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Antioxidant potential and phenolic profiles of Pearl millet (Pennisetum glaucum)

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ABSTRACT

Pearl millet (Pennisetum glaucum), known as poor man's cereal, besides being packed with classic nutrients, have recently been emerged to contain plethora of phytochemicals. This study was undertaken to evaluate antioxidant potential and phenolic profiles of aqueous-organic extracts from pearl millet. Results of total phenols and flavonoids, as 136.6 mg GAE/100g and 33.33mg CE/100g respectively, and of antioxidant properties expressed through DPPH (2,2-Diphenyl-1-picrylhydracyl) radical scavenging assay, ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay and FRAP (Ferric reducing antioxidant power) assay as 450.00 mg TE/100g, 506.66 mg TE/100g, 450.28 mg FeSO4/100g respectively revealed this cereal to contain significant antioxidant activity, which on correlation analysis was found to be emanated from phenolic fractions. Therefore, pearl millet, being cheap and excellent dietary vehicle of protective antioxidants along with a strong matrix of dietary fibre, protein, vitamins and minerals, is strongly recommended to be incorporated in diet as to manifest biological effects linked with health promotion and disease prevention.

Introduction

Food is cardinal for life and wellbeing. Currently, fundamental changes in nutritional approaches are being witnessed to better understand the mechanisms of health. Whereas earlier reflections on wholesome diet considered to be comprised of traditional nutrients, emerging literature is stressing non-nutritive phyto chemicals to play exemplary role in health and disease. Owing to their antioxidant properties i.e. free radical scavenging, pro-oxidant metals chelation, antioxidant vitamins regeneration, mediation of cell signaling pathways, phytochemicals have been extolled to provide protection against 120 degenerative diseases implicated to be caused due to oxidative insult (Liu RH, 2004). Pearl millet (Pennisetum glaucum), popularly known as poor man's cereal, not merely fulfill the qualifications for nutrient-rich food in terms of macro- and micro-nutrients, but also have been cited to provide an elaborate range of phenolic classes to culminate in chemopreventive and cardioprotective health effects.

Materials and Methods

Sample procurement: The pulse variety Varieties RHB-173 for pearl millet (Pennisetum Glaucum; bajra) was procured from Indian Institute of Pulse Research, Durgapura (Jaipur). Samples were washed, dried in shade and then hand sorted to remove wrinkled seeds and foreign material and thereafter, stored in air tight container for further use.

Chemicals: Folin-ciocalteau reagent, sodium carbonate, gallic acid, aluminium chloride hexahydrate (AlCl3.6H2O), NaNO2, sodium hydroxide (NaOH), catechin, Ethanol, DPPH, ABTS, TROLOX, potassium persulphate, sodium acetate trihydrate, glacial acetic acid, 2,4,6-tripyridyl-S-triazine (TPTZ), Conc. hydrochloric acid (HCl), ferric chloride hexahydrate (FeCl3.6H2O), Ferrous sulphate heptahydrate (FeSO4.7H2O), deionized water were obtained from Sigma Chemicals and Merck.

Sample preparation: the sample was pulverized using home grinder and was used immediately for phenolic extraction.

Sample extraction: Pulverized samples (0.25g) were placed in test tubes with 10ml of methanol/water (50:50, v/v). The pH was adjusted to 2 using 2M HCl. The tubes were thoroughly shaken, using orbital shaker, at room temperature for 1 hr, and then centrigued at 2500 g for 10 mins. Supernatants were collected in clean dry test tubes. Then the residues were extracted again with 10 ml of an acetone/water mixture (70:30, v/v). The methanol and acetone extracts were combined and subsequently used for various assays. Extracts produced in duplicate. In case of non-usage of extracts on the same day, they were stored at 4°C and used within a week for all analysis (Pérez-Jiménez et al 2008).

Phytochemical analysis:

Determination of Total phenolic content (TPC)

Principle: It is based on the single electron transfer (SET) in alkaline medium (7% NaCO3) from phenolic compound to molybdenum, forming blue complex which is measured spectrophotometrically at 750-765nm.

Procedure: The mixture of sample solution (0.1 ml), deionized water (6 ml), FCR solution (0.5 ml), 7% NaCO3 (1.5 ml) was vortexed for 1 min and incubated for 8 min at room temperature. Then a dose of 1.9 ml of deionized water was added. The mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 765 nm against blank (Xu and Chang, 2007).

Calculations: TPC was expressed as gallic acid equivalent (mg GAE/100g sample) through the calibration curve of gallic acid. Linearity range of the calibration curve was 100 to 1000 µg/ml.

Determination of Total flavonoid content (TFC)

Principle: Aluminum chloride forms acid complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonois. In addition, aluminum chloride forms acid labile complexes with orthodihydroxyl groups in the A or B ring of flavonoids.

Procedure: 0.5 ml of sample was mixed with 2.5 ml of deionized water in a test tube followed by adding 0.15 ml of 5% sodium nitrite (NaNO2) solution. After 6 minutes, 0.3 ml of 10% aluminum chloride hexahydrate (AlCl3.6H2O) solution was added and allowed to stand for another 5 minutes before adding 0.1 ml of 1 M NaOH. The mixture was brought to 5 ml with the addition of 0.55 ml of deionized water and mixed well. The absorbance was measured immediately against the blank (the same mixture without the sample) at 510 nm using UV-spectrophotometer (Xu and Chang, 2007).

Calculations: TFC was expressed as catechin equivalents (mg CE/100g sample). Linearity range of catechin calibration curve was 62.5-375 µg/ml.

