**Phytochemical Profiling of Pakistani Grown Barley Varieties**

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**Abstract**

 Barley varieties in Pakistan are cultivated covering wide ranging agro-climatic region and as such are anticipated to exhibit differences in yield, physico-chemical composition and nutritional value. Mainly barley is used as source of carbohydrates but they also comprehend appreciable quantity of different bioactive compounds. Despite having significant influence on human health, phenolic compounds of Pakistani barley varieties have not been studied up till now. In the present work, phytochemical profile of 20 Pakistani grown barley varieties was explored. Antioxidant activity of different barley varieties ranged from 30.09±0.571% to 89.39±2.324%. Total phenolic content in different varieties ranged from 69.2±1.66 mg GAE/100g to 177.8±5.68 mg GAE/100g. Mean values for quercetin content of barley varieties ranged from 0 ppm to 38.02±0.32 ppm. All barley varieties were characterized for 11 free phenolic acids (gallic acid, benzoic acid, m-coumeric acid, cinamic acid, caffeic acid, chlorogenic acid, syringic acid, p-coumeric acid, ferulic acid, vanillic acid and sinapic acid). In the present study gallic acid was detected as most abundant free phenolic acid in Pakistani grown barley varieties. Significant differences in phytochemical profile were found in all barley varieties grown in different locations. Results from this preliminary study helped to screen barley varieties with higher phytochemical profile.

**Keywords:** Barley**,** varietal differences,antioxidants, phenolic acids, total phenolic content, flavonoids, HPLC

**Introduction**

Among major cereal crops; barley ranks at 4th position after wheat, corn and rice; widely planted in the world, and is one of the ancient crop plants. In this context, although barley is an important crop, however, the primary utilization is restricted to poultry feed and it's human consumption is very limited. Primarily barley grains are used as source of carbohydrates (70%), but they also comprehend appreciable quantity of different bioactive compounds (Baik and Ullrich, 2008). Due to higher amount of bioactive components like β-glucans, phenolic acids, flavonoids, tocotrienols and tocopherols, barley is acquiring renewed attention to be used as a constituent in manufacturing of functional food products (Holtekjølen *et al.,* 2008a). Antioxidants have been defined as those molecules involved in prevention or delaying the oxidation of any substances that are prone to be oxidize while using in very low concentrations. Since, barley contains appreciable amounts of phenolic compounds making it an exceptional source of antioxidants that prevents the outbreaks of diseases and thus promotes health being (Zhao *et al*., 2008).

Antioxidants in barley formulated either as free (easily extractable complexes) or bounded (lesser extractable) as the later ones are covalently connected to macromolecules like arabinoxylans. Free phenolics in barley exist in form of tocopherols and flavanols while bound phenolics be present primarily as phenolic acids *i.e.*, ferulic acid and p-coumaric acid. Furthermore, numerous classifications of phenolic complexes in barley found as derivatives of benzoic acid and cinnamic acid, quinones, flavanones, flavones, flavonols, chalcones, amino phenolic composites and proanthocyanidins (Holtekjølen *et al.,* 2006a). Flavonoids (catechin, quercetin, kaempferol) comprise a major class of phytochemical compounds present in barley grain (Tossi *et al*., 2012; Yang *et al*., 2013). These bioactive constituents are responsible for antioxidant activity and health benefits associated with barley.

A lot of phenolic acids detected in cereals are in bound form, particularly in insoluble bound form and to a much smaller degree present in free form (Bonoli *et al*., 2004). Free phenolic acids constitute outer portion of pericarp, however bound phenolics are allied to cell wall constituents i.e., arabinoxylans, hemicelluloses, lignins and cellulose (Tang *et al*., 2016). Free phenolic acids cover a small percentage of total phenolic content (TPC). As the concentration of phenolic acids in barley varies between 4-23 mg/g in case of free phenolics, 86.5-198.5 mg/g in case of conjugated phenolics, 133-523 mg/g for bound phenolics, and 604-1346 mg/g for total phenolic acid concentration (Holtekjølen *et al*., 2006a; Abdel-Aal *et al*., 2012). The percentage of major free phenolic acids in barley are as follows; 27% ferulic acid, 28% vanillic acid, 17% syringic acid and 22% p-coumeric acid (Gamel and Abdel-Aal, 2012). Thus, it is evident that barley can serve as a potential source of natural antioxidants in cereal based food products.

The two subgroups of phenolic acids comprise benzoic acid, cenamic acid and their derivatives. Now a days, researchers are more interested in health benefits of individual phenolic acids. The probable daily intake of phenolic acids is 25 mg to 1 g. They are involved in disease prevention because of unsaturated carboxylic group present in them (Idehen *et al*., 2017).

