PHYTOCHEMICAL AND ANTIOXIDANT PROFILE OF *MONODORA MYRISTICA* SEED EXTRACT

BY

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A PROJECT WRITTEN IN THE DEPARTMENT OF MEDICAL BIOCHEMISTRY AND SUBMITTED TO THE SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

NOVEMBER, 2025

CERTIFICATION

We the underlisted certify that this project titled "**PHYTOCHEMICAL AND ANTIOXIDANT PROFILE OF *MONODORA MYRISTICA* (NUTMEG) SEED EXTRACT"** was carried out by **CHRISTOPHER TRIUMPH OSADEBAMEN (BMS2101383)** in the Department of Medical Biochemistry,School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city, Edo State, Nigeria.

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DEDICATION

This work is humbly dedicated to God Almighty, whose unfailing grace, wisdom, and strength sustained me throughout my journey at the University of Benin. His divine guidance has been my constant light and foundation. With deep love and gratitude, I dedicate this achievement to my beloved mother, Mrs. Favour Omonigho O. Christopher, whose endless sacrifices, prayers, and unwavering support have shaped every step of my academic journey. To my wonderful siblings, Christopher Wonder and Christopher Honour, your love, encouragement, and faith in me have been a continual source of strength and inspiration. I also extend my heartfelt appreciation to my dear friends, colleagues, and everyone who supported and believed in me along the way. Your kindness, motivation, and companionship made this accomplishment possible.

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ABSTRACT

Monodora myristica (calabash nutmeg) is a widely used West African spice with traditional medicinal and preservative applications. This study presents a deep evaluation of its phytochemical composition and antioxidant potential. Quantitative phytochemical analysis revealed that flavonoids were strongly present (Mean ± SEM: 0.8216 ± 0.00072), indicating a high concentration likely responsible for the extract's potent free-radical scavenging activity. Terpenoids (0.2091 ± 0.2183) and cardiac glycosides (0.5091 ± 0.5136) were moderately detected, suggesting contributions to the spice's aromatic, anti-inflammatory, antimicrobial, and cardiotonic properties. Alkaloids (0.2725 ± 0.2734) and steroids (0.1940 ± 0.2560) were present at low to moderate levels, supporting traditional analgesic, antimicrobial, anti-inflammatory, and membrane-stabilizing activities. Antioxidant activity was evaluated using a multi-assay approach, including DPPH, hydrogen peroxide (H_2O_2) scavenging, ferric reducing antioxidant power (FRAP), nitric oxide (NO) scavenging, total antioxidant capacity (TAC), and reducing power (RP), with ascorbic acid as the standard. The extract demonstrated strong, concentration-dependent DPPH scavenging (21.41–87.27%) and progressively enhanced reducing power (76.05–88.12%), reflecting effective hydrogen donation and electron-transfer capacity. NO scavenging was highest at low concentrations (85.45%) but declined at higher doses, while TAC showed moderate and consistent activity (82.99–86.93%). H_2O_2 scavenging remained relatively low (15.35–17.75%), and FRAP displayed a decreasing trend with increasing concentration (82.49% → 25.03%), likely due to matrix interactions affecting iron reduction. Across all assays, ascorbic acid maintained high and stable antioxidant performance (>95%). These findings suggest that *Monodora myristica* is rich in bioactive phytochemicals with significant antioxidant potential, capable of neutralizing free radicals and supporting cellular redox balance. This study also reinforces the spice's traditional medicinal relevance and highlights its promise as a natural source of antioxidants for nutraceutical, therapeutic, and food preservation applications.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF STUDY

Monodora myristica, commonly known as calabash nutmeg or African nutmeg, is a remarkable tropical plant belonging to the family *Annonaceae*. It is indigenous to the dense rainforests of West and Central Africa, where it has been cultivated and utilized for centuries both as a spice and as a potent medicinal plant. The tree bears large, fragrant fruits that encase aromatic seeds, which, when dried and ground, produce a characteristic nutmeg-like flavour. These seeds are widely employed in indigenous culinary practices across Nigeria, Ghana, and Cameroon to flavour soups, stews, porridges, and traditional cakes, lending both taste and aroma to local dishes (Samuel *et al*., 2024).

Beyond its culinary importance, *M. myristica* holds an esteemed position in African ethnomedicine. In many local communities, the seeds, bark, and fruit are revered for their healing and restorative properties. Traditional healers employ various parts of the plant to treat a wide range of ailments including headaches, stomach aches, fevers, hypertension, diarrhoea, and skin infections. The seeds are also used as stimulants, carminatives, and insect repellents, while decoctions from the bark are administered for haemorrhoids, eye infections, and other inflammatory conditions (Osukoya *et al*., 2021). In some cultures, *M. myristica* is even believed to possess aphrodisiac and rejuvenating properties, and its powdered seeds are incorporated into tonics and herbal formulations for general wellness.

The wide ethnobotanical applications of *M. myristica* underscore its deep cultural and medicinal significance. Yet, beyond its folklore and traditional reputation, there is a growing scientific recognition that this plant may possess real pharmacologic potential. The intersection between its historical use and emerging biochemical findings strongly suggests that *M. myristica* is not merely a flavouring spice but a plant of genuine therapeutic and nutritional promise (Aikpitanyi *et al*., 2025). This dual identity as both a food and medicine makes it an ideal candidate for contemporary scientific exploration, especially in the era of functional foods and natural product research.

In recent years, global interest in plant-derived antioxidants has intensified, driven by the increasing understanding that oxidative stress plays a central role in the development of chronic diseases such as diabetes, hypertension, cancer, and cardiovascular disorders. Plants rich in phenolic compounds, flavonoids, and other secondary metabolites have been identified as powerful natural agents capable of scavenging harmful free radicals and protecting biological systems from oxidative damage. Against this background, *Monodora myristica* stands out as a promising indigenous source of such bioactive compounds (Samuel et al., 2024; Igwe et al., 2025).

Several studies have revealed that *M. myristica* seeds contain a diverse array of phytochemicals—including phenolics, alkaloids, saponins, terpenoids, tannins, glycosides, and steroids—each contributing in varying degrees to its biological activity (Osukoya *et al.,* 2021). Quantitative analyses have confirmed substantial amounts of total phenolic and flavonoid contents, which are directly correlated with its antioxidant properties. These compounds function by neutralizing reactive oxygen species (ROS), chelating metal ions, and enhancing endogenous antioxidant defences. In this way, they mitigate cellular damage and promote overall physiological balance.

For instance, Osukoya *et al.* (2021) reported that seed protein hydrolysates of *M. myristica* demonstrated remarkable antioxidant efficiency, exhibiting high DPPH radical-scavenging and Fe^{2+}-chelating activities in vitro. Similarly, Igwe *et al.* (2025) conducted GC/MS analysis and identified a rich composition of chemical constituents, many of which possess well-documented antioxidant and antimicrobial properties. Their findings confirmed that extracts of *M. myristica* exhibit potent free-radical scavenging activity comparable to standard antioxidants. In another comprehensive review, Aikpitanyi *et al.* (2025) highlighted the relevance of African spices like *M. myristica* in preventing oxidative stress–related diseases and emphasized the urgent need for systematic characterization of their phytochemical profiles.

These findings collectively provide a compelling scientific rationale for further exploration of the antioxidant and phytochemical properties of *Monodora myristica*. Despite the promising reports, significant gaps remain in understanding how the plant's biochemical composition relates to its pharmacological activities and whether specific compounds can be isolated or standardized for therapeutic use. Moreover, environmental factors, extraction methods, and solvent types may all

influence the yield and activity of its bioactive constituents, warranting a more rigorous, comparative investigation (Samuel *et al.,* 2024).

Therefore, this study is conceived with several key motivations. First, it seeks to validate traditional knowledge through modern scientific analysis, transforming anecdotal herbal claims into measurable biochemical evidence. Second, it aims to assess the antioxidant capacity of *M. myristica* in relation to established markers of oxidative stress. Third, the study aspires to generate foundational data that could support the development of functional foods, nutraceuticals, or herbal formulations derived from this underutilized spice. Finally, it seeks to contribute to local resource valorization, aligning with Nigeria's broader goals of promoting indigenous biodiversity, sustainable agriculture, and health innovation.

As noted by Samuel *et al.* (2024), indigenous plants like *M. myristica* represent a largely untapped frontier in the search for safe, affordable, and natural alternatives to synthetic antioxidants. In an age where synthetic additives raise increasing safety concerns, exploring and harnessing the natural antioxidant potential of *M. myristica* is both scientifically relevant and socially beneficial. In sum, this research is driven by a convergence of ethnobotanical tradition, biochemical potential, and the modern demand for sustainable, health-promoting plant resources.

1.1 Justification of the Study

Monodora myristica, commonly known as calabash nutmeg, is a traditional spice widely used in West and Central Africa for both culinary and medicinal purposes. Despite its long history of ethnomedicinal applications, including treatment of hypertension, stomach disorders, inflammation, and microbial infections, there is still limited scientific validation of its antioxidant and phytochemical properties.

Oxidative stress is implicated in the pathogenesis of many chronic diseases such as diabetes, cancer, and cardiovascular disorders. Therefore, identifying natural sources of antioxidants is critical in developing preventive and therapeutic strategies. *M. myristica* has been reported to contain diverse bioactive compounds like flavonoids, tannins, terpenoids, and phenolic acids that possess potential free-radical scavenging activity. However, the quantitative and qualitative

assessment of these constituents in locally sourced samples remains inadequate, especially under varying extraction and solvent conditions.

Furthermore, as Aikpitanyi *et al*. (2025) noted, increased consumption of synthetic antioxidants such as BHT and BHA has raised safety concerns, creating the need for safer natural alternatives derived from indigenous plants. The justification for this study, therefore, lies in the need to scientifically validate and characterize the antioxidant potential and phytochemical composition of *M. myristica* seeds to support their safe inclusion in nutraceutical, pharmaceutical, and food formulations. Such work would also add value to a readily available local plant resource, promoting both public health and indigenous economic growth.

1.2 Aim of the Study

The primary aim of this study is to evaluate the antioxidant activity and phytochemical composition of *Monodora myristica* (calabash nutmeg) seed extracts, in order to scientifically validate its traditional medicinal and nutritional uses.

1.3 Objectives of the Study

The specific objectives of the study are

1. Conduct qualitative and quantitative phytochemical screening of *M. myristica* seed extracts to determine the presence and concentration of bioactive compounds such as phenolics, flavonoids, alkaloids, tannins, terpenoids, and saponins.

2. Evaluate the in-vitro antioxidant activity of *M. myristica* seed extracts using standard assays (e.g., DPPH radical-scavenging, ferric reducing antioxidant power).

3. Correlate the antioxidant activity with the phytochemical composition, to determine which bioactive constituents may contribute most to antioxidant potential.

4. Compare the antioxidant efficacy of different solvent extracts (if applicable) to identify the most efficient extraction method.

5. Provide scientific evidence supporting the traditional use of *M. myristica* in disease prevention and health maintenance.

CHAPTER TWO

LITERATURE REVIEW

2.0 OVERVIEW OF MONODORA MYRISTICA

Plants, through millennia of evolution, have developed a rich arsenal of secondary metabolites; phenolics, flavonoids, alkaloids, terpenoids, saponins, and others many of which possess strong antioxidant properties. According to Dewage *et al.* (2022), plant-based antioxidants (e.g., phenolics, carotenoids, vitamins) are receiving increased attention because they help neutralise free radicals, prevent lipid and protein oxidation, and thus may reduce the risk of inflammation, cancer and other oxidative-stress-mediated conditions.

Figure 1: *Monodora Myristica (*reference: Wikipedia)

Such natural antioxidants present several advantages: they are perceived as safer and more "clean-label" compared to synthetic additives; they often carry multiple bioactivities (antioxidant, anti-inflammatory, antimicrobial) and are derived from renewable plant resources. The modern trend away from synthetic preservatives and toward "functional foods" and nutraceuticals underscores the importance of plant-based antioxidants (Petcu *et al*., 2023).

