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Extraction and determination of bioactive compounds and antioxidant activity of buckwheat seed milling fractions

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Abstract

Buckwheat is a precious source of various bioactive compounds like phenolics, flavonoids, rutin, and quercetin, etc. This research work was performed to harvest the nutraceutical potential of indigenous buckwheat varieties and their milling fractions (fine flour, coarse flour, bran flour, and husk). Common buckwheat (CBW) and Tartary buckwheat (TBW) were analyzed in terms of total phenolic content (TPC), total flavonoid content (TFC), and DPPH scavenging activity. When compared to common buckwheat, Tartary buckwheat had a higher total phenolic content (2101.421 mg GAE/100 g), total flavonoid content (1233.990 mg QEQ/100 g), and DPPH scavenging activity (44.51%).In the same context, throughout comparisons among milling fractions, the highest TPC and TFC were observed in the husk part of Tartary buckwheat while the lowest was found in common buckwheat. During the comparison of different solvents and their concentrations, it was observed that Ethanol 70% extracted a greater quantity of phytochemicals as compared to the rest of the other solvents and concentrations. This study recognized variability among buckwheat varieties and milling fractions for nutraceutical potential and nutritional qualities that can be used in the treatment of different maladies and food products.`

Keywords: buckwheat; milling fractions; extraction; solvents; bioactive compounds.

Practical applications: As the findings demonstrated that buckwheat extract possess important bioactive compounds and antioxidants that may be very useful in the nutraceutical and pharmaceutical industries for the treatment of different types of maladies. Moreover, buckwheat plays a very important role in the maintenance of different nutritional disorders because it contains several important nutrients as well as functional ingredients.

1 Introduction

Buckwheat is traditional, wholesome, and nutritious, among many other pseudo-cereals. The two most common varieties of buckwheat, Tartary buckwheat (*Fagopyrum tartaricum*) and common buckwheat (*Fagopyrum esculentum*) are members of the Polygonaceae family and have long been recognized as nutritious foods. In recent years, the interest in buckwheat has been increasing because of its potential contribution to the sustainable nutritional, nutraceutical, and health benefits of human beings (Ohsawa, 2020).

Buckwheat contains a variety of bioactive compounds, including phenols, flavonoids, antioxidants, rutin, quercetin, and fagopyrin. Tartary buckwheat varieties have low retinoid action that plays a very important role in the retention of a high level of rutin in the buckwheat grain (Luthar et al., 2020; Morishita et al., 2007). Rutin is a very essential flavonol glycoside that has been recognized as a functional and beneficial food due to its antimicrobial, anti-inflammatory, anticancer, and antidiabetic characteristics, whereas quercetin is an aglycone produced later than enzymatic deprivation of rutin by rutinosidase, even as oral administration of quercetin is capable of traversing the blood-brain hurdle and gathering in brain tissue (Kawabata et al., 2015).

Rutin plays a very key role in the continuance of the fragility of blood vessels, and that may be very helpful in the protection of various hemorrhagic and hypertension diseases in humans (Jiang et al., 2007). Among cereal crops, only buckwheat possesses a large quantity of rutin, which can be utilized as a major source of dietary food (Kreft et al., 1999). Compared to both buckwheat varieties, the TBW grains have more rutin content as compared to the TBW grains (Fabjan et al., 2003). Antioxidant activity is a noteworthy characteristic that is beneficial for humans and has numerous biological functions, including anti-mutagenic, anti-carcinogenic, and anti-aging, derived from the antioxidant property (Holasova et al., 2002). Buckwheat bran has different layers, among them bran-aleurone, which consists of a large number of phenolic compounds as well as enormous antioxidant properties (Sun & Ho, 2005).

Buckwheat is gaining popularity due to its flavonoid content, which has functional and medicinal properties. Flavonoids play a

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very important role in the prevention of cancer, viral infections, and cardiovascular disease. Flavonoids are also recognized to reduce the cholesterol level in the blood and help in making capillaries and arteries fragile, strong, and flexible. That can minimize the risks of high blood pressure and arteriosclerosis (Li, 2016). The total flavonoid content of Tartary buckwheat is higher than that of common buckwheat. In the buckwheat grains, six flavonoids have been identified and isolated. All the six flavonoids (rutin, quercetin, vitexin, orientin, isoorientin, and isovitexin) were recognized in the hull of buckwheat grains (S.-q. Li & Zhang, 2001).

