

**INHIBITORY EFFECT OF LIPOXYGENASE AND DPPH RADICAL SCAVENING  
ACTIVITY OF *PERILLA FRUTESCENS* VAR. *ACUTA*****In Sook Kye**Department of Food & Nutrition/Kyungnam  
College of Information & Technology  
**KOREA****Man Kyu Huh**Department of Molecular Biology/Dong-eui  
University  
**KOREA****ABSTRACT**

A reactive oxygen species has been implicated in a range of human pathological diseases such as atherosclerosis and certain cancers. This study is to evaluate *Perilla frutescens* var. *acuta* extracts as sources of natural antioxidants and to examine whether they have significant 1- diphenyl 2-picrylhyorazyl (DPPH) activity and Lipoxygenase (LOX) inhibitory activity or not. The plants of *P. frutescens* var. *acuta* were divided into two parts: leaves and stems. An ethanol method for evaluation of the free radical-scavenging activity of foods by using DPPH is examined. DPPH scavenging activity of leaf extracts of *P. frutescens* var. *acuta* was evaluated at 4.0 mg/ml was 64.1% and that of stem was 51.8% at same concentration. LOX inhibitions of leaf and stem extracts at 4.0 mg/ml were evaluated 44.8% and 28.1%, respectively. The stem of *P. frutescens* var. *acuta* showed maximum inhibition of DPPH activity ( $IC_{50} = 35.7$  ug/ml). The leaf showed maximum inhibition of LOX activity ( $IC_{50} = 45.6$  ug/ml). The degree of inhibition of DPPH by *P. frutescens* var. *acuta* were different among leaf and stem at different concentrations, there was show a statistically significant difference ( $p > 0.05$ ). Strong inhibition of DPPH for *P. frutescens* var. *acuta* makes this pharmacopeial plant material an interesting topic for further biological and phytochemical examination.

**Keywords:** *Perilla frutescens* var. *acuta*, 1, 1- diphenyl 2-picrylhyorazyl (DPPH), lipoxygenase.

**INTRODUCTION**

Antioxidant research is an important topic in the medical field as well as in the food industry. Recent researches with important bioactive compounds in many plant and food materials have received much attention. The antioxidant capacity of most plant food sources is usually associated with their phenolic contents (Kedare and Singh, 2011). The 1, 1- diphenyl 2-picrylhyorazyl (DPPH) is a well-known radical and a trap (scavenger) for other radicals (Brand-Williams et al., 1995). Many research works have also been done for antioxidant activity of leafy vegetables (Dasgupta and De, 2007; Sahu et al., 2013).

Lipoxygenases (LOXs; EC1.13.11.12) are a group of monomeric nonheme iron-containing dioxygenases widely distributed in plants and animals. LOX enzyme catalyzes the hydroperoxidation of polyunsaturated fatty acids containing a cis, cis-1, 4-pentadiene system (Meriles et al., 2000). LOX converts the arachidonic, linoleic and other polyunsaturated fatty acid into biologically active metabolites involved in the inflammatory and immune responses (Azila and Don, 2012). In plants, linolenic and linoleic acids are the most common substrates for LOX (Siedow, 1991). The LOX enzymes are classed into several subcategories including 5-, 12- and 15-lipoxygenases. 15-Lipoxygenases have been found in plants as well as in animal tissue. Commercially obtainable 15-lipoxygenase is isolated from soybeans as the type 1 isoenzyme. There is a good correlation between inhibitory activity towards the mammalian and the soybean-derived enzymes (Mansuy et al., 1998; Nuhn et al., 1991). LOX

activity can show problems both in seed storage and as a result of the off-flavours and odours that it generates (North et al., 1989).

