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IN VITRO ANTIOXIDANT ACTIVITY OF *DIOSCOREA TOMENTOSA* KOEN EX. SPRENG

K. Rajalakshmi, V. R. Mohan*

Ethnopharmocology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin-628008, Tamil Nadu, India.

ABSTRACT

The antioxidant activity of *Dioscorea tomentosa* tuber in methanol extracts are assessed using different models like DPPH, hydroxyl, superoxide, ABTS and reducing power. At different concentrations. Total phenolic and flavoinid contentsan assessed by the standard methods. Methanol extract shows considerable in vitro antioxidant and free radical scavenging activities in a dose dependent manner when compared to the standard antioxidant (ascorbic acid/trolox). The results justify the therapeutic applications of the plant in the indigenous system of medicine, augmenting its therapeutic value.

Keywords: Flavonoid, DPPH, ABTS, Reducing power.

INTRODUCTION

Oxidative stress is responsible for many of today's disease that results from an imbalance between formation and neutralization of pro oxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules Such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These charges contribute to cancer, atherosclerosis, cardiovascular disease ageing and antiinflammatory diseases [1, 2]. Antioxidants may act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and or activations of antioxidative defense enzyme system to suppress the radical damages in biological systems [3, 4]. Antioxidants thus play an important role in the protection of human body against damage by reactive oxygen species [5, 6]. Therefore, inhibition of free radical induced oxidative damage by supplementation of antioxidants has become an alternative

therapeutic strategy for reducing the risk of there diseases. Recently much attention has been directed towards the development of ethnomedicines. With strong antioxidant properties but low cytotoxicities [7, 8].

Yam is the common name of rhizomes of a perennial plant from the genus *Dioscorea* in the family Dioscoreaceae. It is estimated that there are more than 600 species of Yams in the world. Yam has been widely used for the enhancement of health in oriental countries and traditionally considered as a superior Chinese herb to improve gastrointestinal function [9, 10]. Recently Wang *et al* [10] have demonstrated that Yam- rich diets enhance caecal fermentation and proliferation of bifidobacteria Yam has also been implicated in the promotion of the health of postmenopausal women. The wild Mexican Yam has been marketed for treating postmenopausal syndromes [11]. Muthukumarasamy *et al* [12] have reported that ten grams of the boiled stem peeled tuber of *Dioscorea tomentosa* is given to children once a day for three days to get relief from bowel complaints by the Palliyar tribe of Grizzled Giant Squirrel wildlife Sanctuary, Western Ghats, Srivilliputhur, Tamil Nadu, India. Taking into consideration of the medicinal importance of this plant, the methanol extract of tuber of *Dioscorea tomentosa* were analyzed for their *in vitro* antioxidant activity using different models viz., DPPH, hydroxyl, superoxide and ABTS.

MATERIALS AND METHODS

The tuber of *Dioscorea tomentosa* Koen ex. Spreng were collected from Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of Extracts

Ten grams of powdered tuber of *Dioscorea tomentosa* were extracted separately with methanol (100mL) in shaker for 24 h at room temperature. Extract was filtered through

Whatman filter paper. The filtrates were subjected to analysis for total phenolic, flavonoid contents and *in vitro* antioxidant activities.

Estimation of Total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method [13]. Samples (100µL) were mixed thoroughly with 2 mL of 2% Na_2CO_3. After 2 min. 100 µL of Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as gram of gallic equivalents per 100 gram of dry weight (g $100g^{-1}$DW) of the plant samples.

Estimation of Flavonoids

The flavonoids content was determined according to Eom *et al* [14]. An aliquot of 0.5ml of sample (1mg/mL) was mixed with 0.1ml of 10% aluminium chloride and 0.1ml of potassium acetate (1M). In this mixture, 4.3ml of 80% methanol was added to make 5mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

Radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H [15].

