IN VITRO ANTIOXIDANT POTENTIAL OF METHANOL EXTRACT OF *DIOSCOREA OPPOSITIFOLIA*

Paulpriya K and Mohan V.R.

Ethnopharmocology Unit, Reasearch Department of Botany, V.O. Chidambaram College, Tuticorin-628008, TamilNadu, India.
vrmohanvoc@gmail.com

ABSTRACT

The present study was undertaken to find the antioxidant activity of *Dioscorea oppositifolia* tuber. Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in cardiovascular and antiinflammatory diseases. The total phenolic, flavonoid content and the *in vitro* antioxidant activities of methanol extract of *Dioscorea oppositifolia* tuber which had been used in ethnomedicine were investigated. *In vitro* antioxidant activity was assessed by reducing power and the scavenging activity towards DPPH, hydroxyl, superoxide and ABTS radical cation. High radical scavenging activity was observed in the tuber of *Dioscorea oppositifolia*. The results suggest that, phenolic compounds are the significant contributors to the antioxidant activity. This study shows that, the studied plants are good sources of free radical scavenging compound and may explain their traditional medicinal application.

Key words: Antioxidant activity, flavonoid, DPPH, ABTS.

INTRODUCTION

Free radicals such as superoxide anion, hydroxyl and peroxyl radicals, which are produced in biological systems and foods are responsible for oxidation of cell lipids and DNA damage, and they may cause serious diseases e.g. cancer, coronary, arteriosclerosis, diabetes mellitus (Asokkumar *et al*., 2008) Dietary antioxidants may be effective in prevention of oxidative damage. Many scientists have focused on medicinal and edible plants to discover natural antioxidants since some synthetic antioxidants have toxic effects. In addition, natural antioxidants may have an important –role in protecting human health (Ajith and Janardhanan, 2002; Ali *et al*., 2008). Plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites, which are rich in antioxidant activity (Cai *et al*., 2003). Epidemiological and *in vitro* studies suggested that, plants are major constituent in antioxidant based drugs/formulations used for the prevention of complex diseases (Kuo *et al*., 2002).

Dioscorea oppositifolia L. belongs to the family Dioscoraceae. It is commonly known as "Vethalaivalli". The leaves, flowers, tender shoots and tubers of *Dioscorea oppositifolia* are used for cooling and demulcent. They are used in the form of decoction for leprosy and cancerous lesions. The leaves are antiseptic, the paste is applied on ulcers and abscesses. The root is chewed to cure toothache aphthae. The whole plant is used in application for oedematous tumours and the ash extract of flowering twigs along with tender leaves cure cancer and leprosy (Felix *et al.,*2009).Having the potent medicinal uses, this plant has not yet been subjected to *in vitro* antioxidant studies. Thus the present study was aimed to investigate the *in vitro* antioxidant activity of methanol extract of tuber of *Dioscorea oppositifolia.*

MATERIALS AND METHODS

Dioscorea oppositifolia tubers were freshly collected from the Injikuzhi, Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The plants were identified and authenticated in Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of extracts

Ten grams of powdered tubers of *Dioscorea oppositifolia* 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 (Yu *et al*., 2002). 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 ($g100g^{-1}DW$) of the plant samples.

Estimation of Flavonoids

The flavonoids content was determined according to Eom *et al* (2007). 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 vortexes 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.

DPPH 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 (Blois, 1958).

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 (Blois, 1958). 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 absorbances were 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 Halliwell *et al*., 1987. 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, 1988. 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°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 test 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*., 1999. 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 was 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*., 2009 with minor modification to Oyaizu, 1986. 1.0ml 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 (5.0ml, 1.0%): The mixture was incubated at 50°C for 20 minutes. Then 5ml of 10% trichloroacetic acid was added and centrifuged at 980gm (10 minutes at 5°C) in a refrigerator centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0ml of distilled water and ferric chloride and absorbance read at 700nm. 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 Software for windows release 17.5; SPSS Inc., Chicago IL, USA). Estimates of mean, standard error for aforesaid parameters were calculated.

