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Effect of hydrothermal processing on total polyphenolics and antioxidant potential of underutilized leafy vegetables, *Boerhaavia diffusa* and *Portulaca oleracea*

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PEER REVIEW

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Comments

B. diffusa and *P. oleracea* after processing exhibited more potent antioxidant activity and total phenolics, tannins and flavonoid contents. Henceforth these indigenous green vegetables along with blanching and boiling can be suggested as cost effective and beneficial for human diet and animals.

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ABSTRACT

Objective: To investigate the effect of different processing methods on antioxidant properties of acetone extract of aerial parts from *Boerhaavia diffusa* and *Portulaca oleracea*.

Methods: The total phenolic and flavonoid contents were determined by Folin-Ciocalteau and aluminum chloride method, respectively. FRAP, metal chelating activity, DPPH, ABTS, nitric oxide, hydroxyl and superoxide radical scavenging activities, carotene/linoleic acid bleaching activity were used for the determination of antioxidant capacity.

Results: The total phenolics in *Boerhaavia diffusa* (82.79-162.80 mg GAE/g extract) were found to be higher when compared to that of *Portulaca oleracea* (22.94-10.02 mg GAE/g extract). Hydrothermal processing enhanced the level of inhibition on synthetic radicals such as DPPH (3 439-309 549 mmol TE/g extract) and ABTS (17 808-53 818 mmol TE/g extract) as well as biologically relevant radicals such as superoxide anion (70%-90%) and nitric oxide (49%-57%). In addition, boiling of the vegetables were found to be maximum capacity of FRAP (6 404.95 mmol Fe (II)/g extract) and metal chelating activity (1.53 mg EDTA/g extract) than the respective raw samples.

Conclusions: The present investigation suggests that the processing enhance the functionality and improves the availability of bioactive substances of these vegetables. In addition, they also exhibited more potent antioxidant activity. Therefore these natural weeds from the crop land ecosystem could be suggested as cost effective indigenous green vegetables for human diet and potential feed resources for animals. Further extensive studies on role and importance of those weeds in sustaining the agro biodiversity are also needed.

KEYWORDS

Boerhaavia diffusa, *Portulaca oleracea*, Green vegetables, Boiling and bleaching, Phenolics, Antioxidant capacity.

1. Introduction

Unlike in ancient times, nowadays the diet in India is mainly based on rice as in many other tropical Asian countries and the consumption of fruits and vegetables have been reduced. In India National survey reveals that around 95% of the adult men and women have high prevalence of diabetes due to low intake of fruits and vegetables particularly neglecting of indigenous fruits and vegetables. Therefore, it is important to revise and follow the food habit based on the traditional diet. In ancient period, people used a variety of indigenous

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fruits and vegetables in their daily diets. Among the different vegetables, the leafy vegetables play an integral part in their diet. In addition to serving as an important source of minerals, they are also provided provitamin A, carotenoids, folate, and linolenic acid. Nowadays in India the consumption of leafy vegetables has decreased over the years and highly variable depends on factors such as degree of urbanization, adequate availability, seasons and also the taste of the indigenous leafy vegetables, which are inferior to exotic vegetables such as cabbage and spinach[1]. However, traditional green leafy vegetables are rich source of phytochemicals which improves functionality and health benefits of food. Diet rich in fruits and vegetables have long been associated with reduced risk of chronic disease such as obesity and cardiovascular disease. Recently scientists pay more attention on using available experimental techniques, to identify the natural antioxidants from plants along with nutrients which provide new remedies to mankind. In order to enhance the current nutrition education, knowledge of the nutrient composition and its usage in nutraceutical action of the traditional vegetables is essential. *Boerhaavia diffusa* (*B. diffusa*) and *Portulaca oleracea* (*P. oleracea*) are the most common traditional leafy vegetables which have a long history of use by indigenous and tribal people of India and by many other countries.

B. diffusa is an herbaceous weed, commonly known as 'Punarva', is the member of Nynctaginaceae family. It is widely used by tribal people in Uttar Pradesh and Madhya Pradesh for the treatment of rheumatism, leucorrhea, stomach ache and elephantiasis. In the Indo-Nepal Himalayan terrain, the tribal peoples harvest this plant for medicinal purposes mainly for flushing out the renal system, to treat seminal weakness and blood pressure[2]. The plant contains several bioactive compounds -flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, hypoxanthine 9-Larabinofuranoside, ursolic acid, lirodendrin, and glycoprotein that have been accredited to diverse pharmacological properties. Many rotenoids known as boeravinones (A-F) have also been isolated from the roots of the *B. diffusa*[3]. Previous studies have reported that parts of *B. diffusa* such as root, leaf extracts exhibit a wide range of medicinal properties such as anti-inflammatory, diuretic, laxative, antiurethritis, anticonvulsant, antinematodal, antifibrinolytic, antibacterial, anthelmintic, antileprotic, antiasthmatic, antiscabby and antistress, hepatoprotective, antioxidant, antinociceptive, antibacterial and antidiabetic[3]. Further, it is also used as a kidney rejuvenating drug and as an excellent natural remedy for urinary track diseases[4]. The leaves and immature fruits are found to be a potential feed source for birds and poultry. Due to the presence of high nutrition, palatability and non-toxic factors, such weed plants are being grazed by sheep, goats and cows from agroecosystem. Interestingly in West Bengal, it is believed that the plant improves the milk yield when fed to the dairy animals[5].

P. oleracea, an herbaceous member of family *Portulacaceae*, is also known as purslane, Verdolaja, Karikkirai, Brihalloni, Chota Lunia. There are several species of genus *Portulacaceae* which are distributed in tropical, subtropical and temperate regions throughout the world like Africa, Australia, Euroasia, South America, and North America. It has been in use since ancient times by indigenous and tribal people of Europe, Africa, United States, China, India, and also in Australia[6]. The preliminary screening of the *P. oleracea* plant revealed that the presence of protein, soluble carbohydrate, inorganic acids, alkaloids, flavonoids, coumarins, cardiac glycosides, anthraquinone glycosides, saponin and tannins[7]. The leaves are reported to have high amount of iron, omega-3 fatty acids, and α-linolenic acid[8]. The most interesting metabolites, from the therapeutic point of view, ω-3 fatty acid from different parts of *P. oleracea* plays a major role in the regulation of inflammation controlling gene products[8]. The stem, leaves and the whole plant of *P. oleracea* have been employed for the treatment of scorpion sting and also used as antihelmintic, cooling or moistening agent for fever, *etc*. The pharmacological studies have been demonstrated that the aerial parts of *P. oleracea* exhibit a wide range of properties such as nephrotoxicity[9], hepatoprotective[10], antioxidant[11], and neuroprotective[8], *etc*. Moreover, presence of secondary metabolites makes this plant medically more important to be exploited by clinicians and scientists to gain more insight into its biological and medicinal properties[7].

