IJBPAS, November, 2021, 10(11): 4109-4116

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

INHIBITORY PROPERTY OF *BARLERIA CRISTATA* EXTRACT ON A-AMYLASE AND A-GLUCOSIDASE KEY IN DIABETES AND ANTIOXIDANT

VASANTH S[1*], SIVA VIJAYAKUMAR T[2], JASMINE BUELAH. J[1] AND MEENAKUMARI K[1]

1: Research & Development Wing, Sree Balaji Medical College & Hospital, BIHER, Chrompet, Chennai- 600 044

2: Assistant Professor, Department of Biotechnology, Srimad Aandavan Arts & Science College, Tiruvanaikovil

***Corresponding Author: Dr. S. Vasanth: E Mail:** sakthivel.vasanth@gmail.com

Received 22nd Feb. 2021; Revised 20th April 2021; Accepted 6th May 2021; Available online 1st Nov. 2021

https://doi.org/10.31032/IJBPAS/2021/10.11.5733

ABSTRACT

Objectives: To study the *in vitro* anti-diabetic and antioxidant activity of *Barleria cristata* in two extracts hexane and chloroform. **Material and Methods**: hexane and chloroform extract of leaf of *Barleria cristata* was tested for their anti-diabetic activity. The *in vitro* anti-diabetic 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 hexane and chloroform leaf extract from *Barleria cristata* show signs of dose-dependent increases to inhibitory activity on α-amylase, α-glucosidase enzymes and antioxidant activity when compared with control. Hexane leaves to extract produced maximum in-vitro anti-diabetic effect and antioxidant activity when compared to chloroform extract. **Conclusion:** The Hexane leaf extract from *Barleria cristata* may be used in managing of blood glucose in medical condition like diabetes.

Keywords: *Barleria cristata*; hexane extract; chloroform extract; anti-diabetic activity; antioxidant activity; *in vitro* study

INTRODUCTION

Diabetes mellitus (DM) is a metabolic deficiency characterized by failure of glucose homeostasis, is characterized by hypoglycemia, lipidemia and oxidative stress affected by many individuals to longstanding complications **[1]**. In modern world, natural medicines have been brought a huge feasible in herbal medicine with plenty of curative potential to cure many communicable diseases without associated with the side effects not like artificial drugs. A challenge to investigate herbal medicine which may useful in the impediment of diabetes and antioxidant possible by the hexane and chloroform extracts of *Barleria cristata* leaves belongs to the family of acanthaceae. *Barleria cristata* have historically been used to treat a wide range of illnesses, including antimicrobial, antioxidant, hepatoprotective, antidiabetic and anti- inflammatory activity, **[1, 2, 3]**.

The *Barleria cristata* plant contains alkaloids, flavonoid kind rich in phenolic compounds, in particular apigenin, quercetin, naringenin, luteolin and apigenin glucuronide. Recently reported in rats that nontoxic naringenin in high dose could avoid the glucose absorption from the intestine **[4]**. Leaf, stem and roots of *Barleria* species have more widely used against diabetes and respiratory diseases **[5, 3]**. In light of this, the admirable things of species belonging to the same genus, in the aim of the present study was to screen the phytochemical, evaluate radical scavenging and antidiabetic properties of *Barleria cristata* leaf in two different extracts hexane and chloroform.

MATERIALS AND METHODS

Collection of Plant Material

Fresh leaves of healthy *Barleria cristata* Linn after authenticated by Prof. P. Jayaraman, PARC, Chennai. The voucher specimen was given the No. PARC/2016/3326/1. The fresh leaves were used for the study was obtained from Kurumberi, Vellore district, Tamilnadu, India.

Preparation of leaves Extract

The leaves was washed with running tap water, Shadow dried and powdered well using a mixer and stored future use. 100 g powdered of was taken and subjected to successive solvent extraction (500ml) with to hexane and chloroform. The plant extracts were concentrated and stored in an airtight vial for further studies.

