Food Sci. Biotechnol. 19(2): 503-509 (2010)
DOI 10.1007/s10068-010-0070-6

RESEARCH ARTICLE

In vitro Evaluation of the Antioxidant Activities in the Differentially Processed Seeds from Underutilized Legume, *Bauhinia vahlii* Wight & Arn.

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Received: 5 November 2009 / Revised: 8 December 2009 / Accepted: 29 December 2009 / Published Online: 30 April 2010
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Abstract Antioxidant potential and total phenolics content of 70% acetone extracts of the raw and processed seeds of *Bauhinia vahlii* were evaluated. The extract of raw seeds contained higher levels of total phenolics (30.8 g/100 g) and tannins (19.6 g/100 g) compared to dry heated and soaking followed by autoclaving seed extracts. Extracts were screened for antioxidant and free radical scavenging activities using various chemical and *in vitro* model systems. In all the models, except DPPH radical scavenging activity, the extract from raw seeds manifested the strongest antioxidant activity than that from processed seeds. In β-carotene/linoleic acid emulsion system and superoxide scavenging activity, the raw seed extract registered more activity when compared to the standards (butylated hydroxyanisole and α-tocopherol). Whereas, the extract from dry heated seed exhibited higher $DPPH^{\bullet}$ scavenging activity (IC_{50} 70.77 μg/mL) than the raw seeds (IC_{50} 74.4 μg/mL). This study has to some extent validated the antioxidant potential of the seeds of *B. vahlii*.

Keywords: *Bauhinia vahlii*, polyphenol, antioxidant activity, β-carotene, 2,2-diphenylpicryl-1-picryl-hydrazyl ($DPPH^{\bullet}$)

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Introduction

Oxidative stress is thought to result from an imbalance between the generation of free radicals and the antioxidants that scavenge them. Oxidation of cellular constituents by free radicals provokes several human diseases such as diabetes, arteriosclerosis, cardiovascular illnesses, cancer, several neurodegenerative disorders, and the aging process. Consequently, much attention has been directed towards the discovery of new natural antioxidants, including herbal products, aimed at quenching biologically harmful radicals (1,2). Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage, and DNA strand breaking (3). Phenolic compounds in plant-derived foods and beverages have been shown to have important physiological properties and may be responsible for both detrimental and beneficial effects on human health (4,5). Phenolic compounds and antioxidant activities in legume seeds were reported by several earlier communications (6,7), although legumes constitute one of the most abundant and least expensive sources of protein in human/animal diet.

Bauhinia vahlii Wight & Arn. is a very large, usually evergreen, climber and is distributed in deciduous forests of India from Gujarat southwards to Maharastra and Northern Andhra Pradesh, commonly on hillsides and in forest valleys (8). The seeds of this legume are a rich source of crude protein (24.59%), crude lipid (23.26%), crude fibre (6.21%), carbohydrates (41.72%), minerals, and essential amino acids. The ripe seeds, when eaten raw or fried, taste like cashew-nuts (9). The cooked and roasted mature seeds of *B. vahlii* are eaten by the tribes, Kondakapulu and Baagethalu of Araku valley, Visakhapatnam, Andhra Pradesh, and Mundari group of tribes in India (10,11). The

seeds are considered aphrodisiac and tonic. A paste of the seed is applied to boils and given to children suffering from indigestion (12).

The biological utilization of available protein and carbohydrates in legume feedstuffs are limited by the presence of various antinutritional factors such as phytic acid, trypsin inhibitors, protease inhibitors, lectins, and hemagglutinins (13). Therefore, the legumes have to be processed prior to consumption (14). Food processing induces a variety of physical and biochemical changes that could affect the overall acceptability by the consumer, as well as the nutritional quality of the product. However, the extent to which various processing methods affect the nature of antioxidant and free radical scavenging activities of legume seeds are still unanswered. No information is available with respect to the antioxidant activity of the raw and processed seeds of *B. vahlii*. Hence, the present investigation was carried out to evaluate the antioxidant and free radical scavenging potential of the 70% acetone extracts of raw and processed seeds of *B. vahlii*.

