GC-MS PROFILING OF BIOACTIVE COMPOUNDS IN MONODORA MYRISTICA SEED AND SHELL EXTRACT

BY

Esther Osebhahiemen AGBATOR

(BMS2101368)

A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE, BSc. (HONS)MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

NOVEMBER, 2025.

CERTIFICATION

We the undersigned hereby certify that Miss Esther Osebhahiemen Agbator (BMS2101368) carried out this research in the Department of Medical Biochemistry, School of Basic Medical Sciences. University of Benin and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry

Signed:

……………….......................... ……………………
Ukwuonwo – Ediale A.C (Mrs) (Date)

(PROJECT SUPERVISOR)

. . . .
Dr. B. N. AGUEBOR-OGIE (Date)
(Ag HEAD OF DEPARTMENT)

. . . .
External Examiner (Date)

DEDICATION

I dedicate this work to God Almighty who sustained me all through my time in the University of Benin and gave me the wisdom and enablement to successfully carry out this research and my lovely parents, Mr and Mrs. Agbator, my lovely siblings, friends as well as the DLCF Zion chapel for all their encouragements, prayers, advice and immeasurable support and contributions to my academic success.

ACKNOWLEDGEMENT

I will love to give my profound gratitude to God Almighty for his infinite mercy and protection throughout my years in the department. I also specially acknowledge my project supervisor and course advisor Ukwuonwo – Ediale A.C (Mrs) for her support throughout this project.

I want to also appreciate my parents, Mr. and Mrs. Agbator for their unwavering support and prayers. Also my project group members, Vanessa, Debby, Triumph and Godwin for the collective contribution towards the success of the project. Furthermore, I will like to show appreciation to the Department of Medical Biochemistry for the knowledge passed across, the support, and advice from my lecturers.

I also want to recognize and appreciate those who have in one way or another supported me during this project work. To mention but a few, Abegunde Moses, Samson Okwufulueze, Grace, Tega, Ngozi Odega and the entire members of DLCF zion chapel.

Lastly, I want to appreciate my friends, Ay, Israel, Peace, Vincent, James, TomiFavour, Oyin, Fulness, Chrystabel, Onome, Chineneye, for their support and memories made throughout my stay in University of Benin.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

This study investigated the bioactive phytochemical constituents of *Monodora myristica* (African nutmeg) seed and shell extracts using Gas Chromatography–Mass Spectrometry (GC-MS) to validate their traditional medicinal uses and explore their therapeutic potential. *Monodora myristica*, a tropical tree of the Annonaceae family native to Sub-Saharan Africa, is valued for its culinary and medicinal properties, yet its shell remains underutilized. The study aimed to identify, characterize, and compare the phytochemical composition of hydroethanol extracts from the seed and shell, and to evaluate their possible applications in medicine, food preservation, and nutraceutical development. GC-MS analysis revealed 28 compounds in the seed extract and 15 in the shell extract. The seed extract contained linoelaidic acid (29.49%), 9,12-octadecadienoic acid (26.97%), and (E)-9-octadecenoic acid ethyl ester (21.90%), known for antioxidant and anti-inflammatory properties. The shell extract was rich in (E)-9-octadecenoic acid ethyl ester (35.08%) and linoleic acid ethyl ester (22.21%), which possess strong pharmacological effects. These findings confirm the therapeutic potential of *Monodora myristica*, demonstrating that both seed and shell extracts are valuable sources of bioactive compounds. The study recommends further isolation, toxicity testing, and extraction optimization to support drug discovery and sustainable utilization.

CHAPTER ONE

INTRODUCTION

BACKGROUND OF STUDY

Monodora myristica is a tropical perennial tree belonging to the family of Annonaceae, and it occurs mainly in Sub-Saharan Africa and a few countries of Central and South America (Ndumbe *et al.,* 2022). It occurs naturally in rainforests and evergreen forests, particularly in Nigeria, Liberia, Cameroon, Angola, and Uganda, among other countries, but there is minor production in Western Kenya. (Afolabi *et al.,* 2024).

The tree, also known as African nutmeg or Calabash nutmeg, can reach up to 20 meters in height. It has greenish fragrant flowers and large round fruits that are smooth and green when young, becoming woody at maturity. The fruits are round in shape; each has several ovate, pale brown, pungent seeds embedded in a whitish pulp (Nkwocha *et al.,* 2019). The seeds of the plant have a strong and pleasant smell, and they are utilized as a seasoning in various dishes like soups, stews, cakes, and desserts to impart a flavor similar to nutmeg (Okpoghono *et al*., 2025; Afolabi *et al.,* 2024).

This plant has different indigenous names in Nigeria: Ehuru (Igbo), Ariwo (Yoruba), Erhe (Urhobo), Iwor (Itsekiri), Ikposa (Benin), and Guijija dan miya (Hausa).

The most commercially important aspect of the plant is its seeds, which amount to about 5–9% essential oil that imparts the characteristic odor and medicinal properties. These oils can be used for cooking and medicinal purposes, respectively, the predominant components being α-phellandrene, α-pinene, limonene, and eugenol. In Nigerian cooking the whole dried seeds are used to flavor all sorts of dishes, from pepper soup to banga soup, for example where they provide a warm, nutty, slightly peppery aroma.

In addition to being used for culinary purposes*, Monodora myristica* is well known in traditional medicine for its anti-inflammatory, antimicrobial, anti-sickling, diuretic, and cholesterol-reducing activities (Akinwunmi *et al.,* 2015; Igwe *et al.,* 2023). It is employed in managing ailments such as headaches, mild fevers, bronchitis, arthritis, and stomach disorders. The seeds are also used as stimulants, laxatives, and postpartum tonics, while the leaves are used as insect repellents and wound-healing agents (Akinwunmi *et al.,* 2015).

Phytochemical screening of seeds and shells revealed some secondary metabolites such as phenolics, flavonoids, terpenoids, fatty acids, and essential oils (myristicin and β-caryophyllene) responsible for antioxidant, antimicrobial, and anti-inflammatory potential (Igwe *et al.,* 2023; Afolabi *et al.,* 2024). These aspects demonstrate the antioxidant, antimicrobial, and anti-inflammatory value of the plant, making it desirable in the pharmaceutical or nutraceutical industry.

These seeds are known to be a source of proteins, fats, carbohydrates, and fiber and contain minerals such as calcium, magnesium, and potassium. Roasting or fermentation may reduce the effects of anti-nutrients like tannins and oxalates in the seeds (Agiriga *et al.,* 2018; Nkwocha *et al.,* 2019). Although the seeds are highly utilized, the chemical profile of the shell has received little attention, even though it may provide many bioactive compounds that can be explored in pharmaceutics, cosmetics, and food preservation.

Gas chromatography-mass spectrometry (GC-MS) occupies a significant place as a complementary technique to gas chromatography for separation and mass spectrometry for identification. As an analytical approach, GC-MS identifies and quantifies volatile and semi-volatile phytochemicals at trace levels and, thus, develops a chemical fingerprint for the herbal plants concerned (Okechukwu *et al.,* 2022). The design of studies using GC-MS for *Monodora myristica* opens the way toward a scientific basis for validating its traditional uses, as well as finding new bioactive compounds in seeds and shells.

1.1 AIM OF THE STUDY

The aim of this study is to investigate the bioactive components in *Monodora myristica* seed and shell extracts using Gas Chromatography–Mass Spectrometry (GC-MS). It aims to determine and characterize the chemical compounds responsible for the medicinal properties of this plant, compare seeds and shells constituents, evaluate how these can be used as a potential food preservative, medicine, and development of products beneficial to health.

1.2 OBJECTIVES OF THE STUDY

1. To extract the bioactive compounds present in the seeds and shells of *Monodora myristica* using suitable solvent extraction techniques.
2. To carry out both qualitative and quantitative analyses of the chemical components through GC-MS.
3. To identify and describe the key bioactive compounds that contribute to the plant's medicinal and pharmacological activities.
4. To assess the possible uses of the identified bioactive compounds in food preservation, medicine, and the production of nutraceuticals.

1.3 JUSTIFICATION OF STUDY

As global health issues like antibiotic resistance and diseases associated with oxidative stress continue to escalate, there is an increasing need for innovative natural therapeutic agents. *Monodora myristica*, known for its extensive ethnomedicinal history, stands out as a valuable source of bioactive compounds that may have pharmaceutical relevance.

The analysis of its phytochemicals through GC-MS not only provides scientific validation for its traditional applications but also aids in the identification of new compounds that could play a role in drug development.

CHAPTER TWO

LITERATURE REVIEW

Monodora myristica (African nutmeg) could be regarded as a medium-height, reaching 20 meters, evergreen, tropical flowering tree belonging to the Annonaceae family, the "spice tree" family, that inhabits evergreen forests in West Africa, including Nigeria, for culinary and medicinal uses (Igwe *et al.,* 2025). Each tree produces large fragrant seeds having spice qualities, and such seeds find application in the realm of traditional herbal medicine. *Monodora myristica* botanically is a medium-sized tree of about 20 meters in height. It has simple and alternate leaves, typical of the Annona family. The large, fragrant, predominantly yellow-green flowers with red markings are also characteristic of the family and attract pollinators (Okpoghono, 2025). Its fruit is a woody capsule containing seeds, with fragrant, fleshy, and pulpy arils. The seeds and other parts of the plant contain diverse phytochemicals like fatty acids, terpenoids, flavonoids, and alkaloids, which exhibit biological activities such as antioxidant, antibacterial, anti-inflammatory, and antifungal activities that form part of the herbal formulation of the plant (Igwe *et al.,* 2025; Okpoghono, 2025). The medicinal uses of *Monodora myristica* are to treat oxidative stress, infections, hypertension, and other stress-related disorders due to the active pharmacological biocomponents present. The spice is also used in the preparation of traditional meals.

2.1 BIOLOGICAL CLASSIFICATION AND NOMENCLATURE

Monodora myristica (Gaertn.) Dunal better known as African Nutmeg or Calabash Nutmeg grows in humid forests of West and Central Africa, where it is appreciated for economic and traditional medicine (Miediegha *et al.,* 2023). This tree is a very unique member of the

Annonaceae family. It is one of the very first flowering plants and has unusual features which sets it apart from all the others of this family.

2.1.1 TAXONOMY AND NOMENCLATURE

Monodora myristica is classified by the following taxa:

• Kingdom: Plantae

• Clade: Magnoliids

• Order: Magnoliales

• Family: Annonaceae

• Genus: Monodora

• Species: M. myristica (Gaertn.) Dunal

2.2 ORIGIN AND SIGNIFICANCE OF THE NAME

The term *"Monodora"* is derived from Greek and translates to "single gift." This designation underscores the tree's notable characteristic of bearing only one fruit at a time. The species epithet, *myristica*, highlights the plant's aromatic seeds, which possess a fragrance similar to that of true nutmeg (*Myristica fragrans*). As a result, these seeds are frequently utilized as a substitute spice in various local cuisines (Miediegha *et al.,* 2023).

2.3 GEOGRAPHIC DISTRIBUTION AND HABITAT

Monodora myristica is indigenous to the tropical regions of West and Central Africa. Its natural range extends from Sierra Leone in the west to Tanzania in the east and as far south as Angola (Miediegha *et al.,* 2023). The species is commonly encountered in the wild across several countries, including Nigeria, Ghana, Cameroon, Liberia, Uganda, and western Kenya (Agiriga, 2023).

This plant primarily thrives in humid, lowland evergreen rainforests and along the peripheries of deciduous forests. It is typically found near rivers, favoring locations with well-drained, fertile soils. *Monodora myristica* generally grows at elevations below 1,000 meters (Keay, 1989).

2.4 CULTIVATION, MORPHOLOGY, AND TRADITIONAL USES OF CALABASH NUTMEG (MONODORA MYRISTICA)

2.4.1 Cultivation and Historical Background

The calabash nutmeg tree (*Monodora myristica*) is indigenous to the evergreen forests of West and Central Africa, with its natural range extending from Liberia through Nigeria and Cameroon, as well as Ghana, Angola, Uganda, and western Kenya. During the 18th century, the species was introduced to the Caribbean islands as a result of the transatlantic slave trade, where it became established and is now commonly referred to as Jamaican nutmeg. In 1897, the tree was introduced to the Bogor Botanical Gardens in Indonesia. Although the trees flower regularly in this environment, fruit production has not yet been observed. Owing to its large, orchid-like flowers, *Monodora myristica* is also cultivated as an ornamental species (Weiss *et al.,* 2022).

2.4.2 Morphological Description

Vegetative Characteristics

Monodora myristica is a large, perennial evergreen tree, though it may occasionally shed its leaves. Mature specimens typically reach heights between 15 and 35 meters, with trunk diameters up to 2 meters at breast height (Agirigia, 2023).

The trunk is generally straight and supports a broad, rounded canopy formed by horizontally spreading branches.

The bark is initially smooth and greyish-brown, becoming fissured or vertically corrugated with age.

Leaves are arranged alternately, are simple and elliptic to oblong in shape, and possess a leathery texture. They may grow up to 45 centimeters in length and 20 centimeters in width. Young leaves often display a purplish hue before maturing to a glossy dark green, and a characteristic drooping habit is observed (Agirigia, 2023).

Floral Characteristics

The flowers of *Monodora myristica* are notable for their ornamental value. They are solitary, pendulous, and borne on long pedicels that may reach up to 20 centimeters, emerging from the base of new shoots. The flowers are large and fragrant, exhibiting a lantern-like appearance. Each flower consists of two whorls of petals: three large outer petals, approximately 10 centimeters in length, typically yellow-cream with prominent red-brown speckles and undulating margins; and three smaller, nearly triangular inner petals that form a yellowish cone around the flower's center. (Agirigia, 2023). The species is protogynous, with stigmas becoming receptive before the anthers mature, thereby encouraging cross-pollination. Beetles are the primary pollinators, drawn by the scent and structure of the flowers.

Reproductive Characteristics

The fruit of *Monodora myristica* is a large, sub-spherical berry that is initially green and smooth, developing a woody texture as it matures. Fruits typically measure between 15 and 20 centimeters in diameter and are supported by elongated stalks that can extend up to 60 centimeters (Agirigia, 2023). Inside the woody shell, numerous oblongoid, pale brown seeds—

usually numbering between 119 and 122—are embedded in a white, oily, and aromatic pulp. These seeds, measuring 1.5 to 2.5 centimeters in length, are the primary source of African nutmeg, which is widely used both as a spice and in traditional medicine (Agirigia, 2023; Miedieghe *et al.,* 2023).

2.5 ETHNOBOTANICAL SIGNIFICANCE AND TRADITIONAL USES

Monodora myristica holds significant value not only as a culinary spice but also for its applications in traditional African medicine. The seeds and their oil contain bioactive compounds that have been associated with antioxidant, antibacterial, anti-inflammatory, and antifungal properties. (Igwe *et al.,* 2025; Okpoghono, 2025).

