Food ©2008 Global Science Books

Antioxidant Activity and Free Radical Scavenging Capacity of Dietary Phenolic Extracts from Processed Indigenous Legumes, *Macrotyloma uniflorum* (Lam.) Verdc. and *Dolichos lablab* L.

Perumal Siddhuraju[1*] • Vellingiri Maheshu[2] • Nataraj Loganayaki[2] • Sellamuthu Manian[2]

[1] Department of Biotechnology, School of Biotechnology and Genetic Engineering, Bharathiar University, Coimbatore 641046, Tamil Nadu, India
[2] Department of Botany, Bharathiar University, Coimbatore 641046, Tamil Nadu, India

Corresponding author: * **rajupssad@yahoo.com**

ABSTRACT

The antioxidative properties and total phenolic contents of raw, dry heated and pressure cooked seed sample extracts of *Macrotyloma uniflorum* (brown and black variety) and *Dolichos lablab* were examined. The raw, dry heated and pressure cooked samples were extracted with 50% methanol and 70% acetone separately and the extracts were pooled and used for analysis. The raw and processed seed samples of respective legumes had a good source of dietary phenolics including tannins and high molecular condensed tannins. The extracts were screened for their potential antioxidant activities using reducing power, α,α-diphenyl-β-picrylhydrazyl (DPPH$^•$), OH$^•$, ABTS$^{•+}$, antihemolytic, metal chelating and linoleic acid emulsion model systems. All the extracts exhibited reducing power activity; moreover, they were well correlated with phenolic concentration in a dose-dependent manner. Like reducing power activity, a similar dose-dependent trend was also observed in the hydroxyl radical scavenging and DPPH radical scavenging activities. Nonetheless, at a concentration of 1 mg in the reaction mixture, all the extracts showed peroxidation inhibiting activity between 94 to 99% with out any significant differences ($p<0.05$) in the linoleic acid emulsion system. Interestingly, pressure cooked samples of both legumes registered the highest ABTS$^{•+}$ cation radical scavenging activity when compared to the raw and dry heated samples. Although all extracts were found to have potential metal chelating property (31.27-75.78 mg EDTA g^{-1}), antihemolytic activity was very low except in raw and dry heated samples of *D. lablab*. Both dry heated and pressure cooked samples of *M. uniflorum* and *D. lablab* could be advocated for wider consumption as potent antioxidant nutrients to prevent oxidative stress-induced and nutrient-borne diseases.

Keywords: ABTS$^{•+}$, antioxidant activity, DPPH$^•$, legume seeds, pressure cooking

INTRODUCTION

A large number of people living in developing as well as developed countries are affected by chronic diseases that result in increased premature death. Changes of life style and dietary habits affect diseases that include obesity, diabetes mellitus, cardiovascular diseases, hypertension and stroke and some types of cancer (Rui 2004; Espinosa-Alonso *et al.* 2006). In the last decades oxidation mechanisms and the role of free radicals in living systems have gained increased attention. Oxygen uptake inherent to cell metabolism produces reactive oxygen species such as superoxide and hydroxyl radicals. The reaction of these species with lipid molecules produces peroxyl radicals and their interaction with nucleic acids and proteins leads to certain alterations and functional modifications. These processes are recognized as the cause of aging and participate in the pathogenesis of several serious disorders as cancer, atherosclerosis, inflammatory and coronary heart diseases (Halliwell *et al.* 1992; Chaillou and Nazareno 2006).

Antioxidants are substances that are capable of counteracting the damaging but normal effects of the physiological process of oxidation in animal tissue. Antioxidants are nutrients (vitamins and minerals) as well as enzymes (protein in the body that assists in chemical reactions) (Sun *et al.* 2002). They are believed to play a role in preventing the development of chronic diseases and cataracts (Chu *et al.* 2002). Natural polyphenols exert their beneficial health effects by their antioxidant activity, these compounds are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases (Oboh 2006). Flavonoids obtained commercially and isolated from plant species are known to be effective free radical scavengers. Recently condensed and hydrolysable tannins of relatively high molecular weight have also been shown to be effective antioxidants in addition to that of simple phenolics. There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidant activity of these compounds (Hagerman *et al*. 1998; Beninger and Hosfield 2003).

Legume seeds occupy an important place in the human diet all over the world. They are a good source of protein, lipid, polysaccharides (dietary fiber, starch) and micronutrients (vitamins, trace elements). Nonetheless, legumes are also a rich source of bioactive non-nutrient compounds (phytoceuticals) (Anderson *et al*. 1999; Messina 1999). Dietary polyphenols from dry beans may act as antioxidants to inhibit the formation of damaging free radicals that result from the natural degradation of foods (Namiki 1990). The inclusion of such legumes in the daily diet has many beneficial physiological effects in controlling and preventing various metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer. Currently the role of legumes as therapeutic agents in the diets of persons suffering from metabolic disorders is gaining interest (Tharanathan and Mahadevamma 2003). The consumption of whole legumes is very high in several parts of the world, mainly developing countries of Asia, Africa and South America where chronic human diseases are hardly found. Heart disease and cancer researchers are looking at potential benefits of specific foods such as soybean and other plant derived

Received: 21 June, 2008. Accepted: 6 October, 2008.

Original Research Paper

diets. Therefore, the bioactive compounds, such as antioxidants, including tocopherols, carotenoids, vitamin C and phenolic compounds, seem to be associated with these health benefits (Cadenas and Packer 2002; Frias *et al.* 2005).

Dolichos lablab L., commonly referred to as field bean or hyacinth bean, is a legume widespread throughout the tropics. In India it is an important multipurpose legume crop used as a pulse, vegetable and forage. Whereas its derived beans serve as pulse, its tender pod with beans or the green beans alone serve as vegetable. Because of its abundant foliage, the crop is employed as an excellent fodder. Besides India, it is also cultivated as a forage crop in the United States, Hungary, Nigeria and many other countries. Dried seeds of lablab bean contain 20-28% crude protein and the amino acids are moderately well balanced with especially high lysine content (6.1%). Lablab bean leaves are also rich in protein (up to 28%) and among legumes it is considered to be one of the best sources of iron (155 mg per 100 g of leaves, dry weight) (Wealth of India 1992). Some varieties of *D. lablab* beans secrete a fragrant oil on the surface of their pods. The exudate acts as bacteriocide or bacteriostat, which is in conformity with the fact that fatty acids and their derivatives do possess such properties (Sumrell *et al.* 1978; Fernandes and Nagendrappa 1979). Whereas, the potential breast cancer fighting chemical known as kievitone is also found in hyacinth bean (Oboh 2006). Nonetheless, an antifungal protein Dolichin, possessing a molecular weight of 28 kDa and an N-terminal sequence resembling chitinases, has been purified from the seeds of the field bean *D. lablab*. Dolichin was capable of inhibiting Human Immuno-deficiency Virus (HIV) reverse transcriptase and α- and β-glucosidases which are glycohydrolases implicated in HIV infection. It had very low ribonuclease and cell-free translation-inhibitory activities (Ye *et al.* 2000). Further the dietary protein concentrates of *D. lablab* seeds are having potential hypocholesterolemic effect (Chau *et al.* 1998).

Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) is a pulse crop native to the South-East Asian subcontinent and to Tropical Africa. It is extensively cultivated, especially in dry areas of Australia, Burma, India and Sri Lanka (Duke and Reed 1981). It is widely used as animal feed and as minor pulse in human nutrition. The use of dry seeds of horse gram is limited due to their poor cooking quality. However, it is consumed as sprouts in many parts of India. The US National Academy of Sciences identified this legume as a potential food source for the future. Earlier studies showed that horse gram is a good source of protein (17.9–25.3%), carbohydrates (51.9–60.9%), essential amino acids, energy and a low content of lipid (0.58–2.06%) and is an excellent source of iron and molybdenum (Kadam and Salunkhe 1985; Sudha *et al.* 1995; Bravo *et al.* 1999). It also possesses slow digestible starch, which is considered to have low postprandial glucose response when consumed by diabetic patients (Bravo *et al.* 1998). Administration of the methanolic extract of whole plant to rabbits in which oxidative stress had been induced by being fed a high fat diet (HFD), led to an improvement in antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), and an increase in reduced glutathione (GSH) concentration (Muthu *et al.* 2006). In addition to that, the cooking liquor of horse gram seeds with spices is considered to be a potential remedy for the common cold, throat infection and fever and the soup made from the seeds of this plant is said to generate heat and to help dilute renal stones. It contains bioactive substances like isoflavone diglycoside, 5-hydroxy-7, 3', 4'-trimethoxy-8-methylisoflavone; 5-*O*-α-L-rhamnopyranosyl (1→2)-O-β-D-glucopyranoside (Mitra *et al.* 1983; Siddhuraju and Manian 2007). The methanolic extract of *D. biflorus* possesses hypolipidemic activity in high fat diet fed rats. *D. biflorus* has antitumor activity, the root or the plant is used as expectorant in China, it is also used in menstrual problems (Muthu *et al.* 2005). However, the available information regarding the antioxidant activities of bioactive constituents especially from hydrothermally processed *D. lablab* and *M. uniflorum* (brown and black varieties) are found to be meagre. Therefore, the present work has been carried out to evaluate the phenolic constituents, antioxidant potential and free radical-scavenging property of raw, dry heated and hydrothermally processed legume seeds.

MATERIALS AND METHODS

Chemicals

Potassium ferricyanide, ferric chloride, 2,2-diphenyl-1-picryl-hydrazyl (DPPH$^{\bullet}$), potassium persulfate, 2,2'azinobis (3-ethylbenzothiozoline-6-sulfonic acid) disodium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), linoleic acid, ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylenediamine tetra acetic acid (EDTA) disodium salt, 2,2'-bipyridyl and hydroxylamine hydrochloride were obtained from Himedia, Merck or Sigma. All other reagents used were of analytical grade.

Seed samples and processing

The dry seeds of *M. uniflorum* (brown and black varieties) were purchased from the market located at Salem, TN, India during September 2006. The dry seed of *D. lablab* was purchased from the market located at Mettupalayam, TN, India during October 2006. Raw seeds of about 100 g were weighed and dry heated at 160°C for 15 min in an oven. Another 100 g of the raw seeds, which were cooked in a pressure cooker for 20 min, used a seed: water ratio of 1:3 (w/v). Then water was decanted and the cooked seeds were dried at 50°C until a constant weight was reached. The raw, dry heated and pressure cooked seeds were finely powdered using a Willy Mill of 60 mesh size. All the powdered samples were stored separately in a screw capped bottle at a room temperature until further analysis.

Solvent extraction

10 g of each sample was weighed and added 50 mL of 50% methanol (w/v) and using a shaker, the sample was shaken occasionally for 24 h. Then the sample was centrifuged at 5000 rpm for 20 min and the supernatant was collected. Another 10 g of each sample was taken with 50 mL of 70% acetone (w/v) and using a shaker, the sample was shaken occasionally for 24 h. Then the sample was centrifuged at 5000 rpm for 20 min and the supernatant was collected. The two solvent extracts were pooled together and stored in a separate screw capped bottles for further analysis.

Extract recovery percentage

From the seed extract, a known volume was taken, dried in an oven at incubator temperature of 40°C (until sample getting a constant weight) and the recovery percent was calculated as follows:

$$\text{Recovery}\,\% = \frac{(\text{Extract} + \text{container (g)})\ \ (\text{Empty container (g)})}{\text{Sample weight (g)}} \times 100$$

Estimation of total phenolics and tannins

Aliquots of appropriate concentration of the extract were placed in a test tube and made up to a volume of 1 mL with distilled water. Then 0.5 mL of Folin- Ciocalteu phenol reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in the dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The amount of total phenolics was calculated as tannic acid equivalents from a calibration curve (Siddhuraju and Becker 2003). Using the same extract the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP). One hundred milligrams of PVPP was weighed in a 100 × 12 mm test tube and to this 1.0 mL distilled water and then 1.0 mL of tannin containing phenolic extract were added. The content was vortexed and kept in the test tube at 4°C for 15 min. Then the sample was centrifuged (5000 rpm for 10 min at room temperature) and the supernatant was collected.

This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured, as monitored above and expressed as the content of non-tannin phenolics on a dry matter basis (Siddhuraju and Manian 2007). From the above results, the tannin content of the sample was calculated as follows:

Tannin (%) = Total phenolics (%) – Non-tannin phenolics (%).

Estimation of condensed tannins

0.50 mL of the extract was pipetted into a test tube, 0.3 mL of the butanol-HCl reagent and 0.1 mL of the ferric reagent were added sequentially. The contents were vortexed and the mouth of each tube was covered with a glass marble and the tubes were kept in a heating block adjusted at 97 to 100°C for 60 min. After cooling the test tubes, the absorbance was recorded at 550 nm and suitable blank was subtracted, which is usually the absorbance of unheated mixture (Siddhuraju and Becker 2003). The condensed tannins (% in dry matter) as leucocyanidin equivalent were calculated by using the following formula:

Condensed tannins = (OD value at 550 nm × 78.26 × Dilution factor) / (% dry matter).

Reducing power assay

Different concentrations of extracts were dissolved in 1 mL of phosphate buffer in a test tube and 5 mL of 0.2 M phosphate buffer, pH 6.6 was added. To this, 5 mL of 1% potassium ferricyanide solution was added. The mixture was incubated at 50°C for 20 min. After the incubation, 5 mL of 10% TCA was added and the content was centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5 mL) was mixed with 5 mL of distilled water. To this, 1 mL of ferric chloride (0.1%) was added and mixed. Then, the absorbance of the reaction mixture was read spectrophotometrically at 700 nm against water blank (Oyaizu 1986).

Stable free radical scavenging activity using DPPH method

Different concentrations of extracts were taken in different test tubes. The volume was adjusted to 100 µL with methanol. 5 mL of a 0.1 mM methanolic solution of $DPPH^\bullet$ was added to these tubes and shaken vigorously. The tubes were allowed to stand for 20 min at 27°C. The control was prepared as above without any extract, and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm (Blios 1958). Radical scavenging activity was expressed as the inhibition percentage of free radicals by the samples and was calculated using the following formula:

% radical scavenging activity = (Control OD - Sample OD / Control OD) ×100.

Extract required for decreasing 50% of $DPPH^\bullet$ radical scavenging activity under the specified experimental condition was also calculated.

