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ORIGINAL ARTICLE



In vitro antioxidant and antidiabetic activities of *Barleria cuspidata* Heyne ex Nees

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ABSTRACT

Free radicals produced endogenously by oxidation reaction initiate the chain reactions that causes chronic diseases of cancer, diabetes, hepatotoxicity etc. Diabetes Mellitus is a complex and multifarious gathering of metabolic issue with chronic hyperglycaemia that disturbs the metabolism of carbohydrates, lipids and proteins. Traditionally, Barleria cuspidata Heyne ex Nees is utilized for antidiabetic action with absence of logical investigation. Thus, the current examination was attempted to explore for its in vitro antioxidant and antidiabetic activities by various standard models. In this attempt the chloroform and methanol extracts of Barleria cuspidata were evaluated for its in vitro antioxidant activities like DPPH radical scavenging activity, nitric oxide (NO) radical inhibition assay, lipid per oxidation assay, superoxide anion radical scavenging activity, hydroxyl radical scavenging. Further, in vitro antidiabetic activity, glucose diffusion inhibitory study and glucose uptake capacity by yeast cells. The results revealed that the extracts of Barleria cuspidata shows the better radical scavenging capacity for its antioxidant activity on compared with that of standard antioxidants. Likewise, the extracts of Barleria cuspidata shows the in vitro antidiabetic activity by inhibiting alpha amylase, glucose by yeast cells. From the results it is concluded that chloroform and methanol extracts of Barleria cuspidata possess good antioxidant and antidiabetic properties as shown by in vitro assay.

Key words: Barleria cuspidata Heyne ex Nees, in vitro assays, antioxidant activity, antidiabetic activity.

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INTRODUCTION

Diabetes Mellitus (DM) is a complex and multifarious group of metabolic disorder with chronic hyperglycaemia that disturbs the metabolism of carbohydrates, lipids and proteins. The starting point and etiology of DM can differ enormously yet consistently remember deserts for either insulin discharge or response of target tissues or in both [1]. The hallmarks of DM are polyuria(excessive urine production), polydipsia (excessive thirst) and polyphagia (excessive eating). The two principal types of DM are insulin dependent diabetes mellitus (IDDM or Type 1 DM) and non-insulin dependent diabetes mellitus (NIDDM or Type 2 DM). Type 1 DM are fundamentally managed with dietary limitation, exercise and insulin treatment while Type 2 DM are managed with weight decrease, dietary limitation, exercise and medication like oral hypoglycaemics and antihyperglycaemics [2].

In both type 1 and type 2 DM there is an increased oxidative stress [3], which results from an imbalance between the generation of oxygen derived free radicals and the organism's natural antioxidant potential [4]. In DM the imbalance is associated with increased formation of free radicals and decreased in antioxidant potential [3]. It further leads to oxidative disorder of cell components such as protein, lipid and nucleic acid which plays role in the development and progression of DM as well as their complications [5].

Constant utilization of oral hypoglycaemics and antihyperglycaemics in Type 2 DM causes hematological impacts and influences the elements of significant organs of liver, kidney and so on., Worldwide now

daily's number of restorative plants have been accounted for and utilizing for treatment of DM, as they are powerful, nontoxic with practically zero reactions and furthermore amazing material for oral treatment [6].

Barleria cuspidata Heyne ex Nees is one of the important species in *Barleria* belongs to the family Acanthaceae. It is a shrub found in waste places, poor soils and along road ways [7]. The roots and leaves were used traditionally in stomach ache, tonic, febrifuge, cough, bronchitis and in inflammation [8]. Earlier study has proved that the plant contains alkaloids, terpenoids, triterpenoids, esters, aliphatic ketones, β -carotene and so on [9]. *Barleria cuspidata* is proved for wound healing property [10] and hepatoprotective activity [11]. Still there was lack in scientific study of antioxidant and antidiabetic effect of *Barleria cuspidata* to substantiate the traditional claim. Hence, the current work was embraced to assess for its antioxidant and antidiabetic by *in-vitro* models.

