



## TIME DEPENDENT ANTIOXIDANT ACTIVITY IN WHEATGRASS (*TRITICUM AESTIVUM*)

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### ABSTRACT

Wheatgrass is known to exert strong antioxidant capabilities. An experiment was conducted to ascertain the antioxidant activity in time dependent manner in order to find out the best suitable time for the harvesting of the grass. Wheatgrass was grown in the laboratory pots and harvested each day up to the day 15 after first germination. The DPPH activity was studied in a comprehensive manner. Results showed that the DPPH scavenging activity increased with time up to 9 days and gradually decreased afterwards. Highest antioxidant activity was found as 98.8% as compared to ascorbic acid (control) on 9<sup>th</sup> day. There was a positive correlation between total flavonoids content and antioxidant activity.

**KEYWORDS:** Wheatgrass, *Triticum aestivum*, green blood, antioxidant activity, flavonoids, DPPH.

### INTRODUCTION

Wheatgrass juice (also known as “green blood” and “plant super food”) is considered as one of the best natural remedies for good health. In India, wheatgrass has been used as an herbal medicine since ages. Wheatgrass juice is a powerful concentrated liquid nutrient. It contains the full spectrum of vitamins A, B and C, as well as important minerals, i.e., calcium, phosphorus, magnesium, sodium and potassium.<sup>[1]</sup> Wheatgrass is a complete source of protein, supplying all of the essential amino acids, and more. It has about 20% of total calories coming from protein. This protein is in the form of poly peptides, simpler and shorter chains of amino acids that the body uses more efficiently in the blood stream and tissues. In addition to flooding the body with therapeutic dosages of vitamins and minerals, it supplies good doses of antioxidants in the form of phytonutrients which act as a powerful detoxifier, especially of the liver and blood.<sup>[2]</sup>

Wheatgrass juice is prepared from the readily cut leaves by crushing them in a small amount of water. Usually, the plant of *Triticum aestivum* remain in grass stage for initial 15 days, during which the leaves are soft, and the juice in this stage of plant is preferred.<sup>[3]</sup> However, during these 15 days, on which day, the juice is most effective in terms of antioxidant activity has not been studied. The present paper deals with the antioxidant activities in growing wheatgrass on a daily basis, in order to identify the best time when juice will supply the most antioxidants to the body for therapeutic uses. Wheatgrass and other plant-based foods contain natural phytochemicals such as flavonoids that are

antioxidants<sup>[4]</sup>, the levels of flavonoids were also measured on a daily basis in order to correlate with the antioxidant activity.

### MATERIALS AND METHODS

#### *Cultivation of wheatgrass*

The wheatgrass was cultivated from the seeds of *Triticum aestivum* purchased from local market. The seeds were soaked in water for 24 hours and were sowed in household pots in multiples. The day 1 was counted when the germinated seeds achieved a height of 1 inch. Each day, the grass was harvested and 50 g of fresh weight was used to prepare 100 ml wheatgrass juice by crushing the leaves in water using a mixer grinder and filtered. The juice was clarified by addition of 1 g activated charcoal before starting the experiment.

#### *Determination of flavonoids*

The total flavonoid content was estimated by the method developed by Jia et al.<sup>[5]</sup> Briefly, 0.3 mL of 5% (w/v) NaNO<sub>2</sub> was added to every 4 mL of wheatgrass juice solution. After 5 min of incubation, 0.3 mL of 10% (w/v) AlCl<sub>3</sub> was added. After 6 min, 2 mL of 1 M NaOH was added. The total volume was made up to 10 mL with distilled water. After vortex shaking for 1 min, the absorbance was noted at 510 nm. The total flavonoid content was reported in terms of μM of quercetin equivalents ml<sup>-1</sup> of extract.

#### *Antioxidant (DPPH scavenging) activity*

The antioxidant activity was measured as the ability of plant extract to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical.<sup>[6]</sup> For the assay, 2.366 mg of 1,1-

diphenyl-2-picrylhydrazyl radical (Sigma, USA) was dissolved in 100 ml of absolute ethanol to obtain 60  $\mu\text{M}$  DPPH. The scavenging effect of the wheatgrass as well as ascorbic acid (4  $\text{mg ml}^{-1}$ ) corresponding to quenching intensity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was carried out. The sample solution of wheatgrass (500  $\mu\text{l}$ ) was mixed with the same volume of DPPH solution and allowed to stand for 1 h at room temperature in dark or until stable absorption values were obtained. The absorbance was then measured at 517 nm using a spectrophotometer (EI, India). The percentage scavenging effect was determined by comparing the absorbance of the solution containing the test sample to that of negative control solution (ethanol). The absorbance was taken three times, each after 30 min. The result is the mean of three measured values for each sample. The ascorbic acid in the same concentration was used as positive control.

