Indian Journal of Ecology (2023) 50(4): 1220-1224
DOI: https://doi.org/10.55362/IJE/2023/4040

Manuscript Number: 4040
NAAS Rating: 5.79

Comparison of Bioactive Compounds and Antioxidant Potential of Different Cereal Grasses for Potential Therapeutic Use

D Bernice Ekhe, Renuka Aggarwal, Kiran Bains and Amandeep Singh Sidhu

Department of Food and Nutrition, Organic Farming
Punjab Agricultural University, Ludhiana-141 004, India
E-mail: bernicedecember@gmail.com

Abstract: Wheat grass is presumed to contain bioactive compounds and antioxidant activity and has gained popularity as a health drink. The present study was undertaken to explore the possibility of using other cereal grasses in place of wheat as affected by variety and growth length. The bioactive compounds total phenol content (TPC) and total flavonoid content (TFC) increased significantly with the increase in length of the grass in wheat varieties but decreased in rice and barley grasses. Maximum TPC and TFC was observed in the barley grass and rice grass at 6- and 4-inches length, respectively. Wheat grass showed maximum content at 15 and 20 cm inches. Wheatgrass of variety PBW 550 at length of 20 cm showed the highest antioxidant power as evidenced by FRAP (ferric reducing antioxidant power) and ABTS (2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) assay. Rice variety PR 126 at 10 cm and barley variety PL 807 at 15 cm exhibited maximum scavenging activity comparable to wheat grass. Wheat grass is the best cereal grass in terms of bioactive and antioxidant potential. There is a significant variation in these components of cereal grasses that change with increase in length of the grass. These should be harvested at the growth length when the bioactive compounds and antioxidant activities are optimum. Rice and barley grass also exhibit notable antioxidant potentials, which could be considered as a substitute for wheatgrass. The different varieties of these cereal grasses contain non-significantly different bioactive compounds. Hence, any variety which is available at a particular location can be cultivated.

Keywords: Wheat grass, Rice grass, Barley grass, Bioactive compounds, Free radical scavenging activity

The cereal grasses have gained wide attention as a novel and convincing source of natural antioxidants. The young leaves obtained from cereal grasses of the *Poaceae* or *Gramineae* family and are presumed to contain maximum bioactive and freely available compounds such as amino acids, vitamins, and trace elements (Pająk et al 2014). Major cereal grass that has gained popularity today is wheatgrass. Germination and sprouting increase antioxidants, nutrients and phenolic compounds contents of cereal grasses (Fortună et al 2018). The wheat grains sprouted for 6-10 days are considered as 'wheatgrass' (Akbas et al 2017, Benincasa et al 2019). Wheatgrass has been considered as storehouse of nutrients, containing considerable amounts of vitamins, minerals, amino acids, active enzymes antioxidants and chlorophylls (Ahmed et al 2021, Kaur et al 2021). The wheatgrass juice have antioxidant, antimutagenic, antimicrobial, antiallergenic, nephron-protective, anti-inflammatory and diuretic properties (Özköse et al 2016, Hebbani et al 2020, Thakur et al 2020, Choi et al 2021). The evidence also suggests the protective effect of wheatgrass juice against chemotherapy-induced damage (Avisar et al 2020a, 2020b). Its gluten-free properties has been found to be beneficial for those with gluten allergy (Qamar et al 2019). The young grass of the rice plant (*Oryza sativa)* has been introduced in recent times to substitute the use of wheatgrass, mainly in high rice production areas especially Asian countries where rice acts as the major food source of energy (Chomchan et al 2016). Khanthapok et al (2015) reported that rice grass juice possesses DNA protective and antioxidant properties including vitamins, minerals and phytonutrients. Wangchareon and Phimphilai (2016) observed that 6-20 days old rice grass has a high level of antioxidant compounds and contain higher levels of polyphenols while containing comparable antioxidant activities to wheatgrass juice. The use of barley (*Hordeum vulgare* L.) as a cereal grain date back to ancient times. Barley grass is the young leaves of the barley plant. Barley grass juice contain vitamins C and B like wheatgrass juice in addition to minerals like calcium, copper and manganese (Niroula et al 2019). The use of natural substances from plants as a functional component in beverages is on the rise resulting in functional beverages taking the leading spot among functional foods (Wangchareon and Phimphilai 2016). Wheatgrass has been blended with other sources like wheatgrass juice blended with pomegranate (Kashudhan et al 2016) and with kombucha (Sun et al 2015) which showed higher and more stable antioxidant activity suggesting that these could be used as novel beverages with effective, healthy and functional therapeutic properties (Sun et al 2015, Kashudhan et al 2016).

