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Research Article

Antioxidant activities of Pearl millet *(Pennisetum glaucum)* and Little millet *(Panicum sumatrense)* in different *in vitro* models

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Abstract: Several epidemiological studies have shown that consumption of whole grains is associated with reduced risk of chronic diseases, which are attributed in part to their unique phytochemical composition. In the present study three varieties of pearl millet (ICMV 221, CO (CU) 9 and market variety) and one variety of little millet were used. The methanolic extracts of the grains were prepared and the total phenols and flavonoid content were estimated. The antioxidant activities of the extracts were measured using stable 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical by UV visible spectroscopy. The reducing power and metal chelating abilities were also determined. Pearl millet ICMV 221 had highest TPC (106.86±2.57 mg GAE/100g flour) whereas total flavonoid content was high in pearl millet CO (CU) 9 (40.22±2.41 mg CE/100g flour). The DPPH radical scavenging activity is highest for pearl millet CO (CU) 9 (70.39±2.57%) and lowest for little millet (39.87±2.0%). Pearl millet ICMV 221 and CO (CU) 9 showed highest reducing power (0.560 and 0.513 respectively). Pearl millet ICMV 221 showed highest metal chelating activity (88.45 %) compared to other millet varieties. The total flavonoid content (TFC) correlated well with DPPH radical scavenging activity(R=0.97618) and moderate correlation with reducing power ((R=0.55456). The total phenolic content (TPC) moderately correlated with DPPH radical scavenging activity. These findings suggest that pearl millet and little millet are rich source of phenolic antioxidants and their consumption may be useful in combating diseases in which free radicals are involved.

Keywords: Millets, Phenolic compounds, Dietary antioxidants, Free radicals, Oxidative stress

Introduction

Free radicals are the molecular species which are capable of independent existence possessing unpaired electrons in their outer most shell and are produced during the normal cellular metabolism or from the external source such as cigarette smoke, pollution, medication, and also by radiation. These free radicals are highly unstable and very reactive, play beneficial as well as deleterious role in the human body [1]. At moderate concentration the free radicals such as ROS (reactive oxygen species) and RNS (reactive nitrogen species) exerts beneficial effects on immune function and cellular responses [2]. If there is an imbalance between the antioxidant defence mechanism and the production of free radicals, results in oxidative stress, which is a deleterious process and can damage membrane lipids, proteins and DNA, which causes cell injury and development of several chronic diseases such as diabetes, cancer, atherosclerosis, cardiovascular disease, insulin resistance, ischemic stroke and ageing [1]. Dietary antioxidants, particularly polyphenols present in plant foods may protect the human body from such oxidative damage caused by free radicals [3].

Several epidemiological studies have shown that the intake of whole grains is protective against chronic and degenerative diseases [4, 5] including type II diabetes [6], obesity [7], cardiovascular disease and cancer [5]. Whole grains include wheat, maize, barley, buckwheat, sorghum, oats and millets. Whole grains play an important role in our daily diet as they contain high concentration of dietary fiber, resistant starch, antioxidants, trace minerals, phenolic compounds and oligosaccharides. Grains contain lower amount of tocopherols and also contain various phenolic phytochemicals such as benzoic acid, cinammic acid derivatives (phenolic acids), flavonoids, lignans, coumarins and phytoestrogens [5]. All these molecules have various biological properties such as antioxidant, anticarcinogenic, anti-inflammatory and antiatherosclerotic effects. Several studies have shown and confirmed the presence of flavonoids and phenolic acids in grains. The polyphenolic compounds in grains are less than 1% of dry weight of grains.

Millets are inexpensive and nutritionally comparable or even superior to major cereals and are consumed traditionally as health and vitality foods in rural India [8]. The protein, fat, carbohydrates, crude fibre, minerals and vitamins are comparable to other cereals such as rice and wheat. They also contain various phenolic compounds such as benzoic and cinnamic acid derivatives, anthocyanidins, quinines, flavonoids, lignans and phytoestrogens, which play an important role in preventing from diseases [9]. The finger millet extract showed free radical quenching potential [10, 11] antimicrobial effect [12], aldose reductase, amylase inhibition properties [13] and inhibition of the collagen glycation *in vitro* [14]. Feeding of millet diet to diabetic rats showed hypoglycemic effect [15] and also hastened the wound healing process [16]. Supplementation of kodo millet and finger millet whole grain and bran alleviated high fat diet induced changes in mice [17, 18].

Pearl millet and little millet are important minor cereals grown extensively in the tropics and are staple food for the low income groups in some countries of the world [19]. Most of the antioxidant and nutraceutical studies were investigated on major millets such as sorghum, finger millet and foxtail millet [20-23]. Less information is available on pearl millet and little millet. Previous

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studies have reported the nutraceutical and antioxidant properties of little millet (local variety) and pearl millet (dark green cultivar) [24, 25]. However, no reports on antioxidant activity of pearl millet varieties, ICMV 221 and CO (CU) 9 varieties of India. Hence, the present study is aimed at screening three different varieties of pearl millet ICMV 221, CO (CU) 9 and a market variety and one variety of little millet for the antioxidant activity.

