Chiang Mai J. Sci. 2016; 43(1) : 80-88
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Ameliorating Reactive Oxygen Species Induced *in vitro* Lipid Peroxidation in Liver, Carbohydrate and DNA Damage by *Dendrocalamus hamiltonii* Different Leaf Extracts

Arvind Kumar Goyal [a,b], Sushil Kumar Middha [c], Talambedu Usha [c] and Arnab Sen*[a]

[a] Molecular Genetics Laboratory, Department of Botany, University of North Bengal, Siliguri 734013, West Bengal, India.

[b] Bamboo Technology, Department of Biotechnology, Bodoland University, Kokrajhar 783370, Assam, India.

[c] Department of Biotechnology, MLA College for Women, Bangalore, Karnataka, India.

*Author for correspondence; e-mail: senarnab_nbu@hotmail.com

Received: 6 December 2013
Accepted: 15 August 2014

ABSTRACT

Dendrocalamus hamiltonii has been widely used as a traditional remedy for several ailments in India and Taiwan. In the present study, we aimed to evaluate biomembrane lipids, carbohydrate, DNA damage, biochemical constitutes and *in vitro* antioxidant activity of aqueous, methanolic and acetone leaf extracts of *D. hamiltonii*. This study shows that methanol was the most effective solvent to extract phenolic (595.22±0.06 µg GAE/g), flavonoids (827.20±0.01 µg QE/g), flavonols (9.41±0.14 µg QE/g) and proanthocynidin (233.58±0.02 µg Cathechin equivalent/g) compounds as compared to water and acetone. Ferric reducing antioxidant power was comparatively higher for methanolic extract than the other two solvents. Therefore, methanolic extract was considered for further studies. Our findings showed that extract at 30 and 80 mg/mL significantly ($P<0.05$) protected 65 %, 66% of the lipid peroxidation in liver homogenate, respectively. However, the extract alleviated partially the DNA and carbohydrate damage. This is perhaps the first report to provide evidence that the leaf of *Dendrocalamus hamiltonii* is a potential source of natural antioxidants. The protective mechanism can be partially correlated to the radical scavenging property of *D. hamiltonii*.

Keywords: *Dendrocalamus hamiltonii*; antioxidants; lipid peroxidation; carbohydrate and DNA damage.

1. INTRODUCTION

Plants are considered to be a reserve of wide variety of free radical scavenging molecules like phenolics, flavonoids, vitamins etc. having higher antioxidative properties [1, 2] and are much safer as medicine in comparison to the synthetic chemicals [3]. In spite of having several benefits, the use of natural antioxidants is in most part, very restricted mainly due to lack of proper knowledge about their molecular composition

and dynamics. One such plant is *Dendrocalamus hamiltonii* Nees and Arnott ex Munro. *D. hamiltonii* belongs to the family Poaceae and subtribe Bambusinae. These are large plants having long, erect and brown culms reaching a height of about 24.4 m and diameter 17.8 cm and are covered with hairs [4]. Literature survey for their antioxidant properties and their damage reduction capability revealed no such studies on *D. hamiltonii*. However, *D. hamiltonii* has been found to be significant in lowering the blood glucose level and thus acts as a hypoglycemic medicinal plant [5].

Since ancient times regenerative medicinal products from plants are proven templates for natural antioxidants and have many interesting biological activities [6]. To determine the antioxidant activity, several studies have been conducted on different medicinal plants used in traditional medicine [7]. Overproduction of Reactive oxygen species (ROS) and declining of the antioxidant defense mechanism (both enzymatic and non-enzymatic) occurs simultaneously during oxidative stress leading to cell membrane disintegration and degradation of cellular components which ultimately propagate the development of diseases including cancer, cardiovascular diseases, liver damage, aging etc.[8].

In the present study antioxidant activity of aqueous (DAQE), methanolic (DME) and acetone (DAE) extract of *D. hamiltonii* leaves were investigated.

2. MATERIALS AND METHODS

2.1 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 bleomycin sulphate, magnesium chloride ($MgCl_2$), deoxyribose 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), ferrous sulphate, ($FeSO_4{\cdot}_7H_2O$), pyridine (C_5H_5N), ethanol (C_2H_5OH), disodium hydrogen phosphate (Na_2HPO_4), acetic acid (CH_3COOH), n-butanol, bromophenol blue, thiobarbituric acid and methanol were procured from Merck, Mumbai, India. Sodium dodecylsulphate and Calf thymus DNA from Sigma-Aldrich. Folin-Ciocalteu reagent from Sisco research laboratory, Mumbai, India. Aluminium chloride ($AlCl_3$) was obtained from Sd fine chemicals limited, Mumbai, India. All chemicals and solvents used in the present study were of analytical grade.

