EXTRACTION, PHYSICOCHEMICAL ANALYSIS AND BIOCHEMICAL ASSESSMENT OF IRVINGIA GABONENSIS SEED OIL

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CERTIFICATION

This is to certify that this project report entitled "EXTRACTION, PHYSIOCHEMICAL ANALYSIS AND BIOCHEMICAL ASSESSMENT OF IRVINGA GABONENSIS SEED OIL" was written by ABDULWAHAB MARYAM ADENIKE with Matriculation Number HND/23/SLT/FT/0798 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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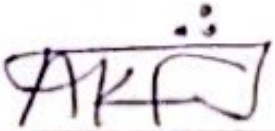

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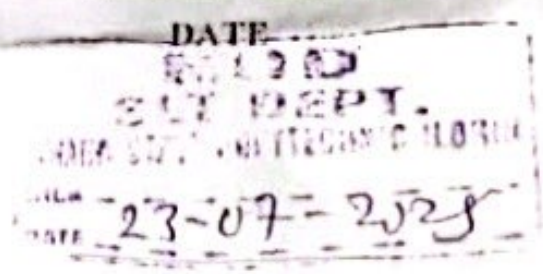

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DEDICATION

This research is dedicated work to Almighty God for his grace and guidance on me upon completing this project, and also to my beloved parent.

AKNOWLEDGEMNT

I would like to express my heartfelt gratitude to my supervisor in person of Mrs. O. E., Amira, mentor, and advisor for their guidance and support throughout this project. Their expertise and feedback were invaluable in shaping this work.

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ABSTRACT

This study investigates the physicochemical and biochemical properties of Irvingia gabonensis (African bush mango) seed oil, extracted using ethanol solvent, to assess its potential for nutritional and industrial applications. Seeds sourced from Ilorin, Nigeria, were processed, and oil was extracted via solvent extraction in two batches, yielding 20.90% and 28.96% oil content. Physicochemical analyses, conducted following AOAC standards, included acid value (17.02 and 16.00 mg NaOH/g), free fatty acid content (12.40% and 11.30%), iodine value (86.29 and 44.16 mg I_2/g), peroxide value (26.00 and 36.00 meq/kg), saponification value (84.02 and 48.01 mg NaOH/g), and ester value (66.02 mg NaOH/g for both). Biochemical assessments revealed significant antioxidant activity, with lycopene (0.019 and 0.026 mg/ml) and β-carotene (0.066 and 0.025 mg/ml) detected in both extracts, alongside low oxalate levels (0.9992 mg/g) in the residual cake.

The high acid and peroxide values indicate hydrolytic and oxidative degradation, likely due to suboptimal storage or processing conditions, which could compromise the oil's shelf life and suitability for direct consumption. However, the elevated iodine value in Extract A suggests a higher degree of unsaturation, potentially beneficial for nutritional applications, while the presence of carotenoids and phenolic compounds underscores the oil's antioxidant potential. These attributes highlight its promise for use in food, cosmetic, and pharmaceutical industries, provided refining techniques are applied to enhance stability.

The findings position Irvingia gabonensis seed oil as a viable alternative to conventional vegetable oils, with implications for sustainable development and economic empowerment in tropical regions. Further research is recommended to optimize extraction methods, reduce degradation, and conduct comprehensive phytochemical and toxicological studies to fully validate its commercial and therapeutic potential.

CHAPTER ONE

1.0 INTRODUCTION

The growing global emphasis on renewable, sustainable, and plant-based bio-resources has intensified research into underutilized oil-bearing seeds, particularly those indigenous to sub-Saharan Africa. Among these, *Irvingia gabonensis*, commonly known as African wild mango, bush mango, or dika nut, has emerged as a multipurpose species of significant economic and nutritional importance. Native to the humid forest zones of West and Central Africa, *Irvingia gabonensis* is a member of the Irvingiaceae family and is prized for its edible fruits and seeds, the latter of which are rich in non-conventional oil (Leakey et al., 2005; Ejiofor et al., 1996).

The seeds of *I. gabonensis* are extensively used in traditional West African cuisines as a thickening agent in soups due to their mucilaginous properties, but beyond their culinary use lies a rich biochemical composition. The seed kernels, also referred to as dika nuts, contain approximately 50–70% oil by dry weight, making them a promising source of plant oil with potential applications in food, cosmetics, and pharmaceutical industries (Ndjouenkeu et al., 1996; Ekpa & Ekpe, 1995). The oil is semi-solid at room temperature, akin to cocoa butter, and is characterized by a high content of saturated and monounsaturated fatty acids, which influence its stability and functionality (Akinoso et al., 2011).

The extraction and characterization of plant oils, such as that from *I. gabonensis*, is essential to understanding their suitability for industrial and nutritional applications. Physicochemical parameters such as acid value, peroxide value, iodine value, saponification value, and refractive index provide crucial insights into the quality, stability, and usability of the oil (Codex Alimentarius, 1999). Moreover, the biochemical assessment of the oil – including its lipid profile, antioxidant capacity, and potential bioactive components – can inform its efficacy as a functional food or nutraceutical (Uhegbu et al., 2009; Egbekun & Ngoddy, 1990).

In light of the increasing demand for alternative oils that are locally available, sustainable, and economically viable, research into *Irvingia gabonensis* seed oil represents both a scientific and socio-economic opportunity. Despite its underutilization in mainstream industrial

production, this indigenous seed oil holds potential not only as a nutritional lipid source but also as a raw material for soaps, cosmetics, and pharmaceutical preparations (Okolo et al., 2020). A comprehensive study involving the extraction methods, physicochemical properties, and biochemical composition of the oil is therefore essential to unlock its full potential and enhance its commercial exploitation.

1.1 BACKGROUND OF THE STUDY

The rising global demand for natural oils with health-promoting benefits has intensified research into underutilized plant species, particularly those indigenous to Africa. Among these is *Irvingia gabonensis*, a tree species native to the humid forest zones of West and Central Africa. Traditionally cultivated for its fleshy fruit, the seeds have become increasingly recognized for their oil content and nutritional richness. The oil, commonly referred to as "dika oil" or "bush mango butter," has been used in local diets and traditional medicine, but its full potential remains underexploited in industrial and scientific contexts.

