In vitro Antioxidative Profiling of Different Fractions of *Dendrocalamus strictus* (Roxb.) Nees leaf Extracts

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ABSTRACT

Introduction: Plant derived natural antioxidants have attracted scientists for many years because of their economic viability and virtually no side effects. Therefore the present study was planned to evaluate the antioxidant activity of the leaf of *Dendrocalamus strictus (Roxb.) Nees*, used as household therapy for cold, cough and fever, extracted with different solvents of increasing and decreasing polarity such as water (DAQE), acetone (DAE) and methanol (DME). **Methods:** The antioxidant profiling of the leaf extracts was done at varying concentrations (20-200 μg/ml), using different assays like 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity, reducing power assay and hydrogen peroxide radical scavenging activity along with their total phenolic and flavonoid contents. **Results:** DME had more antioxidant activity than other extracts. Total phenol and flavonoid content were highest in the DAQE and DME fraction (112.10 ± 11.21 mg GAE/g and 199.20 ± 19.92 mg QE/g) respectively. This result also suggests a close relationship among total phenolic content, flavonoids and antioxidant activity. **Conclusion:** The study shows that leaf of *Dendrocalamus strictus* is a potential source of useful natural antioxidants, which substantiates its use as herbal remedies.

Keywords: Antioxidant, Correlation, *Dendrocalamus strictus*, DPPH, FRP, Reducing power.

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INTRODUCTION

Dendrocalamus strictus (Roxb.) Nees commonly known as "Male bamboo" is one of the most important and commonly found bamboo species, native to India and deciduous in nature.[1] The culm is densely tufted and is often solid at the basal portion. It grows up to 18.5 m in height and 12.7 cm in diameter.[2] This particular species of bamboo finds it's use in both domestic as well as agricultural purposes apart from being extensively used in paper industries.[1] The young shoots are also used for eating purpose in different parts of North-east India because of its high nutritive values.[2] It is assumed that the antioxidant capacity of the bamboo leaves provide the medicinal benefits and help to get rid of certain diseases.[3] The leaf decoction alone is used as abortifacient[4] and when mixed with turmeric powder (*Curcuma longa*) is used to treat cold, cough and fever.[5] Leaf powder has cut and wound healing property.[6]

It is well know fact that reactive oxygen species (ROS) is generated in our body due to various biochemical reactions, which is scavenged by the antioxidant defense system under normal condition. However, sometimes the rate of generation of ROS exceeds the rate of diminution leading to the accumulation of ROS in our body which in turn leads to various ailments such as diabetes, cancer, cardiovascular diseases etc.[7] With the advent of modern technology synthetic substitutes like gallic acid esters, butylated hydroxyl anisol (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone have captured the market which in turn leads to various side effects. Thus, to get rid of these side effects, scientists have now diverted themselves towards the plant derived natural antioxidants due to their economic viability and having no side effects.[8] Plants are considered to be store house of different types of free radical scavenging molecules like phenols, flavonoids, vitamins etc. having high antioxidant properties,[9] but still the use of phytochemicals are limited due to less information available with regard to their chemical composition and dynamics, amount of active chemicals present and relevant toxicity data.[10]

To date there has been no report on the study of potential antioxidant activity of *D. strictus* leaf as such. Therefore, the objective of the present study was to evaluate the *in vitro* antioxidative properties of *D. strictus* leaf in different solvents like water, acetone and methanol and also to determine their correlations.

MATERIALS AND METHODS

Chemicals and reagents

2,2-diphenyl-1-picryl-hydrazyl (DPPH), quercetin, sodium nitrite ($NaNO_2$), trichloroacetic acid (TCA), ascorbic acid, Ferric chloride ($FeCl_3$), gallic acid were obtained from Himedia Laboratories Pvt. Ltd, Mumbai, India. Potassium di-hydrogen phosphate (KH_2PO_4), di-potassium hydrogen phosphate (K_2HPO_4), sodium hydroxide (NaOH), potassium ferricyanide ($K_2Fe(CN)_6$), sodium carbonate (Na_2CO_3), Hydrogen peroxide (H_2O_2) and Methanol were procured from Merck, Mumbai, India. Folin-Ciocalteu reagent from Sisco research laboratory, Mumbai, India. Aluminium chloride ($AlCl_3$) was obtained from SD Fine Chemicals Ltd., Mumbai, India. All chemicals and solvents are analytical grade.