Preliminary Phytochemical Screening

Test for the presence of phytoconstituents such as alkaloids, carbohydrates, flavonides, saponin, steroids,

tannins and phenolic compounds were studied using standard phytochemicals procedures **[6]**.

Antioxidant activity

DPPH Free radical scavenging activity

Hexane and chloroform extract of *Barleria cristata* at different concentrations (20-100µg) were taken (0.4ml) and mix with 1.0ml of 0.2mm DPPH solution, resulting in the absolute concentration of DPPH being 0.1mm. The mixture was shaken well and left to place for 30min, and the absorbance was measured at 517nm **[7]**. The DPPH Free radical scavenging activity was compared with BHT.

Ferric reducing antioxidant power (FRAP)

The FRAP was determined according to the method of Oyaizu. [8] 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 RPM for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1 %) and the absorbance was measured at 700nm. Ascorbic acid was used as the reference. The increased absorbance of the reaction mixture indicated increased reducing power.

***In vitro* anti-diabetic studies**

Inhibition of α- amylase enzyme

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

Inhibition of α- 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 concentration (20- 100µl) of plant extract was added incubation for 5 min at 37°C. The reaction was initiated by adding 1 ml of alpha-glucosidase enzyme (1U/ml) to it followed by incubation for 40mins at 35°C. Then the reaction was terminated by the

addition of 2ml of 6N HCl. Then the color development was measured at 540nm.

RESULT

Phytochemical screening

Table 1 shown the phytoconstituents of *Barleria cristata*, which shows that the alkaloids, carbohydrates, glycosides, steroids, flavanoid, saponins and phenolic compounds are present in different extracts of the leaf. The percentage yield of hexane and chloroform extracts of *Barleria cristata* Leaves 7.5% & 9.0%. In that maximum yield was found in chloroform extract.

Antioxidant activity

DPPH radicals have been used to test the free radical activities of hexane and chloroform extracts. All the samples were analyzed in triplicate. The antioxidant activity of the extracts was estimated by DPPH free radical scavenging, using butylated hydroxytoluene (BHT) as controls were shown **(Table 2)**.

The hexane extract ensured the strongest free radical-scavenging activity with concentration value of 68.6 μg/mL **(Table 2)**. On the other hand the lowest capacity to reduce DPPH was observed in *Barleria cristata* **c**hloroform extract .22.3 μg/mL.

The reducing power of hexane and chloroform extracts of *Barleria cristata* Leaves, and the standard ascorbic acid at concentrations is shown **Table 3**. A significant ($p < 0.05$) dose response relationship is found in the Ferric Reducing Power activity in *Barleria cristata* extract.

Hence the result visibly indicates that the reducing power of the *Barleria cristata* extract increased to increasing the concentration and is comparable with the standard ascorbic acid, hence *Barleria cristata* is having the antioxidant activity.

***In vitro* α- Amylase Inhibitory Activity**

Polysaccharides are breakdown into simple sugar by the action of enzyme α-Amylase and only monosugar are absorbed in the stomach form food. *Barleria cristata* leave extracts (Hexane & Chloroform) were shows the dose-dependent increases from percentage inhibitory activity. Hexane extract from *Barleria cristata* showed a maximum percentage inhibition 60.7% at a concentration of 100µl while its chloroform extract shows 58.3% **(Table 4)**.

***In vitro* α- Glucosidase Inhibitory Activity**

The dose-dependent α-glucosidase inhibitory activities were indicated in **Table 5**. The hexane and chloroform extracts tested showed a concentration-dependent inhibitory activity with a similar progress profile using graded concentrations. The *Barleria cristata* leave extracts revealed a significant inhibitory action of alpha-glucosidase enzyme. The percentage inhibition varied

from 41.4% - 17.3% for the highest concentration on the lowest concentration **(Table 5)**.