Hydroxyl radical scavenging activity

The scavenging activity of the extracts of raw and processed seed samples on hydroxyl radical were measured according to the method of Klein *et al.* (1991). Various concentrations (100, 200, and 300 µg) of extracts were added to 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and incubated at 80-90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1.0 mL of ice-cold TCA (17.5% w/v). Three mL of Nash reagent was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured spectrophotometrically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated by the following formula:

%HRSA=1-(difference in absorbance of sample/difference in absorbance of blank) × 100.

Antioxidant activity determination by using linoleic acid-emulsion system

A sample extract having a concentration of 1 mg was taken. To this 0.5 mL of 2.51% linoleic acid in 99.5% ethanol, 1 mL of 0.05 M phosphate buffer (pH 7), and 0.5 mL of distilled water was placed in a screw capped tube and then incubated in dark oven at 40°C. A control without sample extract was used. Every 6 h, 0.1 mL aliquots of this solution were taken and 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate were added. Precisely 3 min after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm until the absorbance of the control reached the maximum (Kikuzaki and Nakatani 1993). The antioxidant activity (AA) was calculated as percentage of inhibition relative to the control using the following equation:

$$\mathrm{AA}\,\% = 1 - \left\{\frac{(\text{Sample absorbance at 54 h}) - (\text{Sample absorbance at 0 h})}{(\text{Control absorbance at 54 h}) - (\text{Control absorbance at 0 h})}\right\} \times 100$$

Total antioxidant activity assay by radical cation ($ABTS^{\bullet+}$)

ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to 30°C to give an absorbance at 734 nm of 0.700 ± 0.02 in a 1 cm cuvette. The stock solution of the sample extracts in ethanol were diluted such that, after introduction of a 10 µL aliquot of each dilution into the assay, they produced between 20-80% inhibition of the blank absorbance. After the addition of 1 mL of diluted $ABTS^{\bullet+}$ solution to 10 µL of samples or Trolox standards (final concentration 0-15 µM) in ethanol, OD was taken at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration (Re *et al*. 1999) described by Siddhuraju and Becker (2003). The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts on dry matter basis.

Antihemolytic activity

The erythrocytes from cow blood were separated by centrifugation and washed with phosphate buffer (pH 7.4) until the supernatant was colourless. The erythrocytes were then diluted with saline or phosphate buffer to give 4% suspension. 500 µg of sample/mL saline buffer were added to 2 mL of the suspension of erythrocytes and the volume was made up to 3-5 mL with saline buffer. This mixture was pre incubated for 5 min and then 0.5 mL H_2O_2 solutions of appropriate concentration in saline buffer were added. The concentration of H_2O_2 in the reaction mixture was adjusted so as to bring about 90% hemolysis of blood cells after 240 min. After the incubation time the reaction mixture was centrifuged at 1500 rpm for 10 min. and the extent of hemolysis was determined by measurement of the absorbance (at 540 nm) corresponding to haemoglobin liberation (Naim *et al.* 1976).

Metal chelating capacity

Chelating property of the raw and processed seed sample extracts was assessed by bipyridyl assay (Yamaguchi *et al.* 2000). The reaction mixture contained 0.25 mL of 1 mM $FeSO_4$ solution, 0.25 mL of extract, 1 mL of 0.2 M Tris-HCl buffer (pH 7.4), 1 mL of 2, 2' bipyridyl solution, 0.4 mL of 10% hydroxylamine-HCl and 2.5 mL of ethanol. The final volume was made up to 5 mL with deionised water and the absorbance was determined at 522 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g sample extracts.

Statistical analysis

The data of antioxidant activity on linoleic acid emulsion system were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($p < 0.05$) using statistica (Statsoft Inc., Tulsa, USA). Values expressed are mean of three replicate determinations ± standard deviation.

RESULTS AND DISCUSSION

Extract yield percentage and phenolic content of seed extracts

Extract yield percentage, total phenolics, tannins and condensed tannins of extracts obtained from the raw and processed seed samples of *M. uniflorum* (brown and black varieties) and *D. lablab* are shown in **Table 1**. Among the three legumes maximum yield was obtained for the extracts of *M. uniflorum* (black variety) raw sample. The extractable total phenolic content of raw samples was found to be higher than the processed samples. However, the values obtained in pressure cooked samples were significantly lower than the respective raw and dry heated samples. The tannin content of *M. uniflorum* brown variety and *D. lablab* dry heated samples was higher than that of the raw and pressure cooked samples. In the black variety, however, sequential reduction of tannin content was observed from raw samples to dry heated and pressure cooked samples. The highest condensed tannin content was observed in pressure cooked *M. uniflorum* black variety and *D. lablab* samples and raw samples of brown variety of *M. uniflorum*. The hydrothermally treated seed sample had the lowest concentration of phenolic fractions, possibly due to the dissolution of phenolics in the soaking medium and subsequent discarding of them (Siddhuraju and Becker 2006). The dry heated sample had the lower concentration of phenolic fractions possibly due to the poor extractability by the formation of insoluble tannin-protein and tannin-carbohydrate including cell wall polysaccharide complexes (Siddhuraju 2007). The presence of phenolic substances, including tannins, in horse gram was reported in the study of Reddy *et al.* (1985), Sudha *et al.* (1995) and Siddhuraju and Manian (2007). The extractable total phenolics and tannins of the dry heated samples were found to be higher than in raw samples that could be due to the solubility of phenolics and other aroma compounds. Phenolic compounds could easily donate hydroxy hydrogen due to resonance stabilization (Fessenden and Fessenden 1994). In addition, high radical scavenging potential might be explained by their ability to donate a hydrogen atom from their phenolic hydroxy groups (Yen *et al.* 2005). The results suggest that there was a decrease in phenolic content during pressure cooking and dry heating. This is in agreement with the reports of Barroga *et al.* (1985) who found that boiling and cooking reduced the amount of phenolics in legumes by 75%. However this might be caused in part by diffusion of phenolics from the seed coat to cooking water (Rocha-Guzman *et al.* 2007). Similarly, the decrease in total phenolic content on heat treatment of *Beta vulgaris* and *Phaseolus vulgaris* was reported by Jiratanan and Liu (2004). The binding between phenolics and the protein matrix might account for the enhancement of antioxidant capacity in peas. This is because a phenolic-protein interaction is able to stabilize the protein and its antioxidant capacity is increased during heating (Tsai and She 2006). As antioxidants, polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress, by acting directly on reactive oxygen species or by stimulating endogenous defense systems (Scalbert *et al.* 2005).

Table 1 Solvent extract recovery percentage, total phenolics, tannins and condensed tannins of raw and processed seed extracts from *Macrotyloma uniflorum* (brown and black variety) and *Dolichos lablab*.

