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# *In Vitro* Assessment on Biological Activities of Methanol Extracts from Several Compositae Edible Plants

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**Abstract -** Phytotoxicity, antioxidant activity, and cytotoxicity of the aqueous or methanol extracts from the young sprouts of the six Compositae medicinal plants were determined. Aqueous leachates at 40g dry tissue $L^{-1}$ (g $L^{-1}$) *Cirsium japonicum* and *Aster yomena* showed the highest inhibitory effect on alfalfa (*Medicago sativa* L.). Total phenolic content showed the highest amount in methanol extracts from *Ixeris dentata*, and followed by *A. yomena*, and *Cephalonoplos segetum*. Methanol extracts of *C. segetum* and *I. dentata* at 25$\mu$g $mL^{-1}$ exhibited the highest DPPH radical scavenging activity by 87.2, and 52.8%, respectively. By means of HPLC analysis, MeOH extracts of *C. segetum* had the highest amount of antioxidant chlorogenic acid. Based on MTT assay, the methanol extracts from *Y. sonchifolia* ($IC_{50}$ = 65.7$\mu$g $mL^{-1}$) showed the highest cytotoxicity against Calu-6. These results suggest that plant extracts had a dose-dependent biological potentials including phytotoxicity, antioxidant activity, and anticancer activity, and that their activities exhibited differently depending on plant species.

**Key words -** Compositae medicinal plants, Young sprout extracts, Allelopathy, Radical scavenging activity, Cytotoxicity

## Introduction

Plants have many phytochemicals with various bioactivities, including phytotoxic, antioxidant, anti-inflammatory, anticancer, and antidiabetic activities. Recently, there has been a worldwide trend towards the use of the phytochemicals from wild plants. Some plants have biologically-active substances that cause serious yield losses in spring sown-small grains row crops, and pastures (Hodgson, 1968), however, others such as Korean salad plants are being used as promising phytochemicals that are antioxidant to foods. Phenolic compounds occur ubiquitously in plants and are diversified group of phytochemicals derived from phenylalanine and tyrosine (Harborne and Turner, 1984; Shahidi and Maczk, 2004).

Allelopathy was defined by Molisch (1937) as a chemical interaction between plants including stimulatory as well as inhibitory influences. It plays a key role in natural as well as manipulated ecosystems such as agricultural areas (Rice 1984). Most of the studies on allelopathy have focused on its negative impacts, although recent studies have also concentrated on exploitation of its positive role. Improvement in crop productivity and environmental protection through eco-friendly control of weeds, pests, crop diseases, conservation of nitrogen in crop land, and synthesis of novel agrochemicals based on natural products have been the center of attention of scientist engaged in allelopathic research.

Free radical scavenging is generally the accepted mechanism for antioxidants inhibiting lipid oxidation. Antioxidants, inhibitors of lipid peroxidation, are important not only for food protection but also for the defense of living cells against oxidative damage. The model of scavenging stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time (Brand-Williams *et al.,* 1995) compared to other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability (Blois, 1958). 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

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food antioxidants because of their excellent results and low cost. When slightly larger doses (50mg/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 and 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).

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 browing treactions in potatoes, sunflower seed, leaf protein concentrates, milk proteins, and other foods (Deshpande *et al.,* 1984).

Also, a number of studies have suggested that regular consumption of tea decreased the risk of various types of cancers (Yang *et al.,* 2000; Kathiyar and Mukthar, 1996). The Compositae medicinal plants in this study have long been used as traditional seasoned salads, and were screened for biologically-active effects of functional foods (Cho, 2005). Earlier studies showed that extracts from *Areca catechu* var. *dulcissima* possess antidepressant properties (Dar and Khatoon, 2000). Au *et al.* (2001) suggested that the methanol extracts of *Paeonia suffruticosa* potently inhibit human immunodeficiency virus (HIV)-1 integrase. 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.

Therefore, the phytochemicals present in various Compositae medicinal plants may act as preventative or therapeutic agents similar to prescription drugs. The objective of this research was to determine phytotoxicity, total phenolic level, antioxidant activity, and cytotoxicity of aqueous or methanol extracts from young sprouts of the 6 Compositae medicinal plants. This will make attractive the research for antioxidant and scavenger natural compound, especially in Compositae medicinal plants.

# Materials and Methods

## Preparation of extracts

Young sprouts of the six Compositae medicinal plants, *Aster yomena, Cephalonoplos segetum, Cirsium japonicum, Ixeris dentata, Taraxacum mongolicum*, and *Youngia sonchifolia*, grown in a mountain area of the Suncheon City, Korea, were harvested at a vegetative stage in 2005. The samples were directly freeze-dried at -40℃ for 5 days, ground with a Wiley mill to pass a 1-mm screen, and stored in a refrigerator at 2℃ until used.