Materials and Methods

Chemicals 2,2'-Azinobis(3-ethylbenzothiozoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$), 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^{\bullet}$), 2,4,6-tripyridyl-*S*-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nitroblue tetrazolium (NBT), and linoleic acid were purchased from Sigma-Aldrich (Bangalore, Inida). Potassium persulfate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ferrous chloride, hydrogen peroxide, riboflavin, trichloroacetic acid (TCA), Tween 40, and ferric chloride were obtained from Himedia (Mumbai, India). Ethylenediamine tetraacetic acid (EDTA) disodium salt was purchased from Merck (Mumbai, India). All other reagents used were of analytical grade.

Seed samples and processing The seeds of *B. vahlii*, brown in color, were collected from Nallamalai hills, Srisailam, Andhra Pradesh, India during the month of September, 2008. The seeds (100 g) were dry heated in a hot air oven at 150°C for 30 min and the beans were allowed to cool at room temperature. For the pressure-cooking treatment, the seeds (100 g) were soaked in distilled water (seed:water, 1:10 w/v) for 12 hr at room temperature (25°C). After decanting the water, the soaked seeds were subjected to autoclaving (seed:water 1:5 w/v) for 20 min at 120°C. Excess water was drained off and the seeds were rinsed with distilled water and dried at 55°C. The raw and processed seed samples were ground to fine powder (particle size of about 0.25 mm) and stored in separate screw cap bottles at –20°C before analysis.

Solvent extraction Raw and processed ground seed samples (20 g) were extracted by stirring with 100 mL of 70% acetone at 25°C for 24 hr and filtering through Whatman No. 4 filter paper. The residues were re-extracted with an additional 50 mL of 70% acetone, as described above, for 3 hr. The solvent of the combined extract was evaporated under reduced pressure, using a rotary vacuum-evaporator (RE300; Yamato, Tokyo, Japan) at 40°C and the remaining water was removed by lyophilization (4KBTXL-75; VirTis Benchtop K, New York, NY, USA). The freeze-dried extract thus obtained was used directly for total phenolics and tannins estimation and also for the assessment of antioxidant capacity through various *in vitro* assays. One-hundred mg of the sample extract was dissolved in 100 mL of the solvent and generally used in different assays.

Determination of total phenolic and tannin contents The total phenolic content was determined according to the method described by Siddhuraju and Becker (13). Aliquots of extracts were taken in test tubes and made up to the 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 dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents (TAE). Tannins in the extracts were estimated after treatment with polyvinyl polypyrrolidone (PVPP). One-hundred mg of PVPP was weighed in a 100×12-mm test tube and to this 1.0 mL of 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 4 hr. Then the sample was centrifuged (3,000×g 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 mentioned above and expressed as the content of non-tannin phenolics on a dry matter basis (15). From the above results, the tannin content of the sample was calculated as follows:

$$\text{Tannin (\%)} = \text{total phenolics (\%)} - \text{non-tannin phenolics (\%)}$$

Ferric reducing/antioxidant power (FRAP) assay The antioxidant capacity of raw and processed *B. vahlii* seed samples was estimated according to the procedure described by Benzie and Strain (16). FRAP reagent (900 μL), prepared freshly and incubated at 37°C, was mixed

with 90 μL of distilled water and 30 μL of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37°C for 30 min in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5 mL of 20 mmol/L TPTZ solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 mol/L acetate buffer (pH 3.6) as described by Siddhuraju and Becker (6). At the end of incubation the absorbance readings were taken immediately at 593 nm, using a spectrophotometer. Methanolic solutions of known Fe(II) concentration, ranging from 100 to 2,000 μmol/L ($FeSO_4 \cdot 7H_2O$) were used for the preparation of the calibration curve. The parameter equivalent concentration (EC_1) was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L $FeSO_4 \cdot 7H_2O$. EC_1 was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/L concentration of Fe(II) solution, determined using the corresponding regression equation.

