

E-ISSN: 2278-4136 P-ISSN: 2349-8234 JPP 2018; 7(5): 3248-3255 Received: 04-07-2018 Accepted: 06-08-2018

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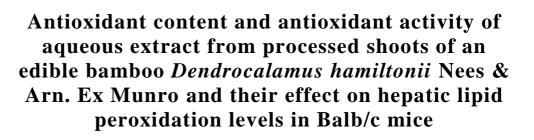
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Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com



Journal of Pharmacognosy and

Phytochemistry

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Abstract

Objective: Bamboo shoots offer some incredible health promoting qualities due to the presence of many important nutrients, bioactive compounds and antioxidants. Freshly collected shoots need to be processed for removal of anti-nutrients and long term usage. This study was conducted to evaluate the impact of boiling, brine preservation and fermentation on the level of antioxidant compounds and antioxidant activities of *Dendrocalamus hamiltonii* shoots.

Methods: Total antioxidant activity of shoots was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities and 2, 2'-Azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activities *in vitro* and lipid peroxidation *in vivo*.

Results: Fermented shoots being a rich source of Fe, Zn and phenols showed highest ABTS and DPPH radical scavenging activity and caused highest decrease in hepatic tissue malondialdehyde (MDA) content.

Conclusion: Fermented shoots as a potential source of antioxidants are very useful to prevent and control oxidative-stress induced diseases and also exhibit potential as raw material for the food and pharmaceutical industries.

Keywords: Bamboo shoot, fermentation, antioxidant, free radical, lipid peroxidation

1. Introduction

Antioxidants play a major role in the protective effect of plant foods exhibiting a variety of biological effects such as anti-oxidants, anti-inflammatory, anti-hypertensive, anti-aging, antiatherosclerosis and anti-tumor ^[1, 2]. Several epidemiological studies have shown that fruit and vegetable consumption is inversely related to all-cause mortality ^[3]. There are numerous types of antioxidants in dietary plants and knowledge of their total antioxidant activity which is the cumulative capacity of food components to scavenge free radicals would be useful for epidemiological purposes ^[1, 4]. Vitamins C, vitamin E, carotenoids, phenols and flavonoids are the major antioxidants present in vegetables. The antioxidants scavenge free radicals and inhibit the chain initiation or break the chain propagation. With increasing awareness of the importance of antioxidants in health maintenance, their retention through food processing and storage has assumed vital importance ^[5]. It is now apparent that the knowledge of food, raw as well as processed is therefore indispensable to good health. Raw foods are generally high in nutrients, vitamins, minerals and bioactive components. But some foods like grains, beans, legumes, cassava and bamboo shoots need to be processed for safe human consumption due to the presence of antinutrients such as phylate, oxalate, alkaloids, saponins and cyanogenic glycosides etc. Antinutrients have been shown to reduce the availability of nutrients and cause growth inhibition and also reduce the blood glucose and insulin responses to starchy foods and the plasma cholesterol and triglycerides ^[6].

Bamboo is a multipurpose plant with every part of the plant- root, rhizome, culm, leaves, seeds and shoots being used for different purposes. The juvenile shoots are delicious and rich in nutrients, antioxidants and bioactive compounds and gaining popularity worldwide as a health food. The potential health benefits of shoots include anti-inflammatory, serum cholesterol lowering, anti-cancer and prevention of cardiovascular diseases ^[7, 8]. But fresh juvenile shoots need to be processed, prior to exploiting them as a food additive due to the presence of anti-nutrients (cyanogenic glycosides, thiocyanate, glucosinolate) ^[9, 10, 11], and perishable nature of fresh bamboo shoots. A number of studies have demonstrated that food processing techniques reduced the nutrients, bioactive compounds and antioxidants of processed foods ^[12].

