PHYSICOCHEMICAL ANALYSIS AND BIOCHEMICAL ASSESSMENT OF MONODORA MYRISTICA SEED OIL

BY

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CERTIFICATION

This is to certify that this project report entitled "PHYSIOCHEMICAL ANALYSIS AND BIOCHEMICAL ASSESSMENT OF MONODORA MYRISTICA SEED OIL" was written by GBADAMOSI ODUNOLA with Matriculation Number HND/23/SLT/FT/0605 in partial fulfillment of the requirement for the Award of Higher National Diploma (HND) in the Department of Science Laboratory Technology, Biochemistry Unit, Kwara State Polytechnic Ilorin, Kwara state.

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DEDICATION

I dedicate this research work to Almighty God for his grace and guidance, and to my families for their constant support and encouragement throughout the duration of this project.

AKNOWLEDGEMNT

Immeasurable thanks goes to Almighty God for the privilege given to me to complete this project work; he has been helping me from the beginning till the end of this project

My sincere gratitude goes to my kind and esteem supervisor in person of Mrs. Amira E.O, for her advice through supervision, moral support and word of encouragement to the success of this project. May God almighty continue to preserve, guide, and shower his blessings on you ma.

My upmost and sincere appreciation goes to my beloved parents Mr. and Mrs. GBADAMOSI for being my backbone and supporters, without them, I have no power to be where we are today. I thank them for their support financially, morally, and spiritually, and for always encouraging me to be of my best behavior. May God in his absolute mercy enrich their course and grant them long life and prosperity to reap the fruit of their labor.

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ABSTRACT

This study investigated the physicochemical properties and biochemical composition of Monodora myristica *(African nutmeg) seed oil to evaluate its potential for nutritional, medicinal, and industrial applications. The oil was extracted using ethanol-based solvent extraction, yielding 19.91%. The oil displayed a dark brown appearance with a strong aroma, and its physical parameters such as density (0.9971 g/cm³) and specific gravity (1.029) were consistent with those of edible seed oils.*

Key physicochemical values included an acid value of 21.61 mg KOH/g, free fatty acid content of 14.83%, iodine value of 69.54 g I_2/100g, saponification value of 60.01 mg KOH/g, and peroxide value of 232.0 mEq/kg. While these values suggest unsaturation and potential industrial use, the elevated acid and peroxide values indicate significant lipid degradation, likely due to oxidation or poor post-harvest handling.

Biochemical assays revealed moderate antioxidant activity (42.84% DPPH inhibition) and total phenolic content of 21.79 mg GAE/g oil. Additionally, carotenoids such as β-carotene and lycopene were present in measurable amounts (β-carotene: 0.0186mg/ml, lycopene: 0.0169mg/ml). The oxalate content in the cake residue (0.5251 mg/g) was low, suggesting minimal antinutritional risk.

In conclusion, Monodora myristica seed oil shows promising bioactive and nutritional properties but requires refining to reduce its acidity and oxidative levels. These findings contribute valuable data supporting the wider utilization of this underexploited seed oil in food, cosmetic, and pharmaceutical industries.

CHAPTER ONE

1. INTRODUCTION

The global demand for plant-based oils has witnessed a significant surge in recent years due to their nutritional, medicinal, and industrial applications. Natural oils are increasingly preferred over synthetic alternatives because they are biodegradable, environmentally friendly, and often possess bioactive compounds that contribute to human health (Akinoso et al., 2011). Among various plant sources, *Monodora myristica* commonly referred to as African nutmeg is a lesser-known tropical plant with high potential for oil extraction and utilization. Indigenous to West and Central Africa, this plant is valued for its seeds, which are widely used as spices in traditional cooking and herbal medicine (Burkill, 2000).

The seeds of *Monodora myristica* are known to contain significant amounts of oil, which is believed to possess various beneficial properties. Traditional practices have linked the seed oil to therapeutic effects, such as analgesic, antimicrobial, and anti-inflammatory activities (Edeoga et al., 2005). However, scientific investigations into the physicochemical and biochemical characteristics of the oil remain limited. Understanding these properties is crucial for assessing the oil's quality, stability, shelf life, and suitability for food, cosmetic, and pharmaceutical applications.

Physicochemical parameters such as acid value, iodine value, peroxide value, and saponification value are essential indicators of oil purity, freshness, and industrial usability (AOAC, 2010). These parameters help determine the oil's resistance to rancidity and its performance under processing or storage conditions. In addition, the biochemical assessment especially the identification of antioxidant compounds like phenolics, flavonoids, and carotenoids provides insight into the oil's

potential health benefits. Such bioactive compounds help combat oxidative stress and may contribute to the prevention of chronic diseases (Halliwell & Gutteridge, 2007).

Given the increasing interest in nutraceuticals and natural food preservatives, *Monodora myristica* seed oil presents a promising candidate for further research and development. Despite its rich ethnobotanical background, there is a notable gap in empirical data that scientifically validates its physicochemical integrity and biochemical richness.

1.1 BACKGROUND OF THE STUDY

The exploration of plant-derived oils has garnered significant global interest, particularly due to the increasing awareness of their potential nutritional, medicinal, and industrial applications. Seed oils from underutilized or indigenous plants have become a focus of research as sustainable alternatives to synthetic compounds and as renewable sources of essential nutrients. One such plant that has recently drawn scientific attention is *Monodora myristica*, commonly known as African nutmeg. This aromatic spice, native to the tropical rainforests of West and Central Africa, is primarily cultivated for its seeds, which are widely used as condiments in traditional African cuisines (Burkill, 2000).

The seed of *Monodora myristica* is rich in oil, and previous studies suggest that it may possess a wide range of bioactive compounds including flavonoids, tannins, alkaloids, and essential fatty acids (Ojezele & Agunbiade, 2013). These compounds contribute not only to the medicinal and antioxidant properties of the oil but also to its possible industrial utility. Furthermore, the oil is reputed to exhibit analgesic, anti-inflammatory, antimicrobial, and hypotensive effects, making it of interest in pharmacognosy and natural product chemistry (Edeoga et al., 2005).

Despite its traditional usage, the scientific basis for the physicochemical and biochemical properties of *Monodora myristica* seed oil remains limited. For any plant oil to be considered for large-scale application—whether in the food, cosmetic, or pharmaceutical industry—it must undergo rigorous analysis to determine its physicochemical characteristics, such as iodine value, saponification value, acid value, and peroxide value. These properties not only determine the oil's stability and usability but also influence its shelf life and reactivity under storage or industrial conditions (Akinoso et al., 2011).

