

Full Length Research Paper

# Antioxidant capacity of bioactive compounds extracted from selected wild and domesticated cereals of Zimbabwe

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Bioactive compounds were extracted from 6 wild and 4 domesticated cereal grains of Zimbabwe, using 50% methanol with the aim of testing their capability to prevent phospholipid peroxidation and  $\beta$ -carotene bleaching. The highest yield of phenolic compounds was obtained from *Eleusine indica* (a wild cereal) with 7.16 mg GA/100 mg sample, while the least yield was obtained from *Amaranthus hybridus* with 1.13 mg GA/100 mg sample. Antioxidant activities of the cereal extracts were studied using the  $\beta$ -carotene-linoleic acid and the inhibition of phospholipid peroxidation assays. It was shown that *Sorghum arundinaceum* had the greatest (77%) increase in inhibition of phospholipid when its concentration was increased from 20 to 80 mg/ml, while *Eleusine corocana*, a domestic cereal grain had the least. Relative to a standard BHA (an artificial antioxidant), *E. indica* was found to have the highest ability (67%) to prevent bleaching of  $\beta$ -carotene, while *Pennisetum* spp with 17.3% inhibition, had the least ability. Owing to the ability of the cereal grain extracts to act as antioxidants, the studies can be further extended to exploit the phenolic extracts as replacements of artificial antioxidants like butylated hydroxyl anisole (BHA) in food and health supplements and nutraceuticals.

**Key words:** Wild cereal grains, antioxidants, phospholipid peroxidation, health supplements.

## INTRODUCTION

Natural plants have received attention as sources of biologically active substances including antioxidants, antimutagens and anticarcinogens (Singh et al., 2004). Antioxidants play a crucial role in preventing diseases because of their ability to capture, deactivate or repair the damage caused by a group of molecules or atoms called free radicals that are implicated in many diseases (Wang et al., 1997). Free radicals, such as superoxide ( $O_2^{\bullet-}$ ,  $OOH^{\bullet}$ ), hydroxyl ( $OH^{\bullet}$ ) and peroxy ( $ROOH^{\bullet}$ ) radicals play an important role in oxidative stress related to the pathogenesis of various important diseases (Slater,

1984). In addition, these molecules are considered to induce lipid peroxidation causing the deterioration of foods. In healthy individuals, the production of free radicals is balanced by the antioxidative defense system. Oxidative stress is generated when the balance is in favor of the free radicals as a result of an increased production or depletion of antioxidant levels. It is common knowledge that oxidative stress, particularly due to aging, may be a contributory factor in neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases.

There is therefore, a marked interest in determining the role of phytonutrients in promoting improved health and in reducing cancer, cardiovascular disease, and the effects of aging. Antioxidant phytonutrients can inhibit the propagation of free radical reactions that may ultimately lead to the development of diseases, especially those

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which are aging related. Many cereal grains have strong antioxidant capacities, and this capacity is due primarily to non-vitamin C phytochemicals (Prior et al., 1998). The presence of a wide range of phytochemicals such as phenolics, thiols, carotenoids, anthocyanins and tocopherol, have been suggested to exert chemo-preventive (Dragsted et al., 1993), cardioprotective (Vita, 2005) effects, and protect the human body against oxidative damage by free radicals (Halliwell and Gutteridge, 1999). The health benefits of cereal grains have notable implications for the improvement of food quality, particularly through applications in functional foods and nutraceuticals (Abdul-Hamid and Luan, 2000; Truswell, 2003). Phytochemicals in plants can function as natural antioxidants and thereby retard or even prevent rancidity of food lipids, improve sensory scores and offer greater consumer acceptance of food products (Nakatani, 1997). As a group, these naturally occurring compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species (ROS), the major cause of many chronic human diseases such as cancer and cardiovascular diseases (Andreasen et al., 2001a; Yu et al., 2002, 2003).

A number of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ) have been added to foodstuffs but, because of toxicity issues, their use is being questioned (Valentaõ et al., 2002). Attention has therefore been directed toward the isolation of natural antioxidants from plant sources, especially edible plants, with the hope of finding non-toxic replacements to the synthetic antioxidants. The use of natural antioxidants in foods is limited, however, on account of the lack of knowledge concerning their molecular composition, the content of active compounds in the raw material and the availability of relevant toxicological data.