Forty grams of dried leaves were separately extracted by soaking in 1L distilled water at 24℃ for 24h in a shaker to give a concentration of 40g dry tissue $L^{-1}$ (hereafter referred to as 'g $L^{-1}$' ). The extract was filtered through two layers of cheesecloth to remove the fibre debris, and centrifuged at 5000rpm (x 4530g) for 2h. The supernatant was vacuum filtered again through Whatman No. 42 paper. The aqueous extracts from each plant were used for bioassay on allelopathic activity. The 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 methanol extracts was about 10% of the original plant sample. The methanol extracts from each plant were used for measuring DPPH radical scavenging activity, total phenolic content and cytotoxicity.

## Phytotoxic effects of aqueous extracts on alfalfa

Each stock extract from the 6 Compositae plant species was diluted appropriately with sterile distilled water to give the final concentrations of 10, 20, 30, and 40g $L^{-1}$. Four milliliters of the extracts were pipetted onto Whatman No. 1 filter paper in a Petri dish. Distilled water was the control. Twenty imbibed seeds of alfalfa (cv. Vernal) were evenly placed on filter paper wetted with extract in each Petri dish. The Petri dishes were placed flat in a growth chamber at 24℃ during the 14-h light period and 22℃ during the 10-h dark period. Plates were illuminated with 400$\mu$mol photons $m^{-2}$ $s^{-1}$ photosynthetically active radiation (PAR), provided by a mixture of incandescent and fluorescent lamps. Root and hypocotyl lengths were measured on all seedlings in each Petri dish 6 days after seeding on the filter paper. Data were transformed to percent of control for analysis as used. There were two experiments, each with four replications.

### 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). Each methanol extract at various concentrations (3.1, 6.3, 12.5, 25, and 50mg $100g^{-1}$) was added to a $1.5 \times 10^{-4}$ M solution of DPPH in methanol and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 520nm, 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 plant extracts was partially expressed as $IC_{50}$, which was defined as the concentration (in mg $100g^{-1}$) of extract required to inhibit the formation of DPPH radicals by 50%.

### Total phenolics content

The concentration of total phenolics (TP) was measured using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Briefly, 5mL of Nanopure water, 0.5~1.0ml of sample, and 1.0mL of Folin-Ciocalteu reagent were added to a 25mL volumetric flask. The contents were mixed and allowed to stand for 5-8min at room temperature. Next, 10mL of a 7% sodium carbonate solution was added, and followed by the addition of Nanopure water filled to volume. Solutions were mixed and allowed to stand at room temperature for 2h. Sample aliquots were filtered through a Whatman 0.45m poly (tetrafluoroethylene) filter prior to the determination of TP concentration using a UV-1650 spectrophotometer (Shimadzu, Japan) monitoring 640nm. TP content was standardized against ferulic acid and expressed as ppm of ferulic acid equivalents (FAE). The linearity range for this assay was determined as 0.5-5.0mg/L FAE ($R^2 = 0.9990$), giving an absorbance range of 0.050-0.555 AU.

### Identification and quantification of chlorogenic acid

The dried methanol extracts were redissolved in HPLC grade MeOH to give 1,000ppm for HPLC analysis. The standard phenol compound used for HPLC analysis was chlorogenic acid (Aldrich Co., USA). The chemical was purchased as high purity standards and the used solvents were HPLC spectral grade. Chlorogenic acid was identified by a high-performance liquid (HPLC) using SPP 10AVP (Shimadzu, Tokyo, Japan) with a flow rate of 1mL $min^{-1}$, the column was CAPCELL PAK C18 SG120 (4.6 × 250mm) and an autoinjector with a 10$\mu$l 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 275nm. Standard compounds were chromatographed alone and as mixtures. Retention times for the standard compound and the major peak in the extract was recorded. Chlorogenic acid was identified by retention times or standard addition, and amounts were calculated by comparing peak area with those of standards.