Many research investigations revealed that the retention of adequate bioactive components in the food material even after subjecting under various cost effective indigenous processing methods could supply the potential nutrient and nutraceuticals. Several studies have been reported that the high antioxidant properties of *B. diffusa* Linn and *P. oleracea*[2,3,11,12]. However, there are no studies on the effect of processing on antioxidant activities of these indigenous vegetables. Therefore, the present study aimed to estimate the effects of different processing methods, like boiling and blanching, on the total phenolics, tannins, total flavonoids and antioxidant activity of *B. diffusa* and *P. oleracea*. Results from this preliminary study may provide a better understanding of the antioxidant properties of this plant for developing value-added foods and neutraceuticals.

2. Materials and methods

2.1. Chemicals

Butylated hydroxyanisole (BHA), 2,2′-diphenyl-1-picrylhydrazyl (DPPH·), potassium persulfate, 2,2 azinobis (3-ethylbenzo-thiozoline-6-sulfonic acid) disodium

salt (ABTS), 6-hydroxy-2,5,7,8-tetra-methylchroman 2-carboxylic acid (Trolox), ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylene diamine tetra acetic acid (EDTA) disodium salt and ferric chloride were obtained from Hi Media, Merck and Sigma. All chemicals were of analytical grade. All analysis was performed with UV-visible spectrophotometer (Cyberlab-UV 100, USA).

2.2. Samples and processing methods

The aerial parts of *B. diffusa* and *P. oleracea* were harvested during or prior to their flowering period at Alappuzha, Kerala State, India. Upon arrival at the laboratory, samples were washed with water to remove debris and damaged portions. The leaves along with stem parts were stripped from the plants and divided into three equal batches. One part of the sample was cut into small pieces and air dried at 45 °C. The second part was boiled in water at 100 °C for 15 min in the ratio of 1:10 (w/v). The third part of the respective sample was blanched in boiling water (at 100 °C) for 10 min in the ratio of 1:10 (w/v). After boiling and blanching, the remaining water was discarded and the respective processed samples were cut into small pieces and dried at 45 °C. After drying, all the raw and processed samples were ground to fine powder and stored in separate screw capped bottles at room temperature for further analysis.

2.3. Solvent extraction

The raw and processed samples (each 15 g) were extracted by stirring with 100 mL of aqueous acetone (70:30) at 25 °C for 48 h and filtering through Whatman No. 4 filter paper. The residues were reextracted with an additional 75 mL of aqueous acetone, as described above, for 3 h. The solvent of the combined extract was evaporated under low temperature at 40 °C in incubator (NSW, New Delhi) respectively. The extract thus obtained was used directly for total phenolics and tannins estimation and also for the assessment of antioxidant activity through various *in vitro* assays. From the 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 by following equation;

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

2.4. Estimation of total phenolics and tannin contents

The total phenolics and tannins were measured as gallic acid equivalents[13] from gallic acid standard curve (3-15 μg range). For the assay, aliquots (100 μL) of extracts were taken in test tubes and the volume was made up to 1 mL with distilled water. Then 0.5 mL of Folin-Ciocalteau phenol reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20% w/v) 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. For tannin estimation, the sample extracts were incubated with polyvinyl polypyrrolidone (PVPP) (100 mg) for 4 h at 4 °C. The phenolics and tannins were expressed as mg gallic acid equivalents (GAE)/g extract. From the above results, the tannin content of the sample was calculated as follows:
Tannin (%)=Total phenolics (%)-Non-tannin phenolics (%).

2.5. Total flavonoids

The total flavonoid content was measured by a spectrophotometric assay[14] outlined by Siddhuraju & Becker[15]. A total of 1 mL aliquot of standard solution of rutin at different concentrations (0-100 mg/L, external calibration with n=6 concentrations) or sample was added to 10 mL volumetric flasks containing 4 mL water. At the onset of the experiment, 0.3 mL of 5% $NaNO_2$ was added to the flask. After 5 min, 3 mL of 10% $AlCl_3$ was added. At 6 min, 2 mL of 1 mol/L NaOH was added to the mixture. Immediately, the solution was diluted to a final volume of 10 mL with water and mixed thoroughly. The absorbance of the mixture was determined at 510 nm against the prepared blanks. Total flavonoid content was expressed as mg rutin (RUT)/g extract.

2.6. Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of phenolic extracts of raw and processed *B. diffusa* and *P. oleracea* were estimated according to the procedure by Pulido *et al*[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 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 $FeCl_3.6H_2O$ and 25 mL of 0.3 mol/L acetate buffer, pH 3.6. 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 2000 μmol/L ($FeSO_4.7H_2O$) were used for plotting the calibration curve. The parameter equivalent concentration (EC1) was defined as the concentration of antioxidant has a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L $FeSO_4.7H_2O$. 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.

2.7. Metal chelating activity

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 results were expressed as mg EDTA equivalent/g extract using the calibration curve of EDTA. Linearity range of the calibration curve was 0.5-2.5 μg[17].

2.8. Stable free radical scavenging activity using DPPH• method

The radical scavenging activity of raw and processed sample extracts was measured using DPPH radical by the method of Brand-Williams *et al.*[18] with slight modification. Extract of 0.1 mL prepared in methanol was mixed with 3.9 mL of DPPH• (6×10^{-5} mol/L methanol). The solution was incubated at room temperature for 30 min and the decrease in absorbance at 515 nm was determined at the end of incubation period with a spectrophotometer. The trolox standard was prepared in the range of 0-2.5 mmol/L. The concentration of DPPH was calculated from trolox standard graph and expressed as mmol trolox equivalents/g extract.

