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Phase II Enzyme-Inducing and Antioxidant Activities of Beetroot (*Beta vulgaris* L.) Extracts from Phenotypes of Different Pigmentation

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Free-radical scavenging, reducing, and phase II enzyme-inducing activities of aqueous and 5% aqueous ethanol extracts of freeze-dried root tissue of four beet (*Beta vulgaris* L.) strains (red, white, orange, and high-pigment (red) phenotypes) were determined. Aqueous and ethanolic tissue extracts of the regular and high-pigment red phenotypes were most capable of inhibiting metmyoglobin/H_2O_2-mediated oxidation of 2-2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH)-mediated bleaching of β-carotene. These same extracts were also most efficient at reducing ABTS radical cation and inducing quinone reductase in murine hepatoma (Hepa 1c1c7) cells in vitro.

KEYWORDS: Quinone reductase; phase II enzyme; antioxidant; chemopreventive; Hepa 1c1c7 cells; beetroot; betalains

INTRODUCTION

Vegetables furnish an abundance of nutrients, especially vitamins and minerals, to the diet of individuals. They also contain nonnutritive constituents, such as fiber and phenolic compounds, the latter which have been implicated in conferring biological and beneficial health effects in test animals and humans (*1*, *2*). In the past, many of the nonnutritive constituents have been ignored because they were considered biologically inert (*3*, *4*). Recent investigations show that there is a profound link between the dietary habits and the incidence of cancer and heart diseases in humans, and the nonnutritive constituents appear to play a role in preventing the development of these diseases (*5*). Many of the beneficial health effects of nonnutritive constituents of vegetables have been known to originate from or be closely associated with their antioxidant properties (*6*).

The development of cancer occurs in three stages: initiation, promotion, and progression (*7*). Initiation can be caused by electrophilic carcinogens reacting with cellular DNA, leading to mutations in the genetic code (*8*). Mechanisms that have evolved to counter the potentially harmful effects of nascent electrophiles (or those activated by phase I enzyme action on procarcinogens) include the set of detoxifiying enzymes or phase II enzymes, such as reductases (e.g., quinone reductase) and conjugating enzymes (e.g., glutathione *S*-transferase) (*9*). The detoxification process involves the conversion of electrophiles into inactive, more water-soluble, and readily excretable conjugates. Phase II enzymes also compete with phase I activating enzymes to limit the generation of electrophiles, thus reducing the risk of initiation (*10*). Therefore, the maintenance of elevated levels of phase II enzymes in bodily tissues provides for a cancer chemopreventive defense against highly reactive electrophiles.

Various synthetic organic compounds, such as β-naphthoflavone, *tert*-butylhydroquinone, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA), have been reported to be potent chemopreventive agents because they can induce phase II enzymes in cultured murine hepatoma cells (*10*). BHT and BHA have also been reported to be carcinogenic, but this risk appears evident only at excessive dietary intakes and when biological defense mechanisms are compromised (*11*). Many of the nonnutritive food components, such as phenolics (*12*) and sulfur-containing compounds (*13*, *14*), including glucosinolates and their metabolites (*15*−*17*), have also been shown to possess cancer chemopreventive properties.

Crude extracts of 25 vegetables were screened for their ability to induce phase II enzymes in vitro (*15*). The activities observed for green onion, broccoli, and other varieties of *Brassica oleracea* were superior among those vegetables investigated. Beetroot extracts (*Beta vulgaris* L.) registered among the least effective phase II enzyme inducers in this study. However, the lack of phase II enzyme induction by beetroot extracts may be conferred by the use of acetonitrile as the extractant (*15*), as we have recently found water and 95% ethanol (5% aqueous) extracts of lyophilized beetroot tissue to possess significant phase II enzyme-inducing activity (*18*).

The hypothesis behind the present study is that a polar, aqueous-extractable component in beetroot tissue is an active

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phase II enzyme inducer in murine hepatoma cells and that phenotype (degree and type of pigmentation) may be related to the presence of this and other antioxidant functions. Therefore, the objective of this work was to screen four beetroot strains (regular (red), white, orange, and high-pigment (red)) for their phase II enzyme-inducing and selected antioxidant properties.

