Eur Food Res Technol (2009) 228:903–911
DOI 10.1007/s00217-008-1003-y

ORIGINAL PAPER

A comparative study of phenolic compounds and antioxidant and antiproliferative activities in frequently consumed raw vegetables

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Received: 28 June 2008 / Revised: 5 December 2008 / Accepted: 19 December 2008 / Published online: 3 February 2009

Abstract The aim of this study was to assess the antioxidant value and antiproliferative activity of some vegetables such as raw garlic (*Allium sativum* L), white and yellow, and red onions (*Allium cepa* L), red and green peppers (*Capsicum annuum* L.), and white cabbage (*Brassica oleracea var. capitata f. alba*), which were harvested in the same year and in the same geographical and climatic conditions. It was found that the highest content of some bioactive compounds (25.19 ± 2.2 mg GAE/g, 3.84 ± 0.3 mg CE/g, 4.88 ± 0.3 mg CE/g, 59.20 ± 0.8 μg CE/g, 1992 ± 99.8 μg AA/g, 452.7 ± 26.1 mg CGE/kg DW for polyphenols, flavonoids, tannins, flavanols, ascorbic acid, and anthocyanins, respectively) and the antioxidant activity (41.32 ± 3.9, 31.05 ± 2.7, 59.17 ± 5.2 and 58.94 ± 5.1 μM TE/g for DPPH, FRAP, CUPRAC and ABTS, respectively) was in red onion. Methanol extracts in concentration of 1,000 μg ml^{-1} of garlic and red onion exhibited antiproliferative activity (83.1 ± 2.1 and 85.0 ± 3.2% of viability, respectively). In spite of relatively high antioxidant activity in methanol extracts of yellow onion, red and green pepper, no antiproliferative activity on both tumor cell lines was registered. In conclusion, among the studied vegetables raw red onion was the preferable. The interrelationship was in the following order: red onion > white onion = yellow onion > red pepper > garlic = green pepper > white cabbage. The antiproliferative activities of these vegetables were different: some samples reacted only on Calu-6 and the others—on SNU-601. Thus, vegetables from the same cultivation place were examined for their antioxidant and antiproliferative activities with four different methods. Based on obtained data a direct comparison between these vegetables was possible for the first time.

Keywords Vegetables · Methanol extracts · Bioactive compounds · Antioxidant · Antiproliferative activities

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Introduction

Epidemiological studies have indicated that the consumption of fruits and vegetables is associated with a reduced risk for the development of chronic diseases, such as cardiovascular disease and cancer [1]. Phytochemicals, including phenolics and flavonoids, are suggested to be the major bioactive compounds contributing to the health benefits of fruits and vegetables [1–6]. It was shown that the health properties of these natural products depend on the contents of bioactive compounds, mainly phenolics, and partly on dietary fibers [7–9]. Some investigators recommend including in disease preventive diets only fruits and vegetables with high antioxidant activity [10, 11]. At present, there is no unified estimation of the real antioxidant activity of the frequently used vegetables and fruits [12, 13]. It is known that the contents of bioactive compounds and related antioxidant activity in fruits and vegetables are influenced by geographical region, climatic and storing conditions, and degree of ripeness. No doubt, that the samples used by the above-mentioned authors [12, 13] were not from the same geographical region, have grown not in the same climatic conditions and most probably were not of the same ripeness. For example, some investigators used fruits purchased on three separate occasions from different local supermarkets [14]. We had a unique opportunity to study raw garlic (*Allium sativum* L), white, yellow and red onions (*Allium cepa* L), red and green peppers (*Capsicum annuum* L.), and white cabbage (*Brassica oleracea var. capitata f. alba*), from the same year of harvest, grown in the same geographical region and climatic conditions and of the same ripeness and to find the most bioactive among them. We suppose that the data will be reliable.

In order to receive the most reliable antioxidant activity data, four tests [2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Ferric-reducing/antioxidant power (FRAP), and Cupric reducing antioxidant capacity (CUPRAC)] were applied in this investigation.

According to Banerjee et al. [15], garlic possesses antiproliferative properties. It was of interest to explore how the antioxidant activity of the studied vegetables correlates with their antiproliferative activity. Most of the authors found such correlation [16–19]; however, in another report the results were different and no correlation was established [20].

In this report it was of interest to investigate the relationship of the reliable antioxidant activity of the studied garlic, onions, peppers and white cabbage, and their antiproliferative activity. Therefore, an experiment of vegetable samples harvested in the same year and in the same geographical and climatic conditions was performed. The antiproliferative activity of methanol extracts of all studied vegetables was tested on human cancer cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma) for the first time in this report.