Plant material and extraction

D. strictus (Roxb.) Nees leaves were collected from the Sukna Forests in July, 2010 and were authenticated by Bamboo taxonomist. A voucher specimen (SUK/KRR/D002) is deposited at Bambusetum, Kurseong Research Range, Sukna, Darjeeling, West Bengal.

Dried, ground leaves of *D. strictus* (10 g each) were extracted by Soxhlation using 80% aqueous methanol (DME), double distilled water (DAQE) and acetone (DAE) separately (the ratio of plant material to solvent was 1:15 w/v).[11] The extraction was carried out for 6 hours in each case at boiling temperature separately. The extracts obtained were evaporated under pressure (12 Torr) at 50 °C. The extracts were stored at –20 °C for further use. The extracts were dissolved in double-distilled water (DDW) in desired concentrations just prior to use.

Determination of Plant Extract Yield

The yield of evaporated extracts based on dry weight was calculated from the following equation:

$$Yield\ (g/100\ g\ of\ dry\ plant\ material) = \frac{W_1 \times 100}{W_2}$$

where, W1 and W2 are the weight of the extract after the solvent evaporation and the weight of the dry plant material, respectively.

Determination of Total Phenolic Content

The total soluble phenol in the extracts were determined by using Folin-Ciocalteau reagent according to the method of Singleton and Rossi.[12] The absorbance of the blue color that developed was read at 760 nm. Gallic acid was used as a standard. The concentration of total phenolic compounds in gallic acid was determined as μg of gallic acid equivalent (GAE) using an equation obtained from the standard gallic acid graph.

Determination of total flavonoid content

The total flavonoid content of the extracts was determined with aluminium chloride ($AlCl_3$) according to Zhishen *et al.*[13] using quercetin as a standard in mg QE (Quercetin equivalent). The absorbance was measured at 510 nm. All tests were performed in triplicates.

In vitro *Antioxidant properties of the extracts*

Free Radical Scavenging Activity 2,2-Diphenyl-1-picrylhydrazyl (DPPH Method): The antioxidant activity of the extracts and standard were assessed on the basis of the radical scavenging effect of the stable DPPH free radical as per the modified protocol by Goyal *et al.*[14] The absorbance of each solution was determined at 517 nm using spectrophotometer (Thermo UV1 Spectrophotometer, Thermo Electron Corporation, England, UK). The corresponding blank reading were also taken and the remaining DPPH was calculated by using the following formula,

$$DPPH\ scavenging\ activity\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Where $Abs_{control}$: Absorbance of the control and Abs_{sample}: Absorbance in the presence of the sample/standard. IC_{50} value is the concentration of the sample required to scavenge 50% of the free radical.

Ferrous Reducing Power Assay (FRP): The ferrous reducing power of the extracts was determined according to the method of Oyaizu.[15] The absorbance was measured at 700 nm and compared with standards. Increased absorbance of the reaction mixture indicated increased reducing power.

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Scavenging of Hydrogen Peroxide: The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch.[16] The percentage scavenging activity of hydrogen peroxide by extract was calculated using the following formula,

$$H_2O_2\ \textit{scavenging activity}\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where, $Abs_{control}$ and Abs_{sample} are absorbance of the control and the extract/standard respectively.

Statistical Analysis

All samples were tested and analyzed in triplicates. Results were calculated as the mean ± SD (standard deviation) for each sample. Statistical analysis was done with one way analysis of variance using Graph pad Prism, Version 4.0 (Graph Pad Software, San Diego, CA, USA).The correlation coefficient (R^2) was used to show correlations. A significant difference was judged to exist at a level of $p < 0.05$ and $p < 0.01$.

RESULTS

Plant Yield

The plant yield of *Dendrocalamus strictus* leaves DAQE, DAE and DME extracts was found to be 8.55%, 5.93% and 5.57% respectively.

Determination of total phenolics

The total phenolic content was found to be highest in DAQE with 112.10 ± 11.21 mg/ml GAE per 100 mg plant extract followed by DME i.e. 68.91 ± 6.90 mg/ml, while DAE had the lowest with 36.16 ± 3.61 mg/ml. Figure 1 shows the amount of phenol present in each extract.

Determination of total flavonoids

The total flavonoid content was found to be maximum in case of DME of *D. strictus* with 619.2 ± 61.92 mg/ml QE equivalent per 100 mg plant extract while DAQE and DAE had 199.2 ± 19.92 mg/ml and 175.2 ± 17.2 mg/ml respectively. The flavonoid content is shown in figure 1.