The biochemical assessment of such oils involves determining the presence and concentration of secondary metabolites and antioxidant activities. These bioactive constituents are essential for evaluating the oil's potential to combat oxidative stress, which is linked to numerous chronic diseases such as cancer, diabetes, and cardiovascular disorders (Halliwell & Gutteridge, 2007). Hence, a comprehensive investigation into *Monodora myristica* seed oil will contribute to the scientific database and provide insight into its quality, safety, and functionality.

Given the widespread traditional use of *Monodora myristica* and the growing need for novel bioresources, it becomes imperative to scientifically assess the oil extracted from its seeds. This study aims to bridge the knowledge gap through systematic analysis and to potentially introduce *Monodora myristica* seed oil as a viable natural resource for health and industrial purposes.

1.2 STATEMENT OF THE PROBLEM

Although *Monodora myristica* is well known across several African cultures for its culinary and ethnomedicinal importance, there is a lack of comprehensive scientific evaluation of its seed oil, particularly regarding its physicochemical and biochemical composition. Most traditional

applications are based on anecdotal knowledge, and without empirical data, the full potential of the oil cannot be harnessed for commercial or therapeutic use (Okwu & Morah, 2007). This knowledge gap poses a challenge to standardizing its application in modern industries and restricts its exploitation in pharmacological innovations.

Moreover, synthetic food additives and preservatives currently used in many products have raised health concerns, ranging from allergies to carcinogenic effects. As a result, there is a growing demand for safer, natural alternatives with antioxidative and antimicrobial properties. However, the absence of standardized scientific data on the biochemical components of *Monodora myristica* seed oil hinders its qualification as a candidate for such substitution (Iwu, 1993).

Another critical problem is the potential underutilization of the plant, despite its richness in oil. Due to the unavailability of verified data on its yield, quality, and stability, the seed oil is not industrially exploited, especially in regions outside Africa. This underexploitation could be linked to the absence of research that comprehensively evaluates its physicochemical indices and antioxidant properties (Nwosu et al., 2008).

Furthermore, most existing studies have focused on either the phytochemical composition of the seed powder or the essential oil derived from the seeds, neglecting the cold or hot-expressed seed oil that could serve food or therapeutic purposes. The physicochemical characteristics such as peroxide and acid values are essential for assessing oil rancidity and quality, especially in industrial production and storage (AOAC, 2010).

Therefore, there is a compelling need to analyze the seed oil of *Monodora myristica* using standardized laboratory methods to determine its safety, stability, and biological relevance. This

research seeks to address these challenges and provide valuable scientific insight that will promote the informed utilization of the oil.

1.3 JUSTIFICATION OF THE STUDY

The increasing trend toward natural remedies and organic products has placed significant value on indigenous plants with nutritional and medicinal potentials. *Monodora myristica*, an underutilized plant species, presents an opportunity for such exploration. Conducting physicochemical and biochemical analysis of its seed oil will provide empirical data essential for validating traditional claims and for exploring new commercial applications (Ajayi et al., 2014).

Given the global health crisis stemming from oxidative stress-related conditions such as cancer, cardiovascular diseases, and neurodegenerative disorders, natural antioxidants are in high demand. The assessment of the oil's biochemical properties such as phenolic and flavonoid content, as well as its free radical scavenging ability, is crucial in determining its therapeutic potential (Halliwell, 2006). If found effective, *Monodora myristica* seed oil may contribute significantly to the nutraceutical and pharmaceutical sectors.

Moreover, this study could serve as a precursor for future pharmacological and toxicological investigations. With a solid physicochemical foundation, researchers can determine its suitability for drug formulation or as a dietary supplement. It can also inform guidelines for proper storage and handling if the oil is to be marketed or integrated into food and cosmetic products (Akinoso et al., 2011).

In addition, the economic benefits of promoting *Monodora myristica* cannot be overstated. Indigenous communities engaged in its cultivation and trade can gain from its commercial

exploitation, contributing to sustainable rural development. Establishing the oil's industrial viability could encourage local processing and value addition, reducing import dependence on foreign oils and additives (Oladele & Oshodi, 2008).

Ultimately, the justification for this study lies in bridging the gap between traditional use and scientific validation. It is a step toward developing a comprehensive profile for *Monodora myristica* seed oil and positioning it as a bioresource of high nutritional, medicinal, and industrial relevance.

1.4 AIM AND OBJECTIVES OF THE STUDY

Aim:

The main aim of this study is to extract, characterize, and evaluate the physicochemical and biochemical properties of *Monodora myristica* seed oil to determine its potential for nutritional, medicinal, and industrial applications.

Objectives:

1. To extract oil from *Monodora myristica* seeds using appropriate laboratory techniques.
2. To determine the physicochemical properties of the extracted oil, including acid value, iodine value, saponification value, peroxide value, Ester value, specific gravity, %Free Fatty Acid, Density and % Oil yield
3. To evaluate the biochemical composition of the oil by determining the presence and concentration of bioactive compounds such as carotenoids.
4. To assess the antioxidant potential of the oil using DPPH radical scavenging assay, and Total Phenolic Content (TPC)

5. To assess the antinutritional content of the oil.
6. To compare the results obtained with standard values and existing literature for possible industrial or pharmaceutical application.

This study's objectives are designed to generate a multidimensional profile of *Monodora myristica* seed oil, offering insights that can support its commercial development and health applications.

1.5 SCOPE OF THE STUDY

This research is limited to the extraction and analytical evaluation of oil obtained from *Monodora myristica* seeds. The oil will be subjected to standard laboratory procedures to analyze its physicochemical and biochemical characteristics. The focus will be on parameters relevant to food, cosmetic, and medicinal applications, particularly acid value, iodine value, saponification value, and antioxidant activity.

The study does not cover the pharmacodynamics, pharmacokinetics, or toxicological effects of the oil on living organisms. Similarly, no clinical or in vivo tests will be conducted; the biochemical assessment will be confined to qualitative and quantitative phytochemical analysis and in vitro antioxidant assays (e.g., DPPH method).

Geographically, the research is localized to seeds obtained from selected markets or locations in Nigeria, and results may not fully represent samples from other regions or countries. Seasonal variation and agronomic factors affecting the oil yield are also not considered within the scope of this work.

Only laboratory-grade reagents and standardized protocols, such as those recommended by the Association of Official Analytical Chemists (AOAC), will be employed. Therefore, deviations from standard conditions, as may be experienced in industrial settings, are outside the scope.