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

Materials and methods

Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (FCR), lanthanum (III) chloride heptahydrate, $FeCl_3 \times 6H_2O$, $CuCl_2 \times 2H_2O$, 2,9-dimethyl-1,10-phenanthroline (neocuproine), and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co., St. Louis, MO, USA. 2,4,6-tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionized and distilled water was used throughout.

Samples

Bulbs without the coats of raw garlic (*Allium sativum* L), cultivar Pallium sativum; and onions (*Allium cepa*): white and yellow cultivar Armstrong and red cultivar Red Baron; and whole fruit without seeds of sweet red and green pepper (*Capsicum annuum* L.), cultivar Red Kinsh, were obtained from the Company "Elena". White cabbage (*Brassica oleracea var. capitata f. alba*) was purchased at the local market. The samples were defatted in a Soxhlet extractor with *n*-hexane for 10 h, and then were stored at 5 °C after the removal of hexane.

Preparation of extracts

Defatted lyophilized vegetable samples were extracted from a 50-mg aliquot with 5 ml of 1.2 M HCl in 50% methanol/water with heating at 90 °C for 3 h for unconjugated plus conjugated (total) polyphenols and without HCl for free polyphenols. The samples were cooled, diluted to 10 ml with methanol, and centrifuged for 5 min at 4,000×*g* with a benchtop centrifuge to remove solids. These extracts were used for determination of antioxidant activity and some of the bioactive compounds [21]. High temperature was used only for the extraction of total and free polyphenols.

Fourier transform infrared (FT-IR) spectra of polyphenols

The presence of polyphenols (flavonoids and phenolic acids) in the investigated vegetables was studied by Fourier

Transform Infrared (FT-IR) spectroscopy. A Bruker Optic GMBH Vector FT-IR spectrometer (Bruker Optic GMBH, Attingen, Germany) was used to record IR spectra. A potassium bromide microdisk was prepared from finely ground lyophilized powder of 2 mg of vegetable sample with 100 mg of KBr [3, 5, 6].

Determination of the contents of the bioactive compounds

The studied bioactive compounds were determined as previously described [22, 23]. To determine the total amount of polyphenols in the studied extracts, the Folin-Ciocalteu reagent (FCR) was used, and measurement was performed at 765 nm with gallic acid as the standard. Results were expressed as mg of gallic acid equivalent (GAE). Flavonoids, extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH, were measured at 510 nm. The total flavanols amount was estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read. The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm. (+)-Catechin served as a standard for flavonoids, flavanols, and tannins, and the results were expressed as catechin equivalents (CE).

Anthocyanins were extracted at room temperature. The absorbances for total anthocyanins were measured in extracts (1 g of the defatted sample was extracted with 1 ml of acetonitrile containing 4% acetic acid) for two pHs (1.0 and 4.5) in a Beckman spectrophotometer at 510 nm, using the pH differential method [24].

Results were expressed as mg of cyanidin-3-glucoside equivalent (CGE). Total ascorbic acid was determined by CUPRAC assay [4]. Water extract was prepared from 100 mg of lyophilized sample and 5 ml of water, and stirred for 24 h and centrifuged. This extract (1 ml) was mixed with 2 ml of 3.0×10^{-3} M of lanthanum (III) chloride heptahydrate. Ethylacetate (EtAc) was used for extraction of flavonoids in order to avoid the interference. Ascorbic acid was quantified in the aqueous phase. One mL of Cu (II)-neocuproine (Nc), in ammonium acetate-containing medium at pH 7, was added to 1 ml of the obtained extract. The absorbance of the formed bis (Nc)-copper (I) chelate was measured at 450 nm [4].

Determination of the antioxidant activity

The following four tests were used:

1. Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants contained in the samples to reduce ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}) which absorbs light at 593 nm [25].
2. 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt (ABTS+) was generated by the interaction of ABTS (7 mmol/l) and $K_2S_2O_8$ (2.45 mmol/l). This solution was diluted with methanol until the absorbance reached 0.7 at 734 nm [26].
3. 1,1-Diphenyl-2-picrylhydrazyl method (DPPH) solution (3.9 ml, 25 mg/l) in methanol was mixed with the samples extracts (0.1 ml). The reaction progress was monitored at 515 nm until the absorbance was stable [26].
4. Cupric reducing antioxidant capacity (CUPRAC) is based on utilizing the copper (II)-neocuproine [Cu(II)-Nc] reagent as the chromogenic oxidizing agent. To the mixture of 1 mL of Cu(II), Nc, and NH_4Ac buffer solution, extracts of vegetable sample (or standard) solution (x ml) and H_2O [$(1.1 - x)$ mL] were added to make the final volume of 4.1 ml. The absorbance at 450 nm was recorded against a reagent blank [27].

