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Assessment on *In Vitro* Antioxidant Properties of Common Thistle (*Cirsium pendulum* Fisch.) Plant Parts

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ABSTRACT : Antioxidant activity of the extract fractions from leaves, stems, roots and flowers of *Cirsium pendulum* Fisch. was investigated. The results showed the greatest antioxidant activities in leaves by Rancimat, TBA and DPPH methods. Extracts of common thistle plants dose-dependently increased DPPH free radical scavenging activity. The extract from flowers and its hexane fraction showed the strongest antioxidant activity. HPLC analysis showed that BuOH fraction of the leaves had the highest amount of antioxidant chlorogenic and *p*-coumaric acids at 5.38 and 9.71 mg 100 g^{-1}, respectively. It implies that common thistle plants had potent antioxidant activity, and their activities were differently exhibited depending on plant part and solvent fraction.

Key words : antioxidant activity, DPPH radical scavenging activity, plant parts, extracts, natural antioxidant

INTRODUCTION

Antioxidants, inhibitors of lipid peroxidation, are important not only for food protection but also for the defense of living cells against oxidative damage. The toxic and otherwise unfavorable effects of synthesized food antioxidants have been widely noted. Phenolic compounds, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ), have been widely used as synthetic antioxidants in food lipid. Although those antioxidants are considered as safe natural antioxidants, they do not always provide effective protection against in vitro oxidation (Frankle, 1980). Nevertheless, the phenolic antioxidants are still used extensively as food antioxidants because of their excellent results and low cost. When slightly larger doses (50 mg/kg/day) of these phenolic antioxidants are administered to rodents and monkeys, however, certain pathological, enzyme and lipid alterations as well as carcinogenic effects have been observed (Branen, 1975). Therefore, research on other natural antioxidants has gained momentum as they are considered, rightly or wrongly, to pose no health risk to consumers (Wanasundara & Shahidi, 1994; Wanasundara *et al.*, 1997). The development of alternative natural antioxidants has, therefore assumed as increased importance. Many investigators have found different types of antioxidants in various sources of plants (Larson, 1988).

Recently, there has been a worldwide trend towards the use of the phytochemicals from wild plants. Common thistle is an annual herbaceous plant of Compositae, one of the largest and most diverse families of flowering plants. Compositae plant in Korea is known to be an increasingly important medicinal resources and new functional agent, mainly due to antioxidant activity (Lee *et al.*, 1997). Phenolics are ubiquitous compounds found in all plants as their secondary metabolites. Naturally-occurring antioxidative components in foods or plants include flavonoids, phenolic acids, lignan precursors, terpenes, mixed tocopherols, phospholipids, polyfunctional organic acids and also plant extracts such as those of rosemary and sage (Schuler, 1990; Wanasundara *et al.*, 1997). Chlorogenic acid, a naturally-occurring polyphenol compound, is reported as a clastogenic agent in hamster cells (Stich *et al.*, 1981) and to participate in enzymatic browning reactions in potatoes, sunflower seed, leaf protein concentrates, milk proteins, and other foods (Deshpande *et al.*, 1984).

Probable major biosynthetic pathways leading to production of natural antioxidants have been known to be shikimic acid or acetate pathway (Rice, 1984). The objective of this research was to determine their antioxidant activities of the dried samples or extracts through Rancimat, TBARS, and DPPH methods. The research will make attractive the research for antioxidant and scavenger natural compound.

MATERIALS AND METHODS

Fractionation of methanol extracts

Common thistle plants grown in a pasture of the Suncheon area, Korea were harvested at a reproductive stage in July

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2005. The plant samples were separated into leaves, stems, roots and flowers. The four plant parts were directly freeze-dried at −40 ℃ for 5 days, and ground with a Wiley mill to pass a 1-mm screen, and stored in a refrigerator at 2 ℃ until used. The ground samples from each plant part were use for measuring antioxidant activity by Rancimat and TBA methods.

The ground samples were extracted with 95% methanol at room temperature. The extract was then filtered through a Whatman No. 1 filter paper. The collected filtrate was evaporated to dryness under vacuum at 40 ℃ using a rotary evaporator (N-1000V-W, Eyela, Japan). After evaporation, the yield of dried extracts (methanol extract) was about 10% of the original plant sample. The methanol extracts from each plant part were use for measuring DPPH radical scavenging activity.

