ANTIOXIDANT AND PHYTOCHEMICAL ANALYSIS ON ETHANOLIC EXTRACT OF *Psidium guajava*

By:

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SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

INSTITUTE OF APPLIED SCIENCE (IAS), KWARA STATE POLYTECHNIC, ILORIN, KWARA STATE, NIGERIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF NATIONAL DIPLOMA (ND)

IN SCIENCE LABORATORY TECHNOLOGY

AUGUST, 2025.

CERTIFICATION

This is to certify that this project was carried out by **JAMES, EMMANUELLA FITIMI** with matriculation number **ND/23/SLT/PT/0369** and it was read and approved as meeting the requirements of Department of Science Laboratory Technology, Institute of Applied Science, Kwara State Polytechnic, Ilorin for the Award of National Diploma (ND) in Science Laboratory Technology.

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DEDICATION

I dedicate this project to the Almighty GOD whose unending mercy has guided my thoughts and efforts throughout this work. This work is also dedicated to my beloved parents, Mr. and Mrs. James, for the great sacrifices that was made on me, whose sacrifices, prayers, and unwavering faith in me have been my greatest strength. May this achievement be a reflection of their love and guidance.

ACKNOWLEDGEMENT

First and foremost, I give all glory and thanks to Almighty God for His unwavering guidance, grace, and strength throughout the course of this project. His divine wisdom and provision sustained me through moments of challenge and clarity alike. Without His favor, the successful completion of this project would not have been possible.

I am deeply grateful to my parents Mr. & Mrs. James and all my siblings for their constant encouragement, support, and prayers. Their belief in my abilities, even in moments when I doubted myself, served as a source of motivation. Their sacrifices, both seen and unseen, played a vital role in making this academic journey a reality for me.

I extend my heartfelt appreciation to my project supervisor, Mrs. Abdulrahman, R.A., whose guidance, constructive criticism, and patience greatly shaped the direction and quality of this work. I also express gratitude to the Head of Department Dr. Usman, A., and the entire staff and lecturers of the Department of SLT at Kwara State Polytechnic for imparting knowledge and providing an environment conducive to learning.

Finally, I acknowledge everyone who contributed in one way or the other towards the success of this project. Your input, no matter how small, is deeply appreciated. May God bless you all abundantly.

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ABSTRACT

Psidium guajava is a widely cultivated tropical plant known for its rich phytochemical profile and diverse medicinal applications, particularly in traditional medicine systems where it is used to manage oxidative stress-related disorders. Antioxidants from plant sources are increasingly sought after as natural alternatives to synthetic agents due to their potential health benefits and lower toxicity. This study aimed to evaluate the phytochemical composition and in vitro antioxidant properties of the ethanolic extract of *P. guajava* leaves. The justification for this investigation lies in the growing scientific and industrial interest in identifying potent natural antioxidants that could be developed into nutraceutical or therapeutic agents, as well as in addressing the variation in reported antioxidant activities due to differences in extraction methods and environmental conditions. Phytochemical screening revealed the presence of tannins, flavonoids, terpenoids, glycosides, alkaloids, and phenols, while saponins, steroids, phlobatannins, and amino acids were absent. In vitro assays demonstrated that the ethanolic extract exhibited strong free radical scavenging activity, with ABTS, DPPH, hydroxyl, and nitric oxide radical inhibition values comparable to but slightly lower than the synthetic antioxidant BHT. However, the extract consistently showed higher IC_{50} values, indicating lower potency than BHT, and exhibited significantly lower ferric reducing power and total antioxidant capacity. These findings confirm that *P. guajava* ethanolic extract is a rich source of bioactive compounds with substantial antioxidant potential, supporting its possible application in the development of natural antioxidant formulations, though further optimization and clinical validation are recommended.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Psidium guajava L., commonly known as guava, is a tropical fruit-bearing tree widely cultivated in many regions worldwide. Its leaves and fruit have been the subject of considerable scientific interest due to their rich content of phytochemicals including phenolic acids, flavonoids, tannins, terpenoids, steroids, and saponins (Kareem & Kadhim, 2024). In particular, ethanolic extracts of guava leaves have demonstrated high levels of total phenolic content (TPC) and total flavonoid content (TFC), both key contributors to antioxidant potential (Kim *et al*., 2024)

Antioxidant phytochemicals also contribute to cosmetics and health-food applications, with studies showing ethanol extracts inhibiting tyrosinase, collagenase, and trans-2-nonenal activity—suggesting skin-whitening and anti-aging potential (Kim *et al*., 2021).

Scaling these findings, industrial interest in encapsulation and stabilization of guava polyphenols is growing, to improve bioavailability and shelf-life (Molecules report, 2023). Thus, the integration of antioxidant and phytochemical analysis supports potential uses in food, pharma, cosmetic, and therapeutic domains.

The ethanolic extraction of Psidium guajava leaves yields a complex mixture of phenolics and flavonoids with well-documented antioxidant capacities. Recent comparative studies show that Soxhlet ethanol extraction offers superior recovery of TPC and TFC compared to other methods such as ultrasonic or microwave-assisted techniques (Fang *et al*., 2023). This variability

underscores the importance of choosing a robust extraction protocol when analyzing bioactive phytochemicals.

Detailed phytochemical profiling by LC–MS and HPLC has identified key compounds including catechin, quercetin, gallic acid, rutin, kaempferol, and guaijaverin. These constituents are known to contribute significantly to radical scavenging, metal chelation, antimicrobial effects, and potential wound healing benefits (Xu *et al*., 2022; Gutiérrez-Montiel *et al*., 2023).