Determination of the antiproliferative activity

The antiproliferative activities of 100% methanol extracts of all studied vegetables on human cancer cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma) were measured using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. The cell lines were purchased from Korean Cell Line Bank (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), and transferred to a 96-well plate, and incubated for 24 h prior to the addition of methanol extracts of vegetables. Serial dilutions of the extracts were prepared by dissolving compounds in Dimethyl Sulfoxide (DMSO) followed by dilution with RPMI-1640 medium to give final concentration at 10, 30, 100, 300, and 1,000 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 it was added to each of the 96 wells. The wells were wrapped with aluminum foil and incubated at 37 °C for 4 h. The solution in each well containing media, unbound MTT and dead cells were removed by suction and 150 μl of DMSO was added to each well. The plates were then shaken and optical density was recorded using a micro plate reader at 540 nm. Distilled water was used as positive control and DMSO as solvent control [17, 28]. The effect of the vegetable extract on the proliferation of cancer and normal cells was expressed as relative cell viability: percent viability = OD of vegetable extract treated sample/OD of none treated sample) × 100, where OD is optical density [29].

Statistics

The results of this investigation are means ± SD of five measurements. Differences between samples were tested by two-way ANOVA using GraphPad Prism, version 2.0. (GraphPad Software, San Diego, CA), followed by Duncan's new multiple range test to assess differences between group means. The P values of <0.05 were considered significant.

Results and discussion

Bioactive compounds

Figure 1a–c shows band assignments for polyphenols in the standards and investigated vegetable samples. The wavelengths numbers of FTIR spectra for catechin (Fig. 1a, line 1) at 827, 1,039, 1,115, 1,143, 1,286, 1,478, 1,511 and 1,610 cm^{-1} were assigned to C–H alkenes, –C–O alcohols, C–OH alcohols, –OH aromatic, C–O alcohols, C–H alkanes, C=C aromatic ring, and C=C alkenes. Gallic acid showed (Fig. 1a, line 2) the following wavelength numbers (cm^{-1}) of 866, 1,026, 1,238, 1,450, 1,542, and 1,618. White onion, garlic, green pepper and cabbage (Fig. 1b, lines 1–4, respectively) showed slightly different bands than the standards, but the wavelengths of the bands were similar in this group of noncolored vegetables.

Figure 1c shows the group of vegetables with color: red onion (line 1), yellow onion (line 2), and red pepper (line 3) and the spectra were similar in this group. The absorption bands at 827 cm^{-1} were absent in white onion and did not shift for garlic and red onion, which were assigned to C–H alkenes. Only in red pepper was found exact band of 1,032 cm^{-1} as –C–O alcohols, and in cabbage of 1,147 cm^{-1} as –OH aromatic. Other bands in the vegetable samples were slightly shifted in comparison with the standards.

Our results were in accordance with others [3, 5, 6]. FTIR spectroscopy can be used as an additional tool to screen vegetables for their content of phenolic compounds.

The amounts of total and free polyphenols in methanolic extracts of the studied vegetables are summarized in Tables 1 and 2, respectively, and were significantly higher in extracts, where 1.2 M HCl was added to 50% methanol/water ($P < 0.05$). It was found that the highest content of the determined bioactive compounds (25.19 ± 2.2 mg GAE/g, 3.84 ± 0.3 mg CE/g, 4.88 ± 0.3 mg CE/g and 59.20 ± 0.8 μg CE/g for polyphenols, flavonoids, tannins and flavanols, and 1,992 ± 99.8 μg AA/g and 452.7 ± 26.1 mg CGE/kg DW) for ascorbic acid and anthocyanins, respectively, was in red onion, followed by white onion = yellow onion > red pepper > garlic = green pepper > white cabbage (Tables 1 and 2, Fig. 2).

Our data were confirmed by other authors [30, 31]. So, Ninfali et al. [30] determined that polyphenols in garlic were 86.07 mg GAE/100 g FW = 3.1 mg GAE/g DW; and flavonoids—12.4 mg CE/100 g FW = 44.76 mg CE/100 g DW. Polyphenols in onions [30] were about 6.40 mg GAE/100 g FW = 77.12 mg GAE/100 g DW; and flavanols—0.28 mg CE/100 g FW = 3.37 mg CE/100 g DW.

In cabbage [30], total phenols were about 105.2 mg caffeic acid (CA)/100 g FW = 12.81 mg GAE/g DW; flavonoids −45.70 mg CE/100 g FW = 5.25 mg CE/g DW; and flavanols −0.66 mg CE/100 g FW = 7.58 mg CE/100 g DW.

In green pepper [30] polyphenols were estimated as 44.6 mg CA/100 g FW = 8.92 mg GAE/g DW; flavonoids −9.90 mg CE/100 g FW = 1.86 mg CE/g DW; and flavanols −0.56 mg CE/100 g FW = 10.56 mg CE/100 g DW.