Sample	Extract yield (%)	Total phenolics		Tannins		Condensed tannins	
		g/100 g sample	g/100 g extract	g/100 g sample	g/100 g extract	g/100 g sample	g/100 g extract
M. uniflorum **(brown variety)**							
Raw	1.90	0.816 ± 0.062	42.962 ± 3.279	0.169 ± 0.033	8.882 ± 1.761	0.377 ± 0.055	19.855 ± 2.914
Dry heated	1.85	0.790 ± 0.051	42.676 ± 2.767	0.193 ± 0.029	10.440 ± 1.558	0.183 ± 0.028	9.871 ± 1.490
Pressure cooked	0.80	0.173 ± 0.019	21.662 ± 2.364	0.036 ± 0.005	4.482 ± 0.659	0.127 ± 0.020	15.931 ± 2.505
M. uniflorum **(black variety)**							
Raw	1.95	0.782 ± 0.075	49.845 ± 2.973	0.145 ± 0.039	7.453 ± 2.022	0.416 ± 0.017	21.346 ± 0.877
Dry heated	1.80	0.771 ± 0.073	42.825 ± 4.044	0.112 ± 0.020	6.241 ± 1.111	0.349 ± 0.015	19.374 ± 0.824
Pressure cooked	0.75	0.374 ± 0.022	40.107 ± 3.861	0.036 ± 0.010	4.781 ± 1.345	0.172 ± 0.002	22.987 ± 0.236
D. lablab							
Raw	1.60	0.412 ± 0.025	38.312 ± 0.820	0.028 ± 0.016	1.725 ± 1.029	0.336 ± 0.027	20.992 ± 1.695
Dry heated	1.45	0.402 ± 0.009	25.770 ± 1.558	0.028 ± 0.004	1.929 ± 0.267	0.286 ± 0.028	19.750 ± 1.906
Pressure cooked	1.05	0.296 ± 0.018	20.424 ± 1.208	0.016 ± 0.003	1.537 ± 0.271	0.346 ± 0.033	32.968 ± 3.156

Values are mean of triplicate determinations ($n = 3$) ± SD (standard deviation).

Table 2 Reducing power activity of seed extracts from *Macrotyloma uniflorum* (brown and black varieties) and *Dolichos lablab*.

Sample	Raw		Dry heated		Pressure cooked	
	Conc. (μg)	OD value	Conc. (μg)	OD value	Conc. (μg)	OD value
M. uniflorum (brown variety)	1.90	0.378 ± 0.009	1.85	0.372 ± 0.002	1.60	0.388 ± 0.003
	3.80	0.614 ± 0.003	3.70	0.550 ± 0.004	3.20	0.586 ± 0.004
	5.70	0.870 ± 0.005	5.55	0.712 ± 0.005	4.80	0.745 ± 0.004
	7.60	1.099 ± 0.006	7.40	0.955 ± 0.002	6.40	0.880 ± 0.006
	9.50	1.395 ± 0.005	9.25	1.148 ± 0.055	8.00	1.070 ± 0.003
M. uniflorum (black variety)	1.95	0.067 ± 0.002	1.80	0.077 ± 0.001	1.50	0.279 ± 0.017
	3.90	0.406 ± 0.001	3.60	0.405 ± 0.005	3.00	0.434 ± 0.005
	5.85	0.627 ± 0.006	5.40	0.595 ± 0.009	4.50	0.663 ± 0.003
	7.80	0.831 ± 0.016	7.20	0.754 ± 0.006	6.00	0.798 ± 0.007
	9.75	1.113 ± 0.011	9.00	0.917 ± 0.003	7.50	1.037 ± 0.012
D. lablab	1.60	0.177 ± 0.003	1.45	0.205 ± 0.005	2.10	0.204 ± 0.009
	3.20	0.250 ± 0.004	2.90	0.301 ± 0.007	4.20	0.275 ± 0.003
	4.80	0.312 ± 0.022	4.35	0.402 ± 0.005	6.30	0.342 ± 0.005
	6.40	0.396 ± 0.008	5.80	0.522 ± 0.008	8.40	0.409 ± 0.007
	8.00	0.480 ± 0.006	7.25	0.576 ± 0.008	10.50	0.459 ± 0.003

Values are mean of triplicate determinations ($n = 3$) ± SD (standard deviation).

Reducing power assay

Antioxidant can be explained as reductants, which inactivate oxidants. They are involved in redox reactions in which one reaction species (oxidant) is reduced at the expense of the oxidation of the antioxidant (reductant). The yellow colour of the test solution changes to various shades of green and blue depending upon the reducing power of each extract. The presence of reductants (antioxidants) in the extracts causes the reduction of Fe^{3+}/ferric cyanide complex to ferrous form. Therefore the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Chung *et al.* 2002). **Table 2** shows the reducing power of raw and processed seed samples of *M. uniflorum* (brown and black varieties) and *D. lablab*. In general the increasing absorbance value is directly proportional to the concentration of extracts. Therefore the increasing OD value implies increasing trend of reducing power. The raw seed samples of *M. uniflorum* brown (9.5 µg; 1.395 OD_{700}) and black variety (9.8 µg; 1.113 OD_{700}) and dry heated samples of *D. lablab* (7.3 µg; 0.576 OD_{700}) showed highest ferric reducing antioxidant activity, as was noted in the DPPH· method. In the present study, the reducing power activity of seed sample extracts is in the following order: *M. uniflorum* brown variety; RA > DH > PC, black variety; RA > PC > DH, *D. lablab*; DH > RA > PC. Both raw and processed seed samples had potent reducing power. The stability of antioxidant potent of dry heated samples might partly be due to the formation of products from Maillard reaction. Nicoli *et al.* (1997) reported that medium dark roasted coffee brews had the highest antioxidant properties due to the development of products as a result of Maillard reaction. Tsai and She (2006) concluded that there was a change in the phenolic compounds after heating which contributed to the increase in reducing power. The decrease in reducing power of pressure cooked samples correlates with the low level of phenolic contents since, during cooking, a part of phenolics diffuse from the seed coat to cooking water (Rocha-Guzman *et al.* 2007). Yen and Duh (1993) reported that the reducing power of bioactive compounds, particularly phenolics extracted from peanut hulls, was associated with antioxidant activity, specifically scavenging of free radicals. The results revealed that processed bean extracts could act as an electron donor and react with free radicals and convert them to stable products, thus terminating the radical chain reaction.

DPPH• radical scavenging activity

This method is based on the reduction of DPPH, a stable free radical. Because of the odd electron of DPPH, it gives a strong absorption maximum at 517 nm by visible spectroscopy (purple colour). As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, that is, a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolourisation is stoichiometric with respect to the number of electrons captured (Blios 1958). This reaction has been widely used to test the ability of compounds act as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Soares *et al.* 1997; Porto *et al.* 2000). The percentage DPPH radical scavenging activities of all the extracts were dose dependent. The DPPH radical scavenging activities of raw and processed seed samples of *M. uniflorum* (brown and black varieties) and *D. lablab* and concentration of the sample which was necessary to decrease initial concentration of DPPH• by 50% (IC_{50}) under the experimental conditions has been calculated and represented in **Fig. 1**. The lower value of IC_{50} indicates a higher antioxidant activity. The IC_{50} values of raw seed samples of *M. uniflorum* brown (0.78 µg) and black (0.63 µg) varieties and dry heated samples of *D. lablab* (1.86 µg) registered the highest DPPH· radical scavenging activity. Interestingly, *M. uniflorum* (brown and black variety) and *D. lablab* raw and processed seed samples were found to have a higher hydrogen-donating ability or DPPH activity than the positive standards like ascorbic acid (30.12 µg), tannic acid (5.22 µg), pyrogallol (3.86 µg), catechol (9.50 µg) and L-Dopa (7.53 µg). The DPPH· radical scavenging efficiency of extracts from dry heated seed samples might have also been partly attributed to Maillard reaction products other than the phenolic constituents because they also effectively participate as a radical scavengers. Siddhuraju and Manian (2007) reported that a high concentration of phenolics and tannins extracted from seeds of *M. uniflorum* brown and black varieties was found to have DPPH• radical quenching capacity. This antiradical scavenging activity of untreated and treated seed extracts would be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability (Brand-Williams *et al.* 1995). According to Tsai and She (2006) a change in phenolic compounds after heating might be contributed to a decrease in DPPH-scavenging ability. Duenas *et al.* (2003) reported that proanthocyanidins from mocan seeds, had high ability to quench DPPH and that their effect was dependent on the molecular weight: higher molecular weight compounds had a greater effect. The increase in activity during cooking might release compounds bound in the cell wall or they might be bound to an insoluble fraction of other compounds during cooking of seeds (Herker *et al.* 2007). The condensed and hydrolysable tannins and phenolics in the seed coat of *P. vulgaris* have been found to be potent antioxidants (Beninger and Hosfield 2003). However, an extensive investigation on antiradical and antioxidant activities of small phenolics including flavonoids and phenolic acids has also been reported (Heim *et al.* 2002).