Traditional Uses of the Seed (Kernel)

The seeds are used in a manner similar to true nutmeg, imparting a warm, aromatic flavor to a variety of dishes. They are commonly added to soups, stews, and porridges in local cuisines. (Miediegha *et al.,* 2023). Traditionally, the crushed seeds serve as remedies for several conditions such as in treatment of infections, inflammatory conditions (Oluwasegun *et al.,* 2025; Igwe *et al.,* 2025). The oil extracted from the seeds is a principal component in traditional medicinal preparations. (Oluwasegun *et al.,* 2025).

(Source: Okechukwu, 2022)

Fig2.1 *M.myristica seed (kernel)*

2.5.2 Traditional Uses Of The Shell And Other Plant Parts

Although the seed is often the primary focus of research, the shell (pericarp), leaves, and bark of the plant have also been utilized in traditional medicine. These components, while less extensively studied, play a significant role in various traditional remedies.

1. **Medicinal Applications**

   - **Antimicrobial and Antioxidant Properties**:

   Extracts from the shell are recognized for their high levels of secondary metabolites, particularly phenolic compounds. These substances enhance the plant's medicinal value and are traditionally used for their antibacterial effects (Ejiofor *et al.,* 2013).

   - **Treatment of Fevers and Body Aches:**

Decoctions prepared from the bark or leaves are sometimes employed to address fevers and general body aches. This practice is based on the plant's known anti-inflammatory properties (Okpoghono, 2025).

   - **Primary Sources:**

The shell and leaf extracts serve as the main sources for these traditional medicinal uses (Ejiofor *et al.,* 2013).

Fig2.2 *Other Parts of M.myristica plant (Source: Sela seeds, 2019)*

2.6 HEALTH BENEFITS OF MONODORA MYRISTICA SEED AND SHELL

Monodora myristica, commonly known as African Nutmeg, is recognized for its rich nutritional profile and diverse range of bioactive phytochemicals. Traditionally used across Africa, particularly as a spice and in medicinal applications, its benefits are increasingly supported by scientific research. The health advantages of this plant extend from general nutritional support to specific therapeutic and pharmacological effects.

2.6.1 Health Benefits Of Monodora Myristica Seed (Kernel)

The seed of *Monodora myristica* is the most thoroughly studied and widely utilized part of the plant. Its value in human nutrition and disease management is attributed to its high content of essential oils, fatty acids, and potent phytochemicals (Miediegha *et al.,* 2023).

1. **Cardiovascular and Lipid Health**

- ***Cholesterol and Hypolipidemic*** **Activity**: Research involving animal models indicates that aqueous extracts of the seeds can significantly reduce serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol, while increasing high-density lipoprotein cholesterol (Onyenibe *et al.,* 2015). This supports its traditional use in managing chronic cardiovascular conditions.
- ***Anti-Hypertensive Activity***: The essential oil, which is rich in monoterpenoids such as p-Cymene and α-Phellandrene, has demonstrated a hypotensive effect in experimental studies (Koudou *et al.,* 2007), aligning with its traditional application for hypertension.

- ***Mineral Balance:*** The seed contains a high concentration of potassium and maintains a favorable sodium-to-potassium ratio (less than 1), which is recommended for the prevention and management of high blood pressure (Daniel *et al.,* 2024).

2. Antioxidant and Anti-inflammatory Properties

- ***Potent Antioxidant Activity***: The seed is a source of antioxidants such as flavonoids, phenols, vitamin C, and β-carotene (Daniel *et al.,* 2024). These compounds are effective in neutralizing free radicals, protecting cells from oxidative damage, and modulating lipid peroxidation (Ukachukwu *et al.,* 2018).
- ***Anti-inflammatory Effects***: Scientific evidence supports the seed's traditional use as an anti-inflammatory agent. Compounds like linoleic acid and p-Cymene contribute to this effect by modulating inflammatory pathways (Igwe *et al.,* 2025). The root bark has also been shown to reduce acute inflammation (Isiogugu *et al.,* 2023)

3. Gastrointestinal and Metabolic Health

- ***Aids Digestion and Relieves Constipation***: The seed provides dietary fiber (up to approximately 21.9%), which supports digestive health, alleviates constipation, reduces bloating, and helps prevent metabolic and digestive disorders (Daniel *et al.,* 2024). Traditionally, the powdered kernel is used as a stimulant to relieve constipation.
- ***Blood Sugar Regulation***: Recent studies indicate that *Monodora myristica* may help regulate blood sugar levels and improve glucose metabolism, making it relevant for individuals with or at risk of diabetes (Smoky Hazel, 2023).

- ***Hepatoprotective Activity:*** Extracts from the seed have demonstrated the ability to reverse liver toxicity induced by high-cholesterol diets, suggesting a protective effect on liver health (Onyenibe *et al.,* 2015)

4. Reproductive and Sexual Health

- ***Enhanced Male Sexual Function***: Animal studies suggest that seed extracts can improve male sexual function by increasing sexual excitement and libido, as well as delaying ejaculation (Adienbo *et al.,* 2024). This supports its traditional use in addressing sexual dysfunction.
- ***Postpartum Tonic:*** Traditionally, the powdered kernel is included in pepper soup as a stimulant to help control passive uterine hemorrhage in women immediately after childbirth (Daniel *et al.,* 2024).

5. Antimicrobial and Germicidal Applications

- ***Antibacterial and Antifungal Properties:*** The essential oil and extracts exhibit significant activity against various pathogens, including both Gram-positive and Gram-negative bacteria, supporting their use in managing enteric infections and in pharmaceutical preparations (Okechukwu *et al.,* 2022).
- ***Skin Applications:*** Roasted and ground seeds are traditionally applied to the skin for the treatment of skin diseases, indicating germicidal or antiseptic properties (Nkwocha *et al.,* 2018; Okafor, 2003).

6. Nutritional Value and General Wellness

- ***Rich in Micronutrients***: The seed is a valuable source of essential minerals such as calcium, iron, magnesium, manganese, zinc, and copper, as well as vitamins including vitamin C and B-complex vitamins (thiamin, riboflavin, niacin, and folic acid) (Daniel *et al.,* 2024).
- ***Brain Function***: Compounds found in the spice have been used traditionally to relieve headaches and are reported to enhance brain function, improve memory, and increase focus (Ishola *et al.,* 2016). The oil has also demonstrated anti-depressant effects in animal studies (Afolabi *et al.,* 2024).

2.6.2 Health Benefits of Monodora myristica Shell and Other Parts

Although less extensively studied than the seed, the shell, bark, and root of *Monodora myristica* are important components of the plant's traditional medicinal uses. These parts offer distinct health benefits due to their unique phytochemical profiles.

1. **High Antioxidant Source**

- ***Polyphenolic Concentration***: The shell (pericarp) and bark often contain higher levels of bound phenolics and tannins compared to the seed. These compounds provide strong antioxidant capacity, which is important for neutralizing free radicals and potentially reversing oxidative damage associated with various diseases (Ejiofor *et al.,* 2013).

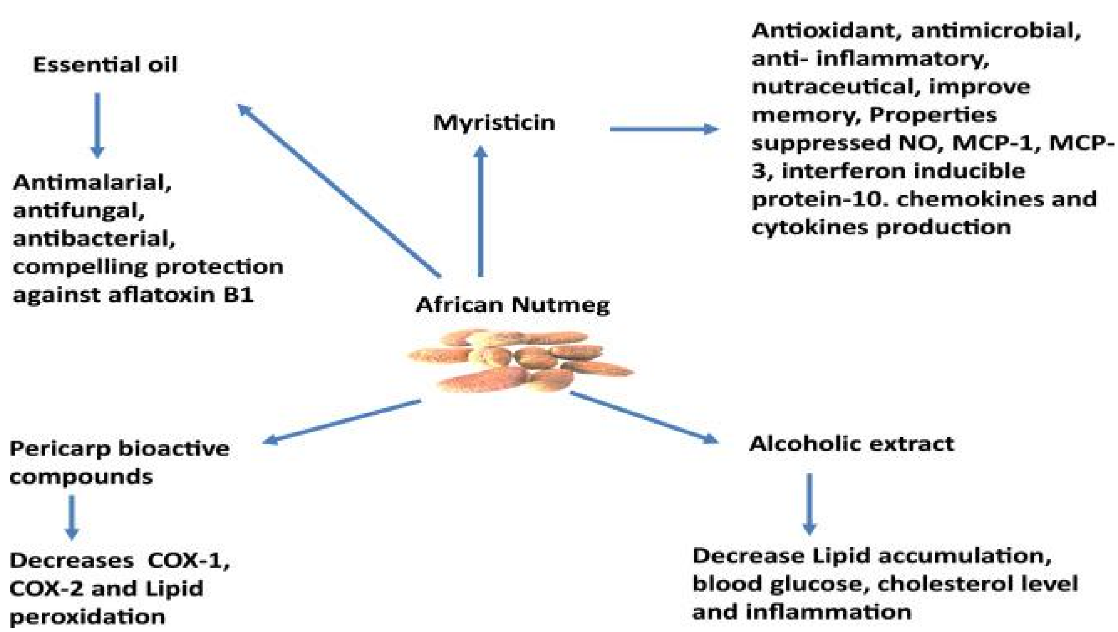
2. **Traditional Treatment of Specific Ailments**

- ***Hemorrhoids and Stomach Aches***: The stem bark is traditionally used to treat hemorrhoids, stomach aches, fever pains, and eye diseases (Daniel *et al.,* 2024). This suggests the presence of anti-inflammatory and analgesic compounds in the bark.

- ***Anti-inflammatory (Root Bark***): Research on the root bark has shown effectiveness in reducing both acute and chronic inflammation, further supporting the plant's use in managing inflammatory conditions (Isiogugu *et al.,* 2023).

3**. Potential for Natural Medicine and Preservatives**

- ***Antimicrobial and Antifungal Activity***: Extracts from the shell and bark possess antimicrobial and antifungal properties, which corroborates their traditional use in remedies for infections and skin conditions (Okechukwu *et al.,* 2022; Enabulele *et al*., 2014).
- ***Insect Repellent:*** The seeds and other parts are traditionally crushed and used as repellents for insects, fleas, and lice, indicating the presence of volatile compounds with insecticidal properties (Ileke,2018).



Source: Science Direct 2024

Fig2.3 Miscellaneous health benefits of African nutmeg

2.7 EXISTING GC-MS REPORT ON MONODORA MYRISTICA SEED AND SHELL EXTRACT

Gas chromatography–mass spectrometry (GC-MS) is a powerful analytical technique used for separating, identifying, and quantifying volatile and semi-volatile compounds in complex mixtures, widely applied in phytochemistry, forensics, pharmaceuticals, and environmental analysis. GC-MS combines two major technologies:

Gas Chromatography (GC): Which separates mixture components based on differences in their volatility and interactions with the stationary phase inside a capillary column. The sample is vaporized and carried by an inert gas (such as helium) through the column. Compounds with distinct properties move through the column at different speeds (retention times), leading to their separation.

Mass Spectrometry (MS): As compounds elute from the GC, they enter the mass spectrometer, where they are ionized (usually via electron impact). The mass spectrometer fragments the molecules and sorts the charged ions according to their mass-to-charge (m/z) ratio. Detectors record the abundance of each fragment, generating a mass spectrum that acts as a molecular fingerprint for each compound.

2.7.1 GC-MS ANALYSIS OF MONODORA MYRISTICA SEED AND SHELL EXTRACTS

Gas Chromatography-Mass Spectrometry (GC-MS) remains the benchmark analytical method for characterizing the complex chemical composition of essential oil and solvent extracts from *Monodora myristica* (African nutmeg) seeds and shells. Through this technique, researchers can identify and quantify volatile and semi-volatile phytochemicals that contribute to the plant's distinctive aroma, flavor, and pharmacological properties.

2.7.2 Phytochemical Profiling Of Seed Extracts

Recent studies have utilized GC-MS to examine the bioactive compounds present in *Monodora myristica* seed and shell extracts, emphasizing their phytochemical diversity and potential therapeutic applications.

2.7.2.1 Major Constituents Identified

One investigation, employing an Agilent GC-MS 7890B/5970MSD system with a DB-WAX capillary column, identified 26 compounds in the seed oil. Notable constituents included aromatic hydrocarbons such as p-cymene and unsaturated fatty acids like linoleic acid. These compounds are associated with the seed oil's antimicrobial and anti-inflammatory properties, with significant antibacterial activity observed against Staphylococcus aureus and Escherichia coli (Oluwasegun *et al.,* 2025). The study also reported that aromatic hydrocarbons, such as metal-cymene, had the lowest molecular weight, while linoleic acid had the highest.

Another GC-MS study detected approximately 25 related compounds in the seed extract, including several previously unreported in the species. Key compounds identified were α-Limonene, which is recognized for roles in weight management, cancer prevention, bronchitis treatment, and as a pharmaceutical excipient. Germacrenes, which is known for antimicrobial and insecticidal effects. Fatty acids including, cis-oleic acid and oleic acid (in triglyceride form), which are common in the diet and have applications in soap, pharmaceuticals, and aerosols. Other fatty acids identified include n-hexadecanoic acid, pentadecanoic acid, n-octadecanoic acid, eicosanoic acid, hexeicosanoic acid, docosanoic acid, and 9,15-octadecadienoate. These contribute to the nutritional value and potential anti-inflammatory benefits of the seeds. The study concluded that the detection of both known and novel compounds expands the phytochemical profile of *Monodora myristica* (Adewole *et al.,* 2013).

2.7.2.2 Toxicity and Physicochemical Properties

Further research, utilizing the Shimadzu GCMS-QP2010SE, assessed acute toxicity and physicochemical properties of the seed oil. The oil contained fatty acids such as n-hexadecanoic acid, cis-vaccenic acid, and 9,12-octadecadienoic acid, as well as terpenoids like alpha-terpineol and alpha-cadinol (Miediegha *et al.,* 2023). Toxicity evaluation classified the oil as moderately toxic, with an LD50 of 316 mg/kg, indicating the need for caution in therapeutic use but supporting its potential in food and pharmaceutical formulations (Akor *et al.,* 2023).

2.7.3 Analysis Of Shell Extracts

Although less frequently studied than the seeds, shell extracts have demonstrated promising profiles, particularly in secondary metabolites. Antimicrobial assessments indicate that both seed and shell extracts contribute to the medicinal value of *Monodora myristica*, suggesting that comprehensive analysis of all plant parts may facilitate the discovery of new therapeutic agents (Ejiofor *et al.,* 2013).

GC-MS profiling of shell extracts confirmed the presence of phenolic compounds, such as Phenol, 2,4-bis(1,1-dimethylethyl), which significantly enhance the plant’s antioxidant activity (Ejiofor *et al*., 2013). Solvent fractionation studies have identified up to 97 bioactive compounds in butanol, dichloromethane, and ethyl acetate fractions, further confirming the presence of alkaloids, terpenes, fatty acids, and phenolic compounds (Oluwole *et al.,* 2025).