In red pepper [30] polyphenols were found to be 76.5 mg CA/100 g FW = 1039.6 GAE/100 g DW; flavonoids −7.91 mg/100 g FW = 101.4 mg CE/100 g DW; flavanols −0.42 mg/100 g FW = 5.38 mg/100 g DW. As can be seen, the above-cited data of Ninfali [30] are in agreement with most results of our present investigation, based on all bioactive compounds in hydrolyzed and non-hydrolized vegetable methanolic extracts (Tables 1, 2).

Also Ou et al. [31] studied inter alia the contents of total phenols and flavonoids in three onion cultivars (white, red and yellow). They found that the content of phenolics was 115, 133 and 107 and of flavonoids −0.4, 0.5 and 0.2 μg/mg DW for white, red and yellow onions, respectively. Therefore, also these authors found that the contents of total phenols and total flavonoids were higher in red onion in comparison with white and yellow ones. According to us and the cited authors [30, 31], red onion is the preferable among the studied vegetables.

The antioxidant activity

The obtained results of antioxidant activities in hydrolyzed and non-hydrolyzed methanolic extracts of the studied vegetables are summarized in the Tables 3 and 4, respectively. The antioxidant activities were significantly higher in hydrolyzed methanolic extracts with 1.2 M HCl ($P < 0.05$). It was found as well that the highest antioxidant activity (41.32 ± 3.9, 31.05 ± 2.7, 59.17 ± 5.2 and 58.94 ± 5.1 μM TE/g for DPPH, FRAP, CUPRAC and ABTS, respectively) was in red onion, followed by white onion = yellow onion > red pepper > garlic = green pepper > white cabbage. It must be underlined that the antioxidant activity of vegetables determined by different antioxidant assays could give different results [31]. So, Ou et al. [31] investigated a total of 927 freeze-dried vegetable samples, including 111 white cabbages, 59 carrots, 51 snap beans, 57 cauliflowers, 33 white onions, 48 purple onions, 130 broccoli,