Antioxidants are vital constituents in foods, promoting human health by neutralizing cell damage caused by reactive oxygen species or reactive nitrogen species. Bamboo shoot is a good source of natural antioxidants ^[13] and can plays key role in scavenging of free radicals. Free radicals are associated with damage to a large number of molecular species such as proteins, lipids and nucleic acids ^[14]. Oxidative degradation of lipids is a major cause of atherosclerosis and many degenerative diseases such as cataract and the aging process ^[15]. When free radicals attack lipids containing carbon-carbon double bonds, especially polyunsaturated fatty acids (PUFAs) ^[16], the various direct products like malondialdehyde (MDA) are formed which is considered as the most important biomarkers of oxidative stress in tissues. It is anticipated that natural antioxidants might augment the body for its antioxidant defense mechanism. Bamboo shoots are very good reservoir of number of antioxidants including phenols, flavonoids, vitamin C, vitamin E, minerals like selenium, iron, manganese, copper and zinc. Processing of bamboo shoot improves palatability, increases shelf-life and, detoxifies shoots by removing anti-nutrients ^[17]. However, shoots also lose some of their nutrients and bioactive compounds, which further affect the therapeutic qualities of processed shoots ^[18]. Hence, it is necessary to test the therapeutic quality of processed bamboo shoots using efficient techniques. The aim of the present study was to investigate the impact of boiling, brine preservation and fermentation on the antioxidant compounds and antioxidant activity of shoots and also on lipid peroxidation (LPO) level in hepatic tissue of Balb/c mice with a view to giving preliminary information toward effective utilization of Dendrocalamus hamiltonii shoots in the food and pharmaceutical industry.

2. Material and Methods

2.1 Collection and processing of bamboo shoots

The juvenile shoots of *Dendrocalamus hamiltonii* were collected from Shillong, Meghalaya. The shoots were harvested two weeks after emergence above the ground, packed properly and transported to Botany Department, Panjab University, Chandigarh, India by air. In the laboratory, shoots were washed properly under tap water, hard basal portion of the shoots was discarded and the culm sheaths were removed carefully until the milky white portion of the shoots were then cut into thin slices, divided into four equal portions and subjected to processing (boiling (15 min), brine treatment (5%), fermentation). Thereafter, shoots were dried at 40°C and pulverized to fine powder using electrical blender.

2.2 Preparation of bamboo shoots extract

For preparation of aqueous extract, 10 g of each kind of bamboo shoot powder was weighed in a conical flask; 100 ml of distilled water was added, plugged with cotton wool and then kept on rotary shaker at 120 rpm for 24 hrs. Thereafter, extract was filtered and dried using hot air oven at 40°C. The dried crude extract was weighed and stored at 4°C in air tight containers for further use.

2.3 Determination of total phenols

Total phenolic content was estimated by using Folin-Ciocalteu's method as previously reported ^[19], to 0.5 ml of extract, 0.1 ml of Folin-Ciocalteu's reagent was added and the reaction mixture was incubated for half an hour at room

temperature and then absorbance was measured at 760 nm. Gallic acid (10-100 μ g/ml) solution prepared in distilled water was used for making the calibration curve. Total phenolic content is expressed in terms of gallic acid equivalent (mg/g of extracted compounds).

2.4 Determination of total flavonoids

Total flavonoid content was estimated by using aluminium chloride method as previously reported ^[20]. 0.5 ml of extract was taken in a test tube and 0.5 ml aluminium chloride was added to it. The solution was then incubated at room temperature for 60 min and absorbance was measured at 420 nm. Quercetin (10-100 μ g/ml) solution prepared in methanol was used for making the calibration curve. The flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compounds).

2.5 Determination of vitamin C

The amount of vitamin C was estimated by using High Performance Liquid Chromatography (HPLC) technique. HPLC analysis was conducted with a Shimadzu chromatograph equipped with UV-VIS detector and a 250 mm×4.6 mm reverse phase column. Methanol extract of shoots was dissolved in appropriate volume of HPLC grade methanol and injected into the apparatus. The sample analysis was performed at room temperature (25°C), in the wavelength of 245 nm. The injection volume of the sample was 20 µl, the total run time was 10 min, flow rate was 1.2 ml/min and the mobile phase used was 0.5% phosphoric acid and acetonitrile in the ratio of 93:7. The standard compound, ascorbic acid was run for comparative detection and optimization. All the samples were injected in duplicate and results (mg/100g, dry weight) were obtained by comparison of peak areas of the samples with that of standards.