Antioxidant activity by the $ABTS^{\bullet+}$ assay The total antioxidant activity of the samples was measured by ABTS radical cation decolorization assay according to the method of Re *et al.* (17) described by Siddhuraju and Manian (15). $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 hr at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to give an absorbance at 734 nm of 0.700±0.02. The stock solution of the sample extracts were diluted such that after introduction of 10 μL aliquots into the assay, they produced between 20 and 80% inhibition of the blank absorbance. After the addition of 1 mL of diluted ABTS solution to 10 μL of test sample or Trolox standards (final concentration 0-15 μM) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. The percentage inhibition was calculated of the blank absorbance at 734 nm and then was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as μmol/g of dry sample extract.

Metal chelating activity The chelating of ferrous ions by the 70% acetone extracts of raw and processed seeds of *B. vahlii* was estimated by the method of Dinis *et al.* (18). Briefly the extracts (100 μL) were added to a solution of 2 mmol/L $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

Phosphomolybdenum assay The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex according to the method of Prieto *et al.* (19). An aliquot of 100 μL of sample solution (in 1 mM dimethyl sulfoxide, DMSO) was combined with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a 4-mL vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported are mean values expressed as g of ascorbic acid equivalents/100 g extract (ascorbic acid equivalent antioxidant activity).

Assay of superoxide radical scavenging activity Superoxide radicals were generated by a modified method of Beauchamp and Fridovich (20). Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 μg riboflavin, 12 mM EDTA, 0.1 mg nitroblue tetrazolium (NBT), and 100 μL extract. Reaction was started by illuminating the reaction mixture with sample extract for 90 sec. Immediately after illumination, the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminium foil in the dark. The percentage inhibition of superoxide anion generation was calculated as: % Inhibition=$[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample extract/standard.

Hydroxyl radical scavenging activity The scavenging activity of raw and processed *B. vahlii* seed extracts on hydroxyl radical was measured according to the method of Klein *et al.* (21). One-hundred μL of extract was added with 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulfate 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 (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity (HRSA) is calculated by the following formula: % HRSA=$[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the

absorbance of the extract/standard.

Free radical scavenging activity on DPPH˙ The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Blios (22). Sample extracts at various concentrations was taken and the volume was adjusted to 100 μL with methanol. Five mL of a 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula: % DPPH radical scavenging activity=(control OD−sample OD/control OD)×100.

β-Carotene/linoleic acid antioxidant activity One mL of a β-carotene solution in chloroform (1 mg/10 mL) was pipetted in to a flask containing 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator (RE300; Yamato) at 45°C for 4 min and, 50 mL of oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion. A 5 mL aliquot of the emulsion was added to a tube containing 200 μL of the antioxidant (extracts, BHA, or α-tocopherol) solution at 1 mg/mL concentration and the absorbance was measured at 470 nm immediately, against a blank consisting of the emulsion without β-carotene (23). The tubes were placed in a water bath at 50°C and the absorbance was monitored at 15 min intervals until 180 min. The antioxidant activity of the sample extracts and standard was evaluated in terms of bleaching of β-carotene using the following formula: % of antioxidant activity=$[1-(A_0-A_t)/(A^1_0-A^1_t)]\times100$, where A_0 and A^1_0 are the absorbance measured at 0 time of incubation for the test sample and control, respectively, and A_t and A^1_t are the absorbances measured in the test sample and control, respectively, after incubation for 180 min.

Statistical analysis The data 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, OK, USA). Values expressed are means of 3 replicate determinations ($n=3$)±standard deviation (SD).