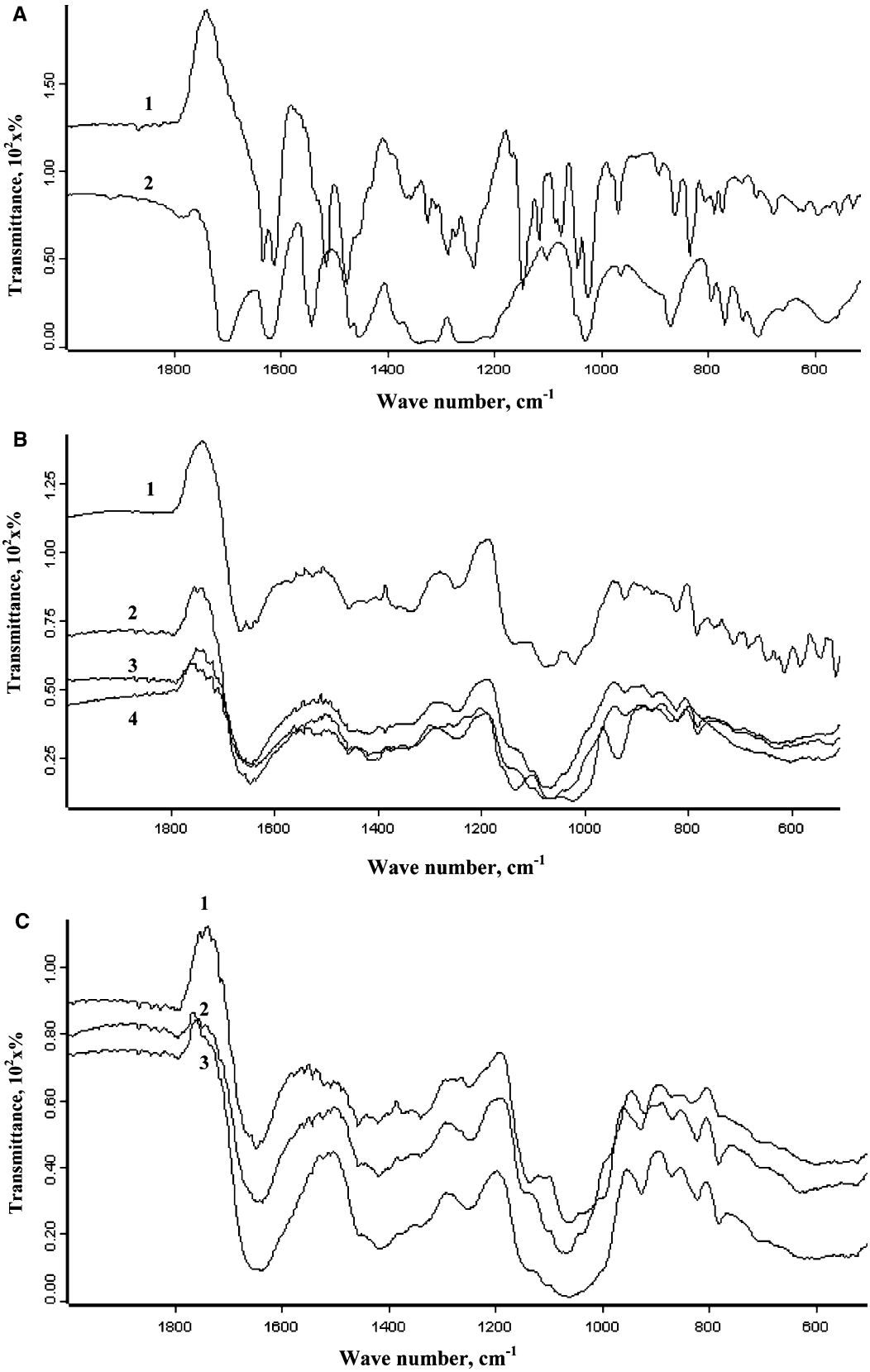

Fig. 1 FTIR spectra of A: catechin (l), gallic acid (2). B: white onion (1), garlic (2), green pepper (3), cabbage (4). C: red onion (1), yellow onion (2), red pepper (3)

Table 1 The amounts of bioactive compounds (dry weight) in raw vegetables, extracted with 50% methanol/water

	WC	Garlic	RO	WO	YO	GP	RP
Polyphenols (mg GAE/g)	3.16 ± 0.3^{a}	6.36 ± 0.5^{b}	15.56 ± 1.3^{d}	11.92 ± 0.9^{c}	10.05 ± 0.8^{c}	10.30 ± 0.8^{c}	2.63 ± 1.1^{a}
Flavonoids (mg CE/g)	1.67 ± 0.1^{d}	0.41 ± 0.03^{a}	0.98 ± 0.08^{c}	0.76 ± 0.06^{b}	1.61 ± 0.14^{d}	0.76 ± 0.06^{b}	2.60 ± 0.18^{e}
Tannins (mg CE/g)	1.15 ± 0.09^{a}	1.40 ± 0.1^{a}	3.67 ± 0.3^{c}	1.78 ± 0.1^{a}	3.19 ± 0.3^{c}	0.98 ± 0.08^{a}	2.11 ± 0.2^{b}
Flavanols (μg CE/g)	12.15 ± 1.2^{b}	17.45 ± 1.5^{e}	14.64 ± 1.6^{d}	5.93 ± 0.5^{d}	7.24 ± 0.3^{c}	13.34 ± 1.3^{a}	28.56 ± 2.3^{f}

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

WC white cabbage, *Garlic* garlic, *RO* red onion, *WO* white onion, *YO* yellow onion, *GP* green pepper, *RP* red pepper, *CE* catechin equivalent, *GAE* gallic acid equivalent

Table 2 The amounts of bioactive compounds (dry weight) in raw vegetables, extracted with 1.2 M HCl in 50% methanol/water

	WC	Garlic	RO	WO	YO	GP	RP
Polyphenols (mg GAE/g)	10.17 ± 0.8^{a}	19.40 ± 1.7^{b}	25.19 ± 2.2^{c}	20.49 ± 1.9^{b}	19.69 ± 18^{b}	18.68 ± 1.6^{b}	19.23 ± 1.8^{b}
Flavonoids (mg CE/g)	3.22 ± 0.3^{b}	3.37 ± 0.3^{b}	3.84 ± 0.3^{b}	3.79 ± 0.3^{b}	2.34 ± 0.2^{a}	2.34 ± 0.2^{a}	3.68 ± 0.3^{b}
Flavanols (μg CE/g)	27.54 ± 2.2^{a}	67.05 ± 0.5^{e}	59.20 ± 0.8^{d}	45.60 ± 0.7^{c}	41.33 ± 0.6^{c}	37.50 ± 0.3^{b}	45.23 ± 0.4^{c}
Tannins (mg CE/g)	1.23 ± 0.1^{a}	2.40 ± 0.2^{b}	4.88 ± 0.3^{d}	2.64 ± 0.2^{b}	4.16 ± 0.3^{d}	1.52 ± 0.1^{a}	3.04 ± 0.3^{c}

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

WC white cabbage, *Garlic* garlic, *nd* not detected, *RO* red onion, *WO* white onion, *YO* yellow onion, *GP* green pepper, *RP* red pepper, *CE* catechin equivalent, *GAE* gallic acid equivalent

