Plant Foods Hum Nutr (2009) 64:25–31
DOI 10.1007/s11130-008-0092-x

ORIGINAL PAPER

Total Phenolics Level, Antioxidant Activities and Cytotoxicity of Young Sprouts of Some Traditional Korean Salad Plants

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Published online: 19 November 2008

Abstract The aim of this investigation was to study the antioxidant and anticancer activities of young sprouts of some traditional Korean salad plants. Total phenolics, antioxidant and anticancer activities of the methanol extracts from young sprouts of 11 salad plants were determined. The highest amount of phenolics was found in methanol extracts of *Euonymus alatus* (235.7 mg kg^{-1}), followed by *Hypericum ascyron* (197.1 mg kg^{-1}), *Zanthoxylum piperitum* (194.1 mg kg^{-1}) and *Zanthoxylum schinifolium* (142.5 mg kg^{-1}). Methanol extracts of *E. alatus*, *H. ascyron*, and *Z. piperitum* at 63 mg kg^{-1} exhibited the highest dose-depend DPPH radical scavenging activity by 91.2, 91.2 and 83.9%, respectively. According to the MTT results, the methanol extracts from *Stellaria aquatica*, *Eleutherococcus sessilifolrus* and *Z. schinifolium* showed the highest anticancer activities against Calu-6 (IC50< 25.0 μgml^{-1}) and from *S. aquatica*—the highest anticancer activities against SNU-601 (153.3 μgml^{-1}), following by *E. sessilifolrus* (196.7 $\mu g\ ml^{-1}$) and *Amaranthus mangostanus* (303.1 μgml^{-1}). Total phenolics were highly correlated with the DPPH, suggesting that they contribute to the antioxidant properties of the studied plants. In conclusion: young sprouts of Korean salad possess antioxidant and anticancer properties and could be used as a supplement to proper drugs.

Keywords Antioxidant and anticancer properties · Methanol extracts · Salad plants

Abbreviations

BHA	butylated hydroxyanisole—synthetic antioxidant
BHT	butylated hydroxytoluene—synthetic antioxidant
Calu-6	human pulmonary carcinoma—human cancer cell line
DMSO	dimethyl sulfoxide—commercial solvent
DPPH, 1	1-diphenyl-2-picrylhydrazyl method—free radical scavenging activity test
FAE	ferulic acid equivalents—determination unit
LDL	low density lipoprotein—one of the plasma lipids
MTT	(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)—assay for determination of anticancer activity
RPMI-1640	A medium developed by Moore et. al. at Roswell Park Memorial Institute, and used for the culture of human normal and neoplastic leukocytes

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SMU-601	human gastric carcinoma—human cancer cell line
TBHQ	*tert*-butylhydroquinone—synthetic antioxidant
TP	total phenolics—one of the major natural antioxidants

Introduction

Because of the effectiveness and minimal side effects in prevention and treatment of some diseases, we are witnesses of the growing use of natural products [1–4]. The effectiveness of the plant products is connected to their bioactive compounds, mainly antioxidant phenolics [5–8]. Antioxidant phenolics showed many important properties hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, anti-thrombotic and anti-hyperhomocystic and others [9–14]. It was shown that some plants possess also cytotoxic properties [15–20]. The anticancer properties of some plants were known from ancient times [17]. According to Madari and Jacobs [17] these plants were used by traditional healers of ancient Persia. To support the ancient claims and to evaluate the reported cytotoxic effects these authors investigated six herbal formulations that contain 39 different species from 21 plant families. Previous phyto-chemical analyses have shown that a number of plant species are rich in coumarin compounds that have potential antineoplastic or cytotoxic properties.

