

Original Research Article

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Nutrient and Antioxidant Evaluation of Four Underutilized Minor Millets

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ABSTRACT

The antioxidants of cereals are usually not given importance as vegetables and fruits. Though its health benefits are known from the olden days they are not given importance in the area of Research. The importance of cereals as sources of antioxidants is often undervalued because of the relatively low content of antioxidants, but the consumption of cereals is high. In the present research work, the less explored, underutilized, nutrient rich, commercially available minor millets Foxtail millet (*Setaria italica* (L.) Beauv), Kodo millet (*Paspalum scrobiculatum* L.), Little millet (*Panicum sumatrense* Roth ex Roem. and Schultz) and Barnyard millet (*Echinochloa frumentacea* (Roxb.) Link) were analysed for their phytochemical constituents and antioxidant activity. The aqueous extract of the five samples were subjected to proximate, qualitative and quantitative phytochemical and antioxidant activity analysis. It was found that the chosen minor millets are rich in crude fibre and protein. It was also found that all the four grains are found to contain Phytochemicals like phenolic compounds, steroids, tannins, Flavanoids, and many. They are found to be rich in calcium, iron and phosphorous. The grains also showed increasing antioxidant activity with increasing concentrations. Thus the revealed nutritional property and antioxidant activity of the four selected underutilized minor millets may benefit the people to make them overcome or survive from various chronic diseases like diabetes mellitus, cardiovascular diseases, etc.

Keywords

Barnyard millet,
Little millet,
Foxtail millet,
Kodo millet,
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Phytochemicals,
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Introduction

One of the basic needs of human being is food and it is the food that provides us the necessary nutrition. As said by Hippocrates” Let food be thy medicine and medicine be thy food”, in recent years people concentrate more on the functional foods (Hardy, 2000).The present century people are facing problems like water scarcity, increasing

population, climatic changes, rise in food price and other factors leads to great demand for food worldwide and a great threat to food security. Cereals are consumed a lot and it plays an important role in human diet. Among cereals it is Minor millets that contributes a lot to the potential health benefits and national food security and it has

recently gained interest of scientists and nutritionists (Ahmed, *et al.*, 2013).

Phytochemicals and phenolic compounds in minor millets enhances its antioxidant activity and makes it nutritionally superior to other cereals.

Minor millets like finger millets and pearl millets are more exploited for research but there is a need to find the nutritional and clinical importance of other less explored, underutilized minor millets like kodo millet, foxtail millet, little millet and barnyard millet (Vandana Mishra *et al.*, 2014).

Minor millets in general is rich in essential aminoacids, good source of range of micronutrients like iron, calcium and dietary fibre, high in important vitamins like folic acid, thiamine, niacin and riboflavin, low in glycemic index (Mal, *et al.*, 2013, Yenagi, *et al.*, 2010). India ranks first in the minor millets production but its health and nutritional benefits are not aware among the people (Ahmed, *et al.*, 2013(b)).

The food concept previously emphasized on hunger satisfaction, survival and health maintenance but currently it emphasizes on the 'functional foods' and especially on the 'Antioxidants', that can neutralize the free radicals which is getting popularized recently among people (Handelman, *et al.*, 1996). Many synthetic antioxidants are available in the market and due to potential hazards they must be used under strict regulations.

So, interest has turned towards 'Natural Antioxidants' (Husrev, *et al.*, 2012). Much attention has to be paid on the antioxidants of minor millets by closely examining its Phytochemicals because it has potential role in preventing chronic diseases.

Materials and Methods

Plant Materials

Minor millets viz. Foxtail millet (*Setariaitalica* (L.) Beauv), Kodo millet (*Paspalum scrobiculatum* L.), Little millet (*Panicum sumatrense* Roth ex Roem. and Schultz) and Barnyard millet (*Echinochloa frumentacea* (Roxb.) Link) were purchased from a local shop in Erode, Tamilnadu, India and was identified and authenticated by Dr. K.Althaf Ahamed Kabeer, Scientist-D, Botanical survey of India, Southern regional centre, Coimbatore, Tamilnadu, India. The minor millets were cleaned under running tap water for 10 min, rinsed twice with distilled water and air-dried in an oven at 40° C overnight and were ground to a powder separately using an electric grinder for 10 min. Since this research work is to evaluate the minor millets just as a 'Nutritious diet', the phytochemical analysis was done with the powdered form of the minor millets i.e., only with the aqueous extract and not with the other extracts.

