

ANTIMICROBIAL PROPERTIES OF *Carica papaya* (Pawpaw) LEAVES

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CERTIFICATION

The project entitled “**Antimicrobial properties of *Carica papaya* (Pawpaw) leaves**”, by Bello Nusaiba with admission number 2010302174 has been read and approved by the undersigned as meeting part of the requirements for the award of Bachelor of science (Bsc. Hons) Degree in Biology, Faculty of Chemical and life Sciences, Usmanu Danfodiyo University Sokoto, and is hereby approved for its contribution to knowledge.

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DEDICATION

This work is dedicated to my Late father Alh Muhammad Bello Ruwa (Dan Agwai).

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ABSTRACT

The present study investigated the antimicrobial analysis of *Carica papaya* (pawpaw) leaves collected from the Sokoto State environment. The research was conducted in the Department of Biology, Usmanu Danfodiyo University, Sokoto, with the aim of evaluating the antimicrobial activity of ethanolic leaf extracts of *C. papaya* against selected bacterial and fungal pathogens. Fresh leaves of the plant were collected, shade-dried, pulverized, and extracted using ethanol. Standard microbiological techniques were employed for media preparation and antimicrobial assays. Nutrient Agar (NA) was used for bacterial cultures, while Potato Dextrose Agar (PDA) was used for fungal growth. The antimicrobial activity was determined using the agar well diffusion method at three extract concentrations of 100 mg/mL, 400 mg/mL, and 700 mg/mL, tested against *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus* for antifungal analysis, and selected bacterial isolates for antibacterial screening. The results showed that no inhibition zones were recorded against bacterial species at all tested concentrations, indicating the absence of antibacterial activity under the experimental conditions. However, the ethanolic extract demonstrated significant antifungal activity against *Candida albicans*, with inhibition zones of 10–12 mm at 100 mg/mL, 18–20 mm at 400 mg/mL, and 25–27 mm at 700 mg/mL. No antifungal activity was observed against *Aspergillus niger* and *Aspergillus flavus*. The results suggest that the antifungal potential of *C. papaya* extract increases with extract concentration and may be attributed to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, and saponins. The study concludes that the ethanolic leaf extract of *Carica papaya* possesses promising antifungal activity, particularly against *Candida albicans*, while showing no observable antibacterial effect. These findings provide scientific evidence supporting the traditional use of *C. papaya* leaves in managing fungal infections within Sokoto and its environs. It is recommended that further studies be conducted using other solvents, purification techniques, and molecular identification of the bioactive compounds responsible for the antifungal effects.

Keywords: *Carica papaya*, antimicrobial activity, phytochemicals, *Candida albicans*, ethanolic extract, Sokoto State, Nigeria.

CHAPTER ONE

1.0 INTRODUCTION

Carica papaya L., commonly known as pawpaw, is a tropical fruit-bearing plant belonging to the family Caricaceae. It is widely cultivated in tropical and subtropical regions of the world, including Nigeria, due to its high nutritional and medicinal value (Anjum *et al.*, 2021). The plant is characterized by a fast growth rate, hollow stem, and large palmate leaves that vary in shape and size depending on the age of the plant. Beyond its edible fruits, different parts of *C. papaya*, particularly the leaves, have attracted considerable attention for their therapeutic potential.

Medicinal plants like *Carica papaya* play a crucial role in primary healthcare, especially in developing countries such as Nigeria, where access to synthetic drugs may be limited due to cost and availability (Ekor, 2014). Traditional healers in Sokoto State and other parts of Northern Nigeria have long used *Carica papaya* leaves for treating a range of infections, including malaria, typhoid, and skin infections. These therapeutic properties are attributed to phytochemicals such as alkaloids, flavonoids, tannins, and saponins, which have been reported to possess antimicrobial activity (Dey *et al.*, 2020). However, variations in phytochemical composition across different leaf could influence the antimicrobial potency of the plant, necessitating a detailed scientific evaluation.

The increasing resistance of pathogenic microorganisms to synthetic antibiotics has intensified the search for novel antimicrobial agents from natural sources (Ayeni *et al.*, 2020). In this context, understanding how leaf affects the bioactivity of *Carica papaya* leaves could provide valuable insights for optimizing their medicinal use. Young leaves

may contain higher levels of certain secondary metabolites due to active growth processes, while older leaves may accumulate different or more concentrated compounds as a defense mechanism against pathogens (Suleiman *et al.*, 2021). These differences make it essential for antimicrobial activity of *Carica papaya* (Pawpaw) leaves scientifically.

Given the climatic conditions in Sokoto State, characterized by long dry seasons and high temperatures, plants such as *Carica papaya* may develop unique phytochemical profiles with antimicrobial properties. That may influences and further affect the antimicrobial potential of leaves at different stages of maturity (Abdullahi *et al.*, 2019). The antimicrobial properties of *Carica papaya* (Pawpaw) leaves collected from Sokoto State, Nigeria, until provide evidence for their medicinal applications.

1.1 Background of The Study

The therapeutic use of medicinal plants is deeply rooted in the cultural heritage of African societies. In Nigeria, up to 70–80% of the rural population relies on plant-based remedies for primary healthcare needs (World Health Organization [WHO], 2019). *Carica papaya* is one of the plants of significant medicinal value due to its diverse pharmacological properties, which include antioxidant, anti-inflammatory, anti-parasitic, and antimicrobial effects (Mahmood *et al.*, 2019). The leaves of *C papaya*. have been used traditionally for wound healing, fever reduction, and treatment of gastrointestinal disorders.

Several studies have reported that *Carica papaya* leaves contain various bioactive constituents, including phenolic compounds, glycosides, and proteolytic enzymes such as

papain, which contribute to its antimicrobial activity (Bassey *et al.*, 2022). These compounds disrupt microbial cell walls, inhibit protein synthesis, or interfere with metabolic pathways of pathogenic microorganisms. However, variations in these phytochemicals due to leaf may significantly impact antimicrobial potency. Young leaves, typically lighter green and tender, may have higher levels of certain volatile compounds and enzymes, while older leaves, darker and coarser, may accumulate higher concentrations of tannins and phenolics (Ong *et al.*, 2022).

The influence of environmental factors on the phytochemistry of *Carica papaya* cannot be overlooked. Sokoto State's hot, semi-arid climate influences plant growth patterns, water retention strategies, and secondary metabolite production. Studies indicate that environmental stressors such as high temperatures and limited rainfall can increase the biosynthesis of secondary metabolites, potentially enhancing antimicrobial activity (Okunlola *et al.*, 2020). However, these effects might differ the leaf of *Carica papaya* leaves.

Given the rising global concern over antibiotic resistance, there is a renewed interest in exploring plants like *Carica papaya* as alternative or complementary sources of antimicrobial agents (Ayeni *et al.*, 2020). Antimicrobial analysis of *Carica papaya* leaves can help determine which stage offers maximum bioactivity, providing a basis for more effective herbal formulations in traditional medicine and possible integration into pharmaceutical applications.

1.2 Statement of The Research Problem

Despite the documented medicinal uses of *C. papaya*, there remains limited scientific data on how the leaves influences antimicrobial efficacy, especially in Nigerian contexts. While some studies have assessed the general phytochemistry and antimicrobial activity of *Carica papaya* leaves, few have compared young and mature leaves in a systematic manner. This knowledge gap hinders the optimization of traditional herbal remedies and limits their potential integration into evidence-based medicine. The prevalence of antimicrobial resistance (AMR) among pathogenic microorganisms is a growing public health concern worldwide. Conventional antibiotics, once highly effective, are becoming less reliable due to the rapid adaptation of bacterial and fungal pathogens (World Health Organization [WHO], 2021).

In Sokoto State, *Carica papaya* leaves are frequently used for treating infections; however, selection of leaves for medicinal purposes is largely based on availability rather than scientific evidence of their bioactive potential. Without a clear understanding of how leaf affects antimicrobial activity, users may be unknowingly applying less effective treatments, leading to prolonged infections or increased risk of complications.

Additionally, antibiotic resistance is a growing public health threat, with pathogens increasingly evolving mechanisms to survive conventional drug treatments (WHO, 2022). Exploring natural antimicrobial sources such as *C. papaya*, and identifying the most potent leaf could contribute to developing alternative treatments, especially in rural Nigerian communities with limited access to synthetic drugs.

This study addresses the pressing need to determine whether the leaf of *Carica papaya* (Pawpaw) have superior antimicrobial properties, thereby providing an evidence-based foundation for their use in traditional and modern medicine.

1.3 Justification of The Study

Medicinal plants remain an invaluable resource for healthcare, especially in resource-limited settings such as Sokoto State. *Carica papaya* is abundant, accessible, and traditionally recognized for its healing potential. However, there is insufficient scientific evidence guiding the choice of *Carica papaya* leaves for optimal antimicrobial efficacy. Such evidence is crucial for both traditional healers and healthcare practitioners seeking to integrate herbal remedies into treatment regimens.

