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An *in vitro* new vista to identify hypoglycemic activity

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Abstract

Objective & Methodology: The present study aimed to detect a novel *in-vitro* method for some of indigenous plant extracts which demonstrate inhibitory effect to glucose oxidase and are in use of our traditional remedial system for hypoglycemic impending.

Result: *Bambusa vulgaris* possessed hypoglycemic activity of varying degree. *Dendrocalamus hamiltonii, Dendrocalamus sikkimensis* had shown the better activity in neutral and basic media than others. Whereas, *Bambusa balcooa and Bambusa pallida* showed prominent result in acidic media. The result in three different media revealed that, acidic medium had less prominent hypoglycemic activity as compared to neutral and basic medium.

Conclusion: This may be the first report for using glucose oxidase as *in vitro* method to prove their antidiabetic properties, can save a hugh preclinical burden for the budding herbal products.

Keywords: *Diabetes; Indigenous plants; Antihyperglycemic activity; Glucose oxidase.*

1. Introduction

Plants, a therapeutic source are a prehistoric knowledge[1]. Past investigations have indicated that the consumption of natural antioxidants is related with reduced risks of numerous ailments like diabetes[2]. Freshly, there is an increasing attention in finding natural antioxidants to substitute manmade ones[3,4]. India has a vast biodiversity due to its topographical and climatic circumstances. In India about 139 species of bamboos are encountered belonging to 36 genera[5]. North east region holds two third of the countries bamboo reserve with 58 species included in 10 genera. Ethano-pharmacological survey studies in this region have revealed a large number of plant species are traditionally used in North east and surround regions for many ailments[6]. There are a few or almost none studies on these plants that have been recorded. Also, these plants were recommended by the natural healers for treatments of diabetic symptoms and its complications[1,7,8].

Numerous *in vivo* methods are there to investigate anti-hyperglycemic activity of these herbs. But there are rare specific studies[9] to verify their *in vitro* anti-hyperglycemic activity. The current contemporary practice could be used to diminish animal sacrifices in the preliminary screening of anti-hyperglycemic activity of plant species. Neither previous reports provide any demonstration on *in vitro* anti-hyper glycemic studies of these ethnic plants nor on which medium or pH does the plant illustrate remedial activity. Therefore, this study is premeditated to witness the inhibition of glucose oxidase, a marker of anti-hyperglycemic activity of therapeutic plants used in north east traditional system in India.

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2.Materials and Methods

2.1 Chemicals

Glucose, Glucose oxidase, Potassium mono hydrogen phosphate, Hydrochloric acid, Potassium dihydrogen phosphate, and EDTA were purchased from Merck and Sigma. All chemicals and reagents used were of the analytical grade and highest commercially available purity.

2.2 Plant collection

Leaves of *Dendrocalamus hamiltonii* (DH), *Dendrocalamus sikkimensis* (DS), *Bambusa balcooa* (BB), *Bambusa pallida* (BP), *Bambusa vulgaris* (BV) were collected from the Sukna forest after taxonomic identification and authentication. The live accessions are deposited at the Bambusetum in Kurseong Research Range, Sukna, Siliguri

2.3 Extract Preparation

After shade dried (Temp<40°C.), plant material was grounded into a moderately coarse powder was boiled with sixteen parts of methanol for a period of 15 minutes[10] and filtered was evaporated under reduced pressure and dried .The yield of the extract was 1.2-2.06% (w/w).

2.4 Procedure for glucose oxidase method

Trinder (1969)[12] method with a slight modification was used to determine inhibition of glucose oxidase level. 0.05 ml glucose solution of 20, 40, 60, 80& 100 mg/dl was added with 0.05ml of extract in to different test tubes and incubated in dark for 4hrs. Then 5 ml of glucose oxidase enzyme was added in all test tubes and kept for 30 minutes in dark at RT. Results were spectrophotometrically (Thermo scientific Multiscan Go) recorded at 546nm. Concentration of glucose was measured as following

$$\text{Concentration of glucose} = \frac{Ak}{Au} - X \quad C$$

Where Ak = Absorbance of known (standard glucose) ; Au = Absorbance of unknown (extracts of plants), C = concentration of standard glucose.

