EVALUATION OF SOME *IN VIVO* ANTIOXIDANT ACTIVITIES OF AQUEOUS ROOT EXTRACT OF *Anthocleista djalonensis* ON THE LIVER OF WISTAR RATS

BY

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LSC2007287

PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY FACULTY OF LIFE SCIENCES UNIVERSITY OF BENIN BENIN CITY

OCTOBER, 2025

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCE, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA. IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE BACHELOR DEGREE (BSc.) IN SCIENCE LABORATORY TECHNOLOGY DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY (PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES) FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY

OCTOBER, 2025

CERTIFICATION

This is to certify that this project work, titled "**EVALUATION OF SOME *IN VIVO* ANTIOXIDANT ACTIVITIES OF AQUEOUS ROOT EXTRACT OF *Anthocleista djalonensis* ON THE LIVER OF WISTAR RATS"** was carried out by Francess Ofuezeme EKPADA (Miss) with Matriculation Number LSC2007287 of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo state under the supervision of Dr. D.O. Uwaya and Mr. Marvelous Uwamusi.

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DEDICATION

This work is dedicated to God Almighty for his grace and mercies and also to my parents Mr. and Mrs. J.J Ekpada and my uncle Rev. Fr Amos Paul.

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ABSTRACT

Medicinal plants have long been integral to healthcare, forming the foundation of traditional medicine across many cultures. This study evaluated the in vivo antioxidant activity of the aqueous root extract of *Anthocleista djalonensis* (Loganiaceae) in the livers of adult Wistar rats. Twenty-four rats were randomly divided into four groups of six. Group I served as the control and received 2 ml of distilled water orally. Groups II, III, and IV were administered 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the aqueous root extract, respectively, for 28 days. After sacrifice, livers were isolated, weighed, and homogenised in cold normal saline. Catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were measured. The extract had no significant effect on CAT and SOD activities compared to control ($p > 0.05$), but significantly reduced MDA levels ($p < 0.05$). Liver weight remained unchanged ($p > 0.05$). These results indicate that *Anthocleista djalonensis* root extract exhibits promising antioxidant properties, particularly through direct free radical scavenging.

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CHAPTER ONE

INTRODUCTION

1.1Background of Study

Medicinal plants have played a vital role in healthcare for thousands of years, serving as a necessary and relevant foundation for traditional medicine across various cultures. Their longstanding therapeutic importance is evident in the continuous use of natural remedies from ancient times to modern practice (Khan, 2014). The influence of natural products on modern pharmacology is substantial, as many contemporary medicines have evolved from herbal sources, highlighting the enduring link between traditional and modern/complementary healthcare (Patwardhan *et al.,* 2008). Globally, the popularity of herbal and plant-based remedies as complementary and alternative medicine (CAM) continues to grow (Welz *et al.,* 2018). According to the World Health Organization (2021), approximately 10–50% of the population in developed nations regularly use herbal products. Scientific evidence supports the growing global popularity of medicinal plants, highlighting their therapeutic potential with minimal adverse effects. While the natural origins of the products often lead to perceptions of inherent safety, it is crucial to recognise that plant-based remedies comprise complex chemical profiles that can interact with other substances, yielding comparable benefits or risks as synthetic pharmaceuticals (Asher *et al.,* 2017). Thus, researchers have improved their studies on the safety and efficacy of medicinal plants and their metabolites, both alone and with other drugs. Medicinal plants have been found to be a treasure trove of bioactive chemicals, which have been shown to play a significant role in preventing chronic diseases such as cancer, diabetes, and heart disease. These phytochemicals can be classified into key categories, including dietary fibres, anticancer agents, detoxifying agents, neuropharmacological agents, antioxidants, etc. (Mamta *et al.,* 2013). Medicinal plants with potent antioxidant properties can help reduce oxidative stress, which is associated with various diseases, including inflammatory conditions

and cancer. By combating oxidative stress, these antioxidants may play a crucial role in disease prevention and treatment (Duduku *et al.,* 2011).

Oxidative stress can be described as a state in which the natural balance between molecules that generate Reactive Oxygen Species (ROS) and the systems that protect or repair the body from their effects is disrupted, leading to gradual wear or injury at the cellular or molecular level, which can cause inflammation and damage lipids, proteins, and nucleic acids, leading to cellular dysfunction and potentially contributing to various diseases (Yasueda, 2016). In simpler terms, oxidative stress happens when the body makes more harmful oxygen molecules than it can safely control or cleanup, which can damage cells and tissues over time.

This imbalance can be restored by antioxidants, which help bring equilibrium by neutralising reactive oxygen species (ROS) or free radicals and reducing oxidative damage. By intervening in the oxidation process, antioxidants can effectively slow down or halt the damage. They do this by interrupting the oxidation chain reactions by donating electrons or hydrogen atoms to stabilise reactive molecules, thereby preserving cellular function (Goodarzi, 2018). By eliminating radical intermediates and undergoing self-oxidation, antioxidants effectively terminate chain reactions and suppress oxidation reactions, safeguarding other molecules from oxidative damage (Awuchi and Okpala, 2022). The human body is equipped with a complex system that counters free radicals, leveraging antioxidants from both internally produced

(Endogenous antioxidants) and external/dietary intake (exogenous antioxidants) to minimise oxidative damage (Khaled, 2014). Endogenous antioxidants are produced in the body through the action of enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxide; they all play crucial roles in combating oxidative stress by acting enzymatically to convert reactive species into less

harmful compounds (Cheng *et al.,* 2017). Exogenous antioxidants can be obtained from external sources; some of those external sources are foods that are rich in vitamin A (retinol), vitamin E (tocopherol), and vitamin C (ascorbic acid); minerals (zinc, manganese, selenium, iron, etc.); and phenols, with a subclass called polyphenols (flavonoids). These external sources are predominantly found in phytochemical-rich (plant-derived) foods (Sen *et al.,* 2013; Lawal *et al.,* 2016).

Phenolic antioxidants work by inhibiting the initiation and propagation of oxidation by donating hydrogen atoms and scavenging free radicals, thereby blocking free radical formation and disrupting the autoxidation process (substance reaction with oxygen leading to the formation of free radicals), which helps to prevent oxidative damage. Many plant extracts, valued for their flavouring properties, exhibit potent antioxidant activity due to their ability to donate hydrogen atoms (H). This antioxidant effect is primarily attributed to compounds like phenolic acids (rosmarinic acid), phenolic diterpenes (carnosol), flavonoids (quercetin), and volatile acids (thymol). Additionally, certain plant pigments (anthocyanin) can slow down oxidation through a dual antioxidant mechanism: metal chelation and hydrogen donation to oxygen radicals (Brewer, 2011).

1.2 Aim of the study

The aim of this study is to investigate some in vivo antioxidant activities of *Anthocleista djalonensis* extracts of liver of Wistar rats.