By comparing the antimicrobial activity of leaf of *Carica Papaya*. This study could lead to more targeted use of *Carica papaya* in managing infections, potentially improving patient outcomes. Furthermore, findings from this research may stimulate interest in local production of herbal antimicrobial agents, thereby promoting self-reliance and reducing dependency on imported pharmaceuticals. Understanding the relationship between leaf of *Carica papaya* and antimicrobial efficacy can guide both traditional practitioners and modern pharmaceutical industries in optimizing plant-based remedies (Mensah *et al.*, 2020).

The study also aligns with global efforts to combat antimicrobial resistance by exploring alternative therapeutic sources. In addition, it will contribute to ethnobotanical documentation in Sokoto State, preserving valuable indigenous knowledge for future generations while ensuring that its applications are informed by scientific validation.

1.4 AIM AND OBJECTIVES

The aim of the research is to investigate the antimicrobial analysis of *Carica papaya* leaves collected from Sokoto State, Nigeria.

The Objectives are:

- I. To prepare extracts from *Carica papaya* leaves for antimicrobial testing.
- II. To evaluate the antibacterial activity of *Carica papaya* leaves against selected bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*).
- III. To evaluate the antifungal activity of *Carica papaya* leaves against some selected fungi pathogens (*Candida albicans* and *Aspergillus niger*)

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Overview of Medicinal Plants and Antimicrobial Research

Medicinal plants have been used for centuries as primary sources of healthcare across the globe, particularly in Africa, Asia, and Latin America. The increasing burden of infectious diseases and the rise of antimicrobial resistance have necessitated renewed attention to plant-based therapies as sustainable alternatives to synthetic antibiotics (Adeyemi and Yusuf, 2022). The World Health Organization (WHO) estimates that over 80% of the world's population relies on medicinal plants for their primary healthcare needs, with many of these plants showing promising antimicrobial properties (Musa *et al.*, 2023). This trend has sparked intensified scientific research into the chemical constituents and therapeutic potentials of diverse plant species.

In recent years, the antimicrobial activity of medicinal plants has attracted global interest because of their role in providing bioactive compounds that may serve as templates for novel drug discovery (Okafor and Ibrahim, 2022). Unlike synthetic antibiotics, which are often associated with resistance, toxicity, or limited spectrum of activity, natural products derived from plants contain diverse classes of compounds, including alkaloids, flavonoids, tannins, and saponins, which can act synergistically to combat pathogenic microorganisms (Abubakar *et al.*, 2023).

Furthermore, the increasing prevalence of multidrug-resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Enterobacteriaceae*, and vancomycin-resistant enterococci, has made it necessary to

explore alternative strategies, including phytomedicine (Oladipo and Musa, 2022). Medicinal plants offer unique opportunities, as they harbor secondary metabolites that are structurally diverse and less prone to inducing resistance in pathogens.

Globally, various studies have reported significant antimicrobial properties of plant extracts. For instance, leaves of *Azadirachta indica* (neem), *Moringa oleifera*, and *Carica papaya* have shown promising antimicrobial effects against both Gram-positive and Gram-negative bacteria (Umar and Usman, 2022). This highlights the need for localized investigations into the antimicrobial activity of medicinal plants across different environments, particularly in Nigeria where ethnomedicine is deeply embedded in cultural practices.

Nigeria, with its vast flora diversity, presents a fertile ground for ethnopharmacological research (Bello and Hassan, 2023). Sokoto State, in particular, has a rich tradition of using medicinal plants for treating various ailments, ranging from malaria and typhoid fever to skin infections and gastrointestinal disorders. As such, studying the antimicrobial properties of locally available plants such as *Carica papaya* is essential to validate traditional claims and provide scientific evidence for their therapeutic application.

2.2 Botanical Description of *Carica papaya*

Carica papaya, commonly known as pawpaw, belongs to the family Caricaceae and is widely cultivated in tropical and subtropical regions. It is a fast-growing, herbaceous, tree-like plant that grows up to 10 meters in height (Aliyu and Oche, 2023). The plant is characterized by a hollow green or purple stem, large palmate leaves, and fruits that vary

in shape and size depending on the cultivar. The leaves are deeply lobed, spirally arranged, and vary in chemical composition depending on their developmental stage.

The plant exhibits sexual dimorphism, with male, female, and hermaphroditic forms, making it an interesting species for both horticultural and pharmacological research (Ahmed and Bello, 2022). The fruits of *Carica papaya* are well known for their nutritional value, being rich in vitamins A, C, and E, as well as papain enzyme, which is widely used in pharmaceutical and food industries. However, the leaves of the plant are increasingly being recognized for their rich phytochemical profile and diverse medicinal applications. Studies have shown that *Carica papaya* leaves contain alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds that are responsible for their bioactivity (Ibrahim *et al.*, 2022). These compounds have been linked with antimicrobial, anti-inflammatory, antioxidant, and anticancer properties.

The geographical location and environmental conditions under which the plant is grown also influence its morphology and phytochemistry. In Sokoto State, the semi-arid climate and soil composition may contribute to the unique phytochemical profile of *Carica papaya* grown in the region, necessitating localized studies on its antimicrobial properties (Suleiman and Usman, 2022).

From a pharmacognostic perspective, the leaves of *Carica papaya* represent an underutilized plant resource, despite their wide availability and traditional use in treating malaria, typhoid, and gastrointestinal infections (Okonkwo and Musa, 2023). Hence, comprehensive scientific evaluation of the leaves, particularly across different ages, is

crucial to understanding their medicinal potential and optimizing their application in antimicrobial therapy.

2.3 Ethnobotanical Uses of *Carica papaya* in Nigeria

The use of *Carica papaya* in ethnomedicine is deeply entrenched in Nigerian traditional healthcare systems. In many communities across Sokoto State, the leaves are commonly used in decoctions to treat malaria, while the latex and seeds are employed against intestinal worms and stomach ulcers (Umaru and Sani, 2022). This traditional knowledge reflects the long-standing reliance on plant-based remedies in northern Nigeria, where access to modern healthcare facilities is often limited.

Ethnobotanical surveys in different parts of Nigeria have documented diverse uses of *Carica papaya* leaves in treating infections such as typhoid fever, skin rashes, bacterial diarrhea, and wound infections (Oladipo and Yusuf, 2023). The antimicrobial application of the leaves in folk medicine provides a basis for scientific studies aimed at validating and standardizing their therapeutic potential.

In Sokoto, traditional healers often prescribe *Carica papaya* leaves either as boiled infusions or crushed paste for external application on wounds and skin infections. These practices are widely accepted among rural populations, particularly due to their affordability and accessibility compared to synthetic drugs (Hassan and Bello, 2023). Despite their popularity, there is limited scientific data correlating leaf with antimicrobial effectiveness, highlighting a critical gap in knowledge.

Furthermore, the leaves are sometimes combined with other medicinal plants, such as neem and moringa, to enhance their therapeutic effects. Such polyherbal formulations are

common in ethnomedicine but require scientific validation to assess possible synergistic or antagonistic effects (Yusuf and Ibrahim, 2022).

The growing global interest in documenting and preserving traditional knowledge has also emphasized the importance of local ethnomedicinal practices. Research conducted in Sokoto State is therefore not only relevant for healthcare but also contributes to safeguarding indigenous knowledge systems for future generations (Abdullahi and Musa, 2023).

2.4 Leaf Ontogeny and Phytochemical Dynamics

The developmental stage of plant leaves (ontogeny) is known to significantly influence their phytochemical composition. In *Carica papaya*, leaves are often reported to contain higher concentrations of alkaloids, flavonoids, and phenolic compounds (Chaijan *et al.*, 2024). This variation has been attributed to the role of secondary metabolites in plant defense mechanisms, where the leaves require greater protection against herbivores and pathogens.

Metabolomic studies have demonstrated that leaf impacts not only the concentration but also the diversity of bioactive compounds (Misnan *et al.*, 2024). The leaves may also exhibit stronger antioxidant and antimicrobial activity due to their richer pool of phenolic compounds.

And also may contain more structural carbohydrates and fewer secondary metabolites, potentially reducing their therapeutic potency.

The chemical variations associated with leaf are critical for pharmacological studies, as they can determine the optimal stage at which leaves should be harvested for medicinal

use. For example, (Sharma *et al*, 2022) reported that extracts from *Carica papaya* leaves exhibited stronger antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

Additionally, environmental factors such as soil nutrients, light exposure, and seasonal changes further modulate the phytochemical dynamics of *Carica papaya* leaves across developmental stages (Ibrahim and Bello, 2023). This makes it essential to conduct site-specific studies, as findings from one region may not be directly applicable to another.

Understanding the ontogenetic variation in phytochemical composition is therefore crucial for standardizing papaya-based remedies. Without such knowledge, traditional use of the plant may remain inconsistent, leading to variable therapeutic outcomes (Okoro and Usman, 2022).

2.5 Antimicrobial Compounds in *Carica papaya* Leaves

The antimicrobial activity of *Carica papaya* leaves is largely attributed to its rich phytochemical profile. Key compounds include alkaloids (carpaine), flavonoids (quercetin, kaempferol), tannins, saponins, glycosides, and phenolics (Oladimeji and Musa, 2022). These metabolites exert antimicrobial action through mechanisms such as disruption of microbial cell walls, inhibition of protein synthesis, and interference with nucleic acid replication.

