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In Vitro Anti Diabetic Potential of *Pithecellobium dulce* Leaf Extract Via α-Amylase Inhibition Assay

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Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels, necessitating effective therapeutic strategies for its management. The present study was designed to evaluate the in vitro antidiabetic potential of methanolic leaf extract of *Pithecellobium dulce* through α-amylase inhibition assay. The plant leaves were collected, shade-dried, powdered, and extracted using methanol by maceration. The extract was prepared in different concentrations (1–4 mg/mL) and tested for its inhibitory activity against salivary α-amylase using starch as a substrate. Acarbose was used as the standard drug for comparison. The results demonstrated that the extract exhibited a concentration-dependent increase in α-amylase inhibition. The percentage inhibition ranged from 27.05% at 1 mg/mL to 63.52% at 4 mg/mL. The IC_{50} value of the extract was calculated to be 3.04 mg/mL, indicating moderate inhibitory activity. Although the extract showed lower potency compared to acarbose, it displayed a similar trend of enzyme inhibition. The observed activity may be attributed to the presence of bioactive phytoconstituents such as flavonoids and phenolic compounds. These findings suggest that *Pithecellobium dulce* possesses potential as a natural source of α-amylase inhibitors and may be useful in controlling postprandial hyperglycaemia. However, further studies are required to isolate active compounds and evaluate *in vivo* efficacy.

Keywords

Pithecellobium dulce, α-Amylase inhibition, Antidiabetic activity, Methanolic extract, Postprandial hyperglycemia, Acarbose, Enzyme inhibition, Medicinal plants

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (1,2). It has become a major global health concern, with a rapidly increasing prevalence across both developed and developing nations. The disease is broadly classified into type 1 diabetes, which results from autoimmune destruction of pancreatic β-cells, and type 2 diabetes, which is primarily associated with insulin resistance and impaired insulin secretion (3). Chronic hyperglycaemia is linked to long-term complications such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy, significantly affecting patient quality of life (2).

Postprandial hyperglycaemia plays a critical role in the progression of diabetes and its complications. Carbohydrate-digesting enzymes such as α-amylase are responsible for the breakdown of complex

carbohydrates into absorbable glucose units. α-amylase catalyses the hydrolysis of starch into smaller molecules such as maltose and oligosaccharides, which are further converted into glucose (4). Therefore, inhibition of α-amylase delays carbohydrate digestion and reduces glucose absorption, thereby helping to control postprandial blood glucose levels. This mechanism has been widely recognized as an effective therapeutic approach in the management of type 2 diabetes (4).

Although synthetic α-amylase inhibitors such as acarbose are widely used in clinical practice, their use is often associated with adverse gastrointestinal effects, including bloating, flatulence, and diarrhoea (4). In addition, long-term use of these agents may lead to reduced patient compliance and increased treatment costs. These limitations have encouraged the search for alternative therapeutic options that are both effective and safer.

Medicinal plants have been extensively utilized in traditional systems of medicine for the management of diabetes. They are rich in bioactive compounds such as flavonoids, phenolics, tannins, and alkaloids, which exhibit diverse pharmacological activities. Plant-derived compounds have been reported to exert antidiabetic effects through multiple mechanisms, including inhibition of carbohydrate-digesting enzymes, antioxidant activity, and enhancement of insulin secretion (5,6). Due to their natural origin and relatively lower incidence of side effects, medicinal plants are considered promising candidates for the development of novel antidiabetic agents.

Pithecellobium dulce, commonly known as Manila tamarind, is a tropical medicinal plant traditionally used for various therapeutic purposes. Different parts of the plant have been reported to possess pharmacological activities such as antioxidant, antimicrobial, and anti-inflammatory effects. The plant contains phytoconstituents such as flavonoids and phenolic compounds, which are associated with its biological activities (7). These compounds may contribute to its potential antidiabetic effects by inhibiting carbohydrate-digesting enzymes and reducing oxidative stress. However, scientific studies evaluating its α-amylase inhibitory activity remain limited.

