ANTROXIDANT AND PHYTOCHEMICAL ANALYSIS ON ETHANOLIC EXTRACT OF PSIDIUM GUAJAVA

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2025/2026 SESSION

CERTIFICATION

This is certify that this project is the original work carried out and reported by

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Submitted to the Department of Science Laboratory technology, Biochemistry unit, Institute Of Applied Sciences (IAS) Kwara State Polytechnic Ilorin and it has been approved In partial fulfillment of the requirements of the Award of National Diploma (ND) In Science Laboratory Technology

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(HOD SLT)

DEDICATION

We dedicate this project work to Almighty God and our parents

ACKNOWLEDGEMENT

Our Appreciation goes to God, Our Supervisor in person of MRS. ABDULRAHMAN R. A. and our parents for making this project work a success

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ABSTRACT

Psidium guajava (Guava.) belongs to the Myrtaceae family and it is an important fruit in tropical areas like India, Indonesia, Pakistan, Bangladesh, and South America. The leaves of the guava plant have been studied for their health benefits which are attributed to their plethora of phytochemicals, such as quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid. Extracts from guava leaves (GLs) have been studied for their biological activities, including anticancer, antidiabetic, antioxidant, antidiarrheal, antimicrobial, lipid-lowering, and hepatoprotection activities. 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.

CHAPTER ONE

1.0 Introduction

1.1 Background of the study

Psidium guajava, commonly known as guava, is a plant of significant medicinal importance belonging to the Myrtaceae family. Widely distributed across tropical and subtropical regions, including Asia, South America, and Africa, guava has been traditionally utilized for its therapeutic properties in managing conditions such as inflammation, diabetes, hypertension, gastrointestinal disturbances, and febrile illnesses. Its broad-spectrum pharmacological relevance is attributed to its diverse phytochemical composition, which includes alkaloids, flavonoids, tannins, saponins, sterols, glycosides, and phenols. These bioactive compounds have been extensively studied for their potential roles in antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities, further supporting their integration into modern medicinal practices. Emerging research continues to validate the phytochemical richness of Psidium guajava leaves, demonstrating their efficacy in modulating various biological processes. For instance, flavonoids such as quercetin and its derivatives have shown promise in antimicrobial and anticancer studies, while tannins exhibit potent astringent and antioxidant properties, making them valuable in managing infections and oxidative stress-related disorders. Saponins and glycosides, commonly present in guava, are associated with membrane-permeabilizing effects and glycemic control, highlighting their pharmacodynamic potential. Ethanol and hydroalcoholic extractions of guava leaves have been consistently reported as the most effective methods for isolating these bioactive compounds due to their polarity and solubility characteristics (Biomed Pharmacol J., 2024)

.Despite the abundant evidence of its pharmacological benefits, the application of *Psidium guajava* in evidence-based medicine remains underexplored. Current investigations focus on qualitative and quantitative profiling of phytochemicals, aiming to establish a robust scientific basis for its use in clinical formulations. Studies have also identified limitations in the methodological standardization of phytochemical analyses, with variability in solvent selection, extraction techniques, and regional differences affecting outcomes (Fang *et al.*, 2023). Addressing these gaps is critical to enhancing the therapeutic potential of guava leaves and ensuring their safe application in healthcare. This study builds upon existing knowledge by analyzing the phytochemical composition of *Psidium guajava* (guava) leaf extracts using a range of solvents. Through systematic profiling, the research aims to identify key bioactive constituents, emphasizing their pharmacological significance and providing foundational data for future preclinical and clinical investigations. This approach seeks to contribute to the scientific validation of guava leaves as a versatile medicinal resource, bridging traditional knowledge with contemporary medical science (Aliya, F. A., 2023).

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*., 2022).

Scaling these findings, industrial interest in encapsulation and stabilization of guava polyphenols is growing, to improve bioavailability and shelf-life (Molecules report, 2021). Thus, the integration of antioxidant and phytochemical analysis supports potential uses in food, pharma, cosmetic, and therapeutic domains.

