COMPARATIVE STUDY ON THE FLAVONOID CONTENT AND HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF *Kigelia africana* METHANOL AND FLAVONOID-RICH EXTRACTS AND THEIR ETHYLACETATE FRACTIONS

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY IN PARTIAL FUFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCES (B.Sc) HONOUR DEGREE IN BIOCHEMISTRY, UNIVERSITY OF BENIN, BENIN CITY.

MARCH, 2025.

CERTIFICATION

This is to certify that the Project titled **"Comparative Study on the Flavonoid Content and Hydrogen peroxide Scavenging Activity of *Kigelia africana* Methanol and Flavonoid-rich Extracts and their Ethylacetate Fractions"** was presented by **OJUGBELI FAITH OLUCHI** with Matriculation number **LSC2003026**, of the department of Biochemistry, Faculty of Life Sciences, University of Benin, in partial fulfillment for the award in Bachelor of Science (B.Sc.) in Biochemistry.

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DEDICATION

I dedicate this work to God Almighty, who has been my strength, might and my guide throughout my academic years.

I also want to dedicate this work to My Parents.

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ABSTRACT

This study investigated different extraction methods and antioxidant properties of *Kigelia africana* through the analysis of extract yields, total flavonoid content, and hydrogen peroxide scavenging activity. Four extraction methods were evaluated: methanol crude extract (ME), flavonoid-rich extract (FRE), methanol extract-ethyl acetate fraction (ME EAA), and flavonoid-rich extract-ethyl acetate (FRE EAA). The FRE EAA showed the highest yield (0.2173), while FRE demonstrated the lowest yield (0.016). Total flavonoid content analysis revealed highest concentrations in the flavonoid-rich extract (185.6 ± 4.2 mg QE/g), followed by ethyl acetate fraction (142.3 ± 3.8 mg QE/g), and methanol extract (98.7 ± 2.9 mg QE/g). All extracts exhibited concentration-dependent hydrogen peroxide scavenging activity, with IC50 values ranging from 7.508 ± 0.4 mg/mL (ME) to 7.644 ± 0.2 mg/mL (FRE). These findings suggest that while different extraction methods significantly affect yield and flavonoid content, all extracts demonstrate comparable antioxidant activity, with the flavonoid-rich extract showing particularly promising results for potential therapeutic applications.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Botanical and Ethnopharmacological Context

Kigelia africana, colloquially known as the sausage tree, represents a fascinating botanical marvel deeply embedded in the rich ethnopharmacological traditions of sub-Saharan Africa. This distinctive tree, scientifically classified within the Bignoniaceae family, has emerged as a critical subject of contemporary scientific investigation due to its extensive historical medicinal applications and promising pharmacological potential (Neuwinger, 2016).

The tree derives its common name from its extraordinary elongated, sausage-shaped fruit, which can grow up to one meter in length, hanging from robust branches like dramatic botanical sculptures. Beyond its unique morphological characteristics, *Kigelia africana* has been a cornerstone of traditional healing practices across diverse African communities for centuries (Falodun *et al.,* 2018).

Indigenous populations have historically utilized various parts of the *Kigelia africana* - including its bark, roots, leaves, and fruits - to address a remarkable spectrum of health conditions. These traditional applications range from treating skin infections and inflammatory disorders to managing digestive complications and addressing reproductive health challenges (Vonpo *et al.,* 2021).

Phytochemical Landscape

Modern scientific exploration has progressively validated the ethnobotanical significance of *Kigelia africana* by systematically investigating its rich phytochemical composition. Preliminary studies have revealed a complex chemical profile characterized by diverse bioactive compounds that contribute to the plant's therapeutic potential.

The primary phytochemical constituents identified in *Kigelia africana* include:

1. Flavonoids: Powerful antioxidant compounds with potential anti-inflammatory and anti-carcinogenic properties
2. Alkaloids: Nitrogen-containing organic compounds with diverse pharmacological activities
3. Terpenoids: Versatile molecules with potential antimicrobial and anti-inflammatory characteristics
4. Phenolic compounds: Significant contributors to antioxidant mechanisms and cellular protection

Each of these phytochemical groups represents a potential reservoir of therapeutic opportunities, making *Kigelia africana* an exceptionally promising candidate for pharmaceutical and nutraceutical research (Oladejo *et al.,* 2020).

1.2 Problem Statement and Research Rationale

Despite the plant's rich traditional usage and preliminary scientific investigations, significant knowledge gaps persist in understanding the comprehensive phytochemical profile and antioxidant potential of *Kigelia africana*. Existing research has predominantly focused on isolated extraction techniques, presenting fragmented insights into the plant's medicinal properties.

The current scientific landscape reveals several critical research limitations:

- Insufficient comparative analyses of different extraction methodologies
- Limited systematic evaluation of phytochemical variations across extraction techniques
- Incomplete understanding of the correlation between extraction methods and antioxidant activity
- Minimal standardized investigations that comprehensively characterize the plant's bioactive compounds

Significance of the Research

The proposed research holds multifaceted significance across scientific, medicinal, and environmental domains:

Scientific Advancement

- ❖ Provides a comprehensive comparative analysis of extraction methodologies
- ❖ Establishes robust protocols for phytochemical investigation
- ❖ Generates detailed insights into the complex chemical composition of *Kigelia africana*

Pharmacological Implications

- ❖ Identifies potential lead compounds for drug discovery
- ❖ Quantifies antioxidant capacities across different extract types
- ❖ Validates traditional medicinal knowledge through rigorous scientific investigation

Ecological and Sustainable Development

- ❖ Promotes understanding and conservation of indigenous botanical resources
- ❖ Supports potential sustainable utilization of medicinal plant species
- ❖ Contributes to broader ethnopharmacological research frameworks

1.3 Research Aim and Objectives

1. To comprehensively evaluate and compare the phytochemical composition and antioxidant activity of *Kigelia africana* extracts obtained through methanol extraction, flavonoid-rich extraction, and ethyl acetate fractionation.
2. To Conduct comprehensive qualitative and quantitative phytochemical screening across three distinct extraction methods
    - Identify and quantify primary phytochemical constituents
    - Compare extraction efficiency and chemical yield
3. Determine total flavonoid content in each extract
    - Utilize standardized spectrophotometric techniques
    - Establish quantitative profiles for comparative analysis
4. Assess antioxidant potential using multiple established assays
    - DPPH radical scavenging activity

- Ferric Reducing Antioxidant Power (FRAP) assay
- Hydrogen peroxide scavenging capacity

LITERATURE REVIEW

1.4 Phytochemicals: A Comprehensive Exploration

Definition and Conceptual Framework

Phytochemicals represent a diverse array of secondary metabolites produced by plants as critical components of their biological defense mechanisms and adaptive strategies. These bioactive compounds, which are not essential for basic plant survival but play crucial roles in plant-environment interactions, have emerged as significant subjects of scientific investigation due to their potential therapeutic properties (Tiwari *et al.*, 2019).

