Characteristics of the leaf parts of some traditional Korean salad plants used for food

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Abstract

BACKGROUND: Total phenolics content, antioxidant activity and cytotoxicity of the methanol extracts from leaf parts of 13 Korean traditional salad plants were investigated in order to determine their properties.

RESULTS: The highest phenolics content (mg ferulic acid equivalents kg^{-1} dry weight (d.w.), omit one) was found in methanol extracts from *Polygonum aviculare*, at 293.7 ± 6.0, followed by *Euonymus alatus*, at 250.7 ± 3.3, *Saxifraga stolonifera*, at 125.0 ± 8.1 and *Ligularia fischeri*, at 122.5 ± 5.9. The methanol plant extracts dose-dependently increased free radical scavenging activity. Methanol extracts of *Polygonum aviculare, Euonymus alatus* and *Saxifraga stolonifera*, at 31 mg kg^{-1}, exhibited the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (%) by 90.8 ± 4.2, 85.7 ± 3.9 and 64.1 ± 3.2, respectively. According to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the methanol extracts from *Portulaca oleracea* ($IC_{50} < 25.0\,\mu g\,mL^{-1}$) showed the highest cytotoxicity against Calu-6, followed by *Plantago asiatica* ($49.2\,\mu g\,mL^{-1}$) and *Osmunda japonica* ($89.6\,\mu g\,mL^{-1}$).

CONCLUSION: Total phenolics content of the tested plant extracts was correlated with the DPPH radical scavenging activity, suggesting the phenolics compounds are contributing to the antioxidant properties of Korean salad plants. The leaf parts of the 13 Korean traditional salad plants described here that are currently used as foods may also provide some benefit to human health, and research into their potential benefits as preventative and/or therapeutic agents is warranted.
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Keywords: Korean salad plants; methanol extracts; total phenolics; radical scavenging activity; cytotoxicity

INTRODUCTION

Some plants exhibit various bioactivities, including antioxidant, anti-inflammatory, anticancer and anti-diabetic.[1–3] Recently, there has been a worldwide trend towards the use of wild plants due to their bioactive phytochemicals, in particular, phenolics.[4,5] Phenolic compounds are secondary metabolites that are synthesized by plants during normal development and in response to stress conditions such as infection, wounding and UV radiation.[3–5] These compounds occur ubiquitously in plants and are a diversified group of phytochemicals derived from phenylalanine and tyrosine.[6,7]

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.[8,9] Therefore, not only antioxidants of natural products, but also synthesized antioxidants are widely used.[10–12] The toxic and other unfavorable effects of synthesized food antioxidants have been widely noted.[13,14] Phenolic compounds, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ), have been widely used as synthetic antioxidants in food. These substances are considered as safe natural antioxidants; however, they do not always provide effective protection against oxidation *in vitro*.[15] Nevertheless, these phenolic antioxidants are still used extensively as food antioxidants because of their low cost. However, it must be mentioned that as early as in 1975 it was

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reported that administration of these phenolic antioxidants in doses of 50 mg kg^{-1} per day to rodents and monkeys led to certain pathological changes, including enzyme and lipid alterations as well as carcinogenic effects.[16] Therefore, research on new natural antioxidants has gained momentum as they are considered, rightly or wrongly, not to pose any health risk to consumers.[17,18]

Antioxidants in plants include flavonoids, phenolic acids, lignan precursors, terpenes, mixed tocopherols, phospholipids and polyfunctional organic acids, and also plant extracts such as those of rosemary and sage.[18,19] It was reported that the antioxidant activity of the antioxidants mentioned is in the following order: rosmarinic acid > caffeic acid phenethyl ester > caffeic acid > chlorogenic acid > tocopherol > ferulic acid > ferulic acid phenethyl ester > BHT.[20]

Vegetables, fruits and whole grains contain a wide variety of phytochemicals that have the potential to interfere with the development of cancer.[1–3] These phytochemicals are isothiocyanates (cruciferous vegetables), carotenoids, including α-carotene, γ-carotene, β-cryptoxanthin, zeaxanthin(e), lutein, lycopene (tomatoes), resveratrol (grapes and wine), ellagic acid (various berries), glutathione-*S*-transferase (garlic), diallyl sulfide (garlic), genestine (soybean), curcumine (turmeric), indole-3-carbinol, inositol, organosulfur compounds, sulforaphane, squalene, and terpenes.[21] Also regular consumption of tea decreased the risk of various types of cancer.[22,23]