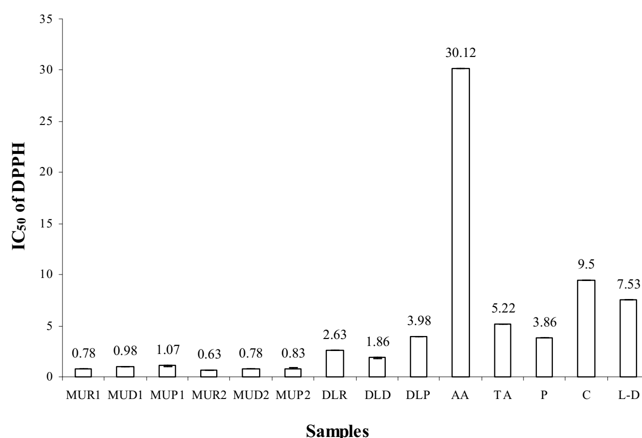

Fig. 1 DPPH• free radical scavenging activity (IC_{50}) of raw and processed seed samples of *Macrotyloma uniflorum* (brown and black variety) and *Dolichos lablab*. Microgram of sample necessary to decrease initial DPPH• concentration by 50% (IC_{50}) under specified experimental condition. Values are mean of three independent determinations (n = 3) ± standard deviation. 1, *M. uniflorum* brown variety; 2, *M. uniflorum* black variety; MUR, *M. uniflorum* raw sample; MUD, *M. uniflorum* dry heated sample; MUP, *M. uniflorum* pressure cooked sample; DLR, *D. lablab* raw sample; DLD, *D. lablab* dry heated sample; DLP, *D. lablab* pressure cooked sample; AA, ascorbic acid; TA, tannic acid; P, pyrogallol; C, catechol; L-D, L-Dopa.

Hydroxyl radical scavenging activity

The hydroxyl radical induces damage to biomolecules such as proteins, DNA, PUFA, nucleic acid and almost all biological molecules it touches. Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using ascorbic acid-iron EDTA. The hydroxyl radical formed by the oxidation react with dimethyl sulfoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent (Singh *et al.* 2002). The hydroxyl radical scavenging abilities of raw and processed seed samples of *M. uniflorum* (brown and black variety) and *D. lablab* are shown in **Fig. 2**.

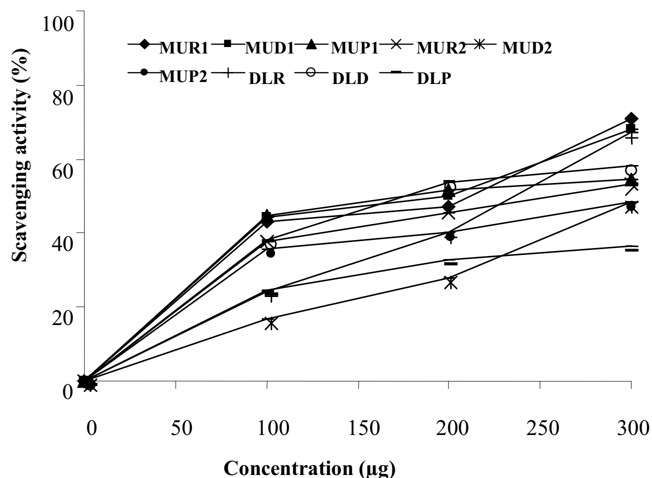

Fig. 2 Hydroxyl radical scavenging activity of raw and processed seed samples of *Macrotyloma uniflorum* (brown and black variety) and *Dolichos lablab*. Values are mean of three independent determinations (n = 3); ± standard deviation. 1, *M. uniflorum* brown variety; 2, *M. uniflorum* black variety; MUR, *M. uniflorum* raw sample; MUD, *M. uniflorum* dry heated sample; MUP, *M. uniflorum* pressure cooked sample; DLR, *D. lablab* raw sample; DLD, *D. lablab* dry heated sample; DLP, *D. lablab* pressure cooked sample.

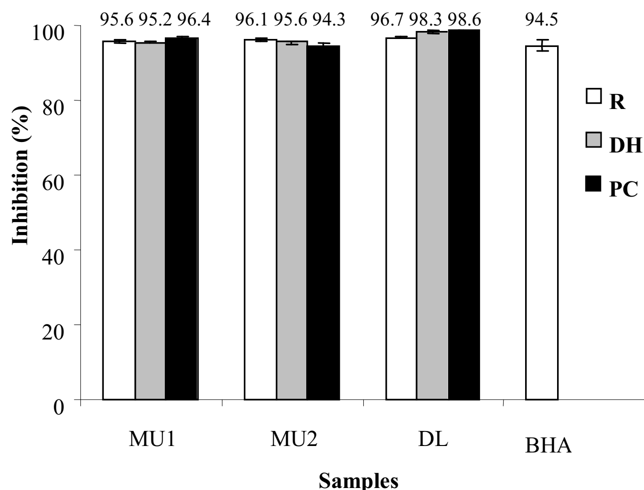

Fig. 3 Peroxidation inhibiting property of raw and processed seed samples of *Macrotyloma uniflorum* (brown and black variety) and *Dolichos lablab*. MU1, *M. uniflorum* brown variety; MU2, *M. uniflorum* black variety; DL, *D. lablab*; BHA, butylated hydroxyanisole; R, raw seed sample; DH, dry heated seed sample; PC, pressure cooked seed sample. Values are mean of three independent determinations (n = 3) ± standard deviation.

All the samples showed hydroxyl radical-scavenging activities (36.67-70.75%) at 300 µg in the reaction mixture. However, among the various samples, raw seed extracts of *M. uniflorum* (brown and black varieties) and *D. lablab* had the highest hydroxyl radical scavenging activity. The comparable values were obtained from raw and dry heated samples than pressure cooked samples of respective legumes. A study on antioxidant properties of faba bean tannins indicated that the antioxidant activity was accounted for by the direct interaction of tannin with hydroxyl radical rather than to a metal chelating activity (Carbonaro *et al.* 1996). These results show that the potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution. Yen and Hsieh (1995) reported that xylose and lysine Maillard reaction products had scavenging activity on hydroxyl radical that depended on dose response manner and which might have been attributed to the combined effects of reducing power, donation of hydrogen atoms and scavenging of active oxygen. Hagerman *et al.* (1998) also explained that high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the hydroxyl radical scavenging activity by tannins than their specific functional groups. According to Yen *et al.* (2005) the peanut seed testa and its antioxidative component protocatechuate might be used to provide a good hydroxyl radical scavenger. Similarly, raw and dry heated seed extracts of horse gram found to have potent hydroxyl radical scavenging activity and this might have been attributed to combined effects of Maillard reaction products (Siddhuraju and Manian 2007).