1.3 Objectives

- To prepare extracts of *Anthocleista djalonensis* roots using a maceration method.
- To evaluate the antioxidant activities of the extracts on liver homogenates of Wistar rats like Superoxide dismutase (SOD), Malondialdehyde (MDA) and catalase (CAT) using spectrometric method.
- To evaluate the Malondialdehyde (MDA) using spectrometric method.
- To evaluate the organ weight after sacrificing by weighing the organ with an analytical weighing balance.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Anthocleista djalonensis*

Anthocleista djalonensis, commonly known as cabbage plant, is a widely used traditional medicine in Africa to treat variety of diseases/ conditions. It is a small tree that is found from west tropical Africa to South Chad and it leans towards savanna and forest habitats. Native to the African continent, it is widely found in West African countries, including Ghana, Guinea, Ivory Coast, Liberia, Nigeria, and Sierra Leone (GBIF, 2000). *Anthocleista djalonensis* (A.chev) is a modest sized tree typically reaching heights of up to 15m, with a trunk diameter of approximately 40cm. Its twigs may sometimes bear a pair of erect, spine-like projections or small cushion-like structures at the leaf axils. The Leaves are arranged in opposite pairs, each simple and entire, with petioles measuring from 1 to 9cm and possessing auricled (ear-like) bases. Leaf blades range from oblong-elliptical to obovate-elliptical, typically 9-35cm long and 5-17cm wide though juvenile plants may present much larger leaves, up to around 115cm by 50cm. The leaf base may be cordate, rounded, or cuneate, while the apex is rounded. Flower clusters form as erect terminal dichasial cymes reaching 15-50cm in length. The peduncles and branching stalks are pale to greenish-white with darker green spots, thickened at the nodes. Flowers are bisexual and radially symmetrical; each has four free, orbicular sepals (6-10mm long). The corolla features a tubular structure 20-32mm long, topped by 11-14 spreading oblong lanceolate lobes (10-18mm), colored white or creamy. The same number of stamens alternate with the corolla lobes; their filaments are fused into a ring, and the excerted anthers are creamy or pale yellow. The ovary is superior, obovoid, measuring roughly 6-7mm by 3-4mm and comprising four

chambers; the stigma is a two-lobed, obovoid-cylindrical structure positioned at the apex (De Ruijter, 2007).

Plate A: *Anthocleista djalonensis* plant

Source: Catalogue of life

2.1.1 Taxonomic hierarchy of *Anthocleista djalonensis*

Kingdom – plantae

Subkingdom - Embryophyta

Division – Tracheophyta

Subdivision – Spermatophytina

Class – Magnoliopida

Subclass – Asteridae

Order – Gentianales

Family - Gentianaceae

Genus – *Anthocleista*

Species – A.chev (Adebayo and Olamide, 2022)

2.1.2 Description

Anthocleista djalonensis is a tree species that can grow up to 15m tall, with a cylindrical bole and spreading crown that can reach 40cm in diameter. The twigs sometimes have 2 erect spines or small cushions above the leaf axils. The leaves exhibit varied shapes, including elliptic, oblong, obovate, and oblanceolate forms, and are thick and leathery with prominent veins and are often glabrous with lengths spanning 8-70 cm and widths of 3-28 cm. The stomata types are predominantly anisocytic or anomocytic, with some species displaying amphistomatic characteristics. Additionally, the leaves feature trichomes that are stellate, dendritic, or a combination of both, with varying arm numbers and lengths. The leaf epidermal cuticle is adorned with distinct ornamentation, including soft wax or scale and plate patterns (Olusola, 2013). The leaves are oppositely arranged, simple, and entire, with petioles ranging from 1-9 cm in length and featuring auricles. The leaf blades are typically oblong-elliptical to obovateelliptical in shape, measuring 9-35 cm x 5-17 cm, although younger plants can have larger leaves up to 115 cm x 50 cm. The base of the leaf can be cordate, rounded, or cuneate, and the apex is rounded. The inflorescence is characterized by an erect terminal dichasial cyme, 15-50 cm long, with numerous flowers. The peduncle and branches are greenish-white or pale green with darker green dots and are thickened at the nodes. The flowers are bisexual and regular, with four free sepals that are orbicular in shape and 6-10 mm long. The corolla tube is cylindrical, measuring 20-32 mm in length, and has 11-14 oblong-lanceolate lobes that are 10-18 mm long and spreading, with a white or creamy color. The stamens are exserted, with filaments fused into a ring, and the anthers are creamy or pale yellow. The ovary is superior, obovoid in shape, and

4celled, with a stigma that is obovoid-cylindrical and apically 2-lobed. The fruit is an ellipsoid berry, measuring 3.5-5 cm x 2-3.5 cm, with a rounded apex and thick walls, and is dark green in color with numerous seeds. The seeds are obliquely ovoid, brown in color, and measure 2.5 mm x 1.5-2 mm x 1 mm (Okoli and Iroegbu, 2004).

2.2 Distribution of *Anthocleista djalonensis*

Anthocleista djalonensis, commonly known as the cabbage tree, is a small to medium-sized tree species native to tropical Africa. It occurs predominantly across West Tropical Africa, extending eastward to southern Chad, and thrives mainly in savanna and forest habitats (GBIF, 2000). The species is widely distributed in Ghana, Guinea, Ivory Coast, Liberia, Nigeria, and Sierra Leone, where it grows naturally in the wild and is extensively utilized in traditional medicine (GBIF, 2000).

According to Adebayo and Olamide (2022), *A. djalonensis* belongs to the family Gentianaceae and typically flourishes in lowland tropical regions characterized by moderate rainfall and welldrained soils. Its adaptability to both savanna and secondary forest zones explains its wide presence across West African ecological regions.

The broad geographical distribution of *A. djalonensis* enhances its accessibility and supports its extensive ethnomedicinal applications across West Africa, particularly in Nigeria and neighboring countries, where it is recognized as an important medicinal plant in local traditional practices.

2.2.1 Habitat and Ecology of *Anthocleista djalonensis*

Anthocleista djalonensis typically grows in open woodlands, secondary forests, and savanna regions, where it benefits from seasonally dry tropical climates. It prefers well-drained loamy or sandy soils and is most commonly found at low altitudes, often below 500 meters above sea level (Adebayo and Olamide, 2022).

The plant thrives in areas receiving moderate to high annual rainfall and can tolerate both full sunlight and partial shade, making it well adapted to disturbed or regenerating habitats. Its ability to survive under variable moisture conditions contributes to its resilience and broad ecological range within the tropical zones of West Africa.

Ecologically*, A. djalonensis* plays a role in maintaining soil stability and providing shade and habitat for smaller plant species. Its adaptability to different ecological conditions supports its conservation and sustained availability for medicinal and ethnobotanical use in rural communities across West Africa.

