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The evaluation of the antioxidant activity of ethanolic leaf extract of Psidium guajava: a preliminary study toward its potential antidiabetic properties.

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Report On

The Evaluation of the Antioxidant Activity of Ethanolic Leaf Extract of Psidium guajava: A Preliminary Study Toward It's Potential Antidiabetic Properties.

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Submitted to

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DECLARATION by the Students

I hereby declare that the research work entitled "Evaluation of the Antioxidant Potential of Ethanolic Leaf Extract of Psidium guajava Using DPPH Radical Scavenging Assay" is an original work carried out by me. This report has been completed under proper academic guidance and reflects my own effort and investigation.

To the best of my knowledge, no part of this work has been previously published or submitted to any other university or institution for the award of any degree or diploma. All sources of information and assistance have been properly acknowledged.

I further declare that upon publication in a peer-reviewed or indexed journal, this research work will be available for public access.

_____

Name: Meherun Mahmud
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Certification by the Supervisor

This is to certify that the undergraduate research project entitled “Evaluation of the Antioxidant Potential of Ethanolic Leaf Extract of Psidium guajava Using DPPH Radical Scavenging Assay” has been successfully conducted by Meherun Mahmud (Student ID: 2010548) under my direct supervision in the Department of Pharmacy, Independent University, Bangladesh.

To the best of my knowledge, the work presented in this report is original and has been carried out independently by the student. The study meets the required academic standards and is suitable for submission in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (B.Pharm).

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Naima Nusrat Jahan
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ABSTRACT

Medicinal plants serve as vital reservoirs of bioactive molecules with strong therapeutic potential. Among these, Psidium guajava L. (guava) holds a significant place in traditional medical practices due to its wide-ranging pharmacological effects, notably in treating metabolic conditions such as diabetes. This study aimed to investigate the bioactivity of the ethanolic leaf extract of Psidium guajava L. by quantifying total phenolic levels and measuring radical scavenging capacity via the DPPH assay.

To prepare the sample, freshly collected leaves of Psidium guajava L. were dried in the shade, ground into a powder, and subjected to cold maceration in ethanol over a 72-hour period. The resulting liquid was filtered and condensed under reduced pressure using a rotary evaporator. Serial dilutions were prepared from the concentrated extract to evaluate antioxidant potential through DPPH radical scavenging, utilizing ascorbic acid as a standard benchmark. The Folin-Ciocalteu colorimetric method, calibrated against gallic acid, was employed to measure total phenolic content.

The ethanolic leaf extract demonstrated a dose-dependent DPPH radical scavenging capacity, where higher concentrations yielded greater percentage inhibition. High linearity in the gallic acid standard curve ($R^2 = 0.9934$) confirmed the accuracy and reliability of the method used to determine total phenolic content.

These results indicate that the ethanolic leaf extract of Psidium guajava L. exhibits notable antioxidant capability, largely driven by its rich phenolic profile. Because oxidative stress plays a key role in the breakdown of pancreatic \beta-cells during diabetes progression, these scientific findings validate the traditional therapeutic application of the plant and support more in-depth exploration into its antidiabetic properties.

Keywords: Psidium guajava L., Antioxidant activity, Antidiabetic potential, DPPH assay, Total Phenolic Content, Phenolic compounds, Ethanolic leaf extract.

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CHAPTER 1

INTRODUCTION

Introduction

Oxidative stress is defined as a state of physiological imbalance between the production of reactive oxygen species (ROS) and the efficiency of the endogenous antioxidant defense system. The excessive accumulation of ROS can result in oxidative damage to critical biological macromolecules, including lipids, proteins, nucleic acids, and cellular membranes. Such damage is strongly implicated in the pathogenesis and progression of numerous chronic and degenerative diseases, such as diabetes mellitus, cardiovascular disorders, cancer, neurodegenerative diseases, and inflammatory conditions. Consequently, the exploration and development of effective antioxidant agents have become a central focus in contemporary biomedical and pharmaceutical research. In this regard, naturally derived antioxidants from medicinal plants have attracted significant scientific interest owing to their relative safety, biocompatibility, and rich composition of bioactive phytochemicals, as well as their extensive history of use in traditional medicine.

Psidium guajava L., commonly known as guava, is a tropical plant belonging to the family Myrtaceae and is widely distributed in many regions of the world. The plant is recognized not only for its nutritional value but also for its diverse therapeutic applications. Various parts of P. guajava, particularly the leaves, have been traditionally utilized for the management of a wide range of ailments, including diabetes mellitus, gastrointestinal disturbances, microbial infections, and inflammatory disorders. Contemporary pharmacological studies have demonstrated that P. guajava possesses multiple bioactivities, such as antioxidant, antidiabetic, antimicrobial, anti-inflammatory, and anticancer effects. These pharmacological properties are primarily attributed to the presence of significant phytoconstituents, including flavonoids, tannins, phenolic compounds, and vitamins, which contribute to its therapeutic efficacy.

The present study was undertaken to evaluate the antioxidant potential of the ethanolic leaf extract of Psidium guajava through the determination of its free radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) in vitro assay. This assay is widely recognized for its reliability and simplicity in assessing the radical scavenging ability of plant extracts. The findings of this investigation are expected to provide scientific evidence supporting the antioxidant efficacy of P. guajava and to further substantiate its potential as a natural source of antioxidant agents for future pharmaceutical and therapeutic applications.

1.1 Medicinal Plants

Medicinal plants have been utilized since ancient times as natural sources for the prevention and management of a wide variety of diseases. These plants are enriched with numerous biologically active constituents, including flavonoids, phenolic compounds, alkaloids, tannins, saponins, and terpenoids, which are responsible for their therapeutic efficacy. Such phytochemicals are known to exhibit a broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer effects.

