INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *BARLERIA GRANDIFLORA*

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Article Received on: 10/06/15 Revised on: 30/07/15 Approved for publication: 19/08/15

DOI: 10.7897/2230-8407.069124

ABSTRACT

Introduction: The juice of leaves of *Barleria grandiflora* has been used by folk across central region of India for the treatment of oral ulcers. The objective of the study is to determine the antioxidant and the antibacterial activity of ethanolic extract of leaves of *Barleria grandiflora* Dalz (Acanthaceae). Materials and Methods: Phytochemical screening was carried out as per the usual chemical tests. The TLC was carried out to determine the constituents which might be similar to *Barleria prionitis*. Total phenolic content was determined using Folin-Ciocalteau method. Antioxidant activity was performed by using hydrogen peroxide radical scavenging method and DPPH (1, 1-Diphenyl-2- picrylhydrazyl) radical scavenging activity. Antibacterial activity was carried using agar well diffusion method. Results: Phytochemical screening had shown the presence of carbohydrates, proteins, alkaloids, glycosides, phenolic compounds and flavanoids in ethanolic extracts of leaves of the plant. The total phenolic content was found to be 75.44 mg/10ml. *Barleria grandiflora* was found to have antioxidant potential comparable to ascorbic acid (vitamin c). It was also found that the ethanolic extract of *Barleria grandiflora* shows significant antibacterial activity. Zones of inhibition of ethanolic extract of *Barleria grandiflora* were found comparable to that of standard. Conclusion: Ethanolic extract of leaves of *Barleria grandiflora* exhibited antioxidant and antibacterial activity which may be the reason for the use of juice of *Barleria grandiflora* leaves in the treatment of mouth ulcers by some folk.

Keywords: *Barleria grandiflora*, Phytochemical Screening, Thin Layer Chromatography, Antioxidant Activity, Antibacterial Activity.

INTRODUCTION

The genus *Barleria* includes 28 taxas and 23 species. *Barleria grandiflora* Dalz (Acanthacae) commonly known as *Dev koranti*, *Kate koranti*, *Shemmuli* in Marathi and Tamil, is frequently distributed in North India especially in Vidarbha region of Maharashtra[1]. The leaves extracts of the plant Barleria grandiflora have been reported with antioxidant activity[2, 3] and anticancer activity[4, 5]. Oral ulcers or apthous ulcers are common with an estimated point prevalence of 4% in the world wide. Apthous ulcers may affect as many as 25% of population worldwide. Most ulcers are benign and resolve spontaneously but small proportions of them are malignant[6]. Ethanobotanical survey reveals the use of *Barleria grandiflora* (B.G.) leaves juice as a medicine in the treatment of oral ulcer, tumours by the tribal people of Maharashtra in Vidarbha region[5, 7]. The aim of this study is to perform TLC fingerprinting, antioxidant activity and antibacterial activity of alcoholic extracts of the herb. Antioxidants play major role in the living system and it prevents oxidative damage. 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 non free-radical species such as hydrogen peroxide (H_2O_2)[8, 9]. 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 [10-13]. When produced in excess, ROSs can cause tissue injury. However, tissue injury can itself cause ROS generation [14]. Also, antioxidants are commonly prescribed agents in treatment of oral ulcers. Bacteria like *Staphylococcus aureus, streptococcus mutans* and *Lactobacillus sporogens* are reported to be one of the causes for oral ulcers[15]. 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 amongst certain folk of Vidarbha region of Maharashtra, prompted us to carryout antioxidant activity and antibacterial activity.

MATERIALS AND METHODS

Plant Material

The leaves of *Barleria grandiflora* Dalz (Acanthaceae) were collected from tribal region of Amravati (Maharashtra) in the month of July and were authenticated by Dr. Mrs Ranjana Shrivastava, Professor, Department of Botany, Durg Science College, Durg (Chattisgarh). Leaves were collected from the shrub, dried in shade and used for further work.

Microorganisms

Staphylococcus aureus, streptococcus mutans and *Lactobacillus sporogens* were kindly made available by pathology department of Jawahar Lal Nehru Hospital, Sector-9, Bhilai, Chhattisgarh.

Chemicals and Reagent

Ethanol, petroleum ether, ethyl ether, acetone, phosphate buffer saline (PBS) pH 7.4, gallic acid, Folin's-ciocalteu reagent, sodium carbonate, ascorbic acid, DPPH (Diphenyl Picryl Hydrazine), H_2O_2, Dimethyl sulphoxide (DMSO), chlorhexidine etc.

