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Research Article

ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF *PSIDIUM GUAJAVA* LEAF ETHANOLIC EXTRACT IN DIABETIC RATS

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ABSTRACT

The present study investigates the antidiabetic, hypolipidemic, and antioxidant effects of ethanolic leaf extract of *Psidium guajava* (Linn.) in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg body weight), and rats exhibiting fasting blood glucose levels above 250 mg/dL were considered diabetic. The extract was administered orally at doses of 200 mg/kg and 400 mg/kg for 21 days, and its effects were compared with the standard drug glibenclamide (5 mg/kg). Biochemical parameters such as fasting blood glucose, serum insulin, total cholesterol, triglycerides, HDL, LDL, VLDL, malondialdehyde (MDA), and superoxide dismutase (SOD) were evaluated. The extract exhibited a significant ($p < 0.05$) reduction in blood glucose and lipid levels, along with a marked improvement in antioxidant enzyme activity. Histopathological analysis of pancreatic tissues confirmed partial regeneration of β-cells in extract-treated groups.These findings suggest that *Psidium guajava* ethanolic leaf extract possesses potent antidiabetic and antioxidant properties, potentially attributed to its flavonoids, tannins, and phenolic compounds. The study supports the traditional use of *P. guajava* in the management of diabetes and oxidative stress–related complications.

Keywords: *Psidium guajava*, Antidiabetic activity, Antioxidant, Streptozotocin, Hypolipidemic.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from insulin deficiency, insulin resistance, or both. It is associated with severe complications affecting the cardiovascular, renal, and nervous systems, making it one of the major global health challenges of the 21st century. According to the International Diabetes Federation (IDF, 2024), over 540 million adults are affected worldwide, and this number continues to rise, particularly in developing countries where lifestyle and dietary changes accelerate disease progression. Conventional therapies such as insulin injections and oral hypoglycemic agents provide temporary glycemic control but are often limited by side effects, cost, and reduced long-term efficacy. As a result, there is a growing scientific interest in identifying plant-based natural products that offer multifunctional therapeutic potential with minimal adverse effects.

Psidium guajava (Linn.), commonly known as guava, is a member of the family *Myrtaceae* and has been traditionally used in folk medicine for the management of diabetes, diarrhea, inflammation, and hypertension. Phytochemical analyses of guava leaves have revealed the presence of flavonoids (quercetin, kaempferol), tannins, terpenoids, and phenolic acids, all known for their antioxidant and glucose-lowering properties. These compounds are believed to modulate carbohydrate metabolism by enhancing insulin secretion, glucose uptake, and antioxidant defense mechanisms. Experimental studies have shown that extracts of *P. guajava* possess significant free radical scavenging

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activity, inhibit α-amylase and α-glucosidase enzymes**,** and improve lipid metabolism. However, there is limited comprehensive evaluation of the ethanolic leaf extract in STZ-induced diabetic models, which closely mimic human Type 1 and Type 2 diabetes. Hence, the present study was designed to investigate the antidiabetic and antioxidant effects of ethanolic leaf extract of *Psidium guajava* in streptozotocin-induced diabetic rats, focusing on biochemical, histopathological, and oxidative stress parameters. The results are expected to provide scientific validation for its traditional use and highlight its potential as a natural therapeutic agent for diabetes management.

