International Journal of Pharmacological Research
ISSN: 2277-3312 (Online)
Journal DOI: https://doi.org/10.7439/ijpr **Research Article**

In vitro Antidiabetic activity of *Piper betel*

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Abstract

Leaves of *Piper betel* possess several bioactivities and are used in traditional medicinal systems. However, its antidiabetic activity has not been scientifically investigated so far. The aim of this study, therefore, was to investigate the antidiabetic activity of Piper betel. An aqueous extract of the leaves was prepared and subjected to various in-vitro anti diabetic assays such as, In vitro Alpha Amylase Inhibition Assay, Yeast Glucose Uptake Assay, Alpha Glucosidase Enzyme Inhibition Assay, Haemoglobin Glucose Inhibition Assay. The aqueous extract proved to possess significant anti-diabetic activity All assays showed a maximum inhibition of either the enzyme or uptake of glucose into the cells. Thus, concluding that leaves of Piper betel possess significant antidiabetic activity.

Keywords: Antidiabetic, α-Amylase Inhibition, α-Glucosidase Inhibition, Haemoglobin Glucose Inhibition Assay, Piper betel, Traditional medicine, Yeast Glucose uptake.

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***Article History:**
Received: 02/03/2020
Revised: 26/03/2020
Accepted: 28/03/2020
DOI: https://doi.org/10.7439/ijpr.v10i3.5382

QR Code

How to cite: Ravichandran R, Nivedha H, Sumaya R, Kumar P, Lashmansamy A and Krishnaraj. *In vitro* Antidiabetic activity of *Piper betel*. *International Journal of Pharmacological Research* 2020; 10(03): e5382. Doi: 10.7439/ijpr.v10i3.5382 Available from: https://ssjournals.com/index.php/ijpr/article/view/5382

1. Introduction

Diabetes has caused a major burden to the health sector in the developing countries and has shown an increasing trend among the urban population.[1] The increase in health loss from diabetes since 1990 in India is the highest among major non-communicable diseases, with this increase observed in every state of the country [2]. Type-2 diabetes results when the body does not make enough insulin, or the body cannot use the insulin it produces. Type-2 diabetes is the leading cause of premature deaths. Improperly managed, it can lead to several health issues, including heart diseases, stroke, kidney disease, blindness, nerve damage, leg and foot amputations, and death [3].

The management of diabetes is a global health care problem until now and successful treatment is not yet discovered. Currently, there are nine classes of orally available pharmacological agents to treat Type 2 diabetes sulfonylureas, meglitinides, metformin, thiazolidinediones, alpha glucosidase inhibitors, dipeptidyl peptidase inhibitors, bile acid sequestrants, dopamine agonists, and sodium-glucose transport protein inhibitors [4]. But these are observed to produce serious adverse side effects such as liver problems, lactic acidosis and diarrhoea. We have long history Ayurvedic medicines which uses plants for treating many diseases. Plants especially have shown to possess significant antidiabetic activity and could become a source of a new range of side effect free effective medication.

Piper betel is one such plant which possesses many bioactive compounds which are responsible for its antioxidant, antibacterial, antidermatophytic, antitumour, gastroprotective, anti-inflammatory activity and much more [5].

2. Materials and Methods

2.1. Extraction:

Healthy leaves of Piper betel were taken during the month of January in and around Chennai, Tamil Nadu, India. They were shade dried for five days and ground to a coarse

powder. 50g of powdered leaf and flower were taken and extracted using 500ml of water for about 20 min at 100°C. The extract was then filtered using a Whatman no 1 filter paper and dried under reduced pressure to make a concentrated paste. The concentrated extract was and stored in sterile microfuge tubes at -4°C until further use.

2.2. In Vitro Alpha Amylase Inhibition Assay:

This assay was conducted in two methods, a well assay and by the Dinitrosalicylic acid method. The well assay evaluated the activity of the extract qualitatively and the latter, quantitatively.