Results and Discussion

Recovery percent, total phenolic, and tannin contents of extracts The extract yield, total phenolics, and tannin contents of 70% acetone extracts of raw and processed seeds of *B. vahlii* are shown in Table 1. Soaking followed by autoclaving of seeds gave a low extraction yield (7.2%) which was independent from raw (17.5%) and dry heated seeds (14.0%). The results indicated that raw seeds contained higher amounts of total phenolics (30.8 g/100 g extract) and tannins (19.6 g/100 g extract) than processed seeds. The processing method of soaking followed by autoclaving highly decreases the levels of total phenolics and tannins (48 and 63.8%, respectively) when compared to raw seeds. Siddhuraju and Becker (6) suggest that the reduction of phenolics and tannins in the processed seed sample is possibly due to the dissolution of phenolics in the soaking and autoclaving medium and their subsequent discarding both of them. In the dry heated seeds, the phenolics are not extracted by solvent due to the formation of insoluble tannin-carbohydrate, including cell wall polysaccharide complexes. Tannins are water soluble polyphenols present in many foods. They have been recognized as antioxidants. Plant phenolics have received considerable attention because of their potential antioxidant activity. Natural polyphenols have chain-breaking antioxidant activities and are believed to prevent many degenerative diseases, including cancer and atherosclerosis (24). The results of this study clearly indicate that phenolics are important component of *B. vahlii* seed, and its free radical scavenging and antioxidant effects could be attributed to the presence of these valuable constituents.

Table 1. Extract yield percentage, total phenolics, and tannin content of the 70% acetone extracts of raw and processed seed extracts of *B. vahlii*

Sample[1)]	Extract yield (%)	Total phenolics (g/100 g extract)	Tannin (g/100 g extract)
T1	17.5	30.8±0.8[2)]	19.6±0.6
T2	14.0	29.2±0.6	15.0±0.1
T3	7.2	16.0±0.4	7.1±0.3

[1)]T1, Raw; T2, Dry heated; T3, Soaking followed by autoclaving
[2)]Values are mean±SD ($n=3$).

FRAP assay The reducing capacity of an extract may serve as a significant indicator of its potential antioxidant activity (25). The FRAP potential of 70% acetone extracts for the raw and processed seeds of *B. vahlii* were estimated from their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II). The extract of raw seeds exhibited the highest FRAP antioxidant activity (1,968.3 mmol Fe(II)/mg extract) (Table 2). The FRAP value for the soaking followed by autoclaved seed extract (1,197.2 mmol Fe(II)/mg extract) was significantly lower than those of raw and dry heated seed extracts. According to Oktay *et al.* (26), a highly positive relationship between total phenolics and antioxidant activity appears to be the trend in many plants. These results indicate that the raw and processed seed

Table 2. FRAP, $ABTS^{\bullet+}$ scavenging, metal chelating, and phosphomolybdenum activity of the 70% acetone extracts of raw and processed seed extracts of *B. vahlii*

Sample[1)]	FRAP[2)] (mmol Fe (II)/mg extract)	TAA[3)] (μmol/g extract)	Metal chelating (mg EDTA/g extract)	AEAC[4)] (g AA/100 g extract)
T1	1,968.3±129.8[a5)]	2,0385.7±287[a]	6.5±0.3[a]	38.5±1.1[a]
T2	1,244.4±983.8[b]	1,9116.1±253[b]	4.5±0.2[c]	34.5±0.5[a]
T3	1,197.2±229.9[c]	1,2217.3±852[c]	5.1±0.2[b]	29.0±1.1[b]

[1)]T1, Raw; T2, Dry heated; T3, Soaking followed by autoclaving
[2)]Concentration of substance having ferric-TPTZ reducing ability expressed as mmol Fe (II) equivalents
[3)]Total antioxidant activity (μmol equivalent Trolox performed by using $ABTS^{\bullet+}$ radical cation)
[4)]Ascorbic acid equivalent antioxidant capacity (g equivalent of ascorbic acid/100 g extract) through the formation of phosphomolybdenum complex.
[5)]Values are mean±SD (n=3); Mean values followed by different superscripts in a column are significantly different (p<0.05).

extracts of *B. vahlii* have a potency to donate electron to deactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction.

$ABTS^{\bullet+}$ radical scavenging activity The decolorization of $ABTS^{\bullet+}$ radical reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate this radical species. The $ABTS^{\bullet+}$ radical cation is generated from the reaction of ABTS with potassium persulfate overnight in water (17). The ABTS radical cation scavenging potential of the acetone extracts of raw and processed seeds of *B. vahlii* is presented in Table 2. The extract of raw seeds is highly potent in neutralizing ABTS cation radicals (20,385.7 μmol/g extract). This scavenging ability of the autoclaved seeds (12,217.3 μmol/g extract), however, showed a 40% decrease when compared to raw seeds. Phenolics of this seed extracts are probably involved in the ABTS radical scavenging activity. This study indicated that the extracts have the hydrogen donating ability and could serve as free radical scavengers by acting as primary antioxidants.