Barley varieties greatly differs in their chemical composition because of genetic variations along with environmental factors or their combine effects (Andersson *et al*., 1999; Rodehutscord *et al*., 2016). So, we need to figure out those barley varieties having high nutritional profile. Literature has shown some studies on phytochemical characterization of barley varieties (Lahouar *et al*., 2014; Zhu *et al*., 2015; Marecek *et al*., 2017; Suriano *et al*., 2018; Holtekjølen *et al*., 2006a; 2011; Legzdiņa *et al*., 2018). However, no such attempt has been undertaken to characterize Pakistani grown barley varieties for their phytochemical profile.

Chemical composition and physical attributes of cereal grains are strongly affected by environmental factors such as temperature, rainfall, soil conditions, and fertilization (Rodehutscord *et al*., 2016). Balasundram *et al*. (2006) stated that the variation in total phenolic acids depends mainly on intrinsic factors such as genus, species, line and cultivars along with other extrinsic factors such as environmental, ecological and agronomic etc. Moreover, it was reported that the presence of phenolic acid varied significantly due to the presence of hull in barley varieties. Several studies reported the positive impact in terms of elevated level of total phenolics of organic barley crops as compared to conventional farming practices (Johansson *et al*., 2014). A group of researcher Konopka *et al*. (2012), observed increased total polyphenols and decreased phenolic acids contents in cereals grown by organic farming practices in comparison to conventional farming. Moreover, in case of total polyphenols concentration the environmental effects possess significant effects in comparison to genotype of barley and wheat varieties (Mpofu *et al*., 2006; Menga *et al*., 2010; Bellato *et al*., 2013). Legzdiņa *et al*. (2018) examined the influence of genes and environmental factors along with farm practices on concentration of total phenolics in covered and hull-less spring barley varieties. It was evident from results that barley grain contains 13 phenolic acids with the highest content of ferulic acid followed by syringic acid and p-coumaric acid. It was also found that crop management system had non-significant effects on antioxidants contents. On the other hand, growing year showed pronounced effects on total phenolic contents. Farm management system had effects on total polyphenols with highest values observed in organic farming. When compared the barley varieties on the basis of hull it was found that covered varieties exhibits higher total polyphenols as compared to its counterpart.

In Pakistan, barley is considered as one of the underutilized and neglected food crop, possessing high nutritive/dietary value but its part in accomplishing nutrition and food security is not implicated sufficiently, thus it doesn’t attribute in nutrition and dietary policies and strategies of the country. The production of barley recorded during 2015-16 was 61 thousand tones that decreased to 58 thousand tones during 2016-17 (GOP, 2018). Although the abundant concentration of phenolic compounds in barley can serve as an excellent dietary source of natural antioxidants, the phytochemical profile of different Pakistani barley varieties has not been studied up till now. Nowadays there is a need for reliable and updated data on nutritional profiling and food composition as nutrition is the key adaptable determining factor of chronic diseases. Keeping in view the above mentioned facts; this study aimed to characterize the phytochemical profile of Pakistani grown barley varieties from different geographical regions. Results from this preliminary study will help to screen barley varieties with higher phytochemical profile.

**Materials and Methods**

**Procurement of raw material**

Barley grains of twenty varieties/lines were procured from different ecological regions during 2017-2018. The samples were stored in labeled plastic jars at room temperature to ensure preserve integrity. All the available barley varieties/lines in four provinces of Pakistan were procured (Fig. 1). Haider-93, Jau-17, and Sultan-17 were procured from Ayub Agriculture Research Institute (AARI), Faisalabad, Punjab; Rakhshan-10 and Sanober-96 were procured from Balochistan Agricultural Research and Development Center (BARDC), Quetta, Balochistan; AAJ-2013, Bajwar-2000, Jau-e-Paghmbari, MPT-V2, MPT-V4, MPT-V5, MPT-V6, MPT-V7, MPT-V9, MPT-V11, MPT-V12 were procured from Cereal Crops Research Institute, Pirsabak, Nowshera, Khyber Pakhtunkhwa; Sadabahar, Upcoming-1, Upcoming-2 and Upcoming-3 were procured from Barley and Wheat Research Institute, Hyderabad, Tandojam, Sindh.

**Chemicals**

The chemicals, reagents and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

**Sample preparation**

The grains were dehulled using a laboratory-scale barley dehuller and milled to flour by a laboratory-scale milling machine (Perten Instruments Mill 3100).