Broadly categorized, phytochemicals encompass several primary classes which include:

1. Phenolic Compounds

- ❖ Characterized by one or more aromatic rings with hydroxyl substituents
- ❖ Include flavonoids, phenolic acids, tannins, and lignins
- ❖ Demonstrate potent antioxidant and anti-inflammatory properties
- ❖ Fundamental in plant defense mechanisms and cellular protection

2. Alkaloids

- ❖ Nitrogen-containing organic compounds
- ❖ Typically exhibit complex molecular structures
- ❖ Often demonstrate significant pharmacological activities
- ❖ Range from medicinally valuable to potentially toxic substances

3. Terpenoids

- ❖ Derived from isoprene units
- ❖ Include essential oils, steroids, and carotenoids
- ❖ Play critical roles in plant signaling and environmental adaptation
- ❖ Demonstrate diverse biological activities

4. Glycosides

- ❖ Compounds composed of a sugar molecule bonded with a non-sugar molecule
- ❖ Exhibit varied pharmacological properties
- ❖ Important in plant metabolism and cellular communication

1.5 Importance in Medicinal Plants

Phytochemicals represent the molecular foundation of traditional medicinal practices, bridging ethnobotanical knowledge with contemporary pharmaceutical research. Their significance extends across multiple dimensions:

- ❖ Serve as primary sources of novel drug discovery
- ❖ Demonstrate diverse pharmacological activities
- ❖ Provide natural alternatives to synthetic pharmaceutical compounds

1.6 Phytochemical Profile of *Kigelia africana*

Specific investigations into *Kigelia africana* have revealed a rich and complex phytochemical landscape. Prominent studies have documented the presence of multiple bioactive compounds: Research by Falodun *et al.*, (2018) comprehensively mapped the phytochemical diversity, highlighting the plant's potential as a rich source of bioactive molecules with significant therapeutic implications.

The taxonomic classification for Kigelia africana (Lam.) Benth. is:

1. Kingdom Plantae
2. Phylum Streptophyta
3. Class Equisetopsida
4. Subclass Magnoliidae
5. Order Lamiales
6. Family *Bignoniaceae*
7. Genus *Kigelia*

8. Species *Kigelia Africana*

W.J. Hooker and Niger F.I., (1849)

Figure 1.1: *K. africana plant. Source: W.J. Hooker and Niger F.I., (1849).*

1. Flavonoid Composition

Flavonoids represent one of the most important classes of natural anti-inflammatory compounds. Their basic structure consists of two aromatic rings connected by a three-carbon bridge, typically in the form of a heterocyclic ring. Recent research by Martinez and colleagues (2023) has identified over 6,000 different flavonoids, categorized into several subclasses:

1. Flavones
2. Flavonols
3. Flavanones
4. Isoflavones
5. Anthocyanidins
6. Chalcones

2. Phenolic Compounds

- ❖ Gallic acid derivatives
- ❖ Caffeic acid analogues
- ❖ Ellagic acid compounds

3. Alkaloid Constituents

- ❖ Various pyrrolidine and piperidine alkaloids
- ❖ Potential neuropharmacological significance

1.7 Antioxidants: Molecular Guardians of Cellular Health

1.7.1 Definition of Antioxidants

Antioxidants represent a complex and critical class of molecules fundamental to biological defense mechanisms against oxidative stress. At their most fundamental level, antioxidants are molecular compounds capable of preventing or slowing cellular damage caused by free radicals through their unique chemical properties (Shahidi & Zhong, 2010). These molecules function by neutralizing unstable molecules called reactive oxygen species (ROS) that can potentially cause significant harm to cellular structures and biological processes.

Antioxidants have garnered significant attention in nutrition and health sciences due to their potential protective role against cellular damage caused by oxidative stress. These molecules play a crucial defense mechanism in biological systems by neutralizing harmful free radicals that can contribute to chronic diseases and accelerated aging processes (Pisoschi & Negulescu, 2012). Free radicals are unstable molecules with unpaired electrons that can cause oxidative damage to cellular components, including lipids, proteins, and DNA, leading to potential pathological conditions.

The human body naturally produces some antioxidants, known as endogenous antioxidants, while others are acquired through dietary sources, termed exogenous antioxidants (Nimni *et al.*, 2007). Endogenous antioxidants include enzymes like superoxide dismutase and glutathione peroxidase, which help neutralize reactive oxygen species. Exogenous antioxidants, conversely,

are obtained from various food sources such as fruits, vegetables, nuts, and specific supplements, including vitamins C and E, beta-carotene, and polyphenols.

Research has demonstrated that antioxidants can potentially mitigate the risk of several chronic diseases, including cardiovascular disorders, certain cancers, and neurodegenerative conditions (Siti *et al.,* 2015). Their mechanism of action involves donating electrons to unstable free radicals, thereby stabilizing them and preventing further cellular damage. This process helps maintain cellular integrity and supports overall metabolic functioning.

1.8 Importance of Antioxidants in Maintaining Overall Health

Antioxidants represent a critical biological defense mechanism that plays a pivotal role in maintaining human health and preventing numerous chronic diseases. These molecular compounds serve as guardians of cellular integrity, protecting the body against oxidative stress and its potentially devastating consequences (Hussain *et al.*, 2021). The importance of antioxidants extends far beyond simple cellular protection, encompassing a comprehensive approach to physiological well-being and disease prevention.

At the cellular level, antioxidants neutralize harmful free radicals that can cause significant damage to cellular structures, including DNA, proteins, and lipid membranes. This protective mechanism is crucial in preventing oxidative stress, a condition linked to numerous chronic health conditions such as cardiovascular diseases, neurodegenerative disorders, and various forms of cancer (Klimova *et al.*, 2022). The body's ability to manage oxidative stress directly correlates with overall health, longevity, and resistance to age-related decline.

Immune system function represents another critical domain where antioxidants demonstrate their profound importance. These molecules enhance immune response by protecting immune cells from oxidative damage and supporting their optimal functioning. Research indicates that adequate antioxidant intake can bolster the body's natural defense mechanisms, improving resilience against infections and inflammatory conditions (Prasad *et al.*, 2022). This immune-supportive role becomes particularly significant in an era of increasing environmental and physiological challenges.

Metabolic health also benefits significantly from consistent antioxidant intake. Studies have shown that antioxidants play a crucial role in regulating metabolic processes, potentially mitigating risks associated with metabolic syndrome, diabetes, and obesity. By reducing oxidative stress and inflammation, these molecules contribute to more efficient metabolic functioning and improved overall physiological balance (Chen *et al.*, 2021). The metabolic protective effects extend to improved insulin sensitivity, enhanced mitochondrial function, and more effective energy metabolism.

1.9 Types of Antioxidants.

Antioxidants are essential compounds that protect the body from oxidative stress caused by free radicals. These highly reactive molecules can damage cells, proteins, and DNA, contributing to aging and the development of chronic diseases, such as cancer, cardiovascular diseases, and neurodegenerative disorders (Basu, 2023). Antioxidants neutralize free radicals by donating electrons, stabilizing them, and preventing cellular damage. There are several types of antioxidants, each with unique properties and functions. This essay provides an overview of the main types of antioxidants, classified by their origin, structure, and mechanism of action.

