PHYTOCHEMICAL AND ANTIOXIDANT ANALYSIS OF *MYRISTICA FRAGRANS* (NUTMEG) SEED EXTRACT

BY

AVRE VANESSA EREZI

(BMS2101381)

DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCE

COLLEGE OF MEDICAL SCIENCE

UNIVERSITY OF BENIN

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

NOVEMBER, 2025

CERTIFICATION

We the underlisted certify that this project titled "**PHYTOCHEMICAL AND ANTIOXIDANT ANALYSIS OF *MYRISTICA FRAGRANS* (NUTMEG) SEED EXTRACT**" was carried out by **AVRE VANESSA EREZI** (**BMS2101381)** in the Department of Medical Biochemistry, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

.

....

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, my Creator, my constant source of strength, and the wellspring of my inspiration, wisdom, and understanding. It is by His grace alone that I have been able to embark on and complete this journey. Through His guidance, I have found direction; on His wings, I have soared.

This work is also lovingly dedicated to my parents Mr James Avre and Mrs Philo Avre, whose unwavering support, encouragement, and belief in me have been the foundation of my perseverance. Their steadfast presence has fueled my determination and carried me through every challenge. May God's abundant blessings continue to be with them now and always.

ACKNOWLEDGEMENT

I wish to express my profound gratitude to God Almighty for His guidance, wisdom, provision and strength throughout the course of this project work.

My deepest appreciation goes to my project supervisor, Mrs. Ukwuonwo-Ediale, for her invaluable guidance, patience, and constructive criticisms which were instrumental to the successful completion of this work. Her dedication, mentorship, and encouragement have been a great source of inspiration. I also sincerely thank my Head of Department, Dr. B. N. Aguebor-Ogie, for his leadership, support, and for providing a conducive academic environment that made this project possible and all the academic and laboratory staff of the Department of Medical Biochemistry.

My deepest gratitude goes to my parents, Mr James AVRE and Mrs Philo AVRE for their continuous support, prayers, sacrifices and encouragement throughout my academic journey.

I appreciate my siblings, Barbara, Naomi and Divine for their care and motivation which has shaped my journey in school.

Shout out to my project group colleagues, Godwin, Esther, Triumph and Deborah for their amazing contributions to the success of this project

To my amazing friend, Engr. Charles, Thank you for being a steady source of support and inspiration throughout my academic journey. Your constant belief in me has meant so much to me.

Special thanks to my wonderful friends¸ Nosakhare, Favour, Osanor and Ada. I'm grateful for your constant love and motivation throughout my academic journey. Shout out to everyone who has one way or the other contributed to the success of my stay in this university.

TABLE OF CONTENTS

REFERENCES

APPENDIX

ABSTRACT

Nutmeg (Myristica fragrans) is a widely used culinary spice known for its diverse therapeutic properties. This study investigated the phytochemical constituents and antioxidant activity of nutmeg seed extract to provide scientific insight into its medicinal potential. Phytochemical screening revealed the presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and Cardiac glycosides. These compounds are associated with various biological activities including antimicrobial, anti-inflammatory, and free-radical scavenging effects. The antioxidant capacity of the extract was evaluated using standard assays like DPPH(2,2-diphenyl-1-picrylhydrazyl), which had an IC_{50} value of 2.965. FRAP (Ferric Reducing Antioxidant Power)which had an IC_{50} value of 2.228. TAC(Total antioxidant capacity) which had an IC_{50} value of 1.209. The Antioxidant Scavenging activity of this plant suggests that this plant *Myristica Fragrans* has good antioxidant capacity and can help combat oxidative stress.

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND OF STUDY

Over the past few decades, numerous scientific studies have shed light on the vital roles of Reactive Oxygen Species (ROS) in maintaining normal physiological functions such as cell signaling and immune defense. However, when ROS levels surpass the body's natural antioxidant capacity, they cause oxidative stress, leading to structural and functional damage of cellular components, including lipids, proteins, and DNA (Gupta *et al*.,2019). Such oxidative damage is closely associated with the onset and progression of chronic disorders such as cancer, diabetes, cardiovascular diseases, and neurodegenerative conditions (Morsy *et al*.,2016). To mitigate these harmful effects, researchers have increasingly focused on naturally occurring antioxidants, particularly those derived from medicinal plants, as promising alternatives to synthetic compounds (Ginting *et al*., 2018).

Medicinal plants are known to be rich sources of phytochemicals—bioactive secondary metabolites that include phenols, flavonoids, alkaloids, tannins, terpenoids, and saponins—which contribute significantly to their pharmacological potential (Abourashed *et al*., 2016). These compounds possess remarkable free radical scavenging and metal chelating abilities, thereby enhancing the body's defense mechanisms against oxidative damage (Abourashed *et al*., 2016). Studies on various medicinal plants have confirmed their antioxidant capacity through biochemical assays and in vivo investigations, supporting their traditional roles in promoting health and longevity (Ginting *et al*. , 2018).

Among these valuable medicinal plants, *Myristica Fragrans*, commonly known as nutmeg, holds a prominent position due to its rich historical, culinary, and therapeutic importance. Nutmeg is an evergreen tree native to the Maluku Islands (the Spice Islands) of Indonesia, which were once the world's exclusive source of this highly prized spice (Ginting *et al*.,2018). Historically, nutmeg played a pivotal role in global trade during the 15th and 16th centuries, fueling European exploration and the famous "spice wars." It was regarded as a luxury commodity used not only for flavoring food but also for its perceived medicinal and preservative properties [Ginting *et al*.,2018]. Over time, cultivation of *Myristica Fragrans* spread to other tropical regions, including India, Sri Lanka, Grenada, and Malaysia, making it one of the most widely recognized and economically important spice crops (Ginting *et al*.,2018).

The seed of *Myristica Fragrans*—commonly referred to as nutmeg seed—is a rich source of essential oils and diverse phytochemical constituents responsible for its distinct aroma and bioactivity. Traditionally, nutmeg has been used to treat digestive disorders, pain, insomnia, and infections, and to enhance appetite and blood circulation (Ginting *et al*.,2018). Modern pharmacological studies have reported that nutmeg seed extracts possess antioxidant,

antibacterial, anti-inflammatory, analgesic, antiulcer, anticancer, and hepatoprotective properties (Ginting *et al*.,2018). These therapeutic effects are largely attributed to its phytochemical constituents such as myristicin, elemicin, eugenol, safrole, and various flavonoids, which have demonstrated significant radical scavenging activity (Ginting *et al*.,2018).

Despite these promising findings, it is important to recognize that certain plant-derived compounds may exert toxic, mutagenic, or teratogenic effects at inappropriate doses or under prolonged exposure (Panggabean *et al*., 2018). This has led to the increasing need for scientific validation and safety assessment of herbal remedies and plant-based antioxidants through phytochemical characterization and antioxidant testing (Panggabean *et al*.,2018). Such analyses are crucial in identifying the specific compounds responsible for antioxidant activity, understanding their mechanisms of action, and determining safe dosage ranges for therapeutic use (Panggabean *et al*.,2018).

In view of this, this research is designed to conduct a thorough phytochemical and antioxidant analysis of the seed extract of *Myristica Fragrans*. This study seeks to identify and characterize the bioactive constituents responsible for its pharmacological effects and to evaluate its free radical scavenging potential using standard analytical assays. The findings will contribute valuable scientific evidence supporting the traditional use of nutmeg as a natural source of antioxidants and will provide a foundation for further exploration into its possible applications in the prevention and management of oxidative stress-related diseases.

1.2 JUSTIFICATION OF STUDY

The increasing global concern over oxidative stress and its role in chronic diseases such as cancer, diabetes, and cardiovascular disorders has intensified the search for safe, natural antioxidants. Synthetic antioxidants like BHA and BHT, though effective, have raised health concerns due to their potential toxicity. This has prompted greater interest in plant-derived antioxidants known for their safety, accessibility, and multiple health benefits. *Myristica Fragrans* (nutmeg), a widely used spice and traditional remedy, contains numerous bioactive compounds, yet its seed extract remains underexplored compared to its essential oil. Investigating the phytochemical composition and antioxidant potential of the seed extract is therefore essential to provide scientific validation for its traditional uses and to uncover its potential as a natural antioxidant source.

1.3 AIMS OF STUDY

The main aim of this research is to evaluate the phytochemical constituents and antioxidant potential of the seed extract of *Myristica Fragrans* in order to establish a scientific basis for its traditional medicinal and nutritional applications.

1.4 OBJECTIVES OF STUDY

1. To perform qualitative and quantitative phytochemical analyses of *Myristica Fragrans* seed extract to determine the presence and concentration of key bioactive compounds such as phenols, flavonoids, alkaloids, tannins, saponins, and terpenoids.

2. To evaluate the antioxidant activity of the seed extract using standard in vitro assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power), or TAC(Total Antioxidant Capacity) methods.

3. To establish the correlation between the total phenolic and flavonoid contents and the measured antioxidant activity of the extract.

4. To provide baseline data that will guide further research on the potential pharmacological and nutraceutical applications of nutmeg seed extracts.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical Background, Origin, and Botanical Overview of *Myristica Fragrans*

Nutmeg (*Myristica Fragrans*.) is among the most iconic spice trees globally, belonging to the family Myristicaceae, which includes over 300 species of tropical trees (Kusuma *et al*., 2023). The plant's precise origin has long been debated. While many authors recognize the Maluku or Moluccas Islands of Indonesia—often called the "Spice Islands"—as the broader region of origin, others have specifically identified the Banda Islands, a small archipelago within the southern Moluccas, as the true center of domestication and early cultivation (Kusuma *et al*., 2023).

Historical records show that nutmeg was known to Asian traders centuries before European exploration. Chinese writings from the 13th century, notably the work of Zhao Rugua (c. 1225), appear to make early reference to nutmeg and its source islands, although the descriptions were not precise enough to confirm botanical identity (Kusuma *et al*., 2023). The Portuguese became the first Europeans to reach the region in the early 16th century, describing Banda as the primary

site of nutmeg cultivation. Later, the Dutch, through the Dutch East India Company (VOC), established a trade monopoly from the 17th to 18th centuries to control this lucrative spice (Kusuma *et al*., 2023). During this time, nutmeg became one of the world's most sought-after commodities, driving global maritime trade and colonial competition.

Attempts by other colonial powers, notably the French and the British, to break the Dutch monopoly led to the introduction of nutmeg trees to other tropical regions, including India (Kerala), Sri Lanka, the Caribbean, and South America (Kusuma *et al*., 2023). These introductions marked the beginning of nutmeg's global distribution, transforming it from a regional Indonesian spice to an internationally cultivated crop.

Archaeobotanical findings further indicate that nutmeg's association with human use dates back thousands of years. Evidence from Pulau Ai (Ai Island), one of the Banda Islands, suggests that nutmeg was used during the Neolithic period (approximately 3500–2000 BP), implying that the species has been known and consumed for over a millennium before European arrival (Kusuma *et al*., 2023). This early evidence highlights nutmeg's longstanding role in local diets and traditional practices within the Indonesian archipelago.

Today, *Myristica Fragrans* is cultivated widely in Indonesia, India, Malaysia, Sri Lanka, China, Taiwan, and South America, where it thrives in tropical rainforests with warm, humid conditions (Sultan *et al*., 2023). Botanically, the tree is evergreen, reaching 10–20 meters in height, and produces a yellowish fruit that splits open when mature to reveal the seed (nutmeg) encased in a bright red aril (mace)—both of which are valuable spice components (Ashokkumar *et al*., 2022).

2.2 Traditional, Cultural, and **Ethnomedicinal Relevance of *Myristica Fragrans***

Beyond its culinary fame, *Myristica Fragrans* holds deep traditional, cultural, and medicinal significance across Asia, Africa, and the Caribbean. In Ayurvedic, Chinese, and Unani medicine, nutmeg is valued as a warming agent, digestive tonic, and remedy for ailments such as abdominal pain, diarrhea, insomnia, and nervous disorders (Sultan *et al*., 2023; Elfia and Susilo, 2023). In Indonesia and India, powdered nutmeg has been used for toothache relief, rheumatism, and respiratory problems, while its essential oil serves as a topical analgesic and antiseptic.

In various African and Middle Eastern traditions, nutmeg has long been regarded as a symbol of vitality and purification, incorporated into folk remedies, aphrodisiac preparations, and culinary rituals. Its distinctive flavor and aroma have also made it a spiritual and ceremonial ingredient, often believed to promote clarity of mind and ward off negative energy.

Phytochemical investigations have revealed that the therapeutic efficacy of nutmeg arises from a rich array of bioactive compounds such as phenols, terpenoids, alkaloids, flavonoids, lignans, tannins, and essential oils (EOs) (Ashokkumar *et al*., 2022; Tutuarima *et al*., 2024). These compounds exhibit diverse biological effects including antioxidant, anti-inflammatory, anticancer, antimalarial, antifungal, and antibacterial activities (Ibrahim *et al*., 2020; Fernando and Senevirathne, 2021; Okiki *et al*., 2023).

In addition, nutmeg's pharmacological spectrum extends to antiplatelet (Arunachalam *et al*., 2018), antispasmodic (Prashant *et al*., 2013), anticonvulsant (Kumar and Samanta, 2018), antidepressant (Dhingra and Sharma, 2006), antidiabetic (Pashapoor *et al*., 2020), and antihepatotoxic (Zhao *et al*., 2020) properties. Other studies have reported its diuretic, immune-modulatory, and analgesic effects (Bhuiyan, 2019; El Shanawany *et al*., 2024).

These findings provide scientific support for the traditional ethnomedicinal uses of nutmeg, bridging ancient knowledge and modern pharmacological understanding. Its dual identity as a spice and medicine has made it an invaluable plant in global food and healthcare systems. Moreover, the continued exploration of its phytochemical and antioxidant profile is essential for validating its therapeutic potential and ensuring its safe and effective application in modern phytotherapy and nutraceutical industries.

2.3 Botanical Description and Distribution of *Myristica Fragrans*

Myristica Fragrans., commonly known as nutmeg, belongs to the family Myristicaceae, which comprises more than 15 genera and over 300 species distributed mainly in tropical regions of Southeast Asia and the Pacific (FAO, 2018; Kusuma *et al*., 2023). The plant is an evergreen, aromatic tree characterized by its dense canopy and glossy dark-green leaves. It typically grows to a height of 10–20 meters and develops a pyramidal or oval crown when mature (Sultan *et al*., 2023).