The genus *Perilla* contains only one species and three varieties. *Perilla* contains the lignan-family anti-oxidation element beside such general elements as fat, protein, sugar and minerals, its oil will effectively resist the oxidation of food (Choi, 1995). Meanwhile, it was reported that sesame has such medical efficacies as reduction of cholesterol, activation of liver, immunity, prevention of diseases, slow-down of aging, etc. According to our classic literature, sesame has the efficacies of longevity, prevention of diseases, skin beauty, earlier healing of wound and prevention of stopped blood vessel. They are native to Eastern Asia. *Perilla frutescens* is well-known in Chinese medicine and has a long history of cultivation in China. *Perilla frutescens* (L.) Britt. var. *acuta* (Thunb.) Kudo is a species of family (Lamiaceae) and has been used as an edible biologic medicine in Eastern Asia for more than a thousand years. This species is widely cultivated as a source of medicine and spices. The leaves, stems, and fruit of this plant are used individually to treat a variety of diseases (Chinese Pharmacopoeia Commission, 2005). *Perilla* oil is extracted from the seed and the oil can be used for cooking or food. Leaf of *Perillae* is used to induce perspiration and dispel chills, and to regulate stomach function. *Caulis Perillae* is traditionally used as an analgesic and anti-abortive agent, while *Fructus Perillae* is employed for dyspnea and cough relief, phlegm elimination, and the bowel relaxation (Chinese Pharmacopoeia Commission, 2005). In addition, *Perillae* oil has high iodine content, is rich in polyunsaturated fatty acids, and is used as a preservative. This variety grows along roadsides, in disturbed areas, hillsides, and forest edges, and may be seen near houses.

The purpose of the present study is to evaluate plant extracts as sources of natural antioxidants for DPPH and to examine whether the herbal medicine (*P. frutescens* var. *acuta*) having significant LOX inhibitory activity.

## MATERIALS AND METHODS

### Sample extract

The plants of *P. frutescens* var. *acuta* were divided into two parts: leaf (folium) and stems. Each sample (100 g) of *P. frutescens* var. *acuta* was ground in a mortar and pestle with liquid nitrogen at  $-70^{\circ}\text{C}$  and homogenized prior to beginning extraction experiments. The ground powders were dissolved in 1000 ml ethanol and treated with ultrasound at room temperature for a given duration. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was further stirred with a magnetic bar at  $65^{\circ}\text{C}$  for 12 hours. Extracted sample was filtered. The sample was evaporated to remove solvent under reduced pressure and controlled temperature by using a rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan) at  $60^{\circ}\text{C}$ . To get dry powder, the remaining water was removed by lyophilization in a low temperature vacuum chamber.

### DPPH free radical

1 ml of 0.1 mM DPPH solution in ethanol was mixed with 1 ml of plant extract solution of various concentrations (0.5, 1.0, 2.0 and 4.0 mg/ml). The antioxidant activity of the *P. frutescens* var. *acuta* extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williams et al. (1995) with slight modifications. DPPH was added to the solutions prepared with plant extracts and standard antioxidant substances and stirred. Briefly, a

solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of ethanol, and the solution was kept in the dark at 4°C. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 µl of ethanol DPPH solution (final concentration 300 µM) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. After 30 min, the optical density (OD) of the solution was read using the UVmini-1240 Reader (Shimadzu, Kyoto, Japan) at the wavelength 517 nm. Absorbance changes for the samples were measured using the UV mini-1240 Reader (Shimadzu, Kyoto, Japan) at 517 nm. Corresponding blank sample was prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard (positive control). The inhibition % was calculated using the following formula. Percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = [1 - \text{OD (DPPH + sample)} / \text{OD (DPPH)}] \times 100\%$$

To determine the IC<sub>50</sub> value of the active component, the technique using 96-well microplates was employed (Lee et al., 1998). A dose response curve was plotted to determine the 50% inhibition (IC<sub>50</sub>) values. IC<sub>50</sub> is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity.