Materials and Methods

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid and catechin were purchased from Sigma. All other reagents and solvents used were of analytical grade.

Grain samples and sample preparation

Two varieties of pearl millet *viz* pearl millet ICMV 221 (PMI), pearl millet CO (CU) 9 (PMC) were procured from Tamil Nadu Agricultural University, Coimbatore, India. Pearl millet market variety (PMM) and little millet (LM) were procured from Vellore market, Vellore district, Tamil Nadu, India. All the millets were cleaned from soil particles, debris and finely powdered using blender.

Extraction of polyphenols

Twenty five grams of millet flour was mixed with 125ml of hexane and kept for overnight on orbital shaker with continuous stirring. Then the hexane was removed without disturbing the flour and flour was dried. Then the dried flour was mixed with 100 ml of methanol and then refluxed using condenser at 60°C for 2hrs. The supernatant was removed and the residual flour was subjected for extraction by adding 50 ml of methanol for 1hr at 60°C. Both the supernatants (extracts) were pooled and filtered through Whatmann No 1 filter paper and concentrated in a rotary evaporator. The high vaccum was applied to remove the residual methanol solvent and the extracts weights were recorded. Finally the extracts were redissolved in methanol and made up to 10 mL with the same solvent and stored in brown glass bottles at 8 °C till use [11]. This extract was used for the estimation of total phenols (TPC), flavonoids (TFC), antioxidant potential, reducing power and metal chelating activity.

Determination of total phenolic content (TPC)

The TPC of the extracts were determined by spectrophotometric method using gallic acid as a standard [26]. Briefly, 100µl of the millet extract (1/10 dilution) was made up to 3ml by adding distilled water; to this 0.5ml of Folin- Ciocalteau's reagent (1:2 dilution with water) was added and the mixture was incubated for 3 mins at room temperature; to this mixture 2ml of (20%) Na_2CO_3 was added; then kept in boiling water bath for exactly 1min. The reaction mixture was then cooled and the absorbance was measured at 650 nm using UV-Vis spectrophotometer.

Determination of total flavonoid content (TFC)

The total flavanoids content in the extract was determined by $AlCl_3$ method using catechin as standard [27]. Briefly, 50µl of the extract was made up to 5ml by adding 4.9 ml of distilled water; at 0th min 0.3ml of 5% NaNO2 was added; then at 5th min 0.3ml of 10% $AlCl_3$ was added to this mixture and mixed well; at 6th min 2ml of 1M NaOH was added; to this 2.4ml of double distilled water was added and then mixed well. The absorbance was measured at 510nm using UV-Vis spectrophotometer. The TFC was expressed as mg catechin equivalents (CE)/100 g of flour.

Determination of DPPH radical scavenging activity

The antioxidant activity of millet extracts was determined by the DPPH radical scavenging method according to the Brand-Williams *et al.,* [28] with modifications. Briefly, 100µl of extract (1/10 dilution) was made up to 500µl by adding methanol; then 3 ml of DPPH (0.1mM in methanol) solution was added. The mixture was thoroughly mixed and the decrease in absorbance of the solution was measured at 515 nm continuously at different time intervals (30 sec) until reaction reaches plateau (10 min). The result was expressed as DPPH radical scavenging activity (%) by the extract. The radical scavenging activity (RSA) of extracts was calculated using the following formula. DPPH radical scavenging activity (%) = [Ac –As/Ac] x 100. Where Ac is absorbance of control and As is absorbance of sample.

Determination of reducing power

The reducing power of millet extracts was determined by the method of Suma *et al.,* [23]. It is based on Fe^{3+} - Fe^{2+} transformation by the antioxidants. In this assay compounds with reducing ability, react with potassium ferricyanide (Fe^{3+}) and gives potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex with absorption maximum at 700 nm. Briefly, 200µl of extract (1/10 dilution) was made up to 1 ml with methanol, to this 2.5ml of the 0.2M phosphate buffer (pH: 6.6) and 2.5ml of 1% $K_3Fe(CN)_6$ was added and incubated for 20mins at 50°C. Then 2.5ml of TCA (10%) was added to the reaction mixture to arrest the reaction and then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water, 0.5 ml of freshly prepared 0.1 % ferric chloride solution and the absorbance was measured at 700 nm. A blank was prepared without adding standard or extract. The increased absorbance at 700 nm of the reaction mixture indicates increase in reducing power of the extract. Ascorbic acid was used as standard.