Most investigations of the cytotoxic properties of plants were conducted by the scientists of the Far East [15–20]. So, Ginseng radix the root of Panax ginseng, the best-known oriental medicinal herbs with numerous therapeutic applications, was investigated by Kim et al. [15]. They used 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, flow cytometry, DNA fragmentation assay, reverse transcription-polymerase chain reaction (RT-PCR), Western blotting, and caspase-3 enzyme assay in order to assess whether Ginseng radix possesses a protective effect against 1-methyl-4-phenylpyridine (MPP1)-induced cytotoxicity in PC12 neuronal cells. Cells treated with MPP1 exhibited various apoptotic features, while cell pretreated with *Ginseng radix* prior to MPP1 exposure showed a decrease in the occurrence of apoptotic features. The SRB assay was used to test cytotoxicity against three human cancer cell lines and one normal cell line of 11 Thai medicinal plant species used by traditional doctors in treating cancer patients [16]. The extraction procedures used were similar to those practiced by Thai traditional doctors (ethanolic and water extracts). Extracts were tested against the human large cell lung carcinoma cell line COR-L23, the human breast adenocarcinoma cell line MCF-7 and human colon adenocarcinoma cell line LS-174T and normal human keratinocytes SVK-14. The results showed that three plants; *Dioscorea membranacea* Pierre ex Prain & Burkill, *Dioscorea birmanica* Prain & Burkill (Dioscoreaceae) and *Siphonodon celastrineus* Griff. (Celastraceae), exhibited high cytotoxic activity showing a certain degree of selectivity against the different cell types.

In the below cited study the anticancer potential of 11 plants used in Bangladeshi folk medicine was evaluated [20]. The plant extracts were tested for cytotoxicity by the brine shrimp lethality assay, sea urchin eggs assay, hemolysis assay and MTT assay using tumor cell lines. The extract of *Oroxylum indicum* showed the highest toxicity on all tumor cell lines tested, with an IC_{50} of 19.6 μg/ml for CEM, 14.2 μg/ml for HL-60, 17.2 μg/ml for B-16 and 32.5 μg/ml for HCT-8. On the sea urchin eggs, it inhibited the progression of cell cycle since the first cleavage (IC_{50}=13.5 μg/ml). The extract of *Aegle marmelos* exhibited toxicity on all used assays, but in a lower potency than *Oroxylum indicum*.

The Korean medicinal plants have been used for a long time as traditional seasoned salads, and were already studied for their bioactive properties [21]. Recently we published two papers describing inter alia anticancer activity of leaf parts of some traditional Korean salad plants [22, 23]. It was interesting to know if also young sprouts of these plants possess the above mentioned properties. For this purpose we decided to determine their total phenolics level, antioxidant activities and cytotoxicity. In order to receive reliable data Folin–Ciocalteu, DPPH and MTT assays were used for determinations of total phenolics content, for assessment of the radical scavenging and anticancer activities, respectively. In addition, we decided to compare the results of the investigation of the same salad plants harvested in 2006–2007 years. As far as we know, there are no published results of similar investigation.

Materials and Methods

Materials

Chemicals

Folin–Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ascorbic acid were obtained from Sigma Chemical Co., St. Louis, MO, USA. The cell lines were purchased from Korean Cell Line Bank for MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay.

Plant Material

Young sprouts of 11 Korean medicinal salad plants (*Stellaria aquatica*, *Amaranthus mangostanus*, *Solidago virgaurea*, *Hypericum ascyron*, *Cephalonoplos segetum*, *Eleutherococcus sessilifolrus*, *Syneilesis palmata*, *Zanthoxylum schinifolium*, *Ainsliaea acerifolia*, *Zanthoxylum piperitum*, and *Euonymus alatus*) grown in a mountain area of the Suncheon City, Korea, were harvested at a vegetative stage on April to June, 2006 and 2007. The samples were directly freeze-dried at −40 °C for 5 days, ground with a Wiley mill to pass a 1-mm screen, and stored in a refrigerator at 2 °C until used. The samples were extracted with 95% methanol at room temperature of 22 °C. Then the extracts were 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). After evaporation, the yield of dried methanol extract was about 10% of the original plant sample. The methanol extracts from each plant were used for determination of total phenolic content, DPPH radical scavenging activity and cytotoxicity.

Methods

Total Phenolics Content

The concentration of total phenolics (TP) was measured by Folin–Ciocalteu assay [24], using a UV-1650 spectrophotometer (Shimadzu, Japan) monitoring 640 nm. TP content was standardized against ferulic acid and expressed as mg kg^{-1} of ferulic acid equivalents (FAE). The linearity range for this assay was determined as 0.5–5.0 mg/l FAE (R^2=0.9990).

