GC-MS CHARACTERISATION OF BIOACTIVE COMPOUNDS IN *Myristica fragrans* SEED EXTRACT AND HYDROETHANOLIC PRECIPITATE

BY

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CERTIFICATION

We the undersigned certify that Deborah Braimoh Eghonghon (BMS2106197) carried out this work titled "GC-MS Characterisation of Bioactive compounds in *Myristica fragrans* seed extract and hydroethanolic precipitate" 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.

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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 parents, Mr and Mrs Kadiri Braimoh, for their immeasurable support and contributions to my academic success so far, to my siblings and freinds for all their encouragements and advice.

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ABSTRACT

This study investigated the bioactive phytochemical constituents of *Myristica fragrans* (nutmeg) seed extracts and the hydroethanolic precipitate using Gas Chromatography-Mass Spectrometry (GC-MS) to validate their traditional medicinal uses and explore their therapeutic potential. *Myristica fragrans,* an aromatic evergreen tree, widely cultivated in Nigeria mainly in the southern and rainforest regions, is valued for its culinary and medicinal properties. The study aimed to identify, characterize and compare the phytochemical composition of hydroethanolic extracts from the seed, and the hydroethanolic precipitate of the seed extract, and to evaluate their possible applications in medicine, food preservation, and nutraceutical development. GC-MS analysis revealed 62 compounds in the seed extract and 55 in the hydroethanolic precipitate. The seed extract and the hydroethanolic precipitate contained tetradecanoic acid (myristic acid) in abundance, 1,2,3-trimethoxy-5-2-propenyl, palmitic acid, cis-vaccenic acid, and 1,3-benzodioxole derivatives, known for anti-inflammatory, antimicrobial, hypocholesterolemic, antioxidant, and analgesic properties. These findings confirm the therapeutic potential of *Myristica fragrans,* demonstrating that both the seed extracts and hydroethanolic precipitate are valuable sources of bioactive compounds.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Myristica fragrans (Nutmeg) seeds are widely known as a spice but also contain a variety of chemical compounds with potential pharmacological effects. These compounds include secondary metabolites such as alkaloids, flavonoids, phenols, glycosides, and terpenoids that may serve as candidates for new drugs in treating various ailments. The chemical composition and yield of extracts can differ depending on the extraction methods used, including hydroethanolic extraction, which is known to efficiently precipitate bioactive constituents for analysis. Recent studies show that the chemical constituents of *Myristica fragrans* seeds have been effectively analyzed through GC-MS (Gas chromatography-Mass spectrometry). This analysis have confirmed the presence of multiple bioactive compounds such as myristicin, elemicin, methoxyeugenol, sabinene, and various phenolic compounds with antioxidant, anti-inflammatory, antimicrobial, and therapeutic properties (Ojo *et al;* 2022). Hydroethanolic extraction is noted for efficiently isolating a broad spectrum of these phytochemicals, enhancing the quality of the extract for analytical characterization by GC-MS. For example, Ojo *et al;* (2022) observed that aqueous extracts of *Myristica fragrans* seeds contained higher concentrations of phenols and flavonoids, compared to dichloromethane extracts, highlighting variations in extract composition based on solvent polarity. Their GC-MS results identified 6 bioactive compounds in the aqueous extract and 18 in the dichloromethane extract, many associated with biological activities, relevant to medicinal use. Furthermore, studies confirm the seed's traditional use in treating inflammation and oxidative stress, supported by detected antioxidant compounds (Al-Qahtani, 2022). This scientific validation underlines the rationale for

further GC-MS analysis as an effective method for bioactive compound identification and potential therapeutic exploitation.

1.2 JUSTIFICATION OF THE STUDY

The study of GC-MS Characterization of Bioactive compounds in *Myristica fragrans* seed extract and hydroethanolic precipitate is justified by the plant's rich bioactive profile with pharmacological potentials such as antioxidant, anti-inflammatory, antidiabetic, antimicrobial activities (Trifan *et al;* 2023). This study is important because traditional uses of *Myristica fragrans* suggests medicinal benefits. However, the full chemical characterization using advanced techniques like GC-MS to fully harness these properties for pharmaceutical and nutraceutical applications. Hydroethanolic extraction specifically improves the yield and diversity of compounds extracted, making it suitable for tracing bioactive compounds. Therefore, characterizing these bioactive compounds supports drug discovery, development of novel therapies, and validates ethnomedicinal claims associated with the plant (Matulyte, 2019). GC-MS enables comprehensive identification of compounds such as myristicin, elemicin, and malabaricone derivatives, which show promise in therapeutic applications and drug development (Asika, 2016). Moreover, this study provides essential baseline data for future clinical research.

1.3 AIMS AND OBJECTIVES

1.3.1 AIM OF THE STUDY

- To identify and characterise the bioactive compounds present in *Myristica fragrans (n*utmeg) seed extract and its hydroethanolic precipitate using Gas Chromatography-Mass Spectrometry (GC-MS).
- To evaluate the phytochemical profile of M. fragrans seed extract relevant to potential pharmacological or therapeutic applications.

1.3.2 OBJECTIVES

- To prepare *Myristica fragrans* seed extracts using appropriate solvent extraction method (hydroethanolic extraction).
- To perform GC-MS analysis to detect and identify the chemical constituents within both the seed extract and hydroethanolic precipitate.
- To analyze the potential biological activities of the identified compounds.
- To provide information based on the results that promote pharmacological and therapeutic research of *Myristica fragrans.*

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF *Myristica fragrans* (NUTMEG)

Myristica fragrans, commonly known as Nutmeg, is an aromatic evergreen tree. It is widely cultivated in Nigeria mainly in the southern and rainforest regions around Ado-Ekiti, parts of the Niger Delta and southeastern states such as Bayelsa, Rivers, Delta, and Akwa Ibom states. It is alo cultivated in Benin City, Edo State and frequently sold in Benin City markets. The seed of this plant is used as a spice and also for medicinal purposes including the treatment of digestive disorders such as diarrhea, nausea, and flatulence, producing two major spices: Nutmeg (the seed) and mace (the aril covering the seed). The seed is rich in essential oils and bioactive compounds such as myristicin, elemicin, safrole, eugenol, and various lignans which contribute to its distinctive aroma and medicinal properties (Ashokkumar *et al;* 2022).

Studies have shown that *Myristica fragrans* possesses a variety of pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiarrheal, anticancer, anticonvulsant, hepatoprotective, and aphrodisiac properties. These properties are largely attributed to its phytochemical constituents, which include essential oils, phenolic compounds, neolignans, and flavonoids (Kuete, 2017). The essential oils extracted from nutmeg seeds have been examined extensively through GC-MS, revealing variations in chemical composition influenced by extraction methods and geographical origin (Ashokkumar *et al;* 2022).

Traditionally, Nutmeg is used as both a spice and a medicinal plant to manage various ailments. Ethnomedical uses include its application as an aphrodisiac, carminative (relieving flatulence), nerve stimulant and tonic. It's analgesic and anti-inflammatory effects make it useful for

relieving muscle aches, neuralgia, arthritis, and rheumatism. (Akinduko *et al;* 2023). Nutmeg seeds are commonly prepared as aqueous extracts or oils and used in treating digestive disorders and nervous system conditions. The high-fat content of the seed supports its use in topical formulations for muscle relaxation and pain relief. Additionally, nutmeg's antimicrobial and antioxidant properties are recognized in traditional Nigerian medicine, contributing to its use in managing infections and inflammatory conditions (Al-Qahtani *et al*; 2022). Nutmeg's traditional use also extends to skin diseases like eczema and it enhances blood circulation and heart contractility. (Akinduko *et al;* 2023).

Fig. 2.1 Representation of Nutmeg seeds (Wikipedia).

2.2 CHEMICAL COMPOSITION OF Myristica fragrans SEEDS

1. ESSENTIAL OILS: The essential oil extracted from nutmeg seeds contains approximately 38-40 compounds, with major constituents including sabinene, alpha-pinene, beta-pinene, myristicin, eugenol, caryophyllene and limonene (Kapoor *et al;* 2013). Sabinene is often the predominant compound, comprising up to 20-30% of the essential oils, followed by alpha-pinene, beta-pinene, and myristicin. GC-MS analysis reveals that these constituents contribute contribute to the characteristic aroma, as well as biological activities such as antioxidant and antimicrobial effects.

2. PHENOLIC AND LIGNAN COMPOUNDS: The seed contains significant quantities of phenolic compounds, including neolignans such as malabaricone C, macelignan, and other lignans, which are linked to antioxidant and anti-inflammatory activities (Elfia *et al;* 2023). These phenolic compounds contribute to the seed's total antioxidant capacity, with malabaricone C being the most potent, showing strong radical scavenging activity.
3. OTHER PHYTOCHEMICALS: Alkaloids like myristicin, safrole, and elemicin are present in high amounts and are responsible for several bioactivities including psychoactive and antimicrobial effects (Gopala Krishnan, 2022). Tannins, saponins, and proteins are also detected, contributing to the seed's pharmacological potential.
4. FATTY ACIDS AND FIXED OIL CONTENT: The fixed oil of Nutmeg seeds constituting around 40% of the seed weight, is rich in fatty acids such as myristic acids, oleic acid, and linoleic acid (Shamsun *et al;* 2020).
5. MINERALS: The mineral content includes high levels of calcium (512 mg/g), iron (56 mg/g), phosphorus (135 mg/g), and magnesium, supporting its nutritional value (Al-Qahtani *et al;* 2015).

2.3 PHARMACOLOGICAL AND BIOACTIVE POTENTIAL OF MYRISTICA FRAGRANS SEED

1. ANTIOXIDANT CAPACITY: The high phenolic and lignan content endows the nutmeg seed with robust antioxidant potential, effectively neutralizing reactive oxygen species (ROS) and protecting biomolecules from oxidative damage. Specifically, malabaricone C and related compounds show superior radical scavenging abilities and lipid peroxidation inhibition, supporting cardiovascular and cellular health. This potent antioxidant property explains the seed's therapeutic effect in oxidative stress-related diseases (Krishnakumari *et al;* 2014).

2. ANTI-INFLAMMATORY EFFECTS: Nutmeg seed extracts have been shown to significantly reduce the production of inflammatory cytokines such as TNF-ALPHA, IL-6, and IL-1BETA, and inhibit inducible nitric oxide synthase (iNOS) activity. These effects dampen inflammatory signaling pathways, which are relevant to conditions like arthritis, inflammatory bowel disease, and other chronic inflammatory disorders. (Ha, 2020).
3. ANTIMICROBIAL ACTIVITIES: Essential Oil components (e.g; eugenol, myristicin) display broad-spectrum antimicrobial activity against various bacterial and fungal pathogens. This antimicrobial property underpins traditional uses of Nutmeg in managing infections and preserving food. It also highlights its potential as a natural antimicrobial agent for Healthcare and food industries (Elfia *et al;* 2023).
4. ANTICANCER POTENTIAL: Several phenolic compounds and lignans isolated from nutmeg seed extracts have demonstrated anticancer effects by inducing apoptosis, inhibiting cell proliferation, and modulating oxidative stress in tumor cells. Macelignan and related neolignans contribute to these effects by targeting molecular pathways involved in cancer progression, suggesting potential as complementary agents in cancer therapy.
5. ANTIDIABETIC EFFECTS: Compounds such as malabaricone C and alpha-amylase, which are responsible for carbohydrate digestion and glucose absorption, thereby contributing to blood sugar regulation. This enzymatic inhibition suggests promising therapeutic potential for managing type 2 diabetes mellitus and metabolic syndrome.
6. HEPATOPROTECTIVE AND NEUROPROTECTIVE PROPERTIES: Myristicin and other seed constituents effects by reducing liver damage markers and oxidative stress in

hepatocytes. Neuroprotective activities are linked to antioxidant effects and modulation of neurotransmitter levels, which may explain nutmeg's mood-enhancing and cognitive benefits (Sultan *et al;* 2023).

7. SAFETY AND TOXICITY:
    - Culinary use: Safe at 0.1g/day.
    - High doses (>5-15g): Causes Nutmeg intoxication; hallucinations, palpitations, nausea, dry mouth, delirium, due to myristicin metabolism to MMDA (amphetamine-like).

2.4 HYDROETHANOLIC EXTRACTION

Hydroethanolic extraction is a widely used green chemistry technique for isolating bioactive compounds from plant materials, particularly in food and pharmaceutical applications. It involves using a binary solvent mixture of ethanol (an organic solvent) and water (e.g 80:20 ethanol:water) to dissolve and extract target compounds from the plant matrix. It is a preferred approach over single solvents because it balances polarity to capture a broader spectrum of bioactive compounds, such as polyphenol, flavonoids, tannins, and alkaloids, which vary in solubility. Hydroethanolic extraction is particularly suitable for nutmeg seeds due to their oily and fibrous nature.

2.4.1 PROCEDURE

1. Plant preparation: The plant material e.g, dried and powdered seeds, is pre-treated via drying, milling, or enzymatic hydrolysis, to increase surface area and release bound compounds, preventing degradation and microbial growth.

2. Extraction: The material is soaked in the hydroethanolic solvent at often mild controlled temperatures.
3. Filtration and Concentration: The hydroethanolic mixture is filtered to separate the liquid extract from the insoluble residue (precipitate). The filtration is then evaporated under vacuum to concentrate the crude extract.

2.4.2 IMPORTANCE OF ANALYSING THE CRUDE EXTRACT AND PRECIPITATE OF MYRISTICA FRAGRANS SEED

The crude extract refers to the concentrated soluble fraction (filtrate after evaporation), while the precipitate (residue), is the insoluble solid left after filtration. Analysing both is crucial for a holistic characterisation, as they represent complementary fractions of the seed's chemical profile.

1. Comprehensive Bioactive Profiling: The crude extract captures soluble compounds like polyphenols, flavonoids, and glycosides which are key for antioxidant and antimicrobial activity (Helen P.M *et al*; 2012). Hydroethanolic extracts of *Myristica fragrans seeds*, show high phenolic content and bioactivity. However, not all compounds dissolve fully; 20-30% of total bioactive compounds (e.g, bound tannins or high molecular weight terpenes) may remain in the precipitate due to low solubility. Analysing the precipitate reveals insoluble or partially soluble components. In Nutmeg, the precipitate often retains essential oil precursors and non-volatile terpenes, contributing to overall yield optimization.
2. Yield and efficiency assessment: For *Myristica fragrans,* studies show that hydroethanolic extracts have superior antimicrobial effects compared to water extracts, but precipitates can contain up to 40% residual bioactive compounds, indicating

complete extraction(Piras *et al;* 2012). This prevents underestimation of the seed's potential.