In recent decades, there has been a growing scientific interest in medicinal plants due to their potential as sources of natural antioxidants. These compounds play a crucial role in neutralizing reactive oxygen species (ROS), thereby minimizing oxidative stress and protecting cellular components from damage associated with chronic and degenerative diseases. As a result, medicinal plants continue to hold significant importance in modern drug discovery and in the development of safer and more effective therapeutic agents. In this context, Psidium guajava is considered a promising medicinal plant due to its rich phytochemical composition and diverse biological activities.

1.2 Important Constituents of Medicinal Plants

Medicinal plants are abundant in a variety of naturally occurring bioactive substances, commonly referred to as phytochemicals, which are primarily responsible for their pharmacological and therapeutic properties. These secondary metabolites not only contribute to the plant's defense mechanisms but also exhibit significant biological activities that are beneficial in human health. Due to these properties, medicinal plants have gained considerable attention as potential sources for the development of novel pharmaceutical compounds.

The major categories of phytochemicals present in medicinal plants include alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, glycosides, and steroids. Alkaloids are widely recognized for their potent biological activities, such as analgesic, antimicrobial, antimalarial, and anticancer effects. Flavonoids and phenolic compounds are particularly noted for their strong antioxidant capacity, as they effectively scavenge reactive oxygen species and protect against oxidative damage. Tannins possess antimicrobial, antiviral, antioxidant, and astringent properties, making them useful in disease prevention and wound healing. Terpenoids have demonstrated a range of activities, including anti-inflammatory, antimicrobial, antioxidant, and anticancer effects, whereas saponins are associated with immunomodulatory, cholesterol-lowering, and antimicrobial functions. Glycosides are especially important in the treatment of cardiovascular conditions, while steroidal compounds play a role in hormonal regulation and exhibit anti-inflammatory and cardioprotective activities.

The combined and synergistic effects of these phytochemicals contribute to the extensive range of therapeutic benefits observed in medicinal plants. Their diverse

biological activities underscore the importance of medicinal plants as integral components of traditional healthcare systems and as valuable sources for the discovery and development of new drugs and natural antioxidant agents, including those found in Psidium guajava.

CHAPTER 2
LITERATURE REVIEW

2.1 Antidiabetic Activity and Diabetes Mellitus

Diabetes Mellitus (DM) is a chronic, multi-factorial metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, defective insulin action, or a combination of both. As a major global public health concern, chronic elevations in blood glucose levels lead to progressive cellular damage, ultimately causing severe microvascular and macrovascular complications, including nephropathy, neuropathy, retinopathy, and cardiovascular disease. The condition is primarily categorized into Type 1 Diabetes Mellitus (T1DM), which stems from the autoimmune destruction of pancreatic \beta-cells, and Type 2 Diabetes Mellitus (T2DM), which accounts for the vast majority of clinical cases and is driven by peripheral insulin resistance paired with progressive \beta-cell dysfunction.

A pivotal mechanism driving both the onset and secondary complications of diabetes is the generation of systemic oxidative stress. Under sustained hyperglycemic conditions, excess intracellular glucose accelerates the production of Reactive Oxygen Species (ROS) through multiple pathways, including glucose autoxidation, the formation of Advanced Glycation End-products (AGEs), and over-activation of the polyol pathway. Because pancreatic \beta-cells naturally possess relatively low levels of endogenous antioxidant defense enzymes—such as superoxide dismutase, catalase, and glutathione peroxidase—they are exceptionally vulnerable to ROS-mediated damage. This oxidative imbalance damages lipid membranes, induces cell apoptosis, and worsens overall insulin resistance.

Given the close relationship between oxidative damage and glycemic dysregulation, counteracting ROS with exogenous antioxidants has become an important strategy in managing diabetes. Antioxidants directly scavenge free radicals, suppress lipid peroxidation, and shield pancreatic islet cells from structural breakdown, thereby helping to preserve baseline insulin production. While conventional synthetic antidiabetic drugs remain standard therapy, their long-term use is frequently accompanied by adverse gastrointestinal side effects, hypoglycemic risks, or secondary treatment failure. Consequently, research has shifted toward natural, plant-based alternatives that offer both potent antioxidant protection and complementary antidiabetic activity.

In this context, Psidium guajava L. (Guava) presents strong therapeutic potential due to its rich composition of bioactive phytochemicals, particularly polyphenols, flavonoids (such as quercetin), and tannins. Evaluating the antioxidant capacity of its ethanolic leaf extract serves as an essential preliminary step for this project. Demonstrating its ability to neutralize free radicals establishes a mechanistic baseline for how P. guajava may protect pancreatic tissue from oxidative damage, providing a rational foundation for its potential anti-hyperglycemic properties.

2.2 Oxidative Stress and Free Radicals

Free radicals are unstable, highly reactive atoms or molecules that possess one or more unpaired electrons in their outer orbitals. To achieve chemical stability, these molecules rapidly extract electrons from neighboring biological macromolecules, such as cellular lipids, proteins, and DNA. In biological systems, the most prominent class of these species is Reactive Oxygen Species (ROS), which includes both free radical species—such as the superoxide anion (\text{O}_2^{\bullet-}), hydroxyl radical (\text{}^{\bullet}\text{OH}), and peroxyl radical (\text{ROO}^{\bullet})—and non-radical oxidizing agents like hydrogen peroxide (\text{H}_2\text{O}_2). Under normal physiological conditions, ROS are continuously generated as natural byproducts of cellular respiration within the mitochondrial electron transport chain, as well as through enzymatic activities like NADPH oxidase.

