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Evaluation of the Antioxidant Activity of Wheatgrass (*Triticum aestivum* L.) as a Function of Growth under Different Conditions

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The antioxidant activity of wheatgrass, which is consumed as a dietary supplement, was estimated at different levels. The methods employed include FRAP (ferric reducing antioxidant power), ABTS (2,2′-azobis-3-ethylbenzthiazoline-6-sulfonic acid) and DPPH (1,1′-diphenyl-2-picrylhydrazyl) assays. Aqueous and ethanol extracts of wheatgrass grown under different conditions over a period of 6, 7, 8, 10 and 15 days were used. Lipid peroxidation and oxygen radical absorbance capacity (ORAC) were determined and utilized to check the potency of a few selected extracts. Different conditions used for growth were (1) tap water, (2) tap water with nutrients, (3) soil and tap water, and (4) soil with nutrients. For comparison, a commercially available wheatgrass tablet was analysed. To explain the reasons behind the observed differences, the total phenolic and flavonoid contents of the extracts were measured. These contents increased with growth under all the conditions. The ethanol extracts were found to have a higher phenolic and flavonoid content than the aqueous extracts. The highest FRAP values occurred on day 15 of growth under condition 4, the values being 0.463 and 0.573 mmol of ascorbic acid and Trolox equivalents/100 g fresh wheatgrass for aqueous and ethanol extracts, respectively. In the aqueous extracts no specific trend was observed with the DPPH assay for the different conditions nor for the growth period. In the case of ethanol extracts, however, it increased with the growth period and the wheatgrass grown in condition 4 was found to be the most effective. These extracts were also found to inhibit significantly ascorbate-Fe^{2+} induced lipid peroxidation in rat liver mitochondria. The ORAC values of aqueous and ethanol extracts of day 10 with condition 4 were found to be 39.9 and 48.2, respectively, being higher than those reported for many natural extracts or vegetables. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: wheatgrass; antioxidant potential; growth period; free radicals; lipid peroxidation; ORAC values.

INTRODUCTION

Reactive oxygen species (ROS) are generated continuously in living organisms due to various metabolic processes as well as to exposure to various physico-chemical agents. At normal physiological concentrations they are required for cellular activities. But, at higher concentrations, they can be toxic leading to oxidative stress. They can damage major cellular components and have been implicated in various human diseases such as different forms of cancers, neurogenerative disorders, cardiovascular diseases (CVDs) and diabetes mellitus as well as in the process of aging (Harman, 1981; Droge, 2002). Antioxidants are capable of neutralizing the deleterious effects of free radicals. In a normal healthy state endogenous antioxidants act as the body's effective defense system against free radicals. However, in the diseased state, additional antioxidants from the diet and other sources such as medicinal plants are required for effective recovery.

In the past two decades, research in nutrition and food science has focused on plant products with potential antioxidant activities. Such products are also rich in fibre, have no cholesterol and contain antioxidants such as carotenoids and flavonoids. The compounds, which are mainly responsible for the antioxidant effect, are a class of phenolic compounds including flavonoids and their derivatives besides carotenoids and tocopherols (Nocole *et al.*, 1996; Sergio *et al.*, 1999). The search is on for plant products with high antioxidant activities.

Germination/sprouting causes extensive changes in the seeds. During this stage, the synthesis of useful compounds such as vitamins and phenolics occurs. Wheat (*Triticum aestivum* L.) germinated over a period of 6–10 days is generally called 'wheatgrass'. In recent years, in some European countries, USA and India, wheatgrass, in the form of a ready-made juice or tablet is being consumed as a 'health food'.

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It is presumed that the wheatgrass is a rich source of vitamins, antioxidants and minerals in a bioavailable form**.** Wheatgrass contains vitamins C and E, β-carotene, ferulic acid and vanillic acid whose concentration increases with the germination period and reaches a maximum on day 7 of growth (Hanninen *et al.*, 1999). There are reports on the antimutagenic effect of wheatgrass extracts towards benzo(a)pyrene induced mutagenicity. Wheatgrass extracts also possess superoxide scavenging and ferric reducing power (Peryt *et al.*, 1992). Their ability to inhibit oxidative DNA damage was also demonstrated (Falcioni *et al.*, 2002). Chlorophyll, one of the active components in the wheatgrass extract, was found to be responsible for inhibiting the metabolic activation of carcinogens (Lai *et al.*, 1978; Lai, 1979). Recently, some clinical trials have indicated the healing properties of wheatgrass in different diseases. It was shown to reduce transfusion requirements in patients suffering from thalassaemia (Marwaha *et al.*, 2004). It is effective for the treatment of distal ulcerative colitis besides having a significant ability to reduce the overall disease activity index and the severity of rectal bleeding (Ben-Arye *et al.*, 2002).

The observed beneficial effects can possibly be ascribed to the antioxidant properties. Although there are some preliminary studies (Peryt *et al.*, 1992), the antioxidant activity of wheatgrass, at various levels of protection, has not been studied in detail. It is also not known at what period of growth wheatgrass has the maximum antioxidant potential. The effect of different germination conditions used for the cultivation of wheatgrass such as nutrients and soil has not been studied. To fulfil these lacunae, the present study assessed the antioxidant potential of wheatgrass, at different levels of action, during its germination period under different growth conditions. The possible factors responsible for the differences observed, in terms of the chemical composition, were also examined.

MATERIALS AND METHODS

Materials. Ascorbic acid, aluminium chloride, 2,2′-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) diammonium salt, β-phycoerythrin, 1,1′-diphenyl-2-picrylhydrazyl (DPPH), ethylene diamine tetra acetic acid (EDTA), ferric chloride, Folin-Ciocalteu reagent, hydrogen peroxide, myoglobin, potassium ferricyanide, potassium phosphate (monobasic and dibasic), sodium carbonate, 1,1,3,3-tetramethoxypropane, 2,4,6-tripyridyl-s-triazine (TPTZ), 2-thiobarbituric acid and trichloroacetic acid were from Sigma Chemical Co., USA. 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) and Trolox (6-hydroxy 2,5,7,8 tetramethyl chroman 2-carboxylic acid) were from Aldrich Chemical Co., USA. Other chemicals used in the studies were of the highest quality commercially available from local suppliers. The wheatgrass tablets, used in our studies, were purchased from local suppliers in Mumbai and the growth condition of the corresponding wheatgrass is unknown. These tablets constitute 98% of wheatgrass, 1.5% silica and 0.5% vegetable stearates.

