Research Article [Suriyavathana & Indupriya, 2(4): April, 2011]
ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

# Screening of antioxidant potentials in *Dioscorea bulbifera*

**M. Suriyavathana* and S. Indupriya**
Department of Biochemistry, Periyar University, Salem, (Tamil Nadu) - India

## Abstract

*Dioscorea bulbifera* is a major staple food crop which is a species of yam widely distributed around the world in tropical and subtropical regions. *Dioscorea bulbifera* have been traditionally used to lower glycemic index, thus providing a more sustained form of energy and better protection against obesity and diabetes. It *also* has anti-cancer properties. The present study was undertaken to investigate the antioxidant activity of *Dioscorea bulbifera.* The ethanolic extracts of tuber *Dioscorea bulbifera* were screened for their enzymatic and nonenzymatic antioxidant activity. The level of enzymatic antioxidant namely Glutathione peroxidase (GPx), Catalase (CAT), Superoxide dismutase (SOD), Glucose-6-phosphate dehydrogenase (G6PD) and glucose-s-transferase (GST) was found to be very impressive. *Dioscorea bulbifera* contains good and commendable store of non enzymatic antioxidants namely reduced glutathione (GSH), Vitamin – C and Vitamin – E. Our results have good significance, as this increase the innate antioxidant potential of *Dioscorea bulbifera*, which is useful in providing the antioxidants needs in the diet and thereby *Dioscorea bulbifera* accomplishes high value nutritive and natural store of antioxidant.

**Key-Words:** *Dioscorea bulbifera,* Antioxidant, Enzymatic, Non-enzymatic, Nutritive

## Introduction

Free radicals are produced as a part of normal metabolic processes. They are extremely reactive, highly unstable and potentially damaging transient chemical species. Under physiological conditions, the cellular redox state is tightly controlled by antioxidant enzymatic systems and chemical scavengers such as endogenous enzymes, dietary antioxidants as well as some hormones[1]. Antioxidants scavenge free radicals and quench the subsequent reactions, hence protecting the macromolecules and cellular environment from toxicity and degeneration[2]. *Dioscorea bulbifera*, the "air potato", is found in both Africa and Asia, with slight differences between those found in each place. It is a large vine, 6 meters (20ft) or more in length. It produces tubers; the bulbils which grow at the base of its leaves are the important food product. They are about the size of potatoes, weighing from 0.5 to 2kg (1 to 5 lbs). The common names for *Dioscorea bulbifera* are air potato, air yam and bitter yam[3].

*** Corresponding Author:**
E-mail:keerthimv87@yahoo.in,
indupriya@gmail.com, suriyaveda@yahoo.co.in
Mob. 91-98943 29286

In traditional Chinese medicine, *Dioscorea bulbifera* is used in the treatment of sore throat, gastric cancer and carcinoma of the rectum[4]. It was used in the treatment of haemoptysis, epistatis, pharyngitis, goiter, pyogenic infection, serofula, architis, sprains and injuries[5]. The present study was designed to evaluate the antioxidant potentials in *Dioscorea bulbifera*. The ethanolic extract of the tuber *Dioscorea bulbifera* was screened for the presence of enzymatic antioxidants namely GPx, CAT, SOD, G6PD and GST and Non-enzymatic antioxidants such as GSH, Vit-C and Vit-E.

## Material and Methods

### Selection of Samples

The tuber species was collected from Kanjamalai hills, Salem district, Tamilnadu, India. The tuber species was authendicated by the taxonomists Balasubramanian.A, Consultant Central Siddha Research, ABS garden, Karipatty – 636 106, Salem district, Tamilnadu, India.

### Preparation of ethanolic extract

The tuber of *Dioscorea bulbifera* was collected and washed well in tap water first and then with the distilled water. The cleaned tubers were sliced and allowed for the complete shade drying and then made to fine powder with homogenizer. About 1.0 gram of clean dried sample was taken along with 10 ml ethanol and mashed well in a homogenizer and filtered, then used for analysis.

