Allelopathic Potential and Antioxidant Activity of Leaf Extracts from Several Wild Plant Species

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ABSTRACT

Several wild plant species are known to contain biologically active substances that are allelopathic to weed species as well as antioxidant to foods. Plant extracts or residues from leaves of 4 species, *Achyranthes japonica* (speedwell), *Cucumis sativus* (Cucumber), *Trifolium repens* (white clover), and *Vicia angustifolia* (narrowleaf vetch) were bioassayed against *Medicago sativa* (alfalfa) or *Echinochloa crus-galli* (barnyard grass) to determine their allelopathic effects, and used for measurement of antioxidant activities. The aqueous extracts applied on filter paper significantly inhibited root growth of alfalfa. Aqueous extracts or residues from *V. angustifolia* showed the most inhibitory effect on alfalfa or barnyard grass seedling growth and followed by *A. japonica* and *T. repens*. Oxidative stability by Rancimat method, antioxidant activity by TBA (2-thiobarbituric acid) method and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity for the ground samples or methanol extracts were the greatest in *V. angustifolia*, although were less than those of commonly used antioxidants, BHT (butylated hydroxytoluene) and ascorbic acid. These results suggest that the wild plant species had potent allelopathic and antioxidant activities, and that their activities differed depending on plant species.

Key Words : Wild plant species, plant extracts, bioassay, allelopathy, antioxidant activity, Rancimat, TBA, DPPH scavenging activity.

INTRODUCTION

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 was later defined as any direct or indirect harmful or beneficial effect of one plant (donor plant) on another (recipient plant) through the production of chemical compounds that escape into the environment (Rice, 1984). Allelopathy plays an important role in both natural and agricultural 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

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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 (*Glycine max* L.).

Studies on the antioxidant activity of wild plant species have not been documented well. Phenolic compounds, such as butylated hydroxyanisole (BHA), 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 4 plant species including 3 wild plants and a vegetable crop cucumber, and b) to determine their antioxidant activities of the dried samples or methanol extracts through Rancimat method, TBAR method, and DPPH free radical scavenging method. This research would promote better understanding of allelopathy mechanisms in the natural ecosystem and of maximizing utilization of biological resources as a natural antioxidant.

MATERIALS AND METHODS

Sampling and Preparation of Extracts

Four plant species including *A. japonica, C. sativus, T. repens*, and V. angustifolia grown in pastures or in greenhouse of Suncheon area, Korea, were harvested at a vegetative stage on May to September 2001. The leaf samples were immediately oven-dried at 60℃ 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℃ until required. Forty grams of dried leaves were separately extracted by soaking in 1L distilled water at 24℃ for 24 hours in a shaker to give a concentration of 40 g dry tissue L^{-1} (hereafter referred to as 'gL^{-1}'). The extract was filtered through two layers of cheesecloth to remove the fiber debris, and centrifuged at 5000 rpm (x 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℃ 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 four 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℃ 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℃ during the14-h light period and 22℃ during the 10-h dark period. Plates were illuminated with 400 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) and provided by a mixture of incandescent and fluorescent lamps. Root and hypocotyl lengths were measured on all seedlings in each Petri dish at 6 days after placing seeds on the filter paper. The experiments were duplicated with four replications.

Effect of Residue Incorporation on Barnyard Grass Seedling Growth

Residues of each plant species were incorporated with a high organic matter-potting medium (Hanter 21, Seoul Korea) that contained 30% sphagnum peat moss, 50% vermiculite, 18% zeolite, and 2% sand v/v per 200 cm^3 pot, by vigorously shaking the components in plastic bags. The amount of plant residues in a soil medium used were; 0, 25, 50, 75 and 100 g kg^{-1}. After mixing, pots were filled with the medium mixture and five barnyard grass seeds per pot were planted. The pots were saturated with water by subsurface irrigation. During plant growth, the growing medium was maintained near field capacity by sub-irrigation without nutrition solution. The experiments were conducted in greenhouse for 20 days under greenhouse temperatures at 28℃ day/22℃ night. All plants were harvested to determine plant height, root length, and fresh and dry weights 20 days after seeding. Data were transformed to percent of control for analysis.

Antioxidant Activity of Dried Samples and Methanol Extracts

Ground samples and methanol extracts from four 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℃, with a flow rate of 20 L h^{-1}. Oxidative stability was expressed as the oxidation induction time (hour).