**Preparation of extracts**

In current study barley sample extracts were prepared following Lahouar *et al*. (2014) for determination of total phenolic content (TPC) and antioxidant activity (DPPH assay). Barley flour (200 mg) from each variety was extracted in 4 mL acidified methanol (HCl/methanol/water, 1:80:10, v/v/v) using orbital shaker at 25°C for 2 h. The obtained mixture was centrifuged in a centrifugation machine for 10 min at 3000 g and the supernatant was used for quantification of total phenolic content (TPC) and DPPH.

**Total phenolic content**

Total phenolic content in different barley varieties were quantified following Folin–Ciocalteu spectrophotometric method as explained by Sharma and Gujral (2010). Freshly diluted (10 fold) Folin–Ciocalteu reagent (1.5 mL) was added to sample extract (200 µL) and the contents were allowed to equilibrate for 5 min at room temperature. After that 1.5 mL sodium carbonate solution (60 g/L) was added and incubated at room temperature for 90 min. The absorbance was read at 765 nm using spectrophotometer against blank. The results were calculated using following expression and expressed as mg GAE/g extract.

T = C× V / M

Where,

T = Total phenolic contents (mg/g plant extract, in GAE)

C = Concentration of gallic acid (mg/mL)

V = Volume of extract (mL)

M = Weight of extract (g)

**Antioxidant activity using DPPH test**

Antioxidant activity of different barley varieties was determined using free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as outlined by Sharma and Gujral (2010). Sample extract (100 µL) was allowed to react with 3.9 mL freshly prepared DPPH (in methanol solution) and the solution was kept in dark for 30 min at room temperature. Absorbance was read at 515 nm using UV/vis spectrophotometer at 0 and 30 min. Blank sample absorbance was also measured against BHT as standard. Free radical scavenging activity of barley varieties was measured using following formula as percentage reduction in absorbance attributable to presence of antioxidants present in each sample extract.

Antioxidant activity (%) = AB-AA × 100

AB

Where,

AB = Absorbance of blank at 0 min

AA = Absorbance of sample extract at 30 min

**HPLC quantification of phenolics**

Phenolic acids present in barley varieties were detected and quantified following Zhu *et al*. (2015) with little modifications using HPLC system equipped with ODS2 C-18 reversed phase column (25 cm x 4.6 mm, 5.0 μm particle size) and UV/Vis detector. Mobile phase consisted of 99.5% acidified acetonitrile with a constant flow rate of 1 mL/min in isocratic mode. Sample (20 μL) was injected and detection were carried out at 280 nm. Phenolic acids in each barley variety was detected by comparing each sample’s retention time with standard chromatogram. The quantification of each individual phenolic acid was calculated based on peak area measurements and results were presented as µg phenolic acid/g sample weight.

**Statistical analysis**

All analyses were carried out in triplicate and mean values are presented as mean ± standard deviation (SD). The obtained results were statistically analyzed using completely randomized design (CRD) using Statistix 8.1 software. To check the level of significance, analysis of variance technique (ANOVA) was applied, followed by Tukey’s HSD multiple comparison test for separation of mean values (Montgomery, 2008).

**Results and Discussion**

**Phytochemical profiling**

Several phenolic compounds (free and bound) and flavonoids are present in barley grain contributing towards its antioxidant activity and provide health benefits. The results for antioxidant activity, total phenolic content, free phenolic acids and flavonoids present in Pakistani grown barley varieties are presented and discussed in following sections.

