Pharmacologia 3 (5): 138-143, 2012
ISSN 2044-4648 / DOI: 10.5567/pharmacologia.2012.138.143

Free Radical Scavenging and Lipid Peroxidation Inhibition Potential of Various Fractions of *Murraya paniculata*

[1]R. Paramaguru, [1]P. Silambu Janaki, [2]M. Bavani Eswaran, [2]Ch.V. Rao, [2]A.K.S. Rawat and [2]M. Vijayakumar
[1]Department of Pharmacology, SRM College of Pharmacy, Kattankulathur, Chennai-603 203, India
[2]Pharmacognosy and Ethnopharmacology Division, CSIR-National Botanical Research Institute, Lucknow-226 001, India

Abstract: Background: The high antioxidant activity compounds derived from plants are paramount demand for the pharmaceutical, food industries, mitigating their use as replacements for synthetic antioxidants which are playing significant role in preventing many diseases. This research study was designed to investigate the free radical scavenging and lipid peroxidation inhibition potential of 50% ethanolic extract of leaves of *Murraya paniculata* (MPET) and its various fractions of hexane (MPHF), chloroform (MPCF), ethyl acetate (MPEF) and methanol (MPMF). **Materials and Methods:** The free radical-scavenging potential of all fractions of *Murraya paniculata* extract were determined by β-carotene bleaching assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, Nitric oxide (NO), Hydroxyl radical (OH) and ferryl bipyridyl complex scavenging activity. Similarly, Lipid Peroxidation inhibition (LPO) using Thiobarbituric Acid Reactive Substances (TBARS) in rat liver homogenate and total antioxidant capacity. In addition to that integral anti oxidative capacity was studied by Photochemiluminescence assay (PCL). **Results:** In β-carotene bleaching assay, MPEF showed acme percentage of antioxidant activity, MPET showed moderate percentage of antioxidant activity, good radical scavenging activity at various concentrations (200-1000 μg mL^{-1}) against DPPH and the same showed modest scavenging activity against NO, OH, ferryl bipyridal complex and LPO inhibition. Further among the fractions, MPEF showed potent scavenging activity against DPPH, NO, OH, ferryl bipyridal complex and LPO Inhibition. MPMF showed moderate scavenging activity against DPPH, OH, NO Radicals. **Conclusion:** The 50% ethanolic extract of leaves of *Murraya paniculata* and its ethyl acetate fraction (MPEF) were very effective antioxidants.

Key words: Free radical scavenging activity, lipid peroxidation, *Murraya paniculata,* photochemiluminescence assay

INTRODUCTION

Oxidative stress is an imbalance between oxidants and antioxidants and it is caused by free radical damage (Ali *et al*., 2008). Abnormally high levels of free radicals which cause membrane damage due to the peroxidation of membrane lipids and protein glycation and the simultaneous decline of antioxidant defense mechanism leads to cell damage (Maritim *et al*., 2003; Tang *et al*., 2006). Oxidative stress is the important causative factor in various chronic diseases such as cardiovascular diseases, cardiac failure (Jha *et al*., 1995), alcohol induced liver disease, ulcerative colitis (Ramakrishna *et al*., 1997), cancer, several neurogenerative diseases, ageing process (Vijayakumar *et al*., 2006). Due to the over production of ROS such as O_2, H_2O_2 and OH induced by exposure to external oxidant substances or a failure in the defense mechanisms, damages to the cell structure, DNA, lipids and proteins (Valko *et al*., 2006). The concentration of ROS (reactive oxygen species) is modulated by the antioxidant enzymes and the non enzymatic scavengers (Saxena *et al*., 1993). Normal levels of antioxidants defense mechanism are not sufficient for the eradication of the free radical induced injury. Therefore administration of antioxidants from a natural origins have a promising role to play several anti oxidants of plant material are experimentally proved and widely used as a more effective agents against oxidative stress (Bhattacharya *et al*., 1997; Ilavarasan *et al*., 2001; Manonmani *et al*., 2002).