RESULTS AND DISCUSSION

The free radical scavenging activity of *Dioscorea oppositifolia* tuber extract was detected and compared with reference compound ascorbic acid and trolox on the DPPH, hydroxyl, superoxide and ABTS *in vitro* free radical scavenging assay models. The *Dioscorea oppositifolia* extract showed a dose dependent free radical scavenging activity in all *in vitro* assay models (Figure 1-4). IC_{50} values of *Dioscorea oppositifolia* extract and standard ascorbic acid/trolox for DPPH, hydroxyl, superoxide and ABTS scavenging activity were found to be (21.47 µg/ml) and (18.26 µg/ml); (26.33 µg/ml) and (18.46 µg/ml); (31.59 µg/ml) and (72.08 µg/ml); (26.33 µg/ml) and (20.67 µg/ml) respectively (Figure 5). There was also a dose dependent increase in reductive ability with increase in concentration of *Dioscorea oppositifolia* extract and ascorbic acid /trolox. Figure 6 showed the reductive ability of *Dioscorea oppositifolia* compared to ascorbic acid. Absorbance of the solution was increased when the concentration increased. A higher absorbance indicates a higher reducing power. The *Dioscorea oppositifolia* extract was found to contain-0.56g/$100g^{-1}$ total phenolic and 0.51 g/$100g^{-1}$ flavonoids.

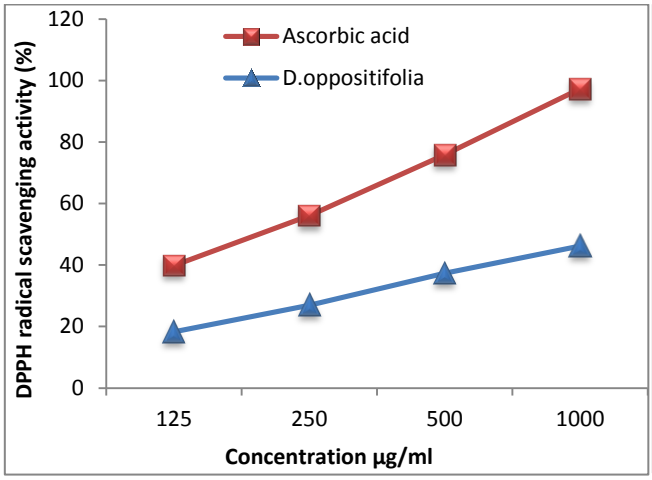

Fig 1: DPPH radical scavenging activity of methanol extract of *Dioscorea oppositifolia*.

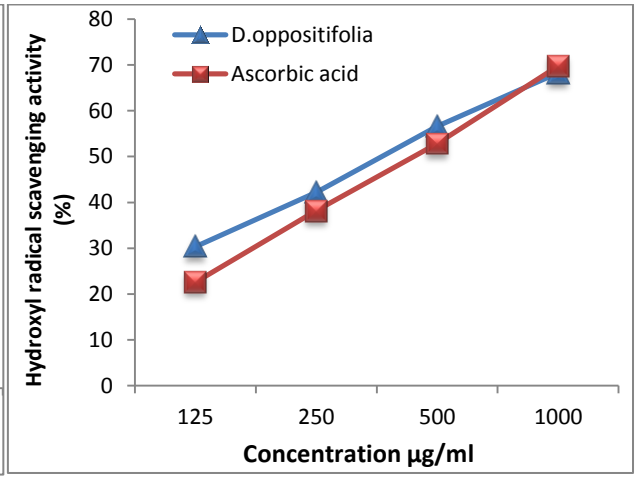

Fig 2: Hydroxyl radical scavenging activity of methanol extract of *Dioscorea oppositifolia*.

