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Antioxidant and Antiproliferative Activities of Common Fruits

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Consumption of fruits and vegetables has been associated with reduced risk of chronic diseases such as cardiovascular disease and cancer. Phytochemicals, especially phenolics, in fruits and vegetables are suggested to be the major bioactive compounds for the health benefits. However, the phenolic contents and their antioxidant activities in fruits and vegetables were underestimated in the literature, because bound phenolics were not included. This study was designed to investigate the profiles of total phenolics, including both soluble free and bound forms in common fruits, by applying solvent extraction, base digestion, and solid-phase extraction methods. Cranberry had the highest total phenolic content, followed by apple, red grape, strawberry, pineapple, banana, peach, lemon, orange, pear, and grapefruit. Total antioxidant activity was measured using the TOSC assay. Cranberry had the highest total antioxidant activity (177.0 ± 4.3 μmol of vitamin C equiv/g of fruit), followed by apple, red grape, strawberry, peach, lemon, pear, banana, orange, grapefruit, and pineapple. Antiproliferation activities were also studied in vitro using $HepG_2$ human liver-cancer cells, and cranberry showed the highest inhibitory effect with an EC_{50} of 14.5 ± 0.5 mg/mL, followed by lemon, apple, strawberry, red grape, banana, grapefruit, and peach. A bioactivity index (BI) for dietary cancer prevention is proposed to provide a new alternative biomarker for future epidemiological studies in dietary cancer prevention and health promotion.

KEYWORDS: Phytochemicals; phenolics; cancer; antioxidant; antiproliferation; fruits

INTRODUCTION

Epidemiological studies have shown that dietary patterns were significantly associated with the prevention of chronic diseases such as heart disease, cancer, diabetes, and Alzheimers's disease (*1*, *2*). Consumption of fruits and vegetables has been highly associated with the reduced risk of cancer (*3*, *4*).

In the status of normal metabolism, the levels of oxidants and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions (*1*, *5*). Overproduction of oxidants in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and proteins (*6*). More and more evidence suggests that this potentially cancer-inducing oxidative damage might be prevented or limited by dietary antioxidants found in fruits and vegetables. Phytochemicals in fruits and vegetables can have complementary and overlapping mechanisms of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral effects (*6*). Recent studies showed that the phytochemicals, especially phenolics, in fruits and vegetables are the major bioactive compounds with human health benefits (*7*, *8*). There was a direct relationship between the total phenolic contents and the antioxidant activities in fruits and vegetables (*8*, *9*). Eberhardt et al. (*10*) demonstrated that the vitamin C in apples only contributed less than 0.4% of total antioxidant activity, suggesting that the complex mixture of phytochemicals in fruits and vegetables provided protective health benefits mainly through a combination of additive and/or synergistic effects.

In the human gastrointestinal system, food is digested in the stomach (acid environment with enzymes), small intestine (mild base environment with enzymes), and colon (neutral pH environment with intestinal microflora). Phenolics in fruits are in both soluble free and bound forms. Bound phenolics, mainly in the form of β-glycosides, may survive the human stomach and small intestine digestion and reach the colon intact, where they are released to exhibit their bioactivity with health benefits (*11*). However, most of the previous research mainly determined the soluble free phenolics on the basis of the solvent-soluble extraction. Therefore, the total phenolic contents of fruits and their antioxidant activities were underestimated in the literature because the bound phenolics were not included. The objectives of this research were (1) to determine the profiles of total phenolics, including both soluble free and bound forms in common fruits, (2) to determine the total antioxidant activities of common fruits, (3) to determine the antiproliferative activities of common fruits on human liver cancer cell growth, and (4)

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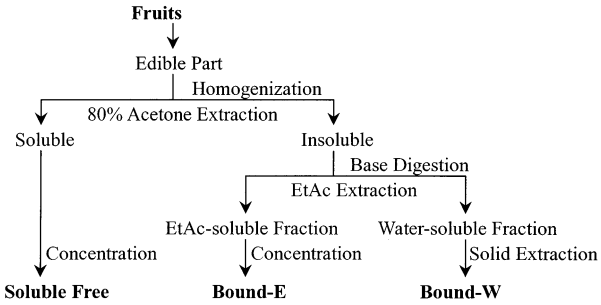

Figure 1. Flowchart of phytochemical extraction of fruits.

to estimate the bioactivity index (BI) of common fruits for dietary prevention of cancer.