Antioxidant activity analysis:

DPPH radical scavenging activity

Principle: It is based on antioxidant-catalyzed reduction of purple-colored DPPH radical to its yellow-colored non-radical form which is measured spectrophotometrically at 517nm.

Procedure: A dose of 0.2 ml of tested legume extract was added to 7.6 ml ethanol solution of DPPH radical (final concentration was 0.1 mM). The mixture was shaken vigorously for 1 min by vortexing and left to stand at room temperature in the dark for 30 min. Thereafter, the absorbance was measured at 517 nm against ethanol blank (Xu and Chang, 2007).

Calculations: DPPH scavenging activity was expressed as trolox equivalents (mg TE/100g sample). Linearity range of trolox calibration curve was 0.05 μM to 0.4 mM trolox.

ABTS radical scavenging activity

Principle: It is based on antioxidant inhibition of the absorbance of blue-green coloured ABTS radical, generated via persulfate-induced ABTS oxidation, measured spectrophotometrically at 734nm.

Procedure: The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS•+ solution with 30 ml methanol to obtain an absorbance of 1.1 ±0.02 units at 734 nm using the spectrophotometer. In sample (0.3 ml), prepared ABTS •+ solution (5.7 ml) was added and kept it for 2 hrs in a dark condition. Then the absorbance was taken at 734 nm using spectrophotometer (Thaipong et al., 2006). Calculations: ABTS scavenging activity was expressed as trolox equivalents (mg TE/100g sample). Linaerity range of trolox calibration curve was 0.1-0.7 mM trolox.

FRAP assay

Principle: It is based on antioxidant-catalyzed reduction of ferric-TPTZ complex (colorless) to ferrous form (intensely blue coloured) which is measured spectrophotometrically at 593nm

Procedure: The working FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10mM TPTZ (2,4,6-Tripyridyl-s-triazine) in 40mM HCl and with 1 volume of 20mM FeCl3 ×6H2O (ferric chloride hexahydrate). Prepared working FRAP reagent was warmed to 37□C. In 6 ml FRAP reagent, 0.2 ml of sample and 0.6 ml of deionized water were added and the absorbance was taken at 593 nm against reagent blank after 4 min (Xu and Chang, 2007).

Calculations: FRAP value was expressed as Fe^{2}+ (Iron (II) sulfate heptahydrate or ferrous sulfate) equivalent (mg FRAP/100g sample). Linearity range of the calibration curve was 0.1-0.9 mM.

Result and discussion

The present study was carried out to estimate phenolic constituents and antioxidant activity of pearl millet (Pennisetum glaucum). Total phenols and flavonoids, as presented in table 1, were found to be present in pearl millet, although former witnessed significant concentration. The results are in agreement with Ragaee et al, 2006 and Pushparaj and Urooj, 2014. Various phenolics deciphered to be present in pearl millet include *flavonoids* viz. tricin, acacetin, 3,4-dimethoxy luteolin, 4-methoxy tricin, vitexin, glucosylvitexin, glucosylorientin; *phenolic acids* viz. vanilic acid, syringic acid, melilotic acid, para-hydroxyl benzoic acid, salicylic acid, ferulic acid, cis/trans p-coumaric acid, *trans*-cinnamic acid, protocatechuic acid, hydroxybenzoic acid (Daniel et al, 2012; Dykes and Rooney, 2007, Nambiar et al, 2012, Radhouane et al, 2013). Besides to contain various classes of phenolics and flavonoids, simulated digestive and enzymatic studies have documented pearl millet to be bio-available in human body to exert antioxidant effects (Prajapati et al, 2013). Results pertaining to antioxidant activity of aqueous-organic extracts from pearl millet, as shown in table 2, revealed considerable amounts. The results of antioxidant activity of pearl millet explored through DPPH radical scavenging activity, ABTS radical scavenging activity and FRAP assay are in line with Ragaee et al 2006 and Prajapati et al 2013. This signifies that pearl millet has profound free radical quenching and reducing ability to arrest oxidative damage and consequent degenerative diseases. Phenolics in this maverick millet have been documented to confer chemopreventive and cardio-protective properties through their high free radical scavenging activity (Odusola et al, 2013). The correlational analysis between phenolic components and antioxidant assays signified positive and good correlation with statistically significant correlation between ABTS and DPPH (.999, <0.05), pinpointing that phenolic fractions are responsible for the antioxidant activity of pearl millet.

Table 1: Phytochemical profile of aqueous-organic extracts of Pearl millet

Total phenols (mg GAE/100g)	Total flavonoids (mg CE/100g)
136.66±20.81	33.33±5.77

[Values are mean ± SD; N = 3]

Table 2: Antioxidant activity of aqueous-organic extracts of Pearl millet

DPPH (mg TE/100g)	ABTS (mg TE/100g)	FRAP (mg FeSO4/100g)
450.00±17.32	506.66±11.54	450.28±20.88

[Values are mean ± SD; N = 3]

Table 3: Correlations between phenolic contents and antioxidant activities of aqueous-organic extracts from Pearl millet

	TPC	TFC	DPPH	ABTS	FRAP
TPC	-	-	-	-	-
TFC	.993	-	-	-	-
DPPH	.655	.737	-	-	-
ABTS	.693	.771	.999*	-	-
FRAP	.444	.543	.968	.953	-

*Correlation is significant at 0.05 level (2-tailed)

Conclusion

Pearl millet (Pennisetum glaucum) besides being economical and easily available food stuff, present itself to be an excellent functional ingredient not only for its rich nutrient profile, but also due to assortment of phenolics studied to be present in it considerably. Phenolic estimation and antioxidant activity analysis in this study validates this ancient millet to be a potential food source for protection against chronic, degenerative diseases associated with oxidative stress.

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