2.3 Ethno-medicinal uses of *Anthocleista djalonensis*

Anthocleista djalonensis has been traditionally used to treat wide ranges of health issues, including wounds, constipation, diarrhea, dysentery, and abdominal pain (Okoli and Iroegbu 2004). Additionally, it's used for hepatitis, jaundice, cirrhosis, fungal skin infections, filarial worm infections, acute inflammation, and boils on the skin (Aiyeloja and Bellow, 2006). The plant has also been reported to have anti-diabetic, antimalarial, anti-pyretic, anthelmintic, antimycobacterial, antibacterial, and wound healing properties (Okoli and Iroegbu, 2004; Nweze and Ngongeh, 2007). It's widely used as a strong purgative and diuretic (Neuwinger, 2000).

A root decoction is commonly used to treat constipation, regulate menstruation, and as an abortifacient, while a root infusion is taken to treat intestinal problems, acute inflammations, and boils on the skin (Okoli and Iroegbu, 2004). The plant also have free radical scavenging activities (Awah *et al.,* 2010). *A. djalonensis* have been observed to contain a high capability to scavenge hydrogen peroxide radicals, thus stopping the chain reaction that causes cell damage (Igbinaduwa and Benjamin, 2019).

A study in 2019 showed that the root extract of *A. djalonensis* possesses high antioxidant activity. The antioxidant activity of the plant suggest that it is a rich source of natural antioxidants, this can be often traced to the phenolic and flavonoid constituents of the plant (Igbinaduwa and Benjamin, 2023).

2.3.1 Phytochemical composition of *Anthocleista djalonesis*

The phytochemical composition of *A. djalonensis* from the roots to the leaves and stem bark contains a wide range of bioactive compounds which includes alkaloids, terpenoids, flavonoids, tannins, saponins, and glycosides (Nsofor, 2024). These compounds have been reported to have antioxidant, anti-inflammatory, and antimicrobial effects.

The plant's extracts have shown promising antioxidant potentials, antidiabetic properties, and potential therapeutic applications (Igbinaduwa and Okeke, 2023). Phenolic compounds and carotenoids have also been identified in A. djalonensis, which may contribute to its antioxidant activity (Igbinaduwa and Okeke, 2023). The presence of these phytochemicals supports the plant's traditional use in medicine.

The medicinal properties of A. djalonensis have been attributed to its diverse phytochemical composition, making it a valuable and promising resource/candidate for the development of new therapeutic agents (Anyanwu *et al.,* 2015).

2.4 PHARMACOLOGICAL ACTIVITIES

2.4.1 Antioxidant activity

Anthocleista djalonensis, native to tropical Africa, exhibits potent antioxidant activity due to its diverse range of bioactive compounds, which effectively neutralize free radicals, underscoring its potential as a strong antioxidant agent (Monon *et al.,* 2022).

Antioxidants play a crucial role in protecting the body from oxidative stress caused by free radicals. These unstable molecules can trigger a cascade of detrimental reactions, leading to chronic conditions like cardiovascular disease, neurodegenerative disorders, and cancer (Meydani, 2001). The body naturally combats oxidative stress with antioxidants, which can be internally produced or obtained from dietary sources such as fruits, vegetables, and nuts. Consuming these antioxidant-rich foods has been shown to mitigate the harmful effects of free radicals. By leveraging natural antioxidants, individuals can potentially reduce the risk of oxidative stress-related diseases (Datel *et al.,* 2010).

In 2017 Oyinlade *et al* did a study on the stem bark of *A. djalonensis*, the study proved that the ethanol extract of stem bark of the plant exhibited high antioxidant activity and cytotoxic effect as a result of its ability to scavenge free radicals (DDPH), inhibit the growth of onions bulb and bring about the death of newly hatched brine shrimp (nauplii). Therefore, the stem bark of *A. djalonensis* not only antioxidant but also chemotherapeutic agents that may be useful in managing free radical provoking disease such as cancer.

Previous phytochemical analyses of *Anthocleista djalonensis* have also revealed a diverse array of bioactive compounds, including alkaloids, flavonoids, triterpenes, phenolic compounds, phthalides, xanthones, and iridoid glucosides, as well as potentially carotenoids and tocopherols. These compounds have garnered significant attention due to their potential biological properties, including antioxidant, anticonvulsant, immunomodulatory, and anti-inflammatory effects. The antioxidant properties of *Anthocleista djalonensis* can be attributed to the presence of these compounds, which work together to neutralize free radicals and protect against oxidative stress (Okoli and Iroegbu, 2004). Ongoing research aims to understand the mechanisms behind these properties and identify the specific active substances responsible for the plant's anticonvulsant and antioxidant activities (Taiwe *et al.,* 2017).

2.4.2 Anticonvulsant activity

Grand mal seizures, a severe form of epilepsy, occur when a group of brain neurons discharge abnormally and synchronously. This can lead to loss of consciousness, intense muscle contractions, and potentially, oxidative stress causing neuronal damage. These seizures are a type of neurological disorder that can affect individuals worldwide regardless of geographical location or other factors, but commonly found in tropical regions of Africa (Valentin and Alarcon, 2012). Current epilepsy treatment primarily involves long-term use of anticonvulsant medications to manage seizures. However, this approach has limitations, as seizures persist in approximately 30% of patients, and side effects are frequent (Taiwe *et al.,* 2016). For individuals with brain injuries at high risk of developing epilepsy, preventive pharmacological interventions may be possible (Becker, 2018). With numerous antiepileptic drugs available, each with its own side effect profile, selecting the right medication can be challenging (Devinsky *et al.,* 2005).

While newer medications have improved safety records, they can still impact quality of life. As a result, researchers are exploring natural products as potential sources for new antiepileptic compounds with better efficacy and tolerability. One such promising natural product is *Anthocleista djalonensis*, a plant species that has shown potential anticonvulsant and antioxidant properties, making it a valuable candidate for further investigation in the development of novel antiepileptic therapies (Shorvon, 2009).

Anthocleista djalonensis's anticonvulsant properties stem from its rich phytochemical composition. Phenolic compounds in the plant exhibit antioxidant and neuroprotective effects, potentially reducing seizure activity. Tannins may influence neuronal excitability by interacting with neurotransmitters. Terpenoids could modulate GABA receptors, contributing to the plant's anticonvulsant effects. Alkaloids may impact neurotransmitter systems involved in seizure regulation, including GABA, glutamate, and dopamine. Additionally, flavonoids like catechin, rutin, and quercetin possess antioxidant and potential anticonvulsant properties. The combined action of these phytochemicals is believed to contribute to the plant's therapeutic potential, warranting further research to fully understand its mechanisms and constituent effects (Igdbinaduwa and Benjamin, 2023).