In this study, we sought to investigate inhibitory effects of phenolic extracts from selected wild and domesticated cereal grains found in Zimbabwe, against oxidation of phospholipids and fatty acids. The cereal grains we chose for the study are usually consumed in times of drought and famine by poor families in Zimbabwe. These cereal grain extracts can potentially be used as replacements of artificial antioxidants in the food industry.

## MATERIALS AND METHODS

### Chemicals

The chemicals were all of high purity grade and were all from Sigma – Aldrich Chemie (Steinheim, Germany).

### Collection of samples

Mature cereal grains were randomly collected from areas in and around Harare in Zimbabwe. Samples were sundried and stored in brown bottles away from direct sunlight until needed for use in assays. The samples collected were *Eleusine indica* (Crowfoot

grass), *Sorghum arundinaceum* (common wild sorghum), *Rottboellia cochinchinensis* (itchgrass), *Brachiaria brizantha* (upright brachiaria), *Panicum maximum* (Guinea grass), *Amaranthus hybridus* (smooth pigweed or slim amaranth), *Eleusine corocana* (finger millet), *Sorghum bicolor* (Red) (sorghum), *Sorghum bicolor* (White) (sorghum) and *Pennisetum americanum* (bulrush millet).

### Extraction method for total phenolic compounds

Total phenolic compounds were extracted from the fruit samples as described by Makkar (1999). The finely ground sample (2 g) was extracted twice with cold 50% aqueous methanol (10 ml, 1:1 v/v) in a 50 ml test tube suspended in ice subject to ultrasonication for 20 min. The two extracts were combined, made up to 20 ml with 50% aqueous methanol, centrifuged at 3000 rpm for 10 min and supernatant transferred into small sample bottles ready for analysis. Analyses were done on freshly extracted samples.

### Folin Ciocalteu assay for total phenolics

The Folin Ciocalteu method for determination of total phenolic compounds was carried out following the method described by Singleton and Rossi (1965). A sample (50 µl), distilled water (950 µl) was added to make up to 1 ml, then 1 N Folin reagent (500 µl) was added followed by sodium carbonate (2.5 ml). At the end of 40 min absorbencies at 725 nm were read using a spectronic 20® genesys™ spectrophotometer against a blank which contained methanol instead of sample. Gallic acid (0.5 mg/ml) was used as the standard and concentration of sample was expressed as mg GA/100 mg.

### Ability to prevent oxidation of β-carotene

The β-carotene-linoleic acid assay was done following the method by Shon et al. (2003). β - carotene (2 mg) was dissolved in chloroform (10 ml). An aliquot (1 ml) of the solution was taken and chloroform was removed by vacuum on a rotary evaporator. Linoleic acid (40 mg) was added to the almost dry material followed by Tween 80 (400 mg) and distilled water (100 ml) with rigorous shaking. Aliquots (3 ml) of the mixture were combined with samples (100 µl, at a concentration of 80 mg/ml). The reaction mixture was shaken until an emulsion was formed and the absorbance at zero time was measured at 470 nm using a Shimadzu UV-1601 UV-visible spectrophotometer. Measurement of absorbance was continued at 5 min intervals for 2 h. A blank without β-carotene was used as a negative control. Butylated hydroxyl anisol (BHA) (0.5%) was used as a standard and its extent of protection of β-carotene was treated as 100% protection.

### Ability to inhibit phospholipid peroxidation

Female Sprague Dawley rats (*Rattus norvegicus*) were obtained from the Animal House, University of Zimbabwe and dissected to obtain the brain. The rat brain was stored at -85°C until it was used. Homogenization of rat brain (2 g) was done in chloroform: methanol mixture (2:1, v/v) followed by centrifugation at 3000 x g for 5 min. The supernatant obtained was used as the source of phospholipids. The test run contained the phospholipids solution (50 µl), the sample extract (0.5 ml), 50% methanol (0.2 ml) and FeSO<sub>4</sub> (0.5 ml). The blank contained the phospholipid solution (50 µl) mixed with distilled water (0.5 ml) instead of the phenolic compound containing sample and methanol (0.2 ml, 50%). Ascorbic acid (0.5%) was used as the control. Incubation of the reaction mixture at 37°C for 1 h

**Table 1.** Total phenolic content expressed as gallic acid equivalence as determined by Folin Ciocalteu assay for wild and domesticated cereal grains on dry weight basis. The results are presented as mean  $\pm$  standard deviation of three independent measurements.

