Comparative Phytochemical, Antioxidant and Antimicrobial Profiling of the Aqueous and Ethanolic extracts of *Piper guineense* (African Pepper) Fruit and Seed

BY

Cynthia Ilekenri IKONOMWAN (Mrs)
PG/LSC2415204

UNIVERSITY OF BENIN
BENIN CITY

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A THESIS WRITTEN IN THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY AND SUBMITTED TO THE COLLEGE OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF POSTGRADUATE DIPLOMA (PGD) IN SCIENCE LABORATORY TECHNOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

DECEMBER, 2025

CERTIFICATION

We certify that this work was carried out by **Cynthia Ilekenri IKONOMWAN (Mrs)** in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

_____ _____

Prof. E.O. Oshomoh **Date**
Supervisor

_____ _____

Prof. E.O. Oshomoh **Date**
PG. Coordinator

_____ _____

Prof. J.O. Osarumwense **Date**
Head of Department

APPROVAL

I hereby certify that this work was accepted in partial fulfillment for the award of Postgraduate Diploma (PGD) in the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City

_____ _____

PROF. D.N. IZEKOR **Date**
Provost, College of Post Graduate Studies

CERTIFICATION OF THESIS

We attest and declare that the thesis titled **Comparative Phytochemical, Antioxidant and Antimicrobial Profiling of the Aqueous and Ethanolic extracts of *Piper guineense* (African Pepper) Fruit and Seed** has successfully passed the anti-plagiarism test and does not violate any copy right regulation.

_____ _____

Prof. E.O. Oshomoh **Date**
Supervisor

_____ _____

Cynthia Ilekenri IKONOMWAN (Mrs) **Date**
(Student)

DEDICATION

This project work is dedicated to God Almighty, who has made it possible for me to be alive till this day and has provided for me and sustained me.

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ABSTRACT

The relentless rise of multidrug-resistant pathogens, coupled with the deleterious health impacts of oxidative stress, has intensified the global search for novel, safe, and effective bioactive compounds from natural sources. *Piper guineense* (Schumach. and Thonn), (Family- Piperaceae) commonly known as African black pepper, West African black pepper**,** Ashanti pepper, or uziza is renowned for its rich phytochemical profiles and antimicrobial activities. The aim of this study was to access the phytochemicals, antioxidants and antimicrobial profiling of the aqueous and ethanolic extracts of *Piper guineense* fruit and seed.

Samples of commercial *Piper guineense* fruits and seeds were sourced from Local Markets in Benin City and identified by lecturers in the Plant Biology and Biotechnology laboratory, University of Benin while the microbial isolates used were obtained from stock cultures of clinical isolates stored from cases of Nosocomial infections from University of Benin teaching Hospital (UBTH). The phytochemical, antioxidants and antimicrobial properties were assayed using standard methods. Also antimicrobial activities of the plant extracts were investigated using agar well diffusion methods. Data obtained for the different parameters were subjected to statistical analysis using the analysis of variance.

The results of the qualitative phytochemical composition of ethanolic and aqueous extracts of *P. guinense* revealed the presence of alkaloids, flavonoids, phenols, saponins, terpenoids, glycosides and steroids were present in ethanolic and aqueous extracts of *P. guinense* while anthraquinones was present in ethanolic extract and absent in aqueous extract. The quantitative phytochemical composition of ethanolic and aqueous extracts of *P. guinense* shows that Saponins (10.05±1.05 mg/g) had the highest composition, followed by phenolics (8.51±2.56 mg/g) in aqueous extract while flavonoids (12.10±2.17 mg/g) had the highest composition in ethanolic extract of *P. guinense.*. Also, aqueous extract (22.82%) had the highest percentage yield compared to ethanolic extract (17.87%). Zone of inhibition of the aqueous fruit and seed extract of *Piper guineense* on bacterial isolates ranged from 6.6±1.6 - 12.9±1.1mm while zone of on fungal isolates ranged from 4.5±1.5 - 11.3±1.5 mm respectively. Zone of inhibition of the ethanolic fruit and seed extract of *Piper guineense* on bacterial isolates ranged from 6.6±1.6 - 23.5±1.6 mm while zone of on fungal isolates ranged from 3.5±1.6 - 21.5±2.5 mm respectively. The Minimum Inhibitory Concentration ranged from 0.9 – 8 mg/ml for aqueous extract and 0.9 - 8 mg/ml for ethanolic extract. Minimum Bactericidal/Fungicidal concentrations (MBCs/MFCs) in aqueous ranged from 4 – 12 mg/ml and 0.9 – 8 mg/ml for ethanolic extracts. DPPH for aqueous extract ranged from 0.756±0.66 (10 µg/mL) to 0.306±0.21 (200 µg/mL), ethanolic extract range from 0.623±0.31 (10 µg/mL) to 0.226±0.05 (200 µg/mL) compared to control (Ascorbic acid) which range from 0.421±0.13 (10 µg/mL) to 0.110±0.04 (200 µg/mL). Percentage inhibition of aqueous extract ranged from 13.5±0.41 (10 µg/mL) to 56.12±0.66 (200 µg/mL), ethanolic extract ranged from 17.65±0.41 (10 µg/mL) to 78.62±0.66 (200 µg/mL) while control (ascorbic acid) range from 49.81±0.11 (10 µg/mL) to 98.01±0.35 (200 µg/mL). ethanolic extract (262.2 ± 7.3 µmol Fe^{2+}/g) had the highest reducing power compare to ethanol extract (133.6 ± 11.3 µmol Fe^{2+}/g) when compared to control (ascorbic acid) 615.5 ± 10.6 7.3 µmol Fe^{2+}/g. The antimicrobial and antioxidant activities observed in *Piper guinense* fruit and seeds aqueous and ethanolic extracts is due to the presence of phytochemicals. The use of *Piper guinense* in folk medicine is therefore recommended

CHAPTER ONE

1.0 INTRODUCTION

The relentless rise of multidrug-resistant pathogens, coupled with the deleterious health impacts of oxidative stress, has intensified the global search for novel, safe, and effective bioactive compounds from natural sources (World Health Organization [WHO], 2021). In this context, medicinal and culinary plants from the Piperaceae family, renowned for their rich phytochemical profiles, have garnered significant scientific interest. *Piper guineense* Schumach. and Thonn., commonly known as African black pepper, West African pepper, or Ashanti pepper, is a climber indigenous to the tropical rainforests of West and Central Africa (Okwu and Ighodaro, 2010). Beyond its widespread use as a spice and food preservative, *P. guineense* holds a venerable position in traditional African medicine, where it is employed to treat a myriad of ailments including rheumatism, bronchitis, gastrointestinal disorders, and infectious diseases (Nwozo *et al*., 2021). This traditional usage strongly implies the presence of potent secondary metabolites with significant biological activities.

Piper guineense is a rich reservoir of diverse phytochemicals, notably alkaloids (such as piperine and piperanine), flavonoids, tannins, saponins, lignans, and essential oils, which are believed to underpin its pharmacological properties (Oloyede *et al*., 2020). These secondary metabolites are integral to the plant's defense mechanisms and are increasingly recognized for their therapeutic potential in humans. Notably, many of these compounds, particularly flavonoids and phenolic acids, are established antioxidants capable of neutralizing free radicals, chelating metal ions, and inhibiting lipid peroxidation, thereby offering protection against oxidative stress-related pathologies like cancer, cardiovascular diseases, and neurodegeneration (Almeida *et al*., 2021).

Concurrently, extracts from *P. guineense* have demonstrated broad-spectrum antimicrobial activity against a range of bacteria and fungi, including resistant strains. This activity is often attributed to the synergistic or individual actions of its key phytoconstituents, such as piperine, which can disrupt microbial cell membranes and inhibit vital enzymes (Dzoyem *et al.*, 2014). The convergence of antioxidant and antimicrobial properties in a single plant makes *P. guineense* an exceptional candidate for development as a source of natural preservatives, nutraceuticals, or complementary therapeutic agents. Therefore, a systematic investigation into the phytochemical composition, antioxidant potential, and antimicrobial efficacy of *P. guineense* extracts is crucial. Such research not only validates its ethnomedicinal uses but also provides a scientific foundation for its potential application in modern food, pharmaceutical, and cosmetic industries to address contemporary health challenges.

1.1 Background to the Study

The escalating crisis of antimicrobial resistance (AMR) poses a profound threat to global public health, rendering many conventional antibiotics increasingly ineffective and necessitating the urgent search for novel therapeutic agents (WHO, 2021). Concurrently, the role of oxidative stress in the pathogenesis of numerous chronic and degenerative diseases has driven scientific interest in compounds that can mitigate cellular damage (Munteanu and Apetrei, 2021). In this dual quest for new bioactive compounds, plant-derived metabolites have re-emerged as a cornerstone of scientific investigation, offering a vast, untapped reservoir of chemical diversity with potential therapeutic applications.

Plants have co-evolved with pathogens and environmental stressors, producing a sophisticated array of secondary metabolites, collectively known as phytochemicals, as part of their defense mechanisms (Tungmunnithum *et al*., 2018). These compounds, which include alkaloids,

flavonoids, tannins, saponins, and phenolic acids, are not only crucial for the plant's survival but also exhibit a wide spectrum of biological activities relevant to human health. Many of these phytochemicals demonstrate significant antimicrobial properties by disrupting microbial cell membranes, inhibiting enzyme activity, or interfering with nucleic acid synthesis (Tungmunnithum *et al*., 2018; Sharma *et al*., 2022). Furthermore, a substantial proportion of these metabolites, particularly phenolics and flavonoids, are potent antioxidants. They function as free radical scavengers, metal chelators, and reducing agents, thereby protecting biological systems from oxidative damage (Sharma *et al*., 2022).

Fruits and seeds represent particularly rich and specialized repositories of these bioactive compounds. Fruits often synthesize phytochemicals to attract dispersers and protect against pests, while seeds amass concentrated reserves of defensive compounds to ensure the survival of the embryo (Almeida *et al*., 2021). Investigating the extracts of these plant parts, therefore, provides a strategic approach to discovering novel antimicrobial and antioxidant agents. Evaluating the antimicrobial efficacy, profiling the phytochemical constituents, and quantifying the antioxidant capacity of such extracts forms a fundamental triad in ethnopharmacological and phytochemical research. This integrative analysis not only validates traditional medicinal uses but also provides a scientific basis for the potential development of standardized plant-based therapeutics, functional food ingredients, or preservatives to combat infectious diseases and oxidative stress-related pathologies.

Piper guineense Schumach. and Thonn., commonly known as West African black pepper or ashanti pepper, is an aromatic climbing plant widely cultivated and utilized across West and Central Africa. Its fruit (dried berries) and seeds serve not only as culinary spices but also as culturally significant medicinal ingredients in traditional healing systems. Across Nigeria, Ghana,

Cameroon, and Benin, the fruits and seeds of *Piper guineense* are routinely incorporated into remedies for postpartum care, respiratory ailments, gastrointestinal disturbances, and infectious diseases (Isikhuemen *et al.,* 2020). The rich ethnomedicinal usage of this species highlights the importance of understanding its chemical constituents, which are responsible for its flavour, aroma, and bioactivity.

Phytochemicals plant-derived secondary metabolites play crucial ecological and physiological roles in plants and also exhibit diverse biological activities relevant to human health. Classes such as alkaloids, phenolics, flavonoids, terpenoids, tannins, saponins, and steroids are known to modulate antimicrobial, antioxidant, anti-inflammatory, analgesic, and chemoprotective pathways. Profiling these compounds in *Piper guineense* fruit and seed facilitates a scientific understanding of its therapeutic potential. Although several preliminary studies have identified major secondary metabolite classes in the plant, phytochemical expression varies with plant organ, environmental conditions, and extraction technique, making systematic profiling essential for accurate characterization (Kpomah *et al.*, 2019). Advanced analytical technologies, particularly gas chromatography mass spectrometry (GC–MS), have enhanced the ability to characterize individual chemical constituents in spices such as *P. guineense*. GC–MS investigations of its seeds have revealed numerous volatile and semi-volatile compounds, including monoterpenes (beta-myrcene, alpha-pinene), sesquiterpenes (caryophyllene, germacrene D), phenylpropanoids (dillapiol, myristicin), fatty acids (oleic acid, hexadecanoic acid), and various alcohols and esters (Kpomah *et al.*, 2019). These compounds are associated with pronounced antimicrobial, antioxidant, and anti-inflammatory properties that support the traditional uses of the plant.

Beyond volatile compounds, qualitative and quantitative screenings have also reported abundant flavonoids, alkaloids, tannins, phenols, carotenoids, and saponins in extracts of *Piper guineense* fruit and seed (Tungmunnithum *et al*., 2018). Alkaloids and flavonoids, in particular, are strongly linked to antimicrobial and cytoprotective functions, while phenolic compounds contribute significantly to antioxidant potential. The combination of these phytochemicals makes the fruit and seed of *Piper guineense* promising sources of natural therapeutic agents and functional food components. Given the global interest in plant-based bioactive compounds and the increasing use of indigenous spices in nutraceutical and pharmaceutical formulations, an in-depth phytochemical profiling of *Piper guineense* fruit and seed is not only timely but necessary. A comprehensive chemical characterization can help standardize its medicinal preparations, authenticate its biological properties, and provide baseline data for future pharmacological studies. Furthermore, understanding the distribution of phytochemicals between the fruit and seed may reveal organ-specific bioactivities that could influence cultivation, processing, and industrial applications.

Previous phytochemical studies have revealed that *Piper guineense* contains diverse classes of secondary metabolites known for strong antioxidant activities. Qualitative and quantitative analyses have consistently reported the presence of alkaloids, phenolics, flavonoids, tannins, and terpenoids in extracts of the fruit and seed (Oloyede *et al*., 2020). Phenolic compounds and flavonoids, in particular, are recognized as major contributors to antioxidant capacity due to their ability to donate electrons and hydrogen atoms while stabilizing reactive species. In addition, GC MS analyses have identified numerous terpenes and aromatic compounds in the seeds including caryophyllene, dillapiol, myristicin, and various fatty acids which may also contribute to antioxidant and anti-inflammatory activities (Kpomah *et al.*, 2019).

Despite these findings, comprehensive antioxidant profiling of *Piper guineense* fruit and seed remains limited. Variations in extraction solvent, plant part, geographical origin, and analytical methods can influence antioxidant outcomes, making systematic assessment necessary. A detailed evaluation using assays such as 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, Ferric Reducing Antioxidant Power (FRAP), 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and total phenolic and flavonoid quantification can provide deeper insights into the antioxidant strength and phytochemical contributions of each plant organ (Nwozo *et al*., 2021). Understanding how antioxidant constituents differ between the fruit and the seed is particularly valuable for optimizing their application in herbal formulations, functional foods, and natural preservatives.

Given the rising global demand for safe, natural antioxidants and the increasing recognition of African indigenous spices as reservoirs of bioactive compounds, investigating the antioxidant profile of *Piper guineense* is both relevant and scientifically significant. A thorough antioxidant profiling will not only enhance knowledge of its bioactive components but also support its potential integration into pharmaceutical, nutraceutical, and food industries. Plants naturally produce a wide range of secondary metabolites with antimicrobial potency, including alkaloids, phenolics, flavonoids, terpenoids, and essential oils. These compounds can disrupt microbial cell walls, inhibit protein synthesis, interfere with nucleic acid replication, or disrupt membrane integrity. Studies have reported that extracts of *Piper guineense* fruit and seed exhibit inhibitory activity against various bacterial and fungal pathogens, suggesting broad-spectrum antimicrobial potential (Adomi *et al.*, 2024). Such bioactivity is consistent with the rich phytochemical profile of the plant, which includes constituents known for antimicrobial action such as piperine, dillapiol, eugenol, and caryophyllene.

