

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

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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 (O_2^-) and hydroxyl radicals (OH^-), as well as nonfree-radical species such as hydrogen peroxide (H_2O_2)^{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 pesticides³⁻⁵. 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 cancer⁸⁻¹¹. When produced in excess, ROSs can cause tissue injury. However, tissue injury can itself cause ROS generation¹². 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 disease²⁰. *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-ligulate²¹. 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 Soxhlet 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 methods²². 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 40⁰ 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 C²³.

Thiobarbituric acid (TBA) method

TBA method used for evaluating the extent of lipid peroxidation. At low pH, and high temperature (100⁰C), 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 (100⁰C) 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 plaques 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.

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