For fractionation, crude methanol leaf extracts were diluted with distilled water and n-hexane to collect hexane fraction using a separating funnel. After hexane collection, the distilled water fractions were added with ethylacetate (EtOAc) to obtain EtOAc fraction in the same way. The same procedure was used in preparing butanol and water fractions. The fractions were taken to dryness on a rotary evaporator at 40-50 ℃, and transferred into vacuum freeze dryer to obtain dry matters. The four fractions used for measuring DPPH radical scavenging activity.

Measurement of antioxidant activity

Dried-ground leaves, stems, roots, and flowers were exploited for investigation of antioxidant activity. Oxidative stability was evaluated by the Rancimat method (Kajimoto *et al.*, 1995) and measured with the Rancimat 743 apparatus (METROHM, AG, CH-9101 Herisau, Switzerland), using a soybean oil of 3.0 ㎖ at 120 ℃, with an air flow rate of 20 L/h. Oxidative stability was expressed as the oxidation induction time (h).

Antioxidant activity for the samples was investigated by a TBA method. The ground samples of 0.1 g were mixed with 10 g storing pork meat and then stored at refrigerator. At 24 and 144 h after storage, the mixed samples were added with 25 ㎖ of 20% trichloroacetic acid (TCA), homogenized at 14000 rpm for 2 min, and diluted with distilled water to give final volume into 100 ㎖. The diluted solution was filtered with Whatman No.1 filter paper. The 5ml-filtered solution was mixed with 5 ㎖ 2-TBA (0.005 M) and transferred into test tube. The test tube was placed into dark room for 15 h at 25 ℃. Then the solution was measured the absorbance at 550 ㎚ of UV-VIS Spectrophotometer. To determine the antioxidant activity of plant extracts kept in meat, TBARS values of methanol extracts from common thistle plant samples were measured at 1 and 7 days after storing, and compared with control, two synthetic antioxidants, ascorbic acid and BHT. TBA-reactive substance (TBARS) value tests were used to indicate the extent of lipid oxidation according to the method of Witte (1970). The values were calculated as follows; TBA (MA ㎎/1000 g) = Absorbance *5.2. All measurements were replicated with 3 times.

DPPH radical scavenging activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay was carried out according to the procedure described by Blosi (1958). Methanol extracts from each plant part, and four fractions from leaf extracts at various concentrations (0.10, 100, 250, 500 and 1000 ㎍ ㎖$^{-1}$) were added to a 1.5×10^{-4} M solution of DPPH in methanol and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 520 ㎚, and the radical scavenging activity was obtained from the following equation: Radical scavenging activity (%) = $\{(OD_{control} - OD_{sample})/OD_{control}\}\times100$. The antioxidant activity of plants extracts was expressed as IC_{50}, which was defined as the concentration (in ㎍ ㎖$^{-1}$) of extract required to inhibit the formation of DPPH radicals by 50%. When the F-test was significant ($P < 0.05$) means were separated on the basis of least significant difference (LSD) (SAS Institute, 2000).

Quantification of chlorogenic and *p*-coumaric acids

The standard phenol compounds used for HPLC analysis were chlorogenic and *p*-coumaric acids, which are well documented to be antioxidant to food (Deshpande *et al.*, 1984; Naczk and Shahidi, 2004). The standard chemicals were purchased as a high purity standard and the used solvents were HPLC spectral grade (Aldrich Co., CA, USA). All solvents and distilled water were degassed before use. All solvent ratios were based on volume. Chlorogenic and *p*-coumaric acids were identified by a HPLC system (SPP 10AVP, Shimadzu, Japan) with a flow rate of 1 ㎖ min^{-1}, the column was CAPCELL PAK C18 SG120 (4.6×250 ㎜) and an autoinjector with a 10 ㎖ sample loop was employed. The mobile phase consisted of water, methanol and acetic acid in the ratio of 12 : 15 : 1 volume, respectively. The UV detector wavelength was set at 275 ㎚. Standard compound was chromatographed. Retention time for the standard compound and the major peaks in the extract were recorded. Chlorogenic and *p*-coumaric acids from each fraction were identified by retention times or standard addition, and their amounts were calculated by comparing peak area with that of standard (Banwart *et al.*, 1985).

RESULTS AND DISCUSSION

Measurement of antioxidant activity by Rancimat and TBA methods

Even though plant samples had less oxidative stability than

Table 1. Effects of ground samples from different plant parts of common thistle on oxidative stability and 2-thiobarbituric acid-reactive substances (TBARS) value.

