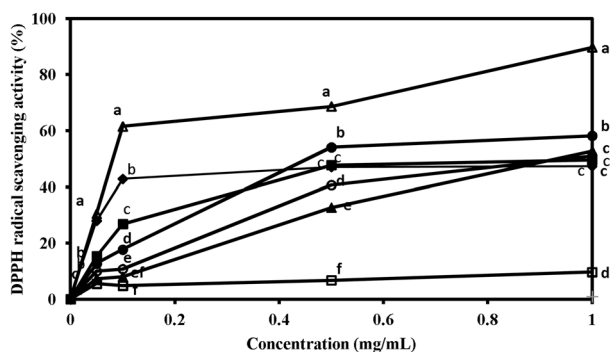
The total flavonoids were determined using the method of Jia *et al.* (1999). A volume of 0.5 ml of 2% AlCl<sub>3</sub> ethanol solution was added to 0.5 mL of extract solution. The mixture was incubated for 1 hr at room temperature for yellow color appearance; the absorbance was measured at 420 nm.

### 4. DPPH radical scavenging activity of fractions of EtOH-extracted *Prunella vulgaris* var. *lilacina*

The antioxidant activity of the extract and fractions, on the basis of their ability to scavenge the stable DPPH free radical, was determined using the method described by Braca *et al.* (2001). The absorbance at 517 nm was determined, and Vit C was used as positive controls. The DPPH radical scavenging activity was calculated by the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A) / A_0] \times 100$$

where  $A_0$  is the absorbance of the control and  $A$  is the absorbance of the *Prunella vulgaris* var. *lilacina* or the standard. The IC<sub>50</sub> values are calculated as the concentration required to inhibit DPPH radical formation by 50%.



**Fig. 2.** DPPH radical scavenging activity of various fractions from 70% EtOH crude extract of *Prunella vulgaris* var. *lilacina*. (■) 70% EtOH crude extract, (□) *n*-Hexane fraction, (●) *n*-BuOH fraction, (○) CHCl<sub>3</sub> fraction, (▲) H<sub>2</sub>O fraction, (△) Vit. C, (◆)  $\alpha$ -Tocopherol. Different superscripts indicate significant differences at  $p < 0.05$  in the each concentration.

### 5. ABTS radical scavenging activity of fractions of EtOH-extracted *Prunella vulgaris* var. *lilacina*.

The spectrophotometric analysis of ABTS<sup>+</sup> radical scavenging activity of *Prunella vulgaris* var. *lilacina* was determined according to the method described by Re *et al.* (1999). The absorbances at 734 nm were determined for each concentration. The ABTS<sup>+</sup> radical scavenging activity was calculated using the following equation:

$$\text{ABTS}^{+} \text{ scavenging effect (\%)} = [1 - (A_{\text{sample}} - A_{\text{control}})] \times 100$$

where  $A_{\text{sample}}$  is the absorbance of the *Prunella vulgaris* var. *lilacina* or the standard and  $A_{\text{control}}$  is the absorbance of the control. The IC<sub>50</sub> values are calculated as the concentration required to inhibit ABTS radical formation by 50%.

### 6. FRAP assay of fractions of EtOH-extracted *Prunella vulgaris* var. *lilacina*.

The FRAP assay was performed according to the modified Benzie and Strain method (Benzie and Strain,

1996). The stock solutions included 300 mM acetate buffer (3.1 g C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub> · 3H<sub>2</sub>O and 16 mL C<sub>2</sub>H<sub>4</sub>O<sub>2</sub>), pH 3.6, 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM FeCl<sub>3</sub> · 6H<sub>2</sub>O solution. The fresh working solution was prepared by mixing 25 mM acetate buffer, 2.5 mL TPTZ solution and 2.5 mL FeCl<sub>3</sub> · 6H<sub>2</sub>O solution and then warmed at 37°C before using. Different concentrations of various fractions (50  $\mu$ L) were allowed to react with 150  $\mu$ L of FRAP solutions for 30 min in the dark condition. Regarding of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. The standard curve was linear between 0.15 and 5 mM FeSO<sub>4</sub>. Results are expressed in FeSO<sub>4</sub> equivalents mM.

### 7. Statistical analysis

Data were expressed as means  $\pm$  S.D. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. A  $p$  value  $< 0.05$  was considered as statistically significant. Correlations among data obtained were calculated using Pearson's correlation coefficient ( $R$ ).