With the increase in the possibilities of wheatgrass to be used in the food industry, exploration to include other cereal grasses, importance to determine the appropriate variety and harvesting stage is needed. This study aimed to investigate the effect of different cereal grasses harvested at varied lengths on the bioactive compounds and antioxidant potential to determine the optimum harvesting stage for their potential therapeutic use.

MATERIAL AND METHODS

Plant materials, cultivation and harvesting: Three varieties each of wheat (Unnat PBW 550, PBW 343, PBW 550), rice (PR 121, PR 114, PR 126) and barley (PL 807, PL 89 and DWRB 123) were procured from the Department of Plant Breeding and Genetics and School of Organic Farming, Punjab Agricultural University, Ludhiana, India. The seeds were soaked for 10 hours and germinated for 12 hours, then washed under tap water and broadcasted in plastic trays using the same soil for each of the varieties with no fertilizer application. The trays were kept indoor at room temperature 20-22°C. The seeds were watered 3-4 times at an interval of 4 hours daily. The grasses were cut with scissors 1cm above the soil when they reached the desired length i.e., 10 cm, 15 cm and 20 cm. The different cereal grasses attained the desired length at different durations of days. Wheat varieties attained the length of 10 cm by 5-6 days, 15 cm by 7-8 days and 20 cm by 10-11 days. Rice varieties attained 10 cm by 8-9 days, 15cm by 10-11 days and 20 cm by 15-16 days. Whereas, barley varieties attained 10 cm by 6-7 days, 15 cm by 8-9 days and 20 cm by 10-11 days.

Total phenol and total flavonoid content: TPC was determined using Folin Ciocalteu method (Singleton et al 1999) and expressed as GAE (Gallic acid equivalent)/100g. TFC was estimated using the method of (Zhishen et al 1999) and expressed as mg QE (Quercetin equivalent)/100g.

Antioxidant potential of cereal grasses: Antioxidant potential of cereal grasses was estimated by FRAP, ABTS and DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. FRAP assay of the extract (Tadhani et al 2007). The radical scavenging assay was determined by ABTS and DPPH assay following the protocol of (Re et al 1999) and (Brand-Williams et al 1995), respectively. The results were expressed as mg ferrous sulphate equivalent per 100 g (mg $FeSO_4$ equivalent/100g) for FRAP and % RSA (radical scavenging activity) for ABTS and DPPH assay.

Statistical analysis: The mean and standard deviation were computed using Microsoft Excel (2010) Statistical Analysis Tool Pack. Two-way Analysis of Variance was done using SAS (Statistical Analysis System, version 9.3 for windows) Institute Inc. A p-value of less than 0.05 was considered statistically significant in all cases.

RESULTS AND DISCUSSIONS

The TPC and TFC of different cereal grasses has been shown in Table 1. There was no significant difference in TPC and TFC among the wheat varieties with mean values ranging from 110.41-117.38 mg GAE/100g and 90.76-101.25 mg QE/100g fresh weight respectively (Table 1). Among the rice varieties a higher TPC and TFC was observed in variety PR 126 with a mean value of 56.78 mg GAE/100g and 81.99 mg QE/100g fresh weight respectively. Barley varieties showed significant difference in both TPC, with variety DWRB 123 exhibiting higher mean value of 91.05 mg GAE/100g fresh weight and TFC with variety PL 891 showing higher mean value of 72.63mg QE/100g fresh weight.

Table 1. Total phenol content (mg GAE/100g) and total flavonoid content (mg QE/100g) of different cereal grasses

Crop/variety	TPC	TFC
Wheat		
Unnat PBW 550	114.11^{a}	101.25^{ab}
PBW 343	110.41^{a}	90.76^{b}
PBW 550	117.38^{a}	95.78^{b}
Rice		
PR 121	48.87^{a}	72.83^{a}
PR 114	45.67^{a}	65.64^{b}
PR 126	56.78^{b}	81.99^{c}
Barley		
PL 807	71.61^{a}	69.20^{ac}
PL 891	88.97^{b}	72.63^{b}
DWRB 123	91.05^{c}	67.79^{c}

Values in columns followed by different superscripts differ significantly ($p \leq 0.05$)

Table 2. Total phenol content (mg GAE/100g) and total flavonoid content (mg QE/100g) of different cereal grasses harvested at different growth lengths