2.3.1 Morphological Features

The leaves of *Myristica Fragrans* are simple, alternate, and elliptic-lanceolate in shape, measuring about 5–15 cm long and 2–7 cm wide, with a leathery texture and a shiny upper surface. The tree produces small, pale-yellow flowers that are dioecious, meaning individual trees bear either male or female flowers (Ashokkumar *et al*., 2022). Pollination is generally entomophilous, aided by small insects attracted to the flowers' scent.

The fruit is a smooth, fleshy drupe, approximately 5–7 cm long, which turns yellowish-orange upon ripening. When mature, it splits longitudinally into two valves to expose a single shiny brown seed (nutmeg) enveloped in a bright red, lacy aril (mace) (FAO, 2018). The seed is oval, hard, and aromatic, containing a rich oily endosperm that serves as the source of nutmeg spice. Both nutmeg (seed) and mace (aril) are highly valued as spices and medicinal ingredients due to their distinct aroma and bioactive compounds such as myristicin, elemicin, and eugenol (Kusuma *et al*., 2023).

2.3.2 Parts Used and Their Industrial Importance

Nearly every part of *Myristica Fragrans* has economic or medicinal value.

The seed (nutmeg) is primarily used as a culinary spice, flavoring agent, and source of essential oil and nutmeg butter.

The aril (mace) is another prized spice, possessing a milder flavor and often used in baking, confectionery, and perfumery industries (FAO, 2018).

The essential oil extracted from both nutmeg and mace has applications in pharmaceutical formulations, aromatherapy, perfumery, and traditional medicine.

The kernel oil, also known as nutmeg butter, is employed in cosmetics, ointments, and soap manufacturing, while waste residues are sometimes used as animal feed or compost (Ashokkumar *et al*., 2022).

The bark and leaves, though less commonly used, contain aromatic compounds that have been studied for their antibacterial and antioxidant properties (Sultan *et al*., 2023).

Geographical Distribution and Major Producing Regions

The true nutmeg tree (*Myristica Fragrans*) is native to the Banda Islands of the Maluku (Moluccas) archipelago in Indonesia, historically known as the "Spice Islands" (FAO, 2018; Kusuma *et al*., 2023). Due to centuries of global spice trade and colonial expansion, nutmeg cultivation has spread to numerous tropical regions worldwide. Today, it is extensively cultivated in:

1. Indonesia (Maluku, North Sulawesi, Aceh, anX d Java)
2. India (Kerala and Karnataka)
3. Sri Lanka
4. Malaysia
5. China (Hainan and Taiwan)
6. Caribbean islands such as Grenada (often called the "Island of Spice")
7. South America (Brazil, Guyana, and Suriname) (Sultan *et al*., 2023; FAO, 2018).

Indonesia remains the world's leading producer and exporter of nutmeg and mace, accounting for over 70–80% of global production, followed by Grenada, India, and Sri Lanka. The species thrives best in humid tropical climates, at altitudes below 700 meters, with well-drained, loamy soils, and annual rainfall of 1500–2500 mm (FAO, 2018).

2.4 Phytochemistry and Antioxidant Activity of *Myristica Fragrans*

Myristica Fragrans, commonly known as nutmeg, is a rich source of both volatile and non-volatile phytochemicals that contribute to its diverse pharmacological and therapeutic properties. Numerous studies have identified a wide range of bioactive compounds in its seed, including essential oils, phenolic compounds, flavonoids, lignans, alkaloids, tannins, saponins, and terpenoids, which collectively account for its biological activities such as antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Das *et al*., 2020; Hiranrat and Hiranrat, 2019; Kholibrina and Aswandi, 2021; Nikolic *et al*., 2021).

Phytochemicals are naturally occurring plant metabolites that often play protective roles in the plant and can exhibit significant physiological effects in humans. In *Myristica Fragrans*, these compounds are broadly divided into volatile and non-volatile constituents. The volatile components are primarily responsible for the characteristic aroma and flavor of nutmeg, while the non-volatile compounds contribute largely to its medicinal and nutritional benefits. In addition, methanolic and ethanolic extracts have shown remarkable antioxidant properties in both in-vitro and in-vivo models, primarily due to their capacity to scavenge reactive oxygen species and inhibit lipid peroxidation (Rastegari *et al*., 2022).

2.5 Volatile Components (Essential Oil)

The essential oil of nutmeg, often referred to as *Myristica Fragrans* essential oil (MFEO), contains several important volatile constituents, including myristicin, elemicin, eugenol, sabinene, safrole, and β-pinene (Valente *et al*., 2015; Özkan *et al*., 2018).

1. Myristicin and elemicin are phenylpropanoids known for their antioxidant and psychoactive properties.
2. Eugenol exhibits potent antimicrobial and anti-inflammatory effects, contributing to the oil's use in dental and topical applications.
3. Sabinene and β-pinene, which are monoterpenes, impart the characteristic warm and spicy fragrance of nutmeg and possess notable antioxidant activity.
4. Safrole, another key component, has been reported to show insecticidal and antifungal properties, although its use is limited due to potential toxicity in high concentrations.

Collectively, these volatile compounds form the backbone of nutmeg's essential oil profile, supporting its traditional use in aromatherapy, perfumery, and herbal medicine for their soothing and protective effects (Thileepan *et al*., 2018).

2.5.1 Non-Volatile Components

Beyond the essential oils, nutmeg seeds also contain a variety of non-volatile phytochemicals, which significantly enhance its pharmacological potential. These include phenolics, flavonoids, lignans, alkaloids, tannins, saponins, and terpenoids (Purkait *et al*., 2018; Nikolic *et al*., 2021).

1. Phenolic compounds and flavonoids act as strong antioxidants, scavenging free radicals and protecting cellular components from oxidative damage.

2. Lignans are known for their anticancer and estrogenic properties, contributing to nutmeg's therapeutic potential in hormone-related disorders.
3. Alkaloids and terpenoids add to its antimicrobial and analgesic effects, while tannins and saponins enhance its astringent and immune-boosting properties.

Together, these non-volatile compounds complement the activity of essential oils, resulting in a synergistic effect that enhances the pharmacological potency of *Myristica Fragrans* extracts.

2.6 Pharmacological Significance of Phytochemicals in *Myristica Fragrans*

Several scientific investigations have demonstrated that *Myristica Fragrans* seed extract and its essential oil exhibit antioxidant, antimicrobial, anti-inflammatory, antiulcer, anticancer, and aphrodisiac activities, among others (Das *et al*., 2020; Kholibrina and Aswandi, 2021; Valente *et al*., 2015). The antioxidant activity, in particular, is largely attributed to the combined presence of phenolic compounds and essential oil constituents like eugenol and myristicin, which play a crucial role in neutralizing Reactive Oxygen Species (ROS) and reducing oxidative stress.

2.6.1 Antioxidant Properties of *Myristica Fragrans*

Antioxidants are molecules capable of neutralizing or inhibiting the harmful effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated during normal cellular metabolism or under stress conditions (Sung *et al*., 2012). When the balance between ROS production and antioxidant defense is disrupted, it results in oxidative stress, which can damage

lipids, proteins, and nucleic acids—ultimately contributing to chronic diseases such as cancer, diabetes, neurodegenerative disorders, and cardiovascular ailments (Park *et al*., 2013; Toul *et al*., 2015; Maisetta *et al*., 2019). Therefore, identifying natural antioxidants from plant sources has become a major focus in pharmacological and nutraceutical research (Srividya and Chandra, 2014; Arina and Rohman, 2013).

Myristica Fragrans (nutmeg) is one such plant known for its significant antioxidant potential, attributed to its rich array of phytochemicals, including phenolics, flavonoids, lignans, terpenoids, and essential oil constituents such as myristicin, eugenol, and sabinene (Gupta *et al*., 2011; Abourashed and El-Alfy, 2016; Tan *et al*., 2013; Ginting *et al*., 2020). These compounds act synergistically to combat oxidative stress, thereby offering protective effects against cellular damage.

2.6.2 Mechanisms of Antioxidant Action

The antioxidant activity of *Myristica Fragrans* is primarily mediated through three major mechanisms:

1. **Free Radical Scavenging:**

Certain phytochemicals in nutmeg, particularly phenolics and flavonoids, can donate hydrogen atoms or electrons to neutralize free radicals such as superoxide anions and hydroxyl radicals (Gupta *et al*., 2011; Tan *et al*., 2013; Abourashed and El-Alfy, 2016). This reduces chain reactions that cause oxidative damage in biological systems.

2. **Metal Chelation:**

Transition metals like Fe^{2+} and Cu^{2+} can catalyze oxidative reactions through Fenton or Haber–Weiss mechanisms. Nutmeg compounds, especially tannins and flavonoids, can chelate these metal ions, thereby preventing radical formation (Park *et al*., 2013; Toul *et al*., 2015; Canadianti *et al*., 2020).

3. **Reducing Power:**

Some antioxidants act by reducing oxidized intermediates or regenerating other antioxidant molecules such as glutathione and vitamin C. Nutmeg extracts have been shown to exhibit strong reducing power, which is often associated with their total phenolic content (Morsy, 2016; Ginting *et al*., 2018; Arina and Rohman, 2013).

Through these complementary mechanisms, nutmeg extracts demonstrate substantial potential as natural antioxidants, both in vitro and in vivo. Numerous studies have confirmed the antioxidant activity of various parts of *Myristica Fragrans*, including the seed, mace, and fruit pericarp (Periasamy *et al*., 2016; Piaru *et al*., 2012; Sulaiman and Ooi, 2012). Among these, the seed extract is often reported to exhibit the highest reducing power and radical scavenging capacity, making it the most potent antioxidant source within the plant (Panggabean *et al*., 2018). However, some studies, such as Ginting *et al*. (2017), found that the fruit flesh extract demonstrated even stronger antioxidant activity than the seed, suggesting variation based on extraction methods, solvents, and plant maturity.

The antioxidant effects are primarily attributed to the presence of phytoconstituents such as saponins, alkaloids, tannins, flavonoids, α-pinene, β-pinene, 1,8-cineole, carvacrol, terpinen-4-ol, sabinene, camphene, myristicin, elemicin, isoelemicin, eugenol, methoxyeugenol, safrole, and lignans (Sung *et al*., 2012; Maisetta *et al*., 2019; Abourashed and El-Alfy, 2016; Ginting *et al*., 2020). These compounds act synergistically to scavenge radicals and protect cellular macromolecules from oxidative injury.

2.7 Correlation Between Phenolic/Flavonoid Content and Antioxidant Activity

A strong positive correlation has been established between the total phenolic and flavonoid content and the antioxidant activity of *Myristica Fragrans* extracts (Srividya and Chandra, 2014). Tannins, being part of the phenolic group, contribute significantly to antioxidant properties; however, processing steps that reduce tannin levels (such as flocculation or clarification) can result in diminished antioxidant activity (Canadianti *et al*., 2020). Gupta and Rajpurohit (2011) further reported that samples with higher total phenolic content exhibited greater radical scavenging activity, while other studies have confirmed that antiradical efficiency is strongly linked to both phenolic and flavonoid concentrations (Arina and Rohman, 2013; Park *et al*., 2013).

This relationship underscores that the antioxidant strength of *Myristica Fragrans* is largely dependent on its phytochemical composition, extraction solvent, and processing conditions. Consequently, assessing both quantitative and qualitative aspects of phenolic and flavonoid constituents is essential in evaluating the antioxidant potential of nutmeg seed extracts.

2.8 Pharmacological and Biological Significance of *Myristica Fragrans*

Beyond its culinary and aromatic appeal, *Myristica Fragrans* (nutmeg) has drawn scientific attention for its broad pharmacological spectrum, particularly its anti-inflammatory, antimicrobial, anticancer, and hepatoprotective activities,(Park *et al*., 2013). These diverse effects are strongly linked to its phytochemical composition and antioxidant potential, suggesting that oxidative stress modulation plays a central role in its overall therapeutic efficacy.

2.8.1 Anti-inflammatory Activity

Although inflammation is a normal biological reaction to damage or infection, persistent inflammation is linked to a number of degenerative illnesses, including diabetes, cancer, and arthritis (Park *et al*., 2013). According to several studies (Abourashed and El-Alfy, 2016; Gupta *et al*., 2013), nutmeg extracts, particularly those high in phenolic and terpenoid compounds, have potent anti-inflammatory effects by blocking pro-inflammatory mediators like cyclooxygenase (COX), lipoxygenase (LOX), nitric oxide synthase, and cytokines like TNF-α and IL-6. Important modulators of inflammatory pathways include substances like sabinene, eugenol, and myristicin (Gupta and Rajpurohit, 2011; Periasamy *et al.,* 2016). For example, it has been demonstrated that myristicin inhibits the generation of nitric oxide in macrophages, whereas eugenol is recognised for its capacity to prevent prostaglandin synthesis and lessen tissue oedema.

Since oxidative stress is a major cause of inflammation, nutmeg's anti-inflammatory properties are closely linked to its antioxidant capabilities. Nutmeg indirectly lowers inflammation and shields tissues from oxidative damage by scavenging free radicals and blocking lipid peroxidation (Sulaiman and Ooi, 2012).

2.8.2 Antimicrobial Properties

Myristica Fragrans's antibacterial activity has been thoroughly investigated against a variety of bacteria, fungi, and parasites (Gupta *et al.,* 2013; Piaru *et al.,* 2012). Aspergillus niger, Candida albicans, Escherichia coli, and Staphylococcus aureus have all been shown to be inhibited by nutmeg essential oil (MFEO) and seed extracts (Sulaiman and Ooi, 2012; Panggabean et al., 2018).

According to (Abourashed and El-Alfy 2016)and (Periasamy *et al.,* 2016), these effects are ascribed to the presence of bioactive compounds such as eugenol, myristicin, safrole, elemicin, and terpinen-4-ol, which disrupt microbial cell membranes, change permeability, and inhibit enzymatic processes necessary for microbial survival. Remarkably, research shows a connection between antioxidant and antibacterial activity (Gupta and Rajpurohit, 2011). For instance, because phenolic compounds can donate hydrogen atoms and attach to microbial proteins, changing their functionality, they can function as both radical scavengers and microbial growth inhibitors (Sung *et al*., 2012; Maisetta *et al*., 2019). Therefore, nutmeg's dual antioxidant–antimicrobial function helps explain why it has long been used as a food preservative and illness cure.