2.2.1. Well Assay:

A solid medium was prepared by combining 2g of agar and 0.5g of starch. The medium was sterilized and poured onto petri plates and allowed to solidify. Once solidified, five wells were created and 20µl of 20-unit alpha amylase enzyme was added to all wells. Keeping one well as control in each plate the sample extract was added in concentration of 20, 40, 60, 80 and 100 µl. The plates were undisturbed for 3hrs and the plates were flooded with grams iodine solution. The diameters of the various zones of inhibition are mentioned in Table 1.

2.2.2. Dinitrosalicyclic Acid Assay:

The assay was performed according to [6]. 200µl (1%w/w) starch solution was added to 200µl of PBS buffer (pH 7.5mM), 20, 40, 60, 80 and 100µl sample extract were taken in various test tubes and 20µl of 20 units alpha amylase enzyme was added. 600µl of deionized water and 400µl of DNSA reagent was added to the test tube and labelled as blank and for control the sample extract was substituted with 100µl water and the test tubes were incubated at 37°C for 15mins. After incubation 400µl of DNSA reagent was added and all the test tubes and then placed in a boiling water bath (100°C) for 5 mins, then each test tube was made to 15 ml by adding deionized water, then the absorbance was read by means of a UV-vis-spectrophotometer at 540nm.

2.3. Yeast Glucose Uptake Assay:

The assay was performed according to [7]. 1g of active dry yeast was taken in a centrifuge tube and distilled water was added to it and the cells were centrifuged at 300g for 5 min. after centrifugation the supernatant was removed, and this step was repeated until the supernatant was clear. Then a 10% yeast solution was created. 1% glucose solution was prepared. To each test tube 1ml of 10% yeast solution, 100µl 1% glucose solution and 100µl of sample extract at various concentrations (20, 40, 60, 80 and 100µl) was added and for the control 1ml yeast solution, 100µl 1% glucose solution and 100µl deionized water was added, all the test tube were incubated at 37°C for 1hr.

After incubation the contents of the test tube were centrifuged at 2000g for 5 mins. 500µl of the supernatant from each sample was taken in a test tube and 400µl of DNSA reagent was added and kept in boiling water at 100°C for 5 mins, for blank 500µl of deionized water was substituted for the sample extract. Then the test tubes were made up to 15ml by addition of deionized water. The absorbance of each sample was noted in a UV-vis-spectrophotometer at 540nm.

2.4. Alpha Glucosidase Inhibition Assay:

The assay was performed according to [6]. To each test tube, 300µl of PBS buffer solution, 50units of 50µl of α glucosidase enzyme and 100µl of sample extract at concentration of 20, 40, 60, 80 and 100µl were added incubated at 37°C for 15 mins. After incubation to each test tubes 3ml of 50mM NaOH solution was added. The blank was prepared by adding 500µl of PBS buffer and 3ml 50mM NaOH solution and the absorbance was read by means of a UV-vis-spectrophotometer at 410nm.

2.5. Evaluation of haemoglobin glycosylation

2.5.1. Preparation of haemoglobin:

The assay was performed according to [8]. The blood was collected from a healthy human volunteer and transferred into a sterile tube containing EDTA. Hemolysate was prepared by preparing a hyposaline solution. The collected blood was washed thrice with 0.14M NaCl solution and one volume of red blood cells suspension was lysed with two volumes of 0.01M phosphate buffer, pH 7.4 and 0.5 volume of CCl4. The haemolysate was then freed from the debris by centrifugation at 2300 rpm for 15 min at room temperature. The haemoglobin rich fraction was separated and dispensed into sample bottle for storage and refrigerated until required for use

2.5.2. Estimation of haemoglobin glycosylation:

To 1 mL of haemoglobin solution, 5µL of gentamycin and 25 µL of the plant extracts (30µg/mL) were added. The reaction was started by the addition of 1 mL of 2% glucose in 0.01M phosphate buffer (pH 7.4) and incubated in the dark at room temperature. The concentrations of glycated haemoglobin at the incubation period of 0, 24 and 72 hrs were estimated spectrophotometrically at 443nm.