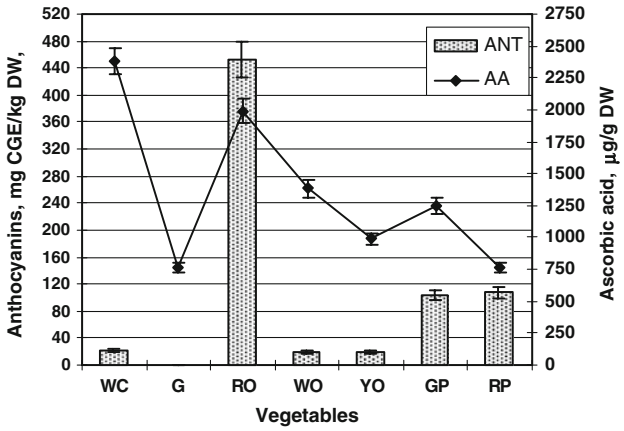

Fig. 2 Amounts of anthocyanins and ascorbic acids in some vegetables. Abbreviations: WC, white cabbage; G, garlic; RO, red onion; WO, white onion; YO, yellow onion; GP, green pepper; RP, red pepper; AA, ascorbic acid; ANT, anthocyanins; CGE, cyanidin–3-glucoside equivalent

169 tomatoes, 25 beets, 88 peas, 88 spinach, 18 red peppers, and 50 green peppers. According to the FRAP test, the above-mentioned vegetables showed the following order (μmol TE/g): red pepper (185), green pepper (157), white cabbage (39), red onion (31), and white onion (17). Similar results were obtained by others as well [32]: the values of phenolic substances and total antioxidant activity obtained by the ABTS, DPPH, and FRAP methods of the sets of samples correlated very well for all used methods. High values of antioxidant activity [32] were found in intensely colored vegetables (red cabbage, red onion and other vegetables).

Other authors [33] suggested an integrated approach to evaluate food antioxidant capacity.

To get a complete and dynamic picture of the ranking of food antioxidant capacity, relative antioxidant capacity index (RACI), a hypothetical concept was created from the perspective of statistics by integrating the antioxidant capacity values generated from different in vitro methods [33]. ABTS and FRAP methods showed that the antioxidant activities in red pepper and cabbage were higher than in white onion [33].

Antiproliferative activity

Combinations of polyphenols naturally found in fruits and vegetables had been suggested to be optimal for cancer prevention and their anticarcinogenic effects [34]. Therefore, it was decided to investigate the antiproliferative activity of the studied vegetables. It was already reported that fruits and vegetables possess antiproliferative properties [35]. Some authors investigated the synergistic effects of flavonoids on cell proliferation in Hepa-1c1c7 and LNCaP cancer cell lines [36]. They found that aglycon flavonoids, such as quercetin, kaempferol, and naringenin (at 12.5–50 μM), inhibited cancer cell proliferation in both cell lines in a dose-dependent manner without cytotoxicity.

Galeone et al. [37] used data from an integrated network of Italian and Swiss case-control studies to analyze the relationship between frequency of the use of onion and garlic and cancer at several sites. They calculated odds ratios (ORs) by using multivariate logistic regression models that were adjusted for energy intake and other major covariates.

Table 3 The antioxidant activities (dry weight) in raw vegetables, extracted with 50% methanol/water

	WC	Garlic	RO	WO	YO	GP	RP
DPPH (μM TE/g)	16.18 ± 1.4^{b}	7.00 ± 0.6^{a}	22.00 ± 1.9^{c}	21.44 ± 1.9^{c}	19.70 ± 1.7^{c}	11.81 ± 1.0^{b}	$23.00 \pm .2.0^{d}$
FRAP (μM TE/g)	11.12 ± 0.9^{b}	6.90 ± 0.5^{a}	19.20 ± 1.7^{e}	14.60 ± 1.3^{d}	19.25 ± 1.7^{e}	10.70 ± 0.8^{b}	12.60 ± 1.1^{c}
CUPRAC (μM TE/g)	11.45 ± 0.9^{a}	15.13 ± 1.4^{a}	39.66 ± 3.2^{e}	32.50 ± 2.9^{d}	39.25 ± 3.2^{e}	20.06 ± 1.8^{b}	27.79 ± 2.2^{c}
ABTS (μM TE/g)	10.50 ± 0.8^{a}	23.71 ± 2.0^{b}	49.68 ± 4.3^{d}	39.27 ± 3.2^{c}	47.24 ± 4.2^{d}	26.95 ± 2.3^{b}	28.62 ± 2.4^{b}

