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ESR spectroscopic studies on free radical quenching action of finger millet (*Eleusine coracana*)

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The free radical quenching action of finger millet (*Eleusine coracana*) on 1,1'-diphenyl-2-picrylhydrazyl (5.407×10^{17} spins/ml^{-1}) and hydroxyl (0.6015×10^{15} spins /ml^{-1}) radicals was studied by electron spin resonance (ESR) spectrometry. A 10 ml concentrate of the methanol extract was prepared using 25 g of the cereal grains and all analysis done after 1:10 dilution. DPPH radical quenching with 50 μl of the extracts showed that the brown finger millet quenched 94% whereas the white finger millet quenched only 4%. The phenolic content of brown finger millet was 96% higher than the white variety. Processing of the brown finger millet by germination and/or fermentation decreased the quenching activity. In comparison, foxtail millet, pearl millet and sorghum, quenched 91, 59 and 52 percent respectively, while wheat, rice (dehusked) and rice husk quenched 18, 1.8 and 20 % respectively. Brown finger millet (50 μl) also quenched 77% of hydroxyl radicals. The results indicate that finger millet is a potent source of antioxidant compounds. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Finger millet, (*Eleusine coracana*) also known as 'ragi' is a popular millet of India, consumed without dehulling either raw or after germination or fermentation. The tiny millet grain has a dark brown seed coat, rich in polyphenols like phenolic acids and its derivatives, flavonoids and tannins. It is also rich in phytic acid, an antinutrient that binds minerals (Hemamalini *et al.*, 1980). All these compounds have been reported to have radical scavenging activity and can therefore serve as antioxidants. Antioxidant compounds are gaining importance due to their dual role in the food industry as lipid stabilisers and in preventive medicine as suppressors of excessive oxidation that causes cancer and ageing (Namikii, 1990).

Several phenolic compounds that have potent antioxidant activity have been isolated — rosmanol and carnosal from rosemary and sage (Chang *et al.*, 1977), isoflavone from soybean (Hammerschmidt & Pratt, 1978), isovitexin from rice hull (Ramarathnam *et al.*, 1989), avenanthramides from oats (Dimberg *et al.*, 1993), phytic acid from wild rice (Kejian *et al.*, 1994) and flavonoids from grapes and wines (Kanner *et al.*, 1994).

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The radical quenching property of finger millet is so far not reported in the published literature. ESR spectrometry, which is one of the standard methods for the detection of free radicals, has been applied only to a limited extent in monitoring antioxidant activity in foods (Santiago *et al.*, 1992). Here we report for the first time the application of ESR as a tool to study the free radical quenching property of finger millet. In a preliminary study the crude methanolic extract of finger millet was shown to have radical quenching activity on DPPH (Sripriya *et al.*, 1995). In this study, the direct quenching activity of finger millet extract on DPPH and on hydroxyl radicals, by competing with the spin trap and comparison with other cereals and the possible involvement of phenols, tannins and phytate in the antioxidant effects is reported.

MATERIALS AND METHODS

Chemicals

Methanol of HPLC grade was used throughout the experiment. 1,1, Diphenyl-2-picrylhydrazyl (DPPH), vitamin E, butylated hydroxy anisole (BHA), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and 4-hydroxy-

2,2,6,6-tetramethyl piperidinyloxy free radical (4-hydroxy-TEMPO) were all purchased from Sigma Chemical Co. (St. Louis, MO, USA.).

Grains

Millets and cereals were cleaned, dried and milled into flour. Finger millet was milled after 12 h soaking and germination and then subjected to fermentation for 24 h, oven dried at 60 °C to constant weight and stored in air-tight containers at 4 °C.

Methanolic extracts

25.0 g of the flour was refluxed with 100 ml methanol for 2 h at 60 °C. The extraction was repeated with 50 ml methanol as above. The extracts were pooled, filtered and concentrated in a rotary evaporator and made up to 10.0 ml with methanol and stored at −20 °C till use. For radical analyses the extract was diluted (1:10) just before use.

