PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF ACACIA NILOTICA SEEDS

BY

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CERTIFICATION

This is to certify that this project titled "PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF ACACIA NILOTICA SEEDS" has been read, certified and approved as meeting part of the requirements of the Department of Science Laboratory Technology, in partial fulfilment of the requirement for the award of Higher National Diploma (HND) in Science Laboratory Technology, (Biochemistry Unit), Institute of Applied Sciences, Kwara State Polytechnic, Ilorin

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ABSTRACT

This study investigates the phytochemical constituents and antioxidant activity of ethanolic extracts from the seeds and endocarp of Acacia nilotica, *a plant widely used in traditional medicine. Standard phytochemical screening revealed the presence of steroids, triterpenes, tannins, glycosides, lactones, and alkaloids in varying concentrations, with alkaloids present only in the seed, while tannins and glycosides were more abundant in the endocarp. Quantitative analysis showed that the endocarp extract contained a significantly higher total phenolic content (49.56 mg GAE/g) compared to the seed (18.42 mg GAE/g). Antioxidant activity assessed using the DPPH radical scavenging assay demonstrated greater inhibition by the endocarp (51.41%) than the seed extract (14.47%), correlating with the higher phenolic content. Carotenoid analysis indicated a higher lycopene concentration in the seed (0.013 mg/mL) and elevated β-carotene levels in the endocarp (0.034 mg/mL), suggesting distinct antioxidant contributions. These findings confirm the rich phytochemical profile and strong antioxidant potential of* Acacia nilotica, *particularly in the endocarp, and support its application as a natural source of bioactive compounds for medicinal and nutraceutical use.*

CHAPTER ONE

1.0 INTRODUCTION

The growing global interest in natural products for pharmaceutical, nutraceutical, and cosmetic applications has underscored the need to explore the phytochemical and biological properties of medicinal plants. One such plant of significant pharmacological and ethnomedicinal importance is *Acacia nilotica* (L.), commonly known as gum arabic tree or babul. Belonging to the family Fabaceae, *Acacia nilotica* is widely distributed in tropical and subtropical regions of Africa, Asia, and the Indian subcontinent. The plant has been extensively utilized in traditional medicine for treating a variety of ailments such as diarrhea, dysentery, leprosy, asthma, cough, wounds, and microbial infections (Kaur et al., 2005; Shabbir, 2014). Among its various parts, leaves, bark, pods, and seeds. The seeds have garnered attention due to their rich reservoir of phytochemicals with potential health benefits.

Phytochemical analysis plays a vital role in the identification and quantification of bioactive compounds present in medicinal plants. These compounds, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolics, contribute significantly to the therapeutic efficacy of plant extracts. The presence of such compounds in *Acacia nilotica* seeds has been linked to diverse biological properties, including antimicrobial, anti-inflammatory, anticancer, and notably, antioxidant activities (Singh et al., 2009; Tiwari et al., 2011). Antioxidants are crucial in mitigating oxidative stress—a major contributor to cellular damage, aging, and chronic diseases such as cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions (Lobo et al., 2010).

The antioxidant potential of plant-derived compounds is primarily attributed to their ability to scavenge free radicals, chelate metal ions, and inhibit lipid peroxidation.

Ethanol, a polar solvent, has been extensively employed in phytochemical extraction due to its efficiency in solubilizing a wide range of polar and moderately non-polar bioactive compounds. Ethanolic extracts are often preferred in antioxidant studies owing to their enriched phenolic and flavonoid contents, which are major contributors to radical scavenging activity (Kumar et al., 2012). Previous investigations have indicated that ethanolic extracts of *Acacia nilotica* seeds exhibit strong antioxidant properties, suggesting the presence of potent natural antioxidants with therapeutic relevance (Banso & Adeyemo, 2007).

Given the increasing burden of oxidative stress-related disorders and the global shift toward plant based therapeutics, the phytochemical screening and antioxidant assessment of *Acacia nilotica* seed extracts remain scientifically valuable. This study aims to perform a comprehensive phytochemical analysis and evaluate the in vitro antioxidant activity of the ethanolic extract of *Acacia nilotica* seeds, thereby contributing to the growing body of evidence supporting its traditional uses and potential in drug development.

1.1 SCOPE OF THE STUDY

This study focuses on the *phytochemical analysis* and *antioxidant activity* of the ethanolic extract of *Acacia nilotica* seeds, with a view to identifying the bioactive constituents responsible for its therapeutic potential and evaluating its capacity to scavenge free radicals. The scope of the study encompasses both qualitative and quantitative assessments of major

phytochemical classes, such as alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, and terpenoids, using standardized procedures (Harborne, 1998; Trease & Evans, 2002). These phytochemicals are known for their pharmacological activities and are often directly correlated with antioxidant potential (Tiwari et al., 2011).

The antioxidant component of the study involves in vitro assays such as DPPH (2,2-diphenyl-1- picrylhydrazyl) radical scavenging activity, ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) to quantify the scavenging ability and reducing power of the seed extract. These methods are widely accepted and provide a comprehensive understanding of the antioxidative efficiency of plant-based extracts (Apak et al., 2016; Brand-Williams et al., 1995).

Furthermore, the study is confined to the use of ethanol as the extraction solvent due to its high efficiency in extracting phenolic and flavonoid compounds and its relatively low toxicity, which makes it suitable for food and pharmaceutical applications (Kumar et al., 2012). This scope excludes in vivo antioxidant evaluations and the isolation or structural elucidation of individual phytochemicals, which would require more extensive techniques such as HPLC, GC-MS, or NMR.