3. Safety and Quality Control in Food Applications: Crude extracts are directly used as natural preservatives, but precipitates may harbor contaminants or undergraduate enzymes/microbes, if pre-treatment is inadequate. Dual analysis ensures purity, stability, and compatibility. In Nutmeg seed applications, analysing both the crude extract and precipitate confirms balanced sensory and nutritional benefits while addressing health concerns like mycotoxins in residues (Ejechi B.O *et al*; 1998).

2.5 REVIEW OF GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) STUDIES ON MYRISTICA FRAGRANS

One study by Matulyte *et al.* (2019) demonstrated that the use of magnesium aluminometasilicate as an excipient during hydrostillation greatly increased the essential oil yield, from 5.25% baseline to between 7.9% and 10.43%. GC-MS profiling revealed modifications in the volatile compound profile, including increased levels of key terpenoids, which could enhance the therapeutic and aromatic qualities of the oil (Matulyte *et al;* 2019). Further research indicates that GC-MS profiling of M.fragrans extracts consistently detects compounds such as elemicin, myristicin, and myristic acid. For instance, Al-Qahtani *et al.* (2022) isolated elemicin as a major component with bioactive properties, including antimicrobial and antioxidant activities against bacteria including E. coli, K. pneumoniae, and fungi like Candida tropicalis and Aspergillus flavus pointing to its potential in developing natural medicinal agents. Similarly, other reports have documented the neuroprotective effects linked to myristic acid and myristicin, emphasizing their therapeutic significance in neurodegenerative conditions such as Alzheimer's disease and cerebral ischemia (Ghorbanian *et al;* 2023). Myristicin plays a role in free radical scavenging

and lipid peroxidation, which are important mechanisms in neuroprotective by reducing oxidative stress and inflammation in neuronal tissues.

Additional GC-MS studies have revealed a complex phytochemical spectrum that varies based on the extraction method, solvent, and plant part. These studies identified compounds like safrole, eugenol, and terpenoids, which are associated with anti-inflammatory, antimicrobial, and anticancer activities. The n-hexane seed extract analyzed by Singh *et al;* (2022), identified elemicin (24.44%), myristicin, eugenol, and safrole as major compounds, which possess antimicrobial, antioxidant, hepatoprotective, and anticancer properties. These findings support the traditional use of nutmeg in relaxing muscles, soothing digestive system disorders, relieving indigestion, bloating, diarrhea, and nausea, while calming the nervous system. Myristicin has been traditionally used for nervous system disorders, including nervousness, insomnia, and muscle relaxation. Its calming and mildly sedative effects contribute to its use in reducing anxiety and promoting restful sleep, enhancing nervous system health. Manimaaran *et al. (2024),* undertook GC-MS profiling of the aril aqueous extract of M.fragrans and recorded multiple volatile compounds (myristicin, elemicin, safrole, eugenol, terpenoids, flavonoids, linalool, 4-Terpinenol, caryophyllene) correlated with anti-inflammatory, neuroprotective, and antioxidant effects. They linked these profiles directly to the traditional uses in treating muscular pain, bronchitis, asthma, and nervous system ailments, bridging phytochemical research with practical folk medicine (Manimaaran *et al;* 2024).

2.6 MAJOR CLASSES OF COMPOUNDS IN MYRISTICA FRAGRANS

2.6.1 MONOTERPENES

The essential oil of Myristica fragrans is dominated by monoterpenes, which constitute the majority of the volatile fraction. GC-MS analysis of Nutmeg grown in India demonstrated that monoterpenes accounted for 53.77-94.82% of the total essential oil, depending on the anatomical source (Mruthunjaya, 2020). Similarly, monoterpenoids comprised 69.15-97.24% of essential oils in traditionally treated nutmeg samples (Beri *et al;* 2017). Among the monoterpenes, sabinene, alpha-pinene, beta-pinene, and D-limonene consistently appear as the major constituents. Oxygenated monoterpenes, such as terpinen-4-ol and linaool, are present in smaller but functionally relevant quantities. In one study, the use of magnesium aluminometasilicate significantly altered the oil profile, increasing compounds such as sabinene and limonene, while also varying the levels of alpha-pinene and beta-pinene (Ibrahim *et al;* 2019). Terpinen-4-ol was also identified in antimicrobial screening of Nutmeg oil (Chaliha *et al;* 2018).

The composition of terpenes varies not only by plant part, but also by the extraction method. Hydroethanolic extraction, hydrodistillation, supercritical CO2 extraction, and modified distillation all yield different proportions of monoterpenes and oxygenated terpenes (Kwon *et al;* 2012). Terpenes contribute strongly to the characteristic aroma and flavor of Nutmeg. Terpenes play a role on the bioactivity of Nutmeg essential oil. Some studies have linked monoterpenes such as gamma-terpinene and terpinen-4-ol to antimicrobial effects (Chaliha *et al;* 2018). They can damage bacterial cell membranes and walls, increasing permeability, leading to leakage of intracellular contents, killing the cell. The toxicity of Nutmeg is commonly associated with Phenylpropanoids such as myristicin rather than terpenes, the volatile terpene fraction may

influence pharmacological outcomes, as demonstrated in mouse behavioral studies involving inhalation of Nutmeg essential oil (Akinburo *et al; 2011).*

2.6.2 PHARMACOLOGICAL SIGNIFICANCE OF MONOTERPENES

1. ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY: Monoterpenes demonstrate potent anti-inflammatory properties. Compounds such as sabinene, alpha-pinene, and beta-pinene have been shown to modulate pro-inflammatory cytokines, including IL-6, IL-1beta, and TNF-alpha, thereby reducing inflammatory signaling pathways (Kaskoniene *et al;* 2020). These effects align with the traditional use of nutmeg preparations for inflammatory conditions like joint pains and rheumatism, muscle aches and sprains, toothache, minor skin inflammation like pimples, and indigestion. Monoterpene-rich extracts contribute to the antioxidant capacity of Nutmeg essential oil. Several studies on oxidative stress models report that monoterpenes enhance the scavenging of reactive oxygen species and unregulated endogenous antioxidant responses, which protect tissues from oxidative injury (Venakutonis *et al;* 2023).
2. ANTIMICROBIAL PROPERTIES: Components including alpha-pinene, beta-pinene, gamma-terpinen-4-ol exhibit inhibitory effects against a wide range of gram-positive (Staphylococcus aureus, Streptococcus pneumonia), and gram-negative bacteria (E. coli), as well as several fungal species (Ozkan *et al;* 2017). Studies have reported synergistic effects between monoterpenes and standard antimicrobial agents, suggesting potential for combination therapy. Alpha-terpinol can disrupt microbial membranes and enhance the efficacy of antibiotics, thereby reducing resistance in certain pathogens (Silva *et al;* 2023).

3. NEUROPHARAMACOLOGICAL AND ENZYME-MODULATORY EFFECTS: Emerging evidence supports the role of Nutmeg (monoterpenes) in neurophramacology, particularly through their modulatory effects on cholinesterase enzymes. Inhibition of butyrylcholinesterase (BChE) and to a lesser degree, acetylcholinesterase (AChE), has been documented in monoterpene extracts 9f nutmeg , suggesting potential applications in the management of cognitive impairment (Barbosa *et al;* 2022). Such actions are significant because BChE inhibition is a known therapeutic mechanism for neurodegenerative conditions such as Alzheimer's disease. Earlier neuropharamacological investigations also indicate that monoterpenes participate in modulating locomotor activity, mood, and central neurotransmission, contributing to mild sedative or antidepressant effects observed in vivo (Hidalgo *er al;* 2011).

2.6.3 ESTERS

Matulyte *et al.* (2019), conducted GC-MS profiling of Nutmeg seed extracts and essential oils by maceration, ultrasound-assisted extraction, and hydrodistillation, including magnesium aluminometasilicate as an excipient. They identified several esters in the essential oil fraction: namely alpha-terpinyl acetate, cis-sabinene-hydrate acetate, and citronella acetate. When magnesium aluminometasilicate was used during hydrostillation, six new compounds were discovered, including citronellyl acetate, which is a long-chain Ester. Muchtaridi *et al.* (2010), performed a pharmacological- bioanalytical study. They inhaled nutmeg seed oil in mice, measured locomotor inhibition, and used GC-MS after solid-phase extraction to identify which compounds appeared in plasma. Among the compounds detected in mouse plasma were fatty acid methyl esters. While myristicin was the most abundant in plasma, the presence methyl esters indicate that they are bioavailable. Esters are not among the quantitatively dominant classes

(Matulyte *et al;* 2019). Their GC-MS data shows that monoterpenes and aromatics make up a much larger portion of the oil. Types of esters reported include;

- Acetate esters: As mentioned earlier, alpha-terpinyl acetate and cis-sabinene-hydrate acetate were identified by Matulyte *et al.* These are acetate esters of monoterpene alcohols, which are relatively volatile and common in essential oils.
- Long-chain esters: The same work reported citronellyl decanoate, a 10-carbon Ester, which is less volatile and more lipophilic.
- Methyl esters of fatty acids: In biological samples, methyl esters derived from fatty acids (e.g; methyl myristate, methyl palmitate) have been detected after inhalation of Nutmeg oil.

2.6.4 PHARMACOLOGICAL SIGNIFICANCE OF ESTERS

Esters in Nutmeg occur primarily in the fixed oil fraction, with trimyristin (a tri-ester of myristic acid and glycerol) being the dominant component. Trimyristin can comprise up to 80% of the fixed oil and serve as a major chemical marker for nutmeg quality (Gupta *et al;* 2013). Nutmeg also contains small amounts of volatile esters in the essential oil as mentioned earlier, although they are less abundant than phenylpropanoids such as myristicin and safrole (Pradeep and Geetha, 2015). Despite their lower concentrations, both volatile and non-volatile esters contribute to the pharmacological profile of Nutmeg seed extract.

1. CHEMICAL SIGNIFICANCE OF ESTERS IN NUTMEG FIXED OIL: The fixed oil is composed largely of fatty acid esters, particularly trimyristin. Upon ingestion, these esters undergo hydrolysis to release myristic acid, which has biological effects on lipid metabolism, cell membranes structure, and fatty acid signaling pathways (Abourashed *et*

al; 2016). Myristic acid increases hepatic synthesis of cholesterol and lipoprotein, raising LDL cholesterol and enhances triglyceride formation. It promotes membrane rigidity and shapes the outer layer of cells enhancing membrane signaling.

2. NEUROPHARAMACOLOGICAL POTENTIAL: Although essential oil constituents receive greater attention, early pharmacological studies indicate that trimyristin exerts mild acetylcholinesterase (AChE) inhibitory activity, contributing to the cognitive and neuromodulatory effects often attributed to nutmeg. (Dohi *et al;* 2009). This activity is weaker than that of phenylpropanoids (e.g myristicin), but esters can act synergistically.
3. ANTI-INFLAMMATORY AND ANTIOXIDANT INFLUENCE: Fatty acid esters modulate inflammation-related pathways, particularly via their hydrolysis products. Studies on nutmeg extracts have demonstrated inhibition of nitric oxide production, scavenging of free radicals, and modulation of COX-2 expression in vitro and in vivo (Rawi *et al;* 2024). Although phenolic compounds contribute strongly to these effects, fatty acid esters also play roles in membrane stabilization and lipid mediator regulation. Extracts containing Ester fractions demonstrated suppression of NO production in macrophage (Matulyte *et al;* 2020).
4. ANTIMICROBIAL ACTIVITY: While most antimicrobial activity is attributed to essential oil constituents, fatty acid esters enhance membrane permeability of microbial cells, indirectly contributing to antimicrobial efficacy (Rema, 2004).
5. PHARMACOLOGICAL AND FORMULATION SIGNIFICANCE: Trimyristicin is used as a model compound in lipid-based drug delivery research because of its defined melting range and stability (Matulyte *et al;* 2020). Sucrose esters have been used to microencapsulate nutmeg essential oil, improving its stability, solubility, and

pharmacokinetics. The high Ester content of Nutmeg Butter supports its use in topical formulations, ointments, and herbal cosmetic products.

6. TOXICOLOGICAL CONSIDERATIONS: High intake of myristic acid (derived from trimyristin hydrolysis) is associated with increased LDL cholesterol, which prevents risk if nutmeg fixed oil I'd consumed excessively (Denke and Grundy, 2000). Nutmeg's toxicity in overdose is primarily due to myristicin and safrole but the Ester fraction influences absorption distribution of thsee compounds.

2.6.5 FATTY ACIDS

Fatty acids represent one of the most abundant chemical compounds in Nutmeg seed, forming the bulk of the fixed oil (nutmeg butter). Unlike the essential oil (rich in volatile phenylpropanoids such as myristicin and safrole), the fixed oil is dominated by non-volatile, long and medium chain fatty acids that significantly contribute to the seed's nutritional, physiochemical, pharmacological properties.

- Myristic acid (C14:0): The predominant fatty acid of Nutmeg. Its exceptionally high concentration (50-60%) of the total fatty acids, making it a chemical marker for nutmeg oil. Liu *et al.* (2021) found myristic acid at 60.8% of the total fatty acid profile in Nutmeg. It contributes to the semi-solid texture, and melting behavior of Nutmeg Butter. Medium-chain saturated fatty acids such as myristic acid have rapid digestibility, use in topical formulations, and roles in lipid metabolism. Because of its abundance, myristic acid also serves as a chemotaxonomic marker for identifying nutmeg species and assessing adulteration.