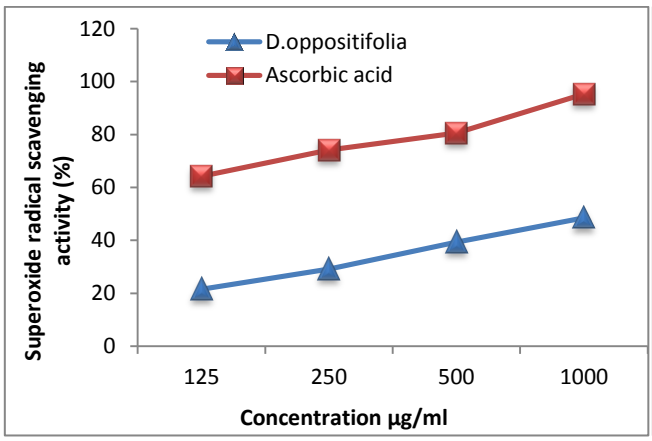

Fig 3: Superoxide radical scavenging activity of methanol extract of *Dioscorea oppositifolia*.

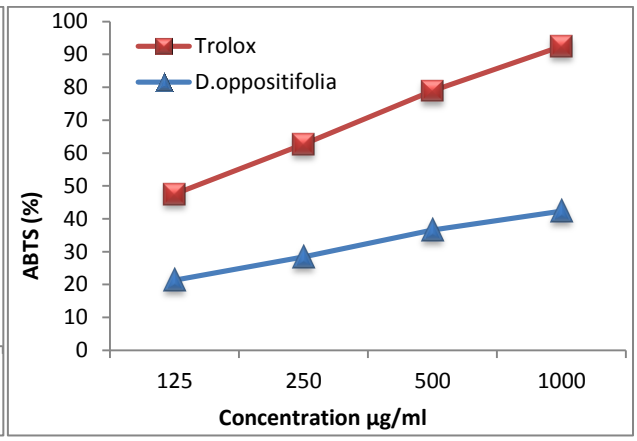

Fig 4: ABTS radical cation scavenging activity of methanol extract of *Dioscorea oppositifolia*.

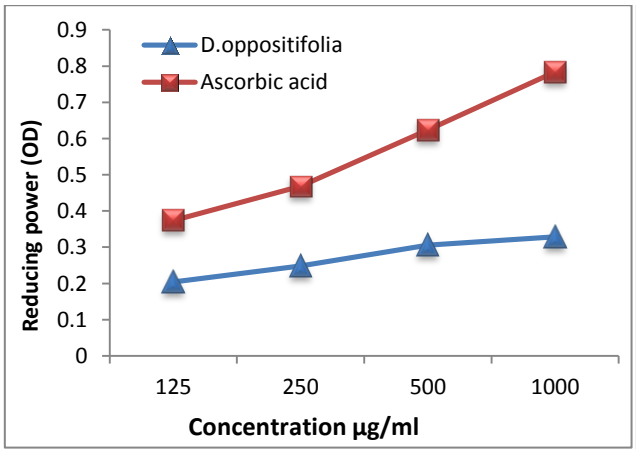

Fig 5: Reducing power ability of methanol extract of *Dioscorea oppositifolia*.

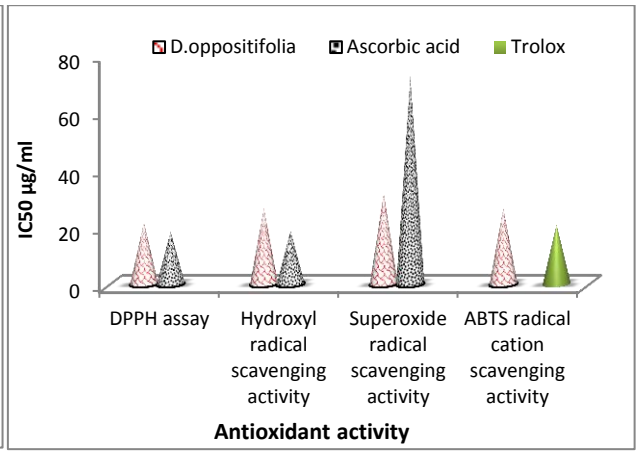

Fig 6: IC 50 values of methanol extract of *Dioscorea oppositifolia*.