Radical-scavenging Activity

Methanol extracts of the studied plants at various concentrations (31, 63, 125, 250, and 500 mg kg^{-1}) were added to a 1.5×10–4 M solution of 1, 1-diphenyl-2-picrylhydrazyl in methanol [25]. 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 plant extracts was partially expressed as IC50, which was defined as the concentration (mg kg^{-1}) of extract required to inhibit the formation of DPPH radicals by 50%.

Anticancer Activity

Anticancer activity of methanol extracts of the studied plants on human cancer cell lines (Calu-6 for human pulmonary carcinoma and SMU-601 for human gastric carcinoma) were measured using MTT assay. Cells were grown in RPMI-1640 medium at 37 °C 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 h 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 μg ml^{-1}. Stock solutions of samples were prepared cell lines at 90 μL and samples at 10 μL, and incubated for 72 h. MTT solution at 5 mg/mL was dissolved in 1 ml of Phosphate Buffer Solution (PBS), and 10 μl of it was added to each of the 96 wells [30]. The wells were wrapped with aluminum foil and incubated at 37 °C for 4 h. The solution in each well containing media, unbound MTT and dead cells were removed by suction and 150 μL of DMSO was added to each well. The plates were then shaken and optical density was recorded using a micro plate reader at 540 nm. Distilled water was used as positive control and DMSO as solvent control. Controls and samples were assayed in duplicate for each concentration and replicated three times for each cell line. 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

To verify the statistical significance, mean±SD of three independent measurements were calculated. Differences between groups were tested by two-way ANOVA. When the means were significant at *F*-test, the means were separated by least significant difference (LSD) test. In the assessment of the antioxidant potential, Spearman correlation coefficient (*R*) was used. Linear regressions were also calculated. The *P* values of <0.05 were considered significant.

Results

Total Phenolics Content

The highest content of total phenolics was in *E. alatus* (235.7 mg kg^{-1}), followed by *H. ascyron* (197.1 mg kg^{-1}), *Z. piperitum* (194.1 mg kg^{-1}) and *Z. schinifolium* (142.5 mg kg^{-1}). The differences were significant (P<0.05).

The lowest content of total phenolics was in *S. aquatica* and *A. mangostanus* (both 48 mg kg^{-1}; Fig. 1). The differences were significant (P<0.05). These results show that contents of total phenolics depend on plant species.

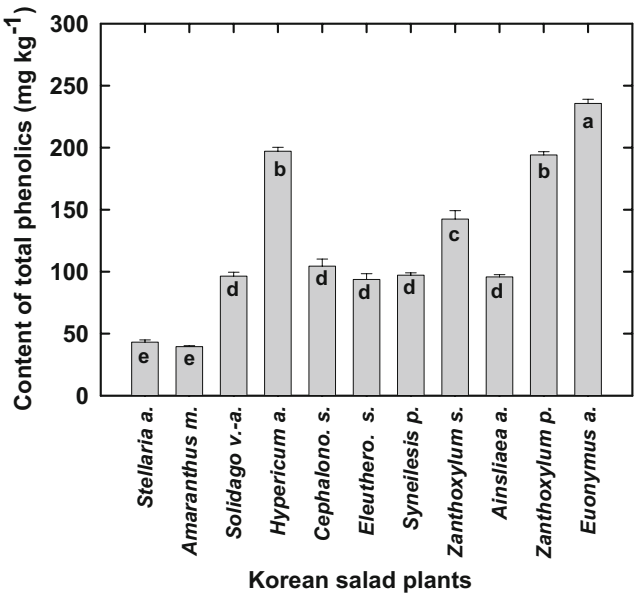

Fig. 1 Total phenolic content of methanol extracts from the young sprouts of 11 Korean salad plants. *Bars* marked with same letters are not significantly different ($P<0.05$)