Crop/Length	10 cm	15 cm	20 cm
Total phenol content			
Wheat	106.16^{a}	113.46^{a}	122.28^{b}
Rice	63.94^{a}	57.10^{b}	30.28^{c}
Barley	77.48^{ac}	94.58^{b}	74.87^{c}
Total flavonoid content			
Wheat	70.36^{a}	97.97^{b}	119.47^{c}
Rice	95.18^{a}	71.28^{b}	54.00^{c}
Barley	65.30^{ac}	75.91^{b}	66.28^{c}

Values in columns followed by different superscripts differ significantly ($p \leq 0.05$)

The maximum TPC (122.28 mg GAE/100g) and TFC (119.47 mg QE/ 100g) , was observed in 20 cm in wheat grass. In rice grass, 10 cm length grass exhibited maximum TPC (63.94 mg GAE/100g) and TFC (95.18 mg QE/100g) . Among the barley varieties maximum TPC was (94.58 mg GAE/100g) and was 75.91 mg /100g) was obtained at 15 cm.

The varieties of cereal grasses used in the study showed significant difference in their FRAP activity (Fig. 1) Among the wheat varieties the highest FRAP activity (198.47 mg $FeSO_4$ /100g) was exhibited by variety PBW 550. PR 126 showed highest value of 134.70 mg $FeSO_4$ /100g among the rice varieties while among the barley varieties the highest value (64.05 mg $FeSO_4$ /100g) was observed in PL 807. When the ABTS and DPPH scavenging activity among wheat varieties was considered, no significant difference was observed (Fig. 2 and 3). The rice variety PL 126 exhibited higher ABTS activity of 19.87% while no significant difference in DPPH activity In barley variety PL 807 exhibited higher ABTS activity of 16.06 % RSA while not showing significant difference in DPPH activity. Considering the effect of length of cereal grasses on the antioxidant activity, wheatgrass showed highest FRAP activity of (186.87 mg $FeSO_4$ /100g) at 20 cm while lowest activity (163.48 mg $FeSO_4$ /100g) was observed at 10 cm. FRAP activity was maximum (140.86 mg $FeSO_4$/100g) at 10 cm in rice grass, while barley grass did not show significant difference when growth length was considered. Similarly, the highest ABTS value was observed at 20 cm in wheatgrass with an activity of 52.09 % RSA. The rice grass did not show significant difference. In barley grass however, higher ABTS activity was observed at 15 cm with activity of 18.30% RSA. No significant difference in DPPH scavenging activity in wheat and rice grass when length was considered whereas, activity at 20 cm was found to be lower in barley grass.

The cereal grasses showed significant difference in bioactive compounds and antioxidant potential. The bioactive compounds, in wheat varieties obtained in the present study are similar to that reported in previous studies (Qamar et al 2019, Devi et al 2020) and the bioactive compounds increased with the increase in length of the grass with optimum bioactive compounds observed at 20 cm which concurs with previous study (Savsatli 2020, Devi et al 2020). Among the rice varieties higher bioactive compounds was observed in 10 cm length grass . Rattanapon et al(2017) also recorded that TPC in rice grass decreased as the seedling time increased.

Antioxidant potential (DPPH) in present study among wheat and barley grasses was lower than that reported by Qamar et al (2019)being 99.29 and 51.69% for DPPH radical scavenging activity in barley and wheatgrass respectively which could be due to varietal differences and the growing condition. The antioxidant activities determined by FRAP was maximum at 10 cm in the rice grass, while ABTS and DPPH scavenging activity did not show significant difference till 20 cm which was similar to results obtained by (Rattanapon et al 2017) where antioxidant activity determined as ABTS and FRAP was higher in rice aged 7 days compared with 14 and 21 days whereas, the DPPH radical scavenging activity was higher on day 14 than on day 7 and 21. The higher content of antioxidant activities in early stages could be attributed to a high oxygen demand during early germination. TPC increased during seedling growth, which results in a significant increase in antioxidant potential

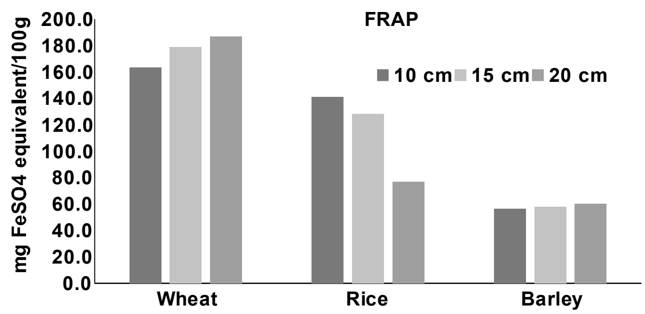

Fig. 1. FRAP-reducing activity of cereal grasses harvested at different growth lengths