MATERIALS AND METHODS

Materials. Beetroots of regular (red), white, orange, and high-pigment (red) strains were developed from a breeding program (*19*) and generously provided by Irwin Goldman of the Department of Horticulture, University of Wisconsin-Madison, WI. Cultivation made use of the herbicides pyrazon at 1.4 kg acre^{-1} and cycloate at 1.8 kg acre^{-1}.

Mono- and dibasic sodium phosphates, sodium chloride, potassium ferricyanide, myoglobin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), H_2O_2, 2-2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2′-azobis-(2-amidinopropane) dihydrochloride (AAPH), β-carotene, linoleic acid, Tween 20, 0.4% trypan blue solution, crystal violet, sodium dodecylsulfate (SDS), digitonin, 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Tris-Cl, flavin adenine dinucleotide (FAD), glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate dehydrogenase, menadione, and acetonitrile were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) while ethanol was obtained from Fisher Scientific, Chicago, IL. Costar microtiter plates were obtained from Corning Inc. (Corning, NY), and minimum essential medium (MEM) and fetal bovine serum (FBS) were obtained from Gibco Life Technologies (Grand Island, NY).

Preparation of Beetroot Powder. Peeled beetroots (400 g) were diced into ~1 cm^3 cubes, frozen at −18 °C, and then lyophilized (Virtis UNI-TRAP freeze dryer, Model 10-100, The Virtis Company, Gardiner, NY) for 72 h at 13.3 Pa. Lyophilized tissue was ground (Type 203 household grinder; Krups North America, Peoria, IL) into a fine powder (300−420 μm) and stored in amber glass bottles at 4 °C until used.

Preparation of Aqueous Extracts. Powdered beetroot tissue (25 g) was blended with 250 mL of boiling water for 2 min, filtered through a double layer of cheesecloth, and the filtrate was centrifuged at 1800 × g for 10 min. The supernatant was collected, frozen, and lyophilized for 48 h at 13.3 Pa. Lyophilized extracts were stored in amber glass bottles at 4 °C until used.

Preparation of Ethanolic Extracts. One hundred milliliters of 95% ethanol (5% aqueous) were added into a round-bottom flask containing 6 g of powdered beetroots and the mixture was held at 60 °C for 25 min with constant stirring. The resulting slurry was filtered through a Whatman No. 3 filter paper and the filtrate was subjected to rotary evaporation (Buchi Rotavapor R110, Flawil, Switzerland) at 40 °C to remove ethanol. The concentrated extracts were frozen and lyophilized for 48 h at 13.3 Pa. Lyophilized extracts were stored in amber glass vials at 4 °C until used.

Inhibition of ABTS Radical Cation (ABTS$^{\bullet+}$) Generation. All reagents used in this assay were prepared in sodium phosphate buffered saline (PBS, 10 mM, pH 7.4). Sample solutions were prepared in either PBS or 95% ethanol. ABTS (2.5 mM, 100 μL), metmyoglobin (50 μM, 180 μL), PBS (790 μL), and test solution (10 μL; final concentrations of 42, 84, and 210 μg mL^{-1} assay medium) were mixed in a disposable cuvette. Reaction was initiated by the addition of H_2O_2 (10 mM, 120 μL) into the cuvette. Absorbance at 734 nm (*20*) of the reaction mixture was recorded up to 10 min (Beckman model DU-65 spectrophotometer, Beckman Coulter, Inc., Fullerton, CA). Data were plotted against a time scale and the areas under the curves were calculated using SigmaPlot software (version 2000, Jandel Scientific, San Rafael, CA) to determine % inhibition as

$$[(\text{Area}_{\text{control}} - \text{Area}_{\text{treated}})/\text{Area}_{\text{control}}] \times 100$$

Measurement of Reducing Power. ABTS radical cation (ABTS$^{\bullet+}$) was generated by mixing 5 mL of an aqueous ABTS solution (7 mM) and 88 μL of a potassium persulfate solution (140 mM), followed by incubation in the dark for ≥6 h at 20−22 °C (*21*). This stock ABTS$^{\bullet+}$ solution was diluted (1.1 mL to 90 mL) with PBS (pH 7.4) such that the absorbance (734 nm) of the diluted solution was ~0.83. Test solution (10 μL; final concentrations of 42, 84, and 210 μg mL^{-1} assay medium) and PBS (pH 7.4, 190 μL) were transferred into a disposable cuvette and the assay was started by the addition of 1 mL of the preformed ABTS$^{\bullet+}$ solution. Absorbance at 734 nm was measured after 10 min and the % reduction of ABTS$^{\bullet+}$ was calculated as

$$[(\text{Absorbance}_{\text{initial}} - \text{Absorbance}_{\text{final}})/\text{Absorbance}_{\text{initial}}] \times 100$$

