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Research Article

EVALUATION OF *IN VITRO* ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF *BARLERIA CRISTATA* LEAVES EXTRACTS

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ABSTRACT

Objectives: The objectives of the study were to study the *in vitro* antidiabetic and antioxidant activity of *Barleria cristata* leaves extracts.

Methods: Ethanol extract and petroleum ether extract of leaf and root of *B. cristata* were tested for their antidiabetic activity. The *in vitro* antidiabetic activity from α-amylase, α-glucosidase, and antioxidant activity was used to assess the potential activity of the fractions.

Results: Our attempt results suggest that ethanol and the petroleum ether leaf extract from *B. cristata* show signs of dose-dependent increases to inhibitory activity on α-amylase, α-glucosidase enzymes, and antioxidant activity when compared with control. Ethanolic leaves to extract produced maximum *in vitro* antidiabetic effect and antioxidant activity when compared to petroleum ether extract.

Conclusion: The ethanol leaf extract from *B. cristata* may be used in managing of blood glucose in a medical condition like diabetes.

Keywords: *Barleria cristata*, Ethanol extract, Petroleum ether extract, Antidiabetic activity, Antioxidant activity, *In vitro* study.

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INTRODUCTION

Diabetes mellitus is a metabolic defect primarily characterized by a loss of glucose homeostasis by a disorder of carbohydrate, fat, and protein metabolism, follow-on from defects in the secretion of insulin or action of insulin, or both [1]. Diabetes mellitus is characterized by hypoglycemia, lipidemia, and oxidative stress affected by many individuals to longstanding complications. In current lots of drugs available to manage and treat diabetic patients, but a total improvement on diabetes may not possible. Remedy for diabetes treatments is not always adequate in maintaining normal levels of blood glucose and avoiding late-stage diabetic consequences [2]. A huge number of medicinal plants are used in the management of diabetes. Medicinal plants have curative property due to the presence of various chemical substances of different composition, secondary metabolites which are found in one or more parts of these plants. The antidiabetic drugs at present clinical use and their similar mechanism of action herbal components are chosen mainly due to lesser side effects and low cost [3].

Barleria cristata is an ornamental perennial shrub belonging to Acanthaceae family. It has been reported on a variety of pharmacological activities such as antimicrobial, hypoglycemic, anti-inflammatory, and hepatoprotective [4,5] an additional shrub of the same family. *Barleria prionitis* has been more widely researched with documented medicinal properties of the whole plant, leaves, and roots against, e.g. diabetes and respiratory diseases [6]. Inhibition of alpha-amylase and alpha-glucosidase enzymes can be an essential approach in the management of postprandial blood glucose level in Type 2 diabetes [7]. Thus, the aim of the present study is to investigate the *in vitro* antidiabetes, antioxidant, and phytochemical property activities of leaf extracts of *B. cristata.*

METHODS

Collection of plant material

Healthy and fresh leaves of *Barleria cristata* Linn used for the study were obtained from Kurumberi, Vellore district, Tamil Nadu, India. The plant was authenticated by Prof. P. Jayaraman, PARC, Chennai. The voucher specimen was given the No.PARC/2016/3326/1. The fresh leaves were washed with running tap water, shadow dried and powdered.

Preparation of leaves extract

The leaves of *B. cristata* L. Was Shado dried and powdered well using a mixer and stored in an airtight container. The leaves powdered (100 g) were taken and subjected to successive solvent extraction (500 ml) with ethanol and petroleum ether. The plant extracts were concentrated and stored in an airtight vial for further studies.

Phytochemical screening

For preliminary phytochemical analysis, the freshly prepared crude with ethanol and petroleum ether extracts of the leaves were tested for the presence or absence of phytoconstituents such as reducing sugar, tannins, flavonoids, steroids, and alkaloids using standard phytochemical procedures [8].

In vitro antidiabetic studies

Inhibition of alpha-amylase enzyme

The inhibition alpha-amylase enzyme was determined by Malik and Singh [9]. Briefly, the total assay mixture containing 200 µl of sodium phosphate buffers (0.02 M), 20 µl of enzyme, and the plant extracts from the range of 20–100 µl were incubated for 10 min at room temperature followed by the addition of 200 µl of 1% starch in all the test tubes. Both control and plant extracts were added with starch solution and left to react with an alpha-amylase solution to alkaline environment at 25°C. The changes in the reaction were measured more than 3 min. The production of maltose was quantified at 540 nm.

Inhibition of alpha-glucosidase enzyme

The inhibition of alpha-glucosidase enzyme activity was determined [10]. Incubating a solution to starch substrate (2% w/v maltose) 1 ml with 0.2 M Tris buffer pH 8.0 and different concentrations (20–100 µl) of plant extract were added incubation for 5 min at 37°C. The reaction

was initiated by adding 1 ml of alpha-glucosidase enzyme (1 U/ml) to it followed by incubation for 40 min at 35°C. Then, the reaction was terminated by the addition of 2 ml of 6 N HCl. Then, the color development was measured at 540 nm.