Marine environments represent a rich reservoir of microorganisms that produce novel enzymes with distinct properties (salt tolerance, cold activity, thermostability) not commonly found in terrestrial isolates. Hassan *et al*. (2018) demonstrated the isolation and characterization of cold-active lipases from marine bacteria, highlighting specific catalytic advantages under low-temperature conditions. Reviews and recent studies emphasize that marine-derived lipases often possess desirable features—halotolerance, solvent stability, and broad pH activity profiles—that make them attractive for industrial applications (Karia (2014); Foronda (2011). Within the genus *Bacillus*, several marine isolates have been reported to secrete extracellular lipases with promising biochemical stabilities and scalability potential (Kaur (2024) and Olusesan (2011). Optimization of fermentation parameters is crucial to maximize extracellular enzyme titers while minimizing costs. Conventional one-factor-at-a-time approaches are less efficient and do not reveal interaction effects among variables. Statistical experimental designs such as Plackett–Burman screening followed by Response Surface Methodology (RSM) and Box–Behnken designs have become standard for identifying critical factors and determining optimal conditions (Vasiee (2016), Mehta (2019) and Açıkel (2018). Multiple studies have successfully applied RSM to increase lipase yields several-fold, validating the utility of these tools for medium composition, pH, temperature, and inducer concentration optimization (Nadaf (2024; Pirghorbani (2021) and Mehta (2019). Carbon and lipid inducers (olive oil, triglyceride substrates), nitrogen sources, salinity, and trace elements strongly influence lipase synthesis. Olive oil or long-chain triglycerides often act as inducer-substrates, enhancing transcriptional activation of lipase genes in many Bacillus strains (Sharma (2017) and Sukohidayat (2018). Nitrogen source quality (organic vs. inorganic), C:N ratio, and the presence of surfactants or emulsifiers can further modulate enzyme secretion and activity (Adetunji (2018) and Ishaq & Pallavi (2012)). Studies on marine isolates additionally note the role of NaCl concentration and osmotic conditions in optimizing enzyme yield and stability (Foronda (2025).

Submerged fermentation (SmF) remains the predominant method for extracellular lipase production due to better process control and downstream processing compatibility; however, solid-state fermentation (SSF) offers advantages for certain Bacillus strains when low-water activities favor secretion (Saranya (2015) and El-Naga (2015). Comparative process studies suggest that SmF coupled with controlled aeration and agitation is generally best for achieving high volumetric productivity for marine Bacillus lipases, while SSF may be advantageous when exploiting agro-industrial residues as low-cost substrates (Adetunji (2018) and Rabbania (2015). Characterization of crude and purified lipases—including substrate specificity, optimal pH and temperature, kinetic parameters, and thermostability—is essential for application tailoring. Classical purification followed by activity assays and stability testing in the presence of solvents, metal ions, and detergents is well described in the literature (Sukohidayat (2018), Nadaf *et al*. (2024) and Olusesan (2011). Marine *Bacillus lipases* often display wide pH optima and retained activity in moderate salt and solvent concentrations, making them suitable for processes like biodiesel synthesis and detergent formulation (Alshehri, (2024) and Abdelaziz (2025).

Immobilization strategies (adsorption, entrapment, covalent binding, carrier-supported methods) improve enzyme reusability, thermostability, and operational lifetime key for industrial uptake. Examples include $CaCO_3$-immobilized *Bacillus* lipases for biodiesel production from waste cooking oil, demonstrating higher conversion and reusability (Alshehri (2024)). Such approaches are particularly relevant for marine-derived lipases which may benefit from carrier stabilization under saline or variable-temperature reaction conditions. Efficient downstream processing (clarification, concentration, aqueous two-phase partitioning) and an understanding of process mass balances are critical for economic viability. Methods to partition and recover lipases at scale while preserving activity have been discussed, alongside techno-economic analyses indicating major cost drivers: substrate costs, fermentation scale, and enzyme recovery efficiency (Sukohidayat, 2018; Magyar (2024) and Nadaf *et al*. (2021). Integrating low-cost feedstocks and energy recovery strategies can significantly improve process economics for enzyme manufacture.

MATERIALS AND METHODS

Plant Material Collection and Extraction

Fresh leaves of *Psidium guajava* were collected from authenticated sources and washed thoroughly with distilled water. The leaves were shade-dried for 10 days and pulverized to a coarse powder. About 200 g of the powder was extracted with 95% ethanol using a Soxhlet apparatus for 8 h. The extract was concentrated using a rotary evaporator under reduced pressure to yield a dark green residue, which was stored at 4 °C until use.

Experimental Animals

Healthy adult male Wistar rats (150–200 g) were obtained from an institutional animal facility. The animals were maintained under standard laboratory conditions (12 h light/dark cycle, 25 ± 2 °C) with free access to a standard pellet diet and water. All experimental protocols were

approved by the Institutional Animal Ethics Committee (IAEC) and conducted following CPCSEA guidelines.