## RESULTS

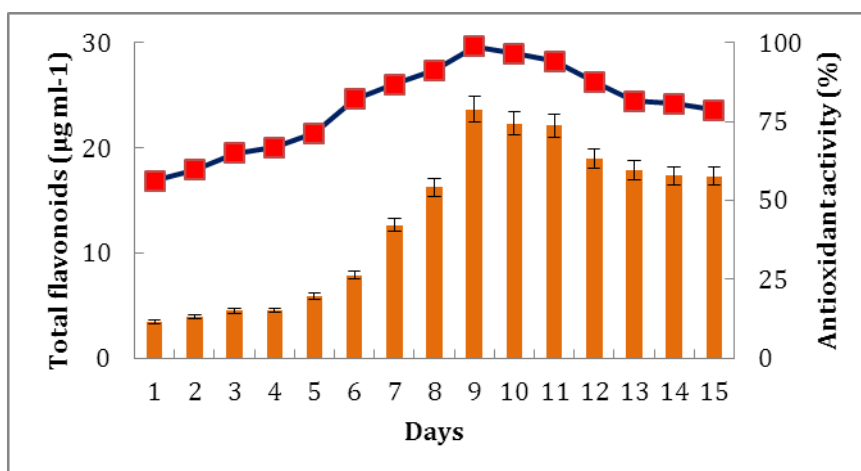
The wheatgrass juice was prepared daily using a standard ration of wheatgrass and water. The juice was clarified and filtered in order to avoid any interference during the spectrophotometric observations for antioxidant activity. Table 1 shows the total flavonoids content and the antioxidant activity in wheatgrass juice from day 1 to day 15. The antioxidant activity was compared with that of ascorbic acid in 4  $\text{mg ml}^{-1}$  concentrations. The data were obtained from the three replicates and presented as mean  $\pm$  standard deviation. The data show that the flavonoids content as well as the antioxidant activity slowly increased for the first week, rapidly afterwards upto day 9 and decreases slowly upto day 15. on day 9, the total flavonoids content was  $23.67 \pm 1.76$   $\mu\text{M QE ml}^{-1}$  and the antioxidant activity was  $98.8 \pm 4.16$  %.

**Table 1: Day wise changes in total flavonoids content and antioxidant activity in wheatgrass juice. data are presented as mean  $\pm$  SD (n=3).**

| Days | Falvonoid content ( $\mu\text{M GE ml}^{-1}$ ) | Antioxidant activity (%) |
|------|--|--------------------------|
| 1    | $3.45 \pm 0.23$                                | $56.2 \pm 2.12$          |
| 2    | $3.98 \pm 0.21$                                | $59.8 \pm 2.01$          |
| 3    | $4.51 \pm 0.33$                                | $65.0 \pm 2.67$          |
| 4    | $4.56 \pm 0.54$                                | $66.8 \pm 3.21$          |
| 5    | $5.87 \pm 0.67$                                | $71.4 \pm 2.56$          |
| 6    | $7.89 \pm 0.45$                                | $82.2 \pm 2.78$          |
| 7    | $12.67 \pm 1.23$                               | $86.8 \pm 4.67$          |
| 8    | $16.24 \pm 2.21$                               | $91.2 \pm 2.51$          |
| 9    | $23.67 \pm 1.76$                               | $98.8 \pm 4.16$          |
| 10   | $22.34 \pm 2.12$                               | $96.6 \pm 3.32$          |
| 11   | $22.11 \pm 1.45$                               | $94.1 \pm 3.61$          |
| 12   | $18.98 \pm 2.11$                               | $87.7 \pm 5.43$          |
| 13   | $17.91 \pm 1.34$                               | $81.6 \pm 2.67$          |
| 14   | $17.34 \pm 2.17$                               | $80.8 \pm 2.11$          |
| 15   | $17.29 \pm 1.56$                               | $78.7 \pm 2.42$          |

Fig 1 compares the flavonoids content with the antioxidant activity. It is very clear that as the flavonoids content increased, there is a increase in the antioxidant

activity too, clearly suggesting that the antioxidant activity in wheatgrass juice is highly dependent on its flavonoids content.



**Fig 1: Dependence of antioxidant activity on total flavonoids content in wheatgrass juice.**

## DISCUSSION

Since ages, herbal medications have been used for relieving the symptoms of many of the diseases as well as for healthy living. In the light of the modern sciences, herbal medications have come out more strongly and make an important contribution to the field of medicine.<sup>[7]</sup> Many of the plants have been investigated for their medicinal properties and especially the antioxidant properties. Natural antioxidants extracted in their crude form contain many chemical constituents, which are very effective to prevent the destructive processes caused by oxidative stress.<sup>[8]</sup>

The use of wheatgrass juice has been proposed as an effective therapeutic agent for various ailments, especially caused by oxidative damage, i.e., for hepatic damages.<sup>[9]</sup> The most active dietary antioxidants belong to the family of phenolic and polyphenolic compounds, also present in wheatgrass juice. Phenolic antioxidants are believed to quench oxygen-derived free radicals as well as the substrate-derived free radicals by donating a hydrogen atom or an electron to the free radical. These phenolic group phytochemicals including the flavonoids, are known to exert strong antioxidant activities *in vitro* as well as *in vivo*.<sup>[10]</sup>

Wheat grass has been used by many of the ayurvedic practitioners since ages. They have been used in many of the ailments such as anemia, liver disorders, digestion problems and cancer related diseases. They are mostly rich in antioxidant properties. However, so far very little work has been done on the activity of these plant extracts. Although in some studies, the levels of flavonoids have been shown higher in methanol and chloroform extracts of the wheatgrass<sup>[11]</sup>, the flavonoids content in aqueous solution is more relevant as the wheatgrass juice is often consumed in this form. This gives a more reliable data for therapeutic use.

The study concluded that the wheatgrass should be harvested after one week of growth, for preparation of juice to get more useful effects. From one week onwards, although there is a change in total flavonoids content as well as antioxidant activity, in each case at least 80% of the antioxidant activity is available.

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