### Lipoxygenase activity

Lipoxygenase (LOX) inhibitor Screening Assay Kit (Abnova, CA, USA) was used and measured the hydroperoxides produced in the lipoxygenation using a purified LOX. Stock solutions of the tested samples 15-lipoxygenase standard (Abnova, CA, USA) were prepared by dissolving the extracts in ethanol or methanol. The reaction was initiated by the addition of aliquots (90 µl) soybean LOX solutions (prepared in potassium phosphate buffer, pH 9.0) in a sufficient concentration to give an early measurable initial rate of reaction to 10 µl of arachidonic acid in phosphate buffer. The enzymatic reaction was performed in presence or absence of inhibitor and their kinetics were compared. Quercetin was used as positive control. Nordihydroguaiaretic acid (NDGA) and Rutin used as negative control. LOX inhibition activity was determined using a spectrophotometric method at 490 nm. Regression analysis was used to calculate IC<sub>50</sub>, defined as the concentration of inhibitor necessary for 50% inhibition of the enzyme reaction (Akular and Odhav, 2008). The percent inhibition was calculated as the decolorization percentage of the test sample using the following formula:

$$\text{Inhibition \%} = (\text{IA} - \text{As}) / \text{IA} \times 100$$

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

### Statistical analysis

All the analysis were performed in triplicate and the results were expressed as the mean ±SD. The data were statistically evaluated using of variance (ANOVA). Duncan's multiple range was carried out in order to test any significant differences between the concentration and treated vegetative organs. Correlation co-efficient (R) to determine the relationship between two or more variables among Radical Scavenging activity tests were calculated using the SPSS software (Release 21.0).

## RESULTS

Bioactivity of *P. frutescens* var. *acuta* extract was significantly varied based the solvents used for extraction, concentration of the extract and stages of the treatment. In this present study combination of both ethanol and ultrasonication was produced good results. The amount of dried powders was also significantly greater in only solvent (ethanol) or water. Table 1 was shown the antioxidant activities of the *P. frutescens* var. *acuta*. Various concentrations of outer bark extracts were higher than those of leaf and endodermis. The maximum high antioxidant activity found on outer bark extracts. DPPH scavenging activity of leaf extracts of *P. frutescens* var. *acuta* was evaluated at 4.0 mg/ml was 64.1% and that of stem was 51.8% at same concentration (Table 1). The degree of inhibition of DPPH by *P. frutescens* var. *acuta* were different among leaf and stem at different concentrations, there was show a statistically significant difference ( $p > 0.05$ ). The stem of *P. frutescens* var. *acuta* showed maximum inhibition of DPPH activity ( $IC_{50} = 35.7$  ug/ml) (Fig. 1). Table 2 was shown the LOX activity of *P. frutescens* var. *acuta* extracts. LOX inhibitions of leaf and stem extracts at 4.0 mg/ml were evaluated 44.8% and 28.1%, respectively. Although the values of LOX inhibition of leaves were higher than those of stems, there were not show a statistically significant difference ( $p < 0.05$ ). The leaf showed maximum inhibition of LOX activity ( $IC_{50} = 45.6$  ug/ml) (Fig. 2).

**Table 1.** Free radical scavenging effects of *Perilla frutescens* var. *acuta* at different concentrations

Concentration (mg/ml)	Leaf	Stem
0.1	17.27±2.67	10.69±6.57
0.5	25.01±3.07	19.17±5.26
1.0	35.14±2.08	27.11±2.88
2.0	54.12±5.70	38.31±5.86
4.0	64.07±5.95	51.78±1.84
F-test	0.855, $p < 0.05$	

Data represent the mean ± SD from three replicates.

**Table 2.** Percent inhibition of lipoxigenase by *Perilla frutescens* var. *acuta* at different concentrations

Concentration (mg/ml)	Leaf	Stem
0.1	17.80±1.60	08.40±5.20
0.5	23.92±1.77	11.65±5.66
1.0	31.59±2.88	16.91±4.24
2.0	37.64±3.30	21.90±2.73
4.0	44.84±1.59	28.06±1.06
F-test	2.314*, $p > 0.05$	

Data represent the mean ± SD from three replicates.