Inhibition of Peroxyl Radical-Mediated β-Carotene Bleaching. A solution of AAPH (0.5 M) was prepared in degassed double-distilled water. A β-carotene working solution was prepared by mixing 10 mg of β-carotene in 10 mL of acetone, centrifuging to yield a clear supernatant, and preparing a 1:1 (v/v) dilution of the supernatant with the same solvent. β-Carotene (60 μL) and 0.6% (w/v) Tween 20 containing 0.3% (w/v) linoleic acid in PBS (120 μL) were mixed with 935 μL of PBS in a quartz cuvette, and this mixture was preincubated for 2 min at 50 °C. Test solution (10 μL; final concentrations of 42, 84, and 210 μg mL^{-1} assay medium) and AAPH (25 μL) were added to the cuvette and the reaction was monitored as changes in absorbance at 452 nm and 50 °C (*6*). Percentage β-carotene retention was calculated as

$$100 - [(\text{Absorbance}_{\text{initial}} - \text{Absorbance}_{5\ \text{min}})/\text{Absorbance}_{\text{initial}}] \times 100$$

Assay of Inducer Potency. This assay used murine hepatoma (Hepa 1c1c7) cells cultured in two 96-well microtiter plates as described by (*22*). One plate was used for the quinone reductase (QR) assay while the other was for the cell protein (cell density) measurement to allow for calculation of QR specific activity in control and induced cells. Each plate containing 10000 cells in MEM per well was incubated for 24 h, emptied, and then 200 μL of serially diluted test materials (0−5 mg mL^{-1}) in MEM were filtered-sterilized and added into the wells. In each plate, several wells were devoted to no-cell blanks and cell controls devoid of test materials, which contained MEM in the absence of the test materials.

After incubating (inducing) cells in both plates for 48 h, the wells of one plate were emptied of free liquid and the cells were lysed using 50 μL of 0.08% (w/v) aqueous digitonin solution (this suspension was initially centrifuged to obtain a particle-free solution). The plate was then incubated for 20 min in a shaker oven at 37 °C, and then an aqueous assay cocktail (150 μL) containing FBS (0.066%, w/v), Tris-Cl (25 mM, pH 7.4), Tween 20 (0.01%, v/v), BSA (0.67%, v/v), FAD (5 μM), glucose-6-phosphate (1 mM), NADP (30 μM), glucose-6-phosphate dehydrogenase (0.0007%, w/v), MTT (0.03%, w/v), menadione (0.0008%, w/v), and acetonitrile (0.1%, v/v; used to prepare menadione solution) was added to each well. The absorbance of the reduced tetrazolium dye was measured over a 10-min period using an optical microtiter plate scanner (SPECTRA MAX plus, Molecular Devices, Sunnyvale, CA) set at 490 nm, and no-cell blanks were used to correct for background absorbance.

The second plate was emptied of liquid contents, then immersed in a crystal violet bath for 10 min, and finally rinsed under cold running water to remove excess stain. A 200-μL aliquot of 0.5% (w/v) SDS (prepared in 50% aqueous ethanol) was added to each well and the plates were incubated for 1 h in a shaker oven at 37 °C. Plates were removed and the absorbance of the crystal violet was measured at 610 nm. The absorbance values of no-cell blanks were subtracted from those of the controls and treated samples. The degree of staining (absorbance) with crystal violet was used as a measure of cell protein (density) and provided the basis for calculating changes in QR specific activity. A reduction in crystal violet staining relative to controls was also regarded as loss in a viability, which may be caused by cytotoxicity or loss of adherent nature of the cells. QR specific activity was not determined in cases where loss of viability exceeded 50%.