Several studies have pointed to the high lipid content of *I. gabonensis* seeds, with oil yields ranging between 50–70%, depending on extraction methods. The seed oil is solid at room temperature, indicating a relatively high content of saturated fats, yet it also contains essential fatty acids and bioactive compounds such as phytosterols, tocopherols, and phenolic compounds. These constituents suggest possible antioxidant and cardioprotective effects, making the oil a candidate for use in functional foods and therapeutic formulations.

Furthermore, the physicochemical properties of seed oils—such as acid value, saponification value, iodine value, and peroxide value—are critical indicators of their quality, stability, and suitability for various applications. Understanding these parameters in *Irvingia gabonensis* seed oil is essential for determining its shelf-life, usability in food products, and potential for conversion into soap, cosmetics, or biodiesel.

Despite its traditional uses, scientific literature on the systematic extraction, physicochemical profiling, and biochemical evaluation of *I. gabonensis* seed oil is still developing. This gap

necessitates focused research to validate traditional knowledge, explore its health benefits, and support its commercial development.

This study, therefore, aims to fill that gap by extracting the seed oil using appropriate techniques, analyzing its physicochemical properties, and conducting biochemical assessments to uncover its potential nutritional and therapeutic value.

1.2 STATEMENT OF THE STUDY

Despite the increasing global interest in plant-based oils for nutritional, medicinal, and industrial purposes, many indigenous African plant resources remain under-researched and underutilized. *Irvingia gabonensis*, widely known for its edible fruit and oil-rich seeds, holds significant promise as a source of natural oil with potential health and economic benefits. Traditionally used in West and Central African communities, the oil extracted from its seeds is believed to possess valuable physicochemical and biochemical properties.

However, scientific data on the composition, quality, and functional potential of *Irvingia gabonensis* seed oil remain limited. There is insufficient documentation on standardized methods of extraction, variation in physicochemical properties, and detailed biochemical profiles, including its fatty acid composition and bioactive compounds. This lack of comprehensive evaluation hinders its wider acceptance and industrial application in the food, cosmetic, and pharmaceutical sectors.

Furthermore, without proper assessment of the oil's physicochemical parameters—such as acid value, saponification value, and iodine value—it becomes difficult to determine its shelf life, usability, and safety for consumption or commercial use. Likewise, understanding its biochemical properties is essential for validating claims of its health benefits, including its antioxidant and lipid-lowering effects.

Therefore, there is a need for systematic research to extract, characterize, and evaluate the oil from *Irvingia gabonensis* seeds. Addressing this gap will not only promote the scientific

understanding of this indigenous resource but also support its potential development into valuable commercial and therapeutic product.

1.3 OBJECTIVES OF THE STUDY

The main objective of this study is to extract and evaluate the physicochemical and biochemical properties of oil obtained from *Irvingia gabonensis* seeds.

1.3.1 SPECIFIC OBJECTIVES

1. **To extract oil** from *Irvingia gabonensis* seeds using an appropriate and efficient extraction method.
2. **To determine the physicochemical properties** of the extracted seed oil, including:
   - Oil yield
   - Ester value
   - Acid value
   - Saponification value
   - Iodine value
   - Peroxide value
   - % Of free fatty acids
3. **To analyze the biochemical composition** of the seed oil, focusing on:
   - Presence of bioactive compounds (e.g., carotenoids)
4. **To assess the anti-nutritional and anti-oxidant value** of the seed oil based on its biochemical and physicochemical characteristics. Including:
   - Anti-nutritional (e.g., determination of oxalate)
   - Anti-oxidant (e.g., DPPH, TPC).

1.4 SIGNIFICANCE OF THE STUDY

This study holds both scientific and socio-economic significance, particularly in the context of promoting sustainable utilization of indigenous plant resources in sub-Saharan Africa. *Irvingia gabonensis*, though widely recognized for its culinary applications, remains

underexploited in terms of its oil potential and industrial value (Ejiofor et al., 1996; Leakey et al., 2005). By investigating the extraction efficiency, physicochemical parameters, and biochemical properties of *I. gabonensis* seed oil, this research contributes valuable data that could inform future development, commercialization, and standardization of this non-conventional oil (Ekpa & Ekpe, 1995; Okolo et al., 2020).

From a scientific perspective, the study enriches the body of knowledge on tropical oilseeds by providing detailed insights into the quality, stability, and functional properties of *I. gabonensis* oil. It also establishes a basis for comparing this oil with conventional oils such as palm oil, coconut oil, and shea butter in terms of nutritional value, shelf-life, and processing potential (Akinoso et al., 2011; Ndjouenkeu et al., 1996). This can support future studies in food science, pharmacology, and cosmetic formulation.

In terms of public health and nutrition, the study offers a potential alternative lipid source that could help diversify diets, improve lipid nutrition, and contribute to food security in regions where malnutrition and limited dietary fat intake are prevalent (Uhegbu et al., 2009; Egbekun & Ngoddy, 1990). The identification of bioactive compounds and antioxidant properties may also position the oil as a candidate for functional food or nutraceutical development (Okolo et al., 2020).

Economically, this research highlights the potential for value addition and income generation through the processing and commercialization of *Irvingia gabonensis* oil. This could benefit rural farmers and entrepreneurs involved in the collection, processing, and trade of the seeds, thereby promoting local industries and contributing to poverty alleviation (Leakey et al., 2005).

Lastly, the project aligns with sustainability goals, as it encourages the use of renewable, locally available natural resources while reducing reliance on imported or environmentally burdensome oils. By promoting the industrial relevance of underutilized species, this study supports biodiversity conservation and the sustainable use of forest products (Leakey et al., 2005; Codex Alimentarius, 1999).

1.5 SCOPE OF THIS STUDY

This study focuses on the extraction, physicochemical analysis, and biochemical assessment of oil derived from *Irvingia gabonensis* (dika nut) seeds. The research is limited to seeds sourced from a specific geographical region within Nigeria to maintain consistency and reduce variability caused by environmental or genetic factors.

The extraction process utilizes the Soxhlet method with *n*-hexane as the solvent to ensure efficient and standardized oil recovery.

The physicochemical parameters analyzed include acid value, peroxide value, iodine value, saponification value, specific gravity, and refractive index, following standard AOAC and Codex Alimentarius methods. These tests are intended to evaluate the quality, stability, and usability of the extracted oil.