Murraya paniculata (L.) jack (family: Rutaceae) is an evergreen shrub or occasionally a small tree, usually 2 to 3 m in height but reaching 7.5 m and 13 cm in stem diameter. The leaves are pinnately compound with three to nine leaflets alternating on the rachis. The leaflets are darkgreen, stiff, ovate and smell of citrus when crushed. Pharmacological studies in the plant is comparatively low

Corresponding Author: M. Vijayakumar, Pharmacognosy and Ethnopharmacology Division, CSIR-National Botanical Research Institute, Lucknow-226 001, India Tel: +91522-2297977

and earlier studies reported leaves of *M. paniculata* possesses both stimulant and astringent properties (Parrotta, 2001) further more it is used to treat venereal diseases (Kinoshita and Firman, 1996). Moreover to date no antioxidant investigations have been reported in this plant. Most of the studies on *M. paniculata* activities have been done using crude extracts. In this current study, the crude extract was further fractionated and the antioxidant potentials of the various fractions determined using various well established antioxidant models. Therefore, the present study was undertaken to investigate the free radical scavenging potential of the ethanolic extract and various fractions of the leaves of *M. paniculata*.

MATERIALS AND METHODS

Plant material: Leaves of *M. paniculata* were collected from Botanical garden, National Botanical Research Institute, Lucknow, India during the month of November 2009. The plants were authenticated from Taxonomy Department of National Botanical Research Institute.

Extraction and fractionation: Air-dried powdered *M. paniculata* leaves (4 kg) were exhaustively extracted with 50% ethanol for 48 h, filtrated, concentrated on rotavapour (Buchi, USA) to get 72 g of the extract (MPET). The extract (50 g) was dissolved in distilled water (100 mL) and subjected to successive extraction with Hexane (2×100 mL), Chloroform (3×150 mL), Ethyl acetate (3X100 mL) and methanol (2×100 mL). The yield obtained after concentration as hexane fraction 5 g (MPHF), chloroform fraction 8.5 g (MPCF), Ethyl acetate fraction 12.5 g (MPEF), Methanol fraction 15 g (MPMF) were collected separately.

Estimation of total phenolic content: Total Phenolic Content (TPC) was analyzed by folin-ciocalteu colorimetric method using gallic acid as standard and expressed as mg g^{-1} gallic acid equivalent (Ragazzi and Veronese, 1973).

β-Carotene bleaching assay: The β-carotene bleaching assay was carried out as described by Emmons and Peterson (1999) and expressed as percentage of inhibition, relative to control Butylated Hydroxy Toluene (BHT).

Free radical scavenging activity using DPPH: The ability of the MPET to scavenge the free radicals was estimated by *in vitro* method using a stable nitrogen centered radical viz. DPPH (Blois, 1958). Scavenging of DPPH free radical determines the free radical scavenging capacity or antioxidant potential of the test sample which shows its effectiveness, prevention, interception and repair mechanism against injury in a biological system. Extract 0.05 mL dissolved in methanol was added to a methanolic solution of DPPH (100 μM, 2.95 mL) at different concentration (200-1000 μg mL^{-1}) and the absorbance was recorded at 517 nm. The above procedure was followed for all the fractions.

$$\text{DPPH scavenging activity (\%)} = \frac{(AC - AS)}{AC} \times 100$$

where, AC is the absorbance value of the control and AS is the absorbance value of the added test samples solution.

The scavenging activity (EC_{50} values) of extracts on DPPH radicals are shown in Table 2. Lower EC_{50} value indicates stronger ability of the extract to act as DPPH scavenger while the higher EC_{50} value indicates the lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction.

Nitric oxide scavenging activity: Nitricoxide (NO) radical scavenging activity was measured by using a spectrophotometer (Sreejayan and Rao, 1997). Sodium nitroprusside (5 mM, 1.5 mL) in phosphate buffered saline was mixed with different concentrations of MPET (200-1000 μg mL^{-1}) dissolved in methanol and incubated at 25°C for 30 min. A control without test compound but with equivalent amount of methanol was taken. 30 min after incubation, 1.5 mL of the incubation solution were removed and diluted with 1.5 mL of Griess reagent (1% sulphanilimide, 2% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilimide and subsequent coupling with naphthyl ethylene diamine was measured at 546 nm. The same procedure was followed for all the four obtained fractions individually.

