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ESR spectroscopic study reveals higher free radical quenching potential in kodo millet (*Paspalum scrobiculatum*) compared to other millets

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Abstract

Six different millets kodo millet (*Paspalum scrobiculatum*), finger millet (*Eleusine coracana*), little millet (*Panicum miliare*), foxtail millet (*Setaria italica*), barnyard millet (*Echinochloa utilis*) and great millet (*Sorghum bicolor*) grown in India and their white varieties were screened for free radical quenching of 1,1, Diphenyl-2-picrylhydrazyl (DPPH) by electron spin resonance (ESR). Methanol extracts of the kodo millet flour showed 70% DPPH quenching in comparison to other millet extracts which showed 15–53%. The white varieties of great millet, finger millet and foxtail millet showed lower quenching than their coloured counterparts, indicating that phenolics in the seed coat could be responsible for the antioxidant activities. However, the content of the phenols and tannin in these grains did not correlate with the antioxidant activities. Kodo millet had the highest DPPH quenching activity followed by great millet and finger millet. Cooking of kodo or finger millet by roasting or boiling reduced the activity. Fractionation of kodo millet in to husk and endosperm also decreased the activity and the phytochemicals appear to act synergistically.

Keywords: Kodo millet; *Paspalum scrobiculatum*; Finger millet; *Eleusine coracana*; Little millet; *Panicum miliare*; Foxtail millet; *Setaria italica*; Barnyard millet; *Echinochloa utilis*; Great millet; *Sorghum bicolor*; 1,1, Diphenyl-2-picrylhydrazyl; Electron spin resonance; Free radical; Antioxidant; Phenolics; Scavenging effects

1. Introduction

Nutritive potential of millets in terms of protein, carbohydrate and energy values are comparable to the popular cereals like rice, wheat, barley or bajra (Malleshi & Hadimani, 1993). Kodo millet (*Paspalum scrobiculatum*) and little millet (*Panicum miliare*) have 37–38% of dietary fibre, which is the highest among the cereals; the fat has higher PUFA (Malleshi & Hadimani, 1993); the mineral content is also higher than rice or wheat. Finger millet (*Eleusine coracana*) has the highest calcium content known in foods (344 mg/100 g; Gopalan, Ramashastri, & Balasubramanian, 1989). However, the millets also contain phytates, phenols, tannins, trypsin inhibitory factors, and dietary fibre which act as "antinutrients" by chelating metals, or inhibiting enzymes (Thompson, 1993). It is now established that phytates, phenols and tannins can contribute to antioxidant activity important in health, aging and metabolic diseases (Bravo, 1998).

Although extensive information is available on millets proximate composition and processing (Sripriya, Usha, & Chandra, 1997; Usha, Sripriya, & Chandra, 1996), their phenolic content and associated antioxidant properties has surprisingly not been investigated to the

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same extent. We reported for the first time antioxidant activity in finger millet extracts by ESR (Sripriya, Chandrasekharan, Murthy, & Chandra, 1996). The extracts showed protection against non-enzymatic glycosylation of rat tail tendon collagen (Prashant, Gowri, & Chandra, 2002). Millets are semi-arid crops grown in India and Asia and form a staple food for the lower socio-economic class especially during drought or famine. They are consumed traditionally as health and vitality foods by the labour class (peasants, artisans) in rural India.

Hence a detailed investigation in to the polyphenols and tannin content of millets and their importance as a source of dietary antioxidants for a large section of Indian population was deemed important.

ESR is a sensitive method for assessing the free radical scavenging activity of antioxidants (Antolovich, Prenzier, Patsalides, McDonald, & Robards, 2002). Hence ESR was used to screen for radical scavenging effects of millets commonly grown in India.