Table 1: Preliminary Phytochemical Screening of *Barleria Cristata*

S. No.	Phytochemical Constituents	Hexane Extract	Chloroform Extract
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Flavonoids	+	+
4	Saponin	+	+
5	Steroids	-	-
6	Tannins	+	+
7	Phenolic compounds	+	+

+ = Present; - = Absent

Table 2: DPPH free radical scavenging activity of *Barleria cristata* extracts

S. No	Concentration of Sample (µl)	% of Inhibition		Control % BHT
		Hexane Extract	Chloroform Extract	
1	20	25.9	22.3	91.8
2	40	37.2	29.6	
3	60	45.5	35.1	
4	80	59.7	50.8	
5	100	68.6	59.7	

Values are given in Mean ± SEM

Table 3: Ferric reducing antioxidant power activity of *Barleria cristata* extracts

S. No	Concentration of Sample (µl)	% of Inhibition		Control % Ascorbic acid
		Hexane Extract	Chloroform Extract	
1	20	0.051	0. 47	0.197
2	40	0.068	0.053	
3	60	0.081	0.070	
4	80	0.095	0.086	
5	100	0.138	0.129	

Values are given in mean ± SEM

Table 4: *In vitro* Anti-Diabetic Activity of Alpha-Amylase

S. No	Sample concentration in µl	Inhibition %	
		Hexane Extract	Chloroform Extract
1	20	27.5	21.4
2	40	32.9	36.6
3	60	41.3	41.9
4	80	54.1	49.7
5	100	60.7	58.3

Values are given in Mean ± SEM

Table 5: *In vitro* Anti-Diabetic Activity of Alpha Glucosidase

S. No	Sample concentration in µl	Inhibition %	
		Hexane Extract	Chloroform Extract
1	20	21.8	17.3
2	40	25.1	24.8
3	60	30.5	29.7
4	80	36.2	33.0
5	100	41.4	38.1

Values are given in Mean ± SEM

DISSCUSION

An important target for the treatment of diabetes includes the development of inhibitors of nutrient digestion and absorption. Inhibition of α-Glucosidase and the associated reduction of glucose absorption is an attractive approach for the discovery of potent agents. In the present study has been accompanied to assess the primary phytochemical examination and the potential for Hexane and Chloroform extract from *B. cristata* leaf in inhibiting alpha-glucosidase and alpha-amylase. The Hexane and Chloroform extract extracts from *B. cristata* were tested for phytochemical constituents such as flavonoid, glycosides, carbohydrates, steroids, proteins, and amino acids. Understanding the chemical components of plants assists in the identification of biological activity.

The extracts of *B. cristata* leaves contain secondary metabolites such as flavonoid, phenol, and tannins, suggesting that they have a wide range of biological activities such as anti-inflammatory, antioxidant, anti-arthritic, antidiabetic, and membrane stabilising properties. This research supports previous research **[3]**. The presence of secondary metabolites suggests the plant's curative ability, as all of the major groups of compounds are described along with their anticancer, anti-inflammatory, and antirheumatic properties **[11-13]**.

B. cristata can effectively inhibit both alpha-amylase and alpha-glucosidase enzymes *in vitro* in a dose-dependent manner, according to the current findings. In a model of alloxan-induced diabetes in rats, ethanol extracts from *B. cristata* seeds had a dose-dependent inhibitory effect on alpha-amylase function, resulting in a substantial decrease in blood glucose levels **[14]**. The alpha-amylase and alpha-glucosidase inhibitory activity of *B. cristata* can also be linked to its anti-diabetic properties. In addition, it is important to decide if *B. cristata* possesses antidiabetic potential *in vivo* in order to validate the plant's proven argument.

CONCLUSION

The existence of flavonoids, phenols, and terpenoids was discovered in preliminary phytochemical studies of *Barleria cristata* using hexane and chloroform extracts. The presence of flavonoids and phenols in the extract can serve as a foundation for scavenging active oxygen species. The observed results concluded that hexane and chloroform extracts of *Barleria cristata* leaves not only possess remarkable inhibitory ability against -glucosidase and -amylase, but also exhibited admirable scavenging activity on the DPPH and FRAP activity. The results

of this study direct further study to assess the curative potentialities of *Barleria cristata* L. in *in vivo* models.