**Antioxidant activity (DPPH)**

Statistical analysis regarding antioxidant activity of different Pakistani gown barley varieties showed a highly significant difference among varieties. Antioxidant activity of different barley varieties ranged from 30.09±0.571% to 89.39±2.324% as indicated by mean values displayed in Table 1. The highest antioxidant activity was observed in hulless barley variety Jau-e-Paghmbri and lowest in MPT-V4, though both the varieties were procured from KPK province. Province wise variation in antioxidant activity of different barley varieties has been presented in Fig. 1a. Antioxidant activity of KPK varieties ranged from 30.09±0.571% to 89.39±2.324%. Among Punjab varieties the values of antioxidant activity ranged from 50.48±1.163% to 60.87±1.765%. Barley varieties procured from Balochistan province showed antioxidant activity ranging from 41.44±0.953% to 57.75±1.155%. The antioxidant activity of Sindh varieties ranged from 40.10±1.002% to 62.38±1.746%. Rakhshan-10 exhibited significantly high antioxidant activity (57.75±1.155%) in comparison to Sanober-96 (41.44±0.953%) while both the varieties were procured from Balochistan province. Jau-17 (60.87±1.765%) and Upcoming-2 (62.38±1.746%) exhibited high antioxidant activity among varieties procured from Punjab and Sindh province, respectively. The differences in the antioxidant activity of different varieties is attributed to weather conditions particularly solar radiation during vegetation period at time of grain formation. A higher solar radiation during vegetation has been reported to increase antioxidant activity (Stratil *et al*., 2007; Marecek *et al*., 2017). Marecek *et al*. (2017) described a negative correlation between antioxidant activity and protein content indicating that soils with higher nitrogen level negatively influence the antioxidant activity. Apart from this impact of zinc fertilizer on antioxidant capacity has been reported in many studies. An increase in phenolic compounds formation was observed in stress environments due to zinc deficiency (Cakmak, 2008; Marecek *et al*., 2017). Genotype difference is 2nd most significant factor influencing antioxidant activity which is attributed to total phenolic compounds in general released during germination period from polysaccharides and protein by hydrolytic enzymes (Dvorakova *et al*., 2008; Zhao and Zhao, 2012). Suriano *et al*. (2018) reported 8.20-13.40 μmol TE g−1 dm DPPH-radical scavenging activity in 20 colored barley genotypes grown in Southern Italy. Gamel and Abdel-Aal (2012) observed 17-25 µmole g−1 DPPH scavenging capacity of whole grain barley flour of Egyptian and Canadian hulless cultivars.

**Total phenolic content (TPC)**

Statistical analysis regarding total phenolic content of different barley varieties indicated highly significant difference among varieties. The mean values for total phenolic content of different barley varieties have been displayed in Table 1. Total phenolic content in different varieties ranged from 69.2±1.66 mg GAE/100g to 177.8±5.68 mg GAE/100g with Haider-93 comprising maximum total phenolic content and MPT-V7 having minimum. Non-significant differences were observed in TPC among barley varieties procured from Sindh province (Upcoming-1, and Upcoming 3) however, barley variety Upcoming-2 comprised significantly high TPC (158.2±4.58 mg GAE/100g). Province wise variation in total phenolic content of barley varieties has been displayed in Fig. 1b. It was observed that total phenolic content in KPK varieties ranged from 69.2±1.66 mg GAE/100 g to 166.4±4.99 mg GAE/100 g. Among Punjab varieties the values of total phenolic content ranged from 137.0±3.83 mg GAE/100 g to 177.8±5.68 mg GAE/100 g. Barley varieties procured from Balochistan province comprised total phenolic content ranging from 132.0±3.82 mg GAE/100 g to 149.4±4.48 mg GAE/100 g. The total phenolic content in Sindh varieties ranged from 115.2±2.88 mg GAE/100 g to 158.2±4.58 mg GAE/100 g. Rakhshan-10 (149.4±4.48 mg GAE/100g) and MPT-V6 (166.4±4.99 mg GAE/100g) contained maximum TPC among barley varieties procured from Balochistan and KPK province.

In a study by Suriano *et al*. (2018) total phenolic content in 20 barley colored genotypes cultivated in Southern Italy ranged from 192.9 mg/100 g to 291.7 mg/100 g which is slightly higher than the findings of present study. Zhu *et al*. (2015) reported 333.9- 460.8 mg GAE/100 g total phenolic content in four highland barley varieties cultivated in China which is higher than the findings of present study. These differences are ascribed to difference in varieties, species, growing or agronomic conditions and analysis method. Holtekjølen *et al*. (2006a) reported 966-1873 µg/g total phenolic content in different barley varieties with hulless barley varieties having lowest and hulled varieties comprising high TPC. The study also observed high TPC in six row genotypes as compared to two row varieties which is in agreement with findings of present study. In a study by Legzdiņa *et al*. (2018) reported total phenolic content ranged from 98.78 mg GAE/100g to 140.13 mg GAE/100g in year 2011 and 81.66 mg GAE/100g to 110.04 mg GAE/100g in year 2012 among different covered and hulless barley genotype cultivated in Latvia which is in agreement with the findings of present study. The study stated a significant influence of cropping year on TPC of barley grain. Apart from this application of organic fertilizer in comparison to synthetic fertilizers significantly increases TPC as stated by Legzdiņa *et al*. (2018). Holtekjølen *et al*. (2011) reported 481-676 mg GAE/100 g dry matter (DM) TPC in whole grain flour of barley varieties grown in Norway.