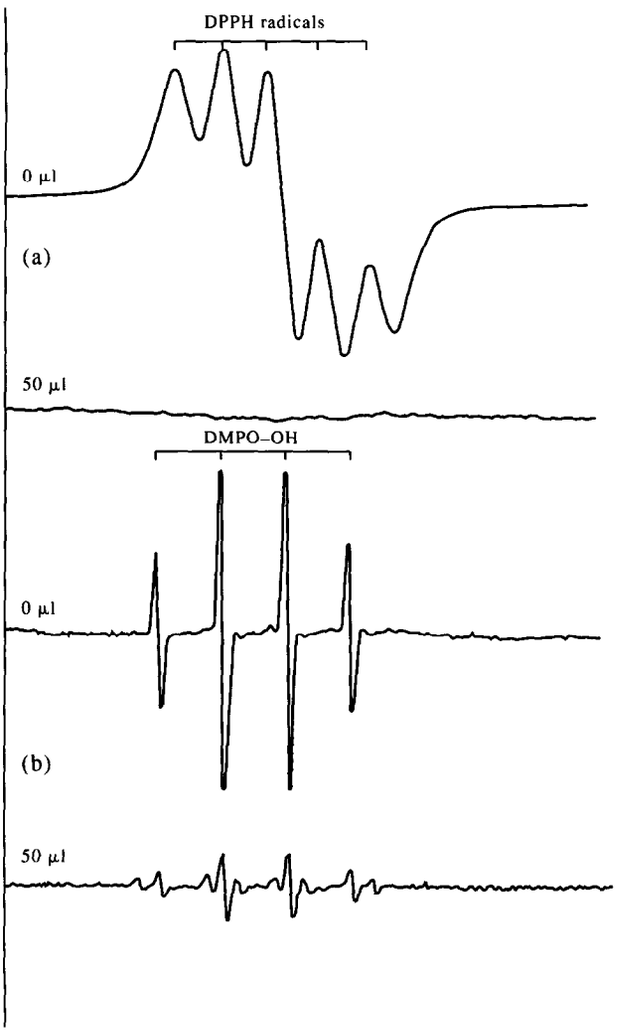

Fig. 1. Electron spin resonance spectra of (a) 1,1,diphenyl-2-picrylhydrazyl (DPPH), (b) hydroxyl radicals as spin adducts of DMPO-OH. $A_N = A_H = 14.792$ G. Spectra are typical of five determinations. (50 µL of 1:10 diluted methanol extract of finger millet was used for quenching. An equal volume of methanol was used in the control.)

Radical analysis

ESR measurements were performed at RT in X-band using a Varian type ESR spectrometer Model E112 with 100 KHz field modulation. The DPPH and hydroxyl radicals were analysed by the method of Santiago *et al.* (1992).

DPPH radical

50 µl of the extract was added to 500 µl of 9.0 µmol DPPH in methanol, mixed and immediately transferred to the special EPR spectrometry aqueous cell and analysed after exactly 60 s. A control was performed with 50 µl of methanol.

Hydroxyl radical

75 µl of 1mM $FeSO_4$ and 1mM DETAPAC, 75 µl of 1M H_2O_2, 50 µl of the extract and 20 µl of 0.092 M DMPO were mixed and the DMPO-OH adducts were analysed at 40 s after addition of DMPO. DMPO served as the spin trap and 4-OH-TEMPO as standard. A control was performed with 50 µl of methanol.

Total phenols

Phenols were estimated by the Folin Denis method (Swain & Hillis, 1959) in the methanolic extracts using chlorogenic acid as the standard.

Phytates

This was estimated by the method of Haug & Lantzsch (1983).

The mean values of two independent extracts and 4–5 determinations are expressed.

RESULTS AND DISCUSSION

Raw finger millet quenched DPPH and hydroxyl radicals (Figs 1–2). Fifty percent of DPPH free radicals (5.4068×10^{17} spins/ml^{-1}) was quenched by vitamin C (1.708 µmoles/ml^{-1}), vitamin E (0.7197 µmoles/ml^{-1}), BHA (1.720 µmoles/ml^{-1}) and by the crude methanol extract of raw finger millet (22 µl) and processed finger millet (89µl).

Raw finger millet extract (50 µl) was also able to quench 77% of hydroxyl radicals (0.6015×10^{15} spins ml^{-1}; $A_N = A_H = 14.791$ G) as seen in Fig. 2.