2.6 Determination of vitamin E

The amount of vitamin E was measured using the method of Baker *et al* ^[21]. Lipid extract was prepared according to the method of Bligh and Dyer ^[22]. using the chloroform methanol mixture (2:1). To 0.5 ml of lipid extract, 1.5 ml ethanol and 2 ml of petroleum ether was added and centrifuged. The supernatant was evaporated to dryness at 80°C, to that 0.2 ml of 2-2'-dypyridyl solution (0.2%) and ferric chloride (0.5%) were added and it was kept in dark for 5 min and then 4 ml of butanol was added. The absorbance was measured at 520 nm and content was expressed in mg/100 g, dry weight of the shoot.

2.7 Determination of trace mineral elements

Mineral content of fresh and processed shoots was determined using WD-XRF Spectrometry. For this, 2 g of powdered bamboo shoot sample was weighed and subjected to pressing at 20 tons cm⁻² for 60 s to obtain a cylindrical pellet of 20–40 mm diameter. Then, each pellet was placed on a sample holder and located directly in the X-ray beam of the X-ray spectrometer used for elemental determination.

2.8 Determination of DPPH free radical scavenging activity

The ability of the fresh and processed shoot extracts to scavenge the DPPH radical was quantified using a spectrophotometric method as previously described ^[23]. The reaction mixture consisting of 1.0 ml DPPH (0.1 mM, OD=0.890) and different concentrations (50-2000µg) of aqueous extract of fresh and processed shoots, was incubated

for 2 to 5 min in dark and then absorbance was measured at 517 nm using UV-spectrophotometer with ethanol as blank. BHT (5 mg/ml ethanol) was used as positive control. Percentage of inhibition was calculated by the following equation: % Inhibition = $\frac{A-B}{A} \times 100$; Where, A is the absorbance of the DPPH solution and B is the absorbance of the sample

2.9 Determination of ABTS radical cation scavenging activity

The antioxidant activity of fresh and processed shoots extract was studied using ABTS radical cation decolourisation assay according to the method of Shirwaikar et al [24]. ABTS radical cations are generated by reaction of ABTS (7 mM) with Ammonium persulfate (2.4 mM) after incubating this mixture at room temperature in dark for 16 hr. This was the working solution which was further diluted with ethanol to give an absorbance of 0.89 ± 0.20 . Thereafter, different concentrations (50-2000µg) of extract were added to 1.0 ml of ABTS working solution. The reaction mixture was incubated at room temperature for 2 to 5 min and then the absorbance was measured at 734 nm with ethanol as blank. BHT (5 mg/ml ethanol) was served as control. Percentage of inhibition was calculated by the following equation: % Inhibition = $\frac{A-B}{A} \times 100$; Where, A is the absorbance of the ABTS solution (ABTS+APS) and B is absorbance of the sample (ABTS + APS + sample).

2.10 Experimental design for in-vivo study

Healthy male Balb/c mice weighing 25-30 g each were procured from Central Animal House, Panjab University, Chandigarh. They were kept in polypropylene cages bedded with sterilized rice husk and maintained at Department of Biophysics, Panjab University, Chandigarh in a 12 hr light/dark cycle at $25 \pm 2^{\circ}$ C. Mice in all the groups had free access to standard animal pellet diet (Ashirwad Industries Ltd., Ropar, Panjab) and clean tap water throughout the experiment. All the experimental protocols were approved by the Institutional Ethics Committee (Panjab University, Chandigarh, India) and conducted according to the Indian National Science Academy Guidelines. Mice were randomly assigned into five groups (N=6). Group-1 served as control group; received tap water and feed ad libitum. Group II animals received the fresh shoots extract, group III animals administered the extract of fermented shoots; group IV animals were given the extract of brine treated shoots while group V animals received the extract of boiled shoots. Fresh doses were prepared every day in distilled water and administered to the animals at the dose levels of 800 mg/kg, body weight in the dose volume of 1 ml/kg, body weight for six weeks. After completion of respective treatments, the animals were sacrificed by cervical dislocation under light ether anesthesia and liver tissue was excised out, perfused with cold normal saline (0.9% Nacl solution), blotted and then weighed carefully.

