DETERMINATION OF THE INVITRO ANTIOXIDANT PROPERTIES OF *COLA NITIDA*

BY

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DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN

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CERTIFICATION

We the undersigned hereby certify that AJOGUNGBE TEMITUOKPE (BMS2004982) carried out this research in the Department of Medical biochemistry, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B. Sc) in Medical biochemistry.

Signed

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DEDICATION

This work is dedicated to God almighty, my comforter, strength, guidance, inspiration, protector, provider, the one who saw me through school and made it possible to complete my Bachelor of Science Degree (B. Sc) program in the Department of Medical biochemistry.

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ABSTRACT

Cola nitida, commonly known as kola nut, is widely recognized for its stimulant and medicinal properties. This study investigates the antioxidant potential of its ethanol extract, highlighting its significance as a natural source of bioactive compounds. Oxidative stress plays a crucial role in the onset of chronic diseases, thereby increasing the need for effective and safe antioxidants. Ethanol extraction, widely regarded for its ability to isolate phytochemicals, was utilized to obtain the active constituents of *Cola nitida.*

Through various invitro assays such as determination of DPPH radical scavenging ability, determination of the ferric-reducing antioxidant property (FRAP), determination of Fe^{2+} chelating ability, determination of hydroxyl radical ($OH^{\bullet}$) scavenging ability and determination of nitric oxide ($NO^{\bullet}$) scavenging ability with quercetin as the control. The result disclosed DPPH radical scavenging capacity of the extract (EC_{50} = 0.539 ± 0.010 mg/mL) was lower than that of Quercetin (EC_{50} = 0.453 ± 0.010 mg/mL). Similarly, the Fe^{2+} chelating ability (EC_{50} = 0.162 ± 0.010 mg/mL) and nitric oxide scavenging activity (EC_{50} = 0.107 ± 0.05 mg/mL) were slightly lower than those of Quercetin (EC_{50} = 0.147 ± 0.009 mg/mL and EC_{50} = 0.103 ± 0.05 mg/mL, respectively). The FRAP assay indicated no significant difference between the extract and the control. However, the extract demonstrated a significant hydroxyl radical scavenging activity (EC_{50} = 0.062 ± 0.008 mg/mL), which was more potent than quercetin (EC_{50} = 0.086 ± 0.009 mg/mL) The ethanol extract exhibited strong free radical scavenging properties and lipid peroxidation inhibition, demonstrating its potential in combating oxidative stress. This research highlights Cola nitida as a promising natural antioxidant source with potential applications in pharmaceuticals, nutraceuticals, and functional foods.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Medicinal plants, also known as medicinal herbs, have been utilized in traditional medicine since ancient times. These plants serve as natural sources of bioactive and therapeutic compounds that contribute significantly to disease prevention and overall human health. They include various plant species whose parts—such as flowers, leaves, roots, stems, fruits, or seeds—are used directly or in formulated preparations to treat illnesses and health conditions (Heinrich et al., 2017).

According to the World Health Organization (WHO), nearly 80% of the population in developing countries relies on traditional and complementary medicine for their primary healthcare needs. In recent years, the use of medicinal plants has grown considerably due to their affordability and widespread acceptance, rooted in centuries of traditional use. Their effectiveness stems from the presence of secondary metabolites with pharmacological properties, many of which serve as key components in treatments for conditions such as cancer, viral infections, epilepsy, bacterial infections, inflammation, pain relief, and diabetes (Rates, 2001).

Cola nitida is a plant species within the Cola genus, widely recognized for its fruit, the kola nut. Native to tropical Africa, it belongs to the Malvaceae family. This species exists in different varieties and is known to contain several bioactive compounds, including catechin, caffeine, epicatechin, polyphenols, alkaloids, tannins, saponins, bromelain, cardenolides,

proanthocyanidins, triterpenes, glycosides, flavonoids, anthraquinones, steroids, anthocyanins, and other phytochemicals (Ajaiyeoba & Fokunang, 2009).

Due to its rich phytochemical composition, *Cola nitida* possesses significant medicinal properties. Various studies have demonstrated its antibacterial, antifungal, antioxidant, anticoagulant, and thrombolytic activities (Lateef et al., 2016). Additionally, research indicates that its high caffeine content makes it valuable in the production of pharmaceuticals, soft drinks, and wines. It is also known for its stimulating effects, boosting energy levels and reducing hunger (Ajaiyeoba & Fokunang, 2009).

Traditional medicine has long attributed Cola nitida with numerous therapeutic benefits, claiming it helps in treating infections, skin conditions, ulcers, toothaches, digestive disorders, headaches, depression, low libido, coughs, asthma, dysentery, constipation, and various eye-related ailments. Given its extensive pharmacological potential, the plant holds promise for the development of new drugs, functional foods, and nutraceuticals, contributing to human health and well-being.

Antioxidants are substances that prevent oxidation by inhibiting the oxidative process when present in small quantities relative to the substrate. Oxidation, often occurring as autoxidation, is a chemical reaction that generates free radicals, leading to the breakdown of organic compounds, including biological materials. The efficiency of an antioxidant system depends on various factors, such as activation energy, chemical structure, metabolism, absorption rate, redox potential, solubility, and volatility (Yogesh & Ali, 2014).

Antioxidants also play a secondary role inslowing down oxidation by mechanisms such as binding metal ions that catalyze oxidation, donating hydrogen to primary antioxidants, capturing oxygen, and neutralizing single oxygen ($^1O^2$) through enzymatic actions of glucose oxidase, superoxide dismutase, and catalase. Additionally, they help absorb UV radiation and break down hydroperoxides, further preventing oxidative damage (Angelo & Jorge, 2007). Based on their source, antioxidants are categorized as either natural or synthetic, with natural antioxidants being widely used in the food industry. Among the richest sources of antioxidants are plant-based foods, particularly fruits and vegetables, which contribute significantly to health and disease prevention.

Cola nitida is a significant natural source of antioxidants, which are essential compounds that help mitigate oxidative stress by neutralizing harmful free radicals in the body. These antioxidants contribute to reducing inflammation and stimulating metabolic processes that boost the body's natural defense mechanisms. The bioactive compounds found in *Cola nitida* work jointly to prevent cellular damage and protect the body from the harmful effects of oxidative stress (Ajaiyeoba & Fokunang, 2009).

1.2 Justification of Study

The increasing interest in natural remedies has brought attention back to *Cola nitida*, a tropical African plant traditionally used for various ailments. This study explores its therapeutic potential, focusing on its antioxidant properties and health benefits. Research shows that *Cola nitida* contains bioactive compounds like polyphenols, flavonoids, and alkaloids, which offer antioxidant, anti-inflammatory, and antimicrobial effects. Its high caffeine content and rich

phytochemical profile make it a valuable resource for developing new pharmaceuticals and functional foods.

1.3 Aim of Study

The study aims to determine the antioxidant capacity of the ethanol extract of *Cola nitida*

1.4 Objectives

1. To identify and determine the antioxidant potential of *Cola nitida*

2. To evaluate the antioxidant effects of *Cola nitida* and identify its effective concentration.

3. To determine the amount of antioxidant present using a control in *Cola nitida*

CHAPTER 2

LITERATURE REVIEW

2.1 THE PLANT *COLA NITIDA*

Cola nitida belongs to the Malvaceae family under the subfamily Sterculioideae. The genus *Cola*, which is native to West Africa, comprises between 100 and 125 species that flourish in various forest ecosystems across the region (Cheek, 2002). Among these, *Cola nitida, Cola acuminata, and Cola verticillata* are particularly notable, with *Cola nitida and Cola acuminata* receiving the most scientific attention. A distinguishing feature between these two species lies in their seed structure; *Cola acuminata* typically has 3 to 6 cotyledons, while *Cola nitida* has only 2 (Cheek, 2002).

