

α -Glucosidase and α -amylase inhibitory activities of *Pithecellobium dulce* bark and leaves

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Abstract

In present study the bark and leaves of *Pithecellobium dulce* were evaluated for α -amylase and α -glucosidases inhibition *in vitro*, compared with acarbose. acetone extracts of bark and leaves showed more sucrase inhibition ($IC_{50} = 1.29 \pm 0.32$ and 1.43 ± 0.84 mg/mL, respectively) than maltase ($IC_{50} = 1.49 \pm 0.18$ and 2.07 ± 0.45 mg/mL, respectively), while methanol extract of bark and leaves showed more sucrase inhibition ($IC_{50} = 2.35 \pm 0.72$ and 2.21 ± 0.28 mg/mL, respectively) than maltase ($IC_{50} = 2.03 \pm 0.91$ and 2.46 ± 0.44 mg/mL, respectively). Amylase inhibitory activity of acetone extract of bark and leaves was found to be 74.78% and 62.43% while methanol extract had 72.23% and 68.32% inhibition at 4 mg/mL, respectively. Acarbose (standard) showed more sucrase inhibition ($IC_{50} = 8.45 \pm 0.21$ μ g/mL) than maltase ($IC_{50} = 27.62 \pm 1.34$ μ g/mL) and had 83.40% α -amylase inhibition at 50 μ g/mL. Acetone extract showed significant activity against α -glucosidases for sucrase than maltase enzyme.

Keywords: *Pithecellobium dulce*; α -glucosidase; α -amylase; diabetes

Introduction

The prevalence and morbidity associated with type 2 diabetes mellitus continues to increase throughout the world. The secondary complications in diabetes mostly from micro and macro vascular changes (Engelgau, et al., 2004). Several studies on the treatment of Type 2 diabetes suggest that improved glycemic control reduces micro vascular risks (Gaster and Hirsch, 1998; Ohkubo, et al., 1995; Vijan, et al., 1997). Glucosidase inhibitors are widely studied and isolated from different sources such as plants (Yoshikawa, et al., 1098) and microbes (Kameda, et al., 1984). In 1970s, it was realized that inhibition of all or some of the intestinal disaccharidases and pancreatic α -amylase by inhibitors could regulate the absorption of carbohydrate and these inhibitors could be used therapeutically in the oral treatment

of the non insulin-dependent diabetes mellitus (Type II diabetes). Acarbose, is a potent pig intestinal sucrase inhibitor with an IC_{50} value of 0.5 mM (Schmidt, et al., 1997). However use of acarbose leads to intestinal disturbances in many patients and there exists a need for development of better and more tolerable α -glucosidase inhibitors.

Pithecellobium dulce (PD) belonging to the Leguminosae family is found throughout India. It is commonly known as 'Vilayati Chinch' or 'Chichbul' and has been documented in the ancient Ayurvedic writings to possess anti diabetic effect. *P. dulce* has been reported to possess multiple activities such as astringent in dysentery, febrifuge, abortifacient, antidiabetic, anticonvulsant, antiulcer and larvicide. It is also useful in dermatitis, eye inflammation, indigestion, intestinal disorder, ear ache, leprosy and tooth ache. The leaves can be applied as plasters for pain and venereal sores (Sugumaran, et al., 2008). The plant is used for hundreds of years in *Ayurvedic* medicine with no reported toxicity.

Phytochemical investigation of bark and leaves had revealed the presence of β -sitos-terol, saponin glycosides, oleanolic and echinocystic acids as sapogenins, echinocystic acid, bisdesmoside, dulcin, triterpenoids, flavanoids, saccharrides, long chain aliphatic hydrocarbons, and tannins (Nigam, et al., 1997; Yoshikawa, et al., 1997; Khatri and Nasir, 1994; Hosmani, 1995; Banarjee, 2005; Saxena and Singal, 1998; Saxena and Singhal, 1999; Zapesoch-naya, et al., 1980; Nigam and Mitra, 1968). Several reports on the phytochemical analysis of *P. dulce* have been recently published but very little information is available about biological activity against α -glucosidase and α -amylase enzymes. In the present study the α -glucosidase and α -amylase inhibitory activity of the *P. dulce* has been evaluated by *in vitro* assays.

