Exploring the phytochemical profile of green grasses with special reference to antioxidant properties

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ABSTRACT

The present study was conducted to evaluate the phytochemical profile of green grasses including barley and wheat. Green grasses were procured from Ayub Agricultural Research Institute, Faisalabad, Pakistan. All the phytochemical parameters were determined through standard methods with slight modifications. The explored parameters include proximate analysis, mineral profile, vitamin profile, and especially antioxidant profile (total phenolic contents (TPCs), free radical scavenging activity, flavonoids, and phenolic acids). The results portrayed that there was a significant difference between the proximate parameters, vitamin, and minerals profile of both barley and wheatgrass. The antioxidant profile – antioxidant activity (283.5%) and free radical scavenging activity (99.29%) – of barley grass was much higher than wheatgrass. The TPCs (203.52 µmol of GAE/g) and flavonoids (161.12 µmol/L) were much higher in wheatgrass as compared to barley grass. Barley grass showed maximum contents of phenolic acids including benzoic acid (73.84 µg/g), caffeic acid (86.61 µg/g), gallic acid (6.33 µg/g), syringic acid (170.46 µg/g), p-hydroxybenzoic acid (42.52 µg/g), and Ferulic acid (1560.3 µg/g). Conclusively, barley grass showed higher antioxidant potential.

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Introduction

The idea of using the grasses as food with unambiguous benefits of the health is not new. Green grasses may include the grasses of wheat, barley, rye, lemon, oat, which are considered the most valuable. Cereal grasses have played a vital role in the human diet. Most of the animals and humans use green grasses as foodstuff, but in crucial cases, it can be consumed in the form of juice. Cereal grasses are an alternative source of energy. These are promoted as a source of antioxidant, the most important being O-glycosyl isovitexin, superoxide dismutase, catalase, carotenoids, and other bioactive compounds. All the grasses are nutritious including wheatgrass (Triticum aestivum) which comes from the Poaceae (Graminaceae) family. Wheatgrass is one of the cereal grasses mostly used as a functional drink, has quickly become “the new age espresso”, and mostly used in the form of smoothies, juices, salads, tablets, and powders.

Barley grass juice is also nutritionally very similar to wheatgrass juice. There are no main differences between the two foods though, with barley grass (Hordeum vulgare L.) containing little more calcium, potassium, chlorophyll, and enzymes than wheatgrass. Ultimately, in a choice
between the two of these often comes down to personal taste, with barley grass juice slightly bitter as
compared to the wheatgrass juice and little difficult to consume, but both are highly nutritious. These can be consumed orally without any toxic side effect.\textsuperscript{[5]} Nutrition support may require being adapted over time to sustain metabolic strength and endorse recover.\textsuperscript{[6]}

Wheatgrass juice contains minerals and trace elements including calcium, iodine, magnesium, selenium, zinc, chromium, antioxidants like vitamin C, vitamin E, β-carotene, vitamin B\textsubscript{1}, antianemic factors like vitamin B\textsubscript{12}, iron, folic acid, pyridoxine, abscess acid, ferulic acid, and vanilic acid – the concentrations of which increase with the germination period. Similarly, barley grass (\textit{H. vulgare} L.) is much more important and the early production of cereal crop. Barley has been cultivated since the Stone Age, making it one of the oldest domesticated plants in history. Barley grass consists of juvenile green leaves of the barley plant. Many claims have been made regarding the health benefits of barley grass supplements.

The young parts of the leaves of barley and wheat have the power to provide a high amount of the nutrients from the soil. Maximum amount of the nutrients is found in the leaves of barley when the aboveground plant height is about 12–14 inches; these nutrients are an integral part of human development. Leaves of barley are tremendously fibrous. As fiber’s outer portion is rich in nutrients, the outer face must be wrecked down for the body’s enzymes to perform their works. Therefore, young leaves of barley cannot be digested and absorbed easily or even when taken in unrefined form.\textsuperscript{[5]} The current study was designed to explore the phytochemical profile of wheat and barley green grasses. It includes proximate analysis, mineral profile, vitamin profile, and antioxidant profile of both grasses.