2.2 Plant Material and Extraction

D. hamiltonii leaves were collected from the Sukna Forests (Latitude N 26° 47 min 33° 4 sec and Longitude E 88° 21 min 55° 1 sec) Darjeeling district, West Bengal, India in the month of July and were authenticated by Bamboo taxonomist. A voucher specimen (SUK/KRR/D004) was deposited at Bambusetum, Kurseong Research Range, Sukna, Darjeeling, West Bengal [9].

The shade-dried leaves were powdered using mechanical grinder and were extracted (10 g each) 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) for 6 hours in each case at boiling temperature. The extracts obtained were evaporated under pressure at 50 °C to acquire the constant weight. The extracts were stored at -20 °C. Prior to use, the extracts were dissolved in double-distilled water (DDW) in desired concentrations.

2.3 Determination of Plant Extract Yield

The yield of evaporated extract based on dry weight was calculated according to the previous study by Goyal *et al.*[2].

2.4 Determination of Biochemical Constituents

The total soluble phenolics (TPC) were determined by Singleton and Rossi [3] method with slight modifications. Briefly, the leaf extract (0.5 mL) was mixed with 0.5 mL of Folin Ciocalteu reagent (diluted 1:1 with distilled water) and incubated for 5 min at room temperature (RT), then 1 mL of 2% Na_2CO_3 solution was added. After incubation at RT for 10 min, the absorbance was measured at 730 nm. The total flavonoid content (TFC) was determined according to Zhishen *et al.*[10] with minor modifications using quercetin as a standard. The method developed by Kumaran and Karunakaran [11] was used to estimate the total flavonols (TFLC) using quercetin as a standard. The total proantocyanidin contents (TPrC) was determined by using the protocol previously reported by Sun *et al.* [12].

2.5 *In vitro* Antioxidant Properties of the Extracts

2.5.1 Free radical scavenging activity (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 using the modified protocol reported by Goyal *et al.* [13].

2.5.2 Reducing power assay

The reducing power of the extracts was determined according to the method of Oyaizu [14].

2.5.3 Hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.* [15].

2.5.4 Lipid peroxidation radical scavenging

Inhibition of lipid peroxidation-induced in rat whole liver homogenate Lipid peroxidation was induced by Fe^{2+} EDTA system in the rat liver homogenate in the presence and absence of *D. hamiltonii* extracts to form thiobarbituric acid reacting substance (TBARS). The TBARS thus formed was determined by using the method of Okhawa *et al.* [16].

2.5.5 DNA damage assay

Bleomycin-Iron dependent DNA damage assay was done using the method described by Aruoma *et al.* [17] with slight modifications as Calf thymus DNA (0.2 mg/mL), bleomycin sulfate (0.05 mg/mL), $FeCl_3$ (25 µM) KH_2PO_4-K_2HPO_4 buffer (20 mM, pH 7.4) $MgCl_2$ (5 mM), ascorbic acid (240 µM) and, *D. hamiltonii* extracts were mixed. The reaction mixtures were incubated for 1 h at 37 °C. After incubation, 0.1 mL EDTA (0.1 M), 1 mL TBA (1%) and 1 mL TCA (2.8%) were added to the reaction mixture and incubated for 20 min. at 90 °C. The absorbance of the mixture was measured at 532nm.

2.5.6 Carbohydrate damage assay

The carbohydrate damage assay was measured by deoxyribose method with a slight modification [18].The reaction mixture contained 0.2 mL of *D. hamiltonii* extract, phosphate buffer, pH 7.4 (20 mM KH_2PO_4-

K_2HPO_4) deoxyribose; ascorbic acid; hydrogen peroxide; EDTA and $FeCI_3$ was incubated at 37 °C for 1 hour. Then 1 mL of 2.8 % TCA was added and the reactivity was developed by adding 1 mL of TBA (l%) followed by heating at 100 °C for 15 min. When the mixture was cool, the absorbance at 532 nm was measured against appropriate blank. All the tests were replicates of six.

