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Research Article

In-vitro antioxidant and anti-inflammatory potential of ethanol extracts (root and aerial parts) of *Barleria noctiflora*

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Abstract: *Barleria noctiflora* L.f. (Acanthaceae) is widely used as folk medicine. In the present study ethanol extracts of root and aerial parts of *Barleria noctiflora* were prepared using soxhlet extractor. Anti-oxidant potential was evaluated by free radical scavenging activity on DPPH and *in vitro* anti- inflammation activity of the selected samples stood determined by inhibition of protein denaturation experiment and inhibition of α-amylase studies. Among the root and aerial parts of *Barleria noctiflora* samples root sample shows significantly high level of antioxidant and anti-inflammatory activity.

Keywords: antioxidant, inflammation, Acanthaceae, *Barleria noctiflora*

Introduction

Plant-based drugs have been used against various diseases since a long time. The nature has provided abundant plants which possess medicinal virtues. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties (Ranjit Singh *et al.*, 2012). Plants with antioxidant activities have been reported to possess free radical scavenging activity. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism (Yogamoorthi and Sathya Priya, 2004; Wetson *et al.*, 2012; Zeng *et al.*, 2006).

Inflammation is a normal protective response shown by living tissue against the injury caused by physical trauma, noxious chemicals or microbiological agents (Tripathi, 2004; Robert, 2009; Sandhya Lakheda *et al.*, 2011), which removes pathogens or other stimuli and further help to restore cells to normal state or replace damaged tissue with scar (Rashmi *et al.*, 2011) Bacterial infection causes an increased numbers of neutrophills, which leads to the production of oxidative burst at the site of microbial invasion (Saima Jalil *et al.*, 2003). However inflammation remains unchecked, it leads to the onset of diseases such as vasomotor rhinitis and atherosclerosis (Duangporn Premjet *et al.*, 2010).

The genus *Barleria* includes 28 taxa and 26 species. It has 3 unique characters calyx 4 partile with 2 large outer segments and 2 smaller inner ones, spheroidal, honey combed pollen grains and the predominant with double cystoliths (Shankar and Yadav, 2010). Most of the *Barleria* species are potent anti inflamatory, analgestic, antileukemic, antitumor, antihyperglycemic, anti-amoebic, virudal and antibiotic (Jassim and Naji, 2003; Suba *et al.*, 2004; Suba *et al.*, 2005). *Barleria noctiflora* L.f. is a shrub and it grows up to 90 cm height (Madhu *et al.*, 2010). It is being widely used as folk medicine. It is widely distributed throughout tropical region of Africa, India, Srilanka and other parts of Asia (Athar *et al.*, 2009). All parts of the *Barleria noctiflora* are used to treat diabeties (Marles and Farnsworth, 1995).

Materials and Methods

Preparation of plant leaf extracts

Fresh healthy plants of *Barleria noctiflora* were collected with root and shade dried for 8-10 days and grinded into powder. The air-dried and powdered plant materials were taken in different amber coloured bottles, 100g dry powder sample was extracted with 80 % ethanol at 55°C for 24 hours in soxhlet apparatus. Solvent elimination was done at room temperature and stored.

Preparation of Drug

The plant material was shade dried and pulverized. Methanol extract of the coarsely powdered material was prepared by employing Soxhlet method. The extract was concentrated and stored in brown bottles for future use.

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Free radical scavenging activity on DPPH

The antioxidant activity of the sample was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blois (1958). The sample extracts at various concentrations (100 - 500µg) were taken and the volume was adjusted to 100 µl with methanol. 5 ml of 0.1 mM methanolic solution of DPPH was added and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm.

Percentage radical scavenging activity of the sample was calculated as follows:

% DPPH radical scavenging activity = (control OD-sample OD / control OD) × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

***In vitro* Anti- inflammation activity**

Inhibition of protein denaturation: The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of the sample extract (100 – 500 µg/ml). pH was adjusted to 6.3 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm. For control tests 0.05 ml of distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

Percentage inhibition =
100–((O.D of test – O.D of product control)/ O.D of Control) x 100

In vitro anti- inflammation activity of the selected samples were determined by inhibition of protein denaturation experiment and inhibition of α-amylase studies. The IC_{50} value was defined as the concentration of the sample extract to inhibit 50% of protein denaturation under assay condition.

Proteinase inhibitory activity

The reaction mixtures (2.0 ml) contained 0.06 mg trypsin, 1.0 ml of 25 mM tris – HCl buffer (pH – 7.4) and 1.0 ml aqueous solution of the sample extract (100 – 500 µg/ml). The mixtures were incubated at 37°C for 5 min then 1.0 ml of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 min. 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Absorbance of the supernatant was read at 280 nm against buffer as blank. The percentage of inhibition was calculated as follows.