In summary, the study emphasizes characterization of *Monodora myristica* seed oil for its potential as a bioresource through in-laboratory physicochemical and biochemical assessments.

1.6 SIGNIFICANCE OF THE STUDY

This study offers both scientific and socioeconomic significance. From a scientific standpoint, it contributes new knowledge to the relatively under-researched area of *Monodora myristica* seed oil. The findings will provide empirical data on its quality and safety, facilitating the understanding of its nutritional and health-promoting potentials (Edeoga et al., 2005).

The study may also serve as a benchmark for future pharmaceutical and nutraceutical research. If the oil demonstrates strong antioxidant and favorable physicochemical properties, it could be explored for use in formulating drugs, dietary supplements, or cosmetic products. This could drive innovation in natural product development and reduce reliance on synthetic substances.

From a public health perspective, the research is aligned with efforts to identify plant-based interventions for managing oxidative stress-related illnesses. As the burden of non-communicable diseases rises, identifying natural products with therapeutic properties becomes increasingly important (Halliwell, 2006).

In terms of economic implications, validating the properties of *Monodora myristica* seed oil could stimulate its commercialization, providing new opportunities for farmers, traders, and local

industries. This supports agricultural biodiversity, conservation, and sustainable use of indigenous resources.

CHAPTER TWO

2.0 LITERATURE REVIEW

Monodora myristica, commonly known as African nutmeg, is a tropical plant that belongs to the family Annonaceae. It is a medium-sized evergreen tree that grows up to 35 meters tall and is primarily found in the rainforests of West and Central Africa, including Nigeria, Cameroon, Ghana, and Angola (Burkill, 2000). The tree has a cylindrical trunk with smooth, grey bark and spreading branches that form a dense crown. Its leaves are simple, alternate, and elliptical, ranging between 10 to 30 cm in length. The tree produces large, solitary, and pendulous flowers that are strikingly beautiful with yellow-green petals marked with reddish or purple blotches.

The fruit of *Monodora myristica* is a woody capsule, about the size of an orange, containing numerous aromatic seeds enveloped in a yellowish pulp. These seeds resemble true nutmeg in appearance and aroma, hence the name "African nutmeg." Upon ripening, the fruit splits open to reveal the seeds, which are harvested, dried, and used as spice or medicine. The seeds are ovoid, dark brown, and covered in a hard seed coat. The inner kernel, which contains the oil, is the part of most scientific and commercial interest (Okwu & Morah, 2007).

The plant thrives in humid tropical climates with adequate rainfall and can be propagated through seeds or vegetative means. Its adaptability to various soil types and resistance to drought make it suitable for cultivation in diverse ecological zones. Despite its potential, *Monodora myristica* remains underexploited compared to other spice crops, primarily due to limited agronomic data and commercial awareness (Nwosu et al., 2008).

Botanically, the plant shares similarities with other members of the Annonaceae family, including *Annona muricata* and *Xylopia aethiopica*, which are also known for their aromatic and medicinal seeds. However, *Monodora myristica* is distinguished by its unique floral structure and highly aromatic seeds. The seed oil extracted from the kernels has been reported to possess a rich profile of volatile oils and fatty acids (Ajayi et al., 2014).

Scientific interest in the plant has increased in recent years due to its potential pharmacological and nutritional benefits. A thorough botanical understanding of *Monodora myristica* is essential for its identification, propagation, and utilization in various scientific and industrial applications.

2.1 ETHNOMEDICINAL AND NUTRITIONAL IMPORTANCE

Monodora myristica has been traditionally employed in various African cultures for its ethnomedicinal benefits. The seeds, in particular, have been used to treat ailments such as headaches, hypertension, stomach disorders, and rheumatism (Iwu, 1993). In local herbal medicine, ground seeds are often mixed with other botanicals to enhance efficacy. The seed's volatile oil is believed to have analgesic, anti-inflammatory, and antimicrobial effects, which support its wide use in traditional healing practices (Ojezele & Agunbiade, 2013).

In addition to its medicinal applications, the seeds are highly valued for their nutritional content. They are rich in proteins, carbohydrates, lipids, and essential minerals such as calcium, potassium, and magnesium. These nutrients make the seeds a potential dietary supplement, especially in regions where protein-energy malnutrition is prevalent (Oladele & Oshodi, 2008). The presence of essential fatty acids in the seed oil further enhances its nutritional value and potential use in food fortification.

The seed oil has also shown promise in reducing oxidative stress, a key factor in the development of chronic diseases such as diabetes and cardiovascular disorders. Its antioxidant properties, attributed to its high content of polyphenolic compounds, support its use in promoting health and preventing disease (Halliwell & Gutteridge, 2007). The oil is also being explored as a functional ingredient in nutraceuticals and health-promoting formulations.

Culinarily, *Monodora myristica* seeds are used as a spice to flavor soups, stews, and sauces. The seeds are typically roasted, ground, and added to dishes to impart a nutmeg-like aroma. Their pleasant taste and aroma have earned them a place in traditional African cuisines, particularly among the Igbo and Yoruba ethnic groups in Nigeria. Beyond its nutritional and medicinal uses, the seeds have also been used in spiritual and cultural ceremonies (Burkill, 2000).

Despite these benefits, the full potential of *Monodora myristica* remains untapped. There is a need for more detailed nutritional profiling and clinical validation of its health effects. Increased awareness and research could promote its cultivation, processing, and commercialization as a valuable functional food and therapeutic agent.

2.2 OVERVIEW OF SEED OILS AND EXTRACTION TECHNIQUES

Seed oils are triglyceride-rich lipids derived from the seeds of various plants and serve as significant sources of edible fats and bioactive compounds. These oils are critical in food, pharmaceutical, and cosmetic industries due to their diverse properties. Extraction of oil from seeds can be achieved through various techniques, each influencing the yield, purity, and composition of the oil obtained (Akinoso et al., 2011).

The most common extraction methods include mechanical pressing (cold or hot), solvent extraction, and supercritical fluid extraction. Cold pressing involves mechanical crushing of the seeds without external heat application, thereby preserving sensitive bioactive compounds such as vitamins and antioxidants. However, the yield from cold pressing is often lower compared to other methods. Hot pressing, though more efficient in yield, may degrade heat-sensitive constituents (Ajayi et al., 2014).

Solvent extraction, typically using organic solvents like hexane, is widely used in industrial settings for its efficiency in recovering oil. The method involves soaking the crushed seeds in a solvent to dissolve the oil, followed by solvent recovery through distillation. Although efficient, concerns about solvent residues and environmental impact have spurred interest in greener alternatives. Supercritical CO_2 extraction is one such method that uses carbon dioxide under high pressure and temperature, offering high purity and minimal thermal degradation (AOAC, 2010).