The Korean medicinal plants that are investigated in this study have been used for a long time as traditional seasoned salads, and were screened for bioactive effects of these functional foods.[24] Earlier studies showed that extracts from *Areca catechu* var. *dulcissima* possess antidepressant properties.[25] Au and Lam[26] suggested that the methanol extracts of *Paeonia suffruticosa* potently inhibit human immunodeficiency virus (HIV)-1 integrase. Lee *el al.*[27] reported that silymarin and silybin purified from *Silybum marianum* have the potential to inhibit activities against oxidation of ^{125}I-LDL by macrophages and endothelial cells. Therefore these Korean plants, used as salad, could act as preventative or therapeutic agents.

The objective of this research was to determine total phenolics content of the methanol extracts from leaf parts of the 13 Korean salad plants, their antioxidant activity and cytotoxicity. In order to receive reliable data the following were used: (a) for determination of total phenolics content: the Folin–Ciocalteu assay; (b) for assessment of the radical scavenging activity: 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, because of its ability in a relatively short time compared to other methods to evaluate antioxidative activities;[28] (c) for anticancer activity: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

As far as we know, there are no published results of similar investigations.

MATERIALS AND METHODS

Chemicals

Folin–Ciocalteu reagent, MTT, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ascorbic acid were obtained from Sigma Chemical Co. (St Louis, MO, USA). All reagents were of analytical grade. Deionized and distilled water were used throughout. The cell lines were purchased from Korean Cell Line Bank (KCLB).

Plant material

Leaf parts of 13 Korean medicinal salad plants (*Saxifraga stolonifera* (L.) Meeb, *Pteridium aquilinum, Hemerocallis minor, Plantago asiatica, Osmunda japonica, Portulaca oleracea, Synurus deltoides, Ligularia fischeri, Rumex acetosa, Polygonum aviculare, Symplocarpus renifolius*, *Chenopodium album*, and *Euonymus alatus*) grown in a mountain area of Suncheon City, Korea, were harvested at a vegetative stage in June 2006. The samples were immediately 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 three times with 95% methanol in a shaker at 24 h intervals for 3 days at room temperature. The extracts were 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). 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.

Total phenolics content

The content of total phenolics (TP) was measured using the classical Folin–Ciocalteu assay.[29] 5 mL of (Thermo Scientific Barnstead NaNOpure™ Water Purification System, Analytical, EW-99277-00) Nanopure water, 0.5–1.0 mL of sample, and 1.0 mL of Folin–Ciocalteu reagent were added to a 25 mL volumetric flask. The above-mentioned substances were mixed and allowed to stand for 5–8 min at room temperature. Next, 10 mL of a 7% sodium carbonate solution was added, followed by the addition of Nanopure water filled to volume. Solutions were mixed and allowed to stand at room temperature for 2 h. Sample aliquots were filtered through a Whatman 0.45 m poly(tetrafluoroethylene) filter prior to the determination of TP content using a UV-1650 spectrophotometer (Shimadzu, Kyoto, Japan) monitoring at 640 nm. TP were standardized against ferulic acid and expressed as ppm of ferulic acid equivalents (FAE). The linearity range for this assay was determined as 0.5–5.0 mg L^{-1} FAE ($R^2 = 0.9990$), giving an absorbance range of 0.050–0.555 AU.

Radical scavenging activity

Free radical scavenging activity of the methanol extracts was determined using the classical DPPH

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method. Each methanol extract at various concentrations (31, 63, 125, 250, and 500 mg kg^{-1}) was added to a 1.5×10^{-4} mol L^{-1} 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 plant extracts was partially expressed as IC_{50}, 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 SNU-601 for human gastric carcinoma) were measured using the MTT assay. Cell lines for the MTT assay were purchased from KCLB. 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^{-1}), and transferred to 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 dimethyl sulfoxide (DMSO), followed by dilution with RPMI-1640 medium to give final concentrations of 25, 50, 100, 200, 400, and 800 μg mL^{-1}. Stock solutions of samples were prepared for cell lines at 90 μL and samples at 10 μL, and incubated for 72 h. MTT solution at 5 mg mL^{-1} was dissolved in 1 mL of phosphate buffer solution (PBS), and 10 μL of this was added to each of the 96 wells.[30] The wells were wrapped with aluminium foil and incubated at 37 °C for 4 h. The solution in each well containing media, unbound MTT and dead cells was 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. Cytotoxicity was established 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 line.