Table 1: Diameter of clearance zone for alpha amylase enzyme inhibition assay

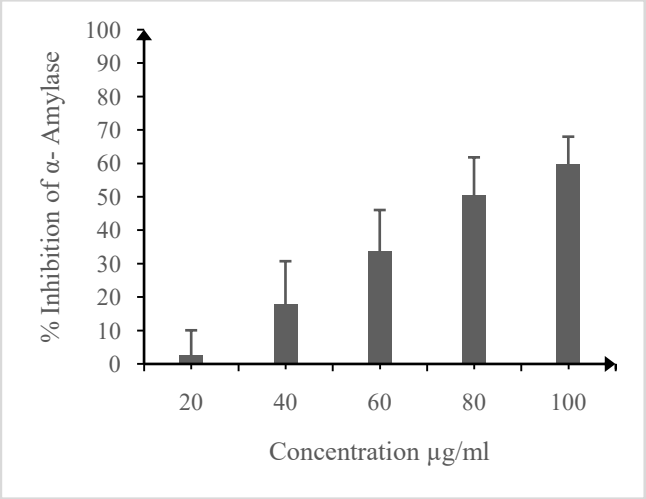
Concentration of Extract (µg/ml)	Diameter of Clearance Zone (mm)
Control	20
25	14
50	11
75	12
100	8

3. Result and Discussion

3.1. *In Vitro* Alpha Amylase Inhibition Assay:

Alpha amylase is a key enzyme excreted by the pancreas whose main function is conversion of starch into absorbable glucose. But this enzyme may pose a threat to people suffering from diabetes, where it will cause an increase in blood sugar. The well assay showed a marked reduction in the clearance zone (Table 1) when compared to the control; this indicates that the extract has significant inhibitory effect of the alpha amylase enzyme. Similarly, the DNSA method also shows a proportionate increase in the inhibition of the a- amylase enzyme by the plant extract as show in Figure 1. This might act as an agent to prevent conversion of starch into glucose thus effectively not allowing blood sugar to increase.

Figure 1: The inhibitory effect of variable doses of aqueous extract of Piper betel. via DNSA assay



Values are expressed as mean ± S.D. The % inhibition was calculated based on control & standard OD at 540 nm. The % inhibition of α- amylase proportionately increases with increase in concentration of extract

3.2. Yeast Glucose Uptake Assay:

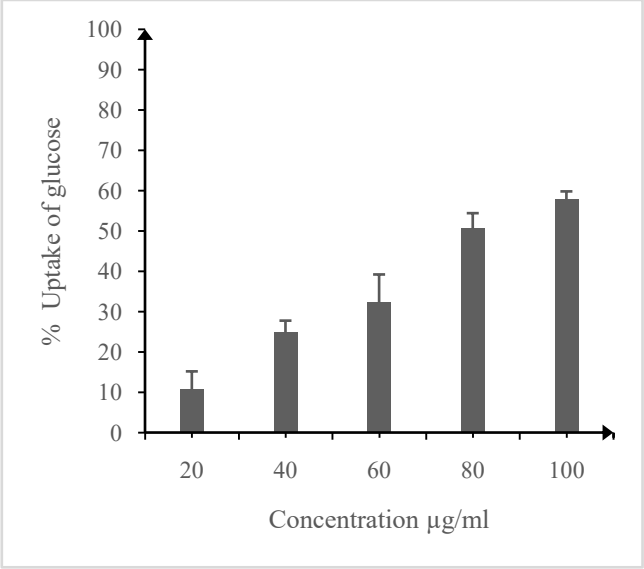
We know the danger it poses when live cells are incapable of up taking glucose and how it affects blood sugar levels in diabetic patients. From Figure: 2 it is evident that the % uptake of glucose by ,live yeast cells markedly increased with increase in concentration of plant extract, this ensures that it has significant antidiabetic activity and proposes a vague mechanism of action for the activity of the plant extract with regards to uptake of glucose by live cells.

3.3. Alpha Glucosidase Inhibition Assay:

Like the alpha amylase enzyme assays, alpha glucosidase also showed a similarly proportionate decrease in activity with the increase in concentration of plant extract, this further supports the previous assays and is shown graphically in Figure 3.

Glucosidase enzymes catalyze hydrolysis of starch to simple sugars. In humans, these enzymes aid digestion of dietary carbohydrates and starches to produce glucose for intestinal absorption, which in turn, leads to increase in blood glucose levels.

