Antioxidant and Antiproliferative Activities of Common Vegetables

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Epidemiological studies have shown that consumption of fruits and vegetables is associated with reduced risk of chronic diseases. Increased consumption of fruits and vegetables containing high levels of phytochemicals has been recommended to prevent chronic diseases related to oxidative stress in the human body. In this study, 10 common vegetables were selected on the basis of consumption per capita data in the United States. A more complete profile of phenolic distributions, including both free and bound phenolics in these vegetables, is reported here using new and modified methods. Broccoli possessed the highest total phenolic content, followed by spinach, yellow onion, red pepper, carrot, cabbage, potato, lettuce, celery, and cucumber. Red pepper had the highest total antioxidant activity, followed by broccoli, carrot, spinach, cabbage, yellow onion, celery, potato, lettuce, and cucumber. The phenolics antioxidant index (PAI) was proposed to evaluate the quality/quantity of phenolic contents in these vegetables and was calculated from the corrected total antioxidant activities by eliminating vitamin C contributions. Antiproliferative activities were also studied in vitro using $HepG_2$ human liver cancer cells. Spinach showed the highest inhibitory effect, followed by cabbage, red pepper, onion, and broccoli. On the basis of these results, the bioactivity index (BI) for dietary cancer prevention is proposed to provide a simple reference for consumers to choose vegetables in accordance with their beneficial activities. The BI could be a new alternative biomarker for future epidemiological studies in dietary cancer prevention and health promotion.

KEYWORDS: Cancer; phytochemicals; phenolics; antioxidant; antiproliferation; vegetables

INTRODUCTION

The presence of phytochemicals, in addition to vitamins and provitamins, in fruits and vegetables has been recently considered of crucial nutritional importance in the prevention of chronic diseases, such as cancer, cardiovascular disease, and diabetes (*1*, *2*). Many of these phytochemicals have been found to provide a much stronger antioxidant activity than vitamins C and E and β-carotene within the same food (*3*, *4*). Synergistically or additively, these dietary antioxidants provide bioactive mechanisms to reduce free radical induced oxidative stress. Oxidative stress results from either a decrease of natural cell antioxidant capacity or an increased amount of reactive oxygen species (ROS) in organisms. When the balance between oxidants and antioxidants in the body is shifted by the overproduction of free radicals, it will lead to oxidative stress and DNA damage. When left unrepaired, it can cause base mutation, single- and double-strand breaks, DNA cross-linking, and chromosomal breakage and rearrangement (*5*, *6*).

Consumption of fruits and vegetables has been associated with the prevention of chronic diseases such as cancer and cardiovascular disease (*5*, *7*, *8*). A significant inverse correlation has also been reported between total fruits and vegetables intake and cerebrovascular disease mortality (*9*). Because prevention is a more effective strategy than treatment for chronic diseases, a constant supply of phytochemical-containing plants with desirable health benefits beyond basic nutrition is essential to furnish the defensive mechanism to reduce the risk of chronic diseases in humans (*6*). Recent research has also shown that, through overlapping or complementary effects, the complex mixture of phytochemicals in fruits and vegetables provides a better protective effect on health than single phytochemicals (*4*). This perspective has been strengthened by the occurrence of inconsistent results in human clinical trials using single antioxidants (*10*−*12*). Although 5,000 plant phytochemicals have been identified, a large proportion remains unknown (*13*). Different plants have different contents of phytochemicals with different structures and thus offer different protective mechanisms to different levels. To obtain optimal health benefits from dietary phytochemicals, it is suggested that humans consume a balanced diet with a variety of phytochemical sources from whole foods, such as fruits, vegetables, and grains as part of whole meals (*6*).

Food going through the human gastrointestinal tract is digested in the stomach (strong acid environment with enzymes),

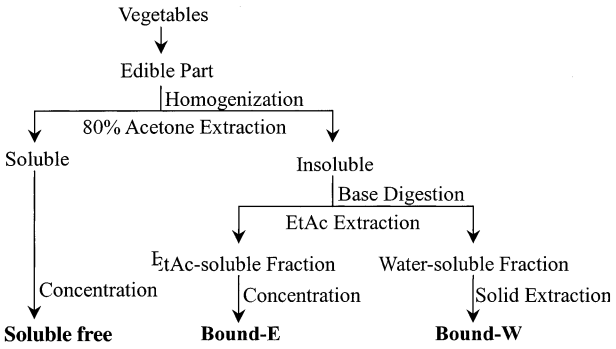

Figure 1. Flowchart of phytochemical extraction of vegetables.

small intestine (mild base environment with enzymes), and then colon (neutral pH with intestinal microflora). Phenolics in vegetables are present in both free and bound forms. Bound phenolics, mainly in the form of β-glycosides, may survive human stomach and small intestine digestion and reach the colon intact, where they are released and exert bioactivity (*14*). However, most of the previous investigations determined primarily free phenolics on the basis of the solvent-soluble extraction. In this respect, the total phenolic contents of vegetables and their antioxidant activities were underestimated in the literature because the bound phenolics were not included.