2.9. Total antioxidant activity assay by scavenging of radical cation (ABTS•+)

ABTS was dissolved in water to a 7 mmol/L concentration, ABTS radical cation (ABTS•+) was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at 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±0.020 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 solution to 10 μL of samples or Trolox standards (final concentration 0-15 μmol/L) in ethanol OD (optical density) 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 standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration[19] described by Siddhuraju and Becker[20]. The unit of total antioxidant activity is defined as the concentration of Trolox having equivalent antioxidant activity expressed as μmol/g sample extracts using the calibration curve of trolox. Linearity range of the calibration curve was 0.25-1.25 mmol/L. The total antioxidant activity of ASC and BHA were also measured by ABTS method for comparison.

2.10. Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside (SNP) was measured by the Griess reaction. Nitric oxide interacts with oxygen to produce nitrite ions that can be observed by Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide[21]. Various concentrations (500 μg) of sample extracts and SNP (5 mmol/L final concentration) in phosphate buffer saline, pH 7.4, in a final volume of 1 mL were incubated at 25 °C for 150 min. A control experiment without samples but with equivalent amount of vehicles was conducted in an identical manner of the sample. After incubation, the reaction mixtures were mixed with Griess reagent (1% sulfanilamide and 0.1% naphthylethylene diamine dihydrochloride in 5% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 540 nm. The total antioxidant activity of ASC (500 μg) and QUE (500 μg) were also measured by nitric oxide scavenging method for comparison. The % nitric oxide scavenging activity was calculated by the following equation;

$$(\%)\ \text{Nitric oxide scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.11. Superoxide anion radical scavenging activity assay

The method used by Martinez *et al.* (2001) for determination of the superoxide dismutase was followed with modification[22] in the riboflavin-light-nitroblue tetra zolium (NBT) system. Each 3 mL of reaction mixture contained 50 mmol/L phosphate buffer (pH 7.8), 13 mmol/L methionine, 2 μmol/L riboflavin, 100 μmol/L EDTA, NBT (75 μmol/L) and 1 mL of sample solution. The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm after 10 min of illumination from a fluorescent lamp. The total antioxidant activity of BHA (150 μg) and TRO (150 μg) were also measured by superoxide radical scavenging method for comparison. The % of superoxide radical scavenging activity was calculated by the following equatio;

$$(\%)\ \text{superoxide scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.12. Hydroxyl radical scavenging activity

The scavenging activities of the extracts of raw and processed samples on hydroxyl radical were measured according to the method of Klein *et al*[23]. Various concentrations (200 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 mol/L 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). 3 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 color formed was measured spectrophotometrically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated by the following equatio; (%)HRSA=1-(difference in absorbance of sample/difference in absorbance of blank)×100

2.13. Linoleic acid/β-carotene bleaching activity

The antioxidant activity of sample extracts and standards (BHA, rutin and trolox) was analyzed according to the method of Taga *et al.*[24] with slight modifications. Two milligrams of β-carotene were dissolved in 1 mL of chloroform containing 40 mg of linoleic acid and 400 mg of Tween 40. The chloroform was removed by rotary vacuum evaporator at 45 °C for 4 min and 100 mL of distilled water was added slowly to the semisolid residue with vigorous agitation to form an emulsion. A 5 mL aliquot of the emulsion was added to a tube containing standards (50 μg) and sample extracts (250 μg) and the absorbance was measured at 470 nm with UV-visible spectrophotometer, immediately against a blank, consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the absorbance measurements were conducted at 120 min. All determinations were carried out in triplicates. The antioxidant activity of the extracts was evaluated in terms of bleaching of β-carotene using the following formula: AA=[1-(A0-At)/(A'0-A't)]×100, where A0 and A'0 are the absorbance of values measured at zero time of the incubation for test sample and control, respectively and At and A't are the absorbance measured in the test sample and control, respectively, after incubation for 120 min.

2.14. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA), and the significance of the difference between means were determined by Duncan's multiple-range test ($P<0.05$) using SPSS (Version 13.0, SPSS Inc., Wacker Drive, Chicago, USA). Values expressed are means of triplicate determinations±standard deviation.

3. Results

3.1. Extract yield and total phenolic content

Recovery is an important step for obtaining extracts with acceptable yields with strong antioxidant activity. The recovery percentage, total phenolics and tannin content of the raw and processed samples of *B. diffusa* and *P. oleracea* are shown in Table 1. The recovery percentage of raw, boiled and blanched samples of *B. diffusa* and *P. oleracea* were ranged from 7.4% to 11.1% and 10.2% to 10.5% respectively. In the present study the levels of phenolic compounds were expressed as gallic acid equivalents (GAE). Higher values of total phenolics and tannin content were registered in *B. diffusa* (162.80 mg GAE/g extract and 68.32 mg GAE/g extract) rather than *P. oleracea* (22.94 mg GAE/g extract and 12.96 mg GAE/g extract). In contrast, the boiled samples of *P. oleracea* have registered with lower phenolic (19.25 mg GAE/g extract) and tannin contents (8.75 mg GAE/g extract) than the raw samples.

Table 1

Total phenolics, tannins and flavonoid contents of raw and processed *B. diffusa* and *P. oleracea*.

Samples	Extract yield	Total phenolics[A]	Tannins[A]	Flavonoids[B]
BDR	11.1	119.17^{b}±2.63	45.09^{b}±4.54	71.08^{c}±0.28
BDBO	7.4	162.80^{a}±1.37	68.32^{a}±6.79	71.08^{c}±0.41
BDBL	9.6	82.79^{c}±1.20	28.27^{c}±0.32	71.40^{c}±0.47
POR	10.5	22.94^{d}±0.27	12.96^{d}±0.75	64.99^{d}±0.58
POBO	10.2	19.25^{e}±0.11	8.75^{d}±0.49	85.14^{a}±0.52
POBL	10.2	10.02^{f}±0.15	ND	81.57^{b}±0.54

Values are means of triplicate determination±standard deviation ($n=3$). Mean values followed by different superscript in the same column are significantly ($P<0.05$) different. BDR: *B. diffusa* raw; BDBO: *B. diffusa* boiled; BDBL: *B. diffusa* blanched; POR: *P. oleracea* raw; POBO: *P. oleracea* boiled; POBL: *P. oleracea* blanched. [A]: mg Gallic acid equivalents/g extract. [B]: mg rutin equivalents/g extract.