2.4.3 Antimicrobial activity

Anthocleista djalonensis are traditionally used to treat various ailments, including diarrhea, wounds, abdominal pain, skin boils, and fungal infections. Phytochemical analysis of the plant's leaf extracts reveals the presence of bioactive compounds such as tannins, saponins, flavonoids, steroids, terpenoids, and cardiac glycosides. These compounds may contribute to the plant's antibacterial properties, supporting its traditional use in treating diseases caused by pathogenic

microorganisms. The varying solvents used in extraction may influence the presence of these metabolites, which possess pharmacological activities (Akinyemi and Ogundare, 2014).

In 2017, a study confirmed the traditional use of *Anthocleista djalonensis* for treating various diseases in Benue State, Nigeria. The plant's leaves were extracted using different solvents, revealing a range of phytochemicals, including glycosides, saponins, terpenes, sterols, flavonoids, anthraquinones, resins, and balsams. The extracts showed significant antimicrobial activity against several microorganisms, including *Methicillin-resistant Staphylococcus aureus*, *Vancomycin-resistant enterococci, Staphylococcus aureus, Helicobacter pylori*, and fungal strains such as *Candida albicans* and *Candida krusei*. Notably, the minimum bactericidal and fungicidal concentrations of the extracts ranged from 1.25 to 5 mg/mL against tested microbes. These findings support the plant's potential as a source of antimicrobial agents and validate its traditional use, highlighting its potential for developing novel therapeutic agents. The results provide scientific evidence for the effectiveness of *Anthocleista djalonensis* in traditional medicine (Ijeoma *et al.,* 2017).

In 2024, Ijeoma *et al.,* did a study on the antimicrobial and anti-HIV-1 properties of *Anthocleista djalonensis, Vernonia cinerea*, and *Pycnanthus angolensis* extracts. The root of *Anthocleista Djalonensis* was used and a methanol extract was made for all three plants. Comparing *Anthocleista djalonensis* methanol extract (MethAD) to *Vernonia cinerea*, and *Pycnanthus angolensis* methanol extract (MethVC and MethPA), it was found that MethAD was the most active. When tested against *Staphylococcus aureus* (MIC: 8 µg/mL), a Gram-positive strain linked to skin conditions, the methanol extract of *Anthocleista djalonensis* (MethAD) produced the greatest results (Ijeoma *et al.,* 2024).

2.4.4 Anti-inflammatory and analgesic activities

In Nigeria, a traditional remedy for asthma involves consuming a cold infusion of *Anthocleista djalonensis* stem bark, often mixed with other herbs, at a dosage of two tablespoons daily. Additionally, poultices made from the plant are applied to swelling, rheumatism, and wounds to relieve pain, reduce inflammation, and promote healing. Studies conducted on albino rats have shown that methanol extracts from the root of A. djalonensis effectively reduce both neurogenic and inflammatory pain through central and peripheral mechanisms, thereby supporting its traditional use as an analgesic (Kagbo and Simon, 2015; Enoghase and Innih, 2025).

2.4.5 Anti-fertility activities

In southwestern Nigeria, *Anthocleista djalonensis* is used as an aphrodisiac to enhance libido, promote erections, increase sperm count, and improve male fertility. Oxidative stress, which can cause sperm membrane damage through lipid peroxidation, is a significant factor in male infertility. Studies have shown that *Anthocleista djalonensis* enhances sperm activity and mitigates oxidative damage, providing scientific validation for its traditional use in supporting male reproductive health, thereby offering potential natural solutions for fertility issues (Enoghase and Innih, 2025).

2.4.6 Anti-helminthic and laxative activities

Anthocleista djalonensis ethanolic extracts demonstrated potent anthelmintic activity against Heligmosomoides polygyrus larvae, inducing concentration-dependent mortality at doses ranging from 25 to 200 mg/mL. Notably, a dose of 100 mg/mL resulted in significant larval mortality (98.45%), comparable to the efficacy of levamisole at 10 mg/mL, thereby validating its

traditional use in treating intestinal worm infections and other parasitic diseases (Enoghase and Innih, 2025).

In various African countries, including Nigeria and Cameroon, the plant has been traditionally used as a potent laxative. To induce purgative effects, decoctions made from its leaves, bark, or roots are commonly ingested, reflecting its long-standing use in local medicine (Enoghase and Innih, 2025).

2.4.7 Toxicity studies

The methanol extracts of *A. djalonensis* stem, roots, and leaves, along with isolated compounds like djalonenol, sweroside, and djalonensone, were evaluated for cytotoxicity against ST-57 brain tumor fibroblasts. Most extracts and compounds showed low cytotoxicity, with ED50 values ranging from 40 to 70 µg/mL, while djalonensone was not significantly active, indicating that A. djalonensis has relatively low toxicity concerns (Enoghase and Innih, 2025).

2.5 ANTIOXIDANTS

Antioxidants are bioactive compounds that protect cells from oxidative damage triggered by free radicals. Even in small amounts, they can slow or halt oxidative reactions, which helps preserve food quality and reduces the risk of chronic diseases like cardiovascular disease, diabetes, and cancer (Gulcin, 2025; Cakmakci *et al.,* 2015). In food systems, antioxidants prevent lipid peroxidation and the formation of secondary oxidative products, thereby maintaining taste, color, and texture. They also protect proteins from oxidation and interaction with lipid-derived carbonyls, which can impair protein function (Gulcin, 2025).

The concept of antioxidants has evolved, with definitions varying between food science and biochemistry. In food science, antioxidants were initially defined as compounds that delay or inhibit oxidation, while in biochemistry, the term encompasses molecules that prevent, delay, or remove oxidative damage (Gulcin, 2025). More recent definitions emphasize their ability to neutralize reactive oxygen species (ROS) or enhance endogenous defense mechanisms (Sadowska-Bartosz and Bartosz, 2022).

It's essential to distinguish between "antioxidant activity" and "antioxidant capacity."
Antioxidant activity refers to the effect of a single compound under specific conditions, whereas antioxidant capacity, often measured as total antioxidant capacity (TAC), assesses the cumulative effect of multiple compounds in complex mixtures (Gulcin, 2025). Antioxidants can be classified based on their origin and mechanisms of action or source, including enzymatic, non-enzymatic, and synthetic antioxidants.

2.5.1 Antioxidant enzymes

The antioxidant enzymatic system is categorized into primary and secondary defenses. Primary enzymes, including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), directly neutralize reactive oxygen species (ROS). GPx reduces hydrogen peroxide and lipid peroxides to water or alcohols using glutathione, while catalase decomposes hydrogen peroxide into water and oxygen at a high turnover rate. SOD converts superoxide anions into hydrogen peroxide, which is then processed by catalase (Gulcin, 2025).

Secondary enzymatic defenses, such as glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PD), support antioxidant function. GR regenerates glutathione, enabling continuous peroxide detoxification, while G6PD generates NADPH, providing reducing power

for antioxidant cycles (Gulcin, 2025). These enzymes work together to protect cells by converting reactive molecules into less harmful products, often relying on cofactors like zinc, copper, and manganese.

