IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 8, April 2026
Impact Factor: 8.2

Comparative Evaluation of α-Amylase Inhibitory Activity of Young, Mature, and Old *Psidium guajava* Leaves

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Abstract: *Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels. Inhibition of carbohydrate-digesting enzymes such as α-amylase is an effective strategy to manage postprandial hyperglycemia. The present study evaluated the in vitro α-amylase inhibitory activity of different developmental stages (young, mature, and old) of Psidium guajava leaves. Leaves were collected, oven-dried, powdered, and extracted using the maceration method. The inhibitory activity was determined using the dinitrosalicylic acid (DNSA) colorimetric assay with acarbose as the standard inhibitor. Absorbance was measured at 540 nm, and percentage inhibition was calculated relative to the control. Young leaves exhibited the highest inhibitory activity (45.9%), followed by old leaves (21.6%), while mature leaves showed comparatively lower inhibition (12.2%). These findings suggest that young Psidium guajava leaves possess greater antidiabetic potential compared to mature and old leaves. Further phytochemical and in vivo studies are recommended.*

Keywords: Psidium guajava; α-amylase inhibition; Antidiabetic activity; Leaf developmental stages; DNSA assay; In vitro study

I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent elevation of blood glucose levels resulting from defects in insulin secretion, insulin action, or both. The increasing prevalence of diabetes worldwide has become a major public health concern. One effective therapeutic strategy for controlling post-prandial hyperglycemia is the inhibition of carbohydrate-digesting enzymes such as α-amylase, which plays an important role in the breakdown of starch into glucose. Inhibition of this enzyme delays glucose absorption and helps in maintaining normal blood sugar levels.

Synthetic α-amylase inhibitors such as acarbose are commonly used for diabetes management; however, their prolonged use may cause side effects including gastrointestinal discomfort. Therefore, there is growing interest in identifying natural plant-based inhibitors that are safer, economical, and easily available. Medicinal plants are rich sources of bioactive compounds such as flavonoids, phenolics, tannins, and alkaloids, which possess significant antidiabetic potential.

Psidium guajava (guava) is a widely distributed medicinal plant belonging to the family Myrtaceae and has been traditionally used in herbal medicine for the treatment of various diseases including diabetes, infections, and inflammation. Guava leaves are known to contain polyphenols and antioxidant compounds that may contribute to inhibition of carbohydrate-hydrolyzing enzymes. The biochemical composition of leaves varies depending on their developmental stage, which may influence their biological activity.

DOI: 10.48175/IJARSCT-33820

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Young, mature, and old leaves differ in metabolic activity and accumulation of secondary metabolites. Young leaves generally contain actively synthesized phytochemicals, mature leaves show stabilized biochemical composition, while old leaves may undergo degradation of active compounds. Comparative evaluation of different leaf stages is therefore important to determine the stage exhibiting maximum therapeutic potential.

The present study was designed to evaluate and compare the α-amylase inhibitory (antidiabetic) activity of young, mature, and old guava leaves using the DNSA method. This investigation aims to identify the leaf developmental stage showing maximum enzyme inhibitory activity, which may contribute to the development of plant-based antidiabetic agents.

Objective:

To evaluate the antidiabetic potential of guava (*Psidium guajava*) leaves using the α-amylase inhibition assay.
To prepare plant extracts from different developmental stages of leaves namely young, mature, and old leaves.
To compare the α-amylase inhibitory activity among young, mature, and old guava leaf extracts.
To determine the leaf stage exhibiting maximum enzyme inhibition activity.
To assess the potential use of guava leaves as a natural alternative source of antidiabetic agents.

II. MATERIALS AND METHODS

2.1 Collection of Plant Material

Young, mature, and old leaves collected from healthy guava plants.
Washed with distilled water, oven-dried, and powdered.

2.2 Preparation of Plant Extract (Maceration)

10 g of powdered leaves soaked in 100 mL methanol for 48 h at room temperature.
Filtered, concentrated, and stored at 4°C.

2.3 α-Amylase Inhibition Assay (DNSA Method)

Reagents: α-amylase, 1% starch solution, phosphate buffer (pH 6.8), DNSA reagent, acarbose (standard).

