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Assessment of Allelopathic Potential and Antioxidant Activity of Leaf Extracts from Three Compositae Plants

Sang-Uk Chon*† and Seong-Kyu Choi**

**Biotechnology Industrialization Center, Dongshin University, #880-4 Ansan-Ri, Noan-Myun, Naju-Si, Jeonnam 520-811, South Korea*
***Department of Oriental Medicine Resources, College of Nature Science, Sunchon National University, South Korea*

ABSTRACT: Some Compositae plants are known to contain biologically active substances that are allelopathic to weeds species as well as antioxidant to foods. Aqueous extracts from leaves of 3 plant species, *Cirsium japonica*, *Kalimeris yomena*, and *Lactuca sativa*, were bioassayed against alfalfa (*Medicago sativa*) to determine their allelopathic effects. The extracts applied on filter paper in bioassay significantly inhibited root growth of alfalfa. Extracts of 20 g dry tissue L^{-1} from *Lactuca sativa* showed the most inhibitory effect on alfalfa seedling growth and followed by *Cirsium japonica* and *Kalimeris yomena*. Oxidative stability by Rancimat method and antioxidant activity by TBA method for the ground samples were the greatest in *Lactuca sativa* although were less than that of a commonly used antioxidant, 1% ascorbic acid. Antioxidant activity of methanol extracts on storing meat was stably kept for 28 days and was excellent compared to control. These results suggest that three Compositae plants have potent allelopathic and antioxidant effects, and that their activities differ, depending on plant species.

Keywords **:** Compositae plant species, plant extracts, bioassay, allelopathy, antioxidant activity

Allelopathy, defined by Molisch (1937), is the chemical interaction between plants (and sometimes microbes and higher plants), including stimulatory as well as inhibitory influences. Allelopathy plays a key role in both natural and agro-ecosystems. Most of the studies on allelopathy have focused on its negative impacts. Recently, however, scientific studies have also concentrated on exploitation of its positive significant roles (Kohli *et al.*, 1998).

The major biosynthetic pathways leading to the production of allelochemicals or natural antioxidants are probably shikimic acid or acetate pathways (Rice, 1984). Einhellig *et al.*, (1970) reported that scopoletin, a coumarin derivative, inhibited dry matter production, leaf area expansion, and photosynthesis in tobacco (Nicotiana tabacum L.), sunflower (Helianthus annuus L.) and redroot pigweed (Amaranthus retroflexus L.). Einhellig and Stille (1979) found that ferulic acid and p-coumaric acid reduced leaf water potential and stomatal diffusive conductance in grain sorghum (Sorghum bicolor (L.) Moench.) and soybean.

A few studies on the antioxidant activity of Compositae plants have been reported. 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). Therefore, research on other natural antioxidants has gained momentum as they are considered, rightly or wrongly, to pose no health risk to consumers (Wanasundara and Shahidi, 1994; Wanasundara *et al.*, 1997). 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).

The objectives of this research were a) to compare allelopathic effects between aqueous extracts from 3 *Compositae* plant species, and b) to determine their antioxidant activities of the dried samples or extracts on storage of meat. This research promote a better understanding of allelopathy mechanisms in the agro-ecosystem and for maximizing utilization of biological resources as a natural antioxidant.

MATERIALS AND METHODS

Sampling and Preparation of Extracts

Three Compositae plant species including common thistle (*Cirsium japonica*), *Kalimeris yomena*, and lettuce (*Lactuca sativa*) grown in pasture and crop fields of Suncheon area, Korea were harvested at a vegetative stage on May to September 2001. The plant samples were separated into leaves, stems, and roots. The leaf samples were immediately oven-dried at 60°C for 5 days (Chon and Nelson, 2001), ground with a Wiley mill to pass through a 1-mm screen, and stored in a refrigerator at 2°C until required. Forty grams of dried leaves were separately extracted by soaking in 1L distilled water at 24°C for 24 hours in a shaker to give a concentra-

†Corresponding author: (Phone) +82-61-336-3109 (E-mail) chosu@lycos.co.kr
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tion of 40 g dry tissue L^{-1} (hereafter referred to as 'g L^{-1}'). The extract was filtered through two layers of cheesecloth to remove the fiber debris, and centrifuged at 5000 rpm (× 4530 *g*) for 2 hours. The supernatant was vacuum filtered again through Whatman No. 42 paper. EC, pH, and osmotic potential (Boyer and Knipling, 1965) were measured on stock extracts 2 days after extraction.

Ground leaf samples, on the other hand, were extracted with 95% methanol for 24 hours 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). The yield of dried extracts from the original plant leaves was 10-15%. Ground plant samples, aqueous and methanol extracts were used for alllelopathy bioassay and examination of antioxidant activity.