Keeping in view the above-mentioned facts, the current study was planned to extract and determined the bioactive compounds and antioxidant activity of buckwheat varieties and their seed milling fractions by using different chemical solvents with different concentrations.

2 Materials and methods

2.1 Sample collection

For this research work, buckwheat samples were collected from Skardu Gilgit-Baltistan, Pakistan with the help of the Agriculture Department Skardu, and transported to the Institute of Food and Nutritional Sciences, PMAS- Arid Agriculture University Rawalpindi for further process.

2.2 Tempering of buckwheat

Buckwheat grains were tempered in the clogged container and water was added to attain 16% moisture by adopting the procedure of (Morishita et al., 2007) with slight modification.

2.3 Milling of buckwheat

To obtain different milling fractions, buckwheat was properly cleaned and subjected to the Quadrumate Senior Mill at the National Agriculture Research Center (NARC), Islamabad. In total, four buckwheat fractions (fine flour, coarse flour, bran flour), including the husks, were obtained. Milling of the buckwheat sample was carried out by adopting the procedure of (Skrabanja et al., 2004). After milling, the buckwheat samples were analyzed for their phytochemical composition.

2.4 Extraction of bioactive compounds

The bioactive compounds were extracted by using different solvents like water, methanol, and ethanol, separately at different levels or concentrations (50, 60, and 70%) and analyzed subsequently following the method developed by (Stankovic, 2011). Solvents and their concentrations are presented in Table 1.

Table 1. Solvents and their concentrations used for the extraction of bioactive compounds.

Solvents	Concentrations	Sample
Ethanol	70%	BW
Ethanol	60%	BW
Ethanol	50%	BW
Methanol	70%	BW
Methanol	60%	BW
Methanol	50%	BW
Water	----	BW

2.5 Total Phenolic Content (TPC)

The total phenolic content of buckwheat samples was evaluated using the Folin-Ciocalteiu reagent method developed by (Al-Farsi et al., 2005). In the first step, 1.5 mL of ten-fold diluted Folin-Ciocalteu reagent was mixed with 200 mL of extract and kept at room temperature for 5 minutes before adding 1.5 mL of aqueous Na2CaO3 (60G/L) and leaving the mixture for 90 minutes. After that, a UV visible spectrophotometer was used to measure the absorbance of all samples at 725 nanometers. Buckwheat extract TPC is measured in GAE/100 g (Galic acid equivalent).

2.6 Total Flavonoid Content (TFC)

The total flavonoid content of buckwheat samples was determined with the help of standard procedure of (Kim et al., 2004). Initially, 0.3 mL sodium nitrate solution (5%) was added to 1mL of each diluted extract (1:4 mL water), pursued by 0.3 mL aluminum chloride (10%) solution, and the test tubes were incubated for 5 minutes at 28 after the addition of 2 mL of 1 M hydroxide and the volume of the solution was made up to 10 mL by adding distilled water (10 mL), the absorbance of the mixture was determined at 510 nm using a UV visible spectrophotometer. The results were expressed as mg QEC/100 g.

2.7 Antioxidant activity

The DPPH solution was prepared for the determination of the antioxidant activity of buckwheat samples according to the method of (Brand-Williams et al., 1995). In brief, 1 mL of DPPH solution (7.8 DPPH in 100 mL methanol) and 1 mL extract of each buckwheat sample were mixed through many shakings and the mixture was kept for 30 minutes in a dark place at room temperature. The absorbance of the extracted mixture was then measured with a spectrophotometer at 517 nm. The absorbance was used to calculate antioxidant activity as a percentage of DPPH by using the formula given below (Equation 1):

$$DPPH\ (\%) = \mathrm{Ao} - \mathrm{Al} \div \mathrm{Ao} \times 100 \quad (1)$$

2.8 Statistical analysis

All these experiments were performed three times and the results of these measurements were reported as means and standard deviation. The data obtained from the study were statistically analyzed by using Minitab version-16 software. Means were compared by using the LSD (least significant difference) test at a 0.05% level of probability as described by Steel & Torrie (1997).