MATERIAL AND METHODS

Chemicals and reagent

All the chemicals and reagents used in these *in-vitro* antioxidant and antidiabetic workswere of analytical grade.

Source and authentication of plant material

Fresh whole plant of *Barleria cuspidata* Heyne ex Nees (Acanthaceae) were pull together from chittoor districts in the areas of Tirumala Hills and Tirupathi surroundings and authentified by Dr. K. Madava Chetty, Professor, Department of Botany, Sri Venkateswara University, Tirupathi. Andhra Pradesh, India. Voucher specimen (No: BB- 1419) of this plant has been kept in the P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

Preparation of plant material

The gathered entire plant of *Barleria cuspidata* was washed with running water, cut into little pieces and shade dried at room temperature to maintain a strategic distance from loss of phytoconstituents of plant. The total shade dried materials pounded for powder and sieved up to 80 meshes. At that point it was homogenized to fine powder and put away in air-tight compartment for additional antidiabetic considers.

Preparation of plant extracts

Whole plant powder of the *Barleria cuspidata* was extracted successively with two different solvents like chloroform (30-60°C) & methanol (50-70°C) in a Soxhlet apparatus in batches of 500 gm each. The overabundance solvent was expelled from extract utilizing a rotary vacuum evaporator and later on concentrated on a water bath. At last dried extracts were put away in desiccators for assess for its antioxidant and antidiabetic by *in-vitro* models[12].

In vitro antioxidant activity

DPPH radical scavenging assay

The DPPH radical scavenging movement of chloroform and methanol concentrates of *Barleria cuspidata* were estimated by 1, 1 diphenyl 2 picryl hydrazyl (DPPH) strategy [13]. A stock arrangement was set up by dissolving chloroform and methanol extracts in refined water. From these stock arrangements various convergences of 5, 10, 20, 40 and 80 μ g/ml working solutions were arranged separately. 0.1 milli molar DPPH solution was set up by dissolving in ethanol. To 1 ml of working solution the 3 ml of individual plant extracts of various fixations were added and afterward the blend was shaken enthusiastically and permitted to remain at room temperature for 20 – 30 minutes. Absorbance was estimated at 517 nm by spectrophotometer. Serial dilutions of standard compound were additionally set up with quercetin as reference standard compound.

Nitric oxide (NO) radical inhibition assay

Various concentrations (5 to 160 μ g/ml) of chloroform and methanol extracts of *Barleria cuspidata* were arranged independently. 2 ml of sodium nitroprusside (10 mM) in 0.5 ml of saline phosphate buffer was blended in with various concentrations of chloroform and methanol extracts of plant and incubated at 30°C for two hours. On finish of incubation period add 1 ml of Griess reagent (1% sulphanilamide, 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 2% orthophosphoric corrosive), phosphate buffer (pH - 7.4) was added. The blend was of course incubated at room temperature for 30 – 50 minutes and its absorbance were estimated at 550 nm. Rutin was filled in as standard [14].

Lipid per oxidation assay

Rat liver microsomal part and chloroform and methanol concentrates of *Barleria cuspidata* in different concentrations $(10 - 160 \ \mu\text{g/ml})$ were set up by the technique for Brouchet *et al* [15] to decide the thiobarbituric acid receptive substances in this examine. 500 μ l of liver microsomal portion, 300 μ l of working arrangement of plant extracts and 100 μ l of FeCl₃ (1mM) were blended. 100 μ l vitamin C (1mM) was added at last. Samples were incubated at 37° C for 1 hour and lipid per oxidation was estimated

utilizing the response with thiobarbituric acid. The absorbance was estimated at 532 nm. All responses were done in three-fold. Vitamin E was utilized as a standard.

Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity of chloroform and methanol concentrates of *Barleria cuspidata* were performed by strategy for Nishimiki *et al.* [16]. Sequential dilutions of 5, 10, 20, 40, 80 and 160 µg/ml were arranged independently from chloroform and methanol extracts of *Barleria cuspidata*. Every dilution was added by 1ml of nitroblue tetrazolium (NBT) solution and 1ml of nicotinamide adenine dinucleotide (NADH). The response was started by adding 100µl of phenazine methosulphate (PMS) solution and afterward incubated at 25° C for 5 min. The absorbance was estimated at 560 nm against blank. Curcumin was taken as reference compound.

