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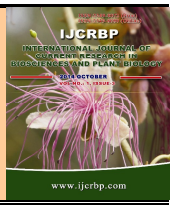

Original Research Article

Antioxidant Activities of *Triticum aestivum* L. Leaves Collected at Different Stages of Growth

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Abstract

Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional system. They are natural sources yielding valuable food or herbal products, which are often used in the treatment of various diseases. One such plant is *Triticum aestivum* L. (wheat grass), the plant known for its nutritive value, has been called one of nature's finest medicines. Wheat grass is fast becoming one of the most widely used supplemented health foods. The objective of the study was to assess the levels of non-enzymatic antioxidants and the activities of enzymic antioxidants in *T. aestivum* leaves collected at three different stages of growth [4, 8 and 12 days after sowing (DAS)]. The results showed that the leaves collected at 4 DAS had higher contents of both enzymic and non-enzymic antioxidants and also exhibited good antioxidant activity. The results of the present study show that the leaves of *T. aestivum* at their early stages of growth are excellent sources of antioxidants, as a primary antioxidant that reacts with free radicals.

Keywords

Enzymic antioxidants
Leaf extracts
Medicinal plant
Non-enzymic antioxidants
Triticum aestivum
Wheat grass

Introduction

Plants and plant products are part of the vegetarian diet and a number of them exhibit medicinal properties. A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties (Aqil et al., 2006). Phytochemicals in fruits, vegetables, spices and traditional herbal medicinal plants have been found to play protective roles against many human chronic diseases including cancer and cardiovascular diseases (CVD). These diseases are associated with oxidative stress caused by excess free radicals and other reactive oxygen species. These compounds have biological properties as antioxidant activity, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of

hormone metabolism (Pan and Ho, 2008). Antioxidant phytochemicals exert their effect by neutralizing the highly reactive radicals.

Among the tens of thousands of phytochemicals found in our diets or traditional medicines, polyphenols and carotenoids stand out as the two most important groups of natural antioxidants (Tsao and Deng, 2004). However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage (Gulcin et al., 2002a).

Increasing evidence indicates that wheat and wheat based food products contain significant levels of natural antioxidants, which may provide health benefits to consumers in addition to general nutrients and energy (Yu et al., 2004). Wheat grass juice is an extract squeezed from the mature sprouts of wheat seeds (*Triticum aestivum* L.). The use of wheat grass juice for therapeutic purposes was developed and popularized by Dr. Ann Wigmore (Aydos et al., 2011). The juice is believed to possess therapeutic qualities only when fresh and this has been attributed to its rich nutritional content, chlorophyll, vitamins (A, C and E), bioflavonoids, minerals (iron, calcium and magnesium) and 17 aminoacids, 8 of which are essential (Walters, 1992).

The objective of the study was formulated to assess the levels and activities of enzymatic and non-enzymatic antioxidants in the extracts of the *T. aestivum* leaves collected at three different stages of growth.

Materials and methods

Plant material

The good quality seeds of *Triticum aestivum* L. (Poaceae) were procured from a local market, Coimbatore. The seeds were sown in earthern pots, in replicates, containing manured garden soil and moistened. The experiments were conducted in the premises of Avinashilingam University, Coimbatore. The leaves of the plantlets were collected fresh at three different stages of growth namely, 4, 8 and 12 days after sowing (DAS). The time point beyond 12 DAS could not be included, because as the age of the plant increased, the fibre content of the leaf increased, resulting in decreased palatability. The leaves were then used for the analysis of enzymic and non-enzymic antioxidants adopting the standard procedures described below.

Determination of antioxidant enzymes

The enzymic antioxidants analyzed were catalase, peroxidase, superoxide dismutase, glutathione S-transferase and polyphenol oxidase. Catalase was analyzed by the method of Luck (1974).

Peroxidase activity

The activity was measured by the change in absorbance at 430 nm caused in 3 ml of 0.05 M pyrogallol solution and 0.5 ml of 1% hydrogen peroxide solution by the addition of the leaf extract in 0.1 M phosphate buffer (Reddy et al., 1995).

Superoxide dismutase activity

The reaction mixture contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of phenazine methosulphate, 0.3 ml of nitroblue tetrazolium, appropriately diluted enzyme preparation in a total volume of 3.0 ml. The reaction was started by the addition of 0.2 ml of NADH. After incubation at 30ºC for 90 sec, the reaction was stopped by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was then shaken with 4.0 ml of n-butanol and the intensity of the chromogen in butanol layer was measured at 560 nm (Kakkar et al., 1984).