Proximate composition of the millet grains

Moisture content was determined by the method of Pearson (Pearson, 1962, Pearson (b) 1962). Total lipid was determined by the methods of Colowick and Kaplan (Colowick and Kaplan 1957). Carbohydrates were estimated spectrophotometrically according to the procedure of Nelson (Nelson N.J 1944). Crude fibre content was determined by the method of Pearson (Pearson, 1973, Pearson D(b) 1973). Crude protein was estimated by "Micro Kjeldahls" method (Nelson, 1944). Total energy was calculated according to Abulude and Folourunso (Abulude and Folourunso, 2003).

The following equation:

$$\text{Energy(Kcal)} = 4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g fat}) \quad (1)$$

Qualitative Analysis of Phytochemicals

The preliminary phytochemical analysis of the powdered minor millets was carried out by using the standard procedures. Alkaloids, carbohydrates, flavonoids, glycosides, phytosterols, proteins, carotenoids and saponins were qualitatively analysed (Kokate, *et al.*, 1995).

After the confirmation of presence of phenols, alkaloids, flavonoids, saponins and tannins by preliminary phytochemical tests, the coarse powder of the selected Minor millets and its combined mixture samples were taken for quantitative estimation.

Quantitative Analysis of Phytochemicals

Calcium estimation

Calcium was determined by precipitating it as calcium oxalate and titrating the solution of oxalate in dilute sulphuric acid against standardized KMnO₄ (Bernard & Hawks, 1965).

Determination of Iron (by Wong's method)

Iron was determined colorimetrically by making use of the principle that ferric iron gives a blood red colour with potassium thiocyanate (NIN, 1983). To a known volume of mineral solution (taken from the total ash as described previously), 1.0 mL each of 30% H₂SO₄ and 7% potassium persulphate solution and 1.5 mL of 40% potassium thiocyanate solution were added with thorough mixing. The red colour that developed was measured within 20 min at 540 nm. Similarly a standard curve was

generated by using ferrous ammonium sulphate. The iron content of sample was then read off from the standard curve.

Estimation of total phenols

Phenols were estimated by the Folin-Ciocalteu method (Malick and Singh, 1980). Exactly 0.5 mg of the sample was weighed and ground with a pestle and mortar in 10 time volume of 80% ethanol. Centrifuged the homogenate at 10,000 rpm for 20min. Re-extracted the residue with five times the volume of 80% ethanol, centrifuged and pooled the supernatants. Evaporated the supernatant to dryness. Dissolved the residue in a known volume of distilled water (5ml). Pipetted out different aliquots (0.2-2 ml) into test tubes. Make up the volume in each tube to 3ml with water. Folin- Ciocalteu reagent about 0.5 ml was added. After 3 min, 2 ml of 20% Na₂CO₃ solution was added to each tube, mixed thoroughly and kept the tubes in a boiling water for exactly one minute. Further cooled and measured the absorbance at 650 nm against a reagent blank.

Antioxidant Assay

DPPH radical scavenging assay

In this assay, free radical scavenging activity of crude extract was determined by measuring the bleaching of purple-colored methanol solution of DPPH. One millilitre from a 0.5 mM methanol solution of the DPPH radical was mixed to 2.0 ml of different concentrations of 95% ethanol; methanol and water extract and were added 2.0 ml of 0.1 M sodium acetate buffer (pH 5.5). The mixtures were well shaken and kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm positive control, whereas methanol was used as negative one.

$$\% \text{ RSA} = [(A_0 - A_S) / A_0] \times 100 \quad (2)$$

Where A₀ and A_S are the absorbance of the control (containing all reagents, except the test compound) and test compound respectively (Veenashri *et al.*, 2011).

