Scientific Research Publishing

Advances in Biological Chemistry, 2024, 14, 196-202
https://www.scirp.org/journal/abc
ISSN Online: 2162-2191
ISSN Print: 2162-2183

Hypoglycemic Activity of Compounds from *Pithecellobium dulce*

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How to cite this paper: Praylin, S.E. and Bakthasingh Lazarus, Y. (2024) Hypoglycemic Activity of Compounds from *Pithecellobium dulce*. *Advances in Biological Chemistry*, **14**, 196-202.
https://doi.org/10.4236/abc.2024.146015

Received: April 8, 2024
Accepted: December 15, 2024
Published: December 18, 2024

Abstract

Medicinal plants have been reported to play an important role in modulating glycemic responses and have preventive and therapeutic implications. The intestinal digestive enzymes play a vital role in the carbohydrate digestion. The present investigation evaluated the possible action of isolated compounds through which *pithecellobium dulce* fruit peel exerts its hypoglycemic effect using suitable *in vitro* techniques. The isolated compounds were subjected to inhibitory effect of non-enzymatic glycosylation of hemoglobin assay and enzymatic alpha-amylase inhibition assay using specific standard *in vitro* procedure. Non-enzymatic glycosylation of hemoglobin assay showed inhibitory activity of 73.7% and 53.9% at 1 mg/ml. The IC_{50} values of amylase inhibitory activity of compounds from *pithecellobium dulce* fruit peel was found to be 80.9% and 56.5% at 1 mg/ml. Results in two different compounds revealed that the compound 1 was found to be more potent than compound 2 at the concentrations 0.2 mg/dl to 1.0 mg/dl. The findings indicate *Pithecellobium dulce* fruit peel possess strong hypoglycemic effect and hence can be utilized as an adjunct in the management of diabetes mellitus.

Keywords

Pithecellobium dulce, *In Vitro*, Phytochemical, *α*-Amylase

1. Introduction

Diabetes is a chronic metabolic disorder in which homeostasis of the carbohydrate, protein and lipid metabolism are improperly regulated by the pancreatic hormone, insulin; resulting in an increased blood glucose level *i.e.,* hyperglycemia. The hyperglycemia is associated with the incidence and progression of micro vascular (diabetic retinopathy, loss of vision and nephropathy) and macro vascular

diseases (amputation and cardiovascular disease mortality) that are difficult to manage [1] [2]. The prevalence of diabetes is increasing annually and the number of diabetics is projected to rise above 300 million before 2025 [3]. Most prevalent form of diabetes is non-insulin dependent diabetes mellitus (NIDDM/type II) caused by impaired secretion of insulin resulting in high postprandial glucose levels. One important factor to result in a postprandial hyperglycemia is the fast uptake of glucose in the intestine by the action of glucosidases, a class of enzymes (α-amylase and α-glycosidase) that helps in the breakdown of complex carbohydrates (starch and oligosaccharides) into simple sugars such as maltose and glucose [4] [5]. The treatment of diabetes involves the decrease postprandial hyperglycemia by causing retardation in absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase. Currently a variety of therapeutic drugs are available for the management of type 2 diabetes; these agents include hypoglycemic agents such as acarbose, miglitol and voglibose that competitively and reversibly inhibit α-glucosidase enzyme from intestine as well as pancreas. However, these drugs are associated with gastrointestinal side effects such as abdominal pain, flatulence and diarrhea in the patients, which might be caused by excessive inhibition of pancreatic α-amylase resulting in fermentation of undigested carbohydrates in the colon by colonic flora [1] [6]. Therefore, a good strategy to manage postprandial hyperglycemia with lesser side effects is to identify the natural inhibitors from dietary sources, which has mild inhibitory effect against α-amylase and strong inhibitory activity against α-glucosidase [7].