Beyond food preservation, the role of these compounds in human health is significant. The capacity to scavenge reactive oxygen species (ROS), chelate metal ions, modulate endogenous antioxidant enzyme systems and thereby prevent oxidative damage to biomolecules positions plant-derived antioxidants as best-in-class candidates for disease prevention. As such, the study of natural products remains a foundational pillar in pharmacognosy, nutrition science and the broader field of integrative medicine.

The global healthcare landscape is witnessing a marked resurgence in the use of medicinal plants not only in traditional and complementary settings but increasingly within mainstream biomedical research and functional-food development. Scholars and practitioners alike recognise that many communities, especially in developing regions, rely on medicinal plants for primary health care; at the same time, the pharmaceutical industry continues to derive drugs from plant sources. For example, medicinal-plant reviews emphasise that a substantial fraction of the world's medicinal-plant species possess antioxidant and other protective capacities that are yet to be fully validated or exploited (e.g., a review noted that about two-thirds of medicinal plant species may have significant antioxidant potential).

This trend is driven by several converging factors: the increasing global burden of non-communicable diseases (NCDs) with oxidative-stress pathogenesis; growing consumer demand for "natural" remedies; concerns about safety and side-effects of synthetic drugs or food additives; and the realisation that many under-utilised plant resources hold high value for health promotion. Indeed, researchers like Aikpitanyi *et al.* (2025) highlight that indigenous African spices and medicinal plants hold untapped promise for functional-food and nutraceutical innovations.

As a result, the interface between ethnobotany, phytochemistry, pharmacology and nutrition is more active than ever. The shift is from "folk remedy" to "evidence-based functional ingredient".

Relevance of *Monodora myristica* in Traditional and Modern Health Systems

Within this broader context, *Monodora myristica* (often referred to as calabash nutmeg or African nutmeg) is a highly relevant case. Native to West and Central African forests, this member of the Annonaceae family has long held dual roles: as a flavouring spice and as a medicinal plant. Its aromatic seeds, bark and fruit have been employed in indigenous cuisines and ethnomedicine for conditions ranging from stomach-aches and fevers to hypertension and skin afflictions (Osukoya *et al.,* 2021).

In modern research terms, *M. myristica* is increasingly recognised for its rich phytochemical profile documented to include phenolic compounds, flavonoids, saponins, terpenoids, alkaloids among others (Igwe *et al.,* 2025) and for demonstrable antioxidant and antimicrobial activities (Samuel *et al.,* 2024). The convergence of its traditional use and emerging phytochemical/antioxidant evidence makes it a prime candidate for deeper scientific inquiry: validating folk wisdom, quantifying bioactivity, and exploring functional-food or nutraceutical applications.

Moreover, the focus on a locally abundant African spice aligns with regional priorities: leveraging indigenous biodiversity, promoting value-addition, supporting agro-economies, and responding to public-health needs in African contexts. As such, *M. myristica* bridges culture, cuisine, medicine and modern phytochemical science and once more justifies my study on it.

2.1 Botanical Description and Distribution of *Monodora myristica*

Taxonomic Classification

Monodora myristica (commonly known as African nutmeg, calabash nutmeg, or ehuru in Nigeria) is a tropical tree species belonging to the family Annonaceae and genus Monodora. Its full scientific classification is as follows:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Magnoliales

Family: Annonaceae

Genus: Monodora

Species: Monodora myristica (Gaertn.) Dunal

The Annonaceae family, also known as the custard apple family, contains several aromatic and medicinally important plants distributed across tropical and subtropical regions. *M. myristica* shares similarities with other Monodora species but is particularly distinguished by its large, fragrant flowers and nutmeg-like seeds, which are valued both as a spice and a traditional remedy (Okpoghono *et al.,* 2023).

Morphological Features

Figure 2: *MONODORA MYRISTICA* flowers(Source: iNaturalist.com)

M. myristica is a medium-sized, evergreen forest tree that can grow up to 25–35 m in height and 2 m in girth. The trunk is straight with smooth to slightly scaly bark, exuding a pleasant aromatic scent when cut. The leaves are simple, alternate, oblong to elliptic, and glossy green, typically measuring 10–25 cm long and 4–10 cm wide, with an entire margin and a pointed apex.

The flowers are solitary, large, and pendulous, arising from leaf axils on long stalks. They are highly distinctive, with greenish-yellow petals marbled or streaked with purple or reddish-brown, giving the tree a striking appearance when in bloom. The flowers are also strongly scented and attract pollinators such as beetles and bees (Daniel-Afolabi *et al.,* 2024).

The fruit is a large, woody, globular capsule (approximately 10–15 cm in diameter), resembling a small calabash hence the name "calabash nutmeg." When mature, the fruit splits open to release numerous brown to dark-brown seeds embedded in a fragrant, mucilaginous pulp. The seeds are hard, ovoid, and aromatic, possessing a flavor and scent reminiscent of true nutmeg (Myristica fragrans), which accounts for the common name. The seeds are the most economically and medicinally important part of the plant, widely used as a spice, condiment, and therapeutic agent (Okpoghono *et al.,* 2023).

Geographic Distribution

M. myristica is indigenous to West and Central Africa, where it thrives in tropical rainforests and humid lowland environments. Its natural range extends from Nigeria, Cameroon, and Ghana to Equatorial Guinea, Angola, and the Democratic Republic of Congo. In Nigeria, it is commonly found in the southern and middle belt regions, particularly in states such as Edo, Delta, Rivers, Cross River, and Enugu (Daniel-Afolabi *et al.,* 2024).

The species typically grows in moist, fertile, well-drained forest soils and prefers semi-shaded environments under the canopy of taller trees. It can also be found in secondary forests and sometimes in home gardens, where it is cultivated for its seeds. The widespread availability of M. myristica across tropical Africa underscores its ecological adaptability and socio-economic importance.

Ecological and Agricultural Significance

Ecologically, *M. myristica* contributes to the stability and regeneration of tropical forest ecosystems. Its large canopy provides shade and habitat for wildlife, while its fruits serve as food for birds and small mammals, aiding in seed dispersal. The species plays a role in maintaining soil fertility and moisture due to its leaf litter and deep root system, which prevents erosion.

Agriculturally, *M. myristica* holds high potential as both a cash crop and agroforestry species. The seeds are traded in local markets as a culinary spice and medicinal ingredient, making it a valuable non-timber forest product (NTFP). Okpoghono *et al.* (2023) noted that the plant's adaptability to diverse forest conditions makes it suitable for domestication and sustainable cultivation, potentially contributing to rural livelihoods and biodiversity conservation. Additionally, its by-products; bark, leaves, and essential oils are being explored for pharmaceutical and cosmetic uses, enhancing its agricultural and industrial prospects.

Historical and Ethnobotanical Background

Historically, *Monodora myristica* has been intertwined with the culinary, spiritual, and medicinal traditions of many West African cultures. Among the Igbo and Yoruba people of Nigeria, the seeds are used to flavor soups, stews, and porridges, providing a warm, nutmeg-like aroma. In traditional medicine, the seeds, bark, and leaves are used to treat headaches, hypertension, stomach disorders, fevers, and rheumatism, among other ailments (Daniel-Afolabi *et al.,* 2024).

The plant also holds ritual and cultural significance; its aromatic seeds are sometimes burned as incense during ceremonies, while powdered seeds are incorporated into herbal mixtures for protection and vitality. Beyond local uses, early European explorers documented *M. myristica* as a spice of trade interest, describing it as "the African nutmeg," highlighting its economic relevance in precolonial and colonial commerce.

Ethnobotanical records affirm that *M. myristica* has remained a staple in both traditional health systems and household diets across West Africa, symbolizing the enduring relationship between people and their natural plant resources. Today, the growing scientific validation of its bioactive

and antioxidant properties continues to bridge the gap between traditional knowledge and modern phytomedicine (Okpoghono *et al.,* 2023; Daniel-Afolabi *et al.,* 2024).

2.2 Phytochemical Constituents of *Monodora myristica*

2.2.1 Overview of Primary and Secondary Metabolites

The seeds, bark and fruit of *Monodora myristica* harbour a rich spectrum of both primary and secondary metabolites, which collectively underlie the spice's nutritional value and medicinal potential. Primary metabolites such as carbohydrates, lipids, proteins and fatty acids provide the bulk nutritional matrix; but it is the secondary metabolites; phenolics, flavonoids, terpenoids, alkaloids, saponins, tannins, glycosides and steroids that largely account for the pharmacological and antioxidant activities observed. For example, early qualitative analyses reported the presence of flavonoids, terpenoids, saponins and tannins in the seeds.

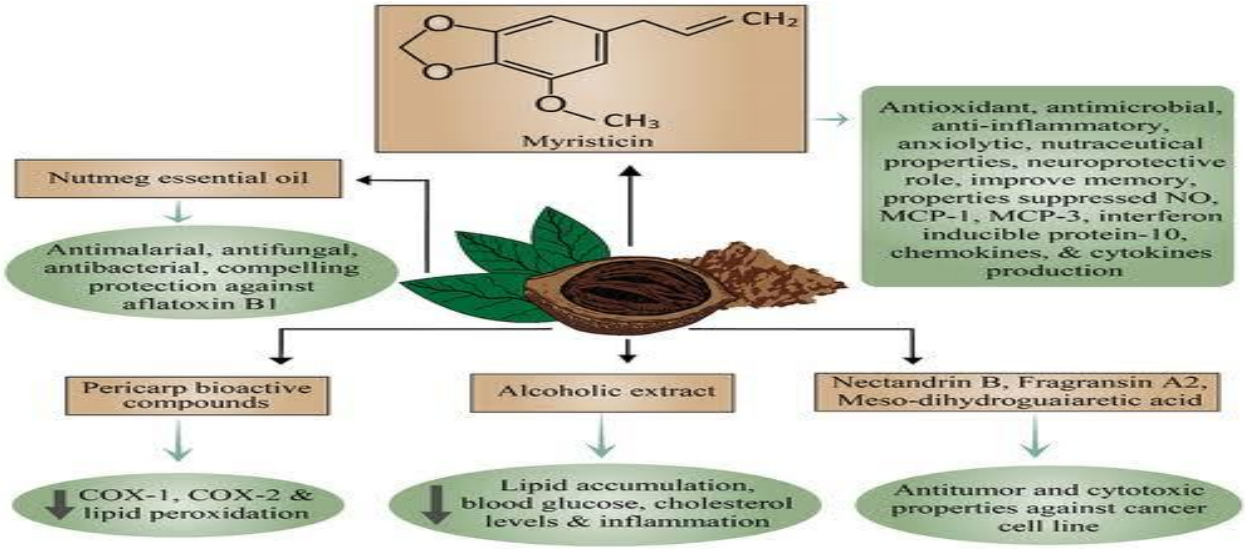

Figure 3: Health Benefits of Monodora Myristica(Source: Wikipedia)

More recently, GC–MS and HPLC-based investigations (e.g., Igwe *et al.,* 2025) have begun to map specific compounds and their relative abundances, thus moving the understanding beyond mere presence/absence to quantitative and structural detail. The work of Samuel *et al.* (2024)

emphasises that to validate the ethnomedicinal uses of *M. myristica* we must systematically profile these bioactives and link them to function — hence the impetus of the present study.

2.2.2 Key Classes of Compounds

Phenolics and flavonoids. Phenolic compounds (including simple phenols, phenolic acids, flavonoids and flavan-3-ols) are widely recognised for their antioxidant, metal-chelating and free-radical-scavenging abilities. In *M. myristica,* quantitative screening has shown measurable amounts of total phenolics and flavonoids in various extracts (e.g., the HPLC screening of seed extracts identified catechin and gallocatechin among the flavonoids) . These compounds contribute to the seed's demonstrated DPPH-scavenging and reducing power activities (Osukoya *et al.,* 2021).

