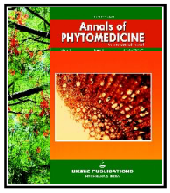
Annals of Phytomedicine 10(2): 354-359, 2021

DOI: http://dx.doi.org/10.21276/ap.2021.10.2.47

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Effects of extraction solvents on cultivars of wheat (PBW-154 and HD-2967) bran antioxidant properties

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Article Info

Article history

Received 10 October 2021

Revised 26 November 2021

Accepted 29 November 2021

Published Online 30 December 2021

Keywords

Wheat bran

Dietary fiber

Solvent polarity

Aantioxidant properties

Abstract

Wheat is an important source of dietary fiber and natural antioxidant. Two cultivars of wheat bran (PBW-154 and HD-2967) were analyzed for their proximate, dietary fibre and antioxidant property. The results indicated that carbohydrates ranged from 75-78.2%, proteins 11.4-13.2%, fats 2.4-2.7% in the wheat bran of different cultivars, and dietary fiber content was between 46.7- 48.2 %. Effect of the uses of different organic solvents (acetone, ethanol and methanol) at various concentrations (50%, 80% and 100%) on the antioxidant properties (total phenolic content, total flavonoid content, free radical scavenging activity (DPPH) and ferric reducing antioxidant power) of selected cultivars of wheat bran extract (PBW-154 and HD-2967) were analyzed by their respective methods. Both cultivars of wheat bran exhibited the maximum value of antioxidant properties at 50% concentration of solvents followed by 80% and 100% concentrations of wheat bran extract. The obtained antioxidants compound and properties were in the following order (from high to low): 50% ethanol > 50% methanol > 50% acetone > 70% ethanol > 70% methanol > 70% acetone > 100% ethanol > 100% acetone > 100% methanol > water for all the selected cultivars of wheat bran and the statistically significant difference was observed at the level of $p \geq 0.05$. The study showed that the extraction of antioxidant of selected cultivars of wheat bran in different organic solvents have significantly different antioxidant properties from each other which might be due to the polarity of extracting solvents and solubility of the antioxidants from wheat bran.

1. Introduction

As a byproduct derived from roller milling of wheat flour production, wheat bran has high dietary fiber content, which contains 44-50% of fiber and can be incorporated into food products to alter the nutritional quality of foods (Onipe *et al*., 2015). Wheat (*Triticum aestivum* L.) is an important raw material in many countries. The grain is composed of a nutritious inner part, the starchy endosperm, and surrounded by numerous layers of pericep, testa and nucellar epidermis. Processing of wheat separates starchy endosperm from embryo and bran layers into flour and this flour is the base of cereal products. The bran which is obtained as a byproduct of milling is significant source of dietary fiber, tocopherol and bioactive compounds. Several health benefits such as reduced risk of diabetes, degenerative and chronic diseases are well established with the consumption of wheat bran (Wu *et al*., 2015; Mellen *et al*., 2009). Bakery products can be supplemented wheat bran for its improved the nutritional and functional value.

Plant antioxidant composites are commonly isolated with solvent extraction method. However, antioxidant activities and extract yields of plant matrix are correlated with the type of recovery solvent, occurrence of diverse antioxidant compounds and polarities of solvent. Polar solvents are repeatedly used for the efficient recovery of polyphenolics from a plant matrix. The commonly employed solvents are aqueous mixtures of organic solvent such as ethanol, methanol, acetone, and ethyl acetate. Methanol and ethanol have been widely used for the recovery of bioactive compounds from various agricultural produce (Aggarwal *et al*., 2020; Sharma and Chakraborty, 2019; Rehman, 2006). Barley flour phenolic compounds were extracted with ethanol and acetone (Bonoli *et al*., 2004), whereas maximum rice bran phenolics were recovered from aqueous methanol (Chatha *et al*., 2006). Similarly, 80% methanol was employed for obtaining complex antioxidants from cereal bran (rice and wheat), oat groats and hull (Anwar *et al*., 2006). Wheat, wheat bran and it's based and supplemented food products were subjected to numerous solvent systems combined with different procedures for the recovery of antioxidants. Extraction efficiency of antioxidants is significantly influenced by number of factors, *viz*., particle size of sample, solvent to sample ratio, time, temperature and composition of solvent (Kim *et al*., 2006; Silva *et al*., 2007). The aim of the work was to examine the effect of three solvents: acetone, ethanol and methanol with different polarities on the antioxidant properties of cultivars of wheat bran.