- Palmitic Acid (C16:0): An important saturated fatty acid that is typically the second most abundant fatty acid in Nutmeg but varies with extraction method. Approximately 6% is found in irradiated nutmeg seeds (Hassan *et al;* 2003). 15-22% is found in some Indian varieties extracted with Petroleum ether (Shamsun Naher *et al;* 2013). Palmitic acid contributes to the structural rigidity of the lipid matrix, thermal stability, and oxidative resistance of the oil. Because it is more resistant to oxidation than unsaturated fatty acids, it helps preserve nutmeg butter during storage.
- Lauric Acid (C12:0): A medium-chain fatty acid with antimicrobial potential. Though typically lower than myristic acid, Lauric acid is a consistent and biologically significant component of Nutmeg oil. Studies show that Lauric acid appears alongside myristic acid in fatty acid-sterol profiles in analytical studies (Ferreira *et al;* 2019). Lauric acid is widely studied for antimicrobial properties, particularly against gram-positive bacteria, skin barrier-enhancing effects, rapid metabolic oxidation, making it energy-rich but less likely to be stored as fat.
- Oleic Acid (C18:1 n-9): The major unsaturated fatty acid. Although nutmeg oil is predominantly saturated, oleic acid is the most significant unsaturated fatty acid present. Reported concentrations include; 7-14% in supercritical CO2 extracts (Gowda *et al;* 2012). 13.4% in a culinary spice survey (Liu *et al;* 2021). Oleic acid improves lipid fluidity, skin absorption properties, and anti-inflammatory activity in topical preparations. Its presence gives nutmeg oil a more balanced fatty acid profile, preventing it from being waxy or brittle.

- Stearic Acid (C-18:0) and Other Minor Fatty Acids: Stearic acid appears in small quantities (<1%) in many analysis (Hassan *et al;* 2003). Other minor fatty acids that contribute to the complexity of Nutmeg oil include:

i. Arachidic acid: Can reach unusually high levels (up to 27%) in some Indian varieties (Shamsun Naher *et al;* 2013).

ii. Petroselinic acid: Detected in trace amounts (0.1%) (Hassan *et al;* 2003).

iii. Behenic acid: found in some solvent extracts.

2.6.6 PHARMACOLOGICAL SIGNIFICANCE OF FATTY ACIDS

1. LIPID METABOLISM AND ANTI-OBESITY EFFECTS: An alcohol extract of Nutmeg has been shown to downregulate lipid synthesis genes in vitro. The extract reduces the expression of fatty acid synthase and sterol regulatory element-binding protein-1c (SREBP-1c) in hepatocyte cells, thereby lowering intracellular lipid accumulation (Zhao *et al;* 2020). In a mouse Mosel of non-alcoholic fatty liver disease, Nutmeg extract treatment reduced body weight gain, ameliorate liver inflammation, decreased lipids, cholesterol, and free fatty acid accumulation, and improved liver function markers.
2. ANTI-INFLAMMATORY ACTIVITY: The same study demonstrated that nutmeg extracts inhibit inflammation triggered by free fatty acids. Treatment suppressed interleukin-6 and tumor necrosis factor-alpha in macrophage cells exposed to a free fatty acid mixture (oleate/palmitate). In vivo, Nutmeg extracts reduced hepatic inflammation, through modulation of lipid-driven inflammatory pathways, which support the concept that nutmeg's fatty acids can directly influence pro-inflammatory cytokine expression.
3. GUT-LIVER AXIS MODULATION: A later study found that nutmeg extract regulate gut microbiota and metabolites, which then attenuated hepatic inflammation and lipid

metabolism disorders. Here, the modulation of fatty acid synthase, a key enzyme in fatty acid synthesis, suggests that nutmeg components impact not only exogenous free fatty acids but also endogenous fatty acid flux, thereby influencing both lipid homeostasis and inflammatory signaling.

4. ANTIBACTERIAL ACTIVITY OF FATTY ACIDS: Nutmeg seed extracts (both crude extract and essential oil) used alongside commensal bacteria produced short-chain fatty acids, such as acetate, propionate, and butyrate, which have antimicrobial effects. (Oo *et al;* 2024). These short chain fatty acids help inhibit bacterial adherence, biofilm formation, and viability of pathogens (e.g Staphylococcus aureus).

2.7 PRINCIPLE OF GC-MS

GC is a separation science technique that is used to separate the chemical components of a sample mixture and then detect them to determine their presence or absence and/or how much is present. GC detectors are limited in the information that they give; this is usually two-dimensional giving the retention time on the analytical column and the detector response. Identification is based on comparison of the retention time of the peaks in a sample to those from standards of known compounds, analyzed using the same method. However, GC alone cannot be used for the identification of unknowns, which is where hyphenation to an MS works very well. MS can be used as a sole detector, or the column effluent can be split between the MS and GC detector(s).

MS is an analytical technique that measures the mass-to-charge ratio (m/z) of charged particles and therefore can be used to determine the molecular weight and elemental composition, as well

as elucidating the chemical structures of molecules. Data from a GC-MS is three-dimensional, providing mass spectra that can be used for identity confirmation or to identify unknown compounds plus the chromatogram that can be used for qualitative and quantitative analysis.

2.7.1 HOW THE GC-MS INSTRUMENT WORKS

The sample mixture is first separated by the GC before the analyte molecules are eluted into the MS for detection.1 They are transported by the carrier gas (Figure 1 (1)), which continuously flows through the GC and into the MS, where it is evacuated by the vacuum system.

1. The sample is first introduced into the GC manually or by an autosampler (Figure 1 (2)) and enters the carrier gas via the GC inlet (Figure 1 (3)). If the sample is in the liquid form, it is vaporized in the heated GC inlet and the sample vapor is transferred to the analytical column (Figure 1 (4)).

2. The sample components, the “analytes”, are separated by their differences in partitioning between the mobile phase (carrier gas) and the liquid stationary phase (held within the column), or for more volatile gases their adsorption by a solid stationary phase. In GC-MS analyses, a liquid stationary phase held within a narrow (0.1-0.25 mm internal diameter) and short (10-30 m length) column is most common.

3. After separation, which for GC-MS analyses doesn’t require total baseline resolution unless the analytes are isomers, the neutral molecules elute through a heated transfer line (Figure 1 (5)) into the mass spectrometer.

4. Within the mass spectrometer, the neutral molecules are first ionized, most commonly by electron ionization (EI). In EI, an electron, produced by a filament, is accelerated with 70

electron volts (eV) and knocks an electron out of the molecule to produce a molecular ion that is a radical cation. This high energy ionization can result in an unstable molecular ion and excess energy can be lost through fragmentation. Bond breakage(s) can lead to the loss of a radical or neutral molecule and molecular rearrangements can also occur. This all results in a, sometimes very large, number of ions of different masses, the heaviest being the molecular ion with fragment ions of various lower masses, depending on:

- the molecular formula
- the molecular structure of the analyte
- where bond breakage has occurred
- which part has retained the charge

5. The next step is to separate the ions of different masses, which is achieved based on their m/z by the mass analyzer (Figure 1 (8)).

There are numerous different mass analyzer types, and this is where the vast differences in mass resolution (and hence instrument price) is seen. Mass resolution is the ability of the mass analyzer to separate ions with very small differences in m/z. Unit mass resolution instruments can only separate nominal masses or those down to a single decimal place, whereas high mass resolution (HRMS) instruments can separate them to four or five decimal places.

The most common type of unit mass instrument is the quadrupole, which is a scanning instrument and varies the voltage to allow only ions of a certain m/z to have a stable trajectory through the four poles to reach the ion detector. Quadrupole instruments are used in two different modes of operation:

Full scan mode, where all ions are acquired across a mass range, useful for identification of unknowns, method development and qualitative and quantitative analysis for higher concentration analytes.

Selected ion monitoring (SIM) mode, where only selected ions that represent the target compound are acquired, useful for trace analysis, as higher sensitivity is obtained, but only of target analytes.

An ion trap is also a scanning instrument but is three-dimensional, trapping the ions in mass-dependent orbits before ejecting them sequentially to reach the ion detector.

Time-of-flight (ToF) mass analyzers separate the ions based on the time they take travel down the flight tube to reach the ion detector. With the same kinetic energy, those with lower masses have a higher velocity and therefore arrive first, whereas those with higher masses have a lower velocity and arrive later. ToFMS instruments can range in mass resolution and acquisition rate: very fast ToFs, with acquisition rates of up to 1000 spectra/second are unit mass resolution, whereas HRMS ToFs have a lower acquisition rate. High acquisition rates are good for two-dimensional GC (GC x GC) applications with peak widths down to 30 ms, however HRMS is very useful to determine the molecular formula. Therefore, there are ToFs on the market that range in speed and mass resolution, the choice of which is dependent on the application, but the GC peak width must match the acquisition rate capabilities of the MS.

Other HRMS instruments that are hyphenated to GC include the magnetic sector mass analyzer, which bends the trajectories of the ions to separate them using electric and magnetic fields. Magnetic sector GC-MS instruments are more commonly found in isotope ratio analyses.

In the HRMS orbitrap, the ions orbit around a central spindle and the frequency that they move up and down the central spindle is m/z-dependent.

6. After the ions have been separated by the mass analyzer based on their m/z, they reach the ion detector.

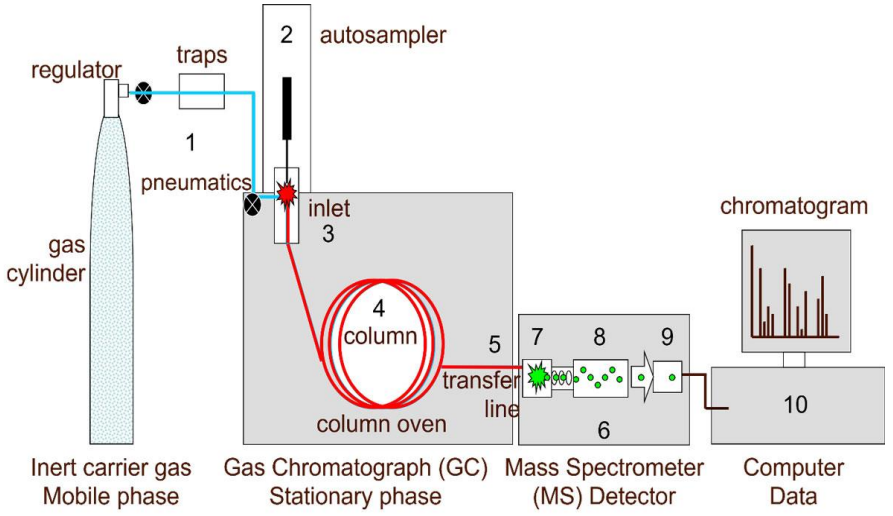

Fig.2.2: A simplified diagram of a gas chromatograph–mass spectrometer showing (1) carrier gas, (2) autosampler, (3) inlet, (4) analytical column, (5) interface, (6) vacuum, (7) ion source, (8) mass analyzer, (9) ion detector and (10) PC. Credit: Anthias Consulting. (Figure 1 (9)) where the signal is amplified by an electron multiplier (for most low resolution MS) or a multi-channel plate (for most HRMS instruments). The signal is recorded by the acquisition software on a computer (Figure 1 (10)) to produce a chromatogram and a mass spectrum for each data point.

Source: (Hernández F, *et al;* 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

These included masking tape, test tunes, holders, tissue papers, hand gloves, and face masks, all of which were essential to keeping the experiment safe and hygienic. For this work, *Myristica fragrans,* often known as Nutmeg, was purchased from Uselu market in Edo State, Nigeria.

Proper sample handling and protection were made easier by tools like aluminum foil, Pyrex conical flasks, universal containers, detergent, and filter papers. Beakers of 500 ml and 1000 ml capacity, a glass rod, and a napkin were used during sample preparation and mixing. The seeds and shells were ground into fine bits using a Kenwood blender, weighed with a balance, and then placed in a stainless steel bucket for efficient maceration. The extract was concentrated using a rotary evaporator (Rotavapor), and lyophillized by freeze-drying. An Agilent GC7890B and the 5977A MSD, both manufactured in the USA, were used for the GC-MS analysis. Ethanol and distilled water were used as solvents in this study.

Fig. 3.1 Image showing some of the materials used (Distilled water, aluminum foil, absolute ethanol, beakers, sieve cloth, stainless steel bucket).

(Source: personal).

3.2 METHODS

3.2.1 PLANT COLLECTION AND IDENTIFICATION

1KG of the *m. fragrans*, was purchased from Uselu market, Ugbowo, Edo State. The sample was identified at the Herbarium unit, in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. A herbarium number was given to the sample (UBH-M350).

3.2.2 SAMPLE EXTRACTION AND PREPARATION OF HYDROETHANOLIC PRECIPITATE

The shells of the nutmeg seeds were broken and separated. The separated seeds were pulverized using a blender to obtain fine particle sizes for efficient maceration and extraction.

Fig. 3.2 Image showing pulverized *Myristica fragrans* (nutmeg) seeds in the stainless steel container.

(Source: personal).

A weighing scale was used to weigh the samples of the powdered nutmeg seed (914.086g) then poured into the stainless steel container to unclump. In a clean beaker,

800 ml of absolute ethanol and 200 ml of distilled water were mixed to form a 1000ml hydroethanolic mixture to serve as the solvent. The mixture was poured into the container of the powdered seeds and mixed with a glass rod (maceration). To minimize evaporation and avoid contamination, the container was sealed with aluminum foil after maceration. To enable complete extraction, the container was labeled for easy identification and left to sit at room temperature for 72 hours. After this period, a whitish and foamy precipitate (hydroethanolic precipitate) was formed on the surface of the extract. The precipitate was decanted in a beaker and covered with aluminum foil paper and kept in the freezer. The extract was filtered in another container to remove insoluble particles, using a sieve cloth, this process was done repeatedly for exhaustive extraction. After extraction, the insoluble remnants (residue) were spread on a flat surface to air dry for several days. The crude extracts were delivered to the Energy Centre in the University of Benin for freeze drying and then to the Pharamaceutical Chemistry Staff Research Laboratory for rotary evaporation.

Fig.3.3a **Fig.3.3b**

Fig.3.3c **Fig.3.3d**

Fig.3.3e **Fig.3.3f**

Fig.3.4(a) Image showing the process of pouring the hydroethanolic mixture into the powdered seeds; (b) image showing the hydroethanolic precipitate formed; (c) image showing precipitate collected in a beaker; (d) image showing the use of sieve cloth for the filtration of the mixture; (e) image of the extract; (f) image showing the residue after extraction.