Determination of metal chelating activity

The metal chelating ability of millet extracts was determined according to the method of Carter, Paul. [29] with slight modification. Briefly, different concentration of extract was made up to 4 ml by adding methanol, to this 50µl of $FeCl_2$ (2mM) was added. The reaction was initiated by the addition of 200µl of Ferrozine (5 mM). The solution was well mixed and allowed to stand at room temperature for 10 mins. Then, the absorbance of sample was measured at 562nm against blank. The blank contains 50µl of distilled water instead of $FeCl_2$. The control contained all the reagents except extract. The % of metal chelating activity was calculated using the following formula: [(Ac-As)/Ac] x 100. Where A_C is the absorbance of the control and As is the absorbance of the sample/standard.

Statistical analysis

Each experiment was performed in triplicates and the results were expressed as the mean values ± standard deviation. The GraphPad prism version 6 was used to analyze data and statistical significance was assessed by using one-way analysis of variance (ANOVA) at a level of $p < 0.05$. Linear regression analysis of the data was

performed using the Origin 6.0 software to see the correlation between the antioxidant activity, total phenolic content (TPC), and total flavonoid content (TFC).

Results and Discussion

Extraction yield

In many reports, methanol was reported as a good solvent compared to other organic solvents for extracting phenolics from natural samples [30, 20, 23]. Hence, in the present study methanol is used as solvent for extraction of soluble phenolics from millets. The yield of the extracts from pearl millet and little millet were found to be 2.1-3.93%. Highest yield was obtained from PMC (3.93 g/100 g flour), followed by PMM (2.95 g), LM (2.52 g) and PMI (2.1 g). Similar range of extraction yields were reported by Pushparaj *et al.,* [31] from pearl millet- Kalukombu and Maharashtra Rabi Bajra (5.8 and 4.4 g/100 g respectively). The yield of extraction depends on the nature of solvent used for extraction and also the extraction conditions [32]. Hence, different authors have reported different extraction yields from millets and other grains.

Determination of total phenolic and flavonoid contents

Phenolic compounds present in plant foods such as fruits, vegetables and whole grains may provide health benefits and contributes for antioxidant activity. Polyphenols have one or more aromatic rings with hydroxyl groups at different positions. Phenolic compounds that are present in grains are of particular importance because of their ability in scavenging free radicals and prevent cells from the oxidative damage [33]. Hence, quantification of phenolic compounds is important to establish health benefits of millets. In the present study the total phenolic content (TPC) of millet extracts ranged from 78.4 to 106.9 mg of gallic acid equivalents/100 g of flour (Figure 1). Among the four millets, PMI had the highest phenolic content of 106.86±2.57mg/100g followed by PMC (91.28±2.72mg/100g), PMM (82.43±0.731 mg/100g) and LM showed least TPC of 78.37±2.72 mg/100g.

The total flavonoid content of millet extracts ranged from 24.97 to 40.22 mg of catechin equivalents/100 g of flour (Figure 2). Among millets, PMC showed high amount of flavonoids (40.22±2.41 mg/100g), followed by PMI (36.56±0.30), PMM (32.64±1.87 mg/100g) and LM showed less TFC (24.97±1.08 mg/100g).

The TPC and TFC results obtained in the present study are in agreement with the previously reported values in different pearl millet varieties [34]. The TPC also vary in different varieties of same grain. Nishani *et al.,* [34] reported that the TPC in pearl millet varieties ranged from 720–874 mg GAE/100g. In another study the TPC of pearl millet PUSA-415 found to be 7.32 mg GAE/g on dry weight basis [35]. The TPC value of pearl millet obtained from the region of Ouled Aïssa contained 1660 µg GAE/g of grains [36]. Pradeep and Manisha [37] extracted phenolics from little millet (CO-Samai-4 variety) using 1% HCl methanol at 60°C and reported higher TPC of 429.9 mg GAE/100g. Chandrasekara and Shahidi reported the TPC in grains ranged from 8.6 to 32.4 µmol ferulic acid equivalents/g [38]. Various phenolic acids and flavonoids from millet grains have been characterized by HPLC [25].

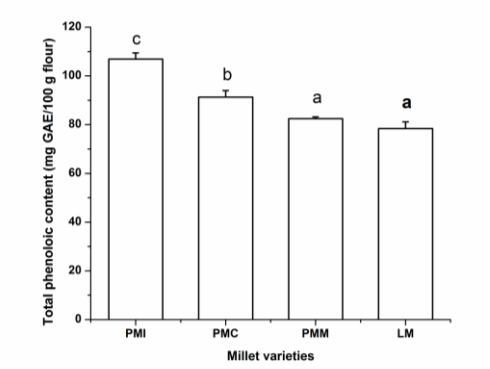


Figure 1: Total phenolic content of methanolic extracts of different pearl millet and little millet varieties. Values are expressed as mean ± SD (n=3). Values marked by different letters are significantly different ($p<0.05$). PMI-Pearl millet ICMV 221, PMC-Pearl millet CO (CU) 9, PMM- Pearl millet market variety, LM-Little millet.

