IDENTIFICATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM THE LEAF, HUSK AND NUT OF ARECA PLANT *(Areca catechu)*

BY

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CERTIFICATION

This is to **c**ertify that this project titled '**Identification and Characterization of the Bioactive Compounds from the Leaf, Husk and Nut of Areca Plant *(Areca catechu)*** was carried out by **ADEJUMO**, Adenike Elizabeth, with the matriculation number **20/10BSM002** and submitted to the Microbiology Programme, Department of Biological Sciences in the Faculty of Computing and Applied Sciences, for meeting the requirements for the award of Bachelor in Microbiology, Thomas Adewumi University, Oko-Irese, Kwara State.

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DEDICATION

This project is dedicated to God Almighty and all those who love the education of an African child.

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ABSTRACT

Areca catechu, commonly known as the betel nut palm, has been traditionally used for its medicinal properties. The aim of the study is to extract, identify, and characterize the bioactive compounds from *Areca catechu* leaf, husk, and nut, and evaluate their antimicrobial and antioxidant activities. Dried plant samples (leaf, husk, and nut) were extracted using acetone, ethanol, methanol, hot water, and cold water solvents. Phytochemical screening was performed to identify key bioactive compounds. Antimicrobial susceptibility tests were conducted against *Candida albicans, Escherichia coli, Staphylococcus aureus, Klebsiella* spp., and *Aspergillus* spp. using the disc diffusion method. Antioxidant activity was evaluated through (DPPH), Ferric reducing antioxidant power (FRAP), and Hydrogen peroxide radical scavenging activity (H_2O_2) scavenging assays. Gas-Chromatography and Mass-Spectrometry (GC-MS) analysis was used to detect specific bioactive compounds in the extracts. Methanol showed the highest extraction yield across all plant parts. Phytochemical screening indicated the presence of alkaloids, saponins, glycosides, and phenols in all extracts, with tannins only detected in the acetone extract. The methanol extract exhibited the most potent antimicrobial activity, especially against *Candida albicans* (20-23 mm) *Escherichia coli* (22-24 mm), and *Aspergillus* spp. (20-24 mm). Antioxidant assays revealed significant free radical scavenging, with methanol extracts showing the highest activity across DPPH, FRAP, and H_2O_2 assays. GC-MS identified compounds like glycerin and 2-hydroxy-gamma-butyrolactone, known for their biological activities. *Areca catechu* extracts, particularly methanol-based, demonstrated strong antimicrobial activity against both fungal and bacterial strains, including *Candida albicans, Escherichia coli,* and *Aspergillus* spp., and exhibited significant antioxidant properties. These findings highlight the medicinal potential of *Areca catechu* for antifungal and antioxidant applications. Further studies should explore the therapeutic potential of these bioactive compounds, especially in pharmaceutical and nutraceutical applications. *In vivo* studies are also recommended to validate the observed bioactivities in clinical settings.

Keywords: Bioactive compounds, Extracts, Phytochemical screening, Antioxidant, GC-MS analysis.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Areca catechu is cultivated in India, Malaysia, Taiwan, and various other Asian countries (Li *et al*., 2020). Traditionally, *Areca* nut commonly known as betel nuts are predominantly chewed and are recognized for their numerous medicinal properties. These include antioxidant, anti-bacterial, anti-fungal, anti-diabetic, antilipidemic, anti-aging, anti-migraine, anti-depressant, and hepatoprotective activities (Sharaf *et al*., 2021, Adesulu-Dahunsi *et al*. 2018a). It is noted that the six major alkaloids which include arecoline, guvacine, arecaine, arecaidine, guvacoline and choline present in *Areca* nut contribute to its drug-like properties (Alotaibi *et al.,* 2021*).*

In general, the *Areca* fruit comprises two primary components, namely, the husk and kernel (Musdja *et al*., 2020). These parts are utilized independently or in combination for both edible and medicinal purposes. The *Areca* nut is reportedly eaten by chewing them alone or wrapped in betel leaves, either with or without slaked lime, tobacco, spices, sweeteners, or other ingredients (Oliveira *et al*., 2020). *Areca* nut is recognized as one of the four major southern medicines in China, as documented in the Compendium of Materia Medica (Sharaf *et al*., 2021). Since 1953, it has been included in the Pharmacopoeia of China (Hung *et al*., 2020). Numerous prescriptions in traditional medicine emphasize the medicinal value of different forms of *Areca* nut, such as decoction pieces, Charred Semen Arecae (CSA), and *Areca* pericarpium, with documented therapeutic effects on conditions like beriberi edema, tenesmus, malaria, abdominal pain, indigestion, and diarrhea (Hung *et al*., 2020). *Areca* nut has also been indicated for treating periodontitis, premature ejaculation, difficulty urinating, and glaucoma (Gupta *et al*., 2020). Combined with other medicinal materials, *Areca* nut plays a crucial role in compound medicines like Da Yuan Yin, renowned for treating various inflammation-related diseases (Sari *et al*., 2020). *Areca* nut has abilities to dissipate phlegm, resolve masses, and relieve dampness were reported (Sari *et al*., 2020).

Research studies have identified numerous bioactive components in *Areca catechu* leaf, husk and nuts, some of which include arecoline, procyanidin, luteolin, and catechin, each exhibiting

diverse pharmacological activities such as anti-inflammatory, anti-depression, anti-tumor, hypoglycemic, and antioxidant effects (Li *et al*., 2020). Arecoline has been found to significantly irritate the ventral tegmental area (VTA) dopaminergic neurons, which can produce dopamine to achieve an excitatory effect having the potential to be used for treating neurodegenerative disease (Sun *et al.,*2024). Bioactive compounds in *Areca* nut seeds can inhibit the enzyme alpha-glucosidase, which plays a role in carbohydrate metabolism, making them a potential alternative treatment for chronic hyperglycemia (Trini *et al.,* 2023). Presence of phenolic compounds, and antioxidants, contribute to immune-boosting and anti-inflammatory effects (Emma *et al.,* 2023; Adesulu-Dahunsi *et al.*, 2018b). Polyphenolics are compounds that feature an aromatic hydroxyl ring derived from L-phenylalanine. Phenolic compounds consist of one (phenolic acids) or multiple (polyphenols) aromatic rings with attached hydroxyl groups. Several types of polyphenols, such as hydrolyzed tannins, lignans, stilbenes, and flavonoids, are classified as phenolic acids. Catechins, which are flavonoids (flavanols) within the polyphenol group, are found in high concentrations in vegetables, fruits, and beverages (Liza *et al.,* 2021). Glycosides exhibit antiproliferative activity, promote wound healing, and have anti-hepatotoxic and analgesic effects. Their anti-inflammatory activity is linked to the down-regulation of inflammatory cytokines like NO, PGE2, and IL-6, as well as the suppression of iNOS and COX-2. Bioactive compounds are naturally occurring substances in plants, animals, and microorganisms that exhibit a wide range of biological activities, contributing to the prevention and treatment of diseases. These compounds include various classes such as alkaloids, phenolics, flavonoids, terpenoids, and glycosides, each with unique therapeutic properties (Liza *et al*., 2021). Alkaloids are nitrogenous compounds that offer significant pharmacological effects, including analgesic, antimicrobial, and neuroprotective activities. Phenolic compounds, which include flavonoids, tannins, and lignans, are recognized for their potent antioxidant, anti-inflammatory, and anticancer properties, helping to neutralize harmful free radicals and protect against cardiovascular diseases (Adesulu-Dahunsi *et al*., 2018). Flavonoids, a subclass of polyphenols, are especially valued for their role in reducing inflammation and oxidative stress, contributing to improved heart health and reduced cancer risk. Terpenoids, another class of bioactive compounds, are known for their antiviral, anti-inflammatory, and antitumor effects, commonly found in essential oils and herbal medicines. Together, these bioactive compounds have gained increasing attention for their role in promoting health, preventing chronic diseases,

and serving as the basis for new therapeutic agents in pharmacology and nutraceutical industries (Li *et al*., 2020).

1.2 STATEMENT OF PROBLEM

Areca catechu, a plant primarily found in Asian countries, is not native to Africa. However, it is found in Thomas Adewumi University (TAU) campus. Understanding the bioactive compounds inherent in the leaves, husks and nuts is crucial to unlocking the full therapeutic potential of *Areca catechu*. By identifying and characterizing bioactive compounds, this research study contributes insights into the medicinal properties of *Areca catechu*. The knowledge could lead to the development of pharmaceuticals, nutraceutical, or complementary medicine for addressing various health conditions. Moreover, this investigation aligns with the broader goal of promoting sustainable and informed utilization of natural resources.

1.3 JUSTIFICATION OF STUDY

This study holds potential economic value by uncovering valuable bioactive compounds that may find applications in pharmaceutical and nutraceutical industries. The identification and characterization of bioactive compounds from *Areca catechu* is crucial due to the plant's rich in traditional medicine.

1.4 AIM AND OBJECTIVES

This study aims to identify and characterize the bioactive compounds from *Areca catechu* leaf, husk and nut of the species of *Areca catechu* present on TAU campus.

The specific objectives of this study are;

i. To collect and identify *Areca catechu* plant present on TAU campus.

ii. To obtain crude extract from *Areca catechu* leaf, husk and nut using different solvents.

iii. To determine the phytochemical components of the plant parts.

iii. To determine the antimicrobial activities of methanolic, ethanolic, acetone, hot and cold water of aqueous *Areca* plant extracts against pathogenic microorganisms.

iv. To evaluate the antioxidant potential of *Areca* extracts.

v. To identify the bioactive compounds in the plant extracts using Gas Chromatography-Mass Spectrometry analysis.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Areca catechu*

The *Areca* nuts have been widely utilized for treating various ailments including malaria, beriberi, typhoid. Many researches have highlighted its therapeutic attributes in addressing a range of conditions like asthma, cough, dermatitis (used on the skin), fainting, glaucoma, impotence, intestinal worms, leprosy, and toothache, encompassing immunomodulation, cytotoxicity, anticancer properties, antibacterial efficacy, and more (Sari *et al*., 2020).