3.2. Total flavonoid content

From the result, it was observed that *P. oleracea* (85.14 mg rutin equivalents/g extract) contained high level of flavonoid compared to *B. diffusa* (71.40 mg rutin equivalents/g extract). As shown in Table 1, up to 10 min of boiling (71.08 mg rutin equivalents/g extract) and blanching (71.40 mg rutin equivalents/g extract) at 100 °C, *B. diffusa* still maintained comparably equal amounts of flavonoids. In the case of *P. oleracea*, boiling (85.14 mg rutin equivalents/g extract) and blanching significantly increased the total flavonoid contents (81.57 mg rutin equivalents/g extract) than raw sample.

3.3. In vitro antioxidant potential

3.3.1. DPPH and ABTS+ radical scavenging activity

As shown in Table 2, *B. diffusa* showed the highest DPPH scavenging activity in all samples while the *P. oleracea* showed lower activity. Among the raw and thermally treated samples, boiled *B. diffusa* had highest DPPH free radical scavenging ability (309 549.90 mmol TE/g extract) followed by blanched *P. oleracea*. Boiled *P. oleracea* (53 818.25 mmol TE/g extract) registered higher ABTS·+ radical scavenging capacity

followed by blanching (53 037.68 mmol TE/g extract) and raw (51 041.34 mmol TE/g extract) samples. The same trend was observed in *B. diffusa*, the boiled samples (53 645.56 mmol TE/g extract) registered higher ABTS·+radical scavenging capacity followed by blanching (50 012.08 mmol TE/g extract) and raw (17 808.51 mmol TE/g extract) samples.

Table 2

Effect of boiling and blanching on the antioxidant activities of *B. diffusa* and *P. oleracea*.

Samples	DPPH (mmol TEA/g extract)[A]	ABTS (mmol TEA/g extract)[A]	FRAP (mmol Fe(II)/g extract)[B]	Metal chelating[C]
BHA	814 172.70±187.00	654 356.10±617.10	350 278.70±735.70	10.49±0.06
RUT	748 175.20±598.00	432 942.70±233.10	172 898.60±272.70	–
TAN	848 540.10±547.00	751 041.70±632.30	565 217.40±427.70	–
BDR	3 575.42^{b}±254.15	53 645.56^{a}±343.23	5 159.65^{c}±26.19	0.98^{c}±0.01
BDBO	309 549.90^{a}±876.92	17 808.51^{c}±643.42	6 404.95^{a}±48.82	1.23^{a}±0.01
BDBL	3 439.29^{b}±79.65	50 012.08^{b}±330.49	6 240.78^{b}±21.81	1.15^{b}±0.01
POR	11 440.39^{a}±20.75	51 041.34^{b}±435.18	5 474.42^{b}±22.82	1.07^{b}±0.01
POBO	3 561.92^{b}±120.45	53 818.25^{a}±787.21	5 971.68^{a}±29.81	1.02^{c}±0.01
POBL	11 452.15^{a}±15.83	53 037.68^{a}±504.36	5 499.16^{b}±22.82	1.53^{a}±0.01

Values are means of triplicate determination±standard deviation (n=3). Mean values followed by different superscript in the same column are significantly (P<0.05) different. BHA: butylatedhydroxyanisole; RUT: rutin; TAN: tannic acid; [A]: mmol of trolox equivalent antioxidant activity; [B]: Ferric reducing antioxidant power assay (mmol Fe (II) equivalent); [C]: mg of EDTA equivalent/g extract.

3.3.2. Ferric reducing/antioxidant power

The reducing power of the green leafy vegetables is shown in Table 2. The reductant capacity of samples are determined by FRAP assay ranged from 6 404.95 to 5 159.65 mmol Fe (II)/g extract and the highest activity of the sample was similar as in DPPH and ABTS assays and the level of activity has been given as follows: BDBO>BDBL>POBO>POBL>POR>BDR.

3.3.3. Ferrous ions chelating capacity

The metal chelating activity of *B. diffusa* and *P. oleracea* were 0.98–1.23 mg EDTA/g extract and 1.02–1.53 mg EDTA/ g extract, respectively (Table 2). However, both the sample extracts were found to be significantly lower than that of synthetic antioxidants such as BHA (10.59 mg EDTA/ g extract). Among the samples, blanched *P. oleracea* showed higher chelating activity with 1.53 mg EDTA/g extract and raw samples of *B. diffusa* registered lower chelating activity (0.98 mg EDTA/ g extract).

3.3.4. Superoxide anion-scavenging activity

The results of superoxide radical scavenging assay are described in Figure 1. All the samples were found to be very good O_2– radical scavengers with the range of 66%–90% and the scavenging ability was increased with the sample after processing. Moreover, the scavenging activity of *P. oleracea* (87%–90%) was much higher than that of common food additives, (BHA (69%) and rutin (76%) but significantly lower than catechin (94%). Blanched *P. oleracea* had registered the highest superoxide anion radical-scavenging activity (90%) when compared to other samples.

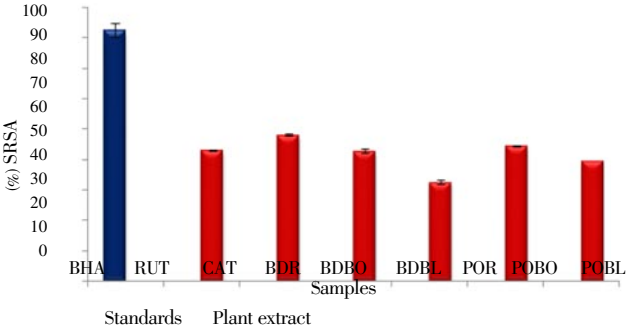

Figure 1. Superoxide anion radical-scavenging activities of raw and processed aerial parts of *B. diffusa* and *P. oleracea*.

Values of triplicate determinations (mean±SD; n=3) with different letters (a–g) are significantly different (P<0.05). CAT: catechin; TRO: trolox.