For a given induced or control sample, QR specific activity was calculated using the linear portion of the reaction progress curve (typically 5 min) from the QR assay relative to the absorbance value of crystal violet stain. The degree of QR induction was then calculated

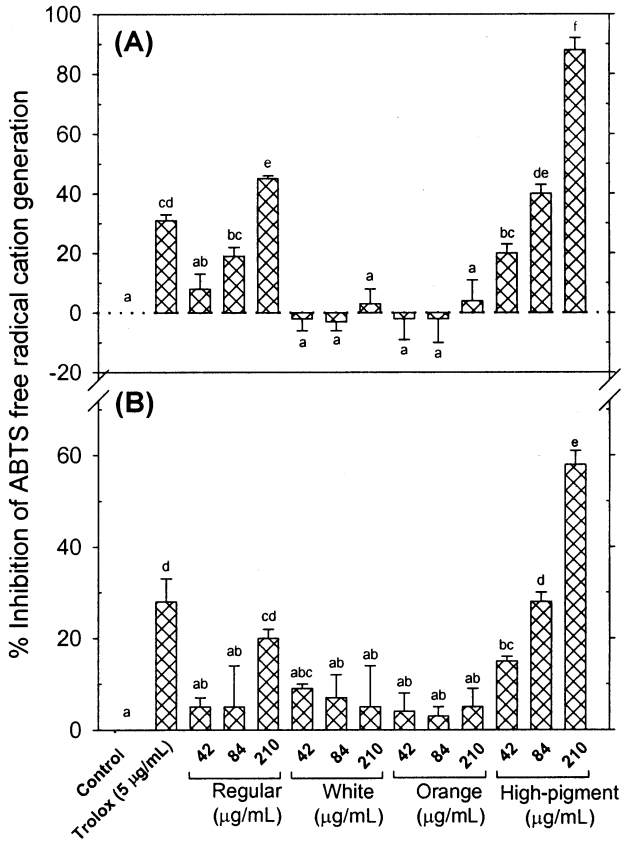

Figure 1. Inhibition of ABTS oxidation by extracts of beetroots. Bars sharing the same letter within each group for aqueous (A) ethanolic (B) extracts are not significantly different ($P > 0.05$) from one another.

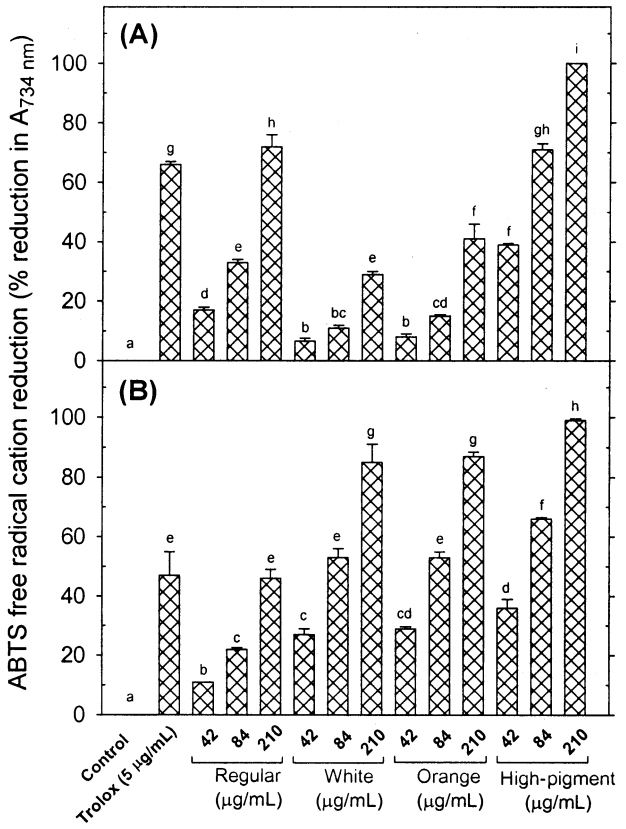

Figure 2. $ABTS^{\bullet+}$ reducing power of extracts of beetroots. Bars sharing the same letter within each group for aqueous (A) ethanolic (B) extracts are not significantly different ($P > 0.05$) from one another.

as the ratio of QR activity in the treated (induced) sample relative to the control sample.

Statistical Analyses. All experiments used completely randomized block designs and the significance of differences among treatment means was established using one-way analysis of variance (ANOVA) followed by the Tukey's studentized range test at the $P < 0.05$ level (*23*).