Therefore, the present study was designed to evaluate the in vitro antidiabetic potential of *Pithecellobium dulce* leaf extract using an α-amylase inhibition assay. The study aims to assess the inhibitory activity of the extract at different concentrations and compare its effectiveness with a standard drug, acarbose. The findings of this study may provide scientific evidence supporting the use of *Pithecellobium dulce* as a potential natural source of antidiabetic agents.

MATERIALS AND METHODS

2.1 Chemicals and Reagents

All reagents used were of analytical grade. Soluble starch, sodium chloride, and buffer constituents were obtained from standard laboratory suppliers. The reference inhibitor Acarbose was procured from a certified pharmaceutical source arogya pharmacy. Distilled water was used for all preparations.

2.2 Plant Material Collection and Authentication

Leaves of *Pithecellobium dulce* were collected from a local area eluru andhraprades and rinsed thoroughly to remove adhering debris. Botanical identity was confirmed by a qualified taxonomist.

2.3 Preparation of Plant Extract

2.3.1 Drying and Powdering

Clean leaves were air-dried under shade at ambient temperature to minimize loss of heat-sensitive constituents, followed by brief oven drying (40–50°C) to remove residual moisture. The dried material was coarsely powdered and stored in airtight containers.

2.3.2 Extraction by Maceration

The powdered sample was immersed in methanol and left to stand for six days at room temperature with intermittent agitation. The mixture was filtered, and the filtrate was concentrated to a semi-solid mass using gentle heating. The extract was preserved at low temperature until use (8).

2.4 Preparation of Extract Concentrations

A primary solution of the extract (100 mg/mL) was prepared in distilled water. From this, working solutions of 1, 2, 3, and 4 mg/mL were obtained through serial dilution.

2.5 Preparation of α-Amylase Enzyme Solution

Salivary α-amylase was used as the enzyme source. Fresh saliva was diluted with distilled water and combined with a sodium chloride solution to maintain ionic strength. A phosphate buffer (pH 7.0) was incorporated to ensure suitable conditions for enzymatic activity (9).

2.6 Preparation of Substrate (Starch Solution)

A 1% (w/v) starch solution was prepared by dissolving soluble starch in distilled water with continuous heating until a clear solution formed. The solution was cooled to room temperature prior to use.

2.7 Preparation of Standard Drug (Acarbose)

A solution of Acarbose was prepared at the required concentration in distilled water and served as the positive control for comparison.

2.8 α-Amylase Inhibition Assay

Aliquots of the plant extract at different concentrations were combined with the enzyme solution and pre-incubated at 37°C for 10 minutes to allow interaction. The starch substrate was then added, and incubation was continued for 30 minutes at the same temperature. The reaction was subsequently assessed by measuring the change in absorbance, reflecting enzyme activity (10).

2.9 Measurement of Absorbance

Absorbance readings were taken at 540 nm using a UV–visible spectrophotometer. A control containing enzyme and substrate without extract was used to represent 100% activity.

2.10 Calculation of Percentage Inhibition

The extent of enzyme inhibition was calculated as:

% Inhibition = [(Ac – As) / Ac] × 100

Ac=Absorbance of control

As=Absorbance of sample

RESULTS

3.1 Effect of Extract on α-Amylase Activity

The methanolic leaf extract of *Pithecellobium dulce* exhibited inhibitory activity against α-amylase enzyme, as indicated by a decrease in absorbance values with increasing extract concentration. This reduction reflects suppression of enzymatic hydrolysis of starch. Similar findings have been reported in studies where plant extracts demonstrated the ability to inhibit α-amylase activity (11).

3.2 Dose-Dependent Inhibitory Activity

A concentration-dependent increase in inhibitory activity was observed for the extract. At 1 mg/mL, the inhibition was 27.05%, which increased to 36.47% at 2 mg/mL and 49.41% at 3 mg/mL. The highest inhibition of 63.52% was recorded at 4 mg/mL. This trend indicates that higher concentrations of the extract enhance enzyme inhibition, consistent with previously reported plant-based α-amylase inhibition studies (11,12).