The biochemical assessment is focused on the carotenoid profiles (using spectrophotometry method), as well as preliminary screening for antioxidant activity and anti-nutritional properties. While the study evaluates the oil for its physicochemical properties and biochemical assessment, its scope is primarily laboratory-based and exploratory in nature.

1.6 LIMITATIONS OF THIS STUDY

1. **Geographic and Sample Limitations**: The seeds used in this study are collected from a limited area, and results may not fully represent the biochemical diversity of *Irvingia gabonensis* across different ecological zones or subspecies.
2. **Extraction Method Constraint**: Solvent extraction (using ethanol as solvent) is used in this research as the only extraction method. Alternative extraction methods such as mechanical pressing, cold pressing or supercritical fluid extraction, which may yield oils with different properties, are not explored.
3. **Absence of Toxicological Studies**: The safety and toxicity profile of the oil, particularly in vivo or clinical assessments, are not part of this study. Hence, claims regarding the oil's health benefits or medicinal properties remain theoretical.

4. **Industrial Application Testing Not Included**: Though the oil is evaluated for physicochemical and nutritional quality, no practical trials are conducted for its application in food, cosmetics, or pharmaceutical formulations. As such, the study provides a scientific basis but not direct industrial proof-of-concept.
5. **Time and Resource Constraints**: Laboratory analyses are limited by available equipment, reagents, and time frame for project completion. This may have influenced the depth and range of tests conducted.

CHAPTER TWO

2.0 LITERATURE REVIEW

Irvingia gabonensis, commonly known as African bush mango, dika nut, or wild mango, is a tropical forest tree native to West and Central Africa. It belongs to the family Irvingiaceae and is predominantly found in the lowland rainforests of countries such as Nigeria, Cameroon, Gabon, and the Democratic Republic of Congo (Leakey et al., 2005). The tree grows up to 30 meters in height and is well adapted to humid tropical environments. It produces mango-like fruits, which contain seeds (kernels) rich in oil and mucilage.

The indigenous uses of *I. gabonensis* are extensive. Traditionally, the fruit pulp is consumed fresh or processed into juices and jams, while the seed kernels are sun-dried and ground into a paste that is widely used as a thickening agent in soups and stews across West African cuisines (Ejiofor et al., 1996). The mucilaginous nature of the ground kernel is due to its high soluble fiber content, which contributes to the texture of traditional dishes like *ogbono* soup in Nigeria.

Beyond its culinary use, *Irvingia gabonensis* holds significant ethnomedicinal value. Various parts of the plant, including the bark, leaves, and roots, have been used in traditional medicine for treating ailments such as diarrhea, diabetes, and wounds (Lowe et al., 2000). The seeds, in particular, have gained attention for their potential metabolic health benefits. Research by Uhegbu et al. (2009) and Ngondi et al. (2005) demonstrated that extracts of *I. gabonensis* seeds could reduce blood glucose and lipid levels in experimental models, highlighting possible hypoglycemic and hypolipidemic effects.

Economically, the tree is classified as a non-timber forest product (NTFP) of growing importance. Leakey et al. (2005) emphasized its role in improving rural livelihoods, as both the fruit and kernels are traded in local and international markets. In Nigeria and Cameroon, for example, *Irvingia* kernels fetch high prices and are often processed into oil or traditional food thickeners, generating income for smallholder farmers and women cooperatives.

Botanical research has also focused on genetic diversity and conservation of *Irvingia gabonensis*. Studies using molecular markers have revealed considerable genetic variation among populations across different ecological zones, indicating the need for conservation strategies to protect this valuable species (Lowe et al., 2000). Its domestication and integration into agroforestry systems have been encouraged to reduce pressure on wild populations and enhance sustainable production.

In recent years, *Irvingia gabonensis* has been the subject of nutritional and pharmacological research, particularly for its potential as a dietary supplement for weight loss and metabolic syndrome. Although commercial products containing *I. gabonensis* extract have emerged in global markets, including Europe and North America, more scientific evidence is required to fully validate its claimed therapeutic effects.

In conclusion, the literature reveals that *Irvingia gabonensis* is a highly versatile and underutilized forest species with significant cultural, nutritional, and economic value. Despite increased scientific interest in recent decades, further research is needed to optimize its domestication, standardize its derivatives, and expand its application in nutrition, medicine, and agro-industrial development.

2.1 TAXONOMICAL CLASSIFICATION OF IRVINGIA BONENSIS

- **Kingdom**: Plantae
- **Phylum**: Magnoliophyta
- **Class**: Magnoliopsida
- **Order**: Malpighiales
- **Family**: Irvingiaceae
- **Genus**: *Irvingia*
- **Species**: *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill.

2.2 OVERVIEW OF OIL EXTRACTION TECHNIQUES

2.2.1 INTRODUCTION TO OIL EXTRACTION TECHNIQUE

Oil extraction is the process of isolating oil from plant materials such as seeds, nuts, or fruits. In the case of *Irvingia gabonensis*, the seeds are rich in lipids and require appropriate extraction techniques to obtain high-quality oil suitable for food, cosmetic, or pharmaceutical use.

2.2.2 CLASSIFICATION OF EXTRACTION TECHNIQUE

The two main categories:

- Mechanical/Physical Methods
- Solvent-Based (Chemical) Methods

Solvent extraction method is employed in this practical for the purpose of laboratory analysis.

2.2.3 SOLVENT EXTRACTION (E.G., ETHANOL)

- **Principle**: Oil is dissolved in an organic solvent like ethanol, then separated and the solvent is evaporated.
- **Advantages**: High yield; efficient extraction of bound oils.
- **Limitations**: Risk of solvent residues; requires skilled handling and equipment.

Common in laboratory and industrial analysis because it provides reproducible results and high recovery.

2.3 PHYSICOCHEMICAL PROPERTIES OF *IRVINGIA GABONENSIS* SEED OIL

Physicochemical properties are essential indicators of the quality, stability, usability, and nutritional value of seed oils. These properties help determine the oil's shelf life, resistance to rancidity, potential for industrial processing, and suitability for human consumption. The seed

oil of *Irvingia gabonensis*—commonly known as dika nut oil—has been extensively studied and found to exhibit favorable physicochemical characteristics comparable to other tropical oils such as coconut and palm kernel oils.