Statistical Analysis[2]

All samples were tested and analyzed in triplicate. Results were calculated as the mean ± SD (standard deviation) for each sample. Statistical analysis was done with one way analysis of variance using Graph pad Prism, Version 4.0 (Graph Pad Software, San Diego, CA, USA). A significant difference was judged to exist at a level of $P < 0.05$.

3. Results and Discussion

Our study reveals the uses of glucose utilization using enzyme glucose oxidase method in a complex mixture or an extract because of its specificity. The phytochemical analyses of five bamboo species of North Bengal region were assessed which revealed their hypoglycemic activity. The results indicate the presence of active constituents in the solvents extracted from medicinal plants material. Special consideration to these effective therapeutic plants will lead us to obtain innovative medicines in controlling diabetes mellitus.

3.1 In vitro Hypoglycemic activity at different pH

Figure I, II & III display anti hyperglycemic activity of the different plants at the different pH 2, 7 and 9 respectively. At pH 7 and 9, extracts showed considerable anti-hyperglycemic activity in comparison to pH 2. At a concentration of 80 µg/ml, *B. pallida and B. vulgaris* at basic and neutral pH however *B. balcooa* showed the highest antihyperglycemic activity at acidic pH. The statistical analysis indicated significant difference between hypoglycaemic activity of plant extracts at $P< 0.05$. One of the possible reasons for these plants could be its adenosine deaminase inhibitory activity, hence reduces glucose levels in hyperglycemic patients as suggested in some other plants studies[13]. Previous studies revealed that *B. vulgaris* repairs damaged β-cells, increases insulin levels, and also enhance the sensitivity of insulin. It might inhibit glucose

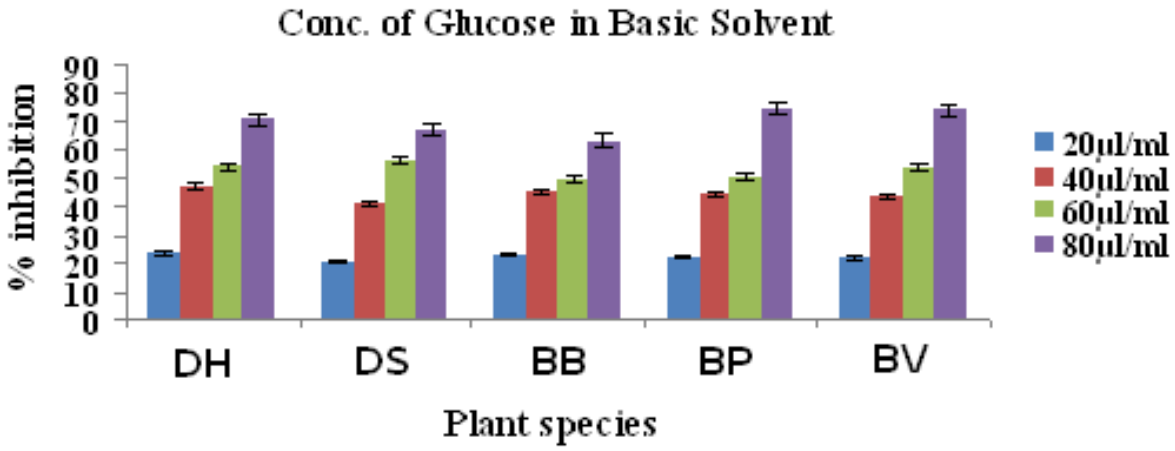

Figure I: Anti hyperglycemic activity of the different plants at the different pH 2.