In contrast, non-enzymatic antioxidants, including vitamins C and E, glutathione, carotenoids, and plant polyphenols, act by scavenging free radicals directly or by interrupting chain reactions, thereby providing additional protection against oxidative stress (Nimse, 2015).

2.5.2 Oxidative stress

Oxidative stress, a concept introduced by Sies in 1985, occurs when 'cells and tissues' antioxidant defenses are overwhelmed by reactive oxygen species (ROS), leading to redox imbalance, molecular damage, and disrupted cellular regulation (Bingöl *et al.,* 2021). This concept has been widely used in medical sciences to explain the imbalance between oxidant production and antioxidant defense. Oxidative stress is typically identified by indicators such as elevated levels of oxidatively modified cellular components or altered antioxidant enzyme activity (Kızıltaş *et al.*, 2021).

Under normal conditions, ROS production and elimination are balanced. However, this equilibrium can be disrupted by factors such as increased autoxidation, depletion of antioxidants, or inactivation of antioxidant enzymes, leading to elevated ROS levels (Yapıcı *et al.,* 2021). A sudden increase in ROS can cause acute oxidative stress, which may be reversible if antioxidant systems restore balance. Chronic oxidative stress, characterized by long-term cellular damage and altered homeostasis, has been linked to various pathologies, including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases (Gulcin, 2025).

The relationship between oxidative stress and disease is complex, and it is often unclear whether oxidative imbalance is a cause or consequence of disease progression. Oxidative stress can result from external factors, such as exposure to oxidants or ischemia/reperfusion injury, and is central to the onset and progression of major chronic conditions (Taslimi *et al*., 2020; Karakaya *et al.,* 2020). ROS play essential roles in signaling and homeostasis, but excess accumulation can damage biomolecules and contribute to disease pathogenesis (Cetin-Cakmak and Gulcin, 2019; Apak *et al.,* 2022).

2.5.3 Oxidative stress biomarkers

Researchers utilize various methods to assess oxidative stress markers, which is crucial for understanding and treating diseases. These markers serve as key indicators of cellular damage and provide insights into the pathophysiological mechanisms of conditions like diabetes, cancer, and metabolic syndromes. Studies suggest a link between diabetes and cancer, with reactive oxygen species (ROS) playing a significant role in this connection (Ayat *et al.,* 2024). Many studies highlight malondialdehyde (MDA) as a key marker of oxidative stress, particularly in type 2 diabetes mellitus (T2DM) patients. Elevated MDA levels indicate increased lipid peroxidation and oxidative damage, contributing to disease complications. Additionally, T2DM patients often exhibit decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which further intensifies oxidative stress.

2.5.3.1 Malondialdehyde (MOD)

Malondialdehyde (MDA) is a byproduct of lipid peroxidation, which occurs when free radicals damage polyunsaturated fatty acids. Elevated MDA levels reflect increased oxidative stress,

making it a reliable marker for various disease conditions. The thiobarbituric acid (TBA) assay is commonly used to measure MDA in biological samples. However, due to MDA's instability and the TBA reaction's limited specificity, many studies measure total thiobarbituric acid-reactive substances (TBARS) as a biomarker of oxidative stress instead (Ayala *et al.,* 2014).

2.5.3.2 Superoxide dismutase

Superoxide dismutase (SOD) is a vital metalloenzyme that plays a crucial role in protecting living organisms from reactive oxygen species (oxidative defense). As the first line of defense, SOD catalyzes the dismutation of superoxide radicals into stable molecules, utilizing metal ions to facilitate the oxidation-reduction reaction. Different isoforms of SOD exist, each dependent on specific metal cofactors such as copper, zinc or manganese that enable its antioxidant function (Maryam *et al.,* 2015).

2.5.3.3 Catalase

Catalase is another crucial enzyme that helps protect the body from oxidative damage by converting hydrogen peroxide into water and oxygen. Alongside superoxide dismutase (SOD), catalase plays a key role in mitigating free radicals and is an essential component of the body's antioxidant defense system. Both enzymes work together by forming a synergistic defense to neutralize reactive oxygen species and maintain cellular homeostasis (Maryam *et al.,* 2015).

2.6 PHYSIOLOGY OF THE LIVER

The liver is a vital multifunctional organ that plays a central role in various bodily functions, including metabolism, detoxification, and storage of essential nutrients (Ozougwu, 2017). Its complex anatomy and structure comprise two primary lobes, further subdivided into hepatic

lobules, with an intricate network of blood vessels and bile ducts (Guyton and Hall, 2016). The liver's multifaceted functions include regulating nutrient (carbohydrate, lipid, and protein) metabolism, eliminating toxins and waste products from the blood, storing essential vitamins and minerals, and contributing to immunological processes (Sherwood, 2015).

The liver receives a unique dual blood supply from the hepatic portal vein and hepatic artery, providing nutrient-rich and oxygen-rich blood, respectively (Boron and Boulpaep, 2016). Its remarkable ability to regenerate after damage is influenced by various factors, including growth factors, cytokines, and transcription factors (Taub, 2004). Supported by ligaments such as the falciform, coronary, triangular, and lesser omentum, the liver's structure enables it to perform a wide range of vital functions (Moore *et al.,* 2016).

The liver's intricate structure and function are essential for maintaining overall health and wellbeing, and its dysfunction can lead to various diseases and disorders (Ozougwu, 2017). Overall, the liver plays a critical role in maintaining homeostasis and ensuring the proper functioning of the body.