Reducing power

The reducing power of minor millets was measured according to Wu *et al.*, (2003). To different concentrations of sample (20 to 100 µg/ml) were added to 2 ml of 0.2 M phosphate buffer (pH 6.6) and 2 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min, and then 2 ml of 10% (w/v) trichloroacetic acid (TCA) added. The mixture was centrifuged for 10 min at 3000 × g, and 2 ml of the supernatant was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) FeCl₃. After reaction for 10 min, the absorbance of the solution was read at 700 nm. Increase in the absorbance of the reaction mixture indicated increased reducing power.

Hydrogen peroxide scavenging assay

The ability of all seeds extracts to scavenge hydrogen peroxide was determined according to the. A solution of hydrogen peroxide (2 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Extracts samples (10 to 50 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of both extracts and standard compounds are calculated by using following equation 3 (Lin *et al.*, 2002).

$$\% \text{ Scavenged H}_2\text{O}_2 = [(A_0 - A_1) / A_1] \times 100 \quad (3)$$

Where A₀ was the absorbance of the control, and A₁ was the absorbance in the Presence of the extracts and Standards.

Statistical analysis

All the experiments were conducted in triplicates and the results were reported as mean values with their respective standard deviations.

Results and Discussion

The nutritional evaluation of selected minor millets has been given in Table 1. The maximum moisture content of the selected minor millets ranged from 10.91 to 11.83 g/100g and lowest in Barnyard (10.91 g/100g) whereas highest in Little millet (13.35 g/100g). The total lipid content was high in Foxtail (4.37g/100g) and the lowest in kodo(3.03g/100g).Crude fibre was high in barnyard(12.5 g/100g) and low in Little (6.7 g/100g).Among the millets the protein content was recorded to be high in Foxtail millet (13.3 g/100g) followed by barnyard millet (11.23 g/100g), Little millet (10.47 g/100g) and kodo millet (8.57 g/100g).The maximum carbohydrate content was found in Barnyard(84.63g/100g) with reducing sugar content high in Little millet (5.2 g/100g) and high non reducing sugar content in Barnyard (81.07g/100g). Thus the energy content was found to be high in Barnyard (412.93 kcal),followed by Little (383.2 kcal),Foxtail (370.37 kcal),Mixed sample (362.2 kcal) and kodo (341.2 kcal).These values are in slightly close proximity with the study of (Sangeeta Gupta *et al.*, 2014). Minor millets are found to possess hypoglycemic and hypolipidemic activity which may be due to high dietary fibre and its resistant starch content (Pathak and Srivastava, 1998). The minor millets

improves its digestibility and helps in the peristaltic movement of the intestine and this is due to their high crude fibre (Davies *et al.*, 1999). The quality of the protein was high in minor millets (Kalinova and Moudry, 2006).

The results of phytochemical screening of selected minor millets are presented in Table 2. Phytochemicals alkaloids, Flavanoids, phenolic compounds, Saponins, Carotenoids, tannins, fatty acids, Coumarins, reducing sugar, protein, carbohydrate and aminoacids were present. Phlobotannins was absent in all the four samples. Phytochemicals are biologically active organic substance of plant origin which are involved in preventing disease and promoting health. The screening of these phytochemicals may help in identifying the active compounds responsible for disease prevention. Most of the phytochemicals have antioxidant activity (Narasinga Rao 2003). In another study, it was also found that the bound phytochemicals of grain prevents colon cancer, other digestive cancers, prostate cancer and breast cancer (Florence Suma and Asna Urooj, 2012).

The results of Calcium, Iron and total phenolics in the four selected minor millets are summarized in Table 3. It was found that calcium was very high in Little millet (39.1 mg/ml) and least in barnyard millet (7.23 mg/ml). Kodo millet was found to contain high iron content (14.1 mg/100g) and the presence of phenolic compounds were found increasing in the order in foxtail (80.5 µg/ml) > barnyard (67.73 µg/ml) > little (42.6 µg/ml) > kodo (40.07 µg/ml). High phenolic content can be linked to the high antioxidant activity (Thippuswamy, B and Akilendar Naidu, 2005). It is also associated

with reduced risk of chronic disease and its antioxidant activity protects against degenerative diseases (Ismail, *et al.*, 2010). Phenolic compounds, an important secondary metabolite of minor millets act as singlet oxygen quenchers, antioxidants, inhibitors of digestive and regulatory enzymes, reducing agents and metal chelators (Truswell, 2002).

