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In Vitro Assay on Antioxidant Activity and Cytotoxicity of Methanol Extracts from Young Sprouts of Several Korean Salad Plants

Sang-Uk Chon*, Chan-Young Ahn[1] and Sook-Young Lee[2]
Callus Co. Ltd., Gwangju Institute of Science and Technology, Gwangju 500-712, Korea
[1]Jangseong High School for Industry, Jangseong 515-800, Korea
[2]Research Center for Proteineous Materials, Chonsun University, Gwangju 501-759, Korea

Abstract - Antioxidant activity, total phenolics level and cytotoxicity of the methanol extracts from the young sprouts of 5 Korean woody salad plants were determined. Methanol extracts of *Kalopanax pictus* had the highest DPPH radical scavenging activity, with an IC_{50} value of 23.5mg $100g^{-1}$, and followed by *Valeriana fauriei* (43.1mg $100g^{-1}$), and *Morus alba* (>100mg $100g^{-1}$). Total phenolic content showed the highest amount in methanol extracts from *Kalopanax pictus* (23.7mg $100g^{-1}$), and followed by *Valeriana fauriei* (22.7mg $100g^{-1}$), *Aralia elata* (16.8mg $100g^{-1}$) and *Morus alba* (14.2mg $100g^{-1}$). In a MTT assay, methanol extracts of *Aralia elata* with IC_{50} values of 151.0 and 140.7μg mL^{-1} showed the most potent cytotoxicity on Calu-6 and MCF-7, respectively. On the other hand, methanol extracts of *Kalopanax pictus* (IC_{50} = 96.5μg mL^{-1}) showed the highest activity against HCT-116, and followed by those of *Aralia elata* (123.3μg mL^{-1}), and *Actinidia arguta* (162.0μg mL^{-1}). Total phenolic content of the tested plant extracts was correlated with the DPPH radical scavenging activity, suggesting the phenolic compounds may contribute to the antioxidant properties of Korean salad plants.

Key words - Korean salad plants, DPPH radical scavenging activity, Total phenolic content, Cytotoxicity

Introduction

Well-being foods have received great attention as functional agents that improve biological functions of human body. Plants have many phytochemicals with various bioactivities, including 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 bio-functional 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 are considered as secondary metabolites that are synthesized by plants during normal development and in response to stress conditions such as infection, wounding, and UV radiation. These compounds occur ubiquitously in plants and are highly diversified group of phytochemicals derived from phenylalanine and tyrosine (Harborne and Turner, 1984; Shahidi and Maczk, 2004).

Free radical scavenging is generally the accepted mechanism for antioxidants inhibiting lipid oxidation. Antioxidants, inhibitors of lipid peroxidation, are important not only for food preservation 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 of food lipid oxidation. Although those antioxidants are considered as safe as 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 (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

*Corresponding author. E-mail : chonsu4100@yahoo.co.kr

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). Chen and Ho (1997) reported that in the study on antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds the scavenging activity order of the compounds was rosmarinic acid > caffeic acid phenethyl ester > caffeic acid > chlorogenic acid > tocopherol > ferulica cid > ferulic acid phenethyl ester > BHT.

The antioxidants present in fruits and vegetables limit the free radical initiation in the body and lower the incidence of various cancers. Vegetables, fruits and whole grains contain a wide variety of phytochemicals that have the potential to interfere with the development of cancer. The phytochemicals present in food and implicated to cancer prevention are isothiocyanates (cruciferous vegetables), carotenoids including alpha-carotene, gamma carotene, beta-cryptoxanthin, zeatxanthin,luttein, lycopene (tomatoes), resveratrol (grapes and wine), ellagic acid (various berries), glutathione-S-transferase (garlic), diallyl sulphide (garlic), genestin (soybean), curcumin (turmeric), indole-3-carbinol, inositol, organosulfur compounds, sulforaphane, squalene, and terpenes (Wattenberg, 1998). 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). Therefore, the phytochemicals present in various Korean medicinal plants, used as salad resources plants, may act as preventative or therapeutic agents similar to prescription drugs. The objective of this research was to determine their antioxidant activity, total phenolic level, and cytotoxicity of methanol extracts from young sprouts of the 5 Korean salad plants.