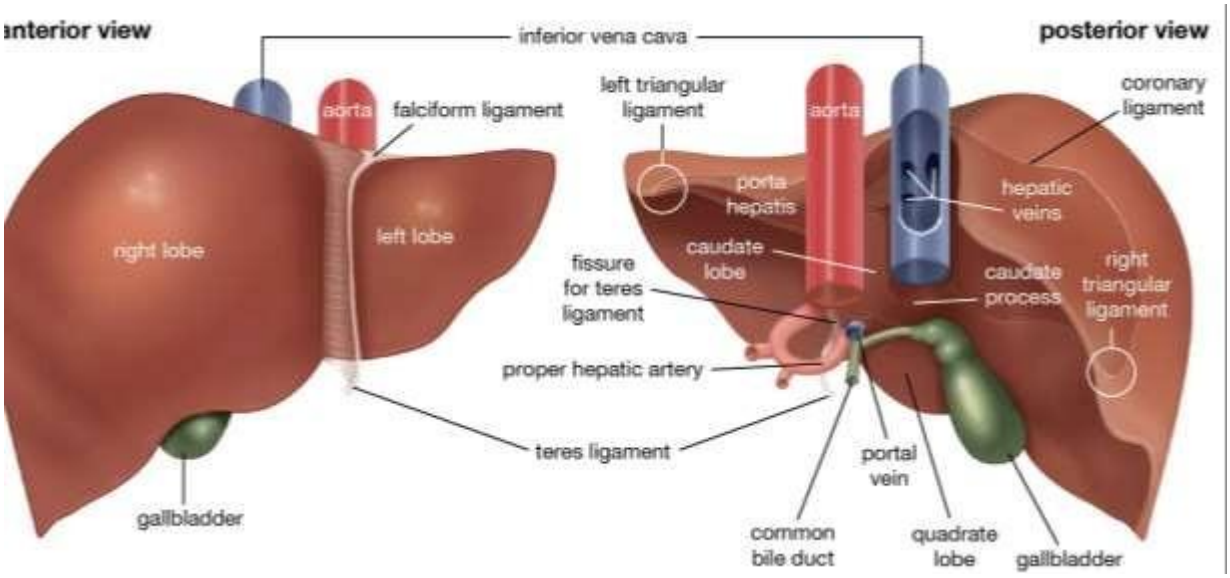

Plate B: The liver

Source: Encyclopaedia Britannica

2.6.1 Liver and its antioxidant activities

The liver is a vital digestive organ that plays a central role in nutrient synthesis, metabolism, storage, and redistribution (Takie-Eldin *et al.,* 2012). Comprising various cell types, including Kupffer cells, hepatocytes, hepatic stellate cells, and hepatic sinusoidal endothelial cells, the liver's functions rely on intricate interactions between these cells through signaling pathways (Payam *et al.,* 2006). However, the liver is susceptible to damage from external factors, such as toxins, viruses, and metabolic disorders, which can lead to various diseases like hepatitis, cirrhosis, and liver cancer. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, is a key mechanism underlying liver damage and disease progression.

Fortunately, the liver has a remarkable ability to regenerate, returning to its original mass after damage (Masato and Michitaka, 2011). Hepatocytes, normally in the G0 phase, can re-enter the

cell cycle in response to stressors, such as drugs or chemicals, and proliferate to restore liver function (Masato and Michitaka, 2011). This process is crucial for liver regeneration, with hepatocyte proliferation and differentiation of other cells, like biliary epithelial cells and liver progenitor cells, playing key roles (Sanae *et al.,* 2005). Additionally, paracrine signaling between different cell types in the liver facilitates hepatocyte proliferation and liver regeneration.

The liver's antioxidant defense system, comprising enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase, plays a critical role in mitigating oxidative stress. However, excessive ROS production can overwhelm these defenses, leading to lipid peroxidation, protein modification, and DNA damage. Understanding the interplay between oxidative stress and antioxidant activities in the liver is essential for developing effective therapeutic strategies to prevent and treat liver diseases.

The liver's regenerative capacity is influenced by various factors, including growth factors, cytokines, and transcription factors. Oxidative stress can impact liver regeneration by modulating signaling pathways and cellular behavior. Conversely, antioxidant therapies have shown promise in promoting liver regeneration and reducing oxidative damage. By elucidating the mechanisms underlying liver regeneration and oxidative stress, researchers can identify potential therapeutic targets to enhance liver regeneration and improve treatment options for liver diseases (Payam *et al.,* 2006).

In the context of liver disease, preserving liver function and promoting regeneration are critical for patient outcomes. This highlights the importance of understanding the complex interactions between different cell types in the liver, signaling pathways, and oxidative stress regulation. By exploring these mechanisms, researchers can develop novel antioxidant therapeutic strategies

particularly those derived from medicinal plants to enhance liver regeneration, reduce oxidative damage, and improve treatment options for liver diseases (Sanae *et al.,* 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Chemicals and reagents

Distilled water, normal saline (0.9% NaCl), hydrogen peroxide (H_2O_2), phosphate buffer saline (PBS, pH 7.4), sodium carbonate (Na_2CO_3), sodium phosphate (Na_3PO_4), muslin cloth, and Whatman No.1 filter paper. Distilled water was used for the preparation of all solutions and sample dilutions.

3.2. Equipment and apparatus

The equipment used included a water bath (Model: HH-S6 water bath, China), a UV–Visible spectrophotometer (Model: unspecified), an analytical weighing balance (Ohaus Corp, Pine Brook, NJ, USA), a centrifuge, an industrial blender (KENWOOD Model: KCB239K), and a tissue homogeniser. Additionally, various glassware such as conical flasks, beakers, pipettes, test tubes, and measuring cylinders were employed. Micro-pipettes with tips, a water bath with temperature control, and an intragastric gavage needle and syringe, as well as animal cages with bedding and feeders, were also utilised. Finally, a refrigerator was used for sample storage.

3.3. Plant collection

Fresh roots of *Anthocleista djalonensis* were collected from Egba Road in the Uhunmwonde local government area of Edo State. The plant specimen was identified and authenticated by Prof. H. A. Akinnibosun from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, at the University of Benin, Benin City. Only healthy, mature roots, free from any signs of disease, were selected for further use.

3.3.1. Root preparation

After collection, the roots were placed in clean, labelled polyethylene bags and transported to the laboratory within 4 to 6 hours. Upon arrival, the roots were thoroughly washed under running tap water to remove any soil particles, followed by a rinse with distilled water. Any extraneous material was meticulously removed. The cleaned roots were then sliced thinly to increase the surface area and were air-dried in a shaded, well-ventilated environment at room temperature (25–30°C) until they reached a constant weight. The dried root slices were pulverised into a coarse powder using a clean industrial blender (KENWOOD Model: KCB239K) and then sieved to achieve a uniform particle size. The resulting powder was stored in airtight glass containers made of amber.

3.4. Sample extraction

A measured quantity of 150 g of the powdered root was placed in a clean glass container and extracted with 5 L of distilled water. The mixture was allowed to macerate for 72 hours at ambient temperature (20–25°C), with stirring conducted at intervals throughout the extraction period. Following maceration, the mixture was sieved and then filtered through muslin cloth, followed by Whatman No. 1 filter paper. The filtrate obtained was concentrated over a water bath (Model: HH-S6) until a viscous extract was achieved. The extract was stored in a sample bottle and kept in a refrigerator at 4°C prior to use.

3.5.1. Experimental animals

Twenty-four (24) healthy adult Wistar rats, each weighing between 150 and 200 g, were obtained from a commercial animal house located in the Oredo local government area of Benin City. The rats were housed in the Faculty of Science Laboratory Technology at the University of Benin, where standard laboratory conditions were maintained. These conditions included a temperature of 22 ± 2°C, relative humidity of 50–60%, and a 12-hour light/dark cycle, all within well-ventilated polypropylene cages

lined with clean wood shavings that were replaced regularly to ensure appropriate hygiene. The animals had unrestricted access to a commercial pellet diet and clean drinking water. All rats were acclimatised to the laboratory environment for 14 days prior to the experiment. All procedures complied with the National Institute of Health (NIH) guidelines for the care and use of laboratory animals. This study received approval from the Science Laboratory Technology Research Ethical Committee, which was assigned a reference number UNIBEN/FSLT/00033.