Hydroxyl radical scavenging activity: The ability of the test extract to scavenge the OH radical was determined using ascorbic acid iron-EDTA (Ethylene diamine tetra aceticacid) model OH generating system. The standard reaction mixture consisted of 100 mM phosphate buffer, pH 7.4, 167 μM iron-EDTA complex, 0.1 mM EDTA, 2 mM ascorbic acid and 33 mM DMSO (Dimethyl sulphoxide) in a final volume of 3 mL. Iron catalyzed oxidation of ascorbic acid at 37°C was used to generate formaldehyde from DMSO. Appropriate controls, reaction mixtures without ascorbic acid, were maintained. MPET was

added to obtain final concentrations ranging from 50-400 μg mL^{-1} separately. D-mannitol (50 mm) was used as standard. The reaction was stopped by the addition of 1ml of ice cooled Trichloroacetic acid (17.5% w/v). The decrease in formaldehyde formation due to scavenging or decreased formation of OH was assayed spectrophotometrically (Nash, 1953). The above procedure was followed for all fractions.

Chelation of Fe^{2+} ions: The concentration of free ferrous ions (Fe^{2+}) was estimated using chelating agent 2,2'bipyridyl (Govindarajan *et al*., 2004b). The same procedure was followed for all fractions.

Assay of lipid peroxidation: Male Sprague Drawley rats (160-180 g) were purchased from the animal house of the Central Drug Research Institute, Lucknow, India. Rats were kept in the departmental animal house at 26±2°C and relative humidity 44-55%, light and dark cycles of 10 and 14 h, respectively for two weeks before the experiment. Animals were provided with rodent diet (Dayal, India) and water *ad libitum*.

Randomly selected rats were fasted overnight and were sacrificed by cervical dislocation, dissected and abdominal cavity was perfused with 0.9% saline. Whole liver was taken out and visible clots were removed and weighed amount of liver was processed to get a clear homogenate in cold phosphate buffered saline, pH 7.4 using glass Teflon homogenizer and filtered to get a clear homogenate. The degree of lipid peroxidation was assayed by estimating the Thiobarbituric Acid Reactive Substances (TBARS) by using the standard method (Govindarajan *et al*., 2004b) with minor modifications (Okahawa *et al*., 1979). Briefly, different concentrations of MPET (200-100 μg mL^{-1}) were added to the liver homogenate. Lipid peroxidation was initiated by adding 100 μL of this reaction mixture and taken in a tube containing 1.5 mL of 0.67% TBA in 50% acetic acid. The mixture was heated in a hot water bath at 85°C for 30 min and in a boiling water bath to complete the reaction. The intensity of the pink coloured complex formed was measured at 535 nm in a spectrophotometer (Perkin Elmer) the values of TBARS were calculated from a standard curve (absorption against concentration of Tetra ethoxy propane) and expressed as nmoles mg^{-1} of protein. Protein estimation was carried out by spectrophotometric method using Follin-ciocalteau reagent (Lowry *et al*., 1951). The same procedure was followed for the all fractions.

Total antioxidant capacity: Total antioxidant capacity was measured according to spectrophotometric method (Govindarajan *et al*., 2004b). Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

Photochemiluminescence assay: For the determination of the integral Antioxidative Capacity (AC) of the water soluble substances in MPET, the method of photochemiluminescence (PCL) was used (Govindarajan *et al*., 2004a). Apparatus used was photochem with standard kit ACW (Analitik jena AG), where the luminal plays a double role of photosensitizer as well as the radical detecting agent. Lyophilized extract was measured at 10 μg mL^{-1} concentration. A standard plot was plotted and the results were calculated in ascorbic acid equivalents (μmol g^{-1}). Same procedure was repeated for all the fractions.

Statistical analysis: All data on all antioxidant activity tests are the average of triplicate analyses. Linear regression analysis was used to calculate the IC_{50} values.

