

**PHYTOCHEMICAL SCREENING AND ANTIOXIDANT MINERAL
CONTENT OF FLESH AND SEEDS EXTRACT OF AFRICAN EBONY**

Project Report

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**In Partial Fulfilment of the Requirements for the Award of B.Sc. (Hons)
Degree in Biochemistry**

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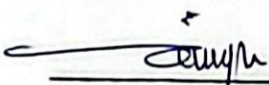
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CERTIFICATION

This is to certify that the research project reported here was conducted by AWITI PATRICIA MAGAJI (2010304002) and has been approved as meeting part of the requirements for the award of Bachelor of Science (B.Sc.) degree in Biochemistry of the Usmanu Danfodiyo University, Sokoto.


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DEDICATION

This project work is wholeheartedly dedicated to the Almighty God, the source of my strength, wisdom, and inspiration, whose divine guidance and unending grace have seen me through every stage of this academic journey. Without His blessings, this achievement would have been impossible. He has been my helper in times of difficulty, my comforter in times of discouragement, and my provider in times of need.

I also dedicate this work to my beloved Mother Mrs. Mustapha Lydia Ikhina and also to my beloved siblings whose unwavering love, prayers, encouragement, and sacrifices have been the backbone of my success. Their belief in my potential and their constant support have kept me motivated, even when the road seemed rough and the challenges appeared overwhelming, I say thank you. This work is as much yours as it is mine. Your encouragement, advice, and companionship made this academic journey worthwhile.

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ABSTRACT

This study examined the chemical makeup and antioxidant mineral content of the flesh and seeds of the African ebony tree *Diospyros mespiliformis*, a plant long used in traditional medicine. The goal was to evaluate the nutritional and therapeutic value of both the commonly eaten flesh and the often-overlooked seeds. Researchers used standard qualitative and quantitative chemical tests and gravimetric methods to profile phytochemicals, measured antioxidant activity with DPPH and FRAP assays, and quantified vitamins A, C, and E plus essential minerals using spectrophotometry. Screening found important bioactive groups tannins, flavonoids, saponins, alkaloids, steroids, and glycosides in both fruit parts, with the seeds richer in tannins and saponins. Saponins were the most abundant metabolites at 6.19–6.50 g per 100 g, followed by alkaloids and flavonoids. The extracts showed strong, dose-dependent free-radical scavenging and ferric-reducing activity, indicating clear antioxidant potential, and Vitamin E registered the highest antioxidant vitamin level at 111.3 percent. Mineral analysis showed more iron in the seeds and higher zinc and magnesium in the flesh. Overall, the fruit appears to be a promising natural source of antioxidants and micronutrients for functional foods and nutraceuticals, and the authors recommend further work on compound isolation, bioavailability, and toxicity to support therapeutic use.

CHAPTER ONE

1.0

INTRODUCTION

1.1 Background to the study

Diospyros mespiliformis, also known as African ebony, is a member of the Ebenaceae plant family. *Diospyros* is a general name that means “divine”, and *mespiliformis* comes from the Greek words “mesos”, which means “half”, and “pilos”, which means “bullets”. The plant produces mature fruits in the form of big yellow berries (Dangoggo *et al.*, 2012). The fruit is a fleshy berry with an enlarged calyx, which is yellow to orange when ripe (Esimone *et al.*, 2009). The plant is widely distributed throughout Africa, Asia, and parts of Europe (Ramadwa and Meddows-Taylor, 2023). It is traditionally used to treat fevers, wounds, malaria, diabetes mellitus, and other diseases in African countries including Nigeria (Ramadwa and Meddows-Taylor, 2023).

The fruits and leaves of *D. mespiliformis* are edible as well as nutritious and reported to contain secondary metabolites like tannins, alkaloids, saponins, flavonoids, glycosides, and steroids (Ebbo *et al.*, 2014). They also have important economic thereby forming a significant part of the diet for local communities (Hegazy and Lovett-Doust, 2016).

Phytochemicals are non-nutritive plant chemicals produced to protect plants against environmental stressors like cold, heat, bacteria, and fungi (Ebbo *et al.*, 2014). They are considered responsible for many positive health outcomes in humans. Plants produce significant amounts of antioxidants to combat oxidative stress induced by oxygen and light in the natural environment (Lobo *et al.*, 2010). These compounds, when extracted from natural sources, have promising applications in the development of functional foods and nutraceuticals (Shikov *et al.*, 2022).

Diospyros mespiliformis represents a nutritionally rich and ethnomedicinally important fruit with diverse bioactive compounds that may play significant roles in promoting health and preventing diseases. Although wild fruits, including African ebony, are known to be reservoirs of phytochemicals and antioxidant minerals, their flesh and seeds remain underexplored compared to other plant parts, such as the leaves and bark. This study, therefore, contributes by providing scientific data on the phytochemical profile and antioxidant mineral composition of the flesh and seeds of African ebony. The findings will not only validate its traditional uses but also reveal the potential of underutilized parts such as the seeds, thereby promoting their nutritional, therapeutic, and economic value.

1.2 Statement of the Problem

The increasing global burden of chronic diseases such as diabetes, cancer, cardiovascular disease, and neurodegenerative disorders is strongly associated with oxidative stress. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used to prevent oxidative damage, but concerns about their possible side effects and health risks have emerged. This highlights the urgent need for safe, natural alternatives derived from plants rich in phytochemicals and minerals with antioxidant properties.

Diospyros mespiliformis (African ebony) is a wild fruit of nutritional and ethnomedicinal importance in Africa, traditionally eaten as food and used to treat various ailments. While its leaves and bark have been studied for their medicinal properties, there is still limited scientific data on the phytochemical components and antioxidant vitamin and mineral contents of its fruit parts, especially the flesh and seeds. In addition, the seeds are often thrown away as waste, yet they could be a hidden source of bioactive compounds with important nutritional and therapeutic potential.

Therefore, it is essential to investigate the phytochemical profile and antioxidant vitamin and mineral contents of the flesh and seeds of African ebony.

1.3 Justification of the Study

The search for natural antioxidants is increasingly important due to health risks linked to synthetic alternatives and the rise of oxidative stress-related diseases. Phytochemicals like flavonoids, tannins, and phenols, along with minerals such as selenium, zinc, and copper, are vital for combating oxidative damage and maintaining cellular health. Investigating the phytochemical and mineral composition of African ebony (*Diospyros mespiliformis*) could unveil its potential in disease prevention and health improvement.

Despite its use in traditional medicine, there is limited scientific data on the bioactive compounds and antioxidant minerals in African ebony fruit and seeds. This study aims to determine the secondary metabolites, antioxidant vitamin and mineral contents of the African ebony.

1.4 Aim and Objectives

The aim of this study was to investigate the phytochemical constituents and antioxidant vitamin and mineral contents of the flesh and seed extracts of *Diospyros mespiliformis*

The objectives of the study are as follows:

- i. To phytochemical constituents of the flesh and seed extracts of *Diospyros mespiliformis*.
- ii. To determine the antioxidant vitamins and mineral contents of the flesh and seed extracts.
- iii. To determine the antioxidant properties of the flesh and seed extracts of *Diospyros mespiliformis*.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Concept of Medicinal Plants and Their Importance

Definition and historical use of medicinal plants.

Medicinal plants are plants used for medical purposes, containing therapeutic compounds that can prevent, treat, or cure diseases.

The World Health Organization defines medicinal plants as those used for medical purposes at some point in history. These plants have been crucial in healthcare for thousands of years, with over 50,000 medicinal plant species identified worldwide (Monib, 2024).

