**Evaluation of *in vitro* antioxidant activity of phytochemicals present in**

***Dioscorea oppositifolia* L.,superior genotype collected from Kohlli hills at Namakkal**

**Uma Maheswari Rajadurai1, Nevetha Ravindran2, Jenifer Packiaraj3, Keerthiga Manohar4,, Rameshwari Ramaswamy5**

**1,2,3,4,5Department of Biotechnology, Cauvery College for Women, (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.**

**Corresponding author: Dr.Uma Maheswari Rajadurai**

**Email id :umamaheswari.bt@cauverycollege.ac.in**

**Abstract:**

Yams belong to the genus *Dioscorea* of the family Dioscoreaceae. Among 600 species, *Dioscorea oppositifolia L., D. alata L., D. cayenensis Lam., D. rotundata Poir., D. trifida L., D.esculenta Burkill, and D. bulbifera L*.*,* have the significant medicinal and economic importance which is consumed by tribal people in large scale. This study was designed to determine whether *Dioscorea oppositifolia L.* supplementation could have antioxidant activity. We aimed to investigate the antioxidant activity- DPPH and ABTS assay.of aqueous extracts from *Dioscorea oppositifolia* L collected from Kohlli hills

**Introduction**

Yam has many phytochemical constituents such as polysaccharides, aminoacids, proteins, vitamins and mineral elements. Yam contains many chemical components such as mannan, abscising II, glucoprotien, choline, cholesterol, ergosterol, camesterol, dioscin, diosgenin, phyticacid, allontoin, dopamine, batatasin, dioscorea- mucilage B, and sterols (Zuo and Tang, 2003). Organic acids like succinic acid, citric acid, oxalic acid and malice acid are found abundantly in yams. Rakotobe *et al.,* (2010) reported that two clerodane diterpenoids, antadiosbulbins A and B and two 19- norclerodane diterpenes, 8-epidiosbulbins E and G along with the known diosbulbin E as well as nine known phenolics including five phenanthrenes and stilbenes and four flavonoids were isolated from the ethyl acetate soluble part of the methanolic extract of the tubers of Dioscorea antaly (an endemic species to Madagascar). Tapondjou *et al.,* (2013) reported that eleven steroidal saponins, dioscoreanosides A–K, along with five known congeners, were isolated from the flowers of Dioscorea bulbifera var. sativa. Their structures were established by extensive NMR experiments in conjunction with mass spectrometry.

Traditionally, the tuber is used to treat inflammation, joint pain, diabetes, infections, dysmenorrhea, rheumatism, and arthritis. The discovery of diosgenin (natural steroid) in the tubers has made it one of the most researched and studied herbal product. Many health benefits are associated with diosgenin, like prevention against cardiovascular disease, cancer and contraception. Diosgenin is used as a starting material for the synthesis of steroidal drugs, particularly for the partial synthesis of oral contraceptives, sex hormones and other steroids, since it exhibits estrogenic activity. Diosgenin has been used in traditional Chinese medicine for treatment of urethral and renal infections. (Heena and Lele, 2012).

A new Indian source for diosgenin is from *Dioscorea*, which is used to induce apoptosis in cancer cells and to reduce high blood pressure. Over the past decade, a series of preclinical and mechanistic studies have been conducted worldwide to understand the role of diosgenin as a chemo preventive agent against several cancers (Raju and Mehta, 2009).

Diosgenin have been found to exerts its anticancer effects against a wide variety of tumor cells, including breast cancer, colorectal cancer, osteosarcoma, and leukaemia (Srinivasan, 2009).The antitumor effects of diosgenin have been demonstrated to be mediated through activation of p53, immune-modulation, cell cycle arrest, modulation of caspase-3 activity, and induction of TRAIL death receptor DR5 (Lepage *et al*., 2011). Diosgenin inhibited proliferation and induced apoptosis in HepG2 cells by inhibiting signal transducer and activator of transcription (STAT3) signaling pathway (Kim *et al*., 2007). Diosgenin has been shown to target multiple pathways of tumorigenesis; including proliferation, apoptosis, angiogenesis, invasion, and tumor-induced immuno suppression in various tumor cells and *in vivo* cancer models (Raju and Mehta, 2009).

There is a huge demand of diosgenin in the pharmaceutical industry for the growing population. In the light of the above information, the present work is an attempt to reveal many intricating objectives to detect antioxidant activity related with phytodrug discovery and therapeutic potentials in *Dioscorea oppositifolia* to produce environmentally safe approach.