India has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. *Pithecellobium dulce* (Roxb.) Benth. (Manila Tamarind) belongs to the Mimosaceae family, mostly grown in India for hedges, street trees and for ornament because of its handsome foliage and curious pods. It is locally called as "Jungal jalebi" and also known as "Vilayati babul" in Hindi and "Vilayati chinch" in Marathi. *Pithecellobium dulce* benth was used traditionally as antidiabetic plant. The presence of steroids, saponins, lipids, phospholipids, glycosides, glycolipids, polysaccharides has been reported in the plant. The bark contains 37% of tannins of catechol type. Quericitin, kaempferol, dulcitol and afzelin have been reported from the leaves [8] [9]. The insulin like principle has been reported in leaves of *Pithecellobium dulce* leaves. Traditionally, the tender leaf paste is mixed with the seeds powder of *Pithecellobium dulce* and is given orally in empty stomach to cure diabetes [10]. The ethyl acetate, methanolic and aqueous extracts of fruit peel of *Pithecellobium dulce* shows significant antioxidant and antibacterial potential [11].

2. Materials and Methods

2.1. Collection of Plant Material

The collected fruits were examined carefully and old, infected and damaged fruits were removed. Initially, the pods were separated the arils were isolated manually

from brown peel and black seed. The fruit peels were washed with tap water and then with distilled water to remove any debris or dust particles, healthy fruit peels were spread out and dried at room temperature for about 15 - 20 days and pulverized by a mechanical grinder and passed through a 40-mesh sieve to get a fine powder and stored in an airtight container [12].

2.2. Extraction of Plant Material

The air dried powdered fruit peel material of *Pithecellobium dulce* was successively extracted with pet ether (60˚ - 80˚) and methanol for 16 hours (thrice) using Sohxlet extractor. The methanol extract was concentrated by rotary vacuum evaporator and then dried.

2.3. Phytochemical Isolation of Compounds 1 & 2 from Fruit Peel Using Column Chromatography

Dried and ground fruit peel was extracted with methanol for 16 hrs in a Soxhlet apparatus. The solvents were concentrated in rotary evaporator at reduced pressure below 40˚C. The crude extract was used for isolation. The crude extract was added to 40 grams of silica gel (60 - 120 mesh) to make admixture. 2.4 diameter columns were packed with the admixture mixed with hexane. The column was eluted with increasing solvent polarity from hexane to ethyl acetate. Column of 2.4 cm, column bed height 20 cm was used for the isolation procedure. Hexane (100% - broad fraction 1), hexane: ethyl acetate (90:10 - broad fraction-2)—**compound 1**, hexane: ethyl acetate (80:20 - broad fraction3), hexane: ethyl acetate (70:30 - broad fraction 4), hexane: ethylacetate (60:40 - broad fraction 5)—**compound 2**, hexane: ethyl acetate (50:50 - broad fraction 6), hexane: ethyl acetate (40:60 - broad fraction 7), hexane: ethyl acetate (30:70 - broad fraction 8), ethyl acetate (100% - broad band 9). Fractions with similar spots were pooled together and concentrated at reduced pressure and temperature. Compounds 1 and 2 answered Molisch test for sugar and glycoside test.

2.4. *In Vitro* Antidiabetic Study

2.4.1. Preparation of Haemoglobin

The blood was collected from a healthy human volunteer and transferred into a blood bottle containing an anticoagulant. Haemolysate was prepared based on the principle of hypotonic lysis [13]. The red blood collected was washed thrice with 0.14 M NaCl solution and one volume of red blood cells suspension was lysed with two volumes of 0.01 M phosphate buffer, pH 7.4 and 0.5 volume of CCl_4. The haemolysate was then freed from the debris by centrifugation at 2300 rpm for 15 min at room temperature. The haemoglobin rich fraction *i.e.*, the upper layer was separated and dispensed into sample bottle for storage and refrigerated until required for use [14].

2.4.2. *In Vitro* Non-Enzymatic Glycosylation of Haemoglobin Assay

Antidiabetic activity of compounds isolated from leaf, fruit and fruit peel of

Pithecellobium dulce were investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520 nm. Glucose (2%), haemoglobin (0.06%) and gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. 0.2 to 1 mg/ml of compounds was added to above mixture. Mixture was incubated in a dark room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetric ally at 520nm. Alpha-Tocopherol (Trolax) was used as a standard drug for assay % inhibition was calculated as the earlier methods [15].