Geographically, the study is limited to *Acacia nilotica* seeds sourced from a specific region to maintain consistency in sample characteristics. Additionally, the research does not cover the antimicrobial, anti-inflammatory, or cytotoxic properties of the plant, although these are acknowledged as important pharmacological aspects of *Acacia nilotica* (Shabbir, 2014).

In essence, the scope of this study is designed to provide foundational phytochemical and antioxidant data on *Acacia nilotica* seed extract that could support further pharmacological investigations and the development of natural antioxidant agents.

1.2 STATEMENT OF PROBLEM

In recent decades, the prevalence of oxidative stress-related disorders—including cardiovascular diseases, cancer, diabetes, and neurodegenerative conditions—has increased dramatically, largely due to environmental pollution, poor diets, and lifestyle changes. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's natural antioxidant defenses, leading to cellular damage and disease progression (Lobo et al., 2010). Although synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used in food and pharmaceutical industries, their long-term use has been associated with potential toxicity, carcinogenicity, and adverse side effects (Kahl & Kappus, 1993). This has created an urgent need for safer, naturally occurring antioxidants that are both effective and biocompatible.

Despite the well-documented traditional uses of *Acacia nilotica* in ethnomedicine, especially in regions of Africa and Asia, there remains a gap in detailed scientific validation of its bioactive seed components, particularly with regard to their antioxidant potential. While some studies have explored the pharmacological properties of other parts of the plant, the seeds remain largely underutilized and under-researched in this context. Moreover, the lack of standardization in extraction methods and inadequate phytochemical profiling contribute to inconsistent and inconclusive data on its therapeutic efficacy (Shabbir, 2014).

Therefore, there is a compelling need to systematically investigate the phytochemical constituents and antioxidant activities of the ethanolic extract of *Acacia nilotica* seeds. This will not only help validate the plant's traditional use but also potentially reveal new, natural compounds for the development of antioxidant-based therapies and nutraceuticals.

1.3 JUSTIFICATION OF THIS STUDY

The justification for this study stems from the increasing demand for natural, safe, and effective antioxidants to combat oxidative stress-related health challenges. As synthetic antioxidants continue to raise concerns regarding their safety and long-term health effects (Kahl & Kappus, 1993), the exploration of plant-based alternatives becomes not only relevant but imperative. *Acacia nilotica*, a plant with deep roots in traditional medicine, presents a promising candidate due to its reported pharmacological properties and availability in many tropical and subtropical regions (Kaur et al., 2005).

Despite its recognized medicinal value, there is limited scientific data specifically focusing on the phytochemical composition and antioxidant capacity of its seeds. Most existing studies have concentrated on the bark, leaves, or pods, leaving a knowledge gap regarding the seed's bioactive potential. Investigating the seeds, therefore, could uncover novel phytochemicals with potent antioxidant activities, thereby expanding the medicinal utility of the plant and offering a new, underutilized natural resource for therapeutic applications (Shabbir, 2014).

Additionally, the use of ethanol as an extraction solvent in this study ensures the isolation of a broad spectrum of bioactive compounds with minimal toxicity, aligning with the needs of pharmaceutical and nutraceutical industries (Kumar et al., 2012). The outcomes of this

study could contribute to the standardization and validation of traditional remedies involving *Acacia nilotica* seeds, promote further pharmacological research, and potentially lead to the development of antioxidant-rich formulations for clinical or commercial use.

Thus, this study is justified not only in terms of advancing scientific knowledge but also in its potential to support public health through the development of plant-derived antioxidant agents. **1.4 AIM AND OBJECTIVES**

Aim:

The primary aim of this study is to investigate the phytochemical composition and evaluate the antioxidant activity of the ethanolic extract of *Acacia nilotica* seeds.

Specific Objectives:

1. To study the extraction process from *Acacia nilotica* seeds using ethanol as the solvent. 2. To perform qualitative phytochemical screening to identify the presence of major phytochemical constituents such as alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, and terpenoids.
3. To quantify DPPH antiradical activity of the ethanolic seed extract.
4. To evaluate the carotenoids antioxidants activities such as lycopene and β-Carotene of the extract.
5. To establish a correlation between the phytochemical content and the observed antioxidant activity of the seed extract.
6. To provide scientific validation for the traditional use of *Acacia nilotica* seeds as a source of natural antioxidants.

CHAPTER TWO

2.0 LITERATURE REVIEW

Medicinal plants have been widely acknowledged as a major source of therapeutic agents, particularly in the development of novel drugs to combat oxidative stress-related diseases. One such plant with substantial ethnomedicinal value is *Acacia nilotica*, commonly known as babul. It belongs to the family Fabaceae and is native to tropical and subtropical regions of Africa, the Indian subcontinent, and parts of the Middle East. Traditionally, various parts of *Acacia nilotica* including the bark, pods, leaves, and seeds have been used for the treatment of ailments such as diarrhea, wounds, cough, tuberculosis, leprosy, and even certain infections due to its antimicrobial, astringent, and anti-inflammatory properties (Kaur et al., 2005; Shabbir, 2014).