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method [15]. Briefly, an 0.1mm solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (125,250,500 &1000µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-

VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of [16]. Stock solutions of EDTA (1mM), FeCl3 (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1mL EDTA , 0.01mL of $FeCl_3$**,**0.1mL H_2O_2, 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (125,250,500 &1000µg/mL)dissolved in distilled water,0.33mL of phosphate buffer (50mM , pH 7.9), 0.1mL of ascorbic acid in sequence . The mixture was then incubated at 37^0c for 1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using the following equation

Hydroxyl radical scavenging activity= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski [17]. The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, P^H 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 ml NADH (0.936mM) solution, 1.0 mL extract of different concentration (125,250,500 &1000µg/ml), and 0.5 mL Tris – HCl buffer (16mM, P^H 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25^{o}C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the following equation

Superoxide radical scavenging activity= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Antioxidant Activity by Radical Cation (ABTS. +)

ABTS assay was based on the slightly modified method of Re *et al* [18]. ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of 100µL of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Reducing Power

The reducing power of the extract was determined by the method of Singh *et al* [19] with minor modification to Oyaizu [20]. 1.0 mL of solution containing 125,250,500 &1000µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH6.6) and potassium ferricyanide (5.0 mL, 1.0%): The mixture was incubated at 50^{o}C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5^{o}C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

RESULTS

Total phenolic content and total flavonoid content

The total phenol content and total flavonoid content of the *Dioscorea tomentosa* tuber extract were found to be 0.41g 100g^{-1}and 1.21g 100g^{-1} respectively.

DPPH radical scavenging activity

The effect of *Dioscorea tomentosa* tuber extracts and standard ascorbic acid on DPPH radical was compared and shown in Figure 1. The scavenging effect increases with the concentration of standard and samples. At 1000µg/mL concentration, of *Dioscorea tomentosa* possessed 56.16% scavenging activity on DPPH. All the concentration of *Dioscorea tomentosa* tuber showed higher activity than the standard ascorbic acid.

Hydroxyl radical scavenging activity

The scavenging effect of OH was investigated using the Fenton reaction and the results shown as the % inhibition of about 58.09 % but this is lower than the standard ascorbic acid (1000 µg/mL).Whose inhibition is 69.73%.

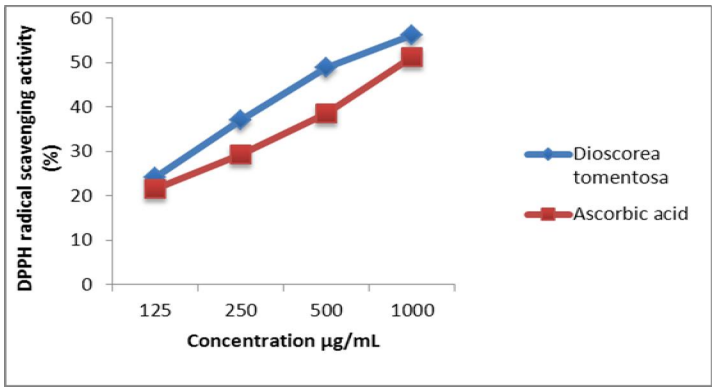

Figure 1
DPPH radical scavenging activity of methanol extract of *Dioscorea tomentosa*

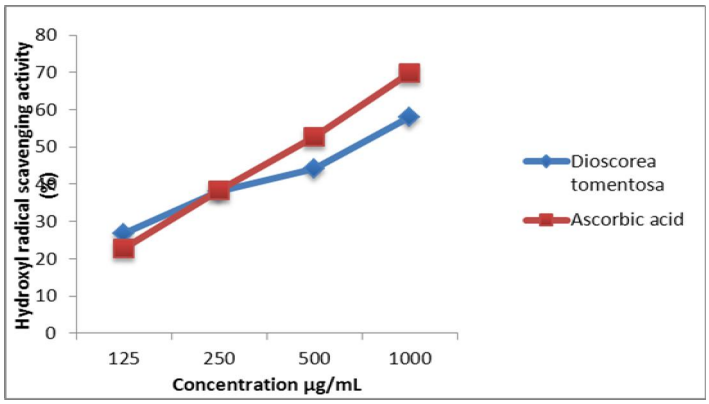

Figure 2
Hydroxyl radical scavenging activity of methanol extract of *Dioscorea tomentosa.*

Superoxide radical scavenging activity

The *Dioscorea tomentosa* tuber extracts were subjected to be superoxide scavenging assay and the results were shown in Figure 3. It indicates that *Dioscorea tomentosa*

(1000µg/mL) exhibited the maximum superoxide scavenging activity of 96.16%. Which is slightly higher than the standard ascorbic acid whose scavenging effect is 95.34%.