Aqueous and ethanol extracts of wheatgrass, prepared from fresh wheatgrass collected at various stages of growth, were studied. Samples were collected on 6, 7, 8, 10 and 15 days after germination. The aqueous extracts were similar to those that people consume as a herbal drink. In order to estimate the total content of the bioactive organic compounds ethanol extracts were prepared as most of the organic compounds are soluble in ethanol. The antioxidant activities of both aqueous and ethanol extracts were determined. The antioxidant activities of wheatgrass were estimated using different assays for its ability to inhibit radical formation (ABTS assay), radical scavenging (DPPH assay), ferric reducing antioxidant power (FRAP) and capacity to inhibit lipid peroxidation in rat liver mitochondria. The levels of total phenolic and flavonoids present were also estimated. In addition, the oxygen radical absorbance capacity (ORAC) of the wheatgrass extracts was checked.

Preparation of wheatgrass extracts. The seeds of wheat (*Triticum aestivum* L. C.V. Pbn-51) were procured and washed with tap water followed by distilled water. The seeds were soaked in distilled water for 8 h and transferred to the containers. The wheat plants were grown in (1) tap water, (2) tap water with nutrients, (3) soil and tap water, and (4) soil with nutrient solution, and termed conditions 1, 2, 3 and 4, respectively. In conditions 1 and 2, the plants were grown in cylinders with perforated plastic tops (h = 5 cm and diameter = 20 cm) called 'wheat-sprout makers'. A total of 100 seeds were placed in each sprout maker. 200 mL of water (pH = 6.3 ± 0.3, Na 4.3 μg/mL, K 3.6 μg/mL, Ca 8.1 μg/mL, Mg 3.8 μg/mL Zn, Mn, Cu < 0.1 μg/mL) was added everyday in condition 1. The same quantity of water along with nutrients was added to the sprout maker in condition 2. The composition of the nutrient solution was 2 mM KNO_3, 2 mM Ca $(NO_3)_2$, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 25 μM H_3BO_3, 2 μM $MnCl_2$, 2 μM ZnSO4, 0.5 μM $CuSO_4$ and 0.5 μM Na_2MoO_4 (Hoagland and Arnon, 1950). In conditions 3 and 4, the seeds were sowed in the trays of dimension 15 × 35 cm^2, containing 7 kg of soil (pH = 7.5 ± 0.4, K 8157 ± 269 μg/g, Na 2415 ± 25, Ca 8657 ± 256, Mg 9845 ± 647, Mn 3520 ± 35, Cu 3.5 ± 0.7, Fe 6190 ± 512, Zn 840 ± 25 μg/g). The trays with soil were provided with sufficient tap water/nutrient solution regularly and were placed in a room where normal airflow and sunlight were available. During the growth of the plants, samples were collected on days 6, 7, 8, 10 and 15.

The samples collected on different days were washed, wiped and cut into small pieces. They were homogenized with a clean pestle and mortar using either distilled water or ethanol (10% w/v). The extracts were centrifuged at 15 000 rpm for 20 min at 4 °C and the supernatants were stored at –20 °C until further use. The extracts were diluted to 1% and 5% as required. The aqueous and ethanol extracts of commercial tablets were prepared in a similar fashion and identified as A1 and E1, respectively.

Ferric reducing antioxidant power of wheatgrass extracts. The ferric complex reducing ability of the extracts was measured by the FRAP assay (Pulido *et al.*, 2000). The calibration curve was plotted with absorbance at 595 nm versus concentration of $FeSO_4$ in the

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range 0–1 mM (both, aqueous and ethanolic solutions). The concentration of $FeSO_4$ was plotted against concentrations of the standard antioxidants (L-ascorbic acid and Trolox).

Inhibition of ABTS$^{\cdot}$ formation assay. In the ferrylmyoglobin/$ABTS^{\cdot+}$. The spectrophotometric assay, the inhibition of radical formation by the extracts, was determined using the ferrylmyoglobin/$ABTS^{\cdot+}$ protocol (Alzoreky and Nakahara, 2001). The calibration curve was plotted with the lag time in seconds versus the concentration of the standard antioxidants (L-ascorbic acid and Trolox).

Radical scavenging assay by using DPPH. The DPPH scavenging effect was determined for the different extracts (Aquino *et al*., 2001). In this method, a commercially available, stable free radical, DPPH$^{\cdot}$, soluble in methanol, was used. In its radical form, DPPH$^{\cdot}$ has an absorption maximum at 515 nm, which disappears on reduction by an antioxidant compound. The calibration curve was plotted with % $DPPH^{\cdot}_{scavenged}$ versus concentration of the standard antioxidants (L-ascorbic acid and Trolox).

Determination of total phenols and total flavonoids. For determining both, total phenolic and flavonoid contents, calibration curves were obtained using known quantities of standard antioxidants. The total phenolic content of the ethanol and aqueous extracts were measured using a modified Folin-Ciocalteu method (Lowry *et al.*, 1951). The absorbance was measured at 750 nm. The measured values were compared with a standard curve of gallic acid concentrations and expressed as millimoles of gallic acid equivalents/100 g fresh wheatgrass. The flavonoid contents of both the extracts were also measured (Luximon-Ramma *et al*., 2002). The absorbance was measured at 368 nm. The values obtained were compared with a standard curve of quercetin concentrations and expressed as millimoles of quercetin equivalents/100 g fresh wheatgrass.

Oxygen radical absorbance capacity (ORAC) assay. The selected wheatgrass extracts were assessed for inhibition of β-phycoerythrin damage induced by peroxyl radicals from thermal decomposition of the azo initiator, AAPH, by fluorescence measurement. The excitation wavelength was 540 nm and the emission wavelength was 565 nm. The fluorescence was recorded after every 5 min, until the last reading was less than 5% of the first (0 min) reading. The ORAC values were expressed in terms of μmol Trolox/g of fresh wheatgrass (Cao and Prior, 2002).

Isolation of mitochondrial fraction from rat liver. Three month old female Wistar rats (weighing 250 ± 20 g) were used for the preparation of mitochondria. The rat livers were excised and homogenized in 0.25 M sucrose containing 1 mM EDTA. To remove cell debris and the nuclear fraction the homogenate was centrifuged at 3000 **g** for 10 min. The supernatant was centrifuged at 10 000 **g** for 10 min to sediment mitochondria. The mitochondrial pellet was washed thrice with 50 mM potassium phosphate buffer, pH 7.4, to remove sucrose (Devasagayam, 1986a) and the protein content was estimated. These pellets were suspended in the above buffer at the concentration of 10 mg protein/mL and stored at –20 °C for further studies.

