

Comparative Phytochemical Profiling and In-Vitro α -Amylase and α -Glucosidase Inhibitory Activity of *Psidium guajava* and *Punica granatum* Leaf Extracts as Potential Anti-Diabetic Agents

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Abstract

Diabetes mellitus, particularly Type 2 diabetes mellitus (T2DM), is a chronic metabolic disorder characterized by persistent hyperglycaemia arising from insulin resistance and progressive β -cell dysfunction. Inhibition of the carbohydrate-hydrolysing enzymes α -amylase and α -glucosidase is a well-established strategy for controlling postprandial hyperglycaemia, and plant-derived inhibitors are of increasing interest as alternatives to synthetic agents such as acarbose, which are often associated with gastrointestinal side effects. The present study aimed to compare the phytochemical profiles and in-vitro α -amylase and α -glucosidase inhibitory activities of *Psidium guajava* (guava) and *Punica granatum* (pomegranate) leaf extracts. Leaves of both plants were collected, authenticated, dried, powdered and extracted, and screened for preliminary phytochemical constituents. Enzyme inhibitory activity was evaluated spectrophotometrically at extract concentrations of 200–1000 $\mu\text{g/mL}$, using acarbose as the reference standard, and IC₅₀ values were derived by non-linear regression of the concentration–inhibition relationship. Both extracts produced concentration-dependent inhibition of α -amylase and α -glucosidase. *P. granatum* showed greater inhibitory activity than *P. guajava* at every concentration tested, with lower IC₅₀ values for α -amylase (524 $\mu\text{g/mL}$ vs. 597.1 $\mu\text{g/mL}$) and α -glucosidase (453.8 $\mu\text{g/mL}$ vs. 604.4 $\mu\text{g/mL}$). Acarbose produced $91.96 \pm 0.65\%$ inhibition at 1000 $\mu\text{g/mL}$ in both assays, exceeding both plant extracts. These findings indicate that *P. granatum* leaf extract possesses comparatively stronger dual-enzyme inhibitory potential, likely attributable to its phenolic and flavonoid constituents, and support further phytochemical and biological investigation of both species as candidate sources of natural antidiabetic agents.

Keywords: Diabetes mellitus; *Psidium guajava*; *Punica granatum*; α -amylase inhibition; α -glucosidase inhibition; postprandial hyperglycaemia; IC₅₀

1. 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. Insulin, secreted by pancreatic β -cells, normally facilitates glucose uptake into peripheral tissues and regulates hepatic glucose production; when this regulation fails, glucose accumulates in the bloodstream, producing hyperglycaemia and, over time, damage to the eyes, nerves, kidneys, heart and blood vessels.

According to the World Health Organization, diabetes affects approximately 9% of the global adult population, with nearly 80% of affected individuals living in low- and middle-income countries. Type 2 diabetes mellitus (T2DM), which accounts for 90–95% of all cases, arises from a combination of insulin resistance and progressive β -cell dysfunction and is closely linked to obesity, physical inactivity and unhealthy dietary patterns. Current pharmacological management includes metformin,

sulfonylureas, SGLT2 inhibitors, GLP-1 receptor agonists and DPP-4 inhibitors; although effective, long-term use of these agents can be associated with hypoglycaemia, gastrointestinal disturbance, weight gain and increased treatment cost, prompting continued interest in plant-derived alternatives with comparable efficacy and fewer adverse effects.

One validated therapeutic strategy for limiting postprandial hyperglycaemia is inhibition of the carbohydrate-hydrolysing enzymes α -amylase and α -glucosidase. α -Amylase initiates starch digestion by cleaving complex carbohydrates into oligosaccharides, while α -glucosidase, located in the intestinal brush border, hydrolyses these oligosaccharides and disaccharides into absorbable glucose. Delaying the action of either enzyme slows glucose liberation and absorption, blunting the postprandial glucose rise and reducing insulin demand. Synthetic inhibitors such as acarbose are used clinically but frequently cause gastrointestinal side effects, including flatulence, bloating and diarrhoea, which has driven the search for natural α -amylase and α -glucosidase inhibitors from medicinal plants.