**Free phenolic acids**

Phenolic acid are basically plant secondary metabolites and broadly present in plant kingdom. All barley varieties were characterized for 11 free phenolic acids (gallic acid, benzoic acid, m-coumeric acid, cinamic acid, caffeic acid, chlorogenic acid, syringic acid, p-coumeric acid, ferulic acid, vanillic acid and sinapic acid) and the results are discussed herein. Statistical analysis regarding free phenolic acids present in Pakistani grown barley varieties indicated a highly significant effect among different varieties. All the analyzed free phenolic acids differed substantially among different varieties and mean values have been displayed in Table 2.

In the present study gallic acid was detected as most abundant free phenolic acid in Pakistani grown barley varieties. The gallic acid in different barley varieties ranged from 1.11±0.01 ppm to 8.71±0.13 ppmwith Bajwar-2000 comprising maximum and Upcoming-1 containing minimum. Gallic acid in free form was not detected in barley variety AAJ-2013. Among KPK varieties gallic acid content ranged from 0 ppm to 8.71 ppm. Gallic acid content in Punjab varieties ranged from 1.94 ppm to 2.5 ppm. The values for gallic acid content ranged from 2.02 ppm to 2.16 ppm in Balochistan varieties. Among Sindh varieties gallic acid content ranged from 1.11 ppm to 2.53 ppm.

Benzoic acid in free form was only detected in MPT-V5, MPT-V6, Bajwar-2000, Sanober-96 and Rakhshan-10 with MPT-V5 comprising minimum (1.56±0.02 ppm) and Rakhshan-10 containing maximum (9.77±0.21 ppm) benzoic acid. Among KPK varieties benzoic acid content ranged from 0 ppm to 5.96 ppm. Among Balochistan varieties benzoic acid content ranged from 9.24 ppm to 9.77 ppm. Benzoic acid was not detected in Punjab and Sindh varieties.

The mean values of m-coumeric acid in Pakistani grown barley varieties ranged from 0.423±0.006 ppm to 0.916±0.01 ppm. The m-coumeric acid in free form was detected only in MPT-V2, MPT-V5, Jau-17, Sultan-17, Sanober-96 and Sadabahar. The highest m-coumeric acid was observed in MPT-V2 and lowest in MPT-V5. The values for m-coumeric acid content ranged from 0.423±0.006 ppm to 0.916±0.01 ppm in KPK varieties. Among Punjab varieties m-coumeric acid content ranged from 0.499 ppm to 0.619 ppm. m-Coumeric acid was only detected in Sanober-96 (0.44 ppm) in Balochistan varieties. Among varieties procured from Sindh province m-coumeric acid was only detected in Sadabahar (0.46 ppm).

The mean values for cinamic acid content in Pakistani grow barley varieties ranged from 2.46±0.04 ppm to 8.42±0.21 ppm. Cinamic acid was detected only in Jau-e-Paghmbari, MPT-V4, MPT-V5, MPT-V7, MPT-V9, MPT-V12, Sultan-17, Sanober-96, Sadabahr and Upcoming-2. The highest cinamic acid content was observed in Sadabahar and lowest in Jau-e-Paghmbri. Among KPK varieties cinamic acid content ranged from 2.46 ppm to 4.64 ppm. Among Sindh varieties cinamic acid content ranged from 5.18 ppm to 8.42 ppm. Cinamic acid was only detected in Sultan-17 (5.84 ppm) and Sanober-96 (3.86 ppm) among varieties procured from Punjab and Balochistan province, respectively.

The concentration of free caffeic acid in different barley varieties ranged from 0.35±0.005 ppm to 33.78±0.91 ppm. Caffeic acid in free form was detected in AAJ-2013, Bajwar-2000, MPT-V5, MPT-V6, MPT-V7, MPT-V9, MPT-V11, Sultan-17 and Rakhshan-10; with MPT-V6 containing minimum and Bajwar-2000 comprising maximum caffeic acid. Among KPK varieties caffeic acid content ranged from 0 ppm to 33.78 ppm. Caffeic acid was only detected in Sultan-17 (1.85 ppm) and Rakhshan-10 (1.17 ppm) among varieties procured from Punjab and Balochistan province, respectively. Caffeic acid was not detected in barley varieties procured from Sindh province.

Chlorogenic acid in free form was detected and quantified in Jau-e-Paghmbri, MPT-V5, MPT-V7, MPT-V9, MPT-V11, Sultan-17, Rakhshan-10 and Upcoming-1. The concentration of free chlorogenic acid ranged from 0.79±0.014 ppm to 13.65±0.36 ppm; with MPT-V5 containing minimum while Sultan-17 comprising maximum chlorogenic acid. Among KPK varieties Chlorogenic acid content ranged from 0 ppm to 9.41 ppm. Chlorogenic acid was only detected in Sultan-17 (13.65 ppm), Rakhshan-10 (5.24 ppm) and Upcoming-1 (4.79 ppm) among varieties procured from Punjab, Balochistan and Sindh province, respectively. Chlorogenic acid was reported among the major phenolic acids present in Korean dehulled barley varieties by Kim *et al*. (2007). Zhu *et al*. (2015) reported 6.09-13.63 mg/100 g chlorogenic acid in free form in different barley varieties.