Exposure of rat liver mitochondria to oxidative stress. Oxidative damage was induced by the ascorbate-Fe^{2+}-system as described elsewhere (Devasagayam, 1986b). The reaction mixture was incubated at 37 °C in a shaker-water bath for 30 min. The samples were then boiled with TBA (thiobarbituric acid) reagent for 30 min. Thiobarbituric acid reactive substances (TBARS) formed were estimated as malondialdehyde equivalents by measuring the absorbance at 532 nm after accounting for appropriate blanks. A malondialdehyde standard was prepared by the acid hydrolysis of tetramethoxypropane. All data are expressed as mean ± 1σ obtained from three independent experiments. Correlation coefficients were calculated by using Microcal Origin 6 software. Student's *t*-test was applied to calculate significant differences between the values and $p > 0.05$ was considered significant.

RESULTS

Ferric reducing and ABTS radical scavenging power of wheatgrass extracts

The results of the ferric reducing power of wheatgrass extracts are given in Fig. 1. On average it was found that the ratio of the fresh to dry weight of wheatgrass was 4. Assuming that there were no additives in the wheatgrass tablet, the content of wheatgrass tablet was normalized by dividing it by 4. The data are expressed as AEAC (ascorbic acid equivalent antioxidant capacity) and TEAC (Trolox equivalent antioxidant capacity) in the cases of the aqueous and ethanol extracts, respectively. The values are expressed as mmol of ascorbic acid/100 g of fresh wheatgrass in the case of AEAC and Trolox in the case of TEAC. Aqueous extracts and ethanol extracts showed similar FRAP values irrespective of their growth conditions. A gradual increase was observed with respect to the growth period in all the conditions. The highest AEAC value of 0.463 was observed for 15 day old wheatgrass grown in condition 4 and the corresponding TEAC value was 0.573.

The values obtained in the ABTS assay are presented in Fig. 2. Both extracts showed similar potential to inhibit ABTS radical formation. Different growth conditions as well as the growth period did not alter the AEAC and TEAC values significantly. However, the values of 0.928 and 0.874 AEAC and TEAC, respectively, obtained for commercially available wheatgrass tablet are higher than those obtained for laboratory grown wheatgrass.

Radical scavenging ability of extracts by DPPH assay

Figure 3 shows a comparison of the DPPH radical scavenging abilities of the different wheatgrass extracts. Ethanol extracts, in general, showed higher DPPH scavenging capacities than the aqueous extracts. The highest DPPH scavenging potential was observed for condition 4, for which the TEAC value increased with

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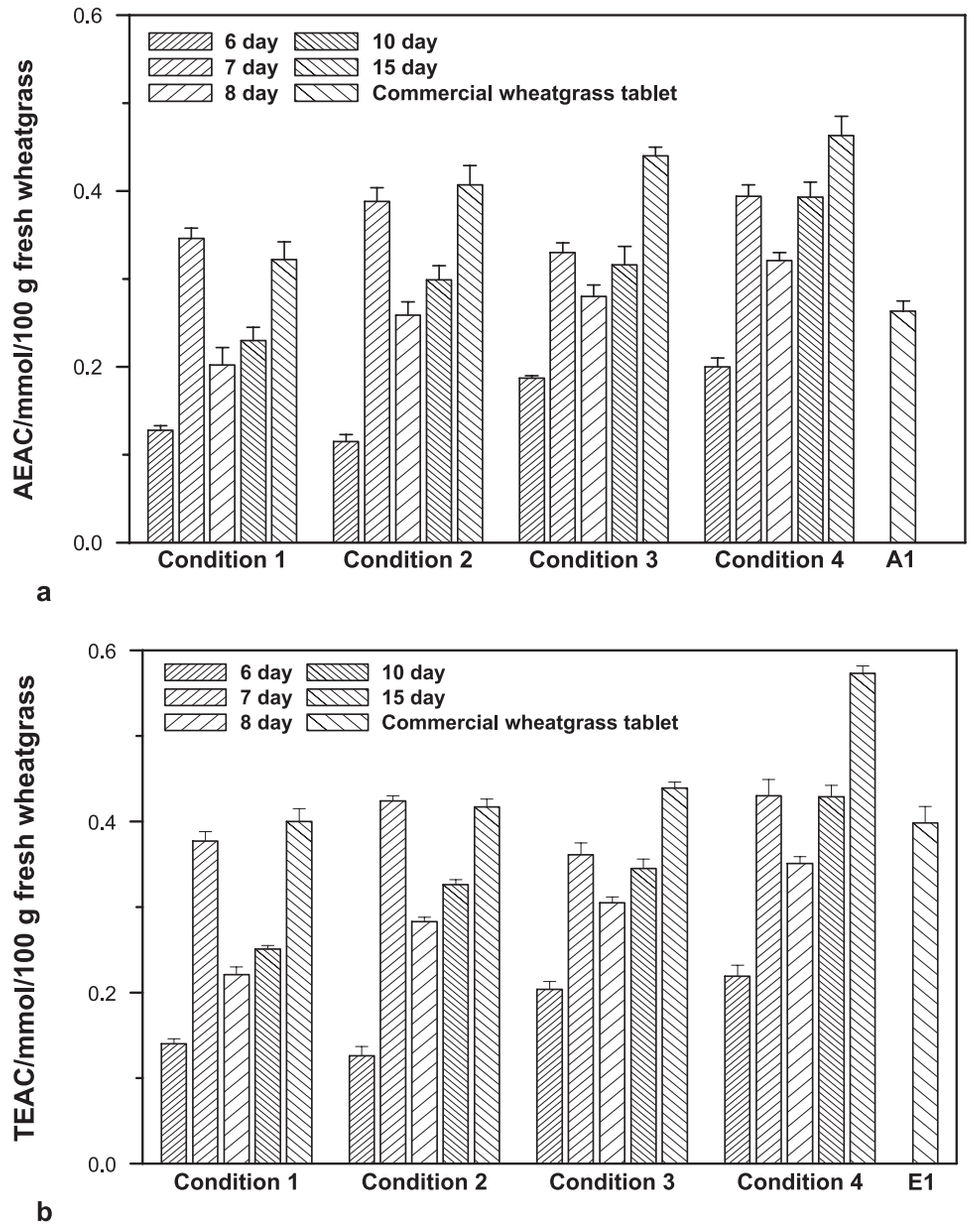

Figure 1. Antioxidant capacity of (a) aqueous extracts and (b) ethanol extracts of wheatgrass grown in different conditions by using ferric reducing antioxidant power (FRAP) assay. Ascorbic acid equivalent antioxidant capacity (AEAC) and Trolox equivalent antioxidant capacity (TEAC) expressed as mmol/100 g fresh wheatgrass. The values are expressed as mean ± SE of three independent experiments.

the plant growth period, with highest the level (1.476) being observed on day 15. In the case of aqueous extracts, different growth conditions had no significant impact on the DPPH scavenging potential. However, the extracts corresponding to day 15 of growth showed the highest DPPH scavenging abilities. Interestingly, the commercially available wheatgrass tablet showed a significantly lower ($p < 0.02$) value than that of the wheatgrass grown in conditions 2, 3 and 4 in the case of the aqueous extracts and in condition 4 ($p < 0.05$) for the ethanol extracts.