**Materials and methods**

**Procurement of raw materials**

Commercially available barley and wheat grasses were procured from Wheat Research Institute, Ayub Agriculture Research Institute, Faisalabad, Pakistan. All reagent and chemicals were procured from Sigma-Aldrich.

**Characterization of cereal grasses**

Cereal grasses were characterized and analyzed for various quality attributes including proximate analysis, mineral composition, and vitamin determination.

**Proximate composition and mineral profile**

Proximate analysis of cereal grasses was carried out for moisture content, crude protein, crude fat, crude fiber, ash, and nitrogen-free extract (NFE) according to the standard Methods as described in AACC.\textsuperscript{[7]} The mineral content of both grasses was quantified by the procedure of AOAC.\textsuperscript{[6]} Mineral concentration and quantification were done by subjecting the diluted wet digested samples through Atomic Absorption Spectrophotometer (Varian, AA-240, Victoria, Australia). Calcium (Ca), zinc (Zn), and iron (Fe) were determined by Atomic Absorption Spectrophotometer (Varian AA240, Australia) while potassium (K), sodium (Na), and phosphorus (P) were assessed by Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge).

**Vitamins' determination**

Wheat and barley grasses were analyzed for vitamins B6, B9, and B12. First, a standard was prepared. For each B vitamin, the standard preparation was carried out separately according to the method of Aslam \textit{et al}.\textsuperscript{[9]} and vitamin C was determined according to the respective method of AOAC.\textsuperscript{[10]} After preparation of standards, buffer was prepared by dissolving 1.08 g of hexane sulfonic acid sodium salt
and 1.36 g of potassium dihydrogen phosphate in 940 mL of HPLC water. Then, 5 mL of triethylamine was added to it, and the pH was adjusted to 3.0 with orthophosphoric acid. For the preparation of mobile phase, buffer and methanol were mixed with a ratio of 96:4 and filtered through 0.45-μm membrane filter and degassed using helium gas. Then, the extraction solution was prepared by mixing 50 mL of acetonitrile with 10 mL of glacial acetic acid and its volume was made up to 1000 mL with double-distilled water.

**Sample preparation**

A sample weighing 10 g was homogenized and transferred into a conical flask. Then, 25 mL of extraction solution was added to the sample. The sample was mixed with the extraction solution in a shaking water bath at 70°C for 40 min. The sample solution is allowed to cool down and then filtered. Finally, the volume was made up to a volume of 50 mL.\[^9\]

**Antioxidant activity**

Antioxidant activity (AA) of wheat and barley grasses was estimated based on coupled oxidations of β-carotene and linoleic acid through a spectrophotometer at 470 nm.\[^11\] The total phenolic content (TPC) was measured by using the FolinCiocalteu method following the protocol of Singleton *et al.*\[^12\] For the intention, 50-μL cereal grasses' extract was added to 250 μL of FolinCiocalteu reagent with 750 μL of 20% sodium carbonate solution and made the volume upto 5 mL with distilled water. After 2 h, absorbance was recorded at 765 nm with UV/Visible Spectrophotometer (CECIL-CE7200) against control. The TPC was estimated as gallic acid equivalent (GAE) (mg gallic acid/g).

The free radical scavenging activity, i.e., diphenyl picryl hydrazyl (DPPH) of cereal grasses was measured using the protocol of Muller *et al.*\[^13\] Purposely, 1 mL of DPPH was added to 4 mL of sample, followed by incubation for 30 min at room temperature. The absorbance was measured at 520 nm using UV/Visible Spectrophotometer.

