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In vitro antioxidant studies of *Dioscorea esculenta* (Lour). Burkill

Manickam Murugan, Veerabahu Ramasamy Mohan*

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

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ABSTRACT

Objective: To evaluate the total phenolic, flavonoid contents and *in vitro* antioxidant activity of methanol extract of *Dioscorea esculenta* (Lour). Burkill. **Methods:** Total phenolic content was estimated using the Folin Ciocalteu method. The flavonoid content was determined using aluminium chloride. *In vitro* antioxidant activities and reducing power capacity were determined using standard methods. **Results:** Total phenolic content in methanol extract of *Dioscorea esculenta* was found to be 0.79g/100g and flavonoids content was found to be 0.26 g/100g. The extract was screened for its potential antioxidant activities using tests such as DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, ABTS radical cation scavenging activity and reducing power activity. **Conclusions:** The present studies confirm the methanol extracts have potential *in vitro* antioxidant activity. The phytochemical phenols and flavonoids could be the reason for its antioxidant activity.

1. Introduction

Free radicals have significant role in the causation of several diseases such as diabetes, cirrhosis, cancer and cardiovascular diseases. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxy nitrite which results in oxidative stress leading to cellular damage. Thus, compounds or antioxidants that can scavenge free radicals have vital role in the improvement of these diseased conditions[1]. Recently, natural antioxidants are in high demand because of their potential in health promotion and disease prevention, and their improved safety and consumer acceptability. Plants are rich sources of natural antioxidants, such as phenolics substance. Several studies have showed that increased dietary intake of natural phenolic antioxidants correlates with decreased coronary heart disease[2]. In the present study, methanol extract of tuber of *Dioscorea esculenta* was secured for its total phenolics, flavonoids and antioxidant activities.

*Corresponding author: Veerabahu Ramasamy Mohan, Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin-628008, Tamil Nadu, India.
Tel: +91 9487279902
E-mail: vrmohanvoc@gmail.com
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2. Materials and methods

The wild edible tubers of *Dioscorea esculenta* (Lour). Burkill were collected from Karaiyar, Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. They were shade dried at room temperature for 10-15 days. 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.

2.1. Preparation of extracts

Ten grams of powdered tuber of *Dioscorea esculenta* was extracted separately with methanol (100 mL) 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.

2.2. Estimation of Total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method[3]. Samples (100 μL) 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 g of dry weight of the plant samples.

2.3. Estimation of flavonoids

The flavonoids content was determined according to Eom *et al*[4]. An aliquot of 0.5 mL of sample (1 mg/mL) was mixed with 0.1 mL of 10% aluminium chloride and 0.1 mL of potassium acetate (1 M). In this mixture, 4.3 mL of 80% methanol was added to make 5 mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415 nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

2.4. 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 nonradical form DPPH-H[5].

The free radical scavenging activity of all the extracts was evaluated by DPPH according to the previously reported method[5]. Briefly, an 0.1 mm solution of DPPH in methanol was prepared, and 1 mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (125, 250, 500 and 1000 μg/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. 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 of scavenging the DPPH radical was calculated by using the following formula:

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

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.

2.5. Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al*[6]. Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), Ascorbic acid (1 mM), H_2O_2 (10 mM) and Deoxyribose (10 mM), were prepared in distilled deionized water.

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

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

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.

2.6. Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski[7]. 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 (0.3 mM), 0.5 mL NADH (0.936 mM) solution, 1.0 mL extract of different concentration (125, 250, 500 and 1000 μg/mL), and 0.5 mL Tris-HCl buffer (16 mM, PH 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12 mM) to the mixture, incubated at 25 ℃ 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:

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

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.

2.7. Antioxidant activity by radical cation

ABTS assay was based on the slightly modified method of Re *et al*[8]. ABTS radical cation ($ABTS.^+$) was produced by reacting 7 mM 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 μL 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 min. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

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

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.