Statistics

To verify the statistical significance, mean ± SD of three independent measurements were calculated. Differences between groups were tested by two-way ANOVA. In the assessment of the antioxidant potential, Spearman correlation coefficients (R) were used. Linear regressions were also calculated. P-values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Total phenolics content

The amounts of TP in methanol extracts (mg FAE kg^{-1} d.w.) for *Polygonum aviculare* and *Euonymus alatus* were 293.7 ± 6.0 and 250.7 ± 3.3, respectively, and were significantly higher ($P < 0.05$) than in *Saxifraga stolonifera* and *Ligularia fischeri* (125.0 ± 8.1 and 122.5 ± 5.9, respectively). The lowest content of TP was in the extracts of *Chenopodium album, Hemerocallis minor*, and *Symplocarpus renifolius* ($P < 0.05$ in all three cases) (Fig. 1). The result was highly consistent with the finding of DPPH radical scavenging activity.[31] Also Zhou and Yu[32] reported that TP content of the tested vegetable extracts was correlated with DPPH radical scavenging activity, suggesting that TP can play a major role in the antioxidant activity of plant materials.

DPPH radical scavenging activity

Table 1 summarizes the results of the determination of DPPH radical scavenging activity. As can bee seen, methanol extracts of *Polygonum aviculare* had the highest DPPH radical scavenging activity, followed by *Euonymus alatus* and *Saxifraga stolonifera*, indicating IC_{50} values below 31.0 mg kg^{-1}. These values of *Polygonum aviculare* and *Euonymus alatus* showed higher activity than those of synthetic antioxidants (vitamin C and BHT), with IC_{50} values of <31.0 and 113.0 mg kg^{-1}, respectively. Methanol extracts of *Polygonum aviculare, Euonymus alatus*, and *Saxifraga stolonifera* at 31.0 mg kg^{-1} exhibited the highest DPPH radical scavenging activity (%) by 90.8 ± 4.2, 85.7 ± 3.9, and 64.1 ± 3.2, respectively.

DPPH radical scavenging activity data for *Chenopodium album* extract was the lowest ($P < 0.05$). All samples of plant species showed that DPPH radical scavenging activity is dose-dependent. The results

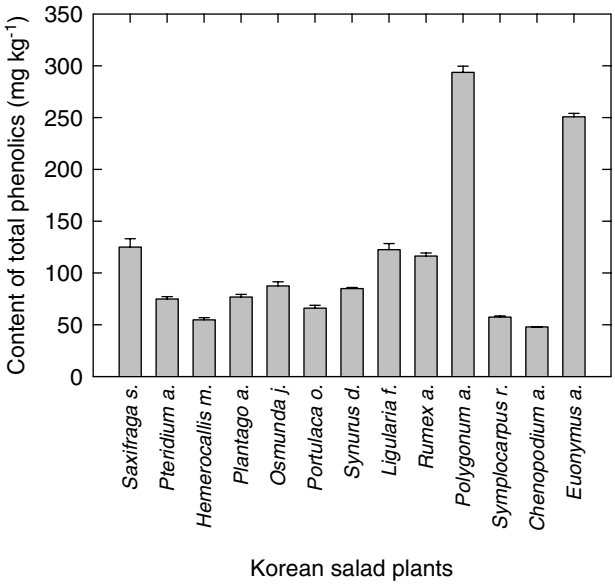

Figure 1. Total phenolic amount (mg FAE kg^{-1} dry weight) of methanol extracts from the leaf parts of 13 Korean medicinal salad plants.