- Alkaloids, saponins, terpenoids, tannins, glycosides, steroids. Beyond phenolics, M. myristica seeds and other parts contain a broad array of secondary metabolites:
- Alkaloids have been reported (albeit in lower quantities) and may contribute to bio-activity through enzyme modulation and receptor interactions.
- Saponins, known for their surfactant properties and ability to modulate cholesterol/lipid pathways, have been found in moderate amounts
- Terpenoids (including monoterpenes and sesquiterpenes) are especially prominent in M. myristica essential oils — compounds such as α-phellandrene, α-pinene, limonene, β-myrcene have been repeatedly detected.
- Tannins (both hydrolysable and condensed) appear in seed extracts; they may impart astringent, antimicrobial and antioxidant properties.
- Glycosides (including cardiac glycosides) have been identified qualitatively in some screening studies.

- Steroids and sterols: Qualitative studies show a high relative concentration of steroids in some seed extracts (for instance, a study reported steroids as “high amount” relative to others)

2.2.3 Summary of Past Phytochemical Screening Results

Multiple investigations have reported both qualitative and quantitative assessments of the phytochemicals in *M. myristica*. For instance, one proximate + phytochemical study found steroids at ~32.75 % (high amount), saponins 12.04 % and terpenoids 19.00 % in seed extracts, with alkaloids and phenol in relatively low amounts.

GC–MS work by Igwe *et al*. (2025) identified 12 compounds in seed extracts: saturated fatty acids (38.897 %), terpenes/terpenoids (29.133 %), salicyclohydrazine (19.626 %), carboxylic acid (8.124 %), flavonoid (2.352 %) and phenolic compound (0.700 %). The two most prominent compounds were n-hexadecanoic acid and p-cymene (each ~24.831 %).

Such data illustrate the complex metabolic makeup of the seed and highlight that multiple compound classes likely act synergistically to yield the observed bioactivities.

2.2.4 Influence of Extraction Method and Solvent Type on Yield and Composition

The solvent and extraction method significantly influence the yield and profile of phytochemicals. Polar solvents (e.g., methanol, ethanol, water) often extract higher amounts of phenolic and flavonoid compounds whereas non-polar solvents (e.g., hexane, petroleum ether) recover fatty acids, terpenoids and lipophilic compounds. For instance, HPLC screening of methanol extract of *M. myristica* reported 2.49 ± 0.77 mg GAE/g extract of phenol and 0.95 ± 0.34 mg QE/g extract of flavonoids.

In GC–MS studies Igwe *et al.* 2025), use of seed extracts prepared with appropriate solvents enabled detection of fatty acids and terpenoids in high relative percentage. These extraction differences point to the need for proper method selection depending on targeted phytochemical classes. Furthermore, Samuel *et al.* (2024) emphasise that standardisation of extraction

conditions is critical in obtaining reproducible phytochemical profiles and linking them meaningfully to bioactivity.

2.5 Notable Research Findings – Linking Phytochemistry to Antioxidant Activity

Several investigations have directly linked the presence of key phytochemical classes in *M. myristica* with antioxidant potential. For instance. A study using DPPH and ferric-reducing antioxidant power assays demonstrated that seed extracts of M. myristica achieved ~72.51 % to ~96.22 % inhibition at concentrations from 25 to 400 µg/mL, values comparable with ascorbic acid standard. (Igwe *et al.,* 2025) Other antioxidant assays (e.g., lipid peroxidation inhibition) showed that methanol extract of M. myristica significantly attenuated oxidative damage in vitro. The essential oil and oleoresin studies of M. myristica reported significant DPPH-scavenging, reducing power and total antioxidant capacity in a concentration-dependent manner. Samuel *et al*. (2024) and Aikpitanyi *et al*. (2025) highlight that the phytochemical diversity of M. myristica especially its terpenoid and phenolic contents positions it as a promising candidate for functional food development, nutraceutical formulation and oxidative-stress mitigation.

Taken together, the phytochemical evidence underscores that *M. myristica* is a reservoir of diverse bioactive compounds especially phenolics, flavonoids and terpenoid/terpene constituents and that its extraction and analytical characterisation have matured in recent years (Samuel *et al.,* 2024; Igwe *et al.,* 2025). The identified compounds and tested antioxidant activities validate many of the traditional uses of the seed (e.g., for treating inflammatory conditions, oxidative stress) and support its potential as a functional food ingredient and nutraceutical source.

2.3 Antioxidant Properties of *Monodora myristica*

Antioxidants are bioactive molecules capable of neutralizing free radicals and reactive oxygen species (ROS), which are harmful byproducts of normal metabolic processes in living organisms. Excess ROS generation causes oxidative stress, leading to cellular damage, aging, and the onset

of chronic diseases such as cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions (Akinyede *et al.,* 2021). Hence, antioxidants play a pivotal role in maintaining redox homeostasis and protecting biological systems from oxidative deterioration.

2.3.1 Mechanisms of Antioxidant Action

The antioxidant mechanism of *Monodora myristica* involves multiple pathways, including:

1. Free-radical scavenging activity, where compounds such as flavonoids and phenolics donate hydrogen atoms or electrons to neutralize radicals like DPPH, hydroxyl, and superoxide radicals.

2. Metal chelation, which prevents transition metals such as Fe^{2+} and Cu^{2+} from catalyzing radical generation via the Fenton reaction.

3. Redox balancing, through enzymatic and non-enzymatic mechanisms that restore the equilibrium between oxidants and antioxidants in biological systems (Okechukwu *et al.,* 2022).

2.3.2 Reported Antioxidant Assays in Literature

Several in-vitro assays have been used to evaluate the antioxidant potential of *M. myristica* extracts, including:

DPPH radical scavenging activity, which measures hydrogen-donating ability. Methanolic and ethanolic extracts of M. myristica seeds have demonstrated high DPPH radical inhibition in a dose-dependent manner (Osukoya *et al.,* 2021).

Ferric reducing antioxidant power (FRAP) assay, indicating the capacity of the extract to reduce Fe^{3+} to Fe^{2+}. The high FRAP values observed suggest a strong reducing capacity attributed to polyphenolic compounds (Igwe *et al.,* 2025).

Hydrogen peroxide and hydroxyl radical scavenging assays, which reflect the ability of phytochemicals to quench reactive oxygen species directly.

Lipid peroxidation inhibition, where extracts of *M. myristica* significantly prevented malondialdehyde formation in tissue homogenates, indicating a protective effect on membrane lipids (Aikpitanyi *et al.,* 2025).

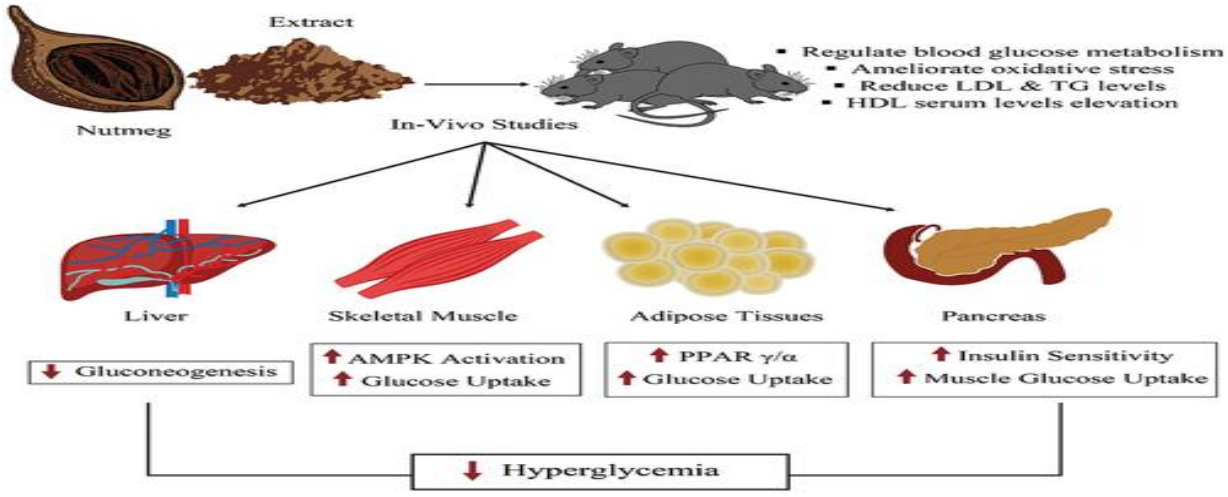

Figure 4: Effects of Extracts For Monodora Myristica reference(TopTropicals.com)

2.3.3 Comparative Antioxidant Potency

When compared with other spices such as Syzygium aromaticum (clove) and Zingiber officinale (ginger), *Monodora myristica* demonstrates comparable or superior antioxidant capacity, particularly in DPPH and FRAP assays (Igwe *et al.,* 2025). This enhanced activity is likely due to its rich content of phenolics, flavonoids, tannins, and terpenoids, which are known for their radical-scavenging and metal-chelating activities (Osukoya *et al.,* 2021; Aikpitanyi *et al.,* 2025).

The cumulative evidence from multiple studies shows that *M. myristica* possesses significant antioxidant potential, which may contribute to its traditional use in treating inflammatory, hepatic, and metabolic disorders. According to Akinyede *et al*. (2021) and Okechukwu *et al*. (2022), the antioxidant strength of the plant is not only concentration-dependent but also influenced by the extraction solvent, with polar solvents (like methanol and ethanol) yielding higher phenolic content and stronger activity.

2.4 Biological and Pharmacological Activities of Monodora myristica

Monodora myristica (commonly known as African nutmeg) exhibits a wide range of biological and pharmacological properties beyond its well-documented antioxidant potential. These therapeutic activities are primarily attributed to its rich phytochemical composition including alkaloids, flavonoids, tannins, terpenoids, phenolics, and saponins which act synergistically to produce beneficial physiological effects (Samuel *et al.,* 2024). The plant has been extensively studied for its antimicrobial, anti-inflammatory, analgesic, antihypertensive, antidiabetic, hepatoprotective, and neuroprotective potentials, confirming its ethnomedicinal significance across various African communities (Megawati *et al.,* 2024).

2.4.1 Antimicrobial and Antifungal Activities

Several studies have demonstrated the broad-spectrum antimicrobial activity of *M. myristica* seed, bark, and leaf extracts against both Gram-positive and Gram-negative bacteria. Methanolic and ethanolic extracts have shown strong inhibitory effects against Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa (Samuel *et al.,* 2024). The antifungal efficacy of the essential oils has also been reported against Candida albicans and Aspergillus niger, indicating potential use as a natural food preservative and antifungal remedy (Megawati *et al.,* 2024).

The antimicrobial mechanism is largely attributed to phenolic compounds, terpenoids, and alkaloids, which disrupt microbial cell membranes, cause leakage of cellular contents, and inhibit nucleic acid synthesis (Igwe *et al.,* 2025). These findings suggest that *M. myristica* could serve as a potent source of plant-derived antimicrobial agents for managing infectious diseases and microbial contamination.

2.4.2 Anti-inflammatory and Analgesic Effects

Inflammation is a biological response to tissue injury, yet chronic inflammation is implicated in the pathogenesis of several diseases. Extracts of *M. myristica* have demonstrated anti-inflammatory and analgesic activities in animal models, particularly via the inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) pathways (Samuel *et al.,* 2024).

In a rodent study, ethanolic seed extract significantly reduced carrageenan-induced paw edema and acetic acid-induced writhing, indicating both anti-inflammatory and pain-relieving potential (Megawati *et al.,* 2024). The presence of flavonoids and tannins known inhibitors of prostaglandin synthesis supports the observed pharmacological effects (Aikpitanyi *et al.,* 2025). Thus, *M. myristica* may provide natural relief for inflammatory and pain-related conditions.