2.6 Statistical Analysis

All the tests were performed in six replicates. And the 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 $p < 0.01$.

3. RESULTS AND DISCUSSION

In this study, the antioxidant activities of different fractions of *D. hamiltonii* leaf were evaluated.

Total yields of the aqueous, methanol and acetone fractions of *D. hamiltonii* leaf were found to be 9.04%, 6.98% and 4.86% respectively. The highest yield was obtained when extracted with water followed by methanol and acetone.

The total phenolic, flaovonoid, flavonol and proanthocyanidin contents of DME, DAQE and DAE are depicted in Table 1. It was elucidated that the DME showed higher phenolic content than the DAQE and DAE in µg/mg as gallic acid equivalent i.e. DME (395.22 ± 0.06) > DAQE (285.26 ± 0.01) > DAE (178.38 ± 0.02). DME also exhibited high content of flavonoid, flavonol and proanthocyanidin (Table 1). The total polyphenolic content with regards to different solvents used for extraction was as follows: DME > DAQE > DAE. The higher polyphenolic content in DME might be due to the difference in the polarity of the solvent and the potential of methanol to release the bound polyphenols present in the cell wall of the plants. Thus, methanol proved to be a suitable solvent for extraction of the plant [19].

Table 1. Polyphenol contents of the aqueous, methanol and acetone fractions of *D. hamiltonii* leaf. (n=6, X ± SEM).

	DAQE	DME	DAE
TPC[a]	285.26±0.01	392.13±0.06	178.38±0.02
TFC[b]	277.20±0.003	827.2±0.007	187.20±0.001
TFLC[c]	0.204±0.01	9.41±0.14	1.02±0.07
TPrC[d]	53.59±0.03	233.58±0.02	49.51±0.04

[a]Total phenol content analysed as gallic acid equivalent (GAE) mg/g of extract; [b]Total flavonoid content analysed as quercetin equivalent (QE) mg/g of extract; [c]Total flavonol content analysed as quercetin equivalent (QE) µg/g of extract; [d]Total proanthocyanidin content analysed as cathechin equivalent µg/g of extract. DAQE, Water fraction; DME, Methanolic fraction; DAE, Acetone fraction.

The DPPH scavenging activity of different leaf extracts compared to the standard ascorbic acid is shown in Figure 1. The radical scavenging activity, using a DPPH generated radical, was tested with different sample extracts. It was observed that the DME exhibited the highest radical scavenging activity IC_{50} (158 µg/mL), followed by DAQE IC_{50} (121 µg/mL) (Figure 1). However, DAE extract showed the weakest activity IC_{50} (51 µg/mL). The results of DPPH scavenging activity reveals that DME contains powerful inhibitor compounds compared to DAQE and DAE, which acts as a potential antioxidants and thus scavenge the DPPH radicals to form stable reduced DPPH molecules.

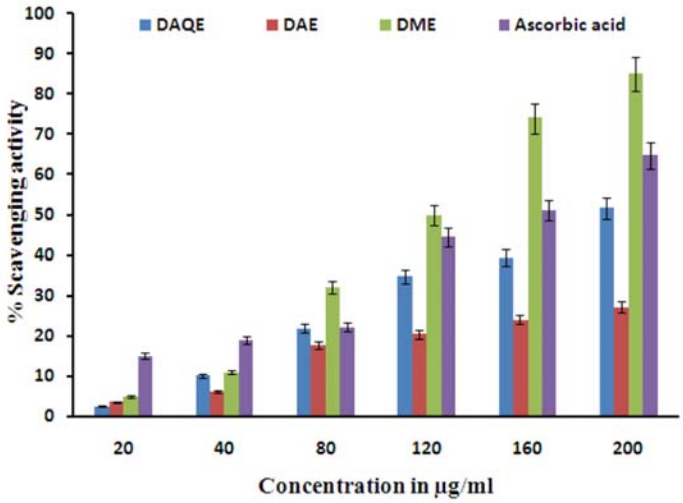

Figure 1. DPPH scavenging activity of *D. hamiltonii* different leaf extracts compared to standard ascorbic acid.

Figure 2 depicts the reductive capability ofthe plant extracts compared to ascorbic acid. The reducing power of *D. hamiltonii* leaf extract was found to be notable, which increased gradually with a rise in concentration. As illustrated in figure 3, Fe^{3+} was transformed to Fe^{2+} in the presence of extract and ascorbic acid, which is a measure of reductive capability. From the figure it is evident that even a low dose of the extract had maximum reducing power, when compared with standard.