MATERIALS AND METHODS

Chemicals. Sodium hydroxide, methyl *tert*-butyl ether, methanol, and acetone were purchased from Fisher Scientific (Pittsburgh, PA). Gallic acid was obtained from ICN Biomedical Inc. (Costa Mesa, CA). 2,2′-Azobis(amidinopropane) (ABAP) was obtained from the Wako Chemicals (Richmond, VA). Folin−Ciocalteu reagent, hyrdrochloric acid, and α-keto-γ-methiolbutyric acid (KMBA) were purchased from Sigma Chemical Co. (St. Louis, MO). All reagents used in the study were of analytical grade.

Sample Preparation. A total of 11 fruits (cranberry, apple, red grape, strawberry, pineapple, banana, peach, lemon, orange, pear, and grapefruit) were chosen on the basis of the consumption per capita data in the U.S. (USDA database). Fresh fruits were purchased from local supermarkets. Fruits were cleaned and dried before extraction. All data collected for each fruit were reported as means ± SD for at least three replications.

Extraction of Soluble Free Phenolic Compounds. Total phenolic extraction of fruits is showed in the flowchart of **Figure 1**. Soluble free phenolics of fruits were extracted using the method reported previously in our laboratory (*10*, *12*). Briefly, 100 g of fresh weight of the edible part of fruits was weighed and homogenized with chilled 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. The homogenates were filtered through no. 2 Whatman paper on a Buchner funnel under vacuum. The acetone in the filtrate was evaporated at 45 °C until approximately 90% of the filtrate had been evaporated. The filtrate was then recovered with water to a final volume of 50 mL. The soluble free phenolic extracts contained both free aglycones and soluble conjugates (glycosylated forms) and were stored at −40 °C until use.

Extraction of Bound Phenolic Compounds (Bound-E and Bound-W). Bound phytochemicals of fruits were extracted by the method reported previously (*13*) and modified in our laboratory (*14*). Bound phenolics consisted of bound-E and bound-W. Briefly, the residues from the above soluble free extraction were collected and hydrolyzed directly with 20 mL of 4 N NaOH at room temperature for 1 h with shaking under nitrogen gas. The mixture was neutralized with concentrated hydrochloric acid and extracted six times with ethyl acetate. The ethyl acetate fraction was evaporated at 45 °C to dryness. Phenolic compounds extracted by ethyl acetate were designated bound-E and were reconstituted in 10 mL of water and stored at −40 °C until use. The remaining water-soluble portion was neutralized to pH 7 and was applied to a column packed with muffled Celite. A solution with 20% methanol/ethyl acetate was used as mobile phase to wash the phytochemicals out of the column. Then the washout was evaporated at 45 °C to dryness. Phenolic compounds in this portion were designated as bound-W and were recovered with 10 mL of water and then stored at −40 °C until use.

Determination of Total Phenolic Content. The contents of total phenolics in samples were analyzed by the Folin−Ciocalteu colorimetric method described previously (*15*) and was modified in our laboratory (*12*). Briefly, the appropriate dilutions of extracts were oxidized with Folin−Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 760 nm after 90 min by a MRX II Dynex plate reader (Dynex Technologies, Inc., Chanilly, VA). Gallic acid was used as standard, and results were expressed as mean (mg of gallic acid equiv/100 g of edible parts of sample) ± SD for triplicates.