(Source): Personal

3.3 GC-MS OPERATING PARAMETERS

The gas chromatography–mass spectrometry (GC–MS) configuration using a front-mounted GC autosampler (ALS) and a mass spectrometer operated in full-scan mode. The GC oven program began at an initial temperature of 110 °C with a 2-minute hold, followed by a two-stage temperature ramp designed for broad-range chromatographic separation. The oven first increased at 10 °C/min to 200 °C with no hold, then ramped more slowly at 5 °C/min to a final temperature of 280 °C, where it was held for 9 minutes. The method used a 3-minute equilibration time and a

maximum oven temperature of 325 °C. Cryogenic cooling and slow-fan modes were disabled. The samples were introduced by the GC ALS using a 10 µL syringe with a 2 µL injection volume. The sequence used multiple pre- and post-injection washes with two solvents, along with defined wash and dispense speeds supporting cleanliness and reproducibility. Standard injection was used with an airgap of 0.2 µL. Sample overlap was disabled, and any ALS errors required user intervention.

The front inlet was configured in splitless mode at 260 °C, with a pressure of 12.675 psi and a total flow of 19.127 mL/min. A septum purge of 3 mL/min was applied, while gas saver was off. Splitless purge to the vent occurs at 15 mL/min after 0.75 minutes, ensuring analyte focusing at the column head during the early part of the run. An Agilent HP-5ms Ultra Inert capillary column (30 m × 250 µm × 0.25 µm), a common low-polarity stationary phase suitable for semivolatile and general-purpose GC–MS analyses was used. The column operated with an initial flow of 1.1268 mL/min, generating an average linear velocity of 39.75 cm/s and a holdup time of approximately 1.26 minutes. The outlet was connected directly to the MSD, and the MS transfer line was set to 260 °C. On the mass spectrometer, data were acquired in scan mode from m/z 50 to 650, beginning at 5 minutes to avoid solvent interference. A solvent delay of 5 minutes matches this start time. The system uses a standard tune calibration file, with electron multiplier gain set to 1.0. Source and quadrupole temperatures were maintained at 230 °C and 150 °C, respectively, both within instrument limits. No timed MS events were programmed. The method collects test-plot signals at 50 Hz, though saving is disabled.

CHAPTER FOUR

RESULTS

4.1 GC-MS RESULTS FOR NUTMEG SEED EXTRACTS

A total of sixty-two (62) compounds were identified in the extract of *Myristica fragrans* seed. Some of the bioactive compounds with their peak values, retention time (RT), percentage composition and their pharmacological roles are shown in Table 1:

Table 4.1: Summary of Results of GC-MS analysis of *Myristica fragrans* seed extract

Retention time	Peak Area (%)	CAS	Compound	Pharmacological Role
10.4696	22.3469	00544-63-899	Tetradecanoic acid (Myristic acid)	Anti-inflammatory; antimicrobial; hypocholesterolemic
7.2984	6.1882	000487-11-699	Benzene, 1,2,3-trimethoxy-5-2-propenyl-	Antioxidant; anti-inflammatory
6.8270	4.7221	000607-91-099	1,3-Benzodioxole, 4-methoxy-6-2-propenyl-	Local anesthetic; analgesic; anti-inflammatory
12.2782	5.3816	000057-10-399	n-Hexadecanoic acid (Palmitic acid)	Antimicrobial; anti-inflammatory; antioxidant
22.430	4.3788	051020-86-1	Licarin A	Antimicrobial; anti-inflammatory
9.9039	3.7948	000106-33-294	Dodecanoic acid, ethyl ester	Antimicrobial; anti-inflammatory
14.2836	2.7931	000506-17-296	Cis-Vaccenic acid	Anti-inflammatory; cardioprotective

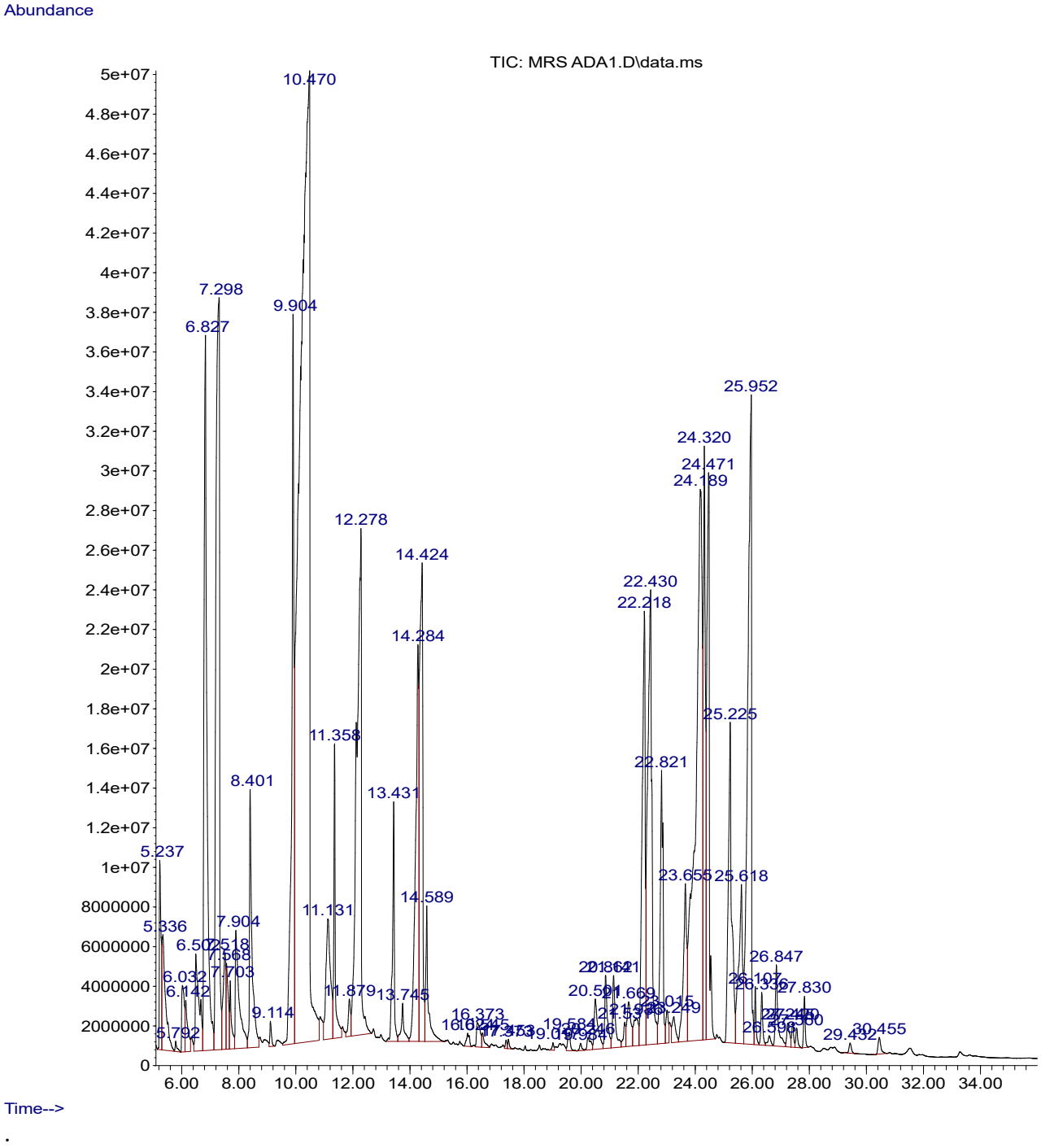

Fig 4.1. Chromatographs of the bioactive compounds of GC-MS analysis of the *Myristica fragrans* seed extracts