Percentage inhibition =
100 – ((O.D of test – O.D of product control)/ O.D of Control) x 100

The IC_{50} value was defined as the concentration of the sample extract to inhibit 50% of protein denaturation under assay condition. (Shravan Kumar *et al.*, 2011).

Results and Discussion

The *in vitro* antioxidant potential

Oxidative stress, the consequence of an imbalance of pro-oxidants and antioxidants in the organisms, is gaining recognition as a key phenomenon in chronic illness like inflammatory and heart diseases, hypertension, and some forms of cancer (Oh, 2001 and Zeynep *et al.*, 2007). Plant based, antioxidant rich foods traditionally formed the major part of the human diet, and plant based dietary antioxidants are hypothesized to have an important role in maintaining human health (Benzie, 2013).

Free radical scavenging activity on DPPH

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule yellow-coloured diphenylpicrylhydrazyl (Soares *et al.*, 1997; Kumaran and Karunakaran, 2007). The *in-vitro* antioxidant potential measured by DPPH inhibition assay method, Alcoholic extracts of aerial parts of ethanol extract of aerial parts of *Barleria noctiflora* is 34.11% to 87.55% and root is 24.81% to 79.86%. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of *Barleria noctiflora* is given in Table 1. This activity was increased by increasing concentration of the sample extract. The IC_{50} value of alcoholic extracts of aerial parts of the *Barleria noctiflora* aerial parts extract shows 84.84 ± 1.15 µg/ml and 101.39 ± 1.18 µg/ml in root, compared with the standard ascorbic acid (3.76 ± 0.07), which is a well-known antioxidant (Table :2). A higher DPPH radical scavenging activity is associated with a lower IC_{50} value (Daya and Mishra, 2012). The reduction capability of DPPH radical is determined by the decrease in absorbance at 516 nm induced by antioxidants. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines (p-phenylenediamine, p-aminophenol, etc.), reduce and decolorize DPPH by their hydrogen donating ability (Blois, 1958; Yokozawa *et al.*, 1998). The antioxidative effect is mainly due to phenolic compounds, such as phenolic acids, and phenolic diterphenes (Shahidi *et al.*, 1992). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). For the measurements of the reductive ability, it has been found that the Fe^{3+}, Fe^{2+} transformation occurred

in the presence of extract samples which were postulated previously by Oyaizu (1986).

Sangilimuthu Alagar Yadav *et al*., (2012) evaluated the antioxidant activity of defatted ethanol extract of *Barleria noctiflora* leaf and root using *in-vitro* models. DPPH radical scavenging activity, ferrous reducing power, Fe^{2+} chelating activity assay, nitric oxide radical scavenging activity, $ABTS^{+}$ radical cation decolourisation assay, superoxide anion and hydrogen peroxide radical scavenging activities were studied. This study clearly showed good antioxidant capacity in DPPH radical scavenging assay, when compared to other in vitro models and the IC_{50} value were found to be 101 µg/mL in root extract of *B. noctiflora*.

In vitro Anti- inflammation activity

Inflammatory diseases like psoriasis, eczema, dermatitis, etc. require topical treatment to get relief from inflammation conditions associated with these diseases. Now a day herbal products are in good demand. The potential of *Barleria* in curing inflammation is well recorded in ancient texts. Studies done by other workers have validated the traditional use of the plant in acute and sub-acute inflammations (Kawal *et al*., 2014).

Protein Denaturation inhibiting activity

Denaturation of proteins is the main cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of the extract to inhibit protein denaturation was studied. Denaturation of proteins is a well-documented cause of inflammation and rheumatoid arthritis (Mizushima, 1966). Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation (Grant *et al*., 1970).

Protein denaturation inhibiting activity of *Barleria noctiflora* is represented in the Table3. Percentage activity in ethanol extract aerial parts of *Barleria noctiflora* is 4.82 ± 0.69 to 54.51 ± 0.58 and in extract of root 5.03 ± 1.08 to 49.28 ± 2.37. This activity is noticed in increasing concentration of extract from 100 µg to 500 µg. The IC_{50} values of aerial parts 163.94 ± 0.93 µg/ml, root 188.17 ± 7.50 µg/ml, which is compared with the standard aspirin 51.10 ± 1.57 µg/ml (Table4).