| Extract                             | Total phenolic compounds (mg GA/100 mg sample) |
|-------------------------------------|--|
| <i>Amaranthus hybridus</i>          | 1.13 $\pm$ 0.01                                |
| <i>Brachiaria brizantha</i>         | 3.18 $\pm$ 0.07                                |
| <i>Eleusine corocana</i>            | 6.31 $\pm$ 0.03                                |
| <i>Eleusine indica</i>              | 7.16 $\pm$ 0.10                                |
| <i>Panicum maximum</i>              | 2.58 $\pm$ 0.02                                |
| <i>Pennisetum</i> spp               | 3.09 $\pm$ 0.02                                |
| <i>Rottboellia cochinchinensis</i>  | 3.31 $\pm$ 0.04                                |
| <i>Sorghum arundinaceum</i>         | 6.18 $\pm$ 0.03                                |
| <i>Sorghum bicolor</i> (Red type)   | 3.98 $\pm$ 0.02                                |
| <i>Sorghum bicolor</i> (White type) | 2.10 $\pm$ 0.03                                |

was followed by the addition of thiobarbituric acid (TBA) (0.5 ml) and trichloroacetic acid (TCA) (4 ml) and the solution was then heated in a boiling water bath for 15 min. After cooling the sample on ice, absorbance was read at 532 nm on a spectronic 20® genesys™ spectrophotometer.

#### Statistical analysis

Results are expressed as the means  $\pm$  standard deviation (vertical error bars) of three replicates. One way analysis of variance (ANOVA) and the Student's *t* test were used to determine the statistical difference. Statistical significance was  $p < 0.05$ , unless otherwise stated.

## RESULTS AND DISCUSSION

### Total phenolics

In Table 1, variations in total phenolic content of wild cereal samples expressed as gallic acid equivalence, are shown. The total phenolic content varied considerably ( $p < 0.05$ ) between cereal samples, with the highest total phenolic content being exhibited by *E. indica* (a wild cereal) with 7.16 mg GA/100 mg sample, while the least amount was found in *A. hybridus* 1.13  $\pm$  0.01 mg GA/100 mg sample). There was also variation within cereals of the same family. *S. arundinaceum*, *S. bicolor* (Red) and *S. bicolor* (White) belong to the same family and their phenolic contents were found to be 6.18, 3.98 and 2.05 mg GA/100 mg sample respectively. This wide variation may be due to the different environmental conditions the three cereal grains are exposed to and their genetic makeup (Singh et al., 2004). The wild cereal, *S. arundinaceum*, had a higher phenolic compound concentration compared to both the domestic varieties that is the red and the white varieties.

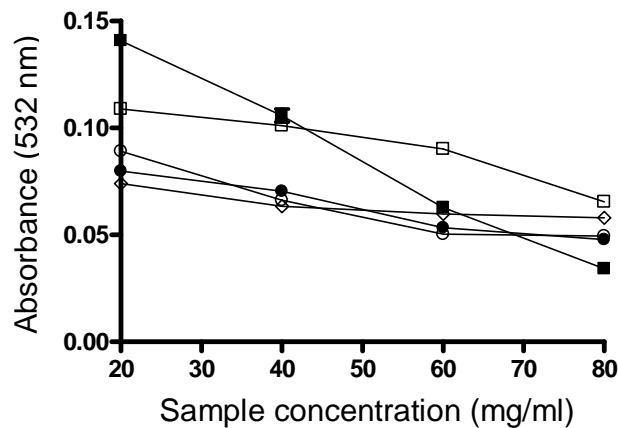
Of the domestic sorghum samples, the red variety also exhibited a larger concentration than the white variety. This may be due to their differences in colour, where the red variety may contain more anthocyanidins than the

white variety (He and Giusti, 2010; Janero, 1990). The total phenolic content follows the order: *E. indica* > *E. corocana* > *S. arundinaceum* > *S. bicolor* (Red) > *R. cochinchinensis* > *B. brizantha* > *Pennisetum* spp > *P. maximum* > *S. bicolor* (White) > *A. hybridus*. The previous results were obtained using the Folin-Ciocalteu method. Characteristics of the phenolic extracts were investigated further using the phospholipid peroxidation and  $\beta$ -carotene-linoleic acid model system assays to measure antioxidant activity.