2.1.1 History of *Areca Catechu*

Areca catechu, commonly known as the *areca* nut or betel nut, has a long history of cultural, medicinal, and economic significance, particularly in Southeast Asia, India, and China. The cultivation and use of the *areca* nut date back thousands of years, with records indicating its role in traditional medicinal systems and cultural practices across Asia. In countries like India, Malaysia, Taiwan, and Indonesia, *areca* nuts have been chewed for their stimulating effects, often wrapped in betel leaves and combined with slaked lime and other ingredients, a practice that continues to this day. The areca nut has also been historically significant in traditional Chinese medicine, where it was documented in ancient texts such as the *Compendium of Materia Medica* and included in the Chinese Pharmacopoeia since 1953. Its traditional uses include treating conditions like indigestion, abdominal pain, diarrhea, and parasitic infections, while also serving as a key ingredient in several compound medicines (Hung et al., 2020; Sharaf et al., 2021)

2.2 Bioactive Components of *Areca* Nut

The key constituents of the *Areca* nut encompass polyphenols (10–30%), polysaccharides (18–25%), fibers (10–15%), fatty acids (10–15%), and alkaloids (0.3–0.7%) (Yang *et al*., 2021). The *Areca* nut is predominantly comprised of cellulose, featuring a higher lignin content that contributes to increased hardness and heightened mechanical friction of the oral mucosa during

chewing. Additionally, the *Areca* nut contains a diverse array of bioactive substances (Yang *et al*., 2021).

2.2.1 Alkaloids

Alkaloids are nitrogenous organic compounds that are commonly present in various higher plants, including *Papaveraceae, Loganiaceae, Leguminosae, Ranunculaceae,* and *Menispermaceae* (Yuan *et al*., 2023). These compounds, often cyclic nitrogenous substances, are recognized as natural psychoactive elements (Casciaro *et al*., 2019). Within the *Areca* nut, the total alkaloid content ranges from 0.3% to 0.7% (Casciaro *et al*., 2019). The main alkaloids found in *Areca* nut include arecoline (N-methyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid methyl ester), arecaidine, guvacoline, and guvacine, with their concentrations varying in fresh seeds (Casciaro *et al*., 2019).Furthermore, new alkaloids, including Arecatemines A-C and acatechu A and B, have been isolated from the nuts and dried fruit of *A*. *catechu*, respectively. Despite these discoveries, the existence of unknown alkaloids in the *Areca* nut remains a possibility, warranting further exploration of their potential physiological activities in future research (Zhao *et al*., 2021).

2.2.2 Polyphenols

The phenolic compounds or polyphenols are secondary metabolites of plants and the most prevalent antioxidant phytochemicals (Cao *et al*., 2019). The Areca nut is abundant in phenolic compounds, including flavonoids and tannins. The total phenol and flavonoid content is significantly higher in the Areca nut compared to the Areca husk and flower. Additionally, the phenolic content in Areca nuts varies depending on ripeness, size, and place of origin. For instance, more mature or longer Areca nuts tend to have a higher concentration of phenolics (Cao *et al*., 2019).

2.2.3 Flavonoids

Flavonoids are compounds characterized by two phenolic hydroxyl benzene rings linked by three central carbon atoms (Sun *et al*., 2023). Extensive research indicates that flavonoids possess inhibitory properties against atherosclerosis and are recognized for their potential as anti-cancer, anti-viral, anti-inflammatory, and antioxidant components (Jianghong *et al*., 2022). *Areca* nut also contains a rich array of flavonoids, predominantly catechins, isorhamnetin, quercetin,

liquiritigenin, 5,7,4′-trihydroxy-3′,5′-dimethoxydihydroflavone, and chrysoeriol (Sun *et al*., 2024; Jianghong *et al*., 2024).

2.2.4 Tannins

Tannins represent a class of intricate water-soluble polyphenolic compounds, abundantly present in legume seeds, cereals, cacao, vegetables, coffee, tea, berries, and nuts (Das *et al*., 2020). *Areca* nuts predominantly contain condensed tannins, with higher concentrations in unripe nuts diminishing as they ripen (Ojo *et al*., 2022). Tannins impart an astringent and bitter flavor, which contribute to the bitter taste of *Areca* nuts (Ojo *et al*., 2022). Tannins exhibit diverse biological functions, encompassing antioxidant, antibacterial, antiviral, antiparasitic, anti-inflammatory, and antidiarrheal activities. These multifaceted functions potentially contribute to the pharmacological activities associated with *Areca* nuts (Yin *et al*., 2021).

2.2.5 Polysaccharides, Lipids, and Other Components

The *Areca* nut encompasses a variety of components, including polysaccharides, triterpenes, steroids, fatty acids, and others, with polysaccharides being particularly abundant (Yin *et al*., 2021). A newly discovered neutral polysaccharide, PAP1b, isolated from the Areca nut and mainly composed of mannose, galactose, arabinose, and xylose, demonstrates significant scavenging activity against 2,2-diphenyl-1-picryhydrazyl (DPPH) and hydroxyl radicals, suggesting its potential as an antioxidant (Ji *et al*., 2022). These properties render it suitable for applications in inflammation treatment, anti-aging, and cardiovascular disease prevention. Triterpenes and steroids present in the *Areca* nut include arborinol, fernenol, arundoin, cycloartenol, and arborinol methyl ether (Zote *et al*., 2021). The predominant fatty acids identified in the *Areca* nut are lauric acid, myristic acid, palmitic acid, stearic acid, and oleic acid. Furthermore, trace elements such as Zn, Fe, Al, Cr, Co, Mn, Cu, and others have been identified in the *Areca* nut (Zote *et al*., 2021).

2.3 Functional Effects of *Areca* plant

Bioactive components derived from *Areca* plants have been identified for their diverse pharmacological functions and biological activities. Similarly, bioactive elements present in *Areca* nuts have demonstrated a range of pharmacologically beneficial effects, primarily influencing the nervous, digestive, and endocrine systems. These effects encompass anti

inflammatory, antitumor, antioxidant, antibacterial, deworming, and antiviral properties (Zote *et al*., 2021).

2.3.1 Antimicrobial Effects

In *Areca* nut extract, compounds such as fernenol, arundoin, and a combination of stigmasterol and β-sitosterol demonstrate inhibitory effects on the growth of *Colletotrichum gloeosporioides* (Yi *et al*., 2022). These compounds have potential applications in preventing and controlling postharvest anthracnose disease in mangoes, showing efficacy comparable to or even surpassing the commercial fungicide benomyl (Yi *et al*., 2022). The methanol extract of *Areca* nut exhibits antibacterial properties against both Gram-positive and Gram-negative bacteria, with notable effectiveness against the Gram-negative bacteria *Escherichia coli*. The volatile components of *Areca* nut also possess antibacterial properties using simultaneous hydrodistillation extraction (SHDE). Yi *et al*., (2022) identified 98 volatile compounds from *Areca* nut, including aliphatic hydrocarbons, alcohols, fatty acids, carbonyl compounds, esters, terpenoids, terpenes, and arecoline. The SHDE extract demonstrated strong bacteriostatic effects, particularly against *Streptococcus canis* (Yi *et al*., 2022). *Areca* nut extract exhibits synergistic antibacterial effects with other substances as well. For instance, when combined with silver nitrate to form silver nanoparticles (AgNPs), *Areca* nut extract displayed enhanced inhibitory activity against both antibiotic-sensitive and drug-resistant bacteria (Jam *et al*., 2022). Moreover, mixed extracts of *Areca* nut and *Punica granatum* L. were found to inhibit the biofilm formation and metabolism of bacteria such as *Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes,* and *Salmonella enterica*. Among these extracts, the ethanolic extract demonstrated the most robust inhibitory effects (Jam *et al*., 2022).

2.3.1.1. Antimicrobial Effect of Methanolic Extract

Methanolic extracts of *Areca catechu* are among the most studied for their antibacterial properties. Methanol, as a solvent, effectively extracts a wide range of bioactive compounds, including alkaloids, flavonoids, and tannins, which are known for their antibacterial activities. Numerous studies have demonstrated that methanolic extracts exhibit significant inhibitory effects against pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* (Ali *et al*., 2020; Sharma *et al*., 2021).

The antibacterial efficacy of methanolic extracts can be attributed to the presence of key phytochemicals like arecoline and various flavonoids, which disrupt bacterial cell membranes, leading to cell lysis. This disruption can inhibit the bacteria's ability to maintain homeostasis and perform essential functions, ultimately resulting in cell death (Saha *et al*., 2022). Furthermore, the synergistic action of multiple compounds present in the methanolic extracts enhances their antibacterial activity, making them a promising candidate for therapeutic applications against resistant bacterial strains (Saha *et al*., 2022).

2.3.1.2 Antimicrobial Effect of Ethanolic Extract

Ethanolic extracts of *Areca catechu* have also shown considerable antibacterial activity. Ethanol serves as an effective solvent for extracting a range of phytochemicals, including essential oils and phenolic compounds, which contribute to the antibacterial effects. Research indicates that ethanolic extracts have significant inhibitory effects against various bacterial strains, including *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Gupta *et al*., 2019; Rai *et al*., 2020).

The mechanism of action for ethanolic extracts involves the disruption of bacterial cell walls and membranes, leading to leakage of intracellular contents. The presence of flavonoids and tannins in the ethanolic extracts has been linked to this antibacterial activity, as these compounds can bind to bacterial cell walls, inhibiting growth and replication. Additionally, the antioxidant properties of these extracts may contribute to their antibacterial effects by neutralizing reactive oxygen species (ROS) produced by bacterial metabolism, further enhancing their efficacy against bacterial infections (Khan *et al*., 2021).