Proteinase inhibiting activity

Result from Table 5 stated that a significant increasing percentage of activity in increasing concentration of sample extracts. Percentage of activity in ethanolic extract of *Barleria noctiflora* aerial parts is 9.68 ± 4.02 to 55.74 ± 0.20, in root 6.09 ± 1.68 to 55.80 ± 2.18. The IC_{50} values of the *Barleria noctiflora* aerial parts 87.80 ± 0.90 µg/ml root extract 88.13 ± 3.05 µg/ml which is compared with the standard aspirin 41.84 ± 0.10 µg/ml (Table : 6). Both aerial parts and root *B. noctiflora* shows a significant protein inhibiting activity.

Table 1. *In vitro* DPPH radical scavenging activity of *Barleria noctiflora* L.f.

Sample	Concentration (µg)	Percentage activity (%)	IC_{50} (µg/ml)
Aerial parts	100	34.11 ± 1.08	84.84 ± 1.15
	200	62.84 ± 5.45	
	300	81.17 ± 0.85	
	400	85.34 ± 0.00	
	500	87.55 ± 0.14	
Root	100	24.81 ± 1.11	101.39 ± 1.18
	200	47.77 ± 1.54	
	300	60.94 ± 2.36	
	400	71.55 ± 0.23	
	500	79.86 ± 1.74	

Values are means of three independent analyses of the extract ± standard deviation (n=3)

Table 2. *Invitro* DPPH radical scavenging activity of standard Ascorbic acid

Sample	Concentration (µg)	Percentage activity (%)	IC_{50} (µg/ml)
Ascorbic acid	2	7.42 ± 0.27	3.76 ± 0.07
	4	17.84 ± 0.40	
	6	32.40 ± 0.33	
	8	39.78 ± 0.11	
	10	52.54 ± 2.00	

Values are means of three independent analyses of the extract ± standard deviation (n=3)

Table 3. Protein Denaturation inhibiting activity of *Barleria noctiflora* L.f.

Sample	Concentration (µg)	Percentage activity (%)	IC_{50} (µg/ml)
Aerial parts	100	4.82 ± 0.69	163.94 ± 0.93
	200	14.53 ± 0.48	
	300	29.05 ± 0.58	
	400	42.05 ± 0.35	
	500	54.51 ± 0.58	
	100	5.03 ± 1.08	188.17 ± 7.50

Root	200	16.52 ± 1.08
	300	24.57 ± 0.75
	400	32.76 ± 1.14
	500	49.28 ± 2.37

Values are means of three independent analyses of the extract ± standard deviation (n=3)

Table. 4. Protein Denaturation inhibiting activity of standard Aspirin

Sample	Concentration (µg)	Percentage activity (%)	IC_{50} (µg/ml)
	25	13.01 ± 0.70	
	50	30.08 ± 2.54	
Aspirin	150	48.78 ± 3.66	51.10 ± 1.57
	200	66.67 ± 2.54	
	250	77.64 ± 0.70	

Values are means of three independent analyses of the extract ± standard deviation (n=3)

Table 5. Proteinase inhibiting activity of *Barleria noctiflora* L.f.

Sample	Concentration (µg)	Percentage activity (%)	IC_{50} (µg/ml)
	100	9.68 ± 4.02	
	200	21.96 ± 1.25	
Aerial parts	300	33.03 ± 1.18	87.80 ± 0.90
	400	46.19 ± 0.81	
	500	55.74 ± 0.20	
	100	6.09 ± 1.68	
	200	23.11 ± 4.19	
Root	300	33.43 ± 1.51	88.13 ± 3.05
	400	45.65 ± 0.64	
	500	55.80 ± 2.18	

Values are means of three independent analyses of the extract ± standard deviation (n=3)

Table 6. Proteinase inhibiting activity of standard Aspirin

Sample	Concentration (µg)	Percentage activity (%)	IC_{50} (µg/ml)
	25	14.15 ± 0.09	
	50	16.22 ± 0.12	
Aspirin	150	30.73 ± 0.15	41.84 ± 0.10
	200	47.59 ± 0.13	
	250	59.11 ± 0.12	

Values are means of three independent analyses of the extract ± standard deviation (n=3)

Conclusion

The present investigation examined the antioxidant potential of various extracts of *Barleria noctiflora* by *in-vitro* methods. The antioxidant activity was determined by DPPH activity. The *in-vitro* studies clearly showed that the ethanol extracts of sample has significant antioxidant activity. These *in-vitro* assays indicate that these plant extracts are a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. The *in-vitro* anti-inflammatory activity conducted Protein Denaturation inhibiting activity and Proteinase inhibiting activity. The result indicates the selected plants have better activity against inflammation.

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