Total phenolic and flavonoid contents

Figures 4a and 4b show the total phenolic contents (TPC) of the aqueous and ethanol extracts of wheatgrass grown in different conditions as a function of the growth period. The TPC is expressed as mmol equivalents of gallic acid/100 g fresh wheatgrass. In both types of extracts the total phenolic content was found to increase with growth. The TPC was found to be highest for condition 4. The values on day 15 of growth were 0.331 and 0.699 for aqueous and ethanol extracts, respectively. The commercially available wheatgrass tablet had TPC values of 0.115 and 0.189 for aqueous and ethanol extracts, respectively.

Figures 5a and 5b present the total flavonoid contents (TFC) of the aqueous and ethanol extracts of wheatgrass as a function of growth in different conditions. TFC is expressed as mmol equivalents of quercetin/100 g of fresh wheatgrass. The TFC of the wheatgrass extracts on day 15 of growth was 0.317 in condition 4 in the case of the aqueous extracts and 0.779 for the ethanol extracts. The commercially available wheatgrass tablet possessed a higher TFC with

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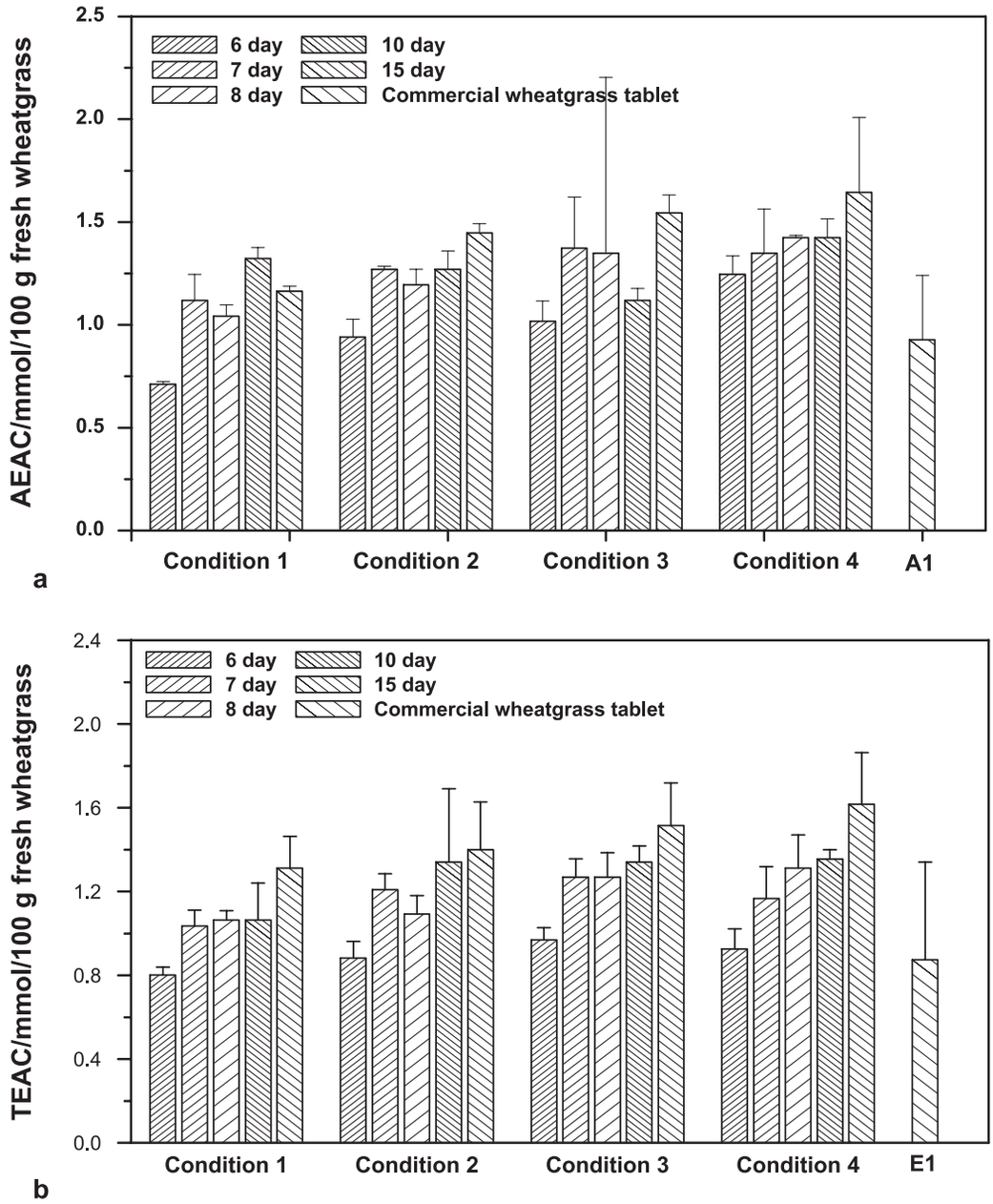

Figure 2. Antioxidant capacity of (a) aqueous extracts and (b) ethanol extracts of wheatgrass grown in different conditions using the ABTS assay. Ascorbic acid equivalent antioxidant capacity (AEAC) and Trolox equivalent antioxidant capacity (TEAC) expressed as mmol/100 g fresh wheatgrass. The values are expressed as mean ± SE of three independent experiments.

values of 0.105 and 0.237 mmol/100 g for the aqueous and ethanol extracts, respectively, which are lower than those for the fresh wheatgrass.