DPPH Radical Scavenging Activity

The results of the determination of DPPH radical scavenging activity were summarized in the Table 1. As can be seen, methanol extracts of *E. alatus* in low extracts concentrations (31 and 63 mg kg^{-1}) had the highest DPPH radical scavenging activity, followed by *H. ascyron* and *Z. piperitum* in the same concentrations. The differences were significant ($P<0.05$). As can be seen their values showed higher activity than the synthetic antioxidant BHT, with IC_{50} values of 104.4 mg kg^{-1}. Methanol extracts of *E. alatus*, *H. ascyron* and *Z. piperitum* at 63 mg kg^{-1} exhibited the highest DPPH radical scavenging activity by 91.2, 91.2 and 83.9%, respectively. DPPH radical scavenging activity for *S. aquatica* and *A. mangostanus* extracts was the lowest ($P<0.05$). All samples of plant species proved that DPPH radical scavenging activity were dose-dependent. Results from this study suggest that that among the studied plants *E. alatus*, *H. ascyron* and *Z. piperitum* are the preferable sources of natural antioxidant.

Anticancer Effect of Methanol Extracts

As can be seen (Table 2) the highest influence on the Calu-6 (human pulmonary carcinoma cell line) was exercised by *S. aquatica*, *E. sessilifolrus* and *Z. schinifolium* (<25.0), following by *Ainsliaea acerifolia* (25.7±1.3) and on SNU-601—by *S. aquatica* (153.3±9.1), following by *E. sessilifolrus* (196.7±5.1). A dose dependent inhibition of cell proliferation was observed in most of methanol extracts tested in this study. It was recorded that the methanol extracts exhibited more inhibition on Calu-6 cell line than of SNU-601. Methanol extracts at 200 μg ml^{-1} from *S. aquatica* exhibited the highest anticancer activity on Calu-6 and SNU-601 tumor cell lines, by 98 and 81%, respectively, and the methanol extracts from *H. ascyron* at the same concentration exhibited the lowest activity by only 44 and 46%, respectively (Fig. 2). These results, however, were not consistent with the findings of DPPH radical scavenging activity or total phenolic content.

Table 1 DPPH radical-scavenging activity in methanol extracts from the young sprouts of 11 Korean traditional salad plants in comparison with synthetic antioxidants

Scientific names	Extract concentration, mg kg^{-1}					
	31	63	125	250	500	IC50[a]
Stellaria aquatica	1.4±0.1a	1.5±0.2a	3.7±0.2a	6.3±0.2a	9.0±0.2a	2751.0
Amaranthus mangostanus	3.3±0.1a	4.1±0.8a	6.5±0.3a	7.8±0.5a	14.0±1.0a	2402.0
Solidago virgaurea	15.2±0.7b	26.9±1.0b	48.7±1.8b	83.6±2.4b	89.3±3.0b	122.8
Hypericum ascyron	63.2±1.6e	91.2±1.9d	91.9±2.9e	91.4±4.0c	90.4±4.1b	16.9
Cephalonoplos segetum	18.8±1.8b	34.0±2.1b	61.7±3.3c	87.2±4.0b	86.1±3.9b	87.8
Eleutherococcus sessilifolrus	16.4±0.3b	26.7±0.7b	50.3±1.6b	83.2±2.2b	86.8±3.1b	113.5
Syneilesis palmata	17.9±0.8b	30.0±1.6b	54.2±2.1b	83.5±3.1b	89.3±3.9b	102.7
Zanthoxylum schinifolium	26.6±2.2c	50.0±3.2c	85.2±4.1d	88.0±4.1b	86.6±4.1b	63.1
Ainsliaea acerifolia	13.9±2.5b	23.3±3.3b	44.3±4.1b	78.3±4.1b	85.6±4.1b	134.4
Zanthoxylum piperitum	49.4±4.2d	83.9±4.2d	91.7±4.2e	92.1±4.2c	90.7±4.3b	27.0
Euonymus alatus	75.9±0.7f	91.2±1.3e	91.8±2.0e	91.1±2.5c	89.2±3.6b	7.2
Ascorbic acid	81.8±3.7g	96.1±4.5f	96.1±4.5e	96.7±4.5d	96.9±4.5c	5.6
BHT	15.6±1.1b	33.5±2.1b	55.2±2.8b	81.3±3.7b	92.4±4.3b	104.4

Values are means±SD of three measurements. Means in columns without letters in common differ significantly ($P<0.05$).
[a] Extract concentrations, which show 50% activity of DPPH radical scavenging, were determined by interpolation.