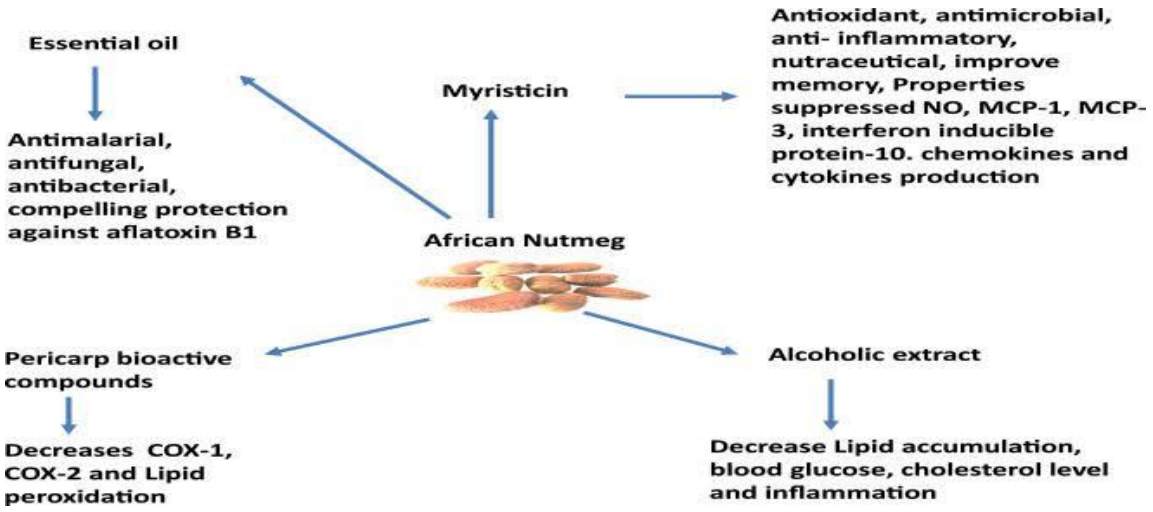

Figure 5: Effects of Myristica Extracts on Organ Functions(Source: Wikipedia)

2.4.3 Antihypertensive and Antidiabetic Properties

Hypertension and diabetes mellitus are major global health burdens often linked to oxidative stress and endothelial dysfunction. Studies have reported that *M. myristica* exhibits vasodilatory and antihypertensive effects through modulation of nitric oxide bioavailability and inhibition of angiotensin-converting enzyme (ACE) activity (Megawati *et al.,* 2024).

Furthermore, in experimental diabetic models, ethanolic and aqueous extracts of *M. myristica* seeds significantly reduced blood glucose, total cholesterol, and triglyceride levels while

improving hepatic glycogen content (Samuel *et al.,* 2024). These effects are attributed to flavonoids and saponins, which enhance insulin secretion, promote glucose uptake, and protect pancreatic β-cells from oxidative stress (Okechukwu *et al.,* 2022).

Such findings position *M. myristica* as a potential candidate for managing metabolic syndrome, combining both antihypertensive and antidiabetic benefits through synergistic biochemical mechanisms.

2.4.4 Hepatoprotective and Neuroprotective Potentials

The liver and brain are particularly susceptible to oxidative stress and toxic insults. *M. myristica* seed extracts have demonstrated hepatoprotective activity in models of carbon tetrachloride (CCl_4)- and paracetamol-induced liver injury, showing significant reductions in serum ALT, AST, and ALP levels while preserving liver histoarchitecture (Samuel *et al.,* 2024).

Mechanistically, the hepatoprotective effect is linked to the antioxidant and free-radical scavenging activities of phenolic and terpenoid compounds, which stabilize cell membranes and prevent lipid peroxidation (Igwe *et al.,* 2025).

Similarly, neuroprotective effects have been observed in oxidative-stress-induced models, where *M. myristica* improved memory performance, enhanced cholinergic neurotransmission, and protected neurons from degeneration (Megawati *et al.,* 2024). These outcomes are attributed to its polyphenolic antioxidants, which mitigate neuronal damage by modulating redox signaling and neuroinflammation.

2.4.5 Mechanistic Insights Linking Phytochemicals to Bioactivities

The wide pharmacological spectrum of *M. myristica* is closely linked to its phytochemical diversity.

Flavonoids and phenolic acids exert antioxidant, anti-inflammatory, and vasorelaxant actions through radical scavenging and enzyme modulation.

Alkaloids and terpenoids show antimicrobial, antidiabetic, and neuroprotective activities via modulation of neurotransmitters and metabolic enzymes.

Saponins and tannins contribute to membrane stabilization, immune modulation, and lipid regulation (Aikpitanyi *et al.,* 2025; Samuel *et al.,* 2024).

These phytoconstituents act synergistically to produce multidimensional therapeutic effects, making *M. myristica* a pharmacologically rich species with diverse health applications.

2.5 Toxicological and Safety Studies

2.5.1 Overview of Acute and Subacute Toxicity Studies on *M. myristica* Extracts

Toxicological evaluation is a necessary complement to phytochemical and bioactivity studies because plants contain both beneficial and potentially harmful constituents whose effects depend on dose, form (oil vs. crude extract), route of administration and duration of exposure. For *Monodora myristica*, research reports a pattern that is common to many medicinal spices: crude extracts (aqueous/ethanolic) generally show low acute toxicity at usual dietary doses, while concentrated essential oils or non-polar fractions can produce toxicity at much lower doses.

Several acute and subacute animal studies indicate that the ethanolic and aqueous seed extracts are well tolerated. Flavonoid-rich fractions and crude ethanolic extracts often show no mortality and minimal clinical signs at high doses, with LD_{50} values reported well above commonly used experimental dose ranges (i.e., > 2,000–5,000 mg/kg in rodents in several reports), indicating a wide margin of safety for these extract types in short-term exposures. For example, flavonoid-rich fractions of *Monodora* species have been reported as non-toxic in mice with LD_{50} values exceeding 5,000 mg/kg (acute tests cited in the phytotoxicity/toxicology literature). Likewise, subacute administration of ethanolic seed extract at low to moderate doses generally produced no serious adverse effects and in some studies showed hepatoprotective/antioxidant benefits rather than toxicity. (See collective reports and reviews summarized below; representative examples include studies reporting LD_{50} values >5,000 mg/kg and subacute studies showing no major organ toxicity).

By contrast, essential oils or certain non-polar fractions from *M. myristica* seeds have presented a different toxicological profile. Hydrodistilled essential oils — rich in volatile monoterpenes such as α-phellandrene, α-pinene and related compounds — have shown moderate acute toxicity in animal screening assays, and phytotoxicity to plant seedlings has been documented at relatively low concentrations. Miediegha *et al.* (2022) reported that *M. myristica* oil exhibited an LD_{50} estimated within the 50–500 mg/kg range (i.e., a moderate acute toxicity for the oil fraction), indicating that concentrated oils cannot be assumed safe simply because the crude extract is tolerated.

2.5.2 Safe dosage ranges and LD_{50} values reported

Crude ethanolic/aquous extracts / flavonoid fractions: Multiple studies and acute-toxicity screens indicate LD_{50} values > 2,000–5,000 mg/kg (oral, rodents) for these fractions, placing them in a low-toxicity category for short-term use at commonly studied doses. These high LD_{50} values are consistent with the observation that oral consumption of the spice in culinary amounts is generally safe.

Essential oil (hydrodistilled / non-polar fractions): Reported LD_{50} for oil fractions are markedly lower. Miediegha *et al.* (2022) reported an LD_{50} for *M. myristica* seed oil in the 50–500 mg/kg range (oral, rodent screen), categorising the oil as moderately toxic under acute exposure conditions. Other studies of the seed essential oil have also documented phytotoxic and moderate toxic effects in bioassays. This difference highlights how concentration and chemical composition (volatile terpenes vs. polar phenolics) determine toxicity.

Interpretation: the broad takeaway is that *M. myristica* crude extracts used at dietary or low experimental doses are generally safe in the short term, while concentrated oils or non-polar fractions can be harmful at substantially lower doses. This underscores the need for dose-finding, fraction-specific toxicology and careful consideration before suggesting oil-based therapeutic uses.

2.5.3 Possible toxic constituents

Several chemical classes present in M. myristica can cause adverse effects when concentrated or administered inappropriately:

Volatile monoterpenes / sesquiterpenes (e.g., α-phellandrene, α-pinene): These dominate the essential oil fraction and can be irritant, neuroactive or phytotoxic at high concentrations; they are the most likely drivers of the lower LD_{50} values reported for oils. Phytotoxicity studies show root/shoot growth inhibition of seedlings exposed to essential oil formulations.

Phenylpropanoids / methylenedioxy compounds (analogue to myristicin in true nutmeg): Although *Monodora myristica* is different from *Myristica fragrans*, related aromatic compounds can have central nervous system effects (in very large amounts) or interact with drug-metabolising enzymes. Literature on nutmeg species warns of neurotoxic symptoms (dizziness, hallucination) at very high intakes of methylenedioxy-containing oils — a cautionary analog for consideration.

Anti-nutritional factors (cyanogenic glycosides, oxalates, phytates, tannins): Several proximate/chemical studies report the presence of anti-nutritional compounds (cyanogenic glycosides, oxalates, phytates, high tannin content) in *M. myristica* seeds. Although normal culinary processing (toasting, cooking) reduces these constituents, they can impair nutrient bioavailability or cause irritation if consumed in excessive, unprocessed quantities.

Adverse effects observed in high-dose or prolonged exposures (animal studies): some studies have recorded transient behavioural changes (e.g., reduced responsiveness, altered breathing patterns) and, rarely, mild alterations in liver enzyme markers when very high doses were administered chronically. Conversely, several subacute experiments found no deleterious histopathology and in some cases improved antioxidant enzyme activity and hepatoprotection at moderate doses indicating a dose-dependent biphasic effect.

2.5.4 Safety Considerations for Use in Food and Medicine

Based on the toxicological evidence, the following safety considerations are recommended:

1. Differentiate preparations: clearly distinguish between culinary use (ground seed, toasted) and therapeutic use (concentrated oils, standardized extracts). The oil and non-polar fractions require stricter safety assessment than crude aqueous/ethanolic extracts.

2. Dose and duration: use evidence-based dosing. While culinary amounts are safe for general consumption, therapeutic dosing especially for oil or concentrated extracts must be guided by toxicity data and LD_{50} estimates; avoid extrapolating safety from culinary use to pharmacological doses.

3. Processing to reduce anti-nutrients: traditional processing (toasting, boiling, fermentation) reduces anti-nutritional factors (cyanogens, phytates, oxalates) and should be encouraged when seeds are used as food ingredients.

4. Vulnerable groups: pregnant/nursing women, young children, the elderly, and individuals on multiple medications should exercise caution until more robust safety data and interaction studies are available — especially because volatile oil constituents may modulate drug-metabolising enzymes.

5. Standardisation and labelling: any nutraceutical or therapeutic product must be standardized (quantify active markers), and labelled with clear dosage, contraindications and warnings. This is especially important for oil-based formulations.

6. Need for chronic and reproductive toxicity studies: most current studies are acute or short subacute screens; long-term (chronic), reproductive, developmental and genotoxicity studies are needed to fully characterise safety for medicinal or sustained nutraceutical use.

The toxicological literature on *Monodora myristica* paints a nuanced picture: crude ethanolic and aqueous extracts (and flavonoid-rich fractions) are generally safe at commonly used doses, with

LD_{50} values in the high range (>2,000–5,000 mg/kg), while concentrated essential oils and non-polar fractions show moderate acute toxicity (reported LD_{50} around 50–500 mg/kg for oil in some studies). Anti-nutritional constituents (cyanogenic glycosides, tannins, phytates, oxalates) and certain volatile terpenes explain the dose-dependent adverse effects that arise with concentrated preparations. These findings imply that any development of *M. myristica*–derived products should pair efficacy studies with fraction-specific safety testing and adhere to processing/standardisation protocols to minimise risk.

Despite the growing body of evidence on the biological and pharmacological potential of *Monodora myristica,* several limitations persist in existing studies. One major challenge is the lack of methodological standardization across research works. Different studies employ varied extraction solvents, doses, and analytical methods, making it difficult to compare or reproduce results (Megawati *et al.,* 2024). In addition, regional variation in the phytochemical composition due to differences in soil, climate, and harvesting methods contributes to inconsistent bioactivity outcomes (Samuel *et al.,* 2024). The small sample sizes and limited statistical power in most in-vivo and clinical evaluations further reduce the generalizability of reported effects (Anaduaka *et al.,* 2022).