Sample	Oxidative stability (Induction time, h)	TBARS (㎎ MDA ㎏$^{-1}$)	
		1 DAS*	7 DAS
Leaves	3.23 ± 0.08 a**	0.041 ± 0.03 ab	0.224 ± 0.02 b
Stems	2.95 ± 0.06 b	0.093 ± 0.02 a	0.280 ± 0.02 b
Roots	2.50 ± 0.03 bc	0.110 ± 0.08 a	0.317 ± 0.07 ab
Flowers	2.96 ± 0.04 b	0.048 ± 0.03 ab	0.244 ± 0.04 b
Ascorbic acid	3.64 ± 0.12 a	0.024 ± 0.00 b	0.066 ± 0.01 c
Blank	1.32 ± 0.02 c	0.163 ± 0.01 a	0.650 ± 0.02 a

* DAS: Day(s) after storing in meat at refrigerator (4℃).
** When the F-test was significant ($P < 0.05$) means were separated on the basis of least significant difference (LSD) (SAS Institute, 2000). Within a column, means followed by the same letter are not significantly different at $p < 0.05$.

ascorbic acid (3.6 h), the difference in stability among common thistle plant parts was apparently exhibited. The oxidative stability determined by the Rancimat technique showed a variation between the different plant parts, ranging from 2.5 to 3.2 h (Table 1). Ground leaf sample was the most stable, while stem and root samples showed the least stability.

TBARS values showed lower in all samples at 1 and 7 days after storing than ascorbic acid as a control antioxidant (0.066), and the values were not equal among plant part extracts. The highest TBARS values at 7 days after storage were obtained from leaf samples (0.224) of common thistle, and followed by flowers (0.244), stems (0.280) and roots (0.317), showing no significant difference in antioxidant activity among plant parts (Table 1). TBARS values of methanol extracts from thistle leave sample were little lower than those of ascorbic acid and BHT as synthetic antioxidants. Lee et al. (1997) reported that silymarin and silybin purified from *Silybum marianum* have potential inhibiting activities against oxidation of ^{125}I-LDL by macrophages and endothelial cells.

DPPH radical scavenging activity of common thistle plant parts and fractions

Extracts of common thistle leaves had the highest DPPH radical scavenging activity, with an IC_{50} value of 335 ㎍ ㎖$^{-1}$, and followed by flowers and roots, with IC_{50} values of 375 and 949㎍ ㎖$^{-1}$, respectively (Fig. 1). All samples of plant parts showed DPPH radical scavenging activity in a dose-dependent manner. After fractionation, methanol extracts of hexane fraction showed the highest DPPH radical scavenging activity, with an IC_{50} value of 125 ㎍ ㎖$^{-1}$, and followed by EtOAc (IC_{50} = 501 ㎍ ㎖$^{-1}$) and BuOH fractions(IC_{50} = 825 ㎍ ㎖$^{-1}$). This value was lower than those of BHT or ascorbic acid, with IC_{50} values of 25 and 92 ㎍ ㎖$^{-1}$, respectively (Fig. 2). However, methanol extracts from water fraction was the

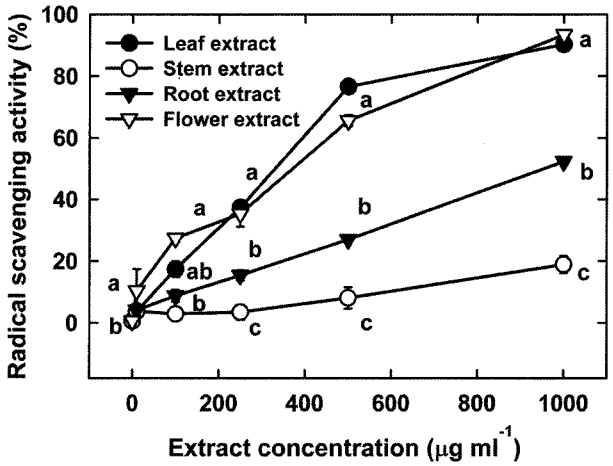

Fig. 1. DPPH radical scavenging activity of common thistle plant parts. Total extracts of each plant part were added to a methanol solution of DPPH and radical scavenging activity was measured at 520 ㎚. Each experiment was performed at least three times and data are expressed as average percent changes versus the control ± S.D. Within an extract concentration, means followed by the same letter are not significantly different at $p < 0.05$. Each bar represents standard error of the mean.

lowest. The results show that causative antioxidant components were present as the highest amount in the hexane fraction and followed by EtOAc, BuOH, and water fractions, resulting in inhibitory effects on DPPH radicals. This also indicates that various compounds that cause antioxidant activity could be produced with different amount from the fractions.