Psidium guajava L. (family Myrtaceae, common name guava) and *Punica granatum* L. (family Lythraceae, common name pomegranate) are widely distributed medicinal plants with long histories of traditional use in Ayurvedic, Unani, Siddha and folk medicine for diarrhoea, hypertension, inflammation, microbial infections and diabetes. Leaves of both species are rich in flavonoids (quercetin, kaempferol, catechin), phenolic acids (gallic acid, chlorogenic acid, ellagic acid) and tannins, compounds repeatedly associated with antioxidant, anti-inflammatory and carbohydrate-enzyme inhibitory activity in the literature. Several prior studies have reported α -amylase and/or α -glucosidase inhibition by extracts of guava leaves, fruit, seed and essential oil, and by pomegranate peel and bark, but direct, side-by-side comparison of the leaf extracts of the two species under identical assay conditions is limited.

The present study was therefore designed to compare the preliminary phytochemical profiles and the in-vitro α -amylase and α -glucosidase inhibitory activities of *P. guajava* and *P. granatum* leaf extracts, with a view to identifying the more promising candidate for further development as a natural antidiabetic agent.

1.1 Plant Profiles

Psidium guajava L. is a tropical fruit-bearing shrub or small tree native to Central America and widely cultivated in India. Its leaves are simple, opposite and decussate, elliptic to ovate in shape, 5–15 cm long and 3–7 cm wide, with an entire margin, acute to obtuse apex, prominent pinnate venation and aromatic essential-oil-bearing glands.

Punica granatum L. is a deciduous shrub or small tree, 5–10 m in height, cultivated for over 4,000 years and widely grown in Maharashtra, Karnataka, Gujarat, Andhra Pradesh, Tamil Nadu and Telangana in India. Its leaves are simple, opposite to sub-opposite, lanceolate to oblong, 3–7 cm long and 1–2 cm wide, with an entire margin, acute apex and a smooth, leathery texture adapted to arid conditions.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh, healthy, disease-free leaves of *P. guajava* and *P. granatum* were collected and authenticated by a qualified botanist. The leaves were washed to remove adhering dust and impurities, shade-dried under controlled conditions until a constant weight was obtained, powdered using a suitable grinder, passed through an appropriate sieve, and stored in clean, labelled, airtight containers until extraction.

2.2 Preparation of Extracts

The powdered leaves of each plant were extracted separately using a suitable solvent by maceration, Soxhlet extraction or another validated technique, following an identical procedure for both species to allow meaningful comparison. Extracts were filtered, concentrated under suitable conditions, dried or reduced to a suitable consistency, and stored in labelled containers. Percentage yield was calculated as (weight of dried extract / weight of powdered plant material) $\times$ 100.

2.3 Preliminary Phytochemical Screening

Both extracts were subjected to qualitative phytochemical screening for flavonoids, phenolic compounds, tannins, terpenoids, saponins and other secondary metabolites using standard chemical tests, in order to enable comparative phytochemical profiling of the two species.

2.4 In-Vitro α -Amylase Inhibition Assay

The α -amylase inhibitory activity of each extract (20–100 $\mu\text{g/mL}$, and up to 200–1000 $\mu\text{g/mL}$ for the comparative assay) was evaluated by preparing a reaction mixture containing sodium phosphate buffer (0.02 M), α -amylase enzyme and the plant extract, incubated at room temperature for 10 minutes. Starch solution was then added to initiate the reaction. After the reaction period, dinitrosalicylic acid (DNS) reagent was added to terminate the reaction, and the tubes were placed in a boiling water bath for 5 minutes for colour development, cooled, and diluted with distilled water. Absorbance was measured at 540 nm using a UV–Visible spectrophotometer, with a control prepared identically but without extract. Acarbose served as the reference standard inhibitor. All experiments were performed in triplicate and results expressed as mean $\pm$ SD.

Percentage inhibition was calculated as: % Inhibition = [(Absorbance of control – Absorbance of extract) / Absorbance of control] $\times$ 100. IC₅₀ values were derived from the percentage inhibition versus log-concentration plot using non-linear regression.