Figure 2: The % uptake of glucose by yeast cells in the presence of variable doses of aqueous extract of Piper betel. via yeast glucose uptake assay



Values are expressed as mean ± S.D. The % inhibition was calculated based on control & standard OD at 540 nm. The % uptake of glucose increases with increase in concentration of extract

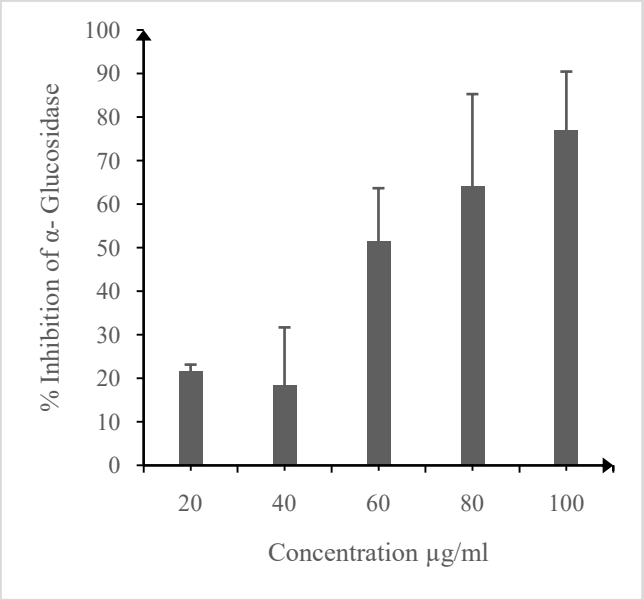
3.4. Evaluation of haemoglobin glycosylation:

From the result shown in Figure 4, we can clearly estimate that the % glycosylation of haemoglobin decreases with increase in concentration of plant extract. This indicates the hypoglycaemic activity of the extract as inhibition of glycosylation of haemoglobin prevents production of glycated end products.

4. Conclusion

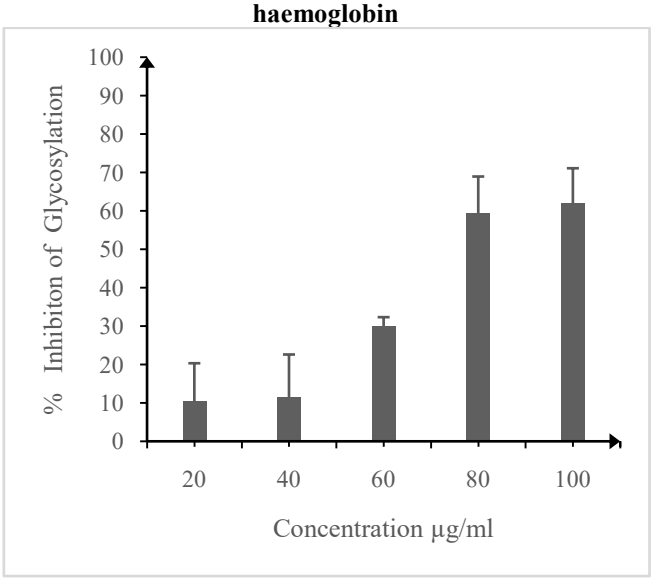
The need for safe, potent and cost-effective drugs for diabetes is a major concern for many families around the world. And from various previously published literature we know that Piper betel possesses various pharmacological properties such as anti-malarial activity, antibacterial activity, antifungal study, insecticidal activities, antioxidant activity, gastro protective activity, antinociceptive activity, cytotoxic activity and anti-platelet [9]. Its antidiabetic activity has yet to proven. This study shows the potent antidiabetic activity of the leaves of the Piper betel by means of various in-vitro antidiabetic assays.

Figure 3: The inhibitory effect of variable doses of aqueous extract of Piper betel. via α- glucosidase inhibition assay



Values are expressed as mean ± S.D. The % inhibition was calculated based on control & standard OD at 540 nm. The % inhibition of α- amylase proportionately increases with increase in concentration of extract as evident from the trend of the graph.

