ANTIOXIDANT ACTIVITY OF DIOSCOREA SPICATA ROTH OF USING VARIOUS IN VITRO ASSAY MODELS
M. Packia lincy, V. R. Mohan*

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

ABSTRACT
In the present study, in vitro antioxidant and free radical scavenging capacity of methanol extract from Dioscorea spicata tuber were evaluated using established in vitro models such as DPPH, hydroxyl radical, superoxide radical, ABTS radical cation and reducing power. Total phenolic and flavonoid contents were estimated. Methanol extract showed considerable in vitro antioxidant and free radical scavenging activities in a dose dependent manner when compared to the standard antioxidant (ascorbic acid/trolox). Thus, this study suggests that D. spicata tuber can be as a potent source of natural antioxidant.

Keywords: In vitro antioxidant, flavonoid, DPPH, reducing power.

INTRODUCTION
Oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. ROS are major sources of primary catalysts that initiate oxidation of in vivo and in vitro. These ROS creates oxidative stress which results in numerous diseases and disorders such as cancer, cardiovascular disease, neural disorders, Alzheimer’s disease, mild cognitive impairment, Parkinson’s disease, alcohol induced liver diseases, ulcerative colitis, ageing and atherosclerosis.[1,2,3,4]. In current herbal drug scenario, plant derived antioxidants are gaining importance because of their potential health benefits, no toxicity and side effects over synthetic antioxidants like butylhydroxyanisole and butylhydroxytoluene[5]. Plants may contain a wide variety of few radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites which are rich in antioxidant activity[6,7].
The genus *Dioscorea* L., a monocotyledon, belongs to the family Dioscoreaceae. It comprises 350 – 400 species and is distributed throughout the tropics and subtropic regions. Especially in West Africa, parts of Central America and the Caribbean, the Pacific islands and Southeast Asia. The genus *Dioscorea* has been the main food sources for tribal people particularly in many parts of Western Africa since prehistoric times because its tubers are rich in essential dietary nutrients [8,9]. *Dioscorea* has been widely used for the enhancement of health in oriental countries and traditionally considered as a superior Chinese herb to improve gastrointestinal function. *Dioscorea* species have also been implicated in the protection of the health of post menopausal woman [10]. So far, no attempts have been made to evaluate the antioxidant properties of *Dioscorea spicata* Roth. Hence the present study was performed to investigate the in vitro antioxidant of methanol extract of *Dioscorea spicata* tuber using different models viz : DPPH, hydroxyl superoxide and ABTS radical cation scavenging activity.

**MATERIALS AND METHODS**

*Dioscorea spicata* tubers were freshly collected from the Injikuzhi, Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The plant 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 *D. spicata* 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 [11]. Samples (100μL) were mixed thoroughly with 2 ml of 2% Na₂CO₃. 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 \[12\]. 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 \[13\].

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 \[13\]. 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 &1000 μg/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance 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.

\[
\text{DPPH scavenging effect (\% inhibition)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]
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 [14]. 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 FeCl3, 0.1mL $H_2O_2$, 0.36ml of deoxyribose, 1.0mL of the extract of different concentration (125,250,500 &1000 μg/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

\[
\text{Hydroxyl radical scavenging activity}\ = \ \frac{(A_0 - A_1)}{A_0} \times 100
\]

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 [15]. The superoxide anion radicals were generated in 3.0 mL of Tris – HCL buffer (16 mM, pH 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 &1000 g/mL), and 0.5 ml Tris – HCl buffer (16mM, pH 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 [16]. 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 100 L 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 [17] with minor modification to Oyaizu [18]. 1.0mL of solution containing 125,250,500 &1000 g/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 (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 phenolic content and total flavonoid content of *D. spicata* tuber extract were found to be 0.26 g 100g⁻¹ and 1.28 g 100 g⁻¹ respectively.

DPPH radical scavenging activity

DPPH radical scavenging activity of *D. spicata* was shown in figure 1. The extract exhibited potent radical scavenging ability against DPPH method in concentration dependent manner. At 1000µg/ml concentration, *D. spicata* possessed 57.51% scavenging activity on DPPH. All the concentration of *D. spicata* showed higher activity than the standard ascorbic acid.

Hydroxyl radical scavenging activity

The effect of *D. spicata* extract and standard ascorbic acid on hydroxyl radical was compared and shown in figure 2. The scavenging effect increases with the concentration of standard and samples. At 1000µg/mL concentration, *D. spicata* exhibited 49.33% scavenging activity on hydroxyl.
Superoxide radical scavenging activity
The D. spicata extracts were subjected to be superoxide scavenging assay and the results were shown in figure 3. It indicates that D. spicata (1000µg/mL) exhibited the maximum superoxide scavenging activity of 62.56%.

ABTS radical cation scavenging activity
The effect of D. spicata tuber extracts and standard trolox on ABTS cation was compared and shown in Figure 4. The scavenging effect increases with the concentration of standard and samples. At 1000µg/ml concentration, D. spicata possessed 66.17% scavenging activity.
activity on ABTS. All the concentration of D. spicata showed higher activity than the standard trolox.

Reducing power

The reducing power of D. spicata was compared with the standard ascorbic acid. The reducing power increases with the increasing concentration. The reducing power of the samples was shown in Figure 5.