The choice of extraction technique can influence the physicochemical and biochemical profile of the oil. For instance, oils obtained through solvent extraction may have higher free fatty acid content due to prolonged exposure to solvents, while cold-pressed oils tend to retain more antioxidants. Therefore, the selection of the extraction method must balance between yield and quality based on the intended use of the oil (Eromosele et al., 1994).

In the case of *Monodora myristica*, both solvent and cold pressing methods have been explored. Recent studies suggest that solvent extraction yields more oil, but cold pressing better retains its flavor and bioactive compounds. Understanding these techniques is crucial for optimizing oil extraction and ensuring the integrity of its nutritional and therapeutic components.

2.3 PHYSICOCHEMICAL PROPERTIES OF SEED OILS

Physicochemical analysis is essential for determining the quality, stability, and suitability of seed oils for various applications. Common physicochemical parameters include acid value, iodine value, saponification value, peroxide value, refractive index, and specific gravity. These properties provide insights into the oil's rancidity, oxidative stability, and usability in food and industrial formulations (AOAC, 2010).

The acid value indicates the free fatty acid content in the oil and reflects the degree of hydrolytic rancidity. A high acid value suggests deterioration, making the oil less suitable for consumption. Iodine value measures the degree of unsaturation in the oil and is directly related to its drying capacity; higher iodine values are characteristic of oils used in paints and cosmetics (Akinoso et al., 2011).

Saponification value quantifies the amount of alkali needed to saponify a given quantity of oil and reflects the average molecular weight of the fatty acids present. Oils with high saponification values are ideal for soap production due to their high fatty acid content. Peroxide value measures the extent of primary oxidation and is used to assess freshness and shelf life. An increase in peroxide value indicates the onset of rancidity (Eromosele et al., 1994).

Refractive index and specific gravity provide additional information about oil purity and composition. These parameters are influenced by the degree of unsaturation and the presence of impurities. For *Monodora myristica* seed oil, studies have shown favorable physicochemical properties comparable to those of established edible oils like soybean and palm kernel oils (Nwosu et al., 2008).

Physicochemical characterization is not only crucial for determining oil quality but also for standardizing its processing and storage. Regulatory bodies such as the Codex Alimentarius and national food safety authorities rely on these parameters to classify oils for food, cosmetic, and pharmaceutical use.

2.4 BIOCHEMICAL CONSTITUENTS OF SEED OILS

Seed oils are reservoirs of diverse biochemical compounds that contribute to their nutritional and therapeutic value. These constituents include essential fatty acids, vitamins, sterols, tocopherols, phenolics, flavonoids, and carotenoids. The composition and concentration of these compounds vary depending on the plant species, extraction method, and storage conditions (Ajayi et al., 2014).

Essential fatty acids such as linoleic acid (omega-6) and alpha-linolenic acid (omega-3) are commonly found in seed oils and are important for maintaining cardiovascular and neurological health. These polyunsaturated fatty acids (PUFAs) help regulate inflammation, lower cholesterol levels, and support cell membrane integrity. Saturated fatty acids, though present in smaller amounts, contribute to the oil's stability and shelf life.

Vitamins E and A, commonly present as tocopherols and carotenoids respectively, function as antioxidants that protect the oil and the body from oxidative damage. Tocopherols not only enhance oil stability but also serve as precursors to vitamin E, an important micronutrient for skin and immune health. Carotenoids such as beta-carotene serve as provitamin A and exhibit antioxidant and anti-cancer properties (Halliwell & Gutteridge, 2007).

Phenolic and flavonoid compounds are responsible for the antioxidant capacity of many seed oils. These polyphenols scavenge free radicals and chelate metal ions, thereby preventing oxidative

stress-related damage. In *Monodora myristica*, significant levels of flavonoids and phenolics have been reported, supporting its traditional use in managing oxidative stress-related ailments (Edeoga et al., 2005).

The biochemical richness of seed oils, including that of *Monodora myristica*, enhances their functional value and supports their inclusion in nutraceuticals, functional foods, and cosmetics. Understanding these constituents is critical for product formulation and for establishing the therapeutic claims of natural oil-based products.

2.5 ANTIOXIDANT AND ANTINUTRITIONAL ACTIVITIES OF SEED OILS

Antioxidants are compounds that inhibit oxidative processes, which are linked to cell damage, aging, and a host of chronic diseases. Many seed oils are rich in natural antioxidants, including tocopherols, polyphenols, and carotenoids, which contribute to their stability and health-promoting properties. The antioxidant potential of seed oils can be evaluated using assays such as DPPH, ABTS, and FRAP (Halliwell, 2006).

In the case of *Monodora myristica*, studies have demonstrated notable antioxidant activity, likely due to its high flavonoid and phenolic content. This activity not only helps protect the oil from rancidity but also offers therapeutic benefits by neutralizing free radicals in biological systems. Regular consumption of antioxidant-rich oils may reduce the risk of cancer, cardiovascular diseases, and neurodegenerative disorders (Iwu, 1993).

On the other hand, some seed oils contain antinutritional factors—compounds that interfere with nutrient absorption or metabolism. These may include phytic acid, oxalates, and tannins. While these compounds can reduce the bioavailability of certain minerals, they may also possess

beneficial properties at controlled levels, such as acting as antioxidants or exhibiting antimicrobial effects (Ojezele & Agunbiade, 2013).

Processing methods such as roasting, fermentation, and solvent extraction can significantly reduce the levels of antinutritional factors. In *Monodora myristica*, the levels of these compounds are relatively low and can be minimized through proper processing, making the oil suitable for consumption and therapeutic use.

The dual presence of antioxidants and trace antinutritional factors in seed oils underscores the importance of comprehensive evaluation. It is essential to balance their health benefits and potential risks to ensure safe and effective use in food and medicine.

CHAPTER THREE

3.0 SAMPLE COLLECTION AND PREPARATION

Seeds of *Monodora myristica* were purchased from Oja-Oba market in Ilorin-West L.G.A, Ilorin, Kwara State, in North central of Nigeria.

The seed coats were peeled, and pulverized (grounded) using a grinding machine.

3.1 MATERIALS USED

The materials utilized for this study include *Monodora myristica* seed, Thimble, Grinding machine, Magnetic stirrer, Tissue paper, White thin rope, UV-Visible spectrophotometer, Distillation apparatus, Heating mantle, Measuring pipette, Analytical balance, Erlenmeyer flask, 250ml volumetric cylinder, Micropipette, Digital thermometer, Mortar, Pestle, Titration setup (burette, pipette, conical, retort stand).