Figure 4: The inhibitory effect of variable doses of aqueous extract of Piper betel on the glycosylation of haemoglobin



Values are expressed as mean ± S.D. The % inhibition was calculated based on control OD at 443 nm. The % inhibition of glycosylation of heamoglonin by aqueous extract of Piper betel, shows an increasing trend signifying it antidiabetic activity.research into the specific active phytocompounds responsible for the activity must be identified to one day make this a potent, easily available and a cost-effective drug for treatment of diabetes.

Acknowledgement

We would also like to thank St. Josephs College of Engineering, OMR, Chennai, India, Especially the Department of Biotechnology and Head of Department Dr. Sreekumar for their continued support and encouragement. We would also like to thank the staffs of APEX Biotech Training and Research Institute for their guidance and support in completion of this project.

Conflicts of interest

No conflict of interest to be declared by any of the authors

Funding

The project was self-funded by the authors and no external funding was provided for the completion of this project.

Compliance with Ethical Standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Reference

[1]. Wickramaratne, M. N., Punchihewa, J. C., & Wickramaratne, D. B. *In-vitro* alpha amylase inhibitory activity of the leaf extracts of *Adenanthera pavonina*. *BMC Complementary and Alternative Medicine*, 2016; 16(1), 466. https://doi.org/10.1186/s12906-016-1452-y

[2]. Radhika, K., Kumaravel, B., Thamizhiniyan, V., & Subramanian, S. Biochemical evaluation of antidiabetic activity of Piper betel leaves extract in alloxan-induced diabetic rats. *Asian Journal of Research in Chemistry*, 2013; 6(1): 76-82.

[3]. Marín-Peñalver, J. J., Martín-Timón, I., Sevillano-Collantes, C., & Del Cañizo-Gómez, F. J. Update on the treatment of type 2 diabetes mellitus. *World Journal of Diabetes*, 2016; 7(17): 354–395. https://doi.org/10.4239/wjd.v7.i17.354

[4]. Feingold KR. Oral and Injectable (Non-insulin) Pharmacological Agents for Type 2 Diabetes. [Updated 2019 Jul 8]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279141

[5]. Dwivedi, V., & Tripathi, S. Review study on potential activity of *Piper betle*. *J Pharmacogn Phytochem,* 2014; 3(4): 93-98.

[6]. Nair, S. S., Kavrekar, V., & Mishra, A. In vitro studies on alpha amylase and alpha glucosidase inhibitory

activities of selected plant extracts. *European Journal of Experimental Biology*, 2013; 3(1): 128-132.

[7]. Santhiya, Nagaraj & Suriyamoorthy, Priyanga & Subrhamanian, Hemmalakshmi & Devaki, Kanakasabapathi. Phytochemical analysis, Anti inflammatory activity, *In vitro* antidiabetic activity and GC-MS profile of *Erythrina variegata* L. bark. *Journal of Applied Pharmaceutical Science*. 2016; 6: 147-155. 10.7324/JAPS.2016.60722.

[8]. Adisa, R., Choudhary, M., Adewoye, E., & Olorunsogo, O. Hypoglycaemic and biochemical properties of *Cnestis ferruginea*. *African Journal of Traditional, Complementary and Alternative Medicines*, 2010; 7(3).

[9]. Rekha V.P.B., Kollipara M., Gupta S.B.R.S.S., Bharath Y. and Pulicherla K.K. A review on *Piper betel* L.: nature's promising medicinal reservoir. *American Journal of Ethnomedicine*. 2014; 1(5): 276-289

[10]. Arambewela, L. S. R., Arawwawala, L. D. A. M., & Ratnasooriya, W. D. Antidiabetic activities of aqueous and ethanolic extracts of *Piper betle* leaves in rats. *Journal of Ethnopharmacology*, 102(2): 239-245.

[11]. Chakraborty, D., & Shah, B. Antimicrobial, antioxidative and antihemolytic activity of Piper betel leaf extracts. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2011; 3(3): 192-199.

[12]. Vikash, C., Shalini, T., Verma, N. K., Singh, D. P., Chaudhary, S. K., & Asha, R. Piper betel Phytochemistry, traditional use & pharmacological activity a review. *International Journal of Pharmaceutical Research and Development* 2012; 4(4): 216-223.