Historically, medicinal plants have been used across diverse cultures to flavor food, preserve health, and treat various ailments (Gyan *et al.*, 2020). They contain secondary metabolites like alkaloids, terpenoids, and phenolics that provide therapeutic effects (Alamgir, 2017). Notably, about 40% of global healthcare relies on traditional medicine, with 85% of traditional medicines being plant-based (Monib, 2024).

Medicinal plants are species whose parts (leaves, bark, roots, seeds, and fruits) contain bioactive chemical compounds used to prevent or treat disease (Ramadwa and Meddows-Taylor, 2023). The development of ideas related to the usage of medicinal plants with awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in the facilitation of man's life (Srivastava, 2018). The use of medicinal plants is deeply embedded historically in many societies African, Asian, Indigenous and

records (oral, written) show their use for fever, infections, pain, and ritual healing long before modern medicine (Diouf *et al.*, 2025).

Medicinal plants is an old treatment method as old as mankind itself. The connection between human and their search for drugs in nature dates from the far past, of which there are enormous evidence from different sources (written documents, preserved monuments, and even original plant medicines). Awareness of medicinal plants' usage is a result of the many years of struggles against diseases and man learned to pursue drugs in barks, seeds, fruits, and other parts of the plant of man's life (Srivastava, 2018).

2.1.1 of plants in traditional medicine and drug development.

Traditional medicine remains a primary source of healthcare for many communities' studies suggest up to 80% of rural populations in some developing countries rely on plant-based remedies (Adamu *et al.*, 2023). Many of the compounds used in modern drugs originated from plants (e.g. alkaloids, terpenoids), and modern bioprospecting continues to explore ethnomedicinal leads. *Diospyros mespiliformis* has been cited in reviews and ethnobotanical studies for diverse therapeutic uses (e.g., for fever, wounds, gastrointestinal disorders) and its bioactive compounds are being isolated and tested (Ramadwa and Meddows-Taylor, 2023; Bababe *et al.*, 2024).

2.1.2 Importance of bioactive compounds in disease prevention and treatment.

Bioactive secondary metabolites such as flavonoids, tannins, alkaloids, saponins, phenolic acids, glycosides, and terpenoids are known to have antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, hepatoprotective, and other effects (Bababe *et al.*, 2024). For example, α - and β -amyrin (triterpenoids) isolated from *D. mespiliformis* leaf extract exhibited strong antioxidant activity ($IC_{50} \approx 8.5 \mu\text{g/mL}$), a value approaching that of standard antioxidants (Bababe *et al.*, 2024). Such

compounds are promising for preventing oxidative stress-mediated diseases (e.g. liver disease, metabolic syndrome).

Bioactive compounds, called phytochemicals, are produced as secondary metabolites in plants that have beneficial effects on health when they are consumed as nutrients. Phytochemicals have an effective role in the formation of the color, smell and taste of the plants (Demir and Akpinar, 2020).

2.2 Phytochemicals in Plants

2.2.1 Definition of Phytochemicals and Types

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients

2.2.2 Role of Phenols

Phenols influence various physiological processes associated with plant growth and development such as seed germination, cell division, synthesis of photosynthetic pigments, improves tolerance and adaptability of plant under trivial conditions, and also participate in defense against ultra-violet radiation or attack by pathogens. Phenols promotes nutrient uptake and mobilization of elements like calcium, potassium, magnesium, zinc , iron by means of chelation of metallic ions, improved active absorption sites and soil porosity.

2.2.3 Roles of Polyphenols

The ability of polyphenols to show a discrepancy in nitrogen availability to the plants exists by means of influencing organisms affecting decomposition and specific N transformations or by complexation with proteins. Polyphenols and phenolic acids possess ability to prevent phosphate sorption and desorbed previously bound phosphate in plants .

2.2.4 Role of Flavanoids

Flavonoids in plants acts as growth regulator, combating agents, signal molecules, phytoalexins, detoxifying agents, stimulants for germination of spores, pollinator attractants, and allelochemical agents.

2.2.5 Role of Lipids

Lipids play pivotal role in plants as signaling molecules to regulate cell metabolism and energy storage compounds. In seeds of some plant speices, lipids are key form of carbon storage and comprise up to 60 % of the dry weight of the seeds. Cuticular lipids produced by epidermal cells acts as hydrophobic barrier preventing water loss and lend protection against pathogens and other environmental stresses. Plant lipids mainly include triacylglycerols, phospholipids, galactolipids and sphingolipids. Triacylglycerols accumulate as oils in seeds and fruits of vegetative tissues. Phospholipid acts as signaling molecules in numerous processes of higher plants such as root growth, pollen and vascular development, hormone effects, cell response to environmental stimuli, stomata closure, embryo development, light and sugar signal transduction.

2.2.6 Role of Anthocyanins

In plants, anthocyanins play essential role in reproduction by attracting pollinators and seed dispersers, and protection against various biotic and abiotic stresses such as solar radiation and ultraviolet radiation, cold temperature and water stress. Anthocyanins assist plant in their defense against pathogenic microorganisms by acting as chemical repellants or antifungal, anti-viral and antibacterial agents. Auxin and gibberellins restrain anthocyanin biosynthesis and thus retards fruit maturing. On the contrary, ethylene, jasmonic acid and abscisic acid uphold anthocyanin biosynthesis and thus improve fruit ripening.

2.2.7 Methods of phytochemical screening (qualitative and quantitative).

Qualitative screening methods include standard tests such as Mayer's or Dragendorff's for alkaloids; ferric chloride or Folin-Ciocalteu for phenolics; foam test for saponins; Bornträger's, or Liebermann-Burchard for steroids/terpenoids. Quantitative methods include measurement of Total Phenolic Content (TPC, often in mg Gallic Acid Equivalents per gram), Total Flavonoid Content (TFC), total tannins, saponins, alkaloids, etc., and use of in vitro antioxidant assays such as DPPH, ABTS, FRAP.

For example, in a study of *D. mespiliformis* stem bark extracts (methanol, petroleum ether, ethyl acetate) from Bauchi, Nigeria, Adamu *et al.* (2023) screened phytochemicals and found that the ethyl acetate fraction had highest antioxidant potential ($IC_{50} = 30.69 \pm 1.28 \mu\text{g/mL}$) via DPPH assay; the methanol extract and petroleum ether extract had higher IC_{50} values (less potent).

2.2.8 Biological and Pharmacological Activities of Phytochemicals (Antioxidant, Antimicrobial, Anti-Inflammatory).

Antioxidant activity: Multiple parts of *D. mespiliformis* have been shown to scavenge free radicals. Bababe *et al.* (2024) reported that the isolated α/β -amyrin had $IC_{50} \sim 8.5 \mu\text{g/mL}$, showing stronger activity than the crude extract ($IC_{50} \sim 10.5 \mu\text{g/mL}$). In a study by Adamu *et al.* (2023), the stem bark ethyl acetate extract had $IC_{50} \sim 30.69 \mu\text{g/mL}$ in DPPH assay.

Antimicrobial activity: Ebbo *et al.* (2019) reported that methanolic leaf extract had broad spectrum antimicrobial activity vs *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium* with minimum inhibitory concentrations (MIC) in the range 156.25–312.5 $\mu\text{g/mL}$.

Anti-inflammatory, hepatoprotective, anti-diabetic etc.: The stem bark extract of *D. mespiliformis* has been shown to have hepatoprotective effect: in a rat model of carbon tetrachloride (CCl₄)- induced liver damage, pre-treatment with methanol stem bark extract reduced marker enzymes, improved histology, reduced lipid peroxidation (Nwaogu *et al.*, 2022). Also, a recent in vivo study “Metabolite Profile, Antioxidant Activity and Ameliorative Efficacy” (Sani *et al.*, 2025) assessed stem bark extract in mice and showed efficacy in reducing oxidative stress induced by CCl₄.