Given the promising antioxidant and biological activities of guava ethanolic extracts, further systematic analysis is warranted. This includes examining extraction yield, phytochemical composition, antioxidant assay results, and linking these to potential applications in medicinal, nutritional, or cosmetic formulations. The present study aims to fill gaps by focusing comprehensively on ethanolic extracts of *P. guajava* leaves, processed via optimized extraction, and analyzed through multiple biochemical assays.

1.2 Statement of problem

The increasing prevalence of oxidative stress-related diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions has driven the demand for safe and effective natural antioxidants. Synthetic antioxidants, though commonly used, have been associated with adverse side effects, raising concerns about their long-term safety. In this context, medicinal plants are gaining attention as rich sources of bioactive compounds with antioxidant and therapeutic properties. *Psidium guajava* (guava) is traditionally used in herbal medicine and is known to contain various phytochemicals, including flavonoids, tannins, and phenols. However, there is limited comprehensive data on the antioxidant capacity and phytochemical profile of its ethanolic

extract, particularly in standardized experimental settings. This knowledge gap poses a challenge in validating and optimizing the use of *Psidium guajava* as a natural alternative to synthetic antioxidants in pharmaceutical and nutraceutical applications. Hence, a scientific investigation into its phytochemical constituents and antioxidant activity is necessary to support its therapeutic potential and promote evidence-based use.

1.3 Justification for the study

The justification for this study lies in the increasing global interest in natural antioxidants and phytochemicals as safer alternatives to synthetic drugs and preservatives, which often pose health risks with prolonged use. *Psidium guajava* (guava) leaves, known for their rich bioactive content, have shown promising therapeutic properties including antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. However, the specific evaluation of the ethanolic extract—particularly its phytochemical composition and antioxidant capacity—remains underexplored in many regions. Ethanol, being both safe and efficient, is an ideal solvent for extracting a wide range of active compounds. By conducting a detailed phytochemical and antioxidant analysis of guava leaf ethanolic extract, this study aims to provide scientific evidence supporting its potential application in pharmaceutical, nutraceutical, and food industries, and contribute to the development of affordable, plant-based therapeutic agents.

1.4 Aim and Objectives

The aim of this study is to evaluate the antioxidant capacity and phytochemical composition of the ethanolic leaf extract of *Psidium guajava*, in order to explore its potential therapeutic and nutraceutical applications.

Specific Objectives:

1. To conduct qualitative and quantitative phytochemical screening of the ethanolic leaf extract of *Psidium guajava*.
2. To determine the total phenolic content (TPC) and total flavonoid content (TFC) of the extract.
3. To evaluate the antioxidant activity of the extract using in vitro assays such as DPPH, FRAP, and ABTS.
4. To identify and quantify specific bioactive compounds present in the extract using analytical techniques such as spectrophotometry or chromatography.
5. To assess the potential health-related applications of the ethanolic extract of *Psidium guajava* based on its phytochemical and antioxidant profile.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Plant Psidium guajava

Psidium guajava L., commonly known as guava, is a perennial tropical fruit tree belonging to the family Myrtaceae, which encompasses more than 140 genera and over 5,500 species, many of which possess significant medicinal and nutritional value (Kumar *et al*., 2021). Taxonomically, it falls under the kingdom Plantae, division Magnoliophyta, class Magnoliopsida, order Myrtales, family Myrtaceae, genus *Psidium*, and species *guajava*. The plant is believed to have originated from tropical America, particularly the region encompassing southern Mexico to Central America, before spreading to Asia, Africa, and other tropical and subtropical areas due to its adaptability and economic importance (Mokhtar & El-Kholy, 2022).

Botanically, *P. guajava* is a small tree or shrub that typically grows 3 to 10 meters in height, although under optimal conditions it can reach up to 15 meters. The stem is hard, with green to reddish-brown bark that exfoliates in thin flakes, a characteristic feature of Myrtaceae species (Egharevba *et al*., 2022). Its leaves are simple, opposite, elliptical to oblong in shape, measuring 5–15 cm in length, with prominent pinnate venation and a rough texture due to the presence of trichomes. The plant produces solitary or clustered white flowers with five petals, numerous stamens, and a pleasant fragrance, followed by berry-like fruits that vary in shape from round to pear-shaped and in color from green to yellow upon ripening. The pulp may be white, pink, or red, containing numerous hard seeds (Patel *et al*., 2021).

The geographical distribution of *P. guajava* spans tropical and subtropical climates worldwide, where it thrives in well-drained soils and tolerates a range of pH conditions from acidic to alkaline. It is cultivated extensively in countries such as India, Nigeria, Brazil, the Philippines, and Thailand, where it serves as an important fruit crop for both domestic consumption and export (Rai *et al*., 2022). Its resilience to drought, pests, and poor soil fertility has contributed to its designation as a hardy fruit tree with minimal cultivation demands (Sharma *et al*., 2023).

Ethnomedicinally, *P. guajava* holds a long-standing role in traditional medicine systems, including Ayurveda, Unani, and traditional African herbalism. Different parts of the plant—leaves, bark, roots, and fruits—are used for various therapeutic purposes. The leaves are most frequently employed for their antimicrobial, antidiarrheal, anti-inflammatory, and antidiabetic properties, often prepared as decoctions or infusions (Omotayo *et al*., 2021). The fruit, rich in vitamin C, dietary fiber, and bioactive phytochemicals, is consumed fresh or processed into juices, jams, and confectioneries, contributing to nutritional health and disease prevention (Rai *et al*., 2022). In folk medicine, guava leaf extracts are traditionally administered for gastrointestinal disorders, wound healing, and respiratory ailments, and recent pharmacological studies have substantiated many of these uses by demonstrating antibacterial, antioxidant, and anti-inflammatory activities (Choudhary *et al*., 2023).

The cultural and medicinal significance of *P. guajava* continues to attract scientific interest due to its abundance of secondary metabolites and its potential applications in functional foods, nutraceuticals, and pharmaceutical formulations. The combination of its adaptability, nutritional richness, and therapeutic value underscores its status as an important medicinal plant with both economic and public health relevance.