Known as "Kola nut" in English, *Cola nitida* is deeply embedded in the cultures of many Nigerian ethnic groups, where it carries different local names; “Goro” in Hausa, “Oji” in Igbo, and “Obi” in Yoruba. The nut is much more than a consumable item; it plays a vital symbolic role in social and ceremonial contexts. In Yoruba tradition, it is associated with unity and peace, often used during rituals, festivals, and important gatherings (Daramola, 2004). Similarly, in Igbo society, the presentation of kola nut marks the beginning of most formal events and is considered a sacred act of goodwill (Ajai, 2011). Geographically, *Cola nitida* is indigenous to countries such as Sierra Leone, Liberia, Ghana, and Nigeria. Although it prefers lowland environments, it can also thrive at elevations of up to 300 meters provided the soil is fertile and rainfall is well distributed

throughout the year (Osei-Bonsu & Asante, 2005). Over time, its cultivation has extended beyond Africa to other tropical regions like Brazil, India, and Jamaica.

Propagation methods for *Cola nitida* include seed planting, the use of stem cuttings, and grafting. Among these seed propagation is the most widespread, with germination typically occurring within one to three months under favorable conditions. Stem cuttings and grafting are also effective, especially for enhancing desirable plant traits (Osei-Bonsu & Asante, 2005).

The processing of kola nuts generates considerable agricultural waste, especially in the form of discarded pods and seed shells. In Nigeria alone, annual waste production from kola nut processing is estimated to exceed 90,000 metric tons (Ogunwusi, 2013). These by-products present an opportunity for sustainable reuse in areas like composting, bioenergy, and industrial applications.

Phytochemical investigations of Cola species have revealed the presence of various bioactive compounds, including tannins, alkaloids, and flavonoids, which underpin their medicinal properties. These compounds contribute to the plant's noted therapeutic potential, which includes antimicrobial, antidiabetic, anti-inflammatory, and anticancer activities (Edeoga et al., 2005).

2.1.1 General Information

Scientific name: *Cola nitida*

Common name(s): kolanut, cola, bitter Kola

Family: Malvaceae

Plant type: tree

Planting month: year round

Origin: native to Sierra Leone, Liberia, Ivory Coast, Ghana and Nigeria

Availability: generally available in areas with deep, rich soils and evenly distributed rainfall

2.1.2 Description

Height: 39 to 66 feet

Spread: 20 to 33 feet

Plant habit: upright

Plant density: dense

Growth rate: moderate

Bark: rough, scaly, and sometimes fissured

Leaf arrangement: alternate

Leaf type: simple

Leaf shape: elliptical, oblong

Leaf venation: pinnate

Leaf colour: dark green on the upper surface, lighter green below

Flower colour: yellowish to reddish with purple streaks

Flower sexuality: hermaphroditism

Fruit shape: star-shaped

Fruit texture: woody, rough

Fruit colour: brown

Seed shape: oval

Seed colour: red

2.1.3 Botanical Description of the Plant

The *Cola* genus comprises perennial, evergreen trees and shrubs, with *Cola nitida* distinguished by its dark green, glossy leaves, which are alternately arranged and possess a leathery texture. These leaves exhibit an elliptical to oblong shape, with a prominent central vein and secondary veins branching outward in a pinnate pattern (Odebunmi et al., 2009). The tree produces flowers in clustered inflorescences, which may be axillary or terminal, often forming panicles. Male inflorescences are dense, containing numerous small flowers supported by bracts, while female inflorescences are more sparsely arranged with fewer flowers per node. In some cases, hermaphroditic inflorescences are observed, where female flowers are positioned at the lower nodes and male flowers at the upper nodes (Odebunmi et al., 2010).

Flowers of *Cola nitida* are unisexual and lack prominent petals. Male flowers are typically small, short-stalked, and rounded in bud form, with a five-lobed calyx enclosing 10 to 15 stamens. The

anthers are elongated and sometimes slightly curved. Female flowers, on the other hand, are either sessile or borne on short stalks, featuring a superior, hairy ovary that is divided into multiple carpels, each containing one or more ovules (Okwu, 2004). The fruit of *Cola nitida* is a large, woody capsule that takes an oblong to ovoid form. Upon maturity, it splits open, revealing multiple seeds commonly referred to as kola nuts. These seeds vary in color from white to reddish-brown or purple, depending on the variety, and have a wrinkled or rough outer texture. Internally, they contain a whitish endosperm, while the embryo is characterized by broad, flat cotyledons (Russell, 1955).

Phytochemical studies have identified various bioactive compounds in *Cola nitida*, including caffeine, theobromine, tannins, flavonoids, and alkaloids. These compounds contribute to its stimulant properties, making the seeds valuable in traditional medicine, cultural practices, and commercial industries such as food, beverages, and herbal remedies (Okwu, 2004).

2.1.4 Taxonomical Classification

Taxonomical classification is a systematic approach used in science to categorize and arrange living organisms according to their shared traits, differences, and evolutionary lineage. The term "taxonomy" originates from the Ancient Greek words taxis, meaning "arrangement," and nomia, meaning "method." It refers to the scientific practice of naming, defining, and classifying organisms based on common characteristics. Organisms are classified into hierarchical groups known as taxa (singular: taxon), each assigned a specific taxonomic rank. The primary taxonomic ranks in modern classification include domain, kingdom, phylum (or division in botanical contexts), class, order, family, genus, and species.

Cola nitida taxonomical classification;

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Malvales

Family: Malvaceae

Genus: Cola

Species: Cola nitida

Figure 2.1: Image of Cola nitida (Micheal Hermann)

Figure 2.2: Image of Cola nitida seed, kolanut (Marco Schmidt)

2.2 USE OF THE PLANT (MEDICINAL BENEFITS AND NON MEDICINAL USES)

2.2.1 Medicinal benefits of *Cola nitida*

Cola nitida, commonly referred to as the kola nut, this plant has been widely valued in traditional medicine for its role in managing various health conditions. Investigating the molecular mechanisms underlying its therapeutic effects is crucial for scientifically validating its medicinal benefits. It contains an abundance of bioactive compounds, such as antioxidants and phytochemicals, which contribute to its stimulant properties, antimicrobial activity, and potential health-enhancing effects.

a**. Weight Management And Metabolic Benefits**

Cola nitida is known for its potential appetite-suppressing effects, which may support weight management. Its caffeine content helps accelerate metabolism, promoting higher energy expenditure and fat breakdown. Consuming *Cola nitida* extract can lead to a reduction in both body weight and food consumption and may enhance thermogenesis, making it a promising natural option for those seeking to control obesity.

b. Digestive Health and Gastrointestinal Benefits

Kola nut has long been used in traditional medicine as a digestive aid, owing to its ability to stimulate the production of gastric acid, which improves digestion and reduces bloating. The bitter compounds found in *Cola nitida* are believed to enhance enzymatic activity, aiding in the breakdown and absorption of food. Also, the alkaloids in kola nut are thought to provide a protective effect on the stomach lining, helping to prevent ulcers.

c. Antioxidant and Anti-Inflammatory Activity

Cola nitida contains bioactive compounds like flavonoids, tannins, and alkaloids, which contribute to its antioxidant and anti-inflammatory effects. These compounds help neutralize free radicals, reducing oxidative stress linked to chronic conditions such as diabetes, neurodegenerative diseases, and cancer. Additionally, kola nut's anti-inflammatory properties may assist in managing inflammation-related disorders like arthritis and inflammatory bowel disease.

d. Cardiovascular Benefits

Cola nitida is linked to cardiovascular health due to its caffeine and theobromine content, which can improve blood circulation. Moderate consumption may enhance circulation and reduce arterial stiffness, but excessive intake could lead to hypertension. Additionally, its polyphenolic compounds may help lower cholesterol, reducing the risk of atherosclerosis.

e. Cognitive and Neurological Enhancement

Cola nitida acts as a stimulant due to its high caffeine content, improving mental alertness, cognitive function, and memory. Theobromine further enhances focus and reduces mental fatigue. Kola nut extract can boost cognitive performance and may help delay cognitive decline linked to aging and neurodegenerative diseases like Alzheimer's.

f. Respiratory Health and Bronchodilatory Effects

Cola nitida has been traditionally used to treat respiratory conditions such as asthma and bronchitis. Theobromine, a key compound, works as a bronchodilator, aiding in the opening of airways and improving breathing. *Cola nitida* extract can improve lung function, reduce airway inflammation, and help relax bronchial muscles, suggesting its potential as a natural remedy for respiratory issues.

g. Aphrodisiac and Reproductive Health Benefits

Cola nitida has been traditionally used in African medicine as a natural aphrodisiac, believed to enhance libido, sexual performance, and reproductive health. Its stimulant properties are thought to boost testosterone levels and improve sperm quality. Furthermore, the

phytochemicals in kola nut may help balance reproductive hormones, potentially aiding individuals facing infertility.