2.3.1.3. Antimicrobial Effect of Acetone Extract

Acetone extracts of *Areca catechu*, while less frequently studied than methanolic and ethanolic extracts, have also demonstrated significant antibacterial properties. Acetone is effective in extracting a unique profile of phytochemicals, including flavonoids and terpenoids, which possess antibacterial activities. Preliminary studies have shown that acetone extracts exhibit inhibitory effects against a range of bacteria, such as *E. coli* and *Staphylococcus aureus* (Nabi et al., 2022). The antibacterial mechanisms of acetone extracts may involve disruption of cell membrane integrity and interference with bacterial metabolic processes. Terpenoids, present in

acetone extracts, have been reported to possess strong antibacterial activity by altering membrane permeability, leading to bacterial cell death (Nadeem *et al*., 2020). Further research is needed to identify the specific compounds responsible for these effects and to explore the full potential of acetone extracts from *Areca catechu* in combating bacterial infections (Nadeem *et al*., 2020).

2.3.1.4. Antimicrobial Effect of Hot and Cold Water Extract

Aqueous extracts of *Areca catechu*, whether prepared using hot or cold water, have also been evaluated for their antibacterial properties. Water extraction is a traditional method commonly used in herbal medicine and allows for the solubilization of hydrophilic phytochemicals. Hot water extracts typically yield a higher concentration of bioactive compounds compared to cold water extracts due to enhanced extraction efficiency (Dhananjaya *et al*., 2018).

Studies indicate that both hot and cold water extracts possess antibacterial activity, though the potency may differ. Hot water extracts have shown effective antibacterial activity against bacteria such as *Staphylococcus aureus* and *Salmonella typhi* (Emma *et al*., 2023). The efficacy of these extracts can be attributed to the presence of soluble tannins and flavonoids that disrupt bacterial cell walls and inhibit growth by interfering with nutrient absorption and metabolic processes (Lavi *et al*., 2020). The antibacterial mechanisms of aqueous extracts involve the inhibition of bacterial growth by altering cell wall integrity and interfering with nutrient uptake. Additionally, these extracts are appealing for their safety profile, making them suitable for use in food products or as natural remedies (Soni *et al*., 2019).

2.3.2 Antioxidant Effects

Areca nut polyphenols exhibit significant antioxidant activity in lipopolysaccharides (LPS)-stimulated RAW264.7 cells, primarily by restraining the MAPK pathway and activating the Nrf2/HO-1 pathway to diminish the generation of reactive oxygen species (ROS) (Weng *et al*., 2022). Key polyphenols found in *Areca* nut, such as epicatechin and syringic acid, also demonstrate remarkable antioxidant capabilities. Experimental findings highlight the anti-fatigue function of polyphenols extracted from *A. catechu* husk (Weng *et al*., 2022). Additionally, oral administration of *Areca* nut procyanidins is effective in preventing Ultraviolet B radiation (UVB) a skin condition such as uneven skin texture, induced photoaging, wrinkles formation, decreased

elasticity and skin pigmentation. Furthermore, the polysaccharide PAP1b derived from *Areca* nut exhibits antioxidant activity, particularly in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals. Notably, *Areca* nut has been identified as a potential collagenase inhibitor, experimentally confirming its anti-wrinkle effect associated with antioxidant properties (Yan *et al*., 2023). Considering antioxidants' recognized therapeutic potential in cancer due to their redox homeostasis capacity, this suggests a promising anti-cancer effect for *Areca* nut extracts. However, caution is warranted, as inappropriate dosage, duration, and the specific type of cancer may lead to potential side effects (Yan *et al*., 2023).

2.3.2.1. Phytochemical Composition Contributing to Antioxidant Activity

The antioxidant effects of *Areca catechu* can be attributed to its rich composition of bioactive compounds, including flavonoids, phenolic acids, tannins, and alkaloids. *Areca catechu's* antioxidant properties are attributed to its rich composition of bioactive compounds, including flavonoids and phenolic acids (Liza *et al*., 2021). These compounds effectively scavenge free radicals and protect cells from oxidative damage, as evidenced by in vitro and in vivo studies (Khan *et al*., 2023). Additionally, Areca catechu can enhance the body's intrinsic antioxidant defenses by upregulating antioxidant enzymes (Dewi *et al*., 2020).

2.3.3 Antifungal Effects

Areca catechu, a traditional Indonesian tooth-whitening agent, has demonstrated potent antifungal properties against various fungal pathogens, including *Candida albicans* and dermatophytes (Hafiz *et al*., 2020). Its extracts have been shown to disrupt fungal cell membranes and induce oxidative stress, leading to fungal death. These findings suggest potential applications for *Areca catechu* as a natural antifungal agent. The extracts' ability to inhibit biofilm formation is particularly noteworthy, as biofilms can confer resistance to conventional antifungal therapies (Dewi *et al*., 2022).

In addition to its antifungal effects, *Areca catechu* also exhibits antibacterial and antioxidant activities, making it a promising natural compound with diverse potential health benefits. Further research is needed to fully explore and understand the mechanisms underlying its biological activities and to develop effective therapeutic applications (Pramesti *et al.,*2022).

2.3.4 Antiviral Efficacy

Areca catechu has shown promising antiviral properties due to its rich phytochemical composition (Javadi *et al*., 2021). Compounds like arecoline and flavonoids have been implicated in inhibiting viral replication and entry into host cells (Ali *et al*., 2020). Studies have demonstrated the antiviral activity of Areca catechu extracts against influenza, hepatitis B, and hepatitis C viruses (Nabi *et al*., 2022). The mechanisms of action include blocking viral entry, interfering with viral replication, and modulating the immune response (Kumar *et al*., 2020). These findings suggest the potential of *Areca catechu* as a natural antiviral agent, though further research is needed to fully understand its antiviral properties and develop therapeutic applications.

2.3.5 Anti-inflammatory Effects

Areca catechu exhibits potent anti-inflammatory properties due to its rich phytochemical composition (Parolini *et al.,* 2020). Alkaloids, flavonoids, phenolic acids, and tannins contribute to these effects(Sang *et al*., 2021). Arecoline is a prominent alkaloid, suppressing pro-inflammatory cytokines like TNF-α and IL-6. Flavonoids and phenolic compounds modulate inflammatory pathways by inhibiting COX and LOX enzymes (Ali *et al*., 2022).

Areca catechu's anti-inflammatory mechanisms include inhibiting pro-inflammatory cytokine production via NF-κB suppression and exerting antioxidant activity to combat oxidative stress. Studies have demonstrated its effectiveness in reducing inflammation in both in vitro and in vivo models. This evidence suggests that Areca catechu holds promise as a potential therapeutic agent for inflammatory diseases (Khan *et al*., 2023).

2.3.6 Anti-osteoporotic Effects

Areca nut has emerged as a versatile natural substance with a broad spectrum of health benefits. Beyond its well-documented anti-inflammatory properties, it has shown promise in addressing various other health concerns. Studies have indicated that *Areca* nut can enhance bone mass, potentially combating osteoporosis.

Other studies have also indicated its high concentration of flavonoids suggests potent anti-cancer effects, operating through mechanisms such as cell cycle arrest and apoptosis induction (Chen *et al*., 2022). Moreover, chewing *Areca* nut has been associated with a range of additional benefits,

including improved well-being, reduced schizophrenia symptoms, decreased seizure frequency, enhanced digestion, and potential anti-parasitic effects (Pradeep *et al*., 2022). These findings collectively highlight the multifaceted potential of *Areca* nut as a valuable natural resource with applications in various health domains (Tchitchek *et al*., 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Material Collection and Preparation

Fresh leaves and mature nuts of *Areca catechu* were collected from Thomas Adewumi University campus and botanical authentication was carried out. The leaves and nuts were washed with distilled water to remove any adhering dirt, debris, or surface contaminants (Makari *et al*., 2021). The plant parts were dried at room temperature to avoid the decomposition of thermolabile compounds. The dried plant materials (leaf, husk and nut) were grounded into a fine powder using a mortar and pestle and a mechanical grinder.

3.2 Extraction Procedure

Acetone, ethanol, methanol, hot and cold water were used as solvents for the extraction of the pulverized samples of the leaf, husk and the nut via maceration (Serina and Castilho 2022. Fifty grams (50 g) of each dried sample (leaf, shaft and nut) were weighed and separately soaked in 300 mL of acetone, ethanol, methanol, hot and cold water respectively. The mixtures were then shaken on a shaker at room temperature for 72 hours for passive diffusion of target compounds, and then filtered twice; initially with a Whatman filter paper No 1 and later with a muslin cloth. The filtrates representing the initial extracts (crude extract), contains a mixture of various compounds extracted.

3.3 Solvent Evaporation and Concentration

The filtrate was evaporated to dryness at 45-100℃ in accordance with the solvent's boiling point using water bath to evaporate the solvent from the crude extract and obtain a dry crude extract. (Serikuly *et al*., 2021). The dried crude extract was kept for further analysis.

3.4 Plant yield and extraction

The percentage yield of the plant samples was calculated with the formula:

$$\frac{\text{Dry weight of extracts}}{\text{Dry weight of samples}} \times 100$$

3.5 Phytochemical Screening

A qualitative test on the acetone, ethanol, methanol, hot and cold water extract of *Areca catechu* nuts, husk and leaf was carried out using the phytochemical standardized screening procedure (Sannidhi *et al.,*2020).

3.5.1 Alkaloids detection

Hager's test: A few drops of Hager's reagent (saturated picric acid solution) was added to 2 ml of the respected plant extract. Bright yellow precipitate formation indicates the existence of alkaloids.

3.5.2 Flavonoids detection

Alkaline reagent test: 1 ml of 2N NAOH solution was added to 1 ml of plant extract. Yellow color appearance indicates the existence of flavonoids in the sample.

3.5.3 Tannins detection

$FeCl_3$ test: 2 ml of 5% $FeCl_3$ was added to 1 ml of plant extract. Appearance of greenish black or dark blue color confirms the existence of tannins in the test samples.

3.5.4 Saponin detection

Two ml of distilled water was added to 2 ml of plant extract. and shaken vigorously for 15 min in a graduated cylinder. A layer of foam 1 cm or more thick confirms the existence of saponins in the test samples.

3.5.5 Anthocyanin and betacyanin detection

An aliquot of 1 ml of 2N NAOH was added to 2 ml of each plant extract and heated at 100℃ for about 5 minutes to assess the presence of anthocyanin and betacyanin. Bluish green color

formation suggests the existence of anthocyanin while yellow color the existence of betacyanin in the plant extract tested.