ISSN: 0976-7126

**Parameters analyzed**

**Enzymatic Antioxidant**

**Assay of Glutathione Peroxidase[6]:** To 0.2 ml of tris buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide and 0.5 ml of homogenate were added. To the mixture, 0.2 ml of glutathione followed by 0.1 ml of hydrogen peroxide were added. The contents were mixed well and incubated at 37°C for 10 minutes along with a tube containing all the reagents except sample. After 10 minutes the reaction was arrested by the addition of 0.5 ml of 10% TCA, centrifuged and the supernatant was assayed.

**Assay of Catalase[7]:** To 0.9 ml of phosphate buffer, 0.1 ml of homogenate and 0.4 ml of hydrogen peroxide were added. After 60 sec. 2.0 ml of dichromate acetic acid mixture was added. The tubes were kept in boiling water bath for 10 minutes and the colour developed was read at 620 nm.

**Assay of Superoxide Dismutase[8]:** 0.5 ml of homogenate was diluted to 1 ml with water. Then 2.5 ml of ethanol and 1.5 ml chloroform (all reagents chilled) was added. This mixture was shaken for 1 minute at 4°C and then centrifuged. The enzyme activity in the supernatant was determined.

**Assay of Glutathione-S- Transferase[9]:** The reaction mixture contained 1.0 ml of phosphate buffer, 0.1 ml of CDNB, 0.1 ml of homogenate and 0.7 ml of distilled water. The reaction mixture was incubated at 37°C for 5 minute then the reaction was started by the addition of 0.1 ml of 30mM glutathione. The absorbance change was read at 340nm for 5 minutes. Reaction mixture without the enzyme was used as the blank.

**Assay of Glucose-6-Phosphate Dehydrogenase[10]:** The incubation mixture contained 1.0 ml of buffer, 0.1 ml of magnesium chloride, 0.1 ml of $NADP^+$, 0.5 ml of phenazine methosulphate, 0.4 ml of the dye solution and the requisite amount of the enzyme extract. The mixture was allowed to stand at room temperature for 10 min to permit the oxidation of endogenous materials. The reaction was initiated by the addition of 0.5 ml of glucose-6-phosphate. The absorbance was read at 640nm against water blank at one min intervals for 3-5 min in a UV Spectrophotometer. The activity of the enzyme was calculated in units by multiplying the change in OD/min by the factor 6/17.6, which is the molar extinction co-efficient of the reduced co-enzyme.

**Non enzymatic antioxidants**

**Estimation Of Vitamin E1[1]:** This method involves the reduction of ferric ion to ferrous ion by α-tocopherol and the formation of a red coloured complex with 2,2′-dipyridyl. Absorbance of the chromophore was measured at 520 nm.

**Estimation of Vitamin C[12]:** 0.5 ml of tissue homogenate was mixed thoroughly with 1.5 ml of 6% TCA and centrifuged for 20 minutes at 3500 g. To 0.5 ml of the supernatant, 0.5 ml of DNPH reagent was added and mixed well. The tubes were allowed to stand at room temperature for an additional 3 hours. Removed, placed in ice-cold water and added 2.5 ml of 85% sulphuric acid and allowed to stand for 30 minutes. A set of standards containing 10-50µg of ascorbic acid were taken and processed similarly along with a blank, containing 0.5 ml 4% TCA. The colour developed was read at 530 nm.

**Estimation of Reduced Glutathione[13]:** A known weight of tissue was homogenized in phosphate buffer. From this 0.5 ml was pipetted out and precipitated with 2.0 ml of 5% TCA. 1.0 ml of the supernatant was taken after centrifugation and added to it 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer. The yellow colour developed was read at 412 nm. A series of standards were treated in a similar manner along with a blank containing 3.5 ml of buffer.