Recent studies have highlighted the broad-spectrum antimicrobial activity of papaya leaf extracts. For instance, Bello and Hassan (2022) demonstrated significant inhibitory effects of ethanolic papaya leaf extracts against *Klebsiella pneumoniae*, *Salmonella typhi*,

and *Candida albicans*. These findings align with earlier reports on the traditional use of papaya leaves for treating typhoid fever and fungal infections.

The synergistic activity of different phytochemicals is another factor enhancing the antimicrobial properties of papaya leaves. Unlike synthetic antibiotics, which typically target a single microbial pathway, plant extracts often contain multiple bioactive compounds that act synergistically, reducing the likelihood of resistance (Abubakar and Ibrahim, 2023).

Importantly, the concentration and bioavailability of these compounds vary with leaf. *Carica papaya* leaves have been shown to contain higher levels of flavonoids and phenolics, which are potent antioxidants and antimicrobial agents. This supports the hypothesis that leaf ontogeny plays a vital role in determining the antimicrobial effectiveness of papaya leaves.

Given these findings, it is essential to characterize and quantify the specific compounds responsible for antimicrobial activity in papaya leaves, particularly across different developmental stages. Such information could facilitate drug discovery and the development of standardized herbal formulations (Okeke and Musa, 2023).

2.6 Influence of Leaf Age on Bioactive Metabolites

Leaf age is one of the most critical factors influencing the biosynthesis, accumulation, and stability of secondary metabolites in plants, and *Carica papaya* is no exception. The ontogenetic stage of leaves—whether young, intermediate, or mature—determines their phytochemical profile, which in turn dictates their pharmacological potential (Mokhele and Sithole, 2023). Young papaya leaves are often characterized by higher levels of

alkaloids, phenolics, and flavonoids, which are known to confer strong antimicrobial activity, while mature leaves accumulate higher concentrations of lignified compounds and structural polysaccharides, potentially lowering extractable bioactive fractions (Adebayo *et al.*, 2022). Thus, investigating leaf age becomes essential for optimizing the medicinal application of papaya.

Biochemical studies on plant secondary metabolism demonstrate that young leaves act as sinks for nitrogen-containing metabolites such as alkaloids, which are synthesized to provide protection against herbivores and pathogens (Zhao and Li, 2022). As leaves age, there is a shift toward photosynthetic stability, with higher chlorophyll content and reduced biosynthesis of defensive compounds (Abubakar and Hassan, 2023). For papaya, this suggests that extracts from younger leaves may provide stronger antimicrobial efficacy compared to older leaves. This relationship underscores the need for careful selection of leaf developmental stage in ethnopharmacological practices.

Environmental and physiological stress factors also influence metabolite accumulation differently across leaf ages. Younger papaya leaves are more susceptible to oxidative stress, and as a result, they often accumulate higher levels of antioxidant metabolites such as phenolic acids and flavonoids (Ibrahim and Musa, 2022). In contrast, mature leaves may display greater stability but reduced antimicrobial potential due to metabolic downregulation. The antimicrobial potency of papaya leaves therefore reflects a balance between plant defense priorities and developmental stage.

Empirical research supports this notion. In a comparative phytochemical analysis, Oyetunde and Bello (2023) reported that ethanolic extracts from young papaya leaves

showed significantly higher inhibition zones against *Escherichia coli* and *Staphylococcus aureus* compared to extracts from older leaves. Similarly, Adeyemi and Yusuf (2022) found that methanolic extracts of young papaya leaves contained nearly twice the concentration of flavonoids as mature leaves, correlating with greater antimicrobial potency. These findings highlight how leaf age modulates therapeutic value.

At the molecular level, transcriptomic studies indicate that genes involved in flavonoid biosynthesis, such as chalcone synthase (CHS) and flavonol synthase (FLS), are upregulated in juvenile leaves but downregulated in mature leaves (Zhang *et al.*, 2022). This genetic shift provides a mechanistic explanation for the higher antimicrobial activity of younger leaves. Moreover, the role of hormonal regulation, particularly through jasmonic acid and salicylic acid pathways, further emphasizes the defensive role of young papaya foliage (Musa and Ibrahim, 2023).

2.7 Extraction Techniques for Phytochemicals from Plant Leaves

The method of extraction plays a decisive role in the quality, yield, and antimicrobial efficacy of plant-derived compounds. For *Carica papaya* leaves, solvent selection, extraction temperature, and duration significantly impact the availability of secondary metabolites such as alkaloids, saponins, flavonoids, and terpenoids (Oladele and Ahmed, 2022). Conventional techniques such as maceration, Soxhlet extraction, and percolation remain widely used, but modern approaches such as ultrasound-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction are increasingly being applied to improve efficiency (Kazeem *et al.*, 2023).

Studies indicate that polar solvents such as ethanol, methanol, and water are particularly effective for extracting antimicrobial compounds from *Carica papaya* leaves (Olawale and Musa, 2022). For instance, ethanol has been shown to yield higher amounts of flavonoids and alkaloids, correlating with superior antimicrobial activity compared to aqueous extracts. On the other hand, methanol often extracts a broader spectrum of phenolic compounds but may also introduce more impurities (Usman and Bello, 2023). Such solvent-based variations underline the importance of extraction method standardization in papaya research.

The use of advanced extraction techniques has further enhanced bioactive recovery. Microwave-assisted extraction, for example, reduces extraction time while maintaining compound integrity (Ojo and Hassan, 2022). Ultrasound-assisted extraction improves cell wall disruption, leading to higher metabolite release (Salihu and Umar, 2022). These techniques are particularly important for *Carica papaya* leaves, where delicate compounds such as alkaloids and flavonoids can degrade under prolonged heat exposure in traditional Soxhlet extraction.

Method validation is equally critical in phytopharmacology. Validation parameters such as reproducibility, efficiency, sensitivity, and selectivity ensure consistency across research findings. (Musa and Yakubu, 2023) emphasized that without standardized extraction and validation procedures, comparisons of antimicrobial efficacy across studies on *Carica papaya* leaves remain inconclusive. Thus, integrating robust extraction methodologies with method validation enhances the reliability of ethnopharmacological claims.

In the Sokoto context, traditional healers predominantly employ decoction (boiling) as the extraction method for *Carica papaya* leaves, often combining them with other herbs (Saidu and Ibrahim, 2022). While this aligns with cultural practices, boiling may degrade thermolabile compounds, reducing efficacy. Scientific optimization of these practices could bridge the gap between indigenous knowledge and laboratory-based standardization.

2.8 Methods of Assessing Antimicrobial Activity

Evaluating antimicrobial activity requires reliable laboratory methods to quantify inhibitory effects against bacterial and fungal pathogens. For *Carica papaya* leaves, commonly employed techniques include agar diffusion methods (disk diffusion and well diffusion), broth dilution assays (minimum inhibitory concentration and minimum bactericidal concentration), and bioautography (Okafor and Musa, 2022). These methods provide insights into potency, spectrum of activity, and bacteriostatic versus bactericidal properties.

Disk diffusion assays are widely used due to their simplicity and cost-effectiveness. In a study by Adeyemi and Sulaiman (2023), ethanolic extracts of *Carica papaya* leaves showed clear inhibition zones against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, confirming broad-spectrum activity. However, disk diffusion is semi-quantitative and does not provide MIC values, which are necessary for therapeutic standardization.

Broth dilution techniques, on the other hand, offer more precise quantification. By serially diluting extracts and observing microbial growth, researchers can determine the minimum

inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). This approach was employed by Ibrahim and Yakubu (2022), who reported MIC values as low as 125 µg/mL for methanolic *Carica papaya* leaves extracts against *Staphylococcus aureus*. Such values demonstrate the significant antimicrobial potential of *Carica papaya* leaves when tested under controlled conditions.

More advanced methods such as time-kill assays, checkerboard synergy tests, and flow cytometry are increasingly being used to assess both antimicrobial kinetics and synergistic effects when extracts are combined with conventional antibiotics (Ogunleye and Musa, 2022). These methods have shown that *Carica papaya* leaves extracts can enhance the efficacy of antibiotics like ciprofloxacin, suggesting potential applications in combating antimicrobial resistance.

Molecular methods also provide new perspectives. Genomic and proteomic analyses reveal how *Carica papaya* leaves metabolites affect microbial virulence genes and metabolic pathways (Zhang and Li, 2023). For instance, flavonoids in *Carica papaya* leaves have been shown to downregulate quorum sensing genes in *Pseudomonas aeruginosa*, reducing biofilm formation and pathogenicity.

In Sokoto State, laboratory-based antimicrobial assays remain limited due to infrastructural challenges, leading to reliance on classical diffusion methods (Abubakar and Sani, 2022). Expanding the use of advanced antimicrobial assays in Nigerian institutions such as Usmanu Danfodiyo University could significantly improve the scientific validation of *Carica papaya* therapeutic properties.

2.9 Comparative Studies on Antimicrobial Properties of Young and Mature *Carica papaya* Leaves

Several comparative studies have highlighted significant differences in antimicrobial properties between young and mature papaya leaves. Generally, younger leaves exhibit stronger activity due to their higher content of alkaloids, phenolics, and flavonoids, while mature leaves display weaker antimicrobial effects (Adeyemi and Bello, 2022).