1.2 Justification of the Study

The ethanol extract of *Psidium guajava* (guava) leaf offers an excellent opportunity to study the plant's bioactive compounds in relation to antimicrobial efficacy. Ethanol is a commonly used solvent in phytochemical extractions, providing a broad spectrum of both polar and non-polar compounds that may contribute to the plant's therapeutic effects (Kumari et al., 2020).

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 (Atanasov. *et al*., 2022).

By addressing the critical gaps in the current literature, this study will contribute valuable insights into the use of natural products as an alternative to synthetic antibiotics. The findings could pave the way for the development of new plant-based therapies, offering a safer, more sustainable solution to the problem of antimicrobial resistance.

1.3 Statement of the 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 (Butler, M. S. 2021).

The urgent need for new antimicrobial agents is more critical than ever. While synthetic antibiotics have been the cornerstone of infectious disease management for decades, there is a growing realization of the limitations and risks associated with over-relying on these agents. In this regard, natural products offer a promising solution. Historically, plant-derived compounds have provided a significant source of antimicrobial agents, with several modern drugs being based on natural plant extracts.

Among the vast array of medicinal plants, *Psidium guajava* (guava) has attracted attention for its diverse pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. Despite its widespread use in traditional medicine, limited scientific research has been conducted to fully explore its antimicrobial potential and the mechanisms underlying its bioactivity. While some studies have suggested its efficacy against bacterial and fungal infections, there remains a gap in understanding the full scope of its antimicrobial activity, particularly against multi-drug-resistant strains (Butler, M. S., & Buss, A. D. 2006).

1.4 Aims and Objectives

The primary aim of this study is to analysis the antioxidant and physicochemical properties of ethanolic extracts of Psidium guajava leaf using a computational approach. This study seeks to evaluate the bioactive compounds present in the plant and their antimicrobial activities, which could contribute to the development of novel natural antimicrobial agents.

Objectives of the Study:

To achieve the aim of this study, the following specific objectives will be pursued:

1. To carry out ethanolic extraction of *Psidium guajava* leaves, isolating the bioactive components from the plant material for further analysis.
2. To analyze the bioactive compounds, present in *Psidium guajava* ethanolic extract using Gas Chromatography-Mass Spectrometry (GC-MS), identifying and quantifying the chemical constituents responsible for the antimicrobial activity.
3. To predict the physicochemical properties of the bioactive compounds in *Psidium guajava* (guava) extract using the SwissADME server (Swiss Analysis of Molecular Drug-like Properties), assessing key parameters such as solubility, lipophilicity, and toxicity, to evaluate their suitability as potential drug candidates.

1.5 Scope of the Study

This study is focused on evaluating the GC-MS analysis, and physicochemical properties of the ethanolic extract of *Psidium guajava* (guava) leaves. The research will be conducted within the following parameters:

This study will be based on the extraction of *Psidium guajava* leaves sourced from [insert location if necessary, or state "locally sourced" or "commercially available"]. The plant material will be authenticated to ensure the correct species is used in the research. Only the leaves of Psidium guajava will be used in this study, as they are traditionally known for their medicinal properties. The extraction will be carried out using ethanol as a solvent, which is effective in isolating both polar and non-polar bioactive compounds (Küfrevioğlu, Ö. İ., 2023).

The GC-MS analysis will be used to identify and quantify the bioactive compounds present in the ethanolic extract of *Psidium guajava*. Only volatile compounds detected through this method will be considered. The analysis will focus on determining the chemical composition and identifying potential active constituents responsible for antimicrobial properties.

The physicochemical properties of the bioactive compounds in the ethanolic extract will be assessed using the SwissADME server. Parameters such as solubility, lipophilicity, toxicity, and drug-likeness will be predicted, and only compounds with high potential for drug development will be considered. The study will not delve into the in vivo toxicity or long-term stability of the compounds. The study will include molecular docking studies to predict the interaction of identified compounds with microbial targets. The research will be limited to computational predictions, and experimental validation of molecular interactions will not be conducted Cowan, M. M. (2016).

CHAPTER TWO

2.0 Literature Review

The literature review serves as the foundation upon which this study is built, providing a comprehensive understanding of previous research related to natural products, medicinal plants, and their applications in drug discovery. It contextualizes the present study within the broader scientific conversation surrounding the use of computational tools in natural product research (Patel *et al*., 2021).