RESULTS AND DISCUSSION

Inhibition of $ABTS^{\bullet+}$ Generation by Crude Beetroot Extracts. Figure 1A shows the antioxidant activity of aqueous extracts of beetroot strains at three different concentrations as measured by this ABTS oxidation assay. Significant antioxidant activity was observed for aqueous extracts of regular beetroot at 84 and 210 μg mL^{-1} but not at 42 μg mL^{-1}, relative to the control. The antioxidant activity of aqueous extracts of regular beetroots at 210 μg mL^{-1} was greater than that afforded by 5 μg Trolox mL^{-1}. Among the aqueous extracts, greatest antioxidant activities were observed for tissue from the high-pigment beetroot strain at all levels tested. At 210 μg mL^{-1}, the antioxidant activity of aqueous extracts of high-pigment beetroots was more than twice that exhibited by 5 μg Trolox mL^{-1}. In contrast, all concentrations tested for the aqueous extracts of white and orange beetroots were ineffective at inhibiting oxidation in this system.

Figure 1B shows the antioxidant activity of ethanolic extracts of beetroots in the ABTS oxidation assay. Significant antioxidant activity was evident for ethanolic extracts of regular beetroots at 210 μg mL^{-1} and for extracts of high-pigment beetroots at all concentrations examined. However, the antioxidant activities observed for ethanolic extracts of regular and high-pigment beetroots were consistently less than those observed for aqueous extracts at the same concentration (**Figure 1A**, **B**). As was found for the corresponding aqueous extracts, ethanolic extracts of white and orange beetroots had no significant antioxidant activity in this assay system (**Figure 1B**).

The presence of antioxidant activity in only the red-pigmented beet phenotypes infers that this activity is conferred by betacyanins or other components associated with the red-pigmented phenotypes. Betalains have been previously shown to be effective antioxidants in this oxidation system (*24*). This antioxidant assay measures the ability of a test compound to either quench H_2O_2, regenerate ABTS from $ABTS^{\bullet+}$, be oxidized (sacrificed) preferentially to ABTS, inhibit metmyoglobin participation in the oxidation reaction, or any combination of these effects. The specific ability to regenerate ABTS from $ABTS^{\bullet+}$ was tested in the next set of experiments.

Reducing Power of Crude Beetroot Extracts. The ability of extracts to reduce preformed $ABTS^{\bullet+}$ into its native form through electron donation was employed to further assess their antioxidant potential. As shown in **Figure 2A**, aqueous extracts of tissue from all beetroot strains exhibited the ability to reduce $ABTS^{\bullet+}$ in a dose-dependent fashion. Greatest reduction (70−100%) of $ABTS^{\bullet+}$ was evident for aqueous-extracted fractions at 210 μg mL^{-1} for regular beetroots and 84 and 210 μg mL^{-1} for high-pigment beetroots, all of which were at least as effective as 5 μg Trolox mL^{-1} (**Figure 2A**). The relative effectiveness of aqueous beetroot extracts in reducing $ABTS^{\bullet+}$ was consistent with the ability to inhibit the generation of $ABTS^{\bullet+}$ from ABTS mediated by metmyoglobin and H_2O_2 (**Figure 1A**).

Ethanolic extracts of tissue from all beetroot strains exhibited the ability to reduce $ABTS^{\bullet+}$ in a dose-dependent fashion (**Figure 2B**). Greatest reduction (50−100%) of $ABTS^{\bullet+}$ was evident for ethanol-extracted fractions at 84 and 210 μg mL^{-1}