3.1.1 REAGENTS USED

Ethanol, N- Hexane: Acetone (1:1), Ethanol:Ether (1:1), Phenolphthalein, 0.1M NaoH (Sodium Hydroxide) solution, Chloroform, Wij's solution, 0.1M $Na_2S_2O_3$ (Sodium Thiosulphate), 5% KI (Potassium Iodide), Starch solution, Ethanolic KOH (Potassium Hydroxide), 0.5M H_2SO_4 (Sulfuric Acid), 0.1M oxalic acid solution, 0.02M $KMnO_4$ (Potassium Permanganate), Methanol, DPPH (2,2- Diphenyl-1-picrylhydrazyl) solution, Standard solution of ascorbic acid, Folin-Ciocalteu Reagent (FCR), 7% Na_2CO_3 (Sodium Carbonate), Distilled water.

3.2 PROCEDURE FOR EXTRACTION

The weight of an empty thimble was measured on a weighing balance and was recorded as W1, then the pulverized sample was placed in the thimble and all were weighed. This was recorded as W2. The weight of the pulverized sample was calculated by subtracting W2-W1.
The samples were wrapped and placed in the extraction chamber and 450ml of ethanol was added as solvent. The beaker was placed on magnetic stirrer for about 8hours for the extraction of the sample oil.

The solvent extraction process was carried out using steam distillation for 4hours. The extracted oil was collected in a beaker. The solvent was removed using a heating mantle.

3.3 PHYSICOCHEMICAL ANALYSIS

The physicochemical properties of the seed oil were performed using standard titrimetric method.

3.3.1 DETERMINATION OF ACID VALUE AND PERCENTAGE FREE FATTY ACID

- 0.2g of oil sample was weighed on an analytical balance into a clean dry Erlenmeyer flask using a micropipette
- 25ml of Ethanol:Ether (1:1) mixture was added
- 0.5ml phenolphthalein was added as indicator

- The solution was titrated against 0.1M NaoH solution to a a faint pink end point.

Acid Value Calculation:

$$\text{Acid Value} = \frac{\text{Mw NaOH} \times \text{Av} \times \text{M NaOH}}{\text{Sample weight}}$$

Where:

- *Mw* = molecular weight of NaOH used (g/mol) = 40.1g/mol
- *Av* = average titer value = 2.70ml
- *M NaOH* = molarity of NaOH used = 0.1M
- *Sample weight* = 0.5g

Percentage Free Fatty Acid:

$$\text{Percentage Free Fatty Acid (based on oleic acid)} = \frac{\text{Acid value} \times \text{Mw Oleic acid}}{10 \times \text{Mw NaOH}}$$

Where:

- Mw Oleic acid = molecular weight of Oleic acid = 282g/mol
- Mw NaOH = molecular weight of NaOH = 40.01g/mol

3.3.2 DETERMINATION OF IODINE VALUE

- 0.5g of sample was weighed on an analytical balance into a 350ml clean and dry Erlenmeyer flask
- 10ml of chloroform was added and swirled to mix
- 25ml of Wij's solution was added, swirled to mix, and all was stood in the dark for 1hour.
- A clean burette was filled with 0.1M $Na_2S_2O_3$ solution and was adjusted to mark
- 20ml of 5% KI solution was added to the solution kept in the dark after 1hour of been kept, then we swirled to mix.
- 0.1M $Na_2S_2O_3$ was titrated against the solution to a faint yellow, 0.5ml of starch solution was added (a blue-black color was observed). The volume of thiosulphate consumed was recorded.
- Blank titration was performed.

Iodine Value:

$$\text{Iodine Value} = \frac{126.90 \times (V_B - V_T) \times \text{Molarity of thiosulphate}}{10 \times \text{Weight of sample}}$$

Where:

- V_B = volume of blank = 30.20ml
- V_T = volume of test = 2.70ml
- Molarity of thiosulphate = 0.1M
- Weight of sample = 0.5g

3.3.3 DETERMINATION OF PEROXIDE VALUE

- 0.5g of oil sample was weighed into an Erlenmeyer flask

- 30ml of NaoH:Chloroform mixture was added and swirled to mix to obtain a homogenous solution
- 0.5ml of saturated KI solution was added
- The solution was shaken carefully for 2minutes
- 0.5ml of starch solution was added
- The solution was titrated against 0.01M $Na_2S_2O_3$ solution to a blue-black solution end point
- The titration was performed on both extract A and B
- Blank titration was performed.

Peroxide value:

$$\text{Peroxide value} = \frac{1000 \times (V_T - V_B) \times M\ Na_2S_2O_3}{\text{Weight of sample}}$$

Where:

- V_B = volume of blank = 15.50ml
- V_T = volume of test = 3.90ml
- M $Na_2S_2O_3$ = molarity of sodium thiosulphate = 0.01M
- Weight of sample = 0.5g

3.3.4 DETERMINATION OF SAPONIFICATION AND ESTER VALUE

- 0.5g of oil sample was weighed into an Erlenmeyer flask
- 25ml of Ethanolic KOH was added, and well shaken
- The mixture was placed on heating mantle for 10minutes
- The mixture was left to cool for 1hour
- Phenolphthalein indicator was added to turn pinkish color
- 0.5M H_2SO_4 was titrated against the mixture until a faint yellow color was observed

Blank titration was performed

Saponification value:

$$\text{Saponification value} = \frac{M_w\ NaoH \times (V_B - V_T) \times \text{Molarity of } H_2SO_4}{0.5}$$

Where:

- V_B = volume of blank = 23.00ml
- V_T = volume of test = 21.50ml
- M H_2SO4 = molarity Of Sulfuric acid = 0.05M
- Weight of sample = 0.5g
- Mw NaOH = Molecular weight of NaOH = 40.01g/mol

Ester value:

Ester value = Saponification value – Acid value

3.4 BIOCHEMICAL ASSESSMENT

The biochemical component of the extracted oil was assessed and evaluated using spectrophotometry technique. This includes, antioxidant activity (DPPH, TPC), anti-nutritional assay (oxalate determination), and carotenoids estimation. Lycopene and β- Carotene were the carotenoids estimated in this experiment.