Hydroxyl radical scavenging activity

Working solutions of different concentrations (10, 20, 40, 80 and 160 μ g/ml) were set up with chloroform and methanol extracts of *Barleria cuspidata* separately [17]. 500 μ l of chloroform and methanol extracts at different concentrations were added with 100 μ l of 2-deoxy 2-ribose and 200 μ l of 1.04 mM ethylene diamine tetra acetic acid (EDTA). Further 200 μ M ferric chloride (1:1, v/v) and 100 μ l of 1.0 mM hydrogen peroxide were added. At last, 100 μ l of 1.0 mM nutrient C was added. All samples were hatched at 37° C. Following one hour 1 ml of 1% thiobarbituric corrosive (TBA) and 1.0 ml 2.8% trichloroacetic corrosive (TCA) were added to the response combination and incubated at 100° C for 20 minutes. The absorbance was estimated at 532 nm against a blank. Nutrient E at different focuses was utilized as appositive control.

In vitro antidiabetic activity

Alpha amylase inhibitory activity

The Assay was done observing the standard protocol with slight alterations. Starch azure (2 mg) was suspended in 0.2 mL of 0.5M Tris–HCl cradle (pH 6.9) containing 0.01 M CaCl₂ (substrate arrangement). The tubes containing substrate solution were boiled for 5 min and afterward preincubated at 37°C for 5 min. Chloroform concentrate of *Barleria cuspidata* was dissolved in DMSO to get concentrations of 5, 10, 25, 50, 75, and 100 µg/mL. At that point, 0.2 mL of *Barleria cuspidata* extract of specific concentration was added to the tube containing the substrate solution. Moreover, 0.1 mL of porcine pancreatic amylase in Tris–HCl cushion (2 units/mL) was added to the tube containing the substrate solution. Moreover, 0.1 mL of porcine pancreatic amylase in 0.5 mL of half acidic corrosive in each tube. The response combination was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of coming about supernatant was estimated at 595 nm utilizing spectrophotometer. Same strategy was followed for methanol concentrate of *Barleria cuspidata* to test their alpha-amylase inhibitory impact. Acarbose, a referred to α -amylase inhibitor was utilized as a standard medication. The Experiments were rehashed threefold. The α -amylase inhibitory action was determined by utilizing following formula:

The alpha amylase inhibitory activity =

(Ac+) - (Ac-) - (As – Ab) / (Ac+) - (Ac-) x 100

where Ac+, Ac-, As, and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme), and a blank (a test sample without enzyme) respectively. The concentration of acarbose and plant extracts required to inhibit 50% of α -amylase activity under the conditions was defined as the IC₅₀ value. The α -amylase inhibitory activities of plant extracts and acarbose were calculated, and its IC₅₀ values were determined [18].

Alpha glucosidase inhibitory activity

For alpha glucosidase inhibitory movement, yeast α -glucosidase was broken up at a concentration of 0.1 U/ml in 100 mM phosphate buffer, pH 7.0, containing bovine serum albumin 2 g/liter and sodium azide 0.2 g/liter which was utilized as enzyme source. Paranitrophenyl- α -d-glucopyranoside was utilized as substrate. Chloroform concentrate of *Barleria cuspidata* (20%, v/v) was gauged and sequential dilutions of 5, 10, 25, 50, 75 and 100 µg/ml were made up with equivalent volumes of dimethylsulfoxide and distilled water. Ten microliters of extract dilutions were incubated for 5 min with 50 µl enzyme source. After incubation, 50 µl of substrate was added and further incubated for 5 min at room temperature. The pre substrate and post substrate expansion absorbances was estimated at 405 nm on a microplate peruser. Same method was followed for methanol concentrate of *Barleria cuspidata* to test their alpha glucosidase inhibitory movement. The expansion in absorbance on substrate expansion was acquired. Each test was performed three times and the mean assimilation was utilized to calculate percentage α -glucosidase inhibition as follows:

Percent α -glucosidase inhibition = (A-B/A) x 100.