The reducing power of the extract was also found to be substantial. The reducing power in different leaf extracts of *D. hamiltonii* ranged from 0.021 to 0.456 abs at concentrations ranging from 20-200 µg/mL. The reducing capacity of extracts was as follows: DME > DAQE >DAE (Figure 2). In the case of reducing power assay, the transformation of Fe^{3+} to Fe^{2+} in the presence of either the extract or the standard (ascorbic acid) is a measure of reducing capability. It is found that the reducing power of the extract increases with increase in concentration. The highest reducing ability was noted in DME (0.456) followed by DAQE (0.417) and DAE (0.102) compared to ascorbic acid (0.041) as standard. The absorbance of DAE and ascorbic acid on one hand and DME and DAQE on the other hand followed similar trend at 20 µg/mL and 40 µg/mL. DME showed the highest antiradical scavenging and FRP as specified

by lower IC_{50} value indicative of higher antioxidative capacity. Similar type of results was also reported by the authors on other bamboo species [6, 20]. It is the presence of these polyphenols in the plants which are responsible for their antioxidant activity along with some of their pharmacological effects [21]. Moreover, the higher the inhibitory action, more powerful is the antioxidant activity [22, 23].

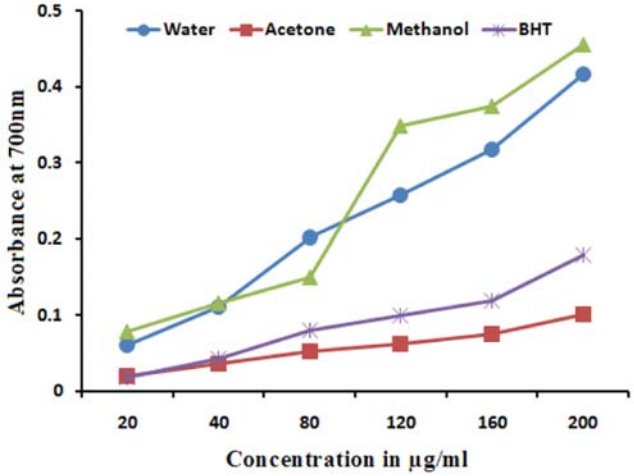

Figure 2. Reducing power assay of *D. hamiltonii* different leaf extracts compared to standard ascorbic acid at 700nm.

Figure 3 depicts the H_2O_2 scavenging activity of DME, DAQE and DAE. A high amount of H_2O_2 scavenging was found. H_2O_2 scavenging activity of *D. hamiltonii* leaf extracts are depicted in Figure 3. Hydrogen peroxide although being a weak oxidising agent has the potential to a few enzymes directly. In the presence of redox active transition metals Fe^{2+} and Cu^{2+}, H_2O_2 is transformed to hydroxyl radical which might be the key to its toxic effect [24]. Thus the amount of H_2O_2 accrue in the cells should be monitored. Figure 3 illustrates the leaf extract are a good scavenger of H_2O_2.

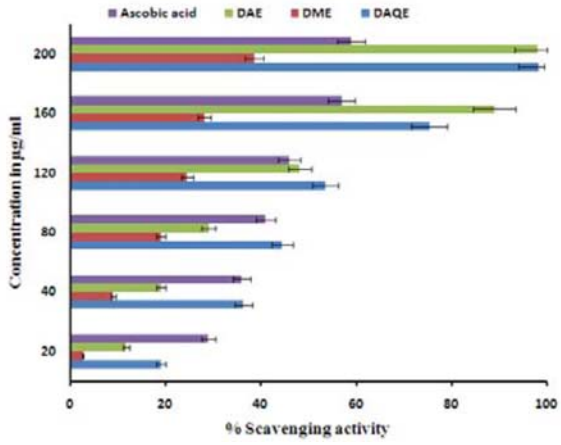

Figure 3. H_2O_2 scavenging activity of *D. hamiltonii* different leaf extracts compared to standard ascorbic acid.

Three additional *in vitro* test systems viz. Lipid peroxidation assay, DNA and carbohydrate damage assay were used to access the antioxidant properties of the extracts in this study. However DAQE and DAE didn't show any significant results (data not shown), the effects of various concentrations of DME (10-80 mg/mL) on Lipid peroxidation, carbohydrate and DNA damage are shown in Table 2.