Oxygen radical absorbance capacity (ORAC) assay

The ORAC is one of the standard assays that food chemists and nutritionists use to check the antioxidant capacity of food products. The ORAC values for the aqueous extracts on day 10 of growth in conditions 1, 2, 3 and 4 were found to be 32.6, 37.4, 35.8 and 39.9, respectively, whereas for the ethanol extracts they were 41.6, 42.4, 42.3 and 48.2, respectively. The corresponding values for the commercial wheatgrass tablet were 13.8 and 17.0, respectively.

Inhibition of lipid peroxidation induced by ascorbate-Fe^{2+} system

The results of the studies on lipid peroxidation assay are presented in Table 1. Both aqueous and ethanol extracts showed significant protection against ascorbate-Fe^{2+} induced lipid peroxidation. The ethanol extracts were found to be more potent than the aqueous extracts. The extracts A1 and E1 gave 56.1% and 74.2% inhibition of lipid peroxidation, respectively. Among the aqueous extracts, the maximum protection (52.9%) was observed with the extracts corresponding to day 10 of condition 4, whereas among the ethanol extracts on day 10 of condition 3 gave the maximum (43.9%) protection against lipid peroxidation.

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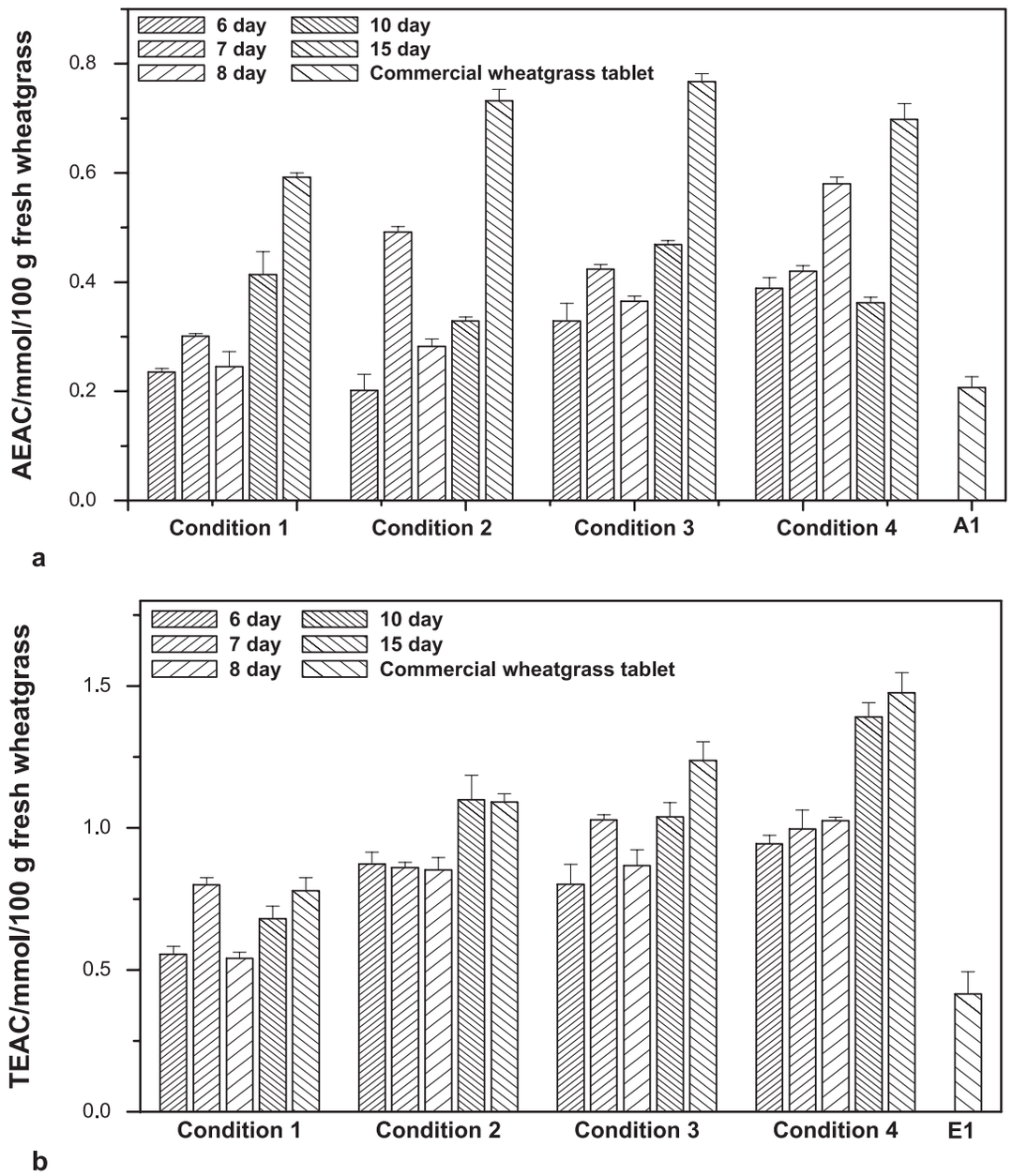

Figure 3. Radical scavenging capacity of (a) aqueous extracts and (b) ethanol extracts of wheatgrass grown in different conditions using the DPPH assay. Ascorbic acid equivalent antioxidant capacity (AEAC) and Trolox equivalent antioxidant capacity (TEAC) expressed as mmol/100 g fresh wheatgrass. The values are expressed as mean ± SE of three independent experiments.

DISCUSSION

It is well known that phenolic compounds including flavonoids of plant origin are mostly responsible for radical scavenging. They possess different antioxidant properties that can be attributed to their therapeutic uses in different diseases. The AEAC and TEAC values obtained from radical scavenging assays for all extracts were correlated with the total phenolic and flavonoid contents. With the DPPH assay, reasonable correlations between the AEAC of the aqueous extracts and the TPC was observed ($r^2 = 0.49$) but the correlation with TFC was better ($r^2 = 0.61$). In the case of the ethanol extracts similar correlations between the antioxidant activity (TEAC) and the TPC and the TFC were observed with r^2 values of 0.64 and 0.53, respectively. With the ABTS free radical formation assay, the aqueous and ethanol extracts, showed values of $r^2 = 0.56$ and $r^2 = 0.73$, respectively with TPC. A strong and significant correlation between the flavonoid content and the antioxidant activity of ethanol extracts ($r^2 = 0.94$) was seen.