## Results and Conclusion

Table - I shows the level of enzymatic antioxidants namely GPx, CAT, SOD, G6PD and GST in *Dioscorea bulbifera.* The proximate analysis conclusively validates *Dioscorea bulbifera* posses high amount of GPx followed by SOD, CAT and very small amount of G6PD and GST. GPx catalyses the decomposition of both hydrogen peroxide and organic peroxide (ROOH) at expense of Reduced Glutathione will be formation of Glutathione disulphide (GSSH) water and organic alcohol[14]. The inference of GPx shows that *Dioscorea bulbifera* posses very good store of important antioxidant and emphasis significant scavenging potential. Catalase is the most significant antioxidant enzyme which protects plants by scavenging free radicals and $H_2O_2$ [15]. Low level of catalase was noted in *Dioscorea bulbifera. Dioscorea bulbifera* contains significant amount of SOD.SOD prevents the formation of ·OH and hence been implicated as an essential defence against the potential toxicity of oxygen. *Dioscorea bulbifera* contains low level of GST and G6PD. GST offers protection against LPO by the conjugation of toxic effect with GSH[16]. G6PD contributes to the maintenance of cellular redox homeostasis by supplying reducing equivalent for efficient glutathione reductase activity and glutathione recycling.[17]

The level of non-enzymatic antioxidants namely Vit-E, Vit-C and GR are presented in the Table - II. *Dioscorea bulbifera* exhibited high level of Vit-C while the level of Vit-E and GR was found to be minimum. Glutathione reductase is a flavour protein enzyme catalysis the reduction of oxidized glutathione to reduced glutathione[18]. Low level of GR is present in *Dioscorea bulbifera.* Vitamin – C is an effective

scavenging of free radicals, which includes $O_2^{\cdot}$,$HO_2^{\cdot}$,$RO_2^{\cdot}$RS$^{\cdot}$and other sulphur and nitrogen radicals[6]. *Dioscorea bulbifera* exhibited high level of ascorbic acid . Vitamin – E resides in the lipid bilayer of the cell membrane. It can transfer its phenolic hydrogen to a peroxyl free radical of peroxidised PUFA, in cellular and subcellular phospholipids[20]. Low level of Vit-E was present in *Dioscorea bulbifera*. The level of antioxidant response in the development of the metabolic state of plant *Dioscorea bulbifera* shown his notable scavenging activity, which emphasis its antioxidant status. *Dioscorea bulbifera* contains good and commendable store of both enzymatic and non-enzymatic antioxidants.

Tubers tend to be very starchy and typically rich in vitamins and minerals. The main nutritional value of roots and tubers lie in the potential ability to provide one of the cheapest source of dietary energy in the form of carbohydrate this make them an excellent addition to the human diet. Since tubers crops form an important stable food crops in tropical country like India. The present study has been initiated with the view and objective to explore the antioxidants store in *Dioscorea bulbifera*. The results arrived were found to be very encouragive and was found to posses valid store of antioxidants. *Dioscorea bulbifera* has the potential to protect the cell against free radical mediated oxidative damage.Hence from the result it may be concluded that posses a promising source of non toxic natural antioxidant. There by to conclude *Dioscorea bulbifera* will certainly serve as a main tuber with high content of nutritive potential and hence can be advocated as a functional food for the future.

## Acknowledgements

We are grateful to Department of Biochemistry, Periyar University, Salem-11, Tamil Nadu, India for supporting the study.