2.8.3 Anticancer Potential

Possibility of Anticancer Myristica Fragrans seed extracts have been shown to have anticancer properties through a variety of mechanisms, including as cell cycle arrest, apoptosis induction, angiogenesis inhibition, and oxidative DNA damage suppression (Piaru *et al.,* 2012; Abourashed and El-Alfy, 2016). It has been demonstrated that phenolic compounds and lignans can influence important cellular signalling pathways such p53, (Ginting et al., 2020; Toul *et al.,* 2015). For instance, it has been documented that myristicin, elemicin, and eugenol are cytotoxic to human leukaemia, breast, and colon cancer cell lines (Ginting *et al*., 2017; Gupta *et al*., 2013). Nutmeg's antioxidants lower DNA alterations and stop cancerous transformation by scavenging free radicals (Gupta and Rajpurohit, 2011; Park *et al.*, 2013). Its potential function as a natural chemopreventive drug is thus mechanistically supported by its antioxidant content.

2.8.4 Hepatoprotective Effects

Impacts In animal models of chemically induced liver injury, nutmeg's hepatoprotective activity has also been noted (Dkhil *et al.,* 2021). Myristica Fragrans extracts have been demonstrated to improve glutathione concentration, lower lipid peroxidation in the liver, and restore altered liver enzyme levels (Al-Olayan *et al.,* 2022). The antioxidant defence system is mostly responsible for these effects, indicating that nutmeg enhances detoxification processes and reduces oxidative stress in the liver.

Nutmeg's antioxidants lower DNA mutations and stop malignant transformation by scavenging free radicals (Gupta and Rajpurohit, 2011; Park *et al.,* 2013). Its potential function as a natural chemopreventive drug is thus mechanistically supported by its abundance of antioxidants.

Additionally, the seed extract's terpenoids and flavonoids may improve hepatic cell membrane stability and promote healing following damage (Singh *et al.,* 2020).

2.8.5 Psychoactive Effects

Nutmeg has several toxic and psychotropic consequences despite its medicinal benefits. When ingested in excess, myristicin, the main phenylpropene component in nutmeg essential oil, is known to have neuroactive and hallucinogenic effects (Demetriades *et al.,* 2005). A syndrome known as "nutmeg intoxication" can result from high doses of nutmeg and include symptoms like nausea, tachycardia, dry mouth, dizziness, and hallucinations (Dolan *et al.,* 2021).

Monoamine oxidase (MAO) inhibition and amphetamine-like activity of myristicin metabolites in the brain are thought to be the causes of this effect (Engelberth *et al.,* 2019). Even though these harmful effects are uncommon at typical dietary levels, they emphasise the necessity of standardisation and regulated dose in pharmaceutical applications. Despite the fact that Myristica fragrans' pharmacological value is widely acknowledged, there are still a number of study gaps. The absence of standardisation in extraction techniques and antioxidant assay procedures is a significant obstacle (Antasonati *et al.,* 2017). It is challenging to compare studies between labs or geographical areas due to unpredictable results caused by variations in solvents, extraction temperatures, and plant component selection. Furthermore, non-volatile fractions of nutmeg (essential oil), which are rich in polyphenols, lignans, and alkaloids, are less studied than volatile components. (Indriaty *et al*., 2015).

The restricted use of sophisticated analytical techniques, such as GC-MS profiling or LC-MS/MS, for measuring and detecting small phytochemicals is another significant drawback (Kikuzaki *et al*., 2020). This gap limits our comprehension of the components' actual contribution to antioxidant activity and their synergistic effects. Furthermore, despite known differences in temperature, soil, and cultivation techniques that can affect phytochemical composition, there are few comparative studies on nutmeg samples from various geographic regions (Aktumsek *et al.,* 2013)

Lastly, most pharmacological and antioxidant research on Myristica fragrans is conducted in vitro and lacks strong in vivo confirmation (Pratama *et al.,* 2019). To validate the bioavailability, metabolism, and safety of its active ingredients, more animal and clinical research is required. Transforming the plant's pharmacological and antioxidant potential into standardised medicinal or nutraceutical products requires an understanding of these factors.

2.9.1 **Key Bioactive Constituents**

Among the numerous phytochemicals isolated from *Myristica Fragrans*, three compounds—myristicin, elemicin, and eugenol—have been consistently identified as major bioactive constituents.

Myristicin, a phenylpropene, exhibits antioxidant, anti-inflammatory, and neuroprotective properties but also shows dose-dependent psychoactive and toxic effects when consumed excessively (Orabi *et al*., 2022).

Elemicin possesses notable free-radical scavenging capacity and contributes to the overall antioxidant effect of the essential oil.

Eugenol, a well-known phenolic compound, has been reported to exert strong reducing power, metal chelation, and lipid peroxidation inhibition, making it one of the most potent antioxidants in nutmeg extracts (Rastegari *et al*., 2022).

These compounds act synergistically with other minor constituents such as safrole, lignans, and flavonoid derivatives to enhance the total antioxidant capacity of the plant.

2.9.2 Methods for Phytochemical Screening and Antioxidant Assays

Phytochemical screening of *Myristica Fragrans* typically involves both qualitative and quantitative analyses. Standard methods include solvent extraction using methanol, ethanol, or aqueous systems followed by chromatographic identification techniques such as thin layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC) (Orabi *et al*., 2022).

Antioxidant activity is usually evaluated using in-vitro radical scavenging assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl), TAC (Total Antioxidant Capacity), FRAP (Ferric Reducing Antioxidant Power), and Hydrogen Peroxide Scavenging tests (Rastegari *et al*., 2022). The total phenolic and flavonoid contents are often quantified using Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively, which correlate strongly with the antioxidant potency of the extracts.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Stainless steel buckets/containers for extraction, nutmeg seeds, ethanol, distilled water, gloves, Aluminum foil, Masking tape

3.2 Equipments and Apparatus:

Rotary evaporator, Spectrophotometer (495, 510, 548, 725, 760 and 780 nm wavelengths)Water bath, Ice bath, Incubator / heating mantle, Weighing balance, Test tubes and racks, micropipettes, Measuring cylinders, Volumetric flasks (10 mL, 25 mL, 50 mL), Conical flasks and beakers, condenser, pump, Muslin cloth, Filter paper, blender, receiving flask, vacuum, Thermometer, Stirring rods, Stop watch, Hisense Freezer (–4 °C)

3.3 Chemicals and Reagents:

All chemicals used, were of analytical grade. They include; 2,4,6-Tripyridyl-s-triazine(Sigma-Aldrich), Ferric chloride hexahydrate(Merck), Acetate buffer, Ferrous sulfate(BDH), Ascorbic acid(Sigma-Aldrich), Ammonium molybdate(Merck), Sulfuric acid(BDH), Sodium phosphate(Sigma-Aldrich), 1,1-Diphenyl-2-picrylhydrazyl(Sigma-Aldrich), Methanol(Fisher Scientific), Sodium nitroprusside (Sigma-Aldrich), Sulfanilic acid(Sigma-Aldrich), Naphthylethylenediamine dihydrochloride(Sigma-Aldrich)

Methodology

3.4 Plant collection and identification:

Fresh *Mystirica Fragrans* seeds were purchased from Uselu market, Ugbowo, Benin city, Edo state. It was transferred to the Department of Plant Biology and Biotechnology at the University of Benin, Benin City, Edo state, Nigeria, for an authenticity test by a botanist. The herbarium voucher number was issued UBH-M575.

Fig 1: *Myristica Fragrans (*Source: Wikipedia)

3.4.1 Grinding

Firstly, the nutmeg seed extra was grinded dry to a fine powder in a Kenwood blender.

Then placed in a beaker to be measured using a weighing balance. The first pack weighed 350.563g while the second pack weighed 563.523g.

The ground nutmeg seed was then put in an iron bucket and it was mashed to ensure that there were no lumps in it.

Figure 2: *Myristica Fragrans* seed being grinded (Source: Personal)

3.4.2 Preparing the solvent:

The solvent used for the extraction process was Hydroethanol solution. A solution prepared by measuring 200ML of water and 800ML of ethanol and then mixing both together to form a hydroethanol solution.

Fig 3: Preparing the solvent (Source: Personal)

3.4.3 Extraction procedure:

The hydroethanol was poured into the bucket containing the nutmeg seed and it was mixed together and set aside for 72 hours.

After 72 hours, the fatty layer of the extra was observed and collected. The nutmeg seed was extracted using a cotton cloth sieve to squeeze the liquid out into a beaker. This process was done twice and the extract was preserved in the refrigerator.

Fig 4: Extraction of *Myristica Fragrans* (Source: Personal)

3.4.4 Freeze drying

All glassware and equipment were thoroughly cleaned prior to use, and the rotary evaporator was assembled with all components properly fitted, including the pump, condenser, water bath, and vacuum flask. The system was connected to a power source and allowed to stabilize. The mixture was shaken thoroughly and transferred into the rotary evaporator flask. The cooling unit was activated and left to chill for approximately 15 minutes, while the water bath was prepared. The receiving flask was positioned beneath the condenser, and the flask containing the extract was attached to the evaporator. The condenser unit was then lowered to make proper contact with the water bath. The extract was gradually introduced and concentrated using the rotary evaporator until the entire sample had been processed. The rate of condensation was noticeably

slow, which was attributed to the presence of 20% (200 mL) water in the mixture.

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

BIOCHEMICAL ASSAYS

DETERMINATION OF *IN VITRO* ANTIOXIDANT ACTIVITY

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

3.5.1 Ferric Reducing Antioxidant Power (FRAP) Assay

3.5.1 Assay for Ferric Reducing Antioxidant Power (FRAP) Benzie and Strain's (1996) modified method was used to perform the Ferric Reducing Antioxidant Power (FRAP) assay. 1.5 ml of freshly made FRAP solution (25 ml of 300 mM acetate buffer pH 3.6), 2.5 ml of 10 mM 2,4,6 tripyridyl-triazine (TPTZ) in 40 mM HCl, and 2.5 ml of 20 mM ferric chloride (FeCl3.6H2O) solution were added to 1 ml of the extracts at concentrations of 100-600 uM. The increase in absorbance at 593 nm was measured after the reaction mixtures were incubated at 370 c for 30 minutes. Ascorbic acid was used as the positive control, while FeSO4 was utilised for the calibration curve. The extracts' FRAP values (represented as mMFe(-/g) of the extract) were then calculated by extrapolating them from the standard curve.

3.5.2 Determination of Total Antioxidant Capacity

Total antioxidant activity was estimated by phosphomolybdenum assay (Prieto *et al*., 1999). The method is based on the reduction of molybdenum (1v) to molybdenum (v) by the extract and the subsequent formation of a green phosphate/molybdenum (v) complex at acid pH.

Three millilitres (3mL) of the extracts (1mg/ml) was added to 1ml molybdate reagent solution. These tubes were incubated at 95^0c for 90min. After incubation, the tubes were normalized to room temperature for 20-30minutes and the absorbance of the reaction mixture was measured at 695nm. Ascorbic acid was used as the standard.

3.5.3 Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the extract against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al.* (1995). Briefly, 0.5ml of 0.3mM DPPH solution in methanol was added to 2ml of various concentrations (0.2-1.0mg/mL) of the extracts. The reaction tubes were shaken and incubated for 15min at room temperature in the dark; absorbance read at 517nm. All tests were performed in triplicate. Ascorbic acid was used as standard control, with similar concentrations as the test samples prepared. A blank containing 0.5mL of 0.3mM DPPH and 2mL methanol was prepared and treated as the test samples.

The radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) = $[(A_0 - A_1) / (A_0)] \times 100$,

Where; A_0 was the absorbance of DPPH radical + Methanol.

A_1 was the absorbance of DPPH radical + sample extract or standard.

The 50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

3.5.4 Estimation of Nitric Oxide Scavenging Ability

The method of Garret (1964) was used to determine the nitric radical scavenging ability of the extracts.

Principle

Nitric oxide generated from sodium nitroprusside in aqueous solution at pysiological pH with oxygen to produce nitrite ions which is measured by Griess reaction. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite.

Procedure

A volume of 2ml of 10mM sodium nitroprusside was prepared in phosphate buffer saline (p7.4) and was mixed with 0.5ml of the extract at various concentrations ranging from 10 to 200ug/ml and ascorbic acid at various concentrations ranging from 10 to 200ug/ml. the mixture was incubated at 25^{o}C. after 150 min, 0.5ml of incubated solution was withdrawn and mixed with 0.5ml of Griess reagent[1.0ml sulfanilic acid reagent(0.33% prepared in 20% glacial acetic acid at room temperature for 5min with 1ml of napthylethylene diamine dihydrochloride (0.1%w/v)]. the mixture was incubated at room temperature for 30min, followed by the measurement of absorbance read at 540nm.

DETERMINATION OF HYDROGEN CONCENTRATION

Hydrogen peroxide levels measured as described by Wolff (1994)

PRINCIPLE

In dilute acid hydrogen peroxide oxidizes Fe (II) to Fe (III) which then selectively forms a blue purple complex with xylenol orange with an absorption maximum at 560nm. The addition of sorbitol initiates a chain reaction with produced hydroxyl radical that increases the yield of Fe (III) and therefore greatly amplifies the response per H_2O_2 molecule present, thereby increasing the sensitivity of the method.

REAGENTS

1. Xylenol orange, 100 uM (MW760.0)
   7.6 mg of xylenol orange was dissolved in 10mL of distilled water.

2. Sorbitol, 100 mM
   1.822 g of sorbitol was dissolved in 10mL distilled water.

3. Sulphuric acid, 25 mM
   140 uL of concentrated H_2SO_4 was added to distilled water and made up to 50mL

4. Ammonium ferrous sulfate (AFS), 250 uM (MW 392.14)
   9.8mg of ammonium ferrous sulfate was dissolved in 50mL of 25 mM H_2SO_4.

5. Hydrogen peroxide stock, 100 uM
   57 uL of 30% H_2O_2 was added to distilled water and the volume made up to 100 mL. 1mL of the resulting solution was taken and made up to 50mL.

   Fox 1 reagent (100 mL) = 10 mL xylenol orange + 10 mL sorbitol + 50 mL AFS + 30 mL distilled water.

Test tube	Blank	1	2	3	4	5

H_2O_2 stock (uL)	-	20	40	60	80	100
Distilled water (uL)	100	80	60	40	20	-
FOX1 reagent (mL)	1.9	1.9	1.9	1.9	1.9	1.9
H_2O_2 conc (uM)	0	1	2	3	4	5

The total reaction mixture was vortexed and incubated at room temperature for 30 minutes before being read against the reagent blank at 560nm.