In this study, 10 common vegetables were selected on the basis of consumption per capita data in the United States (*15*). The objectives of this research were designed to (1) establish the complete distribution profile of phytochemicals that exist in the free and bound forms and evaluate the quantity and quality of phenolics in common vegetables, (2) measure the total antioxidant activities, (3) determine the antiproliferative activity of common vegetable extracts on human liver cancer cell growth in vitro and (4) provide a bioactivity index (BI) of common vegetables for dietary prevention of cancer.

MATERIALS AND METHODS

Chemicals. Sodium nitrite, (+)-catechin, Folin−Ciocalteu reagent, hydrochloric acid, glucagon, insulin, hydrocortisone, Hepes, and α-keto-γ-methiolbutyric acid (KMBA) were obtained from Sigma Chemical Co. (St. Louis, MO). Aluminum chloride, sodium hydroxide, methyl *tert*-butyl ether, methanol, and acetone were purchased from Fisher Scientific (Pittsburgh, PA). Gallic acid was purchased from ICN Biomedical Inc. (Costa Mesa, CA). 2,2′-Azobis(amidinopropane) was obtained from the Wako Chemicals (Richmond, VA). All reagents used were of analytical grade. Williams medium E (WME) and fetal bovine serum were purchased from Gibco Life Technologies (Grand Island, NY).

Sample Preparation. Fresh vegetables (broccoli, cabbage, carrot, celery, cucumber, lettuce, spinach, onion, potato, and red pepper) were purchased from a local supermarket. These 10 vegetables were selected on the basis of consumption per capita data in the United States (*15*). Samples were cleaned and dried before extraction.

Extraction of Soluble Free Phenolic Compounds. The phytochemical extraction of vegetables is shown in the flowchart of **Figure 1**. For the extraction of soluble free phytochemicals, 50 g of the edible part of vegetables was weighed and homogenized with 80% acetone (1:2 w/v) using a chilled Waring blender for 5 min. The sample was then further homogenized using a Polytron homogenizer for an additional 3 min to obtain a thoroughly homogenized sample. The homogenates were filtered through Whatman no. 2 filter paper on a Büchner funnel under vacuum. The residues were saved for extractions of bound phytochemicals. The filtrate was evaporated using a rotary evaporator under vacuum at 45 °C until ∼90% of the filtrate had been evaporated. The vegetable extracts were frozen at −40 °C until analysis.

Extraction of Bound Phenolic Compounds (Bound-E and Bound-W). Bound phytochemicals of vegetables were extracted according to the method reported previously (*16*) and modified in our laboratory (*17*). Bound phenolic contents were composed of bound-E and bound-W, which have distinct extraction properties (**Figure 1**). Briefly, the residues from above soluble free extraction were flushed with nitrogen gas and hydrolyzed directly with 20 mL of 4 N NaOH at room temperature for 1 h with shaking. The mixture was acidified to pH 2 with concentrated hydrochloric acid and extracted six times with ethyl acetate. The ethyl acetate fraction was evaporated at 45 °C under vacuum to dryness. Phenolic compounds extracted by ethyl acetate, designated bound-E, were reconstituted in 10 mL of water and stored at −40 °C until analysis. The remaining water-soluble portion was neutralized to pH 7 and was then applied to a column packed with muffled Celite. The phytochemicals were eluted through the column by 20% methanol/ethyl acetate. Then the eluate was evaporated under vacuum at 45 °C to dryness. Phenolic compounds in this portion, designated bound-W, were recovered with 10 mL of water and stored at −40 °C until analysis.

Determination of Total Phenolic Content. The content of total phenolics was analyzed spectrophotometrically using the Folin−Ciocalteu colorimetric method reported previously (*18*) with modifications (*19*). All vegetable extracts were diluted 1:5 with distilled water to obtain readings within the standard curve ranges of 0.0−600.0 μg of gallic acid/mL. Briefly, 125 μL of the standard gallic acid solution or 1:5 diluted vegetable extract was mixed with 0.5 mL of distilled water in a test tube followed by the addition of 125 μL of Folin−Ciocalteu reagent. The samples were mixed well and then allowed to stand for 6 min before 1.25 mL of a 7% sodium carbonate aqueous solution was added. Water was added to adjust the final volume to 3 mL. Samples were allowed to stand for 90 min at room temperature before the absorbance was measured at 760 nm versus the blank using an MRX II Dynex spectrophotometer (Dynex Technologies, Inc., Chantilly, VA). Absorbance values were compared with those of standards prepared similarly with known gallic acid concentrations. All values were expressed as mean (milligrams of gallic acid equivalents per 100 g of vegetable) ± SD for three replications. All extracts were made in triplicate.