## RESULTS

### 1. Total phenolic and flavonoid contents of *Prunella vulgaris* var. *lilacina* fractions

Plant phenolics and flavonoids, in general, are highly effective free radical scavengers and antioxidants (Mustafa *et al.*, 2010). Therefore, the contents of total phenolics in each fraction determined spectrophotometrically according to the Folin-Ciaccateu method was expressed as gallic acid equivalents (GAE) (Table 1). Total polyphenols ranged from 13.23  $\pm$  0.58 to 127.11  $\pm$  1.14, as mg GAE per 1 g dry residue, and total flavonoids ranged from 5.28  $\pm$  3.12 to 93.31  $\pm$  2.56, as mg rutin equivalents (RTE) per 1 g dry residue. The total phenolic and flavonoid contents of the

**Table 1.** Total phenol and flavonoid contents of various fractions from 70% EtOH crude extract of *Prunella vulgaris* var. *lilacina*.

Fraction	Total phenol contents (mg GAE/dry residue)	Total flavonoid contents (mg RTE/dry residue)
70% EtOH crude extract	90.00 $\pm$ 0.18 <sup>b</sup>	65.23 $\pm$ 1.27 <sup>b*</sup>
<i>n</i> -Hexane	13.23 $\pm$ 0.58 <sup>e</sup>	10.34 $\pm$ 3.12 <sup>d</sup>
CHCl <sub>3</sub>	44.21 $\pm$ 1.02 <sup>c</sup>	5.28 $\pm$ 3.12 <sup>e</sup>
<i>n</i> -BuOH	127.11 $\pm$ 1.14 <sup>a</sup>	93.31 $\pm$ 2.56 <sup>a</sup>
H <sub>2</sub> O	28.69 $\pm$ 1.51 <sup>d</sup>	25.12 $\pm$ 1.32 <sup>c</sup>

\*Means with different superscripts in the same column (a-e) are significantly different at  $p < 0.05$ .

**Table 2.** DPPH and ABTS radical scavenging activity of different fractions from 70% EtOH extract of *Prunella vulgaris* var. *lilacina*.

Fractions	IC <sub>50</sub> (mg/mL) <sup>†</sup>	
	DPPH radical scavenging activity	ABTS radical scavenging activity
70% EtOH crude extract	0.46 ± 0.02 <sup>e</sup>	0.91 ± 0.03 <sup>c*</sup>
<i>n</i> -Hexane	14.84 ± 0.09 <sup>a</sup>	2.82 ± 0.14 <sup>a</sup>
CHCl <sub>3</sub>	0.90 ± 0.10 <sup>b</sup>	2.06 ± 0.06 <sup>b</sup>
<i>n</i> -BuOH	0.50 ± 0.01 <sup>d</sup>	0.69 ± 0.02 <sup>e</sup>
H <sub>2</sub> O	0.92 ± 0.03 <sup>b</sup>	2.01 ± 0.11 <sup>b</sup>
α-Tocopherol	0.98 ± 0.04 <sup>b</sup>	0.82 ± 0.02 <sup>d</sup>
Vit C	0.08 ± 0.02 <sup>c</sup>	0.32 ± 0.01 <sup>f</sup>

<sup>†</sup> The effective concentration at which DPPH and ABTS radicals were scavenged by 50%. \*Means with different superscripts in the same column (a-f) are significantly different at  $p < 0.05$ .

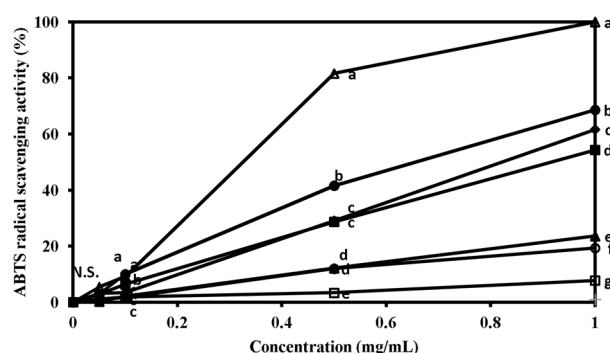
*n*-BuOH fraction were higher than that of the other fractions (Jeong *et al.*, 2011).