Anti-glycation / anti-diabetic potential: *D. mespiliformis* and some other plant leaves have been evaluated for inhibition of protein glycation and oxidative stress (Usman *et al.*, 2023), suggesting implications for diabetes or its complications.

2.3 Antioxidant Minerals and Their Health Importance

Overview of antioxidant minerals (Zn, Se, Cu, Fe, Mn, Mg, Ca, K, etc.).

These minerals serve as essential cofactors in antioxidant enzymes (e.g., Cu, Zn in Cu/Zn-SOD; Mn in Mn-SOD; Se in glutathione peroxidases), are involved in metal chelation, redox reactions, and help in maintaining structural integrity of proteins and membranes (Ramadwa and Meddows-Taylor, 2023).

2.3.1 Mechanisms of action.

Minerals as enzyme cofactors, enabling catalytic removal of reactive oxygen species (ROS).

Direct scavenging / metal chelation (preventing free radicals generated via Fenton reactions).

Stabilization of cellular membranes, reducing lipid peroxidation, supporting structural or signaling roles in antioxidant pathways (e.g. zinc stabilizing thiol groups).

2.3.2 Nutritional and therapeutic importance in human health.

Deficiency in these minerals leads to impaired antioxidant enzyme function, increased oxidative stress, higher prevalence of immune dysfunction, anemia, poor wound healing, neurodegenerative risk. Adequate dietary supply of these minerals is crucial. Plants that combine phytochemicals with mineral content are doubly valuable.

2.4 The Genus *Diospyros mespiliformis* (Ebony Trees)

2.4.1 Botanical description and distribution of *Diospyros* species.

The genus *Diospyros* (family Ebenaceae) comprises over 500 species of trees and shrubs distributed throughout tropical and subtropical regions globally Africa, Asia, Pacific islands. Species vary in habit (tree/shrub), leaf morphology, fruit type, seed hardness. Many produce valuable hardwoods (ebony), edible fruits or medicinal parts (Ramadwa *et al.*, 2023).

The characteristic features of the *Diospyros* species are: trees, rarely shrubs, leaves alternate; flowers green, white or yellow, few to many; axillary cymes or the pistillate solitary; corolla campanulate, urceolate or tubular, the lobes 3–7 (usually 4–5); stamens 4–many (commonly 16); mostly with 4–8 staminodia in pistillate flowers, ovary 4–16 celled, fruit is a large juicy 1–10 seeded berry; the sap wood is white and soft and heartwood is black and hard. The genus is of great economic importance with many species yielding edible fruits, ebony and valuable timbers.

2.4.2 Ethnomedicinal uses of Diospyros plants in Africa and beyond

The genus *Diospyros mespiliformis* (Ebenaceae family) contains species that have been recognized and used in traditional medicine (extended ethnomedical use) and have potential new health benefits supported by in vitro biological, in vivo pharmacological, and clinical tests. Furthermore, within certain cultures or communities, various traditional systems have used all plant parts of this botanical genus (leaf, fruit, bark, twig, hardwood, and root) as herbal medicines.

Beyond their pharmacological value, *Diospyros* spp. have distinct and complementary important qualities, namely valuable wood, and edible fruits, which provide significant economic benefits and are recognized and utilized in various industrial and commercial sectors.

Based on the diverse description in the literature for the human use of the different parts of *Diospyros*, the results are grouped into infectious diseases (antibacterial, antifungal, anthelmintic, and antiviral); gastrointestinal (diarrhea, dysentery, emetic, flatulence, and other gastrointestinal disorders), oral cavity (oral hygiene, healing of oral wounds, and toothaches); urogenital (anti-hemorrhagic, dysmenorrhea, and infertility); skin diseases (dermatitis, fresh wounds, bedsores, and rashes); musculoskeletal (body pain, bruises, painful fractures, and rheumatism); and others conditions (diabetes, internal injuries, antidotes, hemostatic agents, and snake bites).

Among all the different *Diospyros* plant parts used in traditional medicine, the root is the most used part, and is most used to treat infectious diseases. In the treatment of gastrointestinal disorders, it corresponds to 59%, for oral cavity infections, 41%, and for skin diseases.

2.4.3 Reported phytochemicals and mineral composition in *Diospyros* spp.

The medicinal properties of fruits are closely related to their available phytochemicals as well as antioxidant capacity. Many of the indigenous fruits have been traditionally used as folk medicine. This study aims to evaluate the phytochemicals, mineral elements, and antioxidant properties. The phytochemicals, mineral elements, and antioxidants were carried out using standard methods of analysis. The result revealed that phytochemicals such as alkaloids, flavonoids, phenols, tannins, saponins, steroids, terpenoids, and glycosides were present in the fruit. While the mineral elements such as Potassium (K), Magnesium (Mg), Calcium (Ca), Sodium (Na), Iron (Fe), Manganese (Mn), Zinc (Zn), and Copper (Cu) were present indicated that the fruits have substantial mineral elements, however, varied according to the fruits species studied. The DPPH inhibition properties emphasize the diverse antioxidant capabilities of the studied fruits in which *Diospyros mespiliformis* (DS) emerges as a standout in DPPH inhibition activity, exhibiting robust antioxidant activity. Thenitric oxideradical scavenging reveals a concentration-dependent response in the NO radicals scavenging activity for all the fruits and ascorbic acid. The findings conclude that the desert fruits species revealed different phyto-constituents, mineral elements, Nitricoxide radical .

2.5 African Ebony (*Diospyros mespiliformis*)

2.5.1 Description (taxonomy, morphology, habitat).

Diospyros mespiliformis (Ebenaceae) is an evergreen tree, found in the tropical forest of Sub-Saharan Africa;from Ethiopia to Swaziland (Wallnöfer, 2001). It is also found in Angola, Nigeria, South Africa, Tanzania,Uganda, Yemen, Republic of Zambia and Zimbabwe (Arbonnier, 2002). *D. mespiliformis*, commonly known as African ebony tree, is an indigenous fruit tree species

widespread in the Soudano Guinean and Soudanian zones in Benin. The fruits of the species are edible, while the bark, leaves, stem and roots are employed for various purposes (Gnonlonfin *et al.*, 2018). Wide environmental and geographical variation often occurs within the natural range of the plant species (Akoegninou *et al.*, 2006). Adaptation of a species to this variation may produce different morphological and physiological characteristics, resulting in the development of ecotypes. Therefore, the conservation of a forest species requires knowledge on morphological variability to differentiate individuals and target interesting morphotypes. With an increasing demand for products and by-products of ebony trees, their supply and diversity are threatened by increasing deforestation. Therefore, the necessity for domestication (which involves selection of elite trees for multiplication) is paramount. As all species are distributed by habitat fragmentation or other anthropogenic factors, Beninese ebony diversity is declining (Abasse *et al.*, 2011). Therefore, enriching quantitative databases and monitoring programs for conservation is of great significance. Moreover, ebony species play a central role in many ecosystems.

2.5.2 Traditional and medicinal uses of African Ebony (roots, bark, leaves, fruits, seeds).

Traditional uses are expansive. Diouf *et al.* (2025) in their ethnobotanical review list many uses: for coughs, diarrhea, dysentery, toothache, gingivitis, skin fungal infections, ulcers, fever, pneumonia, syphilis, leprosy, wounds, antivenom, purgative, laxative, stimulating childbirth, etc. The stem bark is widely used for fever, wound healing; leaves are used as anti-inflammatory or in digestive disorders; seeds less commonly eaten but used medicinally.