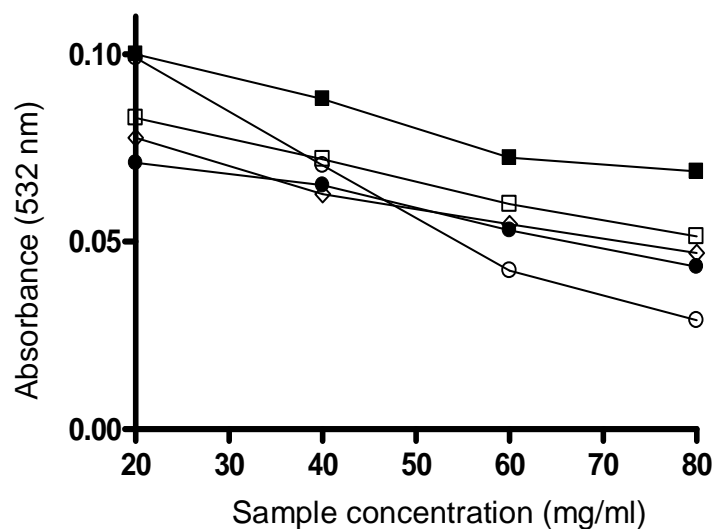
### Inhibition of phospholipid peroxidation assay

Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acid in the cell membranes that generates a number of degradation products. Malonyldialdehyde (MDA), one of the major products of lipid peroxidation, has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress (He and Giusti, 2010; Janero, 1990). In this study, the ability of cereal extracts to prevent lipid peroxidation was followed using a system that contained rat brain homogenates whereas peroxidation was induced by addition of  $\text{FeCl}_2\text{-H}_2\text{O}_2$ . From Figure 1, it is evident that methanolic extracts from *S. arundinaceum*, *E. indica*, *E. corocana*, *S. bicolor* (White), and *S. bicolor* (Red) caused protection of phospholipid as shown by the decrease in absorbance as sample concentration was increased. *A. hybridus*, *P. maximum*, *B. brizantha*, *R. cochinchinensis* and *Pennisetum* spp caused protection of phospholipid from peroxidation as indicated in Figure 2.

Addition of cereal grain extracts to the  $\text{Fe}^{2+}\text{-H}_2\text{O}_2$  system resulted in a decrease in the formation of tissue MDA levels, suggesting that the cereal extracts were scavengers of  $\cdot\text{OH}$ . Therefore, the  $\cdot\text{OH}$ -scavenging activity observed in this study indicates a possible application for the cereal extracts in the management of diseases involving free radicals and oxidative damage, such as lipid peroxidation. Comparatively, the order of



**Figure 1.** Inhibition of phospholipid peroxidation by the methanolic extracts of *Sorghum arundinaceum* (■), *Eleusine indica* (○), *Eleusine corocana* (◇), *Sorghum bicolor* (White) (●) *Sorghum bicolor* (Red) (□). The lower the absorbance reading, the lower the amount of MDA that was formed which in turn was an indication of the extract's ability to inhibit phospholipid peroxidation. The results are presented as mean  $\pm$  standard deviation of three independent measurements.

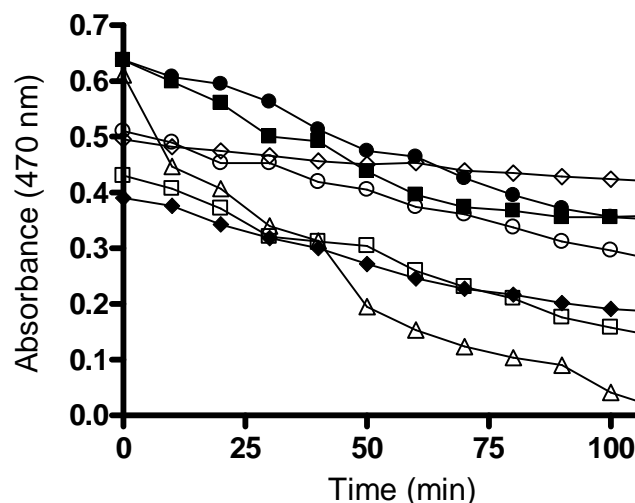


**Figure 2.** Inhibition of phospholipid peroxidation by the methanolic extracts of *Amaranthus hybridus* (□), *Panicum maximum* (●), *Brachiaria brizantha* (■), *Rottiboellia cochinchinensis* (○) and *Pennisetum* spp (◇). The lower the absorbance reading, the lower the amount of MDA that was formed which, in turn, was an indication of the extract's ability to inhibit phospholipid peroxidation. The results are presented as mean  $\pm$  standard deviation of three independent measurements.