2.2 Phytochemicals in Medicinal Plants

Phytochemicals are naturally occurring bioactive compounds produced by plants that contribute to their color, flavor, and resistance to diseases while also providing numerous therapeutic benefits when consumed by humans. They are classified into various categories such as phenolics, alkaloids, terpenoids, glycosides, tannins, saponins, and flavonoids, each with distinct chemical structures and biological activities (Saxena *et al*., 2021). Unlike primary metabolites such as carbohydrates, proteins, and lipids, phytochemicals are secondary metabolites, meaning they are not directly involved in the growth and development of plants but are crucial for defense mechanisms and ecological interactions (Akhtar *et al*., 2022).

Phenolic compounds, including flavonoids and tannins, are among the most studied phytochemicals due to their strong antioxidant properties. These compounds are capable of neutralizing free radicals, thereby reducing oxidative stress and preventing damage to biomolecules such as DNA, proteins, and lipids (Liu *et al*., 2022). Flavonoids, for example, are polyphenolic molecules found abundantly in fruits, vegetables, and medicinal herbs. They possess a wide range of biological effects including anti-inflammatory, antimicrobial, anticancer, and cardioprotective activities (Kumar & Pandey, 2022). Tannins, another major group of phenolic compounds, are known for their astringent properties and their ability to precipitate proteins, making them useful in wound healing and in the treatment of gastrointestinal disorders (Othman *et al*., 2023).

Alkaloids are nitrogen-containing compounds with significant pharmacological activities such as analgesic, antimalarial, and antihypertensive effects. They interact with a variety of molecular

targets in the human body, often influencing the central nervous system and cardiovascular system (Ekor *et al*., 2022). Terpenoids, also known as isoprenoids, represent one of the largest classes of phytochemicals, with functions ranging from antioxidant and antimicrobial activity to anti-inflammatory and anticancer effects (Yang *et al*., 2021). Glycosides are compounds in which a sugar moiety is bound to a non-carbohydrate component, and they often exhibit cardiotonic, anti-inflammatory, and antimicrobial effects (Patel *et al*., 2023).

Saponins are glycosidic compounds known for their ability to form soap-like foams in aqueous solutions. They have been reported to exhibit hypocholesterolemic, anticancer, and immunomodulatory effects, making them valuable in both nutritional and pharmaceutical contexts (Man *et al*., 2022). Phytochemical diversity in medicinal plants not only underpins their therapeutic potential but also contributes to the development of novel drugs and nutraceuticals through bio-prospecting and biotechnological applications.

Advances in analytical techniques such as high-performance liquid chromatography, gas chromatography–mass spectrometry, and nuclear magnetic resonance spectroscopy have greatly improved the identification and quantification of phytochemicals, enabling more accurate correlation between specific compounds and their pharmacological effects (Raza *et al*., 2021). Understanding the complex interactions of these compounds within plant matrices and in the human body remains an active area of research with significant implications for drug discovery and disease prevention.

2.3 Antioxidant Properties of Phytochemicals

Antioxidants are compounds that inhibit or delay oxidative processes by neutralizing reactive oxygen species (ROS) and reactive nitrogen species (RNS) before they can damage biomolecules

such as lipids, proteins, and nucleic acids. Phytochemicals in medicinal plants are well-recognized as natural antioxidants due to their structural features, which enable electron donation, hydrogen atom transfer, and metal chelation (Sharma *et al*., 2022). The antioxidant capacity of these bioactive compounds is essential in reducing oxidative stress, a pathological condition associated with chronic diseases such as cancer, cardiovascular disorders, neurodegenerative diseases, and diabetes (Ayala *et al*., 2022). Vitamin C is known antioxidant with several mechanisms (Figure 2.1).