### Antiproliferative activity on human cancer cell lines

Anticancer activity of methanol extracts from medicinal resources plants on human cancer cell lines, Calu-6 for human pulmonary carcinoma and Calu-6 for human gastric carcinoma, were measured. The cell lines were purchased from Korea Cell Line Bank (KCLB) for MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells were grown in RPMI-1640 medium at 37℃ under 5% $CO_2$ in a humidified incubator. Cells were harvested, counted ($3 \times 10^4$ cells/mL), and transferred into a 96-well plate, and incubated for 24hr prior to the addition of test compounds. Serial dilutions of test samples were prepared by dissolving compounds in DMSO followed by dilution with RPMI-1640 medium to give final concentration at 25, 50, 100, 200, 400, and 800$\mu$g $ml^{-1}$. Stock solutions of samples were prepared cell lines at 90$\mu$l and samples at 10$\mu$l, and incubated for 72h. MTT solution at 5mg/ml was dissolved in 1mL of phosphate buffered saline (PBS), and 10$\mu$l of it was added to each of the 96 wells (Tian *et al.*, 2001). The wells were wrapped with aluminum foil and incubated at 37℃ for 4h. The solution in each well containing media, unbound MTT and dead cells were removed by suction and 150$\mu$l of DMSO was added to each well. The cytotoxicity was obtained by comparing the absorbance between the samples and the control. The values were then used to iteratively calculate the concentration of plant extracts required to cause a 50% reduction ($IC_{50}$) in growth (cell number) for each cell lines.

### Statistical analysis

All experiments had four replications. Data were subjected to analysis of variance. When F test was significant ($p<0.05$), means were separated based on the least significant difference (LSD) at the 0.05 probability level.