3.5.6 Anthraquinone detection

A few drops of 2% HCl was added to the tested plant extract. Red precipitate formation indicates the existence of anthraquinone in the samples.

3.5.7 Glycosides detection

Keller Killani test: A volume of 1ml of glacial acetic acid was added to 1 ml of plant extracts and cooled. After cooling, 2 drops of $FeCl_3$ was added followed by careful addition of concentrated H_2SO_4 along the walls of the test tube. Reddish-brown colour ring formed at the junction of two layers indicated the existence of glycosides.

3.5.8 Phenol test

Ellagic acid test: Few drops of 5% glacial acetic acid were added to 1 ml of plant extract followed by addition of a few drops of 5% $NaNO_2$ solution. Muddy brown color formation reveals the existence of phenols in the test samples.

3.6 Antimicrobial Activity

The antimicrobial activity of *Areca catechu* leaf, husk and nut extracts was evaluated against seven (7) pathogenic microorganisms including; *Staphylococcus aureus, Escherichia coli, Klebsellia spp, Salmonella* sp*, Candida albicans,* and *Aspergillus fumigatus.*

3.6.1 Antimicrobial Screening

Agar well diffusion method was employed. All the selected organisms were cultured overnight in nutrient broth at 37℃ prior to experimentation. The next day, 0.1 g of extract was dissolved in 2 ml of solvent which was used during extraction to make a final concentration of 50 mg/ml. Mueller Hinton agar was prepared and poured on a Petri dish to solidify which were labeled appropriately. Test strains were uniformly distributed on the Petri dish by swabbing with sterile cotton swab in three dimensions to ensure uniform growth on the plate. A cork borer was used to make 4 wells indicating XYZ (leaf, shaft and nut) and a control for each organism per solvent under a sterile condition, 200µl (0.2ml) of liquid extract and controls were distributed into each hole according to its labeling, the Petri dish was left to diffuse for 30 minutes. Afterwards, it

was incubated at 37℃ for 24 hours. The assay was performed and observed against each bacteria strain and the diameter of the zone of inhibition formed around the wells was measured in millimeters (mm) (Oliveira *et al*., 2020).

3.7 Minimum Inhibitory Concentration (MIC) Determination

Further quantitative analysis was performed for the effective plant extracts tested during agar well diffusion assay through determination of minimum inhibitory concentration (MIC). Fungal test was performed on *Candida albicans* in nutrient broth (NB) and about 12 hr-old fungal cultures were used and their suspension turbidity was compared with the turbidity of 0.5 McFarland standard, 0.500g of the plant extract were dissolved in 5ml of solvent each to make a concentration of 100 mg/ml as the stock in sterile test tubes. Six (6) test tubes labeled (50, 25, 12.5, 6.25, 3.125, 1.56) were prepared by distributing 5ml of Mueller Hinton broth (MHB) into each test tubes followed by 5 ml of inoculum from the primarily formed stock of plant extract having a concentration of 100 mg/ml was dispensed into the first test tube labeled 50. Further five consecutive test tubes were dispensed with 5 ml each from serial dilutions and then 5 ml was removed from the 1.56 test tube into another test tube labeled negative control, 100 µl (0.1 ml) of nutrient broth culture was added to the six test tubes each, the test tubes were incubated for 24 hr at 37 ℃. The MIC was demarcated as the sample concentration which stopped the medium from changing color thus demonstrating inhibition of bacterial growth, with MIC values ranging from 6.25 to 25 µg/mL for the organisms (Oliveira *et al*., 2020).

3.8 Minimum Bactericidal/Minimum Fungicidal (MBC/MFC) Determination

Analysis were performed for the effective plant extracts tested during agar well diffusion assay through determination of minimum bactericidal/minimum fungicidal (MBC)/(MFC). Fungal test was performed on *Candida albicans* in nutrient broth (NB) and about 12 hr-old fungal cultures were used. Similarly, MBC/MFC determination broth was picked from each test tube used for the determination of MIC and spread onto a nutrient agar for 24 hr at 37℃. The MBC/MFC are the least concentration of the plant extract to completely eradicate the inoculated organism (Oliveira *et al*., 2020).

3.9 Antioxidant Activity

3.9.1 DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay

Methanolic extracts from the leaf (X), husk (Y), and nut (Z) of *Areca catechu* were mixed with 95% methanol to prepare stock solutions at a concentration of 10 mg/100 ml (100 µg/ml). From these stock solutions, aliquots of 1 ml, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml were transferred into separate test tubes, and serial dilutions were made with the same solvent to achieve final concentrations of 10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml, respectively. Ascorbic acid was used as a standard, prepared at the same concentrations as the sample extracts, using methanol as the solvent.

For the assay, 1 ml of 0.3 mM DPPH methanol solution was mixed with 2.5 ml of the sample solution at different concentrations and allowed to react at room temperature. Absorbance was measured after a 15-minute incubation period at 37°C. A blank was prepared with 1.0 ml methanol and 2.5 ml plant extract solution. The decrease in absorbance of the sample extracts and standard at various concentrations was measured at 517 nm using a double-beam UV spectrophotometer (SHIMADZU UV-1700) (Yan *et al*., 2023).

$$\% \text{ Inhibition} = (\text{A Blank} - \text{A Test}) / \text{A Blank} \times 100$$

3.9.2 Ferric reducing antioxidant power (FRAP) assay

To prepare the FRAP solution, 3.6 mL was mixed with 0.4 mL of distilled water and incubated at 37 °C for 5 minutes. Next, this solution was combined with 80 µL of the plant extract and incubated at 37 °C for 10 minutes. The absorbance of the reaction mixture was then measured at 593 nm. To create a calibration curve, absorbance values were measured for five concentrations of $FeSO_4 \cdot 7H_2O$ (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) using the same procedure as for the sample solutions (Weng *et al*., 2022).

3.9.3 Hydrogen peroxide radical scavenging activity (H_2O_2).

40 mM H_2O_2 solution was prepared in phosphate buffer (50 mM, pH 7.4). 1 ml of test samples at varying concentrations (250–1000 µg/ml) was added to the H_2O_2 solution and incubated for 10 minutes. Absorbance was measured at 230 nm against a blank solution containing phosphate buffer without H_2O_2. Synthetic antioxidant ascorbic acid served as the positive control. The

experiments were conducted and the scavenging activity was calculated as a percentage using the following formula. (Oliveira *et al*., 2020).

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

3.10 Gas-Chromatography and Mass-Spectrometry Analysis

The active compounds in the samples were identified using gas chromatography-mass spectrometry (GC-MS). The analysis was performed with a Varian 3800/4000 gas chromatograph mass spectrometer, equipped with a DB-5MS UI column (30.0 m x 0.25 mm, film thickness 0.25 μm, 5% phenyl methylpolysiloxane) and connected to an Agilent 5975C MSD with a three-axis detector. The system operated in electron ionization mode at 70 eV. Nitrogen gas was used as the carrier gas at a constant flow rate of 1 ml/min. The samples were diluted in an appropriate solvent, filtered, and 1 μl of the particle-free extract was injected into the injector in split mode, with the injector temperature set at 250°C and the ion source temperature at 280°C. The oven temperature was programmed to increase at a rate of 10°C/min from 40°C (held isothermally for 5 minutes) to 300°C, where it was held isothermally for another 5 minutes. Mass spectra were recorded at 70 eV, with a scan interval of 0.5 s and fragment detection ranging from 45 to 450 Da. The total run time for the GC was 43 minutes. The percentage composition of the crude extract components was determined by peak area percentage. The raw GC chromatogram displayed all peaks using MSD Chemstation, and the results were compiled into a single peak table. A library search was conducted for all peaks using NIST/EPA/NIH (version 2.0) to identify the names, structures, and molecular weights of the compounds in the samples. (Li *et al*., 2020).

CHAPTER FOUR

RESULT

4.1 Plant yield and extraction

Table 4.1 shows the percentage yield of *Areca catechu* leaf (X), husk (Y), and nut (Z) extracted using different solvents (acetone, ethanol, methanol, hot water, and cold water). This comparison helps in identifying the solvent that provides the highest yield for each plant part

Table 4.1 Percentage Yield of plant samples.

Samples	X (%)	Y (%)	Z (%)
ACA	5.5	2.8	1.9
ACE	4.6	6.5	11.5
ACM	13.9	7.8	3.4
ACH	12.1	9.9	6.9
ACC	10.5	7.6	7.3

Key:

ACA= Acetone extract of *Areca catechu*

ACE= Ethanol extract of *Areca catechu*

ACM= Methanol extract of *Areca catechu*

ACH= Hot water extract of *Areca catechu*

ACC= Cold water extract of *Areca catechu*

X = Leaf ; Y = Husk, Z = Nut.

4.2 Phytochemical screening

The result of the phytochemical investigation of the acetone, ethanol, methanol, hot and cold water extracts of the plant samples showed that flavonoid, anthocyanin, betacyanin, and anthraquinone while saponins, alkaloids, glycosides and phenol were present in all the plant samples but Tannin were present in only the samples containing the acetone solvent, this is shown in the table below. Table 4.2 highlights the phytochemical constituents that may contribute to the plant's medicinal properties

Table 4.2: Qualitative phytochemical analysis of *Areca catechu* leaf, husk and nut

Plant extract	ACA			ACE			ACM			ACH			ACC		
	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
Alkaloid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Tannnins	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthrocyanins	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Betacyanins	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Glycoside	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Key:

ACA= Acetone extract of *Areca catechu*

ACE= Ethanol extract of *Areca catechu*

ACM= Methanol extract of *Areca catechu*

ACH= Hot water extract of *Areca catechu*

ACC= Cold water extract of *Areca catechu*

X = Leaf ; Y = Husk, Z = Nut.