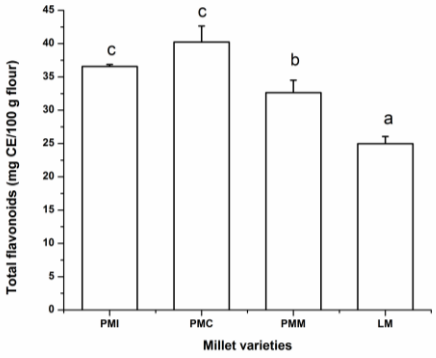


Figure 2: Total flavonoid content of methanolic extracts of different pearl millet and little millet varieties. Values are expressed as mean ± SD (n=3). Values marked by different letters are significantly different ($p<0.05$). PMI-pearl millet ICMV 221, PMC-Pearl millet CO (CU) 9, PMM- Pearl millet market variety, LM-Little millet.

Determination of DPPH radical scavenging activity

The DPPH radical is a nitrogen centered free radical and shows a maximum absorbance at 515 nm in methanol. When a solution of DPPH is mixed with a proton donating substance such as antioxidants, the radicals are scavenged as a result the absorbance decreases. Hence this assay has been widely used to investigate the free radical scavenging ability of natural extracts and pure antioxidant compounds [39, 40]. In DPPH assay the DPPH radical ($DPPH^{\cdot}$) is scavenged by an antioxidant molecule that donates an electron or hydrogen atom and therefore a stable molecule DPPH-H is formed.

Figure 3a shows the kinetics of DPPH radical scavenging by the pearl millet and little millet methanolic extracts. In the absence of extracts the DPPH radical solution was stable with time and showed the absorbance of 1.05. Addition of millet extracts to the DPPH solution, the absorbance was decreased gradually with time, indicating their antioxidant potential. The free radical scavenging activities of the extracts were determined at 10 minutes. Among the millet varieties, PMC showed highest DPPH radical scavenging activity of 70.39% (Figure 3b). All the millet extracts scavenged DPPH radicals in a dose dependent manner (Figure 3c and 3d). These results indicated that methanolic extracts of pearl millet and little

millet contains various phenolic antioxidants with the ability of donating hydrogen and scavenging free radicals. Polyphenols such as phenollic acids and flavonoids are known to scavenge various free radicals by two different mechanisms either by electron transfer or by hydrogen atom transfer and may protect cells and tissues of the body from oxidative stress, thereby prevents from various diseases [1-3]. The present study is in agreement with previous reports where different millet varieties exhibited free radical scavenging activity as evaluated by DPPH radical method [31-34]. Recently Berwal *et al.,* showed antioxidant potential of 92 pearl millet genotypes by DPPH and ABTS radical assays [41].

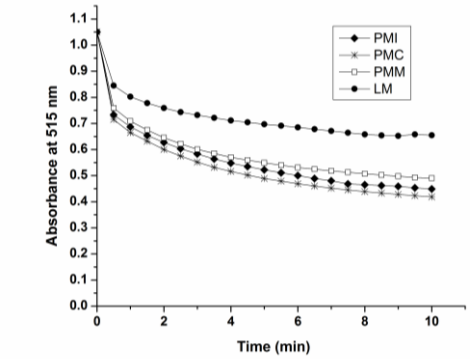


Figure 3a: Reaction kinetics of DPPH radicals with pearl millet and little millet methanolic extracts. PMI-Pearl millet ICMV 221, PMC-Pearl millet CO (CU) 9, PMM- Pearl millet market variety, LM-Little millet.

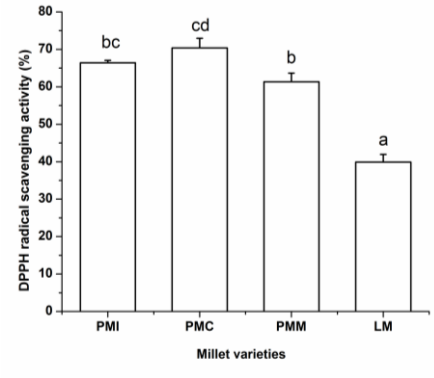


Figure 3b: DPPH radical scavenging activity of pearl millet and little millet methanolic extracts. Concentration of extract: 200 µl (1/10 dilution). Values are expressed as mean ± SD (n=3). Values marked by different letters are significantly different ($p<0.05$). PMI-Pearl millet ICMV 221, PMC-Pearl millet CO (CU) 9, PMM- Pearl millet market variety, LM-Little millet.

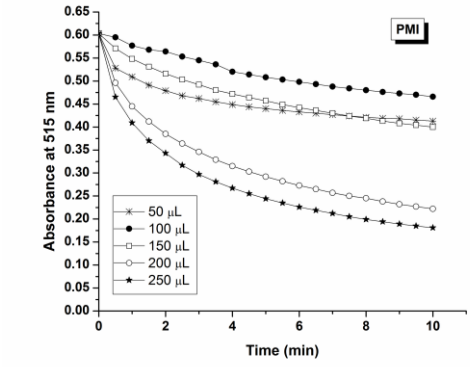


Figure 3c: Reaction kinetics of DPPH radicals with pearl millet ICMV 221(PMI) methanolic extract.