The fruit and seed of *Piper guineense* may differ in their phytochemical composition, extraction efficiency, and antimicrobial potency. Variations in solvent polarity, extraction method, and plant part have been shown to influence antimicrobial outcomes, underscoring the need for systematic profiling of each component. Methods such as agar well diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC) offer valuable insight into the antimicrobial power of plant extracts. Evaluating the antimicrobial properties of both the fruit and seed can help identify which part of the plant offers the greatest therapeutic potential and guide future standardization for herbal formulations (Akinmoladun *et al*., 2019).

1.2 Aim and Objectives

1.2.1 Aim

The aim of this study was to determine the phytochemicals, antioxidants and antimicrobial profiling of the aqueous and ethanolic extracts of *Piper guineense* fruit and seed

1.2.2 Objectives

The objectives of this study were to:

1. determine the phytochemical constituents in extracts of *Piper guineense* fruit and seed
2. evaluate the antimicrobial activity of the aqueous and ethanolic extracts of *Piper guineense* fruit and seed.
3. determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the aqueous and ethanolic extracts of *Piper guineense* fruit and seed
4. determine the antioxidant properties of the aqueous and ethanolic extracts of *Piper guineense* fruit and seed

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of *Piper guineense*

Piper guineense Schumach. and Thonn., commonly known as West African black pepper**,** Ashanti pepper, or uziza, is a culturally and economically important spice plant native to West and Central Africa (Oparaji, Nwachukwu, and Okoli, 2016). While the plant's leaves are popular in traditional cuisine, the fruit and seed are the most valued components due to their potent aroma, flavor, and medicinal properties. Morphologically, it is similar to other members of the *Piper* genus, such as *Piper nigrum*, but can be distinguished by specific leaf and fruit characteristics (Adebayo *et al.*, 2015). The growth habit of *P. guineense* is that of a woody climber or scrambling shrub that extends several meters in length by twining around nearby vegetation (Iwu *et al*., 2025). Its stems are slender, flexible, and jointed, with noticeable nodes from which leaves and inflorescences arise. The leaves are simple, arranged alternately, and broadly ovate to elliptic in shape, typically measuring between 8 and 15 centimeters in length. The leaf apex is acuminate, and the base is rounded to slightly heart-shaped, while the surface is glabrous with a prominent midrib and reticulate venation. When crushed, the leaves emit a characteristic peppery aroma due to their essential oil content (Aké *et al*., 2019).

The plant produces spicate inflorescences that are slender, cylindrical, and generally 2 to 5 centimeters long. The flowers are small, apetalous, and inconspicuous, occurring separately on male and female inflorescences, although both types may be present on the same plant (Adebayo

et al., 2015). Each flower consists primarily of bracts and reproductive structures without well-defined petals or sepals. The fruit develops as small, globose to ellipsoid berries borne densely along the spike. As the fruit matures, it changes from green to reddish-brown and finally black when dried. Each fruit contains a single seed and is rich in bioactive compounds, including piperine and volatile essential oils, which account for its pungency and aroma (Oparaji *et al*., 2016).

The seeds of *P. guineense* are small, hard, and dark brown to black when fully dried. They serve as the primary source of the plant's pungency and are extensively used as a spice. The thin seed coat encloses a small, oily endosperm that contributes to its aromatic and medicinal properties (Essiett *et al*., 2010). Ecologically, *P. guineense* thrives in wet, shaded lowland forests with well-drained loamy soils and requires a tropical climate with high humidity and rainfall. Its distribution spans Nigeria, Ghana, Cameroon, Congo, and other regions of West and Central Africa (Uhegbu *et al*., 2011).

Plate 2.1: (A) fruit of *Piper guineense* (B) Dry Seeds of *Piper guineense*

2.2 Phytochemical Constituents of *Piper guineense*

Phytochemicals are chemicals that are derived from plant. *Piper guineense* contains wide varieties of phytochemicals including essential oils. These phytochemicals are responsible for the various activities of *Piper guineense* such as antimicrobial, antioxidant, anti-inflammatory and analgesic activities (Gazuwa *et al*., 2013).

2.2.1 Piperine

Piper guineense (Schumach. and Thonn), commonly known as West African black pepper or uziza, contains several bioactive compounds, among which piperine is the most notable. Piperine is an alkaloid responsible for the characteristic pungency of the plant's fruits and seeds and contributes to many of its pharmacological effects (Ekanem and Obiekezie, 2016). Chemically, piperine is classified as a nitrogen-containing amide, and it is structurally similar to the piperine found in *Piper nigrum*, though variations in concentration and associated compounds give *P. guineense* a distinct flavor and aroma (Ajayi *et al.,* 2011).

Piperine is found primarily in the dried fruits and seeds of *P. guineense*, where it is concentrated in the outer pericarp and the seed's endosperm (Udofia *et al.,* 2015). Its pungent taste is the basis for the use of the seeds as a spice in West African cuisines, especially in soups, sauces, and traditional spice blends. Beyond its culinary role, piperine exhibits a wide range of pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and bioavailability-enhancing effects. Studies have demonstrated that piperine can increase the

absorption of certain drugs and nutrients by inhibiting metabolizing enzymes in the liver and intestine, which has implications for both traditional medicine and modern pharmacology (Ekanem and Obiekezie, 2016; Udofia *et al.,* 2015).

The concentration of piperine in *P. guineense* varies depending on factors such as geographical location, plant maturity, and processing methods. Dried seeds typically contain higher concentrations compared to fresh leaves, making them the primary source for both culinary and medicinal applications (Akinbuluma *et al*., 2017). In addition to piperine, the seeds and fruits contain other alkaloids, essential oils, and phenolic compounds, which may act synergistically to enhance the plant's therapeutic effects.

Research on piperine in *P. guineense* has expanded interest in the plant beyond traditional spice use. Its potential applications include natural antimicrobial agents, functional foods, and complementary medicine for inflammatory or metabolic disorders. These studies highlight the importance of piperine as a key bioactive constituent that underpins much of the plant's ethnopharmacological relevance (Ogunmefun *et al*., 2017).

2.2.2 Essential Oils of *Piper guineense*

Piper guineense contains a rich array of essential oils, primarily composed of volatile terpenoids, which contribute significantly to the plant's aroma, flavor, and pharmacological properties (Aribo *et al.,* 2019). Essential oils are concentrated in the fruits, seeds, and to a lesser extent the leaves, and they are responsible for the characteristic pungent, spicy, and slightly floral scent that distinguishes *P. guineense* from other *Piper* species (Akinwale *et al.,* 2020).

The volatile terpenoids present in *P. guineense* include monoterpenes, sesquiterpenes, and their oxygenated derivatives, such as β-caryophyllene, limonene, α-pinene, myrcene, and linalool. These compounds are largely responsible for the antimicrobial, antioxidant, and insecticidal

activities observed in the plant (Onwuka *et al*., 2022). For example, β-caryophyllene and limonene have been shown to possess significant antibacterial and antifungal properties, making the essential oil of *P. guineense* a potential natural preservative for food and storage of grains (Akinwale *et al*., 2020).

The composition of essential oils in *P. guineense* varies depending on plant part, maturity, geographic origin, and method of extraction. Studies have shown that the seeds tend to contain higher concentrations of sesquiterpenes, whereas the leaves are richer in monoterpenes (Iwu *et al.,* 2016). Steam distillation and solvent extraction are the most commonly employed methods for isolating these oils for both chemical analysis and practical applications.

In addition to their aromatic and culinary uses, the volatile terpenoids in *P. guineense* play important roles in traditional medicine. They have been associated with anti-inflammatory, analgesic, and insect-repellent effects. The essential oil also contributes to the plant's synergistic pharmacological activity when used in combination with piperine and other alkaloids (Ekanem and Obiekezie, 2016). The growing interest in essential oils of *P. guineense* reflects their potential applications in natural therapeutics, food preservation, and aromatherapy, highlighting the plant as a valuable bioresource.

2.2.3 Lignans and Neolignans of *Piper guineense*

Piper guineense is rich in secondary metabolites, among which lignans and neolignans play an important role in its pharmacological and chemotaxonomic profile. Lignans are a class of phenylpropanoid dimers derived from the oxidative coupling of two cinnamyl alcohol units, while neolignans are related compounds formed through different coupling modes of phenylpropanoid units. Both classes of compounds are known for their diverse biological activities (Oparaji *et al.,* 2016). The fruits and seeds of *P. guineense* contain lignans such as

cubebin, guineensol, and yatein, as well as neolignans, which have been identified through chromatographic and spectroscopic analyses (Uyanwa *et al.,* 2024). Cubebin, for instance, is a major lignan responsible for many of the plant's antimicrobial, anti-inflammatory, and analgesic activities, while neolignans contribute to antioxidant and cytotoxic properties. These compounds are predominantly concentrated in the seeds and dried fruits, although they can also be detected in the leaves.

Pharmacologically, lignans and neolignans from *P. guineense* exhibit broad-spectrum antimicrobial activity, effective against Gram-positive and Gram-negative bacteria, as well as fungi (Ekanem and Obiekezie, 2016). Additionally, lignans have been studied for their reproductive health-promoting properties, as some have been shown to modulate hormonal activity and improve fertility in traditional medicine applications. Neolignans also display antioxidant and anti-inflammatory effects, enhancing the plant's therapeutic potential when used in combination with other bioactive compounds like piperine and essential oils.

The biosynthesis and accumulation of lignans and neolignans in *P. guineense* are influenced by plant part, maturity, and environmental conditions. Studies suggest that seeds harvested from mature fruits contain higher concentrations of these compounds compared to younger fruits or leaves (Oparaji *et al*., 2016). Their presence not only contributes to the plant's medicinal value but also serves as a chemotaxonomic marker distinguishing *P. guineense* from other *Piper* species.

Overall, lignans and neolignans are significant contributors to the ethnopharmacological importance of *P. guineense*, underpinning its use in antimicrobial therapies, anti-inflammatory remedies, and as part of functional foods or nutraceuticals.

2.2.4 Alkaloids

P. guineense is recognized for its rich phytochemical composition, which includes a significant presence of alkaloids. Alkaloids are nitrogen-containing secondary metabolites that are largely responsible for the plant's characteristic pungency, flavor, and pharmacological properties (Ekanem and Obiekezie, 2016). Among the bioactive constituents of *P. guineense*, alkaloids such as piperine, pellitorine, and guineensine are of particular interest due to their diverse biological activities (Emeziem *et al.,* 2024).

Piperine is the principal alkaloid in *P. guineense*, predominantly concentrated in the dried fruits and seeds. It imparts the plant's spicy taste and is associated with various pharmacological effects, including anti-inflammatory, antioxidant, antimicrobial, and bioavailability-enhancing properties. Pellitorine, another alkaloid, contributes to the plant's antimicrobial and insecticidal activity, while guineensine is noted for its central nervous system-modulating and anti-inflammatory effects (Iwu *et al.,* 2019).

The alkaloid content of *P. guineense* varies depending on several factors, including plant part, age, geographic location, and processing method. Seeds generally have the highest concentrations, followed by fruits, while leaves contain lower amounts. These variations influence both the culinary potency and medicinal efficacy of the plant (Patience *et al*., 2024).

Alkaloids in *P. guineense* contribute not only to traditional uses in West African medicine such as treating respiratory infections, digestive disturbances, and inflammatory conditions but also to modern pharmacological applications. Research indicates that these compounds may enhance the absorption of other bioactive molecules, demonstrating synergistic effects when combined with other secondary metabolites such as essential oils and lignans (Ekanem and Obiekezie, 2016).

Overall, alkaloids are central to the ethnobotanical and pharmacological significance of *P. guineense*, underpinning its use as both a spice and a therapeutic agent in traditional and modern applications.

2.2.5 Flavonoids

Flavonoids constitute an important class of polyphenolic secondary metabolites in *Piper guineense*, contributing significantly to its pharmacological and antioxidant properties. These compounds are widely distributed in the leaves, fruits, and seeds of the plant, where they function as protective molecules against oxidative stress and microbial invasion. Studies on the phytochemical composition of *P. guineense* consistently identify flavonoids such as quercetin, kaempferol, rutin, and catechins, although the specific profile may vary depending on extraction method and plant part (Essien *et al*., 2015).

The presence of these flavonoids is associated with the plant's potent antioxidant activity, which is frequently highlighted in laboratory assays involving radical scavenging and lipid peroxidation inhibition. For example, methanolic and ethanolic extracts of *P. guineense* leaves and fruits demonstrate high flavonoid content, correlating strongly with their antioxidant capacity (Alagbe *et al*., 2021). These compounds also contribute to the plant's anti-inflammatory, antimicrobial, and chemoprotective properties, which support its traditional use in West African ethnomedicine for managing respiratory ailments, gastrointestinal disturbances, and inflammatory conditions.

Quercetin and kaempferol, two of the principal flavonoids detected in *P. guineense*, are well-recognized for their broad biological activities, including inhibition of pro-inflammatory enzymes, modulation of oxidative pathways, and antimicrobial effects (Oyemitan, 2017). Rutin and catechin further enhance the therapeutic potential of the plant, particularly through their strong free-radical neutralizing ability and capacity to stabilize cellular membranes. The

combined action of these flavonoids may exert synergistic effects with other phytochemicals in *P. guineense*, such as alkaloids and essential oils, contributing to the plant's overall medicinal profile (Essien *et al*., 2015). Collectively, the flavonoid components of *P. guineense* are central to its ethnopharmacological relevance. Their abundance and bioactivity underscore the plant's therapeutic versatility and support continued scientific interest in its potential as a source of natural antioxidants and anti-inflammatory agents.

2.2.6 Phenolics

Phenolic compounds represent a major class of bioactive constituents in *Piper guineense*, contributing significantly to its therapeutic and antioxidant potential. These compounds, which include phenolic acids, tannins, and complex polyphenols, occur in high concentrations in the plant's leaves, fruits, and seeds. Phenolics in *P. guineense* are well-documented for their ability to neutralize free radicals, chelate metal ions, and modulate oxidative pathways, thereby enhancing the plant's medicinal value (Ojinnaka *et al*., 2016).

Studies analyzing the phytochemistry of *P. guineense* have identified various phenolic acids, including gallic acid, caffeic acid, chlorogenic acid, and protocatechuic acid, although the specific profile varies with extraction solvent and plant tissue. Methanolic and ethanol extracts typically yield the highest concentrations, reflecting the solubility of phenolic compounds in polar solvents (Essien *et al*., 2015). These phenolic constituents form an important basis for the plant's traditional uses, especially in the management of inflammatory and oxidative stress-related disorders.

Phenolics are directly associated with the plant's strong antioxidant capacity, as demonstrated by multiple in vitro assays such as 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), Ferric Reducing Antioxidant Power assay (FRAP), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

assay (ABTS) radical scavenging tests. Extracts rich in phenolics from *P. guineense* fruits and leaves exhibit substantial activity in these assays, showing a positive correlation between total phenolic content and antioxidant performance (Akpangwa *et al*., 2020). This relationship reinforces the idea that phenolics are primary contributors to the overall bioactivity of the plant. Beyond antioxidant effects, phenolics in *P. guineense* demonstrate antimicrobial, anti-inflammatory, and chemoprotective properties. Their antimicrobial activity is thought to arise from interactions with microbial cell walls and proteins, disrupting essential metabolic pathways. The anti-inflammatory effects, on the other hand, are linked to inhibition of pro-inflammatory mediators and suppression of oxidative tissue damage (Allaqaband *et al*., 2022). These bioactivities support the widespread traditional application of the plant in treating infections, gastrointestinal conditions, and respiratory ailments.