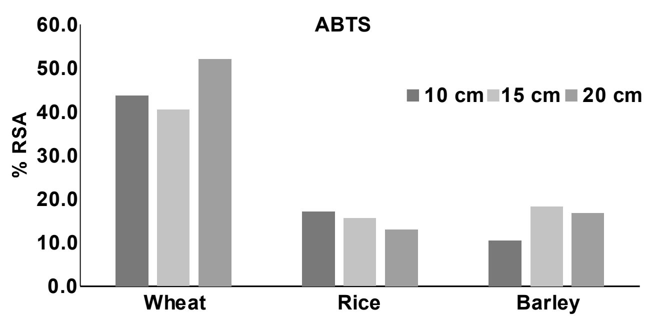

Fig. 2. ABTS- free radical scavenging activity of cereal grasses harvested at different growth lengths

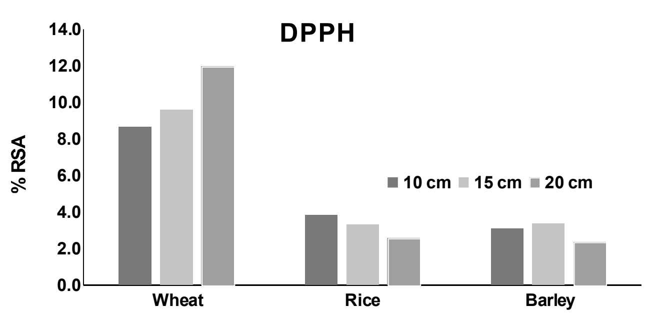

Fig. 3. DPPH- free radical scavenging activity of cereal grasses harvested at different growth lengths

as reported by Zhang et al (2015). Previous studies on wheatgrass by earlier scientist reported correlation between antioxidant activity and bioactive compounds which was also observed in the present study(Shakya et al 2014, Savsatli 2020, Kaur et al 2021).

CONCLUSIONS

The work done in this paper indicates that among the analysed cereal grasses of wheat, rice and barley, significant difference was observed in the bioactive compounds and antioxidant activity of different varieties at different lengths. Wheatgrass had maximum bioactive compounds and exhibited maximum antioxidant activity followed by rice and barley grasses. In addition, the study suggests that different grasses should be harvested at the growth length when the bioactive compounds and antioxidant activities are optimum. Moreover, as grasses of rice and barley varieties also exhibit notable antioxidant potentials, they could be considered as a substitute for wheatgrass to meet the need of the population for health enhancing therapeutic products.

REFERENCES

Ahmed Z, Faisal Manzoor M, Hussain A, Hanif M, Zia-ud-Din and Zeng XA 2021. Study the impact of ultra-sonication and pulsed electric field on the quality of wheat plantlet juice through FTIR and SERS. *Ultrasonics Sonochemistry* **76**: 105648.

Akbas E, Kilercioglu M, Onder ON, Koker A, Soyler B and Oztop MH 2017. Wheatgrass juice to wheatgrass powder: Encapsulation, physical and chemical characterization. *Journal of Functional Foods* **28** :19-27.

Avisar A, Cohen M, Brenner B, Bronshtein T, Machluf M, Bar-Sela G and Arahon A 2020a. Extracellular vesicles reflect the efficacy of wheatgrass juice supplement in colon cancer patients during adjuvant chemotherapy. *Frontiers in Oncology* **10:**1659.

Avisar A, Cohen M, Katz R, Shentzer-Kutiel T, Aharon A and Bar-Sela G 2020b. Wheatgrass juice administration and immune measures during adjuvant chemotherapy in colon cancer patients: Preliminary results. *Pharmaceuticals* **13**(6): 129.

Benincasa P, Falcinelli B, Lutts S, Stagnari F and Galieni A 2019. Sprouted grains: A comprehensive review. *Nutrients* **11**(2): 421.

Brand-Williams W, Cuvelier ME and Berset C 1995. Use of a free radical method to evaluate antioxidant activity. *LWT- Food Science and Technology* **28**(1): 25-30.

Choi MH, Lee M, Yang SH, Shin HJ and Jeon Y 2021. Hydrophobic fractions of *Triticum aestivum* L. Extracts contain polyphenols and alleviate inflammation by regulating nuclear factor-kappa B. *Biotechnology and Bioprocess Engineering* **26**(1): 93-106.

Chomchan R, Siripongvutikorn S, Puttarak P and Rattanapon R 2016. Investigation of phytochemical constituents, phenolic profiles and antioxidant activities of rice grass juice compared to wheatgrass juice. *Functional Foods in Health and Disease* **6**(12): 822-835.