Total Antioxidant Activity Measurement. The total oxyradical scavenging capacity (TOSC) assay (*20*) with modifications (*4*) was used to quantify the total antioxidant activity of the phytochemicals in vegetable extracts. The degree of inhibition of ethylene formation from KMBA in the presence of antioxidant competitors for oxyradicals was determined by a Hewlett-Packard gas chromatograph (series 5790) with a flame ionization detector. Antioxidant activity was measured at 15, 30, 45, and 60 min for four different extract concentrations plus one control. The TOSC value of each concentration was calculated by integrating the area under the kinetic curve and then quantified by the following equation, where $\int$SA and $\int$CA are the integrated areas from the sample and control reaction, respectively:

$$\text{TOSC} = 100 - \left(\int \text{SA} / \int \text{CA}\right) \times 100$$

The TOSC is expressed as micromoles of vitamin C equivalents per gram of sample. All values were presented as mean ± SD for three replicates. All extracts were made in triplicate.

Measurement of Cell Proliferation. Antiproliferative activities of common vegetable extracts were measured according to the method described previously (*21*). $HepG_2$ cells [The American Type Culture Collection, ATCC, Rockville, MD] were maintained in Williams medium E (WME), containing 10 mM Hepes, 5 μg/mL insulin, 2 μg/mL glucagon, 0.05 μg/mL hydrocortisone, and 5% fetal bovine serum. $HepG_2$ cells were maintained at 37 °C in 5% CO_2 in an incubator. Cell concentrations of 2.5×10^4/well in the growth media were placed in each well of a 96-well flat-bottom plate. The cell number was determined from a linear response curve during 96 h of cell growth. After 4 h of incubation at 37 °C in 5% CO_2, the growth medium was removed and media containing various concentrations (1, 5, 10, 20, 30, 40, and 50 mg/mL) of vegetable extracts were added to the cells. Control cultures received the extraction solution minus the vegetable extract, and blank wells contained 100 μL of growth medium with no cells. After 96 h of incubation, cell proliferation was determined using the colorimetric MTS assay (MTS-based cell titer 96 nonradioactivity

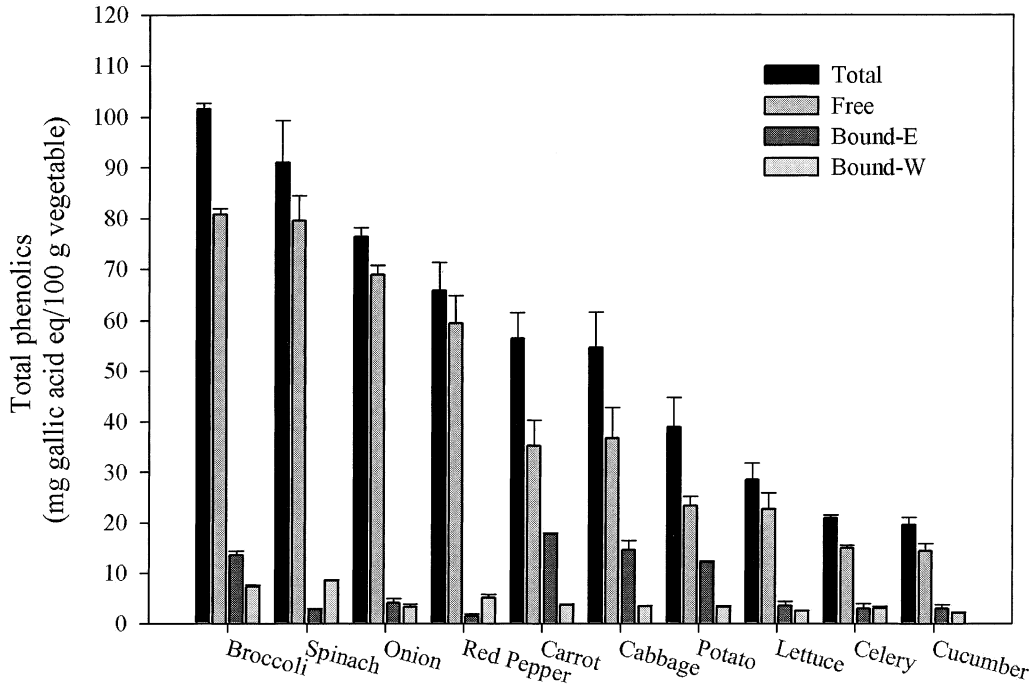

Figure 2. Phenolic contents in vegetables (mean ± SD, $n = 3$).

cell proliferation assay) (Promega, Madison, WI), a colorimetric method utilizing a tetrazolium reagent. Cell proliferation (percent) was determined at 96 h from the MTS absorbance (490 nm) reading for each concentration compared to the control. At least three replications for each sample were used to determine the cell proliferation (percent) value.