Allelopathic Effects of Aqueous Extracts against Alfalfa

Each stock extract from the three *Compositae* plant species was diluted appropriately with sterile distilled water to give the final concentrations of 10, 20, 30, and 40 g L^{-1}. Four milliliters of the extracts were pipetted onto Whatman No 1 filter paper in a Petri dish. Distilled water was used as a control. Alfalfa 'Vernal' seeds were surface sterilized with 0.525 g L^{-1} sodium hypochlorite for 15 min. Seeds were rinsed four times with deionized water, imbibed in deionized water at 22°C for 12 h, and carefully blotted using a folded paper towel. Twenty swelled seeds were evenly placed on filter paper wetted with extract in each petri dish. The Petri dishes were covered, sealed by wrapping in parafilm, and placed in a growth chamber at 24°C during the14-h light period and 22°C during the 10-h dark period. Plates were illuminated with 400 μmol photons m^{-2} s^{-1} Photosynthetically active radiation (PAR), provided by a mixture of incandescent and fluorescent lamps. Root and hypocotyl lengths were measured with all seedlings in each Petri dish at 5 days after placing seeds on the filter paper. The experiments were duplicated with four replications.

Antioxidant Activity of Dried Samples and Methanol Extracts

Dried ground samples and methanol extracts from three plants were exploited for investigation of antioxidant activity. To determine short term 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 ml at 120°C, with an air flow rate of 20 L h^{-1}. Oxidative stability was expressed as the oxidation induction time (hour).

For long storing term, antioxidant activity of the samples was investigated by TBA method. One hundred mg of the ground samples was mixed with 10 g storing pork meat and then stored at refrigerator. At 7 days after storage in meat, the mixed samples were added with 25 ml of 20% trichloroacetic acid (TCA), homogenized at 14,000 rpm for 2 min, and diluted with distilled water to give final volume into 100 ml. The diluted solution was filtered with Whatman No.1 filter paper. The 5ml-filtered solution was mixed with 5ml 2-TBA (0.005 M) and transferred into a test tube. The test tube was placed into dark room for 15 h at 25°C. Then the solution was measured the absorbance at 550 nm of UV-VIS Spectrophotometer.

To know how long the antioxidant activity of plant extracts keeps in meat, TBARS values of methanol extracts from the plant samples were measured every 7 days over 28 days and compared with control and a synthetic antioxidant, 1% ascorbic acid. 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 (MDA mg kg^{-1})=Absorbance× 5.2. All measurements were replicated with 3 times.

RESULTS AND DISCUSSION

Allelopathic Effects of Aqueous Extracts against Alfalfa

Electrical conductivity (EC), pH, and osmotic potential of the plant extracts at 40 g L^{-1} ranged from 1.5 to 2.7 mS m^{-1}, from 5.2 to 6.0, and from -0.01 to -0.11 MPa, respectively (Table 1). It was thought that values of EC, pH, and osmotic potential of plant extracts did not affect seedling growth of test plants, indicating allelopathic effects of plant extracts

Table 1. Electrical conductivity (EC), pH, and osmotic potential of aqueous plant extracts at 40 g L^{-1}.

Scientific name	EC (mS m^{-1})	pH	Osmotic potential (-MPa)
Cirsium japonica	2.68	5.80	0.110
Kalimeris yomena	1.49	5.23	0.004
Lactuca sativa	2.40	6.02	0.009
Mean	2.02	6.89	0.046

could go beyond the EC, pH and osmotic effects. Our experience in another study demonstrated that no significant growth reduction was observed at all concentrations of PEG 8000, corresponding to same osmotic potential of alfalfa leaf extracts. Alfalfa root lengths were similar for osmotic potentials of PEG 8000 below -0.20 MPa and then increased (Chon *et al.*, 2003). Thus, in our assay procedure we can conclude that osmotic stress has little effect on root growth at concentrations of extract normally used.