Metal chelating activity Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury. If they undergo Fenton reaction, these reduced metals may form reactive hydroxyl radicals and thereby contribute to oxidative stress (27). The chelating ability of ferrous ions by the differentially processed seed extracts was estimated by the method of Dinis *et al.* (18). Ferrozine can quantitively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that red color of the complex is decreased. The metal chelating activity of acetone extracts from raw and processed seeds of *B. vahlii* is presented in Table 2. In this assay, the raw seed extract of *B. vahlii*, registered higher metal chelating activity (6.5 mg EDTA/g extract) compared to processed seeds. Among the processed seed extracts, the dry heated extract of seeds showed the lowest metal chelating activity (4.5 mg EDTA/g extract) (Table 2). The scavenging potential and metal chelating ability of the antioxidants are dependent upon their unique phenolic structure and the number of hydroxyl groups (28).

Phosphomolybdenum assay The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The phosphomolybdenum method is quantitative since the antioxidant activity is expressed as the number of equivalents of ascorbic acid (19). The raw and processed seed extracts of *B. vahlii* were used to determine their antioxidant capacities by the formation of green phosphompolybdenum complex (Table 2). The results indicate that the extract of the raw seeds is more powerful antioxidant in the reduction of phosphomolybdenum complex (38.5 g AA/100 g extract) followed by the extract of dry heated seeds (34.5 g AA/100 g extract). However, the value of the autoclaved seed extract was much lower, it might be due to the decreased level of phenolic content.

Superoxide radical scavenging activity Endogenously, superoxides could be produced in large amounts by various biological processes. It is known to be very harmful to cellular components as a precursor of the most reactive oxygen species (ROS), contributing to tissue damage and various diseases (29). The raw seeds of *B. vahlii* showed 82.6% superoxide inhibition at the concentration of 100 μg/mL followed by the dry heated seeds (78.7%) (Table 3). These extracts exhibited higher ability in scavenging superoxide anion radical, when compared to the standards BHT (72.6% at 100 μg/mL) and α-tocopherol (45.2% at 100 μg/mL). Although, the scavenging ability of the extract from autoclaved seeds was significantly (p<0.05) lower, it was better than the standard α-tocopherol. The scavenging ability of the seed extracts on superoxide anion radical was in the decreasing order of T1>T2>BHT>T3> α-tocopherol. The results reveal that the *B. vahlii* seed extracts have superoxide radical scavenging activity which can be of significant interest in health point of view in reducing the level of superoxide radical which is elevated during oxidative stress in the body.

Table 3. Superoxide, hydroxyl, DPPH radical scavenging, and β-carotene/linoleic acid peroxidation inhibition activity of the 70% acetone extracts of raw and processed seed extracts of *B. vahlii*

Sample[1)]	Radical scavenging activity			β-Carotene/linoleic acid (%)
	Superoxide (%)	Hydroxyl (%)	IC_{50} of DPPH (μg/mL)	
T1	82.6±1.2[a2)]	56.0±1.7[b]	74.1[b]	43.0±1.7[a]
T2	78.7±1.5[b]	51.1±1.7[b]	70.8[b]	37.5±2.1[b]
T3	67.1±2.1[c]	35.3±2.2[d]	190.9[c]	35.6±1.3[b]
BHT	72.6±2.4[b]	67.5±3.0[a]	33.9[a]	36.6±1.0[b]
α-Tocopherol	45.2±1.1[d]	41.6±1.4[c]	75.5[b]	41.0±1.9[a]

[1)]T1, Raw; T2, Dry heated; T3, Soaking followed by autoclaving; BHT, Butylated hydroxytoluene
[2)]Values are mean±SD (n=3); Mean values followed by different superscripts in a column are significantly different (p<0.05).