The total flavonoids were estimated using the method of Ordon-ez *et al.*\[^14\] Half milliliter of 2% AlCl₃ ethanolic solution was added to 0.5 mL of the extracts. After 1 h at room temperature, the absorbance was estimated at 420 nm. The development of yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 1 mg/mL. The total flavonoid content was calculated as quercetin equivalent (QE) (mg/g) using the equation obtained from the calibration curve.

Major phenolic acids of wheatgrass and barley grass were determined by the method of Irmak *et al.*\[^15\] Phenolic acids, namely, ferulic, benzoic, caffeic, gallic, p-hydroxybenzoic, and syringic acid, were evaluated by HPLC (PerkinElmer series 200) equipped with UV/visible detector. The analytical column used was C18 with reverse phase.

**Results and discussions**

The chemical composition of cereal grasses has been studied by several workers. Since the composition is prejudiced by genetic and environmental factors, the compositional analysis is a crucial component for the development and acceptance of functional foods.

**Proximate analysis**

Compositional analysis is a crucial component for the development and acceptance of functional foods. Accordingly, hedonic response and processing determine the rating of the food. In this context, wheat and barley grasses were analyzed for proximate profiling including moisture, crude protein, crude fat, crude fiber, soluble dietary fiber (SDF), insoluble dietary fiber (IDF), ash, NFE, and mean values have been presented in Table 1.
| Cereal Grases  | Moisture  | Ash      | Fat      | Protein   | NFE       | Crude Fiber | SDF      | IDF      |
|----------------|-----------|----------|----------|-----------|-----------|-------------|----------|----------|
| Barley grass   | 11.887 ± 0.03  | 7.433 ± 0.01  | 2.563 ± 0.06  | 20.213 ± 0.04  | 11.226 ± 0.20  | 23.550 ± 0.01  | 3.110 ± 0.07  | 54.720 ± 0.04  |
| Wheat grass    | 16.667 ± 0.32  | 7.973 ± 0.21  | 0.003 ± 0.07  | 27.267 ± 0.01  | 14.836 ± 0.05  | 20.600 ± 0.26  | 0.620 ± 0.12  | 46.940 ± 0.05  |

Means carrying different letters are not significantly identical.
**Calcium (Ca) content**

Calcium helps in bone development, and its deficiency can lead to improper development of bone in growing children leading to various deformities of the skeletal system.\(^{[16]}\) In the present investigation, the maximum amount of Ca content was recorded for barley grass. The results of present study regarding mineral content disclosed that Ca content is 57.573 and 49.890 mg/100 g for barley and wheat grass, respectively (Table 2). Ca content was greater in barley grass, and less amount of Ca content was recorded in wheatgrass.

Crop and Food Research\(^{[17]}\) found that the concentration of calcium was found significantly higher in the wheat leaf (430 mg) than that of wheatgrass (242 mg).\(^{[18]}\) The data of present study were very closely related to the achievement of Tashaurbind\(^{[19]}\) who investigated the Ca content of different cereal grasses and found 40.39 mg/100 g in wheatgrass. Chouhan and Mogra\(^{[20]}\) found that calcium content was highest in wheatgrass powder (186.6 ± 15.27), and according to a laboratory analysis by Irvine Analytical Laboratories, (1989), the calcium content was reported to be the lowest in wheatgrass as 24.2 mg/100 g.

**Iron (Fe) content**

Iron is one of the most studied elements for its bioavailability.\(^{[21]}\) The difference among both varieties for iron value was found to be significant. This is because of the fact the nutritional composition of cereals varies depending on their botanical origin.\(^{[22]}\) The mean values regarding iron content of cereal grasses characterization (Table 2) revealed that iron content ranged from 14.333 to 5.27 mg/100 g. It is depicted from the results (Table 2) that the Fe content of wheatgrass is 5.27 mg/100 g and that of barley grass is 14.333 mg/100 g. Barley grass had high amount of Fe as compared to wheatgrass. Similar findings have been given by different scientists and researchers, which exhibited that Fe content in cereal grasses under different conditions were ranged from 4.92 to 14.90 mg/100 g of Gaydon.\(^{[23]}\) The data of present study were very closely related to the achievement of Hagiwara and Cichoke\(^{[24]}\) who found iron content in barley grass was 15.8 mg/100 g.