Statistical Analysis. Statistical analysis was conducted using SigmaStat version 8.0 (Jandel Corp., San Raphael, CA). Differences among vegetable samples were determined using the ANOVA test. For relationship plots, significance of the relationship was determined by regression analysis of variance using Minitab release 12 software (Minitab Inc., State College, PA).

RESULTS

Total Phenolic Contents of Common Vegetables. The total phenolic contents of 10 selected common vegetables are shown in **Figure 2**. Phenolic contents were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight of the edible part of vegetables. Broccoli and spinach had the highest amount of free phenolics (80.76 ± 1.17 and 79.55 ± 8.39 mg/100 g of sample, respectively), followed by yellow onion (68.90 ± 1.84 mg/100 g of sample), red sweet pepper (59.39 ± 5.38 mg/100 g of sample), cabbage (36.66 ± 6.93 mg/100 g of sample), carrot (35.19 ± 5.00 mg/100 g of sample), potato (23.31 ± 5.83 mg/100 g of sample), and lettuce (22.55 ± 3.24 mg/100 g of sample). Cucumber had the lowest free phenolics (14.37 ± 1.48 mg/100 g of sample) of the 10 vegetables, which was just slightly below celery's (14.95 ± 0.51 mg/100 g of sample).

For the phenolic contents in the bound-E fraction, carrot had the highest bound-E phenolics (17.65 ± 0.03 mg/100 g of sample), followed by cabbage (14.64 ± 0.02 mg/100 g of sample), broccoli (13.57 ± 0.06 mg/100 g of sample), potato (12.20 ± 0.05 mg/100 g of sample), yellow onion (4.11 ± 0.07 mg/100 g of sample), lettuce (3.50 ± 0.002 mg/100 g of sample), celery (2.94 ± 0.03 mg/100 g of sample), cucumber (2.92 ± 0.07 mg/100 g of sample), spinach (2.84 ± 0.09 mg/100 g of sample), and red pepper (1.42 ± 0.08 mg/100 g of sample).

Spinach had the highest bound-W phenolics (8.6 ± 0.01 mg/100 g of sample), followed by broccoli (7.3 ± 0.01 mg/100 g of sample), red pepper (5.06 ± 0.09 mg/100 g of sample), carrot (3.58 ± 0.07 mg/100 g of sample), cabbage (3.33 ± 0.07 mg/100 g of sample), potato (3.28 ± 0.05 mg/100 g of sample), yellow onion (3.27 ± 0.06 mg/100 g of sample), celery (3.01 ± 0.05 mg/100 g of sample), lettuce (2.41 ± 0.09 mg/100 g of sample), and cucumber (2.17 ± 0.06 mg/100 g of sample). Generally, free phenolic contents were higher than bound phenolic contents ($p < 0.01$), and the content of bound-E phenolics was higher than that of bound-W phenolics ($p < 0.05$). It is interesting to note that carrot, cabbage, broccoli, and potato had relatively higher amounts of bound-E phenolics compared to the other vegetables in this study ($p < 0.05$). In addition, the bound-W phenolics, usually lower, were significantly higher in spinach and red pepper ($p < 0.05$) than their bound-E counterparts.

The total (free plus bound) phenolic contents followed the same order as the free phenolic contents because free phenolics contributed 76.2% to the total, on average (**Figure 2**). Broccoli and cucumber, again, had the highest and lowest overall phenolic contents with 101.6 ± 1.24 and 19.5 ± 1.61 mg/100 g of sample, respectively.

Total Antioxidant Activity. The total antioxidant activity, determined by TOSC assay, is shown in **Figure 3**. The total antioxidant activities of 10 common vegetables are listed in the order of descending antioxidant activity. The 10 common vegetables could be divided into three major groups on the basis of the significant differences in their total antioxidant activities. Red pepper, broccoli, carrot, and spinach were in the highest group with antioxidant activities of 46.95 ± 1.57, 44.03 ± 1.87, 42.56 ± 1.04, and 42.20 ± 0.71 μmol of vitamin C equiv/g of sample, respectively. The medium group comprised cabbage (17.97 ± 0.52 μmol of vitamin C equiv/g of sample) and yellow onion (14.09 ± 0.1 μmol of vitamin C equiv/g of sample). The remaining four vegetables in the group with lower antioxidant activities included celery (5.08 ± 0.33 μmol of vitamin C equiv/g of sample), potato (4.86 ± 0.2 μmol of vitamin C equiv/g of sample), lettuce (2.73 ± 0.08 μmol of vitamin C equiv/g of sample), and cucumber (1.28 ± 0.05 μmol of vitamin C equiv/g of sample).