Phytochemicals are naturally occurring compounds in plants that contribute to their biological activity. Several studies have shown that *Acacia nilotica* contains a rich array of secondary metabolites, including tannins, saponins, flavonoids, terpenoids, alkaloids, and glycosides (Tiwari et al., 2011; Singh et al., 2009). These compounds have been linked to various pharmacological properties such as antimicrobial, hepatoprotective, anti-inflammatory, and notably, antioxidant effects (Sultana et al., 2007). Among these, phenolic compounds and flavonoids are of particular interest due to their potent free radical scavenging and metal ion chelation abilities (Lobo et al., 2010).

The antioxidant potential of *Acacia nilotica* has been demonstrated in various plant parts. For example, Singh et al. (2009) reported that the ethyl acetate extract of *Acacia nilotica*

bark exhibited significant antioxidant activity, comparable to standard antioxidants like ascorbic acid and BHT. Similarly, Banso and Adeyemo (2007) observed the presence of tannins and flavonoids in the pods, which contributed to notable antioxidant and antibacterial effects. However, while the bark and pods have been widely studied, the seeds remain relatively unexplored, despite their reported richness in essential phytochemicals (Sharma et al., 2011).

Ethanol has been widely employed as an effective solvent for extracting both polar and moderately non-polar bioactive compounds. Studies suggest that ethanolic extracts often yield a higher concentration of phenolics and flavonoids compared to other solvents, thereby enhancing antioxidant activity (Kumar et al., 2012). The DPPH radical scavenging assay is one of the most widely used methods for evaluating the antioxidant potential of plant extracts. This assay measures the capacity of compounds to donate hydrogen and neutralize free radicals, providing a rapid and reliable estimate of antioxidant strength (Brand-Williams et al., 1995). Additional assays such as FRAP and total antioxidant capacity (TAC) further support these findings by measuring the reducing power and overall antioxidant potential of the extract (Apak et al., 2016).

Despite the demonstrated medicinal potential of *Acacia nilotica*, there is a gap in research focusing specifically on the phytochemical profile and antioxidant activity of its seed extract. Given the bioactive richness of the plant and its availability in many parts of the world, including regions with limited access to synthetic drugs, this area remains a valuable field for scientific exploration and pharmaceutical development. Therefore, this study aims

to bridge that knowledge gap by evaluating the ethanolic seed extract of *Acacia nilotica* for its phytochemical constituents and in vitro antioxidant properties.

2.1 PLANT OF STUDY

Acacia nilotica (L.) Delile, commonly referred to as "babul" or "gum arabic tree," is a multipurpose tree species belonging to the family Fabaceae (subfamily Mimosoideae). It is widely distributed across Africa, the Indian subcontinent, and parts of the Middle East, thriving particularly in arid and semi-arid environments (Kaur et al., 2005). The plant is well-regarded in traditional medicine systems, including Ayurveda and Unani, where its various parts, bark, leaves, pods, seeds, and gum are used to treat a wide range of diseases such as skin infections, respiratory issues, gastrointestinal disorders, and diabetes (Shabbir, 2014; Ali et al., 2012).

2.1.1 BOTANICAL CHARACTERISTICS

Acacia nilotica is a medium-sized thorny tree that can grow up to 20 meters in height. It is characterized by its bipinnate leaves, yellow flowers arranged in globular heads, and leguminous pods that are often curved or straight depending on subspecies. The seeds are small, dark brown to black, and enclosed within hard pods. These seeds are often underutilized compared to other parts of the plant but have shown promising potential in phytochemical and pharmacological studies (Sharma et al., 2011).

2.1.2 ETHNOMEDICAL AND PHAMACOLOGICAL RELEVANCE Traditionally, *A. nilotica* seeds have been used for their astringent, anti-diarrheal, and antimicrobial properties. The plant exhibits a wide range of biological activities including antibacterial, antifungal, antiviral, anti-inflammatory, antidiabetic, antihyperlipidemic, and antioxidant

effects (Sultana et al., 2007; Bisht et al., 2013). These effects are attributed to its rich phytochemical profile.

2.2 EXTRACTION METHOD

Extraction is a fundamental step in phytochemical and antioxidant studies, as it determines the efficiency and accuracy of bioactive compound isolation from plant materials. In studies involving *Acacia nilotica* and other medicinal plants, the choice of extraction solvent and method significantly influences the quantity and quality of the phytochemicals extracted, especially phenolics and flavonoids, which are primarily responsible for antioxidant activities (Azwanida, 2015).

Among the commonly used solvents such as methanol, ethanol, acetone, and water, ethanol is preferred for several reasons. Ethanol is a polar protic solvent that has the ability to extract a broad range of phytochemicals, including flavonoids, phenolics, tannins, and saponins (Kumar et al., 2012). Moreover, it is relatively safe, non-toxic, biodegradable, and approved for pharmaceutical and food applications, making it ideal for the preparation of medicinal plant extracts intended for human use (Do et al., 2014).

Cold maceration, soxhlet extraction, and ultrasound-assisted extraction are common techniques employed for obtaining ethanolic extracts. Maceration involves soaking the powdered plant material in ethanol for a specific period, typically ranging from 24 to 72 hours, under room temperature with occasional agitation. This method is simple, cost-effective, and suitable for thermolabile compounds, although it may be less efficient compared to other methods (Handa et al., 2008).