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

WC white cabbage, *Garlic* garlic, *RO* red onion, *WO* white onion, *YO* yellow onion, *GP* green pepper, *RP* red pepper, *CE* catechin equivalent, *ABTS* 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid, *DPPH* 1,1-diphenyl-2-picrylhydrazyl, *FRAP* ferric-reducing/antioxidant power, *CUPRAC* cupric reducing antioxidant capacity

Table 4 The antioxidant activity (dry weight) in raw vegetables, extracted with 1.2 M HCl in 50% methanol/water

	WC	Garlic	RO	WO	YO	GP	RP
DPPH (μM TE/g)	22.33 ± 1.7^{a}	34.86 ± 2.6^{b}	41.32 ± 3.9^{c}	24.65 ± 1.9^{a}	30.24 ± 2.1^{b}	32.00 ± 2.1^{b}	35.25 ± 2.2^{b}
FRAP (μM TE/g)	24.43 ± 1.8^{c}	10.80 ± 0.8^{a}	31.05 ± 2.7^{e}	23.22 ± 1.7^{c}	29.42 ± 1.9^{d}	16.25 ± 1.4^{b}	18.80 ± 1.6^{b}
CUPRAC (μM TE/g)	30.00 ± 2.1^{a}	27.22 ± 1.9^{a}	59.17 ± 5.2^{c}	58.24 ± 5.1^{c}	56.59 ± 4.9^{c}	45.63 ± 4.1^{b}	47.42 ± 4.2^{b}
ABTS (μM TE/g)	21.80 ± 1.6^{a}	37.02 ± 2.9^{b}	58.94 ± 5.1^{d}	58.44 ± 5.1^{d}	54.98 ± 5.0^{d}	46.31 ± 4.1^{c}	47.73 ± 4.2^{c}

Values are means ± SD of five measurements. Means in rows without superscript letters in common differ significantly (P < 0.05). Abbreviations: WC, white cabbage; Garlic, garlic; nd, not detected; RO, red onion; WO, white onion; YO, yellow onion; GP, green pepper; RP, red pepper; ABTS, 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl, FRAP, Ferric-reducing/antioxidant power, CUPRAC, Cupric reducing antioxidant capacity

These authors found that consumption of onions varied between 0–14 and 0–22 portions/week among cases and controls, respectively. The multivariate ORs for the highest category of onion and garlic intake were between 0.12 and 0.74 for cancer of the oral cavity and pharynx, for esophageal, colorectal, laryngeal, breast, ovarian, prostate, and renal cell cancer. Galeone et al. [37] concluded that the uniquely large data set from southern European populations show an inverse association between the frequency of the use of allium vegetables and the risk of several common cancers and are favorably correlated with cancer risk in Europe.

In our present investigation we found that the antiproliferative activity of methanol extracts of most studied vegetables on the tumor cell lines Calu-6 and SNU-601 was different (Fig. 3). So, the antiproliferative activity on SNU-601 was 86.3 ± 4.3, 88.7 ± 4.4, 89.6 ± 4.6, 89.7 ± 2.5 and 93.6 ± 6.1% and 48.4 ± 2.5; 83.0 ± 3.7; 85.2 ± 3.7; 97.2 ± 3.9; 99.7 ± 4.1% of viability for concentrations 1,000, 300, 100, 30 and 10 μg ml^{-1} for white onion, and cabbage, respectively (both vegetables were active only in concentration of 1,000 μg ml^{-1}).

Antiproliferative activity was not found in red onion and garlic on SNU-601 (Fig. 3a, b). However, the antiproliferative activity of these vegetables on Calu-6 was 85 ± 3.2, 92.5 ± 3.4, 93.5 ± 3.7, 95.5 ± 4.4 and 95.5 ± 4.4% and 83.1 ± 2.1, 90.5 ± 2.7, 92.9 ± 2.9, 93.3 ± 2.9 and 94.2 ± 3.1% of viability for concentrations 1,000, 300, 100, 30 and 10 μg ml^{-1}, respectively (both vegetables were active only in concentrations of 1,000 μg ml^{-1}). In spite of high antioxidant activity in methanol extract of yellow onion, red and green pepper, no antiproliferative activity on both tumor cell lines was registered (Fig. 3a, c). Our investigation shows that antioxidant activity of the studied vegetables was not always correlated with the antiproliferative activity. Our data are in accordance with already cited data of Roy et al. [20]. According to the total antiradical activity against the DPPH radical the order was 'komatsuna' > spinach > 'haruna' > 'chingensai' > white cabbage > Chinese cabbage [20]. Antiradical activity against hydroxyl radicals (deoxyribose assay) was the highest for 'komatsuna' and spinach, but white cabbage extract showed the highest antiproliferative activity. Also Kim et al. [18] studied antioxidant and antiproliferative activities of methanol extracts of leafy vegetables consumed in Korea. The antiproliferative activity was tested on breast (MSF7), colon (HCT16), lung (NCI-H460), and gastric (MKN 45) tumor cells. Our results were in correspondence with the cited research, because we have used the same methods: the antiproliferative activity of the vegetables on the tumors cells was measured by evaluating cell viability using MTT assay and the cell viability (%) was obtained by comparing the absorbance viability between the samples and negative control. They found that there was no antiproliferative activity of methanol extracts of some studied vegetables. We also found no antiproliferative activity of methanol extracts of yellow onion, red and green pepper on both studied tumor cell lines in spite of high antioxidant activity. Kim et al. [18] also underlined, as

