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Invitro Antioxidant Activity and *Invitro* A -Glucosidase and A -Amylase Inhibitory Activity of *Barleria Cristata* L.

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ABSTRACT

Medicinal plants are reservoirs of natural products with anti-diabetic potentials. With respect to effective therapeutic approaches to treatment of DM, much effort has being made to investigate potential inhibitors against α-glucosidase and α-amylase from natural products. The aims of this study were to evaluate the antioxidant activity and also evaluate *invitro* inhibitory activity of α-amylase and α-glucosidase of *Barleria cristata* L. extracts . Our assay results suggests that ethanolic and aqueous leaf extract of *Barleria cristata* L. exhibit dose-dependent increase in percentage inhibitory activity on α-glucosidase enzymes and α-amylase when compared with standard drug acarbose. Ethanolic extracts produced highest *invitro* antioxidant activity when compared to aqueous extract. It can be concluded that the ethanolic leaf extract of *Barleria cristata* L. might be used in the control of blood glucose in clinical conditions like diabetes.

Keywords: Antidiabetic activity, *Barleria cristata* L., scavaging activity, α-glucosidase, α-amylase

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INTRODUCTION

Diabetes mellitus (DM) is used to refer to a metabolic disorder of multiple etiologies in which chronic hyperglycemia is caused by defect or alterations in either the secretion or action of insulin. This results in disturbances in carbohydrate, fat and protein metabolism. Oxidative stress is known to play a significant role in the development and progression of DM [1]. Excessive generation of free radicals and depleted levels of free radical scavenging enzymes have been demonstrated in diabetic status. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins [2]. Many diverse therapeutic strategies for the treatment of diabetes are in use. One of the therapeutic approaches for decreasing post-prandial hyperglycaemiais to prevent absorption of glucose by the inhibition of carbohydrate-hydrolysing enzymes, such as alpha-glucosidase (EC 3.2.1.20) and alpha-amylase (EC 3.2.1.1). This action of the retardation might be most effective approaches to control type 2 DM. Oral hypoglycemic agents/drugs may be effective for glycemic control, but they come with their attendant side effects such as liver disorders, flatulence, abdominal pain, renal tumours, hepatic injury, acute hepatitis, abdominal fullness and diarrhea [3]. Therefore, there is an increasing need for the development of a natural and safe product without side effects.

Various medications are available for the treatment of Type 2 diabetes like biguanides, sulphonylureas, thiozolidinediones etc [4]. These drugs however risk inducing hypoglycemia and over time, lose their efficacy, have prominent side effects and fail to significantly alter the course of diabetic complications [5]. Medicinal plants have been always an exemplary source of drugs. Traditional medicinal plants with their various biological constituents have been used effectively by the communities since long time to treat diseases. Plant extracts or bio-active herbal compounds have been reported scientifically for their biological activities [6].

Barleria cristata Linn (family acanthaceae) is a shrub found widely in subtropical Himalaya, Sikkim, Khasi Hills, central, and southern India at a height of 1,350 m. The chemical constituents methanolic extract of the plant have been identified as flavonoid type phenolic compounds, especially apigenin, quercetin, quercetin-3-O-β-D-glucoside, naringenin, luteolin, and apigenin glucuronide. *Barleria cristata* L. have been used traditionally for the treatment of variety of diseases including anemia, toothache, and cough. Leaves were used to reduce swellings in inflammation [7]. The objectives of this research were to establish the aqueous and ethanolic leaf extracts of *Barleria cristata* Linn. *invitro* antioxidant activities and *in vitro* inhibitory potential against α-glucosidase and α-amylase enzymes.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Barleria cristata* Linn used for the investigation was obtained from Coimbatore district, Tamilnadu, India. The plant was authenticated by Botanical survey of India,

TNAU Campus, Coimbatore. The voucher number is BSI/SRC/5/23/2011-12/Tech.-n62. Fresh plant material was washed under running tap water, air dried and powdered.

Preparation of Plant Extract

The leaves of *Barleria cristata* L. was air dried in the absence of sunlight and powdered well using a mixer and stored in an air tight container. The powdered plant material (50 g) was taken and subjected to successive solvent extraction (250ml) with ethanol and water. The plant extracts were concentrated and stored in an airtight vial for further studies.