2.3.1 PERCENTAGE OIL YIELD

Oil yield refers to the amount of oil extracted from a given quantity of seed or kernel, usually expressed as a percentage. It is a critical measure of how efficient the extraction process is. A high oil yield indicates that the seed is a good source of commercial oil and reflects favorable oil-rich content within the material.

2.3.2 ACID VALUE (AV)

Acid value represents the amount of free fatty acids present in an oil sample. It is an important index of oil quality and freshness. A low acid value suggests that the oil is less degraded and suitable for consumption or industrial use, while a high acid value indicates rancidity or poor storage.

2.3.3 SAPONIFICATION VALUE (SV)

Saponification value is the quantity of potassium hydroxide (KOH) required to saponify one gram of oil. It reflects the average molecular weight of the fatty acids in the oil. A high saponification value suggests the presence of short- or medium-chain fatty acids, making the oil suitable for soap and cosmetic production.

2.3.4 PEROXIDE VALUE (PV)

Peroxide value measures the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. It is used to assess the oxidative stability of oil. A low peroxide value indicates that the oil is fresh and has not undergone significant oxidative damage, while a high value is a sign of rancidity.

2.3.5 ESTER VALUE

Ester value is calculated as the difference between the saponification value and the acid value. It reflects the amount of esterified fatty acids (i.e., those that have not been hydrolyzed). A higher ester value generally indicates less hydrolysis and, therefore, better oil quality and stability.

2.3.6 IODINE VALUE (IV)

Iodine value quantifies the degree of unsaturation in the oil by measuring the amount of iodine absorbed. Oils with higher iodine values contain more unsaturated fatty acids, which are often preferred for nutritional purposes. However, higher unsaturation also makes the oil more prone to oxidation and spoilage.

2.3.7 PERCENTAGE FREE FATTY ACID (%FFA)

Free fatty acid percentage indicates the level of fatty acids that have been released from triglycerides, typically due to hydrolytic degradation. A high FFA level may reduce the oil's usability in food and cosmetic applications and is often associated with poor storage or handling conditions.

2.4 BIOCHEMICAL COMPOSITION

The biochemical composition of *Irvingia gabonensis* seed oil, commonly derived from the kernels of the African bush mango, highlights its nutritional and functional value across food, pharmaceutical, and industrial applications. The seed oil is rich in several classes of biomolecules, including fatty acids, proteins, fibers, and natural antioxidants, making it a multifunctional plant-based resource.

2.4.1 LIPID PROFILE

The oil content of *Irvingia gabonensis* seeds is notably high, often forming more than half of the seed's dry weight. The oil is composed primarily of triglycerides, with a high proportion of saturated fatty acids such as lauric and myristic acids. These contribute to its semi-solid consistency at room temperature. Monounsaturated fatty acids, particularly oleic acid, are also present and add to the oil's stability and health-promoting properties. The relatively low presence of polyunsaturated fatty acids gives the oil a high oxidative resistance, extending its shelf life and making it suitable for cooking, food processing, and cosmetic formulations.

2.4.2 PROTEIN CONTENT

Although oil is the primary extractable product, the kernel also contains a significant amount of protein. After oil extraction, the residual cake still retains a moderate level of protein, which includes both essential and non-essential amino acids. These proteins may support dietary supplementation or be used in the formulation of animal feed and protein-enriched foods.

2.4.3 CARBOHYDRATES AND FIBER

The defatted seed cake also contains dietary fiber, primarily soluble fibers that contribute to the mucilaginous texture observed when the ground seed is mixed with water. These fibers are known to aid digestion, support gut health, and modulate blood sugar levels. Carbohydrates present in the seed are mostly structural or non-digestible, contributing more to functional properties than caloric value.

2.4.4 MINERAL COMPOSITION

The seeds contain trace amounts of essential minerals such as calcium, magnesium, iron, potassium, and phosphorus. These minerals are typically concentrated in the non-oil fraction and contribute to the nutritional quality of the defatted seed meal. While not abundant in the oil, some minerals may be carried into the oil fraction depending on the extraction method.

2.4.5 PHYTOCHEMICALS AND ANTIOXIDANTS

The oil contains minor yet biologically active constituents such as phytosterols, tocopherols (vitamin E), carotenoids, and phenolic compounds. These contribute antioxidant, anti-inflammatory, and lipid-lowering effects. The presence of such compounds enhances the oil's functionality in both health-promoting and preservative roles, making it a valuable ingredient in nutraceutical and cosmetic products.

2.4.6 ANTINUTRITIONAL FACTORS

In small quantities, compounds such as tannins or oxalates may also be present. However, these are usually reduced through processing steps such as drying, roasting, or oil extraction, making the final product safe for consumption and industrial use.

CHAPTER THREE

3.0 INTRODUCTION TO MATERIALS AND METHODS USED

This chapter outlines the materials, reagents, and methods employed in the extraction and analysis of oil from *Irvingia gabonensis* seeds. The approach included sample collection, oil extraction via solvent extraction, a series of physicochemical analyses and biochemical assessment. Each method adhered to standard laboratory protocols to ensure the reliability of results.

3.1 SAMPLE COLLECTION AND PREPARATION

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

The seed coats were peeled, then the Dika kernel also known as Ogbono kernel were pulverized (grounded) using a grinding machine.

3.1.1 MATERIALS USED

The materials utilized for this study included *Irvingia gabonensis* seeds, a grinding machine, 250ml and 500ml beakers, a magnetic stirrer, tissue paper, thin white rope, a UV-visible spectrophotometer, distillation apparatus, heating mantle, measuring pipette, analytical balance, Erlenmeyer flasks, volumetric flasks, micropipette, digital thermometer, mortar, pestle, and a titration setup (burette, pipette, conical flask, and retort stand).

3.1.2 REAGENTS USED

The reagents employed in this experiment were Ethanol, Ethanol:Ether (1:1), N-Hexane:Acetone (1:1), Phenolphthalein, 0.1M NaOH, Chloroform, Wij's solution, 5% KI, starch solution, Ethanolic KOH (Potassium Hydroxide), 0.5M H_2SO_4 (Sulphuric Acid), 0.1M Oxalic Acid, 0.02M $KMnO_4$ (Potassium Permanganate), Methanol, DPPH solution, Ascorbic acid, Folin-Ciocalteu Reagent (FCR), 7% Na_2CO_3 (Carbonic Acid), and 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 was 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 extraction was done in duplicate for the sample.