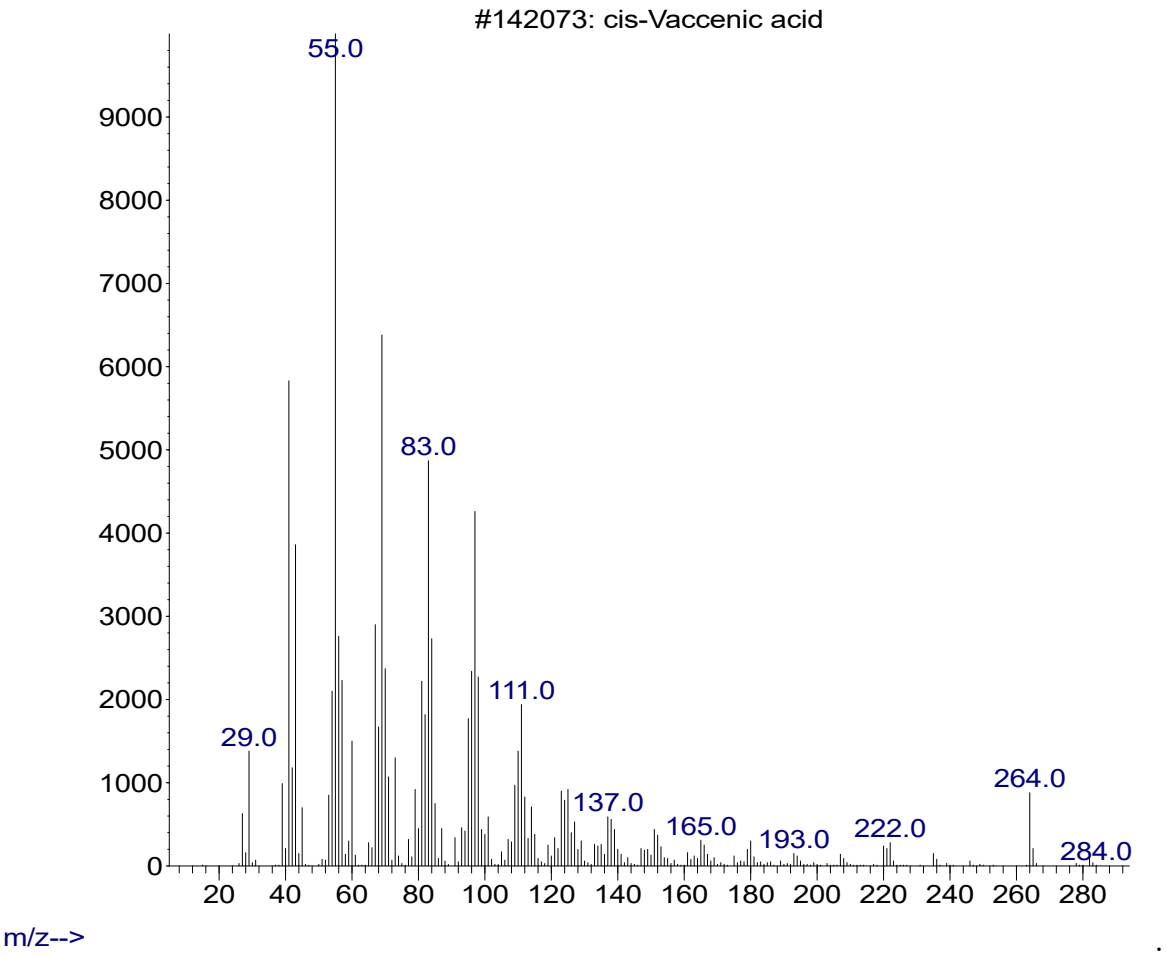

Fig. 4.2 Chromatogram of Cis-Vaccenic acid

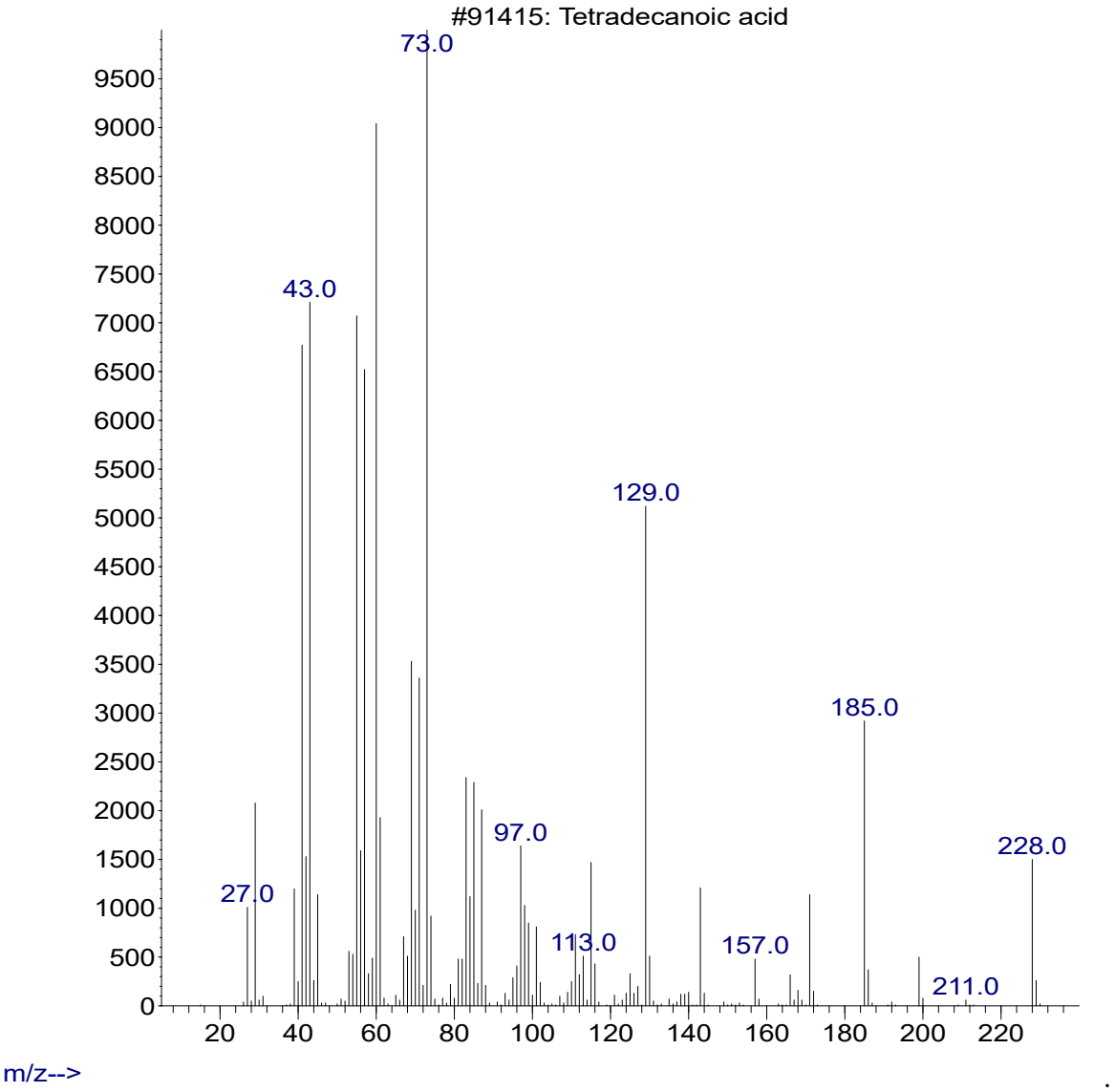

Fig. 4.3 Chromatogram of Tetradecanoic acid

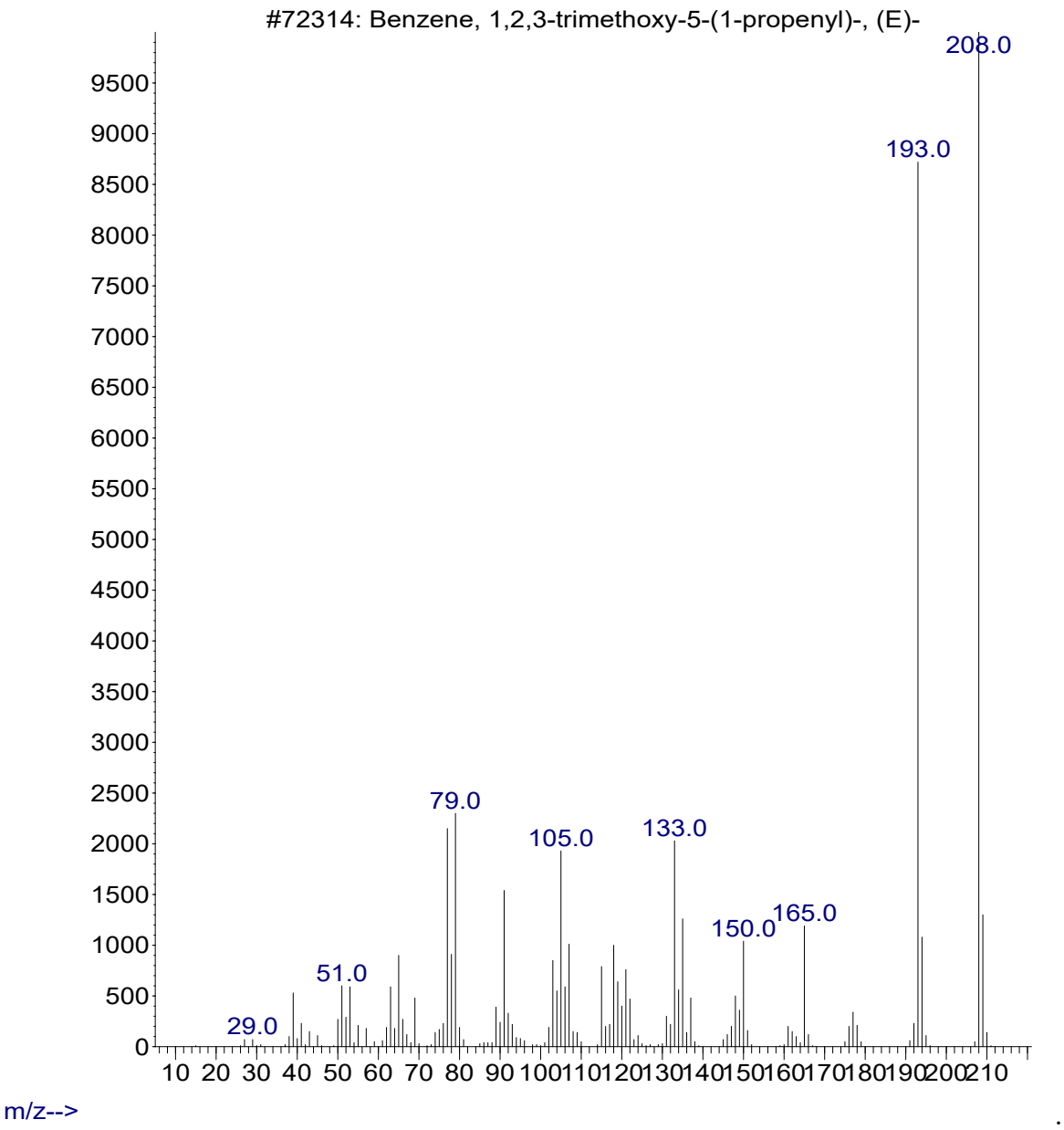

Fig. 4.4 Chromatogram of Benzene 1,2,3-trimetholzy-5-(2-propenyl)

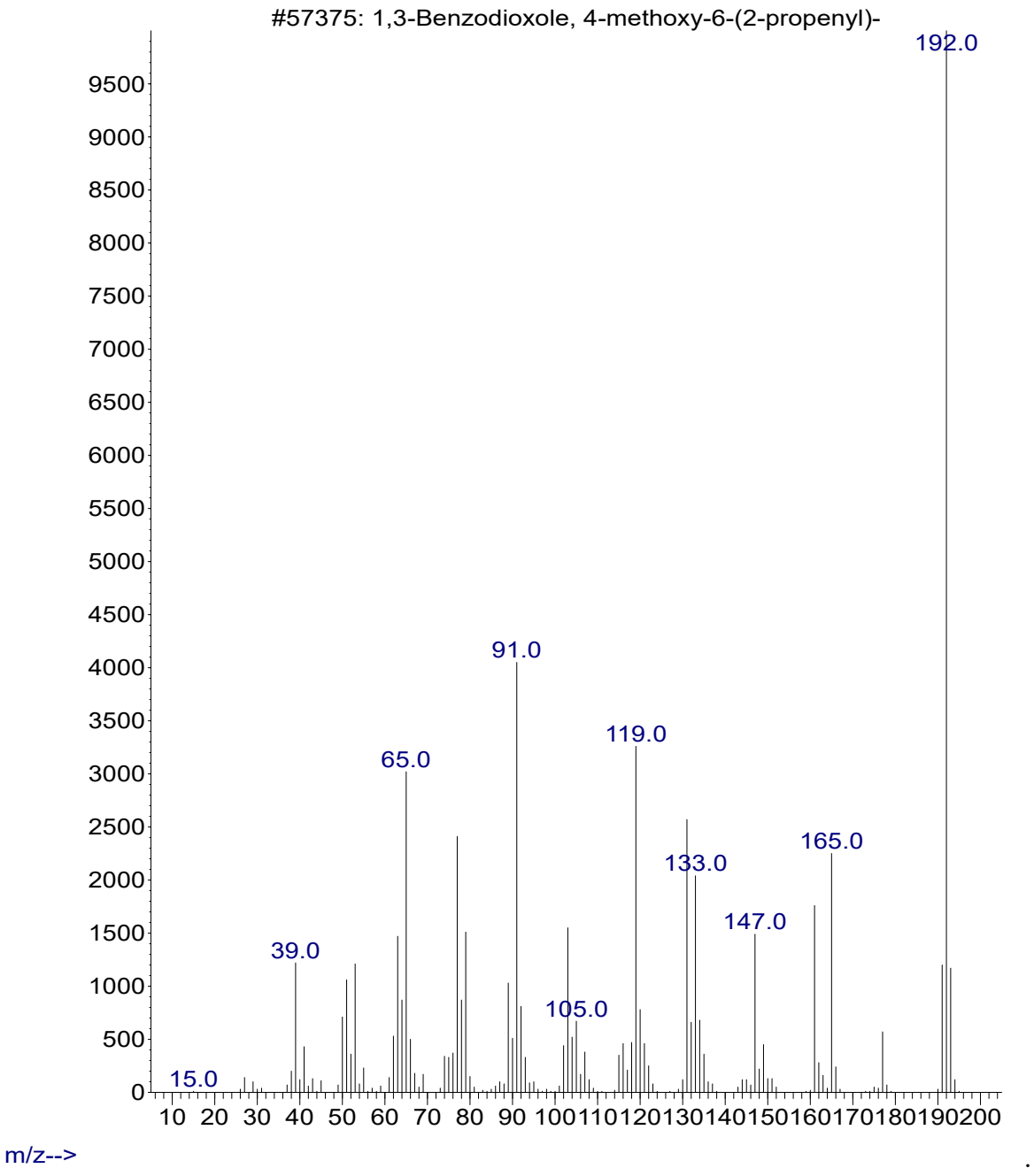

Fig. 4.5 Chromatogram of 1,3-Benzodioxide, 4-methoxy-6-(-2-propenyl)