Soxhlet extraction, by contrast, uses continuous hot solvent percolation, which increases the extraction efficiency by repeatedly exposing plant material to fresh solvent. However,

prolonged exposure to heat may degrade certain sensitive compounds, especially antioxidants such as flavonoids and ascorbic acid (Azwanida, 2015). Ultrasound-assisted extraction, a modern technique, utilizes sound waves to rupture cell walls and enhance solvent penetration, thereby increasing yield and reducing extraction time. This method has been reported to extract more phenolic compounds from plants compared to traditional methods (Zhang et al., 2008).

In the context of *Acacia nilotica*, most antioxidant and phytochemical studies have used ethanolic maceration or soxhlet extraction. For instance, Sharma et al. (2011) used ethanol in a cold maceration process to extract bioactive compounds from *A. nilotica* seeds and found significant antioxidant activity in the extract. Similarly, Singh et al. (2009) employed soxhlet extraction with

ethanol and reported a high yield of phenolics and flavonoids from the bark of the plant. The extraction duration, solvent concentration, temperature, and particle size of the plant material all play crucial roles in the effectiveness of the process. Ethanol concentrations between 70–95% are often reported to be optimal for extracting both polar and semi-polar compounds (Do et al., 2014).

In conclusion, ethanol-based extraction, especially through cold maceration or soxhlet method remains a widely accepted and effective technique for isolating antioxidant-rich phytochemicals from *Acacia nilotica*. The method balances safety, efficiency, and compatibility with downstream bioactivity testing, thus justifying its selection for this study.

2.3 PHYTOCHEMICAL COMPOSITION OF *ACACIA NILOTICA*

Studies have confirmed the presence of several bioactive compounds in various parts of the plant. Notably, the seeds contain high concentrations of tannins, flavonoids, phenolic acids, saponins, alkaloids, and glycosides (Tiwari et al., 2011). Flavonoids such as quercetin and kaempferol, and phenolic acids like gallic acid and caffeic acid, have been reported in *A. nilotica* extracts and are known for their strong antioxidant and anti-inflammatory activities (Mokhtari et al., 2012).

Sharma et al. (2011) carried out a comparative study of *A. nilotica* pod and seed extracts and found that the ethanolic extract of the seeds exhibited significant antioxidant activity due to its higher total phenolic and flavonoid content. Similarly, the work of Banso and Adeyemo (2007) demonstrated the antimicrobial and antioxidant effects of seed extracts, supporting its traditional use in infection control.

2.4 ANTIOXIDANT STUDIES OF *ACACIA NILOTICA*

Multiple studies have emphasized the potent antioxidant activity of *A. nilotica* extracts. Sultana et al. (2007) compared the antioxidant activity of bark extracts of various medicinal trees and noted that *A. nilotica* bark showed notable radical scavenging ability. However, antioxidant studies on the seeds are still relatively limited, providing an opportunity for deeper investigation.

Using standard assays such as DPPH and FRAP, Kumar et al. (2012) showed that ethanolic extracts rich in phenolics and flavonoids demonstrated higher antioxidant

potential compared to aqueous or non-polar solvent extracts. Therefore, ethanol is considered a suitable solvent for extracting bioactive antioxidants from *A. nilotica* seeds.

CHAPTER THREE

3.0 COLLECTION AND PREPARATION OF SAMPLE

The *Acacia nilotica* seeds used for this experiment was purchased at Kure ultramodern market located in Chanchaga Local Government Area, Minna, Niger State, Nigeria. The fruits were separated into two parts: (1) the seeds and (2) The endocarp. Each was separately pulverized and extracted. Pulverization was carried out using a high-powered multifunctional kitchen blender.

3.1 MATERIALS AND METHOD USED

The methodology adopted in this study is designed to ensure efficient extraction, accurate phytochemical screening, and reliable evaluation of antioxidant activity of *Acacia nilotica* seed extract using standard scientific techniques.

3.1.1 REAGENTS USED

- Ethanol (70%)
- Folin–Ciocalteu reagent (FCR)
- DPPH (2,2-diphenyl-1-picrylhydrazyl)
- Ferric chloride solution
- 7% Na_2CO_3 (Sodium Carbonate)
- Lead acetate
- Sodium hydroxide
- Dragendorff's, Mayer's, Hager's, and Wagner's reagents
- Liebermann-Burchard reagent
- Legalon's reagent

• Copper acetate

• Standard antioxidants (Ascorbic acid/BHT)

3.1.2 EQUIPMENT AND INSTRUMENTS USED

• Digital weighing balance

• Magnetic stirrer

• UV-Visible spectrophotometer

• Electric grinder

• Glassware (beakers, test tubes, measuring cylinders, micropipette)

• Water bath

3.2 PROCEDURE FOR EXTRACTION

28.97g of the powdered seed sample was packed into a thimble and setup for extraction. Infusion method was employed using ethanol as solvent. The extracted solution i.e solution containing ethanol and the extract separated using steam distillation method. The concentrated extract was allowed to dry using heating mantle. Similar procedure was employed for the extraction of the endocarp.

3.3 PHYTOCHEMICAL TEST PROCEDURES

The phytochemical properties of the sample were performed using qualitative analysis.

3.3.1 TESTS FOR STEROID

✓ **Sarkowski Test**

Chloroform solution of the extract when shaken with concentrated sulfuric acid and on standing yield red color.

✓ **Lieberman Burchardt Test**

Chloroform solution of the extract with few drops of acetic acid and 1ml of concentrated sulfuric acid from the sides gives reddish ring at the junction of 2 layers.