2.8. Reducing power

The reducing power of the extract was determined by the method of Singh *et al*[9] with minor modification to Oyaizu[10]. 1.0 mL of solution containing 125, 250, 500 and 1 000 μg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%): The mixture was incubated at 50 ℃ for 20 min. Then 5 mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 min at 5 ℃) 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.

2.9 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 and SE for aforesaid parameters were calculated.

3. Results

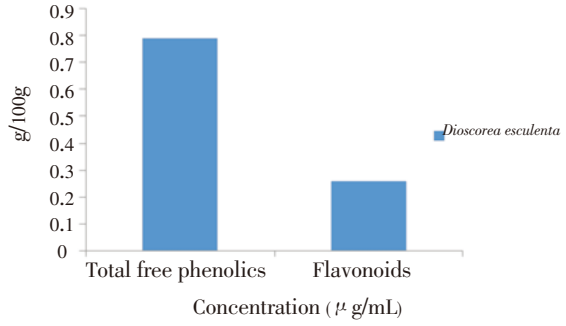

Figure 1. Total phenolic and flavonoid contents of *Dioscorea esculenta*.

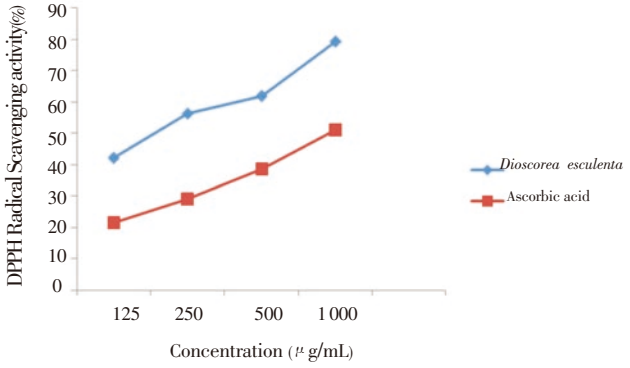

Figure 2. DPPH radical scavenging activity of methanol extract of *Dioscorea esculenta*.

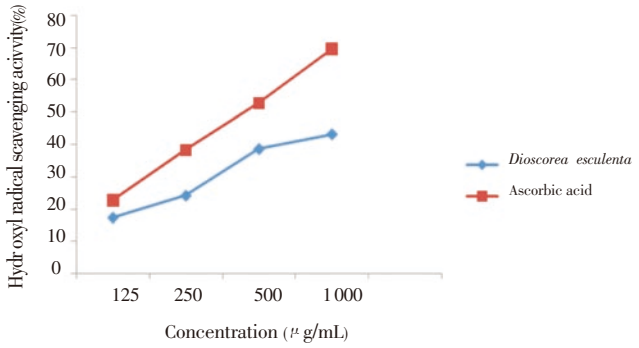

Figure 3. Hydroxyl radical scavenging activity of methanol extract of *Dioscorea esculenta*.

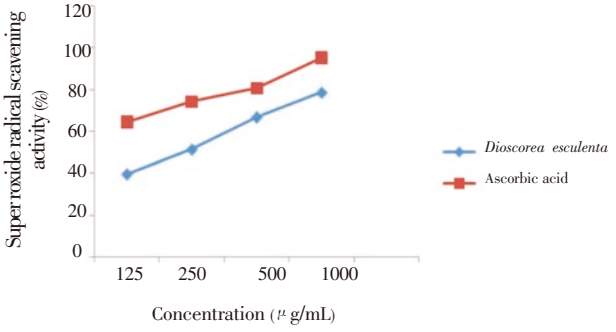

Figure 4. Superoxide radical scavenging activity of methanol extract of *Dioscorea esculenta*.