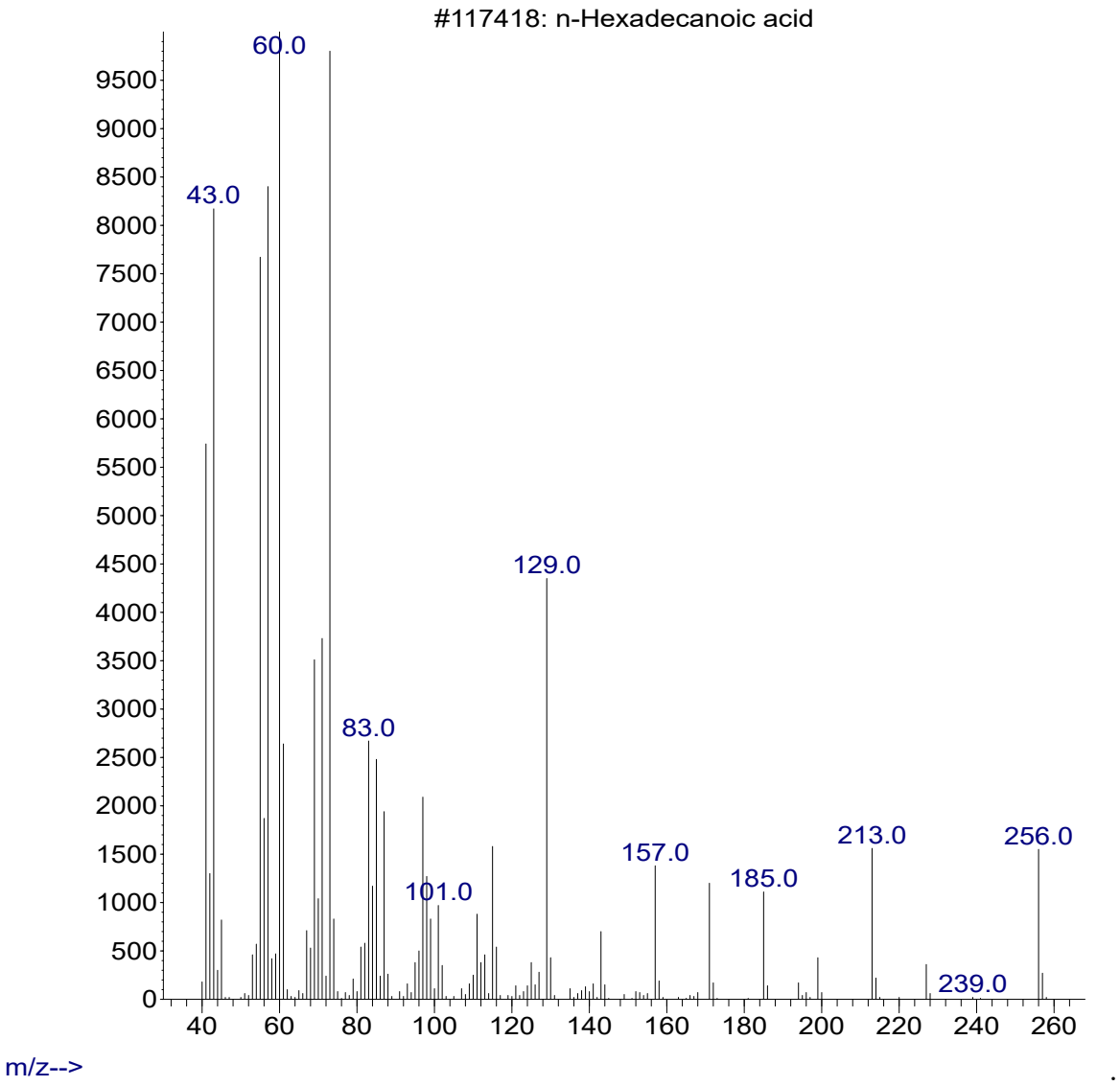

Fig 4.6: Chromatogram of n- Hexadecanoic acid

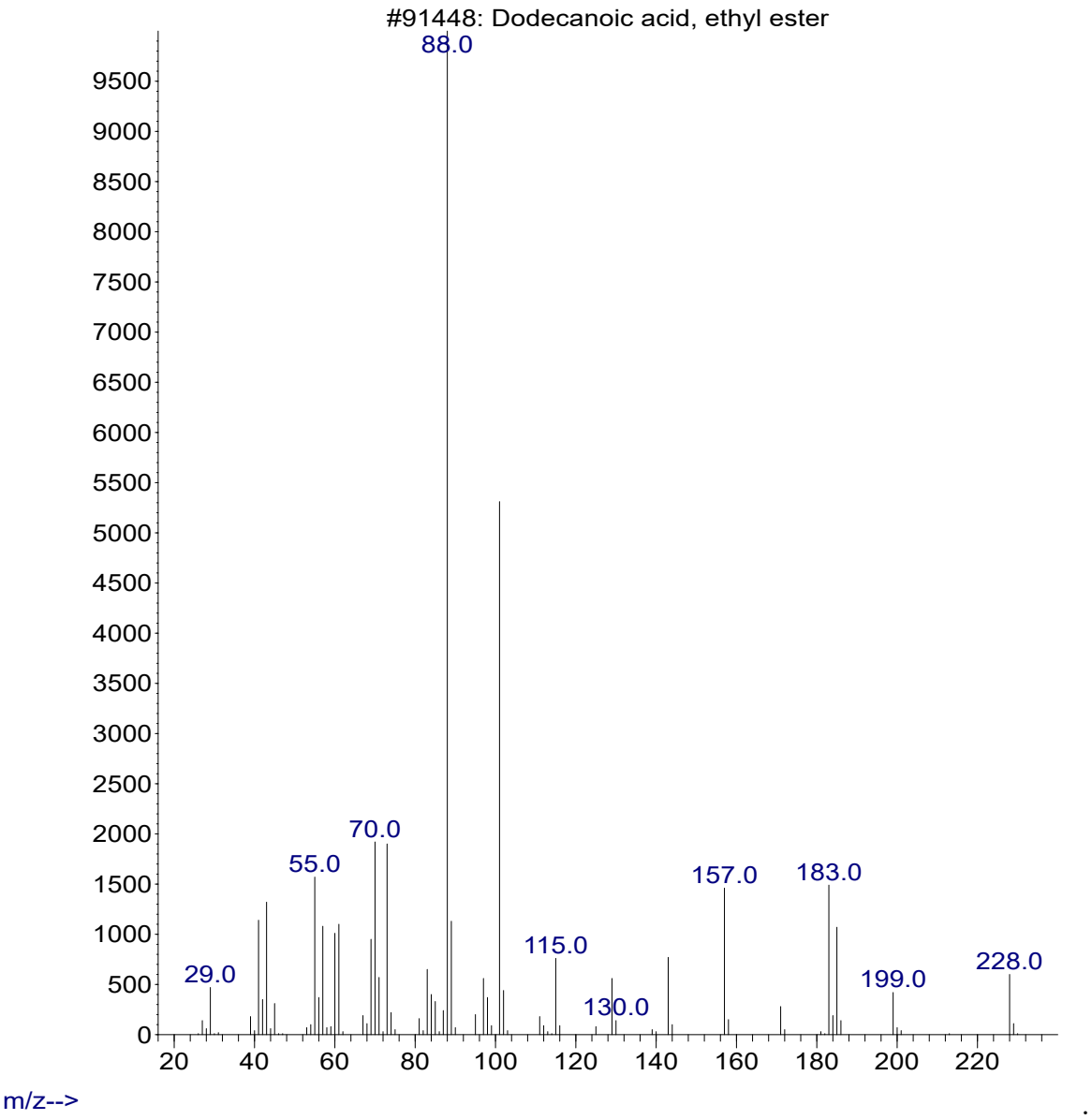

Fig. 4.7 Chromatogram of Dodecanoic acid, ethyl ester

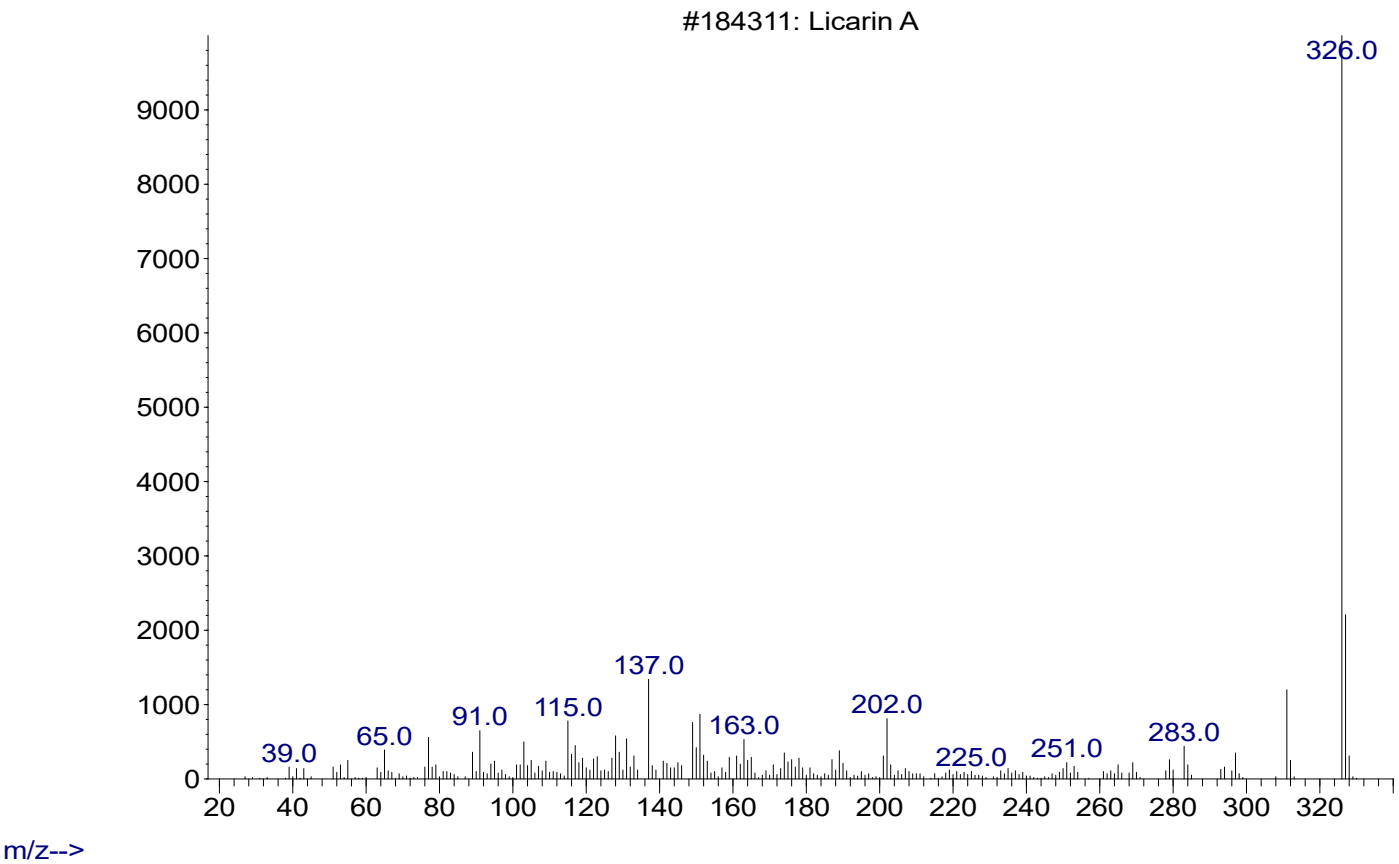

Fig. 4.8 Chromatogram of Licarin A

4.2 GC-MS RESULTS FOR THE HYDROETHANOLIC PRECIPITATE

A total of fifty-five (55) compounds were identified in the extract of the hydroethanolic precipitate of the seed. Some of the bioactive compounds with their peak values, retention time (RT), percentage composition and their pharmacological roles are shown in Table 4.2:

Table 4.2: Summary of Result of GC-MS analysis of the hydroethanolic precipitate of the seed

Retention time (min)	Peak Area (%)	CAS	Compound	Pharmacological Role
10.4472	28.1712	91420000544-63-899	Tetradecanoic acid (Myristic acid)	Anti-inflammatory, antimicrobial, hypocholesterolemic
7.3057	7.4893	72257000487-11-699	Benzene, 1,2,3-trimethoxy-5-2-propenyl-	Antioxidant, anti-inflammatory
6.8525	5.6360	57375000607-91-099	1,3-Benzodioxole, 4-methoxy-6-2-propenyl-	Local anesthetic, analgesic, anti-inflammatory
14.3966	5.0190	142073000506-17-299	Cis-Vaccenic acid	Anti-inflammatory, cardioprotective
24.1616	5.9758	2507551257094-34-090	1S,2R-2-4-Allyl-2,6-dimethoxyphenoxy-1-3,4-dimethoxyphenylpropyl acetate	Anti-inflammatory, antioxidant, antimicrobial
9.9000	4.4339	117418000057-10-394	n-Hexadecanoic acid (Palmitic acid)	Antimicrobial, anti-inflammatory, antioxidant
22.3312	3.1734	184311051020-86-199	Licarin A	Anti-inflammatory, antioxidant, antimicrobial

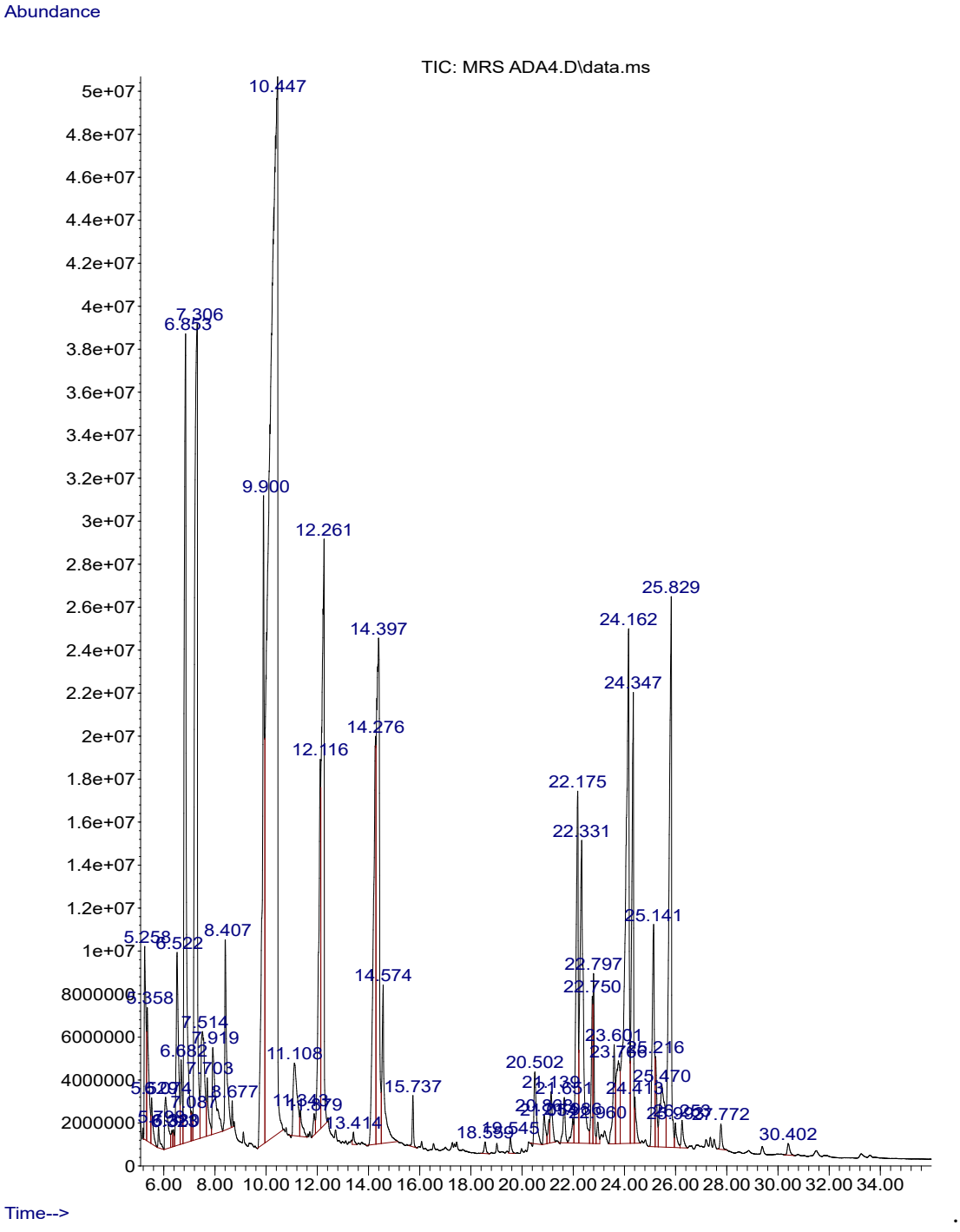

Fig. 4.9 Chromatogram of the bioactive compounds of GC-MS analysis of the hydroethanolic precipitate