2.2.7 Saponins

Saponins are an important class of glycosidic secondary metabolites present in *Piper guineense*, contributing to the plant's medicinal, antimicrobial, and anti-inflammatory properties. These compounds consist of a hydrophobic aglycone linked to one or more sugar moieties, giving them their characteristic foaming ability and enabling interactions with biological membranes. In *P. guineense*, saponins are distributed primarily in the leaves and fruits, where they play a defensive role against pathogens and herbivores (Dibulo *et al*., 2017)

Phytochemical screenings of *P. guineense* consistently confirm the presence of saponins alongside other metabolites such as alkaloids, flavonoids, and tannins. Quantitative analyses reveal that saponins occur in moderate to high concentrations depending on the extraction method, with ethanol and methanol extracts typically yielding the highest levels (Ekanem and Obiekezie, 2016). These variations reflect differences in solvent polarity and plant part

composition, but collectively they support the relevance of saponins to the plant's medicinal potency.

The biological activities of *P. guineense* saponins are broad and pharmacologically significant. They exhibit antimicrobial effects, attributed to their ability to permeabilize microbial cell membranes and inhibit enzymatic functions (Echave *et al*., 2020). This property supports the plant's traditional use in the management of respiratory and gastrointestinal infections. Additionally, saponins contribute to the plant's anti-inflammatory and antioxidant activities by modulating inflammatory mediators and reducing oxidative damage in tissues (Ekanem and Obiekezie, 2016). Saponins are also known for their hypolipidemic and immunomodulatory effects. Although these activities have been more extensively studied in other medicinal plants, preliminary findings suggest similar potential in *P. guineense*, given its significant saponin content (Dibulo *et al*., 2017). Traditional ethnobotanical applications of the plant, particularly for postpartum care, uterine cleansing, and general wellness, may be partly explained by these systemic effects.

2.2.8 Tannins

Tannins constitute one of the key classes of polyphenolic compounds found in *Piper guineense*, where they contribute significantly to the plant's antimicrobial, antioxidant, and astringent properties. These compounds are widely distributed in the leaves, fruits, and seeds, and are known for their ability to precipitate proteins, form complexes with alkaloids and polysaccharides, and modulate oxidative reactions. In *P. guineense*, tannins are often detected alongside other phenolic constituents such as flavonoids and phenolic acids, suggesting a synergistic contribution to the plant's overall pharmacological activity (Essien *et al*., 2015).

Phytochemical studies consistently reveal the presence of tannins in extracts of *P. guineense*, with higher concentrations typically obtained from methanol or ethanol extractions due to the solubility of polyphenolic compounds in polar solvents. The tannin content has been linked to the plant's strong antioxidant capacity, demonstrated in assays such as DPPH and FRAP, where tannin-rich extracts show high radical scavenging and reducing activities (Udoudoakpan *et al*., 2024). These findings support the role of tannins in mediating the plant's protective effects against oxidative stress and cellular damage.

In addition to antioxidant effects, tannins in *P. guineense* possess notable antimicrobial properties, likely arising from their ability to complex with microbial cell walls, inhibit enzyme activity, and disrupt nutrient availability. These mechanisms help explain the plant's traditional use in treating infections, including gastrointestinal and respiratory ailments (Adu *et al*., 2020). Tannins also exhibit anti-inflammatory and astringent effects, contributing to the plant's role in wound healing and management of inflammatory conditions in ethnomedicine.

2.2.9 Anthraquinones

Anthraquinones are a class of aromatic organic compounds widely distributed in many medicinal plants, and their presence has also been reported in *Piper guineense*. Although they typically occur in relatively low concentrations compared with other phytochemical classes such as alkaloids, flavonoids, and tannins, anthraquinones contribute meaningfully to the plant's pharmacological potential. Qualitative phytochemical screenings consistently identify anthraquinones particularly in the leaves and fruits suggesting that *P. guineense* contains free and bound forms of these compounds (Osasemeaga *et al*., 2022). Their detection aligns with broader patterns in the Piperaceae family, where phenolic and quinone-based metabolites are commonly present. Anthraquinones in *P. guineense* are associated with several biological activities,

including antimicrobial, antioxidant, laxative, and anti-inflammatory effects. Their antimicrobial activity is attributed to their ability to inhibit Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA) synthesis in pathogenic microorganisms, leading to impaired cellular function and growth (Osasemeaga *et al*., 2022). This activity may enhance the plant's traditional utility in managing infections of the respiratory and gastrointestinal systems. Although direct isolation of specific anthraquinone structures from *P. guineense* remains limited, their consistent phytochemical detection indicates a contributory role in the plant's broad antimicrobial profile.

Additionally, anthraquinones possess redox-active properties, enabling them to modulate oxidative processes within biological systems. This activity likely supports the antioxidant potential of *P. guineense*, which is frequently demonstrated in assays assessing free-radical scavenging capacity (Essien *et al*., 2015). Through redox cycling and interaction with cellular signaling pathways, anthraquinones may also influence inflammatory responses, contributing to the plant's traditional use in managing inflammation and pain.

2.2.10 Terpenoids

Terpenoids represent one of the most abundant and pharmacologically significant classes of secondary metabolites in *Piper guineense*. These compounds, which include both volatile and non-volatile terpenoids, contribute substantially to the plant's characteristic aroma, flavor profile, and therapeutic properties. The fruits and seeds are particularly rich in monoterpenes and sesquiterpenes, many of which occur in the essential oil fraction. Notable terpenoids commonly identified in the essential oils of *P. guineense* include β-caryophyllene, myrcene, linalool, α-pinene, β-pinene, limonene, and germacrene D (Abd El-Hack *et al*., 2022). These terpenoids not only define the organoleptic qualities of the spice but also underlie its biological activities.

A substantial body of evidence demonstrates that the terpenoids of *P. guineense* possess significant insecticidal, antimicrobial, analgesic, anti-inflammatory, and antioxidant properties. For instance, β-caryophyllene and linalool are well-known anti-inflammatory and analgesic agents that modulate inflammatory pathways and reduce nociceptive responses (Basha and Sankaranarayanan, 2016). Similarly, monoterpenes such as α-pinene and limonene exhibit potent antimicrobial effects, likely through disruption of microbial cell membranes and interference with cellular metabolism. These terpenoid actions help explain the traditional use of *P. guineense* in the treatment of respiratory and gastrointestinal infections.

The terpenoid-rich essential oils of *P. guineense* have been extensively studied for their insecticidal and repellent activities. Abd El-Hack *et al*. (2022) demonstrated that the essential oil, dominated by monoterpenes and sesquiterpenes, showed strong bioactivity against the maize weevil (*Sitophilus zeamais*), indicating its potential for botanical pest control applications. This finding reinforces traditional agricultural uses of the plant in West Africa, where its dried fruits are sometimes used as natural protectants against pests.

Beyond the volatile fraction, *P. guineense* also contains non-volatile diterpenoids and triterpenoids**,** though these have been less extensively characterized. Preliminary phytochemical studies indicate that these compounds contribute to the plant's anti-inflammatory and antimicrobial effects and may act synergistically with polyphenols and alkaloids in whole-plant extracts (Essien *et al*., 2015).

2.2.11 Glycosides

Glycosides are an important group of secondary metabolites detected in *Piper guineense*, contributing to the plant's therapeutic potential and its broad ethnomedicinal applications. These compounds consist of a biologically active aglycone linked to one or more sugar moieties, a

structure that enhances solubility, stability, and transport within plant tissues. Phytochemical screenings of *P. guineense* consistently confirm the presence of glycosides in the leaves, fruits, and seeds, although the quantity and subtype often vary depending on extraction method and plant part (Amadi *et al*., 2019).

Cardiac and flavonoid glycosides appear to be among the most frequently reported classes in *P. guineense*. Their presence aligns with findings from several members of the Piperaceae family, where glycoside derivatives commonly coexist with polyphenols, alkaloids, and terpenoids. In *P. guineense*, glycosides are believed to contribute to biological activities such as anti-inflammatory, antimicrobial, and antioxidant effects (Ekanem and Obiekezie, 2016). Their antioxidant potential is linked to the ability of glycosylated aglycones such as flavonoid glycosides to scavenge reactive oxygen species and stabilize free radicals, thereby supporting the plant's traditional use in treating inflammatory and oxidative stress–related conditions.

Glycosides are also associated with notable antimicrobial properties, which may arise from their capacity to disrupt microbial membranes, inhibit essential enzymes, or interfere with nucleic acid synthesis. These mechanisms support the documented ethnomedicinal use of *P. guineense* in managing infections, including those affecting the gastrointestinal and respiratory systems (Ashokkumar *et al*., 2021). In addition, some studies suggest that certain glycosides may exert mild analgesic and antispasmodic effects, contributing to the plant's role in pain relief and postpartum care in West African traditional medicine.

Although the specific glycoside structures in *P. guineense* have not been fully characterized, their consistent detection in phytochemical analyses underscores their relevance in the plant's overall pharmacological profile. Continued analytical studies, especially those employing

chromatographic and spectrometric techniques, are needed to elucidate the exact types and concentrations of glycosides present.

2.2.12 Steroids

Steroidal compounds constitute an important class of secondary metabolites detected in *Piper guineense*, contributing to the plant's diverse pharmacological activities. Steroids in medicinal plants typically include phytosterols and triterpenoid derivatives, which support various biological functions such as membrane stabilization, anti-inflammatory activity, and modulation of lipid metabolism. Phytochemical investigations of *P. guineense* consistently report the presence of steroids in the leaves, fruits, and seeds, although their concentration often varies depending on the extraction solvent and plant part (Imo *et al*., 2018).

The steroidal constituents identified in *P. guineense* are generally associated with the plant's anti-inflammatory, antimicrobial, antioxidant, and chemoprotective properties. Their anti-inflammatory activity may arise from the inhibition of pro-inflammatory mediators and the stabilization of lysosomal membranes, mechanisms similar to those attributed to common plant sterols such as β-sitosterol (Patel and Mishra, 2011). These actions align with the traditional use of *P. guineense* in managing pain, inflammation, and postpartum recovery, suggesting a biochemical basis for its ethnomedical relevance.

Steroids also play a contributory role in the antimicrobial activity of *P. guineense*. Their ability to interact with microbial cell membranes can lead to membrane disruption, altered permeability, and reduced metabolic activity in bacteria and fungi (Isikhuemen *et al*., 2020). Such effects are consistent with reports of the plant's efficacy against organisms responsible for respiratory, gastrointestinal, and skin infections. Furthermore, steroidal compounds may enhance the

bioactivity of other phytochemical groups in the plant, such as terpenoids, flavonoids, and alkaloids, through synergistic interactions.

In addition, the presence of steroids in *P. guineense* supports its use in traditional wellness practices, including those related to reproductive health. Plant steroids are sometimes associated with mild hormonal or adaptogenic effects, although the specific steroidal structures in *P. guineense* remain to be fully characterized (Madueke *et al*., 2021). Advanced chromatographic and spectrometric analyses are needed to isolate and identify these compounds more definitively.

Overall, steroids form a meaningful component of the phytochemical profile of *Piper guineense*. Their anti-inflammatory, antimicrobial, and supportive physiological actions contribute to the plant's broad medicinal application and highlight its potential value for further pharmacological exploration.

2.3 Medicinal Uses of *Piper guineense*

Piper guineense is widely valued in West and Central African ethnomedicine for its broad therapeutic applications, many of which are supported by phytochemical constituents such as alkaloids, terpenoids, flavonoids, and essential oils. Traditionally, the fruits and leaves of the plant are used to manage various ailments including infections, inflammation, reproductive issues, and gastrointestinal disturbances. These uses are grounded in both indigenous knowledge systems and emerging pharmacological evidence.

One of the most prominent medicinal uses of *P. guineense* is its role as an antimicrobial agent. Extracts of the plant have demonstrated notable antibacterial and antifungal activities against pathogens responsible for gastrointestinal, respiratory, and skin infections. The essential oils—rich in monoterpenes and sesquiterpenes are particularly effective, exhibiting broad-spectrum

inhibitory effects on microorganisms (Ekanem *et al*., 2010). These antimicrobial properties support the traditional use of the plant in treating stomach upset, diarrhea, and infectious diseases. The plant also possesses significant anti-inflammatory and analgesic properties, which contribute to its use in treating rheumatic pain, postpartum discomfort, and general body aches. Phytochemicals such as flavonoids, terpenoids, and steroids are believed to mediate these effects through inhibition of inflammatory mediators and modulation of oxidative processes (Mgbeahuruike *et al*., 2019). This aligns with the common practice of administering *P. guineense* infusions and decoctions to women after childbirth to alleviate pain and promote healing.

Piper guineense is further recognized for its antioxidant activity, attributed largely to its phenolics and flavonoids. These compounds help neutralize free radicals, thereby reducing oxidative stress and potentially lowering the risk of chronic diseases (Adefegha and Oboh, 2012). As a result, the plant is often used in traditional remedies for boosting immunity and supporting general wellness.

Another key medicinal use is in reproductive health. In several African cultures, *P. guineense* is used as an aphrodisiac and fertility enhancer. Studies have reported that its alkaloids and essential oils may stimulate reproductive hormone activity, increase libido, and improve sperm parameters in animal models (Dimo *et al*., 2021). The plant's uterotonic properties also contribute to its application during postpartum recovery.

Additionally, *P. guineense* exhibits insecticidal and antiparasitic effects. Extracts have proven effective against mosquitoes, intestinal worms, and other pests, supporting its ethnobotanical use for controlling parasites and malaria-associated symptoms (Ogunwenmo *et al*., 2017). The

pungent compounds responsible for these activities also make the plant a natural preservative and food protectant.

Overall, the medicinal applications of *Piper guineense* reflect a synergy between traditional knowledge and scientific validation. Its diverse pharmacological properties underscore its potential as a source of natural therapeutic agents and warrant further research into its bioactive compounds and mechanisms of action.

2.3.1 Analgestic Activity

Experimental studies using rodent models have demonstrated that methanol, ethanol, and aqueous extracts of *P. guineense* fruits and seeds exhibit significant analgesic activity. The observed effects are dose-dependent, with higher concentrations of the extracts producing stronger pain-relieving effects. For instance, studies using the hot plate and tail-flick tests, which assess central analgesic effects, showed that *P. guineense* fruit and seed extracts significantly increased the latency period in response to thermal stimuli, indicating a central mechanism of action (Eze *et al*., 2013). Additionally, acetic acid-induced writhing tests, which evaluate peripheral analgesic effects, confirmed that the extracts effectively reduced nociceptive responses, suggesting inhibition of prostaglandin synthesis (Iwalewa *et al*., 2013).

Phytochemical analyses suggest that the analgesic activity of *P. guineense* is attributable to its diverse bioactive compounds. Alkaloids, flavonoids, saponins, and essential oils present in the fruits and seeds are believed to act synergistically to modulate pain perception. Alkaloids may interact with opioid receptors in the central nervous system, while flavonoids and saponins likely inhibit inflammatory mediators such as prostaglandins, nitric oxide, and cyclooxygenase enzymes (Adesegun *et al*., 2016). Terpenoids and essential oil constituents, including β-

caryophyllene and linalool, may further contribute by exerting anti-inflammatory and neuromodulatory effects, enhancing the overall analgesic profile of the plant.