2.11 Determination of the concentration of lipid peroxidation (LPO) product

The lipid peroxide level was determined according to the method as described by Trush *et al* ^[25]. For this, the liver tissues were homogenized in 50 mM Tris buffer (pH 7.4) to obtain 10% homogenate (w/v). The homogenate was then subjected to cold centrifuge at 10000g for 30 minutes. The pellet was discarded and supernatant (PMF) thus obtained was used for the estimation of LPO. To 500 μ l sample, 750 μ l buffer was added and incubated at 37°C for 60 minutes. Thereafter, 0.75 ml of TCA-HCl was added and centrifuged at 3000 rpm for 10 minutes. Supernatant was taken with help of a micropipette and 2 ml of TBA solution was added, boiled at 100°C and absorbance was measured at 535 nm. The lipid peroxide level was calculated using the formula:

$$\frac{OD}{1.56 \times 10^5 M} \times \frac{\text{Total reaction volume}}{\text{Sample volume}} \times \frac{1}{\text{Protein } (\frac{mg}{dl})}$$

2.12 Statistical analysis

The data obtained from the experiments are expressed as mean \pm SD (standard deviation). For statistical analysis, data were subjected to analysis of variance (ANOVA) followed by post-hoc test and values are considered statistically significant at F < 0.05.

3. Results

3.1 Antioxidant compounds

3.1.1 Phenols and Flavonoids

Bamboo shoot processing operations such as boiling, brine preservation and fermentation can have detrimental effects on the phenolic and flavonoid content thereby increasing or decreasing the antioxidant capacity of shoots. The total phenolic and flavonoid content in the fresh shoots was 67.50 milligrams of gallic acid equivalent per gram (mg of GAE/g) and 7.92 milligrams of quercetin equivalents per gram (mg of QUE/g) respectively. Brine preservation caused highest reduction in the phenolic (62%) and flavonoid (77%) content while, boiled shoots retained maximum amount of phenols (62.22 mg of GAE/g) as well as flavonoids (7.78 mg of QUE/g). In contrast, fermentation process of the shoots caused 17% increase in the total phenolic content while 32% decrease in the total flavonoid content (Fig. 1).

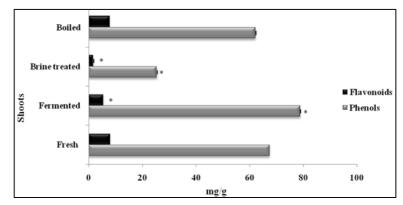


Fig 1: Total phenol and flavonoid content in the fresh and processed shoots of *Dendrocalamus hamiltonii*; (*P< 0.05) significant as compared to fresh shoots

3.1.2 Vitamin C and vitamin E

Processing of bamboo shoots caused a significant (P < 0.05) reduction in the amount of vitamin C and vitamin E content of the shoots (Fig. 2). Vitamin C content ranged from 38.80-69.96 mg/100g, dry weight of which the highest was present in fresh shoots (69.96 mg/100g, dry weight) and the lowest in boiled shoots (38.80 mg/100g, dry weight). Brine preservation

retained the most vitamin C (49.81 mg/100g, dry weight) while, fermentation caused 38% loss in the content. Vitamin E content was also observed to be highest in the fresh form (8.87 \pm 1.42 mg/100g, dry weight) and lowest in the boiled form (4.37 \pm 0.62 mg/100g, dry weight). Brine preservation and fermentation reduced the vitamin E level by 33% and 37% respectively.