### 2. DPPH radical scavenging activity of *Prunella vulgaris* var. *lilacina* fractions

The free radical scavenging activity of its derived fraction of *Prunella vulgaris* var. *lilacina* was assessed by DPPH assay. The IC<sub>50</sub> value is a parameter widely used to measure that activity (Cuveler *et al.*, 1992). As shown in Table 2, 70% EtOH crude extract and *n*-BuOH fractions showed the highest scavenging activity. In addition, the IC<sub>50</sub> values of the *n*-Hexane, CHCl<sub>3</sub>, and H<sub>2</sub>O fraction were 14.84, 0.90, and 0.92 mg/mL, respectively. In this study, Vit C and α-tocopherol were measured as the positive controls, showing the IC<sub>50</sub> values of 0.08 and 0.98 mg/mL, respectively (Table 2).

### 3. ABTS radical scavenging activity of *Prunella vulgaris* var. *lilacina* fractions

ABTS assay is based on the reaction between ABTS and potassium persulfate giving blue/green ABTS radical (ABTS<sup>•+</sup>). With the addition of the antioxidants, decolorization is attained and measured spectrophotometrically at 734 nm. The ABTS radical scavenging activities of different fractions were increased with the sample concentrations (Fig. 3). *n*-BuOH fraction demonstrated the highest scavenging activity for the same concentration. The five fractions in descending order of strength of radical scavenging activity were *n*-BuOH > 70% EtOH crude extract > H<sub>2</sub>O ≥ CHCl<sub>3</sub> > *n*-Hexane fraction (Fig. 3). These results suggested that the *n*-BuOH fraction has potential for use as source of antioxidant material.



**Fig. 3.** ABTS radical scavenging activity of various fractions from 70% EtOH crude extract of *Prunella vulgaris* var. *lilacina*. (■) 70% EtOH crude extract, (□) *n*-Hexane fraction, (●) *n*-BuOH fraction, (○) CHCl<sub>3</sub> fraction, (▲) H<sub>2</sub>O fraction, (△) Vit. C, (◆) α-Tocopherol. Different superscripts indicate significant differences at  $p < 0.05$  in the each concentration.

### 4. FRAP value of of *Prunella vulgaris* var. *lilacina* fractions

The FRAP assay measures the reduction of ferric iron (Fe<sup>3+</sup>) to ferrous iron (Fe<sup>2+</sup>) in the presence of antioxidants, which are reductants with half-reaction reduction potentials above Fe<sup>3+</sup>/Fe<sup>2+</sup>. The ferric complexes reducing ability of different fractions was presented in Fig. 4. Similar to the results obtained for radical scavenging assay, *n*-BuOH fraction showed very strong ferric ion reducing activities for the same concentration. The five fractions in descending order of strength of ferric ion reducing activity were *n*-BuOH > 70% EtOH crude extract > H<sub>2</sub>O > CHCl<sub>3</sub> > *n*-Hexane fraction.

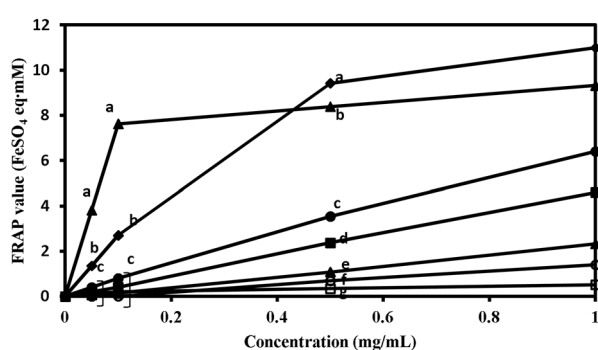
### 5. Correlations analysis

The correlations between antioxidant contents and antioxidant activities were summarized in Table 3. There

**Table 3.** Correlation coefficients (*R*) between the antioxidant properties and content of total phenol and flavonoid in *Prunella vulgaris* var. *lilacina*<sup>†</sup>.

	Total phenol content	Total flavonoid content	DPPH radical scavenging activity	ABTS radical scavenging activity	FRAP value
Total phenol content	1.000				
Total flavonoid content	0.914***	1.000			
DPPH radical scavenging activity	0.702	0.865	1.000		
ABTS radical scavenging activity	0.901***	0.826***	0.771	1.000	
FRAP value	0.916***	0.887	0.894	0.902***	1.000

<sup>†</sup> The correlations of *Prunella vulgaris* var. *lilacina* fractions in the scavenging activity of DPPH radical, ABTS radical, total phenol, and flavonoid contents used. \*\*\**p* < 0.001.