RESULTS AND DISCUSSION

Pro-oxidant conditions in the body dominate either due to the increased generation of the free radicals caused by excessive oxidative stress of the current life, or due to the poor scavenging/quenching in the body caused by depletion of the dietary antioxidants (Schulz *et al*., 2000; Dringen, 2000), further this condition leads to the oxidative stress which may result in tissue injury and subsequent diseases (Finkel and Holbrook, 2000) Free radical scavenging activity of the traditional medicine which may be involved in many diseases like inflammatory diseases, gastric ulcers, diabetes which could act by reducing the oxidative stress that takes place in cells undergoing these processes (Desmarchelier *et al*., 1999; Vijayakumar *et al*., 2005).

The β-Carotene bleaching test is the convenient test used to measure the capability of a compound or mixture to inhibit the oxidation of β-Carotene. In this method, free linoleic acid radical formed up on the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly saturated β-Carotene molecules. Thus β-Carotene loses its chromophore as their double bond loss by oxidation and the loss in orange colour intensity is measured spectrophotometrically (Singh *et al*., 2002). The presence of antioxidants in the extracts can hold back the extent of β-Carotene bleaching by acting on the lipid free radicals formed in the system (Emmons and Peterson, 1999). In present study Antioxidant activity (AOA) showed wide variation from 22.16 to 67.64% (Table 1). MPEF showed higher anti-oxidant activity and other fractions showed moderate activity.

Table 1: Total phenolic content (mg/g GAE dry extract) and antioxidant activity (%) of different fractions of *M. paniculata*

Fractions	TPC mg/g GAE	AOA (%)
MPET	172.61±5.32	51.31±1.92
MPHF	28.36±1.29	22.16±1.49
MPCF	49.36±4.83	38.72±2.32
MPEF	249.42±7.91	67.64±2.36
MPMF	92.61±4.31	48.72±2.32
BHT	-	55.92±3.11

Values are Mean±SEM for n = 3

Table 2: Free radical scavenging activity of 50% ethanolic extract of *M. paniculata*

Free radical method	IC_{50} values of sample μg mL^{-1}	Inhibition % of standard	
		AA 100 μM	TO 10 μM
DPPH	583.43	89.06	-
Hydroxyl radical	927.64	79.84	-
Nitric oxide	956.62	73.20	-
Ferryl-bipyridyl complex	961.53	93.10	-
Inhibition of lipid peroxidation	830.56	-	97.68

Ascorbic acid (AA) and Tocopherol (TO) were used as positive control

The extract MPET scavenged the DPPH radical in a dose dependent manner with IC_{50} value of 583.43 μg mL^{-1} (Table 2). The different fractions of *M. paniculata* showed a wide variation of IC_{50} ranging from 210.07-1097.32 μg mL^{-1} (Table 3). The MPEF was found most powerful DPPH scavenger as evidenced by low IC_{50} 210.07 μg mL^{-1}. The order of scavenging activity of different fractions was found to be MPEF>MPMF>MPCF >MPHF. This method is based on scavenging of the 1,1-Diphenyl-2-Picrylhydrazyl radical (DPPH) from the antioxidants which produces a decrease in absorbance at 517 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of colour (Blois, 1958). Such reactivity has been widely used to test the ability of the plant extracts to act as free radical scavengers.

The extract MPet also inhibited the Hydroxyl radical moderately in the dose dependent manner with IC_{50} value of 927.64 μg mL^{-1} (Table 2). The different fractions of *M. paniculata* also showed an inhibitory action against hydroxyl radical except MPHF, it was evidenced by their respective IC_{50} values (Table 3). MPET exhibited potent hydroxyl radical inhibition with IC_{50} value 417.32 μg mL^{-1}. The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (Halliwell and Gutteridge, 1989). When the hydroxyl radical reacts with polyunsaturated fatty acid moieties of the cell membrane phospholipids, ultimately acquiesce numerous carbonyl products are formed such as malondialdehyde (MDA). That is responsible for the DNA damage (Valentao *et al*., 2002).