3.3.5. Hydroxyl radical scavenging activity

All the samples analyzed were found to be moderate OH· radical scavenging activity with minimal percentage (32%) of inhibition. *B. diffusa* boiled sample was registered with highest hydroxyl radical scavenging activity (48%) whereas the lowest hydroxyl radical scavenging activity was observed in *P. oleracea* raw sample (32%). In general all the hydrothermally processed samples were found to be more hydroxyl radical scavenging activity than the respective raw samples (Figure 2).

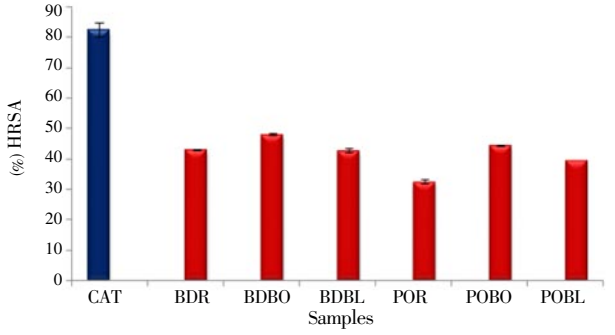

Figure 2. Hydroxyl radical-scavenging activities of raw and processed aerial extracts of *B. diffusa* and *P. oleracea*.

Values of triplicate determinations (mean±SD; n=3) with different letters (a–f) are significantly different (P < 0.05).

3.3.6. Nitric oxide scavenging activity

Figure 3 presents the scavenging ability of different processed samples of *B. diffusa* and *P. oleracea* against NO. The results indicated that the processed samples have found to be better NO scavengers than their raw samples. Among the two processing methods adopted for both samples, boiled sample of *P. oleracea* exhibited highest scavenging activity. Interestingly, when compared to ascorbic acid (57%) and quercetin (62%), the nitric oxide scavenging activity of boiled leaf sample of *P. oleracea* was on par with ascorbic acid.

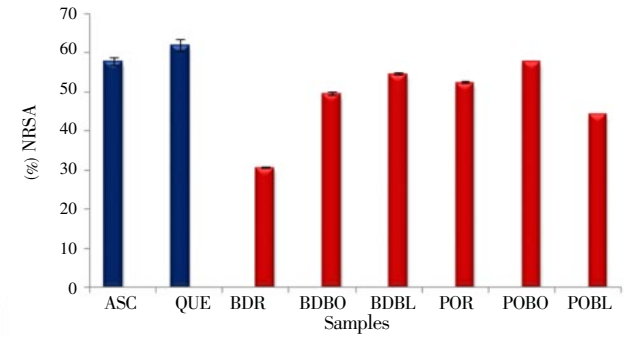

Figure 3. Nitric oxide radical-scavenging activity of raw and processed aerial extracts of *B. diffusa* and *P. oleracea*

Values of triplicate determinations (mean±SD; n=3) with different letters (a-f) are significantly different (P<0.05). ASC: Ascorbic acid; QUE: Quercetin.

3.3.7. β-Carotene-linoleate bleaching assay

Boiled samples of both leafy vegetables showed strong peroxidation inhibiting activity (Figure 4). The inhibiting activity of boiled samples of *B. diffusa* and *P. oleracea* were 53% and 47% respectively, in which the activity of *B. diffusa* boiled sample was higher than that of synthetic antioxidants rutin (33%) and trolox (49%).

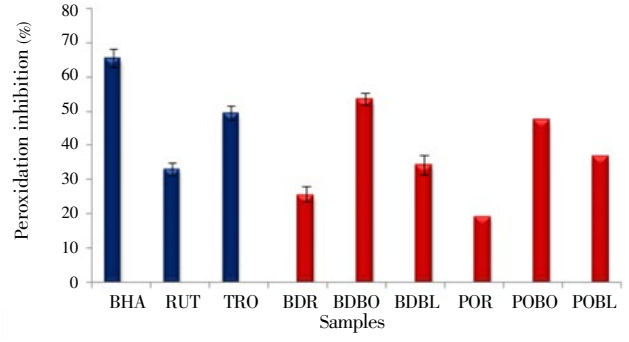

Figure 4. Inhibition of linoleic acid oxidation by raw and processed aerial extract of *B. diffusa* and *P. oleracea*.

Values of triplicate determination (mean±SD; n=3) with the different letters are significantly different (P<0.05).

4. Discussion

Polyphenols are identified as secondary metabolites in plant kingdom. They are receiving much more attention recently by scientific communities due to their antioxidant and disease curing ability[25]. Especially green leafy vegetables contribute the main sources of flavonoids, flavones, and isoflavones. The determination of raw and processed aerial parts from *B. diffusa* and *P. oleracea* revealed that presence of total phenolic and flavonoid contents which are found to have a strong antioxidant activity, free radical scavengers, and metal chelators[26]. The total phenolic content of *B. diffusa* of the present study was similar to the values reported for the same species by Apu *et al.*[12] where as it was lower than to those reported for *P. oleracea*[27]. Many factors influence the total phenolics variation in vegetables such as environmental factors (sunlight, temperature, raining, *etc.*), collection period, variety, chemical composition, maturity at harvest, growing condition, soil state[28] and the main factor is solvent used for the extraction[26]. Kim *et al.*[29] reported an increase in phenolic content up to 4-10-fold after hydrothermal processing in the black garlic cloves. In general, phenolics found in vegetables are bonded to dietary fiber, proteins or to sugars to form complex structures. However, during hydrothermal processing it disrupts the cell membranes, cell walls and hydrolyzes these complexes and making phenolic substances more available[30]. In the present study, the resistant nature of phenolics to thermal treatments was comparable with results of wild banana varieties after pressure cooking[31] and potato tubers after 20-25 min of boiling[32]. In contrary, *P. oleracea* processed samples showed that a significant loss of phenolic content. This is due to temperature induced chemical oxidation whereby water-soluble polyphenols leached into boiled water[33]. Volden *et al.*[34] reported that a loss of 37% in total phenolic content in boiled cauliflower when compared to raw sample. Similarly, Moreno *et al.*[35] also found that a loss of phenolic content in cladodes after 20 min of boiling. Same kind of improvement was observed in flavonoid content after 60 min boiling of lotus and onion varieties[36]. DPPH and $ABTS_+$ radicals are stable organic free radicals. They accept an electron or free radical species, which results to discoloration from purple stable radical DPPH to yellow-coloured diphenyl picryl hydrazine and discoloration of blue-green chromophore $ABTS_+$[37,19]. The radical scavenging activity is related to the total phenolic content. Interestingly the boiled *B. diffusa* sample was found to be higher phenolic content in comparison to the other samples. Some studies have shown that flavonoids are also responsible for the antioxidant capacity of vegetables[38]. So the highest radical scavenging ability of *P. oleracea* might be due to the presence of high total flavonoid content of the samples. The free radical-scavenging activity of processed samples was higher than those of raw samples and this might be due to the enrichment effects of active components during the processing methods. The increased level of total reductant capacity in processed samples by FRAP assay is in good agreement with the report of Gorinstein *et al.*[39] who observed that thermal processing fully preserved their bioactive compounds (polyphenols, flavonoids, flavanols and tannins) and the total antioxidant capacity. The increased reducing activity in processed samples observed in the present study was justified due to the production of redox-active secondary metabolites (Maillard reaction and Amadori rearrangement products), and breakdown of complex phenolic compound by heat treatment that softening or disruption of plant cell walls[40]. Further, Vega-Galvez *et al.*[41] reported that the formation of phenolic compounds at high temperature due to availability of precursors formed by non-enzymatic inter conversion between phenolic molecules. Fe^{2+} has induced the production of oxyradicals and lipid peroxidation. In further, these divalent metals are responsible for the development of neurological disorders. The concentration of Fe^{2+} in the Fenton reaction should be minimized in order to afford protection against oxidative damage[42]. The assay used to determine the