PROCEDURE FOR SAMPLESs

Samples were treated similarly as standard (100 uL 0f sample added to 1.9 mL FOX1 reagent) except that the mixtures were centrifuged at 3000rpm for for 5 min before incubating

3.6 Qualitative phytochemical screening procedure

Phytochemicals are bioactive constituents of medicinal plants which are not nutrients but very useful to the plants. Some bioactive constituents of methanolic extract were analysed qualitatively for Flavonoids, Tannins, Cardiac Glycosides, Saponins, Steroids, Terpenoids, Phenols, Phlobatanins, Coumarin, Anthraquinone and Alkaloids. Phytochemical screening was carried out on the samples after undergoing methanol extraction, using standard procedures to identify the secondary metabolites (Harborne 1973; Trease and Evans, 1989; Sofowora 1993).

3.6.1 Solvent extract preparation:

A pulverised sample (150g) was measured and placed in bottles. 500mL of absolute ethanol was used to soak the samples, subsequently stirred 3 times daily and kept in a dark room for 72 hours. Supernatant was filtered using a muslin cloth and the solvent evaporated using a rotary

evaporator. The dried extracts were weighed and kept in sterile universal bottles for the freezer at a temperature of -4˚C for some time.2g of the crude extract was weighed and used for phytochemical screening.

3.6.2 Test for flavonoids:

5mL of 10% ammonia was added to 1ml portion of an aqueous filtrate of the extract. Then 1ml concentrated sulfuric acid was added. Observed yellow colour indicates the presence of flavonoids.

3.6.3 Test for tannins:

1mL of (0.5g/5mL) ethanol extract was boiled in 2ml of water in a test tube and filtered. A drop of 0.1% ferric chloride was added and observed for brownish green to a blue-black colouration.

3.6.4 Test for cardiac glycosides (Keller-Killiani test):

1mL of 0.5g/5ml aqueous extract was treated with glacial acetic acid containing one drop of ferric chloride solution. 1mL of concentrated sulfuric was gently added. A browning at the interface indicated the presence of a deoxysugar characteristic of cardenolides. Hence, the presence of cardiac glycosides.

3.6.5 Test for saponin (Frothing test):

The ability of saponins to produce frothing in aqueous solution was used as a screening test for saponins. 1mL of extract (0.5g/5mL of distilled water) was mixed with 5mL of distilled water and shaken vigorously for a stable persistent froth, indicating the presence of saponin. This was further confirmed by adding 3 drops of olive oil and shaking vigorously after which it was observed for the formation of an emulsion.

3.6.6 Test for steroids

2mL of concentrated acetic anhydride was added to 0.5mL of (0.5g/5mL) ethanol extract of each sample with 2mLconcentrated sulfuric acid. The colour changed from violet to blue or green colouration was positive for steroids.

3.6.7 Test for terpenoids (Salkowski test):

1mL of the extract in a test tube was mixed with 2mL of concentrated chloroform and 3ml of concentrated sulfuric acid. Reddish brown coloration at the interface confirmed the presence of terpenoids.

3.6.8 Test for phenols:

3 drops of 10% aqueous $FeCl_3$ solution were added in a test tube to 5mL of (0.5g/5mL) ethanol extract. Formation of blue or green coloration indicated the presence of phenols.

3.6.9 Test for phlobatanins:

3mL of (0.5g/5mL) ethanol extract was added to 2mL of 1% HCl and the extract was boiled. Deposition of a red precipitate was taken as evidence for the presence of phlobatanins.

3.6.10 Test for Coumarin:

5mL of (0.5g/5mL) ethanol extract was dissolved in 2mL of hot distilled water and divided into two parts. Half of the volume was a control; the other part 0.5ml of 10% NH_4OH was added.

3.6.11 Test for alkaloids:

Mayer's test: 1mL of (0.5g/5mL) ethanol extract was mixed with 3drops of Mayer's reagent. Cream coloured precipitate formation confirmed the presence of alkaloids.

3.6.12 Test for anthraquinone:

5mL of benzene was added to 1mL of (0.5g/5mL) ethanol extracts in a test tube and shaken vigorously in 2.5mL concentrated NH_3. Formation of pink-red colouration at the lower phase was indicative of the presence of free Anthraquinone.

3.7 Quantitative determination of phytochemicals

3.7.1 Estimation of Alkaloids (This was carried out by Madhu M. *et al*., Method of 2016)

To 1ml of test extract, 5ml of pH 4.7 phosphate buffer was added and 5ml BCG solution and shake a mixture with 4ml of chloroform. The extracts were collected in a 10ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 760nm against blank prepared as the above with extract. Atropine was used as a standard and compared the assay with atropine equivalent.

3.7.2 Estimation of Flavonoids (This was carried out by Madhu M. *et al*., Method of 2016)

Total flavonoid content was determined by the Aluminum chloride method using Quercetin as a standard. 1ml of test sample and 4ml of water were added to a volumetric flask (10ml vol.). After 5mins, 0.3ml of 5% Sodium nitrite and 0.3ml of 10% Aluminum chloride were added. After 6mins incubation at room temperature, 2ml of 1M Sodium hydroxide was added to the reaction mixture. Immediately, the final volume was made up to 10ml with distilled H_2O. The absorbance of the reaction mixture was read at 510nm against a blank spectrophotometrically.

3.7.3 Estimation of Steroids (This was carried out by Madhu M. *et al*., Method of 2016)

1ml of extract of steroid solution was transferred into a 10ml volumetric flask. Sulphuric acid (4N, 2ml) and Iron (III) chloride (0.5% w/v 2ml) were added, followed by potassium

hexacyanoferrate (III) solution (0.5% w/v, 0.5ml). The mixture was heated with occasional shaking and diluted to the mark with diluted water. The absorbance was measured at 780 nm against the reagent blank. Stigmasterol was used as standard.

3.7.4 Estimation of Terpenoids (This was carried out by Alessandra M.P. *et al*., method of 2020)

To 75ul plant extract, 250ul of vanillin solution (50mg/ml) and 500ul of Sulphuric acid (99.5%). The tube was heated in a water bath (60^{o}C) for 20mins and then transferred into an ice bath followed by the addition of 2500ul of acetic acid (99.5%). The resulting solution was cooled for 20mins and absorbance was measured at 548nm. Beta-sitosterol was used as a standard.

3.7.5 Estimation of Coumarin (This was carried out by Ameen, O.A., *et al.* Method of 2021)

A 0.5ml of 5NNaOH was added to the solution of 1 ml of the extract (0.5g in 1 ml methanol). The mixture was heated at 80^{o}C for 5mins. After cooling, 0.75ml of 5N H2SO4 was added and mixed thoroughly, then, 0.25g of anhydrous $NaHCO_3$ was also added and transferred to the extractor and made up to 50 ml with pet-ether for 3hrs. About 20ml of H_2O was added to the pet-ether extract and carefully evaporate the pet-ether in a water bath at 50-55^{o}C. The aqueous solution was transferred to a volumetric flask and made up to 50ml with mixing. 25ml of aqueous solution was pipetted into a flask and 1%. Na_2CO_3solution was added and heated in a water bath at 75% for 15mins and cooled. 5 ml of the diazonium solution was added and stood for 2 hours. The absorbance at 540nm against reagent blank. Esculin was used as standard.

3.7.6 Estimation of Phenols (This was carried out by Tofighi, N. *et al*., Method of 2016)

The methanol solution of each sample (0.2 - 100ug/ml) was mixed with folin-ciocalteu reagent (2 ml, 1:10 diluted with distilled H_2O). After 5mins, saturated $NaHCO_3$ solution (1.5ml, 60g/L distilled water) was added. The mixture was allowed to stand for 90 minutes at room temperature

and absorbance of the solution was measured at 725nm. The same procedure was repeated for different concentrations of gallic acid solution (0.2-1.0ug/ml).

3.7.7 Estimation of Cardiac glycosides (This was carried out by Tofighi, N. *et al*., Method of 2016)

10% extract was mixed with 10ml of freshly prepared Baljet's reagent (95ml of 1% picric of 5ml of 10%NaOH). After an hour, the mixture was diluted with 20ml distilled water and the absorbance was measured at 495nm. Securidaside was used as standard.

3.7.8 Estimation of Tannins (This was carried out by Kritha Chandran C.I. and Indira G. Method of 2016)

Tannins was determined by folin ciocalteu method

0.1ml of sample extract was added to the volumetric flask (10ml) containing 7.7ml of distilled water. The mixture was shaken well and kept at room temperature for 30mins, a set of reference standard solutions of tannic acid (20, 40, 60, 80, and 100ug/ml) in the same manner as described for sample extract. Absorbance for test and standard solutions were measured against reagent blank at 700nm.

CHAPTER FOUR

RESULTS

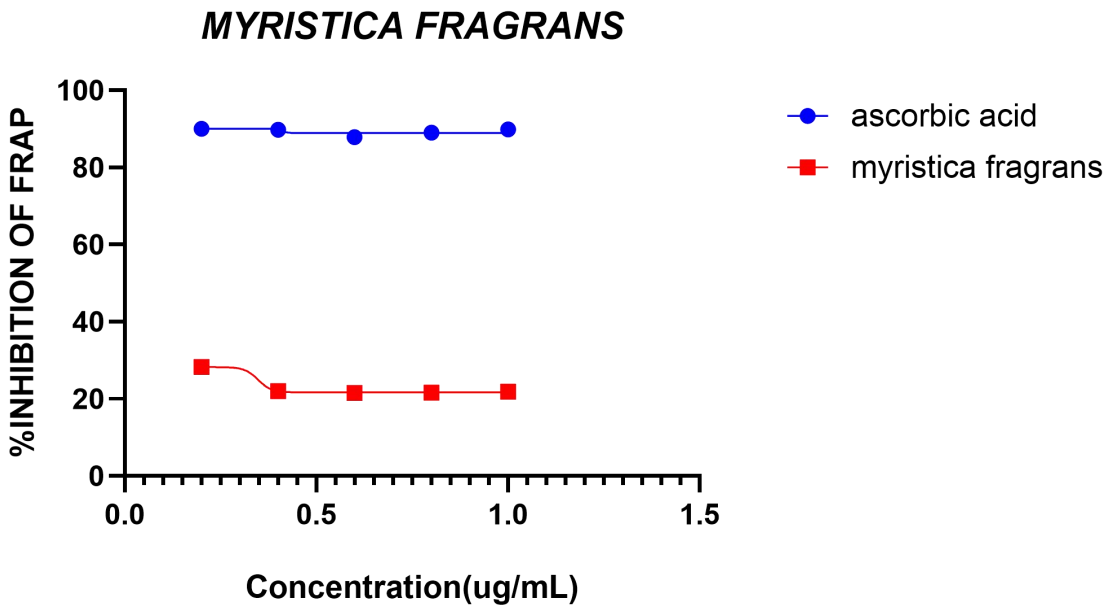


Fig 7:Ferric Reducing Antioxidant Power (FRAP) Scavenging Activity at different concentrations of Ascorbic acid and **aqueous extract of *Myristica Fragrans.***

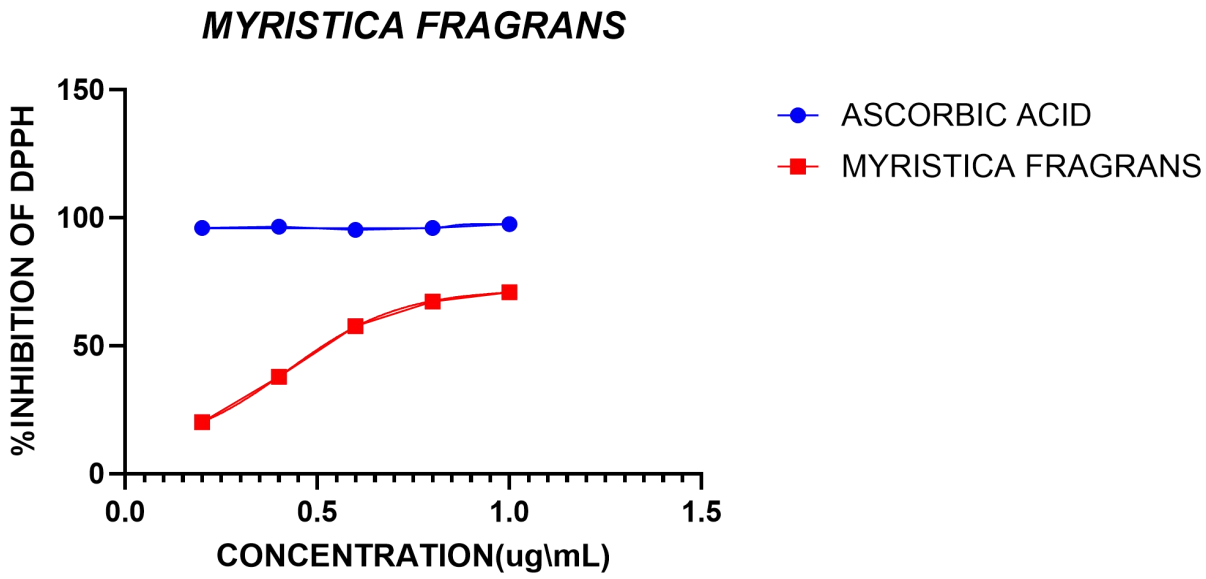


Fig 7.1: Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity at different concentrations of Ascorbic acid and **aqueous extract of *Myristica Fragrans*.**

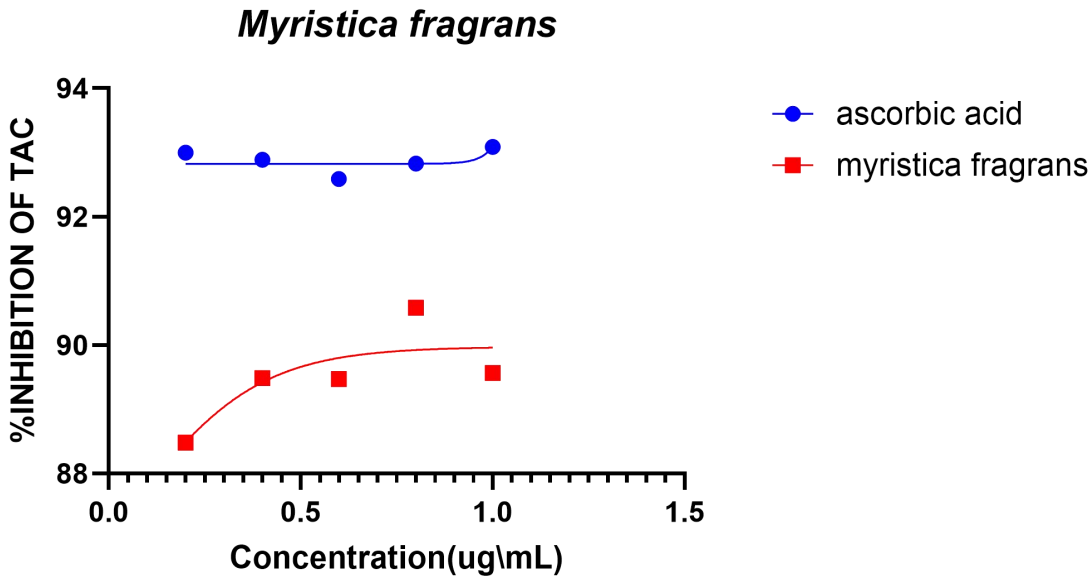


Fig 7.2: Total Antioxidant Capacity (TAC) Scavenging Activity at different concentrations of Ascorbic acid and **aqueous extract of *Myristica Fragrans***

