Korean J. Crop Sci. 50(5): 336~339(2005)

Assessment of Radical Scavenging Activity and Phenolic Compounds of *Xanthium occidentale*

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ABSTRACT: Common thistle contains water-soluble substances that are antioxidative to foods. Antioxidant activities measured by DPPH method for the ground samples were the greatest in leaves, although was less than that of commonly used antioxidants, BHT and ascorbic acid. Methanol extracts and fractions from *Xanthium occidentale* plants dose-dependently increased DPPH free radical scavenging activity, *in vitro* test. The extracts from leaves showed the strongest antioxidant activity. DPPH scavenging activity of the individual fraction was in order of *n*-butanol>water>ethyl acetate>*n*-hexane fraction. By means of HPLC analysis, leaf samples of *Xanthium occidentale* had the highest amount of phenolic compounds, related with antioxidant activity, and followed by stems and roots. Total content of these antioxidant phenolic compounds for leaves extracts were detected in water fraction (36.7 mg 100 g^{-1}) as the greatest amount, especially chlorogenic acid (39.4 mg 100 g^{-1}) was the greatest component. These results suggest that *Xanthium occidentale* plants had potent antioxidant activity, and their activities were differently exhibited depending on plant part and fraction.

Keywords: *Xanthium occidentale*, antioxidant activity, phenolic compounds, DPPH

Antioxidants 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 well known. 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).

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). Samples of various plant parts, including seeds, leaves, stems and roots from *X. occidentale* are known to be associated with antimicrobial activity (Kim & Shin, 1997; Kim *et al.*, 2003), antitrypanosomal activity (Talaki *et al.*, 1995), antitumor activity (Kim *et al.*, 2003), and allelopathic effect on weed species (Chon *et al.*, 2003). 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). Chloro-genic acid, a naturally-occurring polyphenol compound, is reported as a clastogenic agent in hamster cells (Stich *et al.*, 1981) and to participate in enzymatic browing treactions in potatoes, sunflower seed, leaf protein concentrates, milk proteins, and other foods (Despande *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). In this paper, we now report their antioxidant effects of the methanol extracts or their fractions of *X. occidentale* on DPPH radical and describe quantification of the causative components. This research will promote a better understanding of natural chemical production in the natural- and agro-ecosystems through investigating antioxidant activity.

MATERIALS AND METHODS

Extraction of *Xanthium occidentale* plant parts

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X. occidentale plants grown in pastures of the Suncheon area, Korea were harvested at a vegetative stage in May 2001. The plants were separately sampled into leaves, stems, and roots. The samples were immediately oven-dried at 60°C for 5 days, ground with a Wiley mill to pass through a 1-mm screen, and stored in a refrigerator at 2°C until required. Ground leaf 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°C using a rotary evaporator (N-1000V-W, Eyela, Japan). Methanol extracts from leaves, stems and roots were exploited for investigation of antioxidant activity.

Fractionation of methanol extracts from *Xanthium occidentale* leaves

Methanol extracts from ground plant samples were used for the following fractionation. For fractionation, crude methanol extracts were diluted with distilled water and hexane to collect n-hexane fraction using a separating funnel. After n-hexane collection, the distilled water fractions were added with ethyl acetate (EtOAc) to obtain EtOAc fraction in the same way. The same procedure was used in preparing *n*-butanol (BuOH) and water fractions. The fractions were taken to dryness on a rotary evaporator at 40 - 50 °C, and transferred into vacuum freeze dryer to obtain dry matters. The fractions were exploited for investigation of antioxidant activity.

Analysis of 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). Total extracts of each plant at various concentrations (0, 10, 100, 250, 500 and 1000 μg ml^{-1}) were added to a solution of DPPH in methanol (1.5×10^{-4} M) and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 520 nm, and the radical scavenging activity was obtained from the following equation: Radical scavenging activity (%) = $\{(OD_{control} - OD_{sample}) / OD_{control}\} \times 100$. The antioxidant activity of plants extracts was expressed as IC50, which was defined as the concentration (in μg ml^{-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 phenolic compounds from fractions

Phenol compounds used for HPLC analysis were caffeic acid, *p*-coumaric acid, and chlorogenic acid (Aldrich Co., CA, USA). All of the chemicals were purchased as high purity standards and the used solvents were HPLC spectral grade. All solvents and distilled water were degassed before use. All solvent ratios were based on volume. Antioxidant phenolic compounds were identified by a HPLC system (SPP 10AVP, Shimadzu, Japan) with a flow rate of 1 mL min^{-1}, the column was CAPCELL PAK C18 SG120 (4.6 × 250 mm) and an autoinjector with a 10 μl 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 nm. Standard compounds were chromatographed alone and as mixtures. Retention times for the standard compounds and the major peaks in the extract were recorded. Phenolic compounds from each fraction were identified by retention times or standard addition, and their amounts were calculated by comparing peak area with those of standards (Banwart *et al.*, 1985).