3 Results and discussion

The bioactive compounds of all buckwheat varieties and their milling fractions were extracted with different solvents,

water, methanol, and ethanol, separately at different levels or concentrations and analyzed subsequently. The results regarding DPPH, total phenolic, and total flavonoid contents are presented and discussed here:

3.1 Total antioxidant activity

Buckwheat is a good source of antioxidants and plays an important role in the body's health maintenance. The antioxidant activity of different buckwheat and their milling fractions was determined by using the standard DPPH method. The results have shown the significant effect of buckwheat varieties, fractions, solvent concentration, and their relevant interactions on antioxidant activity. The main effects on buckwheat varieties revealed that Tartary buckwheat contained a higher quantity of antioxidant activity in an extract prepared with 70% ethanol (44.51%), whereas the common buckwheat variety had a lower scavenging activity in an extract prepared with 70% (34.47%). In the same way, the Tartary buckwheat extract prepared with pure water contained the maximum quantity of antioxidant activity (17.18%) while the minimum value (15.02%) was found in the common buckwheat extract prepared with pure water (Figure 1).

Similarly, significantly ($p < 0.05$), the highest DPPH scavenging activity was observed in Tartary buckwheat husk extract prepared with 70% ethanol (25.93%) while the lowest was found in the fine flour of common buckwheat (11.67%). In the same context, the Tartary buckwheat fine flour extract prepared with pure water contained a minimum quantity of antioxidant activity (3.59%) compared to the rest of the other fractions (Figure 2).

Similarly, significant variations in common buckwheat milling fractions were found for DPPH scavenging activity. Significantly ($p < 0.05$), the highest DPPH scavenging activity was observed in a common buckwheat husk extract prepared with 70% ethanol (22.59%). In the same context, the common buckwheat fine flour extract prepared with pure water contains a minimum quantity of antioxidant activity (2.92%) (Figure 3).

The findings are in close conformity with the results of (Cao et al., 2008), who reported that Tartary buckwheat contained a higher quantity of antioxidant capacity as compared to common buckwheat wheat. The findings are also consistent with those of (Beitāne et al., 2018), who discovered that the buckwheat flour samples contained DPPH scavenging activity ranging from 21.067 to 22.644 mM TE 100 g^{-1} dry matter. The buckwheat sample contained higher DPPH radical scavenging activity as compared to wheat flour, and the total antioxidant activity ranged from 25.61 mM TE 100 g^{-1} DM for white buckwheat flour to 27.17 mM TE100 g^{-1} DM for raw buckwheat flour (Guo et al., 2011).

3.2 Total phenolic content

The total phenolic content of different buckwheat varieties and their milling fractions were determined. The results have shown the significant effect of buckwheat varieties, fractions, solvent concentration, and their relevant interactions on the total phenolic content. The main effects on buckwheat varieties revealed that Tartary buckwheat contained a higher quantity of total phenolic content in an extract prepared with 70% ethanol (2101.421 mg GAE/100 g), whereas the common buckwheat variety had a higher total phenolic content in an extract prepared with 70% (1980.586 mg GAE/100 g). In the same way, the Tartary buckwheat extract prepared with pure water contained

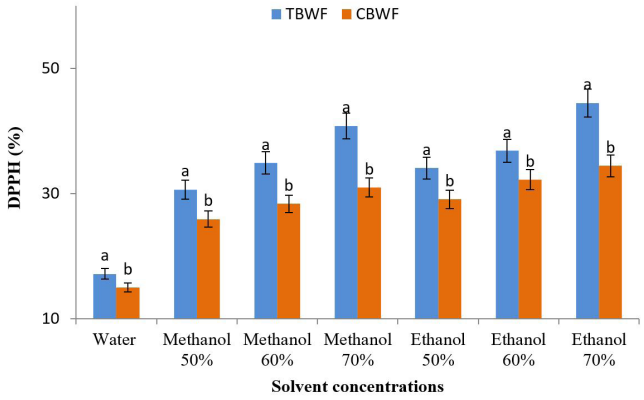

Figure 1. Mean values for DPPH (%) of buckwheat varieties.

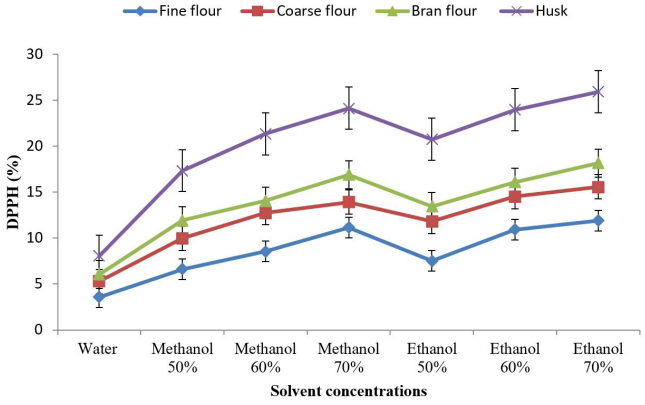

Figure 2. Mean value for DPPH (%) of Tartary buckwheat milling fractions.