## Results and Discussion

### Phytotoxic effects of aqueous extracts on alfalfa

Plant extracts reduced root growth more than shoot growth of

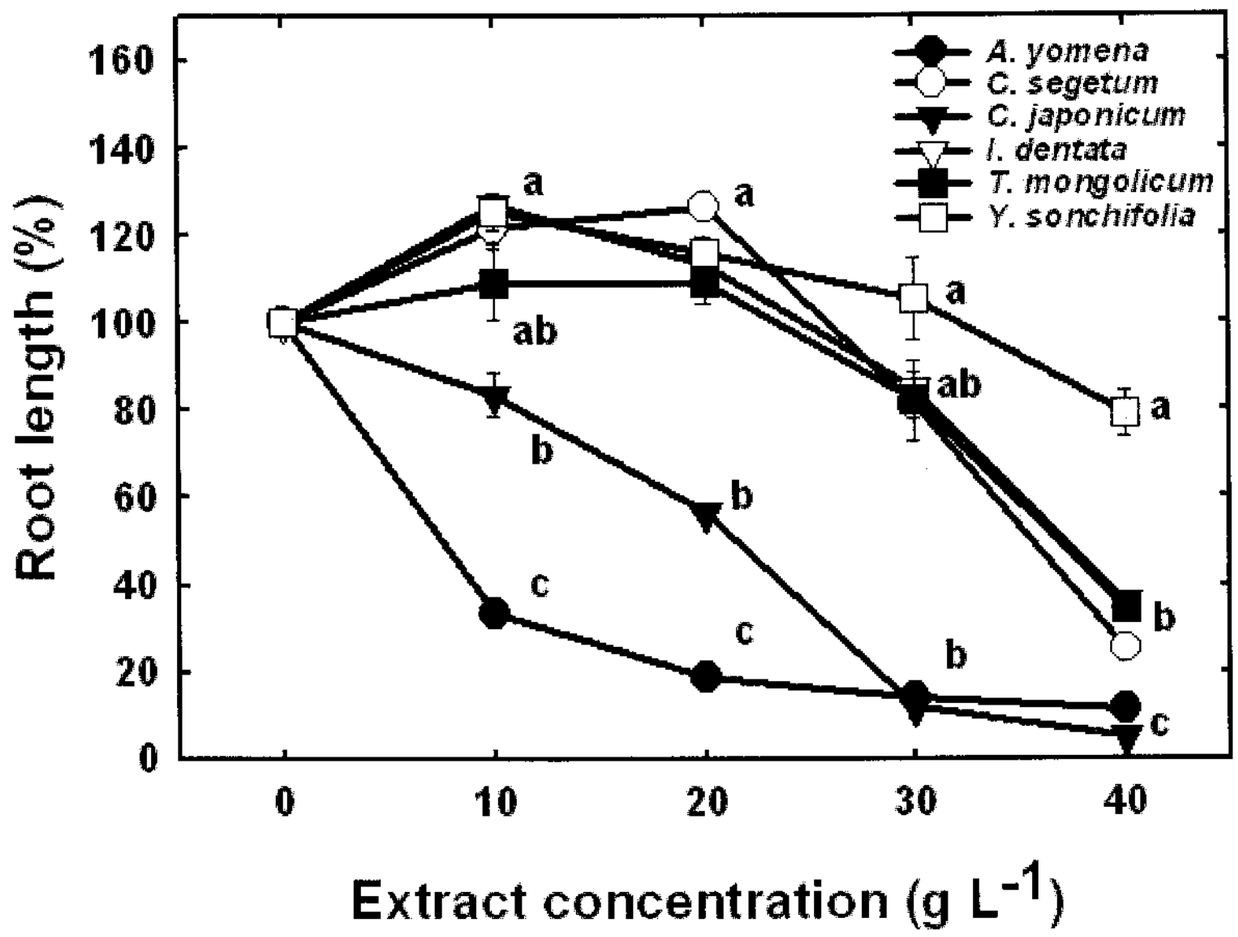


Fig. 1. Effects of aqueous extracts from the six compositae medicinal plants using young sprouts on alfalfa root length (mm) 6 days after seeding. Within an extract concentration, means followed by the same letter are not significantly different at $p<0.05$. Bars represent SE.

alfalfa (Data not shown). Aqueous extracts from different plant species inhibited alfalfa root growth differently (Fig. 1). *A. yomena* extracts above 10g $L^{-1}$ had the greatest inhibitory effect on root growth of alfalfa, and followed by *C. japonicum*. The degrees of their inhibition were increased with increasing concentration of the extract (Fig. 1). At the highest extract concentration of 40g $L^{-1}$, *A. yomena* and *C. japonicum* extracts completely inhibited root length, whereas *Y. sonchifolia, C. segetum, I. dentate and T. mongolicum* extracts at 10 and 20g $L^{-1}$ increased root length of alfalfa up to 9-27% over the control, indicating stimulation effects at low