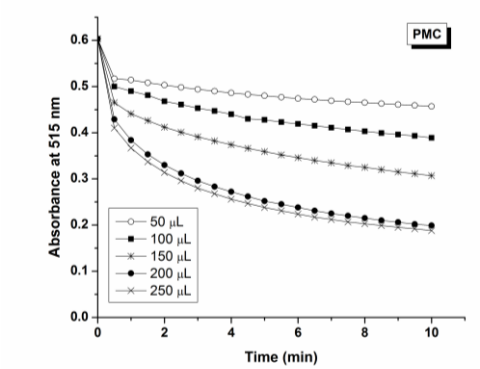


Figure 3d: Reaction kinetics of DPPH radicals with pearl millet CO (CU)9 (PMC) methanolic extract.

Determination of reducing power

The antioxidant activity of reductones is thought to be involved in terminating radical chain reactions by donating hydrogen atoms. Hence determination of reducing power is another method to measure the antioxidant potential of various compounds including polyphenols [39, 40]. The reducing power of plant extracts used as an indicator for their electron donating abilities. In this method, the antioxidant compounds in the sample causes reduction of the Fe^{3+}/ferricyanide complex to the ferrous form, which causes change of test solution color from yellow to green and blue (Perl's Prussian blue; λmax: 700 nm) depending on the redox potential of the sample [39, 40]. The higher absorbance at 700 nm indicates higher reducing power of the sample. In the present study, the reducing power increased with increasing concentration for all four millet extracts (Figure 4a), indicating their hydrogen donating abilities. at the tested concentration (200 µl 1/10 dil) of millet extracts, the reducing power ranged from 0.455 to 0.560 (Figure 4b). The highest reducing power was found in PMI (0.560±0.004), followed by PMC (0.513±0.043), LM (0.478±0.025) and PMM (0.455±0.028).

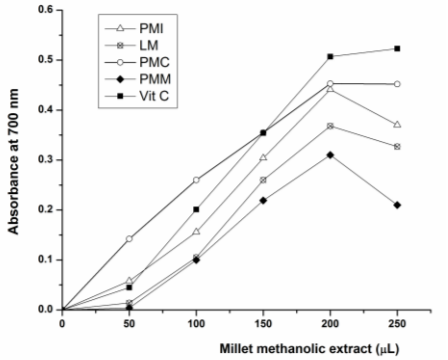


Figure 4a: Reducing power of different pearl millet and little millet methanolic extracts.

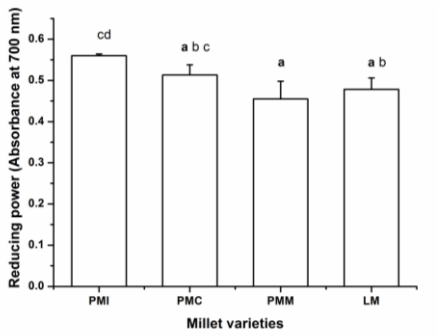


Figure 4b: Reducing power of different pearl millet and little millet methanolic extracts. Concentration of extract: 200µl (1/10 dilution). Values marked by different letters are significantly different ($p<0.05$). PMI-Pearl millet ICMV 221, PMC-Pearl millet CO (CU) 9, PMM- Pearl millet market variety, LM-Little millet.

Determination of metal chelating activity

Ferrous ion in the cell generates hydroxyl radical through fenton chemistry and causes lipid peroxidation and DNA oxidation. The ferrous ion also hastens the decomposition of lipid hydroperoxides and produces peroxyl and alkoxyl radicals. In this study the chelation of ferrous (Fe^{2+}) ions by different millet extracts were estimated using ferrozine reactions. Ferrozine forms a complex with ferrous ions (Fe^{2+}) quantitatively. In the presence of chelators, the ferrozine-ferrous complex is disrupted consequently there is a decrease in the red colour of the complex [39, 40]. This color reduction is measured to estimate the metal chelating activity. Figure 5 shows the metal chelating properties of pearl millet and little millet methanolic extracts. All the millet extracts showed chelation of ferrous ions in a dose-dependent manner. At 100 µl of extract concentration, the metal chelating activities of the extracts were in the following order PMI(88.45%)>PMC(81.12%)>LM(78.31%)>PMM (71.29%).