DPPH scavenging activity

DPPH antioxidant assay is the most commonly used assay to evaluate the antioxidant activity. It is based on the ability of DPPH to decolorize from violet to yellow

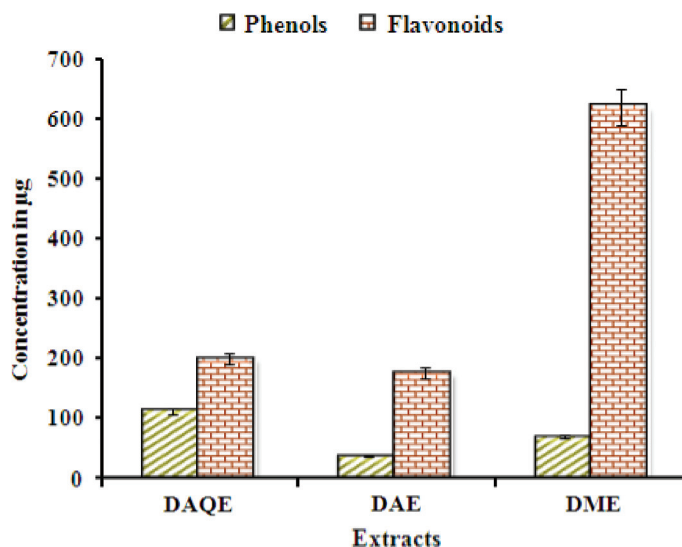

Figure 1. Total phenolics and flavonoids present in different extracts of *D. strictus* leaf.

in presence of antioxidants thus leads to decrease in absorbance at 517 nm. The screening results of the DPPH activity along with standard ascorbic acid is exemplified in figure 2. Three different types of leaves extract i.e. water, acetone and methanol of *D. strictus* have been evaluated.

Different kind of plant extracts at different concentrations exhibited more than 40% scavenging activity (Table 1).

Scavenging of Hydrogen Peroxide

H_2O_2 scavenging activity of *D. strictus* leaf extracts is illustrated in figure 3 which proves the various extracts as good scavenger of H_2O_2.

Reducing power assay

Figure 4 depicts the reductive capabilities of the various plant extracts and fractions compared with ascorbic acid used as positive control.

Linear correlation between different parameters of D. strictus

Linear correlation between the phytochemical constituents and total antioxidant activity was established in order to determine how the antioxidant activity and total phenols or flavonoid level are related in different leaf extracts of *D. strictus*.

DISCUSSION

The highest yield was found to be with water followed by acetone and methanol.

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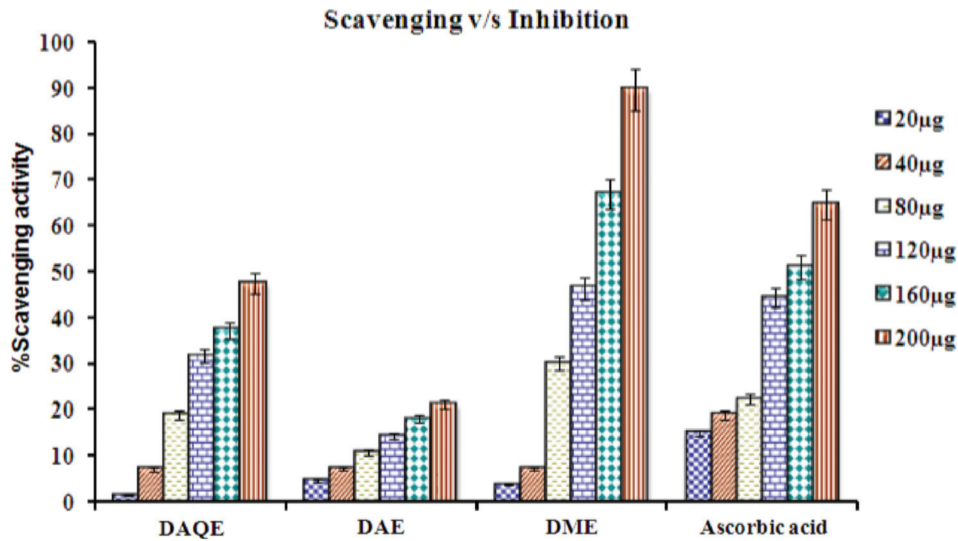

Figure 2. DPPH scavenging activity (I%) of different leaf extracts of *D. strictus* along with standard ascorbic acid. Values are in triplicates ± standard deviation.