2.4.3. *In Vitro* Enzymatic Alpha-Amylase Inhibition Assay

A starch solution (0.1% w/v) was obtained by stirring 0.1 g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 dinitro salicylic acid solution 96 mM. Both control and plant compound were added with starch solution and left to react with alpha-amylase solution under alkaline conditions at 25˚C. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3-amino-5-nitro salicylic acid. This reaction is detectable at 540 nm [13].

2.4.4. Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration of the plant compound required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the compounds. Percentage inhibition (I%) was calculated by

$$I\% = \left(Ac - As\right)/Ac \times 100 .$$

Ac is the absorbance of the control;

As is the absorbance of the sample.

2.5. Discussion

Diabetes mellitus is a metabolic disorder with increasing incidence throughout the world. Insulin is a key player in the control of glucose homeostasis. Lack of insulin affects carbohydrate, fat and protein metabolism [16]. Management of diabetes without side effects are still challenge to the medical community. It was proposed that inhibition of the activity of such alpha-amylase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose; as a result there is a reduction of postprandial blood glucose level. *In vitro* refers to the technique of performing a given procedure in a controlled environment outside the living organism. The purpose of *in vitro* testing is to demonstrate the hypoglycemic activity of compounds isolated from the fruit peel of *pithecellobium dulce*. The report was tabulated in (**Table 1** and **Table 2**). The present research has been carried out to isolate the new compounds present in the fruit peel of *Pithecellobium dulce* and their effect in inhibiting glycosylation of haemoglobin and alpha-amylase. At the concentration of 0.2 ml, compound 1 &

compound 2 showed a percentage inhibition of 26.1% and 7.2% for non-enzymatic glycosylation of haemoglobin assay. In the case of 1.0 ml concentration inhibition level was 73.7% & 53.9% (Table 1) (Figure 1) for non-enzymatic glycosylation of haemoglobin assay. The compounds isolated from *pithecellobium dulce* revealed a significant inhibitory enzyme action. The percentage inhibition at 0.2 - 1.0 ml concentrations of *Pithecellobium dulce* compound showed a dose dependent increase in percentage inhibition. The percentage inhibition for compound 1 and compound 2 varied from 11.8% to 80.9% and 7.1% - 56.5% from the highest concentration to the lowest concentration for alpha-amylase inhibitory activity. (Table 2) (Figure 2).

Table 1. *In vitro* non-enzymatic glycosylation of haemoglobin assay.

S.No	Concentration of sample (mg/ml)	% Inhibition of Compound 1	% Inhibition of Compound 2
1	0.2	26.1	7.2
2	0.4	50.4	16.8
3	0.6	61.3	32.4
4	0.8	68.0	44.1
5	1.0	73.7	53.9

Table 2. *In vitro* antidiabetic activity of alpha-amylase method.

S.No	Concentration of sample (mg/ml)	% Inhibition of Compound 1	% Inhibition of Compound 2
1	0.2	11.8	7.1
2	0.4	20.4	20.8
3	0.6	42.5	35.3
4	0.8	77.2	47.1
5	1.0	80.9	56.5

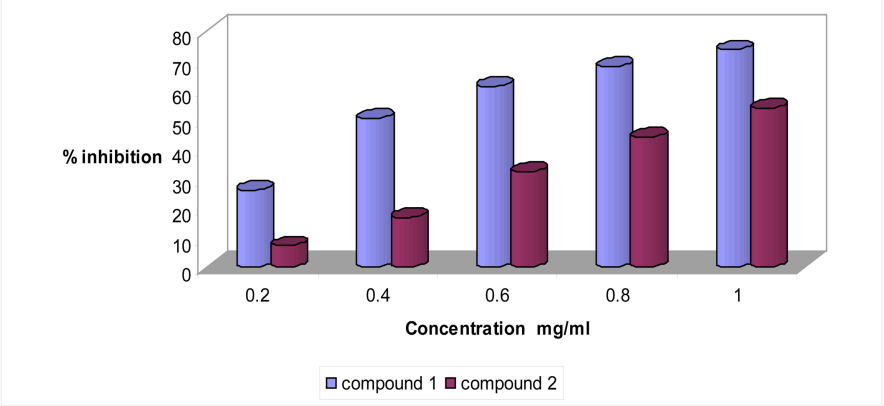

Figure 1. *In vitro* non-enzymatic glycosylation of haemoglobin assay.