GC-MS analyses consistently demonstrate that *Monodora myristica* seed and shell extracts contain a wide range of bioactive chemical classes, including fatty acids, aromatic hydrocarbons, terpenoids, and phenolics. These compounds are responsible for the plant's notable antimicrobial, antioxidant, and anti-inflammatory activities. The findings underscore the importance of integrated toxicological and pharmacological evaluations to ensure the safe application of these natural products in food preservation and drug development

2.8 KEY FINDINGS IN SEED OIL AND OLEORESIN

GC-MS analysis consistently reveals that the seed extracts are rich in **aromatic hydrocarbons**, **terpenoids**, and **fatty acids**.

Table 2.1 *Key Findings In Seed Oil And Oleoresin.*

Extract Type	GC–MS Findings and Key Compounds	Source and Notes
Essential Oil (EO)	Dominated by monoterpene hydrocarbons: p-Cymene, α-Phellandrene, α-Pinene, β-Myrcene. Used for essential oil profiling and highly volatile components.	Oluwasegun *et al.,* (2025)

Extract Type	GC–MS Findings and Key Compounds	Source and Notes
Seed Oil/Oleoresin	Contains major fatty acids (requires derivatization): n-Hexadecanoic acid (Palmitic acid), Linoleic acid (9,12-Octadecadienoic acid), cis-Oleic acid. Also detects terpenoids like α-terpineol..	Ijaz *et al.,* (2023), Miediegha *et al.*, (2023)
Phenolic/Polar Extracts	Earlier GC-FID work (2014) focusing on phenolics reported high levels of phenylpropanoids typical of *Myristicaceae* relatives: Myristicin (42.60%), Caffeic acid (23.39%), Elemicin (3.82%) (Feyisayo & Oluokun, 2014).	Feyisayo & Oluokun (2014)

Source: (Igwe et al., 2025)

2.9 MAJOR COMPOUNDS IDENTIFIED BY GC–MS AND THEIR PHARMACOLOGICAL ROLES

Research across different regions and analytical methods consistently shows that the essential oils (EOs) of *Monodora myristica* seeds are dominated by a core group of monoterpenes.

The most commonly reported major constituents include p-cymene (and its o/p-isomers), α-phellandrene, α-pinene, β-myrcene, as well as varying levels of oxygenated monoterpenes such as α-terpineol and linalool. In addition to these, analyses of oleoresins and solvent extracts reveal the presence of fatty acids—such as hexadecanoic acid (palmitic acid) and linoleic acid—and semi-volatile phenolic derivatives, provided that suitable sample preparation and derivatization are performed. For example, a 2024 study from Côte d'Ivoire identified approximately 32 compounds in the seed essential oils, with ortho/p-cymene and α-phellandrene as dominant peaks. Similarly, a 2022 Nigerian study established a correlation between monoterpene content in the essential oils and its antibacterial and antioxidant activities.

2.9.1 Pharmacological Roles of Major Compounds

1. Monoterpenoids and Aromatic Hydrocarbons (Volatile Fraction)

These compounds are characteristic of the essential oil derived from *M. myristica* seeds. They contribute significantly to the oil's distinctive aroma and are responsible for its immediate biological effects.

❖ **p-Cymene**

Description: An aromatic monoterpene hydrocarbon, often present as a dominant peak in the seed essential oil, sometimes constituting over 24% of the total composition.

Pharmacological Roles:

- **Anti-inflammatory**: Preclinical studies indicate that p-cymene acts as an anti-inflammatory agent by modulating cytokines and pathways such as NF-κB and MAPK. (Balahbib *et al.,* 2021).
- **Antimicrobial**: This compound contributes to the oil's antimicrobial and antifungal properties, often working in synergy with other terpenes. (Balahbib *et al.,* 2021).
- **Antioxidant:** p-Cymene demonstrates notable radical scavenging activity, supporting the traditional use of the seed in managing oxidative stress. (Igwe *et al.,* 2025).

❖ **α-Phellandrene**

Description: A bicyclic monoterpene hydrocarbon frequently identified as a major constituent in various regional EOs. (Traoré *et al.,* 2024).).

Pharmacological Roles:

- **Antimicrobial and Antifungal:** Exhibits activity against a range of microbial and fungal pathogens.
- **Anti-inflammatory**: Contributes to the anti-inflammatory effects of the oil, supporting its traditional use for inflammation and pain (Ishola *et al.,* 2016; Okechukwu *et al.,* 2022; Afolabi *et al.,* 2024).
- **Pest Control**: Noted for its insecticidal properties in ethnobotanical contexts (Ileke, 2018; Wandjou *et al.,* 2022).

❖ **Oxygenated Monoterpenes (α-Terpineol, Linalool)**

Description: These oxidized monoterpenes, including linalool (with a floral scent) and α-terpineol, are commonly found in the essential oil. (Ijaz *et al.,* 2023).

Pharmacological Roles:

- **Enhanced Bioactivity**: Oxygenated monoterpenes generally exhibit higher bioactivity compared to their hydrocarbon counterparts, contributing significantly to the oil's functional profile.
- **Antimicrobial/Antioxidant**: Laboratory studies confirm that these compounds possess strong antimicrobial and antioxidant properties, which are critical to the efficacy of the essential oil.

2. **Fatty Acids (Non-Volatile/Oleoresin Fraction)**

Long-chain carboxylic acids are prominent in the fixed oil fraction of the seeds, where they primarily influence nutritional value, physicochemical characteristics, and prolonged therapeutic effects.

❖ **n-Hexadecanoic Acid (Palmitic Acid)**

Description: A saturated long-chain fatty acid, often accounting for over 24% of the total fat content in the seed oil. (Miediegha *et al.,* 2023).

Pharmacological Roles:

- **Nutritional Value**: Essential for energy storage and cell membrane integrity.
- **Anti-inflammatory Potential**: Although it is a saturated fat, its presence in plant oils is linked to various biological activities, including potential anti-inflammatory and antioxidant effects. (Adewole *et al.,* 2013).

❖ **Linoleic Acid (9,12-Octadecadienoic Acid)**

Description: An essential polyunsaturated omega-6 fatty acid, often present in high abundance (up to 17.5%). (Ijaz *et al.,* 2023).

Pharmacological Roles:

- **Anti-inflammatory**: As a precursor to eicosanoids, linoleic acid plays a key role in regulating inflammation and immune responses, supporting traditional uses against inflammatory conditions. (Adewole *et al.,* 2013).
- **Cardiovascular Health**: Polyunsaturated fatty acids such as linoleic acid are associated with cardiovascular benefits, including the management of hypertension.

- **Antimicrobial**: This fatty acid also demonstrates antimicrobial activity, contributing to the oil's effectiveness against certain pathogens. (Ijaz *et al.,* 2023).

3. Phenylpropanoids (Specialized Components)

Phenylpropanoids are aromatic compounds structurally related to cinnamic acid. They are notable for their strong biological activity, significant presence in some extracts, and their relevance to safety considerations, particularly in relation to true nutmeg.

❖ **Myristicin**

Description*:* A methoxy derivative of allylbenzene, myristicin is a characteristic phenylpropanoid of the Myristicaceae family. It has been detected at high concentrations (up to 42.6%) in some seed extracts. (Feyisayo *et al.,* 2014).

Pharmacological Roles:

- **Aromatic/Flavouring**: Myristicin is a major contributor to the nutmeg-like aroma and flavor of the seeds.

Safety Concern: Due to its high abundance, caution is warranted. While myristicin has insecticidal and antimicrobial properties, it is also responsible for the psychoactive and toxic effects associated with excessive consumption of true nutmeg. (Feyisayo *et al.,* 2014). Its detection is therefore important for establishing safety guidelines.

Table2.2: *Major Compounds and Their Pharmacological Roles*

Compound	**Role and Activity**	**References**

Compound	Role and Activity	References
p-Cymene	Anti-inflammatory, antioxidant, antimicrobial; modulates inflammatory pathways (NF-κB, MAPK); synergistic in essential oils	Balahbib *et al.,* 2021; Oluwasegun *et al*., 2025
α-Phellandrene	Antimicrobial, antifungal, anti-inflammatory; potential for pest control	Abbas *et al.,* 2024
α-Pinene, β-Myrcene	Antibacterial, anti-inflammatory, bronchodilatory; impacts microbial membranes	Salehi *et al.,* 2019; Surendran *et al.,* 2021; Masyita *et al.,* 2022
Oxygenated Monoterpenes	(α-terpineol, linalool) More bioactive per molecule; strong antimicrobial and antioxidant effects	Marei *et al.,* 2019; Badawy et al., 2019
Myristicin, Elemicin	Phenylpropanoids with psychoactive effects; potential toxicity; contribute aroma and medicinal properties	Feyisayo *et al.,* 2014
Fatty acids (palmitic, linoleic, oleic acids)	Nutritional value; anti-inflammatory and antimicrobial	Miediegha *et al.,* 2023; Ijaz *et al.,* 2023

These bioactives explain the species' reported efficacy in managing hypertension, infections, oxidative stress-related ailments, and supporting antimicrobial and anti-inflammatory applications (Igwe *et al.,* 2025; Okpoghono, 2025; Balahbib *et al.,* 2021).

2.10 EVIDENCE FROM FUNCTIONAL STUDIES: ANTIMICROBIAL, ANTIOXIDANT, TOXICOLOGICAL, AND OTHER ACTIVITIES

Between 2020 and 2024, several studies have combined gas chromatography–mass spectrometry (GC–MS) analysis with both in vitro and in vivo assays to investigate the properties of *Monodora myristica*. In 2022, research conducted in Nigeria demonstrated that extracts exhibited antibacterial activity against Escherichia coli and Staphylococcus aureus. The study also assessed DPPH radical scavenging capacity, noting that oleoresin and essential oil (EO) displayed different levels of activity, likely due to variations in their chemical composition. The antioxidant potential was further supported by findings that seed extracts enhanced endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which collectively help mitigate oxidative stress.

A 2023 phytotoxicity screening evaluated the effects of seed essential oil on the growth of crop seedlings and established a link between chemical composition and observed herbicidal properties. Additional studies in rodents examined neuroprotective and metabolic effects, using GC–MS to document extract composition prior to biochemical analysis. These investigations reported beneficial changes in antioxidant enzyme activities and lipid profiles, although the effects were not always attributable to individual EO constituents.

Toxicological assessments have shown that *Monodora myristica* oil can cause moderate acute toxicity in rodents at certain doses, with reported LD50 values in the mid-range. This finding highlights the importance of conducting dosing studies and comprehensive safety profiling before considering the oil for food or therapeutic use. Collectively, these functional studies indicate that GC–MS profiles are valuable for predicting biological activity, but must be complemented by toxicological and dose–response evaluations to ensure safe application.

2.11 IMPORTANCE OF ANALYZING BOTH EDIBLE AND NON-EDIBLE PARTS

A comprehensive analysis of both the edible (seed or kernel) and non-edible (shell, pericarp, leaves, bark) parts of *Monodora myristica* is essential for maximizing the plant's potential in food, medicine, and industry (Ejiofor *et al.,* 2013).

This integrated approach elevates the species from a single-use spice to a versatile source of natural products.

1. **Complete Phytochemical Mapping and Scientific Clarity**

Examining all plant organs provides a thorough inventory of chemical diversity, which is fundamental for accurate scientific interpretation and practical utilization.

- **Organ-Specific Biosynthesis**: Different organs serve as distinct biosynthetic and storage centers. Seeds primarily store highly volatile monoterpenoids (such as p-Cymene and α-Phellandrene) and energy-rich fatty oils like linoleic acid, which contribute to the species' characteristic aroma and initial therapeutic effects. (Ijaz *et al.,* 2023). In contrast, shells, bark, and leaves accumulate less volatile compounds, including high molecular weight phenolic acids, tannins, and complex alkaloids. (Ejiofor *et al.,* 2013; Oluwole *et al*., 2025).

- **Targeted Extraction**: Comprehensive chemical mapping enables the strategic selection of plant parts and extraction methods to optimize yields of specific bioactive compounds. For example, seeds are ideal for essential oil extraction, while shells may provide a superior source of potent antioxidants.
- **Chemotaxonomy and Traceability**: Organ-specific GC–MS profiles reveal subtle chemical markers that are critical for verifying species identity and tracing the geographical origin of raw materials.

2. **Safety, Regulatory Compliance, and Quality Control**

Analyzing all plant parts, particularly the edible portions, is vital for establishing safe consumption parameters and ensuring product quality.

- **Toxicological Assessment**: Profiling the edible seed is necessary for public health. GC–MS detection of compounds such as myristicin, which is psychoactive and toxic at high concentrations, allows for accurate quantification and the establishment of safe consumption limits, thereby informing regulatory guidelines. (Feyisayo *et al.,* 2014)
- **Acute Toxicity Rationale**: Chemical data from various organs are required to interpret toxicity reports, such as the LD50 of 2,316 mg/kg for seed oil. This information helps identify which components contribute most to toxicity, guiding the development of safe dosing strategies (Akor *et al*., 2023).

- **Quality Assurance:** Analysis of the fatty acid profile in seed oil, including the ratio of unsaturated to saturated fats, is essential for assessing product quality, stability, and the potential for rancidity during storage.

3. **Economic Valorization and Circular Economy**

Limiting analysis to the seed alone results in the loss of valuable by-products. A comprehensive approach supports a sustainable, waste-minimizing economy.

- **Transformation of By-products**: Shells, husks, and leaves, often discarded, contain significant concentrations of bioactive compounds suitable for non-food applications. GC–MS characterization provides the necessary chemical evidence to convert these materials into value-added products.
- **New Product Development**

  Natural Preservatives: Antioxidant phenolics extracted from the shell can be used as natural preservatives in food or cosmetics, offering alternatives to synthetic additives (Ejiofor *et al.,* 2013).

  Agrochemicals: Insecticidal or fungicidal compounds found in the bark or leaves can be developed into environmentally friendly bio-pesticides.

- **Economic Optimization:** Identifying commercial uses for all parts of the tree maximizes economic yield and supports a more sustainable and profitable supply chain for local communities.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

All equipment utilized were of analytical grade. They include masking tape, tissues, gloves, face masks, aluminum foil, conical flasks(pyrex), universal containers, detergent, beakers of 500 mL and 1000 mL capacity (pyrex), a glass rod, a handkerchief, kenwood blender, weighing balance, stainless-steel bucket, sieve cloth, rotary evaporator (Rotavapor), lyophilizer. The GC-MS analysis was performed using an Agilent GC7890B coupled with the 5977A MSD, made in the USA.

Monodora myristica, commonly referred to as Calabash nutmeg, was specifically sourced from the Uselu market in Edo State, Nigeria. For this study, distilled water and ethanol served as solvents.