ABTS cation scavenging activity

The effect of *Dioscorea tomentosa* extracts and standard trolox on ABTS cation was compared and shown in Figure 5. The scavenging effect increased with the concentration of standard and samples. At 1000µg/mL concentration, *Dioscorea tomentosa* possessed 81.11% scavenging activity on ABTS. All the concentration of *Dioscorea tomentosa* showed higher activity than the standard trolox.

Reducing power

Reducing power of *Dioscorea tomentosa* was compared with the standard ascorbic acid. The reducing power increased with the increasing concentration. The reducing power of the samples was shown in Figure 5 and it was found to be in the following order: ascorbic acid 0.374-0.784% and *Dioscorea tomentosa* 0.483-0.774%.

IC_{50} value

The IC_{50} values of D*ioscorea tomentosa* tuber extracts and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging activity and trolox for ABTS were found to be 28.57µg/mL and 18.26µg/mL; 61.37µg/mL and 72.08µg/mL and 44.93µg/mL and 20.67µg/mL respectively (Figure 6).

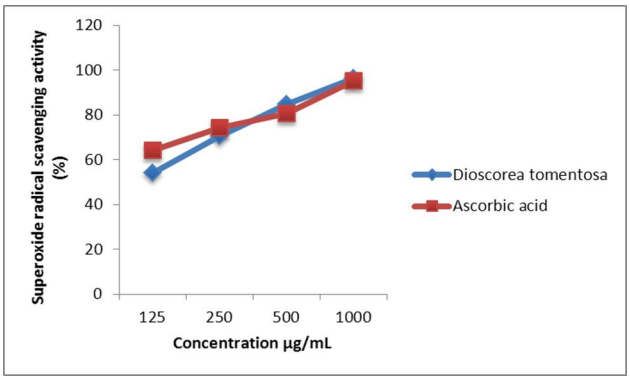

Figure 3
Superoxide radical scavenging activity of methanol extract of *Dioscorea tomentosa*

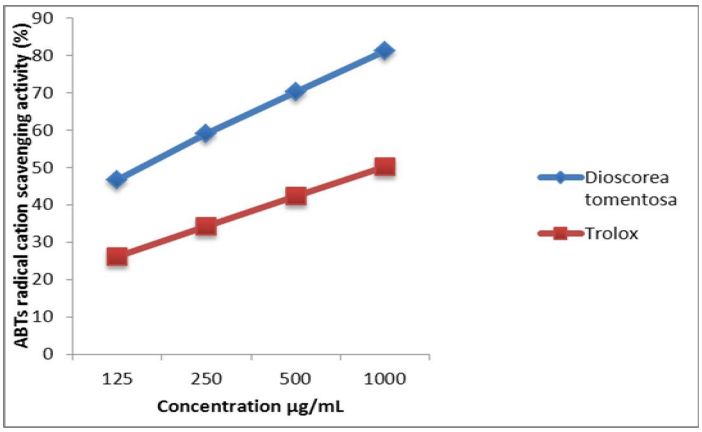

Figure 4
ABTS radical cation scavenging activity of methanol extract of *Dioscorea tomentosa*