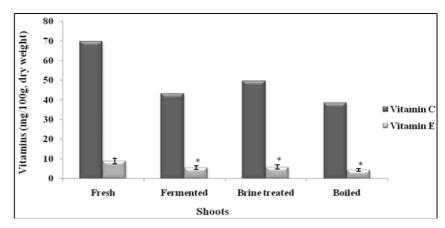


Fig 2: Comparison of vitamin C and vitamin E content (mg/100g, dry weight) in the fresh and processed shoots of *Dendrocalamus hamiltonii*; (*P< 0.05) significant as compared to fresh shoots

3.1.3 Mineral elements

Bamboo shoots are good source of minerals such as copper, zinc, iron and manganese that are necessary for the activities of various antioxidant enzymes. In order to study the effect of processing on the level of mineral elements, fresh and processed shoots were analyzed for manganese, iron, copper and zinc content (Fig. 3). The manganese content in the fresh and processed shoots ranged from 3.0-9.0 mg/100g, dry weight, with highest content in the fresh shoots and lowest in the brine treated shoots. Fermented shoots (8.30 mg/100g, dry weight) retained maximum Mn content followed by boiled shoots (5.6 mg/100g, dry weight). The amount of iron present in the fresh shoots was 4.8 mg/100g, dry weight. Iron content increased significantly after processing with highest increase

in the boiled shoots (10 mg/100g, dry weight) and lowest in brine preserved shoots (5.6 mg/100g, dry weight). Fermentation caused 83% increase in the iron content of the shoots. Copper content ranged from 1.70-2.50 mg/100g, dry weight. Boiling caused the most loss (29%) while, fermentation retained the most (2.20 mg/100g, dry weight) copper content. On the contrary, brine treatment increased the content by 4.3%. Zinc content remained unaffected by 15 min boiling (10 mg/100g, dry weight) but increased drastically after fermentation (30 mg/100g, dry weight). In contrast, brine preservation caused a significant decrease (4.0 mg/100g, dry weight) in the zinc content as compared to the other forms.

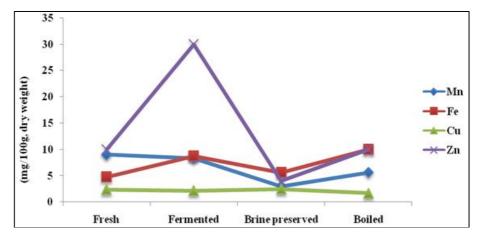


Fig 3: Comparative account of mineral elements present in the fresh and processed shoots of Dendrocalamus hamiltonii

3. 2 Antioxidant studies

In-vitro antioxidant activity of fresh and processed shoots was measured by DPPH free radical scavenging assay and ABTS radical cation assay.

3.2.1 ABTS radical cation scavenging activity

The results of ABTS radical cation scavenging activity of fresh and processed shoots are shown in fig. 4. Butylated 4-hydroxytoluene (BHT) was used as standard and its IC_{50}

value was 13 µg/ml. Fermented shoots showed highest ABTS radical scavenging activity with significantly lower IC₅₀ value (60 µg/ml) while, brine preserved shoots exhibited the least, in a concentration range 25-200 µg/ml with IC₅₀ value 192 µg/ml. Aqueous extract of fresh and boiled shoots was able to scavenge ABTS radicals in a concentration range of 25-125 µg/ml and the IC₅₀ values were 66 µg/ml and 89 µg/ml respectively.

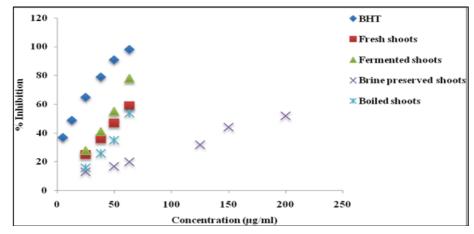


Fig 4: ABTS radical cation scavenging activity of standard BHT and aqueous extract of fresh and processed shoots of *Dendrocalamus hamiltonii*

3.2.2 DPPH free radical scavenging activity

The results concerning DPPH radical scavenging activity of shoots with the standard reference BHT (IC₅₀ value 277 μ g/ml) are shown in fig. 5. It was observed that, aqueous extract of fresh and fermented shoots have higher DPPH radical scavenging activity with IC₅₀ values 568 μ g/ml and

527 μ g/ml respectively. Brine preserved shoots showed poor scavenging activity and its IC₅₀ value was 1667 μ g/ml. Aqueous extract of boiled shoots was able to scavenge radicals in a concentration range of 125-2000 μ g/ml with IC₅₀ value 1281 μ g/ml.