Procedure:

Control: Enzyme + starch solution.
Standard: Enzyme + acarbose + starch.
Test: Enzyme + plant extract + starch.
Pre-incubate at 37°C for 10 min.
Add starch solution, incubate 37°C.
Add DNSA reagent to stop reaction.
Boil in water bath 10 min.
Cool, measure absorbance at 540 nm.
Calculation:
% Inhibition = ((Control − Sample) / Control) × 100

III. RESULT

Sample	Absorbance(540nm)	%Inhibition
Control	0.74	0
Standard(Acarbose)	0.61	17.6
Young leaves	0.40	45.9
Mature leaves	0.65	12.2
Old leaves	0.58	21.6

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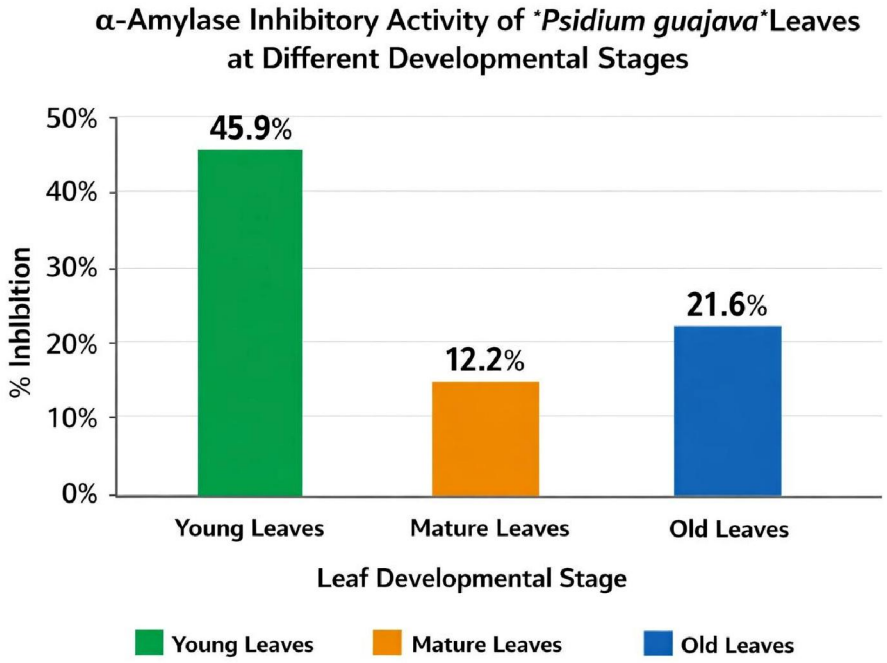
Young leaves showed maximum α-amylase inhibition.
Mature leaves had lowest inhibition.
Old leaves had moderate activity.



IV. DISCUSSION

The present study evaluated the antidiabetic potential of different developmental stages of Psidium guajava leaves by determining their α-amylase inhibitory activity using the DNSA method. Inhibition of α-amylase enzyme is considered an effective approach for controlling post-prandial hyperglycemia, as it slows down the conversion of complex carbohydrates into absorbable glucose.

The experimental results demonstrated variation in enzyme inhibitory activity among young, mature, and old guava leaves. The control sample showed higher absorbance due to maximum starch hydrolysis by α-amylase in the absence of any inhibitor. In contrast, reduced absorbance values observed in plant extract samples indicated inhibition of enzyme activity. The standard inhibitor (acarbose) showed effective inhibition, confirming the reliability of the assay method.

Among the tested samples, young leaves exhibited the lowest absorbance value, indicating stronger α-amylase inhibition compared to mature and old leaves. This finding suggests that young leaves possess higher concentrations of biologically active phytochemicals responsible for enzyme inhibition. Young leaves are metabolically more active and are known to contain elevated levels of phenolic compounds, flavonoids, and tannins, which contribute significantly to antidiabetic activity through enzyme binding and inhibition mechanisms.

Mature leaves showed moderate inhibitory activity, possibly due to stabilization of secondary metabolites during leaf development. Although mature leaves are fully developed, biosynthesis of certain active compounds may decrease compared to younger tissues. Old leaves demonstrated comparatively reduced inhibitory efficiency, which may be attributed to physiological aging, oxidation, or degradation of bioactive constituents over time.

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Variations observed among leaf stages highlight the importance of plant maturity in determining pharmacological effectiveness. These findings support the concept that developmental stage influences phytochemical accumulation and biological activity in medicinal plants. The study therefore emphasizes that selection of appropriate leaf age is a critical factor in maximizing therapeutic efficiency of plant-derived antidiabetic agents.

Overall, the results indicate that guava leaves possess significant natural α-amylase inhibitory potential, with young leaves showing superior antidiabetic activity. This study provides experimental evidence supporting the use of guava leaves as a potential source of natural enzyme inhibitors for diabetes management and encourages further investigation involving phytochemical characterization and in vivo validation.

V. CONCLUSION

Young *Psidium guajava* leaves are more effective α-amylase inhibitors than mature or old leaves.

Leaf developmental stage is critical for antidiabetic activity.

Young leaves can be considered a potential natural source of antidiabetic compounds.

Further studies: phytochemical characterization and in vivo testing.

VI. ACKNOWLEDGEMENTS

Laboratory facilities provided by [Balwant College Vita].

Guidance and support from project mentor [Pawar.P.A].

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