2.2.2 Non Medicinal Use Of Cola Nitida

a. Socio-Cultural and Traditional Uses

Cola nitida plays a vital cultural and social role in West Africa, where it is commonly presented at ceremonies like weddings and naming events as a sign of goodwill and hospitality. Sharing kola nuts among elders symbolizes unity and respect. They are also used in spiritual rituals, including divination and offerings to ancestors and deities. In Igbo and Hausa communities, offering kola nuts is seen as a gesture of peace and is important in resolving conflicts.

b. Economic Importance

The kola nut industry plays a crucial economic role in West African nations such as Nigeria, Ghana, and Ivory Coast, offering employment to rural farmers and contributing to economic development. Kola nuts are highly valued in export markets for their caffeine content and traditional applications. The trade supports businesses in transportation, storage, and retail, helping to drive growth in related sectors of the agricultural economy.

c. Food and Beverage Industry

Cola nitida is used in the production of soft drinks, energy drinks, and traditional beverages because of its caffeine content and unique bitter taste. Although synthetic substitutes have replaced it in products like Coca-Cola, kola nut flavor is still popular in some traditional drinks.

In West Africa, kola nuts are often chewed or made into herbal teas to enhance alertness, while local drinks like "bissap" and kola-based tonics use it for its energizing effects.

d. Industrial and Cosmetic Applications

Caffeine derived from *Cola nitida* is utilized in the pharmaceutical industry for making pain relievers, energy supplements, and weight-loss products. Its stimulating effects are also incorporated into performance-enhancing and sports nutrition formulas. In the cosmetic industry, kola nut extracts are valued for their antioxidant and astringent properties, helping to improve skin elasticity, prevent aging, and protect against environmental damage. These extracts are also used in haircare products to strengthen hair follicles. Additionally, kola nut extracts are being studied as natural dyes and tanning agents, providing an eco-friendly option in leather processing.

e. Agricultural and Environmental Benefits

The kola tree (*Cola nitida*) contributes to agroforestry by offering shade and enhancing soil fertility. It is often grown alongside crops like cocoa and coffee to create a more sustainable farming environment. The byproducts of kola nut processing, such as husks and shells, are used for compost, animal feed, or even as potential biofuels, supporting eco-friendly practices and renewable energy in rural areas.

2.3 PHYTOCHEMICALS

Phytochemicals are compounds produced by plants through their metabolic processes, playing a key role in plant growth and defense mechanisms against threats like pathogens or predators.

Cola nitida contains a variety of bioactive compounds, including catechin, caffeine, epicatechin, polyphenols, alkaloids, tannins, saponins, bromelain, and flavonoids. These compounds contribute to the plant's medicinal properties, justifying its use in treating conditions such as infections, inflammation, diabetes, and malaria. Consequently, *Cola nitida* could serve as a valuable raw material for developing medications to treat various health issues, including sickle cell disease.

a. Alkaloids: Caffeine, Theobromine, and Theophylline

Cola nitida is rich in alkaloids, with caffeine being the most prevalent. This natural stimulant plays a key role in boosting alertness, enhancing cognitive performance, and reducing fatigue (Ajiboye et al., 2020). In addition to caffeine, *Cola nitida* contains other methylxanthines such as theobromine and theophylline, which contribute to cardiovascular and respiratory health by promoting blood vessel dilation and improving airflow. The caffeine content in kola nuts varies based on species, environmental conditions, and processing methods, typically ranging from 1.5% to 2.5%. Theobromine, which has a milder stimulating effect and helps expand blood vessels, is found in concentrations between 0.5% and 1.2%. Although present in smaller amounts, theophylline aids in bronchodilation, making kola nut beneficial for individuals with respiratory issues such as asthma (Ajiboye et al., 2020).

b. Flavonoids: Potent Antioxidants and Anti-inflammatory Agents

Flavonoids, a class of polyphenolic compounds naturally present in Cola nitida, are known for their potent antioxidant, anti-inflammatory, and heart-protective effects (Middleton et al., 2000). These bioactive compounds, including catechins, quercetin, and kaempferol, help

counteract oxidative stress, a major factor in the development of chronic diseases such as cancer, cardiovascular conditions, and neurodegenerative disorders. Research indicates that flavonoid-rich extracts can shield neurons from oxidative damage, which may lower the risk of cognitive impairment and neurodegenerative diseases like Alzheimer's (Spencer, 2008). Additionally, flavonoids play a vital role in reducing inflammation, making them useful in managing inflammatory disorders such as arthritis and metabolic syndrome (Serafini et al., 2010).

c. Tannins: Astringent and Antimicrobial Properties

Tannins, a class of bioactive polyphenolic compounds, are responsible for the distinct bitterness and astringency of kola nuts, *Cola nitida.* These compounds contribute significantly to the plant's medicinal properties, including its antimicrobial, anti-diarrheal, and wound-healing effects. With a tannin content ranging from 5% to 10%, kola nuts have been recognized for their ability to inhibit bacterial growth and reduce the risk of infections. Research suggests that the presence of tannins in Cola nitida supports its traditional use in managing gastrointestinal issues such as diarrhea and dysentery by minimizing intestinal inflammation and suppressing microbial activity (Odebunmi et al., 2009). Tannins hold promise in the food and beverage industry as natural preservatives due to their antimicrobial capabilities.

d. Saponins: Cholesterol-Lowering and Immune-Boosting Compounds

Saponins are known for their cholesterol-lowering, immune-boosting, and antimicrobial properties. These compounds help reduce cholesterol levels by inhibiting its absorption in the digestive tract, which can lower the risk of cardiovascular diseases (Güçlü-Üstündağ & Mazza,

2007). Saponins also have significant effects on the immune system, enhancing the body's ability to combat infections (Avato et al., 2006). In addition to their health benefits, their foaming and emulsifying characteristics make them valuable in industries such as pharmaceuticals, cosmetics, and food processing (Güçlü-Üstündağ & Mazza, 2007).

e. Phenolic Compounds: Anti-Cancer and Cardioprotective Agents

Phenolic compounds such as gallic acid, ellagic acid, and chlorogenic acid are recognized as potent antioxidants in Cola nitida, offering significant health benefits. These bioactive compounds aid in neutralizing free radicals, which helps lower the risk of chronic illnesses like cancer, diabetes, and cardiovascular diseases. Studies have demonstrated that phenolic extracts from Cola nitida possess strong anti-cancer properties, effectively inhibiting the growth of cancerous cells in laboratory experiments. Additionally, the cardioprotective properties of these compounds contribute to blood pressure regulation and improved vascular function, highlighting the potential of kola nuts as a natural remedy for heart-related conditions (Odebunmi et al., 2009).

2.4 ANTIOXIDANTS

Antioxidants play a crucial role in safeguarding cells from damage caused by free radicals—highly reactive molecules that contribute to oxidative stress. These free radicals are byproducts of normal metabolic activities, such as mitochondrial energy production, but they can also arise from external influences like pollution, ultraviolet (UV) radiation, smoking, and poor nutrition (Lobo et al., 2010). When the balance between free radicals and the body’s defense mechanisms is disrupted, cellular structures—including DNA, proteins, and lipids—become

vulnerable to oxidative damage. This imbalance is linked to numerous chronic illnesses, such as heart disease, cancer, neurodegenerative disorders, and premature aging (Pham-Huy et al., 2008).

To combat oxidative stress, the body relies on an intrinsic antioxidant defense system comprising enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). These enzymes neutralize free radicals by converting them into less harmful substances, thereby preventing extensive cellular damage. However, when oxidative stress levels become too high, these endogenous defenses may be insufficient, highlighting the importance of obtaining additional antioxidants through diet (Lobo et al., 2010).