chelating activity of Fe^{2+} was based on the chelating of this metal ion with ferrozine to yield a red colored complex. In the presence of chelating agents, the complex formation is disrupted and the red color of the complex decreases[43]. The chelating activity was estimated by measurement of the rate of color reduction. Loizzo *et al.*[44] suggested that the possible relationship between total flavonoids and Fe- chelating activity and similar results have been observed in *P. oleracea* samples. Gulcin *et al.*[45] reported that tannic acid chelate Fe^{2+} effectively due to the presence of hydroxyl and carboxylate groups. Similarly, boiled sample of *B. diffusa* registered with high tannin content which was responsible for higher iron chelating activity than the raw sample. The present results suggest that the samples were endowed with Fe^{2+}-chelating activity due to the presence of polyphenolic content and in higher extend to the flavonoids and tannins which increased by the thermal processing. Superoxide anion is an initial free radical formed from mitochondrial electron transport systems and macrophage activation due to NADPH-dependant univalent reduction of oxygen to O_2^-[46]. It is an oxygen centered radical deployed by the immune system to kill invading microorganisms. It is biologically toxic due to inactivation of iron-sulfur cluster containing systems, which are most important in the metabolic pathways[45]. It plays an important role in the formation of other reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems in a concentration dependent manner and also observed to directly initiate lipid peroxidation[45,47]. Thus, the higher superoxide radical scavenging activity could be correlated with the estimated flavonoid contents in the extract. Interestingly, the blanched samples of both leafy vegetables showed higher scavenging activity when compared to the boiled and raw samples. The higher activity of processed samples may be due to the gain of phenolics, tannins and flavonoids during the thermal processing. In addition to that flavonoid molecule with polyhydroxylated substitution on ring A or B and a free 3-hydroxyl substitution may increase the superoxide radical scavenging activity[48]. Hydroxyl radicals are one of the major reactive oxygen species generated from superoxide and hydrogen peroxide in the presence of metal ions through Fenton reaction[15]. They attack every non-selective biopolymer such as lipids, proteins, enzymes, DNA, and RNA leading to cell or tissue injury associated with degenerative diseases[49]. The potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution. Nitric oxide is produced from the oxidation process of L-arginine which is initially thought for possible beneficial effects at minimum concentration. While the over production associated with several pathological diseases such as cancer, auto immune diseases and chronic inflammation. This deleterious effect of nitric oxide is due to its reaction with superoxide that forms potent oxidants such as peroxynitrite, OH· and NO_2[50]. Nitric oxide is generated from SNP in an aqueous solution at physiological pH which interacts with oxygen to produce nitrite ions. Plant extracts are the potent competitors for oxygen in the nitrite ion formation and also stops the reaction with other RNS species[51]. Several studies have proved that the NO scavenging ability and neuroprotective effect of *P. oleracea* is due to its antioxidant potential[8]. The antioxidant capacity of raw and processed samples are determined by stabilizing the yellow color of β-carotene and hinder the extent of bleaching by hydrogen peroxide radical through the heat-induced oxidation process of linolenic acid. The biological function of β-carotene was decomposed via free radical mediated oxidation, which breaks the 11 pairs of sensitive double bonds[52]. The antioxidants from plants donate hydrogen atoms to quench radicals and inhibit the bleaching of β-carotene[52]. The presence of phenolics, flavonoids and flavonols, including glycosylated flavones in samples may be responsible for high β-carotene bleaching inhibition[53]. In conclusion all processed samples showed higher content of total phenolics, tannins, and flavonoid contents and exhibited good antioxidant activity than raw samples. The antioxidant activity was related with the phenolic and flavonoid contents present in the extracts. Particularly, *P. oleracea* extracts showed significant amount of phenolic and flavonoid content, which is directly related to the antioxidant activity on FRAP, metal chelating methods and evidenced as scavenging capacity of different free radicals including ABTS, nitric oxide and super oxide. Further, extensive research work on nutritional parameters and nutraceutical evaluation through *in vitro* and in vivo experiments are needed. Finally it can be concluded that after cost effective processing of both indigenous leafy vegetables with enriched antioxidant potential and nutritional components they could be advocated for wider consumption in daily life for possible health benefits. In addition, those non waste weed samples may also be exploited as potential animal feed for future.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

B. diffusa and *P. oleracea* are underutilized green leafy vegetables. They are rich source of different polyphenols. It was hence critical to evaluate the effect of some of the processing methods such as blanching and boiling which

are commonly applied. It is also important to evaluate their impact on the polyphenols and antioxidant activity.