Where A is the absorbance of control and B is the absorbance of samples containing extracts. The inhibitory concentration of the extract required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated. Acarbose was dissolved in distilled water and used as positive control [19].

Glucose diffusion inhibitory study

To test the glucose diffusion across the bio-membrane, 3 cm portions of the dialysis layer (12000MW) were cut and loaded up with 1 ml of 0.15 M NaCl containing 22 mM glucose and 1 ml of chloroform extract of *Barleria cuspidata*. They were then tied at the two closures utilizing a nylon string and put in a 100 ml beaker containing 40 ml of 0.15 M NaCl and 10 ml of refined water to adjust the strength of inner and outside media. These measuring utencils were then positioned on an orbital shaker and kept at room temperature. The control contained 1 ml of 0.15 M NaCl containing 22 mM glucose and 1 ml of distilled water. It was without plant extricate. Tests were taken from every measuring beaker and glucose concentration in them was tried like clockwork by utilizing reagent pack. Three replications of this technique were accomplished for 3 hours [20]. Same technique was followed for methanol extract of *Barleria cuspidata* for considering the glucose diffusion inhibitory action.

Determination of glucose uptake capacity by yeast cells

This test was performed by the very much characterized technique for Cirillo [21]. Commercial baker's yeast was dissolved in distilled water to get ready 1% suspension. The suspension was kept for the time being at room temperature (25°C). On the following days, yeast cells suspension was centrifuged at 4200 rpm for 5 minutes. The cycle was rehashed by the addition of distilled water to the bed until a reasonable supernatant was acquired. Precisely 10 parts of the clear supernatant fluids were blended in with 90 parts of distilled water to get a 10%v/v suspension of the yeast cells. Around 1–5 mg w/v of plant extract was blended in with dimethyl sulfoxide (DMSO) till dissolution. The blend was then enhanced with different concentrations (5, 10, and 25Mm) of 1mL of glucose arrangement and incubated for 10 min at 37°C. To initiate the response, 100 μ L of yeast suspension was poured in the combination of glucose and extract, vortexed, and incubated for an additional hour at 37°C. After incubation, the tubes were centrifuged for 5 minutes at 3800 rpm and glucose was assessed by utilizing a spectrophotometer at 520 nm. Absorbance for the separate control was additionally recorded on a similar frequency. The percent expansion in take-up was determined by the formula:

% increase in glucose uptake =

[(Absorbance of control – Absorbance of sample) / Absorbance of control] x 100

Where control is the solution having all reagents except the test sample. Metronidazole was used as standard drug. These was done for both chloroform and methanol extracts of *Barleria cuspidata* to determine the glucose uptake capacity by yeast cells.

RESULTS AND DISCUSSION *In vitro* antioxidant activity

DPPH radical scavenging assay

The scavenging of DPPH radical by chloroform and methanol concentrates of *Barleria cuspidata* was checked and appeared in Figure 1. The abatement in the absorbance was related with level of colour change from purple to yellow with the scavenging action of *Barleria cuspidata* on DPPH radical. The IC₅₀ estimation of chloroform extract, methanol extract of *Barleria cuspidata* and standard quercetin were discovered to be 12.71 μ g/ml, 12.31 μ g/ml and 8.83 μ g/ml individually. The scavenging movement of *Barleria cuspidata* on DPPH radical might be because of the presence of flavonoid, according to the report of Bors *et al*, 1990.The hydroxyl group joined the B ring of flavonoid particle could go about as decreasing specialist which may signifies the hydrogen molecule for the inactivation of DPPH free radical [22].