2. Materials and Methods

The two cultivars of wheat bran, *i.e.*, PBW-154 and HD-2967 cultivars were procured from the seed store Alopibag Chungi, Prayagraj, U.P.

2.1 Proximate analysis

The proximate composition, *viz*., moisture, fat, protein, ash, and crude fiber was done by using standard methods of analysis (AOAC, 2016). Carbohydrate content was analyzed using different methods.

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2.2 Dietary fiber

Dietary fiber of wheat bran was observed according to the enzyme-gravimetric methods (AACC, 2000).

2.3 Antioxidant properties

2.3.1 Method of extraction of antioxidant

Cultivars of wheat bran were isolated by using three solvents (ethanol, methanol and acetone) at different levels (50%, 80% and 100%). One gram of wheat bran was extracted with 5 ml of solvents in a screw-capped tube in the dark condition at ambient temperature for 24 h and then centrifuged 2000 rpm for 5 min. The collected supernatant was used for further analysis (Moore *et al*., 2006).

2.3.2 Total phenolic content

The total polyphenolic content of the aqueous methanolic extract of wheat bran was done with the 'Folinciocalteu method'. One ml-aliquot of the sample extract was taken in a test tube and mixed 5 ml. of diluted FC reagent and 4 ml 7.5 % sodium carbonate solution. Soon after mixing, the test tubes were placed in the dark for 60 min at ambient temperature and the absorbance was monitored by UV-VIS spectrophotometer (model Evolution 600) at 765 nm against blank as standard. A standard curve was prepared with "Gallic acid" and results were expressed in terms of mg/100 g of polyphenol present in the sample. Samples were analyzed in triplicates and mean was calculated (Matthaus, 2002).

2.3.3 Total flavonoids content (TFC)

The total flavonoids content was analyzed by Boetang *et al.* (2008). Diluted extract (2.0 ml) was added to 150 µl of 5% sodium nitrite and the mixture was allowed to stand for 5 min. Further, 150 µl of 10 % aluminium chloride was mixed and the mixture was kept for 10 min. After that, 1.0 ml of 1M sodium hydroxide and 1.2 ml of distilled water was add on to the solution and mixed well. The absorbance was taken at 510 nm on UV spectrophotometer. Quercetin was used for the standard curve construction (0.05 to 0.5 mg/ml). The results were demonstrated as mg quercetin equivalent (QE)/g of flour.

2.3.4 Per cent free radical scavenging activity (DPPH activity)

The DPPH (2,2-diphenyl 1-pycril hydrazil) radical scavenging activity of wheat bran extracts was measured according to the method given by Sanja *et al.* (2009) with slight modification. 3 ml of extracts of cereal bran of each solvent was mixed with 2 ml of 0.15 mM methanolic DPPH solution. The mixture was shaken and decrease in absorbance was measured at 515 nm with the help of UV/VIS spectrophotometer after 15 min incubation at room temperature. DPPH solution was used as control.

Control use methanol as blank solution.

$$\% \text{ free radical scavenging activity} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

2.3.5 Ferric reducing antioxidant power assay

200 µl of solvent extract of each sample was mixed with 1.3 ml of the FRAP reagent. FRAP reagent, consisted of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mMHCl and 20 mM FeCl3 in a ratio of 10:1:1 (v/v/v). After mixing with FRAP reagent, samples were incubated for 30 min at 37°C and then absorption was measured against blank at 595 using a spectrophotometer. FRAP values, expressed as mmol of Fe (II) equivalent per g flour (Sutharut and Sudarat, 2012).

2.4 Statistical analysis

The data collected from the proximate, dietary fiber and antioxidant analyses were presented as means of triplicates. LSD values were calculated for significant data to find significant difference in proximate composition and dietary fiber. The solvent data were subjected to one-way analysis of variance using SPSS statistical software version 20.0. The mean was separated by applying the Duncan Multiple Range test at 95% confidence level (p<05).