RESULTS AND DISCUSSION

Antioxidant effects of Xanthium occidentale plant parts

Methanol extracts of *Xanthium occidentale* leaves had the highest DPPH radical scavenging activity, with an IC_{50} value of 465 μg ml^{-1}, and followed by stems and roots, with IC_{50} values of 579 and 723 μg ml^{-1} (Fig. 1), respectively. All

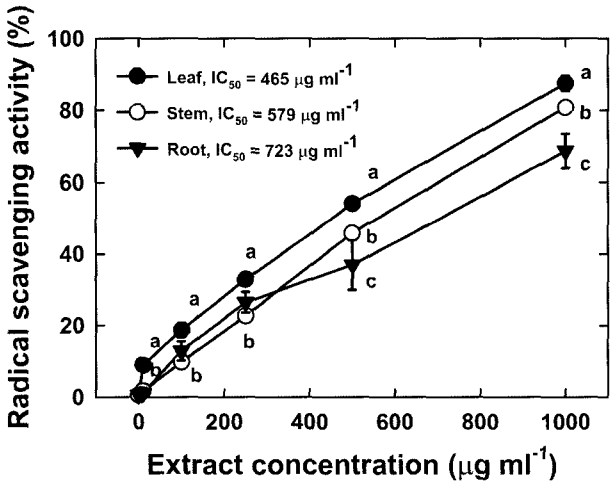

Fig. 1. Radical scavenging effect of the methanol extracts from different plant parts of *Xanthium occidentale* on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Each experiment was performed at least three times and data are expressed as average percent changes versus the control. Within an extract concentration, means followed by the same letter are not significantly different at $p<0.05$. Each bar represents standard error of the mean.

samples of plant parts showed DPPH radical scavenging activity in a dose-dependent manner. Thus, antioxidant effects of methanol extracts of *Xanthium occidentale* at 1000 μg ml^{-1} were ranked in order of leaf, stem, and root. It is generally accepted that the extracts of topgrowth (especially leaves) in plants produce more physiologically-active substances than those from roots and crowns (Miller, 1996).

Antioxidant effects of fractions from methanol leaf extracts

After fractionation, methanol extracts of BuOH fraction showed the highest DPPH radical scavenging activity, with the lowest IC_{50} value of 91 μg ml^{-1}, and followed by water (IC_{50} = 461 μg ml^{-1}) and EtOAc fractions (IC_{50} = 687 μg ml^{-1}). These values were much higher than those of BHT or ascorbic acid, with IC_{50} values of 33 and 85 μg ml^{-1}, respectively. However, IC_{50} value from hexane fraction was the highest (>1000 μg ml^{-1}), meaning the lowest DPPH radical scavenging activity (Fig. 2). Fractions of *Xanthium occidentale* plants dose-dependently increased DPPH free radical scavenging activity at *in vitro* test. The results show that causative antioxidant components were more present in the BuOH fraction than those in the water and EtOAc fractions, resulting in more inhibitory effects on DPPH radicals. These results also indicate that different compounds that cause antioxidant activity could be produced with different amount from different fractions.

Quantification of antioxidant phenolic compounds from fractions

The major phenolic compounds present in the 4 fractions of the methanol extracts from *Xanthium occidentale* leaves were analyzed by HPLC using standard compounds and recorded as a content of each or total phenol compound (s). The individual compounds identified were caffeic acid, *p*-coumaric acid and chlorogenic acid. Of these, chlorogenic acid was detected in all fractions as the greatest component (39.4 mg 100 g^{-1}) and followed by *p*-coumaric acid (3.8 mg 100 g^{-1}). Among total phenol compounds (45.0 mg 100 g^{-1}) major phenolic compounds in leaves were detected in the water fraction (36.7 mg 100 g^{-1}) as the highest amount and followed by BuOH fraction (5.2 mg 100 g^{-1}) (Table 1). The results show that differential antioxidative effect of each plant part would be due to quantitative as well as qualitative matters of the causative oxidant chemicals in plant sample, suggesting various types and amount of antioxidant agents. Radical scavenging effect of phenolic compounds isolated from natural sources has been widely studied (Yioshida *et al.*, 1989). The antioxidative potentials of phenolic acids are inter-related. These 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, an assessment demonstrated that the *X. occidentale* plants had potent antioxidant activity, showing

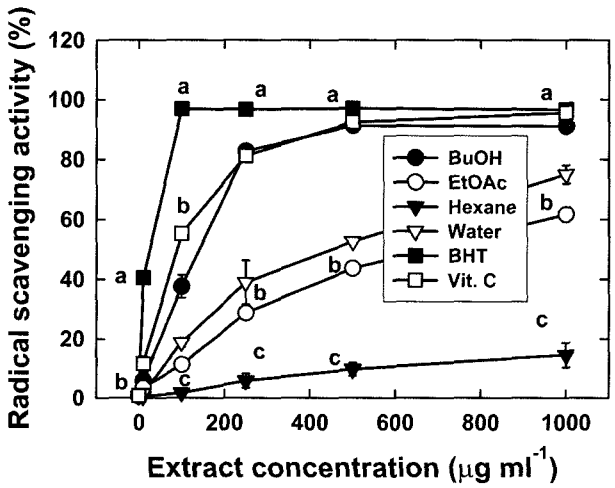

Fig. 2. Radical scavenging effect of different fractions from *Xanthium occidentale* leaf extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Each experiment was performed at least three times and data are expressed as average percent changes versus the control. Within an extract concentration, means followed by the same letter are not significantly different at $p<0.05$. Each bar represents standard error of the mean.