3.3 Comparison with Standard Drug (Acarbose)

The inhibitory activity of the extract was compared with the standard α-amylase inhibitor, acarbose. Although the standard drug typically exhibits higher potency, the plant extract showed a similar pattern of inhibition. Comparable results have been observed in earlier studies evaluating natural inhibitors of carbohydrate-digesting enzymes (12,13).

3.4 Graphical Representation of Results

The graphical representation of concentration versus percentage inhibition showed a steady increase in enzyme inhibition with increasing extract concentration. Such a dose-dependent response is characteristic of enzyme inhibition assays and has been reported in similar experimental studies (13).

3.5 IC_{50} Determination

The IC_{50} value, defined as the concentration required to inhibit 50% of enzyme activity, was calculated using interpolation from the observed data. The IC_{50} of the methanolic extract of *Pithecellobium dulce* was found to be approximately **3.04 mg/mL**, indicating moderate inhibitory potential compared to standard inhibitors (11).

Table 1: Effect of *Pithecellobium dulce* extract on α-amylase inhibition

Concentration(mg/mL)	Control Absorbance	Sample Absorbance	% inhibition
1	**0.850**	**0.620**	**27.05**
2	**0.850**	**0.540**	**36.47**
3	**0.850**	**0.430**	**49.41**
4	**0.850**	**0.310**	**63.52**

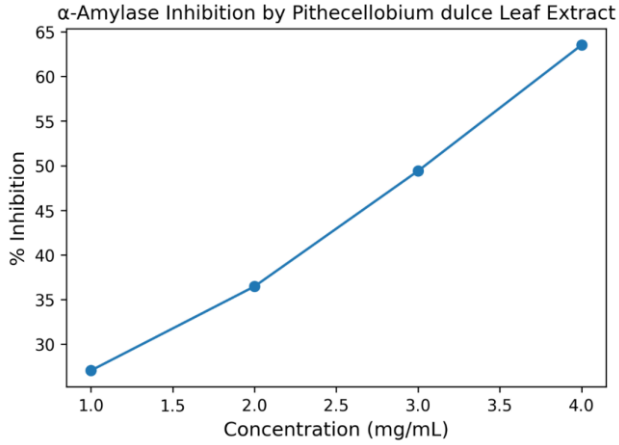


Figure 1: Concentration-dependent inhibition of α-amylase by *Pithecellobium dulce* extract



Figure 2: α-Amylase inhibitory activity of different concentrations of *Pithecellobium dulce* leaf extract.

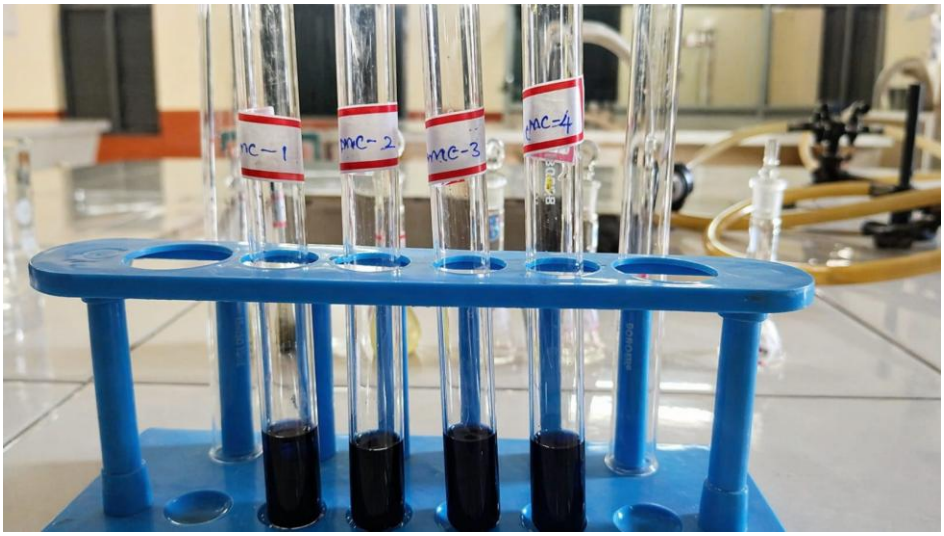


Figure 3: Reaction mixtures showing α-amylase inhibitory assay using different concentrations of *Pithecellobium dulce* leaf extract with enzyme solution, starch substrate, phosphate buffer, and iodine indicator.