Free radicals are chemical species which contains one or more unpaired electrons. They are highly unstable and cause damage to other molecules and extracting electrons from them in order to attain stability. They are formed inside the system, and are highly reactive and potentially damaging transient chemical species. These radicals are continuously produced in the human body because they are essential for detoxification, chemical signaling, energy supply and immune function. Free radicals are regulated by endogenous antioxidant enzyme system, but due to over production of free radicals by exposure to environmental oxidant substances such as cigarette smoking, UV radiation etc or a failure in antioxidant defense mechanism or damage to cell structure, the risk increases for many diseases such as Alzheimer's disease, mild congestive impairment, Parkinson's disease, cardiovascular disorder, liver diseases, ulcerative colitis, inflammation and cancer (Zhu *et al.,*2011). It is possible to reduce this risk of chronic disorders and prevent the disease progression by either enhancing the body's natural antioxidant defense or supplementing with proven antioxidants. For this reason, discovery of natural antioxidants is a major thrust area (Ali *et al.,* 2008).

The antioxidant activity of the methanol extract of *Dioscorea oppositifolia* tuber was investigated against *in vitro* models. Since, free radicals are of different chemical entities, it is essential to test the extract against many free radicals to prove its antioxidant activity. Hence, a large number of *in vitro* methods were used for the screening. IC_{50} values obtained were compared with the standards used.

The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical, to become a stable diamagnetic molecule (Kalaivani and Mathew, 2010). The degree of discoloration indicates the potential of the plant extract to scavenge free radical due to its ability to donate hydrogen proton. The concentration dependent curve of DPPH radical scavenging activity of *Dioscorea oppositifolia* compared well with ascorbic acid. The result is indicative of the hydrogen donating ability of *Dioscorea oppositifolia.* Since the effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability (Conforti *et al*., 2005)

Hydroxyl radical is the most reactive free radical in the biological system and it has been regarded as the highly damaging to almost every molecule found in the biological system. It can conjugate with nucleotides in DNA and cause strand breakage which leads to ultimately mutagenesis, carcinogenesis and cytotoxicity (Pan and Mei, 2010). In the present study, *Dioscorea oppositifolia* tuber extract showed potent hydroxyl radical scavenging activity which is comparable to the standard used. The scavenging activity may be due to the presence of various phyto chemicals including phenolics and flavonoids in methanol extract of *Dioscorea oppositifolia.*

Superoxide anion is also very harmful to cellular components and produced from molecular oxygen due to oxidative enzyme of body as well as via non-enzymatic reaction such as autoxidation by catecholamine (Naskar *et al.,* 2010). The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560 nm, *Dioscorea oppositifolia* extract indicated the ability to quench superoxide radicals in the reaction mixture.

ABTS radical scavenging activity is relatively recent one, which involves a more drastic radical, chemically produced and is often used for screening complex antioxidant mixture such as plant extracts, beverages and biological fluids. The ability in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS for the estimation of antioxidant activity (Huang *et al.,* 2011) Results of the present study, revealed that, methanol extract possesses superior antioxidant activity. The extract showed potent antioxidant activity in ABTS method which is comparable to the standard used.

The reducing properties of the plant extracts are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Dorman *et al.,*2003; Oyedemi and Afolayan, 2011; Sajeesh *et al.,*2011). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The data obtained in the present study suggest that, it is likely to contribute significantly towards the observed

antioxidant effects like the antioxidant activity, the reducing power of the extract increases with increasing concentration. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Osawa, 1994). The methanolic extract of *Dioscorea oppositifolia* showed strong antioxidant activity in various *in vitro* systems tested. The antioxidant effect of *Dioscorea oppositifolia* may be due to the phenolic and flavonoids present in it. In conclusion, the high antioxidant activity exhibited by *Dioscorea oppositifolia* extract provided justification for the therapeutic use of this plant in folkloric medicine due to the phytochemical constituents. The present data suggest that, this extract could be a potential source of natural antioxidant that could be of great importance for the treatment of radical related diseases and age associated diseases.

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