The extract MPET also moderately inhibited NO in dose dependent manner (Table 2) with the IC_{50} being 956.62 μg mL^{-1}. In the case of fractions, MPEF showed potent inhibitory action with the IC_{50} being 423.71 μg mL^{-1} (Table 3). Further, MPMF showed moderate action with the IC_{50} being 906.32 μg mL^{-1} (Table 3). NO is a potent pleiotropic mediator of smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological system including neuronal messenger, vasodilation, antimicrobial and anti-tumor activities. Studies showed a vital role of NO in the pathogenesis of inflammation and pain, NO inhibitors have been shown to have beneficial effects on some aspects of the inflammation and tissue changes seen in inflammatory bowel diseases (Miller *et al*., 1993). Hence present results are the inventiveness for the further pharmacological study on the plant with special emphasis on anti-inflammatory activity.

MPET showed the moderate inhibition of the ferryl-bipyridyl complex with the IC_{50} being 830.56 μg mL^{-1} (Table 2). Among the fractions, MPEF showed potent inhibitory action with the IC_{50} being 427.81 μg mL^{-1}, MPMF showed moderate inhibitory action with the IC_{50} being 920.62 μg mL^{-1} (Table 3). This test is based on the measurement of change in Fe^{2+}/Fe^{3+} ratio. The above results represented that chromogen formation is inhibited in a dose dependent fashion.

Initiation of the LPO by ferrous sulphate takes place either through ferryl perferryl complex (Gutteridge, 1985) or through hydroxyl radical by fenton's reaction (Halliwell, 1978). Ferryl-perferryl complex can also initiate LPO in a similar manner as OH radical. Although it is less reactive than OH radical, in iron induced LPO, role of OH radical is not significant because little effect of tris and mannitol has been reported on this system (Tripathi and Sharma, 1998). MPET showed potent inhibition on MDA formation with the IC_{50} being 830.56 μg mL^{-1} (Table 2). On reference to Table 3, MPEF inhibited MDA formation in concentration dependent manner and the IC_{50} value of inhibition of the lipid peroxidation capacity decreased in the following order: MPEF (346.32 μg mL^{-1}) > MPMF (782.36 μg mL^{-1}) > MPCF (989.67 μg mL^{-1}) > MPHF (996.32 μg mL^{-1}).

Photochemiluminescence (PCL) is an advanced technique for the estimation of the total radical scavenging activity. In the chemiluminescence analysis radicals are generated photochemically by UV irradiation of a photosensitizer compound. These radicals are partially eliminated from the sample by reaction with antioxidants out of the sample. The remaining radicals are

Table 3: Free radical scavenging activity of different fractions of *M. paniculata*

	IC_{50} values of fractions(µg mL^{-1})				Inhibition % of standard	
Free radical method	MPHF	MPCF	MPEF	MPMF	AA100 µM	TO 10 µM
DPPH	1097.32	712.36	210.07	426.37	89.06	-
Hydroxyl radical	1226.36	992.63	417.32	890.32	79.84	-
Nitric oxide	1263.34	1098.73	423.71	906.32	93.10	-
Ferryl-Bipyridyl complex	1492.98	1261.47	427.81	920.62	93.10	-
Inhibition of lipid peroxidation	996.32	989.67	346.32	782.36	-	97.68

Ascorbic acid (AA) and Tocopherol (TO) were positive controls

Table 4: The integral antioxidative capacity of Ethanolic extract and its various fractions of *M. paniculata* by photochemiluminescence assay

Fractions (20 µg)	n moles ascorbic acid/gram equivalents
MPET	2.09
MPHF	0.97
MPCF	1.14
MPEF	3.69
MPMF	1.75

quantified by luminescence generation as a result of a chemical reaction with a detecting chemical in the detection unit. The PCL method is based on a photo-induced chemiluminescence accompanied by antioxidant inhabitable auto-oxidation of luminol. The luminol is a photosensitizer generating superoxide radicals and also a chemiluminogenic probe for free radicals (Govindarajan *et al*., 2004b). The integral antioxidative capacity of the extract and fractions are reported as ascorbic acid equivalents. The value for MPET was found to be 2.09 nmoles ascorbic acid/gram equivalents (Table 4). Along with fractions, MPEF showed the effective antioxidative capacity and its value was found to be 3.69 nmoles ascorbic acid/gram equivalents, MPMF showed moderate antioxidative capacity and its value was found to be 1.75 nmoles ascorbic acid/gram equivalents.