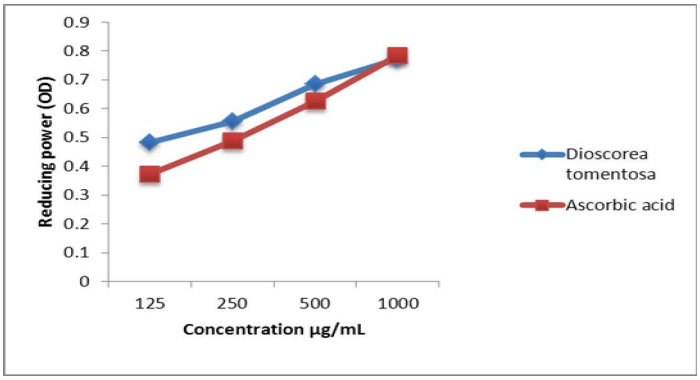

Figure 5
Reducing power ability of methanol extract of *Dioscorea tomentosa.*

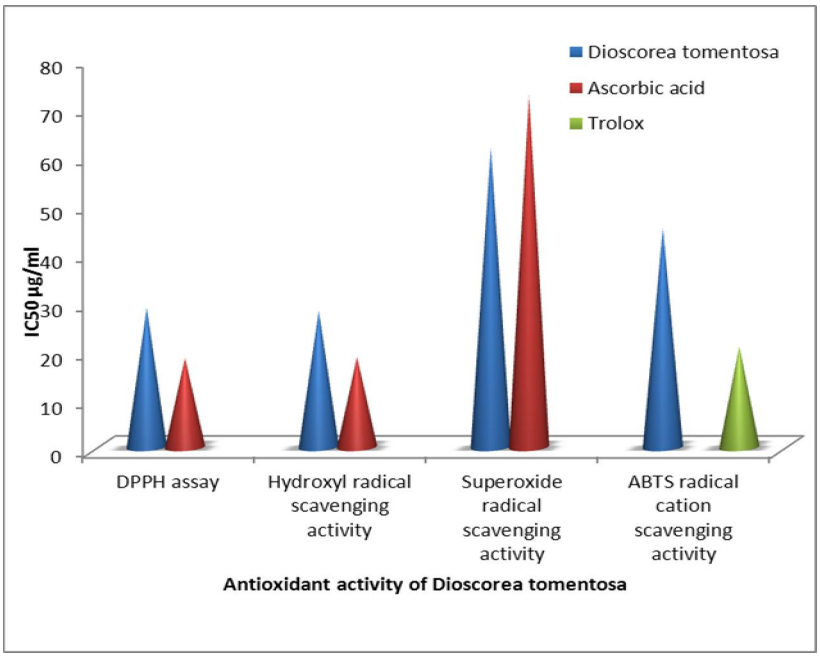

Figure 6
IC $_{50}$ values of methanol extract of *Dioscorea tomentosa*

DISCUSSION

Phenolics have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutageneic, and antiinflammatory activities [21, 22]. Plant derived natural products such as flavonoids, terpenoids, and steroids, etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity [23]. Flavonoids, the major group of phenolic compounds reported for their antimicrobial, antiviral and spasmolytic activity. Flavonoids ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals which highlights may of the flavonoid health-promoting functions in organism, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Flavonoids on the other hand, are potent water soluble

antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity [24, 25].

Dioscorea tomentosa tuber extract exhibited potent *in vitro* antioxidant activity in DPPH radical scavenging assay, superoxide radical scavenging assay, ABTS radical cation scavenging activity and reducing power in comparison to the known antioxidants such as ascorbic acid and trolox.

DPPH is a stable radical that has been used widely to evaluate the antioxidant activity of various natural products. The free radical scavenging activities of test compounds were examined based on their ability to bleach the stable free radical 2, 2, diphenyl-1 picryl hydroxyl (DPPH). Thus, the absorbance of DPPH solution decreases when kept in contact with antioxidant test sample and free radical scavenging activity is inversely proportional to the absorbance of DPPH solution [26]. In the present study, methanol extract showed significant inhibition of DPPH radical. The IC_{50} values were found to be 28.57 and 18.26 µg/mL respectively for methanol extract of *Dioscorea tomentosa* tuber and ascorbic acid.

The hydroxyl radical is the most reactive of the reactive oxygen species, and it induces severe damage in adjacent biomolecules. The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins [27]. In the present methanol extract of tuber of *Dioscorea tomentosa* exhibited concentration dependent scavenging activity against hydroxyl in a Fenton reaction system [28].

In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture [29] in the present study. In the present study, methanol extract showed free radical scavenging activity against superoxide ions. The percentage of scavenging was found to be 96.16. Which is slightly higher than standard ascorbic acid which exhibited 95.34%.