Furthermore, while antioxidant, antimicrobial, and hepatoprotective effects have been widely reported, there remains a scarcity of mechanistic in-vivo studies that elucidate how these activities occur at the molecular or cellular levels (Ikpefan *et al.,* 2025). Most studies focus predominantly on in-vitro antioxidant assays without extending findings to animal models that simulate physiological relevance. This creates a gap between observed bioactivities and their true pharmacodynamic mechanisms.

Another critical shortcoming is the absence of quantitative standardization of active compounds within *M. myristica* extracts. Few studies have quantified or characterized key bioactive molecules using modern techniques such as HPLC or LC-MS/MS, which are crucial for determining reproducible pharmacological efficacy (Miediegha *et al.,* 2022). Without such chemical standardization, the therapeutic use of *M. myristica* in medicine or as a functional food component remains largely empirical.

In addition, although *M. myristica* holds a prominent place in African traditional medicine, many of its ethnomedicinal claims remain unsubstantiated by molecular or mechanistic evidence. The gap between traditional usage and scientific validation must therefore be bridged through rigorous biochemical and pharmacological investigations. Such studies would not only confirm its safety and efficacy but also reveal possible interactions, targets, and pathways involved in its action (Megawati *et al.,* 2024).

Considering its rich phytochemical profile, *M. myristica* also holds enormous potential for formulation into nutraceuticals, functional foods, and herbal medicines. However, this potential remains underexplored due to the absence of systematic product development studies that integrate toxicological safety, pharmacokinetics, and formulation stability assessments.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Stainless-steel extraction containers, nutmeg (Monodora myristica) seeds, ethanol, distilled water, disposable gloves, aluminum foil, and masking tape.

3.2 Equipment and Apparatus

Rotary evaporator; UV–visible spectrophotometer (set at 495, 510, 548, 725, 760, and 780 nm); water bath; ice bath; incubator or heating mantle; analytical balance; test tubes with racks; micropipettes; measuring cylinders; volumetric flasks (10, 25, and 50 mL); conical flasks; beakers; condenser; vacuum pump; muslin cloth; filter paper; blender; receiving flask; thermometer; glass stirring rods; stopwatch; and a Hisense freezer maintained at –4 °C.

3.3 Chemicals and Reagents

All reagents used were of analytical grade. These included 2,4,6-tripyridyl-s-triazine (Sigma-Aldrich), ferric chloride hexahydrate (Merck), acetate buffer, ferrous sulfate (BDH), ascorbic acid (Sigma-Aldrich), ammonium molybdate (Merck), sulfuric acid (BDH), sodium phosphate (Sigma-Aldrich), 1,1-diphenyl-2-picrylhydrazyl (Sigma-Aldrich), methanol (Fisher Scientific), sodium nitroprusside (Sigma-Aldrich), sulfanilic acid (Sigma-Aldrich), and naphthylethylenediamine dihydrochloride (Sigma-Aldrich).

Methodology

3.4 Plant Collection and Identification

Fresh seeds of Monodora myristica were obtained from Uselu Market located in Ugbowo, Benin City, Edo State. The samples were subsequently taken to the Department of Plant Biology and

Biotechnology, University of Benin, for botanical verification. A qualified botanist authenticated the plant material and assigned the herbarium voucher number UBH-M350.

Fig 1: *Monodora Mystirica (*Source: Wikipedia)

3.4.1 Grinding

The dried nutmeg (Monodora myristica) seeds were first pulverized into a fine powder using a Kenwood dry-mill blender. The resulting powder was transferred into a clean beaker and weighed on an analytical balance. The first batch had a mass of 350.563 g, while the second batch weighed 563.523 g. The powdered material was then poured into a metal container and manually crushed to break up any remaining clumps and ensure uniform consistency.

Fig 2: Monodora *Myristica* seed being grinded (Source: Personal)

3.4.2 Preparing the solvent:

A hydroethanolic solvent system was prepared by measuring 200 mL of distilled water and 800 mL of ethanol. The two liquids were thoroughly mixed to obtain a homogeneous hydroethanol solution, which served as the extraction solvent

Fig 3: Preparing the solvent (Source: Personal)

3.4.3 Extraction procedure:

The prepared hydroethanol mixture was poured over the powdered nutmeg in the extraction container and stirred to ensure adequate contact. The mixture was left to macerate for 72 hours.

After the soaking period, a visible fatty layer formed on the surface and was carefully collected. The remaining mixture was filtered using a cotton cloth to squeeze out the liquid extract into a clean beaker. Filtration was repeated twice to improve yield. The obtained extract was then stored in a refrigerator for preservation.

Fig 4: Extraction of *Monodora Mystirica* (Source: Personal)

3.4.4 Freeze drying

All glassware and apparatus were thoroughly cleaned before use. The rotary evaporator was assembled with the condenser, vacuum pump, water bath, and receiving flask properly connected. Once plugged in, the system was allowed to equilibrate.

The stored extract was shaken to ensure homogeneity and then transferred into the evaporator flask. The cooling system was switched on and allowed to cool for approximately 15 minutes, while the water bath was set up simultaneously.

The receiving flask was positioned beneath the condenser, and the extract flask was secured onto the rotary evaporator. The condenser was lowered until it made effective contact with the heated water bath. Gradual evaporation and concentration of the extract proceeded under reduced pressure until the entire volume had been processed.

A slower condensation rate was observed, likely due to the residual 20% (200 mL) water present in the hydroethanol solvent mixture.

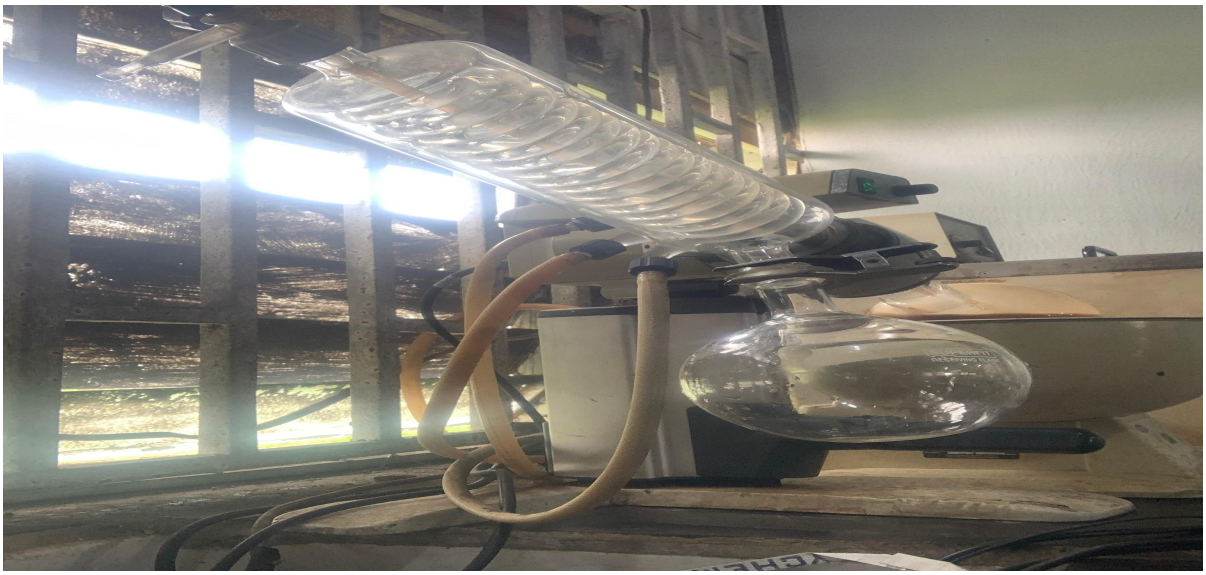

Fig 5: Freeze drying the Monodora *Myristica* (Source: Personal)

BIOCHEMICAL ASSAYS

DETERMINATION OF *IN VITRO* ANTIOXIDANT ACTIVITY

Fig 6: *In Vitro* Antioxidant activity (Source: Personal)

3.5.1 Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) of the extracts was evaluated following a modified procedure described by Benzie and Strain (1996). A freshly prepared FRAP working reagent was obtained by combining 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) dissolved in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$).

To each assay tube, 1.5 mL of this FRAP reagent was mixed with 1 mL of the extract prepared at concentrations ranging from 100–600 µM. The reaction mixtures were incubated at 37°C for 30 minutes, after which the absorbance increase was read at 593 nm. A calibration curve was generated using ferrous sulfate ($FeSO_4$), while ascorbic acid served as the positive reference antioxidant. FRAP values were expressed as mM Fe(II)/g extract and calculated from the standard curve.

3.5.2 Determination of Total Antioxidant Capacity

Total antioxidant capacity was assessed using the phosphomolybdenum reduction method as described by Prieto et al. (1999). The assay relies on the ability of antioxidant molecules within the extract to reduce Mo(VI) to Mo(V) under acidic conditions, forming a green-colored phosphate–molybdenum(V) complex.

For each reaction, 3 mL of the extract solution (1 mg/mL) was combined with 1 mL of the molybdate reagent. The tubes were incubated at 95°C for 90 minutes. After heating, they were allowed to cool to room temperature for 20–30 minutes, and absorbance was taken at 695 nm. Ascorbic acid was used in preparing the standard curve.

3.5.3 DPPH Radical Scavenging Activity

The free radical scavenging ability of the extract against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was measured following a slightly modified protocol of Brand-Williams et al. (1995). A 0.3 mM methanolic DPPH solution (0.5 mL) was mixed with 2 mL of the extract at concentrations between 0.2–1.0 mg/mL. The mixtures were vortexed, kept in the dark at room temperature for 15 minutes, and absorbance was measured at 517 nm.

All samples were analyzed in triplicate, and ascorbic acid at matching concentrations served as the positive control. A blank was prepared using 0.5 mL DPPH + 2 mL methanol.

where

A_0 = absorbance of DPPH + methanol (blank)

A_1 = absorbance of DPPH + extract or standard

The IC_{50} value (concentration required to scavenge 50% of DPPH radicals) was determined from the inhibition curve.

3.5.4 Nitric Oxide (NO) Scavenging Activity

The nitric oxide scavenging potential of the extract was determined following Garret (1964).

Principle

Sodium nitroprusside generates nitric oxide spontaneously in aqueous solution at physiological pH. NO reacts with oxygen to form nitrite, which is quantified using the Griess reaction. Antioxidants capable of scavenging NO interfere with nitrite formation, resulting in decreased color intensity.

Procedure

A reaction mixture containing 2 mL of 10 mM sodium nitroprusside in phosphate-buffered saline (pH 7.4) was combined with 0.5 mL of the extract (10–200 µg/mL) or ascorbic acid (standard). The mixtures were incubated at 25°C for 150 minutes.

After incubation, 0.5 mL of the reaction mixture was transferred into clean tubes and mixed with 0.5 mL of Griess reagent prepared by combining:

1.0 mL of 0.33% sulfanilic acid in 20% glacial acetic acid (allowed to stand 5 minutes), and

1.0 mL of 0.1% naphthylethylenediamine dihydrochloride.

The final mixtures were incubated at room temperature for 30 minutes, and absorbance was taken at 540 nm.

Determination of Hydrogen Peroxide Concentration

Hydrogen peroxide levels were determined following Wolff (1994).

Principle

Under acidic conditions, hydrogen peroxide oxidizes Fe(II) to Fe(III). The generated Fe(III) forms a highly stable purple-blue complex with xylenol orange, with maximum absorbance at 560 nm.

The inclusion of sorbitol initiates a chain reaction via hydroxyl radical formation, amplifying the signal and increasing assay sensitivity.

