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ORIGINAL ARTICLE

Antioxidant activity of extracts from foxtail millet (*Setaria italica)*

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Abstract The less explored, commercially available foxtail millet-milled fractions like whole flour & bran rich fraction were studied for its antioxidant potency. Phytochemicals like alkaloids, phenolics, reducing sugars and flavonoids were found only in methanolic & aqueous extracts, while tannins and terpenoids were present in all the solvent extracts of whole flour & bran rich fraction. Antioxidants were extracted using methanol, ethanol and water. Methanolic extracts of whole flour and bran rich fraction exhibited a significantly higher ($P<0.05$) radical scavenging activity (44.62% & 51.80% respectively) using DPPH model system, and reducing power (0.381 & 0.455 respectively) at 2 mg, than the other solvents used for extraction. As bran rich fraction showed the highest antioxidant activity, suggesting the presence of antioxidant components in the bran layer.

Keywords Antioxidant activity · Commercial foxtail millet · Phytochemicals · Whole flour · Bran rich fraction

Introduction

Natural antioxidants have gained considerable interest in recent years for their role in preventing auto oxidation of fats and oils. Both synthetic and naturally occurring substances may possess health-promoting potential. Since the public call for an 'additive free' and 'natural' diet, major interest has been devoted to substances naturally occurring in the diet (Verhagen 1993; Caragay 1992). Grains contribute to the significant supply of antioxidant to prevent oxidative stress due to the fact that grains are used as a staple food and are consumed in larger amount in our diets (Choi et al. 2007). Recent epidemiological studies have suggested that increased consumption of whole grains, fruits and vegetables is associated with reduced risks of chronic diseases (Hu 2002). This may be attributed to the presence of natural antioxidants from plant foods such as vitamin C, tocopherol, carotenoids and polyphenols which prevent free radical damage (Diplock et al. 1998). Millets are known to contain phenolic acids, which are located in the pericarp, testa, aleurone layer and endosperm (Hahn et al. 1984). Millets are rich in phenolics acids, tannins and phytate which act as 'antinutrients' (Thompson 1993). However, it is now established that these antinutrients are known to reduce the risk for colon and breast cancer in animals (Graf and Eaton 1990). However, millets have been the subject of less interest in any research.

Foxtail millet (*Setaria italica*) is one of the minor millets, containing high amounts of proteins and minerals (Pawar and Pawar 1997). Simple processing methods like dehulling, soaking and cooking are reported to result in significant decreases in antinutrients and improved bioavailability of minerals like iron and zinc and also protein digestibility (Vithal and Machewad 2006). Extensive information on processing and nutrient composition (Geervani and Eggum 1989) and little information on its antioxidant activity (Asharani et al. 2010) are available. However, these reports are on the foxtail millet varieties developed by Agricultural research institutes. But no study has been reported on the commercially available (market sample) foxtail millet. The foxtail millet grains are processed (dehusking and polishing) before they reach the market, to make them edible and suitable for human consumption. In general dehulling or

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dehusking and milling process may cause high dehulling losses due to their small grain sizes and are known to affect the taste and keeping quality of millets (Gulia et al. 2007). Our interest was to know the antioxidant potency of commercially available foxtail millet, as this is what the consumers are eventually going to consume. Therefore, in the present study, the less explored commercially available foxtail millet was analyzed for its antioxidant activity and also screened for the presence of phytochemicals using various solvent extracts.

Materials and methods

Chemicals

1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) was procured from HiMedia Lab Pvt Ltd. (Mumbai, India). BHT was procured form Qualigens fine chemicals (Mumbai, India). All other reagents used were of analytical grade.

Sample preparation

Seeds of foxtail millet were purchased from the local market which was already dehusked and polished (10 degree polishing). It was further cleaned to remove extraneous and foreign material, pulverized in a plate mill into whole flour. A part of the whole flour was sieved through a 44 mesh sieve (BSS) to separate the bran rich fraction ('+' fraction) (Fig. 1). Whole flour and the bran rich fraction was stored in air tight containers for further use.

Preparation of antioxidant extracts

Whole flour and bran rich fraction (15 g) was extracted with 200 mL of solvent (methanol, ethanol and water respectively) in a mechanical shaker for 24 h, filtered and evaporated to dryness under reduced pressure in a rotary evaporator (Superfit, India). The concentrated methanolic, ethanolic and aqueous extracts were re-dissolved with respective solvents, to a concentration of 4 mg/ml and stored in the refrigerator until analysis.