Research frontiers

The present research focuses on the variation in the contents of different bioactive substances such as total phenolics, tannins, flavonoids and also antioxidant activity of *B. diffusa* and *P. oleracea* upon treating them with blanching and boiling processes in comparison to the untreated material. This was experimented through various determination methods of assay.

Related reports

Processing methods are mostly found to have negative effect on various phytochemicals. Some of them showed positive effects as well. Current research confirms the beneficial effect of the processing methods on the recovery of the phytochemicals from the subject green leafy vegetables.

Innovations and breakthroughs

B. diffusa and *P. oleracea* are common traditional leafy vegetables which are rich in several bioactive compounds. The positive effect of common processing methods such as blanching and boiling on these bioactive compounds are revealed.

Applications

B. diffusa and *P. oleracea* are not utilized adequately. Hence with this research the beneficial effects could be concluded and can lead to better utilization in future.

Peer review

B. diffusa and *P. oleracea* after processing exhibited more potent antioxidant activity and total phenolics, tannins and flavonoid contents. Henceforth these indigenous green vegetables along with blanching and boiling can be suggested as cost effective and beneficial for human diet and animals.

References

[1] Uusiku NP, Oelofse A, Duodu KG, Bester MJ, Faber M. Nutritional value of leafy vegetables of Sub-Saharan Africa and their potential contribution to human health: A review. *J Food Compost Anal* 2010; **23**: 499-509.

[2] Olaleye MT, Akinmoladun AC, Ogunboye AA, Akindahunsi AA. Antioxidant activity and hepatoprotective property of leaf extracts of *Boerhaavia diffusa* Linn. against acetaminophen-induced liver damage in rats. *Food Chem Toxicol* 2010; **48**(8-9): 2200-2205.

[3] Khan MS, Ansari IA, Ahmad S, Akhter F, Hashim A, Srivastava AK. Chemotherapeutic potential of *Boerhaavia diffusa* Linn: A review. *J Appl Pharm Sci* 2013; **3**(01): 133-139.

[4] Chaudhary G, Dantu PK. Morphological, phytochemical and pharmacological, studies on *Boerhaavia diffusa* L. *J Med Plants Res* 2011; **5**(11): 2125-2130.

[5] National Institute of Science Communication and Information Resources CSIR. *The wealth of India: raw materials vol. II B*. New Delhi: CSIR; 1988, p. 174.

[6] Chan K, Islam MW, Kamil M, Radhakrishnan R, Zakaria MN, Habibullah M, Attas A. The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp. Sativa (Haw.) Celak. *J Ethnopharmacol* 2000; **73**: 445-451.

[7] Oliveira I, Valentao P, Lopes R, Andrade PB, Bento A, Pereira JA. Phytochemical characterization and radical scavenging activity of *Portulaca oleracea*e L. leaves and stems. *Microchem J* 2009; **92**: 129-134.

[8] Moneim AEA, Dkhil MA, Al-Quraishy S. The potential role of *Portulaca oleracea* as a neuroprotective agent in rotenone-induced neurotoxicity and apoptosis in the brain of rats. *Pestic Biochem Physiol* 2013; **105**: 203-212.

[9] Karimi G, Khoei A, Omidi A, Kalantari M, Babaei J, Taghiabadi E, et al. Protective effect of aqueous and ethanolic extracts of *Portulaca Oleracea* against cisplatin induced nephrotoxicity. *Iran J Basic Med Sci* 2010; **13**(2): 31-35.

[10] Anusha M, Venkateswarlu M, Prabhakaran V, Taj SS, Kumari PB, Ranganayakulu D. Hepatoprotective activity of aqueous extract of *Portulaca oleracea* in combination with lycopene in rats. *Indian J Pharmacol* 2011; **43**(5): 563-567.

[11] Uddin K, Juraimi AS, Ali E, Ismail MR. Evaluation of antioxidant properties and mineral composition of purslane (*Portulaca oleracea* L.) at different growth stages. *Int J Mol Sci* 2012; **13**: 10257-10267.

[12] Apu AS, Liza MS, Jamaluddin ATM, Howlader MA, Saha RK, Rizwan F, et al. Phytochemical screening and *in vitro* bioactivities of the extracts of aerial part of *Boerhavia diffusa* Linn. *Asian Pac J Trop Biomed* 2012; **2**(9): 673-678.

[13] Makkar HPS, Siddhuraju P, Becker K. *Plant secondary metabolites*. USA: Humana press; 2007, p. 130.

[14] Jia ZS, Tang MC, Wu JM. The determination of flavanoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 1999; **64**: 555-559.

[15] Siddhuraju P, Becker K. Studies on antioxidant activities of mucuna seed (*Mucuna pruriens* var. *utilis*) extracts and certain non-protein amino acids through *in vitro* models. *J Sci Food Agric* 2003; **83**: 1517-1524.

[16] Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/ antioxidant power assay. *J Agric Food Chem* 2000; **48**: 3396-3402.

[17] Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salycylate and 5-aminosalycylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch Biochem Biophys* 1994; **315**: 161-169.

[18] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol* 1995; **28**: 25-30.

[19] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999; **26**: 1231-1237.

[20] Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chem* 2007; **101**: 10-19.

[21] Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide

scavenging properties of *Ginkgo biloba* extract EGb76J. *Biochem Biophys Res Commun* 1994; **201**: 748–755.

[22] Dasgupta N, De B. Antioxidant activity of Piper betel L. leaf extract *in vitro*. *Food Chem* 2004; **88**: 219–224.

[23] Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical-generating systems. *Biochemistry* 1981; **20**: 6006–6012.

[24] Taga MS, Miller EE, Pratt DE. Chia seeds as a source of natural lipid antioxidants. *J Am Oil Chem Soc* 1984; **61**: 928–931.

[25] Landete JM. Dietary intake of natural antioxidants: vitamins and polyphenols. *Crit Rev Food Sci Nutr* 2013; **53**: 706–721.

[26] Siwak J, Lewinska A, Wnuka M, Bartosz G. Protection of flavonoids against hypochlorite-induced protein modifications. *Food Chem* 2013; **141**: 1227–1241.

[27] Lim YY, Quah EPL. Antioxidant properties of different cultivars of *Portulaca oleracea*. *Food Chem* 2007; **103**: 734–740.