Glutathione S- transferase activity

The enzyme activity was determined by monitoring the change in absorbance at 340 nm caused in 2.9 ml reaction mixture containing 0.1 ml of both substrates (1mM GSH and 1 mM CDNB) in 0.1 M phosphate buffer (pH 6.5) at room temperature. The reaction was started by the addition of 0.1 ml of the enzyme extract and the readings were recorded for a minimum of 3 min (Habig et al., 1974).

Polyphenol oxidase activity

The polyphenol oxidases comprised of catechol oxidase and laccase. With 2.5 ml of 0.1 M phosphate buffer (pH 6.5), 0.3 ml of catechol solution (0.01 M), the spectrophotometer was set at 495 nm. Then 0.2 ml of the enzyme extract was added and the absorbance was recorded for every 30 sec up to 5 min (Esterbauer et al., 1977).

Determination of non-enzymic antioxidants

The non-enzymic antioxidants analyzed were ascorbic acid, tocopherol, total phenols, glutathione, flavonoids, chlorophyll, total carotenoids and lycopene.

Ascorbic acid

The leaves were extracted in 4% TCA. The extract was treated with activated charcoal and noted the volumes of clear supernatants obtained. The respective aliquots of the supernatants were made up to 2.0 ml with 4% TCA and the same was followed in preparing standard ascorbate. 0.5 ml of 2% dinitrophenyl hydrazine and 2 drops of 10% thiourea were added and incubated at 37˚C for 3 h. The osazone crystals were dissolved using 2.5 ml of 85% sulphuric acid and after incubation for 30 min, the absorbance was measured at 540 nm (Roe and Kuether, 1943).

Tocopherol

Tocopherols were estimated after partitioning into xylene and its reaction with bipyridyl (Rosenberg, 1992). The leaves were extracted overnight into 0.1 N sulphuric acid. 1.5 ml of the extract was diluted to 3 ml with ethanol and extracted with 1.5 ml of xylene. The tocopherol on the xylene layer was estimated by its reaction with equal volume of bipyridyl solution and the absorbance was measured at 460 nm. 0.33 ml of 0.12% ferric chloride solution was added and exactly after 15 min, the absorbance was read at 520 nm.

Carotenoids and Lycopene

Carotenoids and lycopene were extracted after saponification using alcoholic KOH into petroleum ether and estimated by its absorbance at 450 nm and 503 nm using petroleum ether as blank (Zakaria et al., 1979).

Total phenols

Total phenols were estimated by their reaction with Folin-Ciocalteau reagent in the presence of 20% sodium carbonate (Malick and Singh, 1980) after extracting into 80% ethanol.

Reduced glutathione

The leaf extracts were prepared in 5% TCA to prevent aerial oxidation of reduced glutathione. 1 ml of a suitably diluted aliquot was mixed with 2 ml of 0.6mM dithio bis-2-nitrobenzoic acid and the resultant yellow colour formed was measured at 412 nm (Moron et al., 1979).

Flavonoids

The extraction was carried out in two steps in methanol: water, first in 9:1 ratio and then in 1:1 ratio. The lipid soluble contaminants were removed by extracting with hexane or chloroform after concentrating the extract. Colour was developed in a dried aliquot by the addition of 4 ml of 1% vanillin in 70% H_2SO_4, for 15 min. in a boiling water bath and read at 360 nm against catechin standard (Cameron et al., 1943).

Chlorophyll

The leaves were extracted with 80% acetone and the absorption was read at 645 nm and 663 nm in a spectrophotometer. Using the absorption co-efficients, the amount of chlorophyll was calculated (Witham et al., 1971).

Statistical analysis

All values are expressed as Mean ± SD of triplicates. The significance of differences between the mean of the three different stages of growth of *T. aestivum* have been calculated by Anova followed by Fischer's LSD and *p* values less than 0.001 were considered significant.

Results and discussion

Enzymic antioxidants

Table 1 shows the activities of enzymic antioxidants in the leaves of *T. aestivum* at three different stages of growth. The levels and activities of enzymic antioxidants were found to be higher in 4^{th} day plants than the 8^{th} and 12^{th} day plants. The catalase activity (U/g tissue) was 942.50 ± 3.50 in 4 DAS which was higher than that of the results recorded for 8 DAS (681.00 ± 1.41 U/g tissue) and for 12 DAS (568.00 ± 4.24 U/g tissue) and the difference is significant at $p<0.001$ level (Table 1).