Methods and materials

Plant material and chemicals

P. dulce wood bark and leaves were collected from Mumbai, Maharashtra, India and verified by the Dr. G. Iyer, Ruia College, Mumbai, India. Porcine pancreatic α -amylase was procured from Sigma Aldrich Inc., (St Louis, MO). Dinitrosalicylic acid (DNS) and Tris base was obtained from Himedia Laboratory, Mumbai. A glucose estimation kit was procured from Accurex Biomedical Pvt. Ltd., Thane, Mumbai. Starch, Maltose and Sucrose were purchased from Sisco Research Laboratories, (Mumbai, India). Acarbose was obtained from Bayer Medical Co. (Germany). All other chemicals and solvents used are of analytical grade.

Extract preparation

The shade dried plant material chopped into small pieces and pulverized into a fine powder. The plant material (1 kg) was extracted by Soxhlet apparatus for 24 h using methanol as solvent. Same process was used for 70% acetone (aqueous acetone). For each new solvent plant material was air dried. The solvents were concentrated under vacuum.

α -Amylase inhibition assay

α -Amylase activity was performed according to the chromogenic non-pre-incubation method described by Ali *et al.* (Madar, 1989; Kim, et al., 2005; Rhabasa-Lhoret and Chai-

sson; 2004). Briefly 120 μ L of PD (20 mg/mL in DMSO) was mixed with 480 μ L of distilled water and 1.2 mL of 0.5% w/v soluble potato starch in 20 mM phosphate buffer pH 6.9 containing 6.7 mM sodium chloride in a test tube. The reaction was initiated (0 min) by addition of 600 μ L of enzyme solution (4 units/mL in distilled water), 600 μ L of the mixture was withdrawn after 3 min into separate test tubes containing 300 μ L DNSA color reagent (1 g of 3, 5-dinitrosalicylic acid, 30g of sodium potassium tartarate and 20 mL of 2 N sodium hydroxide to a final volume of 100 mL in distilled water) and transferred to a hot water bath maintained at 85-90 °C for 15 min. Afterwards the reaction mixture in each tube was diluted with 2.7 mL distilled water and the absorbance measured at 540 nm (Shimadzu UV-160 spectrophotometer, Kyoto, Japan). Test incubations were also prepared for 2.5, 5 10 and 20 mg/mL of PD to study the concentration dependant inhibition. For each concentration, blank incubations were prepared by replacing the enzyme solution with 600 μ L in distilled water at the start of the reaction. Control incubations, representing 100% enzyme activity were conducted in a similar manner, replacing PD with 120 μ L DMSO. All the tests were run in triplicate. Net absorbance (A) due to the maltose generated was calculated as:

$$A_{540\text{nm}}\text{PD} = A_{540\text{nm}}\text{Test} - A_{540\text{nm}}\text{Blank}$$

From the value obtained the percentage (w/v) of maltose generated was calculated from the equation obtained from the maltose standard calibration curve (0-0.1% w/v maltose). The level of inhibition (%) was calculated as:

$$100\text{-}\% \text{ reaction (at } t=3 \text{ min)}$$

$$\text{Where, } \% \text{ reaction} = \text{Mean maltose in sample} \times 100 / \text{Mean maltose in control}$$

Isolation of α -glucosidase from rat small intestine

The small intestine of male Wistar rats (180 g) was collected after sacrificing the animal under anesthesia. The intestine was thoroughly cleaned with saline and epithelial layer (mucosal tissue was collected by scraping the luminal surface firmly with a spatula. The mucosal scraping were homogenized in phosphate buffered saline (PBS) pH 7.4 containing 1 % triton x 10, and then centrifuged at 12000 rpm for 15 min. The supernatant fraction contained rat small intestinal α -glucosidase. Butanol was added to the supernatant fraction 1:1 proportion and centrifuged at 15000 rpm for 15 min. The aqueous layer was dialyzed overnight against the same buffer. After dialysis, the concentrated enzyme was used as crude α -glucosidase enzyme in the study to observe inhibition by different extracts of *P. dulce*. All the preparations were carried out at 4 °C. The protein content of enzyme preparation was estimated by Lowry method.