2.5.3 Previous research findings on phytochemical constituents of the fruit and seeds.

Seeds: Ilouno *et al.* (2023) conducted proximate, mineral, and anti-nutritional composition study of the seeds. Phytochemicals measured in the seed flour included alkaloids (1.5217/mg/100g),

flavonoids (1.5392/1.5072mg/100g), saponins (1.4969 mg/100g), other anti-nutritional factors (phytate, oxalate) low.

Leaves: Bababe *et al.* (2024) isolated α - and β -amyrin from methanol leaf extract. Preliminary screening detected alkaloids, flavonoids, tannins, saponins, glycosides, etc.

Bark / Stem Bark: Adamu *et al.* (2023) performed phytochemical screening on stem bark; saw presence of alkaloids, tannins, phenolics, flavonoids, saponins, glycosides; their ethyl acetate fraction had better antioxidant activity than crude methanol extract or petroleum ether fraction. Shamsuddeen (2023) studied leaf and bark via methanolic extract: leaves and bark have alkaloids, tannins, saponins, flavonoids, steroids, cardiac glycosides; anthraquinones absent in leaves, terpenoids absent in bark (for that study) (Shamsuddeen, 2023).

2.5.4 Reported mineral composition and antioxidant properties.

Seed mineral content high in Ca (~180 mg/100g), Mg (~92 mg/100g), Fe (~90 mg/100g); low zinc (~0.97 mg/100g) (Ilouno *et al.*, 2023). Antioxidant capacity of seeds has been shown to be relatively strong in certain assays, especially when high phenolic and flavonoid content is present.

Fruit juice/pulp (“Kanya”): moderate Ca, high K; antioxidants (flavonoids, tannins), vitamin C also measured (UMYU Scientifica, 2024).

Bioactivity: antioxidant (DPPH, ABTS), antimicrobial, hepatoprotective, anti-glycation, etc. For instance, in vivo rodent studies with CCl₄-induced oxidative stress show ameliorative efficacy of stem bark extract (Sani *et al.*, 2025).

2.6 Relationship between Phytochemicals, Minerals, and Antioxidant Activity

2.6.1 How phytochemicals and minerals contribute synergistically to antioxidant defense

Phytochemicals such as phenolics, flavonoids, tannins directly scavenge radicals and chelate pro-oxidant transition metals; minerals such as Fe, Mg, Ca serve both structural functions and as cofactors for antioxidant enzymes. In *D. mespiliformis*, seed parts with high mineral content and high phenolic/tannin content show relatively strong antioxidant activity (Ilouno *et al.*, 2023). The presence of both components likely improves antioxidant potential compared to parts low in either minerals or phytochemicals.

2.6.2 Comparative evidence from other fruits with both edible flesh and seeds.

Diospyros mespiliformis (DSM) fruit seeds in human nutrition and health. Research on the potential uses of DSM has focused largely on the ethno-medical potential of the tree's leaf, bark, and root extracts. The bark, root, and leaf extracts are widely employed in ethno-medicine. A leaf decoction is reportedly used in the treatment of malaria and headaches, and as an anthelmintic. The bark extract reportedly provides good relief for coughing. Extracts from the plant have also been shown to have analgesic and antipyretic effects. DSM fruit pulp largely consists of carbohydrates; it has little protein but contains appreciable amounts of minerals as well as vitamin C. It also has low concentrations of oxalate, phytate, saponin, and tannin. As a human food resource, the soluble sugars and starch make DSM seeds a potential source of dietary energy. In drought years when conventional crops fail to thrive, the highly energy-dense seeds could be used to replace calories that would otherwise be derived from cereal grain. There is a need to fully characterize the anti-nutritional factor content of *Diospyros mespiliformis* seed and breed plant varieties with higher seed oil yields but maintaining the current fatty acid profile.

2.6.3 Implications for nutrition, health, and drug development.

Nutrition: consumption of fruit (pulp/juice) provides energy, vitamins, and minerals; seed consumption or use could supplement micronutrients and supply more concentrated phytochemicals. Drug development: isolable bioactive compounds (e.g., α/β -amyrin) can serve as leads. Also, in vivo evidence (hepatoprotection, antioxidant mitigation in induced oxidative stress) supports translational potential. However, safety, anti-nutritional factors, bioavailability, and standardization must be assessed.

2.7 Summary of Literature and Identified Gaps

What is known about phytochemicals and antioxidant minerals in *Diospyros mespiliformis*.

Multiple studies confirm presence of phytochemical classes: alkaloids, flavonoids, tannins, saponins, steroids, glycosides in various parts (leaf, bark, seed, pulp). (Shamsuddeen *et al.*, 2023).

Seeds have high mineral content (Ca, Mg, Fe) and lower anti-nutritional factors, though some variation exists in zinc and other trace elements (Ilouno *et al.*, 2024).

Research gaps.

1. There remain few in vivo human studies to confirm bioavailability, metabolism, safety, and efficacy of phytochemicals and minerals from *D. mespiliformis*.
2. Comparative studies under identical extraction/pulp vs seed vs leaf etc., across seasons/geographical zones, are limited. Variation due to climate/soil is under-explored.
3. Detailed structural identification of many bioactive compounds is still sparse beyond a few (e.g. α/β -amyrin); there may be many more bioactives uncharacterized.

4. Dose-response, toxicity, anti-nutritional factors over long term remain under-studied.
5. Standardization of assays and extraction methods (solvents, part used, age/maturity stage) is inconsistent, making cross-study comparisons difficult.

2.7.1 Justification for the present study.

A comprehensive study is justified that (a) compares flesh/pulp vs seeds vs leaves vs bark using standardized extraction procedures,

(b) quantifies both phytochemicals (TPC, TFC, tannins, etc.) and minerals (macro + trace), (c) conducts multiple antioxidant assays and possibly in vivo models of oxidative stress, (d) isolates and elucidates structures of the most active bioactives, (e) evaluates toxicity / safety / bioavailability, and (f) examines geographical and seasonal variation. This would address the above gaps and strengthen both nutritional and pharmacological understanding of *D. mespiliformis*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Material

The plant material was purchased at central market sokoto, sokoto state ,Nigeria on 3rd August 2025. It was then transferred to the department of plant science and biotechnology for identification and authentication on 4th August,2025.



Figure 3.1.1: African Ebony tree and fruit

3.1.2. Chemical Reagents and Standards

All chemical substances employed throughout the analysis were procured as high-purity analytical grade reagents. Key chemicals included dilute Hydrochloric Acid (HCl), 5% Ferric Chloride (FeCl_3), 10% Sodium Hydroxide (NaOH), 50% Sulfuric Acid (H_2SO_4), Fehling's solution (A and B), Ethyl acetate, 90% and 95% Ethanol, Acetone, Methanol, 0.0025M H_2SO_4 , Strong Ammonia (NH_3) solution, Chloroform, 12.5% Lead acetate, Bayet reagent, PR reagent, Xylene, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and the corresponding reagents for the Ferric Reducing Antioxidant Power (FRAP) assay. Standard reference materials for Vitamin A, Vitamin C, and Vitamin E were utilized for the respective quantification assay.