Figure 1: Scavenging effect of chloroform and methanol extracts of *Barleria cuspidata* and standard quercetin on DPPH radical

Nitric oxide (NO) radical inhibition assay

Sodium nitroprusside in presence of phosphate buffer saline delivered to nitric oxide. The created nitric oxide goes through expansion response with oxygen to deliver stable nitrates and nitrites. The presence of cell antioxidants can diminish the concentration of nitric oxide. A progressive reduction in absorbance was recorded which was straightforwardly identified with the concentration of nitrites. The diminishing in absorbance might be because of the plant extract which rivals oxygen to respond with nitric oxide prompting reduction of nitric oxide accessibility. This might be because of the presence of flavonoids in plant extract, which restrains the nitric oxide discharge just as productively went about as nitric oxide scavenger [23]. The nitric oxide extremist scavengingactivity of chloroform and methanol extracts of *Barleria cuspidata* and standard rutin were recognized from IC_{50} estimations of 52.22 µg/ml, 45.71 µg/ml and 37.24 µg/ml separately and the outcomes were posted on Figure 2.



Figure 2: Scavenging effect of chloroform and methanol extracts of *Barleria cuspidata* and standard rutin on nitric oxide radical

Lipid per oxidation assay

A blend of ferrous sulphate and ascorbic acid demonstrates a decent promoter system for inciting oxidative stress. The ferrous ascorbate complex produces responsive hydroxyl radical. Hydroxyl

radicalattacks the unsaturated fats of liver microsomes causing lipid per oxidation. This outcome in generation of carbonyl parts called malonodialdehyle which respond with thiobarbituric corrosive to shape a pink compound which is consumed at 532 nm. The chloroform and methanol extracts of *Barleria cuspidata* and standard vitamin E displayed a consistent scavenging effect of hydroxyl group at various concentration, which were attracted Figure 3. The IC₅₀ estimation of chloroform and methanol extracts of *Barleria cuspidata* and standard vitamin E were distinguished as 80.26 µg/ml, 70.28 µg/ml and 70.28 µg/ml individually. The marked inhibition of lipid peroxidation by the extracts of *Barleria cuspidata* might be because of the presence of reductants like phenols and steroids in the extracts [24].



Figure 3: Scavenging effect of chloroform and methanol extracts of *Barleria cuspidata* and standard vitamin E on lipid peroxidation of liver microsome induced by ascorbate

Superoxide anion radical scavenging activity

Phenazine methosulfate responds with nicotinamide adenine dinucleotide prompts the development of superoxide radical. Nitro blue tetrazolium gains electrons from superoxide anion radical and gets decreased to blue formazan. Staining of formazan with diminished in absorbance in presence of plant extracts shows the utilization of superoxide anion by plant terpenes in the reaction mixture. Figure 4 speaks to the superoxide anion radicalscavenging activity of chloroform and methanol extracts of *Barleria cuspidata* and standard curcumin with IC_{50} estimations of 34.40 µg/ml, 31.01 µg/ml and 13.05 µg/ml individually.



Figure 4: Scavenging effect of chloroform and methanol extracts of *Barleria cuspidata* and standard curcumin on superoxide anion radical formation

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was estimated by fenton response and the outcomes are attracted Figure 5. In this expansion of iron salts to ascorbic acid produces hydroxyl radical which assaults the hydrogen atom of deoxyribose. The degraded sugar moleculereacts with thiobarbituric acid to shape thiobarbituric acid receptive substances. The presence of EDTA expands the arrangement of thiobarbituric acid receptive substances to four-crease higher than its nonappearance. The concentrations of half hindrance (IC₅₀) were discovered to be 42.19 μ g/ml, 36.89 μ g/ml and 26.91 μ g/ml for chloroform and methanol concentrates of *Barleria cuspidata* and standard vitamin E.



Figure 5: Scavenging effect of chloroform and methanol extracts of *Barleria cuspidata* and standard vitamin E on hydroxyl radical

*In vitro*antidiabetic activity Alpha amylase inhibitory activity

The alpha amylase inhibitory action of chloroform and methanol concentrates of *Barleria cuspidata* were contrasted and basic standard alpha amylase inhibitor acarbose with IC₅₀esteems and appeared in Figure 6. The IC₅₀ estimations of chloroform and methanol extracts of *Barleria cuspidata* were 10.66 μ g/ml and 8.42 μ g/ml individually which were the better on contrasted and standard acarbose 5.38 μ g/ml. The plant-based α -amylase inhibitor offers a forthcoming helpful methodology for the management of diabetes.