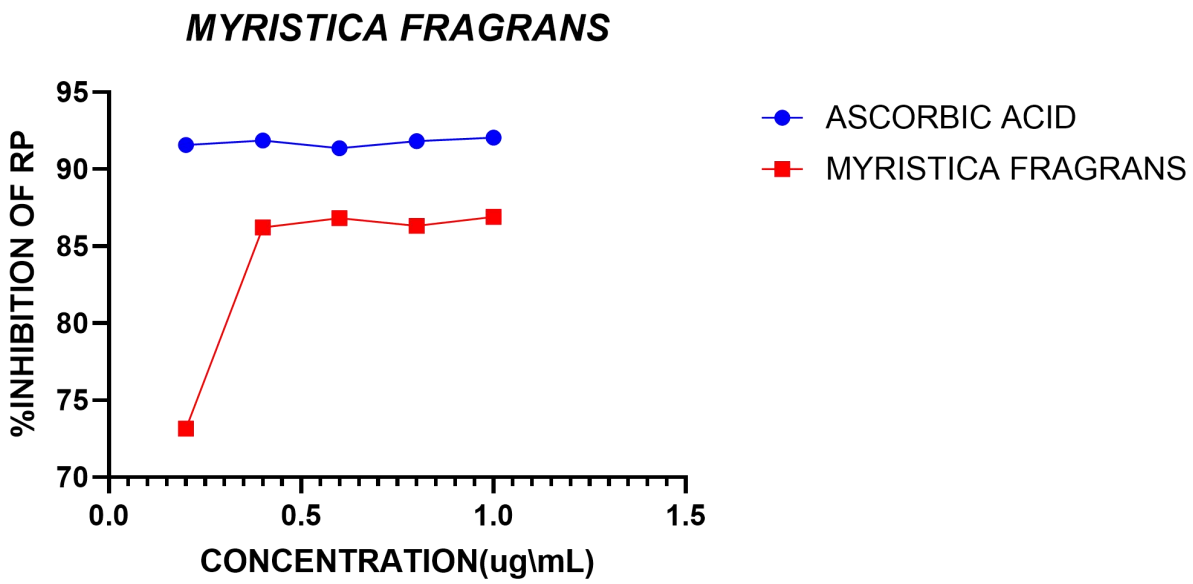


Fig 7.3: Reducing Power (RP) Scavenging Activity at different concentrations of Ascorbic acid and **aqueous extract of *Myristica Fragrans.***

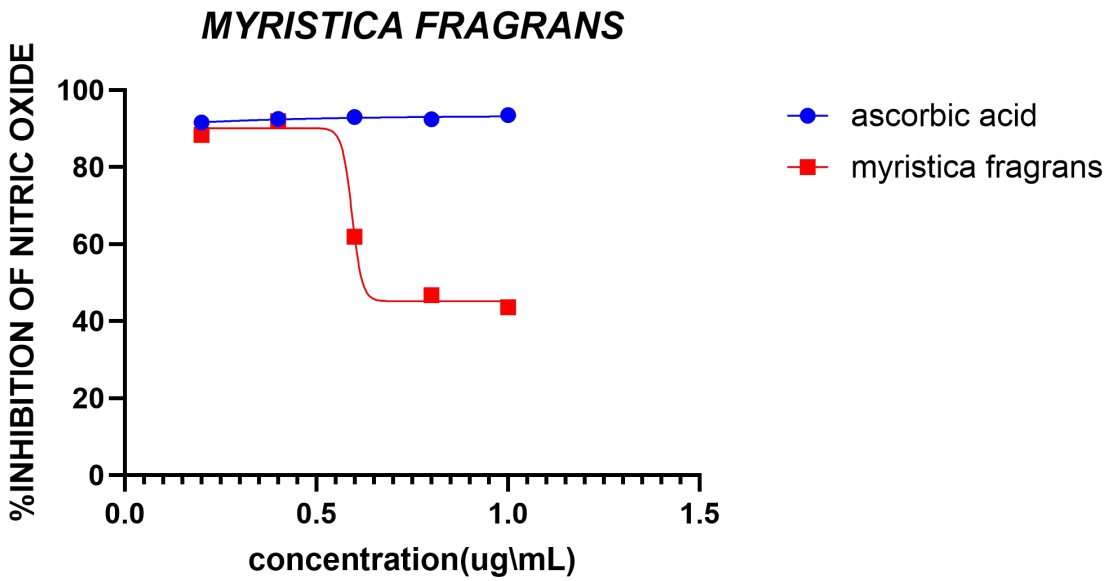


Fig 7.4:

Nitric Oxide Scavenging Activity at different concentrations of Ascorbic acid and **aqueous extract of** ***Myristica Fragrans***

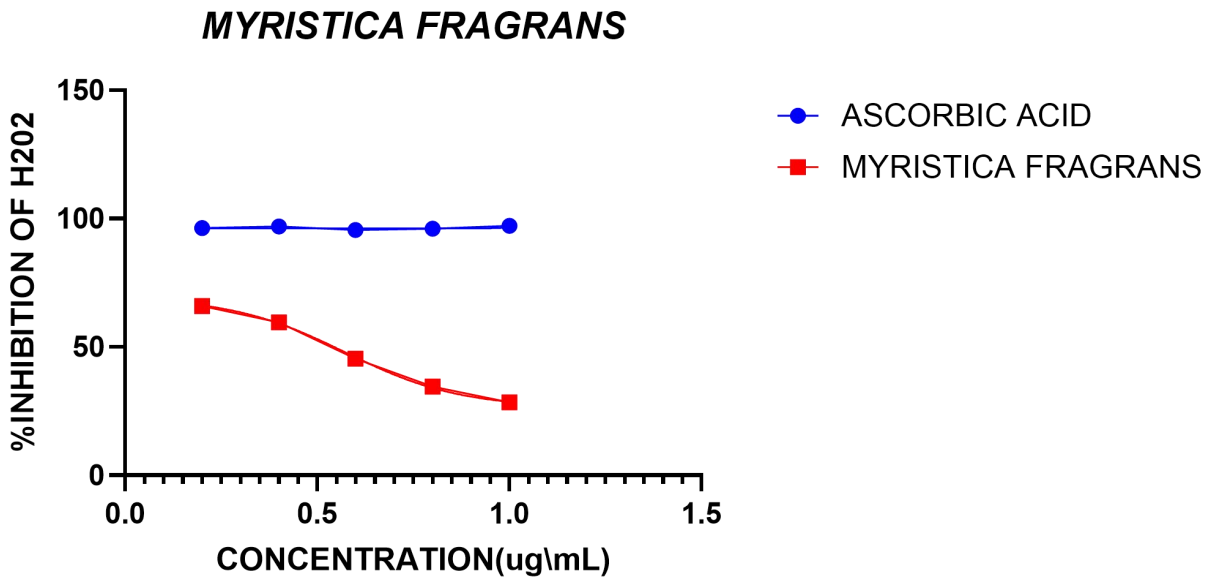


Fig 7.5: Hydrogen Peroxide (H202) Scavenging Activity at different concentrations of Ascorbic acid and **aqueous extract of *Myristica Fragrans*.**

Table 1: The IC_{50} Value of the standard and **the aqueous extract of *Myristica Fragrans***

Inhibitory Assay	Standard	Standard IC_{50} (ug/ml)	Aqueous Extract IC_{50} (ug/ml)
Diphenyl-2-Picryl-Hydrazyl(DPPH)	Ascorbic acid	6.969	2.965
Ferric Reducing Antioxidant Power (FRAP)	Ascorbic acid	2.559	2.228
Nitric Oxide	Ascorbic acid	0.01300	3.919
Total Antioxidant Capacity(TAC)	Ascorbic acid	15.24	1.209
Hydrogen Peroxide (H202)	Ascorbic acid	12.06	3.743
Reducing Power(RP)	Ascorbic acid	6.315	2.173

Data was collected from the linear graph of percentage inhibition versus concentration of standard or samples as appropriate. Values carrying different superscript letters with a row are statistically different.

Table 1: From the above results, the antioxidant strength varies depending on the specific inhibitory assay, as indicated by the IC_{50} values. Since lower IC_{50} values correspond to stronger antioxidant activity. For the DPPH assay, the aqueous extract showed a lower IC_{50} value (2.965 µg/mL) than the standard, indicating that the extract had stronger free radical scavenging activity.

A similar trend was observed in the FRAP assay, where the extract also recorded a slightly lower IC_{50} value (2.228 µg/mL) compared to the standard, demonstrating greater ferric reducing antioxidant power. In the TOC assay, the extract again exhibited a much lower IC_{50} value (1.209 µg/mL), confirming higher total antioxidant capacity relative to the standard. In contrast, the nitric oxide scavenging assay showed the opposite pattern. Here, the extract recorded a higher IC_{50} value (3.919 µg/mL), indicating weaker nitric oxide radical inhibition compared to the standard. The hydrogen peroxide scavenging assay, however, showed stronger activity from the extract (3.743 µg/mL) compared to the standard, suggesting better H_2O_2 detoxification ability. Overall, the aqueous extract of Myristica fragrans demonstrated stronger antioxidant activity in the DPPH, FRAP, TOC, and H_2O_2 assays, while nitric oxide showed weaker antioxidant power.

PHYTOCHEMICAL SCREENING (QUALITATIVE)

Table 2: This table shows the results of the various phytochemicals present and **absent in *Myristica Fragrans***

PHYTOCHEMICALS	SAMPLE A (*Myristica Fragrans)*
FLAVONOIDS	+
TANNINS	-
CARDIAC GLYCOSIDES	+++
SAPONINS	+
STEROIDS	+++
TERPENOIDS	++
PHENOLS	+
PHLOBATANNINS	-
COUMARIN	+
ALKALOIDS	+
ANTHRAQUINONE	-

KEY

- = Absent

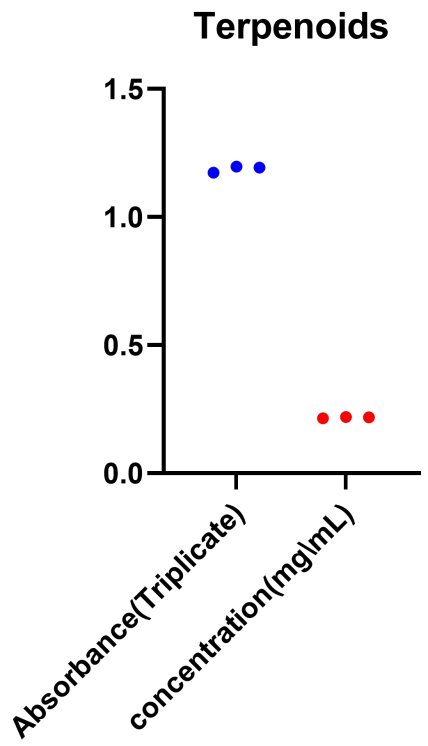
+ = present (Low Conc)

++ = Present (High Conc)

+++ = Present (Very High Conc

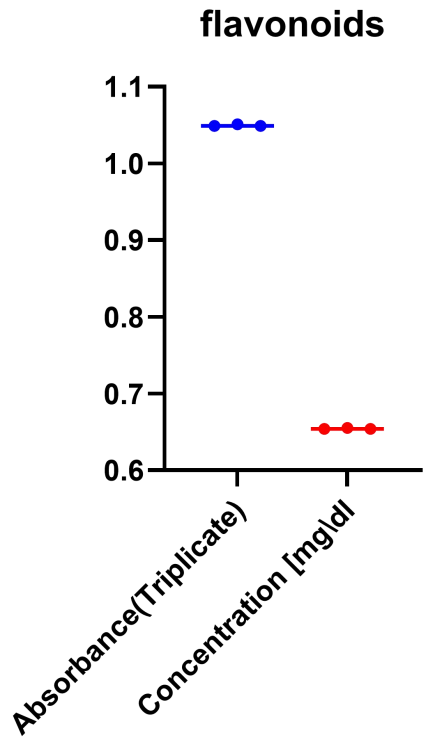
Table 2: From the Quantitative photochemical results above, Cardiac glycosides and steroids were present in very high amounts, while terpenoids were found in high concentration. Flavonoids, saponins, phenols, coumarins, and alkaloids were present in low amounts, indicating moderate antioxidant and antimicrobial potential. Tannins, phlobatannins, and anthraquinones were absent. Overall, *Myristica Fragrans* contains several beneficial phytochemicals that may contribute to its medicinal properties.

PHYTOCHEMICAL SCREENING (QUANTITATIVE)



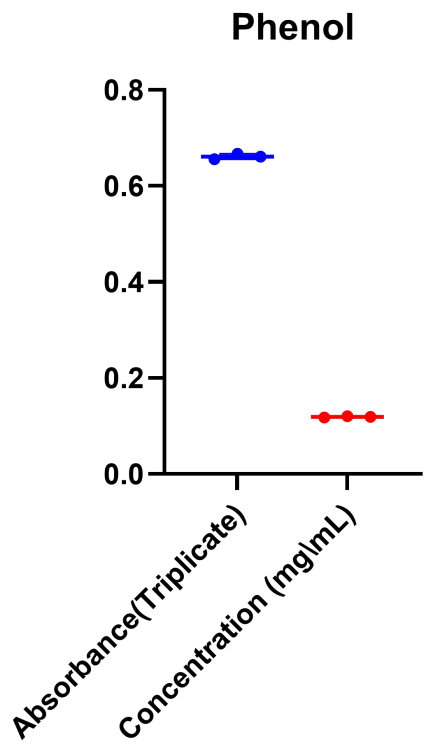
Terpenoids = 0.2170 ± 0.002422mg LE/g (mean ± SD, n = 3)

Fig 8: The terpenoid content was 0.2170 mg LE/g, suggesting a moderate presence of terpenoid compounds in the extract. The low standard deviation (± 0.002422) indicates minimal variation across the triplicates.