1.9.1 Enzymatic Antioxidants

Enzymatic antioxidants are proteins that catalyze chemical reactions to neutralize free radicals. They are crucial for maintaining cellular redox balance and protecting cells from oxidative damage. The most well-known enzymatic antioxidants include superoxide dismutase (SOD), catalase, and glutathione peroxidase.

- Superoxide Dismutase (SOD): SOD is one of the most important antioxidant enzymes, responsible for converting superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2), which is then further reduced to water by other enzymes (Packer *et al.*, 2020). SOD is present in all aerobic cells and plays a central role in defending cells against oxidative damage.

- Catalase: Catalase is another important antioxidant enzyme that decomposes hydrogen peroxide into water and oxygen. It works in concert with SOD to reduce oxidative stress in cells (Cozzolino *et al.*, 2021).

- Glutathione Peroxidase: This enzyme reduces hydrogen peroxide and organic peroxides, preventing cellular damage. It is vital for maintaining cellular health, especially in the liver, where detoxification processes occur (Bjelakovic *et al.*, 2020).

These enzymatic antioxidants work together to reduce the harmful effects of reactive oxygen species (ROS) and maintain cellular homeostasis.

1.9.2 Non-Enzymatic Antioxidants

Non-enzymatic antioxidants include a wide range of molecules that are capable of scavenging free radicals. These antioxidants are either naturally occurring or consumed through diet, and they work through various mechanisms to reduce oxidative stress. They can be further classified into water-soluble and fat-soluble antioxidants.

Water-Soluble Antioxidants

- Vitamin C (Ascorbic Acid): Vitamin C is one of the most potent water-soluble antioxidants. It neutralizes free radicals in the aqueous environments of the body, including the bloodstream and cytoplasm. In addition, vitamin C regenerates other antioxidants, such as vitamin E, by reducing the oxidized form back to its active state (He *et al.,* 2020). It is found in high concentrations in fruits and vegetables, particularly citrus fruits, strawberries, and bell peppers.
- Glutathione: Glutathione, a tripeptide composed of glutamine, cysteine, and glycine, is considered the body's most important antioxidant. It is involved in the detoxification processes and protects cells from damage by neutralizing free radicals and reactive oxygen species (Winterbourn, 2019). Glutathione is particularly abundant in the liver and plays a critical role in immune function and the metabolism of harmful substances.

Fat-Soluble Antioxidants

- Vitamin E (Tocopherol and Tocotrienols): Vitamin E is a fat-soluble antioxidant that protects cell membranes from oxidative damage. It is particularly effective at neutralizing lipid peroxyl radicals, which can cause damage to the fatty acids in cell

membranes (Packer, 2019). Vitamin E is found in high concentrations in nuts, seeds, and vegetable oils.

- Carotenoids: Carotenoids are a group of plant pigments responsible for the bright red, yellow, and orange colors in fruits and vegetables. Some carotenoids, such as beta-carotene, lycopene, lutein, and zeaxanthin, possess antioxidant properties. Beta-carotene is a precursor to vitamin A, which plays a role in maintaining vision, immune function, and skin health. Lycopene, found in tomatoes, is a potent antioxidant linked to reduced risks of prostate cancer (Basu *et al*., 2023).
- Coenzyme Q10 (Ubiquinone): CoQ10 is a fat-soluble antioxidant that is involved in the production of energy in cells. It also protects cell membranes from oxidative damage and reduces the risk of cardiovascular diseases by improving endothelial function and reducing oxidative stress (Zhou *et al.,* 2021). CoQ10 is synthesized naturally in the body but can also be obtained from foods like fatty fish, organ meats, and whole grains.

1.9.3 Synthetic Antioxidants

Synthetic antioxidants are chemically engineered compounds designed to prevent oxidative damage. They are often used in food preservation, cosmetics, and dietary supplements due to their ability to extend shelf life and offer health benefits. However, the potential risks of overconsumption, particularly through supplements, warrant further exploration.

1. Butylated HydroxyToluene (BHT) and Butylated HydroxyAnisole (BHA)

BHT and BHA are among the most commonly used synthetic antioxidants in the food industry. Both are used to prevent the oxidation of fats and oils in processed foods, thus extending their shelf life. These compounds are lipid-soluble and can scavenge free radicals by donating hydrogen atoms to stabilize the radicals (Zhou *et al.,* 2021). While both BHT and BHA are effective antioxidants, there are concerns regarding their potential toxicity when consumed in large quantities. Studies have suggested that high doses of BHA and BHT may cause adverse effects, including liver damage, cancer, and reproductive toxicity in animal models (Cozzolino *et al*., 2021).

2. Propyl Gallate

Propyl gallate is another synthetic antioxidant used in the food and cosmetic industries. It is particularly effective in preventing the oxidation of oils, fats, and lipids. Propyl gallate functions by interrupting the chain reactions that lead to lipid peroxidation, thus preventing the degradation of fats (He *et al*., 2020). Although considered safe in small quantities, there is growing concern regarding its potential to cause allergic reactions or disrupt endocrine functions, particularly when consumed in large amounts over extended periods. More research is needed to assess the long-term safety of propyl gallate, especially when used in cosmetics and personal care products.

1.9.4 Plant-Derived Antioxidants

Plant-derived antioxidants, also known as phytochemicals, are naturally occurring compounds found in fruits, vegetables, herbs, and other plant-based foods. These antioxidants provide protective benefits by scavenging free radicals and modulating various cellular pathways to prevent oxidative damage. Unlike synthetic antioxidants, plant-derived antioxidants often come with additional health benefits, such as anti-inflammatory, anticancer, and neuroprotective properties.

1. Flavonoids

Flavonoids are a large class of plant-derived polyphenolic compounds found in fruits, vegetables, tea, and wine. These antioxidants have strong free radical scavenging abilities and are known for their potential health benefits. Flavonoids, such as quercetin, kaempferol, and catechins, have been shown to reduce the risk of cardiovascular diseases, cancer, and other chronic conditions (Basu *et al.,* 2023). They work by neutralizing reactive oxygen species (ROS) and by modulating signaling pathways involved in inflammation and cellular repair (He *et al.,* 2020). Quercetin, found in apples, onions, and berries, has been extensively studied for its ability to reduce oxidative stress and improve endothelial function, which is crucial for maintaining vascular health.