Preparation of Extract

The leaves of plant *Barleria grandiflora* Dalz were powdered first. The powdered plant material (leaves) was subjected to Soxhlet extraction in order to get the ethanolic extract. In Soxhlet extraction process the powdered material was kept in Soxhlet apparatus using ethanol as solvent at 68-70ºC for 7-8 hrs, after that filtration was

done with vacuum pump. The filtrate was subjected to distillation for removing the solvent, after distillation the semi solid material was kept in vacuum desiccators for drying (EBG).

Preliminary Phytochemical Screening

EBG was subjected to preliminary phytochemical screening as per the standard chemical tests, in order to determine the presence of various phyto-constituents [16-19].

Thin Layer Chromatography

Thin-layer chromatography or TLC is a solid-liquid form of chromatography where the Stationary phase is normally a polar adsorbent and the mobile phase can be a single solvent or combination of solvents. TLC is a simple, quick, and inexpensive procedure. TLC is used to reveal identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound (preferably both run on the same TLC plate) [19]. TLC was carried out using mobile phase- Pet-ether: chloroform: acetone (3:1.5:0.5)[3]. Thin layer chromatography was carried using ethanolic extract of *Barleria grandiflora* and compared with ethanolic extract of *Barleria prionitis* as a reference drug. It might be possible that it contains some constituents which may be similar to that of *Barleria prionitis*.

Total Phenolic Content

Total Phenolic content was determined according to Folin-Ciocalteau method [20]. The reaction mixture was composed of 0.1 ml of extract, 7.9 ml of distilled water, 0.2 ml of Folin-Ciocalteau reagent and 1.5 mL of 20% sodium carbonate. The resultant solution was mixed and allowed to stand for 2 hrs. The Absorbance was measured at 765 nm with Schimadzu UV- spectrophotometer. The total phenolic content was determined as gallic acid equivalent per mg of extract.

Scavenging of Hydrogen Peroxide Radicals

The hydrogen peroxide radical scavenging activity was assessed by Ruch et al method with slight modifications[3, 21]. 30 mg of each extracts and the standard (ascorbic acid) were accurately weighed and separately dissolved in 10 ml of methanol. These solutions were serially diluted with methanol to obtain the lower dilutions. A solution of H_2O_2 (20 mM) was prepared in PBS (pH 7.4). Various concentrations of 1 ml of the extracts or standards in methanol were added to 2 ml of H_2O_2 solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without H_2O_2. The percentage of inhibition was calculated based on the formula:

$$\% \text{ of inhibition} = (A1\text{-}A2)/A1 \times 100$$

Where, A1 - Absorbance of the H2O2 and A 2 -absorbance of the reaction mixture with extract.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was determined by using ethanolic extract of B. G. leaves. The extract was mixed with 3.0 ml DPPH (0.5 m mol/ L) in methanol, the resultant absorbance was recorded at 517 nm after 30 minute incubation at 37°C [22-24]. The percentage of scavenging activity was derived using the following formula,

$$\% \text{ of inhibition} = (A_1 - A_2)/A_1 \times 100$$

Where A_1 – Absorbance of DPPH, A_2 – Absorbance of the reaction mixture with extract (DPPH with Sample)

Antibacterial Activity

The antibacterial activity of the extract of *B. grandiflora* was carried out against *Staphylococcus aureus, streptococcus mutans* and *Lactobacillus sporogens* by agar well diffusion method. The zone of inhibition was determined by using ethanolic extract of *B. grandiflora*. The zones of inhibition obtained with different concentration of ethanolic extract were compared with to that of standard drug (Chlorohexidine) [25-27].

RESULTS

Phytochemical Screening

Qualitative chemical tests were performed on EBG had shown presence of phytoconstituents such as carbohydrates, protein, alkaloids, glycosides, tannins, Phenolic compounds, saponins etc.

Thin Layer Chromatography

Thin layer chromatography was carried using ethanolic extract of *Barleria grandiflora* and compared with ethanolic extract of *Barleria prionitis* as a reference drug. Thin layer chromatography of *Barleria prionitis* shows nine different spots with nine Rf values. TLC of *Barleria grandiflora* shows six different spots with six Rf values [Figure 1, Table 1]. When compared, *Barleria grandiflora* shows four different spots whose Rf values matches to that of *Barleria prionitis*. This reveals the possibility of four chemical constituents in *Barleria grandiflora* may be similar when compared to that of ethanolic extract of *Barleria prionitis*.