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. This process was carried out in two batches in which the extracted oils were labeled A and B. The differences in color, odor, and appearance observed in both extracts were recorded.

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 faint pink end point.
- The titration was performed on both extract A and B.

Acid value is calculated using the formula:

Acid Value = (M × V × MW) / W

Where:

- *M* = Molarity of NaOH,
- *V* = Volume of NaOH used in titration (ml),
- *MW* = Molecular weight of NaOH (40 g/mol),
- *W* = Weight of oil sample (g).

Percentage Free Fatty Acid is calculated as:

% FFA = (Acid value × MW of oleic acid) / (10 × MW of NaOH)

Where the molecular weight of oleic acid is 282 g/mol.

This test estimates the extent of triglyceride hydrolysis. Higher values suggest lipid degradation due to poor storage or enzyme activity.

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 $Na_2S_2O_3$ (Sodium Thiosulphate) 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 was 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.
- The titration was performed on both extract A and B

- Blank titration was performed.

The iodine value is determined using:

Iodine Value = (126.9 × (VB – VT) × M) / (10 × W)

Where:

- *126.9* = Atomic weight of iodine,
- *VB* = Volume of sodium thiosulphate used for blank,
- *VT* = Volume used for test sample,
- *M* = Molarity of sodium thiosulphate,
- *W* = Weight of sample in grams.

Iodine value indicates the level of unsaturation in the oil. A higher iodine value signifies more double bonds, suggesting higher nutritional value but also susceptibility to rancidity.

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 is calculated with:

PV = (1000 × (VT – VB) × M) / W

Where:

- *VT* = Volume of $Na_2S_2O_3$ used for titration,
- *VB* = Volume of blank,
- *M* = Molarity of sodium thiosulphate,
- *W* = Weight of oil sample in grams.

This test measures the extent of primary oxidation. A high value indicates significant lipid peroxidation and poor oxidative stability.

3.3.4 DETERMINATION OF SAPONIFICATION VALUE 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 is calculated as:

SV = (MW × (VB – VT) × M) / 0.5

Where:

- *MW* = Molecular weight of NaOH,
- *VB* and *VT* = Volume of H_2SO_4 for blank and test, respectively,
- *M* = Molarity of acid used.

This value represents the total amount of base required to saponify the fat. It helps assess the average molecular weight of the fatty acids in the oil.

Ester value is obtained by:

Ester Value = Saponification Value – Acid Value

This calculation determines the amount of esterified fatty acids, with higher values indicating greater oil stability and lower hydrolysis.

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. The experiment was conducted on both extract A and B separately.

3.4.1 ESTIMATION OF ANTIOXIDANT ACTIVITY (e.g., DPPH, TPC)

3.4.1.1 2,2- DIPHENYL-1-PICRYLHYDRAZYL (DPPH)

- 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

3.4.1.2 TOTAL PHENOLIC CONTENT (TPC)

- 0.5ml of the extracted oil was weighed and added to 5ml of Folin-Ciocalteu Reagent 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 Folin-Ciocalteu Reagent 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.

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 standardize Potassium Permanganate ($KMNO_4$). 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 standardize 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 ground 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.

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
- The process was performed on both extract A and B
- A blank solution containing 12.5ml of N- Hexane:Acetone mixture was performed
- Absorbance was measured at 453nm, 505nm, and 663nm.

CHAPTER FOUR

4.0 RESULTS

In the first batch of extraction, 221.4g of pulverized seed sample yielded 9.44g of oil while in the second batch of extraction, 118.90g of the pulverized seed sample yielded 24.36g of oil.

The weight of oil yield in both batches is first calculated, and this is used to know the % oil yield of both batches of extraction.

The results of the oil physical properties and % yield are shown in Table 1. Table 2 shows the physicochemical properties of *Irvingia gabonensis* oil extract A and Table 3 shows the physicochemical properties of *Irvingia gabonensis* oil extract B. Table 4 shows the antioxidant and anti-nutritional activity as well as the standardization of $KMNO_4$. Table 5 shows lycopene and β- carotene in oil extract A and B.

TABLE 1: % OIL AND SOME PHYSICAL PROPERTIES

Parameters	Extract A	Extract B
% Oil yield	4.26%	20.49%
Color	Dark brown	Pale yellow
Odor	Strong and appetizing	Nutty
Appearance	Slightly sticky	Liquid form

TABLE 2: PHYSICOCHEMICAL PROPERTIES OF OIL EXTRACT A

Parameters	Results
Acid value	17.02mg/NaoH/gOil
% Free fatty acid	12.40%
Iodine value	86.29mgI_2/gOil
Peroxide value	26.00mEquiv.I_2/gOil
Saponification value	84.02mg/NaoH/gOil
Ester value	66.02mgNaoH/gOil

TABLE 3: PHYSICOCHEMICAL PROPERTIES OF OIL EXTRACT B

Parameters	Results
Acid value	16.00mg/NaoH/gOil
% Free fatty acid	11.30%
Iodine value	44.16mgI_2/gOil
Peroxide value	36.00mEquiv.I_2/gOil
Saponification value	48.01mg/NaoH/gOil
Ester value	66.02mgNaoH/gOil

TABLE 4: ANTIOXIDANT AND ANTI-NUTRITIONAL ACTIVITY

Parameters	Results
% DPPH antiradical activity (control)	29.14%
% DPPH antiradical activity (standard)	15.02%
TPC	59.21mgGAEquiv/g
Standardization of $KMNO_4$	0.018M
Oxalate determination in cake	0.9992mg/g

TABLE 5: 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 DISCUSSION

The extraction and subsequent analysis of *Irvingia gabonensis* seed oil from two separate batches yielded important insights into the oil's physicochemical properties, antioxidant activity, antinutritional content, and carotenoid composition. While both batches 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 PHYSICOCHEMICAL PROPERTIES

The **acid values** recorded for both extracts—17.02 mg NaOH/g (Extract A) and 16.00 mg NaOH/g (Extract B)—were notably high. Correspondingly, the **free fatty acid (FFA)** values of 12.40% and 11.30% respectively suggest significant hydrolytic breakdown of triglycerides. This level of acidity typically implies that the oil may be undergoing deterioration, possibly due to enzymatic activity (lipase action) or prolonged exposure to moisture during post-harvest handling or storage. The elevated acidity also reduces the shelf life and palatability of the oil, indicating a need for better processing or preservation practices.