Dietary antioxidants, derived from plant- and animal-based sources, provide an extra layer of protection against oxidative damage. Essential dietary antioxidants include vitamin C, vitamin E, flavonoids, carotenoids, polyphenols, and coenzyme Q10 (CoQ10), which are abundant in fruits, vegetables, nuts, whole grains, herbs, and teas. Research suggests that consuming a diet rich in antioxidants can significantly lower the risk of oxidative stress-related diseases, including cardiovascular conditions, type 2 diabetes, and neurodegenerative diseases like Alzheimer's and Parkinson's (Ames et al., 1993).

Apart from disease prevention, antioxidants contribute to overall well-being by enhancing immune function, supporting healthy aging, and promoting skin health. For instance, polyphenols and flavonoids, commonly found in foods such as berries, green tea, and dark chocolate, have been associated with improved brain function and cognitive performance. Similarly, carotenoids like beta-carotene, lutein, and lycopene—present in carrots, spinach, and

tomatoes—aid in maintaining eye health and protecting skin from UV-induced damage (Pham-Huy et al., 2008).

2.4.1 Types Of Antioxidants

Enzymatic Antioxidants

These are naturally produced by the body and work by breaking down harmful free radicals:

- **Superoxide Dismutase (SOD)** – Converts superoxide radicals into less harmful molecules.
- **Catalase (CAT)** – Decomposes hydrogen peroxide into water and oxygen.
- **Glutathione Peroxidase (GPx)** – Neutralizes hydrogen peroxide and lipid peroxides.

Non-Enzymatic Antioxidants

These are obtained from food and supplements, directly neutralizing free radicals.

- **Vitamine C (Ascorbic Acid)** – A water-soluble antioxidant that protects tissues from oxidative damage.
- **Vitamin E (Tocopherols & Tocotrienols**) – Fat-soluble and safeguards cell membranes.
- **Vitamin A (Beta-Carotene & Retinol)** – Neutralizes reactive oxygen species and supports vision health.
- **Polyphenols (e.g., Resveratrol, Curcumin)** – Present in red wine, grapes, and turmeric, known for their protective effects.
- **Carotenoids (e.g., Lutein, Lycopene, Zeaxanthin)** – Found in colorful vegetables, supporting eye health.

2.4.2 Sources Of Antioxidants

- **Fruits and vegetables:** apples, citrus fruits, berries, pomegranates, grains, leafy greens, cruciferous vegetables, garlic, onions, carrots and tomatoes
- **Nuts and seeds:** brazil nut, walnuts, flaxseeds, almonds and sunflower seeds
- **Whole grains and legumes:** oats, whole wheat, beans and lentils
- **Herbs and spices:** tumeric, ginger, oregano and cinnamon
- **Beverages:** green tea, black tea, coffee and red wine

2.4.3 Mechanism Of Antioxidants Action

1. Neutralization of Free Radicals

Free radicals are unstable molecules with unpaired electrons, making them highly reactive. To stabilize themselves, these radicals steal electrons from nearby molecules such as proteins, lipids, and DNA, leading to oxidative damage and cellular dysfunction. Antioxidants counteract this process by donating electrons, effectively neutralizing free radicals and preventing further damage including oxidative stress, while remaining stable themselves. Oxidative stress arises when there is an excessive accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which overwhelm the body's ability to neutralize them.

2. Metal Ion Chelation

Certain metal ions, particularly iron (Fe^{2+}) and copper (Cu^{2+}), contribute to free radical production through the Fenton reaction, increasing oxidative damage. Some antioxidants act as chelators, binding to these metal ions and rendering them inactive, preventing oxidative stress.

3. Regeneration of Other Antioxidants

Some antioxidants help revive oxidized antioxidants, restoring their functionality and extending their protective role. This process enhances the body's long-term antioxidant defense, ensuring continuous cellular protection.

4. Activation of antioxidant enzymes

Some antioxidants stimulate the production of detoxification enzymes that help eliminate toxins and repair damaged cells. These enzymes play a critical role in maintaining cellular balance and detoxifying harmful substances.

2.4.4 Benefits Of Antioxidants

1. Supporting Brain Function & Preventing Cognitive Decline: Oxidative stress contributes to neurodegeneration and memory loss. Antioxidants help protect brain cells and reduce neuroinflammation.

2. Enhancing Heart Health: Antioxidants help prevent inflammation, arterial plaque buildup, and LDL oxidation, reducing the likelihood of cardiovascular diseases.

3. Strengthens Immune System: Enhances the body's ability to fight infections by reducing inflammation and supporting white blood cells.

4. Reduces Risk of Cancer: Neutralizes DNA-damaging free radicals, preventing mutations and tumor growth.

5. Improves Skin Health & Slows Aging: Reduces UV damage, wrinkles, and skin aging by preventing collagen breakdown.

2.4.5 Assays For Antioxidants

An assay is an analytical method used in laboratory medicine, mining, pharmacology, environmental biology, and molecular biology to determine the presence, concentration, or functional activity of a specific substance, either qualitatively or quantitatively. Several laboratory techniques are employed to evaluate the antioxidant potential of compounds, measuring their ability to neutralize free radicals, inhibit oxidation, or reduce oxidative stress. Assays used in this study include;

DPPH Assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical compound that appears as a dark-colored crystalline powder. It has two primary uses in laboratory research: one as a probe for chemical reactions involving free radicals, particularly in the widely recognized antioxidant assay, and another as a standard reference for electron paramagnetic resonance signals. The DPPH assay is a popular and effective spectrophotometric method used to evaluate the antioxidant activity of a variety of substances, including plant extracts, synthetic compounds, and food products (Brand-Williams, Cuvelier, & Berset, 1995). This assay works on the principle that antioxidants neutralize DPPH, a stable free radical, by donating electrons or hydrogen atoms. This reaction causes the DPPH solution to change from a deep violet color to yellow, and

the absorbance can be measured at 517 nm using a spectrophotometer. A reduction in absorbance reflects the radical-scavenging potential of the sample, allowing the comparison of antioxidant activities across different substances (Molyneux, 2004).

To perform the DPPH assay, a DPPH solution is prepared in an organic solvent (typically methanol or ethanol), then mixed with the sample of interest, and incubated in the dark for 15–30 minutes at room temperature. Afterward, the absorbance is measured, and the percentage inhibition of DPPH is calculated to assess the antioxidant capacity (Sharma & Bhat, 2009). This method is simple, quick, and requires minimal equipment, making it a preferred choice for preliminary antioxidant screening in various fields such as food science, pharmaceuticals, and natural product research.

FRAP Assay

The Ferric Reducing Antioxidant Power (FRAP) assay is a commonly used technique for evaluating the antioxidant potential of different substances, including plant extracts, food items, pharmaceuticals, and biological samples. This method works by detecting the ability of antioxidants to convert ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}) under acidic conditions. This reaction produces a blue Fe^{2+}-TPTZ (2,4,6-tripyridyl-s-triazine) complex, whose intensity is measured spectrophotometrically at 593 nm (Benzie & Strain, 1996). A stronger color change indicates a higher reducing capacity of the sample, which reflects its antioxidant activity.

The FRAP assay is widely applied due to its ease of use, efficiency, and cost-effectiveness, making it especially suitable for large-scale antioxidant screening. Unlike radical scavenging assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl), which assess an antioxidant's ability to

neutralize free radicals, FRAP is specifically designed to measure the reducing power through electron transfer (Prior, Wu, & Schaich, 2005). However, the assay does not evaluate hydrogen-donating antioxidants or directly measure the neutralization of reactive oxygen species (ROS). Additionally, since it operates under acidic conditions (pH 3.6), it may not fully represent physiological environments, and its lack of specificity means it does not distinguish between different antioxidant types (Benzie & Strain, 1996). To obtain a more comprehensive assessment of antioxidant activity, the FRAP assay is often used alongside other methods such as DPPH, ABTS, ORAC (Oxygen Radical Absorbance Capacity), and TEAC (Trolox Equivalent Antioxidant Capacity) (Prior, Wu, & Schaich, 2005).

Hydroxyl Radical (OH•) Scavenging Assay

The hydroxyl radical (OH•) scavenging assay is a common technique used to measure a substance's ability to neutralize hydroxyl radicals, which are highly reactive oxygen species that cause significant cellular damage. These radicals rapidly interact with biomolecules such as lipids, proteins, and DNA, leading to oxidative stress, which contributes to inflammation, aging, and chronic conditions like cancer, neurodegenerative diseases, and cardiovascular disorders (Halliwell, 2007).