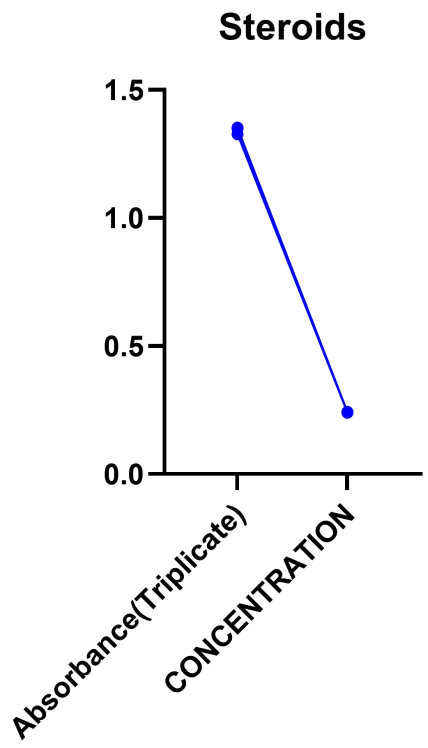
Total flavonoids = 0.6543 ± 0.0007159mg QE/g (mean ± SD, n=3)

Fig 8.1 The total flavonoid content of *Myristica Fragrans* was 0.6543 mg QE/g, indicating a moderate amount of flavonoids in the extract. The very small standard deviation (± 0.0007159) shows that the triplicate measurements were highly consistent and precise.



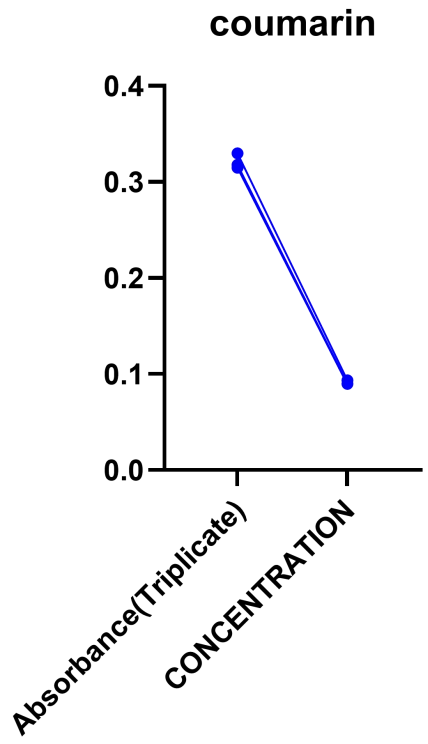
Total phenolics = 0.1191 ± 0.001172mg GAE/g (mean ± SD, n = 3)

Fig 8.2 The total phenolic content of *Myristica Fragrans* was 0.1191 mg GAE/g, indicating a low level of phenolic compounds in the extract. The small standard deviation (± 0.001172) shows that the replicate readings were consistent.



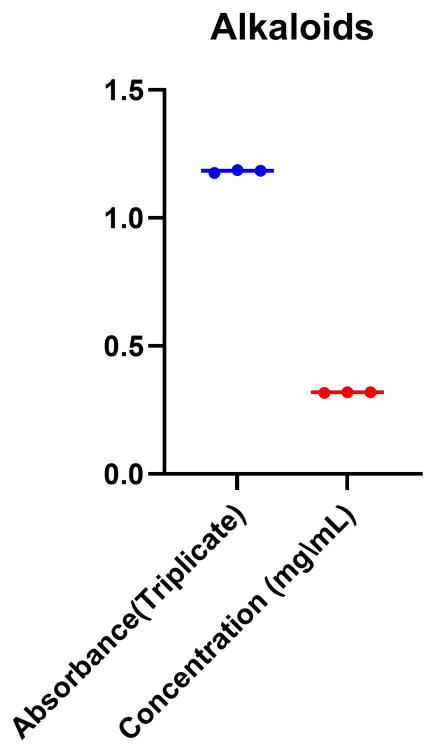
Steroids = 0.2417 ± 0.002604mg CE/g (mean ± SD, n = 3)

Fig 8.3 The total steroid content was 0.2417 mg CE/g, showing that steroids are present in a relatively higher amount compared to some other phytochemicals. The standard deviation (± 0.002604) is very small, meaning the results were stable and reproducible.



Coumarins = 0.09083 ± 0.002270mg CE/g (mean ± SD, n = 3)

Fig 8.4 The Coumarin content was 0.09083 mg CE/g, indicating a low concentration of coumarins in the extract. The small standard deviation (± 0.002270) shows that the results were consistent across replicates.



Alkaloids = 0.3185 ± 0.001494mg AE/g (mean ± SD, n = 3)

Fig 8.5 The alkaloid content was 0.3185 mg AE/g, representing one of the higher concentrations among the quantified phytochemicals. The very low standard deviation (± 0.001494) indicates strong consistency in the measurements.

CHAPTER FIVE

5.1 DISCUSSION

This research focused on examining the phytochemical makeup and antioxidant capabilities of the aqueous extract from *Myristica Fragrans*, commonly known as nutmeg seeds. The primary goal was to establish both the qualitative and quantitative phytochemical profile of the extract. The qualitative analysis identified a broad spectrum of bioactive compounds, showcasing very high levels of cardiac glycosides and steroids (+++), substantial amounts of terpenoids (++), and minimal quantities of flavonoids, saponins, phenols, coumarins, and alkaloids (+). Notably, tannins, phlobatannins, and anthraquinones were not detected (-). Quantitative assessments supported these observations, revealing that flavonoids (0.6543 ± 0.0007159 mg QE/g) and alkaloids (0.3185 ± 0.001494 mg AE/g) were the most prevalent compounds, whereas phenolics (0.1191 ± 0.001172 mg GAE/g) and coumarins (0.09083 ± 0.002270 mg CE/g) were found in much lower concentrations. These findings suggest that the seeds of *Myristica Fragrans* are rich in a variety of secondary metabolites, many of which are known for their antioxidant, anti-inflammatory, and cardioprotective properties (Sultan *et al*., 2023; Orabi *et al*., 2022).

A significant discovery from this study was the strong antioxidant activity of the aqueous extract, evaluated through various inhibitory assays (DPPH, FRAP, nitric oxide, TOC, H_2O_2, and reducing power). The extract demonstrated lower IC_{50} values compared to ascorbic acid in multiple tests, including DPPH (2.965 µg/mL vs. 6.969 µg/mL) and TOC (1.209 µg/mL vs. 15.24 µg/mL), indicating that the extract has impressive free-radical scavenging abilities and can effectively neutralize oxidants. However, in the nitric oxide assay, the extract's IC_{50} was higher than that of ascorbic acid (3.919 µg/mL vs. 0.01300 µg/mL), suggesting a lower effectiveness in this specific mechanism. These differences across the assays highlight how various phytochemicals contribute uniquely to different antioxidant pathways.

The extract's strong performance in the DPPH, FRAP, and reducing power assays is likely due to its high levels of flavonoids and alkaloids, which are recognized for their ability to donate electrons or hydrogen atoms and stabilize free radicals (Sultan *et al*., 2023). In contrast, the lower phenolic content may help explain its relatively weaker activity in the nitric oxide scavenging assay, which heavily relies on the presence of phenolic hydroxyl groups. Additionally, the significant presence of steroids and terpenoids could further enhance the extract's antioxidant capacity by promoting membrane stabilization and influencing oxidative stress responses (Tariq, 2025).

When compared to the earlier research conducted by Nkwocha et al. (2018), there were some notable similarities. Both investigations found high levels of steroids and moderate to substantial amounts of terpenoids, indicating a common trend regarding the significance of these phytochemicals. Additionally, both studies detected low concentrations of saponins, phenols, and alkaloids, reinforcing a consistent pattern in these findings.

On the other hand, there were several important differences. The current study revealed very high levels of cardiac glycosides, which were not detected at all in the previous research. Furthermore, while anthraquinones were absent in both studies, the earlier research also noted the lack of anthocyanins, which were not analyzed in the present study. In terms of quantities, the measurements from this study were significantly lower than those reported by Nkwocha et al. (for example, terpenoids were at (19.00 ± 3.18%) and steroids at (32.75 ± 5.42%). This discrepancy might be attributed to variations in extraction methods, differences in plant sources, or the analytical techniques used. Factors such as the maturity of the plants, environmental conditions, soil composition, solvent polarity, and processing methods may also explain these differences. Specifically, the varied quantification standards used (e.g., mg QE/g, mg AE/g, mg GAE/g) in comparison to percentage compositions (%) in the earlier study could further account for these numerical discrepancies.

A key aspect of this study was to assess how these phytochemicals contribute to the overall antioxidant activity through the inhibitory assays. In the IC_{50} evaluations (Table 1), ascorbic acid served as a standard for comparison across the various tests. As a well-known antioxidant, ascorbic acid provided a useful benchmark for gauging the effectiveness of the extract. Notably, the aqueous extract demonstrated lower IC_{50} values than ascorbic acid in several assays, particularly in DPPH and TOC, indicating superior efficacy in scavenging free radicals and neutralizing oxidants under those specific conditions. This points to a synergistic effect among flavonoids, alkaloids, terpenoids, and steroids that enhances the extract's overall antioxidant properties.

These results align with the growing body of literature that recognizes *Myristica Fragrans* as a valuable source of bioactive compounds with various therapeutic applications, including antioxidant, anti-inflammatory, antimicrobial, and cardioprotective effects (Orabi *et al*., 2022; Sultan *et al*., 2023). While the aqueous extraction method successfully isolated water-soluble phytochemicals, future research could investigate the use of different solvents to extract lipophilic components, such as essential oils, which also play a role in antioxidant activity.

5.2 CONCLUSION

In conclusion, our findings indicate that *Myristica Fragrans* seeds are not only rich in diverse bioactive compounds but also exhibit strong antioxidant activity across a range of mechanisms.

REFERENCES

Abourashed, E. A., and El-Alfy, A. T. (2016). Chemical diversity and pharmacological significance of *MyristicaFragrans*. Phytochemistry Reviews, **15**(5), 927–951.

Aktumsek, A., Zengin, G., Guler, G. O., Cakmak, Y. S., andDuran, A. (2013). Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic Centaurium species. Food and Chemical Toxicology, 55, 290–296.

Al-Olayan, E. M., El-Khalik, M. S. A., Dkhil, M. A., Al-Shaebi, E. M., Al-Quraishy, S., and Abdel Moneim, A. E. (2022). Protective effects of nutmeg on carbon tetrachloride-induced liver toxicity in rats. Biomedicine and Pharmacotherapy, 150, 113014.

Gupta, A., Mumtaz, S., Li, C. H., Hussain, I., and Rotello, V. M. (2019). Combatting antibiotic-resistant bacteria using nanomaterials. *Chemical Society Reviews*, *48*(2), 415-427

Arina, N., and Rohman, A. (2013). Antioxidant properties of various plant extracts using DPPH assay. *Indonesian Journal of Chemistry***, 13**(2), 176–181.

Arunachalam, G., Ramesh, M., and Subramanian, N. (2018). Antiplatelet activity of nutmeg extract. *Journal of Ethnopharmacology*, 220, 15–22.

Ashokkumar, K., Murugan, M., Dhanya, M. K., Raj, S., andKamaraj, D. (2022). Nutmeg (*Myristica Fragrans*): A comprehensive review on morphology, phytochemistry andpharmacological activities. Industrial Crops and Products, 181, 114850.

Bhuiyan, M. (2019). Pharmacological potentials of *MyristicaFragrans*. Asian Pacific *Journal of Tropical Medicine*, **12**(3), 125–132.

Brand-Williams, W., Cuvelier, M. E., and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology**, 28**(1), 25–30.

Rastegari, A., Manayi, A., Rezakazemi, M., Eftekhari, M., Khanavi, M., Akbarzadeh, T., and Saeedi, M. (2022). Phytochemical analysis and anticholinesterase activity of aril of Myristica fragrans Houtt. *BMC chemistry*, *16*(1), 106.

Das, M. P., Habeebullah, S. F. K., and Krishnan, K. M. (2020). Phytochemical analysis and antimicrobial activity of nutmeg. *International Journal of Pharmaceutical Sciences Review*, **64**(1), 45–50.

Demetriades, A. K., Wallman, P. D., McGuiness, A., andGavalas, M. (2005). Nutmeg poisonings: A retrospective review. *Emergency Medicine Journal*, **22**(3), 204–206.

Dhingra, D., and Sharma, A. (2006). Antidepressant-like activity of nutmeg. *Journal of Medicinal Food*, **9**(1), 84–89.

Dkhil, M. A., Al-Quraishy, S., and Abdel-Moneim, A. E. (2021). Protective role of nutmeg extract on liver oxidative stress. Saudi Journal of Biological Sciences, **28**(4), 2256–2262.

Alkhatib, R. Q., Almasarweh, A. B., Abdo, N. M., Mayyas, A. S., Al-Qudah, M. A., and Abu-Orabi, S. T. (2022). Chromatographic analysis (LC-MS and GC-MS), antioxidant activity, antibacterial activity, total phenol, and total flavonoid determination of Cleome arabica L. growing in Jordan. *International Journal of Food Properties*, *25*(1), 1920-1933.

Elfia, F., and Susilo, J. (2023). Ethnomedicinal relevance of *Myristica Fragrans* in Southeast *Asia. Journal of Ethnobotany*, **57**(2), 77–89.

Sultan, M. T., Saeed, F., Raza, H., Ilyas, A., Sadiq, F., Musarrat, A., ... and Al JBawi, E. (2023). Nutritional and therapeutic potential of nutmeg (Myristica fragrans): A concurrent review. *Cogent Food & Agriculture*, *9*(2), 2279701.

Ashokkumar, K., Simal-Gandara, J., Murugan, M., Dhanya, M. K., and Pandian, A. (2022). Nutmeg (Myristica fragrans Houtt.) essential oil: A review on its composition, biological, and pharmacological activities. *Phytotherapy Research*, *36*(7), 2839-2851.

FAO. (2018). Nutmeg and mace: Production, trade and markets. Food and Agriculture Organization of the United Nations.

Fernando, C. D., and Senevirathne, M. (2021). Antifungal activity of nutmeg extracts. Mycobiology, **49**(4), 319–327.

Garret, D. C. (1964). Spectrophotometric determination of nitric oxide. Analytical Chemistry, **36**(2), 271–275.

Bhuiyan, M. S. A. (2019). Pharmacological and biochemical investigations of methanolic extract of Myristica fragrans seeds.

