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Comparative antioxidant study of ethanol and aqueous leaves extracts of *Barleria gibsoni* dalz

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Abstract
Barleria gibsoni Dalz. (Acanthaceae) is commonly known as Neel Koranti, and traditionally used in cataract, ulcer and fever. The Present study was carried out to investigate antioxidant potential of ehanolic and aqueous extracts of *Barleria gibsoni*, by DPPH, Nitric oxide and hydroxyl radical scavenging activity. The DPPH radical inhibition (%) was 85.79, 66.34 and 71.33 for BGLE, BGLA and ascorbic acid respectively. The *Barleria gibsoni* extracts (BGLE and BGLA) showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at the concentrations 1000µg/ml, showing 71.19% and 61.22% of NO inhibition, respectively. The BGLE and BGLA extracts significantly scavenged the hydroxyl radical generated by the EDTA/H_2O_2 system, when compared to that of ascorbic acid.

Keywords: *Barleria gibsoni*, antioxidant activity, DPPH, nitric oxide, EDTA/H_2O_2

Introduction
Antioxidants are essential substances which have the potential to protect the body from destruction create by free radical or reactive oxygen species (ROS). This ROS which includes free radicals such as superoxide anion radicals, hydroxyl radicals, and non-free-radical species such as hydrogen peroxide and singlet oxygen, is various forms of activated oxygen can destruct the DNA and lead to the oxidation of lipid and proteins in cells [1, 4]. Nearly all of the antioxidant presents in vascular plants such as Vitamin C and E, carotenoids, flavonoids, and tannins [5]. Mainly, these natural antioxidants, especially polyphenols and carotenoids, revel a broad range of biological results, such as anti-inflammatory, antibacterial, antiviral, anti-aging, and anticancer activity [6, 7]. These phenolic compounds are attractive to in the food industry because they hold up oxidative degradation of lipids and thereby improve the quality and nutritional value of food [8]. So, importance of natural phenolic compounds from plant materials is also uplift attentiveness among scientists, food manufacturers, and consumers due to functional food with specific health effects [9].
Barleria gibsoni Dalz. Belongs to family Acanthaceae is widely distributed throughout Africa, India, Sri Lanka and tropical Asia. It is commonly known as Neel Koranti, the juice of the leaf is used in fever cataract and ulcer. The dried bark is used in cough treatment and the leaves chewed to relieve toothache. The paste of the root is applied to disperse boils and glandular swellings [10, 11].
The present study was an attempt to compare study of antioxidant activity of ethanol and aqueous extracts of *Barleria gibsoni* Dalz leaves.

Materials and Methods
Collection and Identification of plant material
The entire plant of the *B. gibsoni* were collected during the month of May-June when flowering, from Satara region, Maharashtra, India. The plant authenticated by Botanical survey of India, Pune, Maharashtra, India. A voucher specimen has been deposited at the herbarium of same place for further reference.

Preparation of extracts [12]
The collected fresh matured leaves, stem and roots of *B. gibsoni* were washed with tap water, air- dried at room temperature for 2-3 weeks at 35-40^{0}C and then reduced to coarse powder. A 100 gm powdered leaves, stem and roots were obtained after defatted with petroleum ether and successively extracted with ethanol using Soxhlet apparatus finally 12.0 g of extracts were obtained.

Methods
DPPH radical scavenging activity [13]
The ability of *Barleria gibsoni* extracts to scavenge DPPH radical was assessed using Varahalarao Vadlapudi *et al*., 2009 method with modification. Briefly, aliquot of the extract 200-1000 µg /mL was mixed with 3.0 mL DPPH (0.5 mmol/L in methanol), the resultant absorbance was recorded at 517 nm after 30 min. incubation at 37^{0}C. The standard drug ascorbic acid was used. The percentage of scavenging activity was derived using the following formula,
Percentage of inhibition (%) = [(A control - A sample) / A control] x 100 Where A control - absorbance of DPPH; A sample - absorbance reaction mixture (DPPH with Sample).

Nitric oxide free radical scavenging activity [14]
Nitric oxide radical scavenging was carried out as per the method of KR. Nagulendran *et al*., 2007. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25^{0}C for 150 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20%

glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated.

Hydrogen peroxide radical scavenging activity [15]

The hydrogen peroxide radical scavenging activity was assessed by using ethanolic and aqueous extract of *Barleria gibsoni* leaves aliquots of 200- 1000 µg/mL were added to a 0.6 mL hydrogen peroxide (40 mM) with the already prepared phosphate buffer (pH 7.4). The reaction mixtures were incubated at room temperature for 10 mins. After incubation, the reaction mixture read at 230 nm against the blank solution with phosphate buffer (pH 7.4). The percentage of inhibition calculated based on the formula:

% of inhibition = (A1-A2)/A1 x 100

Where A1 -absorbance of the H_2O_2 A2 -absorbance of the reaction mixture with extract

Results

DPPH Scavenging: The ethanolic (BGLE) and aqueous extracts (BGLA) of the *Barleria gibsoni* showed promising free radical scavenging effect of DPPH in a concentration dependent manner upto a concentration of 1000µg/ml. The BGLE showed more scavenging activity than BGLA. The reference standard ascorbic acid also shows a significant radical scavenging potential in the concentration of 1000 µg/ml. The DPPH radical inhibition (%) was 85.79, 66.34 and 71.33 for BGLE, BGLA and ascorbic acid respectively in Table 1.