2. Materials and methods

2.1. Grains

The seeds of one red and one white variety of *Sorghum bicolor* (Great millet; Jowar), one red and one white variety of *Setaria italica* (Foxtail millet; Thenai), one variety of *Panicum miliare* (Little millet; Samai), one variety of *Echinochloa utilis* (Barnyard millet; Sanwa) and *Paspalum scrobiculatum* (Kodo millet; Varagu) were obtained from local market in Theni, Tamilnadu, India. The CO-13 brown variety and CO-9 white variety of *Eleusine coracana* (Finger millet; Ragi) were procured from Tamilnadu Agricultural University Coimbatore; the GPU-26 brown variety of *Eleusine coracana* from University of Agricultural Sciences Bangalore; and Vamban 1 variety of *Paspalum scrobiculatum* (Kodo millet; Varagu) from National Pulses Research Centre, Vamban, Tamilnadu, India.

2.2. Chemicals

1,1, Diphenyl-2-picrylhydrazyl (DPPH), Vitamin E, and Catechin were purchased from Sigma–Aldrich Chemical Company (St Louis, USA); Vitamin C from SISCO Research Laboratories (Mumbai, India). All other chemicals used were of analytical grade.

2.3. Processing of millets

Millets were cleaned, dried and stored in airtight containers at 4 °C. Two hundred grams of Kodo millet (Vamban 1 var) was subjected to dehusking by gentle crushing and the husk and endosperm were separated with a 0.2 mm mesh. Fifty grams of the whole grains of finger millet (CO-13) and kodo millet (Vamban 1) were dry roasted for 5 min and ground to flour. Fifty grams of flour of kodo millet (Vamban 1) was boiled in 250 ml of water for 15 min and dried at 50°C overnight in an oven. Similarly, finger millet (CO-13) was subjected to roasting and boiling.

2.4. Extraction

Twenty five grams of the processed flour and husk were defatted overnight with 125 ml of hexane. The hexane was decanted and the dry flour was refluxed with 100 ml methanol for 2 h at 60 °C. The extraction was repeated with 50 ml methanol as described above. The extracts were pooled, filtered (Whatman No. 1) and concentrated in a rotary evaporator and made up to 10.0 ml with methanol and stored at 0 °C till use.

2.5. Determination of antioxidant activity by ESR spectroscopy

ESR measurements were performed at RT in X band using a Varian type ESR spectrometer Model E 112. The conditions were as follows: magnetic field 3350 G, microwave power 20 mW, modulation frequency 100 kHz, modulation amplitude 0.5×10 G, scan range 10×10 G, time constant 0.5 s, receiver gain 10×10^3, microwave frequency 9.44 GHz, scan time 4 min. The DPPH radicals were analysed by the method of Santiago, Midori, and Akitane, 1992. The extract was diluted to 1/100 and 10 μl of this diluted extract was added to 0.5 ml of DPPH (0.025 μmol) in methanol, mixed and immediately transferred to the special ESR spectrometry aqueous cell and analysed after exactly 60 s. A control was performed with 10 μl methanol.

2.6. Total phenols

Total phenolics in the methanolic extracts was determined using Folin–Ciocalteu reagent with catechol as the standard (Singleton & Rossi, 1965). Appropriate volume of the methanolic extract of the millets was made upto 3 ml with distilled water, mixed with 0.5 ml of Folin–Ciocalteu reagent (1:2 dilution with distilled water) and allowed to stand for 3 min at room temperature; 2 ml of sodium carbonate (20%) solution was added to the mixture. The mixture was thoroughly mixed and kept in boiling water for exactly 1 min, cooled and the absorbance measured at 650 nm. Results are expressed as mg catechol equivalents/100 g of dry flour.

2.7. Tannins

Tannins in the methanolic extracts were measured spectrophotometrically using catechin as standard by

the modified Vanillin–HCl method (Price, Van Scoyoc, & Butter, 1978). Appropriate volume of the methanolic extract of the millets was made upto 1 ml with distilled water and 5 ml of Vanillin–HCl reagent added immediately. The tubes were allowed to stand at room temperature for 20 min and the colour developed was measured at 500 nm. Results are expressed as catechin equivalents in mg/100 g of the dry flour.