At basal physiological concentrations, free radicals play an essential and beneficial role in maintaining cellular homeostasis. They function as vital secondary messengers in intracellular signaling pathways, modulate gene expression, support immune responses by aiding phagocytes in neutralizing invading pathogens, and regulate vascular tone. To prevent these highly reactive molecules from causing unintended cellular damage, the human body relies on an integrated endogenous antioxidant defense system. This network includes enzymatic antioxidants—such as Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx)—and non-enzymatic molecules like reduced glutathione (\text{GSH}), which collectively neutralize excess ROS and maintain redox equilibrium.

Oxidative stress occurs when there is a fundamental breakdown in this balance, characterized by an overproduction of ROS that overwhelms the endogenous antioxidant defenses. This shift can be triggered by internal metabolic dysfunctions (such as sustained hyperglycemia in diabetes) or external factors like radiation, environmental pollutants, and toxic chemicals. When unchecked, excess free radicals initiate cascade reactions such as lipid peroxidation within cell membranes, leading to structural destabilization and loss of membrane fluidity. Furthermore, ROS inflict oxidative damage on structural proteins, disrupt enzymatic function, and cause irreversible single- and double-strand DNA breaks, ultimately leading to cellular dysfunction or programmed cell death (apoptosis).

In the specific context of metabolic disorders like Diabetes Mellitus, persistent oxidative stress serves as a central pathological driver. Chronic elevation of ROS directly impairs downstream insulin signaling, exacerbates peripheral insulin resistance, and selectively destroys vulnerable pancreatic \beta-cells. Because the body's internal antioxidant mechanisms are often depleted during chronic metabolic stress, there is a strong biological rationale for supplementing exogenous antioxidants. Investigating natural sources—such as the polyphenols and flavonoids found in Psidium guajava leaves—provides a critical foundation for evaluating plant-based interventions capable of quenching free radicals and mitigating oxidative cellular damage.

2.3 Antioxidants

Antioxidants are natural or synthetic substances that, even at low concentrations relative to an oxidizable substrate, significantly delay, prevent, or inhibit the oxidation of biological macromolecules such as lipids, proteins, and nucleic acids. They function as the primary defense system of living organisms against oxidative damage inflicted by Reactive Oxygen Species (ROS) and free radicals. Antioxidants exert their protective effects through several biochemical mechanisms, including direct free radical scavenging, hydrogen atom or electron transfer, singlet oxygen quenching, trace metal ion chelation, and upregulation of cellular redox enzymes. By neutralizing reactive intermediates, antioxidants terminate the destructive chain reactions—such as lipid peroxidation—that otherwise compromise cellular membrane integrity and cellular viability.

Biological antioxidants are broadly categorized based on their origin and mechanism of action into endogenous (internally produced) and exogenous (dietary or plant-derived) systems. Endogenous antioxidants include an enzymatic network consisting of Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx), alongside non-enzymatic molecules like reduced glutathione (\text{GSH}), uric acid, and coenzyme Q10. However, during chronic pathological conditions such as Diabetes Mellitus, the rate of ROS generation significantly overwhelms these intrinsic defense mechanisms, leading to local depletion of antioxidant pools. Consequently, the intake of exogenous antioxidants—particularly naturally occurring non-enzymatic compounds like vitamins (C and E), carotenoids, and plant polyphenols—becomes essential to restore redox balance and prevent oxidative tissue damage.

Among natural exogenous sources, plant-derived secondary metabolites—specifically phenolic compounds, flavonoids, and tannins—have gained extensive research interest due to their superior free radical scavenging abilities and structural stability. Flavonoids, for instance, possess hydroxyl groups attached to their aromatic ring structures, allowing them to readily donate hydrogen atoms or electrons to neutralize reactive radicals, forming stable, unreactive phenoxyl radicals. Furthermore, natural antioxidants exhibit low cytotoxicity and minimal adverse side effects compared to synthetic antioxidants like Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT), which have been associated with potential toxicological concerns over long-term consumption.

The study of plant-based antioxidants holds immense significance in the context of therapeutic research for metabolic disorders. In Psidium guajava L. (Guava) leaves, an abundance of bioactive polyphenolic compounds (such as quercetin, kaempferol, and gallic acid) contributes directly to its potent antioxidant behavior. Evaluating these properties using ethanolic leaf extracts provides vital preliminary insight into the plant's potential to quench free radicals, attenuate oxidative stress, and ultimately mitigate the underlying cellular degradation associated with diabetic complications.

2.4 Psidium guajava L. (Guava)

Psidium guajava L., commonly known as guava, is an evergreen shrub or small tree belonging to the family Myrtaceae. Native to tropical regions of South America, it is now widely cultivated across tropical and subtropical zones globally due to its edible fruit and extensive ethnomedicinal properties. In traditional systems of medicine, various parts of the plant—including the bark, roots, fruits, and particularly the leaves—have been utilized for centuries to treat gastrointestinal ailments (such as diarrhea and dysentery), respiratory conditions, inflammation, and metabolic disorders, including diabetes mellitus.

The therapeutic potential of P. guajava leaves is primarily attributed to their rich and diverse phytochemical composition. Phytochemical screenings reveal an abundance of secondary metabolites, including polyphenols, flavonoids (notably quercetin, guaijaverin, kaempferol, and myricetin), tannins, saponins, triterpenes, and essential oils. Among these, the high concentration of phenolic hydroxyl groups enables these compounds to act as effective electron donors and free radical scavengers.

In recent years, P. guajava leaf extracts—particularly those prepared using polar solvents like ethanol—have gained scientific attention for their broad spectrum of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and anti-hyperglycemic properties. Evaluating the ethanolic leaf extract serves as a critical step in isolating these bioactive phytoconstituents, establishing a firm scientific rationale for its traditional use in managing oxidative stress and its potential application in targeting diabetes mellitus.

Figure 1: Psidium Guajava L.

