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In-vitro anti-diabetic activity of root and aerial parts of *Barleria noctiflora* L.f. (Acanthaceae)

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Abstract
Barleria noctiflora (Acanthaceae) is a folk medicine used to treat Diabetes. The present investigation deals with morphological and *in-vitro* anti diabetic study of ethanolic extracts of root and aerial parts of selected plants. The plant material was extracted using soxlet apparatus and ethanol as a solvent. *In-vitro* anti-diabetic activity was determined by inhibition of α-glucosidase and inhibition of α-amylase studies. The extract showed a significant level of anti-diabetic activity when compared with standards. The results of ethanolic extracts of *Barleria noctiflora* are in support of traditional uses of the species to reduce blood glucose levels. It is highly likely that long term treatment may achieve the desired results with diabetes mellitus patients.

Keywords: *Barleria noctiflora*, anti-diabetic, traditional medicine

Introduction
Diabetes mellitus is the most important non-infective epidemic to hit the globe in the present millennium. By the year 2025, India shall have the maximum number of diabetics in the world making it "Diabetic capital of the world" (Hillary *et al*., 1998) [3]. Many medicinal plants have been provided a potential source of anti-diabetic principles and are widely used for the treatment of diabetes mellitus in various traditional systems of medicine worldwide and many of them are known to be effective against diabetes (Reddy *et al*., 2005) [7, 8]. The anti-diabetic drugs from plants in current clinical use and their similar mechanism of action of herbal components are preferred mainly due to lesser side effect and low cost. Conventional drugs treat diabetes by improving insulin sensitivity, increasing insulin production and/or decreasing the amount of glucose in blood. In addition to adverse effects, drug treatments are not always satisfactory in maintaining normal level of blood glucose and avoiding late stage diabetic consequences (Prabhakar and Doble, 2011) [6].
Barleria noctiflora L.f. is a shrub and it grows up to 90 cm height (Madhu *et al*., 2010) [4]. 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) [1]. All parts of the *Barleria noctiflora* are used to treat diabeties (Marles and Farnsworth, 1995) [5].

Materials and Methods
Mature and healthy plants were collected from Tirunelveli District during January to April. The specimens were authentified by Dr. M. Padma Soma Subramanian, Research Officer (Scientist-II) in Botany, (CCRS, Govt.of India), Mettur Dam. Voucher specimens of the collections are deposited at the Herbarium of Medicinal Plants Garden, Mettur Dam, Tamil Nadu, India.

Preparation of extracts
Fresh healthy plants of *Barleria noctiflora* (Plate: 1) 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 ºC for 24 hours in soxhlet apparatus. Solvent elimination was done at room temperature and stored.

In vitro Anti-diabetic activity
In vitro inhibition of α-glucosidase
The enzyme α-glucosidase inhibitory activity of the ethanol extracts of chosen plant was determined by following the method of Miller, 1959. Premix α-glucosidase (0.07 Units) with

different concentrations (100-500µg) of extract. Then 3mM p-nitrophenyl glucopyranoside was added as a substrate. This reaction mixture was incubated at 37 ˚C for 30 min and the reaction was terminated by addition of 2 ml of sodium carbonate. The α- glucosidase activity was determined by measuring the p-nitrophenyl release from p- nitrophenyl glucopyranoside at 400 nm after which the IC_{50} value was calculated.

Invitro anti- diabetic activity was determined by the inhibition of α-glucosidase and inhibition of α-amylase studies.

In vitro inhibition of α-amylase

The α-amylase inhibition assay was adapted and modified from Giancarlo *et al.*, 2006. Different concentrations (100-500 µg) of samples and 1 ml enzyme solution were mixed in a tube and incubated at 25 °C for 30 min. To 1 ml of this mixture was added 1 ml of 0.5% starch solution and the tube incubated at 25 °C for 3 min. Then, 1 ml of the colour reagent (DNSA) was added and the closed tube placed into an 85 °C water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 ml distilled water and the absorbance value determined at 540 nm. Individual blanks were prepared. In this case, the colour reagent solution was added prior to the addition of starch solution and then the tube was placed into the water bath. Controls were conducted in an identical fashion replacing plant extracts with 1 ml DMSO. The inhibition percentage of α-amylase was assessed by the following formula after which IC_{50} was calculated.