IC$_{50}$ values

IC$_{50}$ values of D. spicata tuber extract and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging activity and trolox for ABTS were found to be 26.37µg/mL and 18.26µg/ml; 19.33µg/ml and 18.46µg/mL ;47.31µg/mL and 72.08µg/mL and 32.46µg/mL and 20.67µg/mL respectively(Figure 6)

![Figure 3](image)

Hydroxyl radical scavenging activity of methanol extract of Dioscorea spicata
Figure 4
ABTS radical cation scavenging of methanol extract of *Dioscorea spicata*.

Figure 5
Reducing power ability of methanol extract of *Dioscorea spicata*.
DISCUSSION

Free radicals and other reactive species are thought to play an important role in many human diseases. In the status of normal metabolism, levels of oxidants and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions \[19\]. Over production of oxidants in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA and proteins. Many synthetic drugs protect against oxidative damage but they have adverse side effective \[20\]. Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Data from both scientific reports and laboratory studies show that plants contain a large variety of substances called “plant chemicals” or “phytochemicals” that posses antioxidant activity \[21,22\]. Many
secondary metabolites which include flavonoids, phenolic compounds etc serve as sources of antioxidants and do scavenging activity\textsuperscript{[23,24]}. Phenolics are secondary metabolites that play a role in maintenance of the human body\textsuperscript{[25]}. The presence of phytoconstituents, such as phenols, flavonoids and tannins in plants, indicates the possibility of antioxidant activity and this activity will help in preventing a number of diseases through free-radical scavenging activity\textsuperscript{[26]}. Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant properties. Many studies focused on the biological activities of phenolic compounds, which have potential antioxidant and free radical scavengers\textsuperscript{[2,27]}. Flavonoids, the major group of phenolic compounds are reported for their antimicrobial, antiviral and spasmylytic activity. Flavonoids are able to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which highlights many of the flavonoid health-promoting functions in organisms. They 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 anti-cancer activity\textsuperscript{[28,29,30]}. DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. From the present results it may be postulated that \textit{D. spicata} tuber reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles\textsuperscript{[31]}, DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up\textsuperscript{[5]}. In the present study, methanol extract \textit{D. spicata} tuber exhibited comparable DPPH radical scavenging activity with IC\textsubscript{50} values 26.37µg/mL compared to ascorbic acid 18.26µg/mL.
Hydroxyl radical is one of the potent reactive oxygen species in the biological system it reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell \(^{[32]}\). The cell damaging action of hydroxyl radical is well known as it is the strongest among free radicals and it has short half life \(^{[33]}\). \(D.\ spicata\) exhibited the greatest scavenging effect of \(-OH\) but less than the standard ascorbic acid \(-OH\) is known to the capable of abstracting hydrogen peroxide from membranes and they bring about lipid peroxidation. It is thus anticipated that \(D.\ spicata\) would show antioxidant effects against lipid peroxidation on bio membranes and would scavenge \(-OH\) radicals at the stages of initiation and termination \(^{[34]}\).

Superoxide anion is an oxygen-centered radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions and by non enzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complexes such as cytochrome \(^{[35,36]}\). The present study showed potent superoxide radical scavenging activity for \(D.\ spicata\) tuber extracts. Methanol extracts showed potent superoxide radical scavenging activity with IC\(_{50}\) values 47.31\(\mu\)g/mL.

ABTS assay is based on the inhibition of the absorbance of the radical cation ABTS, which has a characteristic long, wave length absorption spectrum. The ABTS chemistry involves direct generation of ABTS radical mono cation with no involvement of any intermediary radical. It is a decolorization assay, thus the radical cation is performed prior to addition of antioxidant test system, rather than the generation of the radical taking place continually in the presence of antioxidant \(^{[37]}\). Results of the present study revealed that methanol extracts possesses superior antioxidant activities. Methanol extracts of \(D.\ spicata\) showed very potent ABTS radical scavenging activity with IC\(_{50}\) values 32.46\(\mu\)g/mL compare to ascorbic acid 20.67\(\mu\)g/mL.

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 radicals chain by donating a hydrogen atom \(^{[38,39,40,41]}\). The data obtained in the present
study suggest that it is likely to contribute significant towards the observed antioxidant effects. Like the antioxidant activity, the reducing power of the extract increases with increasing concentration.

The methanol extract of *D. spicata* tuber showed strong antioxidant activity in various in vitro systems tested. The antioxidant effect of *D. spicata* may be due to the phenolic compounds present in it. To our knowledge, this is the first report on the antioxidant potential of *D. spicata*. The results from various free radical scavenging systems reveal that methanol extract of *D. spicata* has significant antioxidant activity. The extract is found to have different levels of antioxidant activity in all the methods tested. IC₅₀ values obtained were comparable to that of the standards used i.e., ascorbic acid and trolox.

According to this study, a significant antioxidant activity was found. Total phenolic and flavonoid contents determination indicates that there compounds could be major contributors to antioxidant activity. Further studies on isolating and characterizing the antioxidant substances and their potential as pharmacological agents are in progress.

ACKNOWLEDGEMENT


REFERENCES


21. Ozen T, Turkekul I: Antioxidant activities of Sarcodon imbricatum weidly grown in the back sea region of Turkey. Pharmacon Mag 2010; 6(22); 89-97.


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

www.pharmasm.com IC Value – 4.01 3767