2.4.1 Taxonomic Classification

Kingdom: Plantae (Plants)
Subkingdom: Tracheobionta (Vascular plants)
Superdivision: Spermatophyta (Seed plants)
Division: Magnoliophyta (Flowering plants)
Class: Magnoliopsida (Dicotyledons)
Subclass: Rosidae
Order: Myrtales
Family: Myrtaceae (Myrtle family)
Genus: Psidium
Species: Psidium guajava L.

2.4.2 Botanical Description

Psidium guajava L. is an evergreen, shallow-rooted shrub or small tree that typically grows to a height of 3 to 10 meters. It features a characteristic trunk with a smooth, light reddish-brown to copper-colored bark that peels off in thin, irregular sheets, exposing a smooth, green layer beneath. The young branchlets are distinctly quadrangular (four-angled) and downy, gradually becoming woody and cylindrical as they mature.

The leaves of P. guajava are simple, opposite, and short-petioled (petiole measuring approximately 3 to 10 mm). The leaf blades are oblong-elliptic to oval in shape, measuring 5 to 15 cm in length and 3 to 6 cm in width. They display an entire margin, an acute to obtuse apex, and a rounded-to-obtuse base. The upper leaf surface is dull green and glabrous to finely pubescent, while the lower surface is paler and covered with soft, appressed hairs. A prominent feature of the leaves is their prominent, deeply impressed pinnate venation, featuring 12 to 20 pairs of secondary veins that are conspicuously raised on the underside. Additionally, translucent glandular dots containing aromatic essential oils are scattered across the leaf blade.

The flowers are solitary or borne in small cymes of 2 to 3 blossoms in the leaf axils. They are complete, actinomorphic, and fragrant, measuring about 2.5 to 3 cm in diameter. Each flower possesses 4 to 5 greenish-white sepals and an equal number of white, delicate, obovate petals. The flower is dominated by a prominent cluster of numerous (up to 250) brush-like white stamens with pale yellow anthers, surrounding a single inferior ovary with a capitate stigma.

The fruit is a globose, ovoid, or pear-shaped berry, ranging from 4 to 12 cm in length. It turns from dark green to light yellow or greenish-yellow upon ripening and emits a distinct, strong aromatic odor. The exocarp is thin and tender, while the inner mesocarp consists of a thick, edible pulp ranging in color from white to pink or deep red. Embedded within the central pulp are numerous small, hard, kidney-shaped seeds measuring 3 to 5 mm in length.

2.4.3 Traditional Uses and Therapeutic Applications

Psidium guajava L. occupies a prominent position in traditional medicine systems globally, including Ayurveda, Traditional Chinese Medicine (TCM), and African folk medicine. While various parts of the plant (bark, roots, and fruits) possess medicinal properties, the leaves are the most widely utilized component due to their high concentration of bioactive phytoconstituents.
The traditional therapeutic applications of P. guajava leaf preparations are classified by treatment target below:

1. Treatment of Gastrointestinal Disorders:
- Diarrhea and Dysentery
- Gastric Ulcers and Spasms

2. Treatment of Oral and Dermatological Conditions:
- Oral Infections
- Wounds and Skin Lesions

3. Treatment of Metabolic and Systemic Diseases:
- Diabetes Mellitus
- Cardiovascular and Rheumatic Health

The extensive ethnomedicinal use of P. guajava leaves in managing hyperglycemia provides a strong empirical foundation for evaluating its ethanolic extract to validate its antioxidant capacity and potential antidiabetic properties scientifically.

2.5 Phytochemical Constituents

The therapeutic efficacy of Psidium guajava L. is directly attributed to its rich and diverse profile of secondary metabolites synthesized throughout the plant, particularly within its leaves. Phytochemical screenings of P. guajava leaf extracts reveal a dense concentration of bioactive chemical classes that act synergistically to confer antioxidant, anti-inflammatory, and metabolic-regulatory properties.
Major Phytochemicals Identified in the Plant Include:

- Flavonoids
- Polyphenols and Phenolic Acids
- Tannins
- Triterpenoids and Steroids
- Essential Oils and Volatiles
- Saponins and Glycosides

During the extraction process, polar solvents such as ethanol are particularly effective at extracting high concentrations of these polyphenols and flavonoids. The presence of these major phytochemical groups in the ethanolic leaf extract forms the chemical basis for its antioxidant capacity and potential antidiabetic properties.

2.6 Pharmacological Activities of Psidium guajava

The extensive ethnomedicinal reputation of Psidium guajava L. has been increasingly validated through modern pharmacological research. Extensive in vitro and in vivo studies demonstrate that extracts derived from P. guajava—particularly the leaves—exhibit a broad spectrum of bioactivities. These include potent antioxidant, antidiabetic, antimicrobial, anti-inflammatory, hepatoprotective, antidiarrheal, and cardioprotective properties.

The primary driver of the plant's therapeutic potential lies in its remarkable antioxidant activity. Phytochemicals such as quercetin and gallic acid act as strong free radical scavengers, mitigating oxidative damage to lipid membranes and cellular DNA. This radical-quenching capacity directly supports its antidiabetic activity, where extracts have been shown to inhibit key carbohydrate-hydrolyzing enzymes (\alpha-glucosidase and \alpha-amylase), enhance peripheral glucose uptake, and protect pancreatic \beta-cells from oxidative apoptosis.

Additionally, P. guajava displays significant antimicrobial activity against a wide array of Gram-positive and Gram-negative bacteria (such as Staphylococcus aureus and Escherichia coli), as well as fungal strains. Its anti-inflammatory and antidiarrheal effects are mediated through the inhibition of pro-inflammatory cytokines and the relaxation of intestinal smooth muscle, respectively. Together, these multifaceted pharmacological properties provide a firm scientific rationale for evaluating the ethanolic leaf extract as a therapeutic candidate for managing oxidative stress and metabolic disorders.