To know how long the antioxidant activity of plant extracts keeps in meat, TBAR (TBA-reactive substances) values of methanol extracts from the plant samples were measured at 0, 7 and 14 days after storing and compared with control and a synthetic antioxidant, 1% ascorbic acid. TBARS value tests were used to know the extent of lipid oxidation according to the method of Witte (1970). 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℃. Then the solution was measured the absorbance at 550 nm of UV-VIS Spectrophotometer. The values were calculated as follows; TBA (MDA mg kg^{-1})= Absorbance × 5.2. All measurements were replicated with 3 times.

In order to measure free radical scavenging activity, the DPPH free radical scavenging assay was carried out according to the procedure described by Blosi (1958). Extracts of each plant were added to a 1.5×10^{-4} M solution of DPPH in methanol and the reaction mixture was shaken vigorously and placed to react under dark

room. 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 $_{control}$} × 100. The antioxidant activity of each plants extract was expressed as % of DPPH activity.

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 2.7 to 3.3 mS m^{-1}, from 7.6. to 8.5, and from -0.14 to -0.16 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 could go beyond the EC, pH and osmotic effects. Although it is often assumed that the response of seeds or seedlings to plant extracts is due entirely to allelopathy, the extract may also exert negative osmotic effects on the test species (Bell, 1974). Our experience, in another study, demonstrated that no significant growth reduction was observed at all concentrations of PEG 8000, corresponding to the same osmotic potential of alfalfa leaf extracts. Osmotic stress less than - 0.2 MPa of PEG 8000 has little effect on root growth at the concentrations of extract normally used. This result suggests that reduction of root length can be explained mainly by allelopathic effect from extracts, not by osmotic effect (Chon *et al.*, 2004).

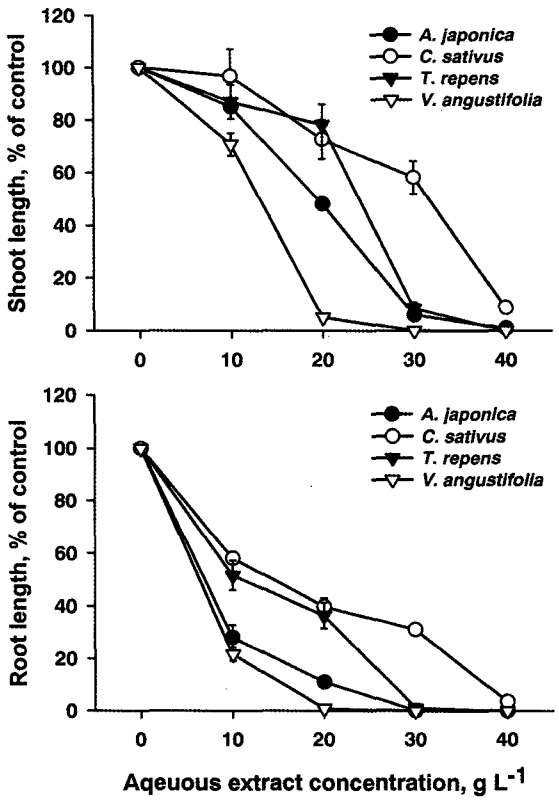

Fig. 1. Effects of aqueous extracts from four different plant species on shoot (A) and root (B) lengths of alfalfa as affected by different extract concentrations. The seedling growth was determined at 6 days after seeding on the filter paper wetted with the various extracts.

All the plant extracts reduced root growth more than hypocotyl growth of alfalfa. Aqueous extracts from different plant species inhibited seedling lengths of alfalfa differently. *V. angustifolia* extracts at all concentrations had the greatest inhibitory effect on the

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)
A. japonica	2. 8	7.8	0.142
C. sativus	3.3	8.5	0.157
T. repens	2.9	7.9	0.152
V. angustifolia	2.7	7.6	0.136
Distilled water	0.0003	6.7	0.001

shoot and root growth of alfalfa and followed by *A. japonica, T. repens*, and *C. sativus*. On the other hand, cucumber extracts were less inhibitory effect on alfalfa seedling growth than other plant extracts. The degree of inhibition for all the extracts was increased with increasing the extract concentration (Fig. 1). At highest extract concentration of 40 g L^{-1}, all the extracts inhibited both hypocotyls and root lengths by above 90%, and hypocotyl 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.).