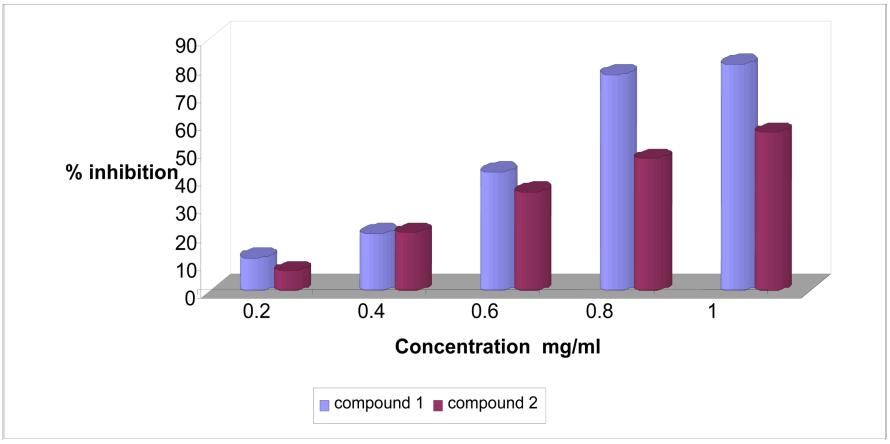

Figure 2. *In vitro* antidiabetic activity of alpha-amylase method.

The present finding reveals that the compound 1 efficiently inhibits both alpha amylase and alpha-glucosidase enzymes in a dose dependent manner compared to compound 2. Manikandan *et al.* (2013) [17] investigated the phytochemical bioactive compounds of the methanol extract of *Psidium guajava leaves,* its *in vitro* anti-diabetic activity. It was proposed by Rhabaso and Chiasson, (2004) [16] that inhibition of the activity of alpha-amylase and alpha-glucosidase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as result the reduction of postprandial blood glucose level elevation. The assay results suggested that the presence of bioactive compounds, could be responsible for the versatile medicinal properties of this plant, including diabetes. The antidiabetic action of compounds isolated from *Pithecellobium dulce* fruit peel can also be attributed due to the presence of polyphenolic compounds in *Pithecellobium dulce* may have a potentially important role in managing diabetes via the inhibition of α-amylase and non-enzymatic glycosylation of haemoglobin. In this present study, we evaluated *in vitro non*-enzymatic glycosylation of haemoglobin and alpha amylase. It was suggested that further studies are required to elucidate the mechanism of antidiabetic potential.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Bhat, M., Zinjarde, S.S., Bhargava, S.Y., Kumar, A.R. and Joshi, B.N. (2011) Antidiabetic Indian Plants: A Good Source of Potent Amylase Inhibitors. *Evidence-Based Complementary and Alternative Medicine*, **2011**, Article ID: 810207. https://doi.org/10.1093/ecam/nen040

[2] Wongsa, P., Chaiwarit, J. and Zamaludien, A. (2012) *In Vitro* Screening of Phenolic Compounds, Potential Inhibition against *α*-Amylase and *α*-Glucosidase of Culinary Herbs in Thailand. *Food Chemistry*, **131**, 964-971. https://doi.org/10.1016/j.foodchem.2011.09.088

[3] Oboh, G., Ademiluyi, A.O., Akinyemi, A.J., Henle, T., Saliu, J.A. and Schwarzenbolz,

U. (2012) Inhibitory Effect of Polyphenol-Rich Extracts of Jute Leaf (*Corchorus olitorius*) on Key Enzyme Linked to Type 2 Diabetes (*α*-Amylase and *α*-Glucosidase) and Hypertension (Angiotensin I Converting) *in Vitro*. *Journal of Functional Foods*, **4**, 450-458. https://doi.org/10.1016/j.jff.2012.02.003

[4] Dong, H., Li, M., Zhu, F., Liu, F. and Huang, J. (2012) Inhibitory Potential of Trilobatin from *Lithocarpus polystachyus* Rehd against *α*-Glucosidase and *α*-Amylase Linked to Type 2 Diabetes. *Food Chemistry*, **130**, 261-266. https://doi.org/10.1016/j.foodchem.2011.07.030