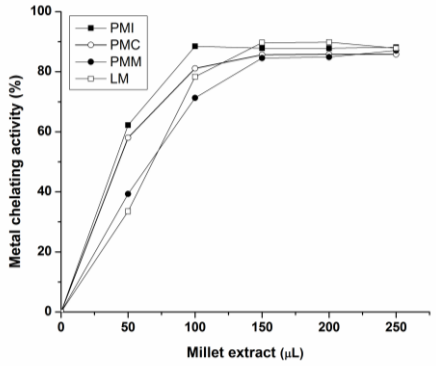
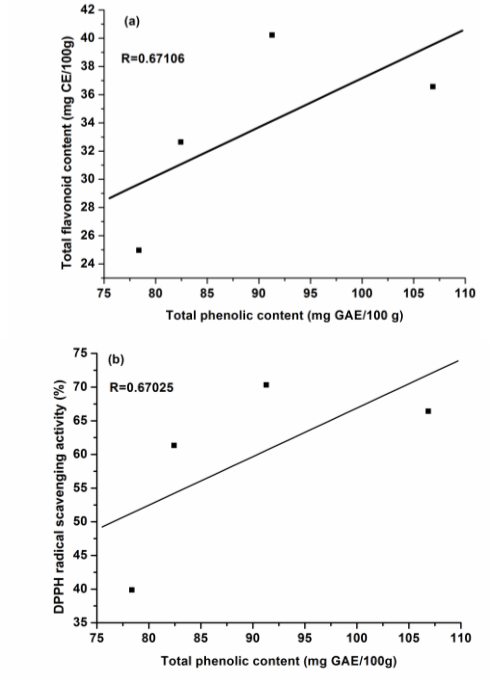


Figure 5: Metal chelating activity of different pearl millet and little millet methanolic extracts. PMI-pearl millet ICMV 221, PMC-Pearl millet CO (CU) 9, PMM- Pearl millet market variety, LM-Little millet.

Correlation between antioxidant molecules and antioxidant activity

The correlation analysis was performed between TPC, TFC and antioxidant parameters such as DPPH radical scavenging activity, reducing power and metal chelation. In some reports a good correlation was shown between TPC and TFC present in grains. In this study moderate correlation was observed between TPC and TFC of millet varieties(R=0.67106). The TPC values of millet extracts moderately correlated with DPPH radical scavenging activity (R=0.67025). Whereas TFC showed good correlation with DPPH radical scavenging activity (R=97618) and moderate correlation with reducing power (R=0.55456) and metal chelation(R=0.398). The results suggest that flavonoids present in these millets are mainly contributing for DPPH radical scavenging activity and reducing power. These results are in agreement with previous studies, where the antioxidant activity of grains correlated well with flavonoid content [31]. The antioxidant activity of millet phenolic extracts is mainly due to the presence of several phenolic acids and flavonoid compounds [25]. These phenolic compounds are mainly present in their ester form and small portion present in their free form. Esterase's present in human gut can cleaves these ester bonds and release free acids in the small intestine and exert their antioxidant activity. The antioxidant activity increases with increasing concentration for any compound. The antioxidant activity of natural extracts depends on the nature of polyphenols present and their structure. Although many reports are available on the antioxidant activity of millets but limited information is available on the detailed study on antioxidant activity of pearl millet ICMV 221 and CO (CU) 9. Pushparaj *et al.,* [31] reported that the antioxidant activity of pearl millet cultivars-Kalukombu and Maharashtra Rabi Bajra was increased by heat processing such as boiling, roasting and pressure cooking compared to raw flour, which was attributed to the presence of higher flavonoid content in processed flours. Pradeep *et al.,* [37] also reported the higher antioxidant activity and nutraceutical properties in processed little millet by germination, steaming and roasting compared to native sample. Phenolics and lipids extracted from pearl millet exhibited immunomodulatory effects by inhibiting mitogen-induced T-cell proliferation [36].



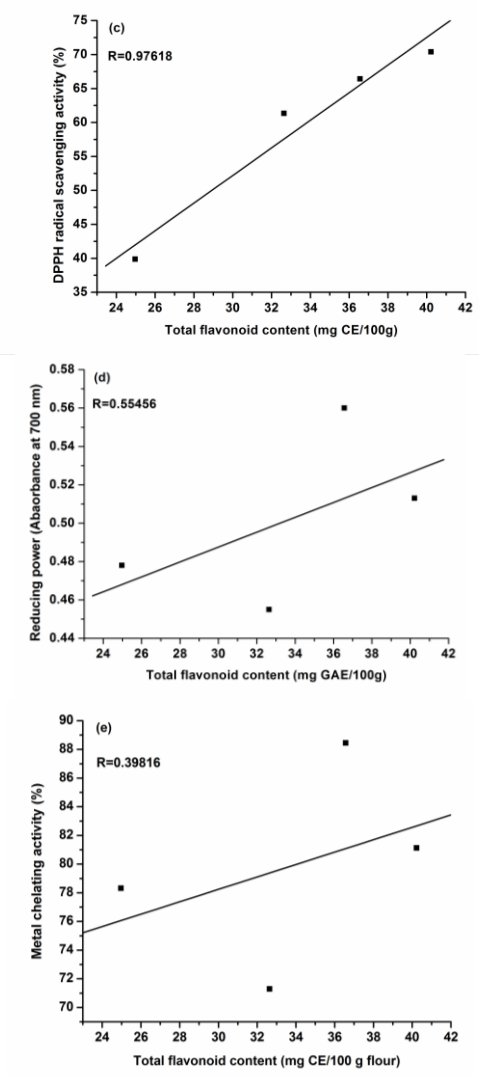


Figure 6: Correlation between the antioxidant activity and TPC and TFC of pearl millet and little millet methanolic extracts.