Syringic acid content in Pakistani grown barley varieties ranged from 1.54±0.02 ppm to 3.46±0.06 ppm. The highest concentration of syringic acid was quantified in Upcoming-2 and lowest in Upcoming-1. Syringic acid in free form was detected only in Rakhshan-10, Upcoming-1 and Upcoming-2. Syringic acid was not detected in KPK and Punjab varieties. Syringic acid was only detected in Rakhshan-10 (2.34 ppm) in Balochistan varieties. Among Sindh varieties syringic acid content ranged from 1.54 ppm to 3.46 ppm. In a study by Andersson *et al*. (2008) detected 0.45-3.74 µg/g syringic acid in different barley varieties and the findings are in agreement with the results reported in present study.

The mean values for p-coumeric acid in Pakistani grown barley varieties ranged from 0.76±0.012 ppm to 3.84±0.055 ppm with Rakhshan-10 comprising lowest and MPT-V11 containing highest. The p-coumeric acid in free form was detected and quantified in MPT-V4, MPT-V9, MPT-V11, Haider-93 and Rakhshan-10. Among KPK varieties p-coumeric acid content ranged from 0.84 ppm to 3.84 ppm. p-coumeric acid was only detected in Haider-93 and Rakhshan-10 (2.34 ppm) among varieties procured from Punjab and Balochistan province, respectively. p-coumeric acid was not detected in varieties procured from Sindh province. The concentration of p-coumeric acid in free form in different barley varieties was reported to be 0.57-7.01 µg/g in a study by Andersson *et al*. (2008) which is higher than the findings of present study.

The concentration of ferulic acid in Pakistani grown barley varieties ranged from 2.57±0.05 ppm to 6.01±0.16 ppm with MPT-V12 comprising minimum and Upcoming-3 comprising maximum ferulic acid. Ferulic acid in free form was detected in MPT-V9, MPT-V11, MPT-V12, Bajwar-2000, Jau-e-Paghmbri, Haider-93, Jau-17 and Upcoming-3. Among KPK varieties ferulic acid content ranged from 2.96 ppm to 4.76 ppm. Ferulic acid in Punjab varieties ranged from 3.63 ppm to 4.27 ppm. Ferulic acid was only detected in Upcoming-3 (6.01 ppm) among Sindh varieties. Ferulic acid was not detected in varieties procured from Balochistan province. In a study by Zhu *et al*. (2015) it was observed that although ferulic acid is most abundant and predominant phenolic acid present in barley grains but it is only present in bound fractions. This observation is in agreement with the findings of present study as very low concentration of free ferulic acid was detected in few varieties and was not detected in most of the varieties analyzed in current study (Table 2). Andersson *et al*. (2008) reported 1.32-5.87 µg/g free ferulic acid in different barley varieties and the results are in agreement with the findings of present study.

The mean values for vanillic acid content of Pakistani grown barley varieties ranged from 0.17±0.003 ppm to 4.89±0.122 ppm. Vanillic acid in free form was detected only in AAJ-2013, MPT-V5, Sultan-17 and Sadabahar; with AAj-2013 comprising maximum while MPT-V5 comprising minimum concentration of free vanillic acid. Among KPK varieties vanillic acid content ranged from 0.17 ppm to 4.89 ppm. Vanillic acid was only detected in Sultan-17 and Sadabahar among varieties procured from Punjab and Sindh province, respectively. Vanillic acid was not detected in Balochistan grown barley varieties. Andersson *et al*. (2008) reported 1.45-4.71 µg/g vanillic acid in free form in barley varieties which is line with the findings of present study.

The concentration of free sinapic acid content in barley varieties ranged from 0.56±0.006 ppm to 3.07±0.073 ppm with MPT-V11comprising minimum and Upcoming-1 containing maximum concentration of sinapic acid. Sinapic acid was only detected in MPT-V6, MPT-V11, Sultan-17 and Upcoming-1. Among KPK varieties sinapic acid content ranged from 0.56 ppm to 1.01 ppm. Sinapic acid was only detected in Sultan-17 and Upcoming-1 among varieties procured from Punjab and Sindh province, respectively. Sinapic acid was not detected in Balochistan grown barley varieties. Andersson *et al*. (2008) reported that sinapic acid was not detected in free form in different barley varieties.