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

Different concentrations of *B. cristata* leaf extract (20–100 µg) were taken (0.4 ml) and mix with 1.0 ml of 0.2 mm DPPH solution, resulting in the absolute concentration of DPPH being 0.1 mm. The mixture was shaken well and left to place for 30 min, and the absorbance was measured at 517 nm [11]. The DPPH free radical scavenging activity was compared with butylated hydroxytoluene (BHT).

Ferric reducing antioxidant power (FRAP)

The FRAP was determined according to the method of Oyaizu [12]. Briefly, various concentrations of extracts (20–100 µg) in 1 ml of distilled water were mixed with 2.5 of ml 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 25 min. An aliquot (2.5 ml) of trichloroacetic acid (10%) was added to the mixture; the mixture was then centrifuged at 3000 revolutions per minute for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml and 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference. The increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis

The values were expressed as mean ± standard error of the mean (n=5). Differences between groups were assessed by one-way analysis of variance using the Statistical Packages for the Social Sciences Software package for Windows (version 16.0). *Post-hoc* testing was carried out for intergroup comparisons using the least significant difference test and the values of $p<0.05$ were considered as statistically significant.

RESULT

Phytochemical screening

The preliminary phytochemical screening result is shown in Table 1, which represent that the alkaloids, carbohydrates, glycosides, steroids, flavonoid, saponins, and phenolic compounds are present in different extracts of the leaf. The amino acids and proteins were not present in extracts from *B. cristata*. The percentage yield of ethanol and petroleum ether extracts of *B. cristata* leaves 10% and 6%. In that maximum yield was found in ethanol extract. One of these secondary metabolites, individually or in combine with others might be responsible for the antidiabetic action of the plant.

***In vitro* alpha-amylase inhibitory activity**

The human digestive system contains lots of enzymes that help in the digestion of food. α-amylase catalyzes the breakdown of polysaccharide into simple sugar and only monosaccharide form of food can be able to absorb in the stomach. *B. cristata* leave extracts (ethanol and petroleum ether) were at the shows the dose-dependent increases from percentage inhibitory activity against the alpha-amylase enzyme. The ethanol extract from *B. cristata* showed a maximum percentage inhibition 67% at a concentration of 100 µl while its petroleum ether extract shows 61% (Table 2).

***In vitro* alpha-glucosidase inhibitory activity**

The *B. cristata* leave extracts revealed a significant inhibitory action of alpha-glucosidase enzyme. The percentage inhibition at 20–100 µl concentrations of *B. cristata* leave showed a dose-dependent increase in percentage inhibition. The percentage inhibition varied from 47.7% to 15.2% for the highest concentration on the lowest concentration (Table 3).

Antioxidant activity

Scavenging activity of with ethanol and petroleum ether extracts was studied against DPPH radicals. All the samples were analyzed in triplicate. The antioxidant activity of the extracts was estimated by DPPH free radical scavenging, using BHT as controls was shown in Table 4. Maximum activity was shown by ethanol leaf extract (76.01) and was observed at 100 µg/ml concentrations followed by a petroleum ether leaf extract (70.57) again at 100 µl. As the concentration of all the extracts increases, the activity also increases. Minimum activity was shown by petroleum ether leaf extract (21.40) from 10 µl concentrations. A significant ($p<0.05$) dose-response relationship is found in the DPPH radical scavenging activity in *B. cristata* extract.

Table 5 reveals that a significant ($p<0.05$) dose-response relationship is found in the ferric reducing power activity in *B. cristata* extract. The result clearly indicates that the reducing power of the *B. cristata* extract increased to increasing the concentration and is comparable with the standard ascorbic acid; hence, *B. cristata* is having the antioxidant activity.

DISCUSSION

Diabetes mellitus is a metabolic disorder with growing incidence of the world. Insulin is a key role in control of glucose homeostasis. Shortage of insulin affects carbohydrate, fat, and protein metabolism [13]. Management of diabetes without side effects is still challenging about the society. It was planned that inhibition of the activity of such alpha-amylase and alpha-glucosidase would postpone in degradation of carbohydrate, which would turn in the absorption of glucose was decreased, as an affect the decline in postprandial blood glucose level high [14].

Table 1: Preliminary phytochemical screening of *Barleria*

CRISTATA			
S. No	**Phytochemical constituents**	**Ethanol extract**	**Petroleum ether extract**
1	Alkaloids	+++	++
2	Carbohydrates	++	++
3	Flavonoids	+++	+++
4	Saponin	++	++
5	Steroids	+	+
6	Tannins	+	+
7	Phenolic compounds	++	++
8	Amino acids	–	–
9	Proteins	–	–

+++=High, ++=moderate, +=present, −=absent

Table 2: *In vitro* antidiabetic activity of alpha-amylase

S. No	Concentration of sample (µl)	% of inhibition	
		Ethanol extract	Petroleum ether extract
1	20	38.3	33.8
2	40	45.4	40.6
3	60	53.7	49.0
4	80	60.9	55.2
5	100	67.1	61.9