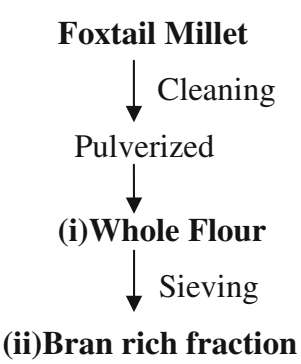

Fig. 1 Flow chart for production of foxtail millet—milled fractions

Screening of phytochemicals

Whole flour & bran rich fraction was subjected to sequential extraction using solvents (petroleum ether ➡ benzene ➡ chloroform ➡ methanol ➡ water) and screened for the presence of various phytochemicals like alkaloids, tannins, phenols, flavanoids and saponins (Mojah et al. 2003), while triterpenoids, steroids and reducing sugars was screened according to the method of Trease and Evans (1996).

DPPH radical scavenging activity

The ability of the extracts (methanolic, ethanolic and aqueous extracts) to scavenge free radicals was determined against a very stable free radical DPPH determined spectrometrically (Blois 1958). Aliquot of the sample extract at different concentrations were added to 1 mM methanolic solution of DPPH. The mixture was vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Reducing power assay

The ability of extracts (methanolic, ethanolic and aqueous extracts) to reduce iron (III) to iron (II) was assessed by the method of Yildrim et al. 2001. The dried extract (125–1,000 μg) in 1 ml of the corresponding solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 10 gl^{-1}), and then the mixture was incubated at 50°C for 30 min. After incubation, 2.5 ml of trichloroacetic acid (100 gl^{-1}) was added and the mixture was centrifuged at 1,650 rpm for 10 min. Finally, 2.5 ml of the supernatant solution were mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (1 gl^{-1}) and the absorbance was measured at 700 nm. High absorbance indicates high reducing power.

Statistical analysis All the experiments were conducted in triplicates and the results were reported as mean values with

Table 1 Phytochemical screening in the sequential extraction of whole flour & bran rich fraction

Phytochemical	Pet. ether		Benzene		Chloroform		MtOH		Water	
	WF	BRF	WF	BRF	WF	BRF	WF	BRF	WF	BRF
Flavonoids	−	−	−	−	−	−	++	++	++	++
Alkaloids	−	−	−	−	−	−	+	++	++	+
Terpenoids	++	++	+	+	+	+	++	++	+	+
Triterpenoids	++	++	−	++	+	++	−	++	−	+
Steroids	−	−	−	−	−	−	−	−	−	−
Tannins	+	+	+	+	+	+	+	+	+	+
Phenolics	−	−	−	−	−	−	++	++	++	++
Saponins	−	−	−	−	−	−	−	−	+	+
Reducing sugars	−	−	−	−	−	−	++	++	++	++

MtOH methanol, *WF* whole flour, *BRF* bran rich fraction, + traces, ++ average, − absence

their respective standard deviations. The data was subjected to analysis of variance (ANOVA) test and the differences between the means were compared for their significance using data analysis tool pack.

Results and discussions

Phytochemical screening

The results of phytochemical screening of foxtail millet whole flour and bran rich fraction are presented in Table 1. Phytochemicals such as terpenoids, and tannins were detected in all the solvent extracts of whole flour and bran rich fractions, while steroids were tested negative in all the solvents used for extraction. Saponin was detected in the aqueous extracts only. Flavonoids, alkaloids, phenolics and reducing sugars were detected in methanol and aqueous extracts. Triterpenoid were detected in the benzene, methanol and aqueous extracts of bran rich fraction; however for petroleum ether and chloroform extracts triterpenoids were detected in whole flour as well as for bran rich fractions. There was a wide variation in detecting the presence of phytochemicals due to differences in the polarity of the extracting solvents. Because methanol is a relatively polar organic solvent compared to other extracting solvents, most of the phytochemicals detected in this study are expected to be polar in nature. The health benefits of whole grains are attributed in part to their unique phytochemical composition (Adom and Liu 2002). Besides the health benefits associated with phytochemicals, these compounds have important functional properties. Phytochemicals in grains contribute to product quality in terms of color, flavor and texture (Holtekjolen et al. 2006). Bound Phytochemicals could survive stomach and intestinal digestion to reach the colon. This may partly explain the mechanism of grain consumption in the prevention of colon cancer, other digestive cancers, breast cancer, and prostate cancer, which is supported by epidemiological studies