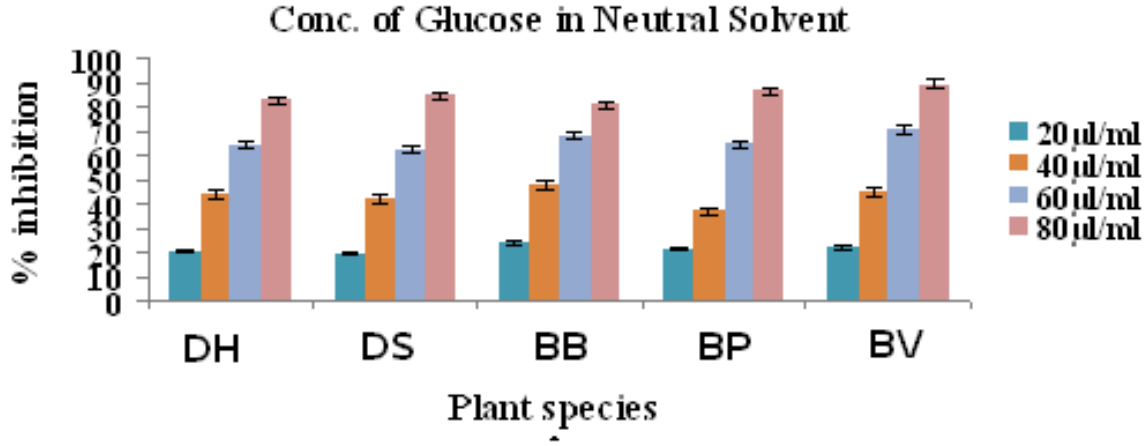

Figure II: Anti hyperglycemic activity of the different plants at the different pH 7.

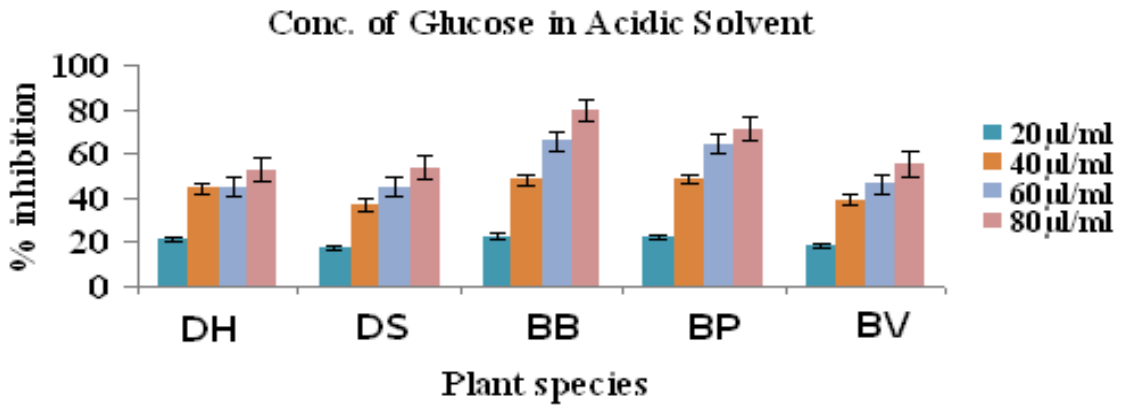

Figure III: Anti hyperglycemic activity of the different plants at the different pH 9.

oxidase and therefore glucose absorption and also suppresses the activity of disaccharides in the intestine[15]. Since these plants are found to have good antihyperglycemic activity *in vitro*. Further, *in vivo* investigations are needed to prove their anti-hyperglycemic activity. Even more comprehensive chemical and pharmacological studies are needed to exploit their ethano-medicinal relevance.

4. Conclusion

B. vulgaris, B. pallida, B. balcooa, D. hamiltonii and *D. sikkimensis* provided the imprint of being noticeable candidates for drug targets for diabetes. This may perhaps be the first strategy using *in vitro* methods to verify their anti-hyperglycemic activity.

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Conflict of interest

We declare that we have no conflict of interest.

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