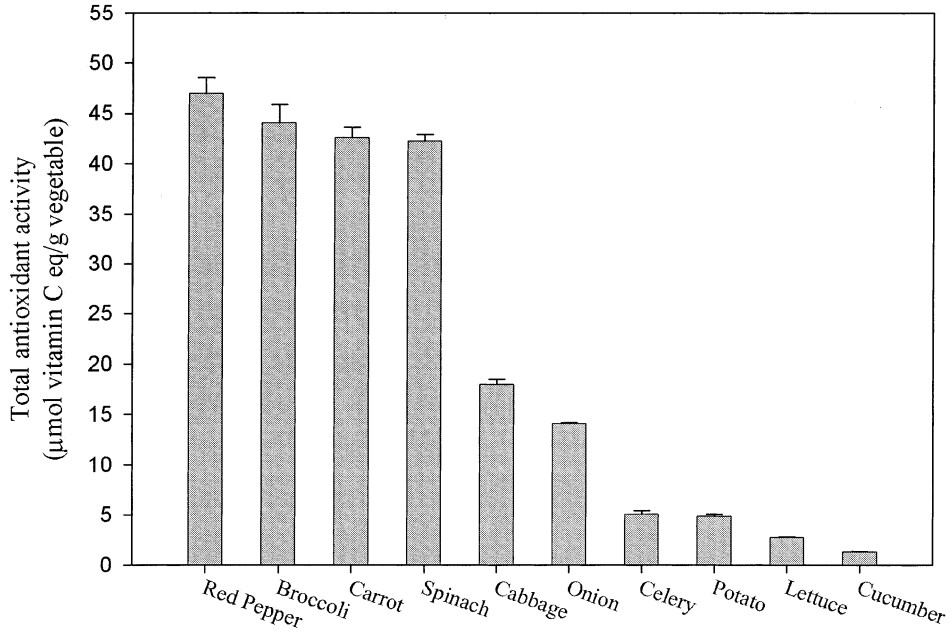

Figure 3. Total antioxidant activity of soluble free phytochemical extracts of vegetables (mean ± SD, $n = 3$).

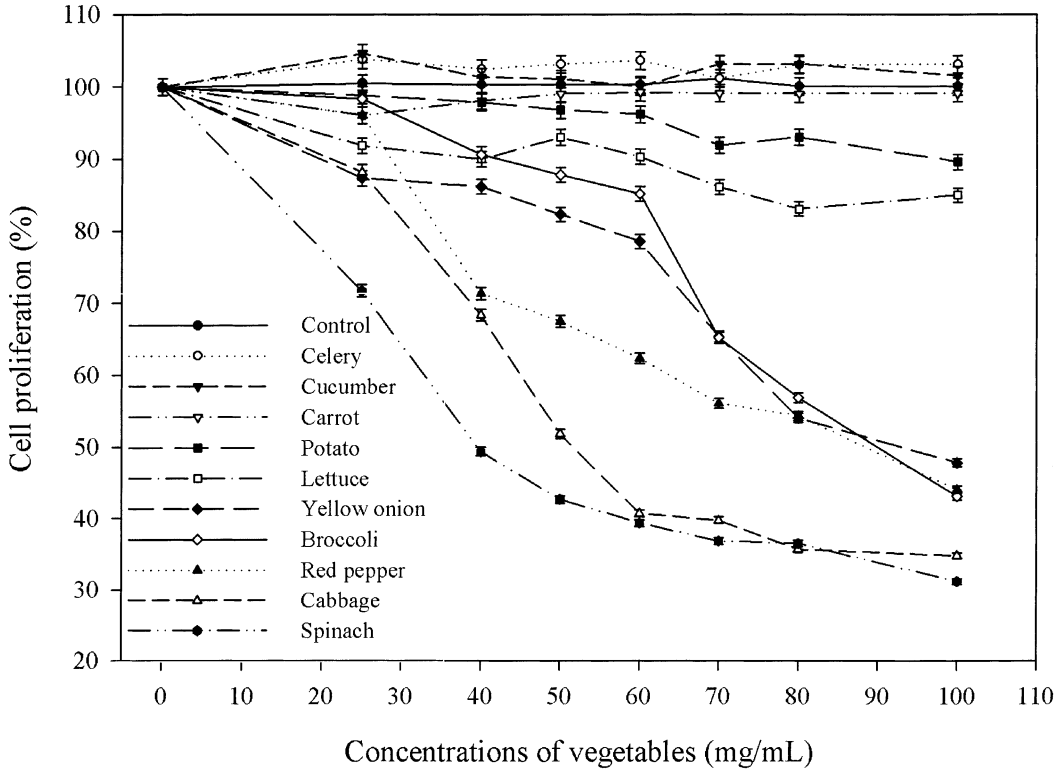

Figure 4. Percentage inhibition of $HepG_2$ cell proliferation by soluble free extracts of selected vegetables (mean ± SD, $n = 3$).