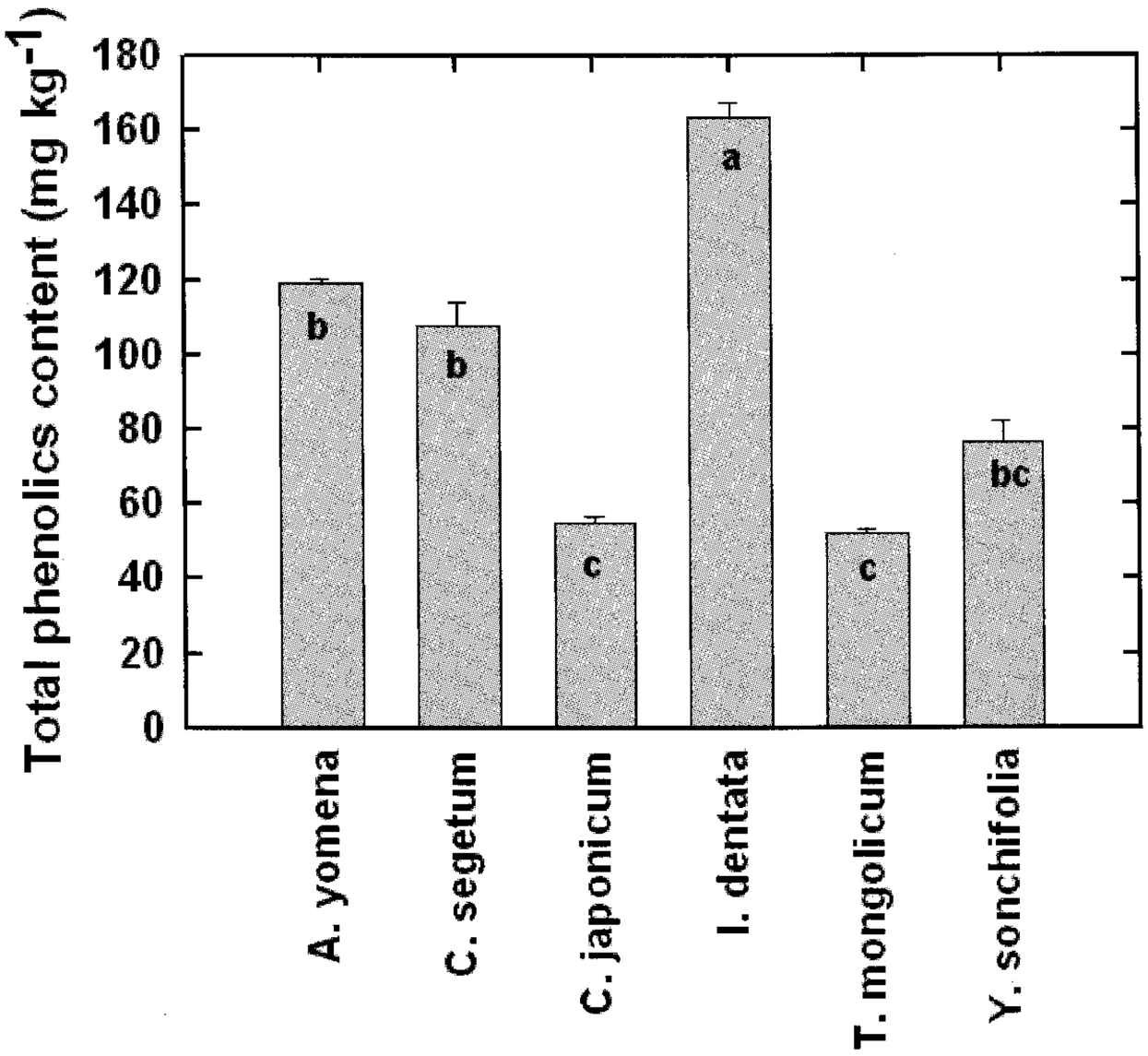


Fig. 2. Total phenolic content of methanol extracts from the young sprouts of the six Compositae medicinal plants using young sprouts. Means followed by the same letter are not significantly different at $p<0.05$. Bars represent SE.

concentration of extracts. Allelopathic activities appeared to be different depending on plant species. Such differences might be related to specific allelopathic compounds being produced in larger quantities in specific same species, imparting a higher level of allelopathy.

## DPPH radical scavenging activity

Methanol extracts of *C. segetum* ($IC_{50}$ = 9.8) had the highest DPPH radical scavenging activity, and followed by *I. dentate* ($IC_{50}$ = 24.6), *C. japonicum* ($IC_{50}$ = 33.0), and *A. yomena* ($IC_{50}$ = 34.1) (Table 1). Their values were much less than those of synthetic antioxidants Vitamin C and BHT, with $IC_{50}$ values of < 3.1 and 8.8 mg $100g^{-1}$, respectively. Methanol extracts of *C. segetum* at 25mg $100g^{-1}$ exhibited the highest DPPH radical scavenging activity by 87.2%. All samples of plant species showed DPPH radical scavenging activity in a dose-dependent manner. Lee *et al.* (2003) reported that the methanol extracts of nine medicinal plants traditionally used in Chinese medicine were screened for antioxidant activity versus resveratrol, and that relatively high levels of DPPH radical scavenging activity were detected in extracts of *Areca catechu* var. *dulcissima*, *Paeonia suffruticosa* and *Cinnamomun cassia* ($IC_{50}$ < 6.0$\mu$g $ml^{-1}$). The extracts of *Areca catechu* var. *dulcissima* showed higher antioxidant activity than resveratrol in all experiments.

## Total phenolics content

Total phenolic content showed the highest amount in methanol extracts from *I. dentata* (163.4mg $kg^{-1}$), and followed by *A. yomena* (119.2mg $kg^{-1}$), *C. segetum* (107.8mg $kg^{-1}$) and *Y. sonchifolia* (76.8mg $kg^{-1}$). However, *C. japonicum* and *T. mongolicum extracts* were the lowest (Fig. 3). The result was considerably consistent with the finding of DPPH radical scavenging activity (Velioglu *et al.,* 1998). Zhou and Yu (2006) also reported that total phenolic content of the tested vegetable extracts was correlated with the DPPH radical scavenging activity, suggesting that total phenolics can play a major role in the antioxidant activity of plant materials.

## Identification and quantification of chlorogenic acid

The major antioxidant substance chlorogenic acid presents in the 6 Compositae plant species was analyzed by HPLC using standard compound. The individual compound chlorogenic acid was identified. Chlorogenic acid was detected in *C. segetum* extracts (49.9mg $kg^{-1}$) as the greatest component and followed by *I. dentate* (34.2mg $kg^{-1}$). The results show that findings of quantification by fraction through HPLC were not associated with the allelopathy or 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 inter-related. 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).

## Antiproliferative activity on human cancer cell lines

A dose dependent inhibition of cell proliferation was observed for most of methanol extracts from Compositae traditional salad

Table 1. DPPH radical-scavenging activity of methanol extracts from the six Compositae medicinal plants using young sprouts. The activities were compared with synthetic antioxidants, Vitamin C and BHT

| Scientific name | Extract concentration, mg $100g^{-1}$ | | | | | $IC_{50}$ † |
|---|---|---|---|---|---|---|
| (Extracts) | 3.1 | 6.3 | 12.5 | 25 | 50 | value |
| *Aster yomena* | 0.8 | 6.5 | 16.8 | 38.2 | 70.3 | 34.1 |
| *Cephalonoplos segetum* | 18.8 | 34.0 | 61.6 | 87.2 | 86.1 | 9.8 |
| *Cirsium japonicum* | 7.5 | 12.9 | 23.1 | 40.9 | 71.1 | 33.0 |
| *Ixeris dentata* | 4.7 | 12.1 | 26.5 | 52.8 | 86.4 | 24.6 |
| *Taraxacum mongolicum* | 0.0 | 0.0 | 0.8 | 1.6 | 5.8 | >50.0 |
| *Youngia sonchifolia* | 13.3 | 13.3 | 20.8 | 37.5 | 61.3 | 39.1 |
| Vitamin C | 81.8 | 96.1 | 96.0 | 96.7 | 96.9 | < 3.1 |
| BHT | 15.6 | 33.5 | 55.2 | 81.3 | 92.4 | 8.8 |