The analgesic efficacy of *P. guineense* fruit and seed extracts is comparable in some models to standard analgesic drugs such as aspirin and morphine, although their mechanisms may involve a combination of central and peripheral pathways. This dual activity supports the traditional use of the plant for managing both localized and systemic pain. Moreover, the relative safety of the plant in acute dosing studies reinforces its potential as a natural alternative or adjunct to conventional analgesics (Iwalewa *et al*., 2013).

In summary, both the fruits and seeds of *Piper guineense* exhibit significant analgesic activity mediated through central and peripheral mechanisms. The presence of alkaloids, flavonoids, saponins, and essential oils underlies this pharmacological effect, corroborating its traditional applications in pain management and highlighting its potential for therapeutic development.

2.4 Antimicrobial Chemotherapy

Most microbiologists distinguish two groups of antimicrobial agents used in the treatment of infectious diseases into Antibiotics, which are natural substances produced by certain group of microorganisms and Chemotherapeutic agents, which are chemically synthesized (Falodun *et al.,* 2011). Chemotherapeutic agents are chemicals used to control or treat diseases. They destroy pathogenic microbes or inhibit their growth within the host. Antimicrobial agents on the other hand are natural or synthesized chemicals that kill or inhibit microorganisms. Antimicrobial agents are usually not intended for therapeutic purposes and are mostly used to sterilize or inhibit microbial growth (Dixon *et al*., 2013).

The modern era of antimicrobial chemotherapy started in 1920, with Fleming's discovery of the powerful bactericidal substance, penicillin and Domagk's discovery in 1935 of synthetic

chemicals with broad antimicrobial activity. Most of the natural antibiotics that are being used in agriculture and medicine are produced by three unrelated groups of microbes. They include; the penicillin and cephalosporin molds, actinomycetes (Streptomyces) species and Bacillus species (Falodun *et al.,* 2011).

2.5 Plant Products as Antimicrobial Agents

Since the advent of antibiotics in the 1950's, the use of plant derivatives as antimicrobials has been virtually non-existent. It is reported that on average, two or three antibiotics derived from microorganisms are launched each year (Barrett, 2014). After a downturn in this phenomenon in recent decades, the pace is again quickening as scientists realize that the effective lifespan of any antibiotic is limited. Newer sources especially plant sources are now being investigated. Also the public is becoming increasingly aware of problems with the over-prescription and misuse of traditional antibiotics (Igbinosa *et al.,* 2011).

Mainstream medicine is increasingly receptive to the use of antimicrobials and other drugs derived from plants, as traditional antibiotics become ineffective and as new, particularly viral, diseases remain intractable to this kind of drug (Mahomed and Ojewole, 2016). It is estimated that there are 250,000 – 500,000 species of plant on earth. A relatively small percentage (1-10%) of these is used as food by both humans and other animal species. It is possible that even more are used for medicinal purposes. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen – substituted derivative (Ghanayem, 2015). Most are secondary metabolites, of which 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defense mechanism against predation by microorganisms, insects and herbivores. Many compounds are

responsible for plant flavour and some of the same herbs and spices are used by humans to season food, yield useful medicinal compounds (Adeboye *et al*., 2018).

2.6 Antimicrobial Activity of *Piper guineense*

Scientific investigations have confirmed that both the fruits and seeds of *P. guineense* possess potent antibacterial, antifungal, and, to a lesser extent, antiviral activities, attributable to the diverse phytochemical constituents present in these plant parts.

Studies have demonstrated that crude extracts of *P. guineense* fruits and seeds, obtained using ethanol, methanol, or aqueous solvents, exhibit significant inhibitory effects against a range of bacterial pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi* (Ekanem *et al*., 2010). The antimicrobial activity has been shown to be dose-dependent, with higher concentrations of extracts producing larger zones of inhibition in agar diffusion assays. The essential oils extracted from the fruits and seeds, which contain monoterpenes and sesquiterpenes such as β-caryophyllene, α-pinene, and linalool, have been particularly effective, suggesting that volatile terpenoids play a major role in microbial inhibition (Ukeh *et al*., 2019).

The antifungal potential of *P. guineense* fruits and seeds has also been reported, with activity observed against pathogenic fungi such as *Candida albicans* and *Aspergillus niger*. These effects are believed to result from the ability of phenolic compounds, flavonoids, and saponins to disrupt fungal cell walls and membranes, impair enzymatic function, and inhibit spore germination (Okigbo and Igwe, 2017). The combined action of multiple phytochemicals in crude extracts likely enhances the spectrum and efficacy of antimicrobial effects, providing a synergistic mechanism that strengthens the plant's ethnomedicinal applications.

Mechanistically, antimicrobial activity in *P. guineense* is attributed to several biochemical interactions. Alkaloids can interfere with microbial DNA and protein synthesis, flavonoids and phenolics can inhibit key metabolic enzymes, and terpenoids can compromise cell membrane integrity, leading to cell lysis (Newman *et al*., 2020). The presence of these bioactive compounds in both the fruit and seed underscores the plant's utility in treating bacterial and fungal infections of the gastrointestinal, respiratory, and integumentary systems.

In addition to laboratory findings, the antimicrobial efficacy of *P. guineense* has practical applications in food preservation. Its pungent fruits and seeds are traditionally used to prevent microbial spoilage in stored foods, demonstrating both medicinal and nutritional relevance. Overall, the antimicrobial activity of *P. guineense* fruits and seeds validates its widespread use in ethnomedicine and supports further exploration for natural antimicrobial agents.

2.7 Some Pathogenic Bacteria

Pathogenic bacteria are bacteria that can cause disease. Although most bacteria are harmless or often beneficial, some are pathogenic, with the number of species estimated as fewer than a hundred that are seen to cause infectious diseases in humans (Graden *et al*., 2022).

2.7.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium known for causing a wide range of infections, particularly in immunocompromised individuals, including urinary tract infections, respiratory infections, and wound infections (Moradali *et al*., 2017). Its intrinsic resistance to multiple antibiotics, coupled with its ability to form biofilms, makes *P. aeruginosa* a major clinical concern. Traditional medicinal plants, such as *Piper guineense*, have been

investigated for their potential to inhibit this pathogen due to their diverse bioactive phytochemicals.

Studies have shown that extracts from *Piper guineense* fruits and seeds exhibit significant antibacterial activity against *P. aeruginosa*. Both methanol and ethanol extracts of the plant have demonstrated inhibitory effects in vitro, as evidenced by disc diffusion and minimum inhibitory concentration (MIC) assays (Ekanem *et al*., 2010). The observed antimicrobial activity is attributed to a combination of bioactive compounds including alkaloids, flavonoids, terpenoids, saponins, and essential oils. These compounds are believed to disrupt bacterial cell membranes, inhibit essential enzymatic pathways, and interfere with nucleic acid synthesis, ultimately suppressing the growth of *P. aeruginosa* (Cowan, 1999).

The essential oils of *P. guineense*, rich in volatile terpenoids such as β-caryophyllene, linalool, and α-pinene, have been particularly effective against *P. aeruginosa*. These compounds can penetrate the bacterial outer membrane, disrupt biofilm formation, and reduce bacterial virulence factors, which is critical given the pathogen's ability to form resilient biofilms in clinical and environmental settings (Ukeh *et al*., 2009). The inhibition of *P. aeruginosa* by *P. guineense* extracts not only supports its traditional use in managing gastrointestinal and wound infections but also suggests its potential as a complementary therapeutic agent in combating antibiotic-resistant strains.

Moreover, the antimicrobial action of *P. guineense* against *P. aeruginosa* is enhanced when used in combination with other plant extracts or conventional antibiotics. Synergistic interactions have been reported, where phytochemicals from *P. guineense* improve the susceptibility of *P. aeruginosa* to standard antimicrobial agents, highlighting the plant's potential role in integrative medicine (Okigbo and Igwe, 2007).

In conclusion, *Piper guineense* exhibits significant inhibitory effects against *Pseudomonas aeruginosa*, attributed to its rich phytochemical profile. These findings validate its ethnomedicinal use against infections associated with this pathogen and underscore its potential in the development of alternative antimicrobial therapies.

2.7.2 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive bacterium responsible for a variety of infections ranging from minor skin infections to life-threatening conditions such as sepsis, pneumonia, and endocarditis. The rise of methicillin-resistant *S. aureus* (MRSA) strains has intensified the search for alternative antimicrobial agents, including those derived from medicinal plants. *Piper guineense*, widely used in West African traditional medicine, has demonstrated significant antibacterial activity against *S. aureus*, supporting its ethnomedicinal applications.

Phytochemical investigations indicate that the antibacterial effects of *P. guineense* fruits and seeds are attributable to a combination of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and essential oils (Ekanem *et al*., 2010). Methanol and ethanol extracts of the fruits and seeds have been shown to inhibit the growth of *S. aureus* in vitro, with zones of inhibition comparable to standard antibiotics in some studies. The mechanism of action is believed to involve disruption of bacterial cell wall integrity, interference with protein synthesis, and inhibition of nucleic acid metabolism (Cowan, 1999).

Essential oils extracted from *P. guineense* are particularly potent against *S. aureus*, with monoterpenes and sesquiterpenes such as β-caryophyllene, linalool, and α-pinene contributing to membrane destabilization and leakage of cellular contents (Ukeh *et al*., 2009). Additionally, flavonoids and phenolic compounds act as enzyme inhibitors and antioxidants, further impairing bacterial metabolism and growth. Studies have also suggested that the combination of different

phytochemicals in whole-plant extracts may produce synergistic effects, enhancing antibacterial efficacy compared to isolated compounds (Okigbo and Igwe, 2007).

The antibacterial activity of *P. guineense* against *S. aureus* has practical implications for both traditional medicine and potential pharmaceutical development. Its effectiveness against clinical isolates, including antibiotic-resistant strains, highlights its potential as a source of natural antimicrobial agents. Furthermore, the plant's use in food preservation is reinforced by its ability to inhibit bacterial growth, reducing the risk of contamination and spoilage.

In conclusion, *Piper guineense* exhibits significant antibacterial activity against *Staphylococcus aureus*, mediated through the synergistic action of its diverse phytochemicals. This activity validates traditional uses of the plant and underscores its potential for integration into modern antimicrobial therapies.

2.7.3 *Bacillus subtilis*

Bacillus subtilis is a Gram-positive, rod-shaped bacterium widely studied as a model organism for bacterial physiology and sporulation. While generally non-pathogenic, *B. subtilis* serves as an important indicator in antimicrobial studies due to its resilience and cell wall structure, which can model responses of pathogenic Gram-positive bacteria to natural antimicrobials. Research on *Piper guineense*, a medicinal plant commonly used in West Africa, has demonstrated significant antibacterial activity against *B. subtilis*, highlighting its potential as a natural antimicrobial agent.

Phytochemical analyses of *P. guineense* fruits and seeds reveal the presence of bioactive compounds such as alkaloids, flavonoids, saponins, tannins, terpenoids, and essential oils, which collectively contribute to its antibacterial properties (Ekanem *et al*., 2010). Both methanol and ethanol extracts of *P. guineense* fruits and seeds have been reported to inhibit the growth of *B. subtilis* in vitro, as evidenced by clear zones of inhibition in disc diffusion assays and low

minimum inhibitory concentration (MIC) values. The antibacterial effects are thought to arise from disruption of bacterial cell walls and membranes, inhibition of key enzymatic pathways, and interference with nucleic acid synthesis (Cowan, 1999).

Essential oils extracted from *P. guineense* fruits and seeds demonstrate particularly strong activity against *B. subtilis*. Monoterpenes and sesquiterpenes such as β-caryophyllene, α-pinene, and linalool interact with bacterial membranes, increasing permeability and causing leakage of cytoplasmic contents, ultimately leading to bacterial cell death (Ukeh *et al*., 2009). Additionally, flavonoids and phenolic compounds in the extracts act as enzyme inhibitors and antioxidants, further impairing bacterial metabolic functions.

The antibacterial activity against *B. subtilis* also supports the traditional use of *P. guineense* as a natural preservative. Its ability to inhibit the growth of Gram-positive bacteria reduces microbial spoilage in foods and enhances the plant's ethnomedicinal applications in treating infections. Furthermore, studies suggest that the combination of multiple phytochemicals in whole-plant extracts may produce synergistic effects, enhancing antibacterial efficacy relative to isolated compounds (Okigbo and Igwe, 2007).

In conclusion, *Piper guineense* exhibits potent antibacterial activity against *Bacillus subtilis*, mediated through the synergistic action of its diverse phytochemicals. These findings validate its traditional use in infection management and suggest its potential as a source of natural antibacterial agents for pharmaceutical and food preservation applications.

2.7.4 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative bacterium that serves as an important opportunistic pathogen, causing severe infections such as pneumonia, urinary tract infections, septicemia, and wound infections. The increasing prevalence of multidrug-resistant strains has heightened

interest in alternative antimicrobial agents derived from medicinal plants. *Piper guineense*, a widely used spice and medicinal plant in West Africa, has demonstrated notable antibacterial activity against *K. pneumoniae*, supporting its traditional medicinal applications.

Investigations into the antibacterial properties of *P. guineense* fruits and seeds have revealed significant inhibitory effects against *K. pneumoniae* in vitro. Methanol, ethanol, and aqueous extracts of the plant effectively suppress bacterial growth, as demonstrated by disc diffusion and broth microdilution assays, with minimum inhibitory concentrations (MICs) reflecting potent antibacterial action (Ekanem *et al*., 2010). The observed activity is largely attributed to the synergistic effect of bioactive phytochemicals, including alkaloids, flavonoids, saponins, tannins, and essential oils, which collectively interfere with the bacterial cell wall, membrane integrity, and key metabolic pathways (Cowan, 1999).

Essential oils derived from *P. guineense* fruits and seeds, rich in monoterpenes and sesquiterpenes such as β-caryophyllene, α-pinene, and linalool, have shown particularly strong activity against *K. pneumoniae*. These volatile compounds disrupt bacterial cell membranes, increase permeability, and inhibit biofilm formation, which is critical for controlling infections caused by this pathogen, especially given its ability to form protective biofilms in clinical and environmental settings (Ukeh *et al*., 2009). Flavonoids and phenolic compounds present in the extracts may further inhibit enzymatic processes essential for bacterial survival and virulence.

The antibacterial activity of *P. guineense* against *K. pneumoniae* aligns with its traditional use in managing respiratory infections, gastrointestinal disorders, and wound healing. Its efficacy against Gram-negative bacteria, which are often more resistant to antimicrobial agents due to their outer membrane barrier, highlights the therapeutic potential of this plant (Okigbo and Igwe, 2007). Moreover, the combination of multiple bioactive compounds in crude extracts may

produce synergistic effects, enhancing antibacterial efficacy compared to isolated compounds alone.

2.7.5 *Candida albicans*

Candida albicans is a common opportunistic fungal pathogen responsible for mucosal and systemic infections, particularly in immunocompromised individuals. The emergence of antifungal-resistant strains has prompted interest in natural products, including medicinal plants, as alternative antifungal agents. *Piper guineense*, traditionally used in West African medicine, has demonstrated significant antifungal activity against *C. albicans*, validating its ethnomedicinal applications.