Antiproliferative Activity. Antiproliferative activities of vegetable extracts on the growth of $HepG_2$ human liver cancer cells in vitro are summarized in **Figure 4**. Among the 10 tested vegetables, spinach, cabbage, red pepper, yellow onion, and broccoli exhibited relatively potent inhibitory activity on $HepG_2$ cell growth in a dose-dependent manner. The results were expressed as the median effective dose (EC_{50}), with a lower EC_{50} value indicating a higher antiproliferative activity (**Figure 5**). Spinach had the highest antiproliferative capacity with the lowest EC_{50} (42.51 ± 1.68 mg/mL), followed by cabbage (56.26 ± 2.24 mg/mL), red pepper (76.75 ± 3.04 mg/mL), yellow onion (100.25 ± 4.0 mg/mL), and broccoli (112.74 ± 4.5 mg/mL). Minor inhibitory activities were observed for potato and lettuce extracts (**Figure 4**), but the EC_{50} could not be accurately calculated because of the possible deviation from the extrapolation estimate. There were no detectable antiproliferative activities for celery, cucumber, and carrot.

DISCUSSION

ROS have been linked to cardiovascular disease, cancer, aging, and several other chronic diseases because of their ability to introduce oxidative damage to biomolecules, for example, lipids, DNA, and proteins. Fruits and vegetables provide a wide variety of ROS-scavenging antioxidants such as phytochemicals

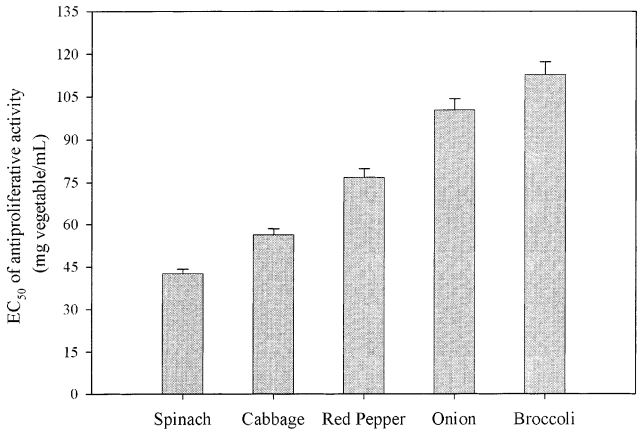

Figure 5. Antiproliferative activity of free phytochemical extracts of vegetables (mean ± SD, $n = 3$).

Table 1. Percentage Contributions of Free and Bound Phenolics in Vegetables

		bound (%)		
vegetable	free (%)	bound-E	bound-W	total
broccoli	79.5	13.4	7.1	20.5
cabbage	67.1	26.8	6.1	32.9
carrot	62.4	31.3	6.3	37.6
celery	71.5	14.1	14.4	28.5
cucumber	73.8	15.0	11.2	26.2
lettuce	79.2	12.3	8.5	20.8
spinach	87.5	3.1	9.4	12.5
onion	90.3	5.4	4.3	9.7
potato	60.1	31.4	8.5	39.9
red pepper	90.2	2.1	7.7	9.8
av	76.2	15.5	8.3	23.8

and antioxidant vitamins. It has been proposed that phytochemicals are the major contributors to the antioxidant capacity of fruits (*4*). Thus, increased consumption of fruits and vegetables containing high levels of phytochemicals has been recommended to prevent or reduce oxidative stress in the human body (*2*, *5*, *6*). Consequently, understanding the phytochemical distribution profile in fruits and vegetables is of primary importance. However, the total phenolic contents of vegetables were underestimated in the literature by not including the bound phenolics.

