

Antioxidant activity and cytotoxicity of methanol extracts from aerial parts of Korean salad plants

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Abstract. The aim of this investigation was to determine the content of total phenolics, antioxidant activity and cytotoxicity of methanol extracts from the aerial parts of 11 Korean medicinal salad plants. The highest total phenolic content of the methanol extracts was found in *Aster scaber* (17.1 mg 100 g⁻¹), followed by *Ixeris dentate* (16.4 mg 100 g⁻¹), *Aster yomena* (12.0 mg 100 g⁻¹) and *Sedum sarmentosum* (9.1 mg 100 g⁻¹) of FW. Methanol extracts of *Ixeris dentate* and *Aster scaber* at 50 µg mL⁻¹ exhibited the highest DPPH radical scavenging activity by 86.4 and 83.3%, respectively. It was registered a dose-dependent increase of DPPH free radical scavenging activity. Total phenolic content of the studied plant extracts was correlated with the DPPH radical scavenging activity. It was found by means of MTT assay, that cytotoxicity of the methanol extracts was the highest against HCT-116. Methanol extracts from *Petasites japonicus* (IC₅₀ < 25.0 µg mL⁻¹) showed the highest activity against HCT-116, following by *Angelica gigas* (34.75 µg mL⁻¹), *Erythronium japonicum* (44.06 µg mL⁻¹), and *Aster scaber* (54.87 µg mL⁻¹).

In conclusion, the studied salad plants have high total phenolics content and high antioxidant activity. These plants dose-dependently increased DPPH free radical scavenging activity. The total phenolics level was highly correlated with the free radical scavenging activity. Most of the studied salad plants have potent cytotoxicity activity. The results of this investigation suggest that the extracts of studied salad plants could be an addition to basic medicine for some diseases.

Keywords: Aerial parts of Korean medicinal salad plants, methanol extracts, natural antioxidants, total phenolic content, DPPH radical scavenging activity, cytotoxicity

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1. Introduction

Epidemiological studies, experiments on laboratory animals and investigations of humans show that consumption of fruits and vegetables is associated with a low risk of some diseases including cardiovascular diseases and cancer [1,2,10,11,25]. This beneficial effect has been attributed to the bioactive compounds of these natural products and first of all to phenolics. Many plants possess antioxidant activities and their consumption was recommended [18,28]. The importance of phenolics, especially flavonoids, is due to their ability to act as efficient free radical scavengers [15,20,30]. Therefore, some of them as isothiocyanates (cruciferous vegetables), carotenoids including alpha-carotene, gamma carotene, beta-cryptoxanthin, zeaxanthin, lutein, 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 are active in cancer prevention [34].

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ), have also been widely used in food, however, some side effects were registered [21]. Therefore, investigations of new natural sources of antioxidants are on the increase [4, 7], and different types of antioxidants were found in various plants, which pose no health risk to consumers [16,31,32].

The above mentioned investigations have shown that an increased consumption of fruit and vegetables reduce significantly the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies [21].

Especially the antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds were studied. It was found that the scavenging activity of these antioxidants was in following order: rosmarinic acid > caffeic acid phenethyl ester > caffeic acid > chlorogenic acid > tocopherol > ferulic acid > ferulic acid phenethyl ester > BHT [31]. Also some Korean medicinal plants were intensively studied [7,8,17]. It was reported that silymarin and silybin purified from *Silybum marianum* have potential inhibiting activities against oxidation of ¹²⁵I-LDL by macrophages and endothelial cells [17]. Extracts from *Areca catechu* var. *dulcissima* possess antidepressant properties [8].

In this study we decided to investigate 11 long used Korean traditional seasoned salad plants and to assess the content of total phenolics, antioxidant activity and cytotoxicity in the aerial parts of their methanol extracts. For this purpose the Folin-Ciocalteu assay, 1,1-diphenyl-2-picrylhydrazyl free radical scavenging assay (DPPH) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay were used.

Free radical scavenging is generally the accepted mechanism for antioxidants to inhibit 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 [1]. The preferable method for evaluation of the scavenging free radicals activities is 1,1-diphenyl-2-picrylhydrazyl test – DPPH [3,33]. DPPH in comparison with other methods is able in a relatively short time evaluate the scavenging free radicals activities [3]. Therefore, in this study the DPPH test was used.