Free radical scavenging activity

DPPH radical scavenging activity [8]

Various concentrations of *Barleria cristata* L. leaf extract (200-1000µg) samples were taken (0.4ml) and mixed with 1.0ml of solution containing DPPH (0.2mM DPPH was dissolved in 80% of methanol), resulting in the final concentration of DPPH being 0.1mM. The mixture were shaken vigorously and left to stand for 30min, and the absorbance was measured at 517nm. BHA was used as control. The percentage of DPPH decolorization of the sample was calculated according to the equation:

% decolorization = $[1-(ABS_{sample}/ABS_{control}] \times 100$

$ABTS^+$ radical scavenging activity [9]

9.6mg of ABTS and 1.6mg of potassium persulphate was dissolved in 2.5ml of double distilled water were incubate for 17 hrs at dark room temperature. From that 1.0 ml of diluted to 80 ml with 80% ethanol (1:79 ratio). Samples were diluted to produce 200 to 1.0mg/ml. The reaction was initiated by the addition of 1.0ml of diluted $ABTS^+$ to 10µl of different concentration of *Barleria cristata* L. leaf extracts of the sample or 10µl of methanol as control. The absorbance was read at 734nm and the percentage inhibition was calculated. The inhibition was calculated according to the equation,

$I = A_o - A_1/A_o \times 100$, where A_o is the absorbance of control reaction, A_1 is the absorbance of test compound.

FRAP Assay [10]

The stock solution of 10mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20mm $FeCl_3$, $6H_2O$ and 0.3M acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5ml TPTZ solution, 2.5ml ferric chloride solution and 25ml acetate buffer. It was freshly prepared and warmed to 37°C. 900µl FRAP reagent was mixed with 90µl water and 30µl test sample/methanol/distilled water/standard antioxidant solution(BHT). The reaction mixture was then incubated at 37°C for 30min and the absorbance was recorded at 595nm. An intense blue

color complex were formed when ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex were reduced to ferrous (Fe^{2+}) form. The absorption at 540nm was recorded. The calibration curve were plotted with absorbance at 595nm vs concentration of ferrous sulphate in the range 0.1mM both aqueous and methanol solutions. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidants**.**

***In vitro* α-amylase inhibition study**

The α-amylase inhibitory activity was determined according to the method described by Jyothi *et al.,* 2011 [11]. Briefly, the total assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme, and the plant extracts in the concentration range 10-100µg/ml were incubated for 10 min at room temperature followed by addition of 200 µl of 1% starch in all the test tubes. The reaction was terminated with addition of 400 µl of 3, 5 dintrosalycylic acid (DNSA) color reagent and placed in boiling water bath for 5 minutes, cooled at room temperature, then diluted with 15 ml of distilled water. The absorbance at 540nm was measured. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing various concentrations of the plant extracts which were prepared with DMSO. The results were expressed as % inhibition calculated using the formula:

$$\text{Inhibition activity (\%)} = [\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{sample})} / \text{Abs}_{(\text{control})}] * 100$$

IC_{50} values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the plant extracts were determined by plotting graph with varying concentrations of the plant extracts on x axis and the percent inhibition on y axis.

α-Glucosidase inhibition assay

The yeast α-glucosidase was dissolved in 100mM phosphate buffer, pH 6.8 was used as enzyme source; 10mM paranitrophenyl-α-D-glucopyranoside was used as substrate. *Barleria cristata* L. leaf extract powder was weighed and mixed with dimethyl sulfoxide to get a concentration of 10-100µg/ml. The different concentrations of plant extracts were mixed with 320µl of 100mM phosphate buffer (pH 6.8) and 50 µl of 10mM PNPG in the buffer and then it was incubated at 30°C for 5 minutes. After the incubation, 20µl of the buffer containing 0.5 mg/ml of the enzyme was added and further incubated at 30°C for five minutes. Finally, 3.0 ml of 50mM sodium hydroxide was added to the mixture and the absorbance (A) was measured at 410nm on a spectrophotometer. The enzyme without plant extract was used as a control [12] IC_{50} values were calculated as above in α - amylase.

$$\text{\% Inhibition} = [A_{410\ \text{control}} - A_{410\ \text{test}} / A_{410\ \text{control}}] * 100$$

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RESULT AND DISCUSSION

Free radical scavenging activity of *Barleria cristata* L.