Wheatgrass showed higher oxygen radical absorbance capacity (ORAC) values, which is used as a standard tool for comparing the antioxidant capacities of food products (Lachnicht *et al.*, 2002). The results presented in Table 2 showed that the values, in the range of 25–68, are similar to or higher than those for some fruits and vegetables. The values were in the range 10–25 for different extracts of turmeric (Tilak *et al.*, 2004), 19.4 for garlic, 12.6 for spinach, 4.5 for onion, 15.36 for strawberry, 9.49 for plum and 2.10 for carrot (Lachnicht *et al.*, 2002).

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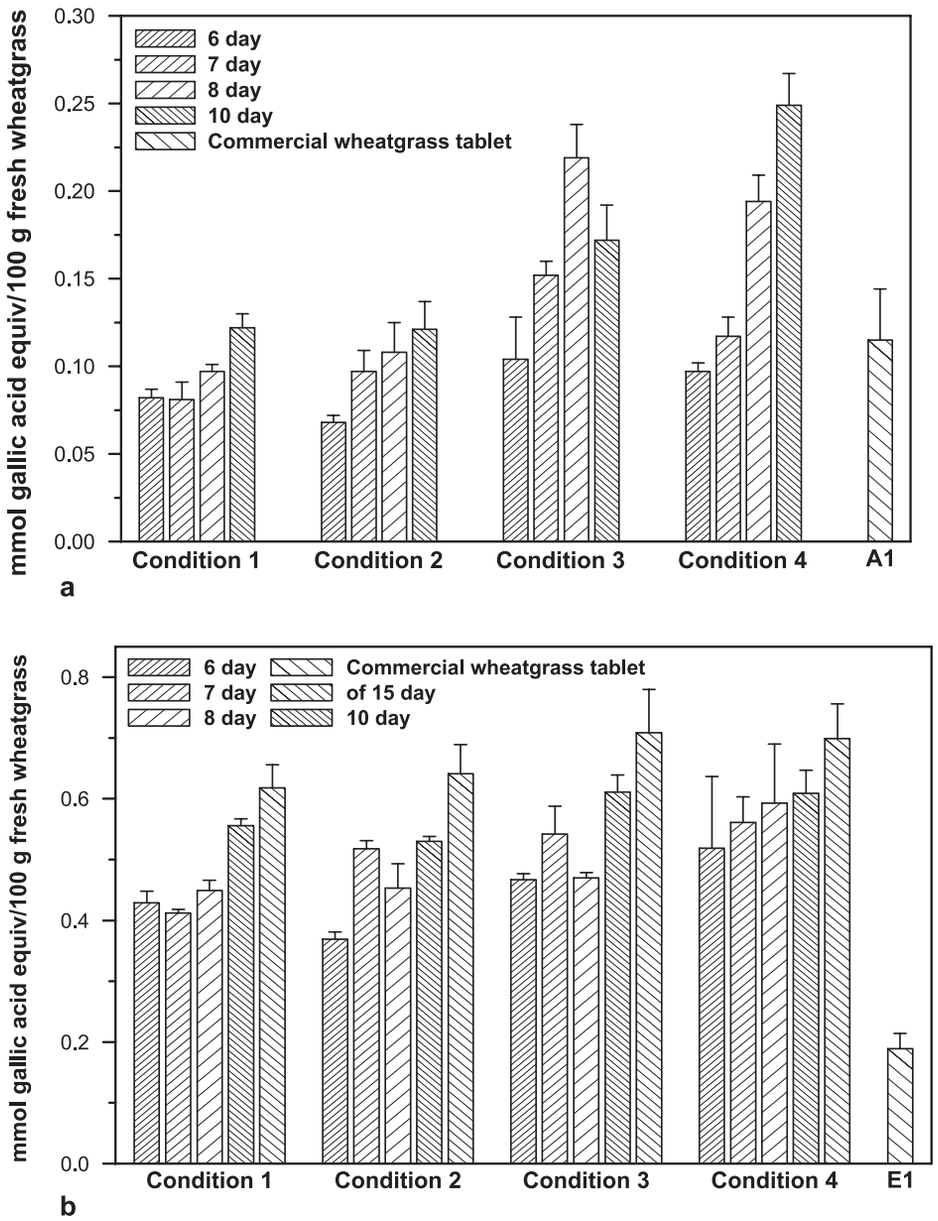

Figure 4. Total phenolic content (TPC) of (a) aqueous extracts and (b) ethanol extracts of wheatgrass grown under condition 1 (tap water), condition 2 (nutrient solution), condition 3 (soil with tap water) and condition 4 (soil with nutrient solution) as a function of its growth period. A1 and E1 represent the aqueous and ethanol extracts of commercial wheatgrass tablet. The concentrations are expressed as mmol gallic acid equivalents/100 g fresh wheatgrass. The values are expressed as mean ± SE of three independent experiments.

The antioxidant activity of wheatgrass extract was observed at various levels of protection such as primary and secondary radical scavenging and inhibition of free radical induced membrane damage. This can possibly be explained on the basis of its chemical content. It has been shown that these extracts contain significant amounts of phenolic compounds including flavonoids. Recently it was shown that during germination, some biologically active compounds were synthesized in the wheat sprouts (Mancinelli *et al.*, 1998; Calzuola *et al.*, 2004). Our results are consistent with Yang *et al.* (2001) who concluded that wheat sprouts reached the maximum antioxidant potential after 7 days of plant growth. Wheatgrass, in general, has been reported to possess therapeutic properties in diseases such as ulcerative colitis and thalassaemia major (Ben-Arye *et al.*, 2002; Marwaha *et al.*, 2004). In addition to this, wheat sprout extracts were found to be antimutagenic in the Ames test (Peryt *et al.*, 1992), capable of inhibiting oxidative DNA damage (Falcioni *et al.*, 2002) and responsible for metabolic deactivation of carcinogens (Lai *et al.*, 1978).