Antioxidant activity in linoleic acid-emulsion system

The antioxidant effects of extracts from raw and processed seed samples of *M. uniflorum* (brown and black varieties) and *D. lablab* and BHA on the peroxidation of linoleic acid were investigated and the results are presented in **Fig. 3**. At 250 µg in the final reaction mixture, BHA inhibited 94.5% peroxidation of linoleic acid after incubation for 54 h. However, raw a sample of *M. uniflorum* black variety (96.11%) and pressure cooked samples of brown variety and *D. lablab* (96.43 and 98.58%, respectively) exhibited the highest inhibition of peroxidation after incubation for 54 h at a concentration level of 1 mg in the final reaction mixture. In summary, the results show an inhibitory potential as follows: BHA > *D. lablab*; PC > DH > RA, *M. uniflorum* brown variety; PC > RA > DH, black variety; RA > DH > PC. However, no significant difference ($p < 0.05$) was observed among the seed sample extracts. Tsuda *et al.* (1993b) also reported that the methanolic extracts of horse gram showed lipid peroxidation inhibiting activity in the linoleic acid. Similar results were also been observed in the present investigation. In general, the seed coat may play an important role in chemical protection from oxidative damage by possessing endogenous antioxidants such as phenolic compounds. Similarly, seed coat extracts containing phenolic substances from red and black beans have been reported to have a strong antioxidant activity against lipid peroxidation (Tsuda *et al.* 1994). Polyphenols from the ethanolic extracts of peanut seed testa were found to effectively inhibit lipid peroxidation (Yen *et al.* 2005). The stability of the antioxidant potential of dry heated samples may be due to the formation of products as a result of the Maillard reaction. Similarly, extract of roasted followed by defatted peanut kernels displayed most remarkable antioxidative activity on linoleic acid emulsion system (Hwang *et al.* 2001). A different range of low and high molecular weight plant polyphenolics presenting antioxidant properties has been studied and proposed for protection against lipid oxidation (Hagerman *et al.* 1998). Jiratanan and Liu (2004) reported that thermal processing of *B. vulgaris* and *P. vulgaris* had no negative impact on total antioxidant activity. Cumulative values of radical scavenging activity for cooked common bean cultivars were higher than those of raw beans. When increasing the cooking time, phenolics from the seed coat diffuses to cooking water and from there to cotyledons and this phenomenon causes outstanding increase of antioxidant activity (Rocha-Guzman *et al.* 2007).

ABTS$^{\bullet+}$ radical scavenging activity

In the ABTS$^{\bullet+}$ radical cation scavenging method, the activity of tested sample extracts was expressed as Trolox equivalent – the micromolar Trolox solution having an antioxidant capacity equivalent to 1 g dry matter of the substance under investigation. The total antioxidant activity of raw and processed seed sample extracts of brown and black varieties of *M. uniflorum* and *D. lablab* are presented in **Table 3**. The pressure cooked samples of *M. uniflorum* (brown: 9094.4 µmol/g and black variety: 14541. 3 µmol/g) and dry heated samples of *D. lablab* (4409.1 µmol/g) exhibited good ABTS cation radical scavenging activity. However, the total phenolic content of processed seed samples of res-

Table 3 $ABTS^{\cdot+}$ radical cation scavenging activity, antihemolytic activity and metal chelating activity of raw and processed seed extracts of *Macrotyloma uniflorum* (brown and black varieties) and *Dolichos lablab*.

Sample	TAA* (µmol/g DM)	Antihemolytic activity	Metal chelating property (mg EDTA/g extract)
***M. uniflorum* (Brown variety)**			
Raw	8412.1 ± 53.8	2.05 ± 0.72	68.56 ± 1.71
Dry heated	7560.5 ± 276.4	2.44 ± 1.09	41.76 ± 1.88
Pressure cooked	9094.4 ± 127.8	8.05 ± 1.18	51.06 ± 2.72
***M. uniflorum* (Black variety)**			
Raw	7662.3 ± 52.4	2.65 ± 0.53	47.66 ± 1.39
Dry heated	6798.2 ± 227.3	9.16 ± 2.37	45.66 ± 1.39
Pressure cooked	14541.3 ± 545.5	4.74 ± 2.43	31.27 ± 1.27
D. lablab			
Raw	3824.0 ± 38.4	17.28 ± 0.10	66.20 ± 1.27
Dry heated	4160.2 ± 311.7	13.81 ± 0.99	63.01 ± 1.18
Pressure cooked	4409.1 ± 28.2	5.86 ± 2.96	75.78 ± 1.26

* Total antioxidant activity (µmol equivalent Trolox performed by using $ABTS^{\cdot}$ radical cation).
Values are mean of triplicate determinations (*n* = 3) ± SD (standard deviation).

pective varieties were found to be relatively low (assumed to be non-harmful); the TAA of such samples seems to be sufficient for functioning as potential nutraceuticals when they are ingested along with nutrients. Hagerman *et al.* (1998) reported that high molecular weight phenolics (tannins) have a greater ability to quench free radicals ($ABTS^{\bullet+}$) and that effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl groups substitution than the specific functional groups. Siddhuraju and Manian (2007) reported the $ABTS^{\bullet+}$ radical cation scavenging activity in raw and dry heated samples of horse gram brown and black varieties. The increase in activity during cooking might be a release of compounds bound in the cell wall or they might be bound to the insoluble fraction of other compounds during the cooking of seeds (Herker *et al.* 2007). On the other hand, the formation of tannin-protein complexes, both in insoluble and soluble complexes, as the result of conventional food/seed processing have also been shown to be potential free radical scavenger and radical sinks. Moreover, such complexes can also be suggested as one of the nutraceutical contributors to prevent free radical-mediated diseases occurring in the gastrointestinal tract (Riedl and Hagerman 2001).

Antihemolytic activity

It is well recognized that the oxidation of polyunsaturated fatty acids in biological membranes can lead to the formation and propagation of lipid radicals, uptake of oxygen, rearrangement of the double bonds in unsaturated lipids, and even destruction of membrane lipids. Many of these biochemical activities can lead to the production of breakdown products that are highly toxic to most cell types. Although hemolysis has a long history of use in measuring free radical damage and its inhibition by antioxidants, only few studies have been performed with erythrocytes in whole blood. In this study, we used a biological test based on free radical-induced erythrocytes lysis in cow blood. This assay is useful either for screening studies on various molecules and their metabolites, especially on the one hand molecules are having an oxidizing or antioxidizing activity and on the other hand molecule having a long-term action (Djeridane *et al.* 2006). Lipid oxidation of cow blood erythrocyte membrane mediated by H_2O_2 induces membrane damage and subsequently hemolysis. The antihemolytic activity or raw and processed seed samples of *M. uniflorum* (brown and black varieties) and *D. lablab* are presented in **Table 3**. The pressure cooked samples of brown variety (8.05%), dry heated samples of black variety (9.16%) showed moderate activity, whereas raw (17.28%) and dry heated (13.81%) samples of *D. lablab* exhibited higher antihemolytic activity. In general, when compared to *M. uniflorum* (brown and black varieties), *D. lablab* was found to have potential antihemolytic activity. Zhu *et al.* (2002) reported that three fractions of Oolong tea were effective against lipid oxidation in erythrocyte membranes and also showed a dose-dependent inhibition toward Red Blood Corpuscles (RBC) hemolysis. Moreover, RBC hemolysis is a more sensitive system for evaluating the antioxidant properties of phytoceuticals.