Nitric oxide Scavenging: The *Barleria gibsoni leaves* extracts (BGLE and BGLA) showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at the concentrations 1000µg/ml, showing 71.19 % and 61.22 % of NO inhibition, respectively. Ascorbic acid was used as reference standard. The % inhibition is shown in Table 2.

Hydroxyl radical scavenging method: The BGLE and BGLA extracts (1000 µg/ml) significantly scavenged the hydroxyl radical generated by the EDTA/H2O2 system, when compared to that of ascorbic acid. (Table 3).

Table 1: *In Vitro* free radical scavenging activity of aqueous and ethanolic leaves extracts of *Barleria gibsoni* by DPPH method

	% Scavenging (Mean ± SEM) of triplicates				
Drug	**200 µg/ml**	**400 µg/ml**	**600 µg/ml**	**800 µg/ml**	**1000 µg/ml**
BGLE	52.89 ± 0.93	66.66 ± 0.99	74.76 ± 1.03	81.28 ± 1.10	85.79 ± 1.10
BGLA	41.65 ± 0.91	52.34± 0.95	53.25 ± 0.94	61.15 ± 0.96	66.34 ± 0.98
Ascorbic acid	48.03 ± 0.99	61.28 ± 0.91	63.24 ± 0.99	68.32 ± 1.02	71.33 ± 0.98

BGLE = *Barleria gibsoni* Ethanolic extract, BGLA = *Barleria gibsoni* Aqueous extract

Table 2: *In Vitro* free radical scavenging activity of aqueous and ethanolic leaves extracts of *Barleria gibsoni* by Nitric oxide scavenging method

	% Scavenging (Mean ± SEM) of triplicates				
Drug	**200 µg/ml**	**400 µg/ml**	**600 µg/ml**	**800 µg/ml**	**1000 µg/ml**
BGLE	36.92 ± 0.93	40.23 ± 0.99	55.76 ± 0.95	68.19 ± 0.99	71.19 ± 1.15
BGLA	45.25 ± 1.92	52.34± 0.95	53.25 ± 0.98	58.13± 0.98	61.22 ± 0.92
Ascorbic acid	42.06 ± 0.99	59.24 ± 0.91	68.26 ± 0.99	74.36 ± 1.02	76.35 ± 0.98

BGLE = *Barleria gibsoni* Ethanolic extract, BGLA = *Barleria gibsoni* Aqueous extract

Table 3: *In Vitro* free radical scavenging activity of aqueous and ethanolic leaves extracts of *Barleria gibsoni* by Hydroxyl radical scavenging method

	% Scavenging (Mean ± SEM) of triplicates				
Drug	**200 µg/ml**	**400 µg/ml**	**600 µg/ml**	**800 µg/ml**	**1000 µg/ml**
BGLE	40.59 ± 1.06	52.51 ± 1.08	61.32 ± 1.10	78.25 ± 1.12	81.26 ± 1.15
BGLA	28.25 ± 0.56	39.59 ± 0.56	52.57 ± 0.49	65.14 ± 0.84	75.29 ± 0.86
Ascorbic acid	48.05 ± 0.99	61.21 ± 0.91	69.25 ± 0.99	84.34 ± 1.02	86.33 ± 0.98

BGLE = *Barleria gibsoni* Ethanolic extract, BGLA = *Barleria gibsoni* Aqueous extract

Discussion

The plants have been investigated for their medicinal value in the recent scientific developments throughout the world, due to their potent antioxidant activities and economic viability. Flavonoids are widely distributed in Plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. The antioxidant activity of leaves of *Barleria gibsoni* extract and standard compounds were compared by using specific *in vitro* methods (Table 1-3).

The free radical scavenging activity was evaluated by various *in vitro* assays. DPPH radical was used as a substrate to evaluate free radical scavenging activities of ethanol and aqueous extract. It involves reaction of specific antioxidant with a stable free radical 2, 2-diphenyl- 1-picrylhydrazyl DPPH. As a result, there is reduction of DPPH concentration by antioxidant, which decreases the optical absorbance of DPPH; this is detected by spectrophotometer at 517 nm. Table 1 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of extracts of *Barleria gibsoni.*

Ascorbic acid was used as standard. The scavenging effect of ethanol extract of *Barleria gibsoni* on the DPPH radical was 85.79%, at a concentration of 1000 µg/ml.

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities. Table 2 illustrates the percentage inhibition of nitric oxide generation by ethanol and aqueous extract of *Barleria gibsoni*. Ascorbic acid was used as a reference compound.

Table 3 shows the H_2O_2 scavenging activity by 1000 µg/ml of ethanol extract of *Barleria gibsoni* extract and comparison with 1000 µg/ml of ascorbic acid. The percentage of H_2O_2 scavenging activity of leaves and ascorbic acid was found as 81.26 and 86.23 respectively. These results indicated that extract has a noticeable effect on scavenging the free radicals. For the measurements of the reducing ability, On the basis of the results of this study, ethanol extract has significant antioxidant activity compared to aqueous extract *in vitro*. In addition, the antioxidant activity may be due to phenolic compounds in extract of *Barleria gibsoni.*

Conclusion

The antioxidant activity of leaves extract of *Barleria gibsoni* and standard compounds were compared by using specific *in vitro* methods *viz*, DPPH, nitric oxide and H2O2 activity. Results showed the percentage inhibition of antioxidant activity by ethanol of *Barleria gibsoni* were very good compared to aqueous extract of *Barleria gibsoni*. Ascorbic acid was used as a reference compound.

Conflicts of Interest

There are no conflicts of interest.

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