\+ = Present

\- = Absent

4.3 Antimicrobial Susceptibility test

Table 4.3 indicates that the methanol extract (ACM) exhibited the highest antimicrobial activity among the extracts tested. It showed activity against *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella* spp. The zone of inhibition for *Candida albicans* ranged from 20 to 23 mm, for *Escherichia coli* from 22 to 24 mm, for *Staphylococcus aureus* from 22 to 24 mm, and for *Klebsiella* spp. from 12 to 22 mm.

The acetone extract (ACA) also showed activity against *Candida albicans* and *Staphylococcus aureus*, with zones of inhibition ranging from 18 to 22 mm for *Candida albicans* and 14 to 18 mm for *Staphylococcus aureus*. The ethanol extract (ACE) exhibited activity against *Candida albicans* and *Klebsiella* spp., with zones of inhibition ranging from 20 to 24 mm for *Candida albicans* and 12 to 14 mm for *Klebsiella* spp. The hot water extract (ACH) showed activity only against *Candida albicans*, with zones of inhibition ranging from 16 to 20 mm. The cold water extract (ACC) did not show any activity against any of the tested microorganisms

Table 4.3: In Vitro Antimicrobial Screening of *Candida albicans*, *Escherica coli*, *Staphylococcus aureus*, *Klebsiella* specie, *Salmonella* specie and *Aspergillus* specie using *Areca catechu* Leaf, Husk, and Nut.

Sample		*Candida albicans*	*Escherichia coli*	*Staph aureus*	*Klebsellia specie*	*Salmonella specie*	*Aspergillus specie*
ACA	X	20	22	18	-	-	-
	Y	22	16	16	-	-	-
	Z	18	-	14	-	-	-
ACE	X	22	20	-	-	13	-
	Y	24	14	-	14	-	-
	Z	20	20	-	-	-	-
ACM	X	23	-	24	-	-	-
	Y	22	-	22	12	-	-
	Z	20	-	-	20	-	-
ACH	X	-	-	16	-	-	-
	Y	-	-	-	-	-	-
	Z	20	-	-	-	-	-
ACC	X	-	-	-	-	-	-
	Y	-	-	-	-	-	-
	Z	-	-	-	-	-	-

Key:

ACA= Acetone extract of *Areca catechu*, ACE= Ethanol extract of *Areca catechu*

ACM= Methanol extract of *Areca catechu,* ACH= Hot water extract of *Areca catechu*

ACC= Cold water extract of *Areca catechu*, X = Leaf ; Y = Husk, Z = Nut, - = No inhibition

4.4 Minimum inhibitory concentration of *Areca Catechu* Extract against *Candida albicans*

The MIC (minimum inhibitory concentration) against *Candida albicans* were determined (Table 4.4). The MIC values ranged from 6.25 to 12.5 µg/ml.

4.4.1 Minimum fungicidal concentration of *Areca Catechu* Extract against *Candida albicans*

MFC (minimum fungicidal concentration) values for the methanol extract (ACM) against *Candida albicans* were determined (Table 4.4.1). The MFC values ranged from 12.5 to 25 µg/ml. The MIC/MFC ratio ranged from 0.125 to 0.5, indicating that the methanol extract has fungicidal activity against *Candida albicans.*

$1 \leq (MFC/MIC) \leq 2$= Fungicidal effect

$4 \leq (MFC/MIC) \leq 16$= Fungistatic effect

4.5 Antioxidant Activity: Result of antioxidant test of DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay, FRAP Assay, Hydrogen peroxide radical scavenging activity (H_2O_2).

The antioxidant analysis demonstrated that the extracts of *Areca catechu* possess significant free radical scavenging abilities. The DPPH assay results indicated high percentage inhibition.

Table 4.4.: Minimum inhibitory concentration of *Areca Catechu* Extract on *Candida Albicans* (50µl/ml)

Sample	MIC (ACA)	MIC (ACE)	MIC (ACM)

X	6.25	3.125	12.5
Y	12.5	6.25	12.5
Z	12.5	12.5	12.5

Key:

ACA= Acetone extract of *Areca catechu*

ACE= Ethanol extract of *Areca catechu*

ACM= Methanol extract of *Areca catechu*

X = Leaf ; Y = Husk, Z = Nut.

MIC= Minimum inhibitory concentration

Table 4.4.1: Antifungal Screening on *Candida Albicans* (50µl/ml)

Sample	**MFC**	**MIC/MFC**	**MFC**	**MIC/MFC**	**MFC**	**MIC/MFC**

X	25	4	12.5	4	25	2
Y	25	2	25	4	25	2
Z	25	2	25	2	25	2

Key:

MIC= Minimum inhibitory concentration

MFC= Minimum fungicidal concentration

X = Leaf ; Y = Husk, Z = Nut.

Table: 4.5: DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay

Table 4.6: Ferric reducing antioxidant power (FRAP) Assay

Concentration (µg/ml)	ACM (X)	ACM (Y)	ACM (Z)	CONTROL	Activity(%) X	Y	Z
1000	0.162	0.125	0.106	0.087	**77.15092**	**82.36953**	**85.04937**
500	0.208	0.183	0.154	0.137	**70.66291**	**74.189**	**78.27927**
250	0.275	0.257	0.215	0.188	**61.21298**	**63.75176**	**69.6756**
125	0.336	0.312	0.275	0.238	**52.60931**	**55.99436**	**61.21298**
62.5	0.383	0.308	0.308	0.293	**45.98025**	**48.80113**	**56.55853**
31.25	0.457	0.425	0.358	0.341	**35.54302**	**40.05642**	**49.50635**
15.625	0.529	0.482	0.396	0.389	**25.38787**	**32.01693**	**44.14669**
7.8125	0.578	0.531	0.465	0.425	**18.47673**	**25.10578**	**34.41467**

Concentration (µg/ml)	ACM (X)	ACM (Y)	ACM (Z)	CONTROL	Activity X	Y	Z
1000	0.168	0.121	0.104	0.091	**76.30465**	**82.93371**	**85.33145**
500	0.204	0.181	0.151	0.132	**71.22708**	**74.47109**	**78.7024**

250	0.271	0.255	0.216	0.184	**61.77715**	**64.03385**	**69.53456**
125	0.337	0.315	0.264	0.232	**52.46827**	**55.57123**	**62.76446**
62.5	0.389	0.361	0.301	0.292	**45.13399**	**49.08322**	**57.54584**
31.25	0.459	0.421	0.354	0.349	**35.26093**	**40.62059**	**50.07052**
15.625	0.531	0.489	0.394	0.384	**25.10578**	**31.02962**	**44.42877**
7.8125	0.575	0.537	0.466	0.428	**18.89986**	**24.25952**	**34.27362**

Table 4.7: Hydrogen peroxide radical scavenging activity (H_2O_2).

Concentration (µg/ml)	**ACM (X)**	**ACM (Y)**	**ACM (Z)**	**CONTROL**	**Activity**		
					X	**Y**	**Z** **C**
1000	0.163	0.122	0.105	0.083	**77.00987**	**82.79267**	**85.19041**
500	0.201	0.184	0.153	0.135	**71.65021**	**74.04795**	**78.42031**
250	0.278	0.257	0.211	0.186	**60.78984**	**63.75176**	**70.23977**
125	0.327	0.313	0.265	0.234	**53.8787**	**55.85331**	**62.62341**
62.5	0.385	0.385	0.305	0.296	**45.69817**	**48.51904**	**56.98166**
31.25	0.453	0.426	0.349	0.349	**36.10719**	**39.91537**	**50.77574**

15.625	0.527	0.487	0.391	0.385	**25.66996**	**31.31171**	**44.8519**
7.8125	0.579	0.535	0.468	0.422	**18.33568**	**24.54161**	**33.99154**

4.8 Gas Chromatography-Mass Spectrometry Analysis

The GC-MS (Gas Chromatography-Mass Spectrometry) analysis of *Areca catechu* extracts identified a variety of bioactive compounds, which are significant for their potential therapeutic applications. Key compounds detected include:

1. Glycerin
2. 2. 2-Hydroxy-gamma-butyrolactone

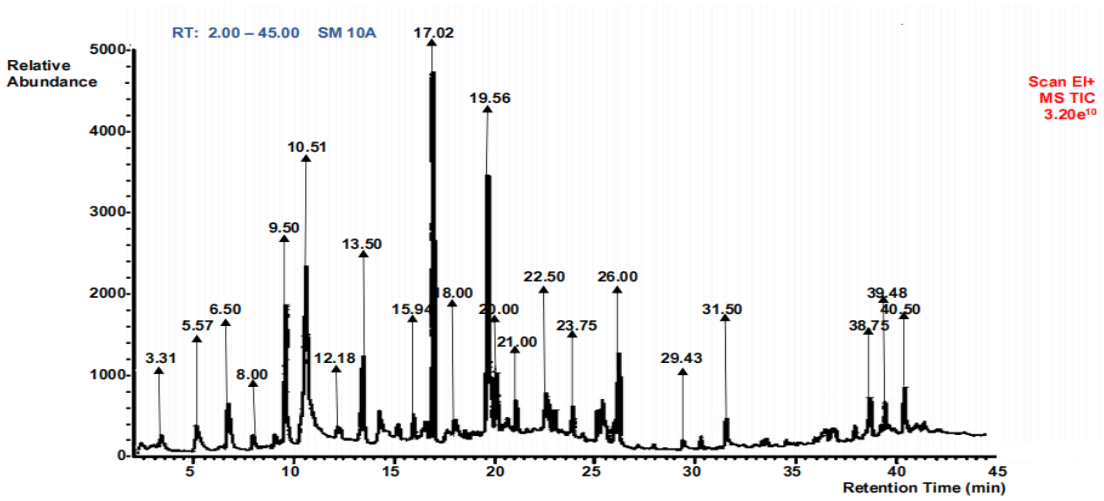
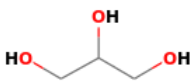
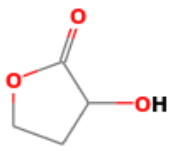
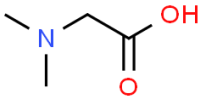
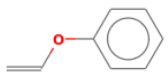
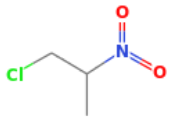
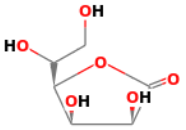
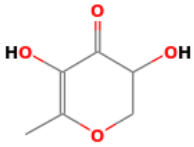
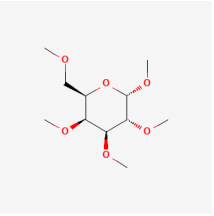
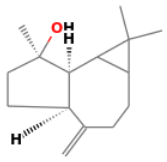
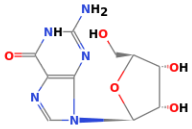
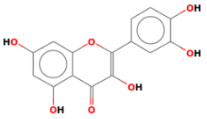
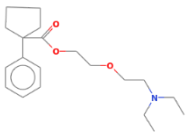
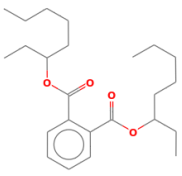