This study established a more complete profile of total phenolic contents in vegetables by further digesting and extracting the bound phytochemicals (**Figure 1**). Phenolics in vegetables are present in both free and bound forms, and bound phenolics consisted of bound-E and bound-W owing to their distinct extraction properties. The percentage contributions of free, bound-E, and bound-W phytochemicals are shown in **Table 1**. The average contribution from bound phenolics was 24%. Thus, total phenolics followed the same concentration trend as their free consitituents (**Figure 2**). Broccoli had the highest amount of total phenolic compounds (80.76 ± 1.17 mg/100 g of sample), whereas cucumber had the lowest (14.37 ± 1.48 mg/100 g of sample). As for the free and bound phenolics distribution, onion had the highest percentage of free phenolics (90.3%), closely followed by red pepper (90.2%). On the other hand, potato had the highest bound (bound-E + bound-W) phenolics (39.9%), and carrot contained the second highest bound fraction (37.6%) of the vegetables tested. Bound phenolics of vegetables, mostly in ester forms, are associated with cell wall components. Most noteworthy among them is ferulic acid (FA) (*22*). FA has been found to be esterified to several polysaccharide families, including pectic polysaccharides (*23*), and cross-linked as a result of peroxidative activity (*24*). Its role in cross-linking polysaccharides has been implicated in conferring stability during the mechanical processing of edible plant tissues (*25*, *26*). Owing to this protective mechanism, it is possible that bound phenolics can survive upper gastrointestinal digestion and may ultimately be broken down in the colon by fermentation by the microflora of the large intestine. On average, approximately a fourth of the fresh vegetable phenolic compounds may be released and absorbed in the colon to furnish additional healthful benefits locally, whereas potato and carrot could release approximately half of their phytochemical contents in the colon. Epidemiological studies have shown an inverse correlation between vegetable consumption and colon cancer incidence (*27*, *28*). We believe this could be attributed partly to the locally released phytochemicals and their consequent healthful functionalities.

The phytochemical extracts of vegetables showed potent antioxidant activities. The total antioxidant activity of 100 g of red pepper was equivalent to that of 826 mg of vitamin C, followed by broccoli (775 mg of vitamin C equiv/100 g), carrot (750 mg of vitamin C equiv/100 g), spinach (737 mg of vitamin C equiv/100 g), cabbage (314 mg of vitamin C equiv/100 g), onion (231 mg of vitamin C equiv/100 g), celery (90 mg of vitamin C equiv/100 g), potato (83 mg of vitamin C equiv/100 g), lettuce (49 mg of vitamin C equiv/100 g), and cucumber (21 mg of vitamin C equiv/100 g). The additive and/or synergistic mechanisms of phytochemicals in the vegetables may contribute to the potent antioxidant activities.

Generally, fruits have higher total phenolic contents than vegetables (*29*, *30*). Although there is a positive correlation between antioxidant activity (TOSC) and free phenolic contents in vegetables observed in this study, it was not strong ($R^2 = 0.57$). Therefore, an objective index to evaluate the total antioxidant activities of free phenolics in vegetables is necessary because free phytochemicals are more readily absorbed and, thus, exert beneficial bioactivities in early digestion. However, antioxidant activities generated by vitamin C, which is prominent in the free water-soluble form, should be calculated and deducted from the total antioxidant values in order to precisely estimate the antioxidant index of phenolics. **Table 2** shows the percentage of antioxidant activities contributed by vitamin C in vegetables. Carrot, onion, and spinach had <5% of the total antioxidant activities contributed from vitamin C with 1.2, 2.6, and 3.8%, respectively. Cucumber (23.5%) and red pepper (23%), on the other hand, had relatively high percentages of vitamin C contribution to the total antioxidant activities. Vinson et al. reported a phenol antioxidant index (PAOXI) by dividing phenol content by inhibitory activity on low-density lipoprotein (LDL) oxidation (*31*). Here we propose the phenolics antioxidant index (PAI), which was calculated by phenolic content divided by the EC_{50} of total antioxidant activity after subtraction of the contribution from vitamin C (**Table 2**). PAI could be used to demonstrate both quality and quantity of phenolics in vegetables. With this evaluation, spinach ranked first with the highest PAI (0.979), followed by broccoli, red pepper, carrot, yellow onion, cabbage, potato, celery, lettuce, and cucumber (PAI = 0.004).

The inhibition of in vitro tumor cell proliferation was studied by adding vegetable phytochemical extracts to $HepG_2$ cell cultures. Five of the 10 selected common vegetables had potent inhibitory effects on $HepG_2$ cell growth in a dose-dependent manner (**Figure 4**). The antiproliferative activities were expressed as EC_{50} values (**Figure 5**). A lower EC_{50} value represents a higher inhibitory ability on cancer cell proliferation.

Table 2. Contribution of Vitamin C to the Total Antioxidant Activity and Phenolic Antioxidant Index (PAI)

	vitamin C					
vegetable	content[a] (mg/g)	antioxidant activity (μmol/g)	contribution to total antioxidant activity (%)	corrected total antioxidant activity[b] (μmol of vitamin C equiv/g)	PAI[c]	PAI rank
spinach	0.28	1.6	3.8	40.60	0.979	1
broccoli	0.93	5.3	12.0	38.73	0.948	2
red pepper	1.90	10.8	23.0	36.16	0.651	3
carrot	0.09	0.5	1.2	42.03	0.448	4
onion	0.06	0.4	2.6	13.73	0.287	5
cabbage	0.32	1.8	10.2	16.15	0.179	6
potato	0.11	0.6	13.3	4.22	0.030	7
celery	0.07	0.4	7.8	4.69	0.021	8
lettuce	0.04	0.2	8.1	2.51	0.017	9
cucumber	0.05	0.3	23.5	0.98	0.004	10

[a] USDA Nutrient Database for Standard Reference. [b] Corrected total antioxidant activity = total antioxidant activity – vitamin C antioxidant activity. [c] PAI = free phenolic content/EC_{50} of corrected total antioxidant activity.