In recent decades, there has been a resurgence of interest in plant-derived compounds due to the rising challenge of antimicrobial resistance and the limitations associated with synthetic drugs. Medicinal plants, such as *Psidium guajava* (commonly known as Guava), have long been valued for their therapeutic properties and are now being re-examined through modern scientific lenses, including phytochemical analysis and computational predictions (Egharevba *et al*., 2022).

According to Huynh (2025), research on guava leaf bioactives showed that 70% ethanol extracts contained diverse phenolic acids and flavonoids—including quercetin, kaempferol, gallic acid, and chlorogenic acid—identified via HPLC–MS/MS methods. These compounds significantly reduced lipid peroxidation and elevated glutathione levels in oxidative stress models. The study emphasized that these bioactives are promising for encapsulation in formulations targeting functional foods, cosmetics, and pharmaceuticals.

According to El-Feky and AbdelRahman (2025), harvest time and soil–plant interactions profoundly influenced phytochemical content and biological activities of *Psidium guajava* leaves. Leaves harvested in March exhibited TPC up to 435 mg GAE/g, compared to ≈294 mg/g in August. March samples also showed stronger DPPH and ABTS scavenging activities and greater enzyme inhibition (COX-2, 5-LOX), suggesting that environmental and temporal factors significantly affect extract potency.

2.1 Overview of Natural Products in Medicine

Natural products have served as a cornerstone in the development of medicinal agents for centuries, with traditional knowledge forming the basis of modern pharmacology. Derived from plants, animals, and microorganisms, natural products are composed of a wide variety of bioactive compounds, many of which exhibit significant pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Newman & Cragg, 2020).

Historically, natural remedies formed the bulk of early healthcare systems, especially in indigenous and traditional medicine. Over time, scientific research has confirmed the medicinal efficacy of many of these substances, leading to the isolation and synthesis of active compounds. Notable all of which have shaped modern drug development (Atanasov et al., 2021).

Natural products continue to play a crucial role in contemporary drug discovery, particularly in the search for novel antimicrobial agents. As antimicrobial resistance (AMR) becomes a critical global health threat, attention has shifted towards bioactive compounds from natural sources, which are perceived to have fewer side effects and are often structurally distinct from synthetic compounds, making them effective against resistant strains (Lahlou, 2013).

Plant-based natural products, especially those extracted from leaves, roots, seeds, and barks, are rich in secondary metabolites such as alkaloids, flavonoids, terpenoids, phenols, and saponins. These compounds contribute to the plants' defense mechanisms and often translate to therapeutic benefits in humans. Current pharmacological research increasingly incorporates modern analytical techniques, such as Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC), to identify these bioactive constituents.

In recent years, the integration of in silico approaches and computational biology into natural product research has further enhanced the efficiency of screening for potential drug candidates. Computational tools now enable researchers to predict drug-likeness, toxicity, bioavailability, and molecular interactions of natural compounds, making natural product research more cost-effective and time-efficient (Jiménez-Luna et al., 2021).

In summary, natural products remain indispensable in the field of drug development, especially as a response to global challenges such as antimicrobial resistance. Their unique chemical structures, coupled with centuries of traditional use and advancing analytical technologies, make them vital candidates for novel therapeutic agents.

2.2 Botanical and Pharmacological Overview of Psidium Guajava

Psidium guajava, commonly known as guava, is a plant of significant medicinal importance belonging to the Myrtaceae family. Widely distributed across tropical and subtropical regions, including Asia, South America, and Africa, guava has been traditionally utilized for its therapeutic properties (Choudhary *et al*., 2023).