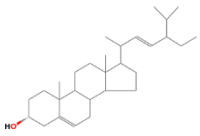
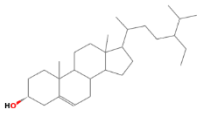
Figure 6: Gas Chromatography and Mass Spectrometry of Methanolic Leaf Extract

Table 4.8. Result of GC-MS of *Areca catechu* of Methanolic Leaf Extract

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp %wt	m/z	Structures
1	3.31	Glycerin	$C_3H_8O_3$	92	1.35	0.52	43, 61, 92	
2	5.57	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102	1.80	0.78	41, 73, 102	
3	6.50	N, N-Dimethylglycine	C_4H_9NO	103	2.70	1.00	41, 69, 103	
4	8.00	Benzene, (ethenyloxy)-	C_8H_8O	120	0.90	1.58	65, 77, 120	

5	9.50	Propane, 1-chloro-2-nitro-	$C_3H_6ClNO_2$	123	8.56	4.30	49, 77, 123	
6	10.51	D-Mannonic acid, γ-lactone	$C_6H_{10}O_6$	178	10.81	11.63	43, 73, 178	
7	12.18	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1.89	1.05	43, 101, 144	
8	13.50	Oxetane, 3-methyl-3-(1,1-dimethylethyl)	$C_8H_{16}O$	128	5.41	3.32	51, 77, 128	
9	15.94	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.26	2.95	43, 73, 200	
10	17.02	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	21.62	16.74	41, 67, 294	
11	18.00	Alpha-D-Galactopyranoside, methyl 2,3,4,6-tetra-O-methyl-	$C_{11}H_{22}O_6$	250	1.71	1.99	75, 88, 250	

12	19.56	5,8,11-Heptadecatrienoic acid, methyl ester	$C_{18}H_{30}O_2$	278	15.32	17.87	41, 67, 278	
13	20.00	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.51	6.13	43, 73, 256	
14	21.00	(-)-Spathulenol	$C_{15}H_{24}O$	220	2.25	3.41	41, 43, 220	
15	22.50	Guanosine	$C_{10}H_{13}N_5O_2$	235	2.79	3.40	43, 57, 235	
16	23.75	11-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	1.96	3.15	41, 55, 282	
17	26.00	Quercetin	$C_{15}H_{10}O_7$	302	5.68	6.43	51, 77, 302	
18	29.42	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.45	2.86	43, 57, 3123	
19	31.50	Carbetapentane	$C_{20}H_{31}NO_3$	333	1.58	2.31	86, 144, 333	
20	38.75	1,2-Benzenedicarboxylic acid bis-(1-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	2.48	2.72	43, 86, 390	

21	39.46	Stigmasterol	$C_{29}H_{48}O$	412	2.03	2.87	43, 66, 412	
22	40.50	β-Sitosterol	$C_{29}H_{50}O$	414	2.93	2.98	43, 107, 414	

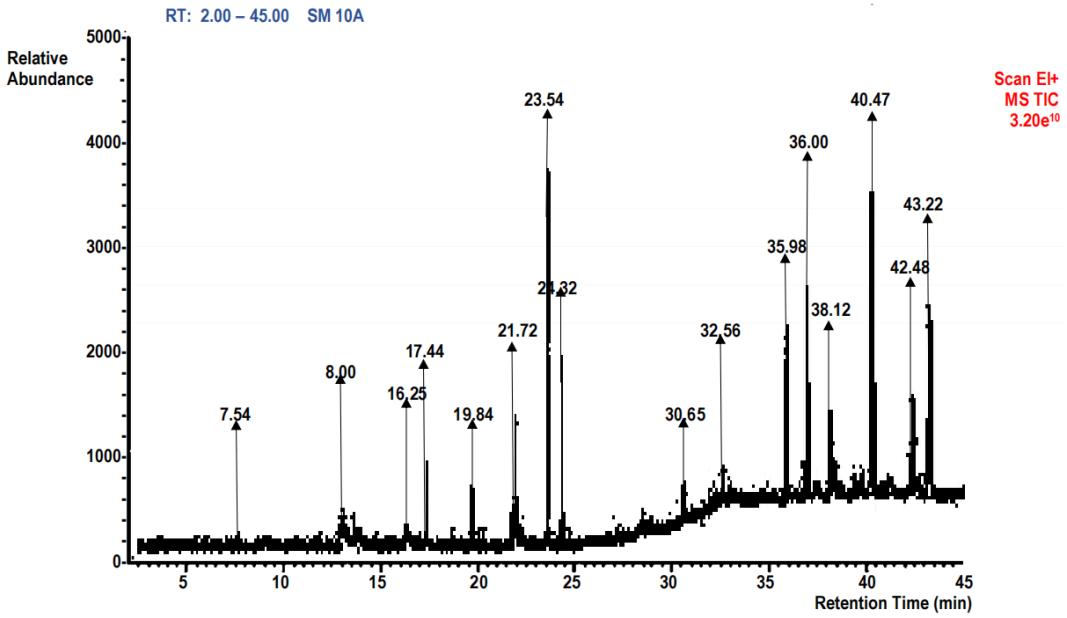
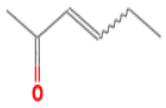
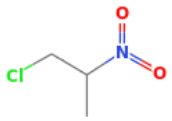
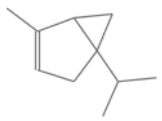
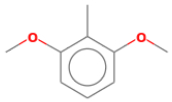
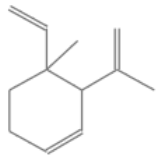
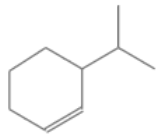
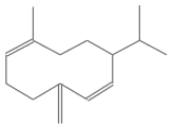
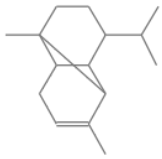
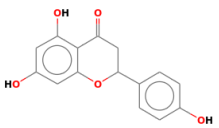
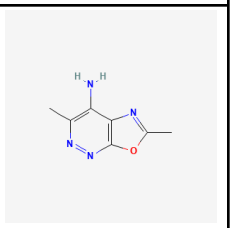
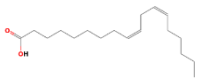
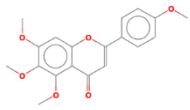
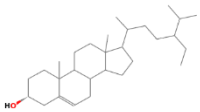
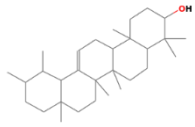


Figure 7: Gas Chromatography and Mass Spectrometry of Acetone Husk Extract

Table 4.9 Result of GC-MS of *Areca catechu* of Acetone Husk Extract

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp %wt	m/z	Structures
1	7.54	3-Hexen-2-one	$C_6H_{10}O$	98	0.80	1.75	43, 55, 98	
2	8.00	Propane, 1-chloro-2-nitro-	$C_3H_6ClNO_2$	123	1.60	2.98	49, 77, 123	
3	16.25	Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	$C_{10}H_{16}$	136	1.20	2.72	41, 93, 136	

4	17.44	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{16}$	136	3.21	4.02	41, 93, 136	
5	19.84	2,6-Dimethoxytoluene	$C_9H_{12}O_2$	152	2.38	3.04	77, 121, 152	
6	21.72	Geijerene	$C_{12}H_{18}$	162	6.61	7.60	51, 77, 162	
7	23.54	Cyclohexene, 3-(1-methylethyl)-	C_9H_{16}	124	14.22	17.42	41,81, 124	
8	24.32	Germacrene D	$C_{15}H_{24}$	204	8.01	6.07	41, 105, 204	
9	30.63	Copaene	$C_{15}H_{24}$	204	3.28	4.16	41, 93, 204	
10	32.56	(-)-Spathulenol	$C_{15}H_{12}O_5$	220	3.92	4.98	41, 67, 272	

11	35.98	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.77	5.90	43, 73, 256	
12	36.00	3,6-Dimethyloxazolo(5,4-c)pyridazin-4-ami ne	$C_7H_8N_4O$	164	10.40	11.40	42, 67, 164	
13	38.12	9,12- Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	5.68	5.08	41, 81, 280	
14	40.47	4H-1-Benzopyran-4-o ne, 5,6,7-trimethoxy-2-(4- methoxyphenyl)-	$C_{19}H_{18}O_6$	342	13.92	6.86	69, 167, 342	
15	42.48	Beta Sitosterol	$C_{29}H_{50}O$	414	6.39	4.73	43, 107, 414	
16	43.22	Alpha-Amyrin	$C_{30}H_{50}O$	426	9.61	10.29	55, 218, 426	