Studies investigating the antifungal potential of *P. guineense* have shown that both fruit and seed extracts possess inhibitory effects against *C. albicans*. Methanol, ethanol, and aqueous extracts of the plant have been tested using disc diffusion and broth microdilution assays, revealing dose-dependent inhibition of fungal growth (Okigbo and Igwe, 2007). The minimum inhibitory concentrations (MICs) reported in these studies indicate that the plant exhibits potent antifungal activity, comparable in some cases to conventional antifungal drugs.

The antifungal effects of *P. guineense* are attributed to its rich phytochemical composition, including alkaloids, flavonoids, saponins, tannins, phenolics, and essential oils (Ekanem *et al*., 2010). Essential oils, particularly monoterpenes and sesquiterpenes such as β-caryophyllene, linalool, and α-pinene, are believed to compromise the fungal cell membrane, leading to increased permeability, leakage of cytoplasmic contents, and cell death (Ukeh *et al*., 2009). Flavonoids and phenolics may further inhibit key fungal enzymes and interfere with ergosterol synthesis, which is critical for maintaining fungal cell membrane integrity.

Additionally, saponins present in *P. guineense* are known to exert antifungal effects by forming complexes with sterols in the fungal membrane, disrupting its structure and function (Cowan, 1999). The synergistic interaction of multiple phytochemicals in the crude extract likely enhances antifungal efficacy, supporting the plant's traditional use in treating infections such as oral thrush, vaginal candidiasis, and other *Candida*-associated conditions.

2.7.7 *Aspergillus niger*

Aspergillus niger is a filamentous fungus commonly associated with food spoilage, agricultural losses, and opportunistic infections in humans, particularly in immunocompromised individuals. The increasing incidence of antifungal resistance has highlighted the need for alternative antifungal agents, including those derived from medicinal plants. *Piper guineense*, a spice and medicinal plant widely used in West African traditional medicine, has demonstrated notable antifungal activity against *A. niger*, validating its ethnomedicinal applications.

Research has shown that extracts from the fruits and seeds of *P. guineense* inhibit the growth of *A. niger* in vitro. Methanol, ethanol, and aqueous extracts have demonstrated significant antifungal activity in disc diffusion and broth microdilution assays, with inhibition zones and minimum inhibitory concentrations (MICs) indicating strong dose-dependent effects (Okigbo and Igwe, 2007). The antifungal efficacy of the plant is largely attributed to its diverse phytochemical profile, which includes alkaloids, flavonoids, saponins, tannins, phenolics, and essential oils (Ekanem *et al*., 2010).

Essential oils from *P. guineense* fruits and seeds, particularly monoterpenes and sesquiterpenes such as β-caryophyllene, linalool, and α-pinene, are considered key contributors to its antifungal activity. These compounds disrupt fungal cell membrane integrity, leading to increased permeability, leakage of intracellular components, and eventual cell death (Ukeh *et al*., 2009).

Additionally, flavonoids and phenolics may interfere with enzymatic systems critical for fungal growth and metabolism, while saponins interact with membrane sterols to further compromise cell viability (Cowan, 1999).

The antifungal activity of *P. guineense* against *A. niger* also supports its traditional use in food preservation and the management of fungal infections. By inhibiting fungal growth, *P. guineense* helps reduce spoilage and contamination in stored foods, while also providing a natural therapeutic option for fungal infections. The combined action of multiple phytochemicals in crude extracts may produce synergistic effects, enhancing antifungal efficacy beyond that of individual compounds (Okigbo and Igwe, 2007).

2.8 Antioxidant properties of *Piper guineense*

The antioxidant properties of its fruits and seeds have been extensively studied due to their potential role in preventing oxidative stress-related diseases, including cardiovascular disorders, diabetes, cancer, and neurodegenerative conditions. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, and natural antioxidants from plants have been recognized for their ability to scavenge free radicals and mitigate cellular damage.

Phytochemical analyses of *P. guineense* fruits and seeds have revealed high concentrations of bioactive compounds, including flavonoids, phenolics, tannins, alkaloids, and essential oils, which are largely responsible for its antioxidant activity (Ekanem *et al*., 2020). Phenolic compounds, in particular, possess hydroxyl groups that can donate hydrogen atoms to neutralize free radicals, while flavonoids can chelate metal ions and inhibit lipid peroxidation, thereby preventing oxidative damage to cellular components (Apak *et al*., 2016).

Several in vitro studies have evaluated the antioxidant potential of *P. guineense* using different assay systems. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay demonstrated that methanol and ethanol extracts of the fruit and seed exhibit strong free radical scavenging activity, comparable to standard antioxidants such as ascorbic acid and trolox (Okigbo and Igwe, 2017). Similarly, the ferric reducing antioxidant power (FRAP) and 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays confirmed the ability of *P. guineense* extracts to reduce oxidative agents and inhibit radical formation.

The antioxidant properties of *P. guineense* are not only beneficial for human health but also contribute to its preservative effects in food systems. The presence of natural antioxidants can prevent lipid oxidation in stored foods, enhancing shelf life and maintaining nutritional quality (Ukeh *et al*., 2009). Moreover, the synergistic action of multiple phytochemicals in the crude extracts may provide superior antioxidant activity compared to isolated compounds, highlighting the importance of whole-plant preparations in traditional medicine.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The following materials were used in this study:

3.1.1 Apparatus/ Equipment

Bench autoclave (Gallenkamp, U.K.), Binocular Microscope (Olympus), Incubator (size 2,

Gallenkamp, U.K.), Hot air oven (size 2, Gallenkamp, U.K.), Weighing balance (H80, Mettler, Switzerland), Centrifuge (MSE High speed 18), Water bath (Gallenkamp, U.K.), Spectrophotometer (SP8-400 uv/ visible, PYE UNICAM England), Soxhlet apparatus, Glass wares (pyrex burettes, pipettes, beakers, microscopic slides, glass petri diches, measuring cylinders, flasks, separating funnels, bijou, universal and Macartney bottles).

3.1.2 Chemicals/Reagents

All chemicals used were of analytical grade and they include, Ethanol (99.89%), Distilled water, n-Hexane, Hydrogen peroxide, 1% Tetramethyl-p-phenylenediamine hydrochloride (Oxidase reagent), Phenolphthalene, 0.1N NaOH, Tween-80 (10%), Picric acid, wagner reagent, Dragendroff's reagent, Methylated spirit, Crystal violet (0.5%$^{w}/_{v}$, BEMA), Safranine (BEMA), Grams iodine, Plasma, Kovac's reagent (Merk 6029259559), 1% Barium chloride, 1% sulfuric acid (H_2SO_4), Dettol, Glycerol, Starch, Glycerin, Sodium chloride, Fehling's solution A and B, Ferric chloride, Sodium picrate, dilute ammonia solution. All media and reagents used were prepared according to the manufacturer's direction.

3.2 Collection and Identification of Plant/ *Piper guineense* Fruit and Seed Samples

The *Piper guineense* were sourced from Local Markets in Benin City. After the purchase/acquisition of sufficient quantity, the samples were transported to the Plant Biology and Biotechnology laboratory, where it was Identified Authenticated by Prof. H. Akinnibosun as well as my supervisor (Prof. Oshomoh) as *Piper guineense* (Africa Pepper).

3.3 Preparation of Plant Extracts

The fruits and seeds of *P. guineense* was thoroughly cleaned and dried at room temperature for two weeks. The dried plant material was then ground to fine powder using an electric blender and stored in sterile containers until needed.

3.3.1 Ethanol extract

One hundred and fifty grams (150 g) of *P. guineense* was weighed into a 250 ml conical flask, then 100 ml of ethanol was added, the mixture was stirred with a glass rod. The mixture was kept at room temperature for 48 hr and rapidly stirred using glass rod every 8 hr. After 48 hr, the extract of the plant was filtered through whatman No.1 filter paper to exclude the powder. Then each filtrate was concentrated using water bath evaporator. A greasy final material obtained for each plant species was transferred to screw cap bottles, labeled and stored under refrigerator (4^0C) condition till use.

3.3.2 Aqueous extraction

A 50 g of the bulb of *P. guineense* was placed in 100 ml distilled water and allowed to stand on the laboratory bench for 48 hr. After 48 hr, the extract of plant was filtered through whatman No.1 filter paper. The filtrate was concentrated using water bath evaporator. A greasy final material obtained for plant was transferred to screw cap bottles, labeled and stored under refrigerator (4^0C) condition till use.

3.4 Qualitative Determination of Phytochemical Constituents of *P. guineense*

Qualitative screening of the phytochemical components of the plant extracts was carried out using the modified method described by sexena *et al*., (2013). Essentially, specific weight of the extracts was made up to 10 ml in a test tube and different reagents were added to specifications. Positive results were indicated by colour change and precipitate formation which were compared against standards. The extracts were tested for the presence of glycosides, alkaloids, tannins, saponins, anthraquinones, phenolics, steroids, resins, terpenoid and flavonoids.

3.4.1 Test for saponins

To 1g of the plant extract was added 20 ml of distilled water and heated for 5minutes. 4ml of the solution was measured into a test tube and 2ml of distilled water was added with vigorous shaking, after which it was allowed to stand for 6 minutes. A stable frothing or foaming indicates the presence of saponins.

3.4.2 Test for anthraquinone

One gram (1 g) of the extract was shaken vigorously with 10 ml of chloroform. To 4 ml chloroform extract was added 10 % ammonium hydroxide solution (2 ml). Observation of color change to orange indicates the presence of anthraquinones

3.4.3 Test for steroids

One gram (1 g) of the extract was extracted with 20 ml methanol, by heating on a water bath. It was filtered and the filtrate evaporated to dryness. A little quantity of the residue obtained from the filtrate was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added by the side of the test tube to form a lower layer.

3.4.4 Test for tannins

One gram (1 g) of the extract dissolved in a tube up to 2ml plus two drops of 5 % ferric chloride. The presence of reddish brown precipitate confirmed the presence of tannins.

3.4.5 Test for flavonoids

To 2 ml of the filtrate obtained above, 1 ml of sodium hydroxide was added, and then 1 ml conc. HCl was added. Formation of cloudy precipitate confirms the presence of flavonoids.

3.4.6 Phenolics

One milliliter of the extract was added to 1 mL of 10% $FeCl_2$ and mixed together. The presence of blue precipitate confirmed the presence of phenols.

3.4.7 Tests for alkaloids

Two grams (2 g) of the extract was dissolved in 5 ml 1 % sulphuric acid and filtered. The filtrate was tested with alkaloidal reagents (Dragendorff, Wagner, Mayer and Hager). In the process, the filtrates are collected in various test tubes. To a tube containing the filterate, a few drops of Wagner's Reagent (Potassium-iodine solution) were added to one part of the filtrate in a test tube. A reddish brown precipitate formation gives a positive result. Generally, the formation of specific precipitate and coloration upon adding drops of Dragendrorff, Wagner, Mayer and Hager's reagent indicates positive results or presence of alkaloids.

3.4.8 Test for terpenoids

A quantity (9ml) of ethanol was added to 1g each of the extracts and refluxed for a few minute and filtered. Each of the filtrates was concentrated to 2.5ml in a boiling water bath. Distilled water, 5ml was added to each of the concentrated solution, each of the mixtures was allowed to stand for 1 hour and the waxy matter was filtered off. Each of the filtrates was extracted with 2.5ml of chloroform using a separating funnel. To 0.5ml each of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicates the presence of terpenoids.

3.4.9 Test for glycosides

To 5ml of the extract in tubes treated with glacial acetic acid containing 1drop of ferric chloride (0.1%) was added to 1ml of concentrated H_2SO_4. A brownish to brick red ring or violet colour at the interphase indicates the presence of glycosides.

3.5 Quantitative Phytochemical Composition

After preliminary analysis to determine presence of these phytochemicals, the samples were further subjected to quantitative analysis to determine the percentage of each of these secondary metabolites present the plant extracts. The following procedures were adopted:

3.5.1. Determination of total phenolics compounds

The total phenol content was determined using a standard caliberation curve as described by sexena *et al*., (2013). To 1ml of samples/extracts in test tube was mixed with methanol (5 g/L) and further mixed with ethanol solution of gallic acid (1 mL; 0.025-0.400 mg/mL) with 5 mL of Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 mL, 0.7 M) solution and ultimately the volume was made up to 8 ml with distilled water followed by vigorous shaking and was allowed to stand for 30mins, after which absorbance values were measured at 765 nm using a spectrophotometer and the standard curve was plotted to determine the total phenolic contents. All experiments were carried out in triplicate. The total phenolics components in the extracts in gallic acid equivalents (GAE) were calculated by the formular:

$T = CxV/M$

Where:

T = total phenolic contents, milligram per gram of sample extract, in GAE

C = the concentration of gallic acid established from the calibration curve, mg/mL

V = the volume of extract, milliliter

M = the weight of sample/extract (g)

Or

Percentage phenol extracted from powdered sample thus:

$$\text{Phenols (\%)} = \frac{100}{W} \times \frac{C}{1000} \times \frac{VF}{VA} \times \frac{D}{1}$$

Where:

W = Weight of sample analysed

C = Concentration of standard in mg/ml

VF = Total filtrate volume

VA = Volume of filtrate analysed

D = Dilution factor where applicable

3.5.2. Determination of tannin content

The tannin content was determined by Folin Denis colorimetric method described by Sexena *et al*. (2013). Briefly, Five grams of the powdered sample was measured into a volumetric flask and 50 mL of distilled water was added to the content of the volumetric flask. The mixture was shaken for 30 min at room temperature and filtered to obtain the filtrate. A standard tannic acid solution was prepared, 2 mL of the standard solution and equal volume of distilled water were dispersed into a separate 50 mL volumetric flasks to serve as a standard and reagent blank respectively. Then 2 mL of each of the respective experimental samples were measured into their respective labeled flasks. The content of each flask was mixed with 35 mL distilled water and 1 mL of the Folin reagent . This was followed by 2.5 mL of saturated Na_2CO_3 solution. Therefore, each flask was diluted to the 50 mL mark with distilled water and incubated for 90 min at room temperature. After which their absorbance was measured at 760 nm in a spectrophotometer with the reagent blank at zero. The tannin content was calculated as shown below:

$$\text{Tannin (\%)} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va}$$

Where: W = Weight of sample

au = Absorbance of test sample

as = Absorbance of standard tanning solution

C = Concentration of standard tannin Solution

Vt = Total volume of extract

Va = Volume of extract analyzed

3.5.3. Determination of total flavonoids

The method is based on the formation of the flavonoids-aluminium complex which has an absorptivity maximum at 415nm. 100µl of the sample/extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorbance at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorbance of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All experiments were carried out in triplicates.

3.5.4. Determination of total alkaloids

To 5g of the sample weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed

Percentage alkaloids were computed as follows:

Alkaloids (%) = $\frac{(W2-W1)}{\text{Weight of sample}} \times 100$

Where:

(W_2- W_1) = Weight of residue

3.5.5. Determination of total saponins

The total saponin was done by the double solvent extraction gravimetric method decribed by Sexena *et al*. (2013). Briefly, 5g of sample was mixed with 50 mL of 20% aqueous ethanol solution and incubated for 12 h at a temperature of 55^{o}C with constant agitation. After that, the mixture was filtered through Whatman No. 42 grades of filter paper. The residue was re-extracted with 50 mL of the ethanol solution for 30 min and the extracts weighed together. The combined extract was reduced to about 40 mL by evaporation and then transferred to a separating funnel and equal volume (40 mL) of diethyl ether was added to it. After mixing well, there was a partition and the other layer was discarded while the aqueous layer was reserved. This aqueous layer was re-extracted with the ether after which its pH was adjusted with drop-wise addition of dilute NaOH solution. Saponin in the extract was taken up in successive extraction with 60 and 30 mL portion of normal butanol. The combine extract was washed with 5% NaCl solution and evaporated to dryness in a previously weighted evaporating dish. The saponin was then dried in the oven at 60^{o}C (to remove any residual solvent) cooled in a desiccators and re-weighed. The saponin was determined and calculated as a percentage of the original samples.