[5] Gray, G.M. (1975) Carbohydrate Digestion and Absorption. *New England Journal of Medicine*, **292**, 1225-1230. https://doi.org/10.1056/nejm197506052922308

[6] Suzuki, Y., Sano, M., Hayashida, K., Ohsawa, I., Ohta, S. and Fukuda, K. (2009) Are the Effects of *α*-Glucosidase Inhibitors on Cardiovascular Events Related to Elevated Levels of Hydrogen Gas in the Gastrointestinal Tract? *FEBS Letters*, **583**, 2157-2159. https://doi.org/10.1016/j.febslet.2009.05.052

[7] Kwon, Y.I., Vattem, D.V. and Shetty, K. (2006) Evaluation of Clonal Herbs of Lamiaceae Species for Management of Diabetes and Hypertension. *Asia Pacific Journal of Clinical Nutrition*, **15**, 107-118.

[8] Shanmugakumaran, S.D., Amerjothy, S., Balakrishnaand, K. and Vasanthakumar, M.S. (2005) Pharmacological Screening of *Pithecellobium Dulce* Benth for Its Antidiabetic and Other Potentials. *Indian Drugs*, **42**, 392.

[9] Shanmugakumaran, S.D., Amerjothy, S. and Balakrishna, K. (2006) Pharmacognostical, Antibacterial and Antifungal Potentials of the Leaf Extracts of *Pithecellobium Dulce* Benth. *Pharmacognosy Magazine*, **7**, 163-167. https://phcog.com/article/view/2006/2/7/163-167

[10] Kirtikar, K.R. and Basu, B.D. (2017) Archeological Survey, Medicinal Plants, Herbs, Plant, Analysis. Indian Medicinal Plants, Periodical Experts Book Agency. https://archive.org/details/in.gov.ignca.2048/page/n5/mode/2up

[11] Sukantha, T.A., Shubashini, K. and Balashanmugam, P. (2011) Evaluation of *in Vitro* Antioxidant and Antibacterial Activity of *Pithecellobium Dulce* Benth Fruit Peel. *International Journal of Current Research*, **3**, 378-382.

[12] Khanzada, S.K., Khanzada, A.K., et al. (2013) Phytochemical Studies on Pithecellobium Dulce Benth: A Medicinal Plant of Sindh, Pakistan. *Pakistan Journal of Botany*, **45**, 557-561. https://www.pakbs.org/pjbot/PDFs/45(2)/31.pdf

[13] Khanzada, S.K., Khanzada, A.K., Shaikh, W. and Abid Ali, S. (2008) Phytochemical Studies on *Pithecellobium Dulce* Benth. A Medicinal Plant of Sindh, Pakistan. *Pakistan Journal of Botany*, **45**, 557-561.

[14] Manikandan, R., Sundaram, R., Srinivasan, P., Beulaja, S. and Arulvasu, C. (2009) Isolation of 1, 2 Disubstituted Idopyranose from Vitex Negundo and Its Effects on Diabetic Rats. *International Journal of Pharmaceuticals Analysis*, **1**, 4-10.

[15] Krishnaveni, S., Balasubramanian, T. and Sadasivam, S. (1984) Sugar Distribution in Sweet Stalk Sorghum. *Food Chemistry*, **15**, 229-232. https://doi.org/10.1016/0308-8146(84)90007-4

[16] Adisa, R.A., Oke, J., Olomu, S.A., Olorunsogo, O. (2005) Inhibition of Human Haemoglobin Glycosylation by Flavonoid Containing Leaf Extracts of *Cnestis ferruginea*. *Journal of the Cameroon Academy of Sciences*, **4**, 351-359. https://www.ajol.info/index.php/jcas/article/view/17676

[17] Gandhi, G.R. and Sasikumar, P. (2012) Antidiabetic Effect of Merremia Emarginata Burm. F. in Streptozotocin Induced Diabetic Rats. *Asian Pacific Journal of Tropical Biomedicine*, **2**, 281-286. https://doi.org/10.1016/s2221-1691(12)60023-9