2.2.1 Biological Activity of Psidium Guajava

Modern-day researchers have been guided to study guava extracts by the long history of guava use. In various clinical trials, its conventional usage against diarrhea, gastroenteritis and other digestive problems has been validated. In a report that included 17 medicinal Thai plants, Guava leaf demonstrated anti-proliferative activity, which was 4.37 times more than vincristine, on antiproliferative effects on human mouth epidermal carcinoma and murine leukemia cells using MIT assays. It was shown that bark and leaf extracts had an in vitro toxic action against various bacteria. Gallo catechin isolated from the guava leaf methanol extract demonstrated antimutagenic activity against E. Coli. Guava extracts of water and chloroform were successful in activating Salmonella typhimurium mutagenecity. Compared to tea tree oil (TTO), doxycycline and clindamycin antibiotics, the antimicrobial activities of P. guajava and leaf extracts, calculated by the disk diffusion method (inhibition zone), were compared. P. guajava leaf extracts have been shown to be helpful in treating acne, especially those that have anti-inflammatory activities. The active ß flavonoid compound extracted from leaves quercetin-3-Oalpha-larabinopyranoside (guaijaverin) - has a high potential for anti-plaque activity by inhibiting Streptococcus mutants formation. In a study performed by the disc diffusion system, Guava leaf extract inhibited the growth of Streptococcus aureus (Kumari, P., *et al.* 2015).

2.2.2 Chemical Composition of Psidium Guajava

i. Fruit

Vitamin C, vitamin A, iron, calcium, Manganese, phosphoric, oxalic and mallic acids, saponins combined with oleanolic acid. Morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside, flavonoids, guaijaverin, Quercetin. Essential oil contains hexanal, -2-hexenal, 2,4- hexadienal, 3-hexenal, 2-hexenal, 3-hexenyl acetate and phenol, while β-caryophyllene, nerolidol, 3-phenylpropyl acetate, caryophyllene oxide, pentane-2-thiol, 3-penten-2- ol and 2-butenyl acetate, 3-hydroxy-2-butano3-methyl-1- butanol, 2,3- butanediol, 3-methylbutanoic acid, (Z)-3- hexen-1-ol, 6- methyl-5-hepten-2-one, limonene, octanol, ethyl octanoate(pink guava fruit).

ii. Leaves

α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, βbisabolene, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene, mallic acids, nerolidol, β-sitosterol, ursolic, crategolic, and guayavolic acids, cineol, quercetin, 3-L-4-4- arabinofuranoside (avicularin) and its 3-L-4-pyranoside (Essential oil), resin, tannin, eugenol, caryophyllene (1a α, 4a α-, 7 α-, 7a β-, 7b α-)]-decahydro-1H-cycloprop[e] azulene, Guajavolide (2 α-,3 β-,6 β-,23-tetrahydroxyurs12-en-28,20 β-olide; 1) and guavenoic acid (2 α-,3 β-,6 β-, 23-tetrahydroxyurs- 12,20(30)-dien-28-oic acid, triterpenes oleanolic acid, triterpenoids, flavinone-2 2'- ene, prenol, dihydro benzophenanthridine and crypto nine.

iii. Root

Tannin, leucocyanidin, sterols, gallic acid, carbohydrates, salts, tannic acid.

iv. Seed

Proteins, starch, oils, phenolic, flavonoid compounds, flavones glycoside, quercetin-3-O-β-D-(2''- Ogalloyglucoside)- 4'-O-vinylpropionate.

2.2.3 Pharmacological Actions of Psidium Guajava Leaf

1. Antibacterial activity

The aqueous and organic extracts of psidium guajava leaves revealed antibacterial activity against Staphylococcus aureus, Proteus spp., and Shigella spp. While no activity against Citrobacter spp, Alcaligenes fecalis, and Aspergillus spawns observed. The aqueous extracts of psidium guajava leaves, roots and stem bark were active against the grampositive bacteria Bacillus subtilis and virtually inactive against the gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa. The aqueous, alcohol and chloroform extracts of leaves were effective against Aeromonas hydrophila, Shigella sppand Vibrio spp, Staphylococcus aureus, Sarcina lutea and Mycobacterium phlei. The antimicrobial activity of P. guajava is attributed to guajaverine, psydiolic acid and the flavonoid compound guaijaverin. Antimicrobial activity against

Propionibacterium acne was demonstrated by the essential oils γ-terpinene and γ-pinene (Daina, A., 2020).