α -glucosidase inhibition assay

The effect of extracts of wood bark and leaves of *P. dulce* on rat intestinal α -glucosidase activity was assayed according to the method of Matsui *et al.*, with slight modifications (Matsui, et al., 1996). Briefly 0.5 mg protein equivalent of crude α -glucosidase enzyme was incubated with different concentrations of PD for 5 min before initiating the reaction with substrates maltose (6 mM) and sucrose (45 mM), in a final reaction mixture of 1 mL of 0.1 M

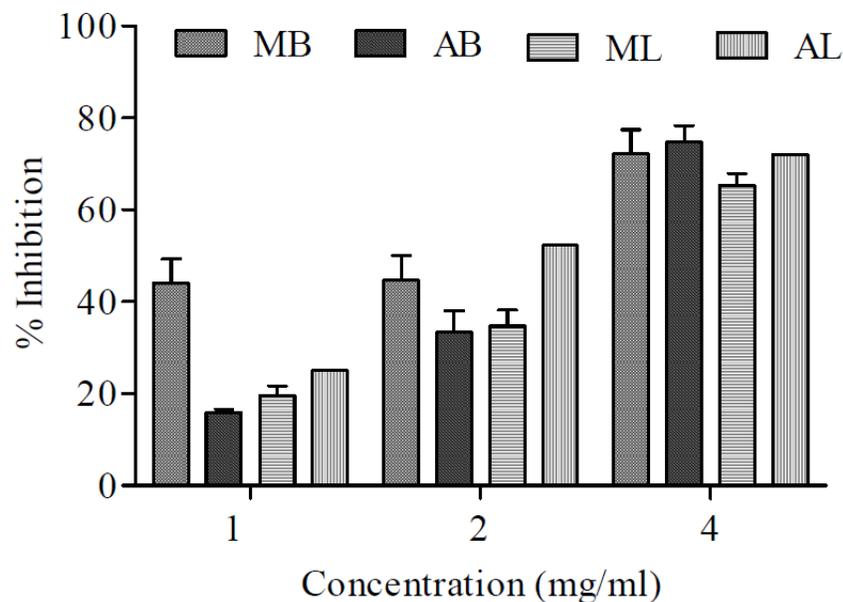


Figure 1. α -amylase inhibitory activity of *P. dulce* wood bark and leaf extracts

phosphate buffer pH 7.2. The reaction mixture was incubated for 20 and 30 min at 37 °C for substrates maltose and sucrose, respectively. The reaction was stopped by adding 1.0 mL of Tris base and α -glucosidase activity was determined by monitoring the glucose released from maltose and sucrose by glucose oxidase method. Enzyme inhibition data were expressed as IC_{50} value (the concentration of PD required to inhibit 50% of α -glucosidase activity).

Statistical analysis

All data are expressed as mean \pm S. D. for six experiments. Linear regression analysis was used to calculate IC_{50} values.

Results

α -Amylase inhibitory assay

Plant derived compounds continue to provide valuable therapeutic agents, in both modern medicine and in traditional system. Methanol and 70% acetone extract of *P. dulce* were studied for their inhibitory effect on α -amylase enzyme involved in starch hydrolysis which is responsible for the increase in postprandial glucose levels in diabetes mellitus. The maximum inhibition of acetone extract of bark was 74.78% and of leaves was 62.42% at the concentration of 4 mg/mL, while the acarbose showed 83.40% inhibition of α -amylase enzyme. The figures 1 represent the α -amylase inhibitory activity of acetone and methanol extracts of bark and leaves respectively.