Table 1. Comparison of DPPH radical scavenging activity (IC_{50}) of *D. strictus* leaf extracts with that of ascorbic acid as standard

Plant extract	Concentration (mg/ml)	% Scavenging[a]
DAQE	0.3	47.59 ± 0.6*
DAE	0.3	21.19 ± 1.08**
DME	0.1	68.11 ± 1.5*
Ascorbic Acid	0.2	64.76 ± 1.4*

[a]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.*Significantly different at $p < 0.05$.**Significantly different at $p < 0.01$.

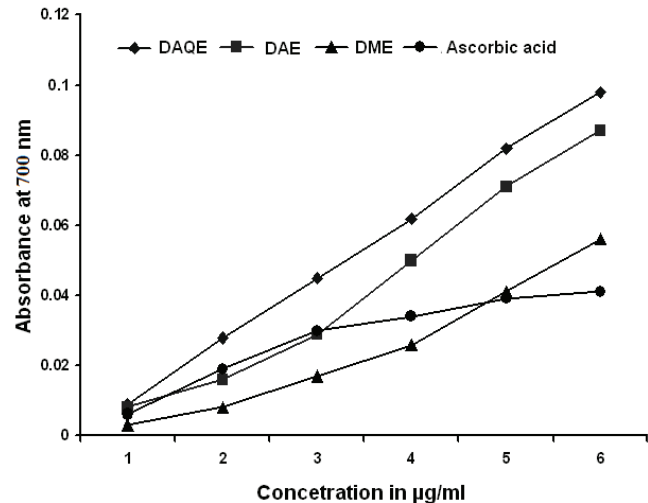

Figure 4. Ferrous reducing power (FRP) activity of different extracts of *D. strictus* leaf compared to ascorbic acid as standard. Values are in triplicates ± standard deviation.

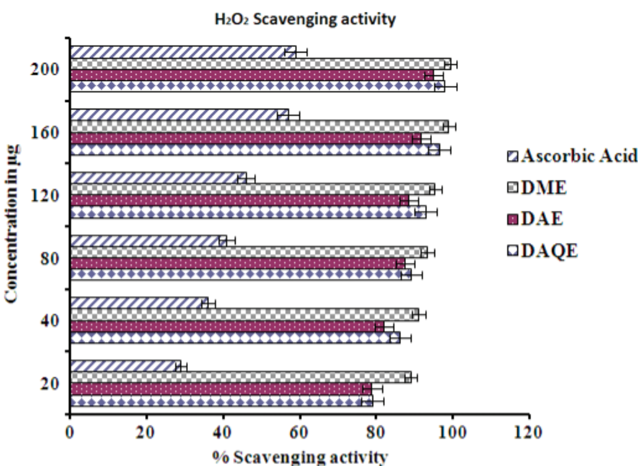

Figure 3. H_2O_2 scavenging activity of *D. strictus* leaf extracts compared to ascorbic acid as satndard. Values are in triplicates ± standard deviation.

The phenolic content was found to be highest in water followed by methanol and acetone. The difference in the phenolic content in different extract may be because of the difference in polarity depending upon which selective phenolic compounds percolate in the extract. This observation is in accordance with that of the earlier workers.[17]

As depicted in figure 1 it can be inferred that the DME had higher flavonoid content as compared to the other extracts. Higher level of flavonoids in DME can be attributed to the fact that methanol is less polar than water and thus has the potential to release the bound flavonoids and polyphenols from the cell wall of the plant.[18]

The main functional components in this type of bamboo are flavonoids, lactones and phenolic acids. The flavone C-glucosides comprise a group of

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representative flavonoids, orientin, homoorientin, vitexin and isovitexin in bamboo, which has also been reported by Zhang and his coworkers in some of bamboo species.[19]

DPPH radical scavenging activity in the plant extracts was found to decrease in the following order: DME > DAQE > DAE. Considerable differences in the antioxidant activity have been noted in different extracts. Since DME extract had the highest antioxidative activity, it can be inferred that methanol could be a suitable solvent for the preparation of extracts since it also inhibit the degradation of polyphenols present in the plants by neutralizing the activity of polyphenol oxidase.[20]

The radical scavenging effect of DME extract at 0.1 mg ml^{-1} was similar to ascorbic acid at 0.2 mg ml^{-1}. Therefore, the antioxidant effect of DME was 2 times greater than that of the synthetic antioxidant, ascorbic acid.