Quantification of chlorogenic and *p*-coumaric acids for the each fraction

Chlorogenic acid and *p*-coumaric acid present in the fractions of methanol leaf extracts of common thistle were analyzed by HPLC using standard. Chlorogenic acid was detected in the

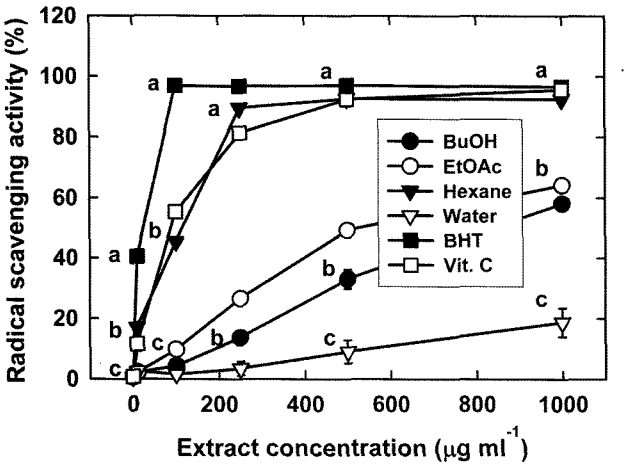

Fig. 2. DPPH radical scavenging activity of four fractions from common thistle plant leaves. Total extracts of each plant part were added to a methanol solution of DPPH and radical scavenging activity was measured at 520 nm. Each experiment was performed at least three times and data are expressed as average percent changes versus the control ± S.D. Within an extract concentration, means followed by the same letter are not significantly different at $p < 0.05$. Each bar represents standard error of the mean.

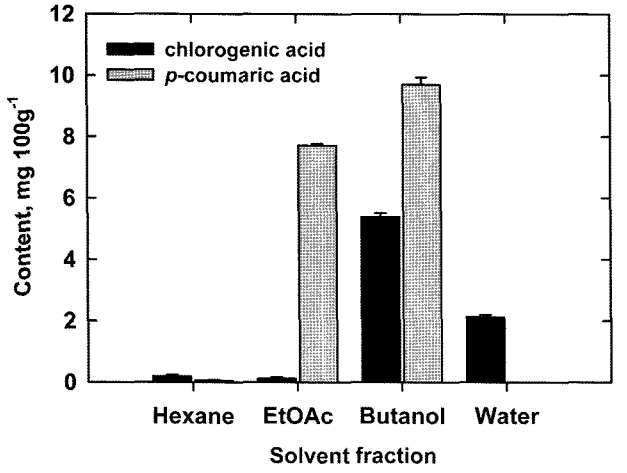

Fig. 3. Quantitative determination of antioxidant chlorogenic and *p*-coumaric acids present in four fractions from leaves of common thistle.

BuOH fraction as the highest amount and followed by water fraction (Table 3). However, level of chlorogenic acid showed the lowest amount in the ethylacetate and hexane fractions. On the other hand, *p*-coumaric acid was more abundant in BuOH and EtOAc fractions than in hexane and water fractions. The results show that findings of quantification by fraction through HPLC were not associated with the antioxidant activity. Radical scavenging effect of phenolic compounds isolated from natural sources has been widely studied (Yioshida *et al.*, 1989). The antioxidative potency and phenolic acids are generally interrelated. These phenolic compounds react with the free radicals formed during autoxidation, and generate a new radical which is stabilized by the resonance effect of the aromatic nucleus (Cuvelier *et al.*, 1992).

In conclusion, common thistle plants showed a potent antioxidant activity through measurement of oxidative stability and 2-thiobarbituric acid-reactive substances (TBARS) value by Rancimat and TBA methods. The methanol extracts of common thistle were found to be able to reduce the production of TBA reactive substances in pork meat homogenates. Extracts of common thistle plants dose-dependently increased DPPH free radical scavenging activity, in vitro. The flower extracts and hexane fraction showed the strongest antioxidant activity. The compounds that cause antioxidant activity could be produced with different amounts depending on plant part. Such differences might be related to antioxidant compounds being produced in larger quantities in certain tissue, imparting a higher level of antioxidant. The results suggest that the extracts of common thistle had antioxidant activity with important values for an alternative natural antioxidant based on natural plant extracts.

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