Quantification of the Total Antioxidant Activity. A modified total oxyradical scavenging capacity (TOSC) assay was used for determining total antioxidant capacity of extracts (*12*, *16*). Peroxyl radicals generated by thermal homolysis of ABAP resulted in the oxidation of KMBA to ethylene, which was monitored by headspace gas chromatographic analysis (*16*). Antioxidant activity was assessed at four different time points (15, 30, 45, and 60 min) and 6 different extract concentrations to determine the TOSC value. The TOSC value for each concentration of fruit sample was calculated using the integration of the area under the kinetic curve. The TOSC value for each concentration was quantified according to the following equation:

$$\mathrm{TOSC} = 100 - (\int \mathrm{SA}/\int \mathrm{CA}) \times 100$$

Here ∫SA is the integrated area from the sample reaction and ∫CA is the integrated area from the control reaction. The median effective dose (EC_{50}) of all samples was calculated from the dose−response curve of fruits versus TOSC values. Total antioxidant activity was expressed as μmol of vitamin C equiv for 1 g of fresh weight of the edible part of fruits. All TOSC values are presented as mean ± SD for at least three replicates.

Measurement of Inhibition Activity on $HepG_2$ Cell Proliferation. Antiproliferative activities of common fruit extracts were measured by the MTS assay (MTS-based cell titer 96 nonradioactivity cell proliferation assay) (Promega, Madison, WI) described previously (*9*). $HepG_2$ 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 (Gibco, Life Technologies, Grand Island, NY). $HepG_2$ cells were maintained at 37 °C in 5% CO_2 in an incubator. A total of 2.5×10^4 $HepG_2$ cells in growth media were placed in each well of a 96-well flat-bottom plate. After 4 h of incubation at 37 °C in 5% CO_2, the growth medium was replaced by media containing different concentrations of fruit extracts. Control cultures received the extraction solution minus the fruit extracts, and blank wells contained 100 μL of growth medium with no cells. After 96 h of incubation, cell proliferation was determined by colorimetric MTS assay. 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.

Statistical Analysis. Statistical analysis was conducted using SigmaStat Version 8.0 (Jandel Corp., San Raphael, CA). Differences among treatments were determined using *t*-tests. 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

Phenolic Content of Common Fruits. Phenolic contents of 11 selected common fruits are expressed as mg of gallic acid equiv/100 g of fresh weight of the edible part of fruits in **Figure 2**. Among all the fruits analyzed, cranberry had the highest soluble free phenolic content (507.0 ± 21.1 mg/100 g, $p < 0.01$), followed by apple (272.1 ± 6.2 mg/100 g), red grape (182.0 ± 2.6 mg/100 g), strawberry (147.8 ± 1.1 mg/100 g), lemon (66.3 ± 3.4 mg/100 g), peach (65.3 ± 0.4 mg/100 g), orange (56.8 ± 0.9 mg/100 g), banana (56.1 ± 2.8 mg/100 g), pear (53.6 ± 2.5 mg/100 g), and pineapple (40.4 ± 1.0 mg/100 g). Grapefruit had the lowest free phenolic content (30.7 ± 0.9 mg/100 g).

The phenolics in soluble free form were higher than that of bound-E form in all the fruits tested except pineapple. Pineapple had the highest bound-E phenolics (43.2 ± 0.4 mg/100 g, $p <$

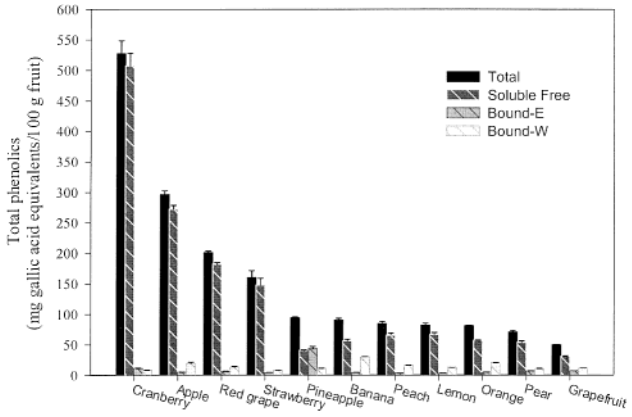

Figure 2. Total phenolics of various fruits (mean ± SD, $n = 3$).