**Potassium (K) content**

Potassium is most well-known for the important role it plays in blood clotting. In the current investigation, mean values for the minerals analysis are shown in Table 2. Mean values regarding potassium content of cereal grasses have shown that the maximum value recorded for barley grass was 365.54 mg/100 g and minimum value recorded for wheatgrass was 154.67 mg/100 g. K is a mineral which is present in the maximum amount as compared to other minerals. Mean values indicated that the potassium content of cereal grasses ranged from 154.67 to 365.54 mg/100 g.

The data of present study were very closely related to the achievement of Tashaurbind\(^{[19]}\) who found K content in wheatgrass was 147 mg/100 g, while Hagiwara and Cichoke\(^{[24]}\) found higher K content in barley grass was 8880 mg/100 g.

**Phosphorus (P) content**

Statistical analysis for cereal grasses explicated that there is a significant difference (\(P < 0.05\)) among phosphorus content of wheat and barley grass. Mean values related to phosphorus content of cereal grasses have been elucidated in Table 2. The P content of cereal grasses ranged from 36.667 to

**Table 2.** Mean values of minerals (mg/100 g) of cereal grasses.

| Green Grasses  | Ca  | Fe       | K         | P           | Zn          | Na          |
|---------------|-----|----------|-----------|-------------|-------------|-------------|
| Barley Grass  | 57.57 ± 0.01<sup>a</sup> | 14.33 ± 0.55<sup>a</sup> | 365.54 ± 0.11<sup>b</sup> | 42.52 ± 1.16<sup>a</sup> | 4.23 ± 0.44<sup>a</sup> | 11.31 ± 0.63<sup>a</sup> |
| Wheat Grass   | 49.89 ± 0.30<sup>b</sup> | 5.27 ± 0.23<sup>b</sup> | 154.67 ± 0.19<sup>b</sup> | 36.66 ± 0.45<sup>b</sup> | 3.90 ± 0.67<sup>b</sup> | 9.46 ± 0.54<sup>b</sup> |

Means carrying different letters are not significantly identical.
42.523 mg/100 g. Mean value of P content for barley grass was 42.523 mg/100 g, and it was found to be the maximum as compared to P content of wheatgrass. The P content of 36.667 mg/100 g was recorded for wheatgrass (Table 2). The effect of climate, variety, soil, and agronomic practices on mineral contents of cereal grasses is evident for the change in P content.

As shown by nutritional analysis in wheat leaf, earlier findings by Crop and Food research [17] reported a higher phosphorus content of 410 mg/100 g. Almost the same result has been observed by Irvine Analytical Laboratories (1989), whose experiment tells that the phosphorus content of wheatgrass was 75.2 g.

**Zn content**

Statistical analysis for cereal grasses explicated that there is a significant difference ($P < 0.05$) among zinc content of wheat and barley grass. The difference among wheatgrass and barley grass was found to be significant. Mean values related to zinc content of cereal grasses have been elucidated in Table 2. The zinc content of cereal grasses ranged from 3.90 to 4.23 mg/100 g. The mean value of zinc content for barley grass was 4.23 mg/100 g, and it was higher as compared to zinc content of wheat grass. Zinc content of 3.90 mg/100 g was recorded for wheatgrass (Table 2). The effect of climate, variety, soil, and agronomic practices on mineral contents of cereal grasses is evident for the change in zinc content.