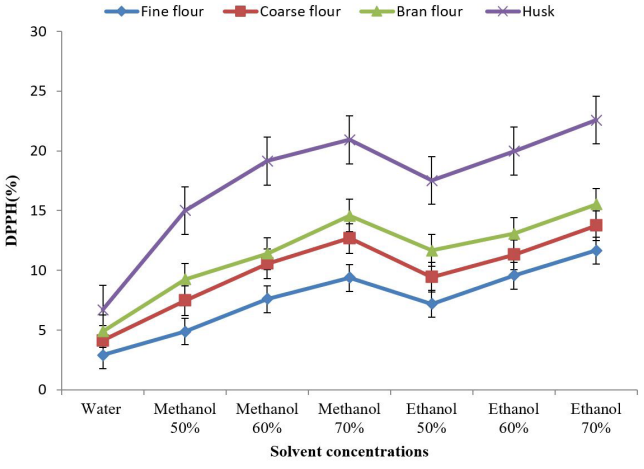

Figure 3. Mean value for DPPH (%) of Common buckwheat milling fractions.

the maximum quantity of total phenolic content (842.146 mg GAE/100 g) while minimal value (704.710 mg GAE/100 g) was found in common buckwheat extract prepared with pure water (Figure 4).

Similarly, significantly ($p < 0.05$), the highest total phenolic content was observed in Tartary buckwheat husk extract prepared with 70% ethanol (1024.186 mg GAE/100 g). The lowest total phenolic content (155.225 mg GAE/100 g) was found in the Tartary buckwheat fine flour extract prepared with pure water (Figure 5). Similarly, significant variations in common buckwheat milling fractions were found for the total phenolic content. Significantly ($p < 0.05$), the highest total phenolic content was observed in common buckwheat husk extract prepared with 70% ethanol (907.702% mg GAE/100 g). The lowest total phenolic content (110.236 mg GAE/100 g) was found in the common buckwheat fine flour extract prepared with pure water (Figure 6).

Our results are closely in conformity with the findings of (Izydorczyk et al., 2014), who reported that the Tartary buckwheat contained a higher quantity of total phenolic content as compared to the common ones. All buckwheat samples had a higher total phenolic content than wheat flour and the highest total phenolic content (974.74 mg GAE/100 g DW) was observed in raw buckwheat flour (Beitāne et al., 2018). Tartary buckwheat contained two times higher amounts of total phenolic than common buckwheat. These variations may be due to varietal and environmental effects (Cao et al., 2008).

3.3 Total flavonoid content

The total flavonoid content of different buckwheat varieties and their milling fractions were determined. The results have shown the significant effect of buckwheat varieties, fractions, solvent concentration, and their relevant interactions on total flavonoid content. The main effects on buckwheat varieties revealed that Tartary buckwheat contained a higher quantity of total flavonoid content in an extract prepared with 70% ethanol (1233.990 mg QEQ/100 g), whereas the common buckwheat variety had a lower total flavonoid content in an extract prepared with 70% (1088.617mg QEQ/100 g). In the same way, the Tartary buckwheat extract prepared with pure water contained the maximum quantity of the total flavonoid content (261.279 mg QEQ/100 g), while the minimum value (214.681 mg QEQ/100 g) was found in the common buckwheat extract prepared with pure water (Figure 1S).

Similarly, the Tartary buckwheat husk extract prepared with 70% ethanol had the highest total flavonoid content (734.892 mg QEQ/100 g), while the Tartary buckwheat fine flour extract prepared with pure water had the lowest total flavonoid content (122.829 mg QEQ/100 g) (Figure 2S). Similarly, significant variations in common buckwheat milling fractions were found for the total flavonoid content. Significantly ($p < 0.05$), the highest total flavonoid content was observed in common buckwheat husk extract prepared with 70% ethanol (693.110 mg QEQ/100 g). The lowest total flavonoid content (103.403 mg QEQ/100 g) was found in the common buckwheat fine flour extract prepared with pure water (Figure 3S).