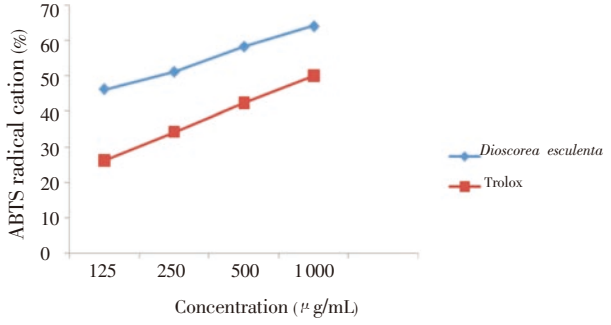

Figure 5. ABTS radical cation scavenging activity of methanol extract of *Dioscorea esculenta*.

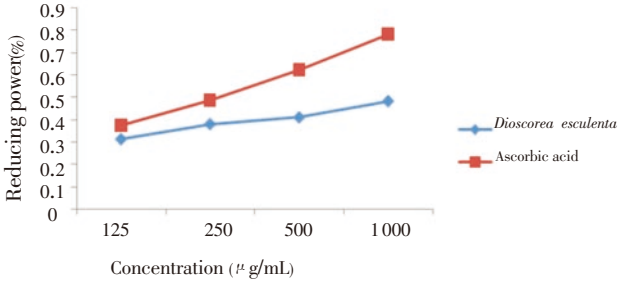

Figure 6. Reducing power ability of methanol extract of *Dioscorea esculenta*.

The quantitative phytochemical analyses of *Dioscorea esculenta* tuber are presented in Figure 1. The total phenolic

and flavonoid contents of *Dioscorea esculenta* were found to be 0.79 g/100 g and 0.26 g/100 g respectively. In this work, the antioxidant properties of tuber of *Dioscorea esculenta* were evaluated by different *in vitro* antioxidant assays such as DPPH, hydroxyl, superoxide, ABTS radical cation scavenging activity and reducing power activity. The DPPH radical scavenging activity of *Dioscorea esculenta* tuber extract increased with increasing concentration, with 79.33% scavenging activity for 1 000 μg/mL extract (Figure 2). The hydroxyl radical scavenging activity was found to be increased in a dose-dependent manner from 17.33% to 43.11% at a concentration of 125-1 000 μg/mL (Figure 3). The superoxide radical scavenger activity of *Dioscorea esculenta* tuber extract increased from 39.40% to 78.56% at 125-1000 μg/mL (Figure 4). The ABTS radical cation scavenging activity was found to be increased in a dose-dependent manner from 46.14 to 64.11% at a concentration of 125-1 000 μg/mL (Figure 5). The reducing power of methanol extracts of *Dioscorea esculenta* tuber was found to be increase with increasing amount of extract concentration (Figure 6). The IC_{50} values of DPPH, hydroxyl, superoxide and ABTS radical cation scavenging activity are presented in Figure 7.

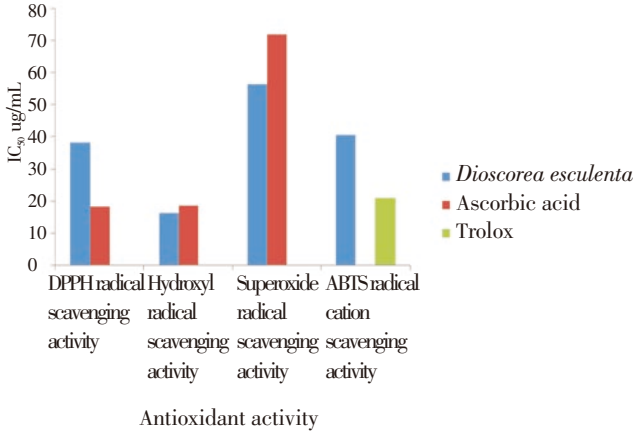

Figure 7. IC_{50} values of methanol extract of *Dioscorea esculenta*.