Aktumsek, A., Zengin, G., Guler, G. O., Cakmak, Y. S., and Duran, A. (2013). Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic Centaurea L. species. *Food and chemical toxicology*, *55*, 290-296.

Gupta, C., Amar, A., and Kaur, H. (2011). Antioxidant andantimicrobial properties of nutmeg. International Research Journal of Pharmacy, **2**(8), 120–126.

Gupta, R., and Rajpurohit, J. (2011). Correlation between phenolic content and antioxidant activity of plant extracts. *Journal of Pharmacognosy*, **3**(1), 25–30.

Kusuma, J., Scarcelli, N., Navascués, M., Couderc, M., Gerard, P., and Duminil, J. (2023). Retracing the center of origin and evolutionary history of nutmeg Myristica fragrans, an emblematic spice tree species. *Authorea Preprints*.

Valente, V. M. M., Jham, G. N., Jardim, C. M., Dhingra, O. D., and Ghiviriga, I. (2015). Major antifungals in nutmeg essential oil against Aspergillus flavus and A. ochraceus. *Journal of Food Research*, *4*(1), 51.

Harborne, J. B. (1973). Phytochemical methods. Chapman andHall.

Hiranrat, A., and Hiranrat, P. (2019). Antioxidant properties of nutmeg extracts. *Journal of Food Biochemistry*, **43**(4), e12776.

Orabi, M., Abdulsattar, J. O., and Nasi, Z. O. (2022). Phytochemical profile, antimicrobial, antioxidant activity and cyclooxygenase 2 inhibitory properties of nutmeg (Myristica fragrans) seeds extract. *Egyptian Journal of Chemistry*, *65*(1), 317-326.

Krishna, S., and Thileepan, T. A review on Pharmacological activity of Nilavembu decoction used in Suram (Fever).

Kholibrina, E., and Aswandi, A. (2021). Phytochemical screening and bioactivity of nutmeg seed extracts. Borneo Journal of Pharmacy, **4**(1), 22–31.

Nikolic, V., Nikolic, L., Dinic, A., Gajic, I., Urosevic, M., Stanojevic, L., ... and Danilovic, B. (2021). Chemical composition, antioxidant and antimicrobial activity of nutmeg (Myristica fragrans Houtt.) seed essential oil. *Journal of Essential Oil Bearing Plants*, *24*(2), 218-227.

Kritha Chandran, C. I., and Indira, G. (2016). Determination of tannin content in medicinal plants. *International Journal of Science and Research*, **5**(4), 1234–1239.

Kumar, S., and Samanta, K. (2018). Anticonvulsant activity of nutmeg extract. Phytomedicine, 45, 120–126.

Tutuarima, T., Noor, E., Rusli, M. S., Setyaningsih, D., and Nurjanah, S. (2024, June). The Effect of Fractionation Temperature Using Spinning Band Distillation on the Myristicin Content of Nutmeg Oil (Myristica fragrans). In *IOP Conference Series: Earth and Environmental Science* (Vol. 1358, No. 1, p. 012017). IOP Publishing.

Morsy, S. (2016). Reducing power and antioxidant capacity of plant extracts. Applied Sciences Reviews**, 3**(2), 55–62.

Antasionasti, I., Datu, O. S., Lestari, U. S., Abdullah, S. S., and Jayanto, I. (2021). Correlation analysis of antioxidant activities with tannin, total flavonoid, and total phenolic contents of nutmeg (myristica fragrans houtt) fruit precipitated by egg white. *Borneo Journal of Pharmacy*, *4*(4), 301-310.

Marey, A. M., Dkhil, M. A., Abdel Moneim, A. A., Santourlidis, S., Abdel-Gaber, R., Alquraishi, M. I., and Abdalla, M. S. (2025). Fighting sepsis-induced liver damage with biosynthesized silver nanoparticles. *Microscopy Research and Technique*, *88*(1), 127-138.

Demetriades, A. K., Wallman, P. D., McGuiness, A., and Gavalas, M. C. (2005). Low cost, high risk: accidental nutmeg intoxication. *Emergency medicine journal*, *22*(3), 223-225.

Sulaiman, S. F., and Ooi, K. L. (2012). Antioxidant and anti food-borne bacterial activities of extracts from leaf and different fruit parts of Myristica fragrans Houtt. *Food Control*, *25*(2), 533-536.

Periasamy, G., Karim, A., Gibrelibanos, M., Gebremedhin, G., and Gilani, A. U. H. (2016). Nutmeg (Myristica fragrans Houtt.) oils. In *Essential oils in food preservation, flavor and safety* (pp. 607-616). Academic Press.

Kim, H., Bu, Y., Lee, B. J., Bae, J., Park, S., Kim, J., ... and Park, J. W. (2013). Myristica fragrans seed extract protects against dextran sulfate sodium–induced colitis in mice. *Journal of medicinal food*, *16*(10), 953-956.

Piaru, S. P., Mahmud, R., Majid, A. M. S. A., and Nassar, Z. D. M. (2012). Antioxidant and antiangiogenic activities of the essential oils of Myristica fragrans and Morinda citrifolia. *Asian Pacific Journal of Tropical Medicine*, *5*(4), 294-298.

Morsy, N. F. (2016). A comparative study of nutmeg (Myristica fragrans Houtt.) oleoresins obtained by conventional and green extraction techniques. *Journal of food science and technology*, *53*(10), 3770-3777.

Tan, K. P., Khoo, H. E., and Azrina, A. (2013). Comparison of antioxidant components and antioxidant capacity in different parts of nutmeg (Myristica fragrans). *International Food Research Journal*, *20*(3).

Prieto, P., Pineda, M., and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity. Analytical Biochemistry, **269**(2), 337–341.

Panggabean, K. A., Rusmarilin, H., and Suryanto, D. (2019, May). The utilization of nutmeg seed (Myristica fragrans Houtt) extract as an antimicrobial on tempeh sausage. In *IOP Conference Series: Earth and Environmental Science* (Vol. 260, No. 1, p. 012087). IOP Publishing.

Piaru, S. P., Mahmud, R., Majid, A. M. S. A., and Nassar, Z. D. M. (2012). Antioxidant and antiangiogenic activities of the essential oils of Myristica fragrans and Morinda citrifolia. *Asian Pacific Journal of Tropical Medicine*, *5*(4), 294-298.

Periasamy, G., Karim, A., Gibrelibanos, M., Gebremedhin, G., & Gilani, A. U. H. (2016). Nutmeg (Myristica fragrans Houtt.) oils. In *Essential oils in food preservation, flavor and safety* (pp. 607-616). Academic Press.

Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books.

Sulaiman, M. R., and Ooi, K. L. (2012). Antioxidant andantimicrobial properties of nutmeg extracts. Journal of Pharmacology and Toxicology, **7**(1), 25–32.

Gupta, A. D., and Rajpurohit, D. (2011). Antioxidant and antimicrobial activity of nutmeg (Myristica fragrans). In *Nuts and seeds in health and disease prevention* (pp. 831-839). Academic Press.

Canadianti, M., Yusiati, L. M., Hanim, C., Widyobroto, B. P., & Astuti, A. (2020). The effect of nutmeg leaves tannin (Myristica fragrans Houtt) as protein protecting agents on in vitro nutrient digestibility. *Buletin Peternakan*, *44*(1), 10-14.

Tariq, M. (2025). Role of terpenoids in antioxidant mechanisms. *Journal of Plant Biochemistry*,), **14**(177–92.

Zaleha Shafiei, Nadia Najwa Shuhairi, Nordiyana Md Fazly Shah Yap, Carrie-Anne Harry Sibungkil, Jalifah Latip

Evidence-Based Complementary and Alternative Medicine 2012 (1), 825362, 2012

Kim, H., Bu, Y., Lee, B. J., Bae, J., Park, S., Kim, J., ... and Park, J. W. (2013). Myristica fragrans seed extract protects against dextran sulfate sodium–induced colitis in mice. *Journal of medicinal food*, *16*(10), 953-956.

Zaleha Shafiei, Nadia Najwa Shuhairi, Nordiyana Md Fazly Shah Yap, Carrie-Anne Harry Sibungkil, Jalifah Latip

Evidence-Based Complementary and Alternative Medicine 2012 (1), 825362, 2012

Tripatih Nagja, KUMAR Vimal, ACHARYA Sanjeev *Int J Pharm Pharm Sci 8 (2), 27-30, 2016*

SS Harshidha, Lubna Fathima, S Sujitha, R Sindhu, D Prabu, M Rajmohan, Dinesh Dhamodhar, Indira Nehru *Journal of Advanced Medical and Dental Sciences Research 13 (4), 31-39, 2025*

Kartika Dewi, Budi Widyarto, Pande Putu Erawijantari, Wahyu Widowati *Int J Res Med Sci 3 (9), 2303-10, 2014*

APPENDIX I

PHYTOCHEMICAL RESULTS (QUANTITATIVE)

STANDARD CALIBRATION GRAPH VALUES (100ug/mL) **Esculin**

Abs @ 760nm	0	0.07	0.141	0.214	0.282	0.345
Amount (ug)	0	20	40	60	80	100

Amount u	Abs
20	0.07
40	0.141
60	0.214
80	0.282
100	0.345

Absorbance test values for **Coumarin** (Extract)

SAMPLE	Abs @ 527nm					
ID	(Y)	CONST	CONST	**X (A) ug/m**	CONST	**X (A) mg/mL**
A1	0.33	0.0035	0.0031	**93.4**	1000	**0.0934**
A2	0.318	0.0035	0.0031	**89.97143**	1000	**0.089971**
A3	0.315	0.0035	0.0031	**89.11429**	1000	**0.089114**

STANDARD CALIBRATION GRAPH VALUES (100ug/mL) **Quercetin**.

Abs @ 510nm	0	0.03	0.078	0.09	0.126	0.164
Amount (ug)	0	20	40	60	80	100

Amount u	Abs
20	0.03
40	0.078
60	0.09
80	0.126

100	0.164

Absorbance test values for **Flavonoids** (Extract)

SAMPLE	Abs @ 510nm					
ID	(Y)	CONST	CONST	**X (A) ug /mL**	CONST	**X (A) mg/mL**
A1	1.049	0.0016	0.0028	**653.875**	1000	**0.653875**
A2	1.051	0.0016	0.0028	**655.125**	1000	**0.655125**
A3	1.049	0.0016	0.0028	**653.875**	1000	**0.653875**
B1	1.316	0.0016	0.0028	**820.75**	1000	**0.82075**
B2	1.318	0.0016	0.0028	**822**	1000	**0.822**
B3	1.318	0.0016	0.0028	**822**	1000	**0.822**
C1	1.545	0.0016	0.0028	**963.875**	1000	**0.963875**
C2	1.568	0.0016	0.0028	**978.25**	1000	**0.97825**
C3	1.572	0.0016	0.0028	**980.75**	1000	**0.98075**

STANDARD CALIBRATION GRAPH VALUES (100ug/mL) **Beta-Sitosterol**

Abs @ 548nm	0	0.158	0.23	0.345	0.468	0.565
Amount (ug)	0	20	40	60	80	100

Amount u	Abs
20	0.158
40	0.23
60	0.345
80	0.468
100	0.565

Absorbance test values for **Terpenoids** (Extract)

SAMPLE	Abs @ 548nm					
ID	(Y)	CONST	CONST	**X (A) ug/m**	CONST	**X (A) mg/mL**
A1	1.173	0.0053	0.0376	**214.2264**	1000	**0.214226**
A2	1.193	0.0053	0.0376	**218**	1000	**0.218**
A3	1.197	0.0053	0.0376	**218.7547**	1000	**0.218755**
B1	1.159	0.0053	0.0376	**211.5849**	1000	**0.211585**
B2	1.175	0.0053	0.0376	**214.6038**	1000	**0.214604**
B3	1.177	0.0053	0.0376	**214.9811**	1000	**0.214981**
C1	1.204	0.0053	0.0376	**220.0755**	1000	**0.220075**
C2	1.213	0.0053	0.0376	**221.7736**	1000	**0.221774**
C3	1.217	0.0053	0.0376	**222.5283**	1000	**0.222528**

STANDARD CALIBRATION GRAPH VALUES (100ug/mL) **Securidaside**

Abs @ 495nm	0	0.068	0.135	0.21	0.275	0.341
Amount (ug)	0	20	40	60	80	100

Amount ug	Abs
20	0.068
40	0.135
60	0.21
80	0.275
100	0.341

Absorbance test values for **Cardiac glycosides** (Extract)

SAMPLE	Abs @ 495nm					
ID	(Y)	CONST	**X (A) ug/mL**	CONST	**X (A) mg/mL**	
A1	1.734	0.0034	**510**	1000	**0.51**	
A2	1.738	0.0034	**511.1765**	1000	**0.511176**	
A3	1.742	0.0034	**512.3529**	1000	**0.512353**	
B1	1.742	0.0034	**512.3529**	1000	**0.512353**	
B2	1.736	0.0034	**510.5882**	1000	**0.510588**	
B3	1.738	0.0034	**511.1765**	1000	**0.511176**	

C1	1.728	0.0034	**508.2353**	1000	**0.508235**
C2	1.742	0.0034	**512.3529**	1000	**0.512353**
C3	1.744	0.0034	**512.9412**	1000	**0.512941**

Abs @ 760nm	0	0.181	0.267	0.334	0.407	0.556
Amount (ug)	0	20	40	60	80	100

Amount ug	Abs
20	0.181
40	0.267
60	0.334
80	0.407
100	0.556

Absorbance of test values for Phenol (Extract)

SAMPLE ID	Abs @ 780nm (Y)	CONST	CONST	**X (A) ug/mL**	CONST	**X (A) mg/mL**
A1	0.667	0.0047	0.1015	**120.3191**	1000	**0.120319**
A2	0.661	0.0047	0.1015	**119.0426**	1000	**0.119043**

A3	0.656	0.0047	0.1015	**117.9787**	1000	**0.117979**

STANDARD CALIBRATION GRAPH VALUES (100ug/mL) **Stigmasterol**

Abs @ 780nm	0	0.059	0.131	0.245	0.367	0.466
Amount (ug)	0	20	40	60	80	100

Amount ug	Abs
20	0.059
40	0.131
60	0.245
80	0.367
100	0.466

Absorbance test values for **Steroids** (Extract)

SAMPLE	Abs @ 780nm					
ID	(Y)	CONST	CONST	**X (ug/mL)**	CONST	**X (mg/mL)**
A1	1.327	0.0053	0.0614	**238.7925**	1000	**0.238792**
A2	1.348	0.0053	0.0614	**242.7547**	1000	**0.242755**

