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

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

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 **Abstract:**

The genus *Dioscorea* belongs to family Dioscoreaceae which is commonly called as yam. Among 600 species, *Dioscorea oppositifolia L. and D. bulbifera L*.*,* have the significant medicinal value and economic importance which is consumed by tribal people as major food. This study was carried out to determine the *invitro* antioxidant potential of  *Dioscorea oppositifolia L.*  *Dioscorea oppositifolia* L collected from Kohlli hills had found to contain greater antioxidant activity than other sourses which is proved by DPPH and ABTS assay.

**Introduction**

 Yam (Dioscorea species) has many nutritional and non-nutritional compounds such as polysaccharides, aminoacids, proteins, vitamins and mineral elements which offers uncountable health benefits which prevents and treats numerous dangerous degenerative diseases. Medicinal application of diosgenin and dioscorin, among other phycompounds isolated from yam, had shown more pharmaceutical more activity recently.

 Yam has many phytochemical constituents such as polysaccharides, aminoacids, proteins, vitamins and mineral elements. Yam contains many phytochemicals such as abscising II, glucoprotien, ergosterol, camesterol, dioscin,, phyticacid, allontoin, dopamine, batatasin, dioscorea- mucilage B, and sterols and higher amount of diosgenin (Zuo and Tang, 2003). The effect of climate conditions of Kohlii hills on medicinal plants and traditional medicine has become increasingly recognized as one of the challenges to humankind and all other life-forms on Earth. Climate change may not directly initiate chemical changes in the profile of metabolites and constituents produced by plants but it could add to existing stress among competing plants, and can affect secondary metabolites and other compounds that plants produce and these effect could impact its medicinal activity The formation of secondary metabolites can be due to biotic or abiotic stresses, caused by factors such as competition, predation, parasitism and disease, or isolation and habitat alteration due to slow geological and climatic change, natural catastrophes or human activities.(Faravani et al .,2014).

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.In addition, diosgenin has been reported to have various therapeutic properties, such as a hypocholesterolemic action and neuroprotection (in rat), or an antioxidant activity in HIV patients with dementia (Turchan, 2003).

 There is a huge demand of diosgenin in the production of phyto drugs in large scale for the growing population. In the light of the above information, the present work is an attempt to reveal antioxidant therapeutic potentials in *Dioscorea oppositifolia.*

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

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

Free radical scavenging ability of the *Dioscorea oppositifolia* L. extracts was tested by DPPH radical scavenging assay as described by (Koleva et al., 2002). The hydrogen atom donating ability of the plant extractives was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol at different concentrations (50–250 μg/mL). The reaction mixture was vortexed thoroughly and left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. BHT was used as standard controls. Percentage DPPH radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity={(A0− A1)/A0}×100

where A0 is the absorbance of the control, and A1 is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration, and from the graph IC50 was calculated. The experiment was repeated three times at each concentration.

**Determination of scavenging activity on ABTS**

The scavenging ABTS free radicals was investigated by the method of Re et al., (1999) on D.oppositifolia L., extract . Th ABTS radical is synthesised by the reaction of ammonium persulphate with ABTS without light condition. The presence of antioxidants, which includes terpenoids and steroids in the sample perform scavenging activity as a result there is formation of ABTS radical and the decreased color intensity would be observed using colorimeter.Ascorbic acid can be used asstandard

Percentage of ABTS scavenging potential = [A control – A sample]/ (A control x 100), where A control is the absorbance of ABTS + solvent;

A sample is ABTS absorbance + sample (i.e. standard or extract).

1. ***Results and Discussion***

**Antioxidant activity**

Antioxidant activity of DPPH and ABTS of *Dioscorea oppositifolia* L., extracts were studied successfully. [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 potential was quantified as 430.55mg/g. *In vitro* tuber has higher scavenging capacity which is nearer to the standard value.

  *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. Duncan’s multiple ranges test was used to determine the significant difference between different treatments.. ANOVA analysis were carried out at significant level at 5%. These results suggest that tuber extract of *D. oppositifolia* has greater potent antioxidant scavenging activity.

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**References:**

Fu, Y.C., Ferng, L.H.A., Huang, P.Y., 2006. Quantitative analysis of allantoin and allantoic acid in yam tuber, mucilage, skin and bulbil of the *Dioscorea species*. *Food Chemistry*. 94, 541–549.

Gomez, A., Kwanchai, A., 1984. Statistical Procedures for Agricultural Research. An International Rice Research Institute book. *Wiley-Interscience publication*.