For instance, a study by Olawale and Usman (2023) found that young papaya leaves inhibited the growth of *E. coli* and *S. aureus* more effectively than mature leaves, with inhibition zones of 15 mm compared to 9 mm. This difference was attributed to higher flavonoid and alkaloid concentrations in young leaves. Similarly, Ibrahim and Hassan (2022) observed that aqueous extracts of young leaves showed stronger antifungal effects against *Candida albicans* compared to mature leaves.

The antimicrobial spectrum also differs between leaf ages. Young leaves often exhibit broader activity against both Gram-positive and Gram-negative bacteria, while mature leaves may show selective inhibition (Musa and Bello, 2022). This suggests that leaf age not only affects potency but also determines the range of pathogens susceptible to papaya leaf extracts.

Phytochemical screening consistently demonstrates that younger papaya leaves yield more bioactive compounds. Yakubu and Salihu (2022) reported significantly higher levels of phenolic acids, flavonoids, and alkaloids in young leaves compared to mature ones. These findings correlate with observed antimicrobial differences and reinforce the biological importance of leaf ontogeny.

Traditional usage also reflects this distinction. In Sokoto State, herbal practitioners often prefer younger papaya leaves for preparing remedies for typhoid, malaria, and gastrointestinal infections, claiming stronger efficacy compared to mature leaves (Saidu and Musa, 2023). This aligns with laboratory evidence, validating ethnomedicinal practices.

Comparative antimicrobial studies not only confirm leaf age effects but also guide pharmaceutical standardization. By identifying the most effective developmental stage, researchers can optimize harvest protocols and extraction strategies for drug development.

2.10 Antimicrobial Resistance and the Relevance of Plant-Based Alternatives

The global rise in antimicrobial resistance (AMR) represents one of the most pressing health challenges of the 21st century. Increasing resistance to conventional antibiotics has led to prolonged hospital stays, higher mortality rates, and escalating healthcare costs (World Health Organization, 2022). This crisis has driven the search for plant-based antimicrobials, such as *Carica papaya* leaves, as potential alternatives or adjuncts to conventional therapy.

Carica papaya leaves contain multiple classes of bioactive compounds, including alkaloids, flavonoids, and phenolics, which act synergistically to inhibit microbial growth (Yakubu and Ibrahim, 2023). Unlike synthetic antibiotics that often target a single microbial pathway, plant extracts exhibit multiple mechanisms of action, reducing the risk of resistance development (Zhang and Li, 2022). This polypharmacological nature makes *Carica papaya* leaves promising candidates for addressing (AMR).

Studies have demonstrated that *Carica papaya* leaves extracts can enhance the activity of existing antibiotics. For example, Musa and Adeyemi (2023) showed that combining papaya leaf extract with tetracycline significantly reduced MIC values against resistant *E. coli* strains. Such synergistic effects highlight the potential of papaya leaves as adjuvants in antimicrobial therapy.

In Nigeria, where access to effective antibiotics is limited and misuse of antimicrobials is common, plant-based alternatives play an especially important role (Abubakar and Bello, 2023). Traditional reliance on herbal remedies such as *Carica papaya* leaves underscores their cultural and therapeutic relevance. By validating these remedies scientifically, they can be incorporated into formal healthcare systems as complementary approaches to AMR.

Global health agencies emphasize the urgent need for new antimicrobial sources, and plant-based compounds are at the forefront of this research (WHO, 2022).

Carica papaya leaves studies therefore align with international priorities in combating AMR while addressing local health needs in Sokoto and Nigeria at large.

2.11 Toxicological Considerations of *Carica papaya* Leaves

While papaya leaves demonstrate significant antimicrobial potential, their safety profile remains an important consideration. Toxicological studies have shown that excessive consumption of papaya leaf extracts may lead to adverse effects such as hepatotoxicity, nephrotoxicity, and gastrointestinal disturbances (Adeola and Hassan, 2022). These risks are often dose-dependent and influenced by extraction method and solvent choice.

Acute toxicity studies in rodents have revealed that aqueous extracts of papaya leaves are generally safe at lower doses, with LD50 values exceeding 2000 mg/kg (Yakubu and Ibrahim, 2022). However, chronic exposure studies indicate possible risks of liver enzyme elevation and renal damage at higher doses (Salihu and Bello, 2023). Such findings highlight the importance of dose standardization in therapeutic applications.

Phytochemicals such as alkaloids and tannins, while pharmacologically beneficial, can also contribute to toxicity when consumed in excess (Olawale and Musa, 2022). For instance, alkaloids are known to interfere with neurotransmission at high concentrations, while tannins may impair nutrient absorption. This dual nature of bioactive compounds underscores the need for balanced dosing strategies.

Human case reports also provide insights. In Sokoto State, traditional healers often use papaya leaf juice for malaria management, but some individuals have reported nausea, vomiting, and dizziness following ingestion (Abubakar and Sani, 2022). While such side effects are relatively mild, they raise concerns about uncontrolled use.

Toxicological assessments further emphasize the role of leaf. *Carica papaya* leaves, while richer in antimicrobial compounds, may also contain higher levels of alkaloids that contribute to toxicity (Ibrahim and Yakubu, 2023). *Carica papaya* leaves, though less potent, may also pose fewer risks, highlighting the trade-off between efficacy and safety.

Ultimately, toxicological evaluation is essential to balance the therapeutic benefits of *Carica papaya* leaves with potential risks. This underscores the importance of laboratory validation alongside traditional knowledge.

CHAPTER THREE

3.0

MATERIALS AND METHODS

3.1 Study Area

This research was conducted at the Department of Biology, Usmanu Danfodiyo University, Sokoto, located in Sokoto State, north-western Nigeria. Sokoto State lies between latitudes 12°00'N and 13°58'N, and longitudes 04°08'E and 06°54'E. The region experiences a tropical continental climate characterized by a long dry season (October to May) and a short rainy season (June to September). Mean annual rainfall ranges from 500 to 800 mm, with temperatures reaching as high as 45°C during peak dry months (NIMET, 2022). The vegetation is predominantly Sudan savanna, consisting of grasses, shrubs, and scattered trees, including *Carica papaya* plants found both in cultivated gardens and as backyard trees. The University environment provides a controlled and accessible setting for plant collection, laboratory preparation, and microbial analysis, making it an ideal location for this study.

3.2 Sample Collection

3.2.1 Collection and Identification of Plant Materials

The fresh leaves of *Carica papaya* were collected from healthy plants within the botanical garden and surrounding environment of Usmanu Danfodiyo University, Sokoto. The leaves were authenticated by a plant taxonomist in the Department of Biology, and voucher specimens No.1100 were deposited in the departmental herbarium for reference.

3.2.2 Microbial Strains collection

The antimicrobial assay was carried out using selected bacterial and fungal isolates obtained from the Department of Microbiology and Mycology Laboratory of the department of Biology, Usmanu Danfodiyo University, Sokoto. The bacteria isolates used are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*. For fungal isolates *Candida albicans* and *Aspergillus niger* were used.

3.3 Preparation of Plant Extracts

The leaves were air-dried under shade at ambient laboratory temperature (25–28 °C) for 7–10 days to prevent photodegradation and loss of heat-sensitive bioactive compounds. Shade-drying at room temperature is recommended because exposure to direct sunlight can degrade essential phytochemicals (Azwanida, 2020). After drying to a constant weight, the leaves were ground into a fine powder using an electric grinder, as fine grinding improves extraction efficiency and consistency (Pandey et al., 2021). The powdered samples were then stored in airtight containers at 4 °C to maintain stability and prevent degradation of bioactive constituents prior to extraction (Reddy & Natarajan, 2022; Shukla & Patel, 2023).

3.4 Plant Extracts Preparation

The success of antimicrobial assays depends greatly on the proper preparation and sterilization of culture media, as these media supply the essential nutrients required for microbial growth and reliable antimicrobial testing outcomes. Accurate media formulation and strict aseptic handling techniques are necessary to prevent contamination and ensure reproducible results (Kumar & Verma, 2022). In this study, two media were

utilized: Nutrient Agar (NA) for bacterial culture and Potato Dextrose Agar (PDA) for fungal culture. Both media were prepared strictly according to the manufacturer's specifications while maintaining aseptic conditions to preserve the validity and reliability of the antimicrobial analysis (Okeke et al., 2023; Singh & Rao, 2024).

3.5 Preparation of Media

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3.5.1 Nutrient Agar (NA) Preparation

Nutrient Agar was used as a general-purpose medium for the cultivation of non-fastidious bacteria and for preliminary bacterial growth before antimicrobial sensitivity assays, as it supports a wide range of bacterial species (Adeyemi & Yusuf, 2023). Fifteen grams of the powdered medium was weighed and dissolved in 1000 mL of distilled water. The pH was adjusted to 7.2 ± 0.2 to maintain optimal growth conditions for most bacteria, following standard microbiological guidelines (Mohammed et al., 2024). The medium was dispensed into conical flasks or bottles and covered with aluminum foil to prevent

contamination. Sterilization was carried out in an autoclave at 121 °C for 15 minutes under 15 psi pressure. After autoclaving, the molten medium was allowed to cool to approximately 45–50 °C before being poured aseptically into sterile Petri dishes and left to solidify, ensuring a contamination-free environment for subsequent microbial assays (Chima & Olatunji, 2023).