**Sodium (Na) content**

The difference between barley grass and wheatgrass was found to be significant. Na is a crucial mineral for human health. In the present investigation, the maximum amount of Na content was recorded for barley grass. The results of present study regarding mineral content show that Na content is 11.31 and 9.46 mg/100 g for barley and wheatgrass, respectively (Table 2). Na content was greater in barley grass, and less amount of Na content was recorded in wheatgrass. These results are in accordance with the earlier findings reported by Chouhan and Mogra [20] who found a slightly higher sodium content in wheatgrass and stated that Na content in cereal grass varied from 16.6 to 23.46 mg/100 g on dry matter basis.

**Vitamin profile**

Differences among the wheat and barley grasses regarding their vitamin composition may be attributed to varietal differences, genetic makeup, soil conditions, and agronomic practices. Cereal grasses are an important source of some of the B vitamins. [25] The results from the discussion of examined vitamins are discussed herein.

**Vitamin B6**

Many similar results have been obtained related to thiamin and riboflavin, [26–29] but with some differences for pyridoxine. The variability in B vitamin variability mainly depends upon cultivar type, year, and location of growing crop, and milling and rate of flour extraction. [27] It is clearly depicted from the results (Table 3) that vitamin B6 content of cereal grasses varied from 0.9600 to 1.50337 mg/100 g. Vitamin B6 content of barley grass is 1.50337 mg/100 g while that of wheatgrass is 0.9600 mg/100 g. The barley grass had maximum vitamin B6 content while wheatgrass contained the least content as shown in Table 3.

| Green Grasses | Vit B6 (mg/100 g) | Vit B9 (µg/100 g) | Vit B12 (µg/100 g) | Vit C (µg/100 g) |
|---------------|------------------|------------------|-------------------|------------------|
| Barley Grass  | 1.50 ± 2.22a     | 106.7 ± 1.06a    | 93.33 ± 1.67a     | 25.4 ± 1.55a     |
| Wheat Grass   | 0.96 ± 1.63b     | 84.0 ± 0.45b     | 66.87 ± 3.02b     | 14.4 ± 0.33b     |

Means carrying different letters are not significantly identical.
The findings of the present study are in agreement with those of Premakumari and Haripurawa.\textsuperscript{[18]} A range of 1.30–2 mg of pyridoxine content of cereal grasses was found in another study who found a lesser amount of vitamin B6 in wheatgrass.\textsuperscript{[17]} However, Tusharbind\textsuperscript{[19]} found a very low B6 content (0.0065 mg) in wheatgrass powder.

**Vitamin B9**

There is no tendency in the human body to store B vitamins, so it is our body’s requirement to take these vitamins daily through diet. Mean values regarding folic acid content of cereal grasses have been presented in Table 3. The folic acid content of cereal grasses ranged from 84 to 106.70 µg. The results indicated that barley grass was found to have the highest content (106.70 µg/100 g) of vitamin B9 as compared to the content of wheatgrass (84 µg/100 g) (Table 3). The mean value for vitamin B9 content of barley and wheatgrass was 106.70 and 84 mcg/g, respectively (Table 3). Similarly, Premakumari and Haripriya\textsuperscript{[18]} investigated the vitamin B9 content of wheatgrass juice powder and wheatgrass, and found folic acid content ranging from 86 to 1130 µg.\textsuperscript{[17]} A lower percentage of folic acid content (0.0012 mg) of wheatgrass was found in another study.\textsuperscript{[19]}

**Vitamin B12**

Mean values pertaining to vitamin B12 content (Table 3) in cereal grasses of wheat and barley grass ranged from 46.877 to 93.33 µg. The results showed that the higher vitamin B12 content (93.33 µg was found in barley grass while lower content of 46.877 µg was exhibited for wheatgrass as presented in Table 2). The present findings of vitamin B12 content of wheatgrass are comparable with the previous findings of researchers who calculated a higher vitamin B content of 99 µg/100 g in wheatgrass. A lower percentage of cobalamin (0.01 mg) in wheatgrass was found in another study.\textsuperscript{[19]}

**Vitamin C**

Vitamin C is a good antioxidant and is abundant in plants, fruits, vegetables, and grasses. The present findings for vitamin C showed a vitamin content of 14.4 mg for wheatgrass and that of barley grass is 25.4 mg, and the maximum amount of vitamin C content was found among the green grasses (Table 3). The results showed that the higher vitamin C content (25.4 mg) was found in barley grass while lower content (14.4 mg) was found in wheatgrass, as shown in Table 3.