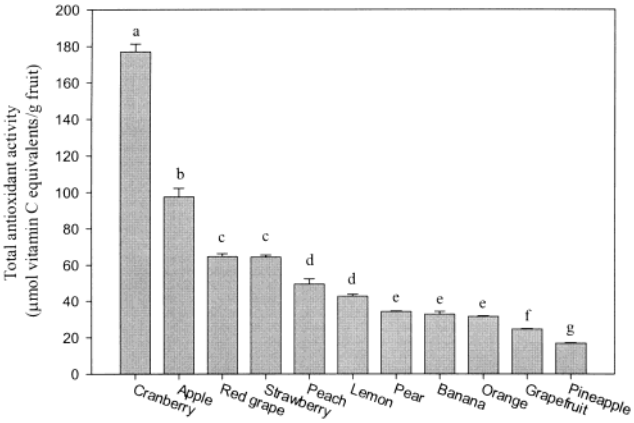

Figure 3. Total antioxidant activity of soluble free phytochemical extracts of fruits (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

0.01) followed by cranberry (11.5 ± 0.4 mg/100 g), grapefruit (7.2 ± 0.01 mg/100 g), pear (6.9 ± 0.02 mg/100 g), red grape (5.9 ± 0.1 mg/100 g), apple (4.9 ± 0.03 mg/100 g), orange (4.6 ± 0.05 mg/100 g), banana (4.4 ± 0.04 mg/100 g), strawberry (4.1 ± 0.04 mg/100 g), peach (3.2 ± 0.02 mg/100 g), and lemon (3.1 ± 0.02 mg/100 g). It is interesting to note that pineapple had an unusually higher bound-E phenolic content than that of its soluble free form.

Banana had the highest bound-W phenolics (29.9 ± 0.4 mg/100 g, $p < 0.01$), followed by orange (19.8 ± 0.1 mg/100 g), apple (19.2 ± 0.2 mg/100 g), peach (16.1 ± 0.4 mg/100 g), red grape (13.1 ± 0.2 mg/100 g), lemon (12.6 ± 0.1 mg/100 g), grapefruit (11.7 ± 0.7 mg/100 g), pineapple (10.7 ± 0.1 mg/100 g), pear (10.1 ± 0.1 mg/100 g), cranberry (8.6 ± 0.01 mg/100 g), and strawberry (8.1 ± 0.1 mg/100 g).

Phenolics in fruits were mainly in soluble free forms, which were significantly higher than bound phenolic contents in all tested fruits ($p < 0.01$) except pineapple ($p > 0.05$). Total phenolic content (soluble free + bound) was the highest in cranberry (527.2 ± 21.5 mg/100 g), followed by apple (296.3 ± 6.4 mg/100 g), red grape (201.0 ± 2.9 mg/100 g), strawberry (160.0 ± 1.2 mg/100 g), pineapple (94.3 ± 1.5 mg/100 g), banana (90.4 ± 3.2 mg/100 g), peach (84.6 ± 0.7 mg/100 g), lemon (81.9 ± 3.5 mg/100 g), orange (81.2 ± 1.1 mg/100 g), pear (70.6 ± 1.6 mg/100 g), and grapefruit (49.6 ± 2.6 mg/100 g).

Total Antioxidant Activity and Antiproliferative Activity. The total antioxidant activities of 11 selected common fruits were expressed as μmol of vitamin C equiv/g of fresh weight of the edible part of fruits and are summarized in **Figure 3**.