Table 3. Bioactivity Index (BI) of Selected Vegetables for Dietary Cancer Prevention

	total antioxidant activity			antiproliferative activity				
vegetable	TOSC (μmol of vitamin C equiv/g)	score	rank	EC_{50} (mg/mL)	score	rank	BI[a]	BI rank
spinach	42.20	0.90	4	42.51	1.00	1	0.95	1
red pepper	46.95	1.00	1	76.75	0.55	3	0.78	2
broccoli	44.03	0.94	2	112.74	0.38	5	0.66	3
cabbage	17.97	0.38	5	56.26	0.76	2	0.57	4
carrot	42.56	0.91	3	ND[b]	0	6	0.45	5
onion	14.09	0.30	6	100.25	0.42	4	0.36	6
celery	5.08	0.11	7	ND	0	6	0.05	7
potato	4.86	0.10	8	ND	0	6	0.05	8
lettuce	2.73	0.06	9	ND	0	6	0.03	9
cucumber	1.28	0.03	10	ND	0	6	0.01	10

[a] BI = $^1/_2$(total antioxidant activity score + antiproliferative activity score). [b] Not detected.

There were significant differences ($p < 0.01$) in antiproliferative activities in any two of these five vegetables (**Figure 5**). Notably, correlations between phytochemical contents and EC_{50} for these five vegetables were not significant ($R^2 = 0.1211$). This correlation suggests that the inhibition of human liver cancer cells by vegetables could not be explained solely by their phenolic contents. Therefore, it is assumed that unique phytochemicals in each vegetable were responsible for the antiproliferative activities. Further investigations should be designed to identify the individual components in the vegetables that inhibit the proliferation of tumor cells.

The bioactivity index (BI) for dietary cancer prevention is proposed here to provide a simple reference for consumers to choose vegetables on the basis of their beneficial activities (**Table 3**). Because red pepper and spinach had the highest antioxidant and antiproliferative activities, respectively, their experimental values were used as the controls to calculate the BI by the following equations:

$$\text{total antioxidant activity score} = \text{sample TOSC value/pepper extract's TOSC value} \quad (1)$$

$$\text{antiproliferative activity score} = \text{spinach extract's } EC_{50} \text{ value/sample } EC_{50} \text{ value} \quad (2)$$

$$\text{BI} = {}^1/_2(\text{total antioxidant activity score} + \text{antiproliferative activity score}) \quad (3)$$

Spinach had the highest BI value (0.95) among the 10 common vegetables tested, followed by red pepper (0.78), broccoli (0.66), cabbage (0.57), carrot (0.45), yellow onion (0.36), celery (0.05), potato (0.05), lettuce (0.03), and cucumber (0.01). Although there was no detectable antiproliferative activity for carrot, its BI value was still higher than that of onion, which possessed a detectable EC_{50}. This is due to carrot's relatively high antioxidant activity, possibly provided by its phytochemical (*32*) and carotenoid contents (*33*). We believe that the BI reported here could serve as a new alternative biomarker for future epidemiological studies in dietary cancer prevention, although the values reported here may be influenced by growing environment, storage duration and conditions in the markets, and processing conditions.

In summary, a more complete profile of phenolic distributions in 10 common vegetables was established in this study using new and modified methods. Because bound phytochemicals contributed ~24% to the total contents, the phenolics in vegetables have been underestimated in the literature. Broccoli possessed the highest total phenolic content (101.6 ± 1.24 mg/100 g of sample), followed by spinach, yellow onion, red pepper, carrot, cabbage, potato, lettuce, celery, and cucumber. Total antioxidant activities were measured using the TOSC assay. Red pepper had the highest total antioxidant activity (46.95 ± 1.57 μmol of vitamin C equiv/g of sample), followed by broccoli, carrot, spinach, cabbage, yellow onion, celery, potato, lettuce, and cucumber. The PAI was proposed to evaluate the quality/quantity of phenolic contents in these vegetables and was calculated from corrected total antioxidant activities by eliminating vitamin C contributions. Antiproliferative activities were also studied in vitro using $HepG_2$ human liver cancer cells. Spinach showed the highest inhibitory effect, followed by cabbage, red pepper, onion, and broccoli. From these results,

the BI for dietary cancer prevention was proposed to provide a simple reference for consumers to choose vegetables on the basis of their beneficial activities. We believe that the BI could be a new alternative biomarker for future epidemiological studies in dietary cancer prevention and health promotion.

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