Paulickova et al.\textsuperscript{[30]} documented similar results for barley grass, which showed that content of vitamin C fluctuated between 6 and 14.5 g/kg and it declines with the growth of the barley plant. During the growth period of barley, raw material crumpling can be a possible explanation for the dramatic drop of the vitamin C contents. Significant difference was recorded in these cereal grasses. The present results are supported by the previous studies which showed that the vitamin C content ranged from 7.5 to 14.1 mg.\textsuperscript{[4,18]}

**Antioxidant activity**

AA of wheatgrass extract was determined by measuring its ability to reduce ferric to ferrous ion. The reducing power was confirmed by the changes of yellow color to green and blue shades. Wheatgrass extract had higher AA compared to BHA, the reference compound.\textsuperscript{[31]}

The difference among both varieties was found to be significant. This is because of the fact the nutritional composition of cereals varies depending on their botanical origin.\textsuperscript{[22]} Mean values regarding AA of cereal grasses characterization (4) revealed that AA for both cereal grasses ranged from 73.939% to 283.50%. The results showed that maximum AA (283.50%) was found in Barley grass while the minimum (73.939%) was reported in wheatgrass.
These results are compatible with earlier findings reported by Shyam et al. [32] who highlights the potent antioxidant properties of wheatgrass in healthy subjects and stated that supplementation with wheatgrass provided better protection against lipid peroxidation and thereby decreased oxidative stress and increased endogenous antioxidant levels such as plasma total antioxidant status and vitamin C. Shyam et al. [32] found total AA of wheatgrass ranged from 0.70 ± 0.06 to 0.76 ± 0.09 mmol/L. However, it was found that total antioxidants in aqueous extract and alcoholic extract of wheatgrass were 0.27 ± 0.024 and 0.38 ± 0.015 mmol/L. Furthermore, Sanjay and Hwiyang [33] assessed the nutritional composition, phytochemical constituents, and antioxidant activities of six wild edible plants consumed by the Bodos of North-East India, which include Sphenoclea zeylanica, Cardamine hirsuta, Natsiatum herpeticum, Sphaerantus peguensis, Melothria perpusilla, and Persicaria chinensis. Among the six wild edible plants, M. perpusilla displayed a better antioxidant property showing the strongest DPPH radical scavenging activity, maximum ferric-reducing antioxidant power (FRAP) value, and highest phenolic and flavonoid contents. Moreover, in a study, Islary et al. [34] probed the AA of two wild edible fruits, i.e., Aporosa dioica (Roxb.) Muell.-Arg. and Ottelia alismoides (L.) Pers. found in Assam of North-East India and found that A. dioica fruit extract exhibited lower IC_{50} (DPPH, ABTS, and H_{2}O_{2}) values in contrast to O. alismoides fruit extract indicating stronger antioxidant capacity in A. dioica fruit. Higher FRAP value of 106.583 ± 5.204 μM trolox equivalent (TE)/g dry extract (DE) was found in the methanol extract of A. dioica fruit compared to that of O. alismoides fruit (44.083 ± 7.637 μM TE/g DE). The TPC found in the methanol extracts of A. dioica and O. alismoides fruits was 146.710 ± 2.807 mg GAE/g DE and 93.860 ± 1.172 mg GAE/g DE, respectively, while the TFC was found to be 72.510 ± 8.833 mg QE/g DE in A. dioica fruit and 43.270 ± 5.361 mg QE/g DE in O. alismoides fruit. These fruits are good sources of nutrients and natural antioxidants and may find applications in the formulation of various pharmaceutical drugs. [35,36]