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases [13]. They are also involved in autoimmune disorders like rheumatoid arthritis etc. Antioxidant compounds may function as free radical scavengers, initiator of the complexes of prooxidant metals, reducing agents and quenchers of DPPH radical.

DPPH has been used extensively to evaluate antioxidant effects. The scavenging ability of BHT, ethanol and aqueous extracts was shown in Figure 1. The free radical scavenging action of ethanolic extracts of *Barleria cristata* L. which showed the maximum scavenaging activity when compared to aqueous extract. Table 1 shows the amount of each extract required for 50% inhibition of DPPH activity (IC_{50}). It can be observed that the ethanolic extract exhibited much higher scavenging ability (IC_{50}) than that of BHT respectively (IC_{50})

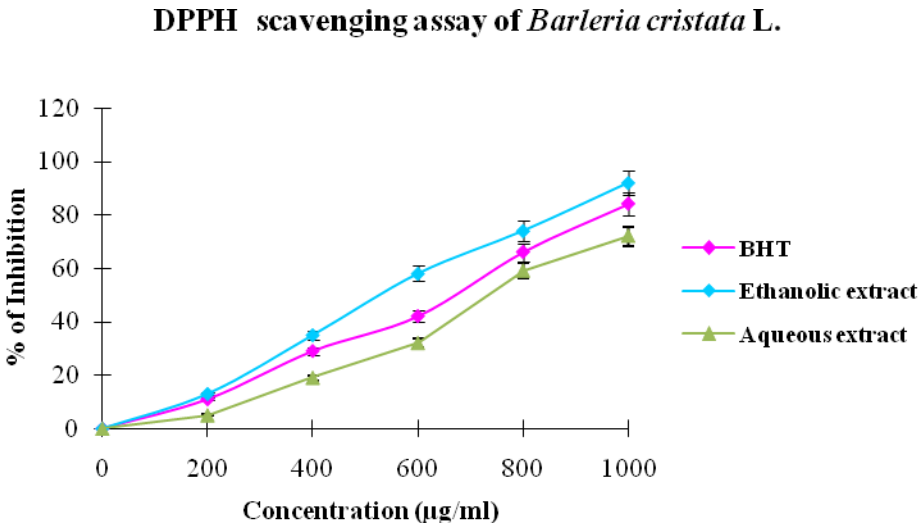

Fig 1. DPPH radical scavenging activity in different extracts of *barleria cristata* l.

Scavenging activity

$ABTS^+$ is more reactive than DPPH and unlike the reactions with DPPH , which involves H atom transfer, the reactions with $ABTS^+$ involve an electron transfer process [14]. Therefore, it was considered necessary to further assess the leaf extract of *Barleria cristata* L . The $ABTS^+$ scavenging activity of ethanol and aqueous extract was depicted in Fig 2. The scavenging activity of IC50values of $ABTS^+$ in BHT, ethanolic extract and aqueous extract respectively were 610 µg, 670 µg and 860 µg/ml . Higher the IC50 value signifies less

antioxidant activity in aqueous extract. Therefore, ethanolic extract showed maximum $ABTS^{+}$ radical scavenging activity.

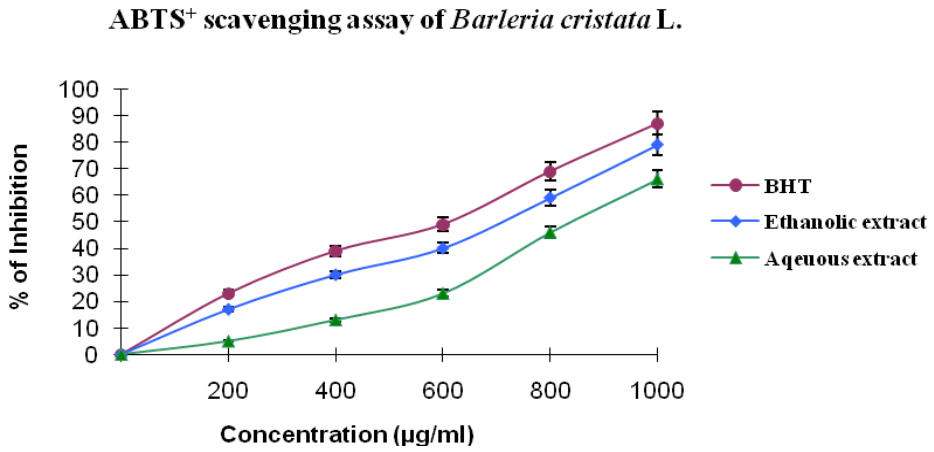

Fig 2. $ABTS^{+}$ radical scavenging activity in different extracts of *barleria cristata* l