3.1.3. Equipments

The analytical instrumentation included a UV-Vis Spectrophotometer, employed for recording absorbance across various wavelengths relevant to specific assays 760nm, 495nm, 700nm, 450nm, and 517nm. A furnace was used for the ashing process required in the preparation of mineral solutions. Sample centrifuging, specifically for the Vitamin A determination, was performed at 2000 revolutions per minute (rpm) for 10 minutes. Boiling under reflux conditions utilized a reflux condenser apparatus, essential for the quantitative determination of saponins and flavonoids.

3.2 Methods

3.2.1 Sample Preparation

The plant sample (African Ebony) was washed twice under a running tap water to ensure absolute reduction of microbial contamination. Afterwards, the plant sample was dried in a hot air oven at 40 to further reduce its moisture contents. The plant was then grinded using mortar and pestle.

3.2.2 Qualitative Phytochemical Screening

The preliminary screening was conducted using 1g of the filtered fruit and seed extract for each test, relying on characteristic color changes or precipitate formation upon reaction with specific chemical reagents.

3.2.2.1. Test for Flavonoids (Alkaline Reagent Test)

A known amount (1g) of the extract was mixed with 10ml of distilled water, followed by the addition of 1ml of 10% Sodium Hydroxide solution. The formation of a yellow color in the mixture indicated a positive result.

3.2.2.2. Test for Tannins (Ferric Chloride Test)

About (1g) of the extract was combined with 10ml of distilled water 5% Ferric Chloride solution (2-3ml) was then added dropwise. A dark green color suggested the presence of condensed tannins, whereas a blue-black color confirmed the presence of hydrolysable tannins.

3.2.2 3. Test for Saponins (Foam Test)

Exactly (1g) of the extract was placed in a test tube with 10ml of distilled water and subjected to strong shaking. The tube was allowed to stand, and the persistence of froth (foam) for several minutes indicated a positive result.

3.2.2.4. Test for Glycosides (Modified Fehling's Test)

A known amount (1g) of the extract was mixed with 10ml distilled water and 2.5ml of 50% H_2SO_4 . The mixture was heated in a boiling water bath for 15 minutes. After cooling, the solution was neutralized using 10% NaOH. Subsequently, 5ml of Fehling's solution was added and brought to

a boil. The formation of a brick-red precipitate confirmed the presence of reducing sugars liberated from glycosides.

3.2.2.5. Test for Alkaloids (Precipitation Test)

About (1g) of the extract was mixed with 10ml of distilled water and 2ml of 10% aqueous Hydrochloric acid. The mixture was divided into two 1ml portions: one portion was treated with a few drops of Wagner's reagent, and the other with Mayer's reagent. The formation of a precipitate in either portion indicated the presence of alkaloids.

3.2.2.6. Test for Cardiac Glycosides

Exactly (1g) of the extract was mixed with 10ml of distilled water, followed by the addition of 2ml of 3.5% ferric chloride solution, and allowed to stand for one minute. 1ml of concentrated H_2SO_4 was then carefully layered down the side of the test tube. The observation of a reddish-brown ring at the interface suggested the presence of cardiac glycosides.

3.2.2.7. Test for Steroids (Salkowski Test Principle)

About (1g) of the extract was dissolved in 2ml of chloroform. 2ml of concentrated Sulphuric acid (H_2SO_4) was gently poured into the tube to form a separate lower layer. The appearance of a reddish-brown color at the interface was interpreted as a positive indication for steroids.

3.3.8. Test for Saponin Glycosides

A known amount (1g) of the extract was combined with 2.5ml of Fehling's solution (Solution A and B). The formation of a bluish green precipitate was used to confirm the presence of saponin glycosides.

3.3.9. Test for Balsams

Exactly (1g) of the extract was combined with an equal volume of 90% ethanol. Two drops of alcoholic ferric chloride solution was added to the mixture. A dark green coloration was interpreted as an indication of balsams.

3.3.10. Test for Anthraquinones (Bornträger's Test)

A known amount (1g) of the extract was shaken with 10ml of benzene. 5ml of 10% ammonia solution was subsequently added, and the mixture was shaken vigorously. The development of a pink, red, or violet color in the ammoniacal phase constituted a positive result.

3.3.11. Test for Volatile Oil

About (1g) of the fractionated extract was mixed with dilute HCl. The formation of a white precipitate was observed as the indication of volatile oil presence.

3.4. Quantitative Phytochemical Determination

The quantitative analysis of major secondary metabolites employed gravimetric and spectrophotometric methods to determine the concentration of compounds, expressed as a percentage weight of the initial sample (g/100g).

3.4.1. Determination of Total Flavonoid Content (Gravimetric Method)

This gravimetric method, adapted for solid recovery, involved placing 1{g} of extract into a 250ml conical flask. 100ml of dilute HCl was added, and the mixture was boiled under reflux for 35 minutes to achieve hydrolysis. The extract was filtered while hot. The resulting filtrate was subjected to precipitation by the dropwise addition of ethyl acetate. The precipitated flavonoid

material was collected by filtration on a pre-weighed filter paper (W1). The filter paper and precipitate were dried and subsequently re-weighed (W3). The total flavonoid content was calculated relative to the initial sample weight (W2 = 1g):

$$\text{Flavonoid (gram \%)} = \frac{W3 - W1}{W2} \times 100 \dots\dots\dots(\text{equation 1})$$

3.4.2. Determination of Total Saponin Content (Gravimetric Precipitation Method)

The method exploits the differential solubility of saponins. 1g of sample extract was placed in a 250ml conical flask with 100ml of 50% alcohol. The mixture was boiled under reflux for 30 minutes and immediately filtered hot through muslin cloth. The cooled filtrate was then mixed with an equal volume of acetone to ensure complete precipitation of the saponins. The precipitate was collected via filtration onto a pre-weighed filter paper (W1), washed with boiling 95% alcohol, dried, and weighed (W3). The percentage saponin content was calculated using the standardized gravimetric formula:

$$\text{Saponin (gram \%)} = \frac{W3 - W1}{W2} \times 100 \dots\dots\dots(\text{equation 2})$$

3.4.3. Determination of Total Alkaloid Content (Acid-Base Gravimetric Method)

The quantification of total alkaloids was achieved via acid-base extraction, followed by gravimetric measurement. 1g of sample was initially extracted using a methanol mixture, and the solvent was evaporated. The resultant residue was dissolved in 200ml of 0.0025M H₂SO₄ and subjected to partitioning with ether to remove neutral and unwanted components. The remaining aqueous fraction was subsequently basified using strong NH₃ solution. This step was followed by repeated extraction with excess chloroform to partition the alkaloidal fraction. The bulk chloroform extract was concentrated to dryness, collected on a pre-weighed filter paper (W1), and

weighed (W3). The percentage alkaloid was calculated based on the initial powder weight (W2 = 1g):

$$\text{Alkaloids (gram\%)} = \frac{W3 - W1}{W2} \times 100 \dots\dots\dots(\text{equation 3})$$

3.4.4. Quantification of Cardiac Glycosides

1g of the sample was extracted with 10ml of 70% alcohol and filtered. 1ml of the filtrate was combined with 1ml of 12.5% lead acetate in a 10ml cylinder, followed by a second filtration step. 2ml of this processed extract served as the sample, and 2ml of distilled water served as the blank. 2ml of Bayet reagent was added to both the sample and the blank, and they were allowed to settle for one hour. Absorbance readings were then obtained at 495nm. The cardiac glycoside index value was calculated

$$\text{as: } \frac{\text{Absorbance} \times 100}{17} \dots\dots\dots(\text{equation 4})$$

3.5. Evaluation of Antioxidant Properties

3.5.1. Determination of Antioxidant Vitamins

The concentration of antioxidant vitamins was determined spectrophotometrically relative to known standards. The general calculation for concentration determination was applied across all vitamin assays:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard} \dots\dots\dots(\text{equation 5})$$

3.5.1.1. Ascorbic Acid (Vitamin C)

0.5g of sample was dissolved in 10ml distilled water and allowed to dissolve for 30 minutes, followed by filtration. 1ml of the filtered sample was reacted with 1ml of PR reagent. Absorbance was recorded at 700nm. The standard used had a concentration of 0.39.