Hu and his coworkers[21] further demonstrated the antioxidant activity of bamboo and its biological responses. It exhibits a good concentration dependent scavenging activity of the DPPH radical, prolong the lag phase and reduce the rate of propagation of liposome peroxidation initiated by the peroxyl radical which in turn is induced by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), protect supercoiled DNA strands against excision induced by the AAPH-Mediated peroxyl radical. Based on all these *in vitro* and *in vivo* antioxidant-related studies, bamboo can be regarded as an effective natural antioxidant.

Figure 3 shows that the leaf extract are a good scavenger of H_2O_2 in a pattern following: DME> DAQE>DAE as compared to ascorbic acid as standard. Antioxidants of *D. strictus* leaf has been reported to be capable of blocking chain reactions of lipid auto-oxidation, chelating transient state metal ions, scavenging nitrite compounds, H_2O_2 and blocking the synthetic reaction of nitrosamine.[22] The results are following the similar trends as described in previous study.[14]

The reducing power was found to be directly proportional to the concentration of the extract and was found to increase steadily with increase in concentration. The reductive capability is determined by the transformation of Fe^{3+} to Fe^{2+} in presence of the extract and the ascorbic acid used as standard. DAQE fractions had the highest levels of reductive ability (OD 700 nm = 0.098) followed by DAE (OD 700 nm = 0.087) and DME (OD 700 nm = 0.056). The absorbance of plant extract (DAQE and DAE) and ascorbic acid was similar at 20μg/ml. It is also noted that the absorbance of DAE and the ascorbic acid shows parallelism at 20, 40 and 80 μg/ml concentration and then differ significantly. At 160 μg/ml concentration the absorbance of the DME and ascorbic acid was found to be almost similar i.e. 0.041 and 0.039 respectively.

A positive linear correlation was found between the phenol and DPPH scavenging activity in all the three different extracts i.e. DAQE (R^2 = 0.946), DAE (R^2 = 0.995) and DME (R^2 = 0.971). Our experimentation on the correlation between the total phenol and reducing power also led to similar results in case of DAQE (R^2 = 0.943) and DME (R^2 = 0.975) while DAE showed moderate correlation between the two i.e. (R^2 = 0.615). Correlation between the total flavonoids and the DPPH was also established for all the extracts: DAQE (R^2 = 0.989), DAE (R^2 = 0.954) and DME (R^2 = 0.950) as well as between flavonoids and reducing power and was found to be DAQE (R^2 = 0.980), DAE (R^2 = 0.965) and DME (R^2 = 0.950). The positive linear correlation between total contents of phenolics and DPPH free radical scavenging activities were in accordance of previous studies.[23,24] Apart from these, other correlations were also established between different parameters as depicted in table 2, 3 and 4.

Table 2. Linear correlation between phenolics, flavonoids and antioxidant acivities (DPPH, FRP, H_2O_2) of *D. strictus* aqueous leaf extract

	Phenol	Flavonoids	DPPH	H_2O_2	FRP
Phenol	1				
Flavonoids	0.976305	1			
DPPH	0.945791	0.9867	1		
H_2O_2	0.874534	0.929196	0.965821	1	
FRP	0.9428	0.98049	0.991793	0.98098	1

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Table 3. Linear correlation between phenolics, flavonoids and antioxidant acivities (DPPH, FRP, H_2O_2) of *D. strictus* acetone leaf extract

	Phenol	Flavonoids	DPPH	H_2O_2	FRP
Phenol	1				
Flavonoids	0.96089	1			
DPPH	0.994882	0.954215	1		
H_2O_2	0.96089	0.654818	0.560788	1	
FRP	0.615314	0.965181	0.978794	0.563844	1

Table 4. Linear correlation between phenolics, flavonoids and antioxidant acivities (DPPH, FRP, H_2O_2) of *D. strictus* methanolic leaf extract

	Phenol	Flavonoids	DPPH	H_2O_2	FRP
Phenol	1				
Flavonoids	0.977965	1			
DPPH	0.971275	0.950206	1		
H_2O_2	0.93081	0.920612	0.98724	1	
FRP	0.975401	0.949808	0.994619	0.98098	1

CONCLUSION

To conclude, the quantitative correlations between the polyphenols and the DPPH, H_2O_2 scavenging activity and reducing power of all the three extracts of *D. strictus* was performed and it was established that they exhibit close linear correlation among each other. This is first report to concur antioxidant activity of *D. strictus*. This study substantiates utilization of this plant as an antioxidant in future. On the other hand, further studies should be continued to obtain appropriate information about the role of *D. strictus* in the other dose-dependent processes.