3. Results

3.1 Proximate composition

Proximate analysis of wheat bran which was generated during the milling process showed that, this ingredient has important nutritional value since it is rich in fiber and protein, while fat and ash as minor components. The proximate composition of two different wheat cultivars bran (PBW-154 and HD-2967) is presented in Table 1.The proximate composition varied significantly (p<0.05) among both cultivars of wheat bran. The ash content, moisture, protein, crude fat and crude fibre contents of wheat bran (HD-2967 and PBW-145) ranged from 3.0 to 3.2%; 4.77 to 6.2%; 11.4 to 13.2%; 2.7 to 2.4%; 7.3 to 10.2%, respectively. In PBW-154 cultivar of wheat bran has the highest value of chemical composition compared to HD-2967 cultivar of wheat bran. Ranhotra *et al.* (1994) reported the ash content of wheat bran was in range from 5.2 to 5.5%. Protein content of wheat bran was 14 to 16% as reported by Halverson and Zeleny (1988). Tian *et al.* (2020) reported that the proximate composition (protein, fat, ash) of wheat bran that was in range from 17.66 to 17.81; 2.89 to 4.16%; and 17.58 to 24.74%. The ash content of selected wheat bran cultivars was similar as descibed by D'hoe *et al.* (2018). Similar results were reported for fat percentage of wheat bran by Curti *et al.* (2013). Elawad, *et al.* (2016) reported moisture, fat, fiber, and carbohydrate contents which were 7.8%, 5.6%, 9.6%, 61.3% in wheat bran, respectively. These compositions were found close to the existing value. Thus, the results of proximate analysis of present study are in line with previous studies. The variation in each nutritional content among all cultivars of wheat bran is due to environmental related factors like maturity period, climate, location, temperature, fertility, diseases, pest exposure, climate, soil condition, *etc*. (Zheng and Wang, 2001).

3.2 Dietary fiber

Dietary fiber is resistive to enzymatic digestion which typically includes non-starch polysaccharides (cellulose, hemicellulose), oligosaccharides (pectic substances, gums, mucilages) and a lignin. Dietary fibre content of selected cultivars of wheat bran (PBW-154 and HD-2967) were analyzed and the results are given in the Table 2. Dietary fibre values was found significantly (p<0.05) different among both cultivars of wheat bran. The soluble dietary fibre, insoluble dietary fibre and total dietary fibre contents of wheat bran (HD-2967 and PBW-145) ranged from 4.8 to 5.7%, 41.9 to 42.5% and 46.7 to 48.2%, respectively. In PBW-154 cultivar of wheat bran has the higher value of dietary fibre content compared to HD-2967 cultivar of wheat bran.Importance of consuming dietary fiber has increased owing to its relation with the reduction of blood cholesterol level,

lower inulin demand and improved laxative properties. The recommended daily intake of total dietary fiber is ranges from 30 to 38 g/day for male and 21 to 26 g/day for female (Gomez *et al.*, 2013). Insoluble dietary fibers helping human well being by stimulating the growth of the intestinal microflora, increase the fecal execration and reducing the intestinal transit (Gomez *et al.*, 2011). Carson and Edwards (2009) reported the dietary fibre values of the whole wheat ranges from 11.6% - 12.7% dry weight.

Table 1: Proximate composition of wheat bran of selected cultivars HD-2967 and PBW-154

Cultivars of wheat bran	Moisture (%)	Protein (%)	Fat (%)	Crude fiber (%)	Ash content (%)	Carbohydrates (%)
PBW-154	$6.20^{b} \pm 0.4$	$13.2^{b} \pm 0.5$	$2.4^{a} \pm 0.3$	$10.2^{b} \pm 0.3$	$3.2^{b} \pm 0.1$	$75.0^{b} \pm 2.1$
HD-2967	$4.77^{a} \pm 0.5$	$11.4^{a} \pm 0.5$	$2.7^{a} \pm 0.2$	$7.3^{a} \pm 0.4$	$3.0^{a} \pm 0.2$	$78.2^{a} \pm 1.2$

Values with different superscript in column are significantly (p<0.05) different.

Table 2: Dietary fibre content of wheat bran of selected cultivars (HD-2967 and PBW-154)

Sample	Soluble dietary fiber (%)	Insoluble dietary fiber (%)	Total dietary fiber (%)
PBW-154	$5.7^{b} \pm 0.2$	$42.5^{a} \pm 1.5$	$48.2^{b} \pm 2.1$
HD-2967	$4.8^{a} \pm 0.2$	$40.4^{b} \pm 1.0$	$45.2^{a} \pm 1.1$

Values with different superscript in column are significantly (p<0.05) different.