(Source: personal)

Fig 3.1 Image showing some materials used (sieve cloth, distilled water, absolute ethanol, stainless steel container, beakers, aluminium foil).

3.2 METHODS:

3.2.1 Sample Collection: A total of 1kg of Monodora myristica was procured from Uselu Market located in Edo State, Nigeria. This specimen was identified and authenticated at the Herbarium unit within the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, bearing the voucher number UBH-M350. The collected samples were properly cleaned to remove dirt, broken seeds, and foreign materials.

(Source: Okechukwu, 2022).

Fig 3.2 Image of Monodora myristica

3.2.2 Preparation of Plant Extract: The calabash nutmegs were cracked open, seeds and shells were separated, Subsequently, and ground using a blender to achieve fine particle size for optimal extraction.

(Source: personal)

Fig 3.3 Image showing blended Monodora myristica seed inside the stainless-steel container.

3.2.3 Hydro-Ethanol Extraction: The powdered seeds and shells samples were weighed using a weighing balance and placed in a stainless-steel container where they were unclumped. A hydroethanol solvent was prepared by mixing 800 mL absolute ethanol with 200 mL distilled water to make 1000 mL solution in a clean beaker. The samples were macerated in the solvent and the containers were covered with aluminum foil and sealed with masking tape to reduce evaporation and prevent contamination. Each container was labeled for identification of the seed and shell samples and left at room temperature for 72 hours to facilitate the thorough extraction of active constituents. During this time, the extract was periodically filtered through a sieve cloth to eliminate insoluble particles, ensuring that only the dissolved bioactive compounds were retained. The insoluble residues were dried on a clean, flat surface. The crude extracts were subsequently sent to the Pharmaceutical Chemistry staff research laboratory and the Energy Center at the University of Benin for rotary evaporation and freeze-drying, respectively.

Fig 3.4a

Fig 3.4b

Fig 3.4c

Fig 3.4d Fig 3.4e Fig 3.4f

Fig 3.4g (Source: personal)

Fig 3.4(a) Image showing the process of pouring the measured hydroethanol solution into the sample; (b) image showing the maceration of sample in solvent; (c) image showing the sealing of the mixture; (d) image showing the filtration of the mixture using a sieve cloth; (e) image showing the extracted product; (f) image showing the extracted product in a beaker to be stored; (g) image showing the residue from the extraction.

3.2.4 Rotatory Evaporation: Upon the conclusion of hydroethanol extraction, the resultant solution underwent rotatory evaporation, facilitated by a Rotatory evaporator, to carefully eliminate solvents such as ethanol and water from the samples through evaporation at reduced pressure and low temperature, thereby preventing the degradation of heat-sensitive compounds. The water bath was calibrated to maintain a temperature between 44° and 47°. All components of the apparatus (pump, condenser, water bath, vacuum) were properly assembled. The rotatory evaporator was connected to the electrical supply. Prior to transferring the mixture into the evaporating flask, it was agitated. The vacuum was filled with water, activated, and allowed to cool for approximately 15 minutes. The water bath was prepared, and the receiving flask was positioned beneath the condenser. The evaporating flask, containing the extract, was secured to the condenser with the flask cap. The condensing section of the apparatus was gently lowered to ensure contact with the water bath. The extract was continuously introduced into the flask until complete condensation of the extract was achieved. Following the rotatory evaporation process, the samples were subsequently lyophilized.

(Source: personal)

Fig 3.5 Image showing the rotavap used

3.2.5 Freeze drying (lyophilization): After the hydroethanol extract had fully condensed, the extracts were subjected to a freeze-drying procedure to concentrate and convert the sample into a powdered form. This procedure entailed freezing the solution under controlled conditions, followed by the sublimation of the solvent, which led to the creation of a stable, dry extract. The resulting powder was subsequently collected and stored at 4°C for further analysis using GC-MS.

Fig 3.6a Fig 3.6b Fig 3.6c

(Source: personal)

Fig 3.6(a) Image showing samples undergoing freeze-drying method; (b) image showing shell sample after freeze-drying; (c) image showing seed sample after freeze-drying

3.2.6 Procedure For Phytochemical Gc-Ms Analysis

The plant extract sample was prepared by dissolving it in a solvent at a ratio of 1:10 v/v. The solution was then filtered through a 0.45 µm nylon microfilter and transferred into a sample vial. Approximately 1 µL of the filtered sample was injected into the gas chromatograph at an injection port temperature of 300°C.

The sample entered the Gas Chromatography (GC) column, which had been conditioned in an oven initially set to 60°C and held for 2 minutes. The temperature was then increased at a rate of 10°C per minute until reaching 280°C, where it was held for an additional 5 minutes. During this process, the sample vaporized and its components were separated based on their mass-to-charge ratio (m/z).

These separated components were then directed to the Mass Selective Detector (MSD), where the resulting mass spectra were analyzed. The compounds were identified and quantified by comparison with a standard reference library using specialized data analysis software, which provided compound names along with their relative quantities expressed as percentages. Analyses were performed using an Agilent Technologies GC-MS system (GC7890B coupled with 5977A MSD, USA).

CHAPTER FOUR

RESULT

4.1 GC-MS Results for seed Extracts

A total of twenty-eight (28) compounds were identified in the extract of *Monodora myristica* seed. Some of the bioactive compounds with their peak values, retention time (RT) and percentage composition are shown in Table 1.

Table4.1: Summary of result of GC-MS analysis of Monodora myristica seed extract

Retention time	Peak Area (%)	Library/ID	Ref	CAS	Qual
8.1935	0.2589	Tau.-Cadinol	85698	005937-11-1	98
10.2564	0.4266	Tetradecanoic acid	91420	000544-63-8	25
12.0321	2.5062	n-Hexadecanoic acid	117418	000057-10-3	99
14.2854	29.4868	Linoelaidic acid	140124	000506-21-8	99
14.3642	21.9022	(E)-9-Octadecenoic acid ethyl ester	169327	006114-18-7	99
14.525	26.973	9,12-Octadecadienoic acid (Z,Z)-	140138	000060-33-3	99
13.7254	0.1711	Methyl stearate	157880	000112-61-8	96

.

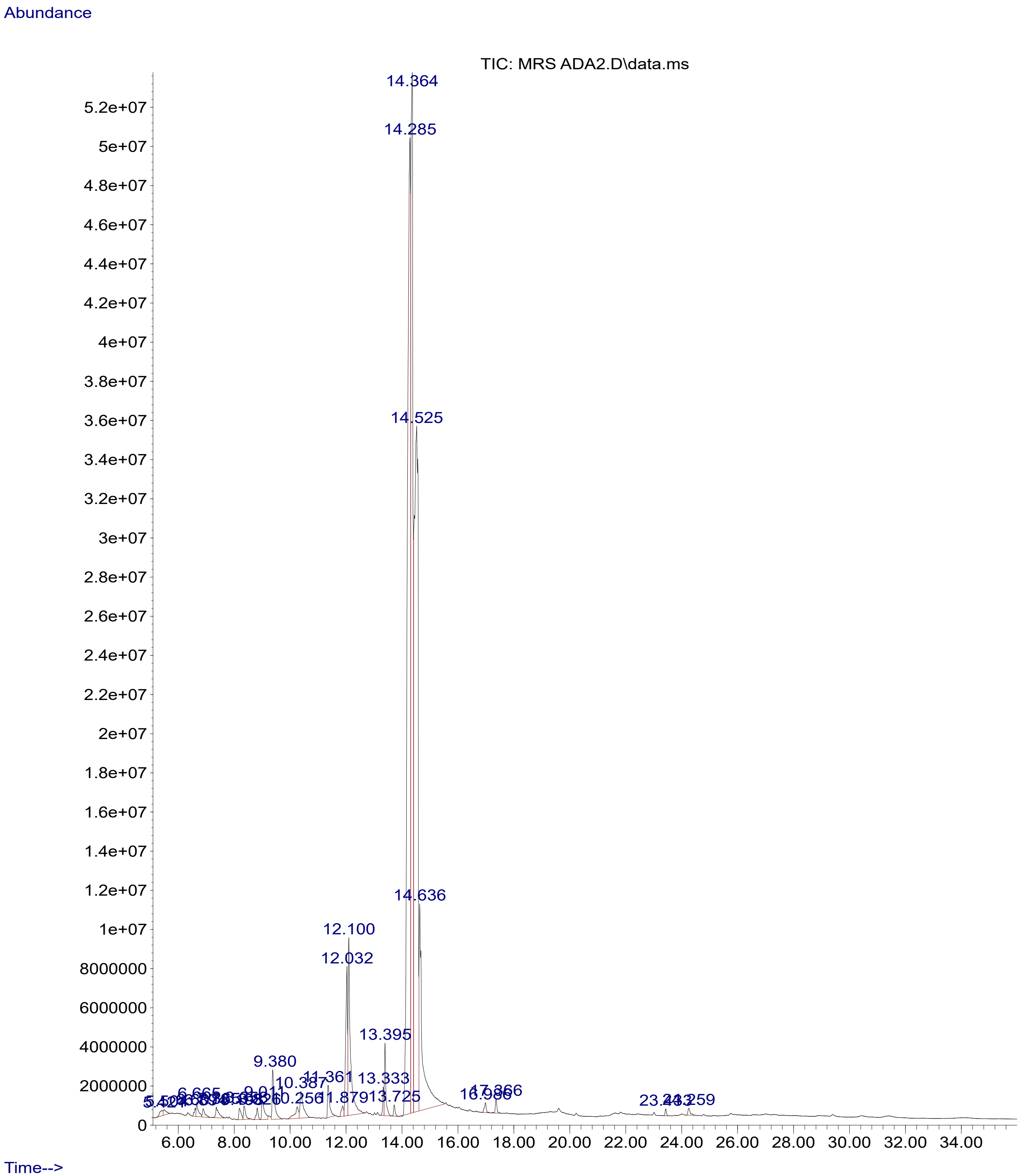


Fig 4.1 chromatograph of the bioactive compounds of GC-MS analysis of monodora myristica seed extract

