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A comparative study on total phenolic content, reducing power and free radical scavenging activity of aerial parts of *Barleria prionitis*

Sunil K. Jaiswal[1*], Mukesh K. Dubey[2], Sanjeeb Das[2], Arti Raj Verma[1], Ch.V. Rao[1]

***Corresponding author:**

Sunil K. Jaiswal

1. Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, (C.S.I.R), Rana Pratap Marg, P.O. Box 436, Lucknow 226001, Uttar Pradesh, India.

Fax No: +91 522 2205836, 2205839.

E-mail address: sunil_pharmachem90@yahoo.co.in

2. Department of Pharmaceutical sciences, Diberugarh University, Diberugarh, Assam, India.

Abstract

Context: *Barleria prionitis* L. (Family Acanthaceae; commonly known as Vajradanti), is an annual shrub, 1–3 feet high, found throughout tropical Asia and in South Africa.

Objective: The aim of the present study was to evaluate the antioxidant activity of 50% ethanolic extract of leaf, flower, and stem of *B. prionitis* by using β carotene bleaching assay, reducing power and free radical scavenging activity (DPPH and hydroxyl radical scavenging activity). Total phenolic content (TPC) was analysed by the Folin–Ciocalteu colorimetric method using gallic acid as standard and expressed as mg/g gallic acid equivalent (GAE).

Results: Total phenolic content (TPC) and antioxidant activity (AOA) in *B. prionitis* leaves were found to be 67.48 mg/g GAE dry plant material and 79.20%. The *B. prionitis* leaves exhibited strong free radical scavenging activity as evidenced by the low IC_{50} values in both DPPH (1,1-diphenyl-2-picryl hydrazyl) (336.15 µg/ml) and hydroxyl radical (568.65 µg/ml) methods.

Conclusions: The leaf of *B. prionitis* possesses high phenolic content, potential antioxidant activity, reducing power & radical scavenging activity in comparison to flower and stem.

Keywords: *Barleria prionitis*, total phenolic content, β carotene bleaching assay, reducing power, free radical scavenging activity.

INTRODUCTION

The research interest is focused on the potential role of antioxidants and antioxidant enzymes in the treatment and prevention of atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus and several other diseases [1]. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation, protein damage and DNA strand breaking [2]. Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radicals

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play a major role. Several plants are known to possess strong antioxidant properties [3, 4].

Barleria prionitis L. (Family Acanthaceae; commonly known as Vajradanti,), is an annual shrub, 1–3 feet high, found throughout tropical Asia and in South Africa. In indigenous system of medicine in India, the aerial parts are used in the fever, toothache, inflammation & gastrointestinal disorders; bark in whooping cough as an expectorant; the whole plant and especially the roots are used as tonic and diuretic [5,6]. Leaves, stem and root of *B. prionitis* possess antibacterial and anti-inflammatory activities [7, 8]. Iridoid enriched fraction of aerial parts (leaves and stems) was reported for hepatoprotective activity in various acute and chronic animal models [9]. Aerial part of *B. prionitis* possesses anti-fertility [10] & *in-vitro* anti viral activity [11]. An aerial part was reported for barlerinoside, shanzhiside methyl ester, 6-*O*-*trans*-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside and lupulinoside [12].

Materials and Methods

Materials

The *B. prionitis* was collected from Botanical Garden of National Botanical Research Institute (NBRI), Lucknow, India in month of January & authenticated by Dr. Sayeeda Khatoon, chemotaxonomist and the voucher specimens (NAB 180023) were deposited in the departmental herbarium (Pharmacognosy and Ethnopharmacology Division, NBRI, Lucknow) for future reference.

Method

Preparation of extract

The freshly collected leaves of *B. prionitis* were washed with distilled water and air-dried under the control conditions and powdered. The powdered plant material was percolated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with of 50% ethanol for 3 days. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure and low temperature obtain solid residue & their extractive value are given in **Table-1**

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Table 1 Percentage yield of different parts of *B. prionitis*

S. No.	Sample	Solvent used for extraction	% Yield (Extract)
1.	Leaves	EtOH : H_2O (1:1)	14.10
2.	Flower	EtOH : H_2O (1:1)	7.5
3.	Stem	EtOH : H_2O (1:1)	10.9

In vitro antioxident activity of B. prionitis (leaf, flower, stem)

Estimation of total phenolic content (TPC)

TPC was analysed by the Folin–Ciocalteu colorimetric method using gallic acid as standard developed by [13] with modification and expressed as mg/g gallic acid equivalent (GAE) on dry weight basis. The 25 mg plant extract was dissolved in 10 ml of 50% MeOH: H_2O (1:1), at room temperature and in its 1.0 ml, 1.0 ml of Folin's Reagent (1N) and 2.0 ml of Na_2CO_3 (20 %) were added subsequently. The test mixture was mixed properly on cyclomixer, left at room temperature for 30 min and maintained to 25 ml with water. The absorbance of test mixture was measured at 725 nm. The reported TPC were expressed as gallic acid equivalent (GAE) mg/g.