The total antioxidant capacity was calculated based on the formation of the Phosphomolybdenum complex which was measured spectrophotometrically at 695 nm. The total antioxidant capacity of MPET was found to be potent and the value obtained was 270.20 nmoles ascorbic acid/gram equivalents. Among the fractions, MPEF was found to be effective and the value obtained was 398.15 nmoles ascorbic acid/gram equivalents. Further, the values for remaining fractions were found to be MPMF (207.18), MPCF (142.72), MPHF (102.61) and all the values were expressed in nmoles ascorbic acid/gram equivalents respectively. Thus establishing the extract as antioxidant.

CONCLUSION

Our study demonstrated that *M. paniculata* extract and its ethylacetate fraction have good potential of scavenging activity. Although, subsequent study is required to evaluate the individually active constituents which responsible for antioxidative activity. Our work, for the first time, revealed the antioxidant potential of *M. paniculata* and this could be the initiative for the further pharmacological investigations on this plant.

REFERENCES

Ali, S.S., N. Kasoju, A. Luthra, A. Singh, H. Sharanabasava, A. Sahu and U. Bora, 2008. Indian medicinal herbs as sources of antioxidants. Food Res. Int., 41: 1-15.

Bhattacharya, S.K., K.S. Satyan and S. Ghosal, 1997. Antioxidant activity of glycowithanolides from *Withania somnifera*. Int. J. Exp. Biol., 35: 236-239.

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature, 181: 1199-1200.

Desmarchelier, C., R.L. Romao, J. Coussio and G. Ciccia, 1999. Antioxidant and free radical scavenging activities in extracts from medicinal trees used in the Caatinga region in Northeastern Brazil. J. Ethnopharmacol., 67: 69-77.

Dringen, R., 2000. Glutathione metabolism and oxidative stress in neurogeneration. Eur. J. Biochem., 267: 4903-4918.

Emmons, G.L. and D.M. Peterson, 1999. Antioxidant activity and phenolic content of oat groats and hulls. Cereal Chem., 76: 902-906.

Finkel, T. and N.J. Holbrook, 2000. Oxidants, oxidative stress and the biology of ageing. Nature, 408: 239-247.

Govindarajan, R., M. Vijayakumar, C.V. Rao, V. Kumar, A.K.S. Rawat and P. Pushpangadan, 2004a. Action of *Asparagus racimosus* against streptozotocin-induced oxidative stress. Nat. Prod. Sci., 10: 177-1781.

Govindarajan, R., M. Vijaykumar, C.V. Rao, A. Shirwaikar, A.K.S. Rawat, S. Mehrotra and P. Pushpangadan, 2004b. Antioxidant potential of *Anogeissus latifolia*. Biol. Pharm. Bull., 27: 1266-1269.

Gutteridge, J.M.C., 1985. Age pigments and free radicals: Fluorescent lipid complexes formed by iron and copper containing proteins. Biochem. Biophys. Acta, 834: 144-148.

Halliwell, B., 1978. Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates: Is it a mechanism for hydroxyl radical production in biochemical systems? FEBS Lett., 92: 321-326.

Halliwell, B. and J.M.C. Gutteridge, 1989. Free Radicals, Aging and Disease. In: Free Radicals in Biology and Medicine, Halliwell, B. and J.M.C. Gutteridge (Eds.). Clarendon Press, Oxford, UK., pp: 416-508.

Ilavarasan, R., S. Mohideen, M. Vijayalakshmi and G. Manonmani, 2001. Hepatoprotective effect of *Cassia angustifolia* Vahl. Ind. J. Pharm. Sci., 63: 504-507.