Plant extracts reduced root growth more than hypocotyl growth of alfalfa. Aqueous extracts from different plant species inhibited seedling lengths of alfalfa differently. *Lactuca sativa* extracts above 20 g L^{-1} had the greatest inhibitory effect on both the shoot and root growth of alfalfa, showing 100% reduction, and followed by *Cirsium japonica* and *Kalimeris yomena*. The degree of inhibition for all the extracts was increased with increasing the extract concentration (Fig. 1 and 2). At highest extract concentration of 40 g L^{-1}, *Cirsium japonica* and *Kalimeris yomena* extracts inhibited root length by 95 and 88%, while hypocotyls growth of alfalfa was less sensitive than was root growth, showing 51-85% reduction at 40 g L^{-1} (Fig. 1). Allelopathic activities were differently exhibited depending on plant species. Such differences might be related to specific allelopathic compounds being produced in larger quantities in certain species, imparting a higher level of allelopathy. Bendall (1975) reported allelopathic effects of water and ethanol extracts and residues in soil, and concluded that an allelopathic mechanism might be involved in the exclusion of some annual thistle, pasture, and crop species from a Compositae plant, Canada thistle (*Cirsium arvense*) areas. In greenhouse experiment, Stachon and Zimdal (1980) found that Canada thistle litter reduced the growth of redroot pigweed (*Amaranthus retroflexus* L.) and green foxtail (*Setaria viridis* L.) more than that of cucumber (*Cucumis sativus* L.) or barley (*Hordeum vulgare* L.).

Antioxidant Effects of Dried Samples and Methanol Extracts

Even though plant samples had less oxidative stability than ascorbic acid (2.4 h), difference in stability among plant species was apparently exhibited. The oxidative stability determined by the Rancimat method showed a variation between the different plant species, ranging from 0.91 to 1.55 h (Table 2). Ground *Lactuca sativa* leaf sample showed the most anti-

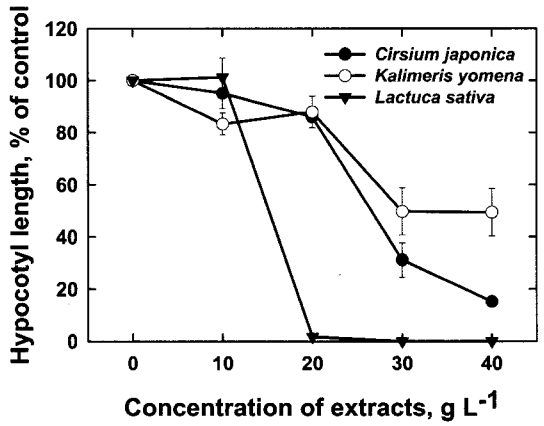

Fig. 1. Effects of aqueous extracts from three Compositae plant species on hypocotyl length of alfalfa at 6 days after seeding. Each bar represents standard error of the mean.

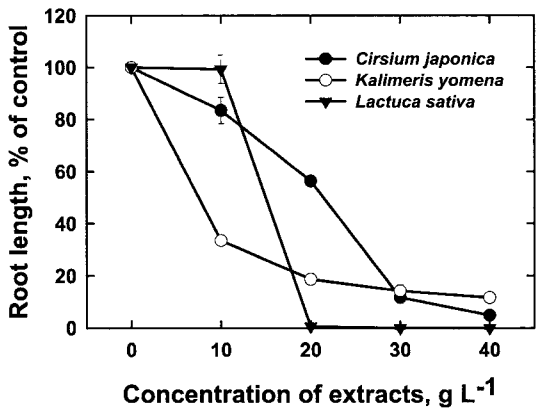

Fig. 2. Effects of aqueous extracts from three Compositae plant species on root length of alfalfa at 6 days after seeding. Each bar represents standard error of the mean.

Table 2. Oxidative stability and 2-thiobarbituric acid-reactive substances (TBARS) values of ground samples from 3 different Compositae plant species in meat.

Scientific name	Oxidative stability (hours)	TBARS values (MDA mg kg^{-1}) at 7 DAS*
Cirsium japonica	0.9091	0.2236
Kalimeris yomena	1.0947	0.2148
Lactuca sativa	1.5492	0.2111
Control	1.0000	0.6500
1% Ascorbic acid	2.4318	0.0655

*DAS: Days after storing meat at refrigerator (4°C).

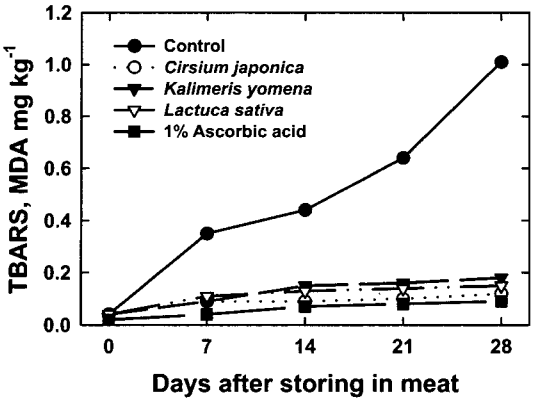

Fig. 3. Change in TBARS value of methanol extracts from leaf samples in meat measured every 7 days over 28 days.

oxidative effect. TBARS values showed higher in all ground samples at 7 days after storing than ascorbic acid as a control antioxidant (0.066). The lowest TBARS values were obtained from leaf of *Lactuca sativa* (0.211) at 7 days after storage, and followed by *Kalimeris yomena* (0.215) and *Cirsium japonica* (0.224). 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.