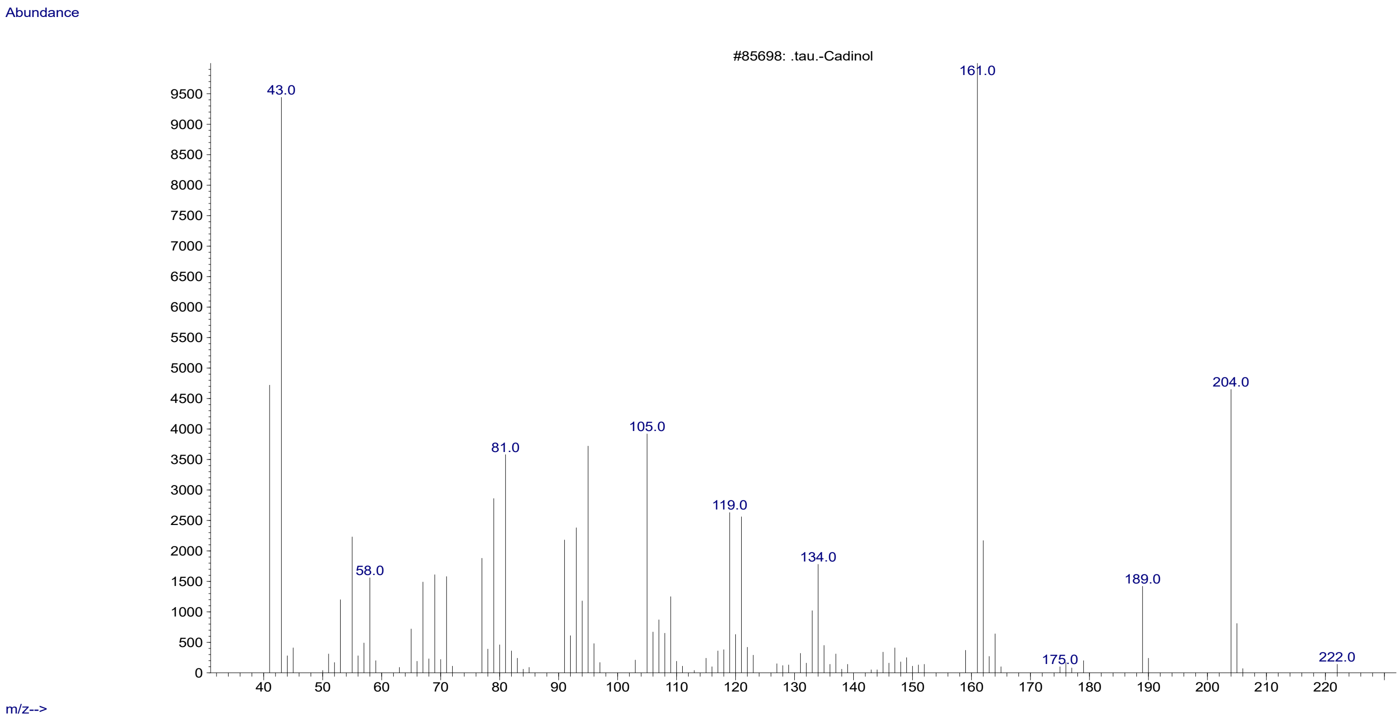


Fig 4.2 chromatogram of tau-cadinol

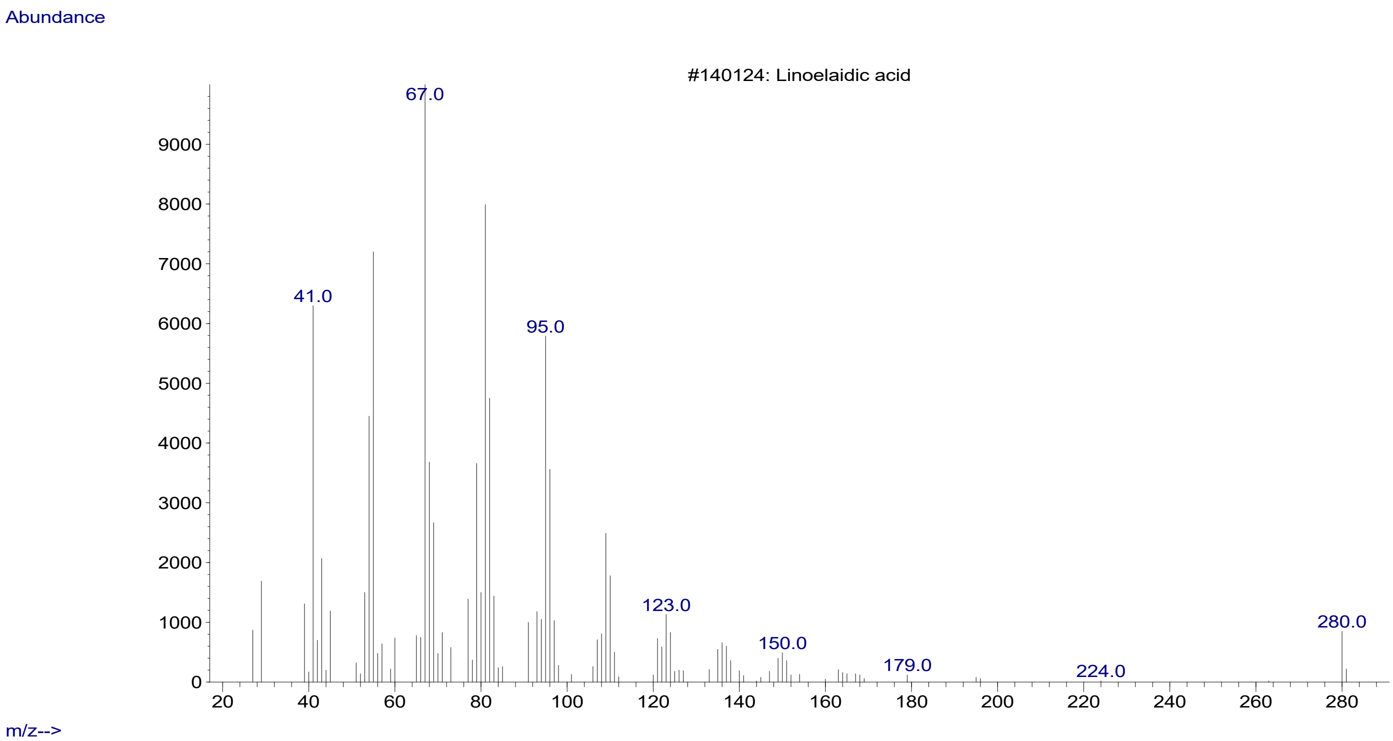


Fig 4.3 chromatogram of Linoelaidic acid

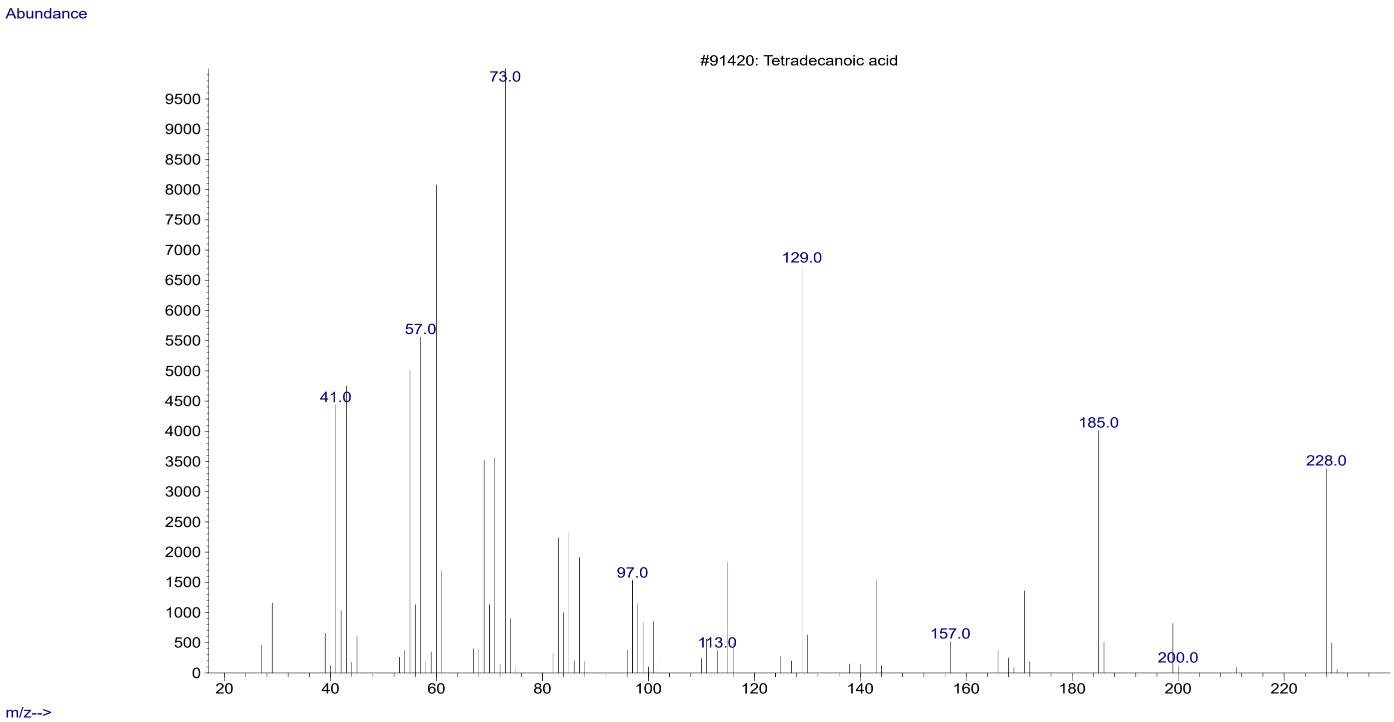


Fig 4.4 chromatogram of tetradecanoic acid

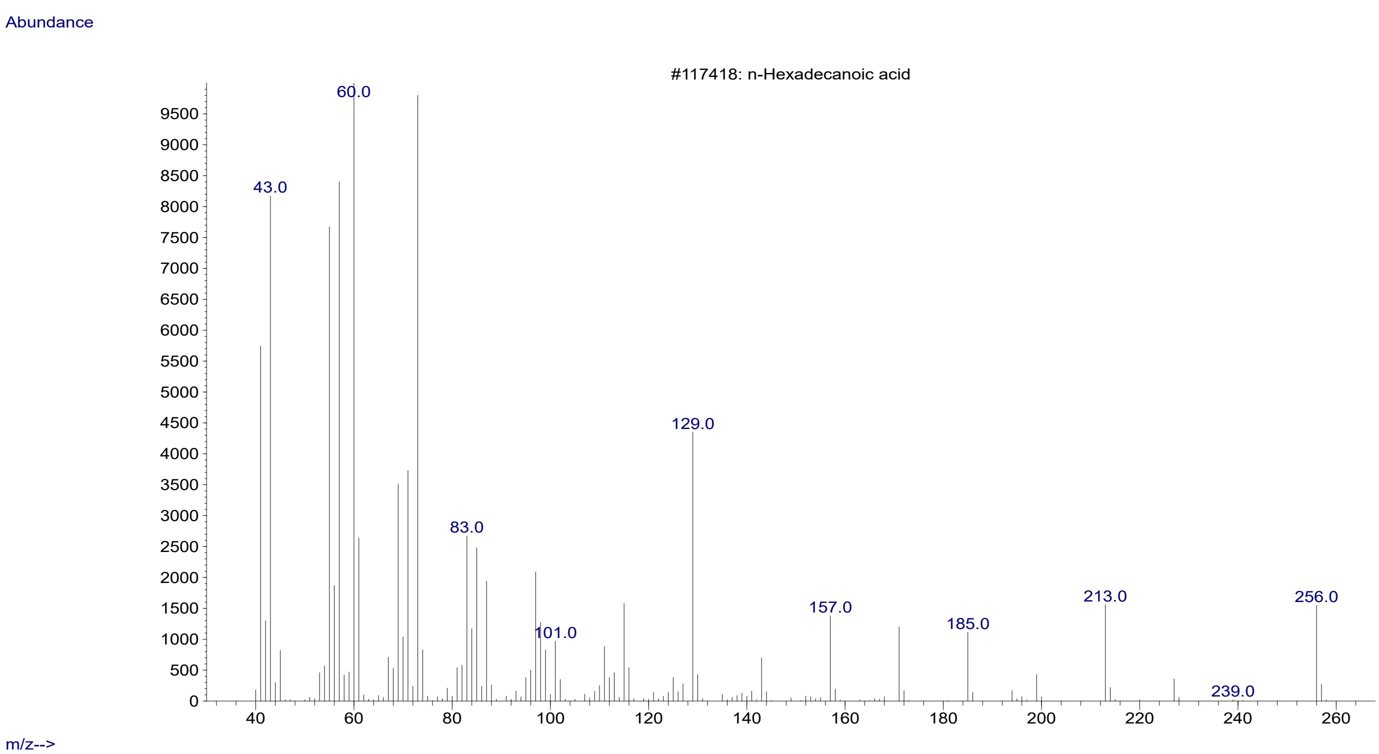


Fig 4.5 chromatogram of n-Hexadecanoic acid

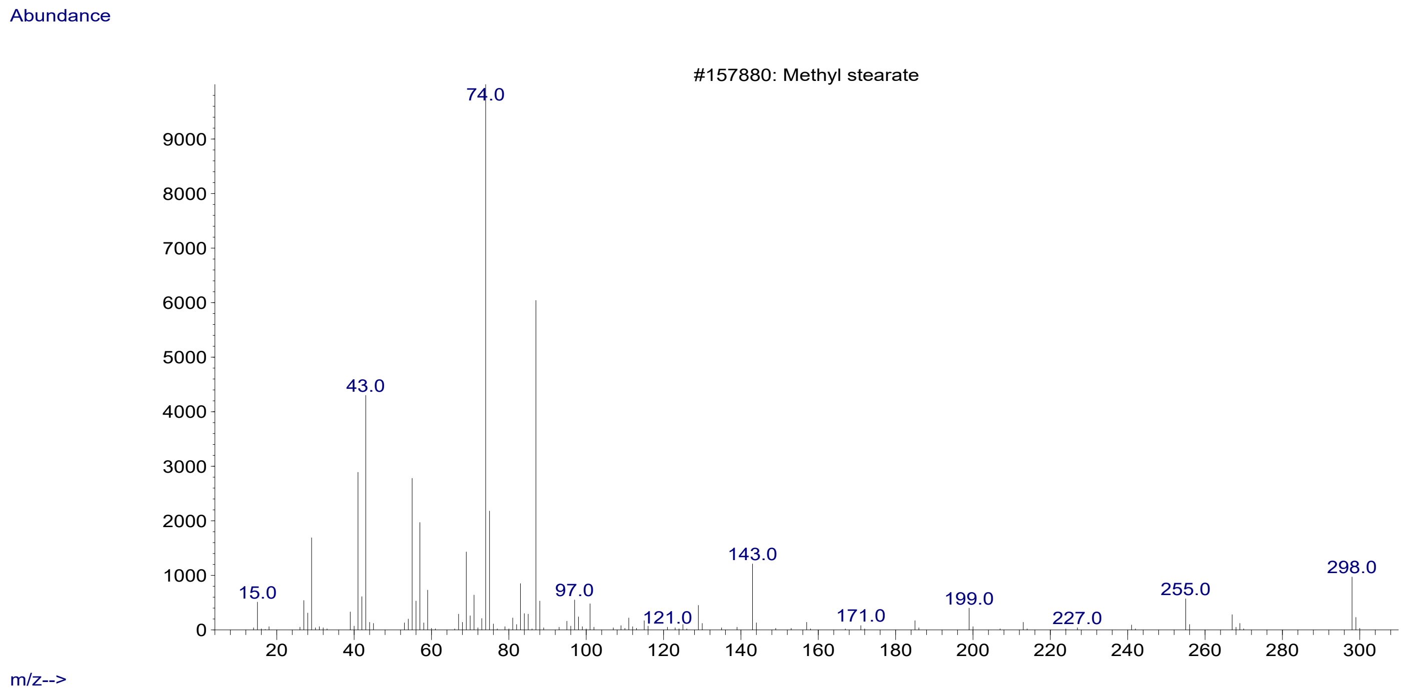


Fig 4.6 chromatogram of methyl stearate

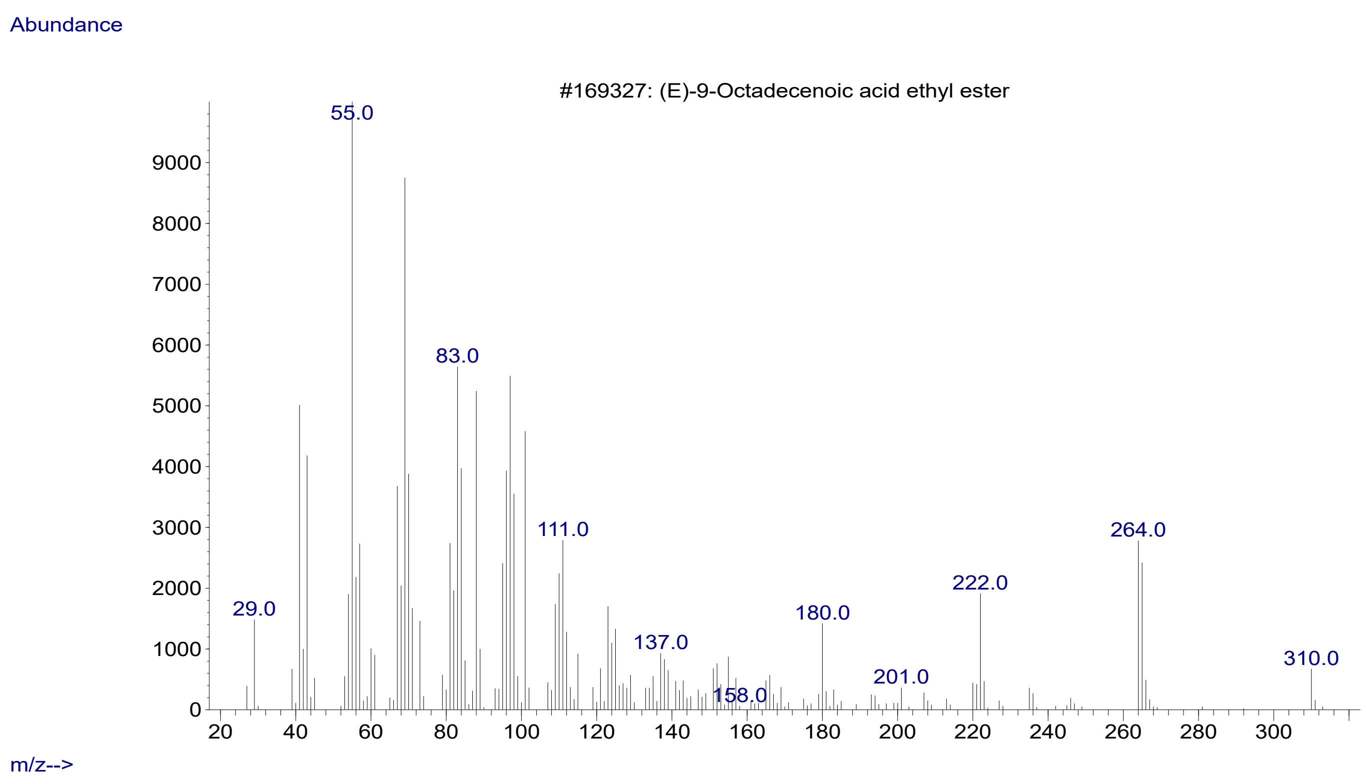


Fig 4.7 chhromatogram of (E)-9-Octadecanoic acid ethyl ester

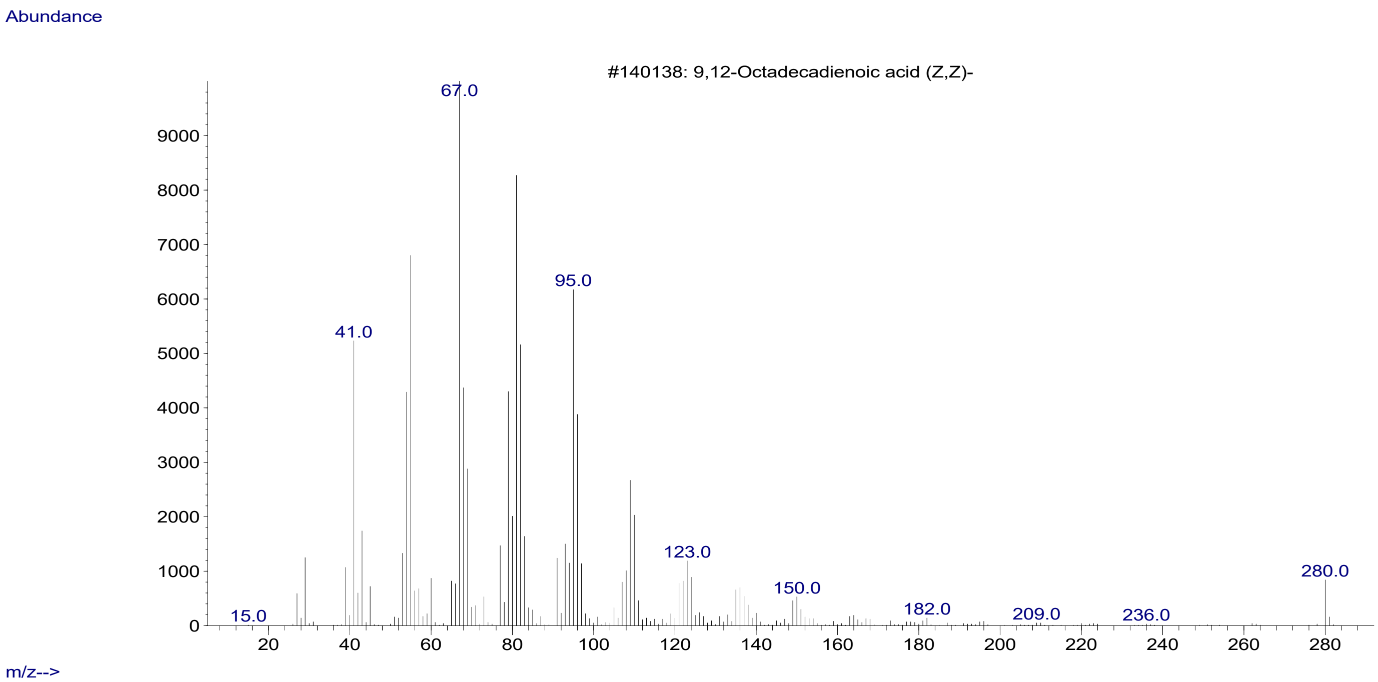


Fig 4.8 chromatogram of 9,12-octaecadienoic acid (z,z)-

Chromatograms generated by the GC-MS were analyzed using the instrument's software to identify and quantify individual components. Relative percentage composition of each constituent was calculated based on peak area normalization.

4.2 GC-MS Results for shell Extracts

A total of fifteen (15) compounds were identified in the extract of *Monodora myristica* shell. Some of the bioactive compounds with their peak values, retention time (RT) and percentage composition are shown in Table 2.