3.3 Effect of extraction solvents on antioxidants compounds (TPC and flavonoids) and antioxidant activity (DPPH and FRAP) from bran of wheat cultivars

The polyphenolics form complexes with other molecules like polysaccharides, protein, pigment such as anthocyanin and chlorophyll. Hence, for the recovery of polyphenolic compounds, appropriate solvent is required which helps in maximum dissolution of these compounds (Michalak *et al.*, 2017). In the present study, different solvent extracts were used for extraction of total phenolics and differences in values were observed with increased in the polarity. The isolation of polyphenolics from different food sample is subjective to the polarity of extracting solvents and solubility of these compounds in the solvent (Nawaz *et al.*, 2020).

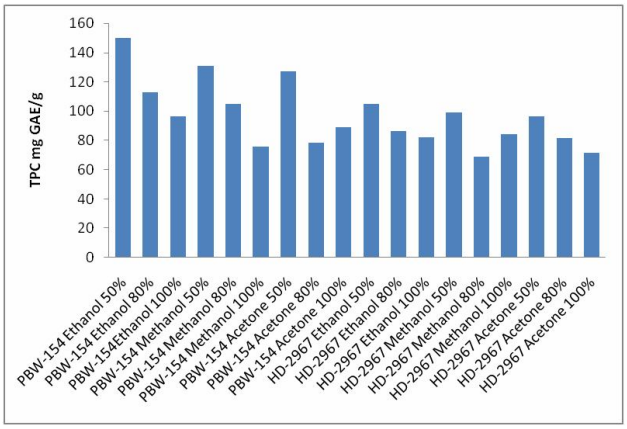

Figure 1: Effect of solvents on total phenolic content of cultivars of wheat bran (PBW-154 and HD 2967).

The obtained TPC values were in the following order (from high to low): 50% ethanol > 50% methanol > 50% acetone > 70% ethanol > 70% methanol > 70% acetone > 100% ethanol > 100% acetone > 100% methanol > water for all selected wheat bran cultivars and the statistical significant difference was observed at the level of p>0.05. The total phenolic content of wheat cultivars in different solvents at various concentrations of extracts varied from 71.3 to 150 GAE mg/g (Figure 1). The highest total phenolic was obtained with 50% ethanol solvents in cultivar of PBW-154 wheat bran, followed by methanol and acetone at 80% and 100% concentration, respectively.

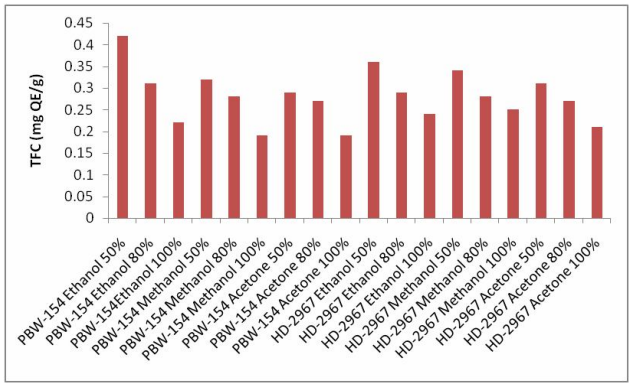

Figure 2: Effect of solvents on total flavonoid content of cultivars of wheat bran (PBW-154 and HD 2967).

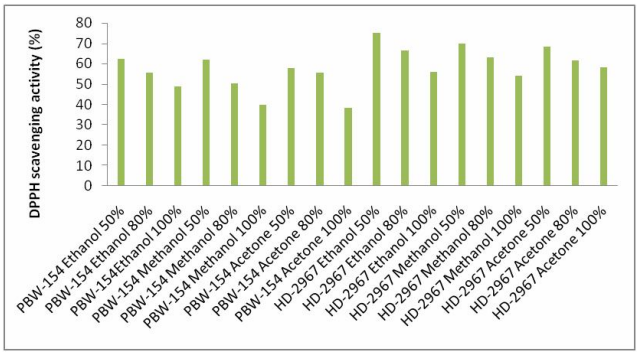

Figure 3: Effect of solvents on DPPH scavenging activity % of cultivars of wheat bran (PBW-154 and HD 2967).