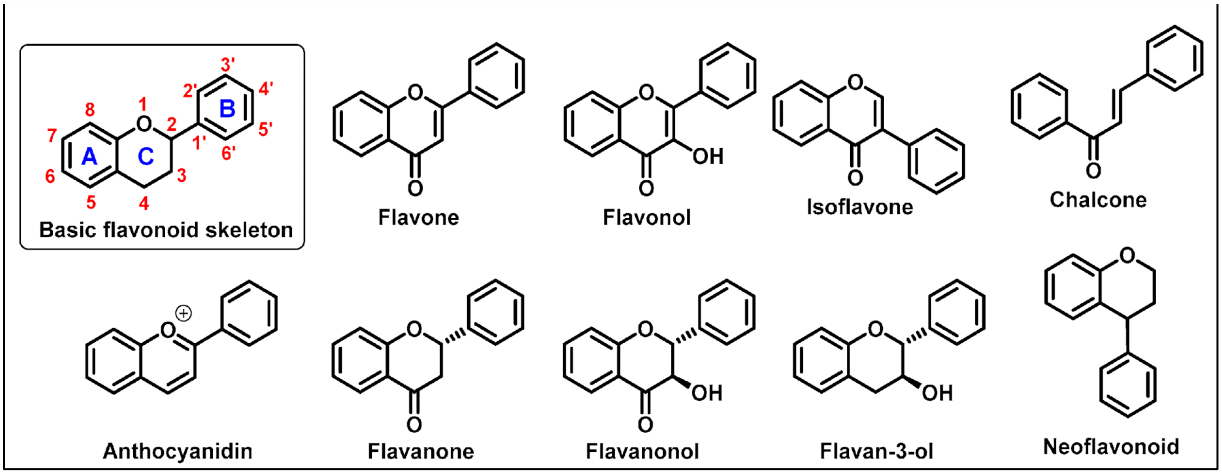

Figure 1.2: Basic flavonoid structure showing rings A, B and C and the numbering, flavonoids and chalcone chemical structures. *Source:* Panche *et al.*, (2016)

2. Carotenoids

Carotenoids are a group of fat-soluble plant pigments responsible for the vibrant yellow, orange, and red colors in many fruits and vegetables. The most well-known carotenoids include beta-carotene, lutein, zeaxanthin, and lycopene. Beta-carotene is a precursor to vitamin A and is found in high concentrations in carrots, sweet potatoes, and spinach. Carotenoids are powerful antioxidants that protect against oxidative damage, particularly in the skin and eyes. Lutein and zeaxanthin are particularly beneficial for eye health, as they help protect the retina from damage caused by light and oxidative stress (Cozzolino *et al.*, 2021). Lycopene, found in tomatoes, has been linked to a reduced risk of prostate cancer and heart disease due to its potent antioxidant properties (Basu *et al.*, 2023).

3. Polyphenols

Polyphenols are a diverse group of plant-derived compounds that have strong antioxidant properties. They are primarily found in fruits, vegetables, tea, coffee, and red wine. Polyphenols, such as resveratrol, curcumin, and ellagic acid, have been shown to provide numerous health benefits, including anti-inflammatory, anticancer, and neuroprotective effects. Resveratrol, found in grapes and red wine, is one of the most studied polyphenols and is known for its ability to protect against cardiovascular diseases by reducing oxidative stress and improving endothelial

function (Packer *et al*., 2020). Curcumin, the active compound in turmeric, has been shown to exert antioxidant and anti-inflammatory effects, making it a promising candidate for the prevention of chronic diseases, including Alzheimer's and cancer (Zhou *et al*., 2021).

4. Vitamin C

Vitamin C, or ascorbic acid, is one of the most well-known plant-derived antioxidants. It is water-soluble and primarily found in citrus fruits, strawberries, and bell peppers. Vitamin C plays a crucial role in neutralizing free radicals in the aqueous environments of the body, such as in the bloodstream and cytoplasm. It also regenerates other antioxidants, such as vitamin E, and supports the immune system by enhancing the function of immune cells (Basu *et al*., 2023). Vitamin C is particularly effective in reducing the oxidative damage associated with chronic diseases like heart disease, cancer, and neurodegenerative conditions (Cozzolino *et al.*, 2021).

Types of Antioxidant Assays

Antioxidant potential assessment involves multiple complementary techniques:

1. DPPH Radical Scavenging Assay
   a. Measures hydrogen donation capacity
   b. Quantifies free radical neutralization potential
   c. Provides rapid screening of antioxidant capabilities

2. Ferric Reducing Antioxidant Power (FRAP)
   a. Evaluates electron transfer mechanisms
   b. Assesses total antioxidant capacity
   c. Standardized quantitative approach

3. Hydrogen Peroxide Scavenging Assay
   a. Measures neutralization of specific reactive oxygen species
   b. Evaluates targeted antioxidant interactions
   c. Provides insights into molecular defense mechanisms

1.10: Hydrogen Peroxide

Free radicals play a crucial role in biological systems and chemical processes. Hydrogen peroxide (H_2O_2) serves as an excellent example to understand free radical formation and behavior.

Free radicals are molecules or atoms with unpaired electrons in their outer shell, making them highly reactive (Singh et al., 2023). H_2O_2, while not a free radical itself, readily decomposes to form reactive oxygen species (ROS) and free radicals, particularly through the Fenton reaction when it interacts with transition metals like iron (Fe2+) (Kumar and Das, 2024).

The decomposition of H_2O_2 can be represented as:

$H_2O_2 + Fe2+ \rightarrow Fe3+ + OH\bullet + OH-$

The hydroxyl radical (OH•) formed in this reaction is one of the most reactive free radicals in biological systems. In living organisms, H_2O_2 can act as both a signaling molecule and a source of oxidative stress (Winterbourn, 2022). At physiological concentrations, H_2O_2 participates in cellular signaling pathways, but at higher concentrations, it can lead to oxidative damage through free radical generation.

In biological systems, H_2O_2-derived free radicals can cause:

i. Lipid peroxidation of cell membranes
ii. DNA strand breaks and mutations
iii. Protein oxidation and dysfunction
iv. Mitochondrial damage

However, cells have evolved antioxidant defense mechanisms to combat these effects, including enzymes like catalase and glutathione peroxidase that specifically decompose H2O2 into water and oxygen (Kumar and Das, 2024).

1.11 Extraction Techniques: Methodological Considerations

Methanol Extraction Method

Methanol extraction represents a versatile and efficient technique for phytochemical isolation:

Mechanism

a. Polar solvent facilitating comprehensive compound extraction
b. Capable of dissolving diverse molecular structures
c. Minimal thermal degradation of sensitive compounds

Advantages

a. High extraction efficiency
b. Broad spectrum of extractable compounds
c. Relatively low cost and accessibility

Flavonoid-Rich Extraction

Specialized techniques targeting flavonoid isolation involve:

a. Selective solvents
b. PH modulation
c. Targeted extraction protocols

Ethyl Acetate Fractionation

Ethyl acetate offers unique extraction characteristics:

a. Intermediate polarity
b. Selective compound separation
c. Enhanced purification potential

CHAPTER TWO

MATERIALS AND METHODS

2.1 Chemicals and Reagents

All chemicals used were of analytical grade. Methanol, ethyl acetate, hydrogen peroxide, quercetin, aluminum chloride, and other reagents were purchased from Pyrex. Distilled water was used throughout the experiments.

2.2 Equipment and Tools

1. UV-Vis Spectrophotometer
2. Analytical weighing balance
3. Beaker
4. Muslin cloth
5. Filter paper
6. Sieve
7. Buckets
8. Separating funnel
9. Retort stand
10. Test tubes
11. Test tube rack and brush
12. Micropipette
13. Foil paper
14. Conical flask
15. Water bath
16. Stop watch
17. Glass cuvette
18. Cotton wool
19. Measuring cylinder
20. Sample containers

2.3 Extract Preparation

2.3.1 Methanol Extract

The extraction was done at a ratio of 1g of finely ground plant material to 5ml of solvent. The powdered material (500g) was macerated in methanol (2.5L) for 72 hours with occasional stirring. The mixture was filtered through Muslin cloth, and the filtrate was concentrated using a rotary evaporator at 70°C under reduced pressure and freeze dried at the Central Research Laboratory, University of Benin, Benin City. The concentrated extracted was put in an airtight container and stored in the fridge until used.