We did not find published data concerning investigations of these 11 Korean traditional seasoned salad plants.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 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).

2.2. Samples

Aerial parts, including leaves and stems, of 11 Korean medicinal salad plants (*Petasites japonicus*, *Hosta longipes*, *Angelica gigas*, *Ixeris dentate*, *Taraxacum mongolicum*, *Aster yomena*, *Capsella bursa-pastori*, *Erythronium japonicum*, *Pimpinella brachycarpa*, *Aster scaber*, *Sedum sarmentosum*), 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°C for 5 days, ground with a Wiley mill to pass a 1-mm screen, and stored in a refrigerator at 2°C until use. The samples were extracted with 95% methanol at room temperature. 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 extracts were about 10% of the original plant samples. The methanol extracts from each plant were used for measuring total phenolics content, DPPH radical scavenging activity and cytotoxicity. All results are for FW.

2.3. Determination of the total phenol content

The content of total phenolics (TP) was measured using the Folin-Ciocalteu assay [24]. Briefly, 5 mL of 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 contents were mixed and allowed to stand for 5–8 min at room temperature. Next, 10 mL 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 2 h. Sample aliquots were filtered through a Whatman 0.45 m poly (tetrafluoroethylene) filter prior to the determination of TP concentration using a UV-1650 spectrophotometer (Shimadzu, Japan) monitoring 640 nm. TP content was standardized against ferulic acid and expressed as $\text{mg } 100 \text{ g}^{-1}$ of ferulic acid equivalents (FAE). The linearity range for this assay was determined as 0.5–5.0 mg/L FAE ($R^2 = 0.9990$), giving an absorbance range of 0.050–0.555 AU.

2.4. Determination of the scavenging activity

1,1-Diphenyl-2-picrylhydrazyl free radical scavenging assay (DPPH) was carried out according to the following procedure. Each methanol extract at various concentrations (3.1, 6.3, 12.5, 25, and 50 $\text{mg } 100 \text{ g}^{-1}$) was added to a $1.5 \times 10^{-4} \text{ M}$ 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 (%) = $\{(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{control}}\} \times 100$. The antioxidant activity of plants extracts was partially expressed as IC_{50} , which was defined as the concentration (in $\text{mg } 100 \text{ g}^{-1}$) of extract required to inhibit the formation of DPPH radicals by 50%.

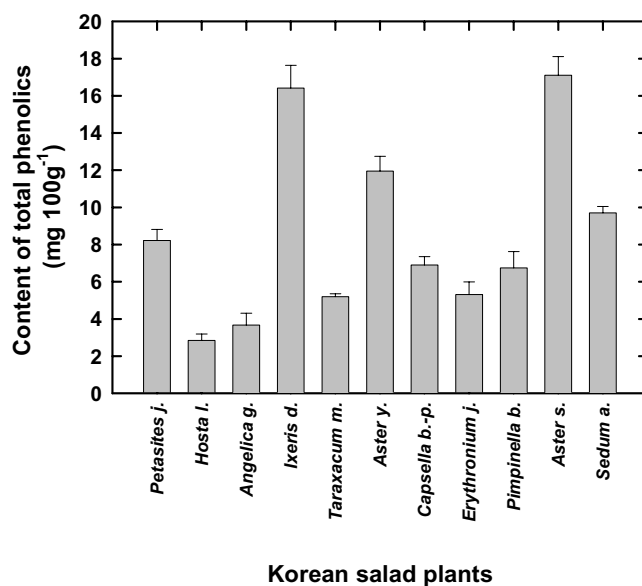


Fig. 1. Total phenolic content of methanol extracts from the aerial parts of 11 Korean medicinal salad plants.

2.5. Determination of the cytotoxicity

For the assessment of the anticancer activity of the studied plants the following human cancer cell lines were used: Calu-6 – for human pulmonary carcinoma, MCF-7 – for human breast adenocarcinoma pleural effusion, and HCT-116 – for human colon carcinoma. 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°C under 5% CO₂ 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 800 $\mu\text{g mL}^{-1}$. 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 [27]. 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. Then the plates were 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 (IC₅₀) in growth (cell number) for each cell lines.