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain-breaking antioxidants [27]. The extract efficiently scavenged ABTS radicals generated by the reaction between 2, 2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) and ammonium persulphate. The activity was found to be increased in a dose-dependent manner from 49.56% to 81.11% at a concentration of 125-1000µg/mL. Therefore, the ABTS radical scavenging activity of methanol extract of *Dioscorea tomentosa* tuber indicates its ability to scavenge free radicals, thereby preventing lipid oxidation via chain breaking reaction.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability is generally associated with the presence of reductions, which breaks the free radical chain by donating a hydrogen atom [30]. In the present study, the tuber extract had reductive ability which increased with increasing concentration of the extract.

The methanol extract of *Dioscorea tomentosa* tuber exhibited strong antioxidant activity by scavenging DPPH, hydroxyl, superoxide anion and ABTS radical cation when compared with ascorbic acid and trolox. In addition to this, the *Dioscorea tomentosa* tuber contains a significant amount of total phenols and flavonoids, which play a major role in controlling oxidation. The phenolic and flavonoid content in the *Dioscorea tomentosa* may be responsible for its free radical scavenging activity. Overall, the plant extract is a source of natural antioxidant that can be important in disease prevention, health preservation and promotion of longevity promoter.

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REFERENCES

1. Rajan S, Mahalakshmi S, Deepa VM, Sathya K, Shajitha S, Thirunalasundari T: Antioxidant potentials of *Punica granatum* fruit rind extracts. Int J Pharma pharmacu Sci. 2011; 3: 82-88.
2. Chew AL, Jessice JJA, Sasidhanan S: Antioxidant and antibacterial activity of different parts of *Leucas aspera*. Asian nat. J Trop Biomed. 2012; **2**: 176-180.
3. Murphy MP, Holmgren A, Coran Larsson N, Halliwell B: Unraveling the biological roles of reactive oxygen species. Cell Metab 2011; 13: 361-366.
4. Venkatesh S, Deecaraman M, Kumar R, Shamri MB, Dada R: Role of reactive oxygen species in the pathogenesis of mitochondrial DNA (mt DNA) mutations in male infertility. Indian J Med Res. 2009; 129: 127-137.
5. Peng KT, Hsu WH, Shih HN, Hsieh CW, Huang TW, Hsu RWW: The role of reactive oxygen species scavenging enzymes in the development of septic loosening after total hip replacement. J Bone Joint Surg. 2011; 93B: 1201-1209.
6. Ling LV, Tan KB, Lin H, Chik CNC: The role of reactive oxygen species and autophasy in Sajingol-induced cell death. Cell death and Disease 2011; 2: 1-12.
7. Kala SMJ, Tresina PS, Mohan VR: Antioxidant, antihyperlipidaemic and antidiabetic activity of *Eugenia floccosa* Bedd leaves in Alloxan induced diabetic rats. J Baric Clin Pharmacy. 2012; 3: 235-240.
8. Shajeela PS, Kalpanadevi V, Mohan VR: Potential antidiabetic, hupolipidaemic and antioxidant effects of *Nymphaea pubercens* extract in Alloxan induced diabetic rats. *J* Appl pharmacu Sci. 2012; 2: 83-88.
9. Chen HL, Hong L, Lee JK, Huang CJ: The bone-protective effect of a Taiwanere Yam. (*Dioscorea alata* L. cv. Tainury No.2) in ovariectomised female BALB/c mice. *J Sci* Food Agri. 2009; 89: 517-522.
10. Wang CH, Tsai CH, Lin HJ, Wang TC, Chen HL: Uncooked Taiwanere Yam (*Dioscorea alata* L. CV. No.2) beneficially modulated the large bowel function and faecal microflora in BLAB/c mice. J Sci Food Agri. 2007; 87: 1374-1380.