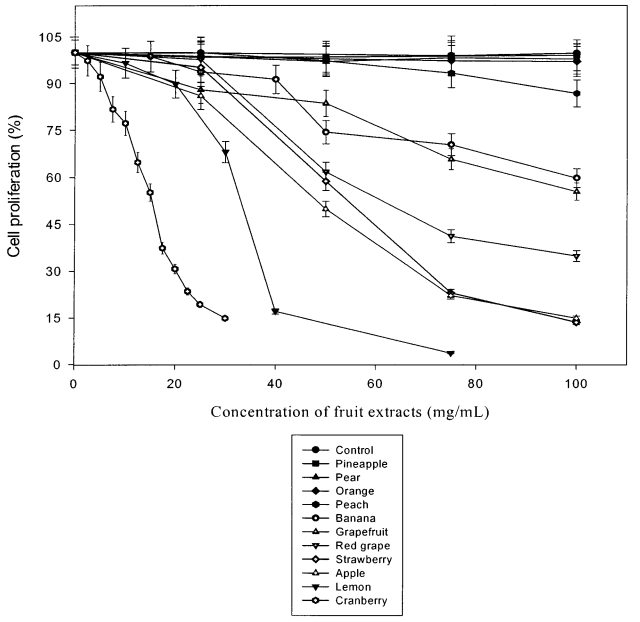

Figure 4. Dose–response curve of antiproliferative activity of soluble free phytochemical extracts of selected fruits (mean ± SD, $n = 3$).

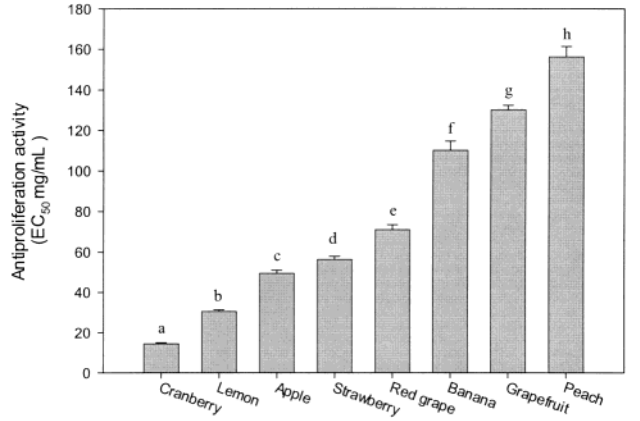

Figure 5. Antiproliferative activity of soluble free phytochemical extracts of fruits (mean ± SD, $n = 3$). Bars with no letters in common are significantly different ($p < 0.05$).

The phytochemical extract of cranberry had the highest total antioxidant activity (177.0 ± 4.3 μmol/g, $p < 0.01$), followed by apple (97.6 ± 4.6 μmol/g), red grape (64.7 ± 1.6 μmol/g), strawberry (64.4 ± 1.1 μmol/g), peach (49.5 ± 2.8 μmol/g), lemon (42.8 ± 1.0 μmol/g), pear (34.2 ± 0.3 μmol/g), banana (32.8 ± 1.5 μmol/g), orange (31.5 ± 0.27 μmol/g), grapefruit (24.7 ± 0.17 μmol/g), and pineapple (16.9 ± 0.3 μmol/g). There was no significant difference ($p > 0.05$) in antioxidant activities between red grape and strawberry, peach, and lemon and banana and orange.

Antiproliferative activities of fruit soluble free extracts on the growth of $HepG_2$ human liver cancer cells in vitro are summarized in **Figure 4**. Among the 11 selected common fruits, cranberry, lemon, apple, strawberry, red grape, banana, and grapefruit showed relatively potent antiproliferative activities on $HepG_2$ cell growth in a dose-dependent manner. The antiproliferative activities of fruits were expressed as the median effective dose (EC_{50}), with a lower EC_{50} value indicating a higher antiproliferative activity (**Figure 5**). The soluble free extract of cranberry had the highest antiproliferative activity with the lowest EC_{50} of 14.5 ± 0.5 mg/mL, followed by lemon (30.6 ± 0.8 mg/mL), apple (49.4 ± 1.6 mg/mL), strawberry