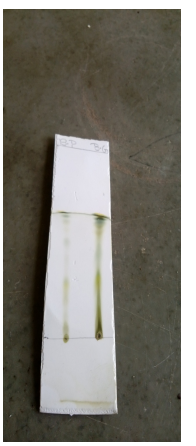


Figure 1: Photograph of TLC plate

Table 1: Rf values of ethanolic extracts of BP and BG

Sample	RF values								
	1	2	3	4	5	6	7	8	9
EBP	0.14	0.17	0.28	0.42	0.6	0.74	0.85	0.91	0.97
EBG	0.51	0.74	0.85	0.91	0.94	0.97	-	-	-

Total Phenolic Content

The total phenolic content is directly related to antioxidant activity. Total Phenolic content for EBG was found to be 75.44 mg/10ml.

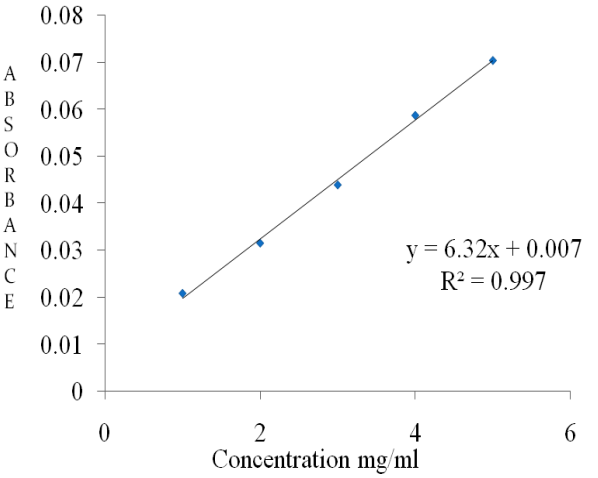


Figure 2: Gallic acid Equivalence curve

H_2O_2 Radical Scavenging Activity

Hydrogen peroxide is generated *in vivo* by several oxidase enzymes and by activated phagocytes and it is known to play an important role in the killing of several bacterial and fungal strains. There is increasing evidences that, H_2O_2, either directly or indirectly, OH^+ can act as a messenger molecule in the synthesis and activation of several inflammatory mediators. Graph 2 shows significant antioxidant activity of EBG in comparison to that of standard vitamin C. Gradual increment in percent inhibition can be observed with increasing concentration of the extract.

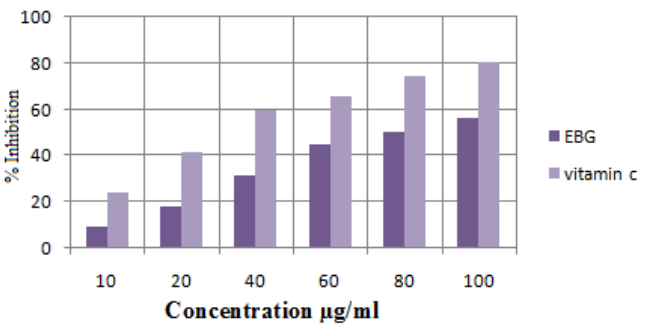
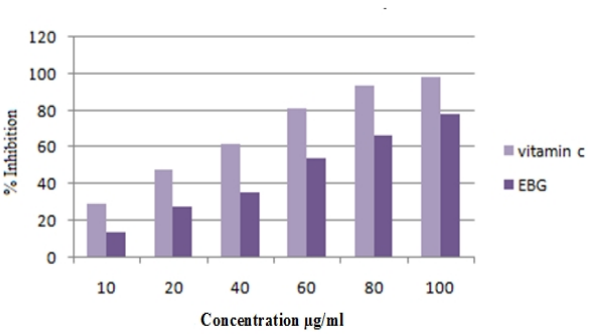


Figure 3: H_2O_2 radical scavenging activity

DPPH Radical Scavenging Activity

Graph 3 shows DPPH radical scavenging activity of EBG compared with Standard vitamin c. EBG shows promising percent inhibition which is comparable to that of vitamin C.



Graph 3: DPPH radical scavenging activity of EBG

Antibacterial Activity

The Antibacterial activity of the extract of *B. grandiflora* was studied against *Staphylococcus aureus, streptococcus mutans* and *Lactobacillus sporogens* bacteria using agar well diffusion method. Zones of inhibition (mm) of EBG were compared with standard (chlorhexidine 10 µg/ml). Accordingly, Antibacterial activity of ethanolic extract was found comparable to standard chlorhexidine [Table 2].