Table 4.2: Summary of result of GC-MS analysis of Monodora myristica shell extract

Retention time	Peak Area (%)	Library/ID	Ref	CAS	Qual
7.3647	1.3521	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol	85774	198991-79-6	97
9.3835	4.0509	1-((1S,3aR,4R,7S,7aS)-4-Hydroxy-7-isopropyl-4-methyloctahydro-1H-inden-1-yl)ethanone	100430	001911-78-0	97
11.4023	3.9151	Pentadecanoic acid, 14-methyl-, methyl ester	130841	005129-60-2	98
12.1407	3.2255	Hexadecanoic acid, ethyl ester	144309	000628-97-7	97
13.3962	12.8067	10-Octadecenoic acid, methyl ester	155731	013481-95-3	99
14.1918	22.2113	Linoleic acid ethyl ester	167366	000544-35-4	99
14.264	35.0846	(E)-9-Octadecenoic acid ethyl ester	169327	006114-18-7	97

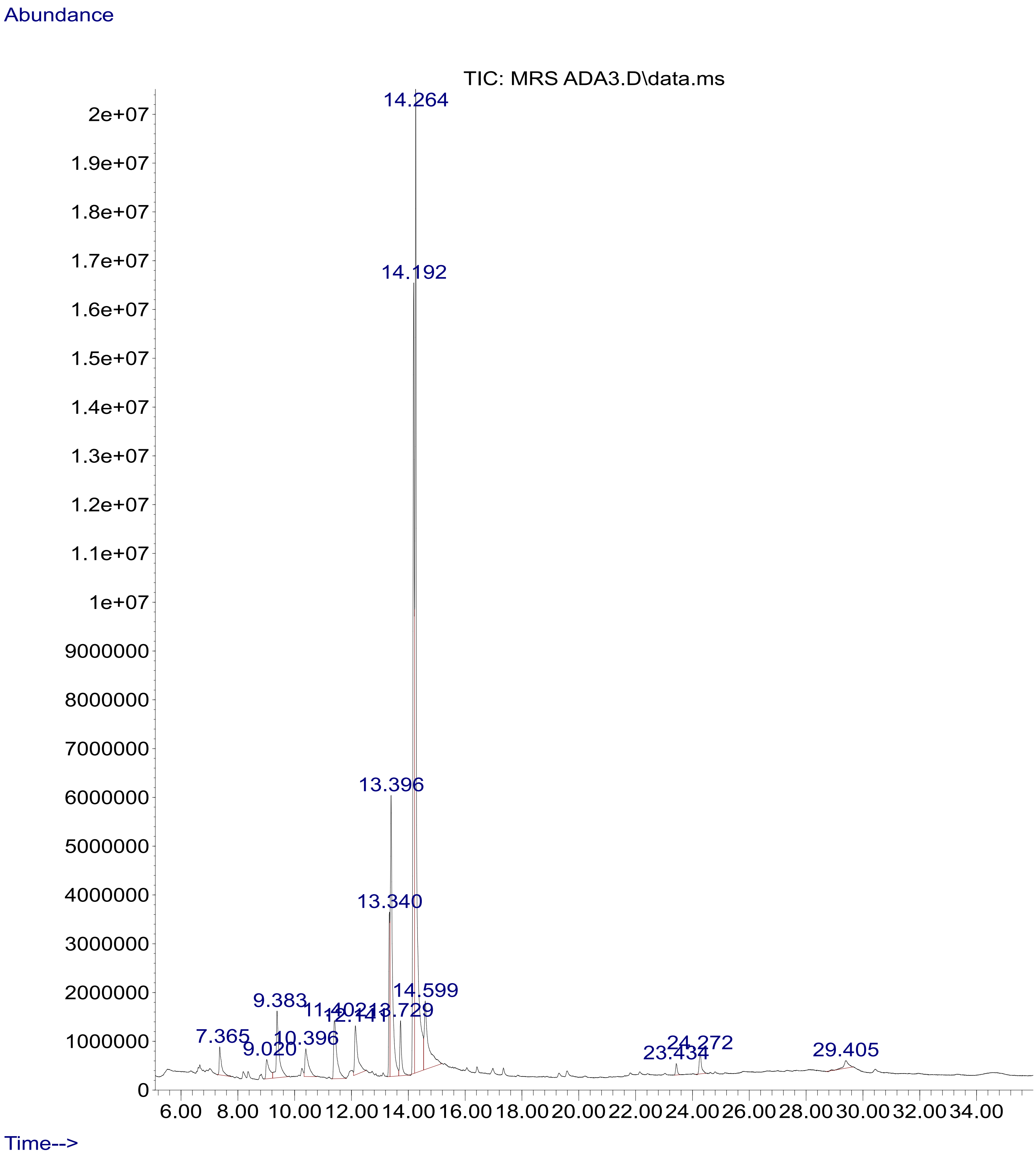


Fig 4.9 chromatograph of the bioactive compounds of GC-MS analysis of monodora myristica shell extract

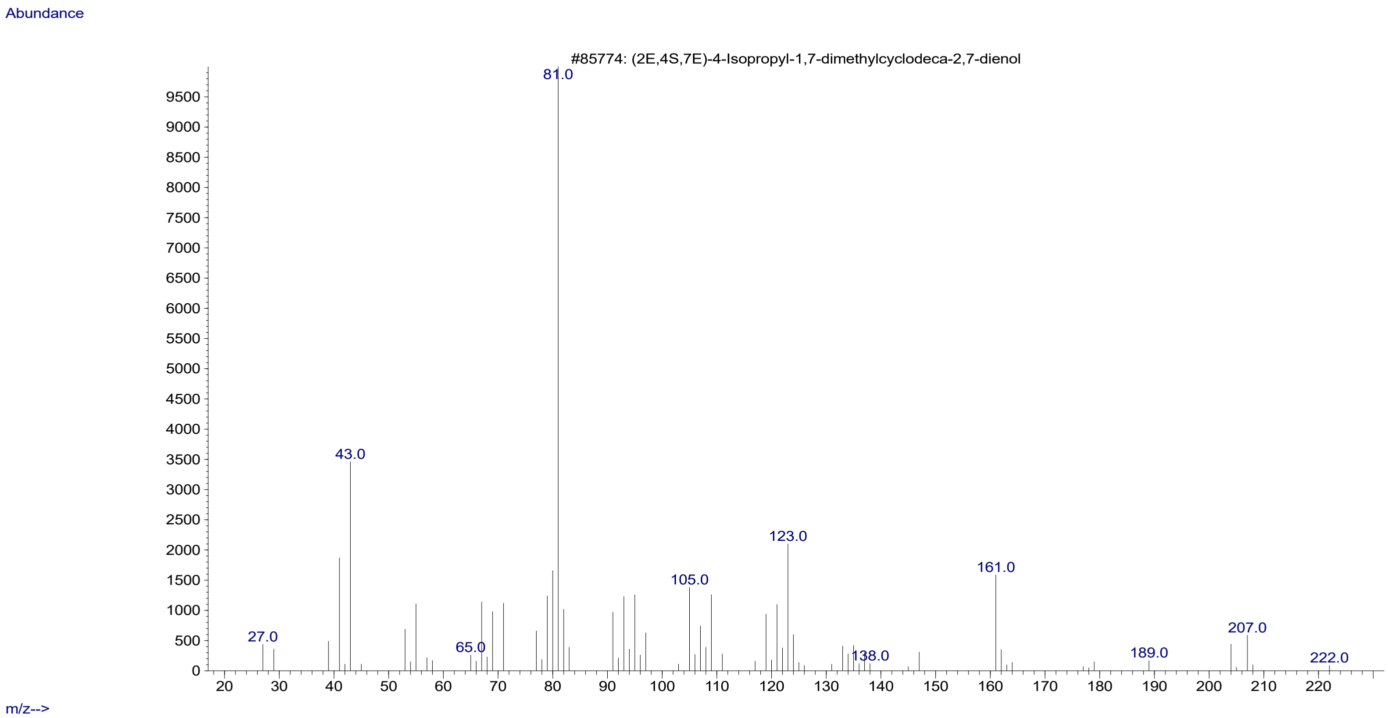


Fig 4.10 chromatogram of (2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol

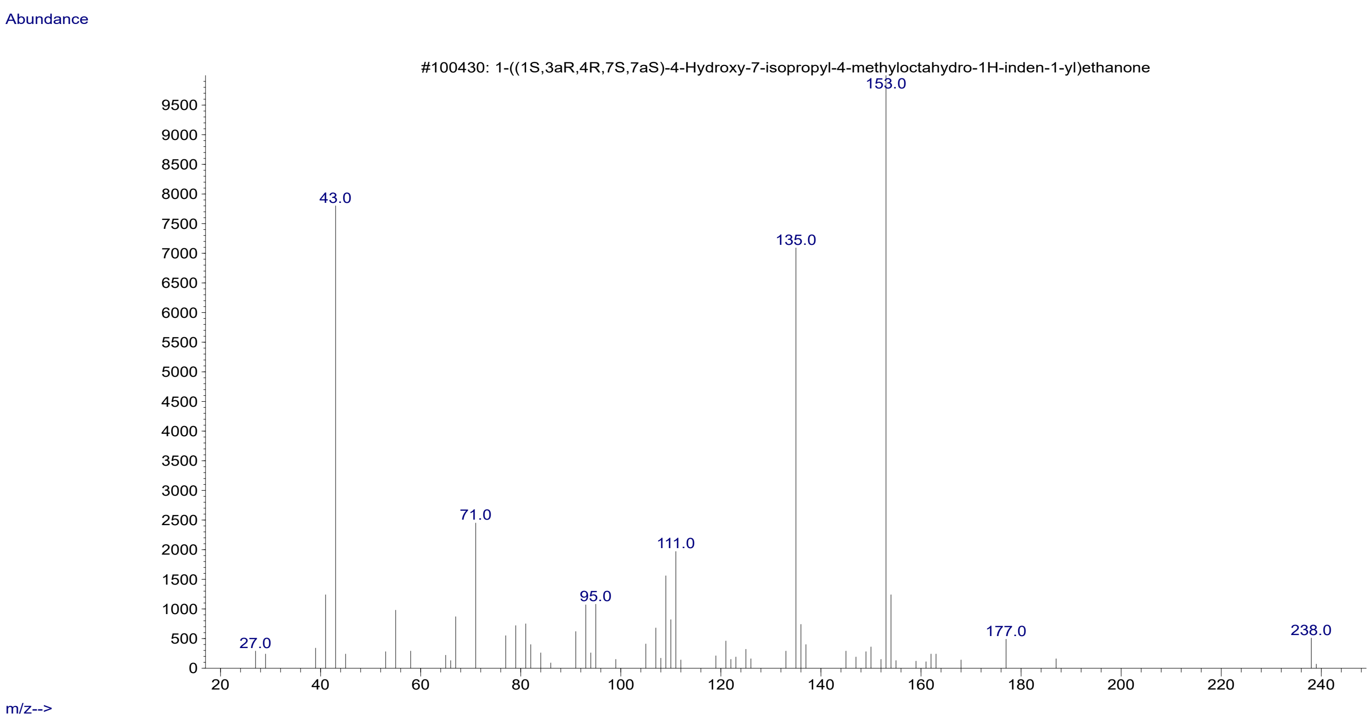


Fig 4.11 chromatogram of 1-((1S,3aR,4R,7S,7aS)-4-Hydroxy-7-isopropyl-4-methyloctahydro-1H-inden-1-yl)ethenone