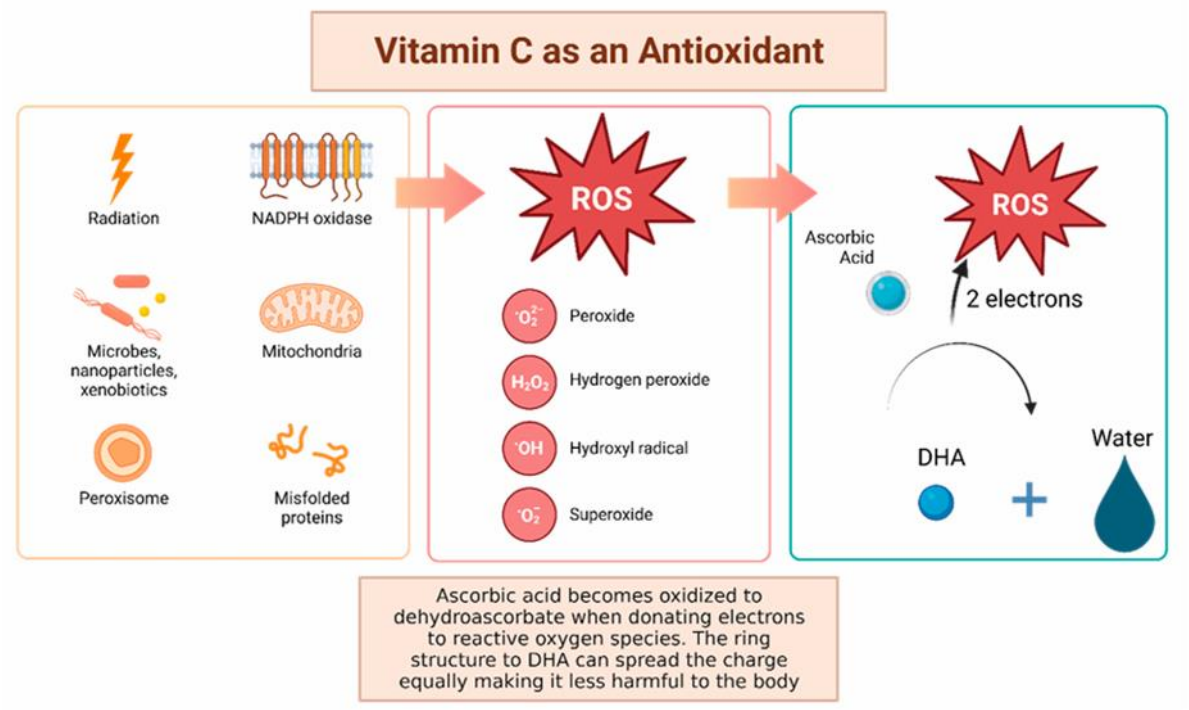

Figure 2.1. Antioxidant Mechanisms of Vitamin C

Source: Didier *et al.* (2023)

Phenolic compounds, including flavonoids, tannins, and phenolic acids, are among the most effective natural antioxidants. Their hydroxyl groups can donate electrons to stabilize free radicals, breaking oxidative chain reactions (Sun *et al*., 2023). Flavonoids in particular can scavenge

superoxide anions, hydroxyl radicals, and peroxyl radicals, and also chelate pro-oxidant metal ions like iron and copper, thereby limiting the Fenton reaction that generates damaging hydroxyl radicals (Wang *et al*., 2021). Tannins also exhibit strong radical-scavenging activity, and their high molecular weight often allows multiple reactive sites for interacting with ROS, enhancing their protective effect against oxidative stress (Kim *et al*., 2022).

Terpenoids, another important class of phytochemicals, have been shown to exert antioxidant effects through modulation of endogenous defense systems such as superoxide dismutase, catalase, and glutathione peroxidase. Certain terpenoids can upregulate the nuclear factor erythroid 2–related factor 2 (Nrf2) signaling pathway, enhancing the expression of antioxidant enzymes and contributing to cytoprotection (Zhang *et al*., 2022). Alkaloids also possess antioxidant potential, though often indirectly, by inhibiting oxidative enzymes and modulating signaling pathways involved in inflammatory and oxidative processes (Akinmoladun *et al*., 2021).

The evaluation of antioxidant activity in phytochemicals commonly employs in vitro assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power (FRAP), nitric oxide scavenging, and hydroxyl radical scavenging assays (Prior *et al*., 2020). These assays measure the capacity of plant extracts or isolated compounds to quench free radicals, reduce oxidized intermediates, or inhibit oxidative processes. Although in vitro tests provide valuable insights, in vivo studies are necessary to fully understand bioavailability, metabolism, and synergistic effects among phytochemicals (Liu *et al*., 2022).

The antioxidant properties of phytochemicals not only protect against oxidative damage but also contribute to their broader pharmacological effects, including anti-inflammatory, anti-aging, and cytoprotective actions. Consequently, medicinal plants rich in phenolics, flavonoids, terpenoids,

and alkaloids are increasingly explored as alternatives to synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which have been associated with potential health risks (Alam *et al*., 2022). This natural antioxidant potential has driven significant interest in the phytochemical composition of plants like *Psidium guajava*, making them valuable candidates for nutraceutical and pharmaceutical applications.

2.4 *Psidium guajava* as a Medicinal Plant

Psidium guajava, commonly known as guava, is a tropical plant widely cultivated in Asia, Africa, and South America for both its nutritional and medicinal values. Its leaves, fruits, bark, and roots have been extensively utilized in traditional medicine for the management of ailments such as diarrhea, diabetes, hypertension, and infections (Roy *et al*., 2023). The therapeutic versatility of *P. guajava* is primarily attributed to its rich phytochemical composition, which includes flavonoids, tannins, phenolic acids, terpenoids, and alkaloids (Gutiérrez *et al*., 2021). These bioactive compounds have been linked to antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer activities, making the plant a valuable candidate for both nutraceutical and pharmaceutical applications (Jiménez-Escrig *et al*., 2022).

Phytochemical analyses have revealed that *P. guajava* leaves are particularly rich in quercetin, catechin, epicatechin, gallic acid, and other phenolic compounds, which confer potent radical scavenging and metal-chelating abilities (Anand *et al*., 2022). These compounds help mitigate oxidative stress by neutralizing reactive oxygen and nitrogen species, thereby reducing the risk of chronic diseases associated with oxidative damage. Additionally, essential oils and terpenoids from guava leaves have demonstrated notable antimicrobial effects against Gram-positive and Gram-negative bacteria, as well as antifungal activity (Nwinyi *et al*., 2021).

The antioxidant properties of *P. guajava* have been confirmed in multiple in vitro assays, including DPPH, ABTS, and FRAP, as well as in vivo models where guava extracts enhanced endogenous antioxidant enzyme activities such as catalase and superoxide dismutase (Shen *et al*., 2021). These effects are thought to be mediated through modulation of oxidative stress-related signaling pathways, such as the Nrf2/ARE pathway, which enhances cellular defense against oxidative injury (Kumar *et al*., 2023). Beyond its antioxidant role, *P. guajava* has been shown to exhibit significant anti-inflammatory effects by inhibiting pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Hossen *et al*., 2022).

The leaves of *P. guajava* have also gained attention for their antidiabetic potential, as they have been reported to inhibit α-glucosidase and α-amylase enzymes, thereby reducing postprandial hyperglycemia (Wei *et al*., 2021). Furthermore, animal studies suggest that guava leaf extract may improve lipid profiles and reduce markers of metabolic syndrome, indicating its potential in cardiometabolic health (Kaur *et al*., 2022). These pharmacological properties are synergistic in nature, meaning that the combined phytochemicals within *P. guajava* may produce greater effects than isolated compounds alone.