**Flavonoids (Quercetin)**

Barley varieties were analyzed for quercetin among different flavonoids present in them. Statistical analysis for quercetin content of different barley varieties indicated a highly significant difference among varieties. Mean values for quercetin content of barley varieties ranged from 0 ppm to 38.02±0.32 ppm as shown in Table 1. Quercetin was detected in all barley varieties except Upcoming-2 procured from Sindh province. The highest concentration of quercetin was observed in MPT-V6. Province wise variation in quercetin content of barley varieties has been shown in Fig. 1c. Among KPK varieties quercetin content ranged from 3.36±0.01 ppm to 38.02±0.32 ppm. The mean values for quercetin content among Punjab varieties ranged from 7.48±0.03 to 14.73±0.08 ppm. Quercetin concentration ranged between 6.02±0.02-7.79±0.03 ppm in Balochistan varieties. Among KPK varieties quercetin content varied from 0 ppm to 4.21 ppm. Overall barley varieties procured from Sindh province found to have poor concentration of quercetin. However, KPK and Punjab varieties comprised significantly higher quercetin content. Yang *et al*. (2013) studied flavonoids profile of normal barley, unhulled and hulled purple barley. The concentration of common flavonoids in different varieties varied substantially. The quercetin content in different varieties was recorded as 60.98 mg/g, 24.35 mg/g and 0.00 mg/g for hulled purple barley, unhulled purple barley and normal barley, respectively.

**Conclusion**

The present study has shown considerable variations in phytochemical profile of Pakistani grown barley varieties. Barley varieties Bajwar-2000, Jau-e-Paghmbri, MPT-V5, MPT-V6, MPT-V12, Rakhshan-10, Haider-93, Jau-17, Sultan-17, Upcoming-2 showed better phytochemical profile. Most of the free phenolics were not detected in MPT-V7 grown in KPK province and this variety presented low phytochemical profile as compared to others. Results from this preliminary study helped to screen barley varieties with higher phytochemical profile. Barley varieties with high phytochemical profile can be used as a potential source of bioactive compounds in barley based food products.

**Table 4.11: Phytochemical profile of Pakistani grown barley varieties**

|  |  |  |  |
| --- | --- | --- | --- |
| **Varieties** | **Antioxidant activity (DPPH)****(%)** | **Total phenolic content (TPC)****(mg GAE/100g)** | **Quercetin (ppm)** |
| AAj-2013 | 30.21±0.574h | 111.8±2.90g | 20.88±0.11b |
| Bajwar-2000 | 50.66±0.911f | 151.6±3.18c | 12.34±0.08e |
| Jau-e-Paghmbri | 89.39±2.324a | 133.2±3.72ef | 10.98±0.06f |
| MPT-V2 | 41.33±0.909g | 97.2±2.23h | 9.57±0.05g |
| MPT-V4 | 30.09±0.571h | 111.2±3.22g | 3.36±0.01o |
| MPT-V5 | 71.12±2.062b | 158.4±4.75bc | 5.91±0.02l |
| MPT-V6 | 59.18±1.301cde | 166.4±4.99ab | 38.02±0.32a |
| MPT-V7 | 39.33±0.983g | 69.2±1.66i | 6.48±0.03k |
| MPT-V9 | 30.12±0.602h | 94.2±2.35h | 6.57±0.03k |
| MPT-V11 | 40.92±0.859g | 137.2±3.84e | 12.71±0.07d |
| MPT-V12 | 69.14±1.936b | 139.2±4.03de | 4.62±0.01m |
| Haider-93 | 50.48±1.163f | 177.8±5.68a | 8.71±0.04h |
| Jau-17 | 60.87±1.765cd | 137.0±3.83e | 7.48±0.03j |
| Sultan-17 | 56.12±1.066e | 149.0±3.87cd | 14.73±0.08c |
| Sanober-96 | 41.44±0.953g | 132.0±3.82ef | 7.79±0.03i |
| Rakhshan-10 | 57.75±1.155de | 149.4±4.48cd | 6.02±0.02l |
| Sadabahar | 42.24±1.013g | 122.4±2.93fg | 4.21±0.01n |
| Upcoming-1 | 40.10±1.002g | 115.2±2.88g | 1.27±0.004q |
| Upcoming-2 | 62.38±1.746c | 158.2±4.58bc | **ND** |
| Upcoming-3 | 51.24±1.434f | 115.4±3.00g | 2.70±0.01p |