† Extract concentrations, which show 50% DPPH radical scavenging activity, were determined by interpolation.

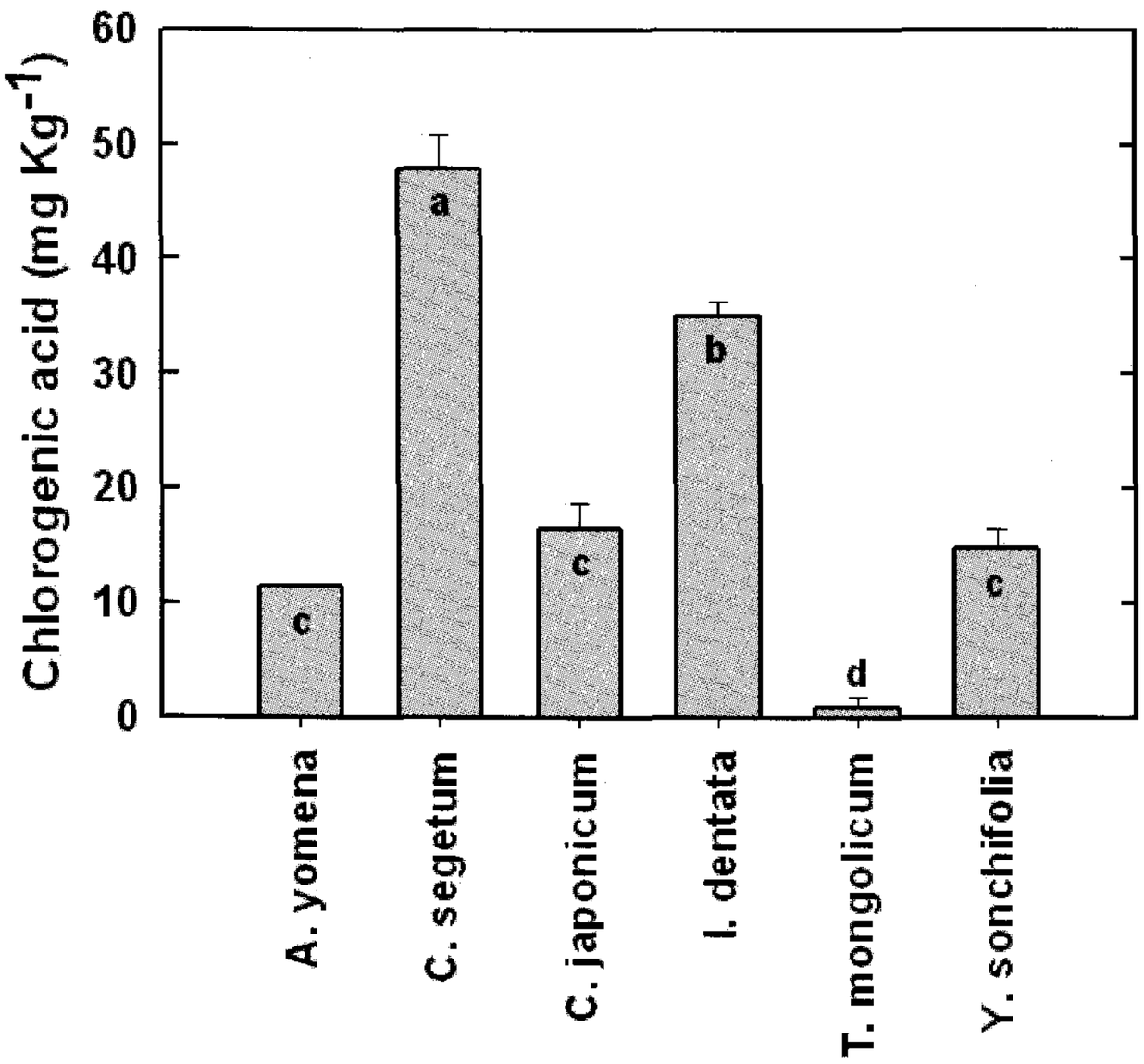


Fig. 3. Content of chlorogenic acid in methanol extracts from of the six Compositae medicinal plants using young sprouts. Means followed by the same letter are not significantly different at $p<0.05$. Bars represent SE.