Given the abundance of phytochemicals and its broad spectrum of bioactivities, *Psidium guajava* represents a promising source of natural antioxidants and therapeutic agents. Its integration into dietary regimens, herbal formulations, and functional food products aligns with current trends toward plant-based health interventions that aim to reduce reliance on synthetic drugs with potential side effects. The current study builds on this evidence base by exploring the phytochemical profile and antioxidant potential of *P. guajava* ethanolic extracts, thereby contributing to the scientific understanding of its medicinal applications.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Material

A whole fresh plant of *Psidium guajava* was purchased from a local herb seller at *Oja-tuntun* Market, Ilorin, Kwara State, Nigeria. Thereafter, they were cleaned and stored at the Science Laboratory, Kwara State Polytechnic, Ilorin, Nigeria.

3.1.2 Chemicals/Reagents

All chemicals such as, NaOH, KOH and ethanol solvent were products of Loba Chemical. All other chemicals were of analytical grade.

3.2 Methods

3.2.1 Preparation of Extract

The method ethanolic extract of *Psidium guajava* was prepared using the method of Wang *et al.* (2018) with slight modifications. The leaves were cleaned with distilled water, cut into pieces and

dried for 24 hours at 60°C. The sample was ground into powder, and 500 g of the sample was dissolved in 2.5 L ethanol. The solution was left for 24 hours. Thereafter, the solution was filtered using Whatman filter paper. The resulting filtrate was concentrated in water bath at 40°C.

3.2.2 Qualitative Phytochemical Screening

The qualitative phytochemical screening of *P. guajava* ethanolic extract was performed using standard methods described by Odebiyi and Sofowora (1978), with additional protocols from Finar (1986), Kokate (1999), and Yasuma and Ichikawa (1953). Alkaloids were detected by heating the extract with 1% HCl, filtering, and adding Wagner's reagent; a reddish-brown precipitate indicated a positive result. Tannins were identified by mixing the extract with 10% KOH, forming a dirty white precipitate. Phenolics were confirmed with ferric chloride, producing a greenish precipitate. Glycosides were tested by acid hydrolysis followed by Fehling's solution; a brick-red precipitate indicated presence. Saponins were confirmed through persistent froth after shaking. Flavonoids gave a yellow color with 10% NaOH. Steroids showed red coloration upon addition of concentrated sulfuric acid. Phlobatannins formed a red precipitate with 1% HCl. Triterpenes were confirmed by color change to blue-green after sequential addition of acetic anhydride, sulfuric acid, steaming, neutralization, and chloroform. Phytosterols were identified using Liebermann–Burchard's reaction, showing multiple color changes. Fixed oils were detected by oil stains on filter paper. Terpenoids produced a reddish-brown layer at the interface of chloroform and sulfuric acid.

3.2.3 *In vitro* Antioxidant Assays

3.2.3.1 ABTS Radical Cation Decolorization Assay

To assess the *in vitro* antioxidant of the partitioned fractions, 2,2′-Azino-bis radical cation ($ABTS^{\bullet+}$) decolorization was measured as described by Re *et al.,* 1999) with minor modifications. $ABTS^{\bullet+}$ solution was prepared by mixing aqueous ABTS (7 mM) solution with 2.45 mM potassium persulfate (1:1 *v*/*v*) and incubating in darkness at room temperature for 16 h. The working solution was then obtained by diluting $ABTS^{\bullet+}$ solution in methanol to an absorbance of 0.70 ± 0.05 at 734 nm. In each well of a 96 well-plate, 25 µL of TDB sample was added to 200 µL of the working solution. After a slight shake, the plate was covered by an aluminum foil and kept at room temperature for 30 min. Subsequently, the absorbance was recorded by a Multiskan^{TM} Microplate Spectrophotometer (Thermo Fisher Scientific, Osaka, Japan). The ABTS radical decolorizing activity was calculated by the following formula

ABTS radical decolorizing activity (%) = $(1 - A_{sample}/A_{control}) \times 100$

3.2.3.2 Free Radical Scavenging Activity (2,2-diphenyl-1-picrylhydrazyl)

Free radical scavenging activity was determined according to the method of Mensor *et al*. (2001). Concisely, 500 µl of 0.3 mM alcoholic solution of DPPH was added to 2.5 mL of test samples at varying concentrations (250–1000 µg/ml). The samples were incubated in dark for 30 min, and absorbance was measured at 518 nm using UV-visible spectrophotometer (Systronics AU-2700, India). Synthetic antioxidant butylated hydroxytoluene (BHT) were used as positive control. The experiments were performed in triplicates, and scavenging activity was expressed as percentage inhibition, using the following formula.

% Scavenging =([$Abs_{control}$ - $Abs_{samples}$]/ $Abs_{control}$)×100

3.2.3.3 Ferric Reducing Antioxidant Power

FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37˚C for 5 min. Then this solution mixed with certain concentration of the plant extract (80 mL) and incubated at 37˚C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of $FeSO_4$, $7H_2O$ (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions (Benzie and Strain, 1996).

3.2.3.4 Nitric oxide scavenging activity

The nitric oxide scavenging activity was determined according to the method of Marcocci *et al*. (1994). Briefly, 2 ml of the test extracts with varying concentrations (250–1000 µg/ml) were incubated with 0.5 ml of sodium nitroprusside (5 mM) for 2 h at 27°C. Aliquot 1 ml of the incubated solution and mixed with 0.6 ml of Griess reagent (1.0 mL sulfanilic acid reagent [0.33%] in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthyl ethylenediamine dichloride [0.1%]). The absorbance was measured immediately at 550 nm, and synthetic antioxidant BHT was used as positive control. The experiments were performed in triplicates, and scavenging activity was expressed as percentage scavenging, using the following formula.