3. Results and discussion

ESR spectrometry is a sensitive technique for the detection of free radical quenching activity. Many studies are reported on the use of ESR for evaluating antioxidant activity in foods. The oxidative stability of processed cheese (Kristensen & Skibsted, 1999); levels of radicals during the progress of oxidation in whole milk powder (Nielsen, Stapelfeldt, & Skibsted, 1997); scavenging properties of three hard winter wheat varieties of wheat (Yu et al., 2002); carbon centred radicals in rhizome extract and rhizome knot extract of edible lotus (Hu & Skibsted, 2002) were evaluated using ESR technique. The DPPH radical quenching was reported in Japanese Soybean paste Miso (Santiago et al., 1992). Subsequently Sripriya et al. (1996) reported DPPH free radical quenching activity of millet extracts by following similar ESR technique. In present study similar technique of ESR was used for screening millet varieties and evaluate the effect of cooking on their antioxidant activity.

The millet extracts quenched DPPH to varying extent (Fig. 1). Kodo millet (Vamban 1) quenched DPPH by nearly 70% (9×10^{19} spins/ml), which was higher than the other millets (15–53%). The quenching by kodo millet was higher than great millet or finger millet CO-13 (Fig. 1). Although great millet (red) showed slightly higher activity than finger millet (Fig. 1) this millet is not presently consumed for human diet and is used more as a fodder (Joseph, Evangeline, & Odette, 1980). Our earlier report indicated finger millet (brown) to be a more potent radical scavenger than rice, wheat, or sorghum (Sripriya et al., 1996; Sripriya, 1998).

The white varieties of the millets (white foxtail and great millet) show distinctly lower activity than their coloured counterparts. This is in agreement with our previous report where the white finger millet variety had almost negligible radical quenching activity (Sripriya et al., 1996). Since the white variety cultivars are being promoted by agriculturists for aesthetic purpose to avoid the dark colour in millet based foods our findings of loss in the antioxidant phytochemical property assumes much significance.

To further evaluate the potency of kodo millet antioxidant activity, various dilutions were tested for DPPH

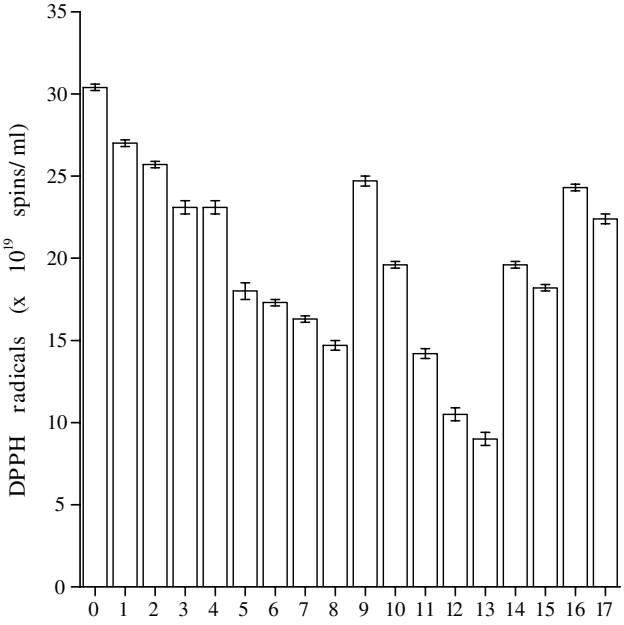

Fig. 1. Comparison of free radical quenching action of millets by ESR. 0 – methanol + DPPH alone; 1 – finger millet (CO-9 white var); 2 – foxtail millet (white var); 3 – little millet; 4 – great millet (white var); 5 – foxtail millet (red var); 6 – barnyard millet; 7 – finger millet (GPU 26 brown var); 8 – finger millet (CO-13 brown var); 9 – finger millet (CO-13 brown var) roasted; 10 – finger millet (CO-13 brown var) boiled; 11 – great millet (red var); 12 – kodo millet; 13 – kodo millet (Vamban 1 var); 14 – kodo millet (Vamban 1 var) husk; 15 – kodo millet (Vamban 1 var) endosperm; 16 – kodo millet (Vamban 1 var) roasted; 17 – kodo millet (Vamban 1 var) boiled; 10 μl of 1/100 diluted methanol extract of the above millets were taken. An equal volume of methanol was used in the control. Values are means ± S.D of three independent experiments.