Table 2. *In vitro* profiles of *D. hamiltonii* methanolic leaf extract in different antioxidant test systems. (n=6, X ± SEM).

Concentration (mg/mL)	Assays		
	DNA damage	Carbohydrate damage	Lipid peroxidation (I%)
Control	$0.197\pm0.01_{a}$	$0.202\pm0.019_{a}$	00.00
10	$0.024\pm0.001_{b}$	$0.161\pm0.02_{b}$	$27.87\pm2.14_{a}$
20	$0.012\pm0.001_{c}$	$0.101\pm0.01_{c}$	$33.65\pm0.30_{b}$
30	$0.009\pm0.00_{d}$	$0.093\pm0.001_{c}$	$64.95\pm0.70_{c}$
40	$0.018\pm0.001_{c}$	$0.099\pm0.001_{c}$	$51.94\pm1.01_{d}$
80	$0.017\pm0.001_{eb}$	$0.083\pm0.001_{c}$	$66.57\pm0.90_{e}$

*Values with different lower case letters in the same line are significantly different at $P<0.05$.

As indicated in the study, DME could inhibit lipid peroxidation effectively than that of the control. Among all the concentrations of *D. hamiltonii*, the concentration of 30 and 80 mg/mL, exhibited the highest activity up to 60% (Table 2). A high concentration of H_2O_2 used to induce the lipid peroxidation DNA fragmentation in liver (0.44 mmol/L) homogenate to overcome the possible higher antioxidant status of the liver cell [25]. DME effectively and dose dependently protected the lipid peroxidation in the liver homogenate. So, it can be inferred that due to high contents of antioxidants in DME, it's extract showed high antioxidant activity on lipid peroxidation at concentration of 3% and 8%. Decrease at 4% in protective effect might be due to the dual behavior of the same polyphenol compounds as antioxidants and prooxidants, depending on the source of free radical and concentration.

The effect of various concentrations of DME on DNA damage has been depicted in Table 2. From the table it is clear that the maximum protection was observed at the concentration of 30mg/mL followed by 20mg/mL, though protection was observed in all the concentrations. However, at a concentration of 40mg/mL the trend of protective effect was wrecked. Increase in the oxidative damage to deoxyribose by the plant extracts might be possibly due to its interaction with the iron ions present in the reaction mixture [26]. But, *D. hamiltonii* leaf extract failed to stimulate the oxidative damage to deoxyribose. These results illustrate that *D. hamiltonii* leaf has a strong protective effect on DNA damage.

In case of carbohydrate damage assay the antioxidant activity was found to be directly proportional to the concentration of the extract used (10-80mg/mL) (Table 2). However, protective effect of *D. hamiltonii* leaf increases slightly at 30mg/mL while significantly at 80mg/mL. The protective activity increased along with increase in concentration expect at the concentration of 40mg/mL where it reduced slightly.

The possible explanation for this behavior might be the dual behavior (as antioxidants and prooxidants) of the polyphenol compound present depending upon the free radicals source and concentration [13].

There are many sources of antioxidants and this would also be one of them, whose antioxidant status was not scientifically revealed. This may be the first report in case of *D. hamiltonii* with regards to DNA damage, carbohydrate damage and lipid peroxidation assays, although this type of work has been reported in other plant species [23].

4. CONCLUSION

To conclude, the results obtained in this study clearly demonstrate that the methanolic extract of *D. hamiltonii* leaf could completely protect the lipid peroxidation but was partially effective to alleviate the DNA damages. However, the concentrations of *D. hamiltonii* leaf selected in this *in vitro* experiment cannot be correlated with that required to render protection *in vivo*. Further detailed *in vivo* experimental models loaded with excess of iron are required to establish the nutraceutical role of this bamboo.

Authors futuristic approach include separation, identification of pure compounds from the extract.

ACKNOWLEDGEMENTS

Authors are thankful to Maharani Lakshmi Ammanni College, Bangalore for providing the interpretation software and research facilties for the work. We would also like to thank Mr. Prasanta Kr. Ghosh, Range Officer, Kurseong Research Range, Sukna, Darjeeling for providing necessary help, support and information. The authors are also obliged to the bamboo taxonomist, Mr. P.P. Paudyal, Consultant, Bamboo Mission, Sikkim for his help in identifying the species of bamboo.