Hydroxyl radical scavenging activity The hydroxyl radical is one of representative reactive oxygen species generated in the body. These radicals are produced through various biological reactions; one of the common reactions is the Iron (II)-based Fenton reaction. The scavenging capacity of the 70% acetone extracts of raw and processed seeds of *B. vahlii* is shown in Table 3. Among all tested extracts, raw seed extract exhibited the strongest hydroxyl radical scavenging activity (56.0% at 100 μg/mL), whilst autoclaved seed extract showed the least radical scavenging activity (35.3% at 100 μg/mL). Hydroxyl radical scavenging activity of differentially processed *B. vahlii* seed extracts and standards is presented in the following descending order: BHT>raw seed>dry heated seed>α-tocopherol> autoclaved seed (p<0.05). The radical scavenging capacity may be attributed to phenolic compounds in seed extracts with the ability to accept electrons, which can combine with free radical competitively to decrease hydroxyl radical (30).

DPPH· radical scavenging activity DPPH radical is one of the few stable organic nitrogen free radicals, which has been widely used to determine the free radical scavenging ability of the various samples (31). The method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction (32). DPPH radical scavenging activity of the tested extracts is concentration dependent and lower IC_{50} value reflects higher scavenging ability. Both the raw and processed seed extracts were able to scavenge the DPPH radical. Interestingly, in the DPPH radical scavenging activity of the dry heated seed extract (IC_{50} 70.77 μg/mL) over the raw seed extract (IC_{50} 74.4 μg/mL) is in contrast with other radical scavenging and antioxidant activities in the present investigation where the latter was more efficient. However, both raw and dry heated seed extracts (IC_{50} 74.4 and 70.8 μg/mL, respectively) exhibited DPPH radical scavenging potential comparable with that of standard α-tocopherol (IC_{50} 75.5 μg/mL) but showed lesser potential when compared to the standard BHA (Table 3). The scavenging effect of the raw and processed seeds of *B. vahlii* and standards on the DPPH radical decreased in the order of BHA>T2>T1>α-tocopherol>T3. The data obtained indicate that *B. vahlii* seed is a powerful free radical scavenger, as well as a primary antioxidant that may prevent free radical damage occurring in the human body.

Antioxidant activity in the β-carotene bleaching assay In this study, the antioxidant efficacy of the differentially processed extracts of *B. vahlii* seeds was evaluated on β-carotene/linoleic acid emulsion system. The mechanism of bleaching of β-carotene is a free radical-mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. β-carotene, in this model system, undergoes rapid discoloration in the absence of an antioxidant. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecules. As β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and characteristic orange color, which can be monitored spectrophotometrically (33). Generally, all the extracts exhibited peroxidation inhibition activity comparable with that of standards BHA and α-tocopherol at the concentration of 200 μg/mL (Table 3). Hence, these seed extracts can hinder the extent of β-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.

The results obtained from this study clearly indicates that the extracts from raw and processed seeds of the underutilized legume *B. vahlii* were found to possess strong antioxidant activity and scavenging effects on free radicals. Further evaluation of the occurrence of tannin-protein interactions in the above said processed samples by testing *in vitro* protein digestibility might be a fruitful approach for advocating them in the diets of humans/ animals as protein and nutraceuticals supplements which may reduce the over dependence on conventional pulses. In addition to their role on supplying macronutrients such

as protein and carbohydrates, *B. vahlii* seeds may be useful as therapeutic agents for treating radical-mediated pathological damages in human system.

Acknowledgments The authors wish to thank the University Grants Commission (UGC), New Delhi, India for providing financial assistance.