% Scavenging =([$Abs_{control}$ - $Abs_{samples}$]/ $Abs_{control}$)×100

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Percentage Yield of *P. guajava*

After the preparation of *P. guajava* ethanolic extract using 500 g of the plant sample, 92 g of the extract was obtained.

$$\% \text{ Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Sample}} \times 100$$

$$= \frac{92 \text{ g}}{500 \text{ g}} \times 100$$

$$= 18.4 \%$$

4.1.2 Phytochemical Screening

The phytochemical screening of *Psidium guajava* ethanolic extract revealed the presence of tannins, flavonoids, glycosides, terpenoids, alkaloids, phenols (Table 4.1). However, steroids saponins, phlobatannins and amino acids were in phytochemical screening were not present.

Table 4.1: Phytochemical Screening of *Psidium guajava* Ethanolic Extract

S/N	Phytochemical Class	Results
1	Tannins	+
2	Saponins	-
3	Flavonoids	+
4	Terpenoids	+
5	Glycosides	+
6	Phlobatannins	-
7	Alkaloids	+
8	Phenols	+
9	Steroids	-
10	Amino acids	-

Keys:

+ = Present

- = Absent

4.1.3 *In vitro* Antioxidant Assays

The extract and BHT showed notable ABTS percentages inhibition of 94.01 % and 94.33 % respectively (Figure 4.1). However, the IC_{50} of the extract (19.95) was significantly higher ($p<0.05$) compared to that of BHT (13.90). In the same pattern, for hydroxyl radical scavenging, the extract and BHT exhibited percentages inhibition of 89.96 % and 95.00 % respectively (Figure 4.2), with the extract (30.07) having a significantly higher ($p<0.05$) IC_{50} compared to BHT (16.82).

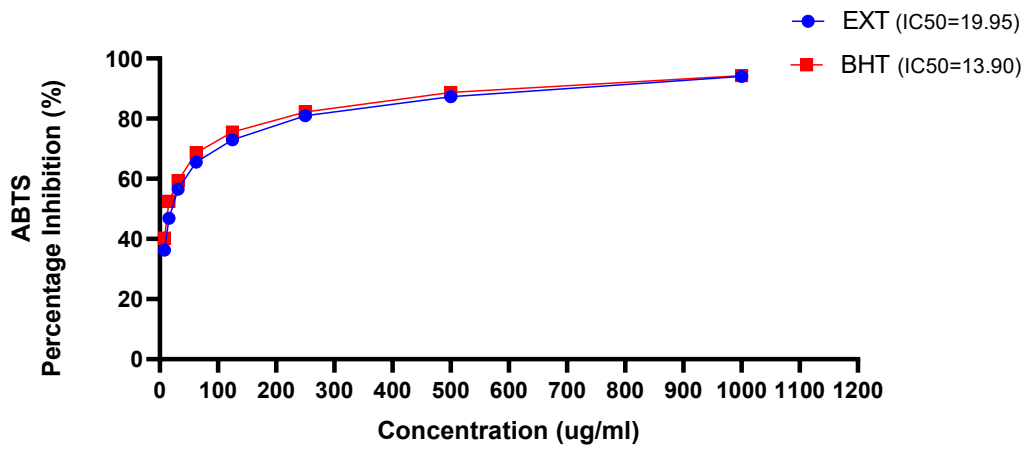

Figure 4.1. ABTS Percentage Inhibition of *P. guajava* Ethanolic Extract

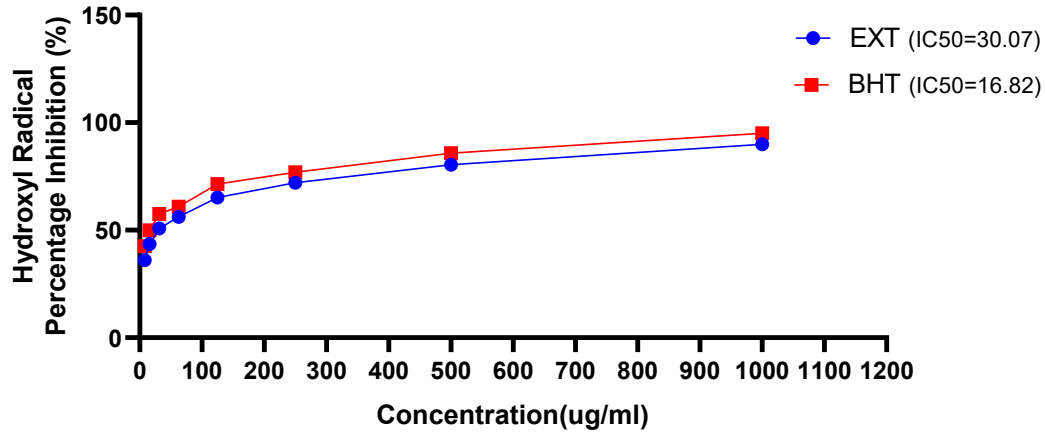

Figure 4.2. Hydroxyl Radical Scavenging Activity of *P. guajava* Ethanolic Extract

The DPPH percentages inhibition of the extract and BHT were of 87.22 % and 89.80 % respectively (Figure 4.3). However, the IC50 of the extract (23.64) was significantly higher ($p<0.05$) compared to that of BHT (15.53). In the same pattern, for nitric oxide radical scavenging, the extract and BHT exhibited percentages inhibition of 87.90 % and 93.85 % respectively, with the extract (33.73) having a significantly higher ($p<0.05$) IC50 compared to BHT (16.35).