2.5 In-Vitro α -Glucosidase Inhibition Assay

Yeast α -glucosidase inhibitory activity was determined using p-nitrophenyl- α -D-glucopyranoside (pNPG) as substrate. The enzyme was dissolved in 100 mM phosphate buffer (pH 6.8); extract concentrations of 20–100 $\mu\text{g/mL}$ (and 200–1000 $\mu\text{g/mL}$ for the comparative assay) were mixed with buffer and incubated with the enzyme solution at 30 °C for 5 minutes. The reaction was terminated with 50 mM sodium hydroxide, and absorbance was measured at 410 nm. A control without extract was prepared identically, and acarbose served as the reference standard. Percentage inhibition and IC₅₀ values were calculated as described for the α -amylase assay. All experiments were performed in triplicate.

2.6 Statistical Analysis

All assays were performed in triplicate, and results are expressed as mean $\pm$ standard deviation (SD). IC₅₀ values were determined by non-linear regression analysis of the concentration–percentage inhibition relationship.

3. RESULTS

Both *P. guajava* and *P. granatum* leaf extracts produced concentration-dependent inhibition of α -amylase and α -glucosidase over the tested range of 200–1000 $\mu\text{g/mL}$, with acarbose used as the positive reference standard (91.96 $\pm$ 0.65% inhibition at 1000 $\mu\text{g/mL}$ in both assays).

3.1 α -Amylase Inhibitory Activity

P. guajava extract showed inhibition increasing from $21.64 \pm 0.64\%$ at 200 $\mu\text{g/mL}$ to $71.21 \pm 0.96\%$ at 1000 $\mu\text{g/mL}$ ($\text{IC}_{50} = 597.1 \mu\text{g/mL}$). *P. granatum* extract showed inhibition increasing from $23.84 \pm 0.58\%$ at 200 $\mu\text{g/mL}$ to $80.96 \pm 0.52\%$ at 1000 $\mu\text{g/mL}$ ($\text{IC}_{50} = 524 \mu\text{g/mL}$). *P. granatum* produced greater inhibition than *P. guajava* at every concentration tested (Table 1).

Table 1. Comparative α -amylase inhibitory activity of *P. guajava* and *P. granatum* leaf extracts (Mean $\pm$ SD, n = 3)

Concentration ($\mu\text{g/mL}$)	<i>P. guajava</i> (% Inhibition)	<i>P. granatum</i> (% Inhibition)
200	21.64 ± 0.64	23.84 ± 0.58
400	35.92 ± 0.76	40.62 ± 0.64
600	50.18 ± 0.89	55.74 ± 0.73
800	62.47 ± 0.98	69.18 ± 0.61
1000	71.21 ± 0.96	80.96 ± 0.52
Standard Acarbose (1000)	91.96 ± 0.65	91.96 ± 0.65
IC_{50} ($\mu\text{g/mL}$)	597.1	524

3.2 α -Glucosidase Inhibitory Activity

P. guajava extract showed inhibition increasing from $23.86 \pm 0.52\%$ at 200 $\mu\text{g/mL}$ to $68.34 \pm 0.47\%$ at 1000 $\mu\text{g/mL}$ ($\text{IC}_{50} = 604.4 \mu\text{g/mL}$). *P. granatum* extract showed inhibition increasing from $29.64 \pm 0.38\%$ at 200 $\mu\text{g/mL}$ to $84.47 \pm 0.12\%$ at 1000 $\mu\text{g/mL}$ ($\text{IC}_{50} = 453.8 \mu\text{g/mL}$). The difference between the two extracts was more pronounced for α -glucosidase than for α -amylase, particularly at 600–1000 $\mu\text{g/mL}$ (Table 2).

Table 2. Comparative α -glucosidase inhibitory activity of *P. guajava* and *P. granatum* leaf extracts (Mean $\pm$ SD, n = 3)

Concentration ($\mu\text{g/mL}$)	<i>P. guajava</i> (% Inhibition)	<i>P. granatum</i> (% Inhibition)
200	23.86 ± 0.52	29.64 ± 0.38
400	37.92 ± 0.49	45.82 ± 0.31
600	49.78 ± 0.51	61.35 ± 0.42
800	59.84 ± 0.46	73.92 ± 0.27
1000	68.34 ± 0.47	84.47 ± 0.12
Standard Acarbose (1000)	91.96 ± 0.65	91.96 ± 0.65
IC_{50} ($\mu\text{g/mL}$)	604.4	453.8