This assay operates on the principle of the Fenton reaction, where ferrous ions (Fe^{2+}) react with hydrogen peroxide (H_2O_2) to generate hydroxyl radicals. These radicals degrade deoxyribose, producing malondialdehyde (MDA), which then reacts with thiobarbituric acid (TBA) to form a pink-colored compound detectable at 532 nm (Halliwell et al., 2000). The presence of antioxidants competes with deoxyribose for hydroxyl radicals, thereby reducing MDA

production and lowering absorbance readings. The percentage of hydroxyl radical scavenging is determined by comparing the absorbance values of the sample and a control without antioxidants.

This method is widely utilized in pharmaceutical and nutraceutical research to identify compounds with strong hydroxyl radical scavenging properties. Natural antioxidants such as flavonoids, polyphenols, and vitamin C have shown remarkable efficiency in scavenging OH•, making them valuable in anti-aging and neuroprotective applications (Yu, 2001). However, some limitations exist, including sensitivity to factors like pH and temperature, as well as potential variations in results due to the presence of iron ions. Despite these challenges, the hydroxyl radical scavenging assay remains a crucial tool for evaluating antioxidant defense mechanisms against oxidative stress.

Nitric Oxide (NO•) Scavenging Assay

The nitric oxide (NO•) scavenging assay is a method used to evaluate the ability of antioxidants to neutralize nitric oxide radicals, which are key players in inflammation, oxidative stress, and cellular signaling. While NO• is essential for physiological functions such as vasodilation, neurotransmission, and immune defense, excessive levels can lead to the formation of peroxynitrite ($ONOO^-$), a reactive nitrogen species associated with oxidative damage, lipid peroxidation, and cell death. This oxidative stress has been linked to inflammatory diseases like arthritis, asthma, and neurodegenerative conditions (Sreejayan & Rao, 1997).

The assay relies on the reaction between sodium nitroprusside (SNP) and oxygen, which generates nitric oxide radicals. These radicals subsequently react with oxygen to form nitrites

(NO_2^-), which can be detected using the Griess reagent. This reaction produces a purple-colored complex that absorbs light at 546 nm (Green et al., 1982). When antioxidants are introduced into the system, they compete with oxygen to react with NO•, reducing nitrite formation and leading to lower absorbance readings. The percentage of NO• scavenging activity is then determined by comparing the absorbance of the antioxidant-treated sample to that of the control.

This assay is particularly valuable for assessing compounds with anti-inflammatory and cardiovascular protective properties. Plant-derived antioxidants, including phenolic compounds, flavonoids, and alkaloids, have been shown to exhibit significant NO• scavenging activity, which may contribute to their therapeutic effects in inflammation-related disorders. However, the method has some limitations. The interaction of nitric oxide with antioxidants in vitro may not fully reflect physiological conditions, and the presence of other bioactive compounds in plant extracts can influence the assay results. Despite these challenges, the NO• scavenging assay remains a crucial tool for identifying natural compounds with potential anti-inflammatory benefits.

2.4 .6 Antioxidants In *Cola Nitida*

Polyphenols: polyphenols in Cola nitida include flavonoids, tannins, and phenolic acids These compounds act as free radical scavengers

Caffeine and Theobromine: exhibit antioxidant properties that reduces oxidative stress in the body

Vitamin C (Ascorbic Acid): an essential water-soluble antioxidant that supports immune function and skin health by combating oxidative stress.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Design

The experiment was designed to assess the antioxidant property of *Cola nitida* extract using a detailed and methodical approach. To enhance accuracy, the extraction process was conducted multiple times, ensuring meticulous sample preparation and thorough filtration. Every stage of the experiment followed a structured procedure, maintaining consistency and reliability in the obtained results.

3.2 Materials

3.2.1 Apparatus

The equipment utilized in this study was obtained from a certified supplier and met experimental standards at the time of acquisition. These tools were essential for sample handling and preparation. Safety and hygiene were maintained with the use of items such as masking tape, test tube holders, tissues, gloves, and nose masks. For this research, *Cola nitida* (commonly known as kolanut) was specifically sourced from a local market, new benin, in Edo, Nigeria. To ensure proper sample preservation and handling, materials such as aluminum foil, conical flasks (Pyrex, England), universal containers, detergent, and filter paper were utilized. Additionally, beakers with 500 ml and 1000 ml capacities, metal buckets, a glass rod, and a handkerchief were employed for sample mixing and preparation. Precise liquid measurements

were achieved using a micropipette, ensuring accuracy throughout the experimental procedures.

3.2.2 Equipment

To ensure accurate and reliable data, several high-quality instruments were utilized in this study. A pH meter from Sigma, Germany was used to determine the acidity or alkalinity of the samples, which is crucial for analyzing the chemical properties of *Cola nitida*. A spectrophotometer, also from Sigma, Germany, was employed to measure light absorption and transmission, enabling the determination of compound concentrations within the samples. Additionally, a centrifuge from the same manufacturer played a key role in separating sample components based on their densities, a necessary step in sample preparation for further analysis. An oven was also used for drying and heating processes, ensuring proper sample treatment before undergoing analytical procedures.

3.2.3 Reagents

Various chemicals and reagents were utilized in this study to facilitate specific reactions and analyses. Distilled water and ethanol served as solvents for dissolving or diluting other substances. DPPH (1g), a widely used compound in antioxidant assays, was employed to evaluate the antioxidant activity of the samples. Additionally, phosphate buffer, dinitrosalicylic acid, and sulfuric acid (H_2SO_4, 500 ml) were used to stabilize the samples and support essential chemical reactions during the experiments. Sodium phosphate and acetonitrile (2.5L) were also included for further testing and in-depth chemical analysis of the samples.

3.3 Procedures

3.3.1 Sample Collection: I obtained *Cola nitida* fruit from local markets in Edo State, Nigeria. The kolanuts were diced and left to air dry under room temperature to remove any excess moisture, ensuring that the samples were not contaminated with unwanted residual water. The dried kolanuts were then grounded into fine particles using a mechanized grinder in the market to enhance the extraction process.

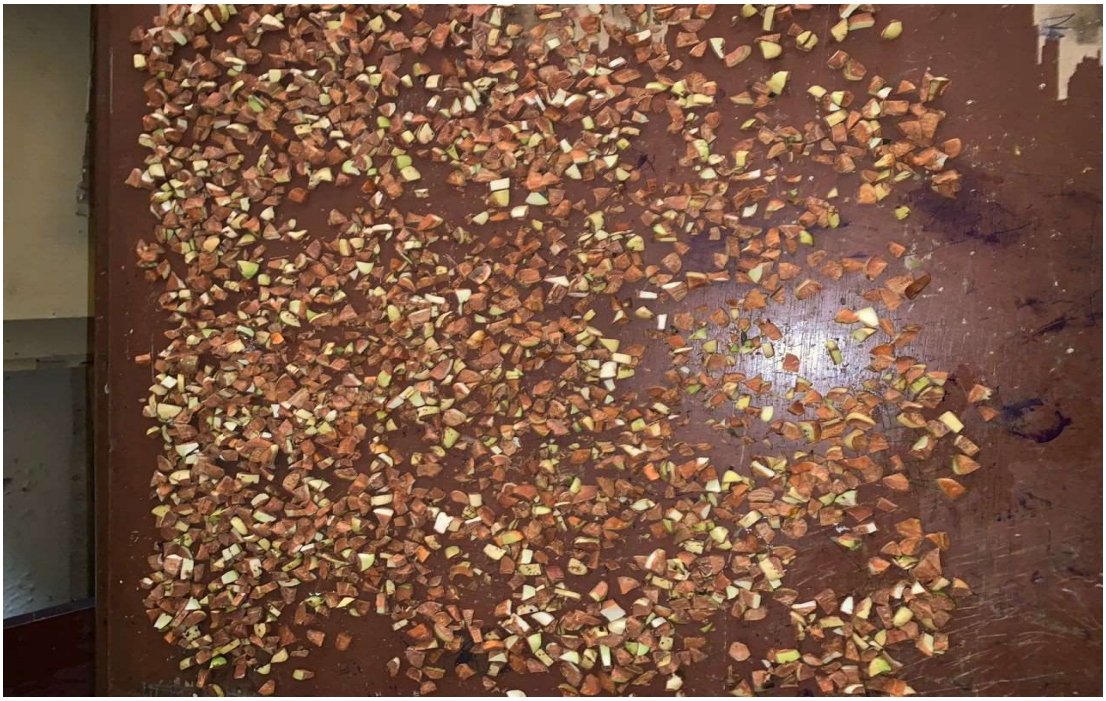

Figure 3.1: Cola nitida being dried (source: personal)