% Inhibition = ((µA Control–µA Sample)/µA Control) x 100

Results and Discussion

Morphological characters of *Barleria noctiflora* L.f.

Barleria noctiflora L.f. (Plate: 1) is a small prickly woody shrub, 75-100 cm tall. Spines are white and shortly forked with 1-4 upward. Branches are yellowish brown young ones appressed with sericeous hairs. Leaves are obovate- spathulate chartaceous, clustered on young branches, 0.8 - 1.5× 0.4 - 0.6 cm. Sericeous hairs are scattered on both surfaces, more in young leaves, base cuneate or shortly decurrent, entire, apex mucronate with spine tip; petiole subsessile. Flowers are solitary, axillary, sessile bracts. Calyx lobes are scarious, whitish conspicuously nerved, outer ones lanceolate, long acuminate. Corolla is white, 4.3 cm long, elongate narrowly cylindric. Capsule are oblong-ellipsoid 1.5 cm long. Seeds 4 appressed hairy.

In vitro Anti- diabetic activity

Barleria lupulina, was reported being used traditionally for diabetes treatments and showed anti-diabetic activities in study using animal models. Study of the alcoholic leaf extract of *Barleria prionitis* showed a significant decrease of blood glucose levels and glycosylated haemoglobin. These observations highlighted the need to test members of the Acanthaceae family for glucosidase inhibitory potentials.

α-Glucosidase inhibitory assay

α-Glucosidase activity was assessed by the release of p-nitrophenol from PNPG *in-vitro*. IC_{50} (µg/ml) values of active extracts are presented in Table:1. The α-glucosidase inhibitory activity of the extracts of both plant species were compared on the basis of their IC_{50} values obtained. α-glucosidase inhibitory activity in alcoholic aerial part extract is observed with IC_{50} values of aerial part extract of *Barleria noctiflora* 161.19 ± 1.22 µg/ml and alcoholic root extract (117.77 ± 0.38 µg/ml). Table:1 showed that the extracts displayed strong α-glucosidase inhibitory activity in a dose dependent manner, with a significant difference in the inhibitory activity. Moreover, the result showed higher α-glucosidase inhibitory activity than the reference compound acarbose (IC_{50} µg/ml) (Table : 2). The percentage inhibition at 100, 200, 300, 400, 500 µg concentrations of *Barleria noctiflora* root extract shows a concentration-dependent increase in percentage activity. Thus the highest concentration of 500 µg tested shows maximum activity of nearly 86.60 ± 0.48. The percentage activity varied from 86.60 ± 0.48 from the highest concentration to the lowest concentration of 36.02 ± 0.56. The results of ethanolic extracts of *Barleria noctiflora* are in support of traditional uses of the species to reduce blood glucose levels. It is highly likely that long term treatment may achieve the desired results with diabetes mellitus patients.

In vitro inhibition of α-amylase

The hypoglycemic effects of pharmacologically active components of plants in diabetes patients have been reported by assessing the lowering effects on alpha amylase (both salivary and pancreatic) for development of diabetes (Reddy *et al*., 2005) [7, 8].

Data from the present study showed the variable inhibitory effect of tested plant extracts on α- amylase activity *in vitro*. Ethanolic extracts of *Barleria noctiflora* were found to significantly inhibit α- amylase at different doses. IC_{50} values of extracts are summarized in Table:3. Alcoholic extract of aerial part of *Barleria noctiflora* shows a maximum percentage inhibition 27.87% whereas in alcoholic extract of root 24.48 percentage of inhibition at the concentration 500 µg. The percentage activity of the control varied from 37.92±0.12 to 93.34±0.14 (Table: 4).