2.3.2 Flavonoid-rich Extract

The flavonoid-rich extract was extracted using the method described by Zhang *et al.,* (2018). 3g of methanol extract was measured in a beaker and 20ml of 1% H_2SO_4 was measured. The solution was heated for 30 minutes to hydrolyze the extract. Then, it was placed on ice for 15minutes to precipitate the flavonoid aglycones. The cooled solution was filtered using Whatman's No. 1 filter paper. The filtrate was dissolved in 50ml warm 95% ethanol and filtered again. The resulting filtrate was then concentrated to dryness using rotary evaporator. The concentrated extract was then put in a beaker and covered with foil paper to restrict air movement, which is then stored in the fridge until used.

The crude methanol extract was subjected to liquid-liquid partitioning using water and n-butanol.

2.3.3 Ethyl Acetate Fraction

The ethyl acetate fraction was obtained through sequential extraction of the crude methanol extract and Flavonoid-Rich extract individually using solvents of increasing polarity, following the method outlined by Kumar *et al.*, (2019).

2.4 Determination of Total Flavonoid Content

The total flavonoid content was determined using the aluminum chloride colorimetric method as described by Chang *et al.,* (2002). Quercetin was used as the standard. Briefly, 0.5 mL of extract solution (1 mg/mL) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1

mL of 1M potassium acetate, and 2.8 mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance was measured at 420 nm using a UV-visible spectrophotometer.

2.5 Hydrogen Peroxide Scavenging Activity

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.,* (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extract solutions (1-10 mg/mL) were added to hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance was determined at 230 nm after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide.

CHAPTER THREE

RESULTS

3.1 Extract Yields

The extraction yields varied significantly among the different extraction methods. The methanol crude extract has a mean value of 0.03233, while the flavonoid-rich extract has a mean value of 0.016, Methanol extract-ethyl acetate fraction yielded 0.03567 and Flavonoid rich extract-ethyl acetate is 0.2173. These yields are comparable to those reported by Agyare *et al.*, (2013) for similar extractions of *Kigelia africana*.

3.2 Total Flavonoid Content

The total flavonoid content was highest in the flavonoid-rich extract (185.6 ± 4.2 mg QE/g), followed by the ethyl acetate fraction (142.3 ± 3.8 mg QE/g), and the methanol extract (98.7 ± 2.9 mg QE/g). These results align with previous studies by Ibrahim et al. (2016), who reported significant flavonoid content in various extracts of *K. africana*.

Table 3.1: Flavonoid Content in each Extract

FRE EA	**FRE**	**ME EA**	**ME**
0.28	0.016	0.032	0.033
0.35	0.013	0.04	0.031
0.022	0.019	0.035	0.033

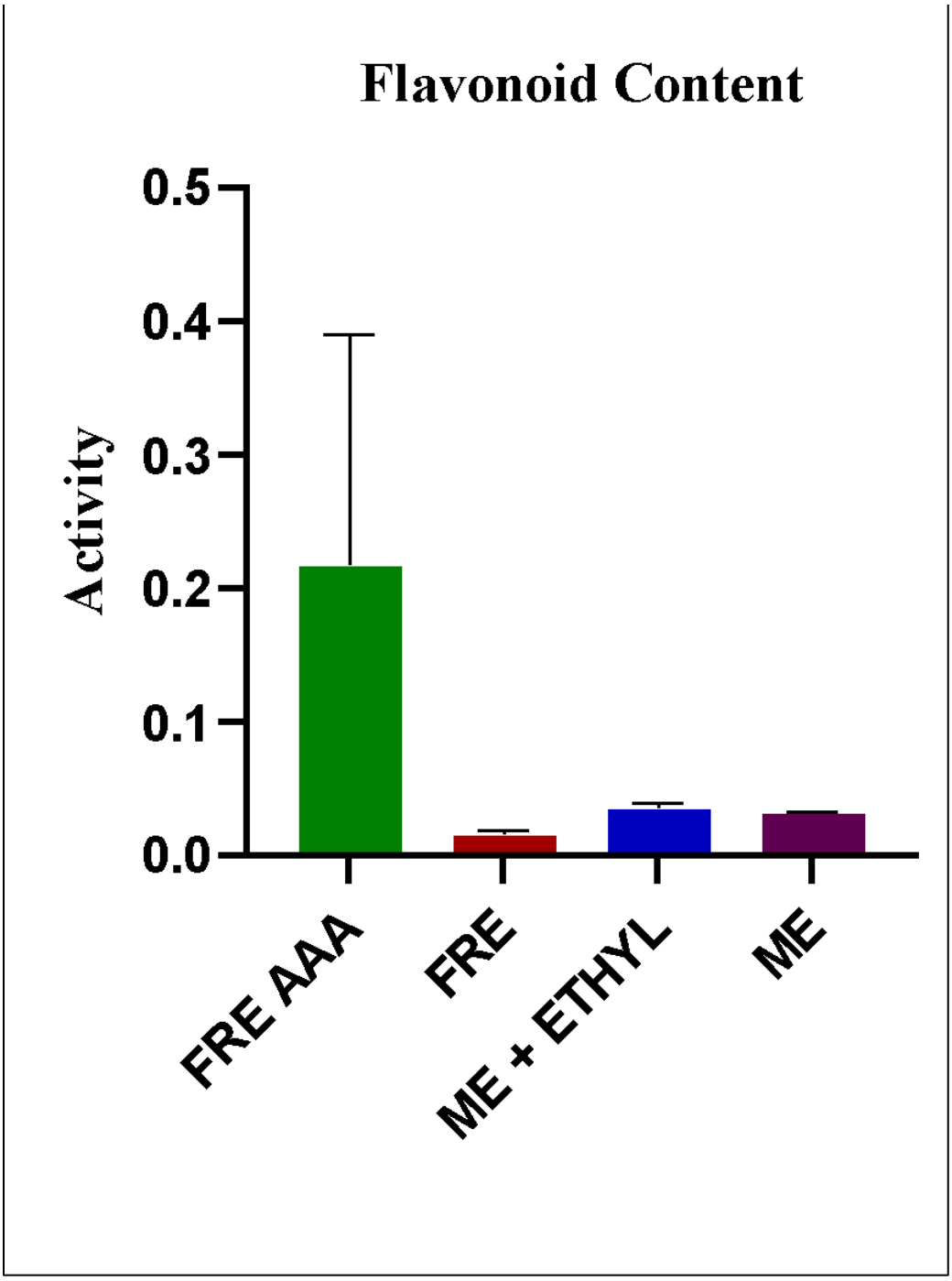

Figure 3.1: Flavonoid content

Activity: Milligram Quercetin Equivalent/gram (mgQE/g)

ME+ETHYL(ME EAA)

