*Ethnobotanical Leaflets 13: 443-49, 2009.*

# The Antioxidant Activity of the Leaves of *Barleria grandiflora* Dalz. (Acanthaceae)

H. A. Sawarkar*, S. S. Khadabadi, M. D. Wandhare, I. A. Farooqui, and U. A. Deokate
Govt. College of Pharmacy, Pharmacognosy and Phytochemistry Department,
Kathora Naka, Amaravati- 444604 (M.S.)
*Address for Correspondence**:** **mrhemant1979@gmail.com**

**Issued 01 April 2009**

**Abstract**

Aqueous and hydro alcoholic extracts of the leaves of *Barleria grandiflora* Dalz. were evaluated for the antioxidant activity by the FTC and TBA methods. The results obtained in the present study indicate that the leaves of *Barleria grandiflora* are potential source of natural antioxidants. Initial phytochemical screenings of the extracts have shown the presence of flavanoids, tannins, saponins, carbohydrates and aminoacids

**Keywords:** Antioxidant activity, *Barleria grandiflora*, FTC and TBA methods.

## Introduction

Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions ($O2^-$) and hydroxyl radicals ($OH^-$), as well as nonfree-radical species such as hydrogen peroxide (H2O2)[1,2]. In living organisms various ROSs can form in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionising radiation, certain pollutants, organic solvents, and pesticides [3-5]. Free radicals can cause lipid peroxidation in foods, which leads to their deterioration [6, 7]. In addition, reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer [8-11]. When produced in excess, ROSs can cause tissue injury. However, tissue injury can itself cause ROS generation[12]. Nevertheless, all aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damages, and numerous damage removal and repair enzymes to remove or repair damaged molecules [4, 13-15]. However, this natural antioxidant mechanism can be inefficient, and hence dietary intake of antioxidant compounds is important [11, 16, 17]. There are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed foods. However, it has been suggested that these compounds have some side effects [18, 19]. In addition, it has been suggested that there is an inverse relationship between dietary intakes of antioxidant rich food and the incidence of human disease [20]. *Barleria grandiflora* Dalz. (Acanthaceae), commonly known as Dev- koranti or Shwet- koranti is a large unarmed shrub, stem branched, branches terrete, quite glabrous except for few small hairs at the node. Leaves are elliptic-lanceolate, acuminate, glabrous, base acutely tapering. Flowers axillary, solitary, bracteoles linear-ligulate[21]. The Literature survey reveals that not much of work has been reported towards the biological activities of the shrub. However, juice of the leaves of the shrub is being used in the treatment of mouth ulcers among certain ethnic groups of Vidarbha region of Maharashtra, prompted us to

carryout antioxidant activity. The purpose of this particular study is to determine antioxidant activities of leaf extracts of *Barleria grandiflora* Dalz. (Acanthaceae).

## Materials and Methods

*Plant material*

The shrub *Barleria grandiflora* Dalz. (Acanthaceae) was collected from Amaravati (Maharashtra) and was authenticated by Dr. Prabha Y. Bhogaonkar, Director, VMV College, Amaravati. Leaves were collected from the shrub, dried in shed and used for further work

*Preparation of Extracts*

250 g of dried leaves were chopped into small parts in a blender and then macerated with 450 ml of boiled water with occasional for 30 min followed by filtration, concentration and drying of the extract. 250 g of dried leaves were chopped into small parts in a blender and then subjected to Soxhelet extraction to obtain hydro alcoholic extract, which is then filtered, concentrated and dried.

*Phytochemical Screening*

The freshly prepared extracts were chemically tested for the presence of different constituents using standard methods[22].The preliminary phytochemical screening carried out for the extracts have shown the presence of flavanoids, tannins, saponins, carbohydrates and aminoacids.

*Antioxidant activity*

*Ferric thiocyanate (FTC) method*

A mixture containing 4 mg of the sample in 4 ml of 99.5% ethanol (final concentration 0.02%). 4.1ml of 2.52% linoleic acid in 99% ethanol, 8 ml of 0.05M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with screw cap and then placed in an incubator at $40^0$ C in the dark. To 0.1 ml of this mixture 9.7 ml of 75% ethanol (v/v) and 0.1 ml of 30% ammonium thiocyanate were added. Precisely 3 minutes later the addition of 0.1ml of 0.02M ferrous chloride in 3.5% hydrochloric acid was added to reaction mixture; (the absorbance of red color indicated the antioxidant activity) was measured at 500 nm for every 24 hours until the absorbance of the control reached maximum. The control and the standard were subjected to the same procedures as the sample except that for the control, only the solvent was used, and for the standard 4mg of the sample was replaced by 4 mg of Vitamin C [23].