Conclusions

The methanolic extracts of the pearl millet and little millet were prepared and the total phenols, total flavonoid contents were estimated. All the millet varieties examined contained significant amount of phenolics, flavonoid content and showed appreciable antioxidant activity by scavenging DPPH radicals. Among the pearl millet varieties, CO (CU) 9 showed highest DPPH radical scavenging activity. Pearl millet ICMV 221 and CO (CU) 9 also showed highest reducing power. These activities were correlated well with flavonoids, and moderate correlation with total phenolic content. These results indicate that millets are potential sources of natural phenolic antioxidants and a diet rich in these grains might contribute its antioxidant potential for preventing diseases in which free radicals play a key role. Further studies are required for the identification of antioxidant active compounds from these extracts to understand the mechanism behind their health benefits.

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References

1. Valko, Marian, *et al.,* "Free radicals and antioxidants in normal physiological functions and human disease." The international journal of biochemistry & cell biology 39.1 (2007): 44-84.

2. Seifried, Harold E., *et al.,* "A review of the interaction among dietary antioxidants and reactive oxygen species." The Journal of nutritional biochemistry 18.9 (2007): 567-579.

3. Scalbert, Augustin, Ian T. Johnson, and Mike Saltmarsh. "Polyphenols: antioxidants and beyond." The American journal of clinical nutrition 81.1 (2005): 215S-217S.

4. Slavin, Joanne. "Whole grains and human health." Nutrition research reviews 17.1 (2004): 99-110.

5. Borneo, Rafael, and Alberto Edel León. "Whole grain cereals: functional components and health benefits." Food & function 3.2 (2012): 110-119.

6. Montonen, Jukka, *et al.,* "Whole-grain and fiber intake and the incidence of type 2 diabetes." The American journal of clinical nutrition 77.3 (2003): 622-629.

7. Liu, Rui Hai. "Whole grain phytochemicals and health." Journal of Cereal Science 46.3 (2007): 207-219.

8. Ragaee, Sanaa, El-Sayed M. Abdel-Aal, and Maher Noaman. "Antioxidant activity and nutrient composition of selected cereals for food use." Food Chemistry 98.1 (2006): 32-38.

9. Shahidi, Fereidoon, and Anoma Chandrasekara. "Millet grain phenolics and their role in disease risk reduction and health promotion: A review." Journal of Functional Foods 5.2 (2013): 570-581.

10. Sripriya, G., *et al.,* "ESR spectroscopic studies on free radical quenching action of finger millet (*Eleusine coracana*)." Food Chemistry 57.4 (1996): 537-540.

11. Hegde, Prashant S., and T. S. Chandra. "ESR spectroscopic study reveals higher free radical quenching potential in kodo millet (*Paspalum scrobiculatum*) compared to other millets." Food Chemistry 92.1 (2005): 177-182.

12. Viswanath, Varsha, Asna Urooj, and N. G. Malleshi. "Evaluation of antioxidant and antimicrobial properties of finger millet polyphenols (*Eleusine coracana*)." Food Chemistry 114.1 (2009): 340-346.

13. Chethan, S., Y. N. Sreerama, and N. G. Malleshi. "Mode of inhibition of finger millet malt amylases by the millet phenolics." Food Chemistry 111.1 (2008): 187-191.

14. Hegde, Prashant S., Gowri Chandrakasan, and T. S. Chandra. "Inhibition of collagen glycation and crosslinking in vitro by methanolic extracts of Finger millet (*Eleusine coracana*) and Kodo millet (*Paspalum scrobiculatum*)." The Journal of nutritional biochemistry 13.9 (2002): 517-521.

15. Hegde, Prashant S., Namakkal S. Rajasekaran, and T. S. Chandra. "Effects of the antioxidant properties of millet species on oxidative stress and glycemic status in alloxan-induced rats." Nutrition Research 25.12 (2005): 1109-1120.

16. Hegde, Prashant S., B. Anitha, and T. S. Chandra. "In vivo effect of whole grain flour of finger millet (*Eleusine*

coracana) and kodo millet (*Paspalum scrobiculatum*) on rat dermal wound healing." Indian journal of experimental biology 43.3 (2005): 254-258.

17. Murtaza, Nida, *et al.,* "Finger millet bran supplementation alleviates obesity-induced oxidative stress, inflammation and gut microbial derangements in high-fat diet-fed mice." British Journal of Nutrition 112.9 (2014): 1447-1458.