Values are given in mean±SEM for n=3. SEM: Standard error of the mean

Table 3: *In vitro* antidiabetic activity of alpha-glucosidase

S. No	Concentration of sample (µl)	% of inhibition	
		Ethanol extract	Petroleum ether extract
1	20	18.3	15.2
2	40	26.7	22.1
3	60	32.3	29.4
4	80	40.5	36.8
5	100	47.7	44.1

Values are given in mean±SEM for n=3. SEM: Standard error of the mean

Table 4: DPPH free radical scavenging activity of *B. cristata* extracts

S. No	Concentration of sample (μl)	% of inhibition		Control % BHT
		Ethanol extract	Petroleum ether extract	
1	20	26.4	21.5	86.4
2	40	41.7	37.3	
3	60	53.9	48.8	
4	80	68.1	63.1	
5	100	76.4	70.5	

Values are given in mean±SEM for n=3. SEM: Standard error of the mean, DPPH: 1,1-Diphenyl-2-picrylhydrazyl, *B. cristata: Barleria cristata*, BHT: Butylated hydroxytoluene

Table 5: FRAP activity of *B. cristata* extracts

S. No	Concentration of sample (μl)	% of inhibition		Control % ascorbic acid
		Ethanol extract	Petroleum ether extract	
1	20	0.037	0.24	0.247
2	40	0.054	0.041	
3	60	0.072	0.057	
4	80	0.097	0.083	
5	100	0.147	0.126	

Values are given in mean±SEM for n=3. SEM: Standard error of the mean, FRAP: Ferric reducing antioxidant power, *B. cristata: Barleria cristata*

In the present study has been conducted to evaluate the preliminary phytochemical investigation and the potential for ethanol and petroleum ether extract from *B. cristata* leaf in inhibiting alpha-glucosidase and alpha-amylase.

The ethanol and petroleum ether extracts from *B. cristata* were tested for phytochemical constituents such as flavonoid, glycosides, carbohydrates, steroids, proteins, and amino acids. Understanding the knowledge of chemical constituents of plants helps to screen for biological activities. Phytochemistry of the extracts of *B. cristata* leaves reveals that they contain flavonoid, phenol, and tannins as secondary metabolites which confirm that the extracts of *B. cristata* leaves have a broad range of biological activities such as anti-inflammatory, antioxidant, anti-arthritic, antidiabetic, and membrane stabilizing activities. This study is in evidence for previous studies [15]. Presence of secondary metabolites indicative of the curative potential for the plant as all the chief class of compounds is identified with their anticancer, anti-inflammatory, and antirheumatic properties [16-18].

The current result reveals that *B. cristata* capably inhibits both alpha-amylase and alpha-glucosidase enzymes *in vitro* in a dose-dependent mode. The ethanol extracts from *B. cristata* seeds showed a dose-dependent inhibitory effect of alpha-amylase activity of oral administering, produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats [19]. The antidiabetic action of *B. cristata* can also be recognized to the alpha-amylase and alpha-glucosidase inhibitory action. In added to this study is necessary to elucidate whether *B. cristata* contained antidiabetic prospective by *in vivo* for validating the established claim on the plant. Dheer and Bhatnagar [20] carried out an antidiabetic activity of alcoholic extract from *B. prionitis* in rats exhibited significantly higher effect.

In this present study, we evaluated the *in vitro* antioxidant activity of ethanol and petroleum ether extract of *B. cristata* by various assays such as DPPH and FRAP. For DPPH activity, it shows that the DPPH free radical scavenging capacity of ethanol extracts from *B. cristata* has proportionally increased from the increase in the concentration of *B. cristata* plant extract. Ferric reducing the ability of plasma assay shows that ethanol extracts from *B. cristata* inhibited more ferric reducing activity compared to petroleum ether extract. Pathy *et al.* [21] reported that *in vitro* antioxidant activities of methanol and acetone extracts of *B. cristata* leaf and bark showed significant antioxidant activities. The same results were observed in petroleum ether and ethanol extracts of *B. prionitis* leaves [22]. Arumugam reported that ethyl acetate fraction of *Barleria noctiflora* showed the highest antidiabetic activity [23].

CONCLUSION

The present study concluded that ethanol and petroleum ether extract of *B. cristata leaves* not only possess remarkable inhibitory potential against α-glucosidase and α-amylase but also exhibited excellent in scavenging activity on the DPPH and FRAP activity. The results of this study direct further study to assess the therapeutic potentialities of *B. cristata* L. *in vivo* in animal models.

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AUTHORS CONTRIBUTION

Sakthivel Vasanth: Designed the experimental study and carried out analysis. Further, the manuscript was drafted. Giridharan Bupesh: Provided suggestions during the work. Tharumasivam Siva vijayakumar: Provided suggestions during the Work. Vellingiri Balachandar: Provided guidance and suggestions during the work and reviewed the article draft. Durai rajan Gunasekaran: Provided guidance and suggestions during the work and reviewed the article draft.

CONFLICTS OF INTEREST

Declared none.

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