DISCUSSION

The present study demonstrated that the methanolic leaf extract of *Pithecellobium dulce* exhibits significant inhibitory activity against α-amylase, suggesting its potential role in the management of postprandial hyperglycemia. The observed inhibition increased progressively with concentration, indicating a clear dose-dependent relationship. Similar findings have been reported in previous studies where plant extracts showed increasing α-amylase inhibition with higher concentrations, supporting the reliability of the present results (14,12).

The mechanism of α-amylase inhibition by plant extracts is often attributed to the presence of bioactive phytochemicals such as flavonoids, tannins, and phenolic compounds. These compounds are known to interact with the active site of the enzyme or form complexes with starch, thereby reducing its availability for enzymatic hydrolysis (15,16). The moderate inhibitory activity observed in this study may therefore be associated with such phytoconstituents present in *Pithecellobium dulce*.

The IC_{50} value obtained in the present study (3.04 mg/mL) indicates that the extract possesses measurable inhibitory potential, although it is less potent than the standard drug acarbose. This is expected, as synthetic inhibitors are typically more specific and purified compared to crude plant extracts. However, plant-based inhibitors are often preferred due to their lower incidence of side effects and additional therapeutic benefits such as

antioxidant activity (16). Previous studies have also reported that crude plant extracts generally exhibit higher IC_{50} values compared to standard drugs, but still demonstrate significant biological relevance (14).

The comparison with acarbose further highlights the therapeutic potential of the plant extract. While acarbose acts as a strong competitive inhibitor of α-amylase, its clinical use is associated with gastrointestinal side effects. In contrast, natural inhibitors derived from plants may offer a safer alternative with fewer adverse effects, making them suitable candidates for long-term management of diabetes (15).

The dose-dependent inhibitory activity observed in this study is consistent with earlier reports on medicinal plants exhibiting α-amylase inhibition. Several studies have demonstrated that plant extracts can achieve inhibition levels above 50% at higher concentrations, indicating their potential usefulness in controlling blood glucose levels (14,12). The results of the present study fall within this range, further validating the antidiabetic potential of *Pithecellobium dulce*.

Overall, the findings suggest that the methanolic extract of *Pithecellobium dulce* leaves contains bioactive compounds capable of inhibiting α-amylase activity. Although the inhibitory effect is moderate compared to standard drugs, the plant extract shows promise as a natural antidiabetic agent. Further studies, including phytochemical characterization and in vivo evaluation, are necessary to identify the active constituents and confirm its therapeutic efficacy.

CONCLUSION

The present study demonstrates that the methanolic leaf extract of *Pithecellobium dulce* exhibits significant in vitro α-amylase inhibitory activity, indicating its potential in the management of postprandial hyperglycemia. The observed dose-dependent increase in inhibition and the IC_{50} value of 3.04 mg/mL suggest moderate antidiabetic efficacy. Similar studies have reported that plant extracts possess the ability to inhibit α-amylase and reduce glucose release from dietary starch (11,17).

The inhibitory activity of the extract may be attributed to the presence of bioactive phytoconstituents such as flavonoids and phenolic compounds, which are known to interact with carbohydrate-digesting enzymes and reduce their activity (18). Although the extract showed lower potency compared to standard drugs, natural inhibitors are often considered beneficial due to their reduced side effects and additional pharmacological properties (17).

Overall, the findings support the potential of *Pithecellobium dulce* as a natural source of α-amylase inhibitors. However, further investigations involving isolation of active compounds and in vivo studies are necessary to confirm its therapeutic applicability and safety profile (11).

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