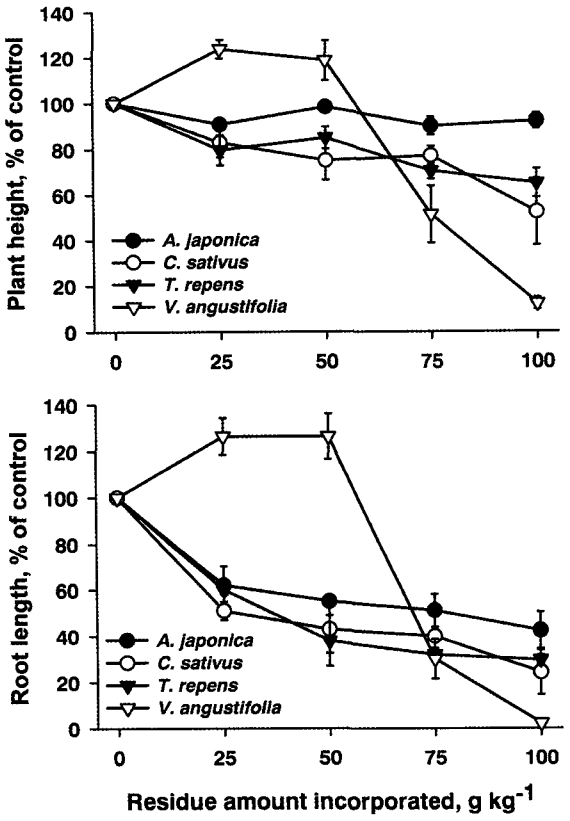

Fig. 2. Effects of plant residue incorporation on plant height (A) and shoot dry weight (B) of barnyard grass. The seedling growth was determined at 20 days after seeding on potting medium. Each bar represents standard error of the mean.

Effect of Residue Incorporation on Barnyard Grass Seedling Growth

Residue incorporation from different plant materials affected seedling growth of barnyard grass. All the residues reduced shoot and root lengths of barnyard grass by 10-90 and 55-100%, respectively, indicating that root growth was more inhibited by the residues than was shoot growth. The degree of inhibition increased with increasing the amount of residue incorporation. Residue incorporation from *V. angustifolia* above 75 g kg^{-1} significantly reduced plant height and root length of barnyard grass, while residues at 25 and 50 g kg^{-1} stimulated plant height and root length of barnyard grass by 20 and 30% over the control, respectively (Fig. 2). The results also indicate that any inhibition of weed growth should be due primarily to the presence of toxic compounds or excessive solutes within the ground plants. Walker and Jenkins (1986) demonstrated that sweet potato field residues were inhibitory to sweet potato (*Ipomoea batatas*) and cowpea (*Vigna unguiculata* (L.) Walp.) growth.

Antioxidant Effects of Dried Samples and Methanol Extracts

Even though plant samples had less oxidative stability than ascorbic acid (2.4 h), difference in

Table 2. Oxidative stability and 2-thiobarbituric acid-reactive substances (TBARS) values and DPPH radical scavenging activity of samples from 4 different plant species.

Scientific name	Oxidative stability	TBARS values (MDA mg kg-1)			Radical scavenging
	(hours)	0 DAS	7 DAS	14DAS	activity (%)
A. japonica	0.7083	0.0629	0.5574	0.6318	14.5
C. sativus	1.1629	0.0551	0.4139	0.6141	13.1
T. repens	1.1326	0.0759	0.4737	0.6235	17.0
V. angustifolia	1.9962	0.0400	0.4025	0.4794	22.8
Control	1.0000	0.1633	0.6500	0.7914	-
1% BHT	2.0903	-	-	-	-
1% Ascorbic acid	-	0.0239	0.0655	0.1238	30.3

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

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 0.71 to 1.99 h (Table 2). Among plant species *V. angustifolia* leaf sample showed the most antioxidative effect. *V. angustifolia* samples showed the lowest TBARS value (0.403) at 7 days after storing, even though was less than ascorbic acid as a control antioxidant (0.066). 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. All samples showed low DPPH radical scavenging activity in a species-dependent manner. Especially, *V. angustifolia* plant extracts had the highest DPPH radical scavenging activity (23%), lower than 1% ascorbic acid (30%). The results indicate that *V. angustifolia* plant extracts with most allelopathic effects showed highest antioxidant activity.

In conclusion, this study demonstrates allelopathic effects of four wild plant extracts on early seedling growths of alfalfa. The plants also showed a potent antioxidant activity through Rancimat, TBA methods and DPPH radical scavenging activity. Allelopathic effects of plant extracts were ranked in order of *V. angustifolia* (highest), *A. japonica, T. repens,* and *C. sativus* (least). Plant extracts from *V. angustifolia* showed the highest antioxidant activity, even though the activity was less than synthetic antioxidants. 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 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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