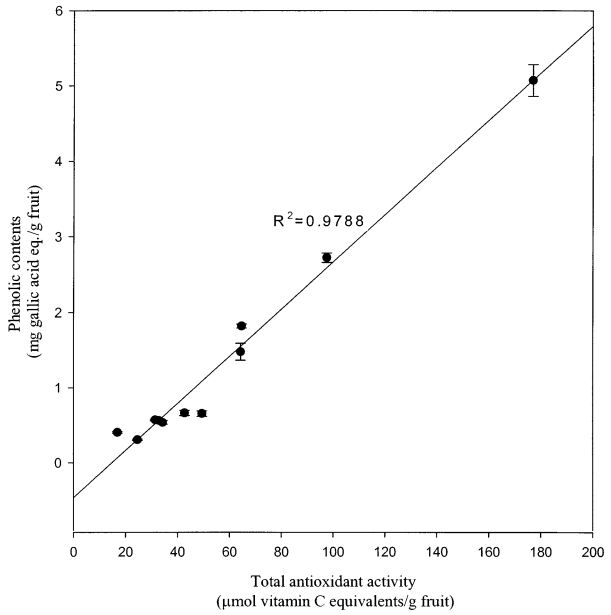

Figure 6. Relationship between total antioxidant activity and phenolic content in fruits.

(56.3 ± 1.5 mg/mL), red grape (71.0 ± 2.2 mg/mL), banana (110.1 ± 2.5 mg/mL), and grapefruit (130.1 ± 4.5 mg/mL). The phytochemical extracts of peach only showed a weak antiproliferative activity at higher doses with the EC_{50} of 156.3 ± 5.1 mg/mL. The phytochemical extracts of orange, pear, and pineapple had no antiproliferative activities under the experimental conditions tested.

Relationship between Total Phenolic Content, Antioxidant Activity, and Antiproliferative Activity. There was a direct relationship between total phenolic content and total antioxidant activity in phytochemical extracts of different fruits (R^2 = 0.9788, $p < 0.01$; **Figure 6**). The higher total phenolic content in fruits resulted in higher total antioxidant activity. There is no obvious linear relationship between total phenolic content and inhibition of $HepG_2$ cell proliferation (R^2 = 0.415, $p > 0.05$). Also there is no significant linear relationship between total antioxidant activity and antiproliferative activity of the fruits tested (R^2 = 0.3693, $p > 0.05$).

DISCUSSION

Cells in humans and other organisms are constantly exposed to a variety of oxidizing agents, some of which are necessary for life. Overproduction of oxidants can cause oxidative stress, which is associated with chronic diseases. Therefore, increased consumption of fruits and vegetables containing high levels of antioxidants (mainly phytochemicals) has been recommended to prevent or slow the oxidative stress caused by free radicals. However, total phenolic contents of fruits in the literature were underestimated because the bound phenolics were not included. It is important to know the profiles of total phenolics, including both soluble free and bound forms in fruits and vegetables, and their potential to improve human nutrition and health.

In this study, we investigated the profiles of total phenolics, including both soluble free and bound forms in common fruits by applying solvent extraction, base digestion, and solid extraction methods. The total phenolic profiles in fruits are summarized in **Table 1**. We showed here that although phenolics in fruits were mainly in soluble free form (62−96%), approximately 2−46% of phenolics existed in bound-E forms and 2−24% in bound-W forms. On average, there were apparently

Table 1. Percentage Distribution Profile of Phenolics in Fruits

		bound (%)		
fruit	free (%)	bound-E	bound-W	tot.
apple	91.8	1.7	6.5	8.2
banana	62.1	4.8	33.1	37.9
red grape	90.5	3.0	6.5	9.5
grapefruit	61.9	14.5	23.6	38.1
lemon	80.9	3.7	15.4	19.1
orange	70.0	5.7	24.3	30.0
peach	77.2	3.8	19.0	22.8
pear	76.0	9.7	14.3	24.0
pineapple	42.9	45.8	11.3	57.1
strawberry	92.3	2.6	5.1	7.7
cranberry	96.2	2.2	1.6	3.8
av	76.5	8.9	14.6	23.5