ACKNOWLEDGMENTS

The authors are thankful to Sree Balaji Medical College & Hospital, Chrompet, Chennai-600044, Tamil Nadu, India for the facilities to carry out the research. The authors wish to record their thanks to Prof. P. Jayaraman Director, Retd, Professor, Presidency College, Chennai for authenticated of plant material.

AUTHOR CONTRIBUTION

The first author carried out the experiment. First author wrote the manuscript with support from other authors.

FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

CONFLICTS OF INTEREST

We declare that we have no conflict of interest.

REFERENCE

[1] Sharma N, Vijayvergia R. A Review on *Digera muricata* (L.) Mart-a great versatile medicinal plant. Int. J. Pharm. Sci. Rev. Res. 2013 May; 20(1): 114-9.

[2] Gambhire M, Juvekar M, Juvekar A, Wankhede S, Sakat S. Evaluation of anti-inflammatory and radical scavenging activity of an aqueous extract of *Barleria cristata* leaves. Planta Medica. 2009 Jul; 75(09): PJ166.

[3] Amutha K, Doss D. *In vitro* Antioxidant Activity of Ethanolic Extract of *Barleria cristata* L. Leaves. Res. J. Pharmacogn. Phytochem. 2009; 1(3): 209-12.

[4] Vasanth, S., Bupesh, G., Siva Vijayakumar, T., Balachandar, V., Gunasekaran, D. R. Evaluation of *In vitro* Antidiabetic and Antioxidant Potential of *Barleria cristata* Leaves Extracts. Asian J Pharm Clin Res, 2018; 11(4): 287-290.

[5] Hemalatha K, Hareeka N, Sunitha D. Chemical constituents isolated from leaves of *Barleria cristata* Linn. Int J Pharma Bio Sci. 2012; 3(1).

[6] Ortiz-Andrade, R. R., *et al*. Antidiabetic and toxicological evaluations of naringenin in normoglycaemic and NIDDM rat models and its implications on extra-pancreatic glucose regulation. Diabetes, Obesity and Metabolism. 2008 Nov; 10(11): 1097-104..

[7] Evans WC, Evans T. Pharmacognosy, 5th Edn., Cambridge University Press, London 2003; 336-93.

[8] Braca, Alessandra, *et al*. "Antioxidant principles from *bauhinia tarapotensis*." J. Nat. Prod. 64.7 (2001): 892-895.

[9] Oyaizu, M. "Studies on products of browning reaction. Antioxidant activities of products of browning reaction prepared from glucoamine." Jap. J. Nutri 986.44: 307-315.

[10] Malick, C. P., and M. B. Singh. "Plant Enzymology and Histo Enzymology Kalyari" Publishers New Delhi pp, 286." (1980).

[11] Krishnaveni, S., Theymoli Balasubramanian, and S. Sadasivam. "Sugar distribution in sweet stalk sorghum." Food chemistry 15.3 (1984): 229-232.

[12] Jyothi, K. S., and M. Seshagiri. "In-vitro activity of saponins of *Bauhinia purpurea*, Madhuca longifolia, Celastrus paniculatus and *Semecarpus anacardium* on selected oral pathogens." Journal of Dentistry (Tehran, Iran) 9.4 (2012): 216.

[13] Kumar, Shashank, and Abhay K. Pandey. "Chemistry and biological activities of flavonoids: an overview." The scientific world journal 2013 (2013).

[14] Atif, Mohammed, *et al*. "Anticataract Potential of *Barleria prionitis*: *In vivo* Study."2014; 72: 100-5. Singh, Ranjit, P. H. Rajasree, and C. Sankar. "Screening for anti-diabetic activity of the ethanolic extract of Bryonia alba roots." Int J Pharm Biol Sci 2.3 (2012): 210-5.