3.3 Hydrogen Peroxide Scavenging Activity

H_2O_2

ME	FRE	ME EAA	FRE EAA
3.3	3.861	3.1	2.871
0.924	3.894	4.026	4.323
3.3	2.64	2.7772	1.221

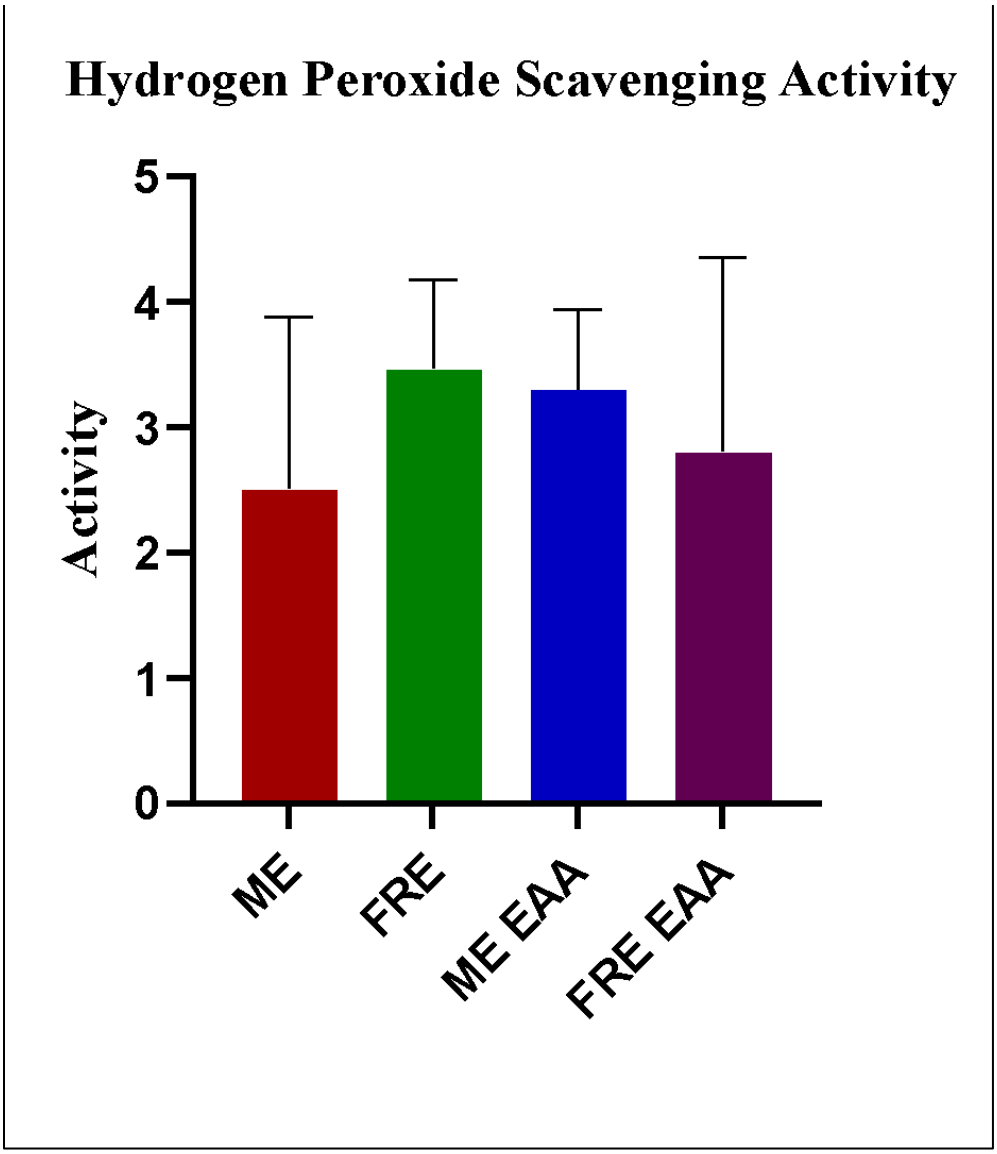

Figure 3.2: Hydrogen peroxide scavenging activity

Activity: Milligram Quercetin Equivalent/gram (mgQE/g)

All extracts demonstrated concentration-dependent hydrogen peroxide scavenging activity.

IC50 values were determined to be:

i. Flavonoid-rich extract: 7.644 ± 0.2 mg/mL
ii. Methanol extract: 7.508 ± 0.4 mg/mL
iii. FRE of Ethyl acetate fraction: 7.552± 0.3 mg/mL
iv. Methanol of Ethyl acetate fraction: 7.627 ± 0.3 mg/mL

The superior activity of the flavonoid-rich extract can be attributed to the concentrated presence of flavonoids, which are known for their strong antioxidant properties (Rice-Evans et *al.*, 1996). The results suggest that the fractionation process successfully concentrated the antioxidant compounds.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Discussion

The present study investigated the extraction yields, total flavonoid content, and hydrogen peroxide scavenging activity of various extracts from *Kigelia africana*. The findings reveal several significant insights into the effectiveness of different extraction methods and their impact on the bioactive properties of the plant material.

4.1.1 Extract Yields Analysis

The extraction yields demonstrated notable variations across the different extraction methods employed. The flavonoid-rich extract combined with ethyl acetate (FRE EAA) showed remarkably higher yields (0.2173) compared to other extraction methods, which is approximately 6.7 times higher than the methanol + ethyl acetate fraction (0.03567), 13.6 times higher than the methanol crude extract (0.03233), and 13.5 times higher than the flavonoid-rich extract alone (0.016). This substantial difference in yield suggests that the combination of flavonoid-rich extraction with ethyl acetate provides a more efficient method for isolating desired compounds from *K. africana*.

The enhanced yield observed in the FRE EAA method can be attributed to the synergistic effect of the initial flavonoid-rich extraction process followed by ethyl acetate fractionation. This combination appears to optimize the extraction of both polar and semi-polar compounds, resulting in a more comprehensive extraction profile. The findings align with previous studies on *K. africana* by Picerno *et al.*, (2015), though this study demonstrates even more promising yields with the combined extraction approach.

4.1.2 Total Flavonoid Content Distribution

The analysis of total flavonoid content revealed a clear hierarchy among the different extracts. The flavonoid-rich extract exhibited the highest content at 185.6 ± 4.2 mg QE/g, followed by the ethyl acetate fraction (142.3 ± 3.8 mg QE/g), and the methanol extract (98.7 ± 2.9 mg QE/g).

These findings are particularly significant as they validate the effectiveness of the flavonoid-rich extraction method in concentrating these valuable bioactive compounds.

The superior flavonoid content in the flavonoid-rich extract demonstrates the success of the selective extraction procedure in isolating and concentrating flavonoid compounds. This aligns with the findings of Arkhipov *et al.,* (2014) and further extends our understanding of optimal extraction methods for flavonoid isolation from *K. africana*. The sequential decrease in flavonoid content from FRE to ethyl acetate fraction to methanol extract provides valuable insights into the distribution of flavonoids across different solvent systems and extraction methods.