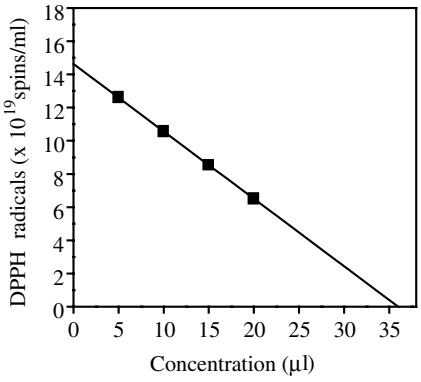

Fig. 2. Effect of kodo millet (Vamban 1) extract on quenching of 1,1, diphenyl-2-picrylhydrazyl (DPPH). 5–20 μl of 1/100 diluted methanol extract was used for quenching.

quenching in comparison with vitamin antioxidants E and C. Kodo millet showed good linearity in quenching DPPH (Fig. 2) and 18.5 μl (1/100 dilution) was required for 50% quenching. For similar 50% quenching of DPPH 0.946 μmol/ml of Vitamin C and 0.348 μmol/ml Vitamin E were required.

Roasting and boiling are normal daily cooking requirements. The millet flours are consumed in two forms, roasted as *chapattis* or pancakes, or boiled as porridge. Hence the flours of finger millet (CO-13) and kodo millet (Vamban 1), which had higher free radical quenching activity, were evaluated after roasting and boiling (Fig. 1). In both the millets the activity reduced on processing and the decrease was higher with roasting.

While the small grained finger millet is consumed as a whole after milling and cannot be dehulled under dry condition, the bigger grains of kodo millet are easily dehusked. The rural population who consume kodo millet generally dehusk it and utilize only the endosperm for cooking as rice or making porridge. From the industrial point of view it becomes necessary to find use for large amounts of husk, which is discarded. There are several reports on the antioxidant activity of residual materials from agro-industrial origin like peanut hulls, potato peel waste, oat hulls, wild rice hulls, mung bean hulls etc (Moure et al., 2001). Our study showed that the husk or endosperm alone of kodo millet (Vamban 1) does not contribute significantly to the radical scavenging effects (Fig. 1). Instead the phytochemicals from both the fractions appear to act synergistically in the whole grain flour (Fig. 1).

Polyphenolic compounds such as flavonoids, phenolic acids, proanthocyanadins are of great interest for the radical scavenging activity and are expected to be effective in the prevention of many diseases and morbid states (Pietta, 2000). The phenolic and tannin contents in the different methanolic extracts of the millets was estimated but did not correlate with the antioxidant activity (Table 1, Fig. 1). However, increasing concentration of extract correlated well with the free radical quenching activity (Fig. 2) indicative of the role of phenolics here. The total phenolic content (TPC) did not correlate with antioxidant activity of 44 different berry and fruit wines and liquors (Heinonen, Lehtonen, & Hopia, 1998); three hard winter varieties of wheat (Yu et al., 2002); 92 phenolic extracts from edible and non-edible plant materials (Kahkonen et al., 1999); malts (Maillard & Berset, 1995); citrus residues (Bocco, Cuvelier, Richard, & Berset, 1998); tea and herbal infusions (Atoui, Mansouri, Boskou, & Kefalas, 2005) and cereals (Sripriya et al., 1996). The TPC correlated with DPPH radical quenching in lotus extract but not with the scavenging of carbon-centred radical (Hu & Skibsted, 2002). The antioxidant activity depends on the type and polarity of the extracting solvent, the isolation procedures, test system and substrate to be protected by the antioxidant (Meyer, Heinonen, & Frankel, 1998).