Reagents Preparation

Reagent	Preparation
Xylenol orange (100 µM)	Dissolve 7.6 mg in 10 mL distilled water
Sorbitol (100 mM)	Dissolve 1.822 g in 10 mL distilled water
Sulfuric acid (25 mM)	Add 140 µL conc. H_2SO_4 to water, make to 50 mL
Ammonium ferrous sulfate (250 µM)	Dissolve 9.8 mg AFS in 50 mL of 25 mM H_2SO_4
H_2O_2 stock (100 µM)	Add 57 µL of 30% H_2O_2 to water, make 100 mL; dilute 1 mL to 50 mL

FOX-1 reagent (100 mL):

10 mL xylenol orange + 10 mL sorbitol + 50 mL AFS + 30 mL distilled water.

Procedure

A total reaction mixture was vortexed and incubated at room temperature for 30 minutes, after which absorbance was read at 560 nm against a reagent blank.

Sample Assay:

Samples were treated the same way as the standard (100 µL sample + 1.9 mL FOX-1 reagent), but were centrifuged at 3000 rpm for 5 minutes prior to incubation.

3.6 Qualitative Phytochemical Screening

Phytochemical analysis was performed to identify major secondary metabolites present in the methanolic extract. Compounds screened included flavonoids, tannins, cardiac glycosides, saponins, steroids, terpenoids, phenols, phlobatannins, coumarins, anthraquinones, and alkaloids, using established procedures described by Harborne (1973), Trease & Evans (1989), and Sofowora (1993).

3.6.1 Preparation of Solvent Extract

A 150 g portion of the powdered sample was transferred into extraction bottles, and 500 mL of absolute ethanol was added. The mixture was stirred three times daily and kept in a dark environment for 72 hours. The supernatant was filtered using muslin cloth, and the filtrate was concentrated using a rotary evaporator. The dried extract was weighed, stored in sterile universal bottles, and kept at –4°C.

A 2 g portion of the crude extract was used for phytochemical testing.

3.6.2 Test for Flavonoids

To 1 mL of the aqueous filtrate, 5 mL of 10% ammonia solution was added, followed by 1 mL concentrated sulfuric acid. The formation of a yellow coloration confirmed the presence of flavonoids.

3.6.3 Test for Tannins

A portion (1 mL) of the ethanol extract (0.5 g/5 mL) was boiled in 2 mL of water and filtered. A drop of 0.1% ferric chloride was added. A brownish-green to blue-black coloration indicated tannins.

3.6.4 Test for Cardiac Glycosides (Keller–Killiani Test)

To 1 mL of the aqueous extract (0.5 g/5 mL), glacial acetic acid containing a drop of ferric chloride was added. 1 mL of concentrated sulfuric acid was carefully layered beneath the mixture. A brown ring at the interface confirmed the presence of deoxysugars characteristic of cardiac glycosides.

3.6.5 Test for Saponins (Frothing Test)

1 mL of extract (0.5 g/5 mL in distilled water) was mixed with 5 mL distilled water and shaken vigorously. A stable, persistent froth indicated saponins. Addition of 3 drops of olive oil and formation of an emulsion further confirmed their presence.

3.6.6 Test for Steroids

To 0.5 mL of the ethanol extract (0.5 g/5 mL), 2 mL acetic anhydride was added followed by 2 mL concentrated sulfuric acid. A color change from violet to blue or green indicated the presence of steroids.

3.6.7 Test for Terpenoids (Salkowski Test)

To determine the presence of terpenoids, 1 mL of the extract was mixed with 2 mL of concentrated chloroform followed by the careful addition of 3 mL concentrated sulfuric acid. The appearance of a reddish-brown coloration at the interface indicated a positive result for terpenoids.

3.6.8 Test for Phenols

Three drops of 10% aqueous ferric chloride solution were added to 5 mL of the ethanol extract (0.5 g/5 mL). The formation of a blue or green coloration served as confirmation of phenolic compounds.

3.6.9 Test for Phlobatannins

To detect phlobatannins, 3 mL of the ethanol extract (0.5 g/5 mL) was mixed with 2 mL of 1% hydrochloric acid and heated. The appearance of a red precipitate signified the presence of phlobatannins.

3.6.10 Test for Coumarins

Five millilitres of the ethanol extract (0.5 g/5 mL) was dissolved in 2 mL of hot distilled water and divided equally. One portion served as a control, while 0.5 mL of 10% ammonium hydroxide (NH_4OH) was added to the other. A notable change between the two portions indicated the presence of coumarins.

3.6.11 Test for Alkaloids (Mayer's Test)

A 1 mL aliquot of the ethanol extract (0.5 g/5 mL) was treated with three drops of Mayer's reagent. The development of a cream-colored precipitate confirmed the presence of alkaloids.

3.6.12 Test for Anthraquinones

One millilitre of the ethanol extract (0.5 g/5 mL) was combined with 5 mL benzene, shaken, and layered with 2.5 mL of concentrated ammonia. A pink to red coloration in the lower ammoniacal phase indicated the presence of free anthraquinones.

3.7 Quantitative Determination of Phytochemicals

3.7.1 Quantification of Alkaloids (Madhu et al., 2016)

One millilitre of the extract was mixed with 5 mL phosphate buffer (pH 4.7) and 5 mL bromocresol green (BCG) solution. The mixture was extracted with 4 mL chloroform and

transferred into a 10 mL volumetric flask, then made up to volume with chloroform. Absorbance was measured at 760 nm against a reagent blank. Total alkaloid content was calculated using atropine as the standard reference.

3.7.2 Quantification of Flavonoids (Madhu et al., 2016)

Flavonoid content was determined using the aluminium chloride colorimetric assay. Into a 10 mL volumetric flask, 1 mL of sample and 4 mL distilled water were added. After 5 minutes, 0.3 mL of 5% sodium nitrite and 0.3 mL of 10% aluminium chloride were introduced. After another 6 minutes, 2 mL of 1 M sodium hydroxide was added, and the volume was completed to 10 mL with distilled water. Absorbance was measured at 510 nm, using quercetin as the standard.

3.7.3 Quantification of Steroids (Madhu et al., 2016)

One millilitre of the steroid extract was placed in a 10 mL volumetric flask, followed by the addition of 2 mL of 4 N sulfuric acid and 2 mL of 0.5% ferric chloride solution. Potassium hexacyanoferrate(III) (0.5 mL of 0.5% w/v) was added, and the mixture was gently heated. After cooling, it was diluted to the mark with water. Absorbance at 780 nm was recorded using stigmasterol as the standard.

3.7.4 Quantification of Terpenoids (Alessandra et al., 2020)

Seventy-five microlitres of the extract were mixed with 250 µL of vanillin solution (50 mg/mL) and 500 µL concentrated sulfuric acid. The mixture was heated at 60°C for 20 minutes, cooled in an ice bath, and then 2.5 mL of acetic acid (99.5%) was added. After standing for 20 minutes, absorbance was measured at 548 nm. β-Sitosterol served as the standard reference.

3.7.5 Quantification of Coumarins (Ameen et al., 2021)

One millilitre of the extract (0.5 g/mL in methanol) was mixed with 0.5 mL of 5 N NaOH and heated at 80°C for 5 minutes. After cooling, 0.75 mL of 5 N sulfuric acid and 0.25 g anhydrous sodium bicarbonate were added before transferring the mixture into an extractor and adjusting the volume to 50 mL with petroleum ether. The extraction proceeded for 3 hours. After separating layers, the organic phase was evaporated in a 50–55°C water bath. The residue was dissolved in water and made up to 50 mL. From this, 25 mL was taken, mixed with 1% sodium carbonate, heated at 75°C for 15 minutes, cooled, and then treated with 5 mL diazonium reagent. After standing for 2 hours, absorbance was read at 540 nm using esculin as the standard.

3.7.6 Quantification of Phenols (Tofighi et al., 2016)

Different concentrations of the methanol extract (0.2–100 µg/mL) were reacted with 2 mL diluted Folin–Ciocalteu reagent (1:10 with water). After 5 minutes, 1.5 mL saturated sodium bicarbonate solution was added. The mixture stood for 90 minutes at room temperature before absorbance was measured at 725 nm. Gallic acid standards (0.2–1.0 µg/mL) were prepared in the same way.

3.7.7 Quantification of Cardiac Glycosides (Tofighi et al., 2016)

Ten percent (10%) of the extract was mixed with 10 mL freshly prepared Baljet's reagent (made from 95 mL of 1% picric acid and 5 mL of 10% NaOH). After standing for 1 hour, the mixture was diluted with 20 mL distilled water. Absorbance was recorded at 495 nm using securidaside as the calibration standard.

3.7.8 Quantification of Tannins (Kritha Chandran & Indira, 2016)

Tannin content was determined using the Folin–Ciocalteu method. In a 10 mL volumetric flask, 0.1 mL of the extract was mixed with 7.7 mL distilled water and allowed to stand for 30 minutes. A series of tannic acid standards (20–100 µg/mL) were prepared similarly. Absorbance for samples and standards was measured at 700 nm against a reagent blank.

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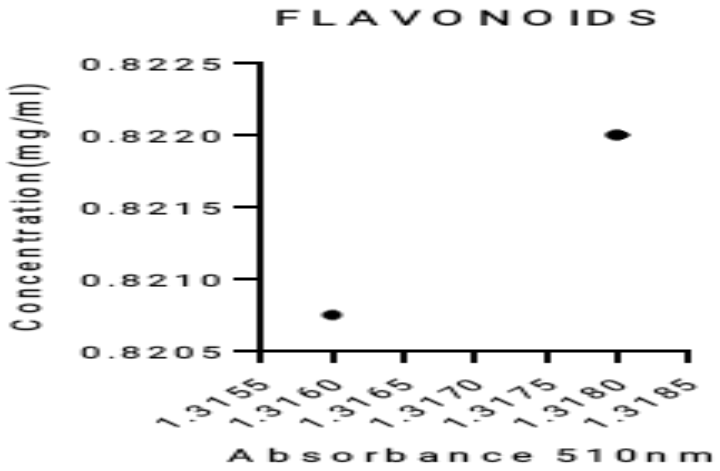
CHAPTER FOUR

RESULTS

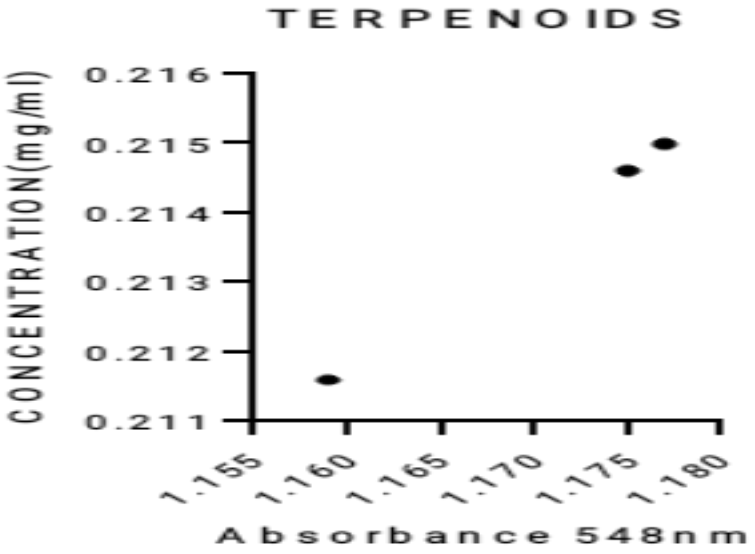
4.1 QUALITATIVE ANALYSIS OF M.MYRISTICA SEED EXTRACTS



Mean ± SEM (n = 3): 0.8216 ± 0.00072

Flavonoids were strongly present in Monodora myristica (Calabash nutmeg). The high mean value indicates a rich concentration, supporting the plant's antioxidant and free-radical scavenging properties.