Means carrying different letters in a column are statistically significant (p<0.01)

**Table 4.13: Free phenolic acids (ppm) profiling of Pakistani grown barley varieties**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variety/line** | **Gallic acid** | **Benzoic acid** | **m-coumeric acid** | **Cinamic acid** | **Caffeic acid** | **Chlorogenic acid** | **Syrnigic acid** | **p-coumeric acid** | **Ferulic acid** | **Vanillic acid**  | **Sinapic acid** |
| AAj-2013 | ND | ND | ND | ND | 10.39±0.22b | ND | ND | ND | ND | 4.89±0.122a | ND |
| Bajwar-2000 | 8.71±0.13a | 5.96±0.13c | ND | ND | 33.78±0.91a | ND | ND | ND | 2.60±0.05g | ND | ND |
| Jau-e-Paghmbri | 3.98±0.04b | ND | ND | 2.46±0.04h | ND | 9.41±0.19b | ND | ND | 4.76±0.11b | ND | ND |
| MPT-V2 | 4.11±0.05b | ND | 0.916±0.01a | ND | ND | ND | ND | ND | ND | ND | ND |
| MPT-V4 | 1.27±0.01k | ND | ND | 2.81±0.05g | ND | ND | ND | 1.59±0.036c | ND | ND | ND |
| MPT-V5 | 1.14±0.01l | 1.56±0.02d | 0.423±0.006f | 4.64±0.1d | 0.48±0.007g | 0.79±0.014g | ND | ND | 2.26±0.04h | 0.17±0.003c | ND |
| MPT-V6 | 2.08±0.02gh | 5.80±0.10c | ND | ND | 0.35±0.005g | ND | ND | ND | ND | ND | 1.01±0.015c |
| MPT-V7 | 3.01±0.03d | ND | ND | 4.30±0.09e | 2.61±0.04d | 2.57±0.05f | ND | ND | ND | ND | ND |
| MPT-V9 | 2.26±0.02f | ND | ND | 3.63±0.07f | 0.5±0.008g | 5.46±0.12c | ND | 0.84±0.016d | 2.98±0.07f | ND | ND |
| MPT-V11 | 3.65±0.04c | ND | ND | ND | 5.88±0.12c | 5.07±0.11de | ND | 3.84±0.055a | 3.35±0.09e | ND | 0.56±0.006d |
| MPT-V12 | 1.96±0.01hi | ND | ND | 4.45±0.1de | ND | ND | ND | ND | 2.57±0.05g | ND | ND |
| Haider-93 | 2.47±0.03e | ND | ND | ND | ND | ND | ND | 3.52±0.091b | 4.27±0.11c | ND | ND |
| Jau-17 | 1.94±0.01i | ND | 0.619±0.01b | ND | ND | ND | ND | ND | 3.63±0.09d | ND | ND |
| Sultan-17 | 2.50±0.03e | ND | 0.499±0.004c | 5.84±0.14b | 1.85±0.03e | 13.65±0.36a | ND | ND | ND | 0.51±0.01b | 1.86±0.027b |
| Sanober-96 | 2.16±0.03fg | 9.24±0.17b | 0.44±0.007e | 3.86±0.07f | ND | ND | ND | ND | ND | ND | ND |
| Rakhshan-10 | 2.02±0.02hi | 9.77±0.21a | ND | ND | 1.17±0.01f | 5.24±0.1cd | 2.34±0.04b | 0.76±0.012e | ND | ND | ND |
| Sadabahar | 1.45±0.01j | ND | 0.464±0.008d | 8.42±0.21a | ND | ND | ND | ND | ND | 0.45±0.009b | ND |
| Upcoming-1 | 1.11±0.01l | ND | ND | ND | ND | 4.79±0.11e | 1.54±0.02c | ND | ND | ND | 3.07±0.073a |
| Upcoming-2 | 2.53±0.04e | ND | ND | 5.18±0.11bc | ND | ND | 3.46±0.06a | ND | ND | ND | ND |
| Upcoming-3 | 2.51±0.04e | ND | ND | ND | ND | ND | ND | ND | 6.01±0.16a | ND | ND |

Means carrying different letters in a column are statistically significant (p<0.01); **ND**: Not Detected

1. **DPPH (%) of barley varieties**
2. **Total phenolic content (TPC) of barley varieties**
3. **Quercetin content of different barley varieties**

**Fig. 1: Province wise variation in phytochemical profiling of Pakistani grown barley varieties (a) Antioxidant activity (b) Total phenolic content (c) Quercetin**

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