Figure 3.2: Ground Cola nitida (source: personal)

3.3.2 Ethanol Extraction: After grinding the dried kola nuts, they were soaked in ethanol for a period of 72 hours. The kolanuts were placed in a metal bucket and ethanol was added in sufficient quantity to fully submerge the sample. The bucket was covered with aluminum foil to prevent evaporation and contamination, and left at room temperature for 72 hours to allow the ethanol to extract the active compounds from the kolanut. The ethanol extract was filtered twice a week using a handkerchief and filter paper to separate the solid material from the liquid extract. This filtration process ensured that only the dissolved ethanol extract was retained for further

analysis. The idea behind the extraction is to exhaustively extract all constituents of the Cola nitida. The crude extracts was then sent to pharmaceutical chemistry staff research laboratory in the university of benin for freeze drying.

Figure 3.3: Ethanol extraction (source: personal)

3.3.3 Freeze-Drying Process: Following ethanol extraction, the solution underwent freeze-drying to concentrate the sample and obtain a pellet. This process involved dissolving the ethanol extract in distilled water, then freezing the solution under controlled conditions to produce a dried powder. The resulting powder was collected and stored for subsequent antioxidant assays.

3.4 BIOCHEMICAL INVESTIGATIONS

Determination Of DPPH Radical Scavenging Ability

One millilitre (1 mL) of diluted samples were combined with 0.4 mM 1-diphenyl-2-picryl hydrazine (DPPH) radicals in a methanolic solution. After 30 minutes of incubation in the dark, the mixture's absorbance value were measured at 516 nm using a spectrophotometer. Likewise, 2 mL of DPPH solution was employed as the control in the absence of the test samples. The samples' capacity to use DPPH to scavenge free radicals was contrasted with the control (Gyamfi *et al*., 1999).

Determination Of The Ferric-reducing Antioxidant Property (FRAP)

The reducing property of the samples were ascertained based on the sample's capacity to reduce $FeCl_3$ solution. 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% w/v potassium ferricyanide were combined with 2.5 mL aliquots. After 20 minutes of incubation at 50^{o}C, 2.5 mL of 10% w/v trichloroacetic acid was added to the mixture. For ten minutes, this mixtures were centrifuged at 8000 rpm. 1 mL of 0.1% w/v ferric chloride and water was added to 5 mL of the supernatants obtained. After measuring the absorbance of the mixture at 700 nm, the ascorbic acid equivalent deduced was used to compute the ferric-reducing power (Oboh *et al*., 2010).

Determination Of $Fe2^{+}$ Chelating Ability

A modified version of the Puntel *et al*., 2005 methodology was employed to evaluate the samples ability to chelate Fe^{2+}. 500 µM $FeSO_4$ was freshly prepared and added in 150 µL to reaction solutions containing each sample. 168 µL of 0.1 MTris-HCl (pH 7.4) and 218 µL of saline

solution were used. After five minutes of incubation, 13 µL of 0.25% 1,10 phenanthroline (w/v) was added to the reaction mixtures. The absorbance at 510 nm was measured using a UV/Visible spectrophotometer. The capacity to chelate Fe^{2+} was then calculated.

Determination Of Hydroxyl Radical ($OH^{\bullet}$) Scavenging Ability

40 µL of 20 mM hydrogen peroxide, 400 µL of 0.1 M phosphate buffer, 40 µL of 20 mM deoxyribose, and 40 µL of 500 mM $FeSO_4$ were added to a reaction mixture containing each sample. Afterwards, distilled water was added to the volume until 800 µL was attained. After 30 minutes of incubation at 37°C, the reaction was halted by adding 0.5 mL of 2.8% trichloroacetic acid (TCA) solution. 400 µL of 0.6% thiobarbituric acid (TBA) solution was subsequently added. The tubes were then submerged in water for about twenty minutes. Using a spectrophotometer, the absorbance was measured at 532 nm. Subsequently, a percentage (%) estimate of the $OH^{\bullet}$ radical scavenging ability was calculated (Ayeni *et al*., 2024)

Determination Of Nitric Oxide ($NO^{\bullet}$) Scavenging Ability

300 µL of 5 mM sodium nitroprusside and 1 mL of the each sample was combined. The test tubes were then incubated for 150 minutes at 25°C. 150 minutes later, 0.5 mL of Griess reagent which is used after 12 hours of preparation and 5 mL of distilled water containing 5% ortho-phosphoric acid were added. This was the same as 1% sulphanilamide and 0.01% naphtyl ethylenediamine. Solubility was measured at 546 nm (Sussanta *et al*., 2006).

3.4.1 Data Analysis

All analysis were carried out in triplicates and presented as mean ± standard deviation (SD) of $n = 3$. The least significant difference (LSD) and one-way analysis of variance (ANOVA) were

used to determine whether significant differences existed between the mean of different treatments $p \leq 0.05$ (Zar, 1984). Linear regression analysis was used to get the EC_{50} and IC_{50} values

CHAPTER FOUR

RESULT

Table 1: EC_{50} of DPPH Scavenging, OH* Scavenging, Fe^{2+} Chelating and NO* Scavenging Capacities of Sample (mg/mL)

	Control	Sample
DPPH*	0.453 ± 0.010^{a}	0.539 ± 0.010^{b}
OH*	0.086 ± 0.009^{a}	0.062 ± 0.008^{b}
Fe^{2+}	0.147 ± 0.009^{a}	0.162 ± 0.010^{c}
NO*	0.103 ± 0.05^{a}	0.107 ± 0.05^{a}

Values represent means ±SD of triplicate determinations. Values with the same alphabet along the same row are not significantly different ($p \leq 0.05$). **Control = Quercetin**

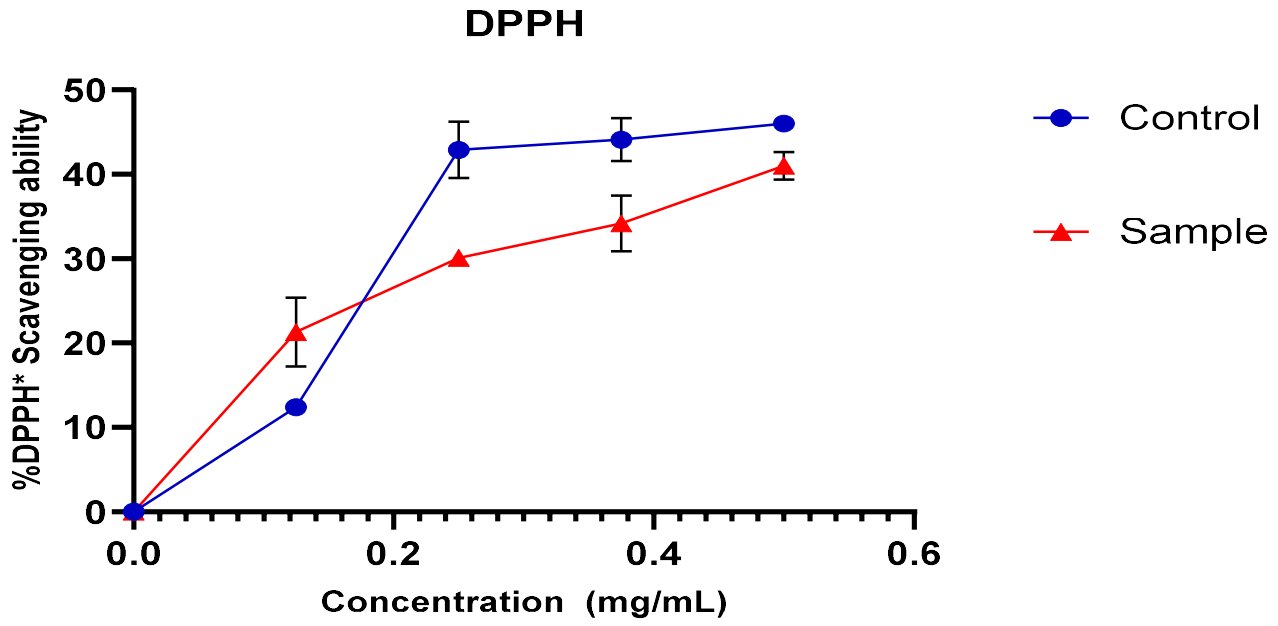

Figure 4.1: % DPPH* radical scavenging ability of Sample. **Control = Quercetin**

The EC_{50} values (graph) indicates the sample (0.539 ± 0.010 mg/mL) had a higher EC_{50} than the control, quercetin (0.453 ± 0.010 mg/mL).