2.7 DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is one of the most widely used methods for evaluating the antioxidant capacity of plant extracts. DPPH is a stable free radical that exhibits a deep violet color due to its unpaired electron. When an antioxidant compound is added to the DPPH solution, it donates hydrogen atoms or electrons to the free radical, converting it into a reduced form that appears pale yellow. This 20 reduction causes a decrease in absorbance, which can be measured spectrophotometrically at 517 nm. The percentage of DPPH radical scavenging activity is calculated using the following equation:

$$\% \text{ Radical Scavenging Activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where:

- Acontrol = absorbance of the DPPH control solution
- Asample = absorbance of the test sample

The concentration required to inhibit 50% of DPPH radicals is referred to as the IC50 value. A lower IC50 indicates stronger antioxidant activity because less extract is required to neutralize half of the free radicals.
The DPPH assay is widely used because it is rapid, simple, inexpensive, reproducible, and suitable for screening natural antioxidants.

2.8 Previous Studies on Psidium guajava

Numerous scientific studies have validated the traditional therapeutic claims of Psidium guajava L., focusing particularly on its antioxidant and antidiabetic properties. Researchers have extensively investigated various solvent extracts of guava leaves to understand their underlying bioactive mechanisms and therapeutic efficacy.

Antioxidant Evaluations: Previous in vitro assays—such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS, and FRAP (Ferric Reducing Antioxidant Power)—have consistently demonstrated that ethanolic and methanolic leaf extracts exhibit superior radical scavenging abilities compared to other plant parts. This high antioxidant capacity strongly correlates with the total phenolic and flavonoid content, specifically driven by active constituents like quercetin and gallic acid.

Antidiabetic and Anti-Hyperglycemic Research: In in vitro and animal models, guava leaf extracts have been shown to significantly lower blood glucose levels. Mechanisms identified in past literature include the inhibition of carbohydrate-digesting enzymes (\alpha-glucosidase and \alpha-amylase), improvement of insulin sensitivity in skeletal muscle, and suppression of postprandial glucose spikes.

Protective Effects Against Diabetic Complications: Studies investigating diabetic rodent models revealed that treatment with P. guajava leaf extract reduces oxidative stress markers (such as malondialdehyde) while restoring endogenous antioxidant enzymes (SOD, CAT, and GSH) in liver, kidney, and pancreatic tissues, thereby offering cellular protection against chronic hyperglycemia-induced damage.

2.9 Research Gap & Justification

While the biological potential of P. guajava is well recognized, preliminary screening of specific ethanolic leaf extracts remains essential to establish standardized baseline antioxidant values. This provides a critical starting point to link free radical scavenging directly with potential antidiabetic applications.

2.10 Summary of Literature Review

The literature reviewed in this chapter highlights the critical role of Diabetes Mellitus as a global metabolic crisis driven by persistent hyperglycemia and progressive cellular dysfunction. A key driver in the development and escalation of diabetic complications is oxidative stress, a state where excess Reactive Oxygen Species (ROS) overwhelm the body's natural antioxidant defenses. Pancreatic \beta-cells are particularly vulnerable to ROS-mediated damage due to their low inherent levels of protective antioxidant enzymes. Consequently, neutralizing free radicals with exogenous antioxidants has emerged as an essential therapeutic strategy to preserve cellular function and manage glycemic dysregulation.
Psidium guajava L. (Guava) possesses a long history of traditional use across tropical regions for managing various ailments, particularly gastrointestinal disorders and hyperglycemia. Phytochemical evaluations confirm that the leaves of P. guajava are rich in bioactive secondary metabolites—most notably flavonoids (such as quercetin), polyphenols, tannins, and triterpenoids. Solvent extraction studies indicate that polar solvents, especially ethanol, efficiently yield these active phytochemicals, providing high Total Flavonoid Content (TFC).

Previous scientific investigations have validated that P. guajava leaf extracts exhibit significant antioxidant capacity (via DPPH, ABTS, and FRAP assays) alongside antidiabetic mechanisms, including the inhibition of carbohydrate-digesting enzymes (\alpha-glucosidase and \alpha-amylase) and the protection of tissue against lipid peroxidation.

CHAPTER 3
MATERIALS AND METHODS

3.1 Study Design

This project employs an in vitro, experimental laboratory-based study design to systematically evaluate the antioxidant potential of the ethanolic leaf extract of Psidium guajava L. as a preliminary step toward assessing its potential antidiabetic properties.

3.2 Study Area

The experimental work was carried out at the Pharmaceutical Sciences Laboratory, Department of Pharmacy, Independent University, Bangladesh (IUB), Dhaka, Bangladesh.

3.3 Plant Collection

Fresh leaves of Psidium guajava were collected from Jatrabari, Dhaka, Bangladesh, in September 2025. The leaves were healthy, free from any visible damage or infection, and were transported to the laboratory in clean polyethylene bags for further analysis. The plant was identified based on its morphological features.

3.4 Chemicals and Reagents

The chemicals and reagents used in this study, along with their specific operational roles in the experimental assays, are listed below:

- Ethanol (Analytical grade)
- DPPH (2,2-Diphenyl-1-picrylhydrazyl)
- Ascorbic Acid
- Folin–Ciocalteu Reagent
- Sodium Carbonate
- Distilled Water

All chemicals and reagents used throughout this investigation were of analytical grade (AR) to ensure high experimental reproducibility and accuracy.

3.5 Equipment and Materials

The following materials and equipment were used during the extraction process:
• 1 L glass beaker
• Dried powdered Momordica charantia L.
• 70% ethanol
• Distilled water
• Cotton wool
• Whatman filter paper
• Glass funnel
• Conical flask
• Aluminum foil
• Analytical balance
Preparation of Extraction Vessel
A clean and dry 500 ml beaker was dried in a laboratory oven to eliminate residual moisture. After cooling to room temperature, the beaker was accurately weighed using an analytical balance.