Devi CB, Bains K, Kaur H and Ram H 2020. Nutritional composition, bioactive compounds and free radical scavenging activity of wheatgrass (*Triticum aestivum* L.) as influenced by harvesting stages and cultivation method. *Indian Journal of Natural Products and Resources* **11**(2): 118-123.

Fortună ME, Vasilache V, Ignat M, Silion M, Vicol T, Patraș X, Miron I and Lobiuc A 2018. Elemental and macromolecular modifications in Triticum aestivum L. plantlets under different cultivation conditions. *PLoS ONE* **13**(8): e0202441.

Hebbani AV, Bulle S, Kanu VR, Balachandrababu Malinin A, Reddy VD and Chakravarthula, VN 2020. Nephro-protective activity of wheatgrass juice against alcohol-induced oxidative damage in rats. *Toxicology Mechanisms and Methods* **30**(9): 679–686.

Kashudhan H, Dixit A and Kumar K 2016. Development of wheatgrass-pomegranate blended therapeutical juice using response surface methodology. *Journal of Food Processing and Preservation* **41**(2): e12869.

Kaur N, Singh B, Kaur, Yadav MP, Singh N, Ahlawat AK and Singh AM 2021. Effect of growing conditions on proximate, mineral, amino acid, phenolic composition and antioxidant properties of wheatgrass from different wheat (*Triticum aestivum* L.) varieties. *Food Chemistry* **341**(1):128201.

Khanthapok P, Muangprom A and Sukrong S 2015. Antioxidant activity and DNA protective properties of rice grass juices. *Science Asia* **41**(1): 119-129.

Niroula A, Khatri S, Khadka D and Timilsina R 2019. Total phenolic contents and antioxidant activity profile of selected cereal sprouts and grasses. *International Journal of Food Properties* **22**(1): 427-437.

Ozköse A, Arslan D and Acar A 2016. The comparison of the chemical composition, sensory, phenolic and antioxidant properties of juices from different wheatgrass and turfgrass species. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **44**(2): 499-507.

Pająk P, Socha R, Gałkowska D, Rożnowski J and Fortuna T 2014. Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chemistry* **143**(1): 300-306.

Qamar A, Saeed F, Tahir-Nadeem M, Hussain AI, Khan MA and Niaz B 2019. Probing the storage stability and sensorial characteristics of wheat and barley grasses juice. *Food Science and Nutrition* **7**(2): 554-562.

Rattanapon R, Siripongvutikorn S, Usawakesmanee W and Thonggruang C 2017. Changes of nutritional value, bioactive compounds and antioxidant activity of primed white rice, Chainat 1, during seedling. *International Food Research Journal* **24**(6): 2563-2571.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* **26**(9-10): 1231-37.

Şavşatli Y 2020. The effects of wheatgrass length on antioxidant activity and total phenolic content in wheatgrass (*Triticum* spp.). *Turkish Journal of Agriculture and Forestry* **44**(3): 271-277.

Shakya G, Pajaniradje S, Hoda M, Durairaj V and Rajagopalan R 2014. GC-MS analysis, in vitro antioxidant and cytotoxic studies of wheatgrass extract. *American Journal of Phytomedicine and Clinical Therapeutics* **2**(7): 877-893.

Singleton VL, Ortherfer R and Lamuela-Raventos RM 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteu reagent. *Methods in Enzymology* **299**(14): 152-178.

Sun TY, Li JS and Chen C 2015. Effects of blending wheatgrass juice on enhancing phenolic compounds and antioxidant activities of traditional kombucha beverage. *Journal of Food and Drug Analysis* **23**(4): 709-718.

Tadhani MB, Patel VH and Subhash R 2007. In vitro antioxidant activities of *Stevia rebuadiana* leaves and callus. *Journal of Food Composition and Analysis* **20**(3-4): 323-329.

Thakur N, Dhaliwal H and Sharma V 2020. Qualitative and quantitative RP-HPLC-PDA method of analysis of polyphenols in lyophilized wheat seedling juice powder. *International Journal on Emerging Technologies* **11**(2): 36-43.

Wangcharoen W and Phimphilai S 2016. Chlorophyll and total phenolic contents, antioxidant activities and consumer acceptance test of processed grass drinks. *Journal of Food Science and Technology* **53**(12): 4135-4140.

Zhang G, Xu Z, Gao Y, Huang X, Zou Y and Yang T 2015. Effects of germination on the nutritional properties, phenolic profiles and antioxidant activities of buckwheat. *Journal of Food Science* **80**(5): 1-9.

Zhishen J, Mengcheng T and Jianming W 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* **64**(4): 555-559.

Received 22 February, 2023; Accepted 20 July, 2023