References

1. Caia Y, Luob Q, Sunc M, Corkea H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 74: 2157-2184 (2004)
2. Romani A, Coinu R, Carta S, Pinelli P, Galardi C, Vincieri FF, Franconi F. Evaluation of antioxidant effect of different extracts of *Myrtus communis* L. Free Radical Res. 38: 97-103 (2004)
3. Ghosal S, Tripathi VK, Chauhan S. Active constituents of *Emblica officinalis*. Part I. The chemistry and antioxidant effects of two new hydrolysable tannins, emblicanin A and B. Ind. J. Chem. 35B: 941-948 (1996)
4. Chung KT, Wong TY, Wie CI, Huang YW, Lin Y. Tannins and human health: A review. Crit. Rev. Food Sci. 38: 421-464 (1998)
5. Singh B, Bhat TK, Singh B. Potential therapeutic applications of some antinutritional plant secondary metabolites. J. Agr. Food Chem. 51: 5579-5597 (2003)
6. Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chem. 101: 10-19 (2007)
7. Fernandez-Orozco R, Frias J, Zielinski H, Piskula MK, Kozlowska H, Vidal-Valverde C. Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. *emmerald*, *Glycine max* cv. *jutro*, and *Glycine max* cv. *merit*. Food Chem. 111: 622-630 (2008)
8. Parrotta JA. Healing Plants of Peninsular India. CABI Publishing, New York, NY, USA. pp. 323-324 (2001)
9. NISC (National Institute of Science Communication). The Useful Plants of India. CSIR, New Delhi, India. p. 69 (1986)
10. Vadivel V, Janardhanan K. Effect of post-harvest treatments on nutritional attributes of the tribal pulse, *Bauhinia vahlii* W. and A. J. Swamy Bot. Club 17: 57-59 (2000)
11. Rajaram N, Janardhanan K. Chemical composition and nutritional potential of the tribal pulses *Bauhinia purpurea*, *B. racemosa*, and *B. vahlii*. J. Sci. Food Agr. 55: 423-431 (1991)
12. Manandhar NP. Plants and People of Nepal. Timber Press, Portland, OR, USA. p. 106 (2002)
13. Siddhuraju R, Becker, K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic orgins of Drumstick tree (*Moringa olifera* Lam.) leaves. J. Agr. Food Chem. 51: 2144-2155 (2003)
14. Vidal-Valverde C, Frias J, Sierra I, Blazquez I, Lambien F, Kuo YH. New functional legume food by germination. Effect on the nutritive value of beans, lentils, and peas. Eur. Food Res. Technol. 215: 472-476 (2002)
15. Siddhuraju R, Manian S. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. Food Chem. 105: 950-958 (2007)
16. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 239: 70-76 (1996)
17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med. 26: 1231-1237 (1999)
18. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315: 161-169 (1994)
19. Prieto P, Pineda M, Aguilar M. Spectophotometric quantitative of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem. 269: 337-341 (1999)
20. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276-277 (1971)
21. Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. Biochemistry 20: 6006-6012 (1991)
22. Blios MS. Antioxidant determinations by the use of a stable free radical. Nature 26: 1199-1200 (1958)
23. Taga MS, Miller EE, Pratt DE. Chia seeds as a source of natural lipid antioxidants. J. Am. Oil Chem. Soc. 61: 928-931 (1984)
24. Roginsky V. Chain breaking antioxidant activity of natural polyphenols as determined during the chain oxidation of methyl linoleate in Triton X-100 micelles. Arch. Biochem. Biophys. 414: 261-270 (2003)
25. Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agr. Food Chem. 43: 1813-1817 (1995)
26. Oktay M, Gulcin I, Kufrevioglu OI. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT. -Food Sci. Technol. 36: 263-271 (2003)
27. Hippeli S, Elstner EF. Transition metal ion-catalyzed oxygen activation during pathogenic processes. FEBS Lett. 443: 1-7 (1999)
28. Pazos M, Gallardo JM, Torres JL, Medina I. Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and fish muscle. Food Chem. 92: 547-557 (2005)
29. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Oxford University Press, Oxford, UK. pp. 617-783 (1999)
30. Thomas SS, Michaelh HG, Jennifer MA. Antioxidant properties of malt model systems. J. Agr. Food. Chem. 53: 4938-4945 (2005)
31. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT. -Food Sci. Technol. 28: 25-30 (1995)
32. Shon MY, Kim TH, Sung NJ. Antioxidants and free radical scavenging activity of *Phellinus baumii (Phellinus* of Hymenochaetaceae) extracts. Food Chem. 82: 593-597 (2003)
33. Jayaprakasha GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem. 73: 285-290 (2001)