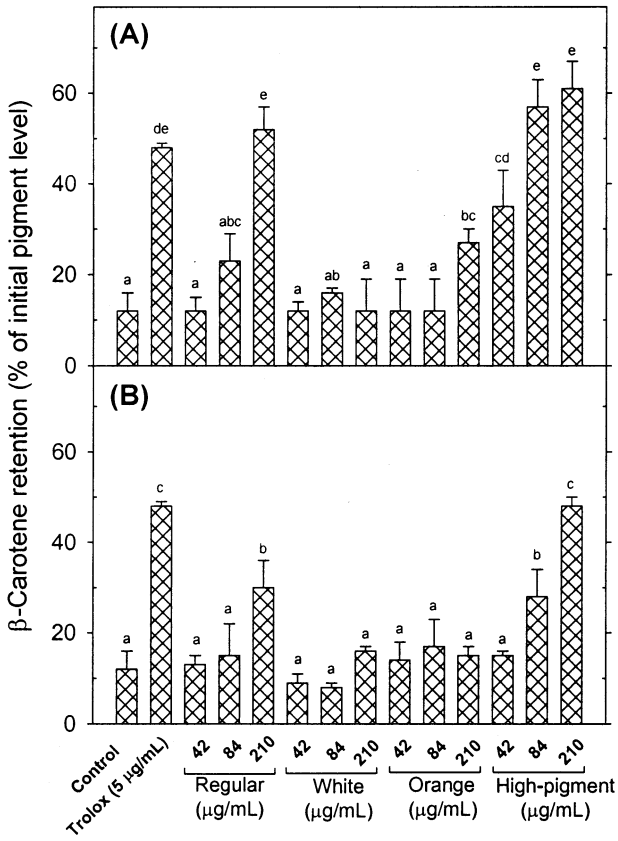

Figure 3. Inhibition of peroxyl radical-mediated β-carotene-bleaching by extracts of beetroots. Bars sharing the same letter within each group for aqueous (A) ethanolic (B) extracts are not significantly different ($P > 0.05$) from one another.

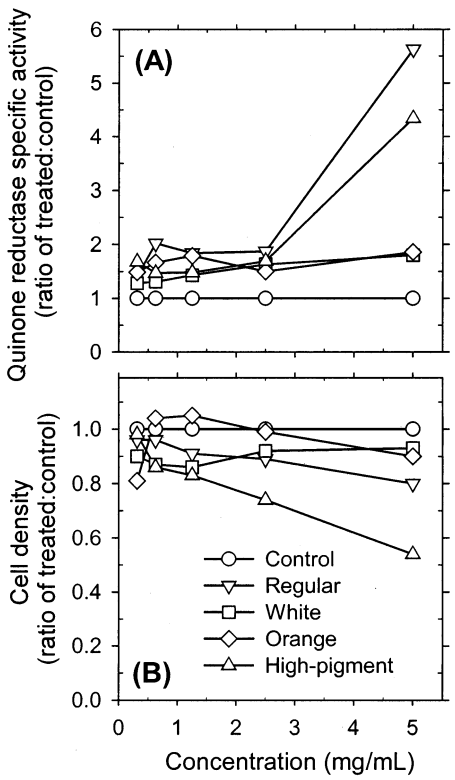

Figure 4. Effects of aqueous extracts of beetroots on Hepa 1c1c7 cells. Induction of quinone reductase is described in panel (A), and the effect on cell density (toxicity) is described in panel (B).

for white, orange, and high-pigmented beetroots, where reducing activity was at least as effective as 5 μg Trolox mL^{-1}. ABTS$^{\bullet+}$ reducing capacity of the ethanolic extracts of tissue from regular beetroots was the least effective of the samples tested. The relative effectiveness of ethanolic extracts of tissue from regular and high-pigmented beetroots in reducing ABTS$^{\bullet+}$ was similar to their respective abilities to inhibit ABTS$^{\bullet+}$ generation (**Figure 1B**). The fact that ethanolic extracts from tissue of white and orange beetroots were effective at reducing ABTS$^{\bullet+}$ (**Figure 2B**) but not at preventing ABTS$^{\bullet+}$ generation (**Figure 1B**) implies that these tissues possess components with significant reducing power that may be easily destroyed or act slow kinetically in the metmyoglobin-H_2O_2 oxidizing system where ABTS$^{\bullet+}$ generation from ABTS takes place. Different reducing compounds can exhibit different kinetics of action, as is the case for compounds that can reduce ABTS$^{\bullet+}$ (*20*) or 1,1-diphenyl-2-picrylhydrazyl radical (*25*, *26*).

Inhibition of β-Carotene Bleaching by Crude Beetroot Extracts. Aqueous-extracted material of regular beetroots at 210 μg mL^{-1} and all levels tested for aqueous-extracted material of high-pigment beetroots were protective of β-carotene bleaching relative to the control (**Figure 3A**). The degree of β-carotene retention for these extracts was similar to that afforded by 5 μg Trolox mL^{-1}. Except for the modest protective effect at the 210 μg mL^{-1} level of aqueous-extracted material from orange beetroots, aqueous extracts of white and orange beetroots were no different than the control in the extent of β-carotene retention.