The extent of antioxidant activity of phenolics depends on the position and extent of hydroxylation of the phenolic rings (Miyake & Shibamoto, 1997). Many other structural features play a significant role in determining the extent of antioxidant activity (Bravo, 1998). Since extracts of natural products have several phenolic compounds, synergistic activity is common. The structure–activity relationships (SARs) seem most important for contributing antioxidative property to any plant product (Bors, Michel, & Stettmaier, 2001; Bravo, 1998; Heim, Tagliaferro, & Bobilya, 2002).

Fractionation of these plant foods for their phenolic profile has shown innumerable compounds. For example over 60 different flavonoids, phenolic acids and their derivatives were identified in herbal infusions

Table 1
Total phenols and tannin content of millets[a]

S. no.	Name of the millet	Total phenols[b]	Tannins[c]
1.	*Eleusine coracana* (Finger millet CO-9 white var)	10 ± 0.25	ND
2.	*Setaria italica* (Foxtail millet, white var)	27 ± 0.76	Nil
3.	*Panicum miliare* (Little millet)	86 ± 2.5	Nil
4.	*Sorghum bicolor* (Great millet, white var)	63 ± 2	70 ± 2
5.	*Setaria italica* (Foxtail millet, red var)	33 ± 1	Nil
6.	*Echinochloa utilis* (Barnyard millet)	22 ± 0.76	Nil
7.	*E. coracana* (GPU-26 brown var)	98 ± 2	524 ± 4
8.	*E. coracana* (CO-13 brown var)	75 ± 2.25	146 ± 5
9.	*E. coracana* (CO-13 brown var) roasted	62 ± 1	140 ± 3
10.	*E. coracana* (CO-13 brown var) boiled	53 ± 0.94	142 ± 7
11.	*Sorghum bicolor* (Great millet, red var)	120 ± 4	250 ± 8
12.	*Paspalum scrobiculatum* (Kodo millet)	368 ± 8.5	Nil
13.	*P. scrobiculatum* (Vamban 1 var)	59 ± 2.75	Nil
14.	*P. scrobiculatum* (Vamban 1 var) husk	43 ± 1.00	Nil
15.	*P. scrobiculatum* (Vamban 1 var) endosperm	13 ± 0.30	Nil
16.	*P. scrobiculatum* (Vamban 1 var) roasted	62 ± 1.00	Nil
17.	*P. scrobiculatum* (Vamban 1 var) boiled	55 ± 1.00	Nil

ND: not determined.
[a] Values are means ± SD of three individual experiments.
[b] mg catechol equivalents/100 g of dry flour.
[c] mg catechin equivalents/100 g of dry flour.

(Atoui et al., 2005; Nanjo, Mori, Goto, & Hara, 1999). Although it is of scientific interest it has limited practical utility when developed as nutraceutical product due to loss of synergistic functions. In this context it is understandable that most researchers have been reporting the antioxidant activity of only the whole extracts of a wide variety of foods without fractionating them. The whole extracts of *Gevuina avellana* hulls (Moure et al., 2000); berry and fruit wines and liquors (Heinonen et al., 1998); edible and non-edible plant materials (Kahkonen et al., 1999); rhizome extract and rhizome knot extract of edible lotus (Hu & Skibsted, 2002); hard winter wheat varieties (Yu et al., 2002); leaf, root, and petiole of *Centella asiatica* (Zainol, Abd-Hamid, Yusof, & Muse, 2003); *Acacia confusa* bark (Chang et al., 2001); fruits in Singapore markets (Leong & Shui, 2002); *Terminalia catappa* leaves (Chyau, Tsai, Ko, & Mau, 2002) and cereals (Sripriya et al., 1996) were evaluated for antioxidant activity. However, it would be of interest to characterize the phenolics in these millets for scientific interest.

In conclusion we report here for the first time that the free radical quenching activity of kodo millet is highest among the millets evaluated. The antioxidant activity is by synergistic action of the phytochemicals, since fractionation in to husk or endosperm caused considerable loss in activity. Cooking by roasting reduced the activity more than by boiling.

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