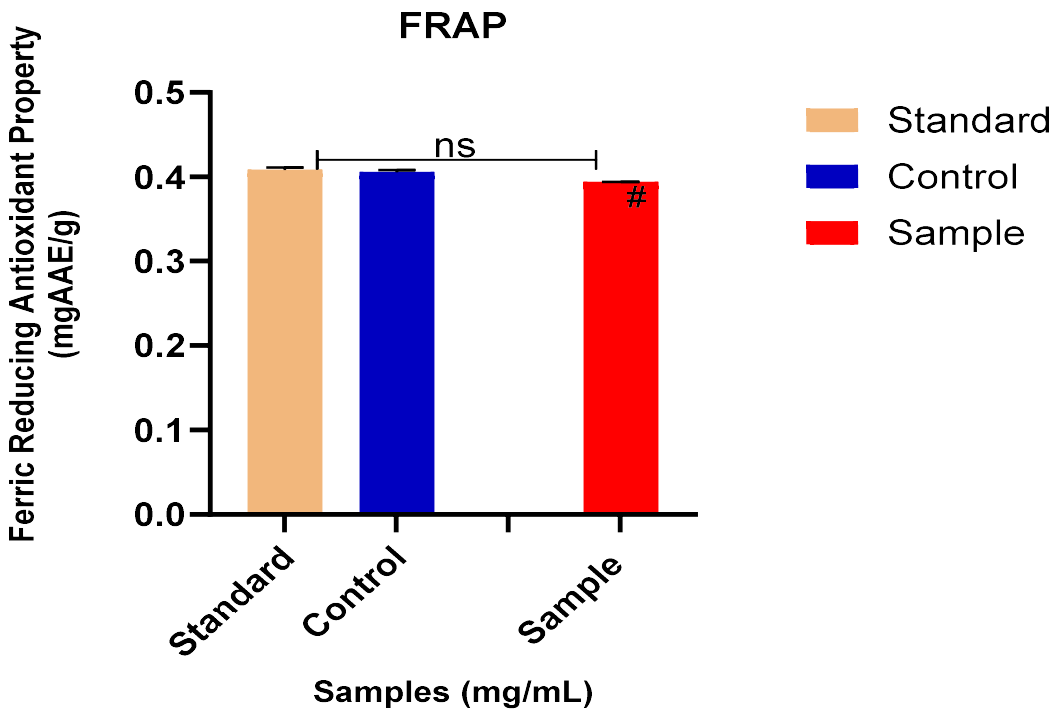

Figure 4.2: Ferric Reducing Antioxidant Potentials (FRAP) of Sample.

Bars represent mean ± SEM (n = 3). Values are statistically different at $^{\#}p< 0.05$ Vs Control. ns = No Significant Difference. **Standard = Ascorbic Acid, Control = Quercetin**

The ferric-reducing antioxidant potential of the sample increased with concentration. Statistical analysis indicates the samples FRAP value is slightly lower than the control, quercetin.

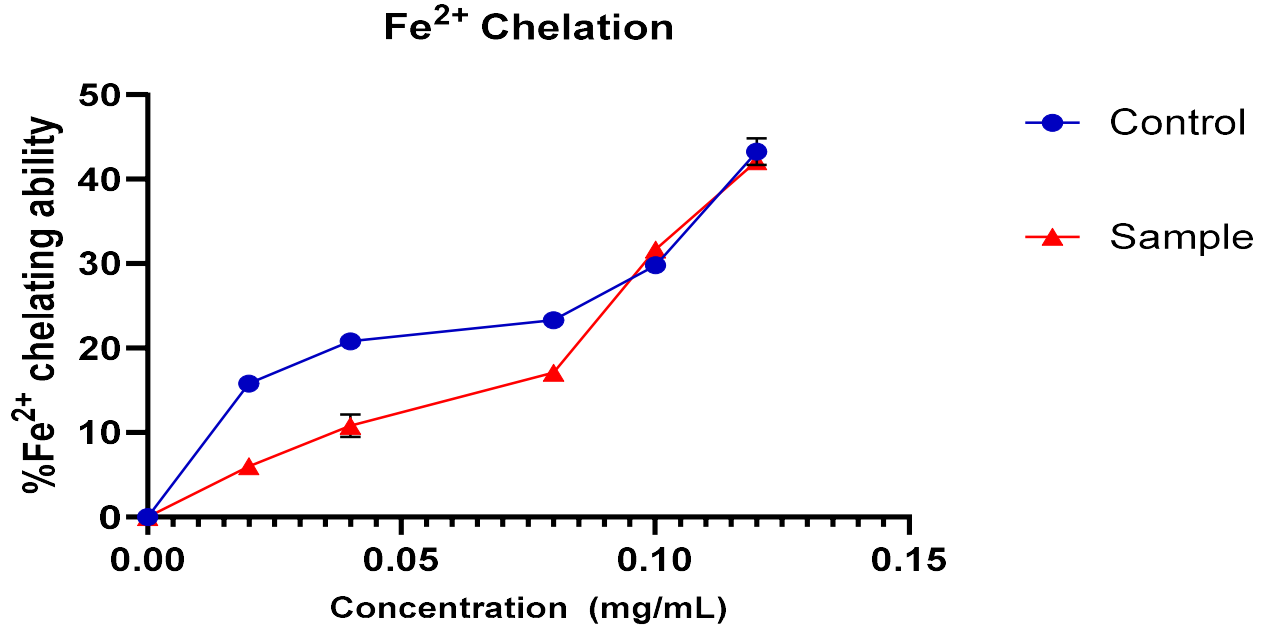

Figure 4.3: % Fe^{2+} chelating abilities of Sample. **Control = Quercetin**

The EC_{50} values (graph) show that the sample had a slightly higher EC_{50} (0.162 ± 0.010 mg/mL) compared to the control, quercetin (0.147 ± 0.009 mg/mL).