α -Glucosidase inhibitory assay

The *in vitro* α -glucosidase inhibitory studies confirmed that both the acetone and methanol extracts had α -glucosidase inhibitory activity. A dose dependant inhibition of α -gl-

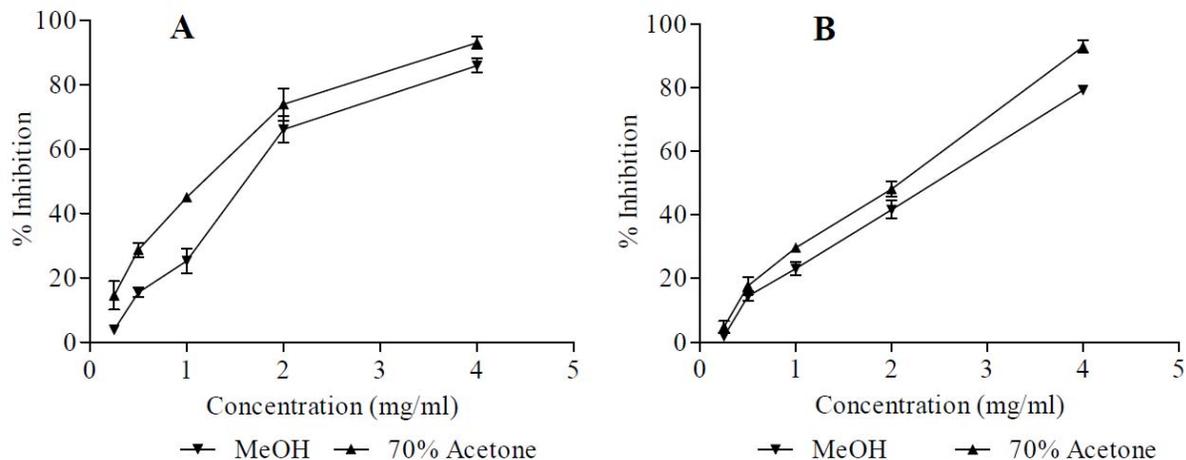


Figure 2. Inhibitory activity of *P. dulce* on maltase enzyme. A, Wood bark extract; B, Leaf extract.

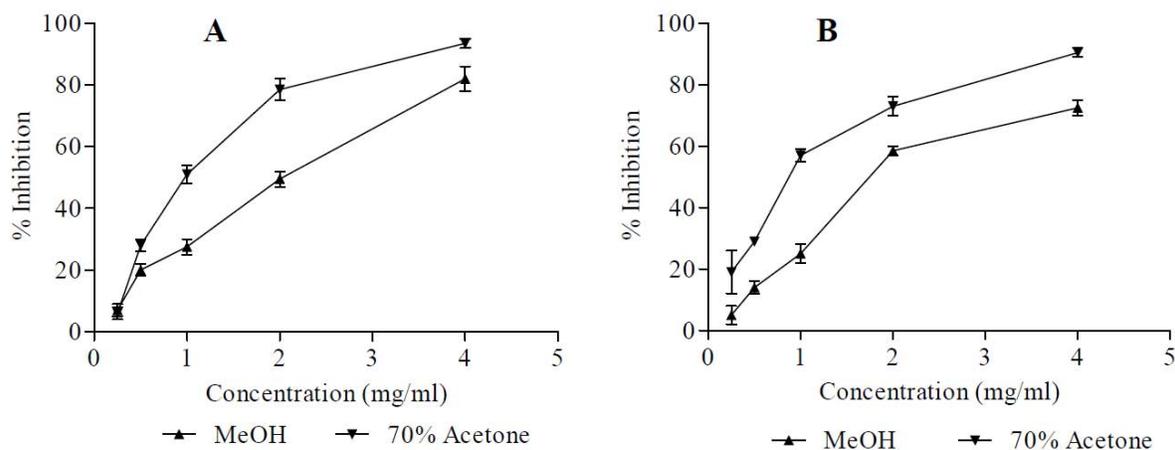


Figure 3. Inhibitory activity of *P. dulce* on sucrase enzyme. A, Wood bark extract; B, Leaf extract.