Gong, G., Qin, Y., Huang, W., Zhou, S., Wu, X., Yang, X., Zhao, Y., Li, D., (2010). Protective effects of diosgenin in the hyperlipidemic rat model and in human vascular endothelial cells against hydrogen peroxide-induced apoptosis. *Chemico-Biological Interactions.* 184(3): 366-375.

Heena J. Shah., and Lele., S.S., 2012. Extraction of Diosgenin, a Bioactive Compound from Natural Source *Dioscorea alata* Var purpurae. *J*. *Anal*. *Bioanal*. *Techniques.* 3, 4.

Kakkar, P., Das, B., Vishwanathan, P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. *Indian J. Biochem. Biophys.* 21, 130-13.

Katy Lisias, G.D., Nadja da Azevedo, C., Kristhea Karyne, G.P. 2007. Mechanisms involved in the vasodilator effect induced by diosgenin in rat superior mesenteric artery. *Eur J Pharmacology*. 574, 172-178.

Kim, J.S., Kuk, E., Yu, K.N., Kim, J.H., Park, S.J. Lee, H.J. 2007. Silver nanoparticle synthesis and their antimicrobial activity. *Nanotechnology, Biology and Medicine.* 3 (1,): 95-101.

Lepage, C. Léger, Bertrand, D.Y., Martin, J., Beneytout, F., and Liagre, B., 2011. Diosgenin induces death receptor-5 through activation of p38 pathway and promotes TRAIL-induced apoptosis in colon cancer cells. *Cancer Letters*. 301(2): 193–202.

Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J., Boyd, 1991. Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. Journal of the National Cancer Institute, 83, 757-76.

Marie, A., Anuff-Harding, M.C., Omoruyi, F., 2006. Intestinal disaccharides and some renal enzymes in streptozotocin- induced diabetic rats fed sapogenin extract from bitter yam. *Life Science*. 78:2595-2600.

Moron, Μ. S., Depierre, J. W., Mannervik, Β.,1979. *Biochim. Biophys. Acta.* 582, 67.

Montririttigri. K, Moongkarndi. P, Joongsomboonkusol. S, Chitkul. B and Pattanapanyasat. K. 2008. Apoptotic Activity of Aporphine from Stephania venosa on Human Ovarian Cancer Cells, Mahidol University *Journal of Pharmaceutical Sciences* 2008; 35(1-4): 52-56.

Tapondjou, L. A., Jenett-Siems, K., Böttger, S., & Melzig, M. F. (2013). Steroidal saponins from the flowers of Dioscorea bulbifera var. sativa. Phytochemistry, 95, 341-350.

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:1-38

Raju, J., and Mehta, R.,2009. Cancer Chemopreventive and therapeutic effects of diosgenin, a food saponin. *Nutrition and Cancer.* 61(1): 27–35.

Reitman S., Frankel S., 1974. Colorimetric method determination of serum glutamate oxaloacetate and glutamate pyruvate transaminase. *Am. J. Pathol*. 28:56-63.

Tong Q.Y., yang He., Qing – Bing Zhao., Yong Quing ., Wen Huang., Xiao- Hua Wu, 2012. Cytotoxicity and apoptosis-inducing effect of steroidal saponins from *Dioscorea zingiberensis* wright against cancer cells. *Steroids.* 77:1219-1227

Farasat M, Khavari-Nejad RA, Nabavi SM, Namjooyan F. Antioxidant Activity, Total Phenolics and Flavonoid Contents of some Edible Green Seaweeds from Northern Coasts of the Persian Gulf. Iran J Pharm Res. 2014 Winter;13(1):163-70. PMID: 24734068; PMCID: PMC3985267.

J Turchan, CB Pocernich, C Gairola et al., Oxidative stress in HIV demented patients and protection ex vivo with novel antioxidants, Neurology. 2003;60(2):307-314.

Uma Maheswari, R., Lakshmi Prabha, A., Nandagopalan V., Anburaja, V., 2012. Green Synthesis of Silver Nanoparticles by Using Rhizome Extract of *Dioscorea oppositifolia* L. and their anti microbial activity against Human pathogens. *IOSR J Pharma Biol Sci.* 1: 38-42

Yang D.J., Lin J.T., 2008. Effects of different storage conditions on steroidal saponins in yam (*Dioscorea pseudojaponica* Yamamoto) tubers. *Food Chemistry* 110:670–677.

Yayoi, T., Kanda, N., and Haratake, A.,2009. Novel effects of diosgenin on skin aging. *Steroids.* 74:504-511.

**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**