Induction of Experimental Diabetes

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) at 55 mg/kg body weight**,** freshly dissolved in 0.1 M citrate buffer (pH 4.5). After 72 h, rats with fasting blood glucose levels above 250 mg/dL were considered diabetic and included in the study. Treatments were administered orally once daily for 21 days**.** Blood samples were collected on days 0, 7, 14, and 21 via retro-orbital puncture. Serum was separated for estimation of: Fasting Blood Glucose (FBG)-GOD-POD method. Lipid Profile**.** Total cholesterol, triglycerides, HDL-C, LDL-C, and VLDL-C. Antioxidant Markers- Superoxide dismutase (SOD), Catalase (CAT), and Malondialdehyde (MDA). Serum Insulin- ELISA kit (Millipore) Pancreatic tissues were fixed in 10% formalin, embedded in paraffin, sectioned (5 μm), and stained with hematoxylin-eosin (H&E) for microscopic evaluation of β-cell morphology.

Table 1. Experimental Design.

Group	Treatment	Description
I	Normal Control	Received vehicle (distilled water)
II	Diabetic Control	STZ (55 mg/kg) only
III	Standard	STZ + Glibenclamide (5 mg/kg)
IV	Test I	STZ + *P. guajava* ethanolic extract (200 mg/kg)
V	Test II	STZ + *P. guajava* ethanolic extract (400 mg/kg)

RESULTS AND DISCUSSION

STZ-induced diabetic rats showed a marked elevation in fasting blood glucose (FBG) levels (≈ 310 mg/dL) compared to normal controls (≈ 95 mg/dL). Treatment with *P. guajava* ethanolic extract (200 mg/kg and 400 mg/kg) resulted in a significant reduction in FBG (to 175 ± 6.2 mg/dL and 132 ± 5.1 mg/dL respectively) after 21 days, comparable to glibenclamide (128 ± 4.8 mg/dL). The glucose-lowering effect may be attributed to flavonoids such as quercetin and kaempferol, which enhance insulin secretion and glucose uptake in peripheral tissues (*Bumrungpert et al., 2021*). Diabetic rats exhibited elevated total cholesterol (TC) and triglycerides (TG) and decreased HDL-C. Extract treatment restored lipid levels significantly: TC decreased by 28.5%, TG by 31.2%**,** HDL-C increased by 22.8% compared to diabetic control. This hypolipidemic action suggests modulation of lipid metabolism via activation of lipoprotein lipase and inhibition of hepatic cholesterol synthesis (*Sathish et al., 2022*). MDA levels (an indicator of lipid peroxidation) were significantly reduced, while SOD and CAT activities were enhanced in extract-treated groups. The 400 mg/kg dose restored SOD to near-normal values, indicating strong antioxidant protection against oxidative stress generated by STZ. Pancreatic sections of diabetic rats showed degeneration and necrosis of β-cells, while extract-treated groups revealed partial restoration and regeneration of islet architecture, similar to glibenclamide-treated rats. This regenerative potential corroborates the biochemical findings and supports the antioxidant-mediated β-cell protection mechanism.

CONCLUSION

The ethanolic leaf extract of *Psidium guajava* exhibited significant antidiabetic and antioxidant activities in STZ-induced diabetic rats. It effectively lowered blood glucose and lipid levels, enhanced antioxidant defense, and protected pancreatic β-cells from oxidative damage. These effects validate the traditional use of guava leaves as a natural remedy for diabetes management. Further investigations should focus on: Isolation and structural elucidation of active phytoconstituents responsible for the observed pharmacological effects. Mechanistic studies to elucidate molecular pathways involved in insulin signaling and oxidative stress modulation. Chronic toxicity and pharmacokinetic evaluations to establish safety profiles. Nanoformulation and drug-delivery studies to enhance bioavailability of the extract. Clinical translation through controlled human trials to validate preclinical efficacy and establish standardized dosing protocols.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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