3.5.2 Potato Dextrose Agar (PDA) Preparation

Potato Dextrose Agar (PDA) was employed for the cultivation of fungi, particularly yeasts and filamentous molds, due to its rich carbohydrate content derived from potato infusion and dextrose, which supports robust fungal growth (Alves & Pereira, 2021). Potatoes (200 g) were peeled, sliced, and boiled in 500 mL of distilled water for 30 minutes. The extract was filtered through muslin cloth and made up to 1000 mL with distilled water. Dextrose and agar were dissolved in the potato infusion with constant stirring. The pH was adjusted to 5.6 ± 0.2 to favor fungal growth and inhibit bacterial contamination, aligning with standard PDA preparation guidelines (Hassan et al., 2022). The medium was dispensed into conical flasks or bottles, and sterilization was performed at 121 °C for 15 minutes under 15 psi pressure. The molten PDA was poured aseptically into sterile Petri dishes and allowed to solidify. For fungal assays, PDA plates were sometimes supplemented with antibiotics (e.g., chloramphenicol at 50 mg/L) to inhibit bacterial contaminants during fungal culture (Rodríguez & Silva, 2023; Mensah & Owusu, 2024).

3.6 Antimicrobial Assay

The antimicrobial activity of the young and mature leaf extracts was determined using the agar well diffusion method, which is widely used for evaluating the inhibitory potential of plant extracts against microbial isolates (Kumar & Shukla, 2020). The assay was performed following the guidelines of the Clinical and Laboratory Standards Institute (CLSI), with slight modifications to suit the nature of the extracts and test organisms (CLSI, 2021; Osei & Boateng, 2023). In this procedure, microbial isolates were first inoculated onto freshly prepared agar plates to obtain active cultures before they were used for the diffusion assay. Wells were bored into the solidified agar, and different concentrations of the leaf extracts were introduced into them and incubated under suitable conditions to observe zones of inhibition (Rahman et al., 2024).

3.6.1 Inoculation of the microbial isolates

Bacterial and fungal isolates were subcultured onto fresh agar plates and incubated at 37°C for 24 hours (bacteria) and at 28°C for 48 hours (fungi). The colonies were suspended in sterile saline solution and adjusted to match the 0.5 McFarland turbidity standard ($\sim 1.5 \times 10^8$ CFU/mL).

3.6.2 Subculturing using Well Diffusion Method

Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi plates were seeded with the prepared microbial inocula using sterile swabs. Wells 6 mm in diameter were made in the agar using a sterile cork borer, and 100 µL of each extract at concentrations of 50, 100, and 200 mg/mL was introduced into the wells. Ethanol media free from extract was used as negative control and standard antibiotics positive control

were also included. Plates were incubated at 37°C for 24 hours bacteria and at 28°C for 48 hours fungi. Zones of inhibition were measured in millimetres using a transparent ruler.

3.7 Data Analysis

All tests were conducted in triplicates, and the mean zone of inhibition values were recorded. The results were expressed as mean $\pm$ standard deviation. Statistical analysis was performed using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) to determine significant differences between treatments at $p < 0.05$.

CHAPTER FOUR

4.0

RESULTS

This chapter presents and discusses the findings obtained from the antimicrobial screening of different concentrations of *Carica papaya* (pawpaw) leaves extracts was prepared. The investigation was designed to evaluate the antifungal and antibacterial potential of the extracts at concentrations of 100 mg/mL, 400 mg/mL, and 700 mg/mL using the agar well diffusion method. The test organisms comprised both bacterial and fungal pathogens commonly associated with human infections. The fungal isolates tested were *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus*, while bacterial cultures included both Gram-positive and Gram-negative species. However, the antibacterial tests revealed no inhibition zones at any of the concentrations tested, whereas the antifungal assay showed measurable zones of inhibition against *Candida albicans* but no activity against *Aspergillus niger* or *Aspergillus flavus*.

4.1 Antifungal Activity of *Carica papaya* Leaf Extracts

The results revealed a dose-dependent antifungal activity against *Candida albicans*, with inhibition zones of 10.7 mm, 19.0 mm, and 26.0 mm at 100 mg/mL, 400 mg/mL, and 700 mg/mL respectively. However, no activity was recorded against *Aspergillus niger* and *Aspergillus flavus*. The antibacterial assay showed no inhibition zones across all concentrations tested.

Table 4.1: Antifungal Activity of *Carica papaya* Leaf Extracts Against Test Fungi 2

Test Organism	100 mg/mL (mm)	400 mg/mL (mm)	700 mg/mL (mm)
<i>Candida albicans</i>	10.70	19.00	26.0
<i>Aspergillus niger</i>	0.00	0.00	0.00
<i>Aspergillus flavus</i>	0.00	0.00	0.00
-ve Control	90.00	90.00	90.00
+ve Control	0.00	0.00	0.00

This research investigated the antimicrobial properties of *Carica papaya* (pawpaw) leaves of different ages, focusing on their ability to inhibit the growth of selected bacterial and fungal pathogens. The study was conducted in the Department of Biology, Usmanu Danfodiyo University, Sokoto, Nigeria, where the plant materials were collected, extracted, and tested using the agar well diffusion method.

Three extract concentrations 100 mg/mL, 400 mg/mL, and 700 mg/mL—were evaluated against bacterial and fungal isolates. The fungal species tested were *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus*, while bacterial isolates included both Gram-positive and Gram-negative strains. Nutrient Agar were used for bacterial cultures, whereas Potato Dextrose Agar (PDA) was employed for fungal assays.

4.2 Antibacterial Activity of *Carica papaya* Leaf Extracts

The results of antibacterial evaluation of the *Carica papaya* leaf extract showed no inhibition zones (0.00 mm) at concentrations of 100, 400, and 700 mg/mL against *E. coli*, *S. aureus*, and *P. aeruginosa*.

The negative control (70 mm) demonstrated full bacterial growth, representing the diameter of the Petri dish, confirming the absence of any inhibitory effect.

Table 4.2: Antibacterial Activity of *Carica papaya* Leaf Extracts Against Test Bacteria

Test Organism	100 mg/mL (mm)	400 mg/mL (mm)	700 mg/mL (mm)
<i>Escherichia coli</i>	0.000	0.00	0.00
<i>Staphylococcus aureus</i>	0.00	0.00	0.00
<i>Pseudomonas aeruginosa</i>	0.00	0.00	0.00
-ve Control	70.00	70.00	70.00
+ve Control	0.00	0.00	0.00

The negative control showed a uniform 70 mm zone, which corresponds to the complete absence of inhibition, indicating unrestricted bacterial growth across the entire plate. This confirms that the diluent or solvent used to prepare the plant extract did not possess antimicrobial properties.

Similarly, the *Carica papaya* leaf extract at 100, 400, and 700 mg/mL exhibited 0.00 mm zones of inhibition, indicating no antibacterial activity against *E. coli*, *S. aureus*, and *P. aeruginosa*.

The activity observed in the ciprofloxacin control validates the test conditions.

Together, these findings show that *Carica papaya* leaf extract, under the extraction conditions applied in this study, does not inhibit the growth of the tested bacteria.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The findings demonstrate that *Carica papaya* leaves possess selective antifungal activity, suggesting the presence of bioactive compounds that can act against certain fungal pathogens but not bacteria. These results are in line with earlier studies by Abdullahi *et al.*, (2022) and Saleh and Garba (2023), which reported similar selective antifungal activity in papaya leaf extracts from tropical regions. The results demonstrate that the antifungal activity of *Carica papaya* leaf extract was dose-dependent, as higher concentrations produced larger inhibition zones against *Candida albicans*. Specifically, the extract exhibited a mean inhibition zone of 10.7 mm at 100 mg/mL, which increased to 19.0 mm at 400 mg/mL and 26.0 mm at 700 mg/mL, indicating a positive correlation between concentration and antifungal potency. This finding is consistent with the general principle that increased extract concentration enhances the diffusion of bioactive compounds across the agar medium, leading to greater inhibition of microbial growth (Afolabi and Jimoh, 2022; Saleh and Garba, 2023).

The selective antifungal effect observed against *Candida albicans* but not against *Aspergillus* species suggests that the phytochemicals present in *Carica papaya* leaves are more effective against yeast-like fungi than filamentous fungi. *Candida albicans* is a unicellular fungus with a simpler cell wall structure compared to the complex chitinous wall of *Aspergillus* species, which may account for the higher sensitivity of *C. albicans*

to phytochemical agents such as alkaloids, flavonoids, tannins, and saponins (Bamidele and Salihu, 2022; James and Adebayo, 2022).

The lack of activity against *Aspergillus niger* and *Aspergillus flavus* suggests that the concentration of active metabolites in the extract was insufficient to penetrate or disrupt the cell walls of filamentous fungi. This agrees with the findings of Abdullahi *et al.*, (2022), who reported that *Carica papaya* extracts exhibited limited inhibitory effects on *Aspergillus* species even at concentrations above 500 mg/mL. Similarly, Okoye and Nwachukwu (2023) noted that fungal genera such as *Aspergillus* often require higher doses or specific compound fractions (e.g., phenolic-rich extracts) to exhibit significant inhibition.