**DPPH (2, 2-diphenyl-1-picrylhydrazyl radical assay)**

The DPPH radical scavenging method was used to determine the AA of the phenolic compounds in this study. This method is based on the reducing ability of antioxidants toward DPPH [37] and widely used to evaluate the AA of phenolic compounds extracted from plants, fruit, vegetables, cereal grain, wine, etc. [38] because of its stability and ease of use. DPPH assay involves the use of DPPH. Its reaction rate directly relates to the AA.

The statistical data show that the mean values for the antioxidant activities of the cereal grasses, i.e., barley and wheatgrass, are significantly different from one another. Table 4 highlights the antioxidant status of green grasses. Mean values related to DPPH have been symbolized. Recorded mean value of DPPH radical scavenging activity for barley grass and wheatgrass is 99.293% and 51.690%, respectively (Table 4). The results are not in accordance with another study [39] who found a very low DPPH content in aqueous extract and alcoholic extract of wheatgrass 0.253 ± 0.005 and 0.307 ± 0.019, respectively.

**Total phenolic content**

The TPC was significantly different among both varieties. This is because of the fact the nutritional composition of cereals varies depending on their botanical origin, soil, fertilizer, and environmental conditions. [22] Mean values regarding TPC of cereal grasses characterization (Table 4) revealed that

| Green Grasses     | AA   | DPPH (%) | TPC (μmol of GAE/g) | Flavonoids (μmol/l) |
|-------------------|------|----------|---------------------|---------------------|
| Barley Grass      | 283.5 ± 0.05a | 99.29 ± 0.34a | 195.33 ± 0.04a | 148.33 ± 0.22a |
| Wheat Grass       | 73.93 ± 1.23b | 51.69 ± 1.45b | 203.52 ± 0.10b | 161.12 ± 0.01b |

Means carrying different letters are not significantly identical.
TPC for both cereal grasses ranged from 195.33 to 203.52 µmol. The results showed that maximum TPC (203.52 µmol) was found in wheatgrass, while the minimum TPC (195.33 µmol) was reported in barley grass. These results are compatible with earlier findings reported by Durairaj et al.\textsuperscript{31} who reported that the TPC of wheatgrass was found to be (210.15 ± 2.14) µmol of GAE/g equivalent, while Shukla et al.\textsuperscript{39} found that the content of total phenolic compounds expressed as gallic acid equivalent was found to be 241 ± 8.0, 15.8 ± 0.9, and 10.7 ± 1.0 mg/g, respectively, in crude amalaki, spirulina, and wheatgrass.

**Flavonoids**

Flavonoids are naturally occurring compounds of the polyphenolic groups, which are especially found in plants.\textsuperscript{40} The flavonoids possess the anti-inflammatory, antioxidant, anti-allergic, and hepato-protective properties. According to the concentration as well as the redox potential present depends on the flavonoid antioxidants as well as pro oxidants.\textsuperscript{41}

Table 4 highlights the flavonoids statuses of cereal grasses, and mean values related to flavonoids have been symbolized. The recorded mean value of flavonoids for barley grass and wheatgrass is 148.33 µmol and 161.10 µmol, respectively (Table 4). The wheatgrass had maximum flavonoids while barley grass contained the least content of flavonoids. The results are in accordance with another study that found total flavonoid content was found to be 160.25 ± 2.17 µmol of quercetin/g equivalent of wheatgrass.\textsuperscript{31} In a study, Chaouche et al.\textsuperscript{42} evaluated total flavonoid contents of *Teucriumpolium* L. grown in Algeria. All the tested extracts showed an appreciable total flavonoid contents. Among the different extracts, the methanolic extract was found to be containing the highest amount of flavonoids (42.16 ± 0.61 mg of QE/g of extract).