18. Sarma, Siddhartha M., *et al.,* "Kodo millet whole grain and bran supplementation prevents high-fat diet induced derangements in a lipid profile, inflammatory status and gut bacteria in mice." Food & function 8.3 (2017): 1174-1183.

19. Serba, Desalegn D., and Rattan S. Yadav. "Genomic tools in pearl millet breeding for drought tolerance: Status and prospects." Frontiers in plant science 7 (2016): 1724.

20. Shobana, S., *et al.,* "Finger millet (Ragi, *Eleusine coracana L.*): a review of its nutritional properties, processing, and plausible health benefits." Adv Food Nutr Res 69.1 (2013): 39.

21. Viswanath, Varsha, Asna Urooj, and N. G. Malleshi. "Evaluation of antioxidant and antimicrobial properties of finger millet polyphenols (*Eleusine coracana*)." Food Chemistry 114.1 (2009): 340-346.

22. Suma, P. Florence, and Asna Urooj. "Antioxidant activity of extracts from foxtail millet (*Setaria italica*)." Journal of food science and technology 49.4 (2012): 500-504.

23. Chethan, S., and N. G. Malleshi. "Finger millet polyphenols: characterization and their nutraceutical potential." Am J Food Technol 2.7 (2007): 582-592.

24. Pradeep, S. R., and Manisha Guha. "Effect of processing methods on the nutraceutical and antioxidant properties of little millet (*Panicum sumatrense*) extracts." Food chemistry 126.4 (2011): 1643-1647.

25. Chandrasekara, Anoma, and Fereidoon Shahidi. "Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MS n." Journal of Functional Foods 3.3 (2011): 144-158.

26. Singleton, Vernon L., and Joseph A. Rossi. "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents." American journal of Enology and Viticulture 16.3 (1965): 144-158.

27. Zhishen, Jia, Tang Mengcheng, and Wu Jianming. "The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals." Food chemistry 64.4 (1999): 555-559.

28. Brand-Williams, Wendy, Marie-Elisabeth Cuvelier, and C. L. W. T. Berset. "Use of a free radical method to evaluate antioxidant activity." LWT-Food science and Technology 28.1 (1995): 25-30.

29. Carter, Paul. "Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine)." Analytical biochemistry 40.2 (1971): 450-458.

30. Choi, Youngmin, Heon-Sang Jeong, and Junsoo Lee. "Antioxidant activity of methanolic extracts from some grains consumed in Korea." Food chemistry 103.1 (2007): 130-138.

31. Pushparaj, Florence Suma, and Asna Urooj. "Antioxidant activity in two pearl millet (*Pennisetum typhoideum*) cultivars as influenced by processing." Antioxidants 3.1 (2014): 55-66.

32. Do, Quy Diem, *et al.,* "Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*." Journal of food and drug analysis 22.3 (2014): 296-302.

33. Chandrasekara, Anoma, and Fereidoon Shahidi. "Inhibitory activities of soluble and bound millet seed phenolics on free radicals and reactive oxygen species." Journal of Agricultural and Food Chemistry 59.1 (2011): 428-436.

34. Nishani, Srikrishna S., *et al.,* "Phenolics, Antioxidant and Pasting Properties of Various Pearl Millet and Finger Millet varieties Varying in Amylose Content." Vegetos-An International Journal of Plant Research 27.2 (2014): 131-145.

35. Salar, Raj Kumar, and Sukhvinder Singh Purewal. "Phenolic content, antioxidant potential and DNA damage protection of pearl millet (*Pennisetum glaucum*) cultivars of North Indian region." Journal of Food Measurement and Characterization 11.1 (2017): 126-133.

36. Nani, Abdelhafid, *et al.,* "Effects of polyphenols and lipids from *Pennisetum glaucum* grains on T-cell activation: modulation of Ca 2+ and ERK1/ERK2 signaling." BMC complementary and alternative medicine 15.1 (2015): 426.

37. Pradeep, S. R., and Manisha Guha. "Effect of processing methods on the nutraceutical and antioxidant properties of little millet (*Panicum sumatrense*) extracts." Food chemistry 126.4 (2011): 1643-1647.

38. Chandrasekara, Anoma, and Fereidoon Shahidi. "Antioxidant phenolics of millet control lipid peroxidation in human LDL cholesterol and food systems." Journal of the American Oil Chemists' Society 89.2 (2012): 275-285.

39. Alam, Md Nur, Nusrat Jahan Bristi, and Md Rafiquzzaman. "Review on in vivo and in vitro methods evaluation of antioxidant activity." Saudi Pharmaceutical Journal 21.2 (2013): 143-152.

40. López-Alarcón, Camilo, and Ana Denicola. "Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays." Analytica chimica acta 763 (2013): 1-10.

41. Berwal, Mukesh Kumar, *et al.,* "Total Antioxidant Potential of Pearl Millet Genotypes: Inbreds and Designated B-lines." Indian J Agric Biochem 29.2 (2016): 201-204.

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