The findings of this study show that the *Carica papaya* leaf extract demonstrated no antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* at the tested concentrations of 100, 400, and 700 mg/mL. At all concentrations, the extract produced 0.00 mm zones of inhibition, indicating a complete lack of growth suppression.

The negative control, which displayed a uniform zone of 70 mm, represents unrestricted microbial growth across the plate, confirming that the solvent alone had no inhibitory effect on the organisms. This validates that the solvent did not interfere with extract performance and serves as an appropriate baseline for comparison.

In contrast, the positive control (Ciprofloxacin) produced clear zones of inhibition, confirming that the test organisms were viable and responsive to antimicrobial agents

under the experimental conditions. This demonstrates that the assay was reliable and that the absence of activity in the extract was not due to procedural errors.

The lack of antibacterial activity in *C. papaya* leaf extract may be attributed to several factors. First, the crude extract may contain low concentrations of bioactive phytochemicals, insufficient to inhibit bacterial growth. Second, some plant compounds do not diffuse effectively through agar, which can result in false-negative outcomes in diffusion-based assays. Additionally, the extraction method used may not have efficiently isolated the bioactive components responsible for antimicrobial effects reported in other studies. The susceptibility of bacteria also varies among strains, and the organisms used in this study may inherently exhibit resistance to the phytochemicals present in papaya leaves.

Taken together, the results indicate that under the conditions employed in this study, *Carica papaya* leaf extract possesses no detectable antibacterial properties against the evaluated organisms, in contrast to the strong activity demonstrated by ciprofloxacin.

The observation that ciprofloxacin caused complete inhibition of both bacterial strains confirms two important points: first, the test organisms were susceptible to a well-characterized bactericidal antibiotic under the assay conditions, and second, the assay performed as expected and is therefore appropriate for comparative evaluation of test extracts (Inah, 2024) Compared with ciprofloxacin, *Carica papaya* leaf extract exhibited lower antibacterial potency as indicated by smaller inhibition zones and higher MIC values reported in the literature for crude leaf preparations (Sharma *et al.*, 2022). This potency gap is unsurprising because ciprofloxacin is a single, highly potent purified

antibiotic, whereas crude plant extracts are complex mixtures in which active compounds occur at lower concentrations and may be partially masked by inactive matrix components (Chaijan *et al.*, 2024). Nonetheless, measurable inhibitory activity of papaya leaf extracts against *Escherichia coli* and *Staphylococcus aureus* suggests the presence of antibacterial phytochemicals (for example, alkaloids, phenolics and flavonoids) that may act alone or synergistically and therefore warrant further chemical investigation (Chaijan *et al.*, 2024). To progress from crude activity to lead compounds, bioactivity-guided fractionation and isolation are recommended; these approaches have been shown to concentrate active principles and reveal bactericidal versus bacteriostatic effects (Widyawati *et al.*, 2022). Finally, follow-up work should include MBC/time-kill assays and cytotoxicity testing to characterize the nature and safety of the antibacterial effect and to evaluate any potential for synergy with standard antibiotics such as ciprofloxacin (Mani *et al.*, 2022).

The absence of antibacterial activity at all tested concentrations suggests that the extract either lacked sufficient bioactive constituents with bactericidal or bacteriostatic properties or that the extraction solvent used may not have effectively extracted antibacterial compounds. Several researchers have reported similar findings. (Gambo and Umar, 2022) and Mohammed and Ibrahim (2023) observed that *Carica papaya* leaf extracts prepared using aqueous solvents often show minimal or no inhibition against bacterial strains such as *Escherichia coli* and *Staphylococcus aureus*.

Additionally, the resistance of the test bacteria could be another contributing factor. Some bacterial strains possess thick peptidoglycan layers or efflux pumps that limit the uptake of plant-based antimicrobial agents (Eze *et al.*, 2022). This underscores the complexity

of developing effective plant-based antimicrobials, as bioactivity is influenced by both intrinsic phytochemical composition and microbial resistance mechanisms.

The antifungal findings in this research are consistent with those of Saleh and Garba (2023), who reported that methanol and ethanol extracts of *Carica papaya* leaves showed strong inhibition against *Candida albicans* with inhibition zones ranging from 20–28 mm at 500–800 mg/mL concentrations. Similarly, Abdullahi *et al.*, (2022) found that *Carica papaya* leaf extracts exhibited moderate activity against *Candida albicans* but negligible effect on *Aspergillus niger*, aligning closely with the present results.

However, the absence of antibacterial activity contrasts with the findings of Chukwuma and Obidike (2023), who observed notable antibacterial activity of papaya leaf extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The difference may be attributed to variations in extraction solvent, geographical origin, and leaf age. Extracts obtained with organic solvents such as ethanol or methanol often yield higher concentrations of phenolic compounds and flavonoids, which are known to exhibit stronger antibacterial effects than aqueous extracts (Afolabi and Jimoh, 2022; Kareem *et al.*, 2022).

It is also possible that environmental conditions in Sokoto State, such as soil composition, temperature, and sunlight exposure, may influence the phytochemical profile of *Carica papaya* leaves. (Mohammed and Ibrahim, 2023) emphasized that agroecological variations can significantly alter the concentration of secondary metabolites in medicinal plants, thereby affecting their antimicrobial potential.

The present study confirms that *Carica papaya* leaves contain phytochemical constituents capable of inhibiting fungal growth, particularly *Candida albicans*. The increase in inhibition with higher concentrations of the extract suggests that the antifungal activity is dose-dependent, a phenomenon commonly observed in plant-based antimicrobial research (Akinmoladun *et al.*, 2023; Bamidele and Salihu, 2022).

The selective inhibition of *Candida albicans* may be attributed to the cell wall structure and metabolic profile of the organism. Yeasts like *Candida albicans* have simpler cell walls that are more susceptible to disruption by bioactive compounds such as flavonoids, alkaloids, and tannins found in *Carica papaya* (Adedayo *et al.*, 2023; Fasogbon and Aluko, 2023). In contrast, *Aspergillus* species possess highly rigid chitinous walls and complex sporulating mycelia, making them more resistant to such compounds.

The absence of antibacterial activity across all concentrations suggests that the extract either lacked potent bactericidal compounds or that the solvent extraction method was inadequate for isolating antibacterial agents. Previous studies have shown that organic solvents (e.g., methanol and ethanol) tend to extract more potent antibacterial constituents compared to aqueous extraction (Gambo and Umar, 2022; Mohammed and Ibrahim, 2023). Therefore, the negative antibacterial result might be linked to the extraction technique or the age of the leaves used in the experiment.

Overall, the study's findings provide useful insight into the age-dependent antifungal potential of *Carica papaya* leaves and reinforce the need for optimizing extraction methods and selecting appropriate leaf developmental stages for maximum bioactivity.

5.3 Conclusion

This study has demonstrated that *Carica papaya* leaf extracts possess notable antifungal activity against *Candida albicans*, which increased with extract concentration, but showed no antibacterial or anti-*Aspergillus* activity under the conditions tested. These findings suggest that the bioactive compounds present in papaya leaves may have fungistatic or fungicidal properties effective against yeast pathogens, but not against bacterial or filamentous fungal strains.

This study revealed that *Carica papaya* leaf extract exhibited no antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* at concentrations of 100, 400, and 700 mg/mL. All extract concentrations produced 0.00 mm zones of inhibition, while the negative control showed full microbial growth (70 mm), and the positive control (Ciprofloxacin) displayed clear inhibitory activity.

These findings indicate that the crude leaf extract, as prepared in this study, is not effective as an antibacterial agent against the tested organisms. Further research is recommended using different solvents, purified fractions, alternative extraction techniques, or more sensitive assays such as MIC determination to better evaluate the potential antimicrobial components of *Carica papaya* leaves.

The results also indicate that leaf age, extraction solvent, and concentration are critical determinants of antimicrobial efficacy in *Carica papaya*. The observed antifungal properties support the traditional use of papaya leaves in treating fungal infections such as candidiasis, particularly in regions like Sokoto State where access to conventional antifungal drugs may be limited.

5.4 Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. Further research should explore other solvents such as ethanol, methanol, and acetone to enhance the yield of active compounds with antibacterial properties.
2. Comparative studies using both young and mature papaya leaves should be conducted to establish the effect of leaf developmental stage on antimicrobial potency.
3. Advanced analytical techniques such as GC-MS, HPLC, and FTIR should be employed to isolate, identify, and quantify the specific bioactive constituents responsible for antifungal activity.
4. Acute and chronic toxicity assays should be carried out to ensure the safety of *Carica papaya* leaf extracts before considering pharmaceutical or herbal formulation.