*Thiobarbituric acid (TBA) method*

TBA method used for evaluating the extent of lipid peroxidation. At low pH, and high temperature (100 $^0$C), melonaldehyde binds TBA to form a red complex that can be measure at 532 nm. 2 ml of 20% trichloroacetic acid and 2 ml of 0.67% TBA solutions were added to 2 ml of the mixtures containing the sample prepared in the FTC method. This mixture was kept in water bath (100 $^0$C) for 10 minutes and after cooling to room temperature, was centrifuged at 3000 rpm for 20 minutes. Antioxidant activity was based on the absorbance of the supernatant at 532 nm on the final day of the assay [24s].The percentage of antioxidant activity was calculated by following formulae for both FTC and TBA methods.

$$\text{Percentage of antioxidant activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

## Results

In FTC method, the total antioxidant activities elicited by the extracts were shown in table 1. in terms

of absorbance at 500 nm. In TBA method, the control produced highest absorbance value (0.141) followed by extracts, aqueous extract (0.131) and hydro alcoholic extract of leaves (0.124) which is shown in table 2.

**Table 1:** Antioxidant activity of aqueous and hydro alcoholic extracts of leaves of *B.grandiflora* (FTC method).

| | Absorbance | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day-1 | Day-2 | Day-3 | Day-4 | Day-5 | Day-6 | Day-7 | Day-8 | Day-9 |
| Control | 0 | 0.199 | 0.260 | 0.280 | 0.319 | 0.441 | 0.480 | 0.593 | 0.550 |
| Vitamin c | 0 | 0.0089 | 0.023 | 0.025 | 0.035 | 0.045 | 0.196 | 0.207 | 0.304 |
| Aqueous leaves extract | 0 | 0.0189 | 0.0242 | 0.0272 | 0.041 | 0.083 | 0.260 | 0.293 | 0.321 |
| Hydro alcoholic leaves extract | 0 | 0.0145 | 0.0233 | 0.0260 | 0.038 | 0.060 | 0.214 | 0.250 | 0.310 |

**Table 2:** Antioxidant activity of aqueous and hydro alcoholic extracts of leaves of *B.grandiflora* (TBA method).

| | Absorbance |
|---|---|
| Control | 0.141 |
| Vitamin C | 0.0916 |
| Aqueous extract of leaves | 0.131 |
| Hydro alcoholic extract | 0.123 |

**Discussion and Conclusion**

The powerful antioxidants including superoxide anions, hydroxyl radicals and hydrogen peroxide are known as free radicals. Free radicals are unguided missiles that bounce around and attack healthy cells, tearing the cell membranes, genetic damage and mutations. They react with serum lipoprotein (LDL) and causes the formation of atheromatous plagues or react with the cell membranes lipid and cause of peroxidation of polyunsaturated fatty acids and cause generation of further free radicals. So, the antioxidants are needed in the different compartments of the body such as the circulating system inside the cells and across the blood-brain-barrier and central nervous system. The leaves of the shrub *Barleria grandiflora* Dalz. (Acanthaceae). were screened for their antioxidant activity by using FTC and TBA methods. FTC method was used to measure the amount of peroxide formed at the primary stage of linoleic acid peroxidation. The peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment. In this method the concentration of peroxide decreases as the antioxidant activity increases. The control showed increase in absorbance values from day 1 and reached on day 8 and dropped on day 9 (Table 1). This reduction is due to the increased level of melonaldehyde compounds from linoleic acid oxidation, which is not stable. Antioxidant activity was based on the absorbance of the final day in TBA method (Table 2). It showed total peroxide values produced by oxidation of linoleic acid. The higher absorbance value indicates the lower level of antioxidant. Based on the absorbance rates, the aqueous and hydro alcoholic

extracts of leaves possesses significant antioxidant activity as compared to the standard Vitamin C. Generally, the hydro alcoholic extract of the leaves showed lower absorbance in both FTC and TBA methods, which indicates that, the hydro alcoholic extract has high antioxidant activity as compared to aqueous extract. The strong antioxidant activity of leaves of the shrub *Barleria grandiflora* may be the reason behind the use of juice of leaves in the treatment of mouth ulcers.