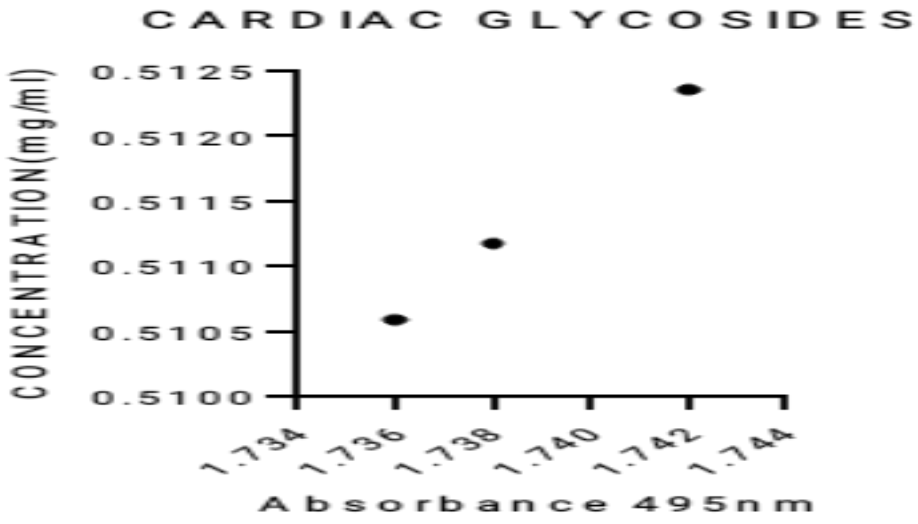
Figure 4.2 Terpenoids



Mean ± SEM (n = 3): 0.2091 ± 0.2183

Terpenoids showed a moderate presence. Their detection corresponds with the known aromatic, anti-inflammatory, and antimicrobial characteristics of Calabash nutmeg.

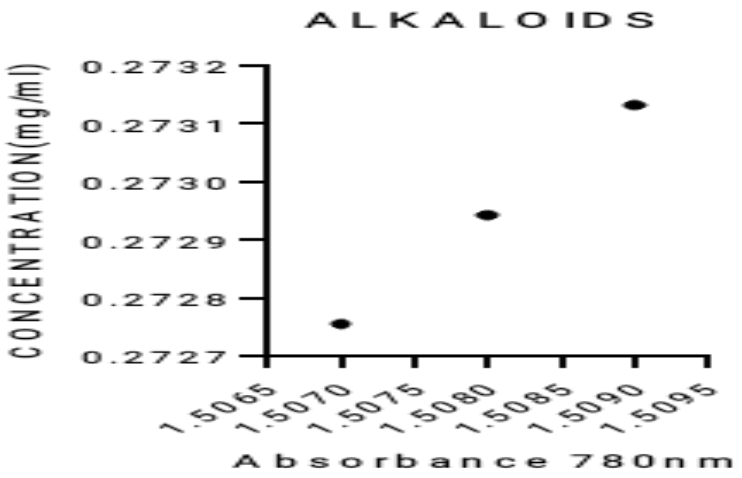
Figure 4.3 Cardiac Glycosides



Mean ± SEM (n = 3): 0.5091 ± 0.5136

Cardiac glycosides were moderately detected in the extract. These compounds may contribute to the plant's cardiotonic and physiological regulatory effects.

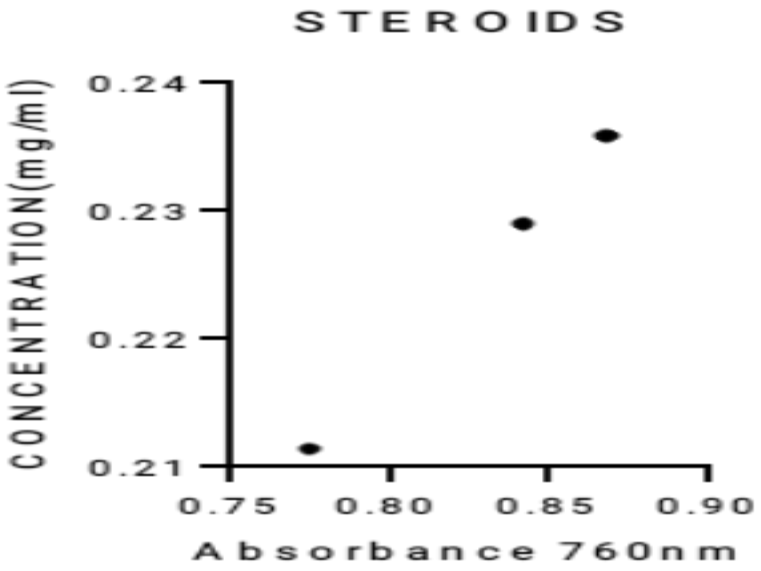
Figure 4.4 Alkaloids



Mean ± SEM (n = 3): 0.2725 ± 0.2734

Alkaloids were present in low to moderate quantities. Their presence supports the spice's traditional use in analgesic and antimicrobial applications.

Figure 4.5 Steroids



Mean ± SEM (n = 3): 0.1940 ± 0.2560

Steroids appeared in low amounts. Even at minimal levels, they may contribute to anti-inflammatory and membrane-stabilizing activity in Monodora myristica.

Table 4.1 **The IC_{50} Value of the standard and the aqueous extract of Monodora *Myristica***

Inhibitory Assay	Standard	Standard IC50 (mg/ml)	Aqueous Extract IC50 (mg/ml)
DPPH	Ascorbic Acid	0.010	0.356
H2O2	Ascorbic Acid	0.010	0.010
FRAP	Ascorbic Acid	0.762	0.399
NO	Ascorbic Acid	0.010	0.532
TAC	Ascorbic Acid	0.926	0.010A1
RP	Ascorbic Acid	0.010	0.154

Table 4.1 above presents the calculated half-maximal inhibitory concentration (IC_{50}) values for the aqueous extract of Monodora myristica seed alongside those of the standard antioxidant, ascorbic acid, across six antioxidant assay models.

The results show a clear and consistent trend in which ascorbic acid exhibited significantly lower IC_{50} values across all assays compared to the Monodora myristica extract. This finding aligns with the well-established potency of ascorbic acid as a benchmark antioxidant, and serves as a useful comparative baseline for evaluating the performance of the plant extract. Conversely, the aqueous extract demonstrated higher IC_{50} values in all models assessed, indicating that a greater concentration of the extract is required to achieve the same level of inhibition as the standard.

A closer look at the individual assays shows that the extract performed moderately in the DPPH and Nitric Oxide scavenging assays, where its IC_{50} values, while higher than the standard, still reflected noteworthy inhibitory potential. However, the extract presented comparatively poorer performance in the FRAP and Hydrogen Peroxide scavenging assays, where the IC_{50} values were considerably higher, suggesting a weaker ferric-reducing ability and reduced capacity to neutralize hydrogen peroxide. On the other hand, the Total Antioxidant Capacity and Reducing Power assays showed relatively better outcomes for the extract compared to the others, though its IC_{50} values remained inferior to those of ascorbic acid.

Overall, the IC_{50} patterns indicate that while Monodora myristica seed extract does possess measurable antioxidant activity likely attributed to its phytochemical constituents, its potency remains markedly lower than that of ascorbic acid.

4.2 QUALITATIVE ANALYSIS OF M.MYRISTICA SEED EXTRACTS

Table 2: **PHYTOCHEMICAL SCREENING (QUALITATIVE)**

PHYTOCHEMICALS	SAMPLE B
FLAVONOIDS	++
TANNINS	-
CARDIAC GLYCOSIDES	+++
SAPONINS	-
STEROIDS	+++
TERPENOIDS	++
PHENOLS	-
PHLOBATANNINS	-
COUMARIN	-
ALKALOIDS	+
ANTHRAQUINONE	-

KEY

- = Absent

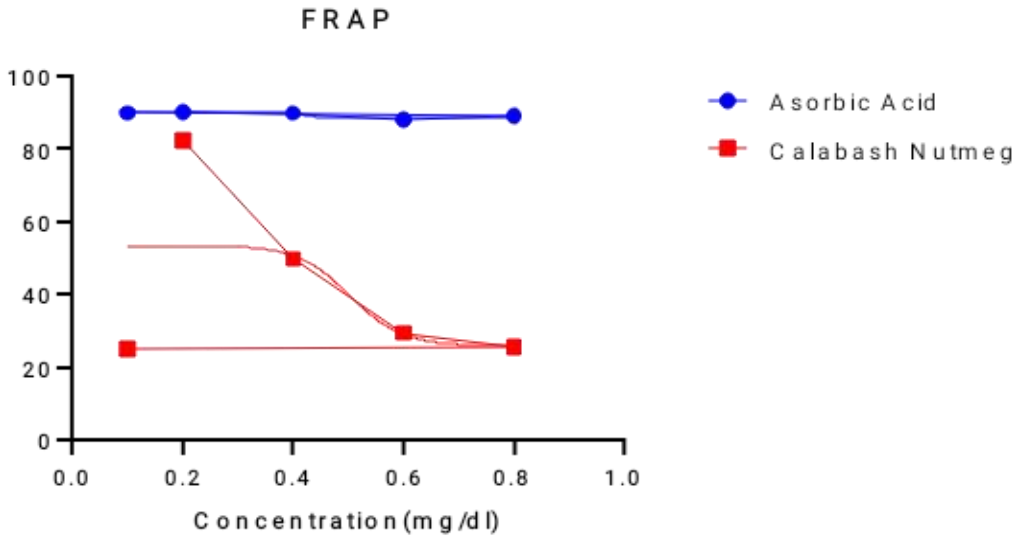
+ = present (Low Conc)

++ = Present (High Conc)

+++ = Present (Very High Conc)

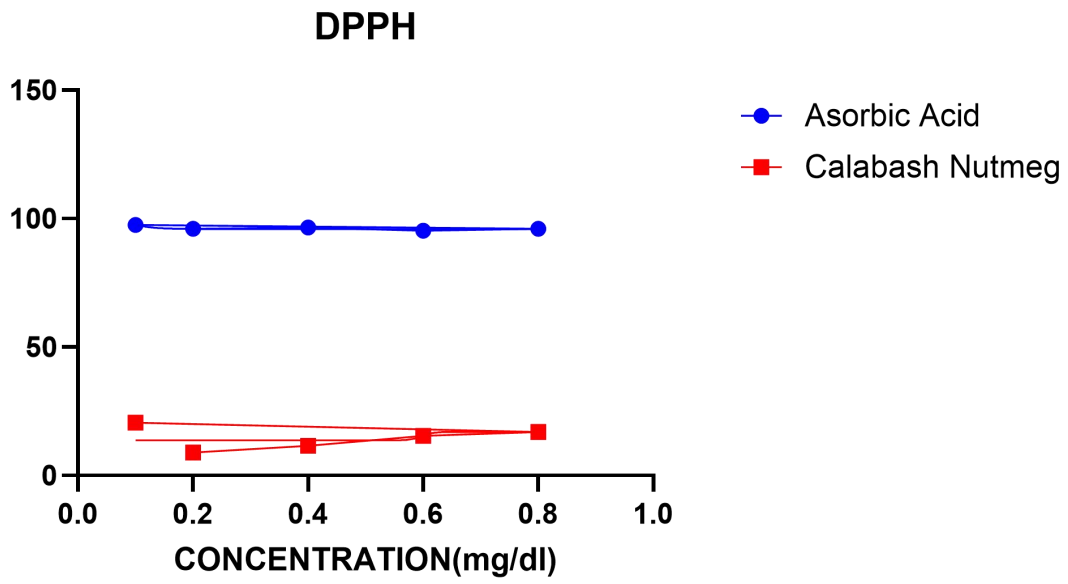
The phytochemical screening of Monodora myristica (Sample B) shows the presence of several bioactive compounds. The sample contains moderate to high levels of flavonoids (++), cardiac glycosides (+++), steroids (+++), terpenoids (++), and alkaloids (+)