3.5.1.2. Vitamin A

An aliquote amount(0.5 mL) of the sample was combined with 0.5ml ethanol. 2ml of Xylene was added to the sample, the standard 1ml, concentration 60, and the blank. The mixture was subsequently centrifuged at 2000rpm for 10 minutes, and the resulting absorbance was read at 450nm.

3.5.1.3. Vitamin E

The concentration of Vitamin E was determined relative to a standard concentration 100 by measuring absorbance at 700nm.

3.5.2. In Vitro Antioxidant Activity Assays

For the antioxidant activity assays, 0.2g of the sample was extracted with 20ml of methanol. This stock extract 10mg /ml was used to prepare serial dilutions for dose-response testing.

3.5.2.1. DPPH Radical Scavenging Activity: Different concentrations

DPPH (2-2-diphenyl-1-picrylhydrazyl) is a stable free radical that absorbs light at 517nm, giving it a deep purple color, it donate an electron to the radical to neutralize it as DPPH radical is scavenged, the absorbance at 517 nm decreases, and the color change is directly proportional to the antioxidant activity of the substance.

An aliquant amount (0.2ml/g to 1.0ml/g) of the samples were prepared by combining 4ml of the methanolic stock extract with varying volumes of distilled water.

The percentage scavenging activity (%ΔPPH) was calculated using the control absorbance:

%ΔPPH =

$$\frac{\text{Absorbance of sample} - \text{Absorbance of control}}{\text{Absorbance of control}} \times 100 \dots\dots\dots (\text{equation 6})$$

3.5.2.2. Ferric Reducing Antioxidant Power (FRAP) Assay:

The FRAP assay, which quantifies the capacity of the extract to reduce Fe^{3+} to Fe^{2+} , was assessed across the same concentration range (0.2ml/g) to 1.0ml/g) as the DPPH assay. The percentage ferric reducing power (FRAB) was calculated relative to the control absorbance: FRAB =

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \dots\dots\dots (\text{equation 7})$$

3.6. Determination of Antioxidant Mineral Content

3.6.1. Sample Preparation and Ashing

The method outlined by Nurudeen et al. (2024) was used to analyze minerals in the sample. Exactly 5 g of the sample e was digested in 20 mL of aqua regia (1:3 HCl/HNO₃) until the cessation of brown fumes evolution. A known quantity (5 g) of the dried sample was weighed in a porcelain crucible and heated at 600 °C for 2 hours in a muffle furnace (Gallenkamp, Model OV 880, London, England). The burnt sample was allowed to cool for 1 hour and then digested in 20 mL of aqua regia. The solution was heated on an electric hotplate until the brown smoke vanished. The solution was then filtered using Whatman No. 1 filter paper and made up to 100 mL with distilled

water. The solution was then subjected to atomic absorption spectrophotometric analysis to obtain the concentrations of heavy metals.

CHAPTER FOUR

4.0 RESULTS, DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

4.1. Results

The analysis provided detailed data on the distribution of phytochemicals, antioxidant activity, and essential minerals between the fruit flesh (Sample A) and the seeds (Sample B) of *D. mespiliformis*.

4.1.1. Qualitative Phytochemical Profile

Table 4.1 shows the qualitative phytochemical results for both the fruits and seeds of African Ebony. The secondary metabolites screening reveals the presence of Tannins, glycosides, alkaloids, steroids, balsams and volatile oil in both samples (fruits and seeds).

It is notable that flavonoids was detected only in seeds of the African Ebony. However, anthraquines was absent in both the fruit and seeds of fruits and seeds of African Ebony.

Table 4.1. Qualitative Phytochemical Screening Results

Phytochemical Class	African Ebony	
	Fruit Flesh	Seed
Flavonoids	Not Detected (ND)	Presence (+)
Tannins	Presence (+)	Very High Presence (+++)
Saponins	Moderate Presence (++)	Very High Presence (+++)
Glycoside	Presence (+)	Moderate Presence (++)
Alkaloids	Presence (+)	Moderate Presence (++)
Cardiac glycoside	Presence (+)	Moderate Presence (++)
Steroids	Presence (+)	Moderate Presence (++)
Saponin glycoside	Not Detected (ND)	Moderate Presence (++)
Balsams	Presence (+)	Moderate Presence (++)
Anthraquines	Not Detected (ND)	Not Detected (ND)
Volatile oil	Presence (+)	Moderate Presence (++)

Keys:

+: Blue-black (Hydrolysable)

++:

+++; Dark green (Condensed)

4.1.2. Quantitative Phytochemical Content

The quantitative phytochemical screening of African Ebony reveals the presence of saponins, alkaloids, flavonoids, and cardiac glycosides in increasing order in both samples (fruits and seeds). Saponins>flavonoids>cardiac glycosides.(Table 4.2).

Table 4.2 Percentage Concentration of Secondary Metabolites (g/100g)

Phytochemicals	Sample A mean	Sample B mean
Saponins	6.19 ± 0.08 ^a	6.50 ± 0.08 ^b
Alkaloids	5.08 ± 0.94 ^a	1.12 ± 0.16 ^a
Flavonoids	4.77 ± 0.04 ^a	ND
Cardiac Glycosides	2.13 ± 0.03 ^a	ND

Keys:

Sample A =Flesh

Sample B = Seed

ND = Not Detected

Values are mean ± standard deviation of three replicates. Values with the same superscripts across the column are not significant (P<0.05).

4.1.3. Antioxidant Vitamin Content

Table 4.3. Summary of Antioxidant Vitamin Content.

		Vitamins (%)		
Sample		Vitamin A	Vitamin B	Vitamin C
African	Ebony	59.56 ± 1.79	111.3 ± 1.74	0.72 ± 0.00153
(fruit)				

4.1.5 Antioxidants Assay of DPPH and FRAP

The antioxidants activities of the methanol extract of *Diospyros mespiliformis* (canya) fruit and standard ascorbic acid are presented in figure 4.1

The methanol extract of *Diospyros mespiliformis* fruit shows significant free radicals scavenging activity and reducing power comparable to the standard ascorbic acid, indicating its potential as a natural antioxidant sources.

The results illustrate that ascorbic acid significantly increases the activity of DPPH and FRAP when compared to the *Diospyros mespiliformis* fruit, In addition, the fruit extracts exhibited significant activity in scavenging hydroxyl radicals when compared to the standard (ascorbic acid) showing its potential as a natural antioxidants source.

a.