Table 2 Anticancer effect of methanol extracts from the young sprouts of 11 Korean salad plants on two human cancer cell lines

Scientific names	IC[50a] (μg mL^{-1})	
	Calu-6[b]	SNU-601[b]
Stellaria aquatica	<25.0±1.1a	153.3±9.1a
Amaranthus mangostanus	176.4±5.2d	303.1±12.2b
Solidago virgaurea	40.9±2.8b	308.8±13.3b
Hypericum ascyron	>800.0±24.2g	738.6±21.8d
Cephalonoplos segetum	141.9±6.1c	412.9±8.1c
Eleutherococcus sessilifolrus	<25.0±1.7a	196.7±5.1a
Syneilesis palmata	120.9±2.2c	304.2±9.3b
Zanthoxylum schinifolium	<25.0±1.7a	345.1±10.0c
Ainsliaea acerifolia	25.7±1.3a	445.6±11.0c
Zanthoxylum piperitum	470.4±13.1f	349.0±9.1b
Euonymus alatus	297.2±6.2e	412.5±12.3c

Values are means±SD of three measurements. Means in columns without letters in common differ significantly ($P<0.05$).
[a] Extract concentrations, which inhibit 50% growth of the cells, were determined by interpolation
[b] Calu-6 is human pulmonary carcinoma and SNU-601 is human gastric carcinoma

Discussion

In the last decades we are witnesses of the growing use of natural products because of their effectiveness and minimal side effects in prevention and treatment of some diseases [1–4, 26]

It was shown that plants products possess not only hypocholesterolemic, hypolipidemic, anti-hypertensive, anti-diabetic, anti-thrombotic and anti-hyperhomocystic, but also anticancer properties [9, 10, 15–20]. Recently we published two papers describing the characteristics of leafs and aerial parts of 11 Korean salad plants [22, 23]. It was found that methanol extracts from these parts of salad plants possess antioxidant and anticancer properties [22, 23]. And it was interesting to know if also young sprouts of some traditional Korean salad plants exercise the above mentioned activities. As was shown, the content of the total phenolics in the methanol extracts of most of the studied plants was high and varied significantly. The highest content of phenolics was in *E. alatus*, and *H. ascyron* and the lowest—in *S. aquatica* and *A. mangostanus*. As other, we have found that the content of phenolics was highly consistent with the finding of DPPH radical scavenging activity [27–30]. Also Zhou and Yu [29] 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. It was observed that also the radical scavenging activity in methanol extracts of most of the studied plants was high and varied significantly. Methanol extracts of *E. alatus* had the highest DPPH radical scavenging activity, which was higher than of synthetic antioxidants BHT, with IC_{50} values of 104.4 mg kg^{-1}. Also other reported that antioxidant activity of plants is higher than of synthetic antioxidants [31, 32]. The lowest DPPH radical scavenging activity was found in *S. aquatica* ($P<0.05$). In was observed a dose-dependent radical scavenging activity in all samples. Also Lee et al., 2003, [32] reported the same results. They investigated methanol extracts of nine medicinal plants traditionally used in Chinese medicine vs. synthetic antioxidant resveratrol and found relatively high levels of DPPH radical scavenging activity in extracts of *Areca catechu* var. *dulcissima*, *Paeonia suffruticosa* and *Cinnamomun cassia* ($IC_{50}<6.0$ μg ml^{-1}). The extract of *Areca catechu* var. *dulcissima* showed in all experiments higher antioxidant activity than resveratrol. We found that the correlation coefficient between the extracted polyphenols and antioxidant activity was 0.974. Also others found high correlation between the content of polyphenols and antioxidant activity [33]. So, Jastrzebski et al. [33] reported that in a mixture of