Our studies showed that water extracts of wheatgrass are a good source of antioxidants. Among the conditions, condition 4 showed relatively higher antioxidant activity in all the cases, the extent of variation among all the conditions was not very significant. The antioxidant activity obtained from different assays in the present study showed higher levels at 7 days onwards, which is in conformity with the use of fresh wheatgrass of growth of 7–8 days. The commercially available wheatgrass tablet, which is prepared from wheatgrass powder also showed significant antioxidant potential. In view of its antioxidant potential and the ease with

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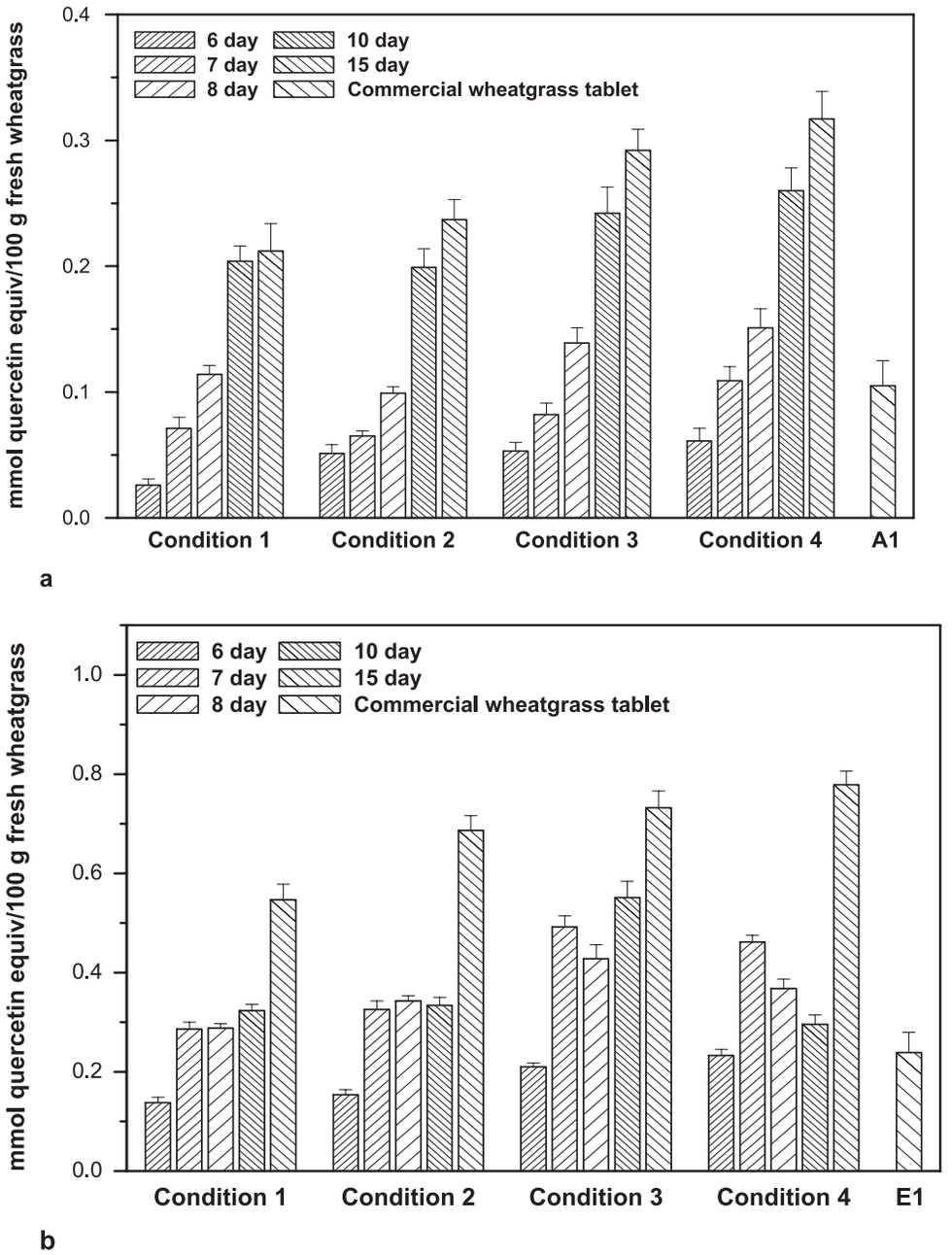

Figure 5. Total flavonoid content (TFC) of (a) aqueous extracts and (b) ethanol extracts of wheatgrass grown under condition 1 (tap water), condition 2 (nutrients solution), condition 3 (soil with tap water) and condition 4 (soil with nutrient solution) as a function of its growth period. A1 and E1 represent the aqueous and ethanol extracts of commercial wheatgrass tablet. The concentrations are expressed as mmol quercetin equivalents/100 g of fresh wheatgrass. The values are expressed as mean ± SE of three independent experiments.

which it can be home-grown under known environmental conditions, wheatgrass extracts can be used as a dietary supplement for antioxidant compounds such as polyphenols and flavonoids. Although wheatgrass from condition 4 showed slightly higher activities as well as a higher elemental content (Kulkarni *et al*., 2006), it appears better to use wheatgrass grown in perforated plastic tops without any additives.

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Table 1. Inhibition of lipid peroxidation in rat liver mitochondria by aqueous and ethanolic extracts of wheatgrass on days 8 and 10 of germination

Condition	Extract	nmol TBARS/mg protein	Inhibition (%)
Damage	–	0.5 ± 0.2	–
Control	–	96.7 ± 1.5	–
Condition 1	8-AT	80.1 ± 5.7	17.2 ± 1.2
	8-ET	71.8 ± 2.6	25.9 ± 2.5
	10-AT	66.9 ± 1.2	30.9 ± 1.1
	10-ET	54.5 ± 9.5	43.9 ± 3.4
Condition 2	8-AN	85.7 ± 2.5	11.4 ± 2.5
	8-EN	66.8 ± 2.4	31.1 ± 2.4
	10-AN	69.6 ± 2.6	28.2 ± 2.6
	10-EN	61.4 ± 1.9	36.7 ± 1.8
Condition 3	8-AST	73.0 ± 1.4	24.6 ± 2.3
	8-EST	64.9 ± 2.8	33.1 ± 2.8
	10-AST	61.0 ± 1.6	37.1 ± 1.5
	10-EST	54.4 ± 1.6	43.9 ± 1.5
Condition 4	8-ASN	64.2 ± 1.4	33.8 ± 1.3
	8-ESN	58.5 ± 5.2	39.7 ± 5.1
	10-ASN	45.8 ± 2.2	52.9 ± 2.2
	10-ESN	54.8 ± 1.1	43.5 ± 1.0
Tablet	A1	42.7 ± 3.4	56.1 ± 3.4
	E1	25.3 ± 1.2	74.2 ± 2.1

Lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) assay and is expressed as nmol malonaldehyde equivalents/mg protein.
Values represented are mean ± SE from triplicate experiments.
Conditions: Condition 1, tap water; Condition 2, tap water + nutrients; Condition 3, tap water + soil; Condition 4, nutrient + soil.
8, 10, plant growth period; A, aqueous extracts; E, ethanol extracts; T, plants grown in tap water; N, nutrient solution; S, soil; A1, aqueous extract of commercial tablet; E1, ethanol extract of commercial tablet.