Ethanol-extracted material from regular beets at 210 μg mL^{-1} and that from high-pigment beetroots at 84 and 210 μg mL^{-1} afforded protection from β-carotene bleaching relative to the control (**Figure 3B**). However, using Trolox as a reference, the inhibitory effect of the ethanol extracts were generally 30−50% less effective than the aqueous-extracted materials at the same levels tested, except that the 210 μg mL^{-1} level of high-pigment beet tissue extract was as effective as Trolox. No capacity for β-carotene retention was observed for ethanolic extracts of white and orange beetroots relative to the control.

Inhibition of AAPH-mediated oxidative reactions is a popular indicator of antioxidant potency of biological materials (*27*, *28*). Using the same oxidation catalyst (AAPH) in this assay, the combined aqueous and acetone extracts of beet tissue was among the top 20% of the 22 vegetables evaluated (*27*). The AAPH used in this assay is a polymerization catalyst, which can generate a peroxy radical intermediate that can bleach β-carotene (*29*). Thus, effective antioxidants in this assay can act by direct reaction with peroxy radicals or by reducing the peroxy radical to the less reactive peroxide and thereby afford protection of β-carotene.

Generally, the antioxidant activities observed in the β-carotene-bleaching assay were consistent with those observed for the ABTS oxidation (**Figure 1**) and ABTS$^{\bullet+}$ reducing (**Figure 2**) assays, in that aqueous and ethanolic extracts from the regular and high-pigmented beetroots were most effective.

Quinone Reductase (QR) Inducing Effect of Crude Extracts. The aqueous extracts of tissue from all four beetroot strains were capable of inducing QR in Hepa 1c1c7 cells (**Figure 4A**). The order of effectiveness was high-pigment > regular > orange ~ white ($P < 0.05$). These extracts also caused losses in viability of these cells (**Figure 4B**; $P < 0.05$) in a pattern inversely related to the ability of the extracts to induce QR.

Only ethanolic extracts from tissue of regular and high-pigment beetroots were capable of inducing ($P < 0.05$) QR (**Figure 5A**). The high-pigment beetroot tissue extract was particularly effective, in that a 4-fold induction was observed at 2.5 mg mL^{-1}. All crude ethanolic extracts of beetroot tissues

Table 1. Yield, Inducer Potency, and Toxicity of Crude Beetroot Extracts[a]

	crude aqueous extract					crude ethanolic extract				
beet phenotype	yield of crude extract (w/w)[a]	level (mg mL^{-1}) to double QR[b]	inducer units[c] gdw^{-1}	level (mg mL^{-1}) at 20% toxicity[b]	equivalent gdw^{-1} for 20% toxicity[c]	yield of crude extract (w/w)[a]	level (mg mL^{-1}) to double QR[b]	inducer units[c] gdw^{-1}	level (mg mL^{-1}) at 20% toxicity[b]	equivalent gdw^{-1} for 20% toxicity[c]
regular (red)	0.58	0.65, 2.60	1120–4460	>5	>8.6	0.30	4.6	326	0.58, 1.5	1.9–5.0
orange	0.54	>5	<540	>5	>9.2	0.23	inactive		0.31, 2.5	1.3–11
white	0.56	>5	<560	>5	>8.9	0.30	inactive		>5	17
high-pigment	0.49	2.80	875	1.7	3.5	0.32	1.2	1391	2.2	7.0

[a] Yields are expressed as gram dry matter recovered as an extract relative to the starting quantity of dry beetroot powder [b] Level of crude extracts required to elicit threshold effect. In cases where progressive increases in extract level did not maintain the threshold effect, two levels are indicated. Cases categorized as ">integer" indicates that a significant effect was observed but the threshold level was not reached. "Inactive" signifies that no significant effect was observed. [c] Levels are expressed on the basis of the amount of original dry beetroot powder required to illicit the threshold effect when prepared as the aqueous or ethanolic extract under the conditions employed.