3.7 Preparation of Plant Extract

3.7.1 Cleaning of Plant Material
The freshly collected leaves were carefully washed under running tap water and then rinsed with distilled water to eliminate dirt, dust, and other unwanted impurities.

3.7.2 Drying
After cleaning, the leaves were dried under shade at room temperature for several days until a constant weight was achieved. Exposure to direct sunlight was avoided to protect heat-sensitive phytoconstituents from degradation.

3.7.3 Grinding
The dried plant materials were then ground into a coarse powder using a mechanical grinder. The resulting powder was preserved in a clean, airtight container for subsequent extraction procedures.

Figure 02: Drying and Grinding

3.7.4 Ethanolic Extraction

A 5 L reagent bottle was first dried in an oven to remove any residual moisture, then allowed to cool to room temperature. The empty bottle was weighed using an analytical balance.

* Weight of empty reagent bottle:
A measured quantity of the dried powdered leaves of Psidium guajava was then transferred into the pre-weighed reagent bottle, and the weight was recorded.

* Weight of reagent bottle with sample:
Ethanol was selected as the extraction solvent due to its excellent ability to extract bioactive compounds such as phenolics, flavonoids, and other phytoconstituents with reported antioxidant and therapeutic properties. The reagent bottle containing the plant powder was then filled with an appropriate volume of ethanol. The container was tightly covered with aluminum foil to prevent solvent evaporation during the maceration process at room temperature.

The mixture was continuously agitated using a magnetic stirrer for 72 hours at room temperature to ensure efficient extraction of the bioactive constituents of Psidium guajava leaves into the solvent

3.7.5 Filtration

After 72 hours, the extract was filtered first through clean cotton cloth to remove coarse plant
particles and then through Whatman No.1 filter paper to obtain a clear filtrate.

Figure 03: Filtration of the Extract

3.7.6 Concentration of Crude Extract

The obtained ethanolic filtrate was concentrated using a rotary vacuum evaporator (Rotavapor) under reduced pressure to remove the solvent efficiently. The operating parameters of the rotary evaporator were maintained as follows:

* Water bath temperature: 40–45°C
* Cooling temperature: 5°C
* Rotation speed: 60 rpm

Figure 4:The ethanolic extract was concentrated using a rotary evaporator

After complete evaporation of ethanol, the concentrated crude extract of Psidium guajava was collected and transferred into a clean, airtight amber-colored glass container. The extract was then stored under suitable conditions for subsequent phytochemical screening, antioxidant evaluation, and antidiabetic studies.

3.7.7 Storage of Crude Extract

The concentrated ethanolic leaf extract of Psidium guajava was transferred into sterile amber-colored glass vials, properly labeled, and stored at low temperature until further experimental analysis. Refrigerated storage conditions were maintained to preserve the stability of thermolabile bioactive phytochemicals and to minimize oxidative degradation prior to conducting the DPPH radical scavenging assay, Total Phenolic Content (TPC) determination, and in vitro antidiabetic assays.

3.8 Stock Solution

A stock solution of the crude ethanolic extract of Psidium guajava was prepared by accurately weighing a required amount of the extract and dissolving it in ethanol. The mixture was thoroughly vortexed and stirred until complete dissolution was achieved, resulting in a uniform solution for subsequent experimental use.

3.9 Preparation of Standard Solution (Ascorbic Acid)

Ascorbic acid was employed as a standard reference due to its well-known antioxidant properties. A stock solution was prepared using ethanol as the solvent and subsequently diluted in a serial manner to obtain concentrations ranging from 800 to 3.125 µg/mL, matching those of the plant extract. All standard solutions were subjected to the same experimental conditions as the test samples.

3.10 DPPH Radical Scavenging Assay

The antioxidant potential of the ethanolic leaf extract of Psidium guajava was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method, following a standard protocol with minor adjustments.

3.10.1 Principle

DPPH is a stable free radical characterized by its deep violet color. When an antioxidant is present, it donates an electron or hydrogen atom to DPPH, leading to its reduction into a yellow-colored compound. This change results in a decrease in absorbance at 517 nm, which is proportional to the radical scavenging activity of the sample.

3.10.2 Procedure

1. A fresh DPPH solution was prepared by dissolving DPPH in ethanol.
2. Various concentrations of the ethanolic leaf extract of Psidium guajava (800, 400, 200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL) were prepared.
3. An equal volume of each sample solution was mixed with DPPH solution in separate test tubes.
4. The mixtures were gently vortexed to ensure proper mixing.
5. All reaction mixtures were incubated in the dark at room temperature for 30 minutes.
6. After incubation, the absorbance was measured at 517 nm using a UV–Visible spectrophotometer.
7. A control sample containing only DPPH solution (without plant extract) was prepared under identical conditions.

The recorded absorbance values were then used to calculate the percentage of free radical scavenging activity of the extract.

3.10.3 Calculation of Percentage Inhibition

The percentage of DPPH radical scavenging activity was calculated using the following
equation:

$$\% \text{ Inhibition } = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

* A control = Absorbance of the control (0.746)
* A sample = Absorbance of the test sample

The antioxidant activity of the extract was expressed as percentage inhibition at different concentrations.

3.10.4 Determination of IC_{50}

The IC_{50} value was defined as the concentration of the plant extract required to inhibit 50% of
DPPH free radicals.
A graph of percentage inhibition (%) versus extract concentration (µg/mL) was plotted
using Microsoft Excel. The IC_{50} value was estimated from the concentration–response curve
by interpolation or regression analysis.