3.3 Overall Comparison

Table 3. Summary comparison of enzyme inhibitory parameters

Parameter	<i>P. guajava</i>	<i>P. granatum</i>	Better Activity
α -Amylase IC_{50}	597.1 $\mu\text{g/mL}$	524 $\mu\text{g/mL}$	<i>P. granatum</i>
α -Glucosidase IC_{50}	604.4 $\mu\text{g/mL}$	453.8 $\mu\text{g/mL}$	<i>P. granatum</i>
α -Amylase inhibition at 1000 $\mu\text{g/mL}$	71.21%	80.96%	<i>P. granatum</i>
α -Glucosidase inhibition at 1000 $\mu\text{g/mL}$	68.34%	84.47%	<i>P. granatum</i>

4. DISCUSSION

Both leaf extracts inhibited α -amylase and α -glucosidase in a concentration-dependent manner, consistent with prior reports of carbohydrate-enzyme inhibitory activity in guava and pomegranate. At the lowest concentration tested (200 $\mu\text{g/mL}$), inhibition by the two extracts was relatively similar, but the gap widened progressively with increasing concentration, indicating a stronger overall inhibitory capacity for *P. granatum*.

For α -amylase, *P. granatum* reached 80.96% inhibition at 1000 $\mu\text{g/mL}$ versus 71.21% for *P. guajava*, with a correspondingly lower IC_{50} (524 vs. 597.1 $\mu\text{g/mL}$). The difference was more pronounced for α -glucosidase, where *P. granatum* reached 84.47% inhibition versus 68.34% for *P. guajava* at 1000 $\mu\text{g/mL}$, and the IC_{50} values (453.8 vs. 604.4 $\mu\text{g/mL}$) differed more substantially than for α -amylase. Within *P. granatum* itself, the lower α -glucosidase IC_{50} relative to its α -amylase IC_{50} suggests comparatively greater potency against α -glucosidase, whereas the two IC_{50} values for *P. guajava* were closer together, suggesting broadly comparable potency against both enzymes.

These differences may reflect variation in the type, concentration or relative proportion of polyphenolic constituents — flavonoids, phenolic acids and tannins — between the two species, which can interact with enzyme active sites through hydrogen bonding and other non-covalent interactions. This is consistent with prior reports identifying ellagic acid, quercetin, kaempferol and related polyphenols as active constituents underlying α -amylase and α -glucosidase inhibition in both guava and pomegranate. Neither extract exceeded acarbose (91.96 $\pm$ 0.65% at 1000 $\mu\text{g/mL}$) in this study, but *P. granatum* approached the standard's activity more closely, particularly against α -glucosidase.

Because α -amylase and α -glucosidase act at successive stages of carbohydrate digestion, simultaneous inhibition of both enzymes provides a plausible biochemical basis for slowing postprandial glucose release from complex carbohydrates. However, these findings represent preliminary in-vitro evidence; enzyme inhibition in a laboratory assay does not by itself establish clinical antidiabetic efficacy. Identification of the specific active constituents, enzyme-kinetic characterisation, and appropriate in-vivo and clinical studies are required before therapeutic conclusions can be drawn.

5. CONCLUSION

Both *Psidium guajava* and *Punica granatum* leaf extracts exhibited concentration-dependent in-vitro inhibition of α -amylase and α -glucosidase. *Punica granatum* demonstrated consistently greater inhibitory activity than *P. guajava*, with lower IC_{50} values for both enzymes (α -amylase: 524 $\mu\text{g/mL}$ vs. 597.1 $\mu\text{g/mL}$; α -glucosidase: 453.8 $\mu\text{g/mL}$ vs. 604.4 $\mu\text{g/mL}$), indicating stronger dual-enzyme inhibitory potential. These findings support *P. granatum* leaf extract as the more promising candidate of the two species for further investigation as a natural antidiabetic agent, while *P. guajava* also showed substantial and consistent activity. Further work involving quantitative phytochemical analysis, isolation and identification of active compounds, enzyme-kinetic studies, and in-vivo/clinical evaluation is warranted to confirm the antidiabetic potential of both species.