[28] Bunning ML, Kendall PA, Stone MB, Stonaker FH, Stushnoff C. Effects of seasonal variation on sensory properties and total phenolic content of 5 lettuce cultivars. *J Food Sci* 2010; **75**(3): S156–S161.

[29] Kim JS, Kanga OJ, Gweon OC. Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps. *J Funct Foods* 2013; **5**: 80–86.

[30] Roy MK, Juneja LR, Isobe S, Tsushida T. Steam processed broccoli (*Brassica oleracea*) has higher antioxidant activity in chemical and cellular assay systems. *Food Chem* 2009; **114**: 263–269.

[31] Sasipriya G, Maria CL, Siddhuraju P. Influence of pressure cooking on antioxidant activity of wild (*Ensete superbum*) and commercial banana (*Musa paradisiaca* var. *monthan*) unripe fruit and flower. *J Food Sci Technol* 2012; doi: 10.1007/s13197-012-0791-z.

[32] Burgos G, Amoros W, Munoa L, Sosa P, Cayhualla E, Sanchez C, et al. Total phenolic, total anthocyanin and phenolic acid concentrations and antioxidant activity of purple-fleshed potatoes as affected by boiling. *J Food Compost Anal* 2013; **30**: 6–12.

[33] Rawson A, Hossain MB, Patras A, Tuohy M, Brunton N. Effect of boiling and roasting on the polyacetylene and polyphenol content of fennel (*Foeniculum vulgare*) bulp. *Food Res Int* 2013; **50**: 513–518.

[34] Volden J, Borge GIA, Hansen M, Wicklund T, Bengtsson GB. Processing (blanching, boiling, steaming) effects on the content of glucosinolates and antioxidant-related parameters in cauliflower (*Brassica oleracea* L. ssp. *botrytis*). *LWT Food Sci Technol* 2009; **42**: 63–73.

[35] Moreno ER, Diaz DC, Mata MCS, Marques CD, Goni I. Effect of boiling on nutritional, antioxidant and physicochemical characteristics in cladodes (*Opuntia ficus indica*). *LWT Food Sci Technol* 2013; **51**: 296–302.

[36] Im MH, Park YS, Leontowicz H, Leontowicz M, Namiesnik J, Hamd KS, et al. The thermostability, bioactive compounds and antioxidant activity of some vegetables subjected to different durations of boiling: Investigation *in vitro*. *LWT Food Sci Technol* 2011; **44**: 92–99.

[37] Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol* 2004; **26**: 211–219.

[38] Cartea ME, Francisco M, Soengas P, Velasco P. Phenolic compounds in *Brassica* vegetables. *Molecules* 2011; **16**: 251–280.

[39] Gorinstein S, Jastrzebski Z, Leontowicz H, Leontowicz M, Namiesnik J, Najmanc K, et al. Comparative control of the bioactivity of some frequently consumed vegetables subjected to different processing conditions. *Food Control* 2009; **20**: 407–413.

[40] Dini I, Tenore GC, Dini A. Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp. *LWT Food Sci Technol* 2013; **53**(1): 382–385.

[41] Vega-Galvez A, Scala KD, Rodríguez K, Lemus-Mondaca R, Miranda M, Lopez J, et al. Effect of air-drying temperature on physico-chemical properties, antioxidant capacity, colour and total phenolic content of red pepper (*Capsicum annuum* L. var. *hungarian*). *Food Chem* 2009; **117**: 647–653.

[42] Ma T, Tian C, Luo J, Zhou R, Sun X, Ma J. Influence of technical processing units on polyphenols and antioxidant capacity of carrot (*Daucus carrot* L.) juice. *Food Chem* 2013; **141**: 1637–1644.

[43] Yamaguchi F, Ariga T, Yoshimira Y, Nakazawa H. Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. *J Agric Food Chem* 2000; **48**: 180–185.

[44] Loizzo MR, Tundis R, Bonesi M, Menichini F, Mastellone V, Avallone L, et al. Radical scavenging, antioxidant and metal chelating activities of *Annona cherimola* Mill. (cherimoya) peel and pulp in relation to their total phenolic and total flavonoid contents. *J Food Compost Anal* 2012; **25**: 179–184.

[45] Gulcin I, Huyut Z, Elmastas M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. *Arabian J Chem* 2010; **3**: 43–53.

[46] González-Perilli L1, Álvarez MN, Prolo C, Radi R, Rubbo H, Trostchansky A. Nitroarachidonic acid prevents NADPH oxidase assembly and superoxide radical production in activated macrophages. *Free Radic Biol Med* 2012; **58**: 126–133.

[47] Li Q, Yub N, Wanga Y, Sunc Y, Lud K, Guan W. Extraction optimization of *Bruguiera gymnorrhiza* polysaccharides with radical scavenging activities. *Carbohydr Polym* 2013; **96**: 148–155.

[48] Nair VD, Panneerselvam R, Gopi R. Studies on methanolic extract of Rauvolfia species from Southern Western Ghats of India-*in vitro* antioxidant properties, characterization of nutrients and phytochemicals. *Ind Crop Prod* 2012; **39**: 17–25.

[49] Murcia MA, Jimenez AM, Martinez-Tome M. Vegetables antioxidant losses during industrial processing and refrigerated storage. *Food Res Int* 2009; **42**: 1046–1052.

[50] Conforti F, Marrelli M, Carmela C, Menichini F, Valentina P, Uzunovc D, et al. Bioactive phytonutrients (omega fatty acids, tocopherols, polyphenols), *in vitro* inhibition of nitric oxide production and free radical scavenging activity of non-cultivated Mediterranean vegetables. *Food Chem* 2011; **129**: 1413–1419.

[51] Nithiyanantham S, Siddhurajua P, Francis GA. A promising approach to enhance the total phenolic content and antioxidant activity of raw and processed *Jatropha curcas* L. kernel meal extracts. *Ind Crop Prod* 2013; **43**: 261–269.

[52] Kubola J, Siriamornpun S. Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts *in vitro*. *Food Chem* 2008; **110**: 881–890.

[53] Pereira C, Barros L, Carvalho AM, Ferreira ICFR. Nutritional composition and bioactive properties of commonly consumed wild greens: potential sources for new trends in modern diets. *Food Res Int* 2011; **44**: 2634–2640.