Table 2. Oxygen radical absorbance capacity (ORAC) values of aqueous and ethanol extracts of wheatgrass on days 8 and 10 of germination

Condition	Extract	ORAC values[a]
Condition 1	8-AT	25.1 ± 2.5
	8-ET	40.3 ± 2.7
	10-AT	32.6 ± 5.3
	10-ET	41.6 ± 1.0
Condition 2	8-AN	25.6 ± 2.8
	8-EN	42.5 ± 0.5
	10-AN	37.4 ± 5.2
	10-EN	42.4 ± 0.7
Condition 3	8-AST	27.3 ± 3.5
	8-EST	42.7 ± 2.2
	10-AST	35.8 ± 1.6
	10-EST	42.3 ± 3.8
Condition 4	8-ASN	33.4 ± 2.0
	8-ESN	45.8 ± 3.7
	10-ASN	39.9 ± 2.6
	10-ESN	48.2 ± 2.2
Tablet	A1	13.82 ± 0.75
	E1	17.01 ± 0.78

[a] ORAC values are expressed as μmol Trolox/g fresh wheatgrass.
Values represent mean ± SE from triplicate experiments
Conditions: Condition 1, tap water; Condition 2, tap water + nutrients; Condition 3, tap water + soil; Condition 4, Nutrient + soil.
8, 10, plant growth period; A, aqueous extracts; E, ethanol extracts; T, plants grown in tap water; N, nutrient solution; S, soil; A1, aqueous extract of commercial tablet; E1, ethanol extract of commercial tablet.

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REFERENCES

Alzoreky N, Nakahara K. 2001. Antioxidant activity of some edible Yemeni plant leaves by ferrylmyoglobin/ABTS^{++}assay. *Food Sci Technol Res* **7**: 141–144.

Aquino R, Morelli S, Lauro MR, Abdo S, Saija A, Tomaino A. 2001. Phenolic constituents and antioxidant activity of an extract of *Anthurium versicolor* leaves. *J Nat Prod* **64**: 1019–1023.

Ben-Arye E, Goldin E, Wengrower D, Stamper A, Kohn R, Berry E. 2002.Wheatgrass juice in the treatment of active distal ulcerative colitis: a random double-blind placebo-controlled trial. *Scand J Gastroenterol* **37**: 444–449.

Calzuola I, Valeria M, Gianfranceschi GL. 2004. Synthesis of antioxidants in wheat sprouts. *J Agric Food Chem* **52**: 5201–5206.

Cao G, Prior RL. 2002. Measurement of oxygen radical absorbance capacity in biological samples. *Meth Enzymol* **299**: 50–62.

Devasagayam TPA. 1986a. Lipid peroxidation in rat uterus. *Biochim Biophys Acta* **876**: 507–514.

Devasagayam TPA. 1986b. Senescence associated decrease of NADPH-induced lipid peroxidation in rat liver microsomes. *FEBS Lett* **205**: 246–250.

Droge W. 2002. Free radicals in physiological control of cell functions. *Physiol Rev* **82**: 47–95.

Falcioni G, Fedeli D, Tiano I *et al.* 2002. Antioxidant activity of wheat sprout extracts *in vitro*: Inhibition of DNA oxidative damage. *J Food Sci* **67**: 2918–2922.

Hanninen O, Rauma AL, Kaartinen K, Nenonen M. 1999. Vegan diet in physiological health promotion. *Acta Physiol Hung* **86**: 171–180.

Harman D. 1981. The aging process. *Proc Natl Acad Sci USA* **78**: 7124–7128.

Hoagland DR, Arnon DI. 1950. The water culture method for growing plants without soil. *Calif Agric Exp Res Station Circ* **347**: 1–39.

Kulkarni SD, Acharya R, Nair AGC, Rajurkar NS, Reddy AVR. 2006. Determination of elemental concentration profiles in tender wheatgrass (*Triticum aestivum L.*) using instrumental neutron activation analysis. *Food Chem* **95**: 699–707.

Lachnicht D, Brevard PB, Wagner TL, DeMars CE. 2002. Dietary oxygen radical absorbance capacity as a predictor of bone mineral density. *Nutr Res* **22**: 1389–1399.

Lai CN. 1979. Chlorophyll: the active factor in wheat sprout extracts inhibiting the metabolic activation of carcinogens *in vitro*. *Nutr Cancer* **1**: 19–21.

Lai CN, Dabney BJ, Shaw CR. 1978. Inhibition of *in vitro* metabolic activation of carcinogens by wheat sprout extracts. *Nutr Cancer* **1**: 27–30.

Lowry OH, Rosebrough NJ, Farr LL, Randall RJ. 1951. Protein measurement with Folin-Phenol reagent. *J Biol Chem* **193**: 265–267.

Luximon-Ramma A, Bahorum T, Soobrattee MA, Aruoma OL. 2002. Antioxidant activities of phenolic, proanthocyandin and flavonoid components in extracts of *Cassia fistula*. *J Agric Food Chem* **50**: 5042–5047.

Mancinelli L, Marsili V, Gianfranceschi G. 1998. Presence of biologically active substances in the wheat bud. *Gastroenterol Int* **11**: 50–51.

Marwaha RK, Bansal D, Kaur S, Trehan A. 2004. Wheatgrass juice reduces the transfusion requirement in patients with thalassemia major: A Pilot study. *Indian Pediatr* **41**: 716–720.

Nocole C, Jean-Luc B, Jean-Pierre C, Pommery J, Jean-Claude W, Emile MG. 1996. Antioxidant properties of hydroxyflavones. *Free Radic Biol Med* **20**: 35–43.

Peryt B, Szymczy KT, Lesca P. 1992. Mechanism of antimutagenicity of wheat sprout extracts. *Mutat Res* **269**: 201–205.

Pulido R, Bravo L, Saura-Calixto F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J Agric Food Chem* **48**: 3396–3402.

Sergio AR, Paiva MD, Russell RM. 1999. β-Carotene and other carotenoids as antioxidants. *J Am Coll Nutr* **18**: 426–433.

Tilak JC, Banerjee M, Mohan H, Devasagayam TPA. 2004. Antioxidant availability of turmeric in relation to its medicinal and culinary uses. *Phytother Res* **18**: 798–804.

Yang F, Basu TK, Ooraikul B. 2001. Studies on germination conditions and antioxidant content of wheatgrass. *Int J Food Sci Nutr* **52**: 319–330.

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