plants tested. Methanol extracts from *Y. sonchifolia* exhibited the highest anticancer activity on Calu-6 tumor cell line, with $IC_{50}$ values of 59.6$\mu$g ml$^{-1}$, while the methanol extracts from *T. mongolicum* at the same concentration exhibited the lowest activity, with $IC_{50}$ values of 300.4$\mu$g ml$^{-1}$ (Fig. 4). These results, however, were not consistent with the findings of other biological activities including phytotoxicity, DPPH radical scavenging activity and total phenolic content.

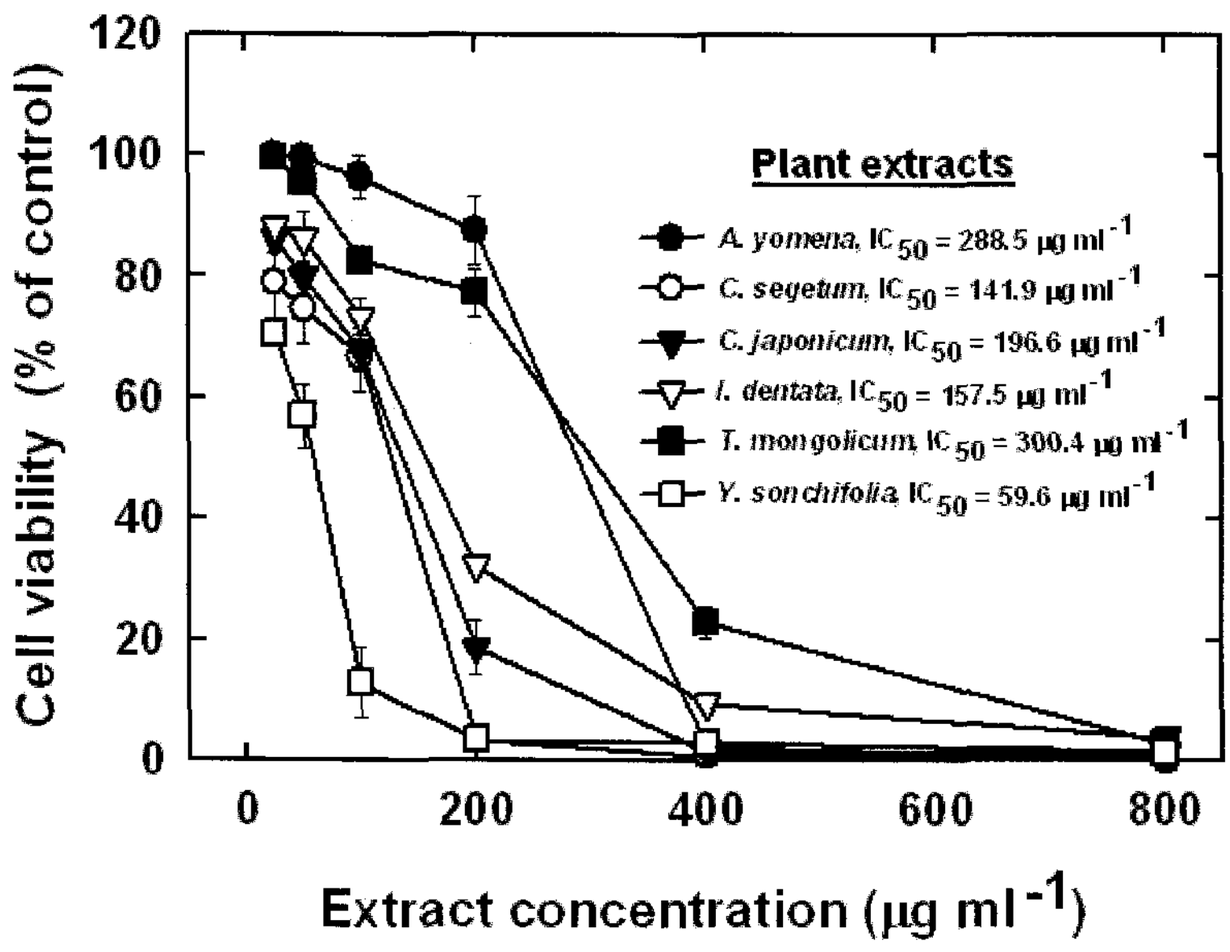


Fig. 4. Cytotoxic effect of methanol extracts from the six Compositae medicinal plants using young sprouts on human cancer cell line, Calu-6 for human pulmonary carcinoma. $I_{50}$ represents extract concentrations, which inhibit 50% growth of the cells, were determined by interpolation. Bars represent SE.