24% of total phenolics coming from the bound fraction. Vinson et al. (*17*) reported bound phenolics in fruits, but the content was much lower compared to our results. This is mainly due to the different methods used. The significance of bound phytochemicals in fruits to human health is not clear. However, it is highly possible that different fruits with different amounts of bound phytochemicals can be digested and absorbed at different sites of the gastrointestinal tract and play their unique health benefits. Bound phytochemicals, mainly in β-glycosides, cannot be digested by human enzymes and could survive stomach and small intestine digestion to reach the colon, providing site specific health benefits (*11*, *18*). For example, banana and pineapple, with a high percentage of bound phytochemicals, may be able to survive the stomach and small intestine digestion to reach the colon and be digested by bacteria flora to release phytochemicals locally to have health benefits. It is interesting to note that banana has been commonly used in traditional Chinese medicine for treating constipation in humans (*19*, *20*). The therapeutic effect of banana to treat constipation may be due to the higher percentage of bound phytochemicals to reach the colon.

The phytochemical extracts of fruits showed potent antioxidant activities. The total antioxidant activity of 100 g of cranberry was equivalent to that of 3120 mg of vitamin C, followed by apple (1740 mg of vitamin C equiv/100 g), red grape (1140 mg of vitamin C equiv/100 g), strawberry (1130 mg of vitamin C equiv/100 g), peach (871 mg of vitamin C equiv/100 g), lemon (753 mg of vitamin C equiv/100 g), pear (603 mg of vitamin C equiv/100 g), banana (578 mg of vitamin C equiv/100 g), orange (554 mg of vitamin C equiv/100 g), grapefruit (434 mg of vitamin C equiv/100 g), and pineapple (298 mg of vitamin C equiv/100 g). The combination of phytochemicals and synergistic mechanisms in the fruit matrix may be responsible for the potent antioxidant activities of fruits (*10*).

Vitamin C has been considered as the major antioxidant in fruits. Our group has shown that vitamin C only contributed 0.4% of the total antioxidant activity in apples (**Table 2**; *10*). Here we further showed that the contributions of vitamin C to the total antioxidant activities in the 11 fruits tested were low, suggesting that the majority of the total antioxidant activity was from other phytochemicals in fruits. Grapefruit had the highest vitamin C contribution to the total antioxidant activity (8.60%), followed by orange (8.16%), lemon (6.15%), pineapple (5.20%), strawberry (3.28%), banana (1.58%), peach (0.76%), pear (0.67%), apple (0.40%), and red grape (0.35%). Interestingly, there is no vitamin C in cranberry though it had the highest total antioxidant activity. We used vitamin C data from the USDA database to estimate the contribution of vitamin C to

Table 2. Contribution of Vitamin C to the Total Antioxidant Activity

fruit	vitamin C			corrected tot. antioxidant activity[b] (μmol of vit C equiv/g)
	content[a] (mg/g)	antioxidant activity (μmol/g)	contribn to tot. antioxidant activity (%)	
cranberry	0	0	0	176.98
apple	0.057	0.33	0.40	97.23
grape	0.040	0.23	0.35	64.35
strawberry	0.370	2.11	3.28	61.08
peach	0.066	0.38	0.76	48.69
lemon	0.460	2.63	6.15	36.61
banana	0.091	0.52	1.58	31.23
pear	0.040	0.23	0.67	33.57
orange	0.450	2.57	8.16	23.32
grapefruit	0.370	2.11	8.57	16.09
pineapple	0.154	0.88	5.20	11.73

[a] USDA nutrient database for standard reference. [b] Corrected total antioxidant activity = total antioxidant activity − vitamin C antioxidant activity.

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

fruit	tot. antioxidant activity			antiproliferative activity				
	TOSC (μmol of vit C equiv/g)	score	rank	EC_{50} (mg/mL)	score	rank	BI[a]	BI rank
cranberry	176.98	1.00	1	14.50	1.00	1	1.00	1
apple	98.56	0.55	2	49.37	0.29	3	0.42	2
lemon	42.75	0.24	6	30.56	0.47	2	0.36	3
strawberry	64.37	0.36	4	56.33	0.26	4	0.31	4
red grape	64.70	0.36	3	71.01	0.20	5	0.28	5
peach	49.45	0.28	5	156.29	0.09	8	0.18	6
banana	32.80	0.19	8	110.10	0.13	6	0.16	7
grapefruit	24.66	0.14	10	130.09	0.11	7	0.13	8
pear	34.24	0.19	7	n/a	0.00	9	0.10	9
orange	31.48	0.18	9	n/a	0.00	9	0.09	10
pineapple	16.93	0.10	11	n/a	0.00	9	0.05	11