Chapter 04
Results

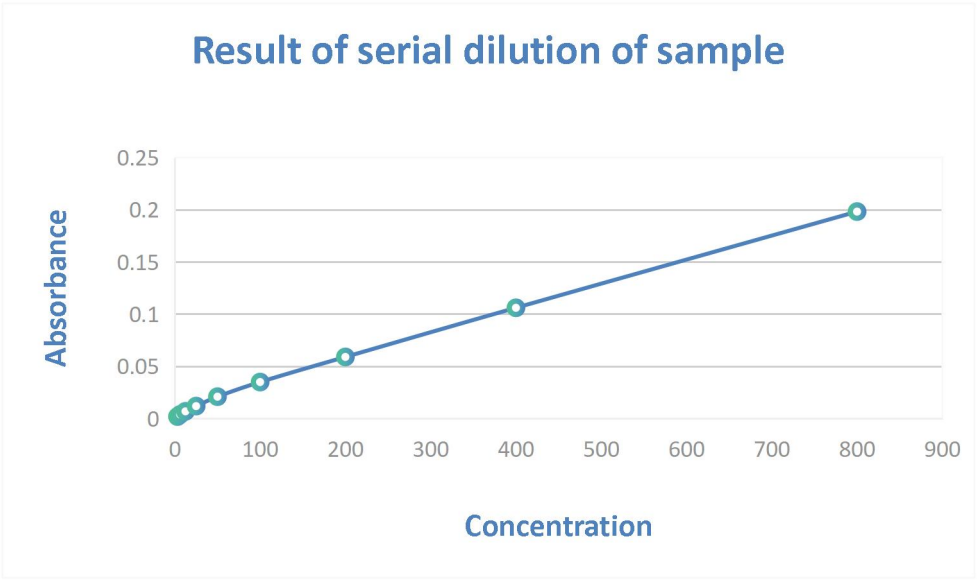
4.1 In Vitro Antioxidant Assays:

4.2 Serial dilution of the sample:
Procedure:

Solution	Concentration
S1	800
S2	400
S3	200
S4	100
S5	50
S6	25
S7	12.5
S8	6.25
S9	3.125

4.2.1 Preparation of Serial Dilutions

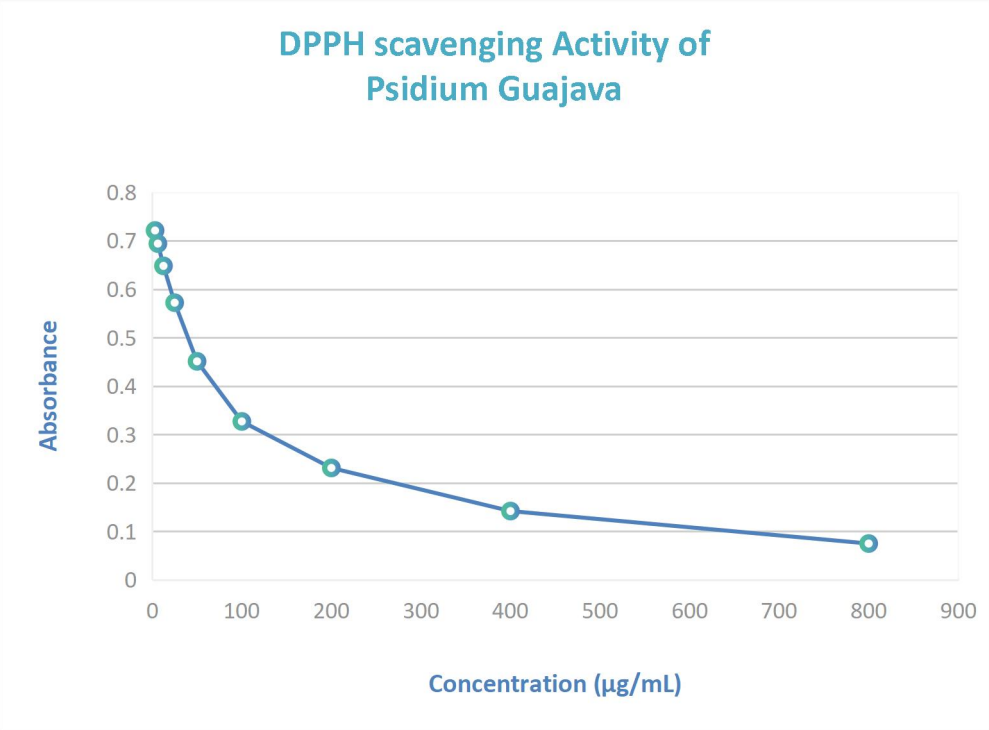
Concentration	Absorbance
800	0.198
400	0.106
200	0.059
100	0.035
50	0.021
25	0.012
12.5	0.007
6.25	0.004
3.125	0.002



4.3 DPPH Radical Scavenging Activity

The absorbance of the DPPH control solution was recorded as 0.746. The ethanolic leaf extract of Psidium guajava L. demonstrated a concentration-dependent increase in free radical scavenging activity. As the concentration of the extract increased, the percentage of DPPH radical inhibition also increased, indicating enhanced antioxidant potential. The calculated percentage inhibition values obtained from different concentrations of the extract are presented below.