Table 1. Quantitative determination of HPLC analysis of some phenolic compounds present in leaves of *Xanthium occidentale*.

Compound	Fractions				
	Hexane	EtOAc	BuOH	Water	Total
			(mg 100 g^{-1})		
caffeic acid	0.175	0.121	1.582		1.878
p-coumaric acid	0.051	0.313		3.390	3.754
chlorogenic acid	2.242	0.241	3.590	33.331	39.404
Total	2.468	0.675	5.172	36.721	45.036

inhibitory effects of their fractions on formation of DPPH radicals. Different compounds that cause antioxidant effect could be produced with different types and amounts of causative antioxidant substances depending on plant part and fractionation method. Such differences also might be related to specific antioxidant compounds being produced in larger quantities in certain plant part or fraction, imparting a higher level of antioxidant component. Our results suggest that the antioxidant activity of *X. occidentale* may be a valuable alternative mean based on natural plant material.

REFERENCES

Banwart, W. L., P. M. Porter, T. C. Granato, and J. J. Hassett. 1985. HPLC separation and wavelength area ratios of more than 50 phenolic acids and flavonoids. J. Chem. Ecol. 11 : 383-395.

Blosi, M. S. 1958. Antioxidant determinations by use of a stable free radical. Nature 26 : 1199-1200.

Branen, A. L. 1975. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. JAOCS 52 : 59-63.

Chon, S. U., Y. M. Kim, and J. C. Lee. 2003. Herbicidal potential and quantification of causative allelochemicals from several Compositae weeds. Weed Res. 43 : 444-450.

Cuvelier, M.-E., H. Richard, and C. Berset. 1992. Comparison of the antioxidative activity of some acid-phenols: Structure-activity relationship. Biosci. Biotech. Biochem. 56 : 324-325.

Despande, S. S., S. K. Sathe, and D. K. Salunkhe. 1984. Chemistry and safety of plant polyphenols. In *Nutritional and toxicological aspects of food safety.* (Fredman, M. Ed.), Plenum: New York. PP. 457-495.

Frankle, E. N. 1980. Lipid oxidation. A review. Progress in Lipid Research 19 : 1-22.

Kim, H. S. and J. O. Shin, 1997. Isolation and antimicrobial activity of Xanthium strumarium L. extract. Kor. J. Appl. Microbiol. Biotechnol. 25 : 183-188.

Kim, H. S., T. S. Yu, I. S. Lee, Y. W. Kim, and S. H. Yeo. 2003. Screening of the antimicrobial and antitumor activity of Xanthium strumarium L. extract. Kor. J. Biotechnol. Bioeng. 18 : 55-61.

Larson, R. A. 1988. The antioxidants of higher plants. Phytochemistry. 27 : 969-978.

Lee, B. C., Y. K. Jeong, and B. H. Ryu. 1997. Antioxidative effect of silymarin and silybin purified from *Silybum marianum* on oxidation of human low density lipoprotein by macrophages. Kor. J. Appl. Microbiol. Biotechnol. 25 : 286-292.

Miller, D. A. 1996. Allelopathy in forage crop systems. Agron. J. 88 : 854-859.

Rice, E. L. 1984. Allelopathy. 2nd ed. Academic Press, New York, USA.

SAS (Statistical Analysis Systems) Institute. 2000. SAS/STAT user's guide. Version 7. Electronic Version. Cary, NC, USA.

Schuler, P. 1990: Natural antioxidants exploited commercially. *In Food Antioxidants*, ed. B.J. F. Hudson, Elsevier Applied Science, London, pp. 99-191.

Stich, H. F., M. D. Rosin, C. H. Wu, and W. D. Powrie. 1981. A comparative genoxicity study of chlorogenic acid (3-O-caffeoylquinic acid). Mut. Res., 90 : 201-212.

Talaki, T. S., S. K. Dwivedi, and S. R. Sharma. 1995. *In vitro* and *in vivo* antitrypanosomal activity of *Xanthium strumarium* leaves. J. Ethnopharmacology 49 : 141-145.

Yoshida, T., K. Mori, T. Hatono, T. Okumura, I. Uehara, K. Komagoe, Y. Fujita, and T. Okuda, 1989. Studies on inhibition mechanism of antioxidation by tannins and flavonoids. V. Radical-scavenging effects of tannins and related polyphenols on DPPH radical. Chem. Pharm. Bull., 37 : 1919-1921.