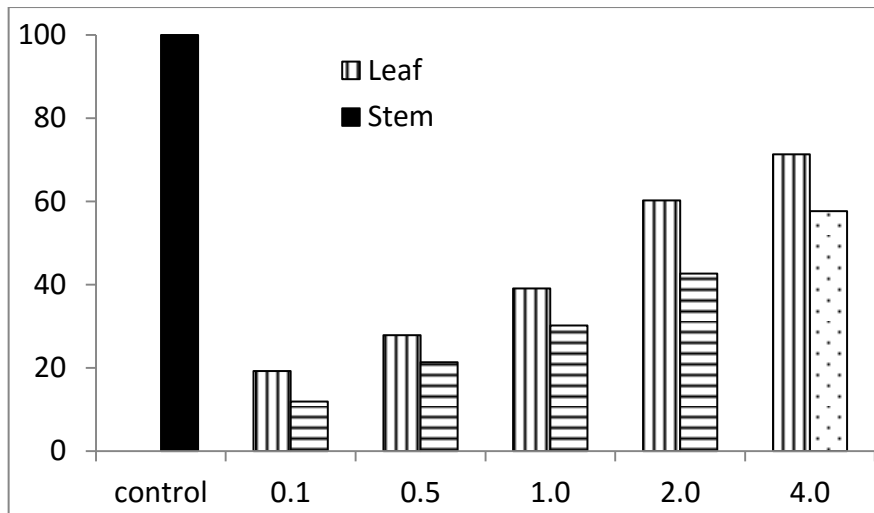


Fig. 1. Relative antioxidant values of the *Perilla frutescens* var. *acuta* extracts for control group (L-Ascorbic acid).

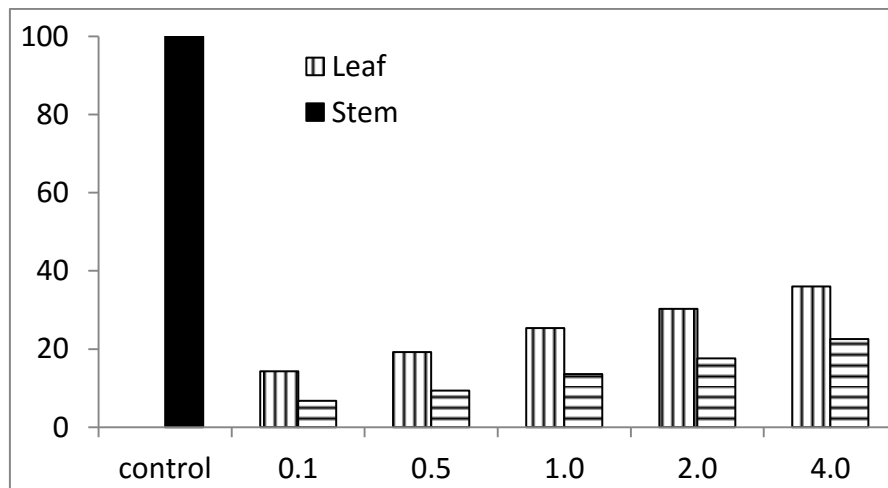


Fig. 2. Relative lipoxygenase inhibitory of *Perilla frutescens* var. *acuta* and nordihydroguaiaretic acid (negative control).