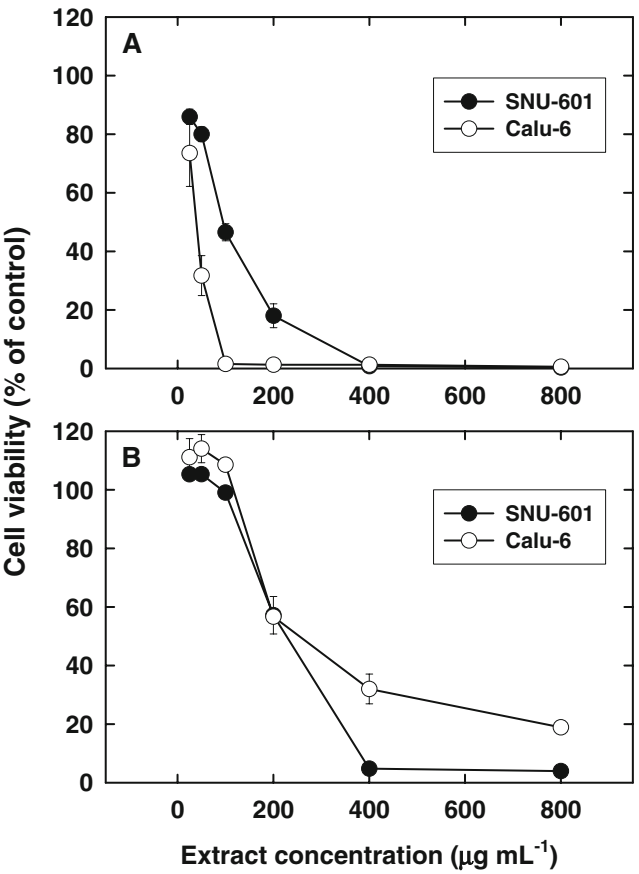

Fig. 2 Cytotoxic effect of methanol extracts from the young sprouts of *S. aquatica* (**a**) and *E. sessilifolrus* (**b**) on human cancer cell lines, Calu-6 and SNU-601

plants the correlation coefficient was between polyphenols and antioxidant activity 0.97.

Results of this investigation showed that traditional Korean salad plants used for food possess higher antioxidant activity. However, the levels of the antioxidant activity in some of the studied plants were different.

Results of the determination of the anticancer activity of methanol extracts of the studied Korean salad plants on two human cancer cell lines (Calu-6 and SNU-601) were summarized in the Table 2. All these extracts were tested for their antiproliferative activity on tumor cell lines Calu-6, and SNU-601 by the MTT assay. A dose dependent inhibition of cell proliferation was observed for most of methanol extracts tested in this study. It was recorded that the methanol extracts exhibited more inhibition on Calu-6 cell line than of SNU-601.

Methanol extracts at 200 μg ml^{-1} from *S. aquatica* exhibited the highest anticancer activity on Calu-6 and SNU-601 tumor cell lines, by 98 and 81%, respectively, and the methanol extracts from *E. sessilifolrus* at the same concentration exhibited the lower activity by only 95 and 75%, respectively. These results, however, were not consistent with the findings of DPPH radical scavenging activity and/or total phenolic content. Also others reported about antiproliferative activities of green tea and essential oil extracted from Thai medicinal plants [34–36]. So, it was shown the 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 [36]. They found that Guava (*Psidium guajava* L.) leaf and Sweet Basil oils exhibited the highest anti-proliferative activity in KB and P388 cell lines, respectively.

In summary, this investigation shows that the content of total phenolics in young sprouts of 11 Korean traditional salads is high: the significantly highest in *E. alatus* and *H. ascyron* and the same plants have the highest DPPH radical scavenging activity. The salad plants dose-dependently increased the free radical scavenging activity. The total phenolics level is highly correlated with the free radical scavenging activity. The highest influence on the Calu-6 is human pulmonary carcinoma cell line was exercised by *S. aquatica*, *E. sessilifolrus* and *Z. schinifolium* (<25.0) following by *Ainsliaea acerifolia* (25.7±1.3) and on SNU-601 (human gastric carcinoma cell line)—by *S. aquatica* (153.3± 9.1), following by *E. sessilifolrus* (196.7±5.1). There was no correlation between plants radical scavenging activity and their anticancer effect. No significant differences were found between the results of the investigation of the same salad plants harvested in 2006 and 2007 years. The young sprouts of the Korean traditional salad plants could be recommend as preventative or/and therapeutic agents in addition to proper prescribed drugs.

Acknowledgment This research was conducted with support from the 2005 ARPC research fund (105088-33-2-HD110). Appreciation is expressed to Dr. Sook-Young Lee at Chonsun University, Gwangju, Korea, for her technical assistance.

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