Jha, P., M. Flather, E. Lonn, M. Farkouh and S. Yusuf, 1995. The antioxidant vitamins and cardiovascular disease: A critical review of epidemiologic and clinical trial data. Ann. Internal Med., 123: 860-872.

Kinoshita, T. and K. Firman, 1996. Highly oxygenated flavonoids from *Murraya paniculata*. Phytochemistry, 42: 1207-1210.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.I. Randall, 1951. Protein determination using Folin-ciocalteau reagent. J. Biol. Chem., 193: 438-448.

Manonmani, G., K. Anbarasi, K. Balakrishna, G. Veluchamy and C.S. Shyamala Devi, 2002. Effect of *Terminalia arjuna* on the antioxidant defense system in alloxan induced diabetes in rats. Biomedicine, 22: 52-61.

Maritim, A.C., R.A. Sanders and J.B. Watkins, 2003. Diabetes, oxidative stress and antioxidants: A review. J. Biochem. Mol. Toxicol., 17: 24-38.

Miller, N.J., C. Rice-Evans, M.J. Davies, V. Gopinathan and A. Milner, 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci., 84: 407-412.

Nash, T., 1953. The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem. J., 55: 416-421.

Okahawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 269: 337-341.

Parrotta, J.A., 2001. Healing Plants of Peninsular India. CABI Publishing, New York, ISBN: 9780851995014, pp: 917.

Ragazzi, E. and G. Veronese, 1973. Quantitative analysis of phenolic compounds after thin layer chromato graphic separation. J. Chromatogr., 77: 369-375.

Ramakrishna, B.S., R. Varghese, S. Jayakumar, M. Mathan and K.A. Balasubramanian, 1997. Circulating antioxidants in ulcerative colitis and their relationship to disease severity and activity. J. Gastroenterol. Hepatol., 12: 490-494.

Saxena, A.K., P. Srivastava, R.K. Kale and N.Z. Baquer, 1993. Impaired antioxidant status in diabetic rat liver. Biochem. Pharmacol., 45: 539-542.

Schulz, J.B., J. Lindnau, J. Seyfriend and J. Dichgans, 2000. Glutathine oxidative stress and neurodegeneration. Eur. J. Biochem., 267: 4904-4911.

Singh, R.P., C.K.N. Murthy and G.K. Jayaprakasha, 2002. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. J. Agric. Food Chem., 50: 81-86.

Sreejayan, D. and M.N.A. Rao, 1997. Nitricoxide scavenging by curcuminoids. J. Pharm. Pharmacol., 49: 105-107.

Tang, L.Q, W. Wei, L.M. Chen and S. Liu 2006. Effects of berberine on diabetes induced by alloxan and a high-fat/high-cholesterol diet in rats. J. Ethnopharmacol., 108: 109-115.

Tripathi, Y.B. and M. Sharma, 1998. Comparison of the antioxidant action of the alcoholic extract of *Rubia cordifolia* with rubiadin. Indian J. Biochem. Biophys., 35: 313-316.

Valentao, P., E. Fernandes, F. Carvalho, P.B. Andrade, R.M. Seabra and M.D.L. Bastos, 2002. Antioxidant activity of *Hypericum androsaemum* infusion: Scavenging activity against superoxide radical, hydroxyl radical and hypochlorous acid. Biol. Pharm. Bull., 22: 1320-1323.

Valko, M., C.J. Rhodes, J. Moncol, M. Izakovic and M. Mazur, 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact., 160: 1-40.

Vijayakumar, M., R. Govindarajan, G.M. Rao, Ch.V. Rao, A. Shirwaikar, S. Mehrotra and P. Pushpangadan, 2005. Free radical scavenging and lipid peroxidation inhibition potential of *Hygrophila auriculata*. Nat. Prod. Sci., 11: 22-26.

Vijayakumar, M., R. Govindarajan, G.M. Rao, Ch.V. Rao, A. Shirwaikar, S. Mehrotra and P. Pushpangadan, 2006. Action of *Hygrophila auriculata* against streptozotocin-induced oxidative stress. J. Ethnopharmacol., 104: 356-361.