Materials and Methods

Preparation of methanol extracts

Young sprouts, including leaves and stems, of 5 Korean medicinal salad plants, *Morus alba, Valeriana fauriei, Actinidia arguta, Aralia elata, and Kalopanax pictus*, grown in a mountain area of the Suncheon City, Korea, were harvested at a vegetative stage on June, 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. 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 extracts (methanol extract) was about 10% of the original plant sample. The methanol extracts of each plant were used for measuring DPPH radical scavenging activity, total phenolic content and cytotoxicity.

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 (63, 125, 250, 500, and 1000μg mL^{-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 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 partially 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 %.

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 5-8 min 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).

Cytotoxicity

Anticancer activity of methanol extracts from medicinal resources plants on human cancer cell lines, Calu-6 for human pulmonary carcinoma, MCF-7 for human breast adenocarcinoma pleural effusion, and HCT-116 for human colon carcinoma, were measured. The cell lines were purchased from Korean 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 (3×10^4 cells/mL), and transferred into a 96-well plate, and incubated for 24 hr 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 800μg mL^{-1}. 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.

Results and Discussion

DPPH radical scavenging activity

Methanol extracts of *Kalopanax pictus* had the highest DPPH radical scavenging activity, with an IC_{50} value of 235μg mL^{-1}, and followed by *Valeriana fauriei* (431μg mL^{-1}), and *Morus alba* (>100 μg mL^{-1}) (Table 1). These values showed much lower activity than those of synthetic antioxidants Vitamin C and BHT, with IC_{50} values of < 63 and 113μg mL^{-1}, respectively. Methanol extracts of *Kalopanax pictus* and *Valeriana fauriei* at 50μg mL^{-1} exhibited the highest DPPH radical scavenging activity by 86.4 and 83.3%, respectively. However, *Actinidia arguta* and *Aralia elata* extracts showed the lowest activity. All samples of plant species showed DPPH radical scavenging activity in a dose-dependent manner. The results showed that various compounds that cause antioxidant activity could be produced with different amount from plant species. Results from this study suggest that *Kalopanax pictus* and *Valeriana fauriei* are the better dietary sources of natural antioxidant activities. 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} < 6.0μg 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 *Kalopanax pictus* (23.7mg 100g^{-1}), and followed by *Valeriana fauriei* (22.7mg 100g^{-1}), *Aralia elata* (16.8mg 100g^{-1}) and *Morus alba* (14.2mg 100g^{-1}) (Fig. 1). The result was highly 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.

Cytotoxicity

All the isolated were tested for their potential *in vitro* human

Table 1. DPPH radical-scavenging activity of methanol extracts from the 5 Korean traditional salad plants using whole part. Their activities were compared with synthetic antioxidants, Vitamin C and BHT

Scientific name (Extracts)	Extract concentration, μg mL^{-1}					IC_{50}† value
	63	125	250	500	1000	
Morus alba	0.0	2.0	6.2	17.1	32.4	> 1000
Valeriana fauriei	5.3	14.3	29.8	57.4	83.9	431
Actinidia arguta	0.0	0.0	0.0	0.6	2.7	> 1000
Aralia elata	0.0	0.0	2.7	4.2	8.7	> 1000
Kalopanax pictus	12.1	26.5	52.8	86.4	89.9	235
Vitamin C	96.1	96.1	96.7	96.9	97.7	< 63
BHT	33.5	55.2	81.3	92.4	95.6	113

† Extract concentrations which show 50% activity of DPPH radical scavenging.

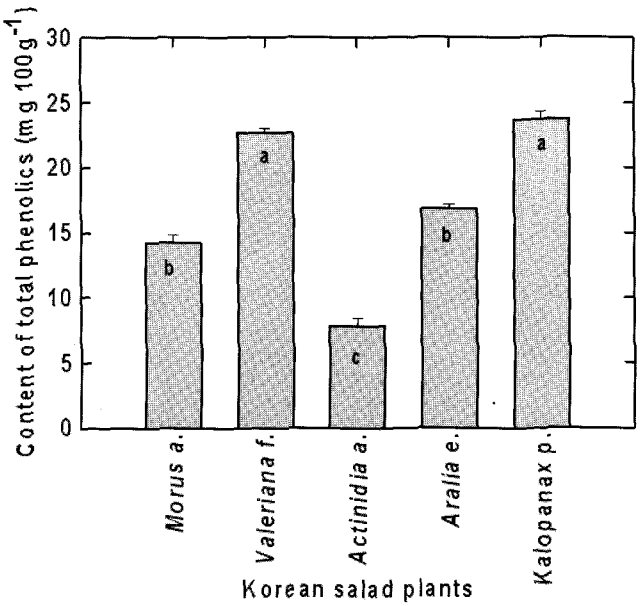

Fig. 1. Total phenolic content of methanol extracts from the aerial parts of 5 Korean medicinal salad plants. Means followed by the same letter are not significantly different at $p<0.05$. Bars represent SD.