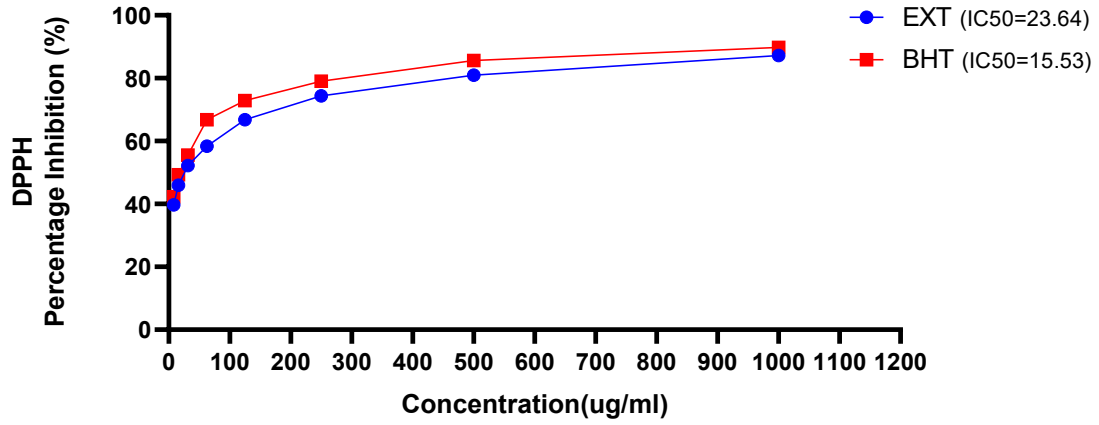

Figure 4.3. DPPH Scavenging Activity of *P. guajava* Ethanolic Extract

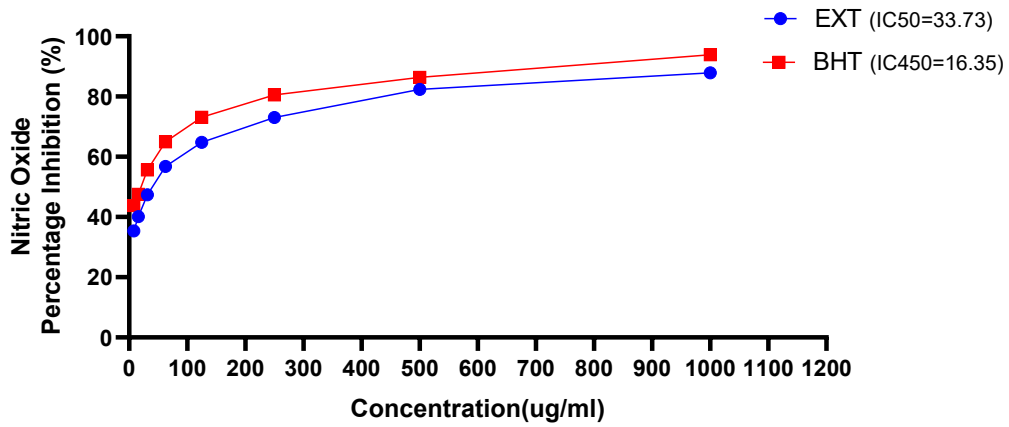

Figure 4.4. Nitric Oxide Radical Scavenging Activity of *P. guajava* Ethanolic Extract

The ferric reducing power of the extract was significantly lower ($p<0.05$) compared to BHT (Figure 4.5). Although the extract showed notable antioxidant properties, its total antioxidant capacity was significantly lower ($p<0.05$) compared to BHT (Figure 4.6).