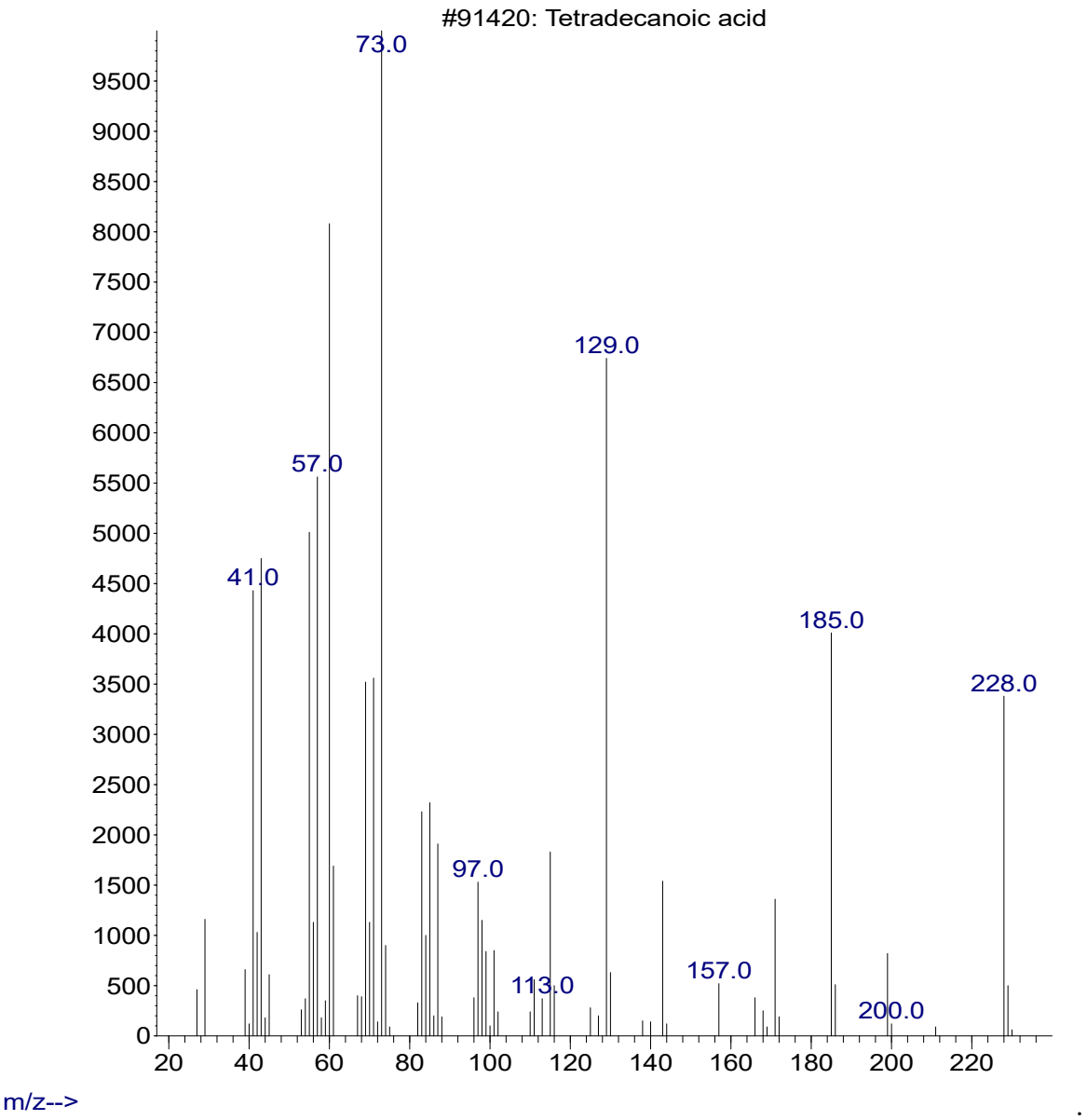

Fig. 4.10: Chromatogram of Tetradecanoic acid

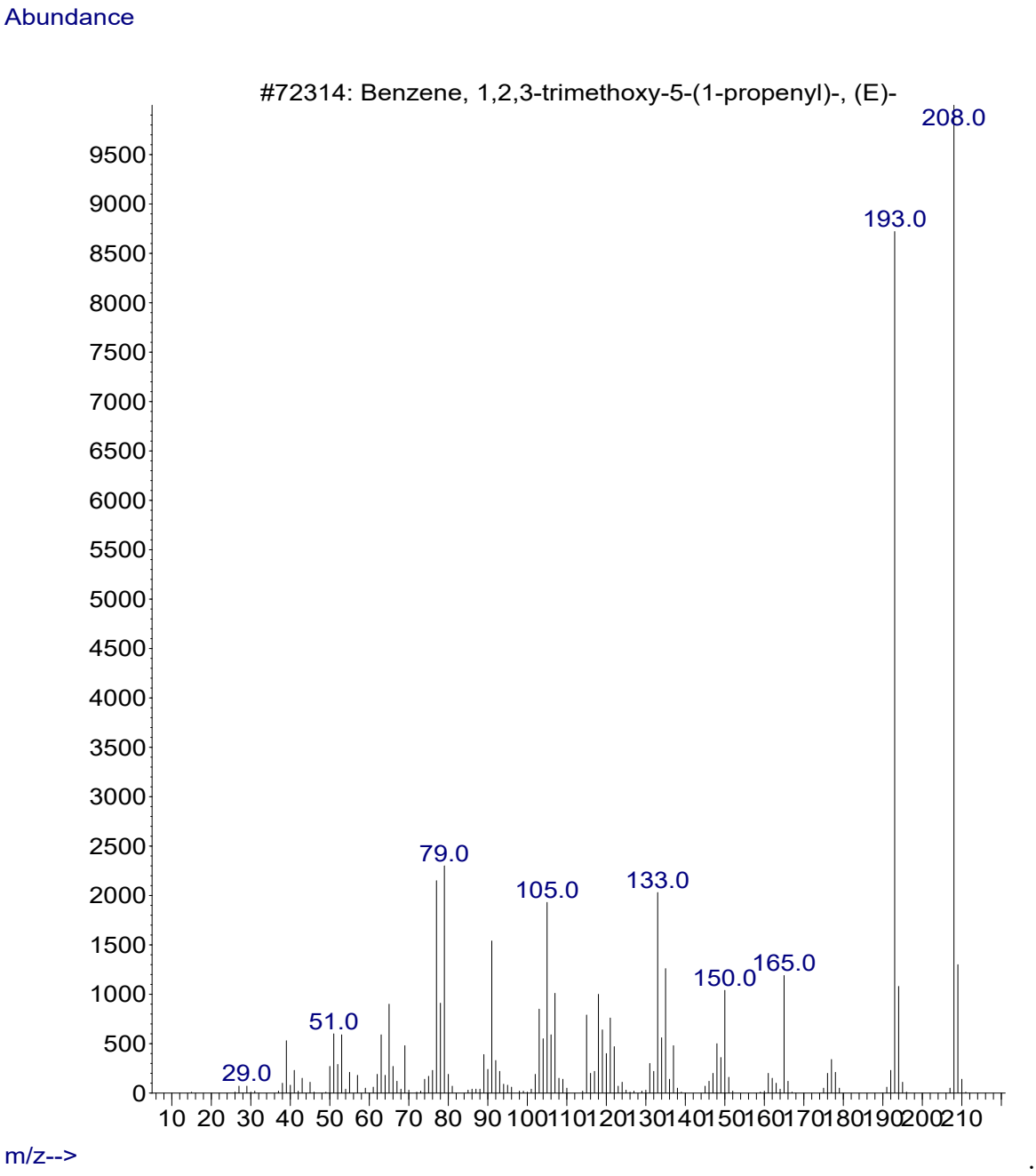

Fig 4.11: Chromatogram of Benzene 1,2,3-trimetholzy-5-(2-propenyl)

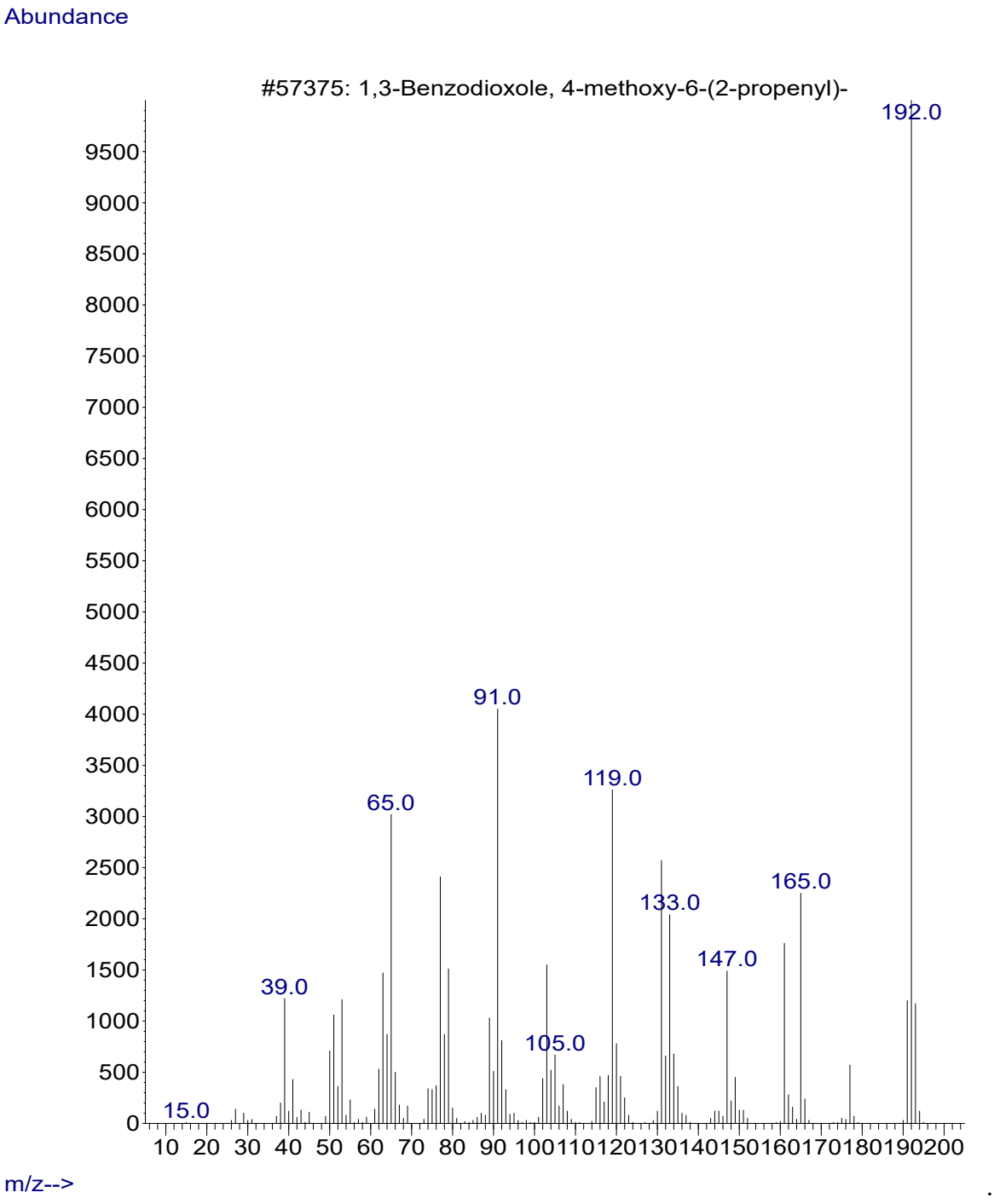

Fig 4.12: Chromatogram of 1,3-Benzodioxide, 4-methoxy-6-(-2-propenyl)