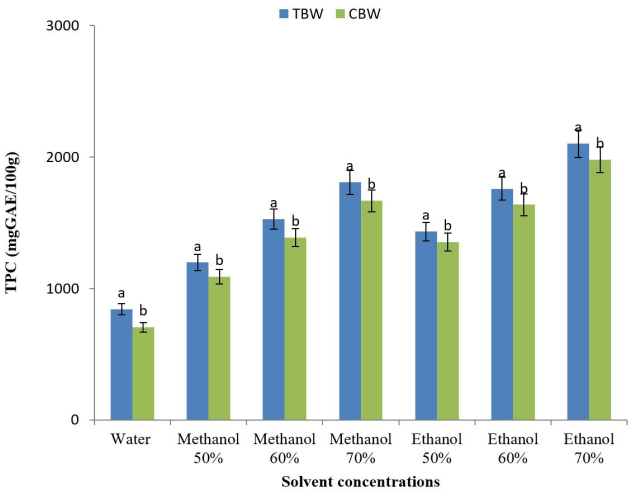

Figure 4. Mean value for (mg GAE/100 g) of buckwheat varieties.

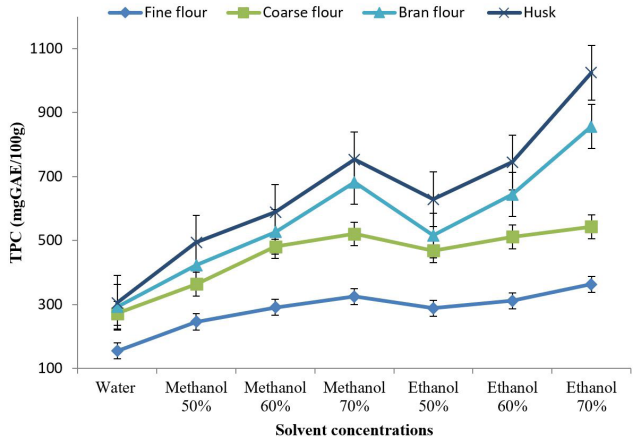

Figure 5. Mean value for TPC (mg GAE/100 g) of Tartary buckwheat milling fractions.

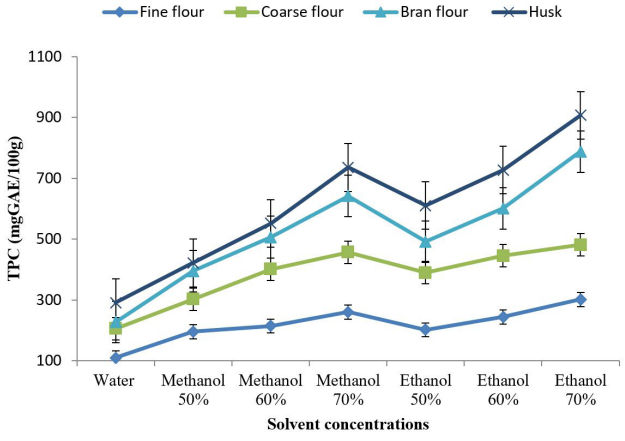

Figure 6. Mean value for TPC (mg GAE/100 g) Common buckwheat milling fractions.

The findings are consistent with those of (Izydorczyk et al., 2014), who discovered that Tartary buckwheat groats contained 40-60 times more total flavonoid content than other varieties

of buckwheat. Similarly, (Uddin et al., 2013) also reported that the total flavonoid content of buckwheat may greatly depend on the cultivar. All buckwheat samples had higher total flavonoid content than wheat flour and the highest total phenolic content (495.31 mg CE/100 g DW) was observed in raw buckwheat flour (Beitāne et al., 2018).

3.4 Conclusions

The results showed that both common and Tartary buckwheat contains a high concentration of bioactive compounds, implying that buckwheat has excellent nutraceutical and medicinal properties. Furthermore, comparative analysis between buckwheat varieties indicated that the Tartary buckwheat contains higher bioactive compounds and antioxidant activity as compared to common buckwheat. All buckwheat seed milling fractions of both varieties were found to have high-quality bioactive compounds and antioxidant activity but the Tartary buckwheat husk extract prepared with 70% ethanol has higher levels of a bioactive compound and antioxidant activity as compared to the rest of buckwheat seed milling fractions.

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Supplementary Material

Supplementary material accompanies this paper.

Figure 1S. Mean value for TFC (mg QEQ/100 g) of buckwheat varieties

Figure 2S. Mean value for TFC (mg QEQ/100 g) of Tartary buckwheat milling fractions

Figure 3S. Mean value for TFC (mg QEQ/100 g) of Common buckwheat milling fractions

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