Table 1. DPPH radical scavenging activity in methanol extracts from the leaf parts of 13 Korean traditional salad plants in comparison with synthetic antioxidants

	Extract concentration (mg kg^{-1})					
Scientific name	31	63	125	250	500	IC_{50}[†]
Saxifraga stolonifera	64.1 ± 3.2g	90.4 ± 4.2h	91.1 ± 4.2h	91.1 ± 4.2h	91.6 ± 4.2h	<31
Pteridium aquilinum	15.6 ± 1.1c	25.8 ± 1.8d	45.5 ± 2.3c	77.2 ± 3.5d	86.4 ± 4.0c	140
Hemerocallis minor	9.8 ± 0.7b	14.8 ± 1.0b	21.7 ± 1.8a	37.8 ± 2.4b	65.8 ± 3.0b	360
Plantago asiatica	20.5 ± 1.6c	31.6 ± 1.9e	56.9 ± 2.9e	88.0 ± 4.0h	90.4 ± 4.1h	106
Osmunda japonica	25.2 ± 1.8d	44.5 ± 2.1f	74.7 ± 3.3h	88.7 ± 4.0h	87.8 ± 3.9c	75
Portulaca oleracea	4.9 ± 0.3a	9.9 ± 0.7a	19.8 ± 1.6a	37.2 ± 2.2a	67.2 ± 3.1b	361
Synurus deltoides	11.3 ± 0.8b	19.5 ± 1.6c	34.2 ± 2.1b	60.3 ± 3.1c	85.4 ± 3.9c	200
Ligularia fischeri	37.2 ± 2.2e	64.8 ± 3.2g	89.4 ± 4.1h	90.5 ± 4.1h	90.6 ± 4.1h	46
Rumex acetosa	49.1 ± 2.5f	75.7 ± 3.3h	89.5 ± 4.1h	90.0 ± 4.1h	90.7 ± 4.1h	32
Polygonum aviculare	90.8 ± 4.2a	91.4 ± 4.2h	91.4 ± 4.2h	91.8 ± 4.2h	92.8 ± 4.3h	<31
Symplocarpus renifolius	9.8 ± 0.7b	16.9 ± 1.3b	30.8 ± 2.0b	51.1 ± 2.5c	79.7 ± 3.6c	242
Chenopodium album	0	0	0	0	4.1 ± 0.3a	–
Euonymus alatus	85.7 ± 3.9h	91.2 ± 4.2h	91.2 ± 4.2h	91.2 ± 4.2h	91.2 ± 4.2h	<31
Ascorbic acid	81.8 ± 3.7h	96.1 ± 4.5h	96.1 ± 4.5h	96.7 ± 4.5h	96.9 ± 4.5h	<31
BHT	15.6 ± 1.1c	33.5 ± 2.1e	55.2 ± 2.8g	81.3 ± 3.7h	92.4 ± 4.3h	113

Values are means ± SD of three measurements. Means in columns without letters in common differ significantly ($P < 0.05$).
1,1-diphenyl-2-picrylhydrazyl (DPPH); butylated hydroxytoluene (BHT).
[†] Extract concentrations, which show 50% activity of DPPH radical scavenging, were determined by interpolation.

show that antioxidant activity compounds could be of different amounts in various plant species. The results of this study suggest that *Ixeris dentate* and *Aster scaber* are to be preferred against synthetic antioxidants. Also others have reported that antioxidant activity of plants is higher than that of synthetic antioxidants.[33] Those authors investigated methanol extracts of nine medicinal plants traditionally used in Chinese medicine *versus* resveratrol. They found relatively high levels of DPPH radical scavenging activity in extracts of *Areca catechu* var. *dulcissima*, *Paeonia suffruticosa* and *Cinnamomun cassia* ($IC_{50} < 6.0\,\mu g\,mL^{-1}$). The extracts of *Areca catechu* var. *dulcissima* showed higher antioxidant activity than resveratrol in all experiments. In some reports[34–36] solvents were applied with different polarity for polyphenols extraction and these solvents had a significant effect on polyphenol content and antioxidant activity. Others have shown that polyphenols and antioxidant capacities in water extracts were higher than in methanol. The correlation coefficients between polyphenols and antioxidant capacities of Prolipid with 1,1-diphenyl-2-picrylhydrazyl radical assay were about 0.97.[34] It was also shown that 50% of ethanol extract from black herbal mate tea had the greatest antioxidant activity.[34–36] The methanol extract of *Ulmus davidiana*[36] exhibited strong antioxidant activity in the tested model systems. *U. davidiana* extracts may be exploited as biopreservatives in food applications as well as for health supplements of functional food, to alleviate oxidative stress. Our results also showed high phenolics content and antioxidant activity in the methanol extracts from *Polygonum aviculare*, *Euonymus alatus*, and *Saxifraga stolonifera*. However, extracts of the plants showed low anticancer activity. The correlation coefficient between the extracted polyphenols with ethanol and antioxidants was about 0.88 (Fig. 2). The relatively high correlation between the above-mentioned variables in the studied extracts was compared with tea, Prolipid and *U. davidiana* extracts.[34–36] Results of this investigation showed that traditional Korean salad plants used for food exercise both antioxidant and anticancer activities. However, the levels of the antioxidant and anticancer activities in some of the studied plants were different.