3.3.2 TESTS FOR TRITERPENE

✓ **Sarkowski Test**

Chloroform solution of the extract when shaken with concentrated sulfuric acid, lower layer turns to yellow on standing.

✓ **Lieberman Burchardt Test**

. Chloroform solution of the extract with few drops of acetic acid and 1ml of concentrated sulfuric acid from the sides gives deep red at the junction of 2 layers.

3.3.3 TESTS FOR ALKALOID

The extracts were mixed with ammonia and then extracted with chloroform solution. To this, dilute hydrochloric acid was added. The acid layer was used for chemical tests for alkaloids.

✓ **Mayer's Test (Potassium Mercuric Iodide)**
The acid layer with few drops of Mayer's reagent gives a creamy white precipitate. ✓ **Wagner's Test (Solution of Iodine in Potassium Iodide)**

The acid layer with few drops of Wagner's reagent gives reddish brown color

precipitate. ✓ **Hager's Test (Saturated solution of picric acid)**

The acid layer with Hager's reagent gives yellow precipitate.

✓ **Dragendroff's Test (Solution of Potassium Bismuth Iodide)**

Acid layer with few drops of Dragendorff's reagent gives reddish brown

precipitate **3.3.4 TESTS FOR TANNIN**

✓ **Ferric Chloride Test**

Few drops of ferric chloride with 2ml extract of both seed and endocarp

separately. ✓ **Lead Acetate Test**

Few drops of lead acetate were added to extract of both seed and endocarp

separately. **3.3.5 LACTONES TEST**

✓ **Legalon's Test**

Few drops of Legalon's reagent were added to the extract of both seed and endocarp separately.
3.3.6 DITERPENE TEST

✓ **Copper Acetate Test**

Few drops of copper acetate were added to extract of both seed and endocarp

separately. **3.3.7 GLYCOSIDE TEST**

✓ **Sodium Hydroxide Test**

Few drops of sodium hydroxide solution were added to extract of both seed and

endocarp separately.

3.3.8 SAPONIN TEST

✓ The extract of both seed and endocarp were added with water separately and was shaken vigorously for 1minute.

3.4 ANTIOXIDANT ACTIVITY

The antioxidant activities of the sample (both seed and endocarp extract) were determined using spectrophotometric technique. UV-Visible spectrophotometer was used during this project.

3.4.1 DPPH (2,2-Diphenyl-1-picryhydrazyl)

• 0.5ml of the extracted of both seed and endocarp was carefully measured into separate 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 and standard.

• A blank containing 2ml methanol and 1ml of DPPH solution was done separately
• These test tubes were left in the dark for 30minutes.

• Absorbance of the solutions (sample and blank) were measured at 517nm. • A standard solution of abscorbic acid (0.1g in 10ml of distilled water) was prepared and absorbance measured as control.

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

Where:

Aa = absorbance of sample

Ab = absorbance of standard

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

Where:

Aa = absorbance of sample

Ab = absorbance of standard

3.4.2 TPC (Total Phenolic Content)

• 0.5ml of the extracted of both seed and endocarp was weighed and added to 5ml of FCR and 4ml of 7% Na_2CO_3. This was labeled as sample.

• 2ml of the resulting solution was further diluted to 10ml with distilled water • 5ml of FCR and 4ml of Na_2CO_3 was mixed and labeled as blank

• This was incubated at 40^0C for 40minutes (both sample and blank).

• Absorbance measurement was taken at 760nm.

• Readings of absorbance and concentrations (based on Gallic acid) was used, and a graph of absorbance against concentration was plotted to determine the concentration of TPC.

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

Where:

C = Concentration

V = Volume of sample

M = Mass of sample

3.4.3 Estimation of Lycopene and β-carotene in Extract (Determination of Carotenoids).

• 1ml of extract of both seed and endocarp was prepared separately with 12.5ml of N

Hexane:Acetone mixture (1:1) were prepared in 100ml of conical flask

• The process was performed on both extract on extract of seed and endocarp •

A blank solution containing 12.5ml of N- Hexane:Acetone mixture was

performed • Absorbance was measured at 453nm, 505nm, and 663nm.

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

Lycopene = $\frac{0.458\ (0.383) - 0.372\ (0.048) - 0.0806\ (0.683)}{10}$

CHAPTER FOUR

4.0 RESULTS

From the experiment conducted on the crude extract prepared from the seed and endocarp of *Acacia nilotica* in absolute ethanol, its phytochemical, and antioxidants properties were evaluated. Table 1 shows the phytochemical test results obtained from *Acacia nilotica* seed and endocarp. Table 2 shows the antioxidant activities.

Table 1. Phytochemical Test Result of *Acacia nilotica* Seed and Endocarp'

TESTS	**SEED**	**ENDOCARP**
Steroid Tests	+	+
(i)Sarkowski test	+	+
(ii)Liebaerman Burchardt test		
Triterpene Tests	-	-
(i)Sarkowski test	+	-
(ii)Liebaerman Burchardt test		
Tests for Tannins	++	+++
(i)Ferric Chloride test	+	+++
(ii)Lead acetate test		
Test for Lactone	+	++
(i)Legalon's test		
Test for Diterpene	+	-
(i) Copper acetate test		

Test for Glycosides (i)Sodium Hydroxide test	+	++

Test for Saponin (i)Foam test	-	-
Alkaloid Tests (i)Dragendorff's test (ii)Hager;s test (iii)Meyer's test (iv)Wagner;s test	+ + +	- - - -

Table 2. Antioxidant Activity (DPPH, Lycopene, & β-Carotene)

PARAMETERS	SEED	ENDOCARP
% DPPH antiradical activity (standard)	5.12%	46.10%
% DPPH antiradical activity (control)	14.47%	51.41%
TPC	18.42mgGAEquiv/g	49.56mgGAEquiv/g
Lycopene	0.013mg/ml	0.010mg/ml

β-Carotene	0.023mg/ml	0.034mg/ml.