11. Wu WH, Lin LY, Chung CJ, Jou HJ, Wang TA: Estrogenic effect of Yam injestion in healthy postmenopausal women. J. Am Coll Nutr. 2005; 24: 235-243.
12. Muthukumarasamy S, Mohan VR, Kumaresan S, Chelladurai V: Traditional medicinal of Palliyar tribe of Srivilliputhur in Antenatal and post- natal care of mother and child. Nat Prod Radi. 2004; 3: 422-426.
13. Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M: Free radical scavenging properties of wheat extracts. J Agri Food Chem. 2002; 50: 1619-1624.
14. Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu C, Cho DH: Far infra red ray irradiation stimulates antioxidant activity in *Vitis flexuosa* Thunb. Berries. Kor J Med Crop Sci. 2007; 15: 319-323.
15. Blois MS: Antioxidant determination by the use of a stable free radical. Nat. 1958; 181: 1199-1200.
16. Halliwell B, Gutteridge JMC, Aruoma OI: The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. Ana Biochem. 1987; 165: 215-219.
17. Robak J, Gryglewski RJ: Flavonoids are scavenges of superoxide anions. Biochem Pharmacol. 1988; 37: 837-841
18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans, E: Antioxidant activity applying an improved ABTS radical cation decolonization assay. Free Radical Bio Med. 1999; 26: 1231-1237.
19. Singh R, Singh B, Singh S, Kumar N, Kumar S, Arora S: Investigation of ethyl acetate extract/fractions of *Acacia nilotica* wild. Ex. Del as potent antioxidant. Rec Nat Prod. 2009; 3: 131-138.
20. Oyaizu M: Studies on product of browning reaction prepared from glucose amine. Jpn J Food Chem. 1986; 44: 307-315.

21. Saidu AN, Mann A, Onuegbu CD: Pytochemical screening and hypoglycemic effect of aqueous *Blighia sapida* root bark extract on normoglycemic albino rats. Bri J Pharmaceu Res. 2012; 2: 89-97.
22. Sasikumar JM, Maheshu V, Aseervatham GSB, Darsini DTP: *In vitro* antioxidant activity of Hedyotis corymbosa (L) Lam aerial plants. Indian J Biochem & Biophy. 2010; 47: 49-52.
23. Mithraja MJ, Johnson MA, Mahesh M, Paul ZM: Phytochemical studies on *Azolla pinnata* R.Br., *Marsilea minuta* L and *Salvinia molesta* Mitch. Asian Pac J Trop Biomed. 2011; 1: S26-S29.
24. Thirunavukkarasu P, Ramanathan T, Ramkumar L, Shanmugapriya R, Renugadevi G: The antioxidant and free radical scavenging effect of *Avicennia officinalis.* J Med Plant Res. 2011; 5: 4754-4758.
25. De Sorsa RR, Queiroz KC, Souza AC, Gurgueira SA, Augustro AC, Miranda MA *et al*: Phosphoprotein levels. MAPK activities and MFK appa B expression are affected by Fisetin. J Enzyme Inhib Med Chen. 2007; 22: 439-444.
26. Patil AP, Patil VR: Evaluation of *in vitro* antioxidant activity of seeds of blue and white flowered varieties of *Clitoria ternatea* Linn. Int J Pharmacy Pharmaceu Sci. 2011; 3: 330-336.
27. Sudha G, Sangeetha priya M, Indhu shree R, Vadivukkarasi S: Antioxidant activity of ripe pepino fruit (*Solanaum muricatum* Aiton). Int J Pharma Pharmaceu Sci. 2011; 3: 257-261.
28. Subhashini N, Nagarajan G, Kavimani S: *In vitro* antioxidant and anticholinesterase activities of *Garcinia combogia***.** Int J Pharma Pharmaceu Sci. 2011; 3: 129-132.
29. Basniwal PK, Suthan M, Rathone GS, Gupta R, Kumar V, Pareek A, Jain D: *In vitro* antioxidant activity of hot aqueous extract of *Helicteres isora* Linn. Fruits. Nat prod Radi. 2009; 8: 483-487.

30. Orech FO, Akenga T, Ochora J, Friis H, Aagaard Hansen J: Potential toxicity of some traditional leafy vegetables consumed in Nyang'oma division, western Kenya. Agri J Food Nutri Sci. 2005; 5: 1-13.

For Correspondence:
V. R. Mohan
Email: vrmohanvoc@gmail.com