Antioxidant activity of the foxtail millet fractions

It is well-known that free radicals cause autoxidation of unsaturated lipids in food. On the other hand, antioxidants are believed to break up the free radical chain of oxidation

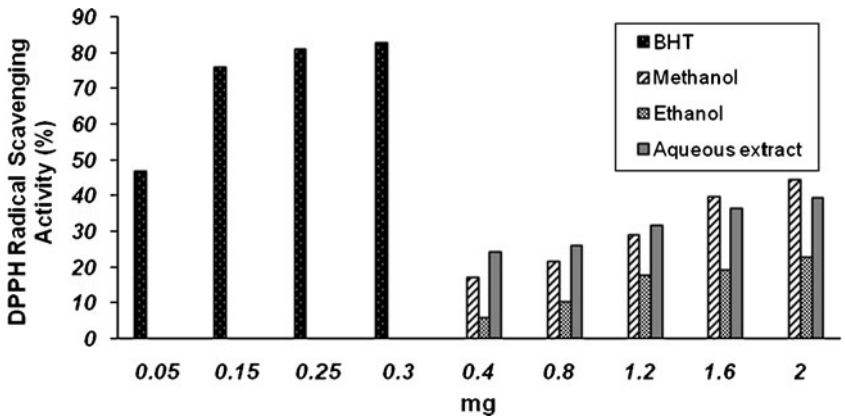

Fig. 2 Radical scavenging activity of foxtail millet—whole flour extracts (4 mg/Ml) & BHT by DPPH method at different concentrations (n=4)

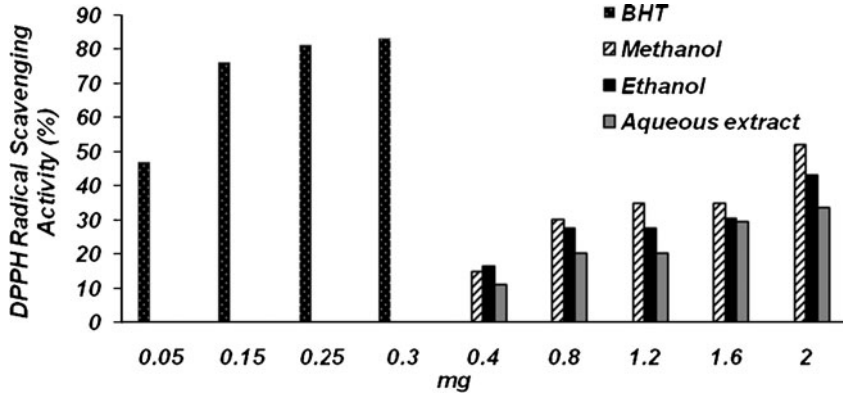

Fig. 3 Radical scavenging activity of foxtail millet—bran rich fraction extracts (4 mg/Ml) & BHT by DPPH method at different concentrations (n=4)

and donate hydrogen there by forming a stable end product, which does not propagate further oxidation of the lipids (Kaur and Perkins 1991; Sherwin 1978). The antioxidants in foxtail millet whole flour and bran rich fraction was extracted using three solvents (Methanol, ethanol and water) at a concentration of 4 mg/ml. The free radical scavenging potentials of various solvent extracts from foxtail millet- whole flour and bran at different concentrations were tested by the DPPH method and the results are shown in Figs. 2 & 3 respectively. Antioxidant reacts with DPPH, which is a stable free radical, and converts it to α,α-diphenyl-β- picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extracts (Singh et al. 2002). There was a significant difference (P<0.05) in the DPPH free radical scavenging activity for each solvent at increasing concentrations (0.4–2.0 mg) for both the milled fractions. At lower concentrations (0.4–1.2 mg) aqueous extract showed a significantly higher (P<0.05) radical scavenging activity when compared to the other solvents in whole flour. While at higher concentrations (1.6–2.0 mg) methanolic extract showed considerably higher radical scavenging activity compared to ethanol and aqueous extracts. Reports have suggested that relatively higher antioxidant activity was observed from methanolic extracts in grains compared to the other solvents including acetone, diethyl ether, ethyl acetate and water (Oki et al. 2000; Ziehnski and Kozlowska 2000). A similar trend was observed in case of bran rich fraction of the foxtail millet where the radical scavenging activity was highest in the methanolic extract (51.8%) followed by alcohol (42.90%) and water (33.60%).This indicates that the methanolic extracts followed by ethanol and aqueous extracts showed a higher radical scavenging activity (2.0 mg), however BHT showed a significantly higher (P< 0.05) radical scavenging activity compared to foxtail millet fraction extracts.