3.4.1 ESTIMATION OF ANTIOXIDANT ACTIVITY (E.G., DPPH, TPC)

3.4.1.1 DPPH (2,2- Diphenyl-1-picrylhydrazyl)

- 0.5ml of the extracted oil was carefully measured into a clean test tube with the aid of a micropipette.
- 2ml of methanol and 1ml of DPPH solution were added respectively and was labeled as sample.
- A blank containing 2ml methanol and 1ml of DPPH solution was done separately
- These test tubes were left in the dark for 30minutes.
- Absorbance of the solutions (sample and blank) were measured at 517nm.
- A standard solution of abscorbic acid (0.1g in 10ml of distilled water) was prepared and absorbance measured as control.

$$\% \text{ DPPH antiradical activity (control)} = \frac{(Ab - Aa)}{Ab} \times 100$$

Where:
Ab = absorbance of control
Aa = absorbance of sample

$$\% \text{ DPPH antiradical activity (standard)} = \frac{(Ab - Aa)}{Ab} \times 100$$

Where:
Ab = absorbance of standard
Aa = absorbance of sample

3.4.1.2 TOTAL PHENOLIC CONTENT (TPC)

- 0.5ml of the extracted oil was weighed and added to 5ml of FCR and 4ml of 7% Na_2CO_3. This was labeled as sample.
- 2ml of the resulting solution was further diluted to 10ml with distilled water
- 5ml of FCR and 4ml of Na_2CO_3 was mixed and labeled as blank
- This was incubated at 40^0C for 40minutes (both sample and blank).
- Absorbance measurement was taken at 760nm.
- Readings of absorbance and concentrations (based on Gallic acid) was used, and a graph of absorbance against concentration was plotted to determine the concentration of TPC.

$$\text{TPC (mgGAEquiv/g)} = C \times \frac{V}{M}$$

Where:
C = Concentration = 47.50
V = Volume of sample = 0.5ml
M = Mass of sample = 1.09g

3.4.2 OXALATE DETERMINATION (ANTI-NUTRITIONAL ACTIVITY)

3.4.2.1 STANDARDIZATION OF KMNO4 USING 0.1M OXALIC ACID SOLUTION

0.1M oxalic acid solution was used to standardized $KMNO_4$. This is done to know the molarity of $KMNO_4$ that will be required in the estimation of oxalate in the cake sample.

12.60g of oxalic acid crystals were carefully weighed and dissolved in small quantity of water. The solution was then made up to 1L using appropriate volumetric flask. This gave a 0.1M oxalic acid solution used to standardized the $KMNO_4$ solution.

25ml of 0.1M oxalic acid was pipetted into a clean, dry Erlenmeyer flask.

15ml of 0.5M H_2SO_4 solution was added and the content heated to 60^0C. Digital thermometer was used to measure the temperature.

The hot mixture was quickly titrated against the $KMNO_4$ solution from a burette until a permanent faint pink color appeared.

The exact concentration of $KMNO_4$ was calculated using mole concept.

3.4.2.2 DETERMINATION OF OXALATE IN CAKE SAMPLE

- The cake sample was grounded into a powder form using a mortar and pestle
- 5g of cake sample was weighed and diluted with 10ml of distilled water. This was labeled as the cake solution.
- 5ml of the cake solution was further diluted with distilled water to 250ml mark of volumetric flask.
- 15ml of 0.5M H_2SO_4 was added to the solution the heated to 60^0C. The temperature was monitored using a digital thermometer.
- 0.02M $KMNO_4$ was quickly titrated against the solution until a pink color appeared to mark the end point.
- The exact concentration of oxalate was calculated using mole concept.

3.4.3 ESTIMATION OF LYCOPENE AND β-CAROTENE IN OIL EXTRACT (DETERMINATION OF CAROTENOIDS).

- 1ml of oil extract was mixed with 12.5ml of N- Hexane:Acetone mixture (1:1) were prepared in 100ml of conical flask
- A blank solution containing 12.5ml of N- Hexane:Acetone mixture was performed
- Absorbance was measured at 453nm, 505nm, and 663nm.

Lycopene and β-carotene Estimation

$$\beta\text{-carotene(mg/ml)} = \frac{0.126(A_{663}) - 0.304(A_{505}) + 0.450(A_{453})}{10}$$

$$\text{Lycopene (mg/ml)} = \frac{0.458(A_{663}) - 0.372(A_{505}) - 0.0806(A_{453})}{10}$$

CHAPTER FOUR

4.0 RESULTS

The results of the oil physical properties and % yield is shown in Table 1. Table 2 shows the physicochemical properties of *Monodora myristica* oil extract. Table 3 shows the antioxidant and anti-nutritional activity as well as the standardization of $KMNO_4$. Table 4 shows lycopene and β-carotene in oil extract.

Table 1: % oil and some physical properties

PARAMETERS	RESULTS
% Oil yield	18.38%
Color	Dark brown
Odor	Aromatic
Appearance	Oil viscous
Density	0.9971g/ml
Specific gravity	1.029

Table 2: Physicochemical properties of oil extract

Parameters	Results
Acid value	21.61mg/NaoH/gOil
% Free fatty acid	14.83%
Iodine value	69.54mgI_2/gOil
Peroxide value	232.0mEquiv.I_2/gOil
Saponification value	60.01mg/NaoH/gOil
Ester value	38.40mgNaoH/gOil

Table 3: Antioxidant and anti-nutritional activity

Parameters	Results
% DPPH antiradical activity (control)	42.84%
% DPPH antiradical activity (standard)	-26.54%
TPC	21.79mgGAEquiv/g
Standardization of $KMNO_4$	0.018M
Oxalate determination in cake	0.5251mg/g

Table 4: Lycopene and B-Carotene in Oil Extract A and B

Parameters	Oil extract A	Oil extract B
Lycopene	0.019mg/ml	0.026mg/ml
B-carotene	0.066mg/ml	0.025mg/ml

4.1 DISCUSSIONS

The extraction and subsequent analysis of *Monodora myristica* seed oil yielded important insights into the oil's physicochemical properties, antioxidant activity, antinutritional content, and carotenoid composition. While the sample displayed valuable characteristics for potential food, nutraceutical, or industrial applications, certain results deviated from the expected or ideal range and warrant further interpretation.

4.1.1 OIL YIELD AND PHYSICAL CHARACTERISTICS

The oil yield of *Monodora myristica* seed was **18.38%**, which is moderately high for underutilized oil seeds. This result aligns with previous studies, suggesting that *Monodora myristica* contains considerable oil content, though not as high as other major oilseeds like soybean or groundnut. The relatively moderate yield may be attributed to the extraction method (ethanol-based solvent extraction), seed maturity, or post-harvest handling. Cold pressing or hexane extraction may have resulted in a higher yield.