Cytotoxic effect of methanol extracts

The results of the cytotoxic effect of methanol extracts from the leaf parts of 13 Korean salad plants on two human cancer cell lines (Calu-6 and SNU-601) are summarized in Table 2. *Portulaca oleracea* exhibited the greatest influence on the Calu-6 human pulmonary carcinoma cell line (<25.0 ± 1.7), followed by *Plantago asiatica* (49.2 ± 4.2). *Osmunda japonica* had the greatest effect on SNU-601 human gastric carcinoma cell line (152.9 ± 8.1), followed by *Portulaca oleracea* (213.9 ± 10.1). All these extracts

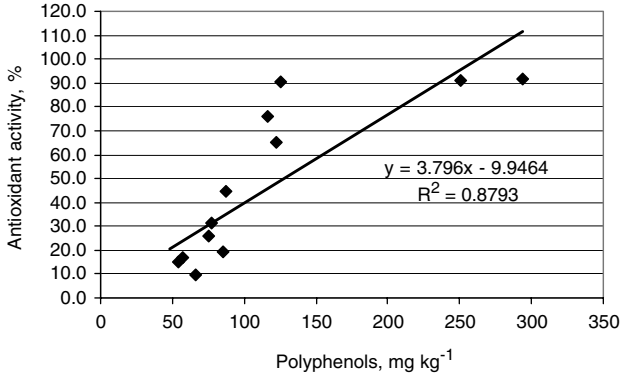

Figure 2. Correlation between amount of polyphenols (mg FAE kg^{-1} dry weight) and antioxidant activity (%) in Korean salad plants.

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Table 2. Cytotoxic effect of methanol extracts from the leaf parts of 13 Korean salad plants on two human cancer cell lines

Scientific name	IC50[a] (μg mL^{-1})	
	Calu-6[b]	SNU-601[b]
Saxifraga stolonifera	449.2 ± 19.1g	471.2 ± 19.1f
Pteridium aquilinum	220.1 ± 10.2d	323.8 ± 12.2d
Hemerocallis minor	181.4 ± 9.1c	344.0 ± 13.3d
Plantago asiatica	49.2 ± 4.2a	276.6 ± 11.8c
Osmunda japonica	89.6 ± 6.1b	152.9 ± 8.11a
Portulaca oleracea	<25.0 ± 1.7a	213.9 ± 10.1b
Synurus deltoides	193.6 ± 9.2c	313.8 ± 12.3a
Ligularia fischeri	195.9 ± 9.3c	283.7 ± 12.0c
Rumex acetosa	344.0 ± 12.3f	424.8 ± 19.0e
Polygonum aviculare	752.4 ± 23.1h	447.1 ± 19.1e
Symplocarpus renifolius	188.7 ± 10.3c	335.8 ± 12.3d
Chenopodium album	378.4 ± 13.2f	339.8 ± 12.3d
Euonymus alatus	297.2 ± 12.1e	412.5 ± 18.9e

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 cells, were determined by interpolation.
[b] Calu-6 is human pulmonary carcinoma and SNU-601 is human gastric carcinoma.