inhibition of lipid peroxidation follows the order: *S. arundinaceum* > *E. indica* > *S. bicolor* (White) = *S. bicolor* (Red) = *P. maximum* > *Pennisetum* spp > *A. hybridus* > *B. brizantha* > *R. cochinchinensis* > *E. corocana* as shown in Figures 1 and 2. This order was obtained by calculating the absorbance of each sample at a concentration of 80 mg/ml as a percentage of its

absorbance at 20 mg/ml.

*S. arundinaceum* showed the greatest (77%) increase in inhibition of phospholipid when its concentration was increased from 20 to 80 mg/ml. The inhibition was dose dependant for all samples and the increase in inhibition as concentration of sample increased was significant ( $P > 0.05$ ) for all cereal grain antioxidant extracts.



**Figure 3.** Antioxidant activity of extracts of *Sorghum arundinaceum* (■), *Eleusine indica* (●), *Eleusine corocana* (◆), *Sorghum bicolor* (White) (□) *Sorghum bicolor* (Red) (○) in  $\beta$ -carotene/linoleic acid system. BHA (◇) was used as the positive control and in the negative control (△), no sample was added. Slow decrease in absorbance signifies protection of  $\beta$ -carotene. Results are presented as the mean  $\pm$  Standard deviation of three independent measurements.

*S. bicolor* (White), *S. bicolor* (Red) and *P. maximum* inhibited phospholipid peroxidation to the same extent when dosage of sample was increased. This may be because the cereal grains contain similar phenolic compounds that are responsible for preventing phospholipid peroxidation. Cetojević-Simin et al. (2010) also reported that plants contain phytochemicals that had protective effects against lipid peroxidation. However, there is need to characterize the phytochemicals by HPLC-MS method to positively identify the constituent phenolic compounds. *S. arundinaceum* is a wild cereal, whereas *S. bicolor* (White) and *S. bicolor* (Red) have been domesticated.

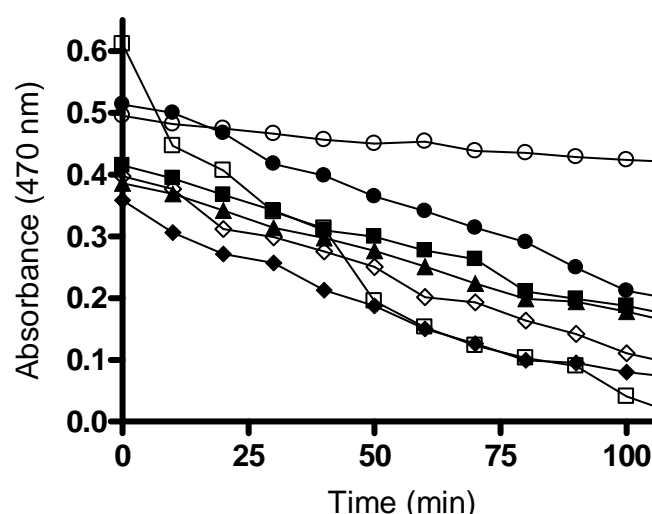
The wild cereal grains exhibited greater potential to prevent lipid peroxidation than its domestic counterparts and the protective capability may be due to the fact that the *S. arundinaceum* has a deeper red colour which is usually indicative of a lot of phenolic compounds, mainly anthocyanidins (Cetojević-Simin et al., 2010; He and Giusti, 2010). The same trend that the sorghums show exists between *E. indica* (45%) and *E. corocana* (23%). *E. indica* is the wild cereal grain which exhibited greater response to increase in dose than *E. corocana*.