4. Discussion

Phenolics compound the principal antioxidant constituents of natural plant products are composed of phenolic acid and flavonoids[11]. These compounds are potent radical terminators by donating a hydrogen atom to the radical and preventing lipid oxidation at the initial step. In this respect, polyphenolic compounds commonly found in plants have been reported to have multiple biological effects like anticancer, antiproliferative, antimicrobial, wound healing and antibacterial activities including antioxidant activity[12,13].

DPPH assay is the most widely reported method for screening antioxidant activity of many plant drugs, based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. DPPH is a stable, nitrogen-centered free radical which produces violet color in ethanol solution. It was reduced to a yellow colored product, diphneylpicryl hydrazine, with the addition of *Dioscorea esculenta* extract in a concentration-dependent manner[14]. The IC_{50} value was found to be 38.33 μg/mL. Ascorbic acid was used as reference standard for the DPPH free radical scavenging assay. The IC_{50} value of ascorbic acid obtained was found to be 18.26 μg/mL. There results indicated that *Dioscorea esculenta* tuber extract exhibited the ability to quench the DPPH radical, which indicated that extract was good antioxidant with radical scavenging activity.

Hydroxyl radicals are major active oxygen species causing lipid peroxidation and enormous biological damage. Hydroxyl radical scavenging capacity of *Dioscorea esculenta* extract is directly related to its antioxidant activity. This method involves *in vitro* generation of hydroxyl radicals using Fe^{2+}/ascorbate/ETDA/H_2O_2 system using Fenton reaction. The oxygen derived hydroxyl radicals along with the added transition metal ion (Fe^{2+}) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid[15]. When *Dioscorea esculenta* extract was added to the reaction mixture, it removed the hydroxyl radicals from the sugar and prevented the reaction. IC_{50} value of ascorbic acid was 18.46 μg/mL. Hence, the *Dioscorea esculenta* extract can be considered as a good scavenger of hydroxyl radicals.

Superoxide anion is also very harmful to cellular components and produced from molecular oxygen due to molecular oxidative enzyme of body as well as via non-enzymatic reaction such as autoxidation by catecholamines[16]. The superoxide radicals generation from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560 nm, *Dioscorea esculenta* extract indicated ability to quench superoxide radicals in the reaction mixture. The IC_{50} value was found to be 56.38% μg/mL. IC_{50} value of ascorbic acid was 72.08 μg/mL. The results of the present study suggested that the methanol extract of *Dioscorea esculenta* is a more potent scavenger of superoxide radical.

ABTS radical scavenging activity is relatively is recent one, which involves a more drastic radical, chemically produced and is often used for screening complex antioxidant mixtures 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[17]. The extracts showed potent antioxidant activity in ABTS method which is comparable to the standard used. Here, the extract's radical scavenging activity showed a direct role of its phenolics compounds in free radical scavenging. The extract exhibited an IC_{50} value of 40.50 μg/mL for ABTS. The IC_{50} value of trolox was 20.67 μg/mL. Therefore, the ABTS

radical scavenging activities its ability to scavenge free radicals, thereby preventing lipid oxidation via a chain-breaking reaction.

The systematic literature collection, pertaining to this investigation indicates that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers. Therefore, it is necessary to determine the total amount of phenols and flavonoids in the plant extract chosen for the study. Flavonoids are the most diverse and widespread group of natural components and are likely to be the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging activity. Phenolics compounds are considered to be most important antioxidants of plants materials. They constitute one of the major groups of compounds acting as primary antioxidant or free radical terminators. Antioxidant activity of phenolics compounds is based on their ability to donate hydrogen atoms to free radicals. In addition, they possess ideal structural properties for free radical scavenging properties[18]. The results of reducing power of presume studies are comparable with Yen and Duh[219]. Who reported that the reducing power of peanut hull extract increased with increase in concentration.

On the basis of the results it is concluded that the extract of *Dioscorea esculenta* contains higher quantities of total phenolic and flavonoid compounds, which exhibit antioxidant and free radical scavenging activity. The present results suggest that *Dioscorea esculenta* could be a promising source of natural antioxidants.

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