Saponin (%) = (W2-W1/W) x 100

Where: W = Weight of sample used

W1 = Weight of empty evaporation dish

W2 = Weight of dish + saponin extract

3.5.6. Determination of total glycosides

The digested glycoside content of the sample was determined using the method described by Gilliani *et al.*, 2007 and Sexena *et al*., 2013. In the process, 5g of the sample was dissolved in 250 ml of distilled water and treated with glacial acetic acid containing 1drop of ferric chloride (0.1%) and introduced into a beaker containing 1ml of concentrated H_2SO_4 with continuous agitated for 3hours using a shaker, followed by filtration. After which 10 ml of freshly prepared 0.10% Anthrone reagent was added, stoppered and mixed thoroughly by gently shaking. The experiment was repeated to obtain a blank using distilled water in place of sample. After which samples obtained were transferred to spectrophotometer and absorbance read at 630 nm against the blank. The total available glycosides was then calculated accordingly:

$$\text{Glycoside (\%)} = \frac{25\ A_1 \times 100}{W \times A_2}$$

Where : W = weight of sample

25 = Constant

A_1 = Absorbance of diluted sample

A_2 = Absorbance of diluted standard

3.6 Invitro Antioxidant Assay

3.6.1. DPPH radical scavenging assay

Free radical scavenging ability of the sample/extracts was tested by DPPH radical scavenging assay as described by Jha*et al*., (2018). Summarily, a solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol making a whole volume of 3mL in per test-tubes of different concentrations (15–960 μg/mL). The reaction mixture was vortexed thoroughly and left in the dark or incubated with complete foil masking in

the dark at ambient trmperature (27±2°C) for 30 min. The hydrogen atom donating ability of the sample was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants which indicates a positive result and characterized by decrease in absorbance readings. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference or positive control while tubes with reagents without sample served as (negative control). The blank correction was a preparation of the extract concentration in the reference solvent (without DPPH reagents). Percentage DPPH radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity (% RSA) = {(A0− A1)/A0}×100

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard.

Or % Inhibition = $\frac{A_{control}-(A_{sample}-A_{sample\ blank})}{A_{control}} \times 100$

Where $A_{control}$ = Absorbance of DPPH in methanol (negative control)

Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. IC_{50} estimation is given by IC_{50} = concentration giving 50% inhibition. Determine by plotting % inhibition vs log(concentration) and interpolate, or rather from a linear interpolation between the two points that straddle 50%. All experiment was done in triplicates for each concentration.

3.6.2. Ferrous reducing antioxidant Potential (FRAP) assay

The ferrous reducing antioxidant Potential (FRAP) of samples was evaluated by the method described by Baydar and Baydar (2013). Accordingly, the freshly prepared stock solution contain 300 mM acetate buffer (3.1g $C_2H_3NaO_2.3H_2O$ and 16 M $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 nMHCl, and 20 mM $FeCl_3.H_2O$ solution. The extracts (1.5 ml) were allowed to react separately with 2.85 ml of the FRAP solution incubated for 5-30 min in the dark in a water bath at 37°C and readings (absorbance) of the coloured product (ferrous tripyridyltriazine complex) were then taken at 593 nm.

The standard curve of $FeSO_4$ (absorbance vs [Fe^{2+}] µM) was made after conversion of sample absorbance to µmol Fe^{2+} equivalent per gram of extract) according to the following:

$$\text{FRAP } (\mu\text{mol}Fe^{2+}/\text{g}) = \frac{\text{X } \mu\text{mol}Fe^{2+}/\text{mL}}{\text{mg sample/mL}} \times 1000$$

3.7 Determination of Percentage Yield

The percentage yield of the plant extracts was determined using the formula:

$$Percentage\ (\%)yield = \frac{Weight\ of\ plant\ extract}{Wt.\ of\ pulverised\ powder} x\ \frac{100}{1}$$

3.7.1. Percentage yield of the ethanol extract:

Mass/weight of extract = 21.5g

Mass of pulverized powder = 120.3g

$$Percentage\ (\%)yield = \frac{21.5}{120.3} x\ \frac{100}{1}$$

Percentage yield = 17.87%

3.7.2. Percentage yield of the aqueous extract

Mass/weight of extract = 27.7g

Mass of pulverized powder = 120.5g

Percentage (%) yield = 27.7/120.5 x 100

Percentage yield = 22.82%

3.8 Collection of Test Organisms

The microbial isolates used were obtained from stock cultures of clinical isolates stored from cases of Nosocomial infections from University of Benin teaching Hospital (UBTH) and stored as stock cultures in Pharmaceutical Microbiology and Biotechnology Department of Faculty of Pharmacy, University of Benin (UNIBEN). The selected isolates include *Staphylococcus aureus*, *Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger* (Cheesbrough, 2006).

3.9 Preparation of Culture Media

All media was prepared according to manufacturer's instruction. The media that was used in this study included Nutrient Agar and Mueller-Hinton agar. All media composition was shown in appendix.

3.9.1 Nutrient Agar (BIOTECH, TM 341, India)

The preparation was by dissolving of 28 g of the nutrient agar powder in 1000 ml of distilled water inside a conical flask sealed with cotton wool and aluminum foil paper. The mixture was shaken in other to attain an almost homogeneous solution. Sterilization was then carried out on the resulting mixture using the autoclave at 121 ^{o}C for 15 min. The medium was cooled to 45-50 ^{o}C which was then dispensed aseptically into sterile Petri dishes.

3.9.2 Mueller Hinton Agar (BIOTECH, TM 339, India)

Mueller-Hinton agar is a microbial growth medium that is commonly used for antibiotic susceptibility test. The medium was prepared from already sold dehydrated powder of Mueller-Hinton agar. The preparation was done by dissolving of 38 g of Mueller-Hinton agar in 1000 ml of distilled water inside a conical flask sealed with cotton wool and aluminum foil paper. The mixture was shaken in other to attain an almost heterogeneous solution. Sterilization was subsequently carried out using the autoclaveat 121^{o}C for 15 min. The medium was cooled to 45^{0}C and then dispensed aseptically into sterile Petri dishes.

3.9.3 Nutrient Broth (BIOTECH, TM 350, India)

Thirteen grams (13g) of nutrient broth powder (CM0001B) was added in 1 litre of distilled water. It was mix to dissolve completely. It was the poured into a conical flask then sterilized by autoclaving at 121°C for 15 minutes.

3.9.4 Sabouraud Dextrose Agar (BIOTECH, TM 361, India)

Sixty five grams (65 g) of the dehydrated powder of **Sabouraud Dextrose Agar** was suspended in 1 liter of distilled water. It was heat to dissolve while the pH was adjusted to 5.6. it was then sterilized by autoclaving at 121°C for 15 min. Finally, it was allowed to cool to 45-50°C then poured into a sterile Petri dishes to solidify.

3.9.5 Potato Dextrose Agar (BIOTECH, TM 387, India),

Thirty-nine grams (39 g) of potato dextrose agar (PDA) powder was dissolved in 1 litre of distilled water in a conical flask covered with cotton wool and aluminium foil paper. It was mixed thoroughly and sterilized by autoclaving at 121^{O}C for 15 minutes. The medium was cooled to 45-50^{O}C and then dispensed aseptically into sterile Petri dishes.

3.10 Determination of Antimicrobial Properties

The modified agar well diffusion method described by Cheesbrough, (2006) and CLSI, (2010) was used to determine the antimicrobial sensitivity/potency of the ethanol and aqueous extracts of *Piper guineense* against test organisms. In the process, wells of 6mm in diameter were made into seeded Mueller Hinton agar (antibacterial sensitivity) and Sabouraud dextrose agar (antifungal sensitivity) plates using a flamed cork borer. Prior to seeding, isolated colonies/spores stored in slants were sub-cultured into nutrient broth/sabouraud dextrose broth, vigorously shaken and adjusted to achieve 1:100 dilution of 0.5 Macfarland tubidity standard (containing approximately 10^6 cfu/spores per mL when counted using a cytometer) previously determined using a spectrophotometer. Sterile swab sticks was then dipped into the standardized microbial suspension and gently spread over (seeding) the surface of the agar plates in even strokes to obtain a uniform growth pattern across the entire surface of the plate. This was achieved by rotating the plate 90 degrees followed by 45 degrees with continuous streaking, and finally by streaking round the diameter of the agar. The 6mm wells were filled with equal volumes (100µL) of the stock concentration and lower dilutions of the sample corresponding to 100, 50 and 25 mg/mL concentrations. The same quantity of sterilized normal saline and 1 µg/mL Ciprofloxacin (bacterial plates)/10µg/mL Nystatin served as negative and positive controls respectively. All plates were appropriately incubated i.e 24hrs, 38^{o}C for bacterial plates and ambient temperature (27±2^{o}C) for 48-72hrs for fungal plates in an upright position to allow proper diffusion of extracts. All experiments were in triplicates. After incubation, the absence or presence of microbial growth around the wells were observed on the plates and the diameter of clear zones were measured using a millimetre (mm) calibrated ruler and the mean Inhibition zone diameters (IZDs) calculated and recorded.

3.10.1 Determination of MICs of the selected antimicrobial agent

The modified broth dilution method described by Firas *et al*. (2008), was used to determine the MICs of the extracts against the test isolates. Varying concentrations of the selected antimicrobial agent ranging from 0.1 -10 mg/mL were constituted in 10 ml of Mueller-Hinton broth in sterile capped tubes from the stock. 100µL of the overnight broth culture of the test standardized microbial suspension. In each round of experiment, a tube without the extract but with same volume of broth and inoculum served as controls. The same experiment was repeated for the fungal isolate but Sabouraud dextrose broth was used in place of Mueller-Hinton. All tubes were appropriately incubated. After incubation, tubes were observed for growth/turbidity. In all cases, the lowest concentration of the extract at which there was no observable bacterial or fungal growth was recorded as the MICs.

3.10.2 Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the Extracts

The broth tubes with no visible growth following MIC determination were inoculated into fresh Nutrient agar/SDA plates using a flamed inoculating loop. Three MIC experimental tubes with concentrations beginning from MIC and progressively higher than the MIC concentrations were considered after which all plates were appropriately incubated (bacterial plates at 38^{o}C for 24 hours and fungal plates at 27±2^{o}C/room temperature for 48 hours). After incubation, all plates were observed for growth and the MBC/MFC was recorded as the lowest concentration of extracts that completely destroyed the microbial cells indicated as no observable growth of test organisms inoculated from tubes into the fresh agar plates (Lalitha, 2004; CLSI, 2010; Dowe *et al*., 2016).

3.11 Data Analysis

Data analysis was carried out using Microsoft excel, Spss and Graphpad prism applications. All data were summarised by descriptive (mean, mean ± standard error of mean, etc.) into table charts and graphs and statistical significance at 0.05 (Ogbeibu, 2015).

CHAPTER FOUR

RESULTS

The results for the phytochemical, antioxidant and antimicrobial profiling of the aqueous and ethanolic extracts of *Piper guineense* fruit and seed are presented in Table 4.1 to table 4.7 and figure 4.1 below.

Table 4.1 shows the qualitative phytochemical composition of ethanolic and aqueous extracts of *P. guinense* revealed the presence of alkaloids, flavonoids, phenols, saponins, terpenoids, glycosides and steroids were present in ethanolic and aqueous extracts of *P. guinense* while anthraquinones was present in ethanolic extract and absent in aqueous extract.

Table 4.2 shows the quantitative phytochemical composition of ethanolic and aqueous extracts of *P. guinense.* Of all the phytochemical identified, Saponins (10.05±1.05 mg/g) had the highest composition, followed by phenolics (8.51±2.56 mg/g) in aqueous extract while Flavonoids (12.10±2.17 mg/g) had the highest composition in ethanolic extract of *P. guinense.*

Results obtained for the yield upon ethanol and aqueous extraction of the *P. guinense* fruit and seeds is presented in Table 4.3. Aqueous extract (22.82%) had the highest percentage yield compared to ethanolic extract (17.87%).

Table 4.1: Phytochemical compounds in the extracts of *P. guinense*

Plant constituents	Aqueous	Ethanol
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Tannins	+	+
Anthraquinones	-	+
Terpenoids	+	+
Glycosides	+	+
Steroids	+	+

Key: + = positive (present), - = negative (absent)

Table 4.2: Quantitative analysis of secondary metabolites in the extracts of *P. guinense* [mean ± SD (mg/g DW)]

Plant constituents	Aqueous	Ethanol
Alkaloids	5.36 ±0.67	9.06± 1.51
Flavonoids	8.35±0.31	12.10±2.17
Phenolics	8.51±2.56	11.06±1.66
Saponins	10.05± 1.05	7.31±0.35
Tannins	7.18±1.21	10.50±1.37
Anthraquinones	0.01±0.00	1.06±1.26
Terpenoids	2.43±0.51	2.13±0.99
Glycosides	3.31±1.33	4.31±0.17
Steroids	3.21±2.03	4.65±1.53

Key: mean ± SD (standard deviation)

Mg/g DW **(**Milligrams per gram of Dry Weight)

Table 4.3: Yield of the ethanol and aqueous extracts of *P. guinense*

Extraction Solvent	Weight of plant material (g)	Weight of extract (g)	Percentage yield (%)
Ethanol	120.3	21.5	17.87
Aqueous	120.5	18.5	22.82

Table 4.4 shows the antimicrobial activities of aqueous extract of *P. guinense* at different concentration. Highest zone of inhibition was observed at 100mg/ml) concentration for *Staphylococcus aureus* (19.3±1.7 mm), *Bacillus subtilis* (30.5±0.5 mm), *Klebsiella pneumoniae* (19.5±0.5 mm), *Pseudomonas aeruginosa* (15.0±1.0 mm), *Candida albicans* (18.3±2.7 mm) and *Aspergillus niger* (13.5±1.5 mm). The zone of inhibition of the aqueous extract were lower than the control (Ciprofloxain 1µg/mL) which had zone of inhibition of 19.3±1.7 mm against *S. aureus*, 30.5±0.5 mm, 35.3±1.6 mm and 15.0±1.0 mm against *B. subtilis, K. pneumoniae* and *P. aeruginosa* respectively for bacterial isolates. Also fungal isolates zone of inhibition were lower than control (Nystatin 10µg/mL) which had 33.5±0.5 mm and 13.5±1.5 mm against *C. albicans* and *A. niger* respectively.