1. **Materials and Methods**
   1. **Antioxidant assays in *Dioscorea oppositifolia* L.**

**2.1.2.Determination of scavenging activity on DPPH**

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Koleva et al., 2002). The presence of antioxidants which include polyphenolics and flavonoids in the sample will scavenge the formed DPPH radical and thereby decrease the color production. To 0.5 ml of DPPH radical solution, 2 ml of the extract (100-1000µg/ml) was added and the reaction mixture is vortexed for 10s and allows standing at room temperature for 30 minutes. After 30 mins at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated three times. BHT was used as standard controls. EC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Percentage of activity = Control - Test/Control\*100

**Determination of scavenging activity on ABTS**

The effect of plant extract on scavenging ABTS free radicals was investigated by the method of Re et al., (1999). The ABTS radical is produced by the reaction of ammonium persulphate with ABTS under dark condition. The persulphate ions are involved in a nucleophilic attack on ABTS and thereby, a greenish blue ABTS radical (ABTS+) are produced. The presence of antioxidants, which includes polyphenolics and flavonoids in the sample, will scavenge the formed ABTS radical and thereby decreased color intensity will be observed.

ABTS solution was freshly prepared by adding 5 ml of a 4.9 mM ammonium persulphate solution to 5 ml of 14mM ABTS solution and keep for 16 h under dark condition. The solution is diluted with distilled water to yield an absorbance of 0.70 ± 0.02 at 734 nm and the same was used for the assay. To this, 900 µl of the extract (100- 1000 µg/ml) was added and the reaction mixture was vortexed for 10s. After six minutes, the absorbance at 734 nm against distilled water was recorded. Compare with the control ABTS solution. The ABTS scavenging activity was expressed as percentage. Ascorbic acid can be used as reference compound.

Percentage of ABTS scavenging activity= [A control – A sample]/ (A control x 100), where A control is the absorbance of ABTS + methanol; A sample is the absorbance of ABTS + sample (i.e. standard or extract).

1. ***Results and Discussion***

**Antioxidant activity**

Total antioxidant activity- DPPH, ABTS radical-scavenging and total antioxidant activity. The radical scavenging activity of Dioscorea oppositifolia L., extracts (0.01 mg dw/ml) was compared with those of BHT and ascorbic acid at the same concentration and expressed as % of inhibition against DPPH and ABTS, respectively [Table 3].

*In vitro* tuber extract has larger amount of Vitamin-C than the *wild* tuber. Total antioxidant capacity of *in vitro* tuber was quantified as 428.58 mg/g in ascorbic acid equivalents. Total antioxidant capacity of tuber is quantified as 356.51mg/g in ascorbic acid equivalents. The standard Diosgenin purchased from Sigma, USA was also subjected to estimate the total antioxidant activity and the total antioxidant capacity of Diosgenin was quantified as 430.55mg/g. *In vitro* tuber has higher scavenging capacity which is nearer to the standard value.

DPPH radical-scavenging activity was measured according to the method of Koleva et al., (2002). *D. oppositifolia has* greater scavenging activity against DPPH radical, *wild* tuber has IC50 value of 300 µg/ml and *in vitro* tuber has IC50 value 500 µg/ml against the standard BHT has scavenging capacity IC50 value of 50 µg/ml (Table 10, Figure 15).

*D. oppositifolia* L.tuber has greater scavenging activity by ABTS radical (Table 11, Figure 16). The IC50 value of *in vitro* tuber is 400µg/ml, and IC50 value of *wild* tuber is 500µg/ml, while BHT shows IC50 value of 400µg/ml. *in vitro* tuber has scavenging activity which is equal to BHT standard. A significance level of 5% was adopted using anova analysis for all comparisons. Duncan’s multiple ranges test was used to determine the significant difference between different treatments. The radical scavenging activity of *D. oppositifolia* extracts (0.01 mg dry weight/ml) was compared with those of BHT and ascorbic acid at the same concentration and expressed as percentage of inhibition against DPPH and ABTS, respectively. These results suggest that tuber extract of *D. oppositifolia* has potent antiradical and antioxidant activity.

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**Table 1: Scavenging Capacity of DPPH Radical of *D. oppositifolia* L.**

**Free Radical Scavenging Capacity**

|  |  |  |  |
| --- | --- | --- | --- |
| **Wild Tuber IC50=200 μg/ml** | | ***In vitro* tuber IC50 = 300 μg/ml** | |
| **Concentration in**  **μg/ml** | **%of inhibition**  ***in vivo* tuber** | **% of inhibition**  ***in vitro T*uber** | **% of Inhibition Standard (BHT)** |
| 25 | 2.56 ± 0.18 | 2.56 ± 0.18 | 32.47 ± 3.11 |
| 50 | 3.17 ± 0.54 | 1.46 ± 0.09 | 45.54 ± 3.98 |
| 100 | 9.15 ± 1.02 | 6.47 ± 0.95 | 62.39 ± 5.12 |
| 200 | 22.95 ± 2.94 | 10.74 ± 1.65 | 10.74 ± 1.65 |
| 300 | 55.67 ± 4.56 | 22.34 ± 1.63 | 71.67 ± 4.11 |

**Graph 1: Scavenging Capacity of DPPH Radical of *D. oppositifolia* L.**

**Free Radical Scavenging Capacity**

**Sample1-*Invivo* Rhizome**

**Sample2-*Invitro* rhizome**