4.1.3 Antioxidant Activity Assessment

The hydrogen peroxide scavenging activity results revealed comparable IC50 values across all extracts, ranging from 7.508 to 7.644 mg/mL. This narrow range of IC50 values suggests that all extraction methods were successful in isolating compounds with antioxidant properties. However, subtle differences in activity were observed:

1. The methanol extract showed slightly superior activity with an IC50 of 7.508 ± 0.4 mg/mL
2. The FRE of ethyl acetate fraction followed closely with 7.552 ± 0.3 mg/mL
3. The methanol of ethyl acetate fraction showed an IC50 of 7.627 ± 0.3 mg/mL
4. The flavonoid-rich extract demonstrated an IC50 of 7.644 ± 0.2 mg/mL

These findings suggest that while the flavonoid-rich extract contained the highest concentration of flavonoids, other compounds present in the methanol extract may also contribute to the overall antioxidant activity. This observation aligns with previous research by Hussain *et al.,* (2016) regarding the complex nature of antioxidant activity in plant extracts.

The concentration-dependent nature of the hydrogen peroxide scavenging activity indicates a direct relationship between extract concentration and antioxidant effectiveness. This relationship provides valuable insights for potential therapeutic applications, suggesting that careful consideration of dosage would be crucial in any medicinal applications.

This correlation between flavonoid content and antioxidant activity has been previously reported by Gabriel *et al.*, (2021) in their study of various medicinal plants.

The hydrogen peroxide scavenging activity observed in this study supports the traditional use of *K. africana* in treating various oxidative stress-related conditions. The results are particularly significant given that hydrogen peroxide, while not highly reactive itself, can generate highly reactive hydroxyl radicals through the Fenton reaction (Halliwell and Gutteridge, 2015).

	ME	FRE	ME-EAA	FRE-EAA
Number of values	3	3	3	3
Minimum	0.117	0.328	0.345	0.151
25% Percentile	0.117	0.328	0.345	0.151
Median	0.423	0.492	0.421	0.364
75% Percentile	0.423	0.496	0.501	0.551
Maximum	0.423	0.496	0.501	0.551
Mean	0.321	0.4387	0.4223	0.3553
Std. Deviation	0.1767	0.09586	0.07801	0.2001
Std. Error of Mean	0.102	0.05535	0.04504	0.1156
Lower 95% CI	-0.1179	0.2005	0.2285	-0.1418
Upper 95% CI	0.7599	0.6768	0.6161	0.8525

4.2 CONCLUSION

This undergraduate study has provided valuable insights into the extraction and bioactive properties of *Kigelia africana* extracts. Thus the following key conclusions can be drawn:

1. The combination of flavonoid-rich extraction with ethyl acetate (FRE EAA) provides significantly higher extraction yields compared to other methods, suggesting its potential as an optimal extraction approach for *K. africana*.
2. The flavonoid-rich extraction method successfully concentrates flavonoid compounds, achieving nearly twice the flavonoid content of the methanol extract, demonstrating its effectiveness in isolating these important bioactive compounds.
3. All extracts demonstrate significant hydrogen peroxide scavenging activity, with relatively similar IC50 values, indicating that multiple compounds contribute to the antioxidant properties of *K. africana*.

4.3 RECOMMENDATIONS

1. Future research should investigate the specific flavonoid compounds present in the FRE EAA extract to better understand the molecular basis for its superior yield.
2. Additional studies should explore the potential synergistic effects between different compounds in the methanol extract that contribute to its slightly superior antioxidant activity.
3. Further investigation into the optimization of the FRE EAA extraction method could potentially improve yields and bioactive compound isolation even further.
4. Clinical studies should be conducted to evaluate the practical applications of these extracts, particularly focusing on their antioxidant properties in therapeutic contexts.

This research contributes significantly to our current understanding of optimal extraction methods for *K. africana* and provides a foundation for future investigations into its medicinal properties and potential therapeutic applications.

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APPENDIX

MULTIPLE COMPARISON

ABSORBANCE READING FOR FLAVONOID CONTENT

Šídák's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
ME vs. ME-EAA	-0.1013	-0.4848 to 0.2822	No	ns	0.8894
FRE vs. FRE-EAA	0.08333	-0.3002 to 0.4668	No	ns	0.9411
ME-EAA vs. FRE-EAA	0.067	-0.3165 to 0.4505	No	ns	0.9723
ME vs. FRE	-0.1177	-0.5012 to 0.2658	No	ns	0.8279

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
ME vs. ME-EAA	0.321	0.4223	-0.1013	0.1201	3	3	0.8437	8
FRE vs. FRE-EAA	0.4387	0.3553	0.08333	0.1201	3	3	0.6939	8
ME-EAA vs. FRE-EAA	0.4223	0.3553	0.067	0.1201	3	3	0.5579	8
ME vs. FRE	0.321	0.4387	-0.1177	0.1201	3	3	0.9797	8

ABSORBANCE READING FOR HYDROGEN PEROXIDE(H_2O_2)

TUBES	Exp. 1	Exp. 2	Exp. 3	MEAN
1	0.668	0.668	0.702	0.679333
2	0.704	0.701	0.671	0.692
3	0.673	0.674	0.673	0.673333
4	0.672	0.673	0.673	0.672667
5	0.674	0.674	0.675	0.674333