Recently, the beneficial effects of the plant extracts and natural products as antioxidant agents, based on reactions with free radicals and other reactive species, has become the focus of interest. In addition, it has been demonstrated that herbal plants

play a protective role in the pathogenesis of various diseases (Halliwell, 1990). The activities all the enzymic antioxidants in the leaves at all three time points was markedly higher in the fourth day plants followed by the eighth and twelfth day plants. Free radicals are produced by a variety of cellular mechanisms and have been shown to play an important role in a wide variety of pathophysiological states. Free radicals can adversely alter lipids, proteins and DNA and have been implicated in aging and a number of human diseases. Lipids are highly prone to free radical damage resulting in lipid peroxidation that can lead to adverse alterations. Free radical damage to protein can result in loss of enzyme activity. Damage caused to DNA, can result in mutagenesis and carcinogenesis (Devasagayam et al., 2004). It is well known that endogenous antioxidant enzymes are responsible for preventing and neutralizing the free radical-induced oxidative damage.

Table 1. Activities of enzymic antioxidants in the leaves of *Triticum aestivum* L. collected at three different stages of growth.

Activities of enzymic antioxidants	Leaves of *T. aestivum* collected at		
	4 DAS	8 DAS	12 DAS
Catalase (U/g tissue)	942.50 ± 3.50	681.00 ± 1.41^{a}	568.00 ± 4.24^{a,b}
Peroxidase (U/g tissue)	20.10 ± 1.60	14.80 ± 0.35^{a}	10.03 ± 0.24^{a,b}
Superoxide dismutase (U/g tissue)	21.72 ± 1.30	18.32 ± 0.28^{a}	18.51 ± 0.53^{a,ns}
Glutathione-S-transferase (U/g tissue)	4.32 ± 0.67	4.30 ± 0.70^{ns}	3.36 ± 0.62^{ns}
Polyphenol oxidase catechol oxidase ($U\times10^{-1}$/g tissue)	2.80 ± 0.12	2.68 ± 0.04^{ns}	2.37 ± 0.09^{a,b}
Laccase activity ($U\times10^{-1}$/g tissue)	2.55 ± 0.18	2.46 ± 0.06^{ns}	2.26 ± 0.06^{ns,ns}

(DAS-Days after sowing; the values are Mean ± SD of triplicates; 'a'-statistically significant (p<0.001) compared to 4 DAS; 'b'-statistically significant (p<0.001) compared to 8 DAS; Catalase 1 Unit = amount of enzyme required to decrease the absorbance at 240 nm by 0.05 Units; Peroxidase 1 Unit = change in absorbance at 430 nm/min; Superoxide dismutase 1 Unit of enzyme is the amount that causes 50% inhibition in NBT reduction; Glutathione-S-transferase 1 Unit = n moles of CDNB conjugated/min; 1 Unit of catechol oxidase/laccase = the amount of enzyme that converts 1µmole of dihydrophenol to 1 mole of quinone/min.).

The antioxidant enzymes, such as catalase, SOD, GPx, GR and GST, constitute a major supportive team of defense against free radicals (Sung et al., 2000). These enzymes work in tandem to scavenge ROS. The SOD catalyzes the dismutation of superoxide anion to H_2O_2. The latter can be converted to the more harmful hydroxyl radicals (·OH). Subsequently, H_2O_2 is reduced to H_2O and O_2 by peroxidases [e.g., GPx) or CAT. GPx scavenges H_2O_2 in the presence of reduced glutathione [GSH) to form H_2O and oxidized glutathione (Michiels et al., 1994). The protective effect of antioxidant rich diets in diseases involving oxidative damage has been reported (Bordoni et al., 2002). There are epidemiological evidences correlating higher intake of components, foods with antioxidant abilities to lower incidence of various human morbidities and mortalities. Natural products from dietary components such as Indian spices and medicinal plants are known to possess antioxidant activity.

Non-enzymic antioxidants

There has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical induced tissue injury (Engelhart et al., 2002). Numerous plant products have been shown to have the antioxidant activity, and the antioxidant vitamins, flavonoids and polyphenolic compounds of the plant origin have been extensively reported as scavengers of free radicals and inhibitors of lipid peroxidation (Devasagayam et al., 2004). The levels of non-enzymic antioxidants observed in the present study at all the three time points of growth were higher on 4th day followed by 8th and 12th day plants, except for chlorophyll, which is present in higher concentrations in the 12th day plant (Table 2). In addition to the constitutive cell antioxidant defense system, there exist various small molecules, vitamins (ascorbic acid and α–tocopherol), carotenoids, flavonoids and simpler phenolic

compounds, exhibiting potent antioxidant properties, are excellent reactive oxygen species scavengers (Halliwell, 1999). Literature data suggest that antioxidants often work by the combination of several mechanisms such as scavenging of lipid alkoxyl and peroxyl radicals through hydrogen donation, regeneration of α-tocopherol by α-tocopheroxyl radical reduction and chelation of transition metal ions (Rice-Evans et al., 1996).