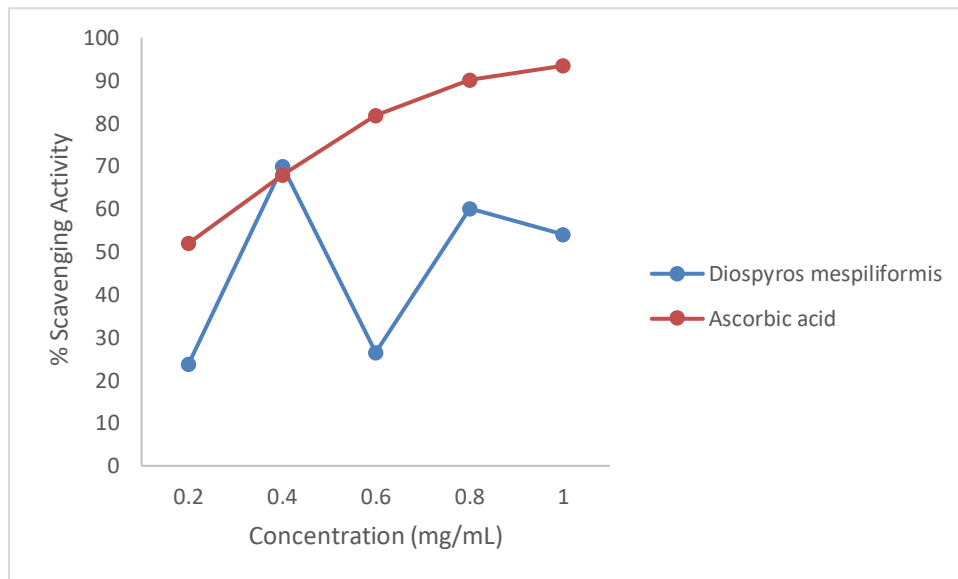


FIGURE 4.1: DPPH Assay of African Ebony (*Diospyros mespiliformis*)

b.

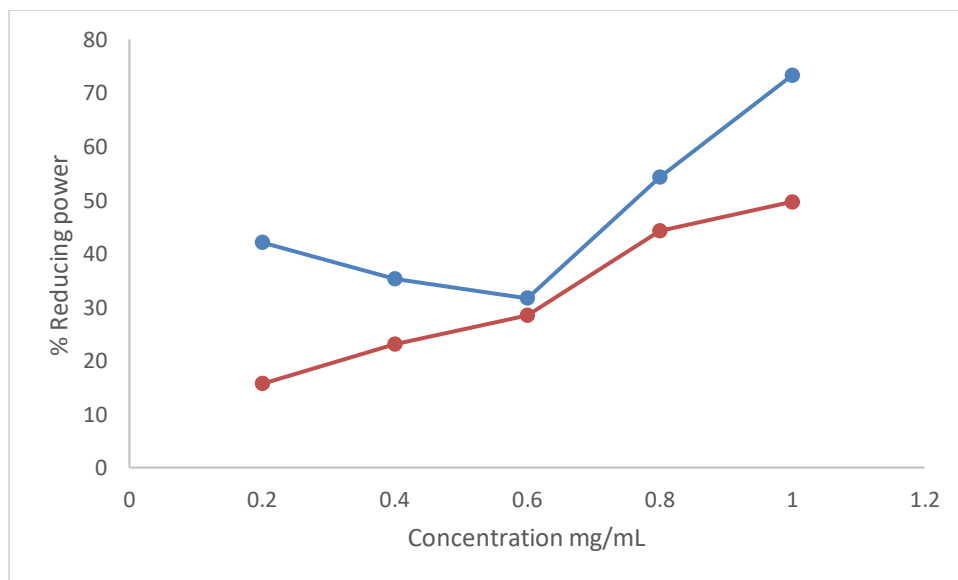


FIGURE 4.1: FRAP Assay of African Ebony (*Diospyros mespiliformis*)

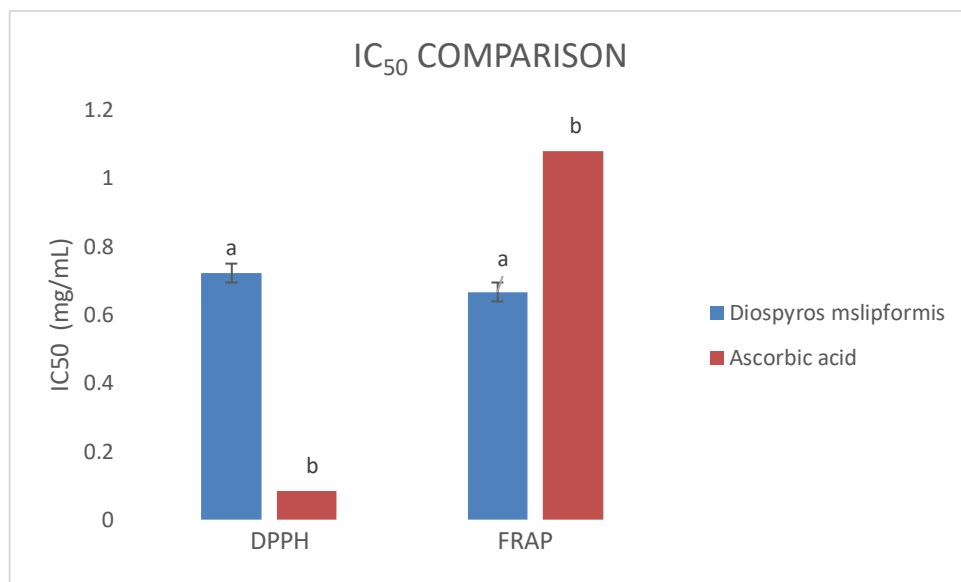


FIGURE 4.2; IC₅₀ OF DPPH and FRAP of African Ebony (*Diospyros mslipformis*)

Figure 4.2; reveals the IC₅₀ of DPPH and FRAP of African Ebony. The results shows a significant increase in the activity of DPPH for African Ebony when compared with the ascorbic acids. Similarly, a significant increase (0.05) was observed in the activity of FRAP for African Ebony when compared with ascorbic acid.

4.1.5. Essential Antioxidant Mineral Content

Figure 4.5 shows the mineral compositions of fruits and seeds of African Ebony. A significant increase ($p < 0.05$) in the fruit for iron (10.50), magnesium (13.00) and selenium (12.41) when compared to seeds in which iron ,magnesium and selenium with 1.10ppm ,11.18, and 11.01 respectively.

However, no significant difference ($p > 0.05$) was observed in the concentration of zinc, and copper in the fruit and seeds of African Ebony.

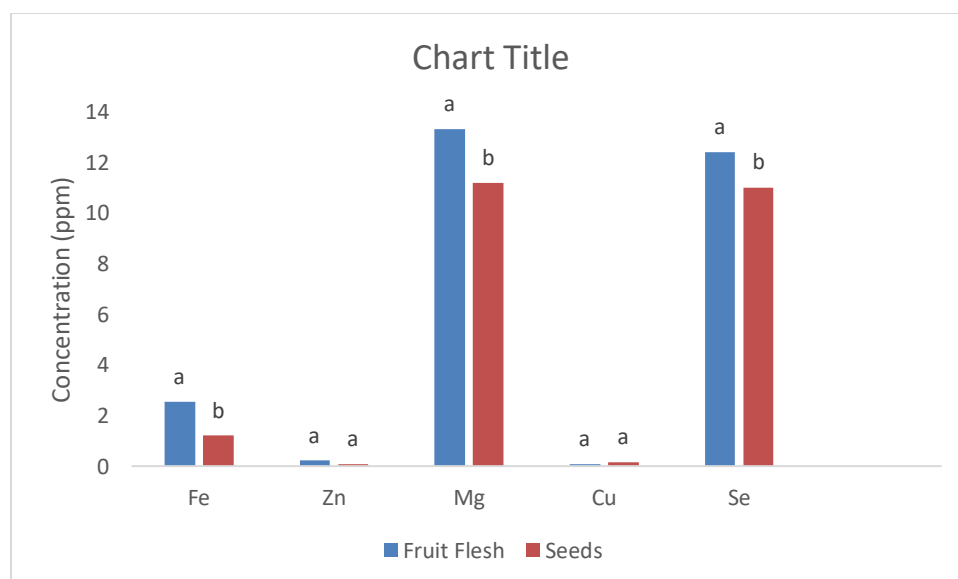


Table 4.5; Essential Antioxidant Mineral Content (Units/g extract)