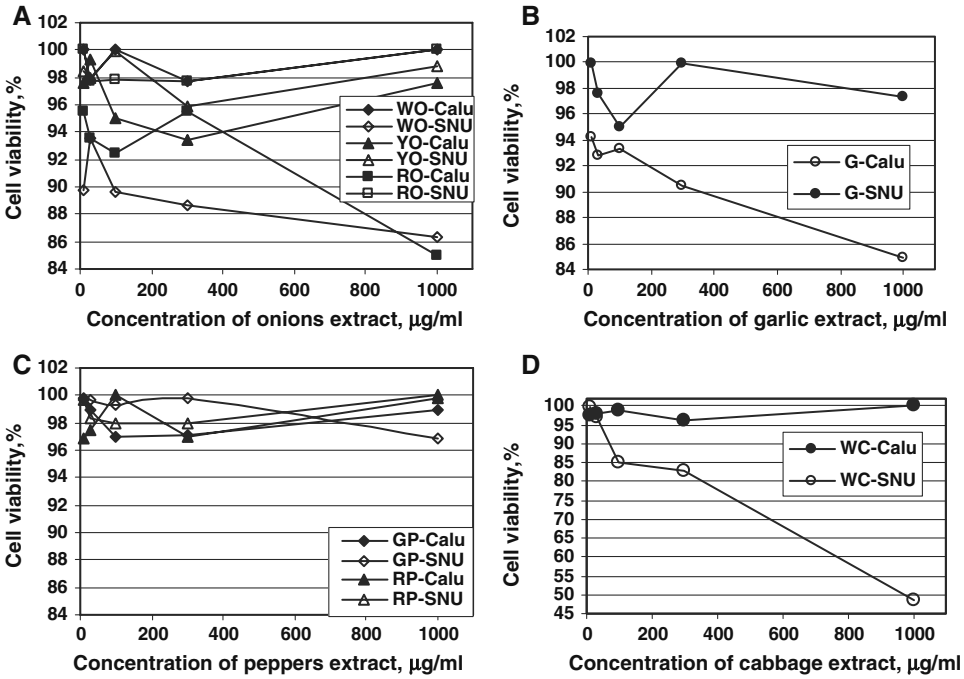

Fig. 3 Cytotoxic effect of methanol extracts from different vegetables on human cancer cell lines, Calu–6 and SNU-601. Abbreviations: WC, white cabbage; G, garlic; RO, red onion; WO, white onion; YO, yellow onion; GP, green pepper; RP, red pepper

we do, that there was no antiproliferative activity of a single vegetable on all studied tumors cells and that there was no correlation between antioxidant and antiproliferative activities [18]. Therefore, the maximal health effect can be achieved by consumption of combination of vegetables [36].

We state once again that the unique features of this investigation are the following:

1. The studied vegetables were harvested in the same year and in the same geographical and climatic conditions and therefore the results of the investigation and the interrelation among the studied vegetables are correct.
2. For the first time that antiproliferative activity of 100% methanol extracts of all studied vegetables was tested on human cancer cell lines (Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma).
3. It was shown that the studied vegetables differently influence the human cancer cell lines and there is not always a direct connection between the antioxidant and antiproliferative activities of the studied vegetables.

Conclusion

1. In the methanol extracts of the studied vegetables the highest contents of most bioactive compounds and the highest level of the antioxidant activity were found in the red onion, followed by white onion = yellow onion > red pepper > garlic = green pepper > white cabbage.
2. The methanol extracts of the studied vegetables possess dose-dependent antiproliferative activity and their influence on the studied tumor cell lines Calu-6 for human pulmonary carcinoma and SNU-601 for human gastric carcinoma was different. Therefore, maximal health effect can be achieved by consumption of combination of vegetables. However, these results must be confirmed by experiments in vivo.

Acknowledgment The authors are thankful to Dr. Elena Katrich (Hebrew University of Jerusalem, School of Pharmacy) for her technical assistance.

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