Table 2. Levels of non-enzymic antioxidants in the leaves of *Triticum aestivum* L. collected at three different stages of growth.

Levels of non-enzymic antioxidants	Leaves of *T. aestivum* collected at		
	4 DAS	8 DAS	12 DAS
Ascorbic acid	2.17 ± 0.07	1.15 ± 0.08^{a}	0.83 ± 0.04^{a,b}
Tocopherol	4.44 ± 0.07	3.70 ± 0.14 ^{a}	2.94 ± 0.12 ^{a,b}
Reduced glutathione	24.40 ± 0.98	22.97 ± 0.96^{ns}	21.76 ± 0.24^{a,ns}
Chlorophyll	0.70 ± 0.02	0.84 ± 0.03 ^{a}	1.07 ± 0.08 ^{a,b}
Total phenol	0.27 ± 0.01	0.22 ± 0.02 ^{a}	0.17 ± 0.007 ^{a,b}
Flavonoid	3.17 ± 0.10	2.89 ± 0.05 ^{a}	2.87 ± 0.10 ^{a,ns}
Total carotenoid	17.92 ± 1.12	17.77 ± 0.88 ^{ns}	12.89 ± 0.38^{a,b}
Lycopene	1.59 ± 0.72	1.01 ± 0.15 ^{ns}	0.72 ± 0.06 ^{ns,ns}

DAS-Days after sowing; the values are Mean ± SD of triplicates; 'a'-statistically significant; (p<0.001) compared to 4 DAS; 'b'-statistically significant (p<0.001) compared to 8 DAS; The Units for the above mentioned parameters are mg/g leaf extract except for reduced glutathione, which is nmoles/g leaf.

More recently, the repair of oxidative damage to proteins or lipoproteins has been shown to also contribute to the flavonoid antioxidant activity (Filipe et al., 2002). GSH is the first line of defense against prooxidant status (Ahmed et al., 2000). GSH is also the most important biomolecule protecting against chemically induced cytotoxicity, by participating in the elimination of reactive intermediates by conjugation and hydroperoxide reduction, or of free radicals by direct quenching (Liu et al., 2002).

Giovannuci et al. (1995) reported that lycopene intake in particular is associated with a decreased incidence of prostate and colorectal cancer. Studies have indicated that the antimutagenicity of certain vegetable extracts is due to the presence of chlorophyll (Ong et al., 1986). Thus all these non-enzymic antioxidants in the present study were found to be higher in the leaves of *T. aestivum* in the 4th day plant, which makes it a potential source of antioxidants and an effective free radical scavenger.

At present, a cocktail of antioxidants is the most reasonable solution for the prevention or treatment of oxidative stress. Reports show that most of the antitumor agents have been shown to act through the inhibition of oxidative stress. The results of the present study show that the leaves of *T. aestivum* at their early stages of growth are excellent sources of antioxidants, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring to the human body. Thus *T. aestivum* leaves can be included in the diet as such or can be consumed orally raw and fresh to combat disorders caused by free radicals.

References

Ahmed, R.S., Seth, V., Banerjee, B.D., 2000. Influence of dietary ginger (*Zingiber officinale* Rosc.) on antioxidant defense system in rat. Comparison with ascorbic acid. Indian J. Exp. Biol. 38, 604-606.

Aqil, F., Ahmed, I., Mehmood, Z., 2006. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk. J. Biol. 30, 177-183.

Aydos, O.S., Ayci, A., Ozkan, T., Karadag, A., Gurleyik, E., Altinok, B., 2011. Antiproliferative, apoptotic and antioxidant activities of wheat grass (*Triticum aestivum* L.) extract on CML (K562) cell line. Turk. J. Med. Sci. 41, 657-663.

Bordoni, A., Hrelia, S., Angeloni, C., Giordano, E., Guarnieri, C., Caldarera, C.M., Biagi, P.L.,

2002. Green tea protection of hypoxia/reoxygenation injury in cultured cardiac cells. J. Nutr. Biochem. 13, 103-111.

Cameron, G.R., Milton, R.F., Allan, J.W., 1943. Measurement of flavonoids in plant samples. Lancet p.179.

Devasagayam, T.P., Tilak, J.C., Boloor, K.K., Sane, K.S., Ghaskadbi, S.S., Lele, R.D., 2004. Free radicals and antioxidants in human health: current status and future prospects. J. Assoc. Physicians India 52, 794-804.