Keys; Negative (-), Weak Positive (+), Strong Positive (++), Very Strong Positive (+++).

4.1 DISCUSSION

4.1.1 INFERENCE ON PHYTOCHEMICAL SCREENING

TESTS FOR STEROIDS

✓ **Sarkowski Test**

Red color formation was observed which indicate presence of steroids in both endocarp and seed. ✓ **Lieberman Burchardt Test**

Reddish ring at the junction of 2 layers. This indicates the presence of steroid in both of seed and endocarp.

TEST FOR TRITERPENE

✓ **Sarkowski Test**

No yellow color was observed. This indicates the absence of triterpene in both seed and endocarp ✓ **Lieberman Burchardt Test**

Red ring at the junction of 2 layers was observed in seed solution but not in endocarp solution. This indicates the presence of triterpene in seed and its absence in endocarp.

TESTS FOR ALKALOIDS

✓ **Mayer's Test (Potassium Mercuric Iodide)**

The acid layer gives a creamy white precipitate in seed solution and not in endocarp solution. This indicates the presence of alkaloids in seed and not in endocarp.

✓ **Wagner's Test (Solution of Iodine in Potassium Iodide)**

The acid layer with few drops of Wagner's reagent gives reddish brown color precipitate in seed solution and not in endocarp solution. This indicates the presence of alkaloids in seed and not in endocarp.

✓ **Hager's Test (Saturated solution of picric acid)**

The acid layer with Hager's reagent gives yellow precipitate in seed solution and not in endocarp solution. This indicates the presence of alkaloids in seed and not in endocarp.

✓ **Dragendroff's Test (Solution of Potassium Bismuth Iodide)**

Acid layer with few drops of Dragendorff's reagent gives reddish brown precipitate in seed solution and not in endocarp solution. This indicates the presence of alkaloids in seed and not in endocarp.

TESTS FOR TANNINS

✓ **Ferric Chloride Test**

Few drops of ferric chloride were added to extract of both seed and endocarp separately. A brownish black color was observed

✓ **Lead Acetate Test**

Few drops of lead acetate were added to extract of seed and endocarp separately. Precipitate formation was slightly observed in seed solution but more concentrated in endocarp solution.

LACTONES TEST

✓ Legalon's Test

Few drops of Lega's reagent were added to extract of both seed and endocarp separately. A pink color was observed in seed solution but more concentrated in endocarp solution.

DITERPENE TEST

✓ Copper Acetate Test

Few drops of copper acetate to were added to oil extract of both seed and endocarp separately. A slight green color change was observed in seed solution but not observed in endocarp solution. This indicates the presence of diterpene in seed and its absence in endocarp.

GLYCOSIDES TEST

✓ Sodium Hydroxide Test

Few drops of sodium hydroxide solution were added to oil extract of both seed and endocarp separately. A yellow color change was observed in both solution but more concentrated in endocarp solution.

SAPONIN TEST

The oil extract of both seed and endocarp were added with water separately and was shaken vigorously for 1minute. No persistent foam formation was observed in both seed and endocarp solution indicating the absence of saponin in both samples

4.1.2 PHYTOCHEMICAL SCREENING:

The phytochemical screening of the ethanolic extracts of *Acacia nilotica* seeds and endocarp reveals a diverse profile of bioactive constituents, with distinct variations in concentration and presence between the two plant parts. These findings provide insight into the pharmacological potentials of each part and validate their traditional uses.

(A) Steroids

Both the Salkowski and Liebermann-Burchard tests returned positive results for steroids in the seed and endocarp extracts, indicating the presence of steroidal compounds. Steroids are essential bioactive constituents known for their anti-inflammatory and hormone-regulating properties. Their presence in both parts suggests potential utility in formulations for inflammation-related conditions.

(B) Triterpenes

The Salkowski test was negative for triterpenes, while the Liebermann-Burchard (L-B) test was positive in both seed and endocarp extracts. This discrepancy might be due to differences in test sensitivity or interference from other compounds. The presence of

triterpenes—known for antimicrobial, hepatoprotective, and anticancer effects—adds value to the therapeutic relevance of the plant parts.

(C) Tannins

A strong positive reaction was observed for tannins, with the ferric chloride and lead acetate tests yielding "++" and "+++" respectively, especially in the endocarp. This indicates a higher concentration of tannins in the endocarp. Tannins possess potent antioxidant, antimicrobial, and astringent properties, and their high concentration in the endocarp may contribute significantly to the plant's free radical scavenging potential.

(D) Lactones

Legalon's test yielded positive results, with stronger intensity in the endocarp (++). Lactones are known to exhibit anti-inflammatory and antimicrobial effects. Their presence in the extract enhances the pharmacological profile of *Acacia nilotica*, especially for treating infections and inflammatory conditions.

(E) Diterpenes

The copper acetate test indicated a weak presence of diterpenes in the seed extract (+), but not in the endocarp (-). Diterpenes are often associated with antioxidant and anti-cancer activity. Their selective presence in the seed suggests that certain bioactivities may be seed-specific.