2.6. Statistical analyses

The results of this investigation are means \pm SD of three measurements. Differences between groups were tested by two-way ANOVA. 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.

3. Results

3.1. Total phenolics content

The highest total phenolic content of the methanol extracts was found in *Aster scaber* (17.1 mg 100 g⁻¹), followed by *Ixeris dentate* (16.4 mg 100 g⁻¹), *Aster yomena* (12.0 mg 100 g⁻¹) and *Sedum sarmentosum* (9.1 mg 100 g⁻¹) (Fig. 1). These results were highly consistent with the finding of DPPH radical scavenging activity [29]. Zhou and Yu [37] 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.

3.2. DPPH radical scavenging activity

The results of the determination of DPPH radical scavenging activity of the studied plants are summarized in the Table 1. As can be seen, the methanol extracts of *Ixeris dentate* had the highest DPPH radical scavenging activity, with an IC₅₀ value of 23.8 mg 100 g⁻¹, and followed by *Aster scaber* (25.8 μg mL⁻¹), *Aster yomena* (33.9 mg 100 g⁻¹) and *Petasites japonicus* (42.9 mg 100 g⁻¹). The results of the determination of the radical scavenging activity of the studied plants were lower than of synthetic antioxidants vitamin C and BHT, with IC₅₀ values of < 3.1 and 11.3 mg 100 g⁻¹, respectively. Methanol extracts of *Ixeris dentate* and *Aster scaber* at 50 mg 100 g⁻¹ exhibited the highest DPPH radical scavenging activity by 86.4 and 83.3%, respectively. The extract of *Hosta longipes* showed the lowest DPPH radical scavenging activity. All samples of the studied plants demonstrated a dose-dependent DPPH radical scavenging activity.

3.3. Cytotoxicity

As was mentioned, the methanol extracts of the studied plants were tested for their antiproliferative activity on Calu-6, MCF-7, and HCT-116 tumor cell lines by the MTT assay [27]. The results of the determination of the antiproliferative activity of the studied plants are summarized in the Table 2. As can be seen, the cell proliferation inhibition was registered for most of the tested plants. The *Petasites japonicus* extract was the most statistically significant inhibitor on HCT-116 cell line (< 25.00 ± 1.7), whereas *Sedum sarmentosum* extract was the less active among the studied plant (491.29 ± 13.4). Methanol extracts at 100 μg ml⁻¹ from *Petasites japonicus* exhibited the highest anticancer activity on Calu-6, MCF-7, and HCT-116 tumor cell lines, by 98.8, 94.3, and 93.7%, respectively. On the other hand, the methanol extracts from *Sedum sarmentosum* at the same concentration exhibited the lowest activity. These results, however, were not consistent with the findings of DPPH radical scavenging activity or total phenolic content.

Methanol extracts from *Petasites japonicus* have shown the most potent cytotoxicity on all three cancer cell lines (26.60 ± 1.9, 34.87 ± 2.1 and < 25.00 ± 1.7), followed by *Angelica gigas* (57.05 ± 3.9, 57.93 ± 3.9 and 34.75 ± 2.1), *Erythronium japonicum* (36.64 ± 2.3, 66.11 ± 5.1 and 44.06 ± 3.2) and *Aster scaber* (75.11 ± 5.9, 177.53 ± 10.1 and 54.87 ± 3.8) for the Calu-6, MCF-7, and HCT-116,

Table 1

DPPH radical-scavenging activity in methanol extracts from aerial parts of 11 Korean traditional salad plants and synthetic antioxidants