Figure 3 shows that finger millet has a potent radical-scavenging activity higher than that of rice, wheat and other millets. When 50 µl of the extracts were used, it was observed that the brown variety, which is com-

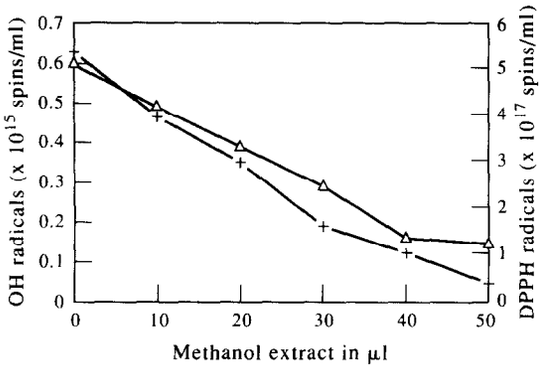

Fig. 2. Effect of finger millet on quenching of 1,1, diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals. △ OH radicals. + DPPH radicals. (10–50 μl of 1:10 diluted methanol extract of finger millet was used for quenching. 50 μl of methanol was used in the control.)

monly available, had a higher activity, 94%, compared to the white variety with only 4%. Polished rice and rice husk showed 1.8 and 20% quenching, respectively.

The brown seed coat of finger millet is rich in polyphenolics like tannins. Tannins act as good radical scavengers, which is supported by the observation of ESR signals of tannins, which persist longer than those of low molecular weight phenols (Namikii, 1990).

The total phenol content of brown finger millet was higher by 96% than the white variety (Table 1). The lower quenching activity of rice, wheat, sorghum and pearl millet corresponds to their lower phenolic content of 2.4, 19.6, 42.2 and 50.3%, respectively, compared to brown finger millet. Finger millet also has a high phytate content (6 mg g^{-1} dry weight). Phytic acid is a strong chelating agent and thus possesses antioxidant activity (Kejian *et al.*, 1994). All these factors may contribute to the radical quenching efficiency of finger millet.

Processing of finger millet decreased its radical scavenging ability (Fig. 3) although phenol content increased (Table 1). While 50 μl of raw finger millet had the maximum DPPH radical quenching ability of 94%, the fermented, germinated and a combination of germinated followed by fermented millet showed 22, 25 and

Table 1. Phenolic content of millets and cereals

Grains	mg/100 g dry wt.
Finger millet (white)	3.47
Finger millet (brown)	
(a) raw	102
(b) germinated	67.4
(c) fermented	137
(d) germinated and fermented	257
Foxtail millet	106
Pearl millet	51.4
Sorghum	43.1
Wheat	20.5
Rice (dehusked)	2.51
Rice husk	159

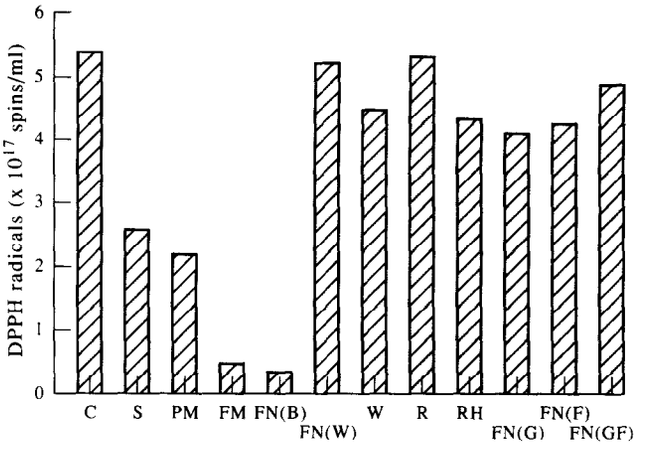

Fig. 3. Comparison of free radical quenching action of millets and cereals by ESR. C — control; W — wheat; S — sorghum; R — rice; PM — pearl millet; RH — rice husk; FM — foxtail millet; FN(G) — finger millet (germinated); FN(B)— finger millet (brown); FN(F) — finger millet (fermented); FN(W)— finger millet (white); FN(GF)— finger millet (germinated and fermented). (50 μl of 1:10 diluted methanol extract of finger millet was used for quenching. An equal volume of methanol was used in the control.)

10% quenching, respectively (Fig. 3). On processing, the phytate content decreased by 58% (6 to 2.5 mg g^{-1}). Similarly, tannins may be hydrolysed resulting in decreased radical quenching of the processed finger millet.

Antioxidant activity of the finger millet has not been studied so far. Here, we report, for the first time, the free radical-quenching activity of finger millet by using the ESR technique. The preliminary finding of a very high free radical quenching activity of finger millet, in comparison with the other grains, indicates that finger millet is a potent source of antioxidant compounds. These bioactive substances have 'extranutritional' properties and a novel role in diet–disease relationships (Kitts, 1994). Further studies are in progress on the extraction, purification and identification of the major compounds responsible for this activity. The antioxidant efficiency has to be tested in a food or biological system.

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