Flavonoids are significant secondary metabolites of plant which include condensed tannins, flavanols and flavones. Various studies advise that the consumption of flavonoid-rich foods guard humans against diseases associated with oxidative stress**.** In order to estimate the potential effect of solvent on the amount of TFC, the wheat bran was subjected to different solvents for the extraction of flavanoids. For both the wheat cultivars, significant difference (p<0.05) in total antioxidant activity was analyzed between the different solvents. These results showed that potential influence of extracting solvents

on total flavonoid content for the tested wheat bran extract. The range of TFC in wheat bran cultivars at different solvents was ranged from 0.19 to 0.42 mg Eqv/g for wheat bran. The results displayed that 50% ethanol containing higher level of flavonoids than methanol and acetone (Figure 2). The TFC values were highest for cultivar PBW-154 (0.42 mg Eqv/g of bran) at 50% ethanol, followed by methanol and acetone at 80% and 100% solvents. Results showed that 50% ethanol was the best medium for the extraction of flavonoid content. The statistical significant (p<0.05) difference in TFC value of wheat bran in different solvents is obtained and ranged from 2.78 to 22.04 mg QCE/g in decreasing order, *i.e.*, 50 % ethanol > 50% methanol > 50% acetone > 80% ethanol >80% methanol > 80% acetone > 100% ethanol > 100% methanol > 100% acetone > water. Present analyzed results are in harmony with those of Safaa *et al.* (2014) who revealed that TFC content was found maximum in 50% ethanol extract of wheat bran.

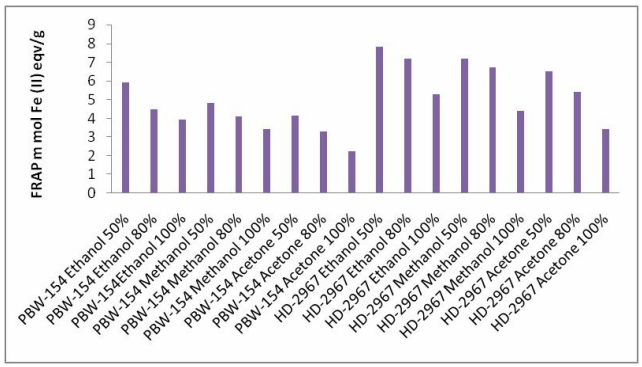

Figure 4: Effect of solvents on FRAP values of cultivars of wheat bran (PBW-154 and HD 2967).

It is usually linked that free radicals formed in the body raised the incidence of degenerative diseases and cancers. Dietary antioxidants help in prevention of chronic illness by reactive oxygen species. Therefore, it is important to determine the radical scavenging effect of antioxidants in cereals. DPPH radicle has the ability to scavenge reactive oxygen species such as hydrogen peroxide, hydroxyl radical and superoxide radical anion. Effect of extracts of both cultivars of wheat bran (PBW-154 and HD-2967) at different concentration of solvents is presented in Figure 3. The DPPH values were statistically (p>0.05) highest in HD-2967 at 50% concentration of ethanol. Results showed a decreasing order in % DPPH activity, *i.e.*, 50 % ethanol > 50% methanol > 50% acetone > 80% ethanol >80% methanol > 80% acetone > 100% ethanol > 100% methanol > 100% acetone > water. The above results were supported by Safaa *et al.* (2014), who found maximum radical DPPH activity of wheat bran in 70% ethanol extract as compared to methanol and acetone extract.

DPPH activities of both cultivars of wheat bran extracts at different concentrations in various solvents showed the similar trend in results as it was seen in TPC and TFC content. Percentage of DPPH activity of both cultivars of wheat bran was found between 38 to 75%. Results showed that the extracting solvent, cultivars and percentage of solvents and water significantly (p<0.05) altered the antioxidant property of all the selected wheat bran cultivars. For both the wheat varieties, significant difference (p<0.05) in total antioxidant activity was observed between the different solvents (Figure 3). These results are in harmony with Safaa *et al.* (2014) and D. Lope *et al.* (2019). The above results were supported by those of Safaa *et al.* (2014), who found maximum radical DPPH activity % of wheat bran in 70% ethanol extract as compared to methanol and acetone extract.