Scientific names	Concentration, mg 100g ⁻¹					IC ₅₀ †
	3.1	6.3	12.5	25	50	
<i>Petasites japonicus</i>	0.6 ± 0.06 ^a	5.3 ± 0.5 ^c	14.3 ± 1.1 ^b	29.8 ± 1.9 ^d	57.4 ± 4.3 ^e	42.9
<i>Hosta longipes</i>	0	0	0	0	0.6 ± 0.01 ^a	–
<i>Angelica gigas</i>	0	0	0	2.7 ± 0.2 ^a	4.2 ± 0.3 ^b	–
<i>Ixeris dentate</i>	4.7 ± 0.3 ^c	12.1 ± 0.9 ^e	26.5 ± 1.7 ^c	52.8 ± 4.1 ^e	86.4 ± 6.9 ^g	23.8
<i>Taraxacum mong.</i>	0	0	0	1.6 ± 0.1 ^a	5.8 ± 0.4 ^b	–
<i>Aster yomena</i>	0.8 ± 0.1 ^a	6.5 ± 0.5 ^c	16.8 ± 1.1 ^b	38.2 ± 2.7 ^d	70.3 ± 5.9 ^f	33.9
<i>Capsella bursa-pastori</i>	0	0.4 ± 0.03 ^a	2.6 ± 0.2 ^a	8.9 ± 0.7 ^b	16.0 ± 1.2 ^c	–
<i>Erythronium japon.</i>	0	1.0 ± 0.07 ^b	2.5 ± 0.2 ^a	5.1 ± 0.4 ^b	12.1 ± 0.9 ^c	–
<i>Pimpinella brach.</i>	0	0	2.0 ± 0.1 ^a	6.2 ± 0.5 ^b	17.1 ± 1.2 ^c	–
<i>Aster scaber</i>	1.9 ± 0.1 ^b	9.5 ± 0.8 ^d	24.9 ± 1.6 ^c	49.3 ± 3.9 ^e	83.3 ± 6.6 ^g	25.8
<i>Sedum sarmentosum</i>	0	0.1 ± 0.01 ^a	5.0 ± 0.4 ^a	14.4 ± 0.9 ^c	28.4 ± 1.7 ^d	–
Ascorbic acid	81.8 ± 6.5 ^e	96.1 ± 7.9 ^h	96.1 ± 7.9 ^f	96.7 ± 8.0 ^g	96.9 ± 8.0 ^h	< 3.1
BHT	15.6 ± 1.1 ^d	33.5 ± 2.6 ^g	55.2 ± 4.2 ^d	81.3 ± 6.5 ^f	92.4 ± 7.2 ^h	11.3

Values are means ± SD of 3 measurements. Means in columns without superscript letters in common differ significantly ($P < 0.05$).

†Extract concentrations, which show 50% activity of DPPH radical scavenging, were determined by interpolation. Abbreviations: *brach*, *brachycarpa*; *japon*, *japonicum*; *mong*, *mongolicum*.

Table 2

Cytotoxic effect of methanol extracts from the aerial parts of 11 Korean salad plants on three human cancer cell lines

Scientific name	IC ₅₀ † (μg mL ⁻¹)		
	Calu-6‡	MCF-7‡	HCT-116‡
<i>Petasites japonicus</i>	26.60 ± 1.9 ^a	34.87 ± 2.1 ^a	<25.00 ± 1.7 ^a
<i>Hosta longipes</i>	128.88 ± 9.7 ^d	182.64 ± 10.2 ^c	66.73 ± 5.1 ^b
<i>Angelica gigas</i>	57.05 ± 3.9 ^b	57.93 ± 3.9 ^b	34.75 ± 2.1 ^a
<i>Ixeris dentate</i>	157.50 ± 9.9 ^d	572.70 ± 14.1 ^f	354.16 ± 12.1 ^e
<i>Taraxacum mongolicum</i>	300.40 ± 11.9 ^e	328.81 ± 12.0 ^d	392.80 ± 12.3 ^e
<i>Aster yomena</i>	288.54 ± 11.8 ^e	300.40 ± 11.9 ^d	229.66 ± 11.3 ^d
<i>Capsella bursa-pastori</i>	135.63 ± 9.8 ^d	312.05 ± 11.9 ^d	481.93 ± 13.4 ^f
<i>Erythronium japonicum</i>	36.64 ± 2.3 ^a	66.11 ± 5.1 ^b	44.06 ± 3.2 ^a
<i>Pimpinella brachycarpa</i>	128.88 ± 9.7 ^d	421.42 ± 13.3 ^e	191.03 ± 10.3 ^c
<i>Aster scaber</i>	75.11 ± 5.9 ^c	177.53 ± 10.1 ^c	54.87 ± 3.8 ^b
<i>Sedum sarmentosum</i>	476.09 ± 13.4 ^f	747.58 ± 15.2 ^g	491.29 ± 13.4 ^f

Values are means ± SD of 3 measurements. Means in columns without superscript letters in common differ significantly ($P < 0.05$).