were tested for their antiproliferative activity on tumor cell lines Calu-6 and SNU-601 by the MTT assay.[30] A dose-dependent inhibition of cell proliferation was observed for most of the 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 *Portulaca oleracea* exhibited the highest anticancer activity on Calu-6 and SNU-601 tumor cell lines, by 100% and 60%, respectively, while the methanol extracts from *Polygonum aviculare* at the same concentration exhibited the lowest activity, by 32% and 60%, respectively (Fig. 3). These results, however, were not consistent with the findings of DPPH radical scavenging activity or total phenolic content. Methanol extracts from *Portulaca oleracea* (IC_{50} < 25.0 μg mL^{-1}) showed the most potent cytotoxicity on Calu-6 cell line, followed by *Plantago asiatica* (49.2 μg mL^{-1}), *Osmunda japonica* (89.6 μg mL^{-1}), and *Hemerocallis minor* (181.4 μg mL^{-1}). However, cytotoxicity of the methanol extracts from all plants against SNU-601 was much lower than that against Calu-6 cell line. Methanol extracts from *Osmunda japonica* with IC_{50} of 152.9 μg mL^{-1} showed the highest activity against SNU-601, followed by *Portulaca oleracea* (213.9 μg mL^{-1}), *Plantago asiatica* (276.6 μg mL^{-1}) and *Ligularia fischeri* (283.7 μg mL^{-1}). Also Manosroi *et al*.[37] reported the antiproliferative 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. They found that guava (*Psidium guajava* L.) leaf and sweet basil oils exhibited the highest antiproliferative activity in KB and P388 cell lines, respectively.

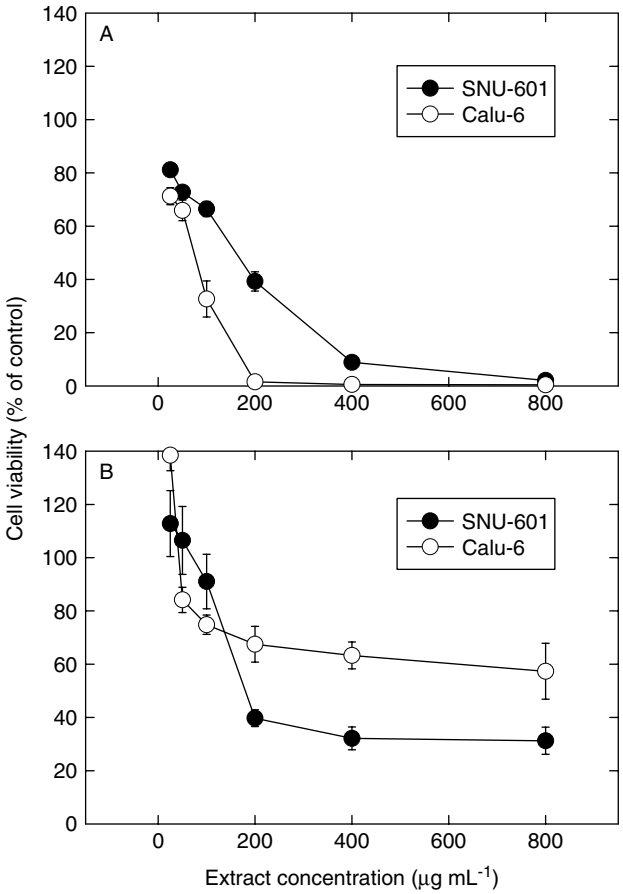

Figure 3. Cytotoxic effect of methanol extracts from the leaf parts of *Portulaca oleracea* (A) and *Polygonum aviculare* (B) on human cancer cell lines, Calu-6 and SNU-601.

CONCLUSION

This investigation shows that the content of total phenolics in 13 Korean traditional salad plants is high: the significantly highest contents were found in *Polygonum aviculare* and *Euonymus alatus*. In addition, methanol extracts of the same plants were characterized by the highest DPPH radical scavenging activity. The salad plants dose-dependently increased free radical scavenging activity. The TP level is highly correlated with the free radical scavenging activity. The highest influence on the Calu-6 human pulmonary carcinoma cell line was found in the methanol extract of *Portulaca oleracea*, followed by that of *Plantago asiatica*, while extracts of *Osmunda japonica* and *Portulaca oleracea* had the highest impact on SNU-601 human gastric carcinoma cell line. Therefore, leaf parts of Korean traditional salad plants could be recommended as preventative or/and therapeutic agents mainly for atherosclerotic heart disease in addition to appropriate prescription drugs.

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