The antimicrobial activities of ethanolic extract of *P. guinense* at different concentration is shown in Table 4.5. The ethanolic extract showed strong activity, especially at 100 mg/mL concentration against *S. aureus* (21.6±2.3 mm), *B. subtilis* (23.5±1.6 mm), *K. pneumoniae* (21.7±1.3 mm) and *P. aeruginosa* (32.1±2.5 mm) but ciprofloxacin remained significantly more potent against *S. aureus* (30.5±1.6 mm), *B. subtilis* (36.3±1.3 mm), *K. pneumoniae* (31.3±1.6 mm) and *P. aeruginosa* (32.1±2.5 mm). The ethanol extract showed strong antifungal activity at 100 mg/mL against *C. albicans* (21.5±2.5 mm) and *A. niger* (15.3±1.6 mm). However, Nystatin exhibited a much larger inhibition zone against *C. albicans* (35.5±3.5 mm) and *A. niger* (29.1±1.6 mm) respectively.

Table 4.4: Antimicrobial activities of the Aqueous extract of *P. guinense* at different concentration

Organisms	**Zones of Inhibition (mean ± S.E.M mm)**					
	Concentrations (mg/mL)			**CIP**	**Nystatin**	**Sterilized**
	25	**50**	**100**	**1µg/mL**	**10µg/mL**	**D.H_2O**
Staphylococcus aureus	12.9±1.1	16.6±2.8	19.3±1.7	33.7±1.3	0.0±0.0	0.0±0.0
Bacillus subtilis	10.4±1.6	18.5±1.6	21.0±1.0	30.5±0.5	0.0±0.0	0.0±0.0
Klebsiella pneumoniae	10.1±2.3	15.5±2.5	19.5±0.5	35.3±1.6	0.0±0.0	0.0±0.0
Pseudomonas aeruginosa	6.6±1.6	12.6±1.7	15.0±1.0	32.1±2.9	0.0±0.0	0.0±0.0
Candida albicans	7.5±2.5	11.3±1.5	18.3±2.7	0.0±0.0	33.5±0.5	0.0±0.0
Aspergillus niger	4.5±1.5	07.1±2.3	13.5±1.5	0.0±0.0	32.1±1.8	0.0±0.0

Key: S.E.M = Standard Error of Mean, 0.0 = No activity, CIP = ciprofloxacin, D.H_2O = Distilled water

Table 4.5: Antimicrobial activities of the Ethanol extract of *P. guinense* at different concentrations

Organisms	Zones of Inhibition (mean ± SD mm)					
	Concentrations (mg/mL)			CIP	Nystatin	Sterilized
	25	50	100	1µg/mL	10µg/mL	D.H_2O
S. aureus	14.5±1.5	18.2±2.3	21.6±2.3	30.5±1.6	0.0±0.0	0.0±0.0
B. subtilis	14.7±1.3	20.7±1.6	23.5±1.6	36.3±1.3	0.0±0.0	0.0±0.0
K. pneumoniae	11.1±2.9	17.6±2.6	21.7±1.3	31.3±1.6	0.0±0.0	0.0±0.0
P. aeruginosa	6.6±1.6	14.2±1.8	17.1±1.9	32.1±2.5	0.0±0.0	0.0±0.0
C. albicans	7.5±2.3	13.5±2.5	21.5±2.5	0.0±0.0	35.5±3.5	0.0±0.0
A. niger	3.5±1.6	10.3±2.3	15.3±1.6	0.0±0.0	29.1±1.6	0.0±0.0

Key: S.E.M = Standard Error of Mean, 0.0 = No activity, CIP = ciprofloxacin, D.H_2O = Distilled water

Table 4.6 shows the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal concentrations (MBCs/MFCs) of the ethanol and aqueous extract of *P. guinense* against the Test microorganisms. The highest MIC (8 mg/mL) for aqueous extract was observed against *K. pneumoniae, P. aeruginosa, C. albicans* and *A. niger* while the highest MBC was against *P. aeruginosa* (10 mg/mL) with *B. subtilis* had the lowest MIC (0.9 mg/mL) and MBC (4 mg/mL). *P. aeruginosa* had the highest MIC (6 mg/mL) for the bacterial isolates while *A. niger* had the highest MIC (8 mg/mL) for the fungal isolates in the ethanolic extract. *P. aeruginosa, C. albicans* and *A. niger* showed highest MBC (8 mg/mL) while *B. subtilis* had the lowest MBC (2 mg/mL).

The result for DPPH radical scavenging activity of African Pepper determined spectrophotometrically at 517nm is shown in Table 4.7. DPPH for aqueous extract ranged from 0.756±0.66 (10 µg/mL) to 0.306±0.21 (200 µg/mL), ethanolic extract range from 0.623±0.31 (10 µg/mL) to 0.226±0.05 (200 µg/mL) compared to control (Ascorbic acid) which range from 0.421±0.13 (10 µg/mL) to 0.110±0.04 (200 µg/mL)

Figure 4.1 shows the percentage inhibition of DPPH radical/Radical Scavenging activity (RSA) of the Aqueous and Ethanol extracts of *P. guinense* at different concentrations. Percentage inhibition of aqueous extract ranged from 13.5±0.41 (10 µg/mL) to 56.12±0.66 (200 µg/mL), ethanolic extract ranged from 17.65±0.41 (10 µg/mL) to 78.62±0.66 (200 µg/mL) while control (ascorbic acid) range from 49.81±0.11 (10 µg/mL) to 98.01±0.35 (200 µg/mL).

The result of FRAP showing the reducing power of the African Pepper extracts by measuring their ability to reduce Fe^{3+} to $Fe^{2+}$is shown in Table 4.8. ethanolic extract (262.2 ± 7.3 µmol Fe^{2+}/g) had the highest reducing power compare to ethanol extract (133.6 ± 11.3 µmol Fe^{2+}/g) when compared to control (ascorbic acid) 615.5 ± 10.6 7.3 µmol Fe^{2+}/g.

Table 4.6: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal concentrations (MBCs/MFCs) of the ethanol and aqueous extract of *P. guinense* against the Test Organisms

Organisms	Aqueous		Ethanol	
	MIC	MBC	MIC	MBC
	(mg/mL)			
S. aureus	4	8	0.9	2
B. subtilis	0.9	4	0.6	0.9
K. pneumoniae	8	8	0.9	2
P. aeruginosa	8	10	6	8
C. albicans	8	8	4	8
A. niger	8	12	8	8

Key:

MIC = Minimum Inhibitory Concentration

MBC= Minimum Bactericidal concentration

MFC = Minimum Fungicidal concentration

Mg/mL = milligram per milliter

Table 4.7: DPPH radical scavenging activity of *P. guinense* determined spectrophotometrically at 517nm

Concentration (µg/mL)	Aqueous Extract	Ethanol Extract	Ascorbic acid (Standard Antiox.)
10	0.756±0.66	0.623±0.31	0.421±0.13
25	0.697±0.71	0.574±0.25	0.363±0.01
50	0.597±0.15	0.381±0.15	0.283±0.05
100	0.448±0.11	0.282±0.13	0.251±0.17
200	0.306±0.21	0.226±0.05	0.110±0.04

Key:

DPPH= 2,2-Diphenyl-1-picrylhydrazyl

Mg/mL = milligram per milliter

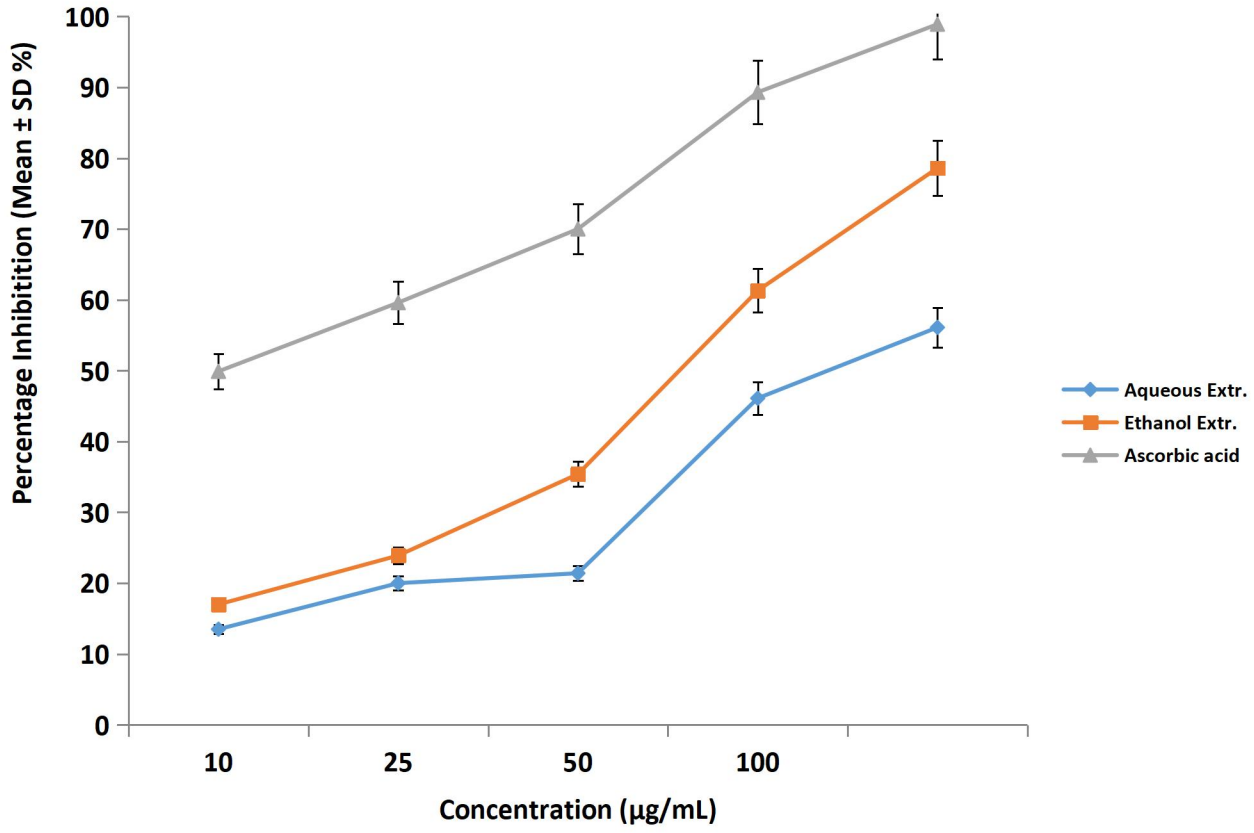

Figure 4.1: Percentage inhibition of DPPH radical/Radical Scavenging activity (RSA) of the Aqueous and Ethanol extracts of African pepper at different concentrations

Table 4.8: FRAP showing the reducing power of the African Pepper extracts by measuring their ability to reduce Fe^{3+} to Fe^{2+}

Sample	FRAP (µmol Fe^{2+}/g extract ± SD)
Ethanol extract	262.2 ± 7.3
Aqueous extract	133.6 ± 11.3
Ascorbic acid (standard)	615.5 ± 10.6

Overall, the ethanol extract exhibited stronger antioxidant potential than the aqueous extract, though both were less active than Ascorbic acid (Standard antioxidant).

CHAPTER FIVE

DISCUSSION

The antimicrobial activity of plant extracts have been linked by many researchers to the presence of phytochemicals in them (Apak *et al*., 2016). The qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, saponins, terpenoids, glycosides, and steroids in both ethanolic and aqueous extracts of *Piper guineense*. This indicates that *P. guineense* is rich in diverse bioactive secondary metabolites, which supports its extensive use in traditional medicine for antimicrobial, anti-inflammatory, and antioxidant purposes. This result is consistent with prior studies of Akinmoladun *et al*. (2019); Ezejiofor *et al*. (2020) who reported that *Piper guineense* contains a wide range of phytochemicals, particularly alkaloids, flavonoids, saponins, and phenolic compounds, which are attributed to its medicinal and antioxidant properties. The presence of anthraquinones exclusively in the ethanolic extract and their absence in the aqueous extract can be attributed to solvent polarity and solubility differences. Anthraquinones are relatively less polar compounds and are more efficiently extracted by organic solvents such as ethanol than by water. Similar observations have been reported in earlier studies on *P. guineense* and other medicinal plants, where ethanolic or methanolic extracts showed a broader phytochemical profile compared to aqueous extracts. These findings agrees with the study of Okigbo *et al*. (2015) indicating that alcohol-based solvents are more effective in extracting moderately polar to non-polar phytochemicals.

Quantitative analysis showed variations in the concentration of phytochemicals depending on the extraction solvent. In the aqueous extract, saponins (10.05 ± 1.05 mg/g) were the most abundant phytochemicals, followed by phenolic compounds (8.51 ± 2.56 mg/g). This suggests that water is

particularly effective in extracting highly polar compounds such as saponins and phenolics. This findings aligns with the study of Olatunji *et al.* (2018); Ejele *et al*. (2012) who reported high saponin and phenolic contents in aqueous extracts of *P. guineense* and related *Piper* species.

In contrast, flavonoids (12.10 ± 2.17 mg/g) were most abundant in the ethanolic extract. Ethanol is known to be a more efficient solvent for extracting flavonoids due to its intermediate polarity, which enhances the solubility of both polar and moderately non-polar compounds. This observation is consistent with reports by Nwankwo *et al*. (2014) and Iqbal *et al*. (2016), who found higher flavonoid contents in ethanolic extracts of *P. guineense* and other medicinal plants compared to aqueous extracts.

The presence of tannins in the extracts of *Piper guineense* implies that the extract can be pharmacologically useful as astringents. Chinonye *et al*. (2025) reported that the astringent activity of tannins is by precipitating proteins, thereby protecting the underlying tissue leading to improvement of wound healing. Tannins inhibit microbial proliferation by denaturation of enzymes involved in microbial metabolism (Aké *et al*., 2019). Tannins also have shown potential antiviral, antibacterial , antiparasitic and anticancer effects (Aké *et al*., 2019).

Flavonoids present in *Piper guineense* fruit and seed extract have been referred to as nature's biological response modifiers because of its efficiency in the modification of the body's response to allergies, viruses, and carcinogens. They display antiallergic, anti-inflammatory microbial and anti-cancer activity (Tungmunnithum *et al*., 2018). The presence of flavonoids in the leaf of *Piper guineense* may also account for its usage as an antinflammatory agent (Patience *et al.*, 2024). It also implies that the plant could be useful in preventing damages caused by free radicals in the body and also the treatment of diarrhoea (Ogunmefun *et al*., 2017).

Saponins have antibacterial activities (Okwu and Ighodaro, 2010) and have been applied in treating microbial infections. Alkaloids have a broad range of pharmacological properties which include antimalarial, antiasthma, anticancer properties as reported by Kittakoop, *et al* (2014). Glycosides have been useful in the treating of congestive heart failure, constipation, edema and microbial infections (Franstisk, 2001).

Phenols present in this study have also been reported to posses antibacterial properties (Adamu *et al*., 2017). It was observed that the quality and quantity of phytochemicals present in *Piper guineense* fruit and seed differ for both extracts. This may be due to the type and nature of extraction solvent used as suggested by Kumar and Pandey (2013). The result suggest that ethanol can extract a wider range and quantity of phytochemicals from *Piper guineense* fruit and seeds than aqueous (water).

The aqueous extract yielded a higher percentage (22.82%) compared to the ethanolic extract (17.87%). This higher yield may be due to the ability of water to extract a wide range of water-soluble constituents, including sugars, proteins, saponins, and some phenolic compounds, which contribute significantly to the total extractable mass. Similar findings have been reported by Akinyemi *et al*. (2016) and Obi *et al*. (2020), who observed higher extraction yields with aqueous solvents than with ethanol when extracting *P. guineense* fruits and seeds. However, despite the higher yield of the aqueous extract, the ethanolic extract demonstrated higher concentrations of certain bioactive compounds, particularly flavonoids and anthraquinones. This supports the findings of Iqbal *et al*. (2016) suggesting that extraction yield does not necessarily correlate with phytochemical potency or diversity.