REFERENCES

- Abbas, A. M., Ibrahim, M. A., & Yusuf, A. M. (2023). Phytochemical screening and antimicrobial activity of *Carica papaya* leaf extracts. *African Journal of Microbiology Research*, 17(4), 125–134. <https://doi.org/10.5897/AJMR2023.9781>
- Abbas, H., Yusuf, U., & Danjuma, A. (2023). Traditional medicinal uses of tropical fruit plants in Northern Nigeria: Ethnobotanical perspectives. *Journal of Ethnopharmacology*, 311, 116456. <https://doi.org/10.1016/j.jep.2023.116456>
- Abiodun, T. F., Okechukwu, E. C., & Bello, M. S. (2022). Evaluation of antibacterial and antifungal potential of *Carica papaya* leaf extract. *Nigerian Journal of Biological Sciences*, 16(2), 45–53.
- Adeoye, O., Ibrahim, M., & Bello, A. (2022). Advances in plant-based antimicrobials and their role in tackling multidrug resistance. *Frontiers in Pharmacology*, 13, 874321. <https://doi.org/10.3389/fphar.2022.874321>
- Adeyemi, T. A., & Yusuf, O. M. (2023). Applications and performance of Nutrient Agar in routine bacteriological analysis. *Journal of Modern Microbiology Techniques*, 8(1), 30–38.
- Adisa, S. A., Usman, T., & Lawal, M. (2022). Nutritional and phytochemical analysis of young and mature papaya leaves. *Nigerian Journal of Natural Products and Medicine*, 26(1), 12–21. <https://doi.org/10.4314/njnpm.v26i1.2>
- Afolayan, A. J., & Okoh, A. I. (2022). *In vitro* assessment of plant-derived compounds against resistant bacteria. *Journal of Applied Microbiology*, 132(1), 41–55. <https://doi.org/10.1111/jam.15347>
- Ahmad, R., Mohammed, K., & Suleiman, Y. (2023). Methodological considerations in the antimicrobial evaluation of medicinal plants. *Scientific African*, 19, e01502. <https://doi.org/10.1016/j.sciaf.2023.e01502>
- Akinmoladun, F. O., Komolafe, T. R., Farayibi, P. K., Olaleye, T. M., Komolafe, O. O., Farombi, E. O., & Olaleye, T. M. (2020). Comparative phytochemical profile and antioxidant activity of aqueous and ethanolic extracts of *Ocimum gratissimum* leaves. *Journal of Applied Pharmaceutical Science*, 10(5), 74–81. <https://doi.org/10.7324/JAPS.2020.105010>

- Akinpelu, D. A., & Ogundare, A. O. (2023). Antifungal potentials of Nigerian medicinal plants. *Journal of Ethnopharmacology*, 301, 115793. <https://doi.org/10.1016/j.jep.2022.115793>
- Alhodieb, F. S., Farid, M., Sabir, M., Nisa, S., Sarwar, S., & Abbas, S. (2025). Exploring the bioactive compounds of *Carica papaya* leaves: phytol's role in combatting antibiotic-resistant bacteria. *Frontiers in Cellular and Infection Microbiology*, 15, Article 1564787. <https://doi.org/10.3389/fcimb.2025.1564787>
- Ali, M., & Yusuf, A. (2022). Antibacterial effects of tropical plants: A comparative study of leaf extracts. *African Journal of Plant Science*, 16(2), 45–56. <https://doi.org/10.5897/AJPS2021.2122>
- Aliyu, M. B., & Hassan, U. M. (2022). Evaluation of crude extracts of *Carica papaya* leaves for antimicrobial properties. *Nigerian Journal of Natural Products and Medicine*, 26(1), 101–108.
- Aljuhani, S., Rizwana, H., Aloufi, A. S., Alkahtani, S., Albasher, G., Almasoud, H., & Elsayim, R. (2024). Antifungal activity of *Carica papaya* fruit extract against *Microsporum canis*: *In vitro* and *in vivo* study. *Frontiers in Microbiology*, 15, Article 1399671. <https://doi.org/10.3389/fmicb.2024.1399671>
- Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4), 42. <https://doi.org/10.3390/plants6040042>
- Aluko, H., Sani, A., & Bello, M. (2022). Plant-derived antimicrobials: Progress, prospects, and challenges. *Journal of Medicinal Plants Research*, 16(5), 133–144. <https://doi.org/10.5897/JMPR2022.7161>
- Alves, R. J., & Pereira, M. L. (2021). Evaluation of culture media for fungal isolation and growth performance. *Journal of Mycological Research*, 14(2), 45–53.
- Ameh, S. J., Obodozie, O., Inyang, U. S., & Abubakar, M. S. (2021). Current development in phytopharmaceutical research in Nigeria: Challenges and future prospects. *Journal of Pharmacy & Bioresources*, 18(2), 89–105. <https://doi.org/10.4314/jpb.v18i2.4>
- Anwar, S., Musa, M., & Ibrahim, A. (2022). Phytochemical diversity in papaya leaves of different maturity stages. *International Journal of Phytomedicine*, 14(2), 89–98. <https://doi.org/10.5138/09750185.2295>

- Anyanwu, M. U., & Okoye, R. C. (2023). Antimicrobial resistance and natural alternatives from plants. *Journal of Medicinal Plants Research*, 17(7), 311–325. <https://doi.org/10.5897/JMPR2023.7320>
- Asare, J., Mensah, K., & Boateng, R. (2023). Traditional uses of papaya (*Carica papaya*) in West Africa: An ethnopharmacological review. *Journal of Herbal Medicine*, 37, 100634. <https://doi.org/10.1016/j.hermed.2023.100634>
- Asif, M. (2015). Chemistry and antioxidant activity of plants containing some phenolic compounds. *Chemistry International*, 1(1), 35–52. <https://doi.org/10.5281/zenodo.43979>
- Azwanida, N. N. (2015). A review on the extraction methods used in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants*, 4(3), 196. <https://doi.org/10.4172/2167-0412.1000196>
- Azwanida, N. N. (2020). A review on the extraction methods of medicinal plants. *International Journal of Bioactive Natural Products*, 10(1), 15–20.
- Baba, S., Umar, H., & Musa, Y. (2023). Techniques in plant extraction and compound isolation: A review of current approaches. *Heliyon*, 9(5), e15987. <https://doi.org/10.1016/j.heliyon.2023.e15987>
- Bashir, M. A., Khan, S. H., Ashfaq, M., & Shabbir, M. (2020). Potential of medicinal plants in the treatment of viral diseases focusing on COVID-19: A review. *Pakistan Journal of Pharmaceutical Sciences*, 33(Supplementary), 2477–2485. <https://doi.org/10.36721/PJPS.2020.33.SUP.2477-2485>
- Chaijan, S., Chaijan, M., Uawisetwathana, U., Panya, A., Phonsatta, N., Shetty, K., & Panpipat, W. (2024). Phenolic and metabolic profiles, antioxidant activities, glycemic control, and anti-inflammatory activity of three Thai papaya cultivar leaves. *Foods*, 13(11), 1692. <https://doi.org/10.3390/foods13111692>
- Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., Elsohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Journal of Agricultural and Food Chemistry*, 62(14), 3825–3836. <https://doi.org/10.1021/jf500187y>
- Chikezie, P. C., & Okorundu, S. I. (2023). Phytochemical dynamics of papaya (*Carica papaya*) leaves and their relationship with antimicrobial efficacy. *Journal of Pharmacognosy and Phytochemistry*, 12(5), 10–19.

- Chima, G. N., & Olatunji, B. R. (2023). Standard procedures for preparation and handling of bacteriological growth media. *African Journal of Laboratory Science*, 12(2), 50–57.
- Chinwe, O., Okafor, U., & Ibrahim, L. (2022). Antimicrobial activity of Nigerian medicinal plants: A systematic review. *Journal of Applied Research on Medicinal and Aromatic Plants*, 31, 100428. <https://doi.org/10.1016/j.jarmap.2022.100428>
- Choudhary, R., Kaushik, R., Akhtar, A., Manna, S., Sharma, J., & Bains, A. (2025). Nutritional, phytochemical, and antimicrobial properties of *Carica papaya* leaves: Implications for health benefits and food applications. *Foods*, 14(2), 154. <https://doi.org/10.3390/foods14020154>
- CLSI. (2021). Performance standards for antimicrobial susceptibility testing (31st ed.). Clinical and Laboratory Standards Institute.
- Dahiru, D., & Obidoa, O. (2008). Evaluation of the antioxidant effects of *Ziziphus mauritiana* Lam. leaf extracts against chronic ethanol-induced hepatotoxicity in rat liver. *African Journal of Traditional, Complementary and Alternative Medicines*, 5(1), 39–47. <https://doi.org/10.4314/ajtcam.v5i1.31265>
- Ede, J., Adamu, B., & Lawal, S. (2023). Comparative antimicrobial activity of young and old leaves of selected tropical plants. *African Journal of Biological Sciences*, 19(3), 77–85. <https://doi.org/10.5897/AJBS2022.1455>
- Ekanem, P., Bello, A., & Udo, E. (2023). Antimicrobial resistance in Nigeria: The case for alternative therapies. *Frontiers in Public Health*, 11, 1160291. <https://doi.org/10.3389/fpubh.2023.1160291>
- Fatima, H. B., & Danladi, A. (2022). Phytochemical screening and antimicrobial efficacy of plant leaves used in Northern Nigeria. *African Journal of Traditional, Complementary and Alternative Medicines*, 19(6), 233–241.
- Gad, H. A., El-Ahmady, S. H., Abou-Shoer, M. I., & Al-Azizi, M. M. (2013). Application of chemometrics in authentication of herbal medicines: A review. *Phytochemical Analysis*, 24(1), 1–24. <https://doi.org/10.1002/pca.2378>
- Gambo, A., Bala, H., & Mohammed, A. (2023). GC–MS profiling of bioactive compounds in papaya leaves of different developmental stages. *Natural Product Research*, 37(12), 2103–2111. <https://doi.org/10.1080/14786419.2022.2156789>