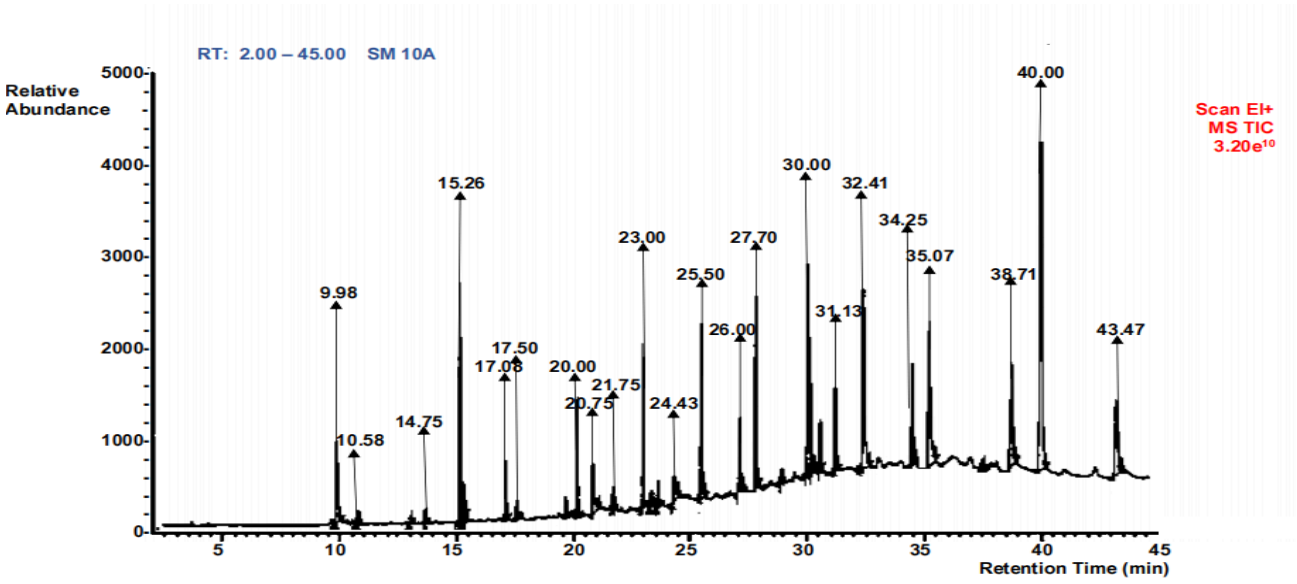
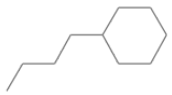
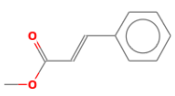
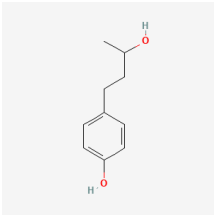
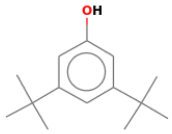
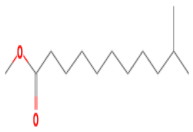
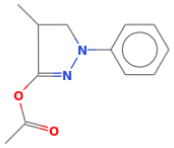
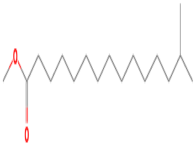
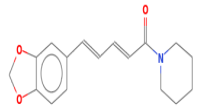
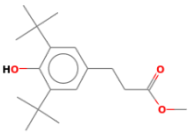
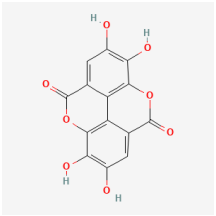
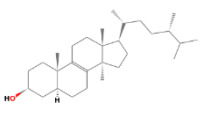


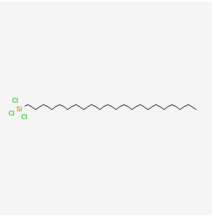
Figure 8: Gas Chromatography and Mass Spectrometry of Ethanolic Nut Extract

Table 4.10 Result of GC-MS of *Areca catechu* of Ethanolic Nut Extract

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp %wt	m/z	Structures
1	9.98	Cyclohexane, butyl-	$C_{10}H_{20}$	140	3.20	2.83	41, 83, 140	
2	10.58	Decane	$C_{10}H_{22}$	142	0.57	1.19	43, 57, 142	
3	14.75	2-Propenoic acid, 3-phenyl-, methyl ester, (E)-	$C_{10}H_{10}O_2$	162	0.63	1.21	51, 131, 162	

4	15.26	4-(3-Hydroxybutyl)phenol	$C_{10}H_{14}O_2$	166	5.36	4.32	51, 105, 166	
5	17.08	Dodecanoic acid	$C_{12}H_{24}O_2$	200	2.33	2.57	43, 73, 200	
6	17.50	Phenol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	0.87	1.74	41, 93, 204	
7	20.00	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	4.09	4.01	43, 73, 228	
8	20.75	Undecanoic acid, 10-methyl-, methyl ester	C13H26O2	214	1.46	2.73	43, 74, 214	
9	21.75	1-Phenyl-3-acetoxy-4-methyl-2-pyrazoline	$C_{12}H_{14}N_2O_2$	218	1.17	2.00	45, 75, 218	
10	23.00	Dodecyl acrylate	$C_{15}H_{28}O_2$	240	5.75	2.98	43, 55, 240	
11	24.43	Octadecane	$C_{18}H_{38}$	254	1.23	2.64	43, 57, 254	
12	25.50	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	5.57	3.17	43, 74, 270	

13	26.00	9-Eicosene, (E)-	$C_{20}H_{40}$	280	3.51	4.01	44, 85, 280	
14	27.70	1-Octadecene	$C_{18}H_{36}$	252	6.60	8.05	43, 55, 252	
15	30.00	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	8.48	7.31	41, 55, 282	
16	31.13	Piperine	$C_{17}H_{19}NO_3$	285	4.74	3.13	41, 115, 285	
17	32.41	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	292	7.84	8.64	43, 77, 292	
18	34.25	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.19	12.41	43, 73, 256	
19	35.07	Quercetin	$C_{15}H_{10}O_7$	302	6.43	7.90	51, 77, 302	
20	38.71	Eicosanoic acid	$C_{20}H_{40}O_2$	312	5.26	6.34	43, 73, 312	
21	40.00	Ergost-8-en-3-ol, 14-methyl-, (3β,5α)-	$C_{29}H_{50}O$	414	12.57	7.85	43, 399, 414	

22	43.47	Docosyltrichlorosilane	$C_{22}H_{45}Cl_3Si$	444	4.15	2.97	43, 57, 444	

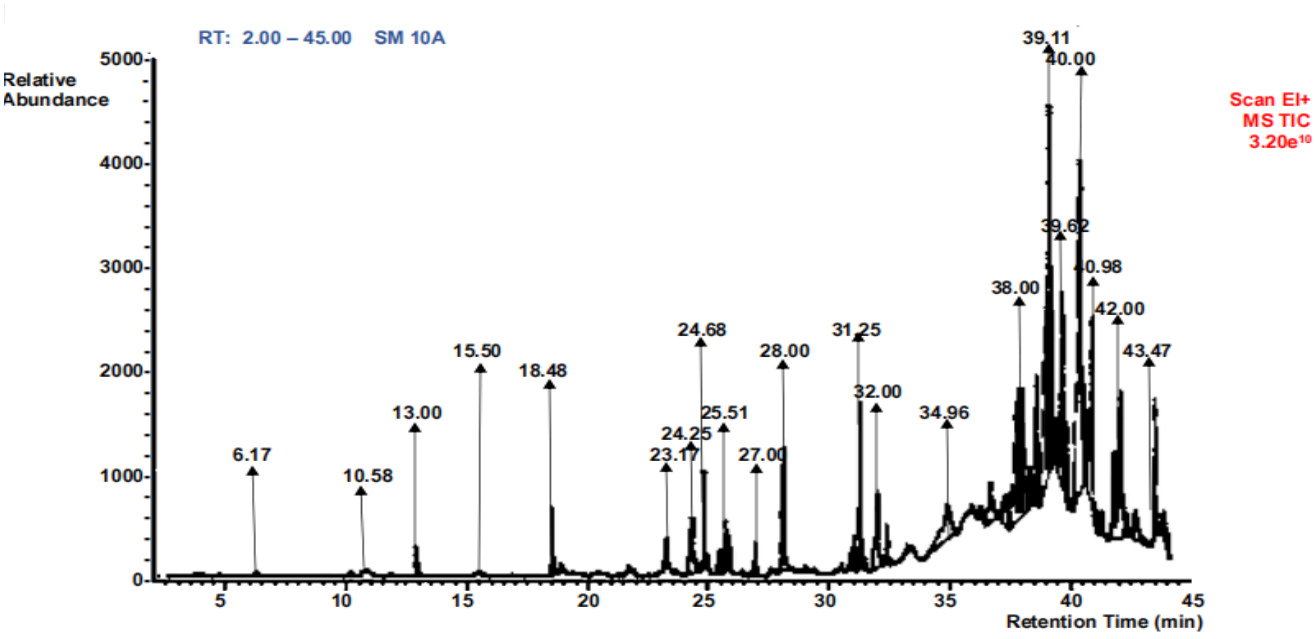
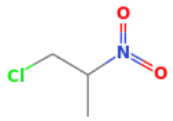
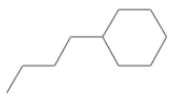
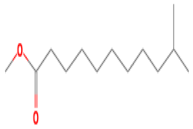
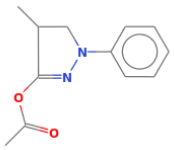
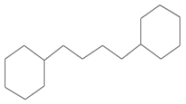
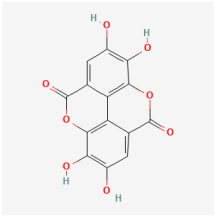
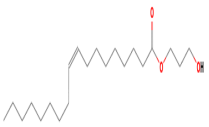
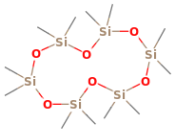


Figure 9: Gas Chromatography and Mass Spectrometry of Methanolic Nut Extract

Table 4.11 Result of GC-MS of Methanolic Nut Extract

Peak #	RT	Compound Detected	Mol. Formula	MW	Peak Area %	Comp %wt	m/z	Structures
1	6.17	Propane, 1-chloro-2-nitro-	$C_3H_6ClNO_2$	123	0.35	1.06	49, 77, 123	
2	10.58	Cyclohexane, butyl-	$C_{10}H_{20}$	140	0.45	1.21	41, 83, 140	
3	13.00	Cyclododecane	$C_{12}H_{24}$	168	1.05	1.67	41, 55, 168	