This study showed that *Piper guineense* fruit and seeds extracts are effective inhibitors of microbial growth. The aqueous extract of *Piper guineense* exhibited measurable antimicrobial

activity against all tested bacterial and fungal isolates, with activity increasing in a concentration-dependent manner. It was observed that, increased concentration of the extracts, led to increased antibacterial activities. The highest zones of inhibition were observed at 100 mg/mL against *Staphylococcus aureus* (19.3 ± 1.7 mm), *Bacillus subtilis* (30.5 ± 0.5 mm), *Klebsiella pneumoniae* (19.5 ± 0.5 mm), *Pseudomonas aeruginosa* (15.0 ± 1.0 mm), *Candida albicans* (18.3 ± 2.7 mm), and *Aspergillus niger* (13.5 ± 1.5 mm). This indicates that the aqueous extract possesses broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungal pathogens.

Despite this activity, the zones of inhibition produced by the aqueous extract were generally lower than those of the standard antibiotics used as controls. Ciprofloxacin (1 µg/mL) produced larger inhibition zones against the bacterial isolates, while nystatin (10 µg/mL) showed superior antifungal activity against *C. albicans* and *A. niger*. This observation is expected, as standard antibiotics are purified compounds with targeted mechanisms of action, whereas plant extracts are crude mixtures containing varying concentrations of bioactive constituents.

These findings are consistent with earlier reports by Ejele *et al*. (2012), who documented moderate antimicrobial activity of aqueous extracts of *P. guineense*, particularly against Gram-positive bacteria. The relatively lower activity of the aqueous extract may be attributed to the limited ability of water to extract non-polar or moderately polar antimicrobial compounds such as alkaloids, terpenoids, and phenolic derivatives, which have been implicated in antimicrobial action (Ejele *et al*., 2012).

The ethanolic extract of *P. guineense* demonstrated stronger antimicrobial activity than the aqueous extract across most tested organisms, particularly at the highest concentration (100 mg/mL). This may be due to the ability of ethanol to extract a wider range of antibacterial

properties than the aqueous solvent. The presence of ethanol in addition to achieving better extraction may also enhance the efficacy of the active ingredients (Ali *et al*., 2018). Notable inhibition was observed against *S. aureus* (21.6 ± 2.3 mm), *B. subtilis* (23.5 ± 1.6 mm), *K. pneumoniae* (21.7 ± 1.3 mm), and *P. aeruginosa* (32.1 ± 2.5 mm). This enhanced activity suggests that ethanol is more effective at extracting antimicrobial phytochemicals from *P. guineense*. These results also agree with prior research indicating that ethanolic extracts of *P. guineense* exhibit stronger antimicrobial activity than aqueous extracts due to ethanol's ability to dissolve both polar and moderately non-polar bioactive compounds such as flavonoids, alkaloids, and phenolics (Akinmoladun *et al*., 2019). The antimicrobial activity of the ethanolic extract observed in this study agrees with previous findings of Akinyemi *et al*. (2016); Okigbo *et al*. (2015) that attribute higher antimicrobial potency to ethanol-based extracts of *P. guineense*. This enhanced activity is likely due to the higher concentration of bioactive phytochemicals such as flavonoids, alkaloids, phenolic compounds, terpenoids, and steroids previously identified in the ethanolic extract. These compounds are known to exert antimicrobial effects through mechanisms such as disruption of microbial cell membranes, inhibition of protein synthesis, enzyme inactivation, and interference with nucleic acid synthesis

Although ciprofloxacin remained significantly more potent against all bacterial isolates, the ethanolic extract displayed comparable activity against *P. aeruginosa*, which is known for its intrinsic resistance to many antimicrobial agents. This finding is particularly significant, as it supports the potential of *P. guineense* as a source of bioactive compounds against drug-resistant pathogens. The ethanolic extract also exhibited strong antifungal activity at 100 mg/mL against *C. albicans* (21.5 ± 2.5 mm) and *A. niger* (15.3 ± 1.6 mm). However, these inhibition zones were smaller than those produced by nystatin, which showed substantially higher antifungal potency.

Similar results have been reported by Nwankwo *et al*. (2014) and Adegoke *et al.* (2010), who observed that ethanolic extracts of *P. guineense* were more effective than aqueous extracts against both bacterial and fungal pathogens.

These findings agrees with previous studies of Akinwale *et al*. (2020); Ezejiofor *et al*. (2020) that reported significant antimicrobial activity of aqueous extracts of *P. guineense* against both Gram-positive and Gram-negative bacteria, as well as fungi. The highest inhibition against *B. subtilis* aligns with reports that Gram-positive bacteria are generally more susceptible to plant extracts due to the absence of an outer membrane that restricts compound penetration (Ekanem and Obiekezie, 2016). However, some previous studies reported lower or negligible activity of aqueous extracts against certain Gram-negative bacteria, such as *K. pneumoniae* and *P. aeruginosa* (Akinwale *et al*., 2020). The current results suggest moderate activity, which could be attributed to differences in extraction methods, plant material source, or assay conditions. Additionally, the antifungal activity observed against *C. albicans* and *A. niger* was lower than the standard (Nystatin), consistent with earlier reports that aqueous extracts generally have weaker antifungal properties compared to standard antifungal drugs (Essien *et al*., 2015).

The potent activity against *P. aeruginosa* is particularly notable, as this Gram-negative bacterium is often resistant to many plant extracts. The enhanced antifungal activity against *C. albicans* is also in line with previous studies that showed ethanol extracts generally have better antifungal efficacy (Ezejiofor *et al*., 2020). Despite the strong antimicrobial activity of the ethanolic extract, standard drugs (Ciprofloxacin and Nystatin) remained significantly more potent. This is consistent with previous studies but contrasts with some reports claiming near-equivalent activity of ethanolic extracts against certain bacteria at high concentrations (Essien *et al.,* 2015). Variations may arise from differences in extract concentration, microbial strains, or methods of

determining the inhibition zones. The finding that both aqueous and ethanolic extracts inhibit Gram-negative bacteria (*K. pneumoniae, P. aeruginosa*), a Gram-positive bacterium (*B. subtilis*), and fungi (*C. albicans, A. niger*) confirms the established broad-spectrum potential of *P. guineense* (Okwu and Ighodaro, 2010).

For the aqueous extract, the highest MIC value (8 mg/mL) was observed against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*, indicating lower susceptibility of these organisms. The highest MBC (10 mg/mL) was recorded against *P. aeruginosa*, suggesting that this organism is particularly resistant to the aqueous extract. In contrast, *Bacillus subtilis* showed the lowest MIC (0.9 mg/mL) and MBC (4 mg/mL), indicating high susceptibility. A similar trend was observed for the ethanolic extract, though with generally lower MIC and MBC/MFC values, reflecting greater antimicrobial potency. *P. aeruginosa* recorded the highest MIC (6 mg/mL) among the bacterial isolates, while *A. niger* showed the highest MIC (8 mg/mL) among the fungal isolates. The highest MBC/MFC (8 mg/mL) was observed for *P. aeruginosa*, *C. albicans*, and *A. niger*, whereas *B. subtilis* again showed the lowest MBC (2 mg/mL). This agrees with the report of Nwinyi *et al*. (2019) identifying *P. aeruginosa* as the least susceptible due to its intrinsic resistance mechanisms. The low MIC for *B. subtilis* is consistent with the report of (Akinmoladun *et al*., 2015) who reported that Gram-positive bacteria often show greater sensitivity to plant extracts compared to Gram-negative bacteria. The difference in activity profiles between aqueous and ethanolic extracts of *P. aeruginosa* is expected, as different solvents extract distinct bioactive compound classes. This solvent-effect correlation is well-documented by Edeoga *et al*. (2020).

These findings of this study are also consistent with the findings of Akinmoladun *et al*. (2019); Ezejiofor *et al*. (2020) prior research indicating that Gram-positive bacteria (*B. subtilis, S. aureus*)

are generally more susceptible to *P. guineense* extracts than Gram-negative bacteria (*K. pneumoniae, P. aeruginosa*) due to differences in cell wall permeability. Ethanolic extracts generally exhibit lower MIC and MBC values than aqueous extracts, reflecting ethanol's superior ability to extract bioactive antimicrobial compounds such as flavonoids, alkaloids, and phenolics (Udofia and Alozie, 2015). Some studies reported slightly lower MIC values for aqueous extracts against Gram-negative bacteria, suggesting higher potency (Akinbuluma *et al*., 2017). The higher MICs observed in this study may be due to differences in extract concentration, microbial strain variations, or extraction methodology.

The DPPH assay results demonstrated that both aqueous and ethanolic extracts of *P. guineense* exhibited concentration-dependent radical scavenging activity. The absorbance values decreased with increasing concentration, indicating increased scavenging of DPPH radicals. The ethanolic extract showed lower absorbance values than the aqueous extract across all concentrations, suggesting stronger antioxidant activity.

Percentage inhibition data further confirmed this trend. The aqueous extract exhibited percentage inhibition ranging from 13.5 ± 0.41% at 10 µg/mL to 56.12 ± 0.66% at 200 µg/mL, while the ethanolic extract ranged from 17.65 ± 0.41% to 78.62 ± 0.66% over the same concentration range. As expected, ascorbic acid showed significantly higher radical scavenging activity, ranging from 49.81 ± 0.11% to 98.01 ± 0.35%. The findings on DPPH potentials of *P. guinense* extracts agrees with the findings of Ezejiofor *et al*. (2020) showing that both aqueous and ethanolic extracts of *P. guineense* exhibit dose-dependent antioxidant activity. Ethanolic extracts showed slightly stronger radical scavenging activity at higher concentrations than aqueous extracts, consistent with studies demonstrating that ethanol extracts more phenolic and flavonoid

compounds, which are major contributors to antioxidant activity (Máthé *et al*., 2022; Tyskiewicz *et al*., 2022).

The study shows that the percentage inhibition of DPPH radicals increased with extract concentration, indicating dose-dependent antioxidant activity. The results are consistent with the findings of Dai & Mumper (2023); Zhang *et al*. (2022) showing that ethanolic extracts of *P. guineense* exhibit stronger DPPH radical scavenging activity than aqueous extracts due to ethanol's superior extraction of phenolic and flavonoid compounds. The dose-dependent increase in percentage inhibition aligns with the findings of Fidrianny *et al*. (2020) indicating that higher concentrations of bioactive compounds enhance radical scavenging activity. Compared to ascorbic acid, both extracts had lower RSA values, which agrees with prior findings but contrasts with reports claiming near-equal activity at high concentrations (Ezejiofor *et al*., 2020).

The FRAP assay results showed that the ethanolic extract possessed significantly higher reducing power (262.2 ± 7.3 μmol Fe^{2+}/g) compared to the aqueous extract (133.6 ± 11.3 μmol Fe^{2+}/g), although both were lower than the control, ascorbic acid (615.5 ± 10.6 μmol Fe^{2+}/g). This indicates that the extracts, particularly the ethanolic extract, have appreciable electron-donating ability and can act as reducing agents. This observation aligns with reports by Adegoke *et al*. (2010) and Oboh *et al*. (2014), who demonstrated that ethanolic extracts of *P. guineense* exhibit higher reducing power than aqueous extracts due to increased concentrations of redox-active phytochemicals such as phenols and flavonoids.

5.1 Conclusion

The findings of this study demonstrate that *Piper guineense* fruits and seeds possess significant antimicrobial and antioxidant activities, which can be attributed to the rich array of phytochemicals present in the extracts, including alkaloids, flavonoids, phenols, tannins,

saponins, glycosides, terpenoids, steroids, and anthraquinones. Both aqueous and ethanolic extracts exhibited broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and fungi. However, the ethanolic extracts consistently showed stronger inhibitory effects, lower MIC and MBC values, and higher antioxidant activity than aqueous extracts. The antioxidant analysis further revealed dose-dependent DPPH radical scavenging activity for both extracts, with ethanolic extracts displaying superior activity, although still lower than that of the standard antioxidant, ascorbic acid. The study therefore confirms the therapeutic potential of *Piper guineense* as a natural source of antimicrobial and antioxidant agents. While standard drugs remain more potent, the observed biological activities highlight the plant's promise as a complementary or alternative remedy. Further studies focusing on isolation, characterization of active compounds, toxicity assessment, and in vivo evaluations are recommended to enhance its potential application in pharmaceutical and nutraceutical development.

5.2 Recommendation

1. Since the ethanolic extract of *Piper guinense* demonstrated higher concentrations of key bioactive compounds (notably flavonoids) and exhibited stronger antimicrobial and antioxidant activities than the aqueous extract, ethanol should be preferred as an extraction solvent in future pharmacological and nutraceutical investigations.
2. Although the extracts showed promising *in vitro* antimicrobial and antioxidant effects, *in vivo* studies and toxicity assessments are recommended to establish safety, efficacy, dosage ranges, and potential side effects before any clinical or therapeutic application.

3. *P. guinense* extracts should be explored as complementary or adjunct antimicrobial agents rather than direct replacements for conventional drugs, especially in the management of drug-resistant infections.
4. The demonstrated antioxidant and antimicrobial properties support the potential use of *P. guinense* in the formulation of herbal products, food preservatives, and nutraceuticals. Standardized formulations and stability studies are recommended to enhance product quality and shelf life.
5. To ensure consistency and reproducibility, standardized protocols for plant material collection, processing, and extraction should be established, considering variations in phytochemical content due to environmental and processing factors.
6. Comparative studies involving different parts of the plant, geographical sources, and harvest periods are recommended to determine variations in phytochemical composition and bioactivity.

5.3 Contribution to Knowledge

The study has contributed to knowledge in the following ways:

The aqueous and ethanolic extract revealed detailed phytochemical components of the fruit and seed of *Piper guinense*.

This work expands existing antimicrobial data on *P. guinense* by systematically comparing its effects against both Gram-positive and Gram-negative bacteria as well as fungal isolates, including *Pseudomonas aeruginosa* and *Aspergillus niger*, which are known for their resistance to antimicrobial agents.

The study showed that the extracts exerts less antimicrobial efficacy than the tested commercially available antibiotics and antifungal drugs. The combined use of DPPH radical

scavenging and FRAP assays provides deeper insight into the dose-dependent antioxidant behavior and reducing power of *P. guinense* extracts.

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APENDIX

Concentration (µg/mL)	Aqueous Extract	Ethanol Extract	Ascorbic acid (Standard Antiox.)
10	13.5±0.41	17.65±0.41	49.81±0.11
25	20.01±0.66	23.91±0.63	59.59±0.31
50	21.4±0.85	34.77±0.75	70.05±0.56
100	46.10±0.63	61.31±0.58	89.3±0.53
200	56.12±0.66	78.62±0.66	98.01±0.35

Aqueous Extr.	Ethanol Extr.	Ascorbic acid
13.5	17	49.9
20	23.9	59.6
21.4	35.4	70
46.1	61.3	89.3
56.1	78.6	98.9

Table 4.6: IC50 of the various *Alium sativum* extracts and positive control for DPPH assay

Extract	IC_{50} (µg/mL)	Relative antioxidant strgt.

Aqueous	108.37±4.67	Moderate
Ethanol	51.51±2.71	Strong
Ascorbic acid	31.16±5.66	Very stong