2. Anti-inflammatory activity

A significant anti-inflammatory activity is provided by the essential oil, the aqueous, the alcoholic, the methanolic and the ethyl acetate extracts. Meanwhile, benzophenone glycosides, sesquiterpenes, and leaf distilled flavonoids are claimed as allergy inhibitors (Jiménez-Luna, J., 2021).

3. Anti cough action

Guava leaf has long been used to treat diseases such as cough and lung diseases in Bolivia and Egypt. The aqueous extract decreased the frequency of capsaicin aerosolinduced cough within 10 minutes after the extract was administered intra peritoneal. More than 5 g/kg was the LD50 of guava leaf extract. These findings indicated that the extract of guava leaf is recommended as a cough remedy Miller, B. (2002).

4. Anti- oxidant activity

Recent studies have shown that P. guajava is an excellent source of antioxidant phytochemical. High antioxidant activity was shown by the methanolic and extract of leaves. The active principles are quercetin, quercetin-3- O-glucopyranoside, Morin, ascorbic acid, carotenoids, and polyphenolics (Newman, D. J., 2024).

5. Anti-diabetic activity

The ethanolic stem bark extract in alloxan-induced hyperglycemic rats displayed statistically significant hypoglycemic activity but was devoid of significant hypoglycemic effect in normal and normal glucose loaded rats (OGTT). The water extract demonstrated statistically significant hypoglycemic activity at an oral dose of 250 mg/kg in both acute and sub-acute studies.

6. Anti-diarrheal activity

The P. guajava L. leaves. This activity is explained by spasmolytic, antibacterial and anti-amoebic effects, and phytochemical such as flavonoids and tannins have been documented to exhibit anti-diarrheal activity by denaturing protein, thereby producing protein-tannate interactions that decrease the permeability of the intestinal mucosa. In addition, the calcium-antagonist properties of quercetin, the biologically active drug, clarify the spasmolytic effect of this popular herbal remedy Parekh, J., & Chanda, S. V. (2018).

7. Antiulcer activity

P.guajava possesses acid secretion inhibitory effect of antiulcer activity in the aspirin-induced gastric ulcer model mediated by prostaglandins (Semalty, M., *et al.* (2017).

8. Spermatoprotective activity

Extracts of Psidium guajava Linn's leaves. They have beneficial effects on the development and quality of sperm 0, and may thus boost the sperm parameters of infertile males with Nono obstructive azoospermia and oligospermia.

2.3 Ethanolic Extraction and Its Relevance

Extraction is a fundamental step in the isolation and identification of bioactive compounds from medicinal plants. It plays a critical role in phytochemical research by helping to isolate compounds of interest in a concentrated and bioavailable form. Among various extraction methods, ethanol is widely recognized as one of the most effective and commonly used solvents due to its ability to extract a broad range of phytochemicals (Taheri, M., *et al.* 2022).

Ethanol as a Solvent: Ethanol is a polar organic solvent that is particularly suitable for extracting both polar and some non-polar compounds, including flavonoids, tannins, phenolics, saponins, alkaloids, and terpenoids (Tiwari et al., 2011). Its relatively low toxicity, ease of availability, and compatibility with food and pharmaceutical applications make it a preferred choice in both laboratory and industrial settings (Banu K.S. & Cathrine, L. 2017)..

Advantages of Ethanolic Extraction:

- Wide Solubility Spectrum: Ethanol can extract a diverse group of phytochemicals, ensuring a more comprehensive phytochemical profile than many other solvents.
- Safety: It is considered safe for human use and suitable for pharmaceutical formulations.
- Antimicrobial Stability: Ethanolic extracts often exhibit greater stability and enhanced antimicrobial activities compared to aqueous extracts due to better solubilization of active compounds (Azwanida, 2025).
- Compatibility with Analytical Tools: Ethanol-based extracts are ideal for subsequent analyses using techniques like Gas Chromatography-Mass Spectrometry (GC-MS), which requires solvent systems that do not interfere with compound separation or detection.