Table 2: Antibacterial activity of ethanolic extract of BG

Sample	Concentration µg/ml	Zone of Inhibition in mm (Mean ± Standad Deviation)		
		E. coli	*E Faecalis*	*L. sporogens*
Standard	10	23.53 ± 0.05	30.53 ± 0.05	25.46 ±0.11
DMSO	-	-	-	-
EBG	10	11.16 ± 0.05	16.2 ± 0.1	11.4 ± 0.05
	20	12.2 ± 0.17	17.5 ± 0.05	12.6 ± 0.05
	40	14.1 ± 0.05	19.7 ± 0.05	14.7 ± 0.05
	60	17.1 ± 0.05	23.0 ± 0.05	17.5 ± 0.05
	80	18.5 ± 0.05	24.2 ± 0.05	18.8 ± 0.05
	100	20.13 ± 0.05	25.6 ± 0.05	20.3 ± 0.05

DISCUSSION

Thin layer chromatography was carried for ethanolic extract of *Barleria grandiflora* and compared with ethanolic extract of *Barleria prionitis* as a reference drug .when compared ethanolic extract of *Barleria grandiflora* shows 4 different spots whose Rf values matching to Rf values of *Barleria prionitis*. This reveals that the possibility of four chemical constituent in *Barleria grandiflora* may be similar to that of ethanolic extract of *Barleria prionitis.* The purpose of TLC was to determine the constituents which might be similar to *Barleria prionitis*. *Barleria prionitis* was reported to contain chemical constituents like barlerin, balarenone, barlerioside, acetyl barlerin, verbascoside etc[28]. Free radicals may be one of the causes for occurrence of oral ulcers. Antioxidant activity was performed by using two methods like Hydrogen peroxide radical scavenging activity and DPPH (1, 1-Diphenyl-2- picrylhydrazyl) activity. The total phenolic content was found to be 75.44 mg/10ml. Barleria grandiflora was found to have antioxidant potential and was found to be comparable with Ascorbic acid (vitamin c). Micro-organisms are reported to cause oral ulcers and hence antibacterial activity was carried out. It was found that the ethanolic extract of *Barleria grandiflora* shows antibacterial activity. Zones of inhibition of ethanolic extract of *Barleria grandiflora* were found comparable to that of standard.

CONCLUSION

Barleria grandiflora was found to have antioxidant and antibacterial activity this could be the reason for the use of juice of *Barleria grandiflora* leaves in the treatment of mouth ulcers by some tribal people. Some of the remedies used by ethnic communities are not part of allied system as they are not reported in literature of medicine; still these remedies are tried by such communities. The study of tribal knowledge of plants is an imperative facet of ethnobotanical research. People healed themselves with traditional herbal medicines and ancient remedies from time immemorial. Human beings have found remedies within their habitat, and have adopted different strategies depending upon the climatic, phyto-geographic and faunal characteristics, as well as upon the peculiar culture and socio-structural typologies. Most of such information is passed on to the following generations by traditional healers through oral communication and discipleship practice. Moreover, the World Health Organization (WHO) has reported that about 80% of the world population relies on traditional medicine to cure ailments. Plants play a major role in the treatment of diseases and still remain the foremost alternative for a large majority of people. This knowledge, if wisely utilized, could draw out promising herbal leads[29-33]. Herbal medicines are assumed to be of great importance in primary healthcare of individuals and communities as the herbal medicines are comparatively safer than synthetic drugs. Plant based traditional knowledge has become a recognized tool in search of new sources of drugs and neutraceuticals[34]. The evaluation of antioxidant activity and antibacterial activity of *Barleria grandiflora* leaves are an attempt to explore a folklore claim. Further, usefulness of the herb in treatment of oral ulcers can be studied using in-vivo studies and clinical trials may be carried out to strengthen the claim. Based on the findings, the extract can be developed to a suitable formulation. Also, isolation of chemical constituents can be carried out to relate them with antioxidant and antibacterial potential of the herb.

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Cite this article as:

H. A. Sawarkar, H. J. Dhongade, Ajit Pandey, Deepak Biswas, Pranita P. Kashyap, C.D. Kaur. Phytochemical and pharmacological evaluation of *Barleria grandiflora*. Int. Res. J. Pharm. 2015; 6(9):635-639 http://dx.doi.org/10.7897/2230-8407.069124

Source of support: Nil, Conflict of interest: None Declared