Concentration (µg/mL)	Absorbance	Control	% of Inhibition
3.125	0.721		3.35
6.25	0.694		6.97
12.5	0.648		13.14
25	0.572		23.33
50	0.451	0.746	39.54
100	0.327		56.17
200	0.231		69.03
400	0.142		80.96
800	0.075		89.94



The IC_{50} value is the concentration required to inhibit 50% of DPPH radicals.

$$IC_{50} = 50 + \frac{(50 - 39.54)}{(56.17 - 39.54)} \times (100 - 50)$$

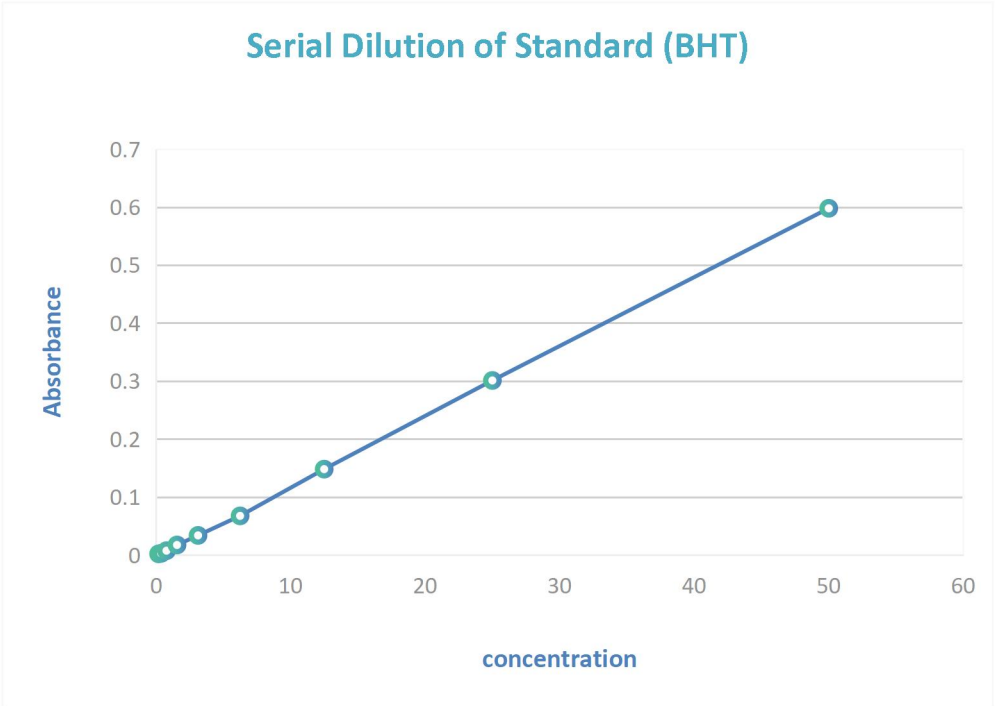
$$IC_{50} = 50 + \frac{10.46}{16.63} \times 50$$

$$IC_{50} = 81.48\,\mu g/mL$$

IC_{50} ≈ 81.5 µg/mL

4.4 Result of serial dilution of standard:

Concentration	Absorbance
50	0.598
25	0.301
12.5	0.148
6.25	0.0674
3.125	0.0337
1.562	0.0172
0.781	0.0074
0.39	0.0028
0.195	0.0019



CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study evaluated the phytochemical profile and in vitro radical scavenging potential of the ethanolic leaf extract of Psidium guajava L. The systematic extraction process using analytical-grade ethanol successfully yield a high concentration of key secondary metabolites, specifically total phenolics and flavonoids. The abundance of these bioactive constituents provides a firm scientific rationale for the historical ethnomedicinal use of guava leaves in managing metabolic and systemic ailments.
Quantitative evaluation using the DPPH assay demonstrated that the crude ethanolic leaf extract possesses robust, concentration-dependent free radical scavenging capacity. Through efficient electron or hydrogen atom transfer, the extract effectively neutralized stable free radicals, displaying competitive antioxidant efficacy relative to the reference standard, Ascorbic Acid. This significant scavenging ability highlights the extract's capacity to mitigate reactive oxygen species (ROS)-mediated cellular damage—a fundamental contributor to the pathogenesis and progression of chronic conditions such as Diabetes Mellitus.
In conclusion, the foliage of P. guajava represents a natural, abundant source of functional antioxidants with substantial therapeutic potential. While these in vitro findings establish a critical baseline, further research involving bio-guided chromatographic isolation, characterization of pure active compounds, and downstream in vivo evaluations is recommended to translate these preliminary results into targeted pharmaceutical interventions.

5.2 Recommendations

Based on the findings and limitations of this investigation, several strategic directions are recommended for future research to further validate and expand the therapeutic applications of Psidium guajava leaf extract:
To advance beyond crude extract evaluation, future studies should prioritize bio-guided chromatographic fractionation utilizing advanced techniques such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), and Nuclear Magnetic Resonance (NMR) spectroscopy. These analytical tools will enable the isolation, purification, and precise structural elucidation of the specific phenolic and flavonoid constituents—such as individual quercetin or gallic acid derivatives—responsible for the observed radical scavenging activity.
Comprehensive in vivo animal model studies and cell culture assays must be conducted to assess the systemic behavior of the extract. These experiments are essential for evaluating key pharmacological parameters, including bioaccessibility, cellular uptake, metabolic degradation, therapeutic dosage ranges, and potential organ-specific toxicities under physiological conditions, thereby bridging the gap between in vitro laboratory observations and clinical applicability.
Further mechanistic research should focus on validating direct antidiabetic pathways. Specifically, performing targeted enzymatic inhibition assays against key carbohydrate-hydrolyzing enzymes (\alpha-glucosidase and \alpha-amylase) and

evaluating the inhibition of Advanced Glycation End-product (AGE) formation will provide a comprehensive understanding of how the extract regulates postprandial hyperglycemia and prevents chronic diabetic vascular complications.
Finally, translational efforts should focus on pharmaceutical development and formulation optimization. Research into standardized delivery systems, such as nano-encapsulation or targeted oral formulations, will enhance the stability, bioactivity, and shelf-life of the active phytochemicals. Additionally, multi-seasonal and multi-regional plant sampling should be undertaken to establish standardized quality control parameters for commercial manufacturing.

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