REFERENCES

[1] Yim H.S., Chye F.Y., Liow M.L. and Ho C.Y., *Chiang Mai J. Sci.*, 2013; **40(1)**: 34-48.

[2] Goyal A.K., Middha S.K. and Sen A., *J. Nat. Pharm.*, 2010; **1**: 40-45. DOI 10.410312229-5119.73586.

[3] Singleton V.L. and Rossi J.A., *Am. J. Enol. Viticult.*, 1965; **16**: 144-158.

[4] Goyal A.K., Middha S.K., Usha T., Chatterjee S., Bothra A.K., Nagaveni M.B. and Sen A., *Bioinformation*, 2010; **5**: 184-185.

[5] Padyana S., Zainab A., Ashalatha M. and Acharya S., *Ann. Biol. Res.*, 2011; **2**: 11-18.

[6] Goyal A.K., Basistha B.C., Sen A. and Middha S.K., *Funct. Plant Biol.*, 2011; **38(9)**: 697-701. DOI 10.1071/FP11016.

[7] Kaewnarin K., Niamsup H., Shank L. and Rakariyatham N., *Chiang Mai J. Sci.*, 2014; **41(1)**: 105-116.

[8] Usha T., Akshya L., Kundu S., Nair R.K., Hussain I. and Middha S.K., *Int. J. Fund. Appl. Sci.*, 2013; **2**: 29.

[9] Goyal A.K., Ghosh P.K., Dubey A.K. and Sen A., *Int. J. Fund. Appl. Sci.*, 2012; **1**: 5-8.

[10] Zhishen J., Mengcheng T. and Jianming W., *Food Chem.*, 1999; **64**: 555-559. DOI 10.1016/S0308-8146(98)00102-2.

[11] Kumaran A. and Karunakaran R.J., *LWT - Food Sci. Technol.*, 2007; **40**: 344-352. DOI 10.1016/j.lwt.2005.09.0.11.

[12] Sun J.S., Tsuang Y.W., Chen I.J., Huang W.C. and Lu F.J., *Burns*, 1998; **24**: 225-231. DOI 10.1016/S0305-4179(97)00115-0.

[13] Goyal A.K., Middha S.K. and Sen A., *Free Rad. Antiox.*, 2011; **1**: 42-48. DOI 10.5530/ax.2011.2.9.

[14] Oyaizu M., *Jpn. J. Nutr.*, 1986; **44**: 307-314.

[15] Ruch R.J., Cheng S.J. and Klaunig J.E., *Carcinogenesis*, 1989; **10**: 1003-1008.

[16] Ohkawa H., Ohishi N. and Yagi K., *Anal. Biochem.*, 1979; **95**: 351-358. DOI 10.1016/0003-2697(79)90738-3.

[17] Aruoma O.I., Murcia A., Butler J. and Halliwell B., *J. Agric. Food Chem.*, 1993; **41**: 1880-1885. DOI 10.1021/jf00035a014.

[18] Halliwell B., Gutteridge J., Aruoma O.I., *Anal. Biochem.*, 1987; **165**: 215-219. DOI 10.1016/10003-2697(87)90222-3.

[19] Nadaroglu H., Demir Y. and Demir N., *Pharm. Chem. J.*, 2007; **41**: 13-18. DOI 10.1007/s11094-007-0089-2.

[20] Nagavani V. and RaghavaRao T., *Adv. Biol. Res.*, 2010; **4(3)**: 159-168.

[21] Nakbi A., Tayeb W., Grissa A., Issaoui M., Dabbou S., Chargui I. et al., *Nutr. Meta.*, 2010; **7**: 80-91. DOI 10.1186/1743-7075-7-80.

[22] Ajith T.A., *Indian J. Clin. Biochem.*, 2010; **25**: 67-73.

[23] Kilicgun H. and Dehen A., *Pharmacogn. Res.*, 2009; **1**: 417-420.

[24] Lapidot T., Walker M.D. and Kanner J., *J. Agric. Food Chem.*, 2002; **50(25)**: 7220-7225. DOI 10.1021/jf020615a.

[25] Mmushi T.J., Masoko P., Mdee L.K., Mokgotho M.P., Mampuru L.J. and Howard R.L., *Afr. J. Tradit. Complement. Altern. Med.*, 2010; **7**: 34-39.

[26] Kumoro A.C., Hasana M. and Singh H., *ScienceAsia*, 2009; **35**: 306-309.