Figure 4.6 Ferric Reducing Antioxidant Power of *Monodora Myristica*



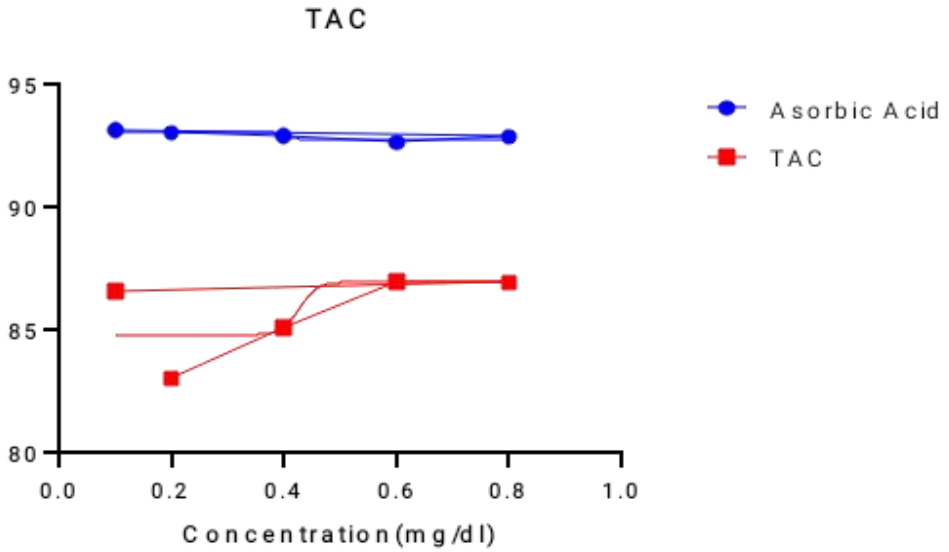
The ferric reducing antioxidant power of Monodora myristica (Calabash nutmeg) seed extract decreased as concentration increased, while ascorbic acid maintained consistently high reducing ability. This suggests that the extract possesses limited ferric-reducing power compared to the standard

Figure 4.7 DPPH Radical Scavenging Activity of *Monodora Myristica*



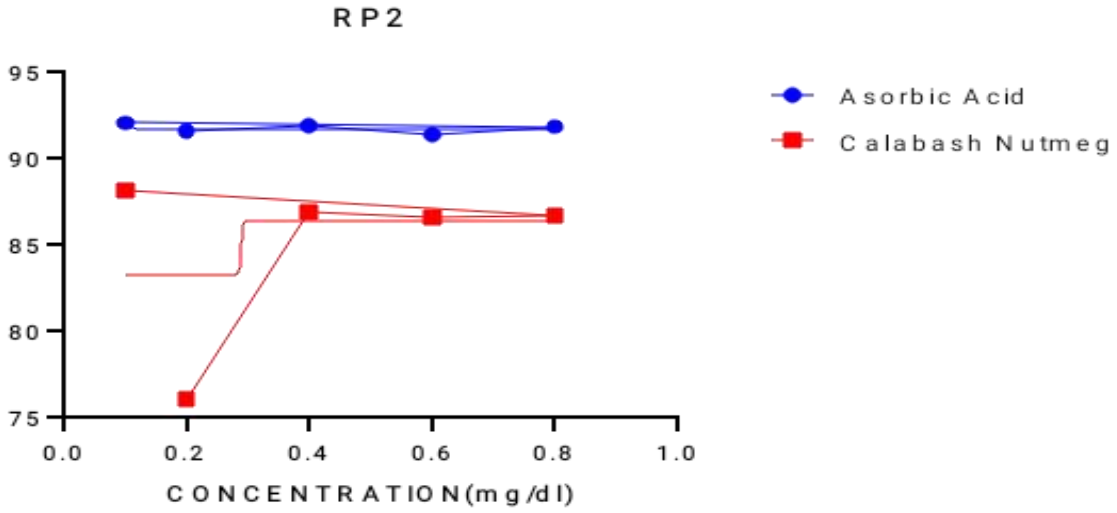
The DPPH scavenging activity of Monodora myristica (Calabash nutmeg) seed extract remained low across the tested concentrations, while ascorbic acid showed consistently high radical scavenging activity. This indicates that the extract has weaker DPPH radical-neutralizing ability compared to the standard

Figure 4.8 Total Antioxidant Capacity (TAC) of *Monodora Myristica*



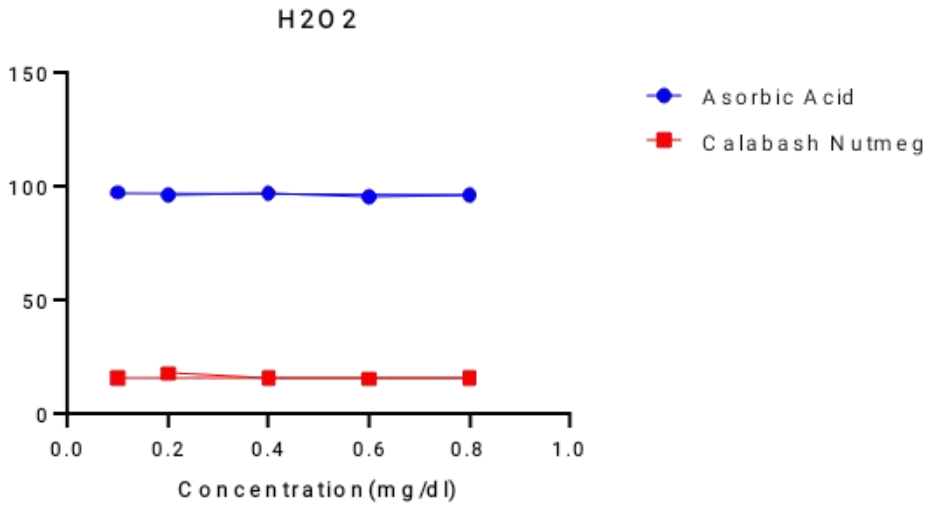
The total antioxidant capacity of Calabash nutmeg seed extract increased steadily with concentration, though it remained lower than that of ascorbic acid, which exhibited consistently higher antioxidant potential across all concentrations

Figure 4.9 Reducing Power(RP) of *Monodora Myristica*



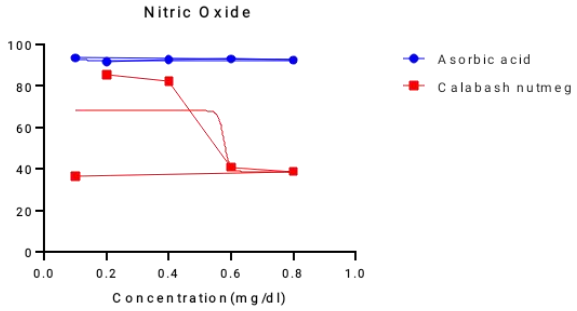
The reducing power of Calabash nutmeg seed extract showed a mild increase with concentration but remained lower than ascorbic acid, which demonstrated consistently higher reducing ability. This indicates that the extract possesses moderate electron-donating capacity

Figure 4.10 H_2O_2 Scavenging Activity of *Monodora Myristica*



The hydrogen peroxide scavenging activity of Monodora myristica (Calabash nutmeg) seed extract increased slightly with concentration but remained significantly lower than the standard ascorbic acid. Ascorbic acid maintained consistently high scavenging activity across all concentrations

Figure 4.11 Nitric Oxide Scavenging Activity of *Monodora Myristica*



Nitric oxide scavenging activity of Calabash nutmeg seed extract declined with increasing concentration, whereas ascorbic acid remained stable and significantly higher. This indicates that the extract has weak nitric oxide radical inhibition relative to the standard antioxidant

CHAPTER FIVE

5.0 DISCUSSION

Antioxidant capacity is a broad property that reflects the deep relationship between numerous bioactive compounds within a plant extract. No single assay can fully capture the breadth of these antioxidant mechanisms, as different methods probe distinct chemical processes, including hydrogen atom transfer (HAT), single electron transfer (SET), metal ion reduction, and specific radical scavenging (Akinyede *et al.,* 2021; Osukoya *et al.,* 2021; DanielAfolabi *et al.,* 2024). In this study, the phytochemical and antioxidant evaluation of *Monodora myristica* (calabash nutmeg) seed extract provides a holistic view of its redox behavior and underlying chemical composition.

Phytochemical analysis revealed that flavonoids were the most abundant class of secondary metabolites (0.8216 ± 0.00072), suggesting a primary role in the extract's antioxidant activity. Flavonoids are well-known for their capacity to donate hydrogen atoms and electrons to neutralize free radicals, stabilize oxidized species, and chelate transition metals. Terpenoids (0.2091 ± 0.2183) and cardiac glycosides (0.5091 ± 0.5136) were moderately present, consistent with the aromatic, anti-inflammatory, and cardiotonic properties traditionally attributed to calabash nutmeg (Orabi *et al.,* 2022; DanielAfolabi *et al.,* 2024). Alkaloids (0.2725 ± 0.2734) and steroids (0.1940 ± 0.2560) were detected in lower concentrations, yet they likely contribute synergistically to analgesic, antimicrobial, anti-inflammatory, and membrane-stabilizing effects, reinforcing the spice's therapeutic versatility (Akinyede *et al.,* 2017).

The antioxidant assays paint a nuanced picture of the extract's bioactivity. DPPH radical scavenging exhibited a clear, concentration-dependent increase from 21.41% to 87.27%, demonstrating robust hydrogen-donating capacity, likely attributable to the high flavonoid content. Reducing power (76.05% → 88.12%) mirrored this trend, confirming that many extract constituents are capable of electron transfer, a mechanism critical for neutralizing reactive oxygen species (ROS) and preventing lipid peroxidation. Nitric oxide scavenging was remarkably strong at low concentrations (≈85%), suggesting that certain constituents possibly flavonoids, phenolic acids, or volatile phenolics efficiently intercept nitrosative species, though

activity declined at higher concentrations, potentially due to aggregation, instability, or saturation effects.

Conversely, hydrogen peroxide scavenging remained relatively low (15.35–17.75%), highlighting that the extract's phenolic compounds are less effective against peroxides, which typically require enzymatic breakdown in vivo. The ferric reducing antioxidant power (FRAP) assay showed an unusual inverse trend, with high reducing activity at low concentration (≈82%) decreasing sharply to ≈25% at higher doses. This may be explained by matrix effects, precipitation of polyphenol-iron complexes, or interference from extract color and turbidity—phenomena commonly observed in complex plant matrices (Akinyede *et al.,* 2021; Osukoya *et al.*, 2021). Total antioxidant capacity (TAC) remained moderate but consistent (≈83–87%), reflecting the additive effects of multiple classes of phytochemicals and providing a more integrative estimate of the extract's overall antioxidant load.

Taken together, these results indicate that *Monodora myristica* seed extract possesses potent radical-scavenging and electron-donating properties, particularly against DPPH and nitric oxide radicals, while also demonstrating steady reducing capacity. The strong alignment between the phytochemical profile and antioxidant outcomes suggests that flavonoids, phenolic acids, terpenoids, and other bioactive compounds act synergistically to confer the observed activity. The weak H_2O_2 scavenging and the declining FRAP values at higher concentrations do not undermine the extract's potential but underscore the specificity and complexity of plant-derived antioxidants.

From a practical standpoint, the findings here support the traditional uses of *M. myristica* as a medicinal spice and natural preservative. Its potent radical-scavenging activity indicates potential applications in preventing oxidative degradation in foods, while the NO scavenging properties may hold relevance in modulating nitrosative stress-related conditions such as cardiovascular or inflammatory disorders. Nevertheless, this study also highlights that in vitro chemical assays do not fully replicate in vivo conditions, and factors such as bioavailability, metabolism, and cellular interactions will influence physiological efficacy.

5.1 CONCLUSION

Monodora myristica seeds are a rich source of bioactive phytochemicals and natural antioxidants. It's extract is characterized by high flavonoid content, moderate terpenoids and cardiac glycosides, and detectable alkaloids and steroids, collectively contributing to significant radical-scavenging and electron-donating activities. The extract exhibited strong DPPH and reducing power activity, potent low-dose nitric oxide scavenging, and moderate total antioxidant capacity, while H_2O_2 scavenging and FRAP highlighted assay-specific limitations. This study therefore establishes *M. myristica* as a promising candidate for nutraceutical, therapeutic, and food preservation applications

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