TBARS values of methanol extracts (0.09-0.18) at 7 days after storing were lower than those of ground samples (0.21-0.22), indicating methanol extracts had stronger antioxidant activity than ground samples (Table 2, Fig. 3). TBARS values of methanol extracts from plant samples were little higher than ascorbic acid as a synthetic antioxidant. Antioxidant effects of all the extracts on meat, however, was steadily kept over 4 weeks (Fig. 3) and not changed for 28 days. The result shows that antioxidative effect of methanol extracts from plant materials can be kept for long period, and the plant extracts would be a promising antioxidant as an alternative mean of synthetic antioxidants.

CONCLUSION

This study demonstrates allelopathic effects of three Compositae plant extracts on early seedling growths of alfalfa. Three Compositae plants also showed a potent antioxidant activity on storing meat through Rancimat and TBA methods. Allelopathic effects of plant extracts above 20 g L^{-1} were ranked in order of *Lactuca sativa* (highest), *Cirsium japonica* and *Kalimeris yomena* (least). Different compounds that cause allelopathy or antioxidant activity could be produced with different amount from each plant species. Such differences might be related to biologically active compounds being produced in larger quantities in certain plant species, imparting a higher level of biological activity. Therefore, the results may have important values for a mean of biological weed control as well as for a mean of alternative natural antioxidant based on natural plant extracts. In this study, however, it is difficult to apply our results to a production directly, because the concentration of allelopathic or antioxidative substances in samples is probably greater than what would be observed under natural conditions. Further investigations are also needed to determine the influence of variations according to growing conditions of sample plants on allelopathic or antioxidant activity, and to verify any correlation between allelopathy and antioxidant activity with identifying the active compounds involved in each phenomenon.

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REFERENCES

Bendall, G. M. 1975. The allelopathic activity of California thistle (*Circium arvense*) in Tasmania. Weed Res. 15 : 77-81.

Boyer, J. S. and E. B. Knippling. 1965. Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. *Proc. N.A.S.* 54 : 1044-1051.

Chon, S. U. and C. J. Nelson. 2001. Effects of experimental procedures and conditions on bioassay sensitivity of alfalfa autotoxicity. Comm. Soil Sci. *Plant Anal.* 32 : 1607-1619.

Chon, S. U., C. J. Nelson and J. H. Coutts. 2003. Responses of seed imbibition, germination and seedling growth to autotoxin from alfalfa leaves. *Agron. J. (in print).*

Einhellig, F. A. and M. L. Stille. 1979. Effects of ferulic and p-coumaric acids on plant water status. Abstract. Bot. Soc. Am., Misc. Ser. Publ. No. 157 : 40-41.

Einhellig, F. A., E. L. Rice, P. G. Risser and S. H. Wender 1970. Effects of scopoletin on growth, CO_2 exchange rates, and concentration of scopoletin, scopolin, and chlorogenic acids in tobacco, sunflower, and pigweed. Bull. *Torrey Bot. Club* 97 : 22-33.

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

Kajimoto, G., M. Nakamura and M. Yamaguchi. 1995. Changes in organic acid components of volatile degradation products during oxidation of oil, and effects of organic acid on increased conductivity determined by the Rancimat method. *J. Japanese Nutrition & Food.* 50 : 223-227.

Kohli, R. K., D. Batish and H. P. Singh. 1998. Allelopathy and its implications in agroecosystems. *J. Crop Production* 1 : 169-201.

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.

Molisch, H. 1937. Der Einfluss einer Pflanze auf die andere -Allelopathie. *Fischer. Jena.*

Rice, E . L. 1984. Allelopathy. 2nd ed. *Academic Press, NY.*

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

Stachon, W. J. and R. L. Zimdal.1980. Allelopathic activitity of Canada thistle (Circium arvense) in Colorado. Weed Sci. 28 : 83-86.

Wanasundara, P. K. J. P. D., F. Shahidi and V. K. S. Shukla.1997. Endogenous antioxidants from oilseeds and edible oils. Food Reviews International 13 : 225-292.

Wanasundara, U. N. and F. Shahidi. 1994. Canola extracts as an alternative natural antioxidant for canola oil. Journal of the American Oil Chemists's Society 71 : 817-822.

Witte, V. C., G. F. Krause and M. E. Bailey. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *J. Food Sci.* 35 : 582-587.