3.5.2. Experimental Design

A total of twenty-four (24) Wistar rats were utilised for the study. The animals were randomly assigned into four groups, with six rats in each group (n = 6 per group). Group I served as the control group and received 2 ml of distilled water orally. Group II was administered 250 mg/kg of the aqueous root extract of *Anthocleista djalonensis*, Group III received 500 mg/kg, and Group IV was given 1000 mg/kg of the extract, all orally, over a period of 28 days.

3.6. Liver preparation

At the conclusion of the 28-day experimental period, the rats were anaesthetised using deep chloroform anaesthesia and subsequently sacrificed. A longitudinal incision was made in the abdomen to expose the liver, which was then promptly removed. The liver was washed in ice-cold normal saline to remove any residual blood, blotted dry, and weighed. Following this, the liver was minced into small pieces and homogenised with 5 ml of cold normal saline. The resulting homogenates were centrifuged at 3,500 RPM for 5 minutes, and the supernatants were collected and stored at −20°C for subsequent antioxidant assays (Golshan *et al*., 2017).

3.7. Determination of Superoxide Dismutase (SOD)

The activity of Superoxide Dismutase (SOD) was assessed using the methodology originally established by Misra and Fridovich (1972) and subsequently refined by Idu *et al.* (2016). In this assay, 0.2 ml of distilled water and 2.5 ml of carbonate buffer were added to the reference tube, followed by the addition of 0.3 ml of freshly prepared sample solution. For the test samples, 2.5 ml of carbonate buffer was placed in labelled test tubes, to which 80 µl of the sample and 120 µl of adrenaline solution were added. The contents were mixed immediately, and the absorbance was recorded at 480 nm every 30 seconds for a duration of up to 120 seconds using a UV–visible spectrophotometer (Model). Distilled water served as the blank for zeroing the instrument.

3.8. Determination of Catalase Activity

Catalase activity was determined following the procedure outlined by Idu *et al.* (2016). In brief, 0.5 ml of the test sample was pipetted into labelled tubes, while distilled water was employed as a blank control. To each tube, 2.5 ml of 30 M hydrogen peroxide was added. After allowing a reaction time of five minutes, both the test and blank solutions were treated with 1 ml of 6 M sulphuric acid and 3.5 ml of 0.01 M potassium permanganate. The absorbance was then measured within 30 to 60 seconds. For the preparation of standards, 3.4 ml of 0.01 M potassium permanganate was combined with 5.5 ml of 0.05 M phosphate buffer (pH 7.0) and 1.0 ml of sulphuric acid. Prior to measurement, the spectrophotometer (model) was calibrated using distilled water.

3.9. Determination of Malondialdehyde (MDA)

Lipid peroxidation, indicated by malondialdehyde (MDA) concentration, was assessed using a modified method from Idu *et al.* (2016). A 0.6 ml aliquot of the tissue homogenate was combined with 3 ml of a thiobarbituric acid–trichloroacetic acid–hydrochloric acid (TA–TBA–HCl) reagent in a 1:1 v/v ratio.

This reagent comprised thiobarbituric acid (0.375 % w/v), trichloroacetic acid (15 % w/v), and hydrochloric acid (0.25 M). The mixture was thoroughly mixed and then heated in a boiling water bath for 15 minutes.

3.10. Statistical Analysis

All the data were expressed as the mean ± standard error of the mean (SEM). Statistical analyses were performed using Graph-pad version 9 software. Group comparisons were conducted using one-way analysis of variance (ANOVA), followed by appropriate post hoc tests where applicable. Differences were considered statistically significant at $p< 0.05$. Mean values sharing the same superscripts were regarded as not significantly different. All data were expressed as the mean ± standard error of the mean (S.E.M). Statistical analyses were conducted using Graph-pad version 9 software, with group comparisons performed via one-way analysis of variance (ANOVA). Appropriate post hoc tests were applied where necessary. Differences were deemed statistically significant at $p < 0.05$. Mean values that shared the same superscripts were considered not significantly different.

CHAPTER FOUR

RESULTS

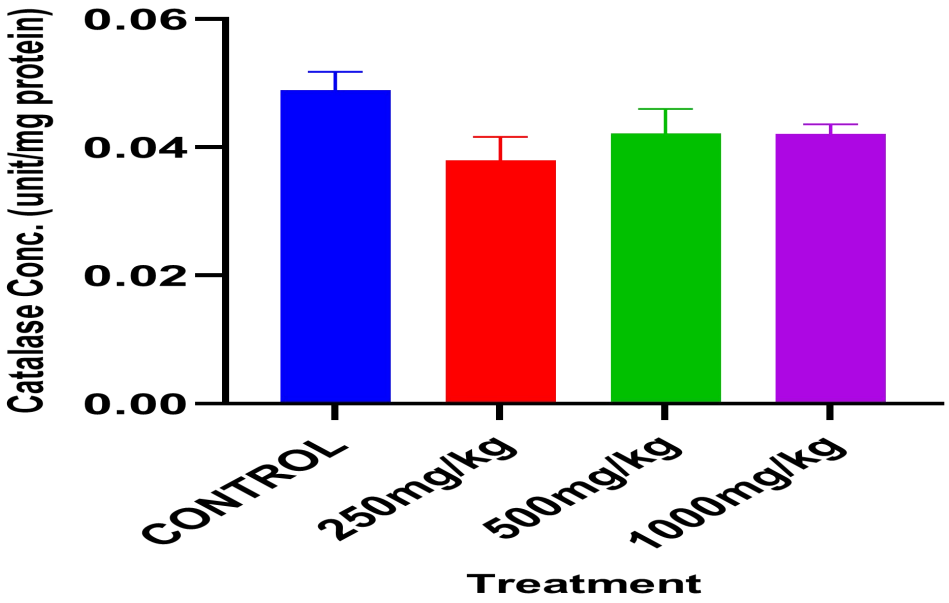

Figure 1: Effect of aqueous root extract of *Anthocleista djalonensis* on catalase activity.

The aqueous root extract of *Anthocleista djalonensis* (250, 500 and 1000 mg/kg) has no effect on the catalase level when compared with control ($p>0.05$). Values are represented as mean ± S.E.M, n=5.