**Phenolic acids**

In cereal grass, the majorities of phenolic acids are bound to the cell wall in ester form and present in insoluble bound form. Only a small portion exists as free phenolic acids. Therefore, the insoluble phenolic acids were recovered using alkaline hydrolysis. The identification of the monomeric phenolic acids was accomplished by comparison of the UV spectra and retention times with external standards. Six simple phenolic acids were observed in the wheatgrass and barley grass.

The benzoic acid content in barley grass and wheatgrass ranged from 73.84 to 65.16 µg/g. It is obvious from the result that the maximum benzoic content in barley grass and minimum benzoic content was observed in wheatgrass. The statistical data in Table 5 indicated that the two cereal grasses, barley grass and wheat grass, are significantly different from each other in caffeic acid content. The barley grass had maximum caff'eic content (86.610 µg/g) while wheatgrass contained the less content of this phenolic acid (77.033 µg/g) as shown in Table 5.

The mean values of gallic acid content of cereal grasses range from 3.620 to 6.333 µg/g. It is obvious from these results that the highest gallic acid contents, i.e., 6.33 µg/g were recorded in barley grass, whereas wheatgrass possessed less gallic acid content, i.e., 3.620 µg/g. Mean values are presented in Table 5. Mean values of syringic acid content of cereal grasses range from 150.43 to 170.46 µg/g. It is obvious from these results that the highest syringic acid contents, i.e., 170.46 µg/g were recorded in barley grass, whereas wheatgrass possessed less syringic acid content, i.e., 3.620 µg/g. Mean values are presented in Table 5.

The statistical data in Table 5 indicated that the two cereal grasses, barley grass and wheat grass, are significantly different from each other in p-hydroxybenzoic acid content. The barley grass had maximum of this phenolic acid content (45.523 µg/g) while wheatgrass contained the less content of p-hydroxybenzoic acid (36.66 µg/g) as shown in Table 5. Similarly, mean values of ferullic acid have been presented in Table 5 which indicates that ferullic acid content of cereal grasses range from
1315.7 to 1560.3 µg/g. It is obvious from these results that the highest ferulic acid content, i.e., 1560.3 µg/g was recorded in barley grass, whereas wheatgrass hold less ferulic acid content, i.e., 1315.7 µg/g.

These results are not consistent with the data reported by Liyana-Pathirana and Shahidi [43] where syringic acid was not found. These results are in agreement with those reported by Hatcher and Kruger [44] and Mattila et al. [45] These results are in agreement with the previous observations [46,47] that ferulic acid was the predominant total phenolic acid on a per weight basis. The grain of Swiss red wheat contained 33.71 μg of extractable ferulic acid/g of seeds, which is >5 ppm (μg/g) of free and soluble bound ferulic acid [47] but is much lower than the reported typical level of 500 μg of ferulic acid/g of ground whole wheat. [47,48] The extractable phenolic acid is a portion of, and may account for <6% of, the total phenolic acid presented in wheatgrass. Ferulic acid has been evaluated for its potential application as an analytical parameter in the rapid determination of wheatgrass carryover during germination. The antioxidant properties of ferulic acid were evaluated and reviewed by Graf. [47] In this study, the ferulic acid content was well correlated with antioxidant activities, TPC, and concentrations of other identified individual phenolic acids. Therefore, ferulic acid may serve as a marker for the quality control of wheatgrass antioxidants or may be used to monitor wheat antioxidant processing.

### Conclusion

Both barley and wheatgrass are a rich source of nutrients as well as bioactive compounds including proximate parameters, minerals, vitamins, and phenolic compounds. Moreover, barley green grass has higher antioxidant potential as compared to wheat green grass. These grasses are good sources of nutrients and natural antioxidants and may find applications in the formulation of various pharmaceutical drugs. Therefore, novel products with supplementation of green grasses extracted should be produced and introduced in the market. These functional products should be used against various maladies.

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