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REFERENCES

1. Saxena S, Dhawan V. Regeneration and large-scale propagation of bamboo (*Dendrocalamus strictus* Nees) through somatic embryogenesis. Plant Cell Rep. 1999; 18:438-3.
2. Goyal AK, Middha SK, Usha T, Chatterjee S, Bothra AK, Nagaveni MB, et al. Bamboo-infoline: A database for North Bengal bamboo's. Bioinformation. 2010; 5(4):184-5.
3. Mee-Hyang K, Han-Joon H, Ha-Chin S. Identification and Antioxidant Activity of Novel Chlorogenic Acid Derivatives from Bamboo (*Phyllostachys edulis*). J Agric Food Chem. 2001; 49:4646-55.
4. Sharma TP, Borthakur SK. Ethnobotanical observations on bamboos of Adi tribes in Arunachal Pradesh. IJTK. 2008; 7(4):594-7.
5. Kamble SY, Patil SR, Sawant PS, Sawant S, Pawar SG, Singh EA. Studies on plant used in traditional medicine by Bhilla tribe of Maharashtra. IJTK. 2010; 9(3):591-8.
6. Mohapatra SP, Prusty GP, Sahoo HP. Ethnomedicinal observations among forest dwellers of the Daitari Range of hills of Orissa India. Ethanobotanical leaflets 2008; 12:1116-23.
7. Xican L, Xiaoting W, Huang L. Correlation between Antioxidant Activities and Phenolic Contents of Radix Angelicae Sinensis (Danggui). Molecules. 2009; 14:5349-61.
8. Middha SK, Mittal Y, Usha T, Kumar D, Srinivasan R, Vashisth L, et al. Phytomellitus: A phyto-chemical database for diabetes. Bioinformation. 2009; 4(2):78-9.
9. Cai YZ, Sun M, Corke H. Antioxidant activity of betalins from plants of Amaranthaceae. J Agric Food Chem. 2003; 51:2288-94.

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10. Shahidi F, Wanasundara UN, Amarowicz R. Natural antioxidants from low- pungency mustard flour. Food Res Int. 1994; 27(4):489-93.
11. Shon YM, Choi SD, Kahng GG, Nam SH, Sung NJ. Antimutagenic antioxidant and free radical scavenging activity of ethyl acetate extracts from white yellow and red onions. Food Chem Toxicol. 2004; 42:659-66.
12. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16:144-58. .
13. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. Food Chem.1999; 64:555-9.
14. Goyal AK, Middha SK, Sen A. Evaluation of the DPPH radical scavenging activity total phenols and antioxidant activities in Indian wild *Bambusa vulgaris* "Vittata" methanolic leaf extract. J Nat Pharma. 2010; 1(1):40-5.
15. Oyaizu M. Studies on products of browning reaction prepared from glucoseamine. Jpn J Nutr. 1986; 44:307-14.
16. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from chinese green tea. Carcinogenesis. 1989; 10:1003-8.
17. Kostic DA, Mitic SS, Mitic MN, Zarubicia AR, Velickovic JM, Dordevic AS, et al. Phenolic content antioxidants and antimicrobial activity of *Papaver rhoeas* L. from southeast Serbia. JMPR. 2010; 4(17):1727-32.
18. Lapornik A, Prosek M, Wondra GA. Comparison of extracts prepared from plants by-products using different solvents and extraction time. J Food Eng. 2005; 71:214-22.
19. Zhang Y. Natural functional extract of bamboo leaves: bamboo leaf flavonoids. China Food Addit. 2002; 13:54-8.
20. Zhang Z, Chang Q, Zhu M. Characterization of antioxidants present in hawthorn fruits. J Nutr Biochem. 2001; 12:144-52.
21. Hu C, Zhang Y, Kitts DD. Evaluation of antioxidant and prooxidant activities of bamboo *Phyllostachys nigra* var. Henonis leaf extract *in vitro*. J Agric Food Chem. 2000; 48:3170-6.
22. Lou DD, Zhang Y, Wu XQ, Qi JJ, Zhuo YX. Application of antioxidant of bamboo leaves (AOB) in Weixin western sausages. Chin. Food Fermentat Ind. 2004; 30:13-7.
23. Tawaha K, Alali FA, Gharaibeh M, Mohammad M, El-Elimat T. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem. 2007; 104:1372-8.
24. Othman A, Ismail A, Ghani NA, Adenan I. Antioxidant capacity and phenolic content of cocoa beans. Food Chem. 2007; 100:1523-30.

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