The FRAP values of the extracts are presented in Figure 4, for both cultivars of wheat bran (PBW-154 and HD-2967). The maximum antioxidant activity was found in cultivar HD-2967 wheat bran at 50% ethanol, followed by methanol and acetone at 80% and 100% concentration. The FRAP values of cultivars of wheat bran extracts are ranked as follows: 50 % ethanol > 50% methanol > 50% acetone > 80% ethanol >80% methanol > 80% acetone > 100% ethanol > 100% methanol > 100% acetone > water. The values of FRAP activity differ significantly (p<0.05) in wheat bran in different solvents of various concentrations and was ranged from 2.25 to 7.8 m M Fe(II)/ /g. Minimum FRAP values were reported in cultivar of PBW-154 wheat bran at 100% concentration of acetone 2.25 m Mol (Fe(II) eqv)/g. Results presented that the isolating solvent, concentration and cultivars was significantly (p<0.05) altered the FRAP assay estimations of both cultivars of wheat bran (Figure 4). Brewer *et al.* (2014) studied the Ferric reducing antioxidant power in different particle size of wheat bran which ranges from 8.93 m Mol (Fe (II) eqv)/g to 23.84 m Mol (Fe (II) eqv)/g. These values are similar to present study.

4. Discussion

Cereals contain variety of compounds showing antioxidant properties. Different methods have been developed to determine the antioxidant property of different cultivars of wheat bran (Moore *et al.*, 2006). The recovery of the compounds is dependent on types of solvent and on the solubility of the antioxidant compounds in solvents used for the extraction. Thus, the isolation of polyphenolic compounds is reliant on the solvents of different polarities. The difference in the isolation efficiency of antioxidants and bioactive compounds from wheat bran might be due to diverse obtainability of extractable components because of its varied chemical components of the cereals. The amount of the antioxidant components that can be extracted is mainly affected by the cultivars, environmental factor, cropping, harvesting, *etc*, which probably may vary from sample-to-sample. Solvent such as aqueous ethanol are mostly used for the extraction of phenolic compounds from plant matrix (Patthamakanokporn *et al.*, 2008; Mc-Cook Russell *et al.*, 2012; Bahorun *et al.*, 2004), as plant phenolics have the ability to dissolve in aqueous ethanol. Hence, it is important to select suitable solvent for the isolation and optimization of polyphenolics and other bioactive compounds. Therefore, the selection of a right solvent is the relevant steps in optimizing the recovery of polyphenolics and other antioxidant compounds from a sample. Nihal *et al.* (2005) reported that 50% acetone has shown maximum polyphenolic content of black mate tea, followed by 80 and 100% and these results were found similar to the present study. Safaa *et al.* (2014) reported the phenolic content of wheat flour and its bran at various conc. (50 % acetone and 70% ethanol and methanol) and found that phenolic content was found to be highest in 50% acetone. Results of discussed studies were in compliance with the results reported in the current study.

The concentration of flavonoid content, DPPH and FRAP activity in both cultivars of wheat bran extracts depends on the various concentration of aqueous solvents (Min and Chun-Hao, 2005). Brewer *et al.* (2014) also reported the extraction of TFC in wheat bran at different particle size (0.177-0.206 mg/gm of sample). This

showed that extraction process may modify the overall efficiency of antioxidant extraction. Therefore, careful selection of solvent is important to maximize the recovery of antioxidants and polyphenolics from wheat bran.

5. Conclusion

The present study has explore the suitability of different extracting solvents with different polarities, as a most relevant step in optimizing the recovery of the total phenolic content and total flavonoid content as well as other antioxidants with potential pharmacological properties and health benefits. The study revealed that extraction of total phenolic, flavonoid content and total antioxidants activity in terms of free radical scavenging activity and ferric reducing power were found maximum in the 50% ethanol followed by 50% methanol, and acetone, respectively. Extractions of polyphenolic compound of both cultivars of wheat bran were strongly correlated with polarity of solvents (ethanol, methanol and acetone). Thus, suitable solvent of appropriate polarity is important for the isolation and optimization of wheat bran plenolics and antioxidant activity.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Citation **Nidhi, Devinder Kaur, Vinita Puranik and Pragya Mishra (2021). Effects of extraction solvents on cultivars of wheat (PBW-154 and HD-2967) bran antioxidant properties. Ann. Phytomed., 10(2):354-359. http://dx.doi.org/10.21276/ap.2021.10.2.47**