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

‡Calu-6 for human pulmonary carcinoma, MCF-7 for human breast adenocarcinoma pleural effusion, and HCT-116 for human colon carcinoma.

respectively. Methanol extracts from *Petasites japonicus* showed the highest activity against HCT-116 (IC₅₀ < 25.0 μg mL⁻¹), following by *Angelica gigas* (34.75 μg mL⁻¹), *Erythronium japonicum* (44.06 μg mL⁻¹), and *Aster scaber* (54.87 μg mL⁻¹). Methanol extracts from *Petasites japonicus* showed the highest activity against Calu-6 (IC₅₀ of 26.6 μg mL⁻¹), following by *Erythronium japonicum* (36.64 μg mL⁻¹), *Angelica gigas* (57.05 μg mL⁻¹), and *Aster scaber* (75.11 μg mL⁻¹). Methanol extracts from *Petasites japonicus* showed the highest activity against MCF-7 (IC₅₀ of 34.87 μg mL⁻¹),

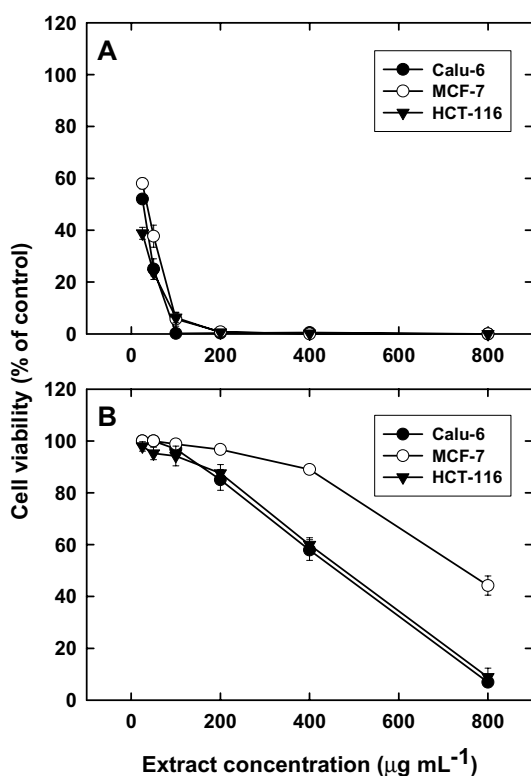


Fig. 2. A and B. Cytotoxic effect of methanol extracts from the aerial parts of *Petasites japonicus* (A) and *Sedum sarmentosum* (B) on human cancer cell lines, Calu-6, MCF-7, and HCT-116.

following by *Angelica gigas* ($57.93 \mu\text{g mL}^{-1}$), *Erythronium japonicum* ($66.11 \mu\text{g mL}^{-1}$), and *Aster scaber* ($177.53 \mu\text{g mL}^{-1}$), (Fig. 2).

4. Discussion

Increased consumption of fruit and vegetables significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies [21,23]. Phytochemicals, especially phenolics of these natural products are suggested to be the major bioactive compounds for these health benefits [26]. It is an established fact that phenolic compounds possess antioxidant properties [13]. As a consequence, consumption of these natural antioxidants is inversely related to some chronic diseases [13]. Antioxidants can delay or inhibit lipids oxidation by inhibiting the initiation or propagation of oxidative chain reactions [29].

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [12].

Now some investigators recommend to include in preventive diets fruits and vegetables only with high contents of phenolics and high antioxidant activity [20].