**Fig. 4.** FRAP value of different fractions from 70% EtOH extract of *Prunella vulgaris* var. *lilacina*. (■) 70% EtOH crude extract, (□) *n*-Hexane fraction, (●) *n*-BuOH fraction, (○) CHCl<sub>3</sub> fraction, (▲) H<sub>2</sub>O fraction, (△) Vit. C, (◆)  $\alpha$ -Tocopherol. Different superscripts indicate significant differences at *p* < 0.05 in the each concentration.

were positively high correlation between phenol contents and ABTS assay ( $R=0.901$ ,  $P<0.001$ ), phenol contents and FRAP assay ( $R=0.916$ ,  $P<0.001$ ), and flavonoid contents and ABTS assay ( $R=0.826$ ,  $P<0.001$ ). These results indicated that a relationship between flavonoid and phenolic compound concentrations in *Prunella vulgaris* var. *lilacina* and their free radical scavenging activities. Therefore, the presence of phenolic and flavonoid compounds significantly contribute to their antioxidant potential. These correlation data are in agreement with previous studies that a highly positive relationship existed between total phenolic contents and antioxidative activity in many plants (Gursoy *et al.*, 2009; Chukwumah *et al.*, 2009).

## DISCUSSION

In this study, total phenolic and flavonoid contents, antioxidative capacities, and their correlations of the various fractions from 70% EtOH crude extract of

*Prunella vulgaris* var. *lilacina* were examined with useful methods.

DPPH and ABTS assays used for testing have been widely used to examine the antioxidative activities of plant extracts. It reported that these assays require relatively standard equipment and yield the fast and most reproducible results (Buenger *et al.*, 2006). From these results, it was demonstrated that the *n*-BuOH fraction from 70% EtOH crude extract of *Prunella vulgaris* var. *lilacina* possessed an excellent antioxidative activity based on the DPPH, ABTS, and FRAP assay. Phenolic compounds are found in both eatable and uneatable plants, which have various biological effects, especially, including antioxidative activity. Phenolic compounds having one or more aromatic rings bearing one or more hydroxyl groups can potentially quench free radicals by forming resonance-stabilized phenoxyl radicals and therefore have redox properties (Rice-Evans *et al.*, 1996; Bors and Michel, 2002). In our results, positive correlations were found between ABTS and FRAP assays and total phenolic and flavonoid contents. These results indicated that a relationship between phenolic compound concentration in various fractions from 70% EtOH crude extract of *Prunella vulgaris* var. *lilacina* and their free radical scavenging capacities. Therefore the presence of phenolic compounds in fractions contributes significantly to their antioxidative potential.

It was reported that the ethanol extract of *Prunella vulgaris* var. *lilacina* ameliorated drug-induced memory dysfunction (Park *et al.*, 2010) and showed anti-allergic and anti-inflammatory activity (Ryu *et al.*, 2000; Psotová *et al.*, 2003). Previous studies indicated that the *Prunella vulgaris* var. *lilacina* contains several bioactive components including rosmarinic acid (Lamaison *et al.*, 1991), anti-herpes simplex virus (HSV) polysaccharide (Xu *et al.*,

1999), and 2, 3-dihydroxyurs-12-ene-28-oic acid (Woo *et al.*, 2011). However, there are few studies on antioxidant effects of various fractions of its extracts. From this study, we could know that antioxidative activity of *Prunella vulgaris* var. *lilacina* was due to constituents including in *n*-BuOH fraction.

In this study, it was demonstrated for the first time examined an excellent antioxidative activity testing assay and total phenolic and flavonoid contents of various fractions of the 70% EtOH crude extract from *Prunella vulgaris* var. *lilacina*. A key finding of the present study is that the *n*-BuOH fraction from 70% EtOH extract of *Prunella vulgaris* var. *lilacina* exhibited the strongest radical scavenging activity which is similar to  $\alpha$ -tocopherol. The major known active components within the *Prunella vulgaris* var. *lilacina* are saponins, triterpenic acids, phenolic acids, and flavonoids (Alamed *et al.*, 2009). Vulgarsaponin A and vulgarsaponin B of *n*-BuOH fraction and rosmarinic acid of CHCl<sub>3</sub> fraction are isolated from the *Prunella vulgaris* var. *lilacina* (Pérez-Fons *et al.*, 2010; Wang *et al.*, 1999). Future studies should focus on the identification of these antioxidants and purification of this plant ingredient into better agents with high efficacy and activity.

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