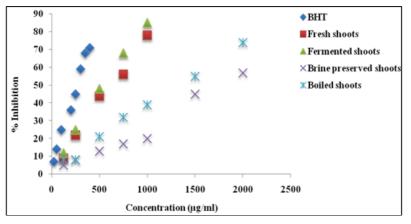


Fig 5: DPPH free radical scavenging activity of standard BHT and aqueous extract of fresh and processed shoots of Dendrocalamus hamiltonii

3.3 Lipid peroxidation (LPO)

The level of MDA content in the liver tissues of control mice was 3.19 ± 0.12 (nmoles/min/mg protein). On fresh and processed shoots extract administration, MDA content decreased significantly (P < 0.05) as compared to the control group (Fig. 6). The group administered with fresh shoot extract showed the highest decrease in MDA content (0.897 ± 0.06 nmoles/min/mg protein). Amongst all the processed shoots, fermented shoots caused highest decrease in MDA content (1.09 \pm 0.21 nmoles/min/mg protein) while, lowest was seen in the mice administered with brine preserved shoot extract (2.63 \pm 0.11nmoles/min/mg protein). The level of MDA content in the liver tissues of boiled shoots extract administered group was 1.56 \pm 0.22 (nmoles/min/mg protein).

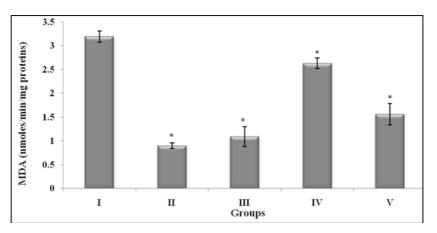


Fig 6: Effect of fresh and processed shoots extract on the level of lipid peroxidation in hepatic tissues, Group I: Control, Group II: Fresh shoots; Group III: Fermented shoots; Group IV: Brine preserved shoots; Group V: Boiled shoots treated mice; (**P*< 0.05) significant as compared to control group

4. Discussion

Bamboo shoots are projected as a new health food due to the presence of nutrients, health-enhancing bioactive compounds and antioxidants ^[26, 13]. But the anti-nutritional factors and toxic influences in freshly harvested bamboo shoots used as food are a matter of concern that needs processing. Bamboo shoot processing procedures are recognized as one of the major factors on the changes of natural phytochemicals, which may affect the therapeutic quality, especially antioxidant properties of processed shoots ^[18, 27]. Antioxidants are highly important molecules that help minimize free radical damage to the body [28]. Thus, the evaluation of bamboo shoot-processing operations influencing the antioxidant activity in processed shoots is imperative in optimizing the conditions to increase or decrease their availability and functionality. The quantitative biochemical analysis of shoots was done for the quantification of antioxidants such as phenols, flavonoids, vitamins and important trace elements which play a critical role in preventing or minimizing oxidative damage in the biological system. Freshly harvested shoots are rich in vitamin C, vitamin E, manganese and flavonoids. Phenolic content increased significantly after fermentation. The increase in total phenolic content during fermentation might be due to the hydrolysis of complex phenolics into soluble-free phenols with the help of proteolytic enzymes. Fermentation not only increase the amount of phenols but also make them more bioavailable ^{[29,} ^{30]}. In addition, bamboo shoot fermentation also increased Fe and Zn availability but did not modify Mn and Cu content of the shoots. Boiled shoots also retained adequate amount of Fe, Zn and flavonoids but the maximum loss of antioxidants occurred during brine preservation due to leaching of components into the brine especially water-soluble antioxidants like phenols, vitamin C and trace elements like Mn and Zn^[31]. Antioxidants act synergistically to show the enhanced efficacy than the sum of the contributions from each single antioxidant [32]. Hence, estimation of a food's total antioxidant activity is important to measure the combined ability of all antioxidants in a given food to neutralize free radicals [33]. Antioxidant activity of fresh and processed shoots was measured by following DPPH free radical scavenging and ABTS radical cation scavenging assays. The ABTS method is based on neutralization of radical cation formed by a single-electron oxidation of a synthetic ABTS chromophore to strongly absorbing ABTS radical cation ^[33] while, in DPPH method the antioxidant activity is proportional to the disappearance of DPPH radicals in the test samples. Fermented shoots showed highest ABTS and DPPH radical scavenging activity while, lowest was seen in the case of brine preserved shoots. The higher antioxidant capacity of fermented shoots may be attributed to the increased amount of phenols, Fe and Zn after fermentation. Several studies have indicated that fermentation improves the antioxidant activity in plant based food primarily due to the increase in phenol and flavonoid content ^[35]. Besides phenolic content, the concentration of minerals like Mn, Zn, Fe and Cu has also a significant influence on the activity of antioxidant enzymes. Even a small change in the concentration of these minerals in the tissue causes a disturbance in their metabolism, which is associated with a number of health problems [36]. Contrarily, high concentration of sodium chloride can damage the liver and also reduces the activity of glutathione peroxidase, catalase and superoxide dismutase [37]. In the present study, brine preservation caused highest decrease in the level of Mn and Zn. Fe content however increased after processing but