Therefore, not only the content of the total phenolics but also the related antioxidant activity were determined in the studied 11 Korean salad plants. We found that the highest total phenolic content

of the methanol extracts was in *Aster scaber* (17.1 mg 100 g⁻¹), followed by *Ixeris dentate* (16.4 mg 100 g⁻¹), *Aster yomena* (12.0 mg 100 g⁻¹) and *Sedum sarmentosum* (9.1 mg 100 g⁻¹). Our results are in accordance with the results of other authors [4,12]. So Javanmardia et al. [12] who studied antioxidant activity and total phenolic content of Iranian *Ocimum* accessions reported that the amount of total phenolics varied in different accessions and ranged from 22.9 to 65.5 mg GAE/g dry weight (DW). These authors found that the highest total phenolic levels were detected in “Babol”, “Isfahan” and “Mahallat”, and the lowest in “Dezful I” and “Dezful II”.

As was shown in our study, the content of total phenolics in plants varies significantly and the cited investigations by others supported our conclusion.

It was found that the methanol extracts of *Ixeris dentate* had the highest DPPH radical scavenging activity, with an IC₅₀ value of 23.8 mg 100 g⁻¹, and followed by *Aster scaber* (25.8 μg mL⁻¹), *Aster yomena* (33.9 mg 100 g⁻¹) and *Petasites japonicus* (42.9 mg 100 g⁻¹). We found that the radical scavenging activity of studied plants was lower than of synthetic antioxidants: vitamin C and BHT, with IC₅₀ values of < 3.1 and 11.3 mg 100g⁻¹, respectively. Methanol extracts of *Ixeris dentate* and *Aster scaber* at 50 mg 100 g⁻¹ exhibited the highest DPPH radical scavenging activity by 86.4 and 83.3%, respectively. The extract of *Hosta longipes* showed the lowest DPPH radical scavenging activity. The studied plants demonstrated a dose-dependent DPPH radical scavenging activity and the strong relation between the contents of total phenolics and the level of radical scavenging activity.

Other authors, who also studied antioxidant activity of medicinal plants, reported similar findings [4, 18]. So, Lee et al. [18] demonstrated that the methanol extracts of nine medicinal plants traditionally used in Chinese medicine have relatively high levels of radical scavenging activity in extracts of *Areca catechu* var. *dulcissima*, *Paeonia suffruticosa* and *Cinnamomun cassia* (IC₅₀ < 6.0 μg mL⁻¹). The extracts of *Areca catechu* var. *dulcissima* showed higher antioxidant activity than resveratrol in all experiments.

Cai et al. [4] studied the content of phenolic compounds, antioxidant and anticancer activities of 112 species from 50 plant families of traditional Chinese medicinal plants. The improved ABTS method was used to systematically assess the total antioxidant activity of the medicinal extracts. They found that TEAC values and total phenolic content for methanolic extracts of herbs ranged from 46.7 to 17,323 Trolox equivalent (TE)/100 g DW and from 0.22 to 50.3 g of gallic acid equivalent (GAE)/100 g DW, respectively. A positive, significant linear relationship between antioxidant activity and total phenolic content (all R² = 0.95) showed that phenolic compounds were the dominant antioxidant components in the tested medicinal herbs. Major types of phenolic compounds from most of the tested herbs were preliminarily identified and analyzed, and mainly included phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids.

Total equivalent antioxidant capacities (TEAC) and phenolics contents in 32 spices extracts from 21 botanical families grown in Poland were investigated [35]. It was found that the total phenolic content is ranged from 0.07 to 15.2 mg GAE/100 g DW. Similar analytical methods were used for the determination of total phenolics and additional radical scavenging assays such as ABTS (2, 2/azinobis-(3-ethylbenzthiazoline-6-sulfonic acid), DPPH and ferric reducing/antioxidant power (FRAP) were also employed in the cited report. Major phenolic acids identified in analyzed species were caffeic, p-coumaric, ferulic and neochlorogenic, while predominant flavonoids were quercetin, luteolin, apigenin, kaempferol and isorhamnetin. Myricetin was detected only in *Epilobium hirsutum*. As we have shown, also these Polish authors found that many investigated spices had high levels of phenolics and exhibited high antioxidant activity. Dastmalchi et al. [9] in their study used aerial material of Moldavian balm collected from Iran. For extraction of bioactive compounds seven solvents of different polarity (petroleum ether, dichloromethane, acetonitrile, ethyl acetate, methanol, n-butanol and water) were used.