Metal chelating property

Iron is essential for life because it is required for oxygen transport, respiration and the activity of many enzymes. However, iron is an extremely reactive metal and will catalyse oxidative changes in lipid, protein, and other cellular components (Decker and Hultin 1992). In addition, liposome peroxidation and oxidative damage of protein model systems were induced by a Fenton reaction in which ferrous ions catalyse the composition of hydrogen peroxide to hydroxyl anion and hydroxyl radical with the production of ferric iron (Yen *et al.* 2005). Although metal chelating agents are not antioxidants, they play a valuable role in the stabilization of fatty acids against rancidity (Yen and Duh 1994). The chelating effect of raw and processed seed sample may be significant because it minimizes the concentration of metal in the Fenton reaction. In this method the metal chelating property of sample extracts were expressed in mg of EDTA equivalent. The metal chelating property of raw and processed seed samples of *M. uniflorum* (brown and black varieties) and *D. lablab* are presented in **Table 3**. Raw samples of brown and black varieties (68.56 and 47.66 mg EDTA g^{-1}, respectively) and pressure cooked samples of *D. lablab* (75.78 mg EDTA g^{-1}) exhibited highest metal chelating property. Among the three legumes, *D. lablab* was found to have a good metal chelating property. The order of metal chelating property of respective seed samples is as follows: *M. uniflorum* brown variety; RA > PC > DH, black variety; RA > DH > PC, *D. lablab*; PC > RA > DH. Yen and Duh (1994) observed that peanut hulls might be the major cause for suppression of chelating metal ions. Similarly, the extract of peanut seed testa showed a significant Fe^{2+} chelating effect (Yen *et al.* 2005). Moreover, Chung *et al.* (2002) implied that fermented red bean extract had an Fe^{2+}-chelating effect and could afford protection against oxidative damage. Study on the antioxidant activity of faba bean tannin content also showed metal chelating activity (Carbonaro *et al.* 1996).

The present study suggests that both raw and processed seed samples have a strong hydrogen donating ability and are good scavengers of ROS, hydroxyl radical, and metal chelating property. Interestingly, the results indicated that not only the phenolic constituent from raw samples but also the phenolics and Maillard products of processed samples are found to be potent antioxidant suppliers. Therefore, consumers may obtain optimal health benefits along with nutrient assimilation without any negative implications. On the other hand, the formation of tannins-protein complexes and phenolics associated with dietary fibre and how these relate to the risk of oxidative injury during gastrointestinal can be

demonstrated through *in vivo* studies. Further, the identification and bulk preparation of bioactive compound-enriched fractions from the studied legume seeds, especially from seed coats, may be exploited as alternative and natural antioxidant nutrient sources for prolonging the shelf life of food and feed products.

ACKNOWLEDGEMENTS

The authors thank the authority of Bharathiar University, Coimbatore, TN for financial support.

REFERENCES

Anderson JM, Smith BM, Washnock CS (1999) Cardiovascular and renal benefits of dry beans and soy bean intake. *American Journal of Clinical Nutrition* **70**, 464S-474S

Barroga FC, Laurena AC, Mendosa EMT (1985) Polyphenols in mung bean (*Vigna radiata* (L.) Wilczek): determination and removal. *Journal of Agricultural and Food Chemistry* **33**, 1006-1009

Beninger CF, Hosfield GL (2003) Antioxidant activity of extracts, condensed tannin fractions and pure flavanoids from *Phaseolus vulgaris* L. seed coat colour genotypes. *Journal of Agricultural and Food Chemistry* **51**, 7879-7883

Blios MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature* **26**, 1199-1200

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology* **28**, 25-30

Bravo L, Siddhuraju P, Saura-Calixto F (1998) Effect of various processing methods on the *in vitro* starch digestibility and resistant starch content of Indian pulses. *Journal of Agricultural and Food Chemistry* **46**, 4667-4674

Bravo L, Siddhuraju P, Saura-Calixto F (1999) Composition of underexploited Indian pulses. Comparison with common legumes. *Food Chemistry* **64**, 185-192

Cadenas E, Packer L (2002) *Handbook of Antioxidants*, Marcel Dekker, New York, 712 pp

Carbonaro M, Virgili F, Carnovale E (1996) Evidence for protein-tannin interaction in legumes: Implications in the antioxidant properties of faba bean tannins. *LWT - Food Science and Technology* **29**, 743-750

Chaillou LL, Nazareno MA (2006) New method to determine antioxidant activity of polyphenols. *Journal of Agricultural and Food Chemistry* **54**, 8397-8402

Chau CF, Cheung PCK, Wong YS (1998) Hypocholesterolemic effects of protein concentrates from three Chinese indigenous legume seeds. *Journal of Agricultural and Food Chemistry* **46**, 3698-3701

Chu YF, Sun J, Wu X, Liu RH (2002) Antioxidant and antiproliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry* **50**, 6910-6916

Chung YG, Chang CT, Chao WW, Lin CF, Chou ST (2002) Antioxidative activity of 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NKI. *Journal of Agricultural and Food Chemistry* **50**, 2454-2458

Decker EA, Hultin HO (1992) Lipid oxidation in muscle foods via redox iron. In: St. Angelo AJ (Ed) *Lipid Oxidation in Food*, ACS Symposium Series 500, American Chemical Society, Washington, DC, pp 34-54

Djeridane A, Yousfi M, Nadjemi B, Vidal N, Lesgards JF, Stocker P (2006) Screening of some Algerian medicinal plants for the phenolic compounds and their antioxidant activity. *European Food Research and Technology* **224**, 801-809

Duenas M, Hernandez T, Estrella I, Rabanal R (2003) Phenolic composition and antioxidant activity of mocan seeds (*Visnea mocanera* L.f). *Food Chemistry* **82**, 373-379

Duke JA, Reed CF (1981) *Macrotyloma uniflorum* (Lam.) Verdc. In: Duke JA (Ed) *Handbook of Legumes of World Economic Importance*, Plenum Press, New York, pp 345

Espinosa-Alonso LG, Lygin A, Widholm JM, Valverde ME, Parades-Lopez O (2006) Polyphenols in wild and weedy Mexican common beans (*Phaseolus vulgaris*). *Journal of Agricultural and Food Chemistry* **54**, 4436-4444

Fernandes AA, Nagendrappa G (1979) Chemical constituents of *Dolichos lablab* (Field Bean) pod exudate. *Journal of Agricultural and Food Chemistry* **27**, 795- 798

Fessenden RJ, Fessenden JS (1994) *Organic Chemistry* (5th Ed), Cole Publishing Company, Belmont, CA, pp 246-260

Frias J, Miranda ML, Doblado R, Vidal-Valerde C (2005) Effect of germination and fermentation on the antioxidant vitamin content and antioxidant capacity of *Vigna sinensis* var *Multolupa*. *Food Chemistry* **92**, 211-220

Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL (1998) High molecular weight polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry* **46**, 1887-1892

Halliwell B, Gutteridge JMC, Cross CE (1992) Free radicals, antioxidants, and human diseases. Where are we now? *Journal of Laboratory and Clinical Medicine* **119**, 598-620

Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry* **13**, 572-584

Herker EM, Ibanoglu S, Oner MD, Bilgicli N, Guzel S (2007) Effect of storage on the phytic acid content, total antioxidant capacity and organoleptic properties of macaroni enriched with cowpea flour. *Journal of Food Engineering* **78**, 366-372

Hwang JY, Shue YS, Chang HM (2001) Antioxidant activity of roasted and defatted peanut kernels. *Food Research International* **34**, 639-647

Jiratanan T, Liu RH (2004) Antioxidant activity of processed table beets (*Beta vulgaris* var. *conditiva*) and green beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry* **52**, 2659-2670

Kadam SS, Salunkhe DK (1985) Nutritional composition, processing, and utilization of horse gram and moth bean. *Critical Reviews in Food Science and Nutrition* **22**, 1-26

Kikuzaki H, Nakatani N (1993) Antioxidant effects of some ginger constituents. *Journal of Food Science* **58**, 1407-1410

Klein SM, Cohen G, Cederbaum AI (1991) Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. *Biochemistry* **20**, 6006-6012

Messina MJ (1999) Legumes and soy beans: over view of their nutritional profiles and health effects. *American Journal of Clinical Nutrition* **70**, 439S-450S

Mitra J, Das A, Joshi T (1983) An isoflavone diglycoside from the seeds of *Dolichos biflorus*. *Phytochemistry* **22**, 1063-1064

Muthu AK, Sethupathy S, Manavalan R, Karar PK (2005) Hypolipidemic effect of methanolic extract of *Dolichos biflorus* Linn. in high fat diet fed rats. *Indian Journal of Experimental Biology* **43**, 522-525

Muthu AK, Sethupathy S, Manavalan R, Karar PK (2006) Antioxidant potential of methanolic extract of *Dolichos biflorus* Linn. in high fat diet fed rabbits. *Indian Journal of Experimental Biology* **38**, 131-132

Naim M, Gestetner B, Bondi A, Birk Y (1976) Antioxidative and antihemolytic activities of soybean isoflavones. *Journal of Agricultural and Food Chemistry* **24**, 1174-1177

Namiki M (1990) Antioxidants/antimutagens in food. *International Journal of Food Science and Nutrition* **29**, 273-300

Nicoli MC, Anese M, Manzocco L, Lerici CR (1997) Antioxidant properties of coffee brews in relation to the roasting degree. *Lebensmittel Wisenschaft und Technologie* **30**, 292-297

Oboh G (2006) Antioxidant properties of some commonly consumed and underutilized tropical legumes. *European Food Research and Technology* **224**, 61-65

Oyaizu M 1986) Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition* **44**, 307-315

Porto CD, Calligaris S, Celloti E, Nicoli MC (2000) Antiradical properties of commercial oognacs assessed by the DPPH$^\bullet$ test. *Journal of Agricultural and Food Chemistry* **48**, 4241-4245

Re R, Pellegirini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* **26**, 1231-1237

Reddy NR, Pierson MD, Sathe SK, Salunkhe DK (1985) Dry bean tannins: A review of nutritional implications. *Journal of American Oil Chemist's Society* **62**, 541-549

Riedl K, Hagerman AE (2001) Tannin-protein complexes as radical scavengers and radical sinks. *Journal of Agricultural and Food Chemistry* **49**, 4917-4923

Rocha-Guzman NE, Gonzalez-Laredo RF, Ibarra-Perez FJ, Nava-Berumen CA, Gallegos-Infanate JA (2007) Effect of pressure cooking on the antioxidant activity of extracts from three common bean (*Phaseolus vulgaris* L.) cultivars. *Food Chemistry* **100**, 31-35

Rui HL (2004) Potential synergy of phytochemicals in cancer prevention; mechanism of action. *Nutrition* **134**, 3479S-3485S

Scalbert A, Manach C, Morand C (2005) Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition* **45**, 287-306

Siddhuraju P (2007) The antioxidant of phenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. *Lebensmittel Wissenschaft und Technologie* **40**, 982-90

Siddhuraju P, Becker K (2003) Studies on antioxidant activities of mucuna seed (*Mucuna pruriens* var. *utilis*) extracts and certain non-protein amino/imino acids through *in vitro* models. *Journal of the Science of Food and Agriculture* **83**, 1517-1524

Siddhuraju P, Becker K (2006) The antioxidant activity and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chemistry* **101**, 10-19

Siddhuraju P, Manian S (2007) The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. *Food Chemistry* **105**, 950-958

Soares JR, Dinis TCP, Cunha AP, Almeida LM (1997) Antioxidant activities of some extracts of *Thymus zygi*. *Free Radical Research* **26**, 469-478

Sudha N, Mushtari Begum J, Shambulingappa KG, Babu CK (1995) Nutri-

ents and some anti-nutrients in horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.). *Food and Nutritional Bulletin* **16**, 81-83

Sumrell G, Mod RR, Magne FE, Novak AF (1978) Antimicrobial activity of some fatty acid derivatives. *Journal of American Oil Chemist's Society* **55**, 395-397

Sun J, Chu Y, Wu X, Liu R (2002) Antioxidant and antiproliferative activity of common beans. *Journal of Agricultural and Food Chemistry* **50**, 7449-7454

Tharanathan RN, Mahadevamma S (2003) Grain legumes a boon to human nutrition. *Trends in Food Science and Technology* **14**, 507-518

The Wealth of India (1992) *Raw Materials* (Vol 3), CSIR, New Delhi, India, pp 59-67

Tsai PJ, She CH (2006) Significance of phenol-protein interactions in modifying the antioxidant capacity of peas. *Journal of Agricultural and Food Chemistry* **54**, 8491-8494

Tsuda T, Makino Y, Kato H, Osawa T, Kawakishi S (1993) Screening for antioxidative activity of edible pulses. *Bioscience, Biotechnology and Biochemistry* **57**, 1606-1608

Tsuda T, Ohshima K, Kawakishi S, Osawa T (1994) Antioxidant pigments isolated from the seeds of *Phaseolus vulgaris* L. *Journal of Agricultural and Food Chemistry* **42**, 248-251

Yamaguchi F, Ariga T, Yoshirmura Y, Nakazawa K (2000) Antioxidative and antiglycation activity of garcinol from *Garcinia indica* fruit rind. *Journal of Agricultural and Food Chemistry* **48**, 180-185

Ye XY, Wang HX, Ng TB (2000) Dolichin, a new chitinase-like antifungal protein isolated from field beans (*Dolichos lablab*). *Biochemical and Biophysical Research Communications* **269**, 155-159

Yen GC, Duh PN (1994) Scavenging effect of methanolic extracts of peanut hulls on free radical and active-oxygen species. *Journal of Agricultural and Food Chemistry* **42**, 629-632

Yen GC, Hsieh PP (1995) Antioxidant activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. *Journal of the Science of Food and Agriculture* **67**, 415-420

Yen WJ, Chang LW, Duh PD (2005) Antioxidant activity of peanut seed testa and its antioxidative component, ethyl protocatechuate. *LWT - Food Science and Technology* **38**, 193-200

Zhu QY, Hackman RM, Ensunsa JL, Holt RR, Keen CL (2002) Antioxidative activities of Oolong tea. *Journal of Agricultural and Food Chemistry* **50**, 6929-6934