The **iodine values** differed considerably between both extracts, with Extract A showing 86.29 mg I_2/g and Extract B 44.16 mg I_2/g. This variation suggests a higher degree of unsaturation in Extract A, which may translate into greater nutritional value but also increased susceptibility to oxidation. In contrast, the lower iodine value in Extract B implies a more saturated oil profile, possibly influenced by environmental or genetic factors affecting the fatty acid composition in the seeds used.

A particularly concerning result was the **peroxide value** in Extract B, which reached 36.00 meq I_2/kg. This is an indicator of primary oxidation products, and such a high value suggests that lipid peroxidation is already in progress, compromising the oil's quality. This could be due to inadequate storage conditions such as exposure to light, air, or heat. Extract A, with a peroxide value of 26.00 meq I_2/kg, also shows signs of oxidation, though to a slightly lesser extent.

The **saponification values** were 84.02 mg NaOH/g for Extract A and 48.01 mg NaOH/g for Extract B. The relatively high saponification value in Extract A suggests a greater presence of short-chain fatty acids or triglycerides, while the significantly lower value in Extract B may indicate longer chain fatty acids or some level of degradation of the oil. These inconsistencies may also result from differences in seed maturity or processing temperature during extraction.

Interestingly, both extracts shared an identical **ester value** of 66.02 mg NaOH/g. This suggests a consistent proportion of esterified fatty acids despite the other variations, hinting at some stability in the structural composition of the oils across both batches.

4.1.2 ANTIOXIDANT AND ANTINUTRITIONAL PROPERTIES

The DPPH assay results for Irvingia gabonensis seed oil indicate moderate antioxidant activity, with the control (oil extract) exhibiting 29.14% antiradical activity and the standard (ascorbic acid) showing 15.02%. These values suggest that the oil has a reasonable capacity to scavenge free radicals, as measured by absorbance at 517 nm after a 30-minute incubation with DPPH and methanol. However, the control's 29.14% activity is relatively low compared to typical benchmarks for potent antioxidant-rich oils, such as olive oil, which often exceed 50% in similar assays. This deviation may stem from factors like seed quality, extraction inefficiencies using ethanol, or oxidative degradation, as evidenced by the high peroxide values (26.00 meq/kg for extract A and 36.00 meq/kg for extract B). These elevated peroxide levels suggest ongoing lipid peroxidation, which likely compromises the oil's antioxidant efficacy, placing it below the optimal range for high-quality edible oils.

The Total Phenolic Content (TPC) of 59.21 mg GAE/g (gallic acid equivalents per gram) indicates a moderate phenolic presence, contributing to the oil's antioxidant potential. This value, determined using the Folin-Ciocalteu reagent with absorbance at 760 nm, is comparable to some vegetable oils like sunflower oil (20–60 mg GAE/g) but lower than antioxidant-rich oils like extra virgin olive oil (100–300 mg GAE/g). The phenolic content supports the oil's potential for nutraceutical applications, but its effectiveness may be limited by the same oxidative instability indicated by the high peroxide values. The presence of phenolics, alongside carotenoids like β-carotene (0.066 mg/ml in extract A) and lycopene (0.026 mg/ml

in extract B), enhances the oil's bioactive profile, yet the observed degradation suggests that improper storage or processing conditions may have reduced the phenolic compounds' stability, deviating from ideal standards for functional oils.

The oxalate content in the defatted seed cake (0.9992 mg/g, measured by titration with 0.018 M KMnO4) is low, indicating minimal anti-nutritional factors that could affect safety or bioavailability. However, the high peroxide values suggest that oxidative damage may have degraded these bioactive compounds, reducing their effectiveness compared to fresh oils, which typically maintain peroxide values below 10 meq/kg. Improved post-harvest handling, such as controlled storage to minimize exposure to light, heat, or air, is critical to preserving the oil's antioxidant properties and aligning its DPPH and TPC results with industry standards for high-quality plant-based oils.

4.1.3 CAROTENOID COMPOSITION

The carotenoid analysis further complements the antioxidant profile, with extract A showing higher β-carotene (0.066 mg/ml) and extract B richer in lycopene (0.026 mg/ml), quantified via spectrophotometry at 453 nm, 505 nm, and 663 nm. These carotenoids contribute to the oil's antioxidant and nutritional value, with β-carotene serving as a vitamin A precursor and a known antioxidant, and lycopene a compound recognized for its role in reducing oxidative stress, offering potential health benefits.

4.1.4 ABOVE-STANDARD OR DEVIANT RESULTS

- The **acid and FFA values** were significantly above what is generally considered acceptable for edible oils. This suggests that hydrolytic degradation may have occurred, possibly due to improper storage or delayed processing after seed harvest.

- The **peroxide value** in Extract B was also higher than ideal, indicating advanced oxidation. This may have been exacerbated by exposure to air or light during extraction or storage.
- On the positive side, the **antioxidant activity** and **phenolic content** were notably high, which could compensate for the oxidative instability of the oil in certain applications. This might be attributed to the naturally rich phytochemical profile of the seed.

4.1.5 POSSIBLE CAUSES OF DEVIATIONS

1. **Storage Conditions**: Prolonged exposure to air, humidity, or light prior to extraction likely led to increased hydrolysis and oxidation.
2. **Delay in Processing**: A time gap between seed harvesting and oil extraction could have allowed enzymatic activity to break down triglycerides.
3. **Environmental Influence**: Variation in soil, climate, or seed origin could account for the differences in iodine, carotenoid, and saponification values.
4. **Extraction Method**: If inconsistent temperature or solvent concentration was applied across the batches, it could affect the oil yield and quality.