## References

1. Marcel Dekker (1996). *Toxicological, Health Perspective*, 1st ed.; New York, NY, USA, 1-32.
2. Vichneveshaia, K.D. and Roy, D.N. (2001). Oxidative stress and antioxidative depense with a emphasis on plants antioxidants. *Enviro. Rev*., **7**: 31-35.
3. Kay D. E. (1987). Root crops. Tropical development and research institute: London.**66 (4):** 86-92.
4. Gao H., Wu L. and Kuroyanagi M. (2001). Seven Compounds from *Dioscorea bulbifera* L. *Natural medicines.,* **55(5)**:277-82.
5. World Health Organization (1997). Medicinal plants in China, A selection and 150 commonly used species, WHO Regional Publications, Western Pacific Series No.2, 110-1.
6. Rotruck J.J., Pope A.L., Gantter H.E. and Swanson A.B. (1973). Selenium: Biochemical role as a component of glutathione Peroxidase. *Science*, **179**: 588 – 590.
7. Sinha A.K. (1972). Colorimetric assay of catalase. *Anal. Biochem.,* **47**: 389 – 394.
8. Kakkar P. and Vishwanathan P.N. (1984). A modified Spectrophotometric assay of Superoxide dismutase. *Indian Journal of Biochem. Biophys.,* **21**: 130 – 132.
9. Habig W.H., Pabst M.J. and Jakoby W.B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem*., **246**: 7130 - 7139.
10. Ellis H.A. and Kirkman H.N. (1961). *Proc. Soc. Exp. Biol*. (N.Y.): 106 - 607
11. Baker H. Frankel, O. De Angelis B. and Feingold S. (1980). Plasma α-tocopherol in man at various time intervals after ingesting free or acetylated tocopherol. *Nutr. Rep. Int.,* **21**: 531 - 536.
12. Omaye S.T., Turnbull T.D. and Sauberlich H.C. (1979). Selected method for the determination of ascorbic acid in animal cells tissues and fluids. *Methods Enzymol* **62:** 3 - 11.
13. Ellman G.L. and Boyne A.F. (1972). A methodology for analysis of tissues sulfhydryl components. *Anal. Biochem*., **46(2)**: 639 - 53.
14. Vural H., Aksoy N. and Ozbilge H. (2004). Alterations of oxidative antioxidative status in human cutaneous leishmanisis. *Cell Biochem. Funct*., **22**:153-156.
15. Vichneveshaia K.D. and Roy D.N. (2001). Oxidative stress and antioxidative depense with a emphasis on plants antioxidants. *Enviro. Rev.,* **7**: 31-35.
16. Jakeby W.B. and Habig W.H. (1980). Glutatjone tramscereals. In : Jokeby, W.B (Ed) *Enzymatic Basis of detoxification.,* Academic press. New York. 63-94
17. Leopold J.A. and Losoalzo J. (2000). Cyclic strains modulates resistance to oxidant stress by increasing G6PD expression in vascular smooth muscle cells. *Am.K. Physiol.,* **279**: 2477-2485.
18. Smith L.L. (1991). Another cholesterol hypothesis: cholesterol as antioxidant. *Free Radic. Biol. Med.,* **11(1)**: 47-61.
19. Jacob R.A. (1995). The integrated antioxidant system, *Nutrition Research*, **15**: 755-766.

20. Sen C., Khanna S. and Roys (2006). Touotrienols: Vitamin-E beyond tocophorols. *Life Science*, **78(18)**:2088-98.

**Enzymatic antioxidants**

**Table – I Level of glutathione peroxidase, catalase and superoxide dismutase in *Dioscorea bulbifera***

| Species | Glutathione peroxidase (µg/mg) | Catalase (µg/mg) | Super oxide dismutase (µg/mg) | Glucose – 6 – phosphate dehydrogenase (MIU/mg) | Glutathione -S-transferase (µg/mg) |
|---|---|---|---|---|---|
| *Dioscorea bulbifera* | 909 ± 0.5941 | 82.64± 0.1880 | 457.74 ± 0.1246 | 0.750 ± 0.0042 | 23.43 ± 0.0272 |

**Non- enzymatic antioxidants**

**Table – II Level of vitamin-e, vitamin-c and reduced glutathione in *Dioscorea bulbifera***

| Species | Vitamin – E (µM/mg) | Vitamin - C (µM/mg) | Reduced Glutathione (µM/mg) |
|---|---|---|---|
| *Dioscorea bulbifera* | 83.33 ± 0.0756 | 92 ± 0.1491 | 4.92 ± 0.0253 |