A3	1.353	0.0053	0.0614	**243.6981**	1000	**0.243698**
B1	1.509	0.0053	0.0614	**273.1321**	1000	**0.273132**
B2	1.508	0.0053	0.0614	**272.9434**	1000	**0.272943**
B3	1.507	0.0053	0.0614	**272.7547**	1000	**0.272755**
C1	1.481	0.0053	0.0614	**267.8491**	1000	**0.267849**
C2	1.48	0.0053	0.0614	**267.6604**	1000	**0.26766**
C3	1.482	0.0053	0.0614	**268.0377**	1000	**0.268038**

CALIBRATION GRAPH VALUES (100ug/mL) **Tannic Acid.** PHLOBATANNIS

Abs @ 725nm	0	0.161	0.319	0.395	0.49	0.542
Amount (ug)	0	20	40	60	80	100

Amount ug	Abs
20	0.161
40	0.319
60	0.395
80	0.49
100	0.542

Absorbance test values for phlobatannins

SAMPLE Abs @

	780nm					
ID	(Y)	CONST	CONST	**X (ug/mL)**	CONST	**X (mg/mL)**
C1	0.581	0.0047	0.1015	**102.0213**	1000	**0.102021**
C2	0.576	0.0047	0.1015	**100.9574**	1000	**0.100957**
C3	0.585	0.0047	0.1015	**102.8723**	1000	**0.102872**

STANDARD CALIBRATION GRAPH VALUES (100ug/mL) **Atropine**(ALKALOIDS)

Abs @ 760nm	0	0.058	0.107	0.204	0.264	0.356
Amount (ug)	0	20	40	60	80	100

Amount ug	Abs
20	0.058
40	0.107
60	0.204
80	0.264
100	0.356

ALKALOIDS CONCENTRATION

SAMPLE

Abs @ 760nm

ID	(Y)	CONST	CONST	**X (A) ug/mL**	CONST	**X (A) mg/mL**
A1	1.187	0.0038	0.0281	**319.7632**	1000	**0.319763**
A2	1.176	0.0038	0.0281	**316.8684**	1000	**0.316868**
A3	1.184	0.0038	0.0281	**318.9737**	1000	**0.318974**
B1	0.868	0.0038	0.0281	**235.8158**	1000	**0.235816**
B2	0.842	0.0038	0.0281	**228.9737**	1000	**0.228974**
B3	0.775	0.0038	0.0281	**211.3421**	1000	**0.211342**
C1	1.563	0.0038	0.0281	**418.7105**	1000	**0.418711**
C2	1.561	0.0038	0.0281	**418.1842**	1000	**0.418184**
C3	1.559	0.0038	0.0281	**417.6579**	1000	**0.417658**

CALIBRATION GRAPH VALUES (100ug/mL) **Tannic Acid.**

Abs @ 725nm	0	0.161	0.319	0.395	0.49	0.542
Amount (ug)	0	20	40	60	80	100

Amount ug	Abs
20	0.161
40	0.319
60	0.395

80	0.49
100	0.542

Absorbance tes values for tannins (Extract)

SAMPLE

Abs @ 780nm

ID	(Y)	CONST	CONST	**X (ug/mL)**	CONST	**X (mg/mL)**
D1	0.78	0.0047	0.1015	**144.3617**	1000	**0.144362**
D2	0.783	0.0047	0.1015	**145**	1000	**0.145**
D3	0.787	0.0047	0.1015	**145.8511**	1000	**0.145851**

APPENDIX II

IN VITRO ANTIOXIDANT RESULTS

SAMPLE ID	DPPH Abs 1	DPPH Abs 2	DPPH Abs 3	Aver Abs	A0	%	**DPPH % Inhib**	FRAP Abs 1	FRAP Abs 2	FRAP Abs 3	Aver Abs	A0	%	**FRAP % Inhib**	NITRIC Abs 1	OXIDE Abs 2	OXIDE Abs 3	Aver Abs	A0	%	**Nitric % Inhib**
A1	1.489	1.342	1.539	1.457	1.826	100	**20.226**	1.007	1.031	1.031	1.023	1.426	100	**28.261**	0.184	0.173	0.218	0.192	1.654	100	**88.412**
A2	1.081	1.136	1.186	1.134	1.826	100	**37.879**	1.107	1.112	1.117	1.112	1.426	100	**22.002**	0.139	0.123	0.139	0.134	1.654	100	**91.919**
A3	0.744	0.764	0.813	0.774	1.826	100	**57.631**	1.112	1.12	1.123	1.118	1.426	100	**21.576**	0.633	0.636	0.618	0.629	1.654	100	**61.971**

A4	0.549	0.642	0.6	0.597	1.826	100	**67.306**	1.115	1.117	1.12	1.117	1.426	100	**21.646**	0.883	0.872	0.886	0.88	1.654	100	**46.775**
A5	0.464	0.572	0.554	0.53	1.826	100	**70.975**	1.112	1.115	1.115	1.114	1.426	100	**21.879**	0.932	0.924	0.941	0.932	1.654	100	**43.632**
B1	1.649	1.707	1.629	1.662	1.826	100	**8.9996**	0.379	0.447	0.464	0.43	1.426	100	**69.846**	0.198	0.218	0.202	0.206	1.654	100	**87.545**
B2	1.677	1.572	1.598	1.616	1.826	100	**11.519**	1.024	1.073	1.054	1.05	1.426	100	**26.344**	0.161	0.178	0.177	0.172	1.654	100	**89.601**
B3	1.518	1.542	1.569	1.543	1.826	100	**15.498**	1.093	1.101	1.099	1.098	1.426	100	**23.025**	0.933	0.933	0.955	0.94	1.654	100	**43.148**
B4	1.558	1.494	1.496	1.516	1.826	100	**16.977**	1.109	1.107	1.107	1.108	1.426	100	**22.324**	0.968	1.002	1.011	0.994	1.654	100	**39.923**
B5	1.422	1.447	1.485	1.451	1.826	100	**20.518**	1.107	1.109	1.117	1.111	1.426	100	**22.09**	1.005	1.013	1.015	1.011	1.654	100	**38.875**
C1	1.598	1.359	1.348	1.435	1.826	100	**21.413**	0.216	0.246	0.287	0.25	1.426	100	**82.492**	0.238	0.241	0.243	0.241	1.654	100	**85.449**
C2	0.861	0.752	0.775	0.796	1.826	100	**56.407**	0.746	0.697	0.706	0.716	1.426	100	**49.766**	0.386	0.258	0.241	0.295	1.654	100	**82.164**
C3	0.516	0.489	0.376	0.460	1.826	100	**74.79**	0.992	1.009	1.021	1.007	1.426	100	**29.383**	0.975	0.955	1.004	0.978	1.654	100	**40.871**
C4	0.338	0.295	0.258	0.297	1.826	100	**83.735**	1.052	1.068	1.061	1.06	1.426	100	**25.643**	1.014	1.012	1.012	1.013	1.654	100	**38.775**
C5	0.25	0.226	0.221	0.232	1.826	100	**87.276**	1.073	1.066	1.068	1.069	1.426	100	**25.035**	1.037	1.056	1.057	1.05	1.654	100	**36.518**
A1 (ASC)	0.07	0.078	0.071	0.073	1.826	100	**96.002**	0.182	0.139	0.104	0.142	1.426	100	**90.065**	0.139	0.135	0.142	0.139	1.654	100	**91.616**
A2	0.064	0.068	0.059	0.064	1.826	100	**96.513**	0.142	0.147	0.149	0.146	1.426	100	**89.762**	0.126	0.126	0.124	0.124	1.654	100	**92.503**
A3	0.086	0.093	0.078	0.086	1.826	100	**95.309**	0.196	0.192	0.129	0.172	1.426	100	**87.915**	0.121	0.118	0.108	0.116	1.654	100	**93.007**
A4	0.068	0.075	0.072	0.072	1.826	100	**96.075**	0.152	0.171	0.146	0.156	1.426	100	**89.037**	0.133	0.129	0.111	0.124	1.654	100	**92.483**
A5	0.037	0.051	0.045	0.044	1.826	100	**97.572**	0.169	0.124	0.139	0.144	1.426	100	**89.902**	0.109	0.106	0.106	0.107	1.654	100	**93.531**

SAMPLE	TAC			Avr			**TAC**	H2O2			Abs			**H2O2**				RP			Avr			**RP**
ID	Abs1	Abs2	Abs3	Abs	A0	%	**%Inhib**	Abs1	Abs2	Abs3	Aver	A0	%	**%Inhib**	Abs1	Abs2	Abs3	Abs	A0	%	**%Inhib**
A1	0.966	0.907	0.862	0.912	0.105	100	**88.483**	0.801	0.551	0.409	0.587	1.726	100	**65.991**	0.498	0.574	0.426	0.499	0.134	100	**73.164**
A2	1.003	0.998	0.996	0.999	0.105	100	**89.489**	0.647	0.691	0.756	0.698	1.726	100	**59.56**	1.035	0.945	0.941	0.972	0.134	100	**86.214**
A3	1.01	0.991	1.001	0.997	0.105	100	**89.472**	0.929	0.913	0.983	0.942	1.726	100	**45.442**	0.958	0.964	1.127	1.016	0.134	100	**86.815**

	1.00	1.34	0.99	1.11	0.10		**90.5**	1.12	1.12	1.13	1.12	1.72		**34.5**	0.98	0.97	0.98	0.97	0.13		**86.3**
A4	4	4	7	5	5	100	**83**	8	8	2	9	6	100	**69**	1	4	3	9	4	100	**17**
	1.01	1.01	0.99	1.00	0.10		**89.5**	1.23	1.24	1.22	1.23	1.72		**28.4**	1.08	1.00		1.02	0.13		**86.9**
A5	1	6	3	7	5	100	**7**	5	5	7	6	6	100	**09**	3	8	0.98	4	4	100	**1**
	0.24	0.26	0.35	0.28	0.10		**63.4**		1.20			1.72		**29.9**	0.64	0.62	0.63	0.63	0.13		**78.9**
B1	2	6	3	7	5	100	**15**	1	2	1.19	1.21	6	100	**15**	7	9	7	8	4	100	**86**
	1.00	1.00	0.99	1.00	0.10		**89.5**	1.30	1.34	1.35	1.33	1.72		**22.6**	1.00		0.96	1.02	0.13		**86.9**
B2	2	2	8	1	5	100	**07**	8	4	4	5	6	100	**34**	6	1.1	7	4	4	100	**18**
	1.00	0.99	0.98	0.99	0.10		**89.4**		1.39	1.39	1.39	1.72		**19.3**	0.98	0.98	1.00	0.98	0.13		**86.4**
B3	1	7	9	6	5	100	**54**	1.39	2	5	2	6	100	**32**	1	4	3	9	4	100	**56**
	1.02	1.00	1.00	1.01	0.10		**89.6**	1.41	1.41	1.41	1.41	1.72		**18.1**	0.97	1.01	1.03		0.13		**86.7**
B4	5	4	3	1	5	100	**11**	4	1	4	3	6	100	**34**	8	9	4	1.01	4	100	**37**
	0.88	1.00	1.00	0.96	0.10		**89.1**	1.43	1.43	1.42		1.72		**17.1**	1.03	1.02	1.01	1.02	0.13		**86.9**
B5	2	4	5	4	5	100	**04**	1	4	6	1.43	6	100	**3**	2	8	8	6	4	100	**4**
	0.94	0.23	0.67	0.61	0.10		**82.9**	1.41	1.42	1.41		1.72		**17.7**			0.50		0.13		**76.0**
C1	5	5	2	7	5	100	**91**	9	6	4	1.42	6	100	**48**	0.6	0.57	9	0.56	4	100	**57**
	0.99	0.37		0.70	0.10		**85.0**		1.45	1.45	1.45	1.72		**15.8**	1.09		0.98	1.02	0.13		**86.8**
C2	6	4	0.74	3	5	100	**71**	1.45	5	2	2	6	100	**56**	1	0.99	5	2	4	100	**88**
	1.00		0.99	0.80	0.10		**86.9**		1.46		1.46	1.72		**15.3**	0.98	0.99	1.00	0.99	0.13		**86.5**
C3	3	0.41	7	3	5	100	**29**	1.46	3	1.46	1	6	100	**53**	9	6	4	6	4	100	**51**
	0.99		1.00	0.80	0.10		**86.8**	1.46		1.45		1.72		**15.3**	1.00	1.00	1.00	1.00	0.13		**86.6**
C4	8	0.4	5	1	5	100	**91**	3	1.46	8	1.46	6	100	**92**	5	6	8	6	4	100	**84**
	0.99	0.34	0.99	0.77	0.10		**86.5**	1.45			1.45	1.72		**15.5**		1.12	1.13	1.12	0.13		**88.1**
C5	9	2	6	9	5	100	**21**	2	1.46	1.46	7	6	100	**66**	1.13	4	1	8	4	100	**24**
A1 (ASC)	1.51	1.49	1.48	1.49	0.10		**92.9**		0.06	0.06	0.06	1.72		**96.3**	1.58	1.59	1.58	1.58	0.13		**91.5**
	8	5	4	9	5	100	**95**	0.06	8	1	3	6	100	**5**	8	5	4	9	4	100	**67**
	1.51	1.46		1.47	0.10		**92.8**	0.05	0.05	0.04	0.05	1.72		**96.8**	1.62	1.66	1.65	1.64	0.13		**91.8**
A2	2	6	1.45	6	5	100	**86**	4	8	9	4	6	100	**91**	2	6	3	7	4	100	**64**
	1.50	1.35	1.38	1.41	0.10		**92.5**	0.07	0.07	0.07	0.07	1.72		**95.6**	1.50	1.55	1.58		0.13		**91.3**
A3	7	5	9	7	5	100	**9**	6	3	8	6	6	100	**16**	7	5	9	1.55	4	100	**57**
	1.51	1.47	1.40	1.46	0.10		**92.8**	0.06	0.06	0.07	0.06	1.72		**96.0**	1.61	1.67	1.62	1.63	0.13		**91.8**
A4	4	5	2	4	5	100	**26**	8	5	2	8	6	100	**41**	4	5	2	7	4	100	**14**
		1.52	1.55	1.51	0.10		**93.0**	0.04	0.05	0.04	0.04	1.72		**97.2**		1.72	1.65	1.68	0.13		**92.0**
A5	1.48	2	4	9	5	100	**86**	7	1	5	8	6	100	**38**	1.68	2	4	5	4	100	**49**