Engelhart, M.J., Geerlings, M.I., Ruitenberg, A., Van Swieten, J.C., Hofman, A., Witteman, J.C., Breteler, M.M., 2002. Dietary intake of antioxidants and risk of alzheimers disease. J. American Med. Assoc. 287, 3223-3229.

Esterbauer, H., Schwarzl, E., Hayn, M., 1977. A rapid assay for catechol oxidase and laccase using 2-nitro-5-thiobenzoic acid. Anal. Biochem. 77, 486-494.

Filipe, P., Morliere, P., Patterson, L.K., Hug, G.L., Maziere, J.C., Maziere, C., Freitas, J.P., Fernandes, A., Santus, R., 2002. Mechanisms of flavonoid repair reactions with amino acid radicals in models of biological systems: a pulse radiolysis study in micelles and human serum albumin. Biochim. Biophys. Acta. 1572, 150-162.

Giovannucci, E., Ascheiro, A., Rimm, E.B., Stampfer, M.J., Colditz, G.A., Willett, W.C., 1995. Intake of carotenoids and retinol in relation to risk of prostate cancer. J. Natl. Cancer Inst. 87, 1767-1776.

Gulcin, I., Buyukokuroglu, Z., Oktay, M.F., Kufrevioglu, O.I., 2002a. On the *in vitro* antioxidant properties of melatonin. J. Pineal Res. 33, 167-171.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139.

Halliwell, B., 1990. How to characterize a biological antioxidant. Free Radic. Res. Commun. 9, 1-32.

Halliwell, B., 1999. Antioxidant defense mechanisms: From the beginning to the end. Free Radic. Res. 31, 261-272.

Kakkar, P., Das, B., Viswanathan, P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophys. 21, 131-132.

Liu, C.L., Wang, J.M., Chu, C.Y., Cheng, M.Y., Tseng, T.H., 2002. *In vivo* protective effect of protocatechuic acid on tert-butyl hydroperoxide-induced hepatotoxicity. Food Chem. Toxicol. 40, 635-641.

Luck, H., 1974, In: Methods in enzymatic analysis 2Ed. Bergmayer, Academic press, New York. 885.

Malick, C.P., Singh, M.B., 1980. In: Plant Enzymology and Plant Histoenzymology, Kalyani Publishers, New Delhi. 286p.

Michiels, C., Raes, M., Toussaint, O., Remacle, J., 1994. Importance of Se-glutathione, peroxidase, catalase, and CU/Zn-SOD for cell survival against oxidative stress. J. Free Radic. Biol. Med. 17, 235-248.

Moron, M.S., Depierre, J.W., Mannervik, B., 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim. Biophys. Acta. 582, 67-78.

Ong, T.M., Whong, W.Z., Stewart, J.D., Brockman, H.E., 1986, Chlorophyllin: A potent antimutagen against environmental and dietary complex mixtures. Mut. Res. 173, 111-115.

Pan, M.H., Ho, C.T., 2008. Chemopreventive effects of natural dietary compounds on cancer development. Chem. Soc. Rev. 37, 2558-2574.

Reddy, K.P., Subhani, S.M., Khan, P.A., Kumar, K.B., 1995. Effect of light and benzyladenine on dark treated graving rice (*Oryza sativa*) leaves II changes in peroxidase activity. Plant Cell Physiol. 26, 987.

Rice-Evans, C.A., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20, 933-956.

Roe, J.H., Kuether, C.A., 1943. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid**.** J. Biol. Chem. 147, 399-407.

Rosenberg, H.R., 1992. Chemistry and physiology of the vitamins. Interscience Publishers Inc, New York. pp. 452-453.

Sung, H., Nah, J., Chun, S., Park, H., Yang, S.E., Min, W.K., 2000. *In vivo* antioxidant effect of green tea. Eur. J. Clin. Nutr. 54, 527-529.

Tsao, R., Deng, Z., 2004. Separation procedures for naturally occurring antioxidant phytochemicals. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 812, 85-99.

Walters, R., 1992. The Alternative Cancer Therapy Book. Avery Publishing Group. pp. 299-308.

Witham, F.H., Blaydes, B.F., Devlin, R.M., 1971. Experiments in Plant Physiology. Van Nostrand, New York. 245p.

Yu, L., Haley, S., Perret, J., Harris, M., 2004. Comparison of wheat flours grown at different locations for their antioxidant properties. Food Chem. 86, 11-16.

Zakaria, M., Simpson, K., Brown, P.R., Krotulovic, A., 1979, Use of reversed phase HPLC analysis for the determination of provitamin A, carotene in tomatoes. J. Chromatogr. 176, 109-117.