Table 1 - IC_{50} value in free radical scavenging activity in different extracts of *Barleria cristata* L

Radical scavenging assays	IC_{50} of plant extracts (µg/ml)		Standard
	Ethanolic	Aqueous	BHT
DPPH	550	760	690
ABTS	670	860	610

Total antioxidant activity (FRAP)

The ability of plant extract to reduce ferric ions was determined in FRAP assay. FRAP measures the antioxidant effect of any substance in the reaction medium as reducing ability. Reducing ability is considered the ability of a natural antioxidant to donate electrons [15]. The change in absorbance at 593 nm owing to the formation of blue colored Fe^{+2}- tripyridyltriiazine (TPTZ) compound from the colourless oxidized Fe^{+3} form by the action of electron donating antioxidants [16]. From the figure 3, the FRAP values of ethanolic extract exhibited more ferric reducing activity than aqueous extract and it was observed that it increased with increasing concentration. Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidant present, thus it can be reported that ethanolic extract of *Barleria cristata* L. may act as free radical scavenger.

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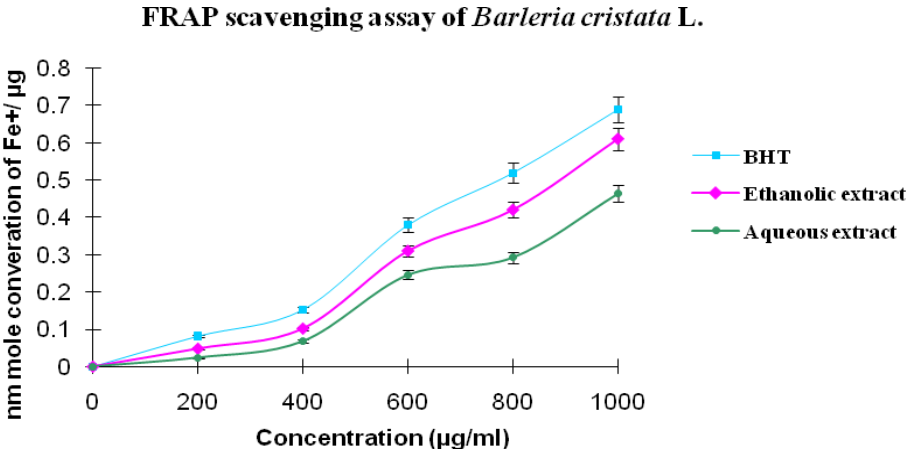

Fig 3. FRAP radical scavenging activity in different extracts of *barleria cristata* l.

Invitro inhibition potential against α-glucosidase and α –amylase

Diabetes is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Recent decades have experienced a sharp increase in the incidence and prevalence of diabetes mellitus. Plant extracts have long been used for the ethnomedical treatment of diabetes in various systems of medicine and are currently accepted as an alternative for diabetic therapy [17]. Recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase both are important in carbohydrate digestion and glucose absorption) have led to the development of newer pharmacological agents. As part of this research, we investigated the inhibitory effects of *Barleria cristata* L. leaf and acarbose against α-amylase and α-glucosidase.

Invitro inhibitory activity on α –amylase

Pancreatic α -amylase is a key enzyme in the digestive system and catalyzes the initial step in the hydrolysis of starch, which is a principal source of glucose in the diet [18] The *in vitro* α-amylase inhibitory studies demonstrated that aqueous and ethanolic extracts of *Barleria cristata* L possess considerable inhibitory action and this will be helpful in the management o diabetes. The percentage inhibition at 100, 80, 60, 40 and 20 µg/ml concentrations of plant extract showed a concentration-dependent reduction in percentage inhibition respectively. Thus the highest concentration of 100µg/ml tested showed an inhibition of 59% by aqueous extract, 74% by the standard acarbose and the maximum inhibition was found to be 68% in ethanolic extract (Figure 4). This study provide an evidence that the ethanolic extract of *Barleria cristata* by its inhibition of α –amylase can acts as agent that prevent the absorption of glucose and helping the reduction of postprandial hyperglycemia.