ACKNOWLEDGEMENT

The authors thank the Department of Pharmacology, Sri Vijay Vidyalaya College of Pharmacy, Dharmapuri, for providing laboratory facilities and support for this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. El-Feky, A.M., et al. (2025). Effect of harvest time and soil–plant relationships on the phytochemical composition and biological activities of *Psidium guajava* leaves.
2. Uuh Narvaez, J.J., et al. (2025). Metabolic profiling and investigation of α -amylase and α -glucosidase inhibitory mechanisms of *Psidium guajava* fruit extract.
3. Karagecili, H., et al. (2023). Chemical profile and biological activities of Zivzik pomegranate (*Punica granatum* L.) investigated using LC-MS/MS profiling. *Life*, 13(3), 735.
4. Patil, P., et al. (2023). Evaluation of total phenolic and flavonoid contents and antioxidant properties of *Psidium guajava* leaves.
5. Zhang, Y., et al. (2023). Chemical constituents and pharmacological activities of *Punica granatum*.
6. Belkacem, N., et al. (2023). Effects of *Punica granatum* fractions on postprandial hyperglycemia and α -amylase inhibition. *Phytothérapie*, 21, 41–49.
7. Zhang, X., et al. (2022). Chemical composition and biological/enzyme-inhibitory properties of *Psidium guajava* leaf essential oil.
8. Nagaraja, B., et al. (2022). Green synthesis of silver nanoparticles using *Psidium guajava* leaf extract and evaluation of their biological and antidiabetic activities. *Molecules*, 27, 4336.
9. Shi, Y., et al. (2022). Screening and identification of potential α -glucosidase inhibitors from *Punica granatum* using bioaffinity ultrafiltration and HPLC-ESI-MS/MS. *Frontiers in Nutrition*, 9.
10. Laaraj, S., et al. (2022). Phytochemical composition and α -amylase and α -glucosidase inhibitory activities of *Punica granatum* extracts.
11. Džamić, A.M. and Matejić, J.S. (2022). Plant products as potential agents for diabetes prevention and management through inhibition of carbohydrate-digesting enzymes.
12. Kumar, M., et al. (2021). *Psidium guajava* leaves: nutritional composition, phytochemical profile and health-promoting biological activities.
13. Huang, X., et al. (2021). Chemical constituents of *Psidium guajava* leaves and investigation of their biological properties.
14. James, M., et al. (2020). Antioxidant and α -amylase/ α -glucosidase inhibitory activities of *Psidium guajava*-derived protein hydrolysates.
15. Dang Kim Thu, H., et al. (2019). α -Glucosidase and protein tyrosine phosphatase-1B inhibitory activities of *Psidium guajava* leaf extracts and fractions.
16. Samejima, K. and Park, S. (2019). Inhibitory effects of guava leaf extract on carbohydrate-digesting enzymes including α -amylase and α -glucosidase.
17. Jacob, J., et al. (2019). Phytochemical and pharmacological potential of *Punica granatum*. *Pharmacognosy Journal*.
18. Simamora, A., et al. (2018). α -Glucosidase inhibitory and antioxidant activities of *Psidium guajava* leaf and fruit extracts.
19. Zhang, Z., et al. (2016). Antioxidant and α -glucosidase inhibitory activities of polysaccharides derived from *Psidium guajava*.
20. Liu, X., et al. (2015). Optimization of ultrasound-assisted extraction of *Psidium guajava* leaves for antihyperglycemic activity. *Food Science and Technology Research*, 21(4), 615–622.
21. Harborne, J.B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (3rd ed.). Chapman & Hall, London.
22. Trease, G.E. and Evans, W.C. (2009). *Trease and Evans' Pharmacognosy* (16th ed.). Saunders Elsevier, London.
23. Kokate, C.K., Purohit, A.P. and Gokhale, S.B. (2010). *Pharmacognosy* (47th ed.). Nirali Prakashan, Pune.

24. Khandelwal, K.R. (2008). Practical Pharmacognosy: Techniques and Experiments (19th ed.). Nirali Prakashan, Pune.
25. Rangari, V.D. (2008). Pharmacognosy and Phytochemistry. Career Publications, Nashik.
26. Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426–428.
27. Worthington, V. (1993). *Worthington Enzyme Manual*. Worthington Biochemical Corporation, Freehold, New Jersey.
28. American Diabetes Association. (2024). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 47(Supplement 1).