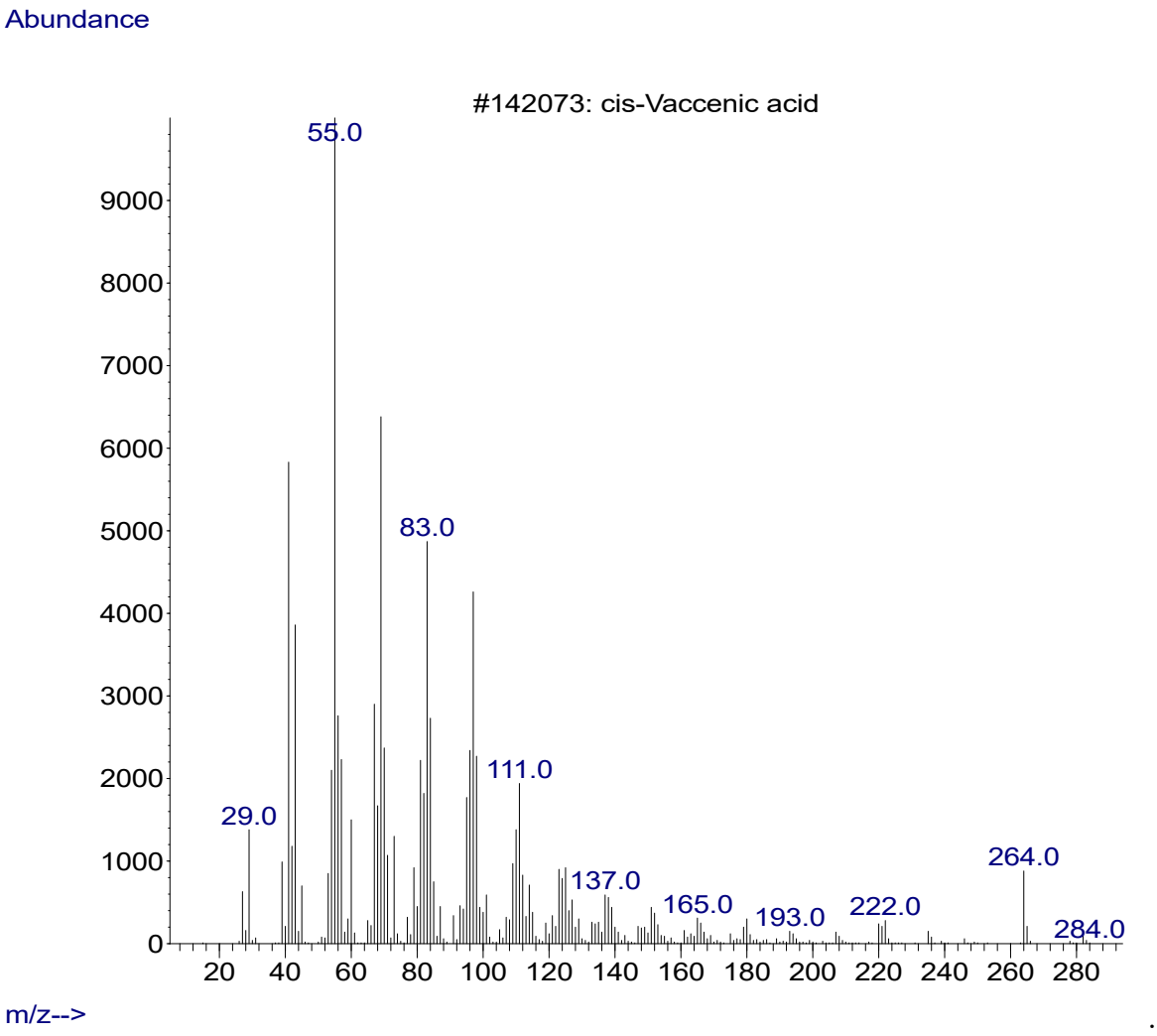

Fig 4.13: Chromatogram of cis-Vaccenic acid

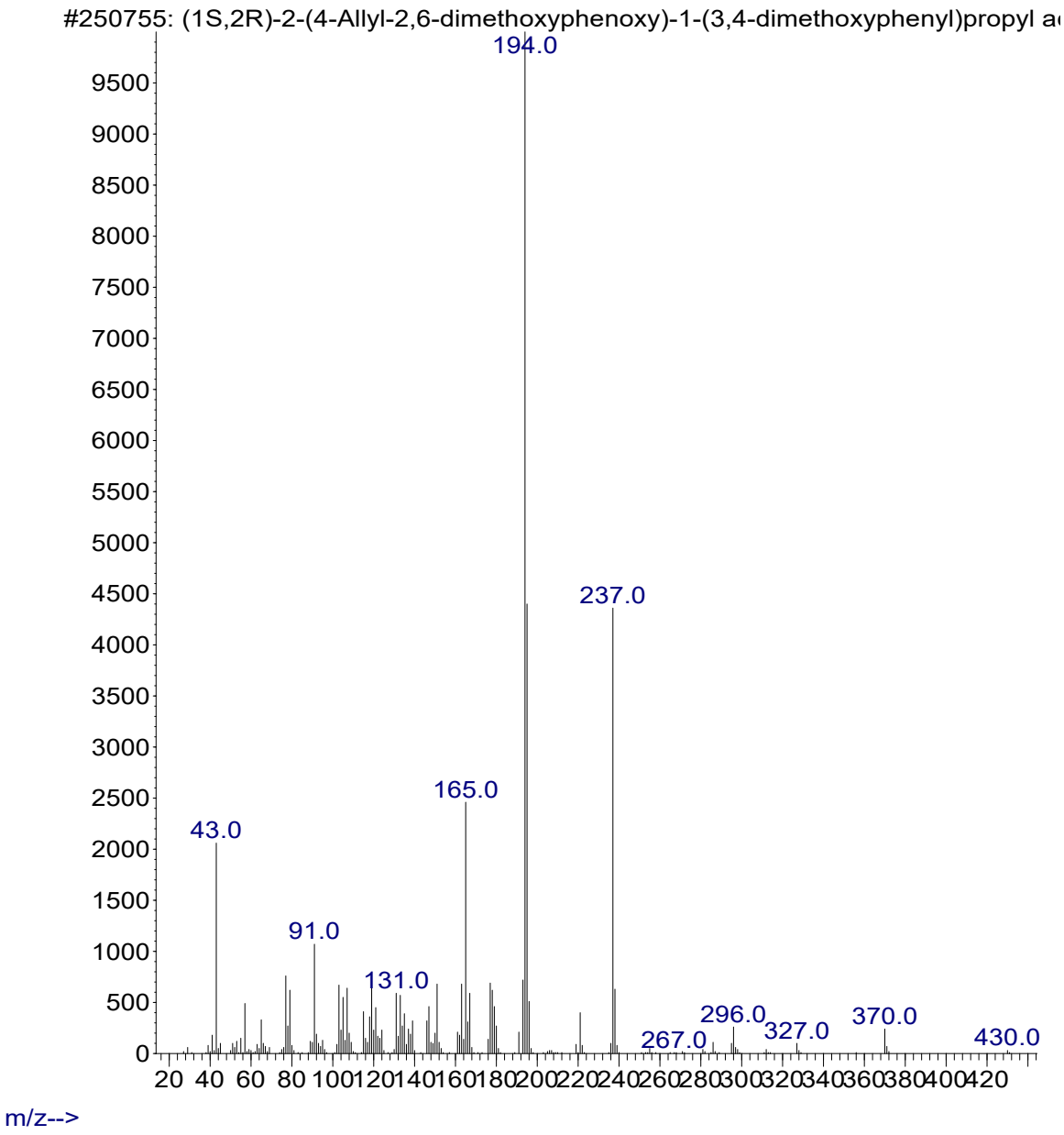

Fig. 4.14: Chromatogram of (1S, 2R) -2- (4- Allyl-2, 6-dimethoxyphenoxy)-1- (3, 4-dimethoxyphenyl) propyl acetate

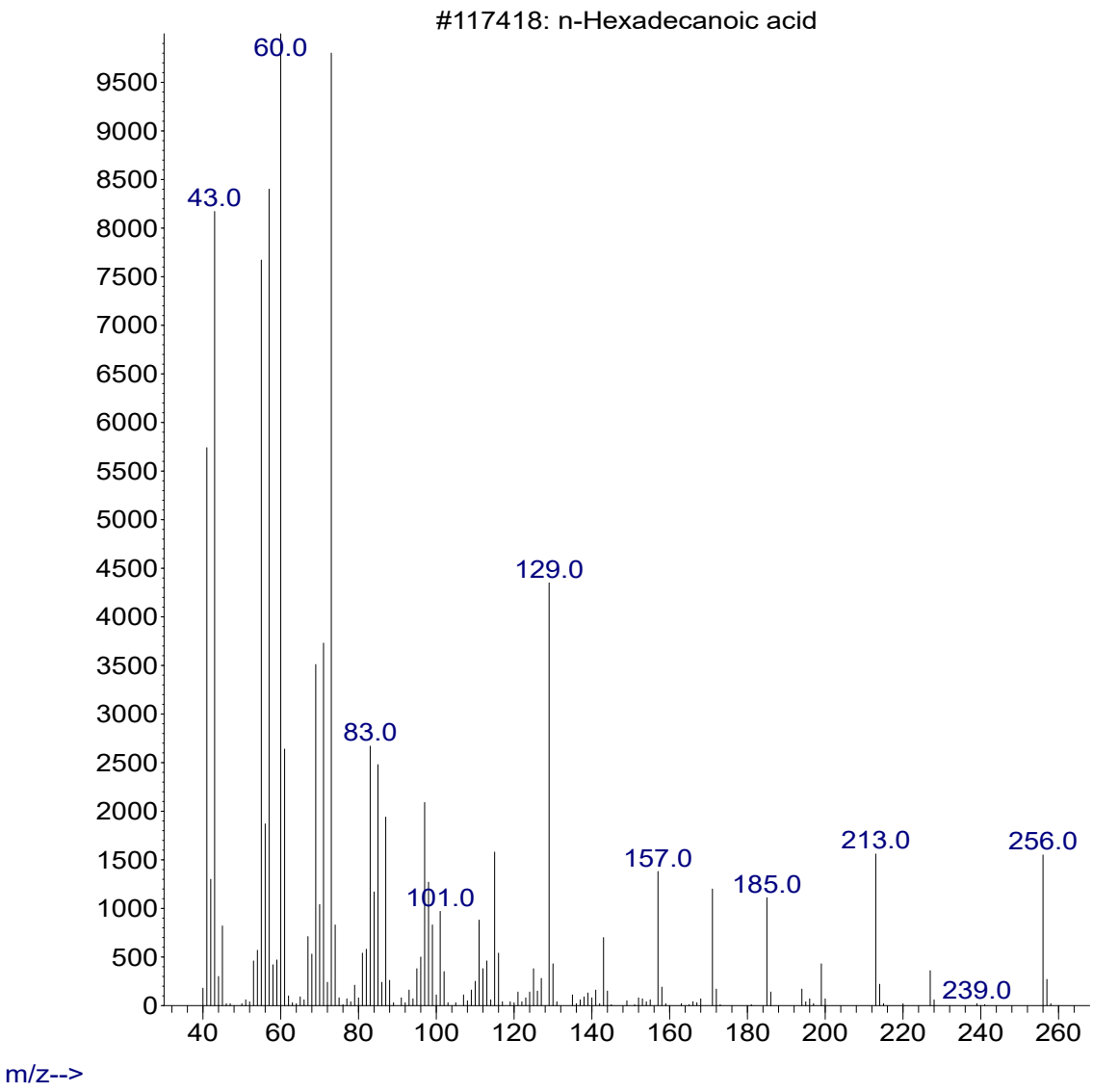

Fig 4.15: Chromatogram of n- Hexadecanoic acid

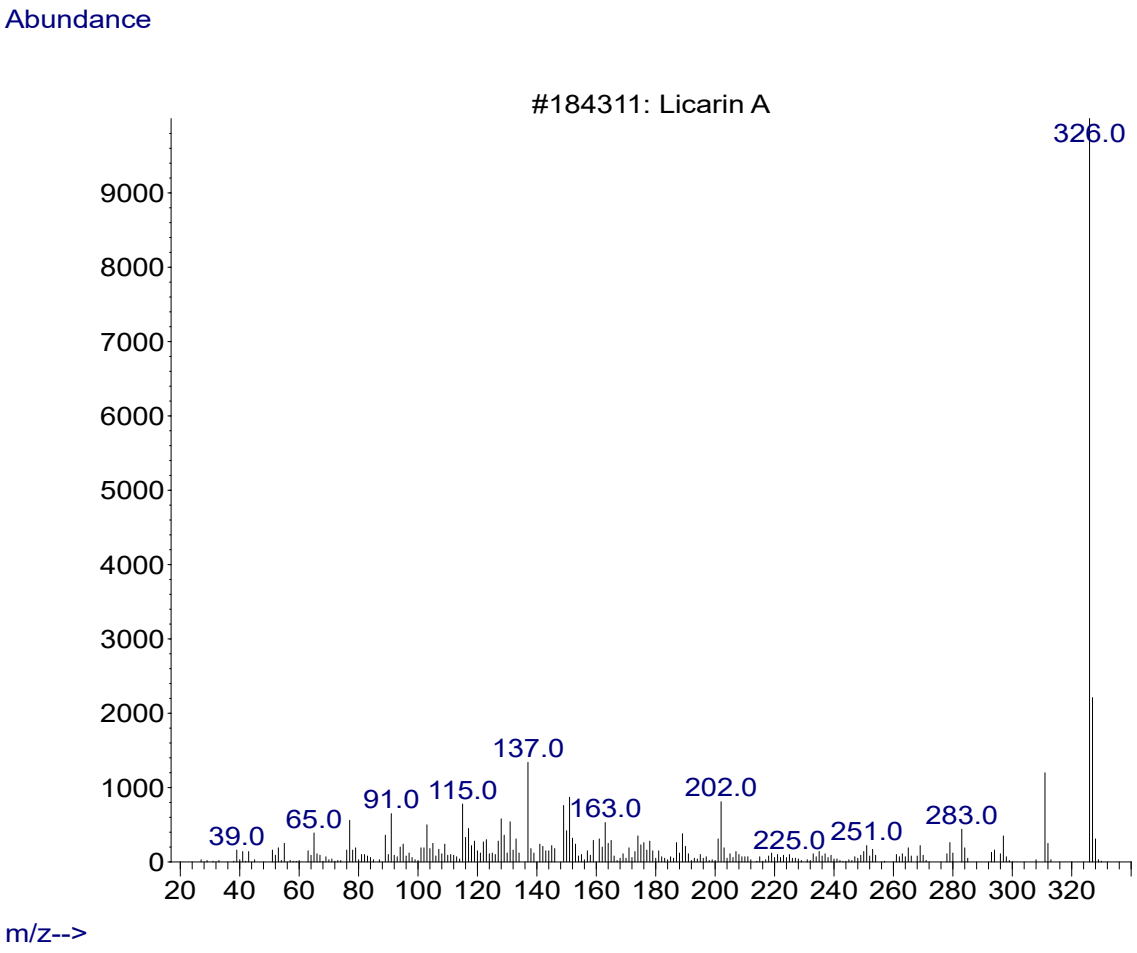

Fig 4.15: Chromatogram of Licarin A

CHAPTER FIVE

5.1 DISCUSSION

The GC-MS analysis of Myristica fragrans seed extract and its hydroethanolic precipitate revealed a diverse array of bioactive compounds, predominantly fatty acids, phenylpropanoids, and lignans, consistent with previous studies on nutmeg phytochemistry. Tetradecanoic acid (myristic acid) was the most abundant compound, which is well-documented for its anti-inflammatory, antimicrobial, and hypocholesterolemic activities. This abundance supports the traditional use of nutmeg for inflammatory conditions and microbial infections. Other significant compounds such as benzene, 1,2,3-trimethoxy-5-2-propenyl- and 1,3-benzodioxole derivatives exhibited antioxidant and analgesic properties, aligning with nutmeg's ethnomedicinal application in pain relief and oxidative stress management.

The presence of cis-vaccenic acid, palmitic acid, and neolignans such as licarin A further highlights the seed's complex pharmacological potential. These compounds confer cardioprotective, anti-inflammatory, and antimicrobial benefits, which corroborate prior pharmacological data emphasizing nutmeg's therapeutic versatility. The hydroethanolic extraction method effectively isolated a broad spectrum of phytochemicals, including both soluble compounds in the extract and less soluble bioactive residues in the precipitate, ensuring comprehensive profiling. This dual analysis reveals that the precipitate contains notable residual bioactives, often overlooked but important for maximizing yield and biological activity.

Importantly, the identified compounds demonstrate mechanistic potential in modulating oxidative stress, inflammation, microbial growth, and lipid metabolism, which are key pathways involved in chronic diseases such as cardiovascular diseases, diabetes, and neurodegenerative

disorders. The biological roles of these compounds provide scientific validation to the traditional medicinal uses of *Myristica fragrans* as well as the hydroethanolic precipitate and suggest prospects for isolated compounds in pharmaceuticals and nutraceuticals. Safety considerations were also noted, particularly concerning myristicin-related toxicity at high doses, underscoring the importance of dose regulation in therapeutic applications.

5.2 CONCLUSION

This study successfully characterized the bioactive chemical constituents of Myristica fragrans seed extracts and hydroethanolic precipitates using GC-MS. The analysis confirmed the presence of significant pharmacologically active compounds including fatty acids (myristic acid, palmitic acid), phenylpropanoids, and lignans with antioxidant, anti-inflammatory, antimicrobial, and cardioprotective properties. The hydroethanolic extraction approach proved efficient in isolating a broad and representative range of compounds, including those retained in the precipitate fraction, supporting its use for comprehensive phytochemical profiling. The findings scientifically support the ethnomedicinal claims and potential health benefits of M. fragrans, positioning it as a valuable source for drug and functional food development. However, caution regarding toxicity at high intake levels is warranted.

5.3 RECOMMENDATIONS

- Further pharmacological and toxicological evaluations should be conducted on the identified major compounds to elucidate their therapeutic efficacy and safety profiles in vivo.
- Clinical studies on standardized extracts of *Myristica fragrans* are recommended to validate the medicinal claims and optimize dosage for safe human use.
- Exploration of synergistic effects among the bioactive compounds could enhance the development of novel herbal formulations with improved therapeutic benefits.
- Development of efficient extraction and purification protocols focusing on both extract and precipitate components will maximize bioactive yield for industrial applications.
- Public awareness about safe consumption levels of nutmeg, especially concerning myristicin-related adverse effects, should be promoted to prevent toxicity.
- Integration of M. fragrans extracts as natural preservatives and additives in pharmaceutical and food industries could be explored, leveraging their antimicrobial and antioxidant properties.

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