Hydroxylated cinnamic acids, their derivatives and flavonoids were identified and quantified within the extracts, with rosmarinic acid being the most abundant component identified. As we found, these authors registered that extracts demonstrated different degrees of potency within each assay (β -carotene-linoleic acid bleaching assay, DPPH and ABTS).

It was an understandable interest to know how high levels of phenolics and exhibited high antioxidant activity influence the anticancer activity of the studied plants. We expected that the studied plants with the high content of the total phenolics and the antioxidant activity will have high anticancer influence. The methanol extracts of *Ixeris dentate* had the highest DPPH radical scavenging activity, with an IC_{50} value of $23.8 \text{ mg } 100 \text{ g}^{-1}$ following by *Aster scaber* ($25.8 \mu\text{g mL}^{-1}$). However, methanol extracts from *Petasites japonicus* has shown the most potent cytotoxicity on all 3 cancer cell lines (26.60 ± 1.9 , 34.87 ± 2.1 and $< 25.00 \pm 1.7$) following by *Angelica gigas* (57.05 ± 3.9 , 57.93 ± 3.9 and 34.75 ± 2.1) for Calu-6, MCF-7 and HCT-116, respectively. These results were different from results of other authors. So, in the already cited investigation of Cai et al. [4] is stated that cancer prevention and treatment using traditional Chinese medicines have attracted increasing interest. These medicinal herbs exhibited stronger antioxidant activity and contained significantly higher levels of phenolics than common vegetables and fruits. Cai et al. [4] concluded that traditional Chinese medicinal plants associated with anticancer might be potential sources of natural antioxidants and beneficial chemopreventive agents. Manosroi et al. [19] reported about 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. Their results showed that Guava (*Psidium guajava* L.) leaf and Sweet Basil oils exhibited the highest anti-proliferative activity in KB and P338 cell lines, respectively. Cho and Leung [7] have studied *in vitro* and *in vivo* anti-tumor effects of *Astragalus membranaceus* a commonly used Chinese medicinal plant. It has been shown to be capable of restoring the impaired T cell functions in cancer patients. Five bioactive fractions were isolated from the root of *Astragalus membranaceus*, the fraction designated as AI was found to be the most potent among the five fractions with respect to its mitogenicity on murine splenocytes. Besides investigating the cytostatic effect of AI, its activities on macrophage function, tumor necrosis factor production, induction of lymphokine-activated killer cell and tumor cell differentiation were also examined. The macrophage-like tumors and the myeloid tumors were found to be more sensitive to the cytostatic activity of AI, whereas the fibroblast-like tumors and the mouse Ehrlich ascites tumor appeared to be relatively resistant. Moreover, AI could effectively suppress the *in vivo* growth of syngeneic tumor in mice. Results showed that murine macrophage pretreated with AI had increased *in vitro* and *in vivo* cytostatic activities towards MBL-2 tumor. AI could also act as a priming agent for tumor necrosis factor production in tumor-bearing mice. Preincubation of mouse splenocytes with AI could induce *in vitro* lymphokine-activated killer-like activity towards WEHI-164 cell. Furthermore, AI was able to induce monocytic differentiation of both human and murine cells *in vitro*. AI administered *in vivo* could even partially restore the depressed mitogenic response in tumor-bearing mice. The authors concluded that *Astragalus membranaceus* could exhibit both *in vitro* and *in vivo* anti-tumor effects, which might be achieved through activating the anti-tumor immune mechanism of the host. All above cited studies support the results of our investigation: salad plants possess anti-tumor properties.

5. Conclusion

The studied salad plants have high total phenolics content and high antioxidant activity. These plants dose-dependently increased DPPH free radical scavenging activity. Their total phenolics level was highly correlated with the free radical scavenging activity. Most of the studied salad plants have potent

cytotoxicity activity. The above mentioned results suggest that the extracts of the aerial parts 11 Korean salad plants could be helpful as addition to basic medicine in treatment of some diseases.

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