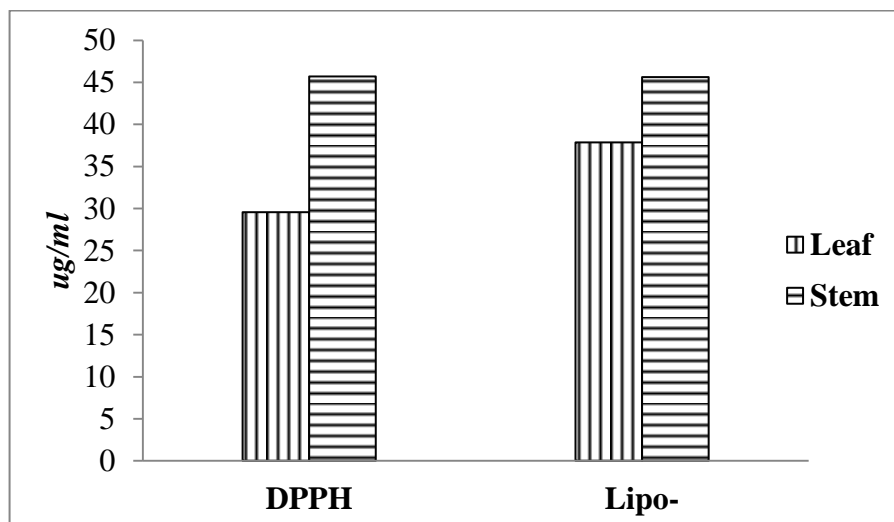


Fig. 3. 50% inhibition {IC<sub>50</sub> (mg/ml)} values on DPPH and lipoxygenase by *Perilla frutescens* var. *acuta* at 4.0 mg/ml concentration.

## DISCUSSION

*Perilla frutescens* var. *japonica* (Hassk.) Hara is the oilseed crop which is the source of perilla oil. This variety is used as an ingredient in Korean cuisine. The plant's Korean name is *deulkkae* or *tülkkae*). The same word is also used when referring to its seed, which has many uses in Korean cuisine, just as the leaves (*ggaennip*) do. The literal translations of *deulkkae* ("wild sesame") and *ggaennip* ("sesame leaf") are in spite of perilla's not being closely related to sesame, and Korean cookbooks translated into English sometimes use these translations. Cans of pickled *ggaennip* can be found in Korean grocery stores all over the world. The other type of edible perilla (*P. frutescens* var. *acuta*) called *Chazoki* or *soyeop*. The leaf color of *P. frutescens* var. *japonica* is green and that of *P. frutescens* var. *acuta* is purple. Fresh leaves of *P. frutescens* var. *japonica* have an aroma reminiscent of apples and mint, and are eaten in salad dishes and with roasted meat. Whereas, *P. frutescens* var. *acuta* do not use edible leaves, but use traditional herbal species in Korea. The Japanese name for the variety of perilla normally used in Japanese cuisine is *shiso*. *Perilla* leaves also occur in red varieties, (*akajiso*), and the flower stalks are used as garnish as well. Japanese *Akajiso* is very similar to Korean *Chazoki*.

The many traditional herbal species in Korea exhibited DPPH-free radical scavenging activity (Choi et al., 2003). Out of 40 species, four species (*Emcommia cortex*, *Moutan radices*, *Paeonine radix*, and *Rubus coreanus*) were shown above 90% inhibition of DPPH radical scavenging activity (Choi et al., 2003). Kim et al. (2008) reported that *P. frutescens* var. *japonica* in Korea showed very high antioxidant activities. In this study, DPPH values of *P. frutescens* var. *acuta* were also relatively high (Table 1).

*Camellia sinensis*, *Rhodiola rosea*, and *Koelreuteria henryi* had notable significant inhibitory activities towards lipoxygenase (Chen et al., 2009). These results show that these plants have some phytochemical constituents which may be active against the lipoxygenase enzyme. We have shown that 4.0 mg/ml weight of ethanol *P. frutescens* var. *acuta* extract has inhibitory effect of lipoxygenase and antioxidants for DPPH. Anti-lipoxygenase activity of chosen antioxidant-rich plant materials can support their traditional use in folk medicine (Wichtl, 1994). Strong inhibition of LOX enzymes by extract from *P. frutescens* var. *acuta* makes this pharmacopeial plant material an interesting topic for further biological and phytochemical examination (Oyetayo, 2008).

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