The results of DPPH radical scavenging effect of the aqueous extract in the range of 0.02 – 0.25 mg/ml increased with an increasing concentration for all the four samples is depicted in figure 1. The degree of discoloration indicates the scavenging capacity of the extract. The effect of antioxidants on the DPPH radical scavenging was thought to result from their hydrogen donating ability.

Five different concentrations of the aqueous extracts of the four samples of minor millet ranging from 20 µg/mL to 100 µg/mL were used to see the effect of the reducing power. The results (Figure 2) showed that the reducing power of all the four samples were concentration dependent. In this assay, the presence of antioxidants caused the reduction of the Fe³⁺/ferricyanide complex to the ferrous form, and the yellow color of the test solution changed to various shades of green and blue depending on the reducing power of each compound. 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). In another study, it was found that water extract of minor millets showed significantly higher reducing power than other extracts (Jignasu P. Mehta and Sohil H. Vadia, 2014).

Table.1 Proximate analysis of the selected four Minor Millets

S.No	Name of the Minor millets	Moisture (g/100g)	Total Lipid (g/100g)	Crude Fibre (g/100g)	Crude Protein (g/100g)	Total Carbohydrates (g/100g)	Reducing Sugar (g/100g)	Non-reducing Sugar (g/100g)	Energy (Kcal)
1	Kodo	12.5±0.09	3.03±0.11	7.39±0.07	8.57±0.12	71.2±0.3	4.1±0.46	67.1±0.35	341.2±9.01
2	Barnyard	10.91±0.2	3.27±0.2	12.5±0.53	11.23±0.25	84.63±0.45	3.57±0.58	81.07±0.4	412.93±2.36
3	Foxtail	12.23±0.12	4.37±0.23	11.37±0.32	13.3±0.26	69.47±0.65	3.7±0.26	65.77±0.67	370.37±4.31
4	Little	13.35±0.39	4.13±0.57	6.7±0.12	10.47±0.25	76.07±0.4	5.2±0.17	70.87±0.51	383.2±5.75

Results are Mean ± SD of three determinants

Table.2 Qualitative phytochemical Analysis of the selected four Minor Millets

S.No	Phytochemicals	Minor millets				
		Kodo millet	Barnyard millet	Foxtail millet	Little millet	Mixture of the four millets
1	Phytosterols	+	+	+	+	+
2	Carotenoids	+	+	+	+	+
3	Phenolic compounds	+	+	+	+	+
4	Flavanoids	+	+	+	+	+
5	Saponins	+	+	+	+	+
6	Alkaloids	+	+	+	+	+
7	Tannins	+	+	+	+	+
8	Fatty acids	+	+	+	+	+
9	Glycosides	+	+	+	+	+
10	Phlbotannins	-	-	-	-	-
11	Coumarins	+	+	+	+	+
12	Reducing sugar	+	+	+	+	+
13	Protein	+	+	+	+	+
14	Carbohydrate	+	+	+	+	+
15	Amino acids	+	+	+	+	+

Table.3 Quantitative Analysis of Calcium, Iron and Phenolic compounds in the selected four Minor millets

S.No	Name of Millet	Calcium (mg/g)	Iron (mg/100g)	Phenolic compounds (µg/ml)
1	Kodo	38.87±0.15	14.1±0.1	40.07±0.12
2	Barnyard	7.23±0.06	3.63±0.06	67.73±0.25
3	Foxtail	11.37±0.21	5.23±0.21	80.5±0.5
4	Little	39.1±0.17	1.47±0.06	42.6±1.22