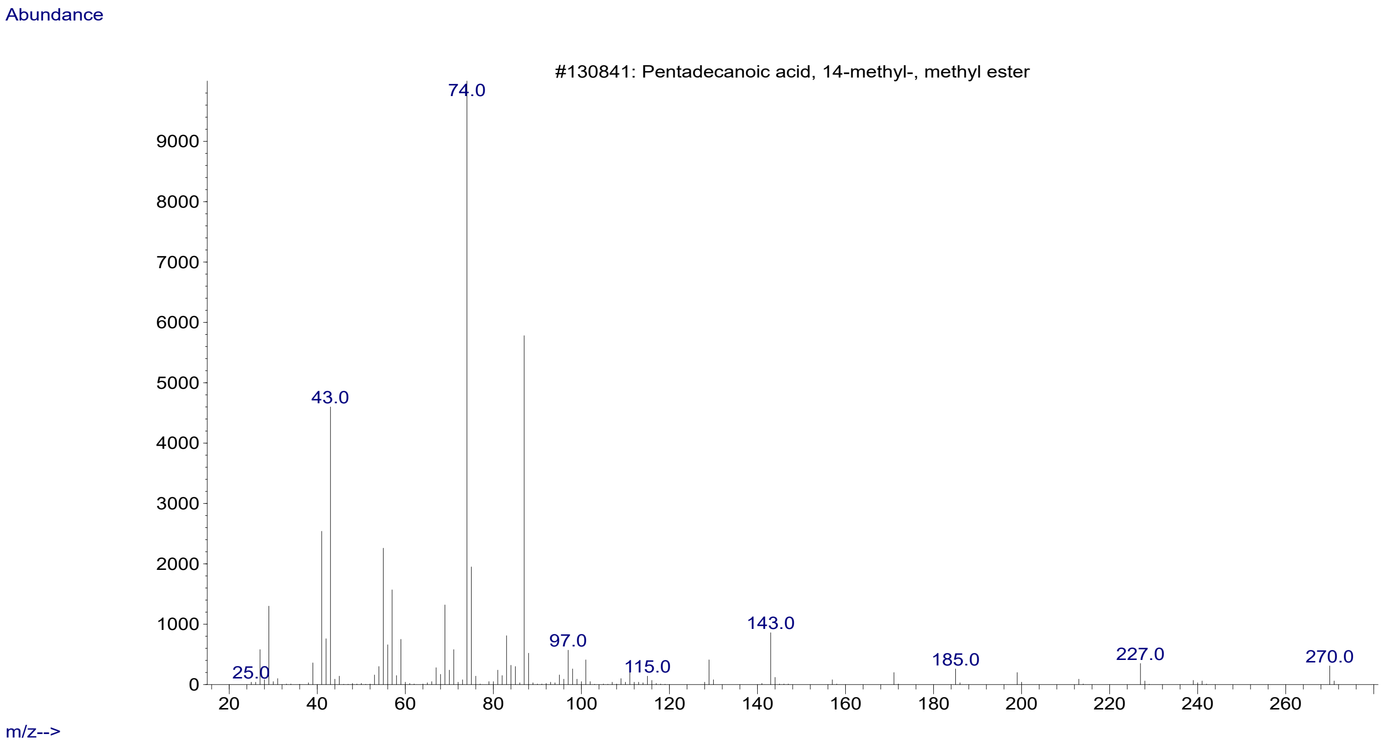


Fig 4.12 chromatogram of Pentadecanoic acid, 14-methyl-, methyl ester

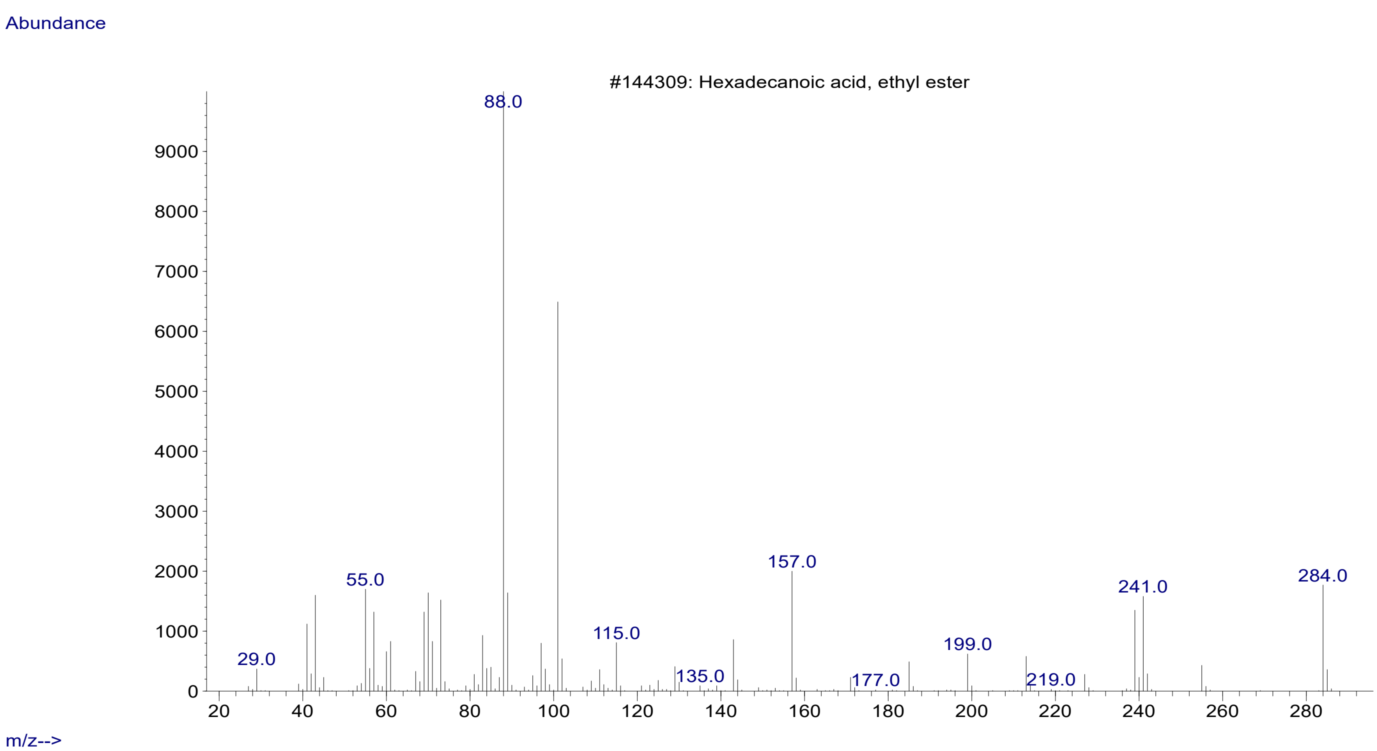


Fig 4.13 chromatogram of Hexadecanoic acid, ethyl ester

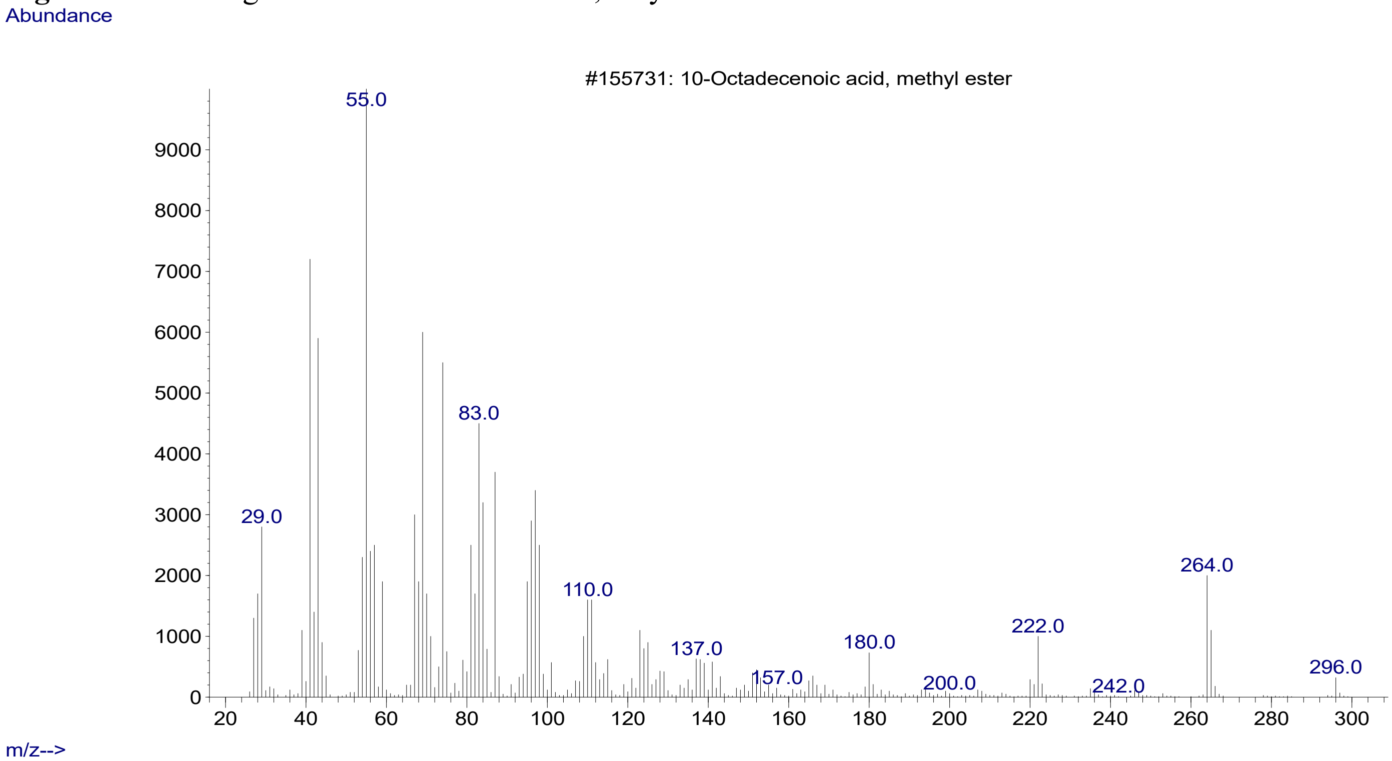


Fig 4.14 chromatogram of 10-Octadecenoic acid, methyl ester

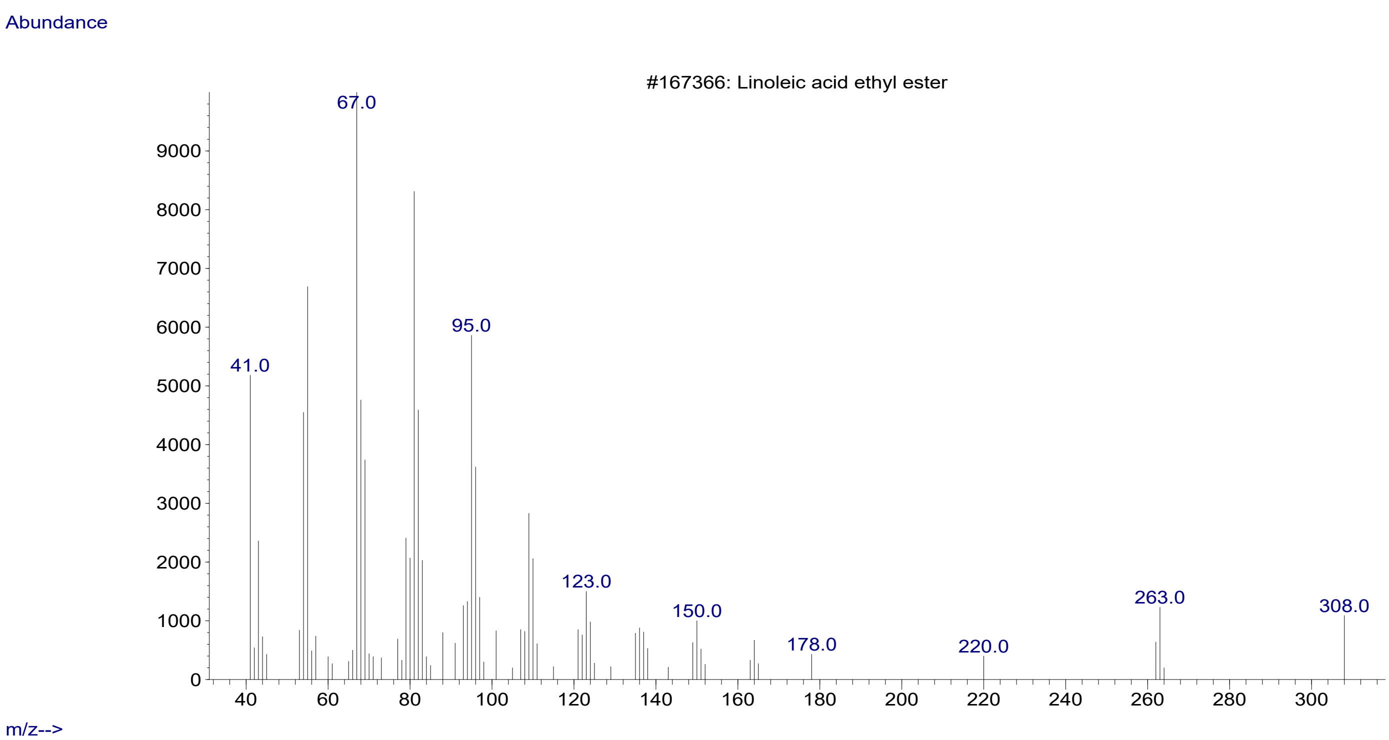


Fig 4.15 chromatogram of Linoleic acid ethyl ester

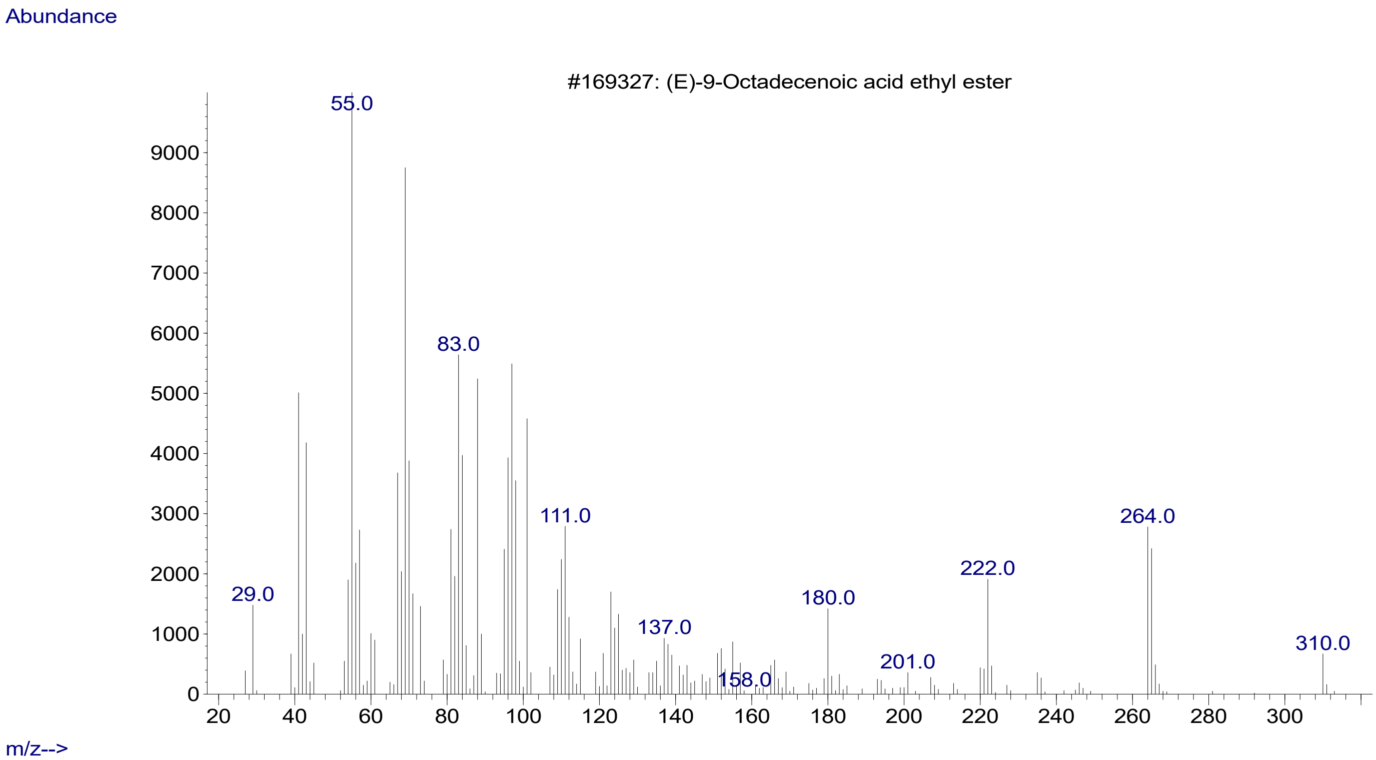


Fig 4.16 chromatogram of (E)-9-Octadecenoic acid ethyl ester

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The main objective of this research was to identify and quantify the bioactive phytochemical constituents present in the hydroethanol extracts of Monodora myristica seed and shell through GC-MS analysis. The GC-MS analysis successfully identified 28 compounds in the seed extract and 15 in the shell extract, thereby confirming that both parts of the plant contain a variety of pharmacologically important compounds. This supports findings from related studies that have documented the existence of numerous bioactive compounds in Monodora myristica extracts with medicinal relevance (Obonga, 2019; Adewole, 2013). The seed extract was distinguished by a significant concentration of fatty acid derivatives, with linoelaidic acid (29.49%), 9,12-octadecadienoic acid (Z,Z)- (26.97%), and (E)-9-octadecenoic acid ethyl ester (21.90%) being the most prevalent. These fatty acids are recognized for their antioxidant, antimicrobial, and anti-inflammatory properties, thereby supporting the traditional application of the seed for medicinal purposes (Obonga, 2019; Adewole 2013). Notably, n-hexadecanoic acid, despite being present in a lower concentration (2.51%), is significant for its antioxidant and antimicrobial effects (Adewole, 2013). Furthermore, the sesquiterpenoid Tau-Cadinol (0.26%) provides strong antimicrobial and antifungal properties while also contributing to the characteristic aroma of the seed, which aligns with ethnomedicinal accounts (Obonga, 2019).

The shell extract, frequently regarded as a byproduct, displayed a comparably rich composition primarily characterized by fatty acid esters. The predominant components included (E)-9-octadecenoic acid ethyl ester (35.08%) and linoleic acid ethyl ester (22.21%), both acknowledged for their potent antioxidant, anti-inflammatory, and antimicrobial properties, thereby enhancing the therapeutic potential of the shell (Obonga, 2019). Additional compounds such as 10-octadecenoic acid methyl ester, hexadecanoic acid ethyl ester, and derivatives of pentadecanoic acid further augment these bioactivities (Obonga, 2019; Adewole, 2013). The efficacy of hydroethanol as a solvent for the extraction of bioactive compounds was distinctly illustrated through a maceration process lasting 72 hours. This technique was found to be particularly effective for phytochemical extraction, successfully isolating essential therapeutic components from the seeds of Monodora myristica. This is corroborated by research indicating that ethanol possesses a superior capacity to extract phenolics and flavonoids, which exhibit significant antioxidant properties (Ogunmoyole et al., 2017). In summary, these results provide scientific support for the traditional applications of both the seed and shell of Monodora myristica in the treatment of infections, inflammation, and conditions related to oxidative stress. The identification of these bioactive compounds also highlights the potential to add value to the shell, an underexploited resource, as a source of natural therapeutic agents, which may have significant implications for sustainable utilization of plant resources and economic advancement (Okechukwu, 2022).

5.2 CONCLUSION

This research effectively delineated the phytochemical composition of hydroethanol extracts from the seeds and shells of Monodora myristica, revealing the presence of 28 compounds in the seeds and 15 in the shells. Many of these compounds are recognized for their antimicrobial, anti-inflammatory, and antioxidant effects. The seed extract was particularly abundant in linoelaidic acid, 9,12-octadecadienoic acid, and (E)-9-octadecenoic acid ethyl ester, whereas the shell extract was primarily characterized by (E)-9-octadecenoic acid ethyl ester and linoleic acid ethyl ester. These results offer substantial scientific support for the traditional medicinal applications of Monodora myristica and underscore the therapeutic potential of the shell, which is typically regarded as waste, for future bioprospecting and drug development efforts.

5.3 RECOMMENDATIONS FOR FUTURE STUDIES

• Isolation and Comprehensive Testing: Subsequent research should focus on isolating key compounds such as (E)-9-octadecenoic acid ethyl ester and linoleic acid ethyl ester from both seed and shell extracts. This approach would facilitate targeted in vitro and in vivo assays to thoroughly validate their antimicrobial, anti-inflammatory, and antioxidant properties.

• Toxicity and Safety Evaluation: A comprehensive toxicological assessment is crucial to determine the safety profile of these extracts, particularly the concentrated shell extract, prior to any clinical or commercial use.

• Extraction Optimization: Additional studies could refine the extraction process by testing various solvents and extraction methods to improve the yield and purity of valuable bioactive compounds derived from Monodora myristica shell and seed.

These recommendations are intended to enhance the pharmacological knowledge and support the practical application of Monodora myristica in both traditional and contemporary medicine.

REFERENCES

Abdel-Shafy, H.I. and Mansour, M.S. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum* 25(1):107–123.

Adolf, O.B., Akwasi, A., Gyang, N.O., Amoah, C.A. and Akorfa, A.A. (2018). Comparative assessment of some physico-chemical properties of seed oils of *Parkia biglobosa* and *Monodora myristica* with some commercial oils. *African Journal of Food Science* 12(1): 1–5.

Afolabi, F.A., Eko, J.A. and Etukudoh, E.E. (2024). The nutritional value of African nutmeg (*Monodora myristica*) seed meal. *African Journal of Research in Biosciences* 1(2):45–53.