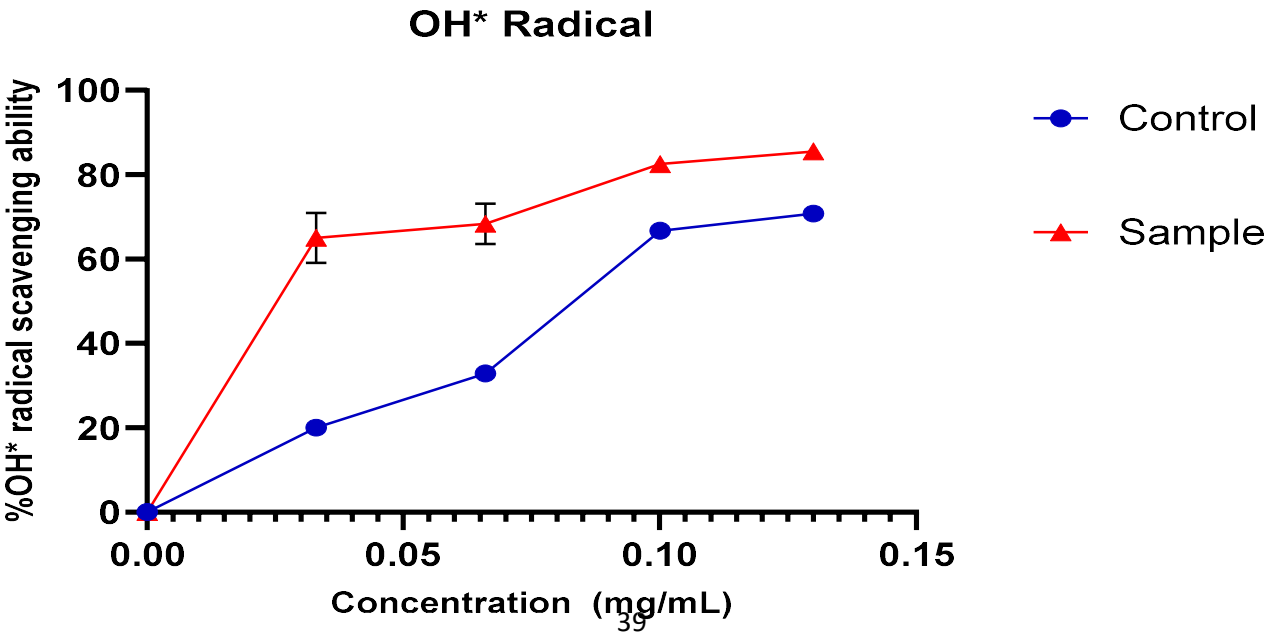

Figure 4.4: % OH* radical scavenging ability of Sample. **Control = Quercetin**

The EC_{50} values (graph) shows the sample (0.062 ± 0.008 mg/mL) exhibited a significantly lower EC_{50} than the control, quercetin (0.086 ± 0.009 mg/mL).

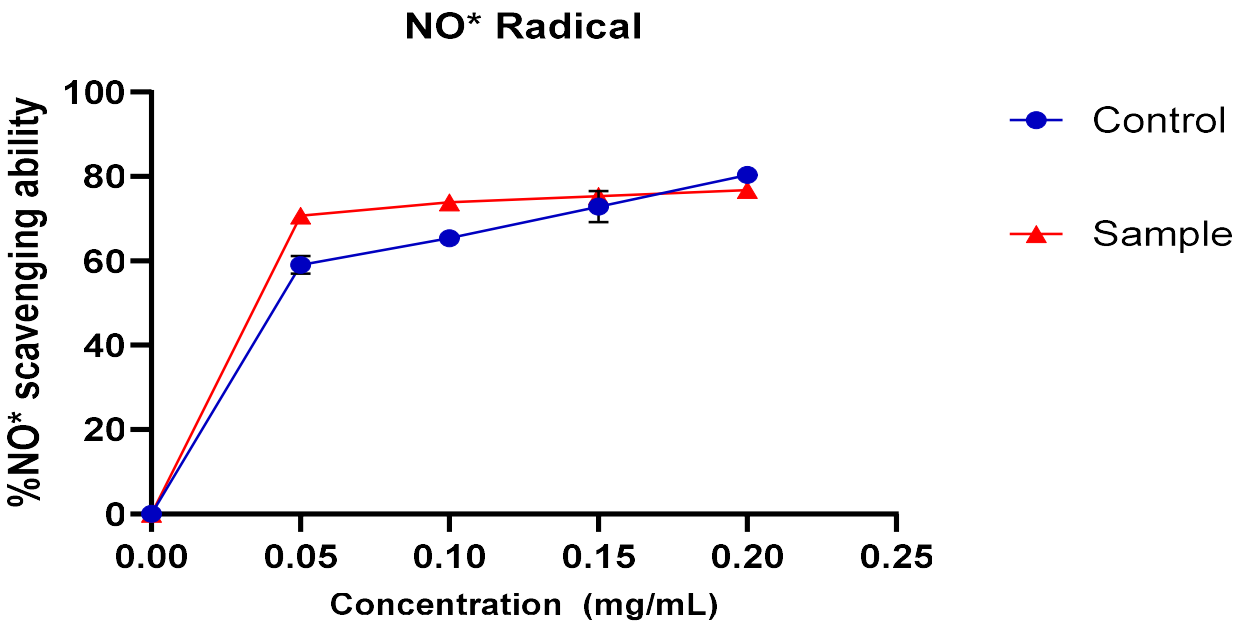

Figure 4.5: % NO* radical scavenging ability of Sample. **Control = Quercetin.**

The EC_{50} values (graph) shows the sample (0.107 ± 0.05 mg/mL) exhibited a slightly higher EC_{50} than the control, quercetin (0.103 ± 0.05 mg/mL).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This research examined the antioxidant potential of *Cola nitida* extract by assessing its ability to scavenge free radicals, chelate metal ions, and prevent lipid peroxidation. Experiments designed to assess the antioxidant property of *Cola nitida* include: determination of DPPH radical scavenging ability, determination of the ferric-reducing antioxidant property (FRAP), determination of Fe^{2+} chelating ability, determination of hydroxyl radical ($OH^{\bullet}$) scavenging ability and determination of nitric oxide ($NO^{\bullet}$) scavenging ability with quercetin as the control.

Results for determination of DPPH radical scavenging ability showed *Cola nitida* extract had a higher EC_{50} value than the control, quercetin, indicating a lower DPPH scavenging capacity as a lower EC_{50} value denotes stronger antioxidant activity. However, the extract showed moderate antioxidants activity due to it's flavonoids and polyphenolic properties.

Outcome of ferric-reducing antioxidant property showed *cola nitida* extract increased with concentration with statistical analysis indicating the sample FRAP value being slightly lower than the control, quercetin, suggesting a comparable reducing power. Ascorbic acid was used as the standard in this assay, known for its strong electron-donating properties and significantly higher reducing power. The comparable reducing ability of the sample and the control indicates that *Cola nitida* extract contains bioactive compounds involved in redox reactions, contributing to its antioxidant potential.

Results for determination of Fe^{2+} chelating ability showed the *Cola nitida* extract had a slightly higher EC_{50} value than that of quercetin This indicates a slightly lower iron-chelating efficiency. Although both demonstrated significant Fe^{2+} chelation, the reduced activity of *Cola nitida* indicates that its polyphenolic compounds may be less effective at binding iron ions compared to quercetin. Nevertheless, its ability to chelate iron still underscores its potential in mitigating oxidative stress-related damage.

The out-turn of the determination of hydroxyl radical ($OH^{\bullet}$) scavenging ability showed the *Cola nitida* exhibiting a significantly lower EC_{50} value than quercetin, demonstrating a greater hydroxyl radical scavenging capacity. This indicates that *Cola nitida* extract effectively neutralizes hydroxyl radicals, likely due to its rich phenolic content. Its strong performance in this assay emphasizes its potential as a natural antioxidant.

Results for determination of nitric oxide ($NO^{\bullet}$) scavenging ability shows the *cola nitida* extract exhibited a slightly higher EC_{50} value than quercetin indicating the sample had a slightly lower NO• scavenging ability. However, the comparable values suggest that *Cola nitida* extract still exhibits effective NO• scavenging ability, making it useful for anti-inflammatory applications.

Previous research shows the testas of *Cola nitida* kolanuts, demonstrated considerable antioxidant activity, comparable to the standard antioxidant, Butylated Hydroxytoluene (BHT). However, *Cola nitida* consistently exhibited lower antioxidant capacity than *Cola acuminata* across all evaluated parameters. This variation is likely due to differences in the concentration of secondary metabolites within the testas, which are essential for scavenging free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). The findings indicate that the fruit testas of Cola species, typically regarded as agricultural waste, have significant

antioxidant potential. Their utilization could serve as a natural antioxidant source for therapeutic purposes, aiding in the management of conditions associated with oxidative stress; According to the research done by Ogunsowo, A. O., et al (2023)

5.2 CONCLUSION

This study confirms that Cola nitida is a rich source of natural antioxidants, exhibiting significant ability to scavenge free radicals such as hydroxyl radicals and inhibit lipid peroxidation. While its antioxidant potential varies across different assays, its effectiveness in key oxidative stress defense mechanisms highlights its promise for applications in health and industry.

The results add to the growing body of research supporting the use of plant-based antioxidants as natural alternatives to synthetic compounds. Enhancing extraction techniques and formulation methods could further improve the bioavailability and practical applications of Cola nitida antioxidants, fostering their use in healthcare, food preservation, and environmental sustainability.

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