Results are Mean ± SD of three determinants

Fig.1 DPPH radical Scavenging Activity of the four selected minor millets

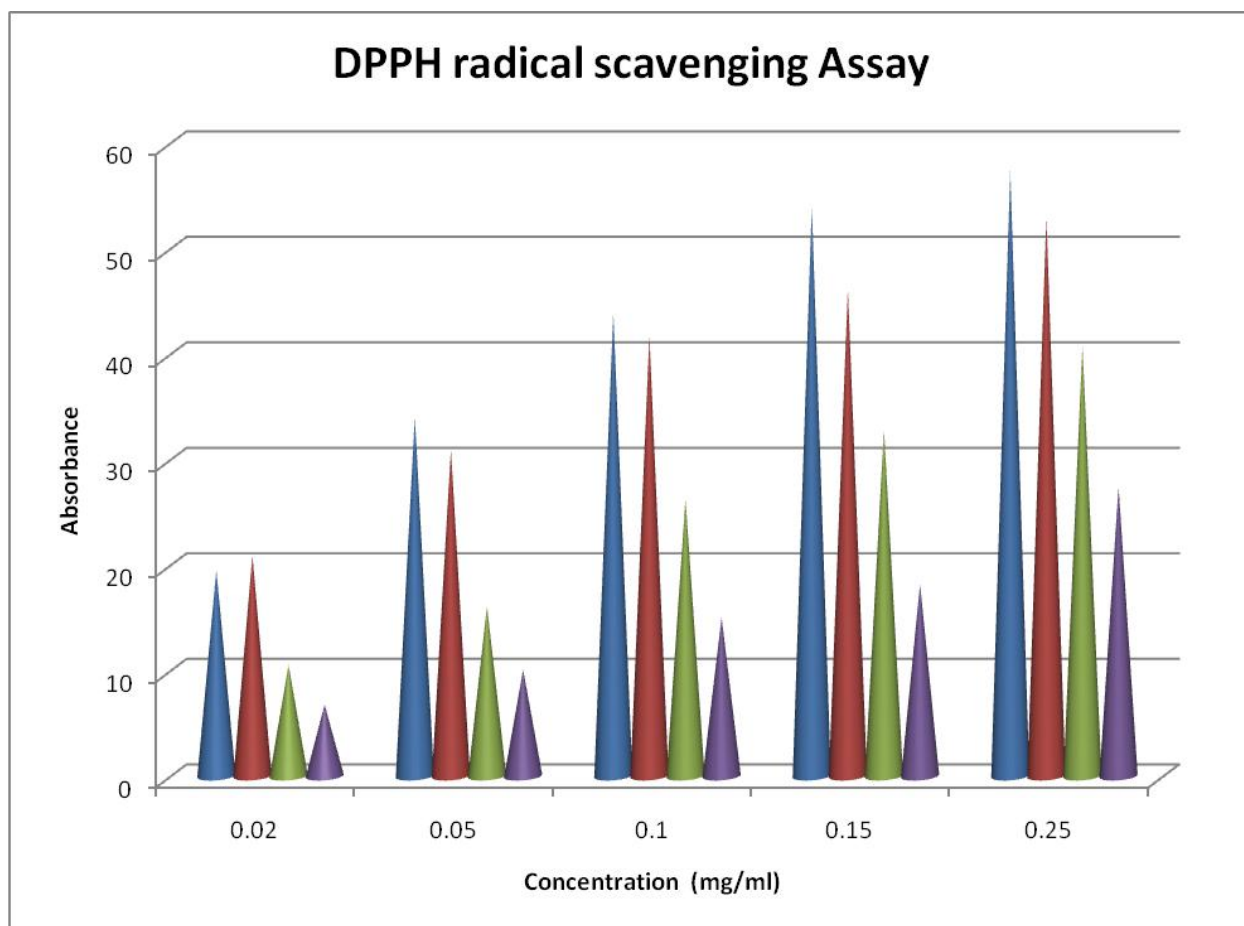


Fig.2 Reducing assay of the selectd four minor millets

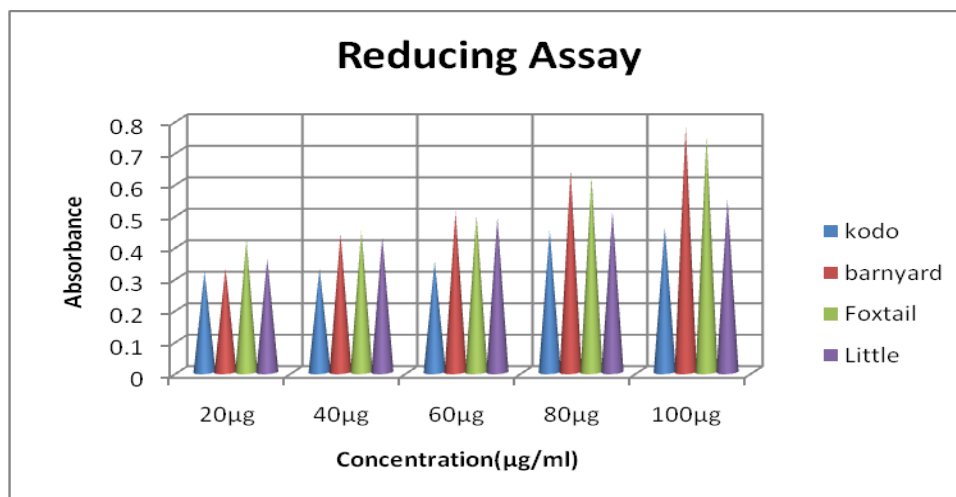
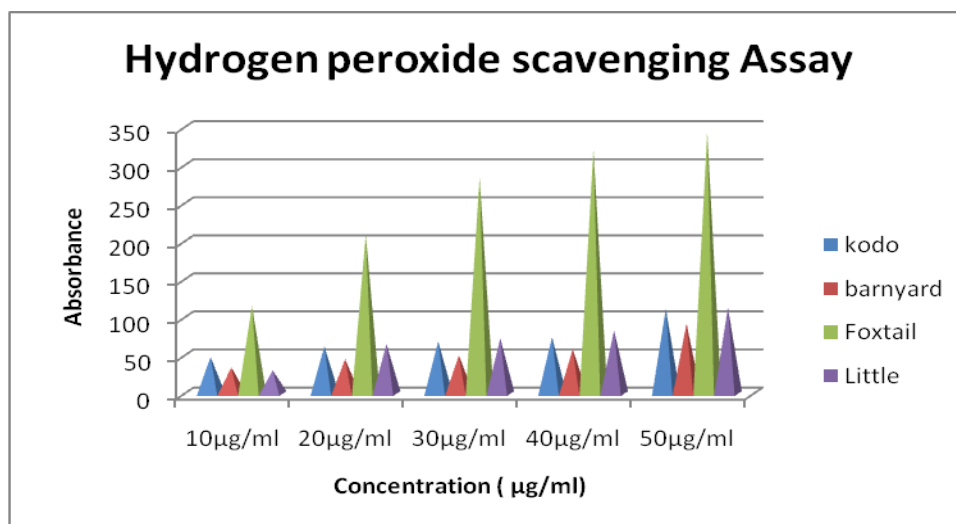


Fig.3 Hydrogen peroxide scavenging assay of the selectd four minor millets



The results (Figure 3) of H₂O₂ scavenging activity indicated that all the minor millets showed excellent H₂O₂ scavenging activities at a concentration range from 10 µg/mL to 50 µg/mL in the reaction mixture and increased steadily with the increased concentration.

In conclusion, minor millets are being consumed by many people nowadays because lot of awareness about its goodness is being created among the people and this research will also be an added step for the people running behind fast and junk foods.

The presence of good amount of iron, calcium and phosphorous will be a boon especially for the menopausal women whose mineral loss is being met by these minor millets. The presence of phytochemicals like flavanoids, phenolic compounds and other antioxidants proves minor millets to be a good nutraceutical food when consumed regularly can protect us from the chronic complications like Diabetes mellitus, cardiovascular diseases and cancer which threatens the people of many countries.

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