4	15.50	Dodecane	$C_{12}H_{26}$	170	0.41	0.99	43, 57, 170	
5	18.48	5-Tetradecene, (E)-acid	$C_{14}H_{28}$	196	2.07	3.07	43, 55, 196	
6	23.17	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.38	2.27	43, 73, 200	
7	24.25	Undecanoic acid, 10-methyl-, methyl ester	$C_{13}H_{26}O_2$	214	1.72	2.54	43, 74, 214	
8	24.68	1-Phenyl-3-acetoxy-4-methyl-2-pyrazoline	$C_{12}H_{14}N_2O_2$	218	3.45	3.98	45, 75, 218	
9	25.51	Cyclohexane, 1,1'-(1,4-butanediyl) bis-	$C_{16}H_{30}$	222	2.17	3.12	41, 83, 222	
10	27.00	Hexadecane	$C_{16}H_{34}$	226	1.21	2.73	43, 57, 226	
11	28.00	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	4.14	4.62	43, 73, 228	
12	31.25	1-Octadecene	$C_{18}H_{36}$	252	6.21	4.34	43, 55, 252	
13	32.00	Hexadecanoic acid, methyl ester,	$C_{17}H_{34}O_2$	270	2.41	3.00	43, 73, 256	

14	34.96	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	1.83	2.17	41, 55, 282	
15	38.00	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	6.31	6.21	43, 65, 282	
16	39.11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	15.86	16.10	43, 73, 256	
17	39.62	1-Docosene	$C_{22}H_{44}$	308	13.10	14.01	43, 57, 318	
18	40.00	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	14.14	12.14	43, 55, 296	
19	40.98	Quercetin	$C_{15}H_{10}O_7$	302	8.97	6.14	51, 77, 302	
20	42.00	Oleic acid, 3-hydroxypropyl ester	$C_{21}H_{40}O_3$	340	6.43	4.08	43, 65, 340	
21	43.47	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444	6.34	4.55	73, 341, 444	

CHAPTER FIVE

DISCUSSION

The findings on the identification and characterization of bioactive compounds from the leaf, husk, and nut of *Areca catechu* has provided valuable insights into its medicinal potential. *Areca catechu* plant collected from Thomas Adewumi University campus was identified and given identification number of UILH/001/1787/2024. The extraction of *Areca catechu* was done using acetone, ethanol, methanol, hot water, and cold water as solvents. 50g of the sample was macerated for extraction. The highest yield was achieved with methanol for the leaf (13.9%), hot water for the shaft (9.9%), and ethanol for the nut (11.5%). These findings reflect the solubility and efficiency of different solvents in extracting bioactive compounds from plant materials. Methanol, known for its polarity, was particularly effective, showing the highest extraction yield, which is consistent with previous studies that demonstrate methanol's superior extraction efficiency for phenolic compounds and flavonoids (Nguyen *et al*., 2020). Ethanol and hot water,

also polar solvents, performed well in extracting compounds from the shaft and nut. In comparison, acetone yielded lower percentages, corroborating research by Singh *et al*., 2018 that highlights acetone's lower efficiency in extracting certain phytochemicals from plant materials.

The phytochemical analysis revealed the presence of alkaloids, saponins, glycosides, and phenols in all samples, regardless of the solvent used. However, tannins were only found in the acetone extracts. Flavonoids, anthocyanins, betacyanins, and anthraquinones were absent across all solvents and plant parts. This phytochemical profile is crucial for understanding the medicinal properties of *Areca catechu*. Alkaloids, known for their antimicrobial and analgesic properties, were present in all samples, aligning with research by (Kumar *et al*., 2019), which found alkaloids to be abundant in *Areca* species. The absence of flavonoids, which are often abundant in plant materials, is notable and suggests that the extraction conditions or the specific plant parts may influence flavonoid availability. Previous studies on other species of *Areca* have identified flavonoids when different parts of the plant or varying extraction techniques were used (Jagtap *et al*., 2021), suggesting that this discrepancy may warrant further investigation.

The antimicrobial activity of the *Areca* catechu extracts was tested against *Candida albicans*, *Escherichia coli, Staphylococcus aureus,* and *Klebsiella* spp. The methanol extract (ACM) showed the most substantial antimicrobial effect, particularly against *Candida albicans* and *Escherichia coli*, with zones of inhibition up to 24mm.

The potency of the methanol extract, especially against *Candida albicans*, aligns with previous studies that underscore methanol's effectiveness in extracting compounds like phenols and alkaloids, known for their antimicrobial activities (Pandey *et al*., 2017). The acetone extract (ACA) exhibited moderate activity, particularly against *Staphylococcus aureus*, while the ethanol extract (ACE) was most effective against *Klebsiella* spp. Comparing these results with earlier studies, particularly those by Arora *et al*., 2019, reveals that *Areca catechu* possesses consistent antimicrobial potential. However, the specific inhibition zones differ across studies, likely due to differences in extraction techniques and the microorganisms tested. The absence of activity in the cold water extract against all tested microorganisms suggests that cold water may not efficiently extract antimicrobial agents (Singh *et al*., 2020),

The methanol leaf, nut and husk extracts exhibited MIC values ranging from 6.25 to 12.5 µg/ml against *Candida albicans*, with corresponding MFC values of 12.5 to 25 µg/ml. The MIC/MFC ratio (0.125 to 0.5) indicates fungicidal activity, aligning with the antimicrobial screening results. These findings are consistent with recent research by (Abou *et al.,* 2021), which also reported low MIC values for methanol extracts against fungal species, indicating potent antifungal properties. The relatively low MIC values suggest that *Areca* catechu has strong antifungal potential, likely due to the presence of phenolic compounds and alkaloids, which have been shown to disrupt fungal cell membranes (Liu *et al*., 2021). The antioxidant activity of *Areca catechu* extracts was measured using three assays: DPPH, FRAP, and hydrogen peroxide scavenging assays. The methanol extract (ACM) demonstrated the highest antioxidant activity in all three assays, particularly at higher concentrations (1000 µg/ml).

The DPPH assay showed percentage inhibition values ranging from 77.15% to 85.05%, indicating strong free radical scavenging activity. The FRAP assay further confirmed the extracts' reducing power, with activity percentages as high as 87.16%. Similarly, the hydrogen peroxide scavenging activity results were consistent, with inhibition values up to 88.29% at the highest concentration tested. These results are in line with previous studies on the antioxidant properties of *Areca catechu* (Lee *et al*., 2017). The presence of phenolic compounds, as revealed in the phytochemical screening, likely contributes to this strong antioxidant activity. Phenols are well-known antioxidants due to their ability to donate hydrogen atoms or electrons, thereby neutralizing free radicals (Halliwell *et al*., 2019).

GC-MS analysis of the methanol, acetone, and ethanol extracts identified several bioactive compounds, including glycerin and 2-hydroxy-gamma-butyrolactone, both of which are known for their therapeutic properties. Glycerin, commonly used for its moisturizing effects, has also been shown to have potential health benefits, including antimicrobial activities and antioxidant properties (Nath *et al*., 2020).

The identification of 2-hydroxy-gamma-butyrolactone is particularly significant, as this compound has been linked to various biological activities, including anticancer and antioxidant properties (Chen *et al*., 2022). The presence of these bioactive compounds suggests that *Areca catechu* has a diverse chemical profile with potential medicinal applications. The findings from the phytochemical screening, antimicrobial activity tests, antioxidant assays, and GC-MS

analysis collectively underscore the significance of *Areca catechu* as a source of bioactive compounds with diverse pharmacological activities (Hung *et al*., 2020; Gupta *et al*., 2020; Sari *et al*., 2020).

5.1 CONCLUSION

This research has successfully identified and characterized several bioactive compounds from *Areca catechu*, demonstrating their promising antimicrobial and antioxidant properties. These findings support the traditional medicinal uses of *Areca catechu* and highlight its potential for further research and development in the pharmaceutical and nutraceutical industries. The significant antimicrobial activity, particularly of the methanol extract, underscores the potential

of *Areca catechu* as a source of natural antimicrobial agents. Additionally, the antioxidant capacity of the extracts highlights their relevance in health promotion and disease prevention.

This study contributes to the growing body of evidence supporting the medicinal value of *Areca catechu* and encourages further exploration of its bioactive compounds for potential therapeutic applications. The present study provides preliminary evidence for the antimicrobial potential of *Areca catechu* extracts. Further investigations are warranted to fully explore the therapeutic potential and focus on isolating and elucidating the mechanisms of action of these compounds, as well as exploring their efficacy in clinical settings. *Areca catechu* represents a valuable resource for the development of novel pharmaceuticals and nutraceuticals, bridging traditional knowledge with modern scientific understanding.

5.2 RECOMMENDATIONS

Based on these findings on the identification and characterization of bioactive compounds from *Areca catechu*, given the promising antimicrobial and antioxidant properties in this study, it is recommended to initiate clinical trials to assess the effectiveness of *Areca catechu* extracts in treating specific infections and oxidative stress-related conditions in humans.

Future studies should focus on isolating the specific compounds responsible for the bioactivities recorded in this work, elucidating their mechanisms of action, and exploring their efficacy in clinical settings.

Enlightening the local communities on the medicinal properties of *Areca catechu* is also important, as this plant is found in TAU environment without exploring the medicinal potential.

Overall, *Areca catechu* represents a significant resource for bridging traditional knowledge with modern scientific understanding, paving the way for novel therapeutic applications and exploring the development of drugs from *Areca catechu* extracts.

5.3 Contribution to Knowledge

This study contributes to knowledge by demonstrating the extraction efficiency of different solvents on *Areca catechu*, with methanol yielding the highest extract quantities, especially from the leaf. It also highlights the plant's rich phytochemical profile, containing bioactive compounds such as alkaloids, saponins, glycosides, and phenols, which are significant for their therapeutic potential. Additionally, the research provides valuable insights into the plant's antimicrobial and antioxidant activities, identifying *Areca catechu* as a promising source of natural bioactive agents. The GC-MS analysis further characterized key bioactive compounds, enhancing understanding of the species on TAU campus.

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