ucosidase enzymes such as sucrase and maltase was observed by acetone and methanol extracts of *P. dulce* bark and leaves extracts (Figure 2 and 3). The IC_{50} values of acetone extracts of bark and leaves for sucrase was found to be 1.29 ± 0.32 and 1.43 ± 0.84 mg/mL while methanol extracts 2.35 ± 0.72 and 2.21 ± 0.28 mg/mL respectively. The IC_{50} values for maltase inhibitory activity was found to be 1.49 ± 0.18 and 2.07 ± 0.45 mg/mL for acetone extract, and 2.03 ± 0.91 and 2.46 ± 0.44 mg/mL for methanol extracts of bark and leaves of *P. dulce* respectively. The standard drug acarbose showed more sucrase ($IC_{50} = 8.45 \pm 0.21$ μ g/mL) than maltase ($IC_{50} = 27.62 \pm 1.34$ μ g/mL) inhibition.

Discussion

Natural α -amylase and α -glucosidase inhibitors from food-grade plant sources offer an attractive therapeutic approach to the treatment of post-prandial hyperglycemia by decreasing glucose release from starch and delaying carbohydrate absorption by inhibiting the activity of the carbohydrate hydrolyzing enzymes in the small intestine and may have poten-

tial for use in the treatment of diabetes mellitus and obesity. On the basis of the prevalence, the delay or inhibition of carbohydrate digestion would contribute to optimize a postprandial blood glucose level (Gallaher and Schneeman, 1986; Murai, et al., 2002; Chiasson, et al., 2002). There are many natural resources with the α -glucosidase inhibitory activity and some of them are more specific for sucrase inhibition rather than maltase inhibition. Inhibition of α -amylase, maltase and sucrase by a polyphenolic extract of green tea has been reported (Hara and Honda, 1990; Honda and Hara, 1993). Our present research suggest that the presence of polyphenolic compounds of *P. dulce* may have a potentially important role in managing diabetes via the inhibition of α -amylase and α -glucosidase enzyme activities. The α -glucosidase and α -amylase inhibitory activity of methanol and 70% acetone extract was confirmed in this study.

Nonetheless, it is important to mention here that α -amylase breaks down starch into disaccharides that are acted upon by isomaltases, especially α -glucosidase to release glucose. The presence of potent α -glucosidase inhibitory activity therefore, appears more important in controlling the release of glucose from disaccharides in the gut than α -amylase inhibition. However, moderate α -amylase inhibition with potent α -glucosidase inhibitory activity may offer better therapeutic strategy that could slowdown the availability of dietary carbohydrate substrate for glucose production in gut. Food-grade phenolic α -amylase inhibitors from dietary plant extracts are potentially safer, and therefore may be a preferred alternative for modulation of carbohydrate digestion and control of glycemic index of food products. Furthermore, the present results demonstrate that the methanol and acetone extract from *P. dulce* bark contained potent α -glucosidase, α -amylase inhibitors and were effective for suppressing post-prandial hyperglycemia.

In conclusion, *P. dulce* wood bark and leaves extract demonstrates good α -glucosidase and α -amylase inhibitory activity. Further attempts at isolation and purification of the active constituent from the extract are ongoing. From previous studies it has been found that leaf contains compounds like Insulin and bark contains catecol type of compounds. *P. dulce* extracts have the dual advantage of having α -glucosidase and pancreatic α -amylase inhibitor action hence could prove to be an effective treatment for diabetes mellitus.

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Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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