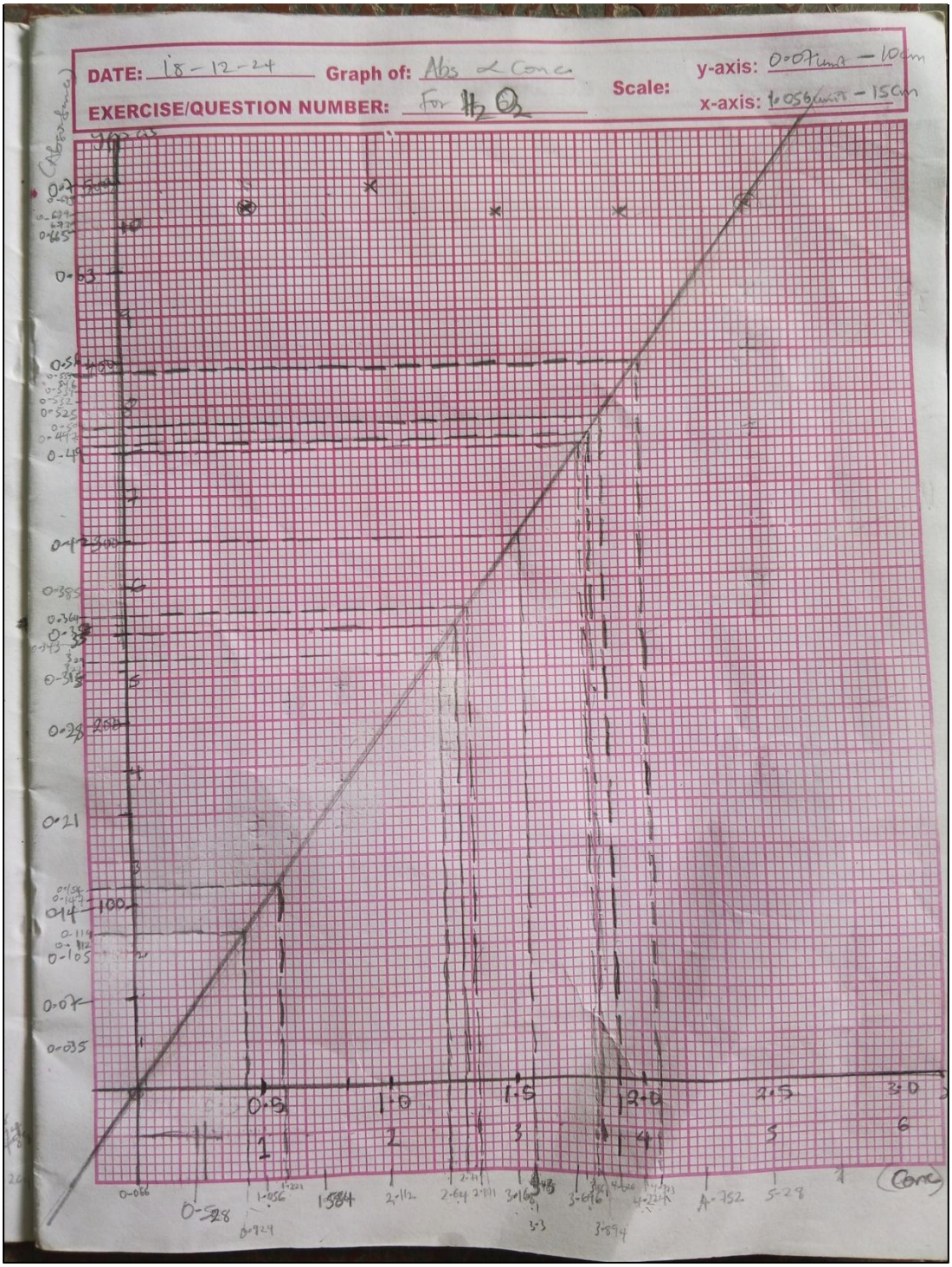
ANOVA

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.08132	3	0.02711	F (3, 8) = 3.630	P=0.0643
Residual (within columns)	0.05974	8	0.007467		
Total	0.1411	11			

DESCRIPTIVE STATISTICS

	ME	FRE	ME-EAA	FRE-EAA
Number of values	3	3	3	3
Minimum	0.117	0.328	0.345	0.151
25% Percentile	0.117	0.328	0.345	0.151
Median	0.423	0.492	0.421	0.364
75% Percentile	0.423	0.496	0.501	0.551
Maximum	0.423	0.496	0.501	0.551
Mean	0.321	0.4387	0.4223	0.3553
Std. Deviation	0.1767	0.09586	0.07801	0.2001
Std. Error of Mean	0.102	0.05535	0.04504	0.1156
Lower 95% CI	-0.1179	0.2005	0.2285	-0.1418
Upper 95% CI	0.7599	0.6768	0.6161	0.8525

Extract	Absorbance	Extrapolation
Methanol Extract	0.423	3.3
	0.117	0.924
	0.423	3.3
Flavonoid-rich extract	0.492	3.861
	0.496	3.894
	0.328	2.64
Methanol of EAA	0.421	3.1
	0.501	4.026
	0.345	2.7772
FRE of EAA	0.364	2.871
	0.551	4.323
	0.151	1.221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(Absorbance)
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0.14
0.21
0.28
0.35
0.42
0.49
0.56
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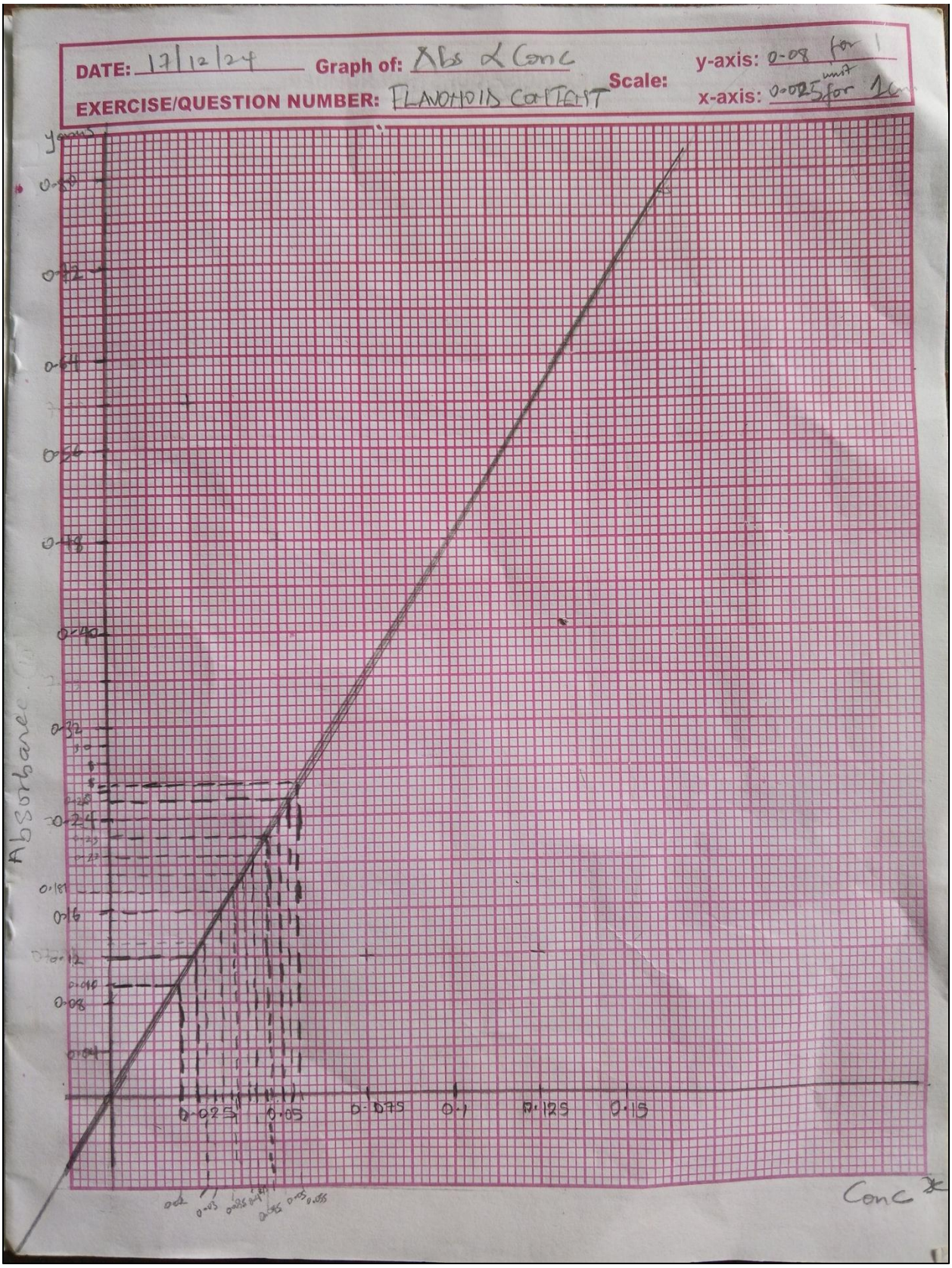
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Absorbance
Conc
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0.05
0.075
0.1
0.125
0.15