**References**


1) Halliwell B. 1995. How to characterize an antioxidant: an update. *Biochem. Soc. Symp.* 61: 73-101.

2) Squadriato Gl. and Pelor WA. 1998. Free Rad. Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite, and carbon dioxide. *Biology and Medicine* 25: 392-403.

3) Halliwell B. and Gutteridge JM. 1989. *Free Radicals in Biology and Medicine.* Clarendon Press Oxford, pp 23-30.

4) Davies K J A. 1994. Oxidative stress the paradox of aerobic life. *Biochem. Symp*. 61: 1- 34.

5) Robinson EE. Maxwell SRJ. and Thorpe GHG. 1997. An investigation of the antioxidant activity of black tea using enhanced chemiluminescence. *Free Rad. Res*. 26: 291-302.

6) Sasaki S. Ohta T. and Decker EA. 1996. Antioxidant activity of water soluble fractions of salmon spermary tissue. *J. Agric. Food Chem.* 44: 1682-1686.

7) Miller NJ. Diplock AT. and Rice-Evans CA. 1995. Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage *J. Agric. Food Chem* 43, 1794- 1801.

8) Tanizawa H. Ohkawa Y. Takino Y. Miyase T. Ueno A. Kageyama T. and Hara S. 1992. Studies on natural antioxidants in citrus species I. Determination of antioxidative activities of citrus fruits. *Chem. Pharm. Bull*. 40: 1940-1942.

9) Hertog MGL. Feskens EJM. Hollman PCH. Katan MB. and Kromhout D. 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: The zupthen elderly study. *The Lancet* 342: 1007- 1014.

10) Alho H. and Leinonen J. 1999. Total antioxidant activity measured by chemiluminescence method. *Methods in Enzymology* 299: 3-15.

11) Duh P-D. 1998. Antioxidant activity of Burdock: Its scavenging effect on free-radical and active oxygen. JAOCS 75: 455- 463.

12) Auroma OI. 1998. Free radicals, oxidative stress, and antioxidants in human health and disease JAOCS 75: 199-212.

13) Granelli K. Bjorck L. and Appelqvist L-A. 1995. The variation of SOD and XO activities in milk using an improved method to quantitate SOD activity. *J. Sci. Food Agric.* 67: 85-91.

14) Fridowich I. 1995. Superoxide radical and superoxide dismutases. *Annu. Rew. Biochem.* 64: 97-112.

15) Sun J. Chen Y. Li M. and Ge Z. 1998. Role of antioxidant enzymes on ionizing radiation resistance. Free Rad. Biology and Medicine 24, 589-593.

16) Halliwel B. 1994. Free radicals, antioxidants and human disease: Curiosity, cause or consequence. *The Lancet* 344: 721- 724.

17) Terao J. Piskula M. and Yao Q. 1994. Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. *Arch. Biochem. Biophys.* 308: 278-284.

18) Branien AL. 1975.Toxicoly and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. JAOCS 52: 59-63.

19) Ito N. Fukushima S. Hassegawa A. Shibata M. and Ogiso T. 1983. Carcinogenicity of butylated hydroxyanisole in F344 rats. *J Natl. Cancer Inst*. 70: 343-347.

20) Rice-Evans CA. Sampson J. Bramley PM. Hollowa DE. 1997. Why do we expect carotenoids to be antioxidants in vivo. Free Rad. Res. 26: 381-398.

21) K.V.Peter . Underutilized and Unexploited Horticultural plants; Vol.1:139.

22) Trease G E. and Evans W.C. 1985. *Pharmacognosy*. London: Bailliere Tindall.

23) Kikuzaki, H N Nakatani, M. 1993. Antioxidant effects of some ginger constituents. *J. Food . Sci.* 58 (6): 1407-1410.

24) Ottolenghi, A. 1959. Interaction of ascorbic acid on mitochondria Lipids. *Arch. Biochem. Biophys.* 79: 355-359.