Alpha glucosidase inhibitory activity

The alpha glucosidase inhibitory activity of chloroform and methanol extracts of *Barleria cuspidata* were contrasted and basic standard alpha glucosidase inhibitor acarbose with IC_{50} esteems and appeared in Figure 7. The IC_{50} estimations of chloroform and methanol extracts of *Barleria cuspidata* were 9.5 µg/ml and 8.1 µg/ml separately which were the better on contrasted and standard acarbose 5.6 µg/ml.



Figure 7: Alpha glucosidase inhibitory activity of chloroform and methanol extracts of *Barleria cuspidata* and standard acarbose Glucose diffusion inhibitory study



Figure 8: Effect of chloroform and methanol extracts of *Barleria cuspidata* on diffusion of glucose out of a bio-membrane over 180 minutes



Figure 9: Relative movement of glucose across bio-membrane with respect to control under the influence of chloroform and methanol extracts of *Barleria cuspidata*

The after effects of the glucose diffusion inhibitory investigation of chloroform and methanol extracts of *Barleria cuspidata* were given in Figure 8 and 9. From the outcomes it can says that chloroform and methanol extracts of *Barleria cuspidata* impedes the diffusion of glucose across the dialysis membrane. Among these the methanol extract shows the greatest hindrance of glucose diffusion across the dialysis layer, at 180 minutes the relative movement concerning control was 54.80 ± 2.78 . In the body there are different carriers which work in synchronization with different molecules to move glucose. For this reason, In the current examination glucose was set up in NaCl, as glucose atoms need a transporter particle to diffuse across cells. Endogenously, this was accomplished by sodium particles [20]. The outcomes show that glucose diffusion inhibition across a membrane is a potential component of antihyperglycemic activity of the plant.









Figure 11: Effect of chloroform and methanol extract of *Barleria cuspidata* on glucose uptake by yeast cell at 10 mM glucose concentration



Figure 12: Effect of chloroform and methanol extract of *Barleria cuspidata* on glucose uptake by yeast cell at 25 mM glucose concentration

The chloroform and methanol extract of *Barleria cuspidata* on uptake of glucose by yeast cell were assessed and results got were appeared in Figure 10, 11 and 12. The glucose uptake at groupings of 5 and 10 mM of chloroform and methanol extracts of *Barleria cuspidata* were practically identical with that of known standard metronidazole. Nonetheless, at 25 mM glucose concentration the impact of metronidazole on glucose uptake by yeast cells were minimal high on contrasted with that of chloroform and methanol extracts of *Barleria cuspidata*. The glucose uptake limit by the yeast cells were expanded regarding increment in concentration of extract of *Barleria cuspidata*. Then again, increment in the molar concentration of glucose shows the reverse relationship on glucose uptake by yeast cells were seen among 5 mM, 10 mM and 25 mM fixations for a similar measure of chloroform and methanol extracts of *Barleria cuspidata*. Regularly the yeast cells will uptake the glucose by facilitated diffusion as opposed to phosphotransferase enzyme system and some other unknow process. Additionally, in yeast cell the majority of the inward sugar is changed over into different metabolites which bring down the interior concentration of glucose and favours for high uptake of glucose to the cell. Similarly, with both the chloroform and methanol extracts of *Barleria cuspidata* the conceivable wase for the yeast cell to uptake the glucose molecule will be both facilitated diffusion and raised glucose metabolism.

CONCLUSION

The results of the present study concluded that chloroform and methanol extract of *Barleria cuspidata* may have *in*-vitro antioxidant and antidiabetic activity. The extracts show comparable results with that of respective standards. Among the chloroform and methanol extract of *Barleria cuspidata*, the methanol extract shows maximum *in-vitro* antioxidant and antidiabetic activity. Present endeavours are composed to separate the dynamic constituents from various extract of plant and explanation of component of activity.

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CONFLICT OF INTERESTS

The authors proclaim that there was no conflict of interest in this research.

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