increase was negligible in the case of brine preserved shoots. These trace elements are a part of the group of superoxide dismutase enzymes (MnSOD, ZnSOD), which catalyse the superoxide anion dismutation into hydrogen peroxide and oxygen ^[36]. Iron-containing enzyme catalase or glutathione peroxidase decomposes hydrogen peroxide into water and oxygen.

It is evident that, significant reduction in the level of antioxidant compounds during brine preservation caused highest decrease in the antioxidant capacity of brine preserved shoots. In vitro antioxidant capacity also provides an insight into the delicate balance in vivo between oxidants and antioxidants. Lipid peroxidation is an important mechanism in free radical mediated cell injury [38]. As a result of lipid peroxidation, malondialdehyde (MDA), a genotoxic harmful degradative byproduct is formed. High level of lipid peroxidation products has been associated with several health such as cancer, cardiovascular problems and neurodegenerative diseases. Bamboo shoot being rich in vitamin C, E, phenols, flavonoids, phytosterols and trace elements can plays significant role in scavenging or quenching of free radicals and ROS ^[13]. When Balb/c mice were administered with aqueous extract of fresh, fermented, brine preserved and boiled shoots, MDA content decreased significantly as compared to the control counterparts. Amongst all the processed shoots, fermented shoots caused highest decrease in MDA content while, lowest was seen in the mice administered with brine preserved shoot extract. It may be inferred from the results of this study that, fermented shoots as a potential source of antioxidant compounds are very useful to prevent and control oxidative-stress induced diseases and other chronic disorders and also exhibit potential as raw material for the food and pharmaceutical, industries.

5. Conclusion

Bamboo shoot being rich in nutrients, antioxidants and healthpromoting bioactive compounds offers protection against a wide range of health problems including cancer, diabetes, cardiovascular disease and age-related degenerative diseases. The young shoots however need to be processed before consumption to remove the anti-nutrients that impart an acrid taste. Soaking, boiling, salting and fermentation are the major processing techniques which are commonly used for removal of anti-nutrients and improvement of organoleptic properties. This study was aimed at determining the *in vitro* and *in vivo* antioxidant properties of boiled, brine preserved and fermented shoots of Dendrocalamus hamiltonii. It is evident that fermented shoots as a potential source of antioxidants are very useful to prevent and control oxidative-stress related chronic and degenerative diseases and also exhibit potential as raw material for the food and pharmaceutical, industries.

6. Acknowledgement

The authors are grateful to the Ministry of Food processing Industries (V45/MFPI/R&D/2000Vol.IV), Department of Biotechnology, New Delhi (BT/475/NE/TBP/20132), and DST PURSE Grant. Govt. of India, American Bamboo Society and Ned Jaquith Foundation, USA, for providing financial assistance to conduct this research work.

7. Conflicts of interest

The authors declare that there are no conflicts of interest.

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