CONCLUSION

In conclusion, the extraction and analysis of *Irvingia gabonensis* seed oil revealed that the oil possesses valuable bioactive properties, including strong antioxidant activity and notable levels of carotenoids such as lycopene and β-carotene. However, the elevated acid, free fatty acid, and peroxide values observed in both oil extracts indicate significant hydrolytic and oxidative degradation, likely due to poor post-harvest handling or inadequate storage conditions. Despite these challenges, the oil's rich phenolic content and favorable biochemical profile highlight its potential for nutritional and industrial applications, provided that proper processing and preservation methods are implemented to enhance its stability and quality.

APPENDIX

Calculations showing the oil % yield. Parameters given are as follows

- For extract A

Weight of pulverized seed (before extraction) W1 = 224.40g

Weight of beaker only W2 = 50.85g

Weight of beaker & oil extract (after solvent extraction) W3 = 60.29g

Weight of oil extract only W4 = W3 – W2 = 60.29g – 50.85g = 9.44g.

Therefore, 221.4g of grounded seed yield 9.44g of oil.

Percentage oil yield = W4/W1 x 100 = weight of oil / weight of sample x 100

$\frac{9.44g}{224.40g} \times 100 = 4.26\%$

- For extract B

Weight of pulverized seed (before extraction) W1 = 118.90g

Weight of beaker only W2 = 99.90g

Weight of beaker & oil extract W3 = 123.26g

Weight of oil extract only W4 = W3 – W2 = 123.26g – 99.90g = 24.36g

Therefore, 118.90g of grounded seed yield 24.36g of oil

Percentage oil yield = W4/W1 x100 = weight of oil / weight of sample x 100

$\frac{24.36}{118.90} \times 100 = 20.49\%$

Raw Data For extract A

A. 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 = 2.15ml
- *M NaOH* = molarity of NaOH used = 0.1M
- *Sample weight = 0.5g*

Acid value = $\frac{40.01 \times 2.15 \times 0.1}{0.5}$ = 17.20mg/NaOH/gOil

2. % Free Fatty Acid:

% 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 = 282.4g/mol

%FFA = $\frac{17.20 \times 282}{10 \times 40.01}$ = 12.40%

3. Saponification Value (SV)

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 = 20.90ml

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

SV = $\frac{40.01 \times (23.00 - 21.50) \times 0.05}{0.5}$ = 84.02mgNaOH/gOil

4. Ester value = Saponification value – Acid value

84.02 – 17.20 = 66.82mgNaOH/gOil

5. Iodine Value (IV)

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

Where:

- V_B = volume of blank = 238.90ml
- V_T = volume of test = 204.50ml
- Molarity of thiosulphate = 0.1M
- Weight of sample = 0.5g

IV = $\frac{126.90 \times (238.90 - 204.90) \times 0.1}{10 \times 0.5}$ = 86.29mEquivI_2/gOil

6. Peroxide Value (PV)

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

Where:

- V_B = volume of blank = 4.90ml
- V_T = volume of test = 6.20ml

- M $Na_2S_2O_3$ = molarity of sodium thiosulphate = 0.01M
- Weight of sample = 0.5g

PV = $\frac{1000 \times (6.20 - 4.90) \times 0.01}{0.5}$ = 26mEquivI_2/gOil

Raw Data on Extract B

A. Calculations of Physicochemical Properties

1. **Acid Value (AV)**

Formula:

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 = 2.00ml
- *M NaOH* = molarity of NaOH used = 0.1M
- *Sample weight = 0.5g*

Acid value = $\frac{40.01 \times 2.00 \times 0.1}{0.5}$ = 16.004mg/NaOH/gOil

2. % Free Fatty Acid:

% 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 = 16.004
- M = Molecular weight of oleic acid = 282.4g/mol

$$\%FFA = \frac{16.004 \times 282}{10 \times 40.01} = 11.30\%$$

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 = 21.800ml
- M H_2SO4 = molarity Of Sulfuric acid = 0.05M
- Weight of sample = 0.5g
- Molecular weight of NaOH = 40.01g/mol

$$SV = \frac{40.01 \times (23.00 - 21.80) \times 0.05}{0.5} = 48.01 \text{mgNaOH/gOil}$$

4. Ester value = Saponification value – Acid value

48.01 – 16.004 = 32.006mgNaOH/gOil

5. Iodine Value (IV)

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

Where:

- V_B = volume of blank = 238.90ml

- V_T = volume of test = 221.50ml
- Molarity of thiosulphate = 0.1M
- Weight of sample = 0.5g

IV = $\frac{126.90 \times (238.90 - 221.50) \times 0.1}{10 \times 0.5}$ = 44.16mEquivI_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 = 4.90ml
- V_T = volume of test = 6.70ml
- M $Na_2S_2O_3$ = molarity of sodium thiosulphate = 0.01M
- Weight of sample = 0.5g

PV = $\frac{1000 \times (6.70 - 4.90) \times 0.01}{0.5}$ = 36mEquivI_2/gOil

7. Carotenoid content calculation

For Extract A

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

Where:

A663 = absorbance at 663nm = 0.308nm

A505 = absorbance at 505nm = -0.169nm

A453 = absorbance at 453nm = 0.227nm

β-carotene = $\frac{0.126\ (0.308) - 0.304\ (-0.169) + 0.450\ (0.227)}{10}$ = 0.066mg/ml

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

Where:

A663 = absorbance at 663nm = 0.308nm

A505 = absorbance at 505nm = -0.169nm

A453 = absorbance at 453nm = 0.227nm

Lycopene = $\frac{0.458\ (0.308) - 0.372\ (-0.169) - 0.0806\ (0.227)}{10}$ = 0.0186mg/ml

For Extract B

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

Where:

A663 = absorbance at 663nm = 0.358nm

A505 = absorbance at 505nm = -0.183nm

A453 = absorbance at 453nm = 0.324nm

β-carotene = $\frac{0.126\ (0.358) - 0.304\ (-0.183) + 0.450\ (0.324)}{10}$ = 0.025mg/ml

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

Where:

A663 = absorbance at 663nm = 0.358nm

A505 = absorbance at 505nm = -0.183nm

A453 = absorbance at 453nm = 0.324nm

Lycopene = $\frac{0.458\ (0.358) - 0.372\ (-0.183) - 0.0806\ (0.324)}{10}$ = 0.026mg/ml

8. DPPH Radical Scavenging Activity

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

Where:

$Ab_{control}$ = absorbance of control = 1.805

Aa = absorbance of sample = 1.279

% DPPH antiradical activity = $\frac{(1.805 - 1.279)}{1.805}$ x 100 = 29.14%

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

Where:

$Ab_{standard}$ = absorbance of standard = 1.505

Aa = absorbance of sample = 1.279

% DPPH antiradical activity (standard) = $\frac{(1.505 - 1.279)}{1.505}$ x 100

= 15.02%

9. Total Phenolic Content (TPC)

TPC (mg GAE/g) = C×VM

- V = volume of extract used = 0.5ml
- M = mass of sample = 1.14g
- C = concentration from standard curve = 135

TPC= 135 x $\frac{0.5}{1.14}$ = 59.21mgEquiv/g

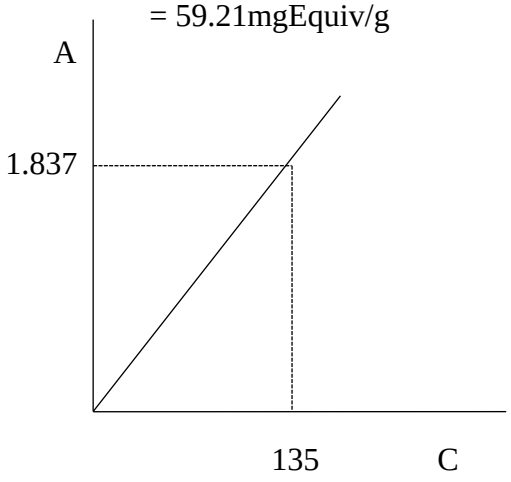

Fig 1. Calibration curve showing Absorbance against Concentration of TPC (1.837 is the absorbance average of sample, while 135 is the concentration obtained from standard curve)

REFERENCES

Akinoso, R., Aremu, A. K., & Balogun, S. A. (2010). *Effect of moisture content on yield and quality of oil from dika nut. Journal of Food Process Engineering, 33*(2), 273–287.

Codex Alimentarius. (1999). *Standard for Named Vegetable Oils (CODEX-STAN 210-1999). Food and Agriculture Organization/World Health Organization.*

Chemical and Amino Acid Composition of Raw and Defatted African Mango Kernel (2014). *Biotechnology Journal International, 4(3), 244–253. reddit.com+15journalbji.com+15isca.in+15*.

Chemical composition and industrial benefits of dikanut (irvingia gabonensis) kernel oil: A review, *Nutrition &Food science (2017), Vol. 47 Issue: 5, pp.741-751, https://doi.org/10.1108/NFS-03-2017-0054*

Ekpa, O. D., & Ekpe, U. J. (1995). *The effects of refining on the physicochemical properties of dika nut* (*Irvingia gabonensis*) oil. *Plant Foods for Human Nutrition*, *48*(3), 209–215.

Ejiofor, M. A. N., Onwubuke, S. C., & Okafor, J. C. (1996). *Developing improved methods of processing and utilization of kernels oils*. *Irvingia gabonensis*. *International Tree Crops Journal*, *9*(2), 109–117.

Effect of Rural Processing on Proximate & Amino Acid Profile of *Irvingia gabonensis* Seeds (Date n.d.). *African Journal of Food, Agriculture, Nutrition and Development*. ajol.info+12ajol.info+12journalbji.com+12

Extraction & Physicochemical Composition of *Irvingia gabonensis* Almond Oil (2021). *MDPI Separations*, 9(8), 207. mdpi.com

GC-MS Analysis of Fatty Acids of *Irvingia gabonensis* Seed Fat (1997). *Nigerian Journal of Natural Products and Medicine*, 1, 43–44. ajol.info.

Irvingia gabonensis Fat: Nutritional Properties & Effects on Growth & Lipid Metabolism in Rats (2011). *Lipids in Health and Disease*, 10, 43. en.wikipedia.org+12lipidworld.biomedcentral.com+12mskcc.org+12

Leakey, R. R. B., Tchoundjeu, Z., Schreckenberg, K., Shackleton, S. E., & Shackleton, C. M. (2005). *Agroforestry tree products (AFTPs): targeting poverty reduction and enhanced livelihoods. International Journal of Agricultural Sustainability*, *3*(1), 1–23.

Lipid Profile of Oils from *Irvingia gabonensis* and Deterioration by Fungal Attack (2019). *Nigerian Journal of Biotechnology*, 36(1). ajol.i-+ nfo.

Lowe, A. J., Gillies, A. C. M., Wilson, J., & Dawson, I. K. (2000). *Conservation genetics of bush mango (*Irvingia gabonensis*): implications from RAPD analysis*. *Molecular Ecology*, *9*(6), 831–841.

Metal Analysis & Fuel Potential of *Irvingia gabonensis* (2024). *Journal of Biological Research and Biotechnology*, 20(3), 1712–1720. bio-research.com.ng

Ndjouenkeu, R., Goycoolea, F. M., Morris, E. R., & Akingbala, J. O. (1996). *Rheology of okra and dika nut polysaccharides. Carbohydrate Polymers*, *29*(3), 263–269.

Ngondi, J. L., Oben, J. E., & Minka, S. R. (2005). *The effect of* Irvingia gabonensis *seeds on body weight and blood lipids of obese subjects in Cameroon. Lipids in Health and Disease*, *4*(1), 12.

Okolo, C. C., Udofia, U. S., & Orukotan, A. A. (2020). *Biochemical characterization of* Irvingia gabonensis *seed oil: Prospects for industrial applications. Journal of Food Biochemistry*, *44*(5), e13190.

Omoti, U., & Okiy, D. A. (1987). *Characteristics and composition of the pulp oil and cake of Irvingia gabonensis*. *Journal of the Science of Food and Agriculture*, *38*(1), 67–72.

Physicochemical Properties & Fatty Acid Composition of Dikanut Seed Oil (2014). *Research Journal of Chemical Sciences*, 4(12), 70–74. isca.in

Uhegbu, F. O., Omodamiro, R. M., & Okechi, R. N. (2009). *Effects of aqueous extracts of Irvingia gabonensis seeds on blood glucose and serum lipid profile of albino rats. Biokemistri*, *21*(2), 85–90.