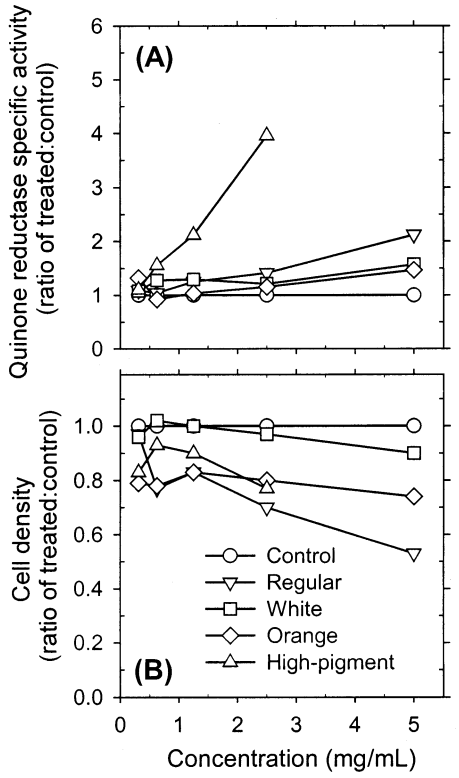

Figure 5. Effect of ethanol extracts of beetroots on Hepa 1c1c7 cells. Induction of quinone reductase is described in panel (A), and the effect on cell density (toxicity) is described in panel (B).

resulted in losses in cell viability (**Figure 5B**). Greatest losses in viability were observed for the two red and orange strains, and limited losses were observed for the white strain ($P < 0.05$).

Using the format that was originally devised to express QR-inducing potential (*15*), the potency of the various tissue extracts was calculated in terms of inducer "units" per gram dry weight (gdw) of the original beetroot powder, taking into account the amount of dry matter extracted by the solvent (**Table 1**). Since this calculation is based on the level of added extract to double QR specific activity in Hepa 1c1c7 cells, two estimates were obtained for the aqueous extracts of regular (red) beetroot tissue extracts (**Figure 4A**) of 1120 and 4460 inducing units gdw^{-1}. The aqueous and 5% aqueous ethanol extracts from high-pigment beetroot powder contained 875 and 1390 inducing units gdw^{-1}, respectively. The fact that we observed a doubling of QR specific activity by the regular and high-pigment beetroot aqueous extracts whereas the previous study did not (*15*) may be conferred by two differences between these studies. One is that we evaluated a slightly greater upper limit of extract testing (equivalent to ~10 mg original beetroot powder mL^{-1} compared to 8 mg mL^{-1}). A more likely explanation is that the aqueous (either water or 5% aqueous ethanol) medium is more effective at extracting the active QR-inducing compounds (this study) than is acetonitrile extraction (*15*). We found that acetonitrile was only 0.4% as effective as water in extracting material from beetroot tissue that absorbs at 537 nm (λ_{max} for betalains; (*30*)) under a standard set of conditions (400 mg powder plus 14 mL extraction solvent (*15*)). If betalains are, or serve as markers for, the active agents, then clearly aqueous extracts would be more effective than acetonitrile extracts at inducing QR activity in Hepa 1c1c7 cells.

The level of beetroot extracts that we observed to confer a 20% loss in viability (referred to as toxicity in other reports) was similar to the 4 mg mL^{-1} levels that was reported in the previous study (*15*). However, toxicity or loss of viability should not necessarily be construed as a negative effect, since Hepa 1c1c7 cells constitute a model for cancer cells and there is a long-standing history of safety in consumption of beets and beet pigment preparations (*31*).

CONCLUSIONS

Previous studies have shown a broad range of antioxidant activities in crude extracts of beetroot tissues (*27*, *32*). Betalain pigments have specifically been shown to possess various antioxidant functions (*24*, *33*, *34*). Extracts of beetroot tissue have also been shown to be antimutagenic in the Ames tester strains (*35*) and antitumor promoting in mouse skin and lung bioassays (*36*). Antioxidant and phase II enzyme (quinone reductase)-inducing activities of beetroots were enriched in regular and high-pigment red phenotypes compared to orange and white phenotypes (**Figures 1**−**5**). Continuing studies on the isolation of the agents conferring these biological activities will focus on red phenotypes to provide an unequivocal assignment of these antioxidant functions to specific beetroot components.

Apparently, the ethanolic extracts of both white and orange beetroots are enriched in reducing agents (**Figure 2B**). The possibility that the active agents in these extracts may be unique compounds provides merit to further research directed toward the elucidation of the structure and function of these unknown antioxidants.

ABBREVIATIONS USED

ABTS, 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid; ABTS$^{\bullet+}$, ABTS radical cation; AAPH, 2,2′-azobis-(2-amidinopropane) dihydrochloride; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole; SDS, sodium dodecylsulfate;

MTT, 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide; FAD, flavin adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; MEM, minimum essential medium; FBS, fetal bovine serum; QR, quinone reductase; PBS, sodium phosphate-buffered saline; gdw, gram dry weight.

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