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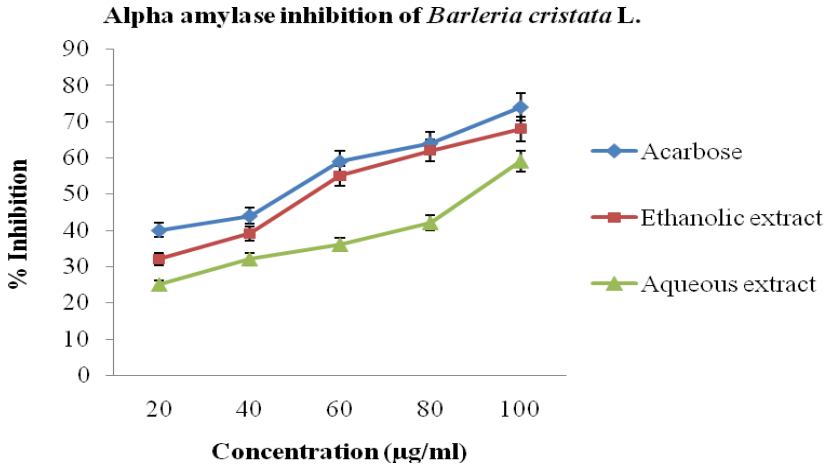

Fig 4. Alpha amylase inhibitory activity of different extracts of *barleria cristata* l.

Inhibitory activity on α –glucosidase

A high postprandial blood glucose response is associated with complications in diabetes and is more strongly associated with the risk for cardiovascular diseases than are fasting blood glucose. α-Glucosidase enzymes in the intestinal lumen and in the brush border membrane play main roles in carbohydrate digestion to degrade starch and oligosaccharides to monosaccharides before they can be absorbed. It was proposed that suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation. Alpha-glucosidase inhibitor retards the digestion of carbohydrates and slows down the absorption [19]. Ethanolic extract showed a maximum inhibition activity of 56% and aqueous extract showed the inhibitory activity of 47% at a concentration of 100 µg/ml. Therefore aqueous extract produced a weak α-glucosidase enzyme inhibition when compared with ethanolic extract and standard acarbose (65%).

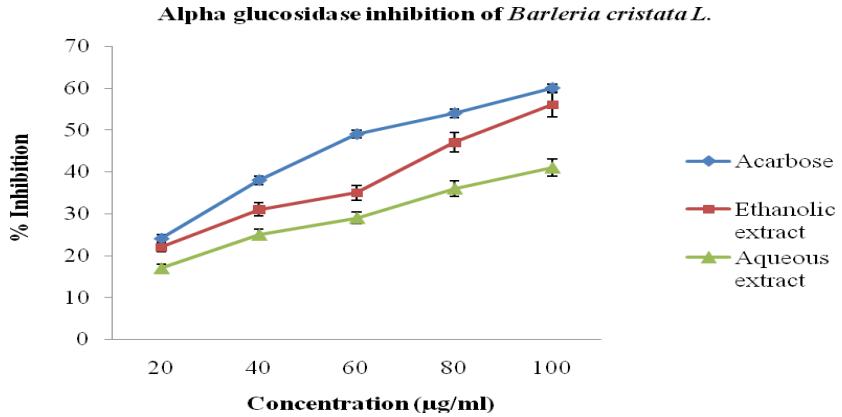

Fig 5. Alpha glucosidase inhibitory activity of different extracts of *barleria cristata* l.

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CONCLUSION

Present study indicated that ethanolic extract of *Barleria cristata* L. leaves not only possessed remarkable scavenging activity on DPPH, $ABTS^{+}$ and FRAP activity, but also exhibited excellent inhibitory potential against α-glucosidase and α –amylase. The results of this study direct further research to evaluate the therapeutic potentialities of *Barleria cristata* L. in the maintenance of postprandial hyperglycemia and management of type 2 diabetes.

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