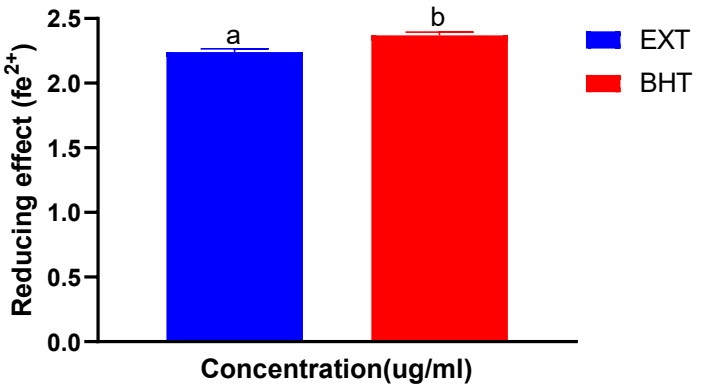

Figure 4.5. Ferric Reducing Effect of *P. guajava* Ethanolic Extract

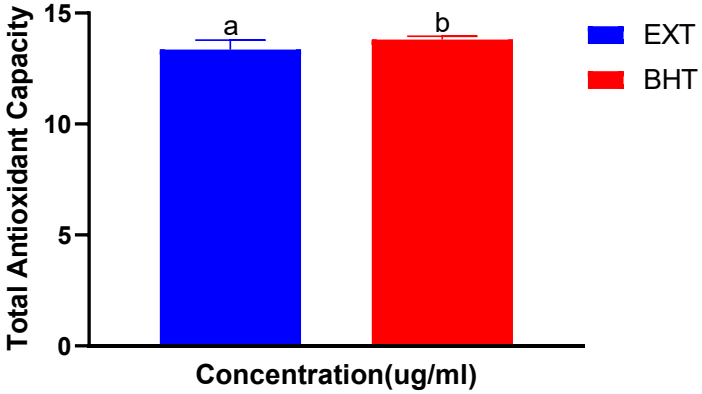

Figure 4.6. Total Antioxidant Capacity of *P. guajava* Ethanolic Extract

4.2 Discussion

The phytochemical composition of the ethanolic extract of *Psidium guajava* obtained in this study confirms the presence of several classes of bioactive secondary metabolites, including tannins, flavonoids, alkaloids, glycosides, terpenoids and phenols. These compounds have been widely documented as the primary contributors to the medicinal value of *P. guajava* and many other tropical plants. Flavonoids and phenolic compounds are of particular interest due to their strong antioxidant potential, which is mainly attributed to their capacity to donate hydrogen atoms or electrons, scavenge reactive oxygen and nitrogen species and chelate pro-oxidant metals (Zhu *et al*., 2023; Salehi *et al*., 2022). Tannins also exert potent free radical scavenging activity and are known to form complexes with metal ions, thereby reducing metal-catalyzed oxidative processes (Baharfar *et al*., 2021). Terpenoids have been shown to modulate oxidative stress by upregulating endogenous antioxidant defenses, while alkaloids can act as radical scavengers and enzyme inhibitors that contribute to redox homeostasis (Niu *et al*., 2022; Vuolo *et al*., 2019). The absence of saponins, steroids and certain nitrogenous compounds such as amino acids in the extract may be linked to the selective solubility of plant constituents in ethanol. This agrees with earlier studies which emphasize that the polarity and extraction efficiency of solvents significantly influence the yield and type of phytochemicals extracted (Do *et al*., 2014; Karak, 2019).

The high radical scavenging potential demonstrated in ABTS, DPPH, hydroxyl and nitric oxide assays suggests that the ethanolic extract of *P. guajava* can effectively neutralize diverse free radicals through multiple antioxidant mechanisms. Although the extract exhibited slightly lower activity than the synthetic antioxidant BHT, such performance is noteworthy given that plant extracts are complex mixtures of various compounds, each contributing synergistically to the overall antioxidant effect rather than acting as single high-potency agents (Shahidi &

Ambigaipalan, 2018). This synergism often translates into broad-spectrum biological activity and greater safety margins compared to synthetic antioxidants, which are sometimes associated with adverse health effects at high concentrations (Shalaby & Shanab, 2013). The higher IC50 values of the extract compared to BHT may reflect the lower specific activity of individual compounds in the extract; however, the substantial inhibition percentages across different radical systems demonstrate its functional relevance as a natural antioxidant.

The moderate ferric reducing power and total antioxidant capacity observed in comparison to BHT could be attributed to the qualitative and quantitative composition of phenolic acids, flavonols and flavones in the extract. Previous analyses of *P. guajava* have shown that while the plant is rich in compounds such as quercetin, kaempferol, gallic acid and catechin, their relative proportions influence the dominant antioxidant mechanism, with some favoring hydrogen atom transfer reactions over single electron transfer (Jiménez-Escrig *et al*., 2001; Barbalho *et al*., 2021). This mechanistic preference explains why high radical scavenging potential does not always correlate directly with high reducing power. The ability of the extract to scavenge nitric oxide radicals is particularly significant, as nitric oxide overproduction is implicated in inflammatory processes and the pathogenesis of cardiovascular and neurodegenerative disorders (Sengupta *et al*., 2022).

The implications of these findings extend to the potential application of *P. guajava* ethanolic extract in functional foods, nutraceuticals and phytotherapeutics aimed at preventing or managing oxidative stress-related diseases. Oxidative stress is a common underlying factor in the pathophysiology of chronic conditions such as diabetes mellitus, atherosclerosis, hypertension, Alzheimer's disease and certain cancers (Liguori *et al*., 2018). Several recent studies have reported that *P. guajava* leaf extracts can significantly reduce oxidative biomarkers, improve lipid profiles and enhance endogenous antioxidant enzyme activities in both in vitro models and animal studies

(Chiari-Andréo *et al*., 2020; Barbalho *et al*., 2021; Zhu *et al*., 2023). The observed phytochemical diversity of the ethanolic extract positions it as a promising source of natural antioxidants that could serve as safer alternatives to synthetic compounds in the food and pharmaceutical industries. Furthermore, the high extraction yield obtained in this study highlights the feasibility of large-scale production, which is essential for sustainable application.

Considering the abundance of *P. guajava* in tropical and subtropical regions, and its cultural acceptance as both a medicinal and dietary plant, the development of antioxidant-rich products from its leaves could have significant public health benefits, particularly in regions where synthetic antioxidants are costly or less accessible. However, the translation of these in vitro antioxidant properties into clinical efficacy requires further in vivo studies, bioavailability assessments and safety evaluations. This aligns with growing interest in plant-derived antioxidants as functional ingredients in chronic disease prevention strategies and as replacements for controversial synthetic agents like BHT and BHA (Kähkönen *et al*., 1999; Shahidi & Ambigaipalan, 2018).

Conclusion

The reviewed literature clearly demonstrates that *Psidium guajava* is a phytochemically rich plant containing bioactive compounds such as flavonoids, phenols, tannins, terpenoids, alkaloids, and glycosides, all of which contribute to its strong antioxidant potential. Both in vitro and in vivo studies have consistently linked these phytochemicals to significant radical scavenging capacity, ferric reducing ability, and modulation of oxidative stress-related pathways. Despite substantial evidence on its pharmacological benefits, there remain variations in reported antioxidant activity that are largely attributable to differences in extraction methods, plant part used, environmental conditions, and analytical techniques. These gaps justify the need for standardized extraction and

comprehensive in vitro evaluation of *P. guajava* ethanolic extracts to establish their comparative efficacy and potential for development into therapeutic and nutraceutical products.

Recommendations

- Future research should focus on optimizing extraction protocols to maximize the recovery of antioxidant-rich phytochemicals from *P. guajava*.
- Comparative studies using standardized antioxidant assays across different geographical sources and plant parts will help to harmonize findings.
- Advanced chromatographic and spectroscopic techniques should be employed for precise characterization of active compounds, coupled with bioassay-guided fractionation to identify the most potent antioxidant constituents.
- Additionally, clinical trials are necessary to validate the efficacy and safety of *P. guajava* extracts in human populations, which will support its integration into evidence-based phytomedicine and functional food industries.

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