(F) Glycosides

The sodium hydroxide test showed glycosides in both extracts, with stronger activity in the endocarp (++). Glycosides play crucial roles in cardioprotective and anti-inflammatory applications. Their higher concentration in the endocarp could be beneficial for cardiovascular and metabolic health support.

(G) Saponins

The foam test was negative in both extracts, suggesting the absence of saponins. Saponins are often linked with cholesterol-lowering and immune-boosting properties. Their absence may indicate that these particular extracts may have limited roles in those biological effects.

(H) Alkaloids

The alkaloid tests (Dragendorff's, Hager's, Wagner's, and Mayer's) showed a consistent presence of alkaloids in the seed extract but not in the endocarp. Alkaloids are well-known for their potent physiological effects including analgesic, antimalarial, and cytotoxic activities. Their exclusive presence in the seed extract implies that this part may have greater neuroactive or analgesic potential than the endocarp.

4.1.2.1 General Interpretation

Overall, the phytochemical screening shows that both seed and endocarp contain important bioactive compounds, but their distribution and intensity vary significantly. The **endocarp** is richer in **tannins, lactones, and glycosides**, which may explain higher antioxidant and antimicrobial properties in this part. On the other hand, the **seed** shows a

broader spectrum of secondary metabolites including **steroids, alkaloids, and diterpenes**, suggesting that it may have a wider range of therapeutic applications.

These differences are critical when selecting plant parts for specific medicinal uses. For antioxidant-focused applications, the **endocarp** may be more potent due to its high tannin and phenolic content. Meanwhile, the **seed** may serve better for neuropharmacological or anti inflammatory formulations, owing to the presence of alkaloids and steroids.

This analysis not only supports traditional knowledge surrounding *Acacia nilotica* but also provides a scientific basis for the differential use of its parts in herbal and pharmaceutical formulations.

4.1.3 ANTIOXIDANT ACTIVITY:

The experimental results present a comparative analysis of the antioxidant potential and carotenoid content specifically lycopene and β-carotene of ethanolic extracts of *Acacia nilotica* seeds and endocarp. These results offer critical insights into the bioactivity of the two plant parts and reflect their distinct phytochemical profiles.

4.1.3.1 DPPH Antiradical Activity

1. Endocarp Extract (51.41%) – High Antioxidant Activity

The endocarp extract exhibited a **high percentage of DPPH radical scavenging activity**, with **51.41% inhibition under control conditions**, and **46.10% under standard conditions**. These values indicate a strong capacity to neutralize free radicals,

which correlates with the high total phenolic content (49.56 mg GAE/g) found in the same extract.

This antioxidant potential is likely due to the high concentration of:

- **Tannins (+++)**: Known for their excellent hydrogen-donating ability.
- **Glycosides and lactones**, which are also known to exhibit redox activity. • **β-carotene** (0.034 mg/mL): A potent lipid-soluble antioxidant particularly effective against singlet oxygen and lipid peroxidation.

Thus, the endocarp stands out as the more potent antioxidant source between the two plant parts studied.

Seed Extract (14.47%) – Moderate Antioxidant Activity

The seed extract showed a **moderate DPPH inhibition** (14.47%) despite the presence of phenolics (18.42 mg GAE/g), alkaloids, and lycopene (0.013 mg/mL). Several reasons explain the comparatively lower antioxidant activity:

• The **phenolic concentration is lower** than that of the endocarp.
• Alkaloids, while pharmacologically potent, are **not highly reactive with DPPH radicals**.
• The contribution of lycopene to DPPH activity is **less pronounced**, as DPPH primarily detects polar antioxidants like phenolics and flavonoids, not lipid-soluble ones.

4.1.3.2 TPC Antioxidant Activity

Interpretation

The **endocarp extract** demonstrated significantly higher total phenolic content than the seed extract. Phenolic compounds are known to be among the most effective plant-based antioxidants due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

The observed TPC pattern correlates well with the phytochemical screening, where tannins and glycosides both phenolic derivatives were found in higher concentration (+++) in the endocarp than in the seed. This suggests that the **endocarp contributes more to the phenolic-based antioxidant capacity** of *Acacia nilotica*.

4.1.3.3 Comparative Analysis

- **Endocarp extract** had **more than 3× higher antioxidant activity** than the seed extract. • This suggests that **phenolic content is the dominant contributor** to the antioxidant power measured by the DPPH method.

• The **positive correlation** between TPC and DPPH inhibition, especially in the endocarp, supports previous research findings (Kumar et al., 2012; Sultana et al., 2007).

4.1.3.4 Possible Cause for Differences:

• The **distribution of phenolics** between the seed and endocarp tissues is naturally uneven due to physiological roles endocarps often contain higher levels of protective compounds like tannins.

• The **extraction efficiency** may also differ due to **tissue density and solubility**, with the endocarp releasing more phenolic compounds in ethanol.

• **Environmental factors** (e.g., sunlight, maturity, and storage) might have influenced phenolic accumulation more significantly in the endocarp tissues.

4.1.3.5 Lycopene Content

- **Seed:** 0.013 mg/mL
- **Endocarp:** 0.010 mg/mL

Lycopene Interpretation:

The seed extract contains a higher concentration of lycopene than the endocarp. Lycopene, a powerful singlet oxygen quencher and lipid-soluble antioxidant, is usually associated with red colored plant parts and contributes significantly to the antioxidant defense mechanism. Its higher content in seeds supports the antioxidant performance observed in the DPPH assay and may account for part of the free radical scavenging effect.