Methanol extracts from *Y. sonchifolia* and *C. segetum* showed the most potent cytotoxicity on Calu-6 cell line at 200$\mu$g ml$^{-1}$, and *A. yomena* and *C. japonicum* at 400$\mu$g ml$^{-1}$, and *T. mongolicum* and *I. dentata* at 800$\mu$g ml$^{-1}$, respectively. Manosroi *et al.* (2006), in the similar study, reported that anti-proliferative activity of essential oil extracted from 17 Thai medicinal plants on human mouth epidermal carcinoma (KB) and murine leukemia (P338) cell lines using MTT assay were investigated and the results showed that Guava (*Psidium guajava* L.) leaf and Sweet Basil oils exhibited the highest anti-proliferative activity in KB and P388 cell lines, respectively.

In conclusion, the six Compositae medicinal plants showed potent phytotoxicity, antioxidant activity, and anticancer activity. The results also suggested that plant extracts dose-dependently had the biological potentials, and that their activities exhibited differently depending on plant species. The plant extracts showed highest inhibitory effect on root growth of alfalfa at a filter paper. Total phenolic level measured was little correlated with biological activities. Difference in chlorogenic acid content may be explained degree of antioxidant activity. Causative phenol compounds exhibited different amounts depending on plant species.

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## Literature Cited

Au, T. K., T. B. Lam and T. B. Ng. 2001. A comparison of HIV-1 integrase inhibition by aqueous and methanol extracts of Chinese medicinal plants. Life Sci. 68: 1687-1694.

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

Brand-Williams, W., M. E. Cuvelier and C. Berset. 1995. Use of free radical method to evaluate antioxidant activity. Food Sci. Technol. (London). 28: 25-30.

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

Cho, J. Y., S. Y. Yang, S. O. Yu, B. W. Kim, H. G. Jang, S. U. Chon, Y. J. Park and B.G. Heo. 2005. The actual distributing states of the fresh wild vegetables at five-day traditional markets in Jeonnam district. Kor. J. Hort. Sci. Technol. 23: 396-401.

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.

Dar, A. and S. Khatoon. 2000. Behavioral and biochemical studies of dichloromethane fraction from the areca catechu nut. Pharmacology, Biochemistry and Behavior. 65: 1-6.

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.

Harborne, J. B. and M. Turner. 1984. Plant chemosystematics. Academic Press, London, UK.

Hodgson, J. M. 1968. The nature, ecology and control of Canada thistle. Tech. Bull. No. 1386, Agri. Res. Serve. USDA, USA.

Kathiyar, S. K. and H. Mukthar. 1996. Tea in chemoprevention of cancer: epidemiologic and experimental studies. Int. J. Onc. 8: 221-238.

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.

Lee, S. E., H. J. Hwang, J. S. Ha, H.S. Ha, H. S. Jeong and J. H. Kim. 2003. Screening of medicinal plant extracts for antioxidant activity. Life Sci. 73: 167-179.

Manosroi, J., P. Dhumtanom and A. Manosroi. 2006. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P338 cell lines. Cancer Letters 235: 114-120.

Molisch, H., 1937. Der Einfluss Einer Pflanze Auf die Andere-Allelopathie. Fischer, Jena, Germany.

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

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

Shahidi, F. and M. Naczk. 2004. Phenolics in food and nutraceuticals:

Sources, applications and health effects. CRC Press. Boca Raton, FL, USA.

Singleton, V. L. and J. A. Rossi. 1965. A colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16: 144-158.

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.

Tian, Q., E. G. Miller, H. Ahmad, L. Tang and B. S. Patil. 2001. Differential inhibition of human cancer cell proliferation by citrus limonoids. Nutr. Cancer 40: 180-184.

Velioglu, Y. S., G. Mazza, L. Gao and B. D. Oomah. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food. Chem. 46: 4113-4117.

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

Wanasundara, U. N. and F. Shahidi. 1994. Canola extracts as an alternative natural antioxidant for canola oil. J. Ame. Oil Chem. Soc. 71: 817-822.

Yang, C. S., J. Y. Chung, G. Yang, S. K. Chhanbra and M. J. Lee. 2000. Tea and tea polyphenols in cancer prevention. J. Nutr. 130 (2S Suppl.): 472S-478S.

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.

Zhou, K. and L. Yu. 2006. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT 39: 1155-1162.

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