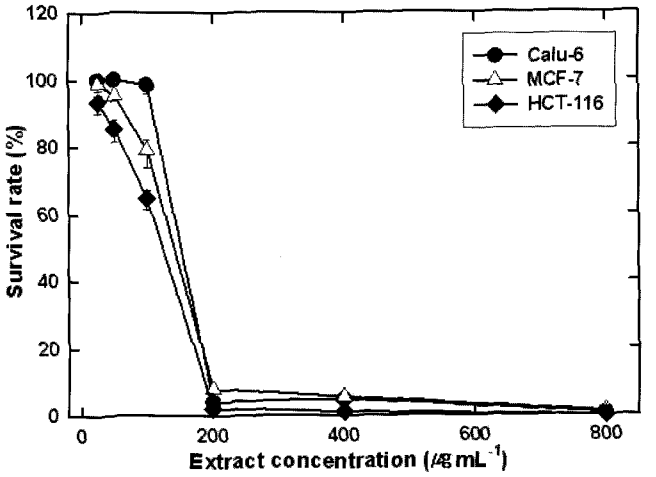

Fig. 2. Cytotoxic effect of methanol extracts from the aerial parts of *Petasites japonicus* (A) and *Sedum sarmentosum* (B) on human cancer cell lines, Calu-6, MCF-7, and HCT-116. Bars represent SD.

tumor cell antiproliferative activity on Calu-6, MCF-7, and HCT-116 tumor cell lines as determined by the MTT assay (Tian *et al.*, 2001). A dose dependent inhibition of cell proliferation was observed for most of methanol extracts from Korean medicinal plants tested. The extract from *Kalopanax pictus* was the most inhibitory on HCT-116 cell line whereas from *Morus alba* was the least. Methanol extracts at 200μg ml^{-1} from *Aralia elata* exhibited the highest anticancer activity on Calu-6, MCF-7, and HCT-116 tumor cell lines, by 95, 90, and 97%, respectively (Fig. 2). These results, however, were not consistent with the findings of DPPH radical scavenging activity or total phenolic content.

Methanol extracts from *Aralia elata* with IC_{50} values of 151.0 and 140.7μg mL^{-1} showed the most potent cytotoxicity on Calu-6 and MCF-7, respectively. On the other hand, methanol extracts from *Kalopanax pictus* (IC_{50} = 96.5μg mL^{-1}) showed the highest activity against HCT-116, and followed by those of *Aralia elata* (123.3μg mL^{-1}), and *Actinidia arguta* (162.0μg mL^{-1}) (Table 2). 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 5 Korean salad plants showed different antioxidant activity through measurement of DPPH free radical scavenging activity. The medicinal salad plants dose-dependently increased DPPH free radical scavenging activity, *in vitro*. The results showed that total phenolics level was highly correlated with the free radical scavenging activity. Phenol compounds that cause

Table 2. Cytotoxic effect of methanol extracts from the aerial parts of 5 Korean salad plants on three human cancer cell lines

Scientific name (Extracts)	IC50[†] (μg mL^{-1})		
	Calu-6[‡]	MCF-7[‡]	HCT-116[‡]
Morus alba	270.1	295.3	332.3
Valeriana fauriei	280.2	380.9	218.0
Actinidia arguta	369.7	666.9	162.0
Aralia elata	151.0	140.7	123.3
Kalopanax pictus	156.5	313.5	96.5

[†]Extract concentrations which inhibit 50% growth of the cells.
[‡]Calu-6 for human pulmonary carcinoma, MCF-7 for human breast adenocarcinoma pleural effusion, and HCT-116 for human colon carcinoma.

the DPPH free radical scavenging activities could be produced with different amounts depending on plant species. Such differences might be related to compounds being produced in larger quantities in certain plant species. The results suggest that 5 Korean salad plants using young sprouts had the potent antioxidant activity and cytotoxicity with important values for an alternative natural based on natural plant extracts.

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