CHAPTER THREE

3.0 Methodology

3.1 Materials

Guava Leaves, Ethanol, Container, Filtration, Rotary Evaporator, Water Bath, Shaker, Centrifuge, Refrigerator, Aluminum Foil or Black Cloth, Muslin Cloth, Distilled water, Latex and rubber gloves, Funnel, Filter paper, Conical flask, Foil paper, Stirring rod.

3.1.1 Plant Material

Psidium guajava materials include compounds like flavonoids, tannins, phenols, essential oils (containing terpenes like limonene and caryophyllene), alkaloids, vitamin C, and fiber. These compounds are found in various parts of the plant, particularly the leaves, but also the bark, roots, and fruit. The leaves are rich in polyphenols and specific flavonoids like quercetin and gallic acid, while the bark contains significant amounts of tannin.

- ***Leaves:*** A rich source of flavonoids, phenols, tannins, and essential oils, which contribute to its diverse medicinal properties.
- ***Bark:*** Contains a high percentage of tannin, along with other polyphenols and resins.
- ***Roots:*** Also contain tannin and other bioactive compounds.
- ***Fruit:*** While not as heavily studied for its phytochemicals as the leaves, the fruit is a significant source of vitamins, carbohydrates, fiber, and fatty acids.

3.1.1.1 Identification of Plant

The plants studied were identified at the Department of Science Laboratory Technology, Kwara State Polytechnic Ilorin, to be known as Psidium guajava commonly known as (guava)

3.1.2 Reagent and Kits of Psidium Guajava

ethanol extract of Psidium guajava (guava), reagents used for analysis are; methanol, chloroform, and ethyl acetate for chromatography, and visualization reagents such as iodine solution and 5% ethanolic ferric chloride for Thin Layer Chromatography (TLC). Commercially available, or laboratory-prepared, kits aren't typically used with the extract itself; instead, standard components of these kits, like antioxidant assays (e.g., DPPH) or antibacterial assays (e.g., with prepared microbial cultures and antibiotics like gentamicin), are employed to test the extract's biological activities.

3.2 Methods

3.2.1 Collection of plant sample

Fresh *Guava* leaves was obtained from Kwara State Polytechnic, Ilorin. The leaves collected were cleaned, rinsed, and air-dried for 15 days. After drying, the leaves will be ground into fine powder using a mortar and pestle and preserved in a refrigerator.

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	-

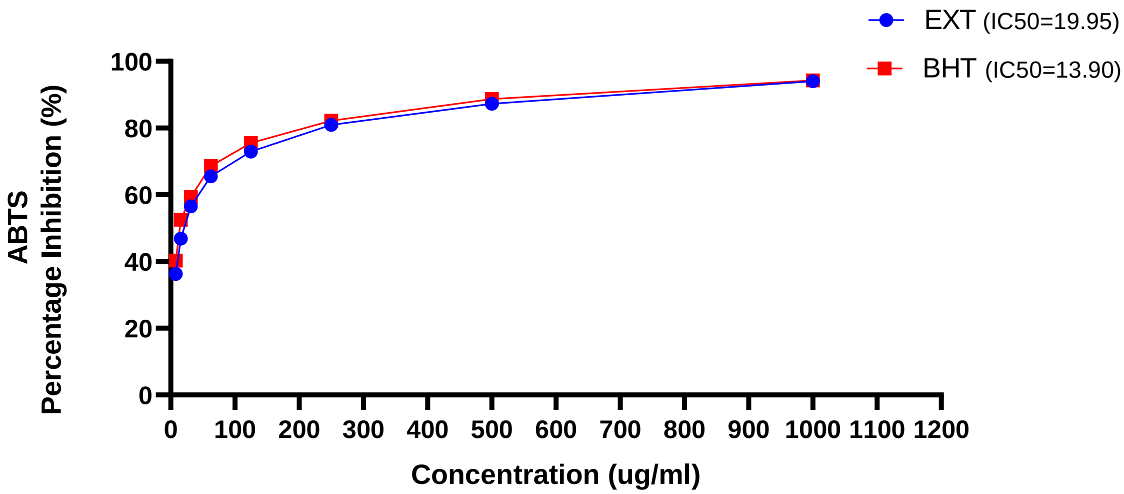
Keys:

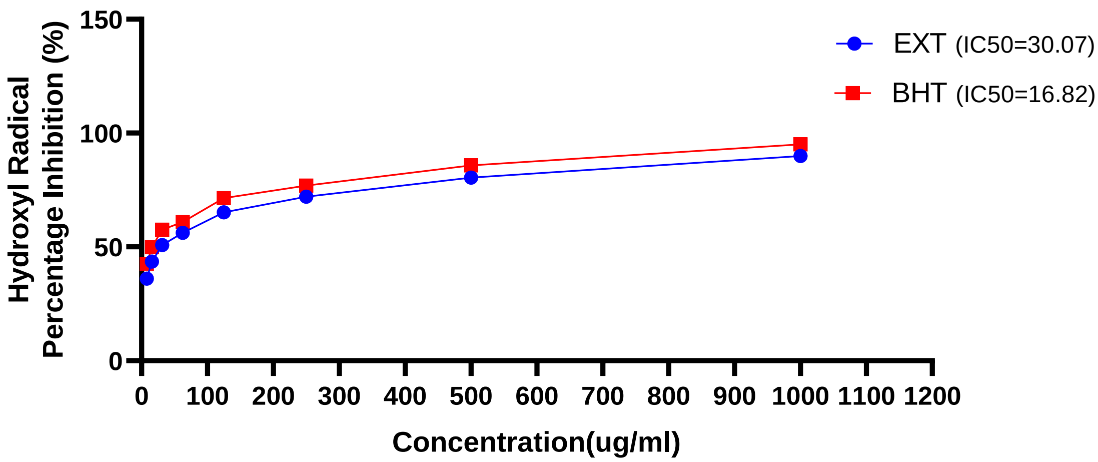
+ = Present

- = Absent

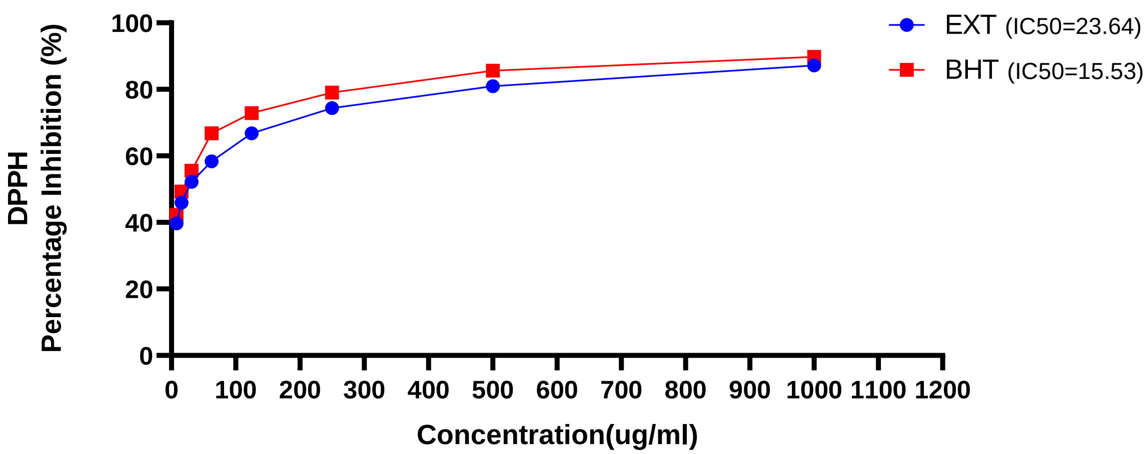
4.1.3 *In vitro* Antioxidant Assays

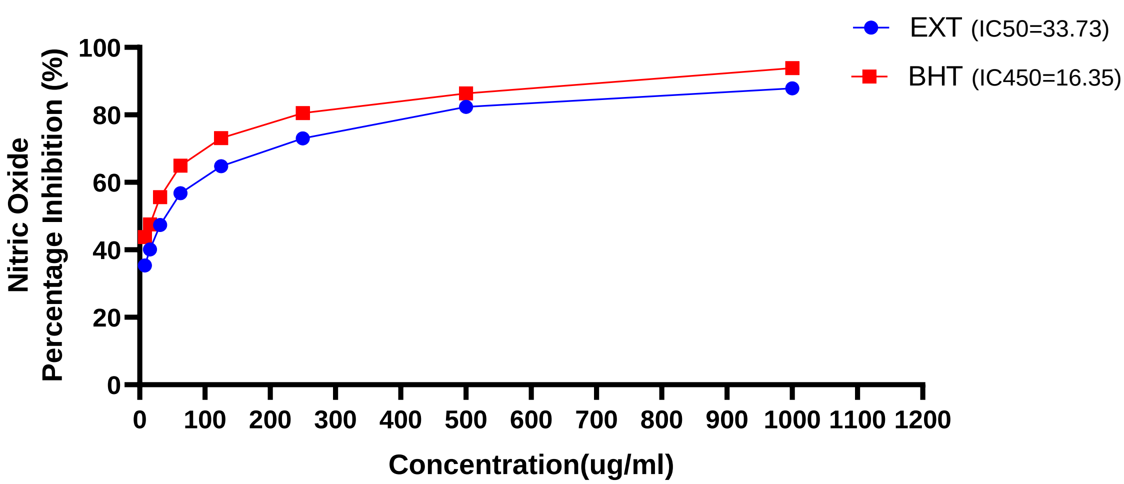
The extract and BHT showed notable ABTS percentages inhibition of 94.01 % and 94.33 % respectively (Figure 4.1). However, the IC_{50} of the extract (19.95) was significantly higher ($p<0.05$) compared to that of BHT (13.90). In the same pattern, for hydroxyl radical scavenging, the extract and BHT exhibited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



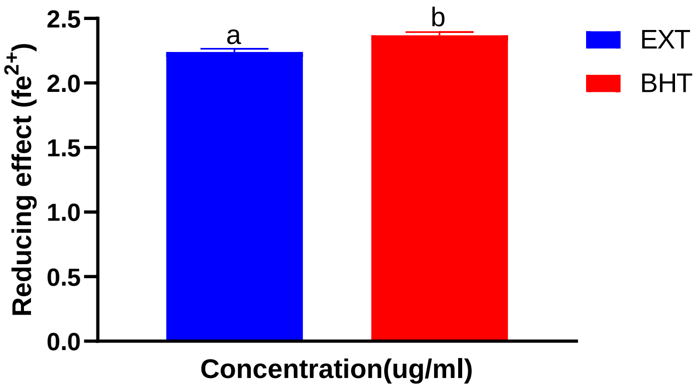


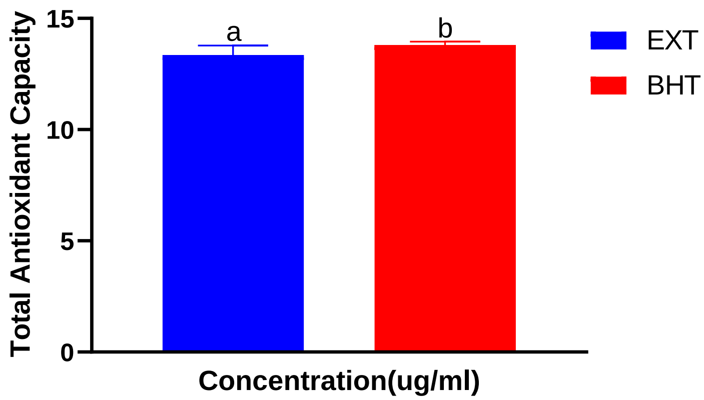
The DPPH percentages inhibition of the extract and BHT were of 87.22 % and 89.80 % respectively (Figure 4.3). However, the IC50 of the extract (23.64) was significantly higher ($p<0.05$) compared to that of BHT (15.53). In the same pattern, for nitric oxide radical scavenging, the extract and BHT exhibited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





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


Reducing effect (fe2+)
2.5
2.0
1.5
1.0
0.5
0.0
a
b
EXT
BHT
Concentration(ug/ml)


Total Antioxidant Capacity
15
10
5
0
a
b
EXT
BHT
Concentration(ug/ml)

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.

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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