Reducing power assay

While iron is essential for oxygen transport, respiration, and activity of enzymes it is a reactive metal that catalyzes oxidative damage in the living tissues and cells (Miller 1996). The ferric reducing antioxidant potential of various extracts (4 mg/ml) of whole flour and bran fraction of foxtail millet is shown in Figs. 4 & 5. In this method ferric-ferricyanide complex is reduced to the ferrous form

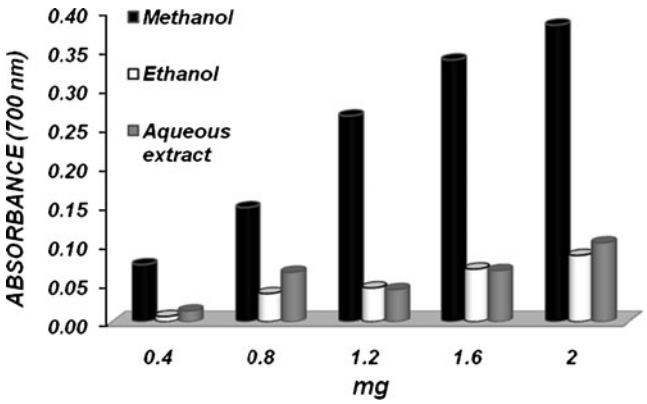

Fig. 4 Ferric reducing power of foxtail millet—whole flour extracts (4 mg/Ml) at different concentrations (n=4)

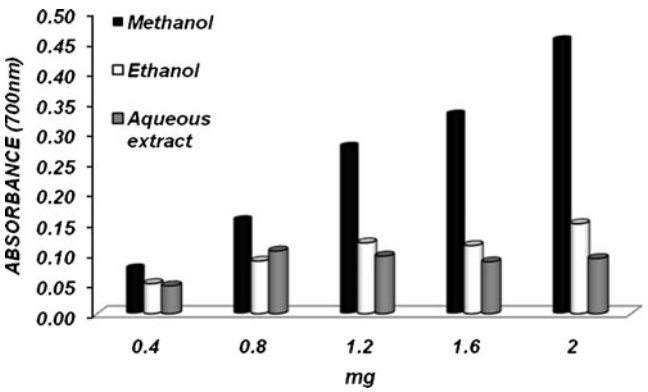

Fig. 5 Ferric reducing power of foxtail millet—bran rich fraction extracts (4 mg/Ml) at different concentrations (n=4)

depending in the presence of antioxidants (Amarowicz et al. 2004).The reducing power assay of methanolic and ethanolic extracts in the whole flour showed a significantly ($P<0.05$) higher reducing power ($A700=0.381$ & 0.341) respectively followed by aqueous extract ($A700=0.120$) at a concentration of 2.0 mg. The reducing power of methanolic extract was relatively higher than that of the previous findings on foxtail millet (Choi et al. 2007). In the bran fraction, methanolic extract showed the maximum activity (A700=0.455) compared to alcoholic and aqueous extracts (A700=0.150 & 0.092 respectively) at 2.0 mg. This indicates that methanolic extracts showed a higher ferric reducing antioxidant potential compared to aqueous and ethanolic extracts.

Studies have shown that consumption of whole grains is associated with reduced risk of chronic diseases. Phytochemicals are biologically active organic substance of plant origin having disease preventing and health promoting properties. The screening of these phytochemicals may help in identifying the active compounds responsible for disease prevention. Most of the phytochemicals like polyphenols, Flavonoids are found to have antioxidant activity (Narasinga Rao 2003). In the present investigation, phytochemical screening revealed the presence of flavonoids, phenolics, tannins along with other components which are potent antioxidants. The results indicated that the extraction with methanol showed high antioxidant activity followed by aqueous and ethanolic extracts. High antioxidant activity was observed in the extracts from bran rich fraction compared to whole flour, suggesting the presence of antioxidant components in the bran rich layer. Hence, it can be concluded that the commercially available foxtail millet is a potent source of antioxidants. Further work is in progress to explore its role as an antioxidant in food system.

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