Antioxidant activity (AOA)

β carotene bleaching assay was carried out according to the method developed by [14]. 2.0 mg of β carotene was dissolved in 20 ml of chloroform and its 3 ml was added to 20 µl of linoleic acid and 200 µl tween 40. After removing under reduced pressure and 100 ml of oxygenated water was added and mixed properly to obtain a stable emulsion. 3 ml aliquot of emulsion were added mixed with 40 µl of sample and incubated for 1 hr at 50°C. The absorbance was regarded at 0 and after 60 min of incubation at 470nm.

Estimation of reducing power (RP)

Reducing power will be determined using ferric reducing - antioxidant power assay and quercetin as reference standard [15]. Different aliquots of sample maintained to 1ml, followed by the addition of 2.5ml phosphate buffer pH (6.6) and 2.5ml of 1 % w/v potassium ferricyanide in each reaction

mixture thus obtained were incubated at 50 ºC for 20 minutes. After incubation, reaction was terminated by additions of 2.5ml of 10% w/v tri choloro acetic acid solution; 2.5ml of above solution from each reaction was diluted with equal amount of distilled water. Aliquot of 0.5ml $FeCl_3$ (0.1%) was added in each and absorbance was recorded after 10 minutes at 700 nm. RP expressed as ascorbic acid equivalent (1mM = 1 ASE). The ASE/ml value is inversely proportional to reducing power.

Free radical scavenging activity by-

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The DPPH radical scavenging activity of *B.prionitis* (leaf, flower, and stem) was determined by using the method proposed by [16]. Different aliquot was added to 2.9ml of freshly prepared (2.8mM), with different concentration of *B.prionitis* (leaf, flower, stem) extract in a final volume of 1ml in potassium phosphate buffer (10mM, pH 7.4). It was incubated at 37 ºC for 1 h and then 1ml of 2.8 % TCA and 1ml of 1 % TBA were added. The mixture was heated in a boiling water bath for 15 min. It was cooled and absorbance was taken at 532 nm.

Results and Discussion

The method used for the determination of TPC is based on the oxidation of the hydroxyl groups of phenols in basic media by Folin-Ciocateu regent (mixture of phosphotungstic and phosphomolybdic acids). For TPC, AOA, reducing power and free radical scavenging activity the results are presented in **Table 2.** In AOA estimation beta carotene undergoes rapid

Table 2.

Antioxidant activity, reducing power & free radical scavenging activity (DPPH and hydroxyl radical) & total phenolic content of different parts of *B. prionitis*

S. No.	Sample	β carotene bleaching (AOA; %)	Reducing Power (ASE/ml)	DPPH (IC_{50} µg/ml)	•OH (IC_{50} µg/ml)	TPC (mg/g GAE)
1.	Leaves	79.20 ± 1.26	0.79 ± 0.08	336.15 ± 7.21	568.65 ± 6.11	67.48 ± 0.72
2.	Flower	62.16 ± 2.56	1.42 ± 0.11	675.07 ± 8.28	809.15 ± 9.21	60.83 ± 0.53
3.	Stem	48.31 ± 1.960	1.91 ± 0.09	1148.63 ± 10.80	862.47 ± 8.32	43.36 ± 0.23
4.	BHT	51.34 ± 1.02	--	--	--	--
5.	Quercetin	--	0.52 ± 0.04	0.021 ± 0.004	0.072 ± 0.007	--

All presented values were calculated from three experiment repetitions at least and reported with ±95% confidence limits.

solution of DPPH (6 x 10^{-5} M in MeOH). The Absorbance was recorded at 517 nm at 0 times & after 1 hour of incubation and inhibitory concentration (IC_{50}) was calculated as described by [17]. IC_{50} value is defined as the concentration of sample required to scavenge 50% of free radical.

Hydroxyl radical scavenging activity

The deoxy ribose method was used for determining the scavenging effect on hydroxyl radicals as describe by [18]. The reaction mixture contained ascorbic acid (50µM), $FeCl_3$ (20µM), EDTA (2µM), H_2O_2 (1.42mM), deoxyribose decoloration in the absence of an antioxidant because of the coupled oxidation of beta carotene and linoleic acid. Highest TPC and AOA were observed in the leave of *B.prionitis*. During the reducing power assay, the presence of reductant in extracts would result in reducing ferric to ferrous. Leaves of *B. prionitis* showed high reducing power as indicated by low ASE/ml value. The disappearance of DPPH is directly proportional to the amount of antioxidant present in the reaction mixture which react with stable free radical i.e. α,α-diphenyl β-picrylhydrazyl and

convert it to α,α-diphenyl β-picrylhydrazine. Leaves of *B. prionitis* were found to have remarkable high DPPH radical and hydroxyl radical scavenging activity as evident by low IC_{50} in comparison to other parts. According to the results of this study, it is concluded that the leaves of *B. prionitis* show high phenolic content, inhibition of β carotene bleaching (AOA), reducing power and free radical scavenging activity in comparison to flower and stem.

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Abbreviations

AOA- Antioxidant activity; ASE-ascorbic acid equivalent; TBA-Thiobarbituric acid; TCA- tetra chloro acetic acid; TPC-total phenolic content.

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