The physical appearance of the oil dark brown, aromatic, and viscous is consistent with oils rich in polyphenolic compounds and essential oils. The **density (0.9971 g/ml)** and **specific gravity (1.029)** are slightly above that of water and fall within the expected range for most vegetable oils. These values suggest a dense oil, likely due to high content of unsaturated fatty acids and bioactive phytochemicals.

4.1.2 PHYSICOCHEMICAL PROPERTIES

The **acid value (21.61 mg KOH/g oil)** and **percentage of free fatty acid (14.83%)** was notably high compared to standard values for edible oils. Typically, acid values below 4 mg KOH/g and FFA below 2% are acceptable for edible oil use. The high values obtained may indicate hydrolytic rancidity, which can occur due to prolonged storage, enzymatic activity, or moisture contamination. It may also result from overexposure to air or heat during processing.

The **iodine value (69.54 mg I_2/g)** reflects a moderate degree of unsaturation, placing *Monodora myristica* oil in the semi-drying category. This level of unsaturation is advantageous for both nutritional and industrial purposes, especially for soap and cosmetic formulations. The iodine value falls within acceptable limits, suggesting potential use in food applications as well.

The **peroxide value (232.0 mEq I_2/g)** is extremely high and significantly exceeds the safe limit for fresh oils (usually below 10 mEq/kg). This indicates advanced oxidation, likely due to prolonged storage, high exposure to light or oxygen, or poor handling conditions. Such high peroxide levels could compromise oil stability and safety, limiting its edible applications unless refined.

The **saponification value (60.01 mg KOH/g oil)** was lower than typical values for common oils (usually between 180–200 mg KOH/g), suggesting the presence of longer-chain fatty acids or complex esters with lower molecular weights. Similarly, the **ester value (38.40 mg KOH/g)**, derived from the difference between saponification and acid values, supports this finding. This indicates potential for industrial use, though its soap-making efficiency may be relatively low.

4.1.3 ANTIOXIDANT AND ANTINUTRITIONAL ACTIVITY

The **DPPH antioxidant activity** recorded was **42.84% (control)**, indicating moderate antioxidant capacity. This aligns with the presence of bioactive compounds like flavonoids and phenolics in the oil. However, the **standard DPPH value** was negative (**–26.54%**), which is unusual. This anomaly might result from incorrect preparation of the standard solution (ascorbic acid), instrumental error, or contamination during spectrophotometric analysis. It would be advisable to repeat this measurement using a fresh standard.

The **Total Phenolic Content (TPC)** was **21.79 mg GAE/g**, which supports the moderate antioxidant activity observed. Phenolic compounds contribute to the oil's radical-scavenging abilities and shelf stability. This value, though decent, could have been influenced by the solvent used in extraction (ethanol), which is known to be effective at recovering polar compounds.

The **oxalate content** in the cake residue was **0.5251 mg/g**, a relatively low level, indicating minimal antinutritional risk. This suggests that *Monodora myristica* oil and its by-products may be safe for dietary use if further processed or refined. However, it is still important to monitor antinutritional factors, especially if the residue is to be used in animal feed or functional food products.

4.1.4 CAROTENOID COMPOSITION: LYCOPENE AND B-CAROTENE

The oil extracts (A and B) revealed variable levels of **lycopene and β-carotene**. Lycopene concentrations were **0.019 mg/ml (A)** and **0.026 mg/ml (B)**, while β-carotene levels were **0.066 mg/ml (A)** and **0.025 mg/ml (B)**. These carotenoids are important for their provitamin A activity and antioxidant function. The observed variation may result from inconsistencies in solvent

distribution, differences in seed particle size during grinding, or instrumental drift during UV-Vis measurement.

Higher β-carotene in extract A and higher lycopene in extract B might also reflect uneven distribution of pigments in the sample batch or incomplete mixing. These results, however, affirm the nutritional potential of *Monodora myristica* oil and its role in promoting visual and immune health.

4.1.5 DEVIANT RESULTS AND POSSIBLE CAUSES

- **High Acid and Peroxide Values**: These strongly suggest oil degradation, possibly due to improper storage, high moisture, or enzyme activity during or after extraction.
- **Low Saponification Value**: May indicate the presence of long-chain fatty acids or impurities that require further purification.
- **Negative DPPH Standard Reading**: Likely caused by errors in standard preparation or instrumental calibration.
- **Variation in Lycopene/β-Carotene Levels**: Could be due to inconsistent sample handling, light sensitivity of carotenoids, or pipetting inaccuracies.

CONCLUSION

The comprehensive analysis of *Monodora myristica* seed oil in this study has provided valuable insights into its physicochemical and biochemical properties, confirming its potential as a nutritionally and industrially relevant plant oil. The seed oil yield of 18.38% reflects its viability as a moderate oil-producing crop, while its dark brown, viscous, and aromatic nature is indicative of a rich bioactive composition. The measured density and specific gravity fall within acceptable ranges, affirming the oil's similarity to other edible and industrial seed oils.

The physicochemical evaluations revealed both strengths and limitations. While the iodine value indicates a favorable level of unsaturation and potential utility in cosmetics and semi-drying applications, the high acid and peroxide values suggest oxidative instability and hydrolytic rancidity likely caused by handling, storage, or processing conditions. These results highlight the importance of refining and preserving *Monodora myristica* seed oil to ensure safety and usability in food and pharmaceutical products.

Biochemical assessments revealed significant antioxidant potential, as evidenced by moderate DPPH radical scavenging activity and appreciable levels of total phenolic content. The presence of vital carotenoids, such as lycopene and β-carotene, further enhances the oil's nutraceutical value, supporting its use in promoting eye and immune health. Meanwhile, the low oxalate content suggests minimal antinutritional risks, enhancing the safety profile of the oil and its by-products.

Despite a few deviant results such as the unusually high peroxide value and a negative DPPH standard, this study successfully establishes *Monodora myristica* seed oil as a promising indigenous resource with multiple applications in food, cosmetics, and pharmaceuticals. Future

research should focus on refining the oil, extending shelf life, and exploring its therapeutic effects through in vivo studies.