#### Antioxidant activity in the $\beta$ -carotene bleaching assay

Oxidation of carotenoids, which is induced by light, heat or peroxy radicals (Ursini et al., 1998) results, in bleaching (Huang et al., 2005). Antioxidants that can donate hydrogen atoms to quench radicals can prevent or reduce decolorisation of carotenoids (Burda and

Oleszek, 2001). All the cereal extracts had the ability to delay the bleaching of  $\beta$ -carotene. For the negative control, no sample was added to prevent bleaching of  $\beta$ -carotene, so rate of discoloration was rapid. Butylated Hydroxy-toluene (BHT) was used as the positive control. In Figures 3 and 4, the extent of protection of  $\beta$ -carotene over 2 h is shown. Relative to BHA, the order of protection of  $\beta$ -carotene can be shown from Table 2 and it is as follows: *E. indica* > *S. bicolor* (Red) > *E. corocana* > *S. arundinaceum* > *A. hybridus* > *S. bicolor* (White) > *P. maximum* > *B. brizantha* > *R. cochinchinensis* > *Pennisetum* spp. There was no notable trend between the wild and the domestic cereal grains in this assay but all cereal grains showed ability to protect  $\beta$ -carotene over time. *E. indica*, a wild cereal grain had 67.5% of the  $\beta$ -carotene remaining unbleached after 2 h, which was the highest percentage of all the cereal grains under study. *Pennisetum* spp, a domestic cereal had the least amount of  $\beta$ -carotene remaining after 2 h. Different extracts of various cereal grains have also been reported to have potential inhibiting activity against the oxidation of  $\beta$ -carotene molecules (Cardador-Martínez et al., 2002).

The differences in ability to protect against bleaching may depend on the phenolic compound constituents in the individual grains. Environmental factors also affect the amount of potential antioxidant in the sample (Osier and Lindroth, 2001). To be able to determine which phenolic compounds were effective as antioxidants in both assays, the active compounds can be isolated by fractionation and then the structures elucidated by mass spectroscopy. All the cereal grains, wild and domesticated contain phenolic compounds. *E. indica* was shown



**Figure 4.** Antioxidant activity of extracts of *Amaranthus hybridus* (■), *Panicum maximum* (▲), *Brachiaria brizantha* (●), *Rottiboellia Cochinchinensis* (◇) and *Pennisetum spp* (◆) in  $\beta$ -carotene/linoleic acid system. BHA (○) was used as the positive control and in the negative control (□), no sample was added. Slow decrease in absorbance signifies protection of  $\beta$ -carotene. Results are presented as the mean  $\pm$  Standard deviation of three independent measurements.

**Table 2.** Antioxidant activity of extracts of wild and domestic cereal grains expressed as a percentage relative to a BHA standard. Assumption is that, after 2 h BHA inhibited bleaching of  $\beta$ -carotene by 100%.

| Sample                              | Relative $\beta$ -carotene remaining after 2 h, expressed as a percentage with 100% meaning that bleaching of $\beta$ -carotene was completely prevented |
|-------------------------------------|--|
| BHA                                 | 100  |
| No sample                           | 0  |
| <i>Amaranthus hybridus</i>          | 46.9   |
| <i>Brachiaria brizantha</i>         | 38.7   |
| <i>Eleusine corocana</i>            | 62.3   |
| <i>Eleusine indica</i>              | 67.8   |
| <i>Panicum maximum</i>              | 40.7   |
| <i>Pennisetum spp</i>               | 17.3   |
| <i>Rottiboellia cochinchinensis</i> | 20.1   |
| <i>Sorghum arundinaceum</i>         | 59.7   |
| <i>Sorghum bicolor</i> (Red)        | 65.5   |
| <i>Sorghum bicolor</i> (White)      | 43.7   |

to have the highest total phenolic content, while *A. hybridus* had the least among the cereals under studied. Only *E. indica* was shown to have a strong positive correlation between total phenolics and antioxidant activity ( $r^2 = 0.934$ ). In the rest of the remaining cereal grains, there was no correlation, suggesting that ability to inhibit phospholipid peroxidation and  $\beta$ -carotene bleaching depended more on the quality than quantity of the constituent phenolic compounds. The highest ability ( $P > 0.05$ ) to prevent phospholipid peroxidation is given by *S. arundinaceum*, while *E. corocana* displayed the least activity. The ability of the wild and domesticated cereal

grains to prevent lipid peroxidation was found to be dose dependant. There was positive correlation between dose of cereal and level of prevention of lipid peroxidation ( $r^2 = 0.967$ ). All cereal grains had the ability to prevent bleaching of  $\beta$ -carotene. The cereal grains may be used as potential sources of natural antioxidants.

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