Agiriga, A.N. and Muthulisi, S. (2018). Effects of thermal processing on the nutritional, antinutrient and in vitro antioxidant properties of *Monodora myristica* (Gaertn.) Dunal seeds.*Acta Scientiarum Polonorum Technologia Alimentaria* 17(4):321–333.

Agiriga, A. and Siwela, M. (2017). *Monodora myristica* (Gaertn.) Dunal: A plant with multiple food, health and medicinal applications – A review. *American Journal of Food Technology* 12:271–284.

Agiriga, A.P. (2023). Morphological and ecological study of *Monodora myristica* in Southern Nigeria. *Journal of Tropical Forestry* 39(2):75–89.

Akinwunmi, K.F. and Oyedapo, O.O. (2015). In vitro anti-inflammatory evaluation of *Monodora myristica* seeds. *European Journal of Medicinal Plants* 8(3):167–174.

Alao, O.O., Awosika, B.S. and Olawoye, M.T. (2025). FTIR, GC–MS, phytochemical, and antimicrobial screening of the seed oil of *Monodora myristica* (African nutmeg). *International Journal of Pharmaceutical Research and Allied Sciences* 14(1):1–13.

Bello, M.O., Adekunle, A.S., Oyekunle, J.A.O. and Yusuf, T.A. (2014). Evaluation of the fixed oil of two commonly consumed spices, *Monodora myristica* and *Myristica fragrans*, as adjunct in food formulations', *Scientific Research and Essays* 9(13):607–610.

Burubai, W., Amula, E., Daworiye, P., Suowari, T. and Nimame, P. (2009). Proximate composition and some technological properties of African nutmeg (*Monodora myristica*) seeds. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 8:396–402.

Daniel, O.C., Ukachukwu, U.A. and Ishola, P.O. (2024). Phytochemical and antioxidant studies of *Monodora myristica* seed. *Journal of Medicinal Plants Research* 18(5):301–312.

Ejiofor, T.I., Uburu, O. and Barber, L. (2013). Phytochemical screening and antioxidant activity of *Monodora myristica* fruit. *Nigerian Journal of Biochemistry and Molecular Biology* 28(2): 89–95.

Ekeanyanwu, C.R., Ogu, I.G. and Nwachukwu, U.P. (2010). Biochemical characteristics of the African nutmeg – *Monodora myristica*. *Agricultural Journal* 5:303–308.

Ezeuko, A.S., Bamgboye, O.A., Jonathan, H. and Uchenna, D. (2017). Extraction, physicochemical, phytochemical analysis, and identification of some important compounds of *Monodora myristica* (African nutmeg) seed oil. *International Journal of Innovative Research and Advanced Studies* 4:406–410.

Enabulele, S.A., Oboh, F.O.J. and Uwadiae, E.O. (2014). Antimicrobial, nutritional and phytochemical properties of *Monodora myristica* seeds. *IOSR Journal of Pharmacy and Biological Sciences* 9:1–6.

Feyisayo, A.J. and Oluokun, A.O. (2014). Comparative analysis of phenolic profile of *Monodora myristica* and *Monodora tenuifolia*. *African Journal of Plant Science* 8(4):183–190.

Feyisayo, A.K. and Oluokun, O.O. (2013). Evaluation of antioxidant potentials of *Monodora myristica* (Gaertn.) Dunal seeds. *African Journal of Food Science* 7:317–324.

Igwe, O.U., Iroha, U.E. and Otuokere, I.E. (2023). Chemical, antioxidant and antibacterial studies of *Monodora myristica* seed extract and molecular docking of the prominent compounds. *Journal of Chemical Society of Nigeria* 50(1):75–91.

Ikem, C.J., Oli, A.N., Nwaigwe, I., Ogbiko, C. and Esimone, C.O. (2024). GC–MS analysis of five commonly used herbal formulations sold in Anambra State, Southeast Nigeria. *Tropical Journal of Phytochemistry and Pharmaceutical Sciences* 3(2):201–207.

Isiogugu, S.G., Ugochukwu, C.A. and Eze, U.M. (2023). Anti-inflammatory effects of *Monodora myristica* root bark extracts. *African Journal of Pharmacology and Therapeutics* 22(1): 44–51.

Miediegha, O., Owaba, A.D.C. and Okori-West, L. (2023). Acute toxicity studies, physicochemical and GC–MS analyses of *Monodora myristica* (Gaertn.) Dunal oil. *Nigerian Journal of Pharmaceutical Research* 18(2):91–99.

Miediegha, V.E., Okpoghono, J.A. and Akinyemi, A.J. (2023). Bioactive components and pharmacological potentials of *Monodora myristica*: Review and prospects. *Phytochemistry Reviews* 22(1):115–134.

Ndumbe, L.J., Ngomba, L.S. and Eva, M. (2022). Harvesting and marketing pattern of *Monodora myristica* and *Afrostyrax kamerunensis* in Wabane Sub-division, South West Region of Cameroon. *Journal of Agriculture and Ecology Research International* 23(2):1–9.

Nkwocha, A.C., Okagu, I.U. and Chibuogwu, C.U. (2019). Mineral and vitamin contents of *Monodora myristica* (African nutmeg) seeds from Nsukka, Enugu State, Nigeria. *Pakistan Journal of Nutrition* 18(3):308–314.

Obonga, W.O., Omeje, E.O., Nnadi, C.O. and Ocheme, W.G. (2019). Phytochemical evaluation of extracts and GC–MS analysis of oil from *Monodora myristica* seed. *Dhaka University Journal of Pharmaceutical Sciences* 18(1):69–73.

Ogbunugafor, H.A., Ugochukwu, C.G. and Kyrian-Ogbonna, A.E. (2017). The role of spices in nutrition and health: A review of three popular spices used in Southern Nigeria. *Food Quality and Safety* 1:171–185.

Okechukwu, P., Eze, C. and Uche, L. (2022). Phytochemical characterization and antimicrobial activity of *Monodora myristica* extracts. *African Journal of Microbiology Research* 16(4): 212–220.

Okpoghono, J., Obi, E.C. and Osemwegie, O.O. (2025). Potentialities of *Monodora myristica* active components as functional ingredients in food. *Current Research in Food Science* 9: 100330.

Oluwasegun, I.O., James, A. and Bolaji, O. (2025). Antibacterial activity of *Monodora myristica* seed oil against *Staphylococcus aureus* and *Escherichia coli*. *Journal of Herbal Medicine* 19:100432.

Omobuwajo, T.O., Omobuwajo, O.R. and Sanni, L.A. (2003). Physical properties of calabash nutmeg (*Monodora myristica*) seeds. *Journal of Food Engineering* 57:375–381.

Onimawo, A., Esekheigbe, A. and Okoh, J.E. (2017). Nutritional evaluation of *Monodora myristica* seeds. *Nutrition and Food Science* 7:1–10.

Owokotomo, I.A. and Ekundayo, O. (2012). Comparative study of the essential oils of *Monodora myristica* from Nigeria. *European Chemical Bulletin* 1(7):263–265.

Ukachukwu, U.A., Daniel, O.C. and Iyayi, E.A. (2018). Antioxidant potentials of *Monodora myristica* seed extracts. *International Journal of Biosciences* 13(3):106–114.

Uwakwe, A.A. and Nwaoguikpe, R.N. (2008). In vitro antisickling effects of *Xylopia aethiopica* and *Monodora myristica*. *African Journal of Biotechnology* 7(3):351–353.