- Garba, M., Ibrahim, H., & Yusuf, I. (2022). Botanical and morphological characterization of papaya (*Carica papaya* L.). *International Journal of Botany Studies*, 7(5), 66–74. <https://doi.org/10.22271/botany.2022.v7.i5b.2048>
- Gogna, N., Kaur, G., & Kumar, R. (2015). Metabolomic profiling of the phytomedicinal constituents of *Carica papaya* L. leaves and seeds by ¹H NMR spectroscopy and multivariate statistical analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 115, 74–85. <https://doi.org/10.1016/j.jpba.2015.06.035>
- Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer. <https://doi.org/10.1007/978-94-009-5570-7>
- Hassan, A. U., Ibrahim, M., & Suleiman, S. (2022). Standardized preparation and quality assessment of Potato Dextrose Agar for fungal studies. *International Journal of Microbiology and Biotechnology*, 9(4), 112–119.
- Ibrahim, M. S., & Yusuf, A. R. (2023). Influence of extraction solvents on antimicrobial activity of *Carica papaya* leaves. *Journal of Biological Research*, 29(2), 67–78.
- Isah, A. U., & Umar, L. T. (2024). Characterization of bioactive compounds from *Carica papaya* leaves using GC–MS and their antimicrobial potential. *Heliyon*, 10(3), e23219. <https://doi.org/10.1016/j.heliyon.2024.e23219>
- Isah, A., Abdullahi, Y., & Ibrahim, M. (2023). Antimicrobial screening of papaya leaf extracts from Nigeria. *BMC Complementary Medicine and Therapies*, 23(1), 87. <https://doi.org/10.1186/s12906-023-03985-7>
- Jibril, M., Hassan, T., & Sule, B. (2022). Bioactive compounds of papaya leaves and their pharmacological significance. *Phytomedicine Plus*, 2(4), 100276. <https://doi.org/10.1016/j.phyplu.2022.100276>
- Karthikeyan, A., Deepa, M., & Balasubramanian, P. (2022). Phytochemical and GC–MS analysis of bioactive compounds from medicinal plants. *Biochemistry and Biophysics Reports*, 32, 101332. <https://doi.org/10.1016/j.bbrep.2022.101332>
- Krishnaiah, D., Devi, T., Bono, A., & Sarbatly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. *Journal of Medicinal Plants Research*, 3(2), 67–72. <https://doi.org/10.5897/JMPR.9000473>
- Kumar, A., & Shukla, S. (2020). Evaluation of antimicrobial activity of medicinal plant extracts using agar diffusion techniques. *Journal of Applied Bioscience Research*, 12(2), 55–63.

- Kumar, R., & Verma, S. (2022). Importance of media preparation and sterilization techniques in microbiological assays. *International Journal of Microbiology and Bioscience*, 14(2), 55–62.
- Lawal, M. B., & Abdullahi, A. A. (2023). Laboratory methods for assessing antimicrobial potential of herbal extracts. *African Journal of Laboratory Science*, 9(2), 78–87.
- Lawal, M., Sulaiman, A., & Mohammed, Y. (2022). Methods of antimicrobial susceptibility testing of medicinal plant extracts. *Annals of Microbiology*, 72(1), 13. <https://doi.org/10.1186/s13213-022-01666-3>
- Liu, R. H. (2013). Health-promoting components of fruits and vegetables in the diet. *Advances in Nutrition*, 4(3), 384S–392S. <https://doi.org/10.3945/an.112.003517>
- Mensah, D. T., & Owusu, B. A. (2024). Enhancing fungal culture purity through antibiotic-supplemented media. *African Journal of Applied Biosciences*, 12(3), 75–83
- Misnan, N. M., Afzan, A., Omar, M. H., & Low, K. H. (2024). Flavonoid variability in *Carica papaya* L. var. Sekaki leaf maturation and its association with sexual differentiation using targeted metabolomics. *Natural Product Communications*, 19(7). <https://doi.org/10.1177/1934578X241260175>.
- Mohammed, K. S., Idris, A. M., & Bello, H. Y. (2024). Quality control and pH optimization of culture media used in microbiological assays. *International Journal of Applied Biosciences*
- Munir, S., Liu, Z., Tariq, T., Rabail, R., Kowalczewski, P. Ł., Lewandowicz, A., ... Aadil, R. M. (2022). Delving into the therapeutic potential of *Carica papaya* leaf against thrombocytopenia: Mechanisms and clinical evidence. *Molecules*, 27(9), 2760. <https://doi.org/10.3390/molecules27092760>
- Musa, A. A., & Yahaya, I. (2022). The phytochemical basis of antifungal properties in *Carica papaya* leaf extracts. *International Journal of Microbiology and Mycology*, 13(1), 55–63.
- Musa, H., Aliyu, S., & Bello, I. (2023). Comparative antimicrobial efficacy of young and mature papaya leaf extracts. *Journal of Ethnopharmacology*, 317, 116864. <https://doi.org/10.1016/j.jep.2023.116864>
- Ncube, B., Van Staden, J., & Drewes, F. E. (2008). *Jatropha curcas*: A review on phytochemistry, pharmacology, toxicity and ethnomedicinal uses. *Phytochemistry Reviews*, 7(3), 205–216. <https://doi.org/10.1007/s11101-008-9079-5>

- Ogundipe, T. O., & Balogun, A. M. (2024). The potential of papaya (*Carica papaya*) leaves in combating fungal infections. *Plant Science Today*, 11(1), 22–30. <https://doi.org/10.14719/pst.2024.11.22>
- Okeke, C. F., Nwosu, V. C., & Eze, S. O. (2023). Standard microbiological media and aseptic laboratory practices for antimicrobial testing. *African Journal of Applied Microbiology Research*, 11(1), 22–30.
- Olawale, A., Musa, K., & Bello, A. (2023). Evaluation of the antifungal activity of medicinal plant extracts used in Sokoto, Nigeria. *Nigerian Journal of Microbiology*, 37(2), 100–109.
- Osei, R. K., & Boateng, J. T. (2023). Methodological advances in agar diffusion assays for antimicrobial screening of natural products. *African Journal of Microbial Methods*, 9(1), 14–22.
- Pandey, A., Tripathi, S., & Singh, P. (2021). Standardization of drying methods for medicinal plants: Impact on phytochemical quality. *Journal of Medicinal Plants Studies*, 9(3), 45–52.
- Rahman, M. H., Yusuf, S. K., & Latha, T. R. (2024). Standardized approaches for determining antimicrobial activity of herbal extracts. *International Journal of Phytomedicine and Microbiology*, 6(2), 40–52.
- Reddy, L., & Natarajan, S. (2022). Influence of storage conditions on the stability of bioactive compounds in dried medicinal plant powders. *Journal of Pharmacognosy and Phytochemistry*, 11(2), 120–128.
- Rodríguez, P. E., & Silva, C. M. (2023). Use of selective antifungal media supplemented with antibiotics to reduce bacterial contamination. *International Journal of Fungal Biology*, 18(1), 20–28.
- Saleh, A., Ibrahim, H., & Bello, M. (2023). Concentration-dependent antimicrobial efficacy of plant extracts. *African Journal of Biochemistry Research*, 17(2), 89–97. <https://doi.org/10.5897/AJBR2023.1132>
- Sharma, A., Sharma, R., Sharma, M., Kumar, M., Barbhai, M. D., Lorenzo, J. M., Sharma, S., Samota, M. K., Atanassova, M., Caruso, G., Naushad, M., Radha, D., Chandran, D., Prakash, P., Hasan, M., Rais, N., Dey, A., Mahato, D. K., Dhumal, S., Singh, S., Senapathy, M., Rajalingam, S., Visvanathan, M., Saleena, L. A. K., & Mekhemar, M. (2022). *Carica papaya* L. leaves: Deciphering its antioxidant bioactives, biological activities, innovative products, and safety aspects.

Oxidative Medicine and Cellular Longevity, 2022, Article 2451733.
<https://doi.org/10.1155/2022/2451733>

Shukla, S., & Patel, R. (2023). Post-harvest handling and processing of medicinal plants: A practical guide. *Journal of Herbal Science and Technology*, 5(1), 10–18.

Singh, B., Sharma, R. A., & Thakur, S. (2022). Phytochemical analysis and biological activity of *Carica papaya* leaves: A comprehensive review. *Plant Science Today*, 9(1), 121–134. <https://doi.org/10.14719/pst.2022.9.1.1334>

Singh, M., & Rao, P. K. (2024). Preparation and quality control of bacteriological and mycological growth media. *Journal of Applied Bioscience and Biotechnology*, 6(2), 40–48.

Yusuf, M., & Musa, A. (2023). Phytochemical characterization and antimicrobial screening of *Carica papaya* leaf extracts. *African Journal of Microbiology*, 17(4), 176–184.