[a] BI = 1/2(score of total antioxidant activity + score of antiproliferative activity).

the total antioxidant activity using a method similar to that previously reported (*8*). This may introduce some added variations in the estimate. However, as Wang et al. (*8*) suggested, the estimated contribution of vitamin C to the total antioxidant activity is relatively low, and the vitamin C values contained in the USDA database generally represent a diverse sampling. Thus, one would not expect a severalfold fluctuation in the amount of vitamin C in the food. Therefore, the major contribution to the total antioxidant activity in fruits was from the combination of phytochemicals, not from the vitamin C, as suggested in our previous report (*10*).

It was reported that the antioxidant activity of raspberry was directly related to the phenolic content (*9*). Here we showed that there was a direct linear relationship between the phenolic contents and total antioxidant activities in the 11 fruits tested ($r^2 = 0.9788$, $p < 0.01$), indicating phenolics may be the major contributor to the total antioxidant activities of fruits. Among the 11 fruits tested, 8 of them showed the ability to inhibit human liver cancer cell growth in vitro. The antiproliferative activities of fruit extracts did not correlate with their antioxidant activities ($R^2 = 0.4150$, $p > 0.05$) or total phenolic contents ($R^2 = 0.3693$, $p > 0.05$). This was consistent with the finding in raspberries that the relationship between total phenolics and EC_{50} of $HepG_2$ cell inhibition was not significant ($R^2 = 0.563$, $p > 0.05$) (*8*). The inhibition of cancer cell proliferation by fruit extracts cannot be explained by the total phenolic contents in the fruits tested. This suggested that a specific phenolic compound or a class of phenolics in fruits was responsible for their antiproliferative activities. Therefore, further identification of specific phytochemicals for their antiproliferative activities is worthy of investigation.

The bioactivity index (BI) for dietary cancer prevention was proposed here to provide a simple reference for consumers to choose fruits on the basis of their beneficial activities (**Table 3**). Because cranberry had the highest antioxidant and antiproliferative activities, its EC_{50} value was used as a control to calculate BI by the following equations:

$$\text{score of total antioxidant activity} = \text{sample TOSC value/cranberry TOSC value} \quad (1)$$

$$\text{score of antiproliferative activity} = \text{cranberry } EC_{50} \text{ value/sample } EC_{50} \text{ value} \quad (2)$$

$$\text{BI} = 1/2(\text{score of total antioxidant activity} + \text{score of antiproliferative activity}) \quad (3)$$

Our results showed that cranberry had the highest BI value (1.00), followed by apple (0.42), lemon (0.36), strawberry (0.31), red grape (0.28), peach (0.18), banana (0.16), grapefruit (0.13), pear (0.10), orange (0.09), and pineapple (0.05). We believe that the bioactivity index reported here could be a new alternative biomarker for epidemiological studies in dietary cancer prevention. The BI could affect the rankings of fruits. If only considering the antioxidant activity of the fruit extracts, lemon was ranked as number 6 out of the 11 fruits tested, but when both antioxidant activity and anticancer cell proliferation activity were considered, such as BI, the ranking of lemon was moved to the number 3 position. Therefore, BI is a better biomarker than either total antioxidant activity or antiproliferative activity alone. However, this was only a simple model, and further research is needed to unveil the real role of phytochemicals of fruits in dietary cancer prevention.

From our study, the profiles of total phenolics that existed both in soluble free and bound forms in common fruits were determined. Approximately 24% of total phenolics in fruits were

present in bound form, which was not reported in the previous literature. Our work clearly showed that phytochemicals in fruits have potent antioxidant and antiproliferative activities. The bioactive index (BI) for dietary cancer prevention was proposed to provide a new biomarker for future epidemiological studies.

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