4.2. Discussion

From this study, it was determined that both the flesh and seeds of *Diospyros mespiliformis* (African ebony) contain important health-giving compounds, including phytochemicals, vitamins, and minerals. This illustrates that the fruit is edible and showcases the potential of the fruit as a natural health food. The qualitative analysis identified the presence of tannins, flavonoids, saponins, alkaloids, steroids, and glycosides in both parts of the fruit, albeit in differing quantities. These compounds are particularly important in protecting the body from oxidative stress, infection, and inflammation, as documented by Adamu *et al.* (2023) and Bababe *et al.* (2024). What is particularly interesting is that the seeds of the *Diospyros mespiliformis* contained more tannins and saponins than the fruit flesh. This demonstrates that seeds, which are typically considered waste, may actually be a biologically active, therapeutic resource. As noted in the study by Demir and Akpinar (2020), tannins are strong antioxidants and have the ability to neutralize and chelate free radicals and saponins exhibit anti-inflammatory and cholesterol-lowering properties. This is in

concurrence with the work of Shamsuddeen (2023) and Ramadwa & Meddows-Taylor (2023) who documented similar phytochemical constituents in other parts of *Diospyros mespiliformis*, including the bark and leaves.

Furthermore, the plant's might help in boosting the immune system due to its high saponin, flavonoids, alkaloids, and cardiac glycosides content. This is constant with the study conducted by Hegazy *et al.*, (2019).

Analysis of antioxidants vitamins revealed A, C, and E vitamins in significant quantities, with E vitamin being the most abundant. Vitamin E, tocopherol, protects cell membranes from oxidative damage, while C vitamin, ascorbic acid, aids in immune function and vitamin E recycling (Kumar *et al.*, 2023). The high amount of these vitamins suggest that these vitamins can mitigate oxidative stress, and improve the overall immune functions (boost the immune system). This is consistent with the previous studies of Ruiz-Rodriguez *et al.*, (2014).

In the DPPH and FRAP tests of antioxidant activity, the methanol extract of *D. mespiliformis* exhibited significant and dose-dependent free radical scavenging and ferric-reducing activities. These activities are due to the fruit possessing certain compounds that can stabilize free radicals by donating electrons or hydrogen, and the activities increase with concentration (Adamu *et al.*, 2023). The extract's activity, while in comparison to ascorbic acid, the standard antioxidant of stronger activity, is nonetheless impressive and suggests potential as a natural antioxidant. These findings align with those of Bababe *et al.* (2024) and Sani *et al.* (2025), who also noted significant antioxidant activity in *D. mespiliformis* extracts. The phytochemicals and antioxidant minerals present in the extracts may explain the antioxidant activity. Also, the presence of minerals may explain the enhancement of the fruit's overall antioxidant ability.

The mineral analysis also showed that iron and magnesium were more concentrated in the flesh of the fruit, and that the seed had more iron, but less zinc and magnesium. For example; Iron is crucial for the formation of blood and the metabolism of energy, and zinc and copper are cofactors in the antioxidant enzyme superoxide dismutase (Ramadwa Meddows-Taylor, 2023).

Strong evidence indicates an abundance of phytochemicals along with other important minerals present in African ebony, indicating its potential importance in supplying essential micronutrients and antioxidants naturally (Li *et al.*, 2016). Hence, this research highlights, *Diospyros mespiliformis* is a wild edible fruit, and, above all, an important source of bioactive compounds. The flesh of the fruit is a concentrated source of vitamin antioxidants, while the seeds have an even greater concentration of minerals and other phytochemicals. Therefore, the plant is an important candidate for preparing products with a potential natural source of antioxidants, functional foods, and nutraceuticals (Shikov *et al.*, 2022; Diouf *et al.*, 2025). The next steps in research involve isolating and defining the most active compounds and assessing their safety and bioavailability for dietary and therapeutic use in managing oxidative stress.

4.3 Conclusions

The study provides scientific backing for the traditional medicinal use of *Diospyros mespiliformis* and highlights its promise as a natural source of antioxidants and micronutrients. The coexistence of phytochemicals, vitamins, and minerals supports its potential role in preventing or managing oxidative stress-related conditions such as cardiovascular disease, diabetes, and cancer. Importantly, the seeds commonly overlooked show substantial nutritional and therapeutic value and should be considered for use alongside the flesh. Overall, the research reinforces the importance of wild fruits in nutrition, health, and sustainable economic use.

4.4 Recommendations

1. Use both flesh and seeds in food and pharmaceutical formulations; process seeds into powders, oils, or extracts for supplements and nutraceuticals.
2. Promote African ebony as a functional food by raising public awareness and encouraging its inclusion in local diets to boost antioxidant intake.
3. Pursue compound isolation using advanced techniques such as GC–MS and HPLC to identify and quantify the specific bioactive constituents.
4. Conduct toxicological and pharmacological studies including in vivo and clinical trials to determine safety, long-term toxicity, and bioavailability for human use.
5. Encourage industry uptake by fostering collaboration between scientists and the food/pharmaceutical sectors to develop natural antioxidant products.
6. Support conservation and sustainable harvesting through cultivation and management practices to prevent overexploitation and protect ecosystems.
7. Provide government and policy support in the form of funding and technical assistance for further research into indigenous wild fruits.
8. Empower communities with value-addition skills so rural populations can process the fruit and seeds into marketable products (dried powders, teas, fortified foods) to improve livelihoods.

In sum, the study validates the antioxidant and nutritional value of *D. mespiliformis* and points to opportunities for research, conservation, and commercial development to fully realize its therapeutic and economic potential.

4.4 Summary

This study carried out a thorough analysis of the phytochemical profile and antioxidant-related mineral content of both the flesh and seeds of *Diospyros mespiliformis* (African ebony). Results show that both fruit components are rich in bioactive compounds and essential nutrients, underpinning their notable antioxidant and health-promoting properties. The research confirms that this commonly eaten and traditionally used fruit contains a range of phytochemicals, vitamins, and minerals that could serve as natural alternatives to synthetic antioxidants.

Phytochemical findings

Qualitative and quantitative tests detected tannins, saponins, flavonoids, alkaloids, glycosides, steroids, and cardiac glycosides in both flesh and seed extracts. Saponins were the most prevalent secondary metabolites, followed by alkaloids and flavonoids. Notably, the seeds often treated as waste had higher levels of tannins, saponins, and flavonoids than the flesh, indicating they are an underused source of valuable bioactive compounds. These observations are consistent with earlier reports on the species' phytochemical composition in other plant parts.

Vitamins and minerals

Analysis of antioxidant vitamins showed vitamin E to be the most abundant, with vitamins A and C present at lower but significant levels; all three contribute to protection against oxidative stress. Mineral profiling revealed that iron and magnesium were more concentrated in the fruit flesh, while the seeds contained relatively more iron but lower zinc and magnesium. These micronutrients act as cofactors for antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, supporting the body's defense against oxidative damage.

Antioxidant activity

Both DPPH and FRAP assays demonstrated that flesh and seed extracts possess strong, concentration-dependent antioxidant activity comparable to ascorbic acid. The extracts effectively scavenged free radicals and reduced ferric ions, indicating capacity to mitigate cellular oxidative damage. The combined presence of phytochemicals and antioxidant minerals suggests a synergistic effect that enhances the fruit's overall antioxidant potential, aligning with similar findings in other studies of the species.

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