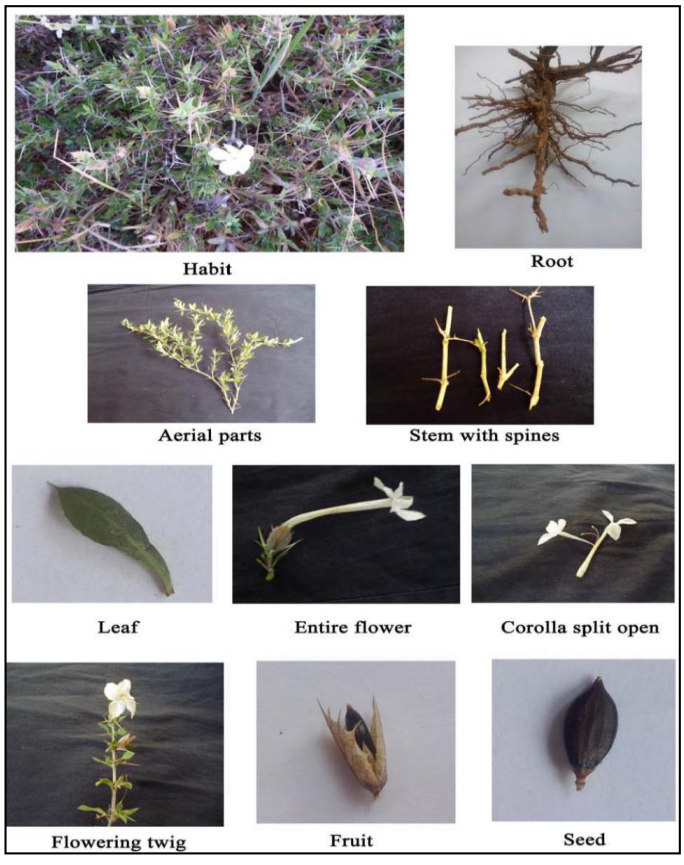

Plate 1: Morphology of *Barleria noctiflora* L.f.

Table 1: α- Glucosidase inhibiting activity of *Barleria noctiflora* L.f.

Sample	Concentration (µg)	Percentage activity (%)	IC_{50} (µg/ml)
Aerial parts	100	16.14 ± 0.84	161.19 ± 1.22
	200	26.11 ± 0.16	
	300	42.67 ± 0.24	
	400	55.59 ± 0.24	
	500	62.08 ± 0.80	
Root	100	36.02 ± 0.56	117.77 ± 0.38
	200	41.30 ± 0.58	
	300	56.22 ± 0.40	
	400	69.09 ± 0.40	
	500	86.60 ± 0.48	

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

Table 2: α- Glucosidase inhibiting activity of standard acarbose

Sample	Concentration (µg)	Percentage activity (%)	IC_{50} (µg/ml)
Acarbose	1	41.29 ± 0.23	28.96 ± 0.45
	2.5	60.01 ± 0.09	
	5	79.90 ± 0.03	
	7.5	88.56 ± 0.11	
	10	98.62 ± 0.14	

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

Table 3: α- Amylase inhibiting activity of *Barleria noctiflora* L.f.

Sample	Concentration (µg)	Percentage activity (%)	IC_{50} (µg/ml)
Aerial parts	100	4.14 ± 0.71	304.39 ± 5.74
	200	9.60 ± 0.28	
	300	15.73 ± 0.59	
	400	21.19 ± 0.28	
	500	27.87 ± 0.59	
Root	100	6.40 ± 0.59	309.98 ± 0.34
	200	11.96 ± 0.43	
	300	16.76 ± 0.16	
	400	20.90 ± 0.28	
	500	24.48 ± 0.43	

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

Table 4: α- Amylase inhibiting activity of standard acarbose

Sample	Concentration (µg)	Percentage activity (%)	IC_{50} (µg/ml)
Acarbose	1	37.92 ± 0.12	80.34 ± 0.28
	2.5	59.91 ± 0.14	
	5	78.40 ± 0.23	
	7.5	87.14 ± 0.11	
	10	93.34 ± 0.14	

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

Conclusion

Natural products have played an important role throughout the world in treating and preventing human diseases. Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. With the changing pattern of life style, most of the diseases are now becoming life style diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. The antidiabetic properties of *Barleria noctiflora* L.f. evaluated *in-vitro* by α-Glucosidase inhibitory assay and inhibition of α-amylase. The results obtained indicate that the extracts possessed significant level of activity in the highest concentration of extract was high effective as an anti-diabetic agent.

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