4.1.3.6 β-Carotene Content

- **Seed:** 0.023 mg/mL
- **Endocarp:** 0.034 mg/mL

β-carotene Interpretation:

In contrast to lycopene, β-carotene is significantly more abundant in the endocarp than in the seed extract. β-Carotene is a potent precursor of vitamin A and an important

antioxidant, particularly effective in quenching singlet oxygen and stabilizing lipid membranes. The higher β-carotene concentration in the endocarp suggests that this part may contribute more robustly to lipid-phase antioxidant activity despite having lower lycopene content.

4.1.4 Deviant Observation:

The inverse relationship observed between lycopene and β-carotene concentrations in the seed and endocarp is **not unusual**. This could be due to:

- **Differential metabolic distribution** in seed tissues.
- **Solvent specificity** in extracting structurally different carotenoids.
- Possible **isomerization** or conversion of one carotenoid type under extraction or storage conditions.

CONCLUSION

This study successfully investigated the phytochemical composition and antioxidant activity of the ethanolic extracts of *Acacia nilotica* seeds and endocarp, highlighting their rich content of bioactive compounds including steroids, triterpenes, tannins, lactones, glycosides, and alkaloids. The antioxidant assays demonstrated that both parts of the plant possess strong radical scavenging potential, which correlates with their phytochemical richness. Notably, carotenoid analysis revealed a higher concentration of lycopene in the seed and β-carotene in the endocarp, suggesting distinct antioxidant contributions from each part. Although both plant parts showed comparable antioxidant strength, slight variations in phytochemical distribution point to their differential therapeutic potentials. These findings scientifically validate the traditional use of *Acacia nilotica* and support its future development as a natural source of antioxidant compounds for nutraceutical or pharmaceutical applications.

APPENDIX

RAW DATA ON SEED

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

Where:

Ab = absorbance of control =1.451

Aa = absorbance of sample = seed 1.241

% DPPH antiradical activity = $\frac{1.451 – 1.241}{1.451} \times 100$ (control)

= 14.47%

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

Where:

Ab = absorbance of standard =1.308

Aa = absorbance of sample = seed 1.241

% DPPH antiradical activity = $\frac{1.308 – 1.241}{1.308} \times 100$

(standard) = 5.12%

RAW DATA ON ENDOCARP

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

Where:

Ab = absorbance of control =1.451

Aa = absorbance of sample (endocarp)= endocarp

0.705 % DPPH antiradical activity = $\frac{1.451 - 0.705}{}$ ×

100 (control) 1.451 = 51.41%

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

Where:

Ab = absorbance of standard =1.308

Aa = absorbance of sample (endocarp) = 0.705

% DPPH antiradical activity = $\frac{1.308 - 0.705}{}$ × 100 (standard)

1.308 = 46.10%

Lycopene and β-carotene Estimation

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

Where:

A663 = absorbance at 663nm = seed (0.287), endocarp (0.383) A505 = absorbance at 505nm = seed (-0.092), endocarp (0.048) A453 = absorbance at 453nm = seed (0.435), endocarp (0.683) Lycopene (mg/ml) =

$\frac{0.458(A_{663}) - 0.372(A_{505}) - 0.0806(A_{453})}{}$ 10

Where:

A663 = absorbance at 663nm = seed (0.287), endocarp (0.383) A505 = absorbance at 505nm = seed (-0.092), endocarp (0.048) A453 = absorbance at 453nm = seed (0.435), endocarp (0.683) **RAW DATA ON SEED**

β-carotene = $\frac{0.126\ (0.287) - 0.304\ (-0.092) + 0.450\ (0.435)}{10} = 0.023\text{mg/ml}$

Lycopene = $\frac{0.458\ (0.287) - 0.372\ (-0.092) - 0.0806\ (0.435)}{10} = 0.013\text{mg/ml}$

RAW DATA ON ENDOCARP

β-carotene = $\frac{0.126\ (0.383) - 0.304\ (0.048) + 0.450\ (0.683)}{10} = 0.034\text{mg/ml}$

Lycopene = $\frac{0.458\ (0.383) - 0.372\ (0.048) - 0.0806\ (0.683)}{10} = 0.010\text{mg/ml}$

RAW DATA ON SEED

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

Where:

C = Concentration = seed (42)

V = Volume of sample = 0.5ml

M = Mass of sample = 1.14g

$$\frac{A}{0.757}$$

42 C

Fig 2. Calibration curve showing Absorbance against Concentration of TPC (0.757 is the absorbance average of sample, while 42 is the concentration obtained from standard curve)

TPC= 42 x $\frac{0.5}{1.14}$ = 18.42mgEquiv/g

RAW DATA ON ENDOCARP

TPC (mgGAEquiv/g) = C × $\frac{V}{M}$

Where:

C = Concentration = endocarp (113)

V = Volume of sample = 0.5ml

M = Mass of sample = 1.14g

$\frac{A}{}$

1.566.

113 C

Fig 2. Calibration curve showing Absorbance against Concentration of TPC (1.566 is the absorbance average of sample, while 113 is the concentration obtained from standard curve)

TPC= 113 x $\frac{0.5}{1.14}$ = 49.56mgEquiv/g

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