APPENDIX

A. Calculations of **% Oil yield** after distillation process. Parameters given are as follows.

Weight of thimble W1 = 11.10g

Weight of thimble containing sample W2 = 105.62g

Weight of sample only W3 = W2 – W1 =105.62g – 11.10g = 94.52g

Weight of beaker W4 = 87.92g

Weight of beaker containing extract W5 = 105.30g

Weight of extract only W6 = W5 -W4 = 105.30g – 87.92g = 17.38g

$$\% \text{ Oil yield} = \frac{\text{weight of oil}}{\text{weight of sample}} \times 100$$

$$\frac{17.38}{96.52} \times 100 = 18.38\%$$

B. Calculation of **oil density**. Parameters is given as follows:

$$\text{Density of oil} = \frac{\text{Weight of oil}}{\text{Volume of oil}}$$

Where:

Volume of oil = 7ml

Weight of oil is calculated as:

Weight of empty cylinder W1 =28.18g

Weight of cylinder containing 7ml of oil W2 = 35.16g

Weight of 7ml of oil W3 = W2 – W1 = 35.16g – 28.18g = 6.98g

$$\text{Density of oil} = \frac{6.98\text{g}}{7\text{ml}} = 0.9971\text{g/ml}$$

C. Calculation of **specific gravity** of oil. Parameters is given as:

$$\text{Specific gravity} = \frac{\text{Density of oil}}{\text{Density of water}}$$

Where density of water is calculated as

$$\frac{\text{Weight of measuring cylinder containing 10ml of water (W1) – weight of empty cylinder (W2)}}{\text{Volume of water}}$$

W1 = 36.33g

W2 = 26.64g

Density of water = $\frac{36.33g - 26.64g}{10ml} = \frac{9.69}{10} = 0.969g/ml$

Specific gravity = $\frac{0.9971}{0.969} = 1.029$

D. Calculations of Physicochemical Properties

1. **Acid Value (AV)**

Formula:

$$\text{Acid Value} = \frac{\text{Mw NaOH} \times \text{Av} \times \text{M NaOH}}{\text{Sample weight}}$$

Where:

- *Mw* = molecular weight of NaOH used (g/mol) = 40.1g/mol
- *Av* = average titer value = 2.70ml
- *M NaOH* = molarity of NaOH used = 0.1M
- *Sample weight = 0.5g*

$$\text{Acid value} = \frac{40.01 \times 2.70 \times 0.1}{0.5} = 21.61\text{mg/NaOH/gOil}$$

2. % Free Fatty Acid:

$$\%\text{ Free Fatty Acid (based on oleic acid)} = \frac{\text{Acid value} \times \text{Mw Oleic acid}}{10 \times \text{Mw NaOH}}$$

Where:

- Mw Oleic acid = molecular weight of Oleic acid = 282g/mol
- Mw NaOH = molecular weight of NaOH = 40.01g/mol
- AV = 21.61
- M = Molecular weight of oleic acid = 282

$$\%FFA = \frac{21.61 \times 282}{10 \times 40.01} = 14.83\%$$

3. Saponification Value (SV)

$$\text{Saponification value} = \frac{M_w\ \text{NaoH} \times (V_B - V_T) \times \text{Molarity of } H_2SO_4}{0.5}$$

Where:

- V_B = volume of blank = 23.00ml

- V_T = volume of test = 21.50ml
- M H_2SO4 = molarity Of Sulfuric acid = 0.05M
- Weight of sample = 0.5g
- Molecular weight of NaOH = 40.01g/mol

SV = $\frac{40.01 \times (23.00 - 21.50) \times 0.05}{0.5}$ = 60.01mgNaOH/gOil

4. Ester value = Saponification value – Acid value

60.01 – 21.61 = 38.40mgNaOH/gOil

5. **Iodine Value (IV)**

Iodine Value = $\frac{126.90 \times (V_B - V_T) \times \text{Molarity of thiosulphate}}{10 \times \text{Weight of sample}}$

Where:

- V_B = volume of blank = 30.20ml
- V_T = volume of test = 2.70ml
- Molarity of thiosulphate = 0.1M
- Weight of sample = 0.5g

IV = $\frac{126.90 \times (30.20 - 2.70) \times 0.1}{10 \times 0.5}$ = 69.54mEquivI_2/gOil

6. **Peroxide Value (PV)**

Peroxide value = $\frac{1000 \times (V_T - V_B) \times M\ Na_2S_2O_3}{\text{Weight of sample}}$

Where:

- V_B = volume of blank = 15.50ml
- V_T = volume of test = 3.90ml
- M $Na_2S_2O_3$ = molarity of sodium thiosulphate = 0.01M
- Weight of sample = 0.5g

PV = $\frac{1000 \times (3.90 - 15.50) \times 0.01}{0.5}$ = 232.0mEquivI_2/gOil

7. Carotenoid Content Calculation

β-carotene(mg/ml) = $\frac{0.126(A_{663}) - 0.304(A_{505}) + 0.450(A_{453})}{10}$

Where:

A663 = absorbance at 663nm = 0.259nm

A505 = absorbance at 505nm = -0.182nm

A453 = absorbance at 453nm = 0.218nm

β-carotene = $\frac{0.126\ (0.259) - 0.304\ (-0.182) + 0.450\ (0.218)}{10}$ = 0.0186mg/ml

Lycopene (mg/ml) = $\frac{0.458(A_{663}) - 0.372(A_{505}) - 0.0806(A_{453})}{10}$

Where:

A663 = absorbance at 663nm = 0.259nm

A505 = absorbance at 505nm = -0.182nm

A453 = absorbance at 453nm = 0.218nm

Lycopene = $\frac{0.458\ (0.259) - 0.372\ (-0.182) - 0.0806\ (0.218)}{10}$ = 0.0169mg/ml

8. DPPH Radical Scavenging Activity

% DPPH antiradical activity (control) = $\frac{(Ab - Aa)}{Ab} \times 100$

Where:
$Ab_{control}$ = absorbance of control = 2.85
Aa = absorbance of sample = 1.63
% DPPH antiradical activity = $\frac{(2.85 - 1.63)}{2.85}$ x 100 = 42.84%

% DPPH antiradical activity (standard) = $\frac{(Ab - Aa)}{Ab} \times 100$

Where:
$Ab_{standard}$ = absorbance of standard
Aa = absorbance of sample
% DPPH antiradical activity (standard) = $\frac{(1.308 - 1.63)}{1.308}$ x 100 = -24.54%

9. Total Phenolic Content (TPC)

TPC (mg GAE/g) = C×VM

- V = volume of extract used = 0.5ml
- M = mass of sample = 1.09g
- C = concentration from standard curve = 47.50

TPC= 47.50 x $\frac{0.5}{1.09}$ = 21.79mgEquiv/g

A

0.650

47.50 C

Fig 1. Calibration curve showing Absorbance against Concentration of TPC (0.650 is the absorbance average of sample, while 47.50is the concentration obtained from standard curve)

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