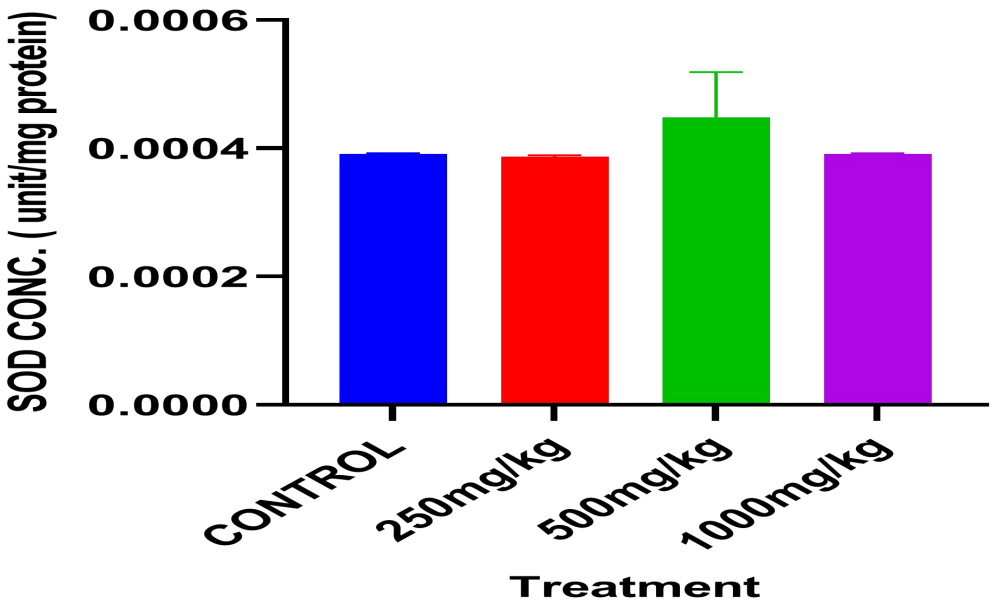

Figure 2: Effect of aqueous root extract of *Anthocleista djalonensis* on superoxide dismutase (SOD) activity.

The aqueous root extract of *Anthocleista djalonensis* (250, 500 and 1000 mg/kg) has no effect on the superoxide dismutase SOD level when compared with control ($p>0.05$). Values are represented as mean ± S.E.M, n=5.

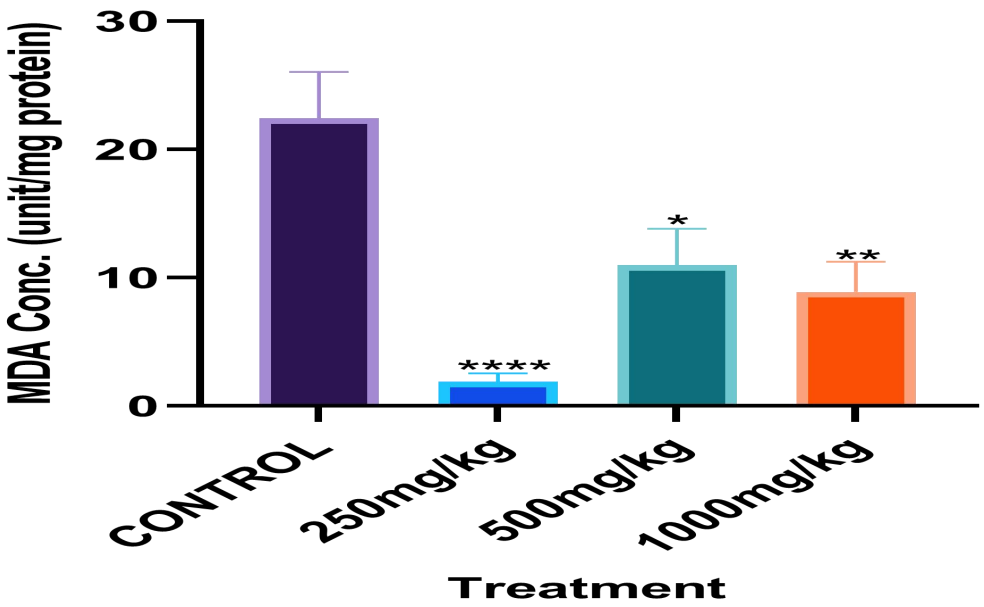

Figure 3: **Effect of aqueous root extract of *Anthocleista djalonensis* on malondialdehyde (MDA) activity**.

The aqueous root extract of *Anthocleista djalonensis* (250, 500 and 1000 mg/kg) significantly reduced the Malondialdehyde (MDA) level when compared with control (*p<0.05; **p<0.01; ****p<0.0001). Values are represented as mean ± S.E.M, n=5.

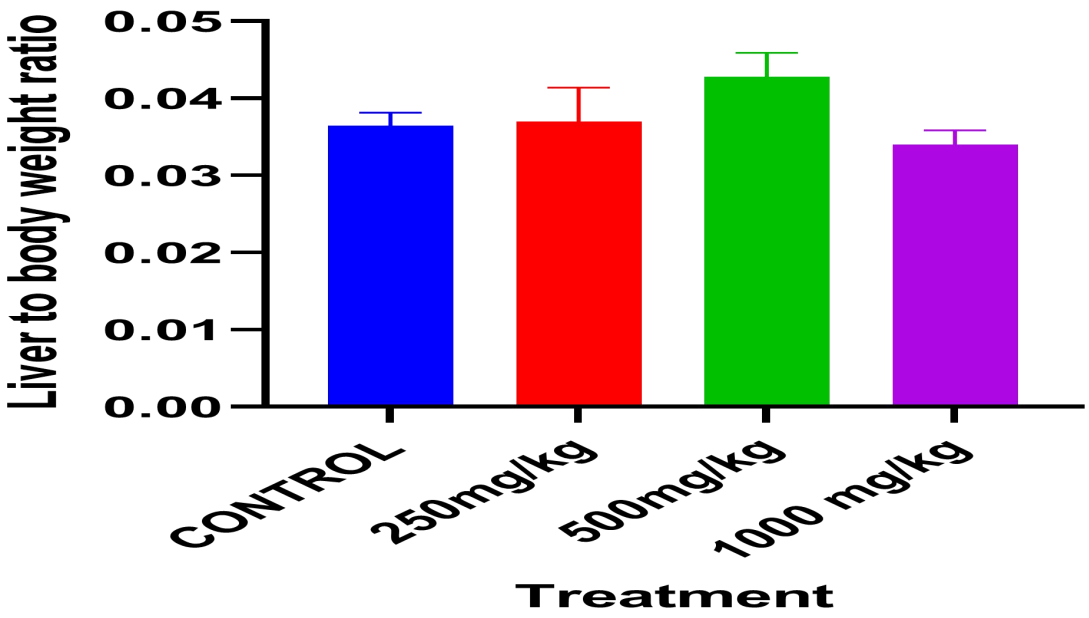

Figure 4: Effect of aqueous root extract of *Anthocleista djalonensis* on Liver to body weight ratio Activity.

The aqueous root extract of *Anthocleista djalonensis* (250, 500 and 1000 mg/kg) has no effect on the Liver to Body weight ratio when compared with control ($p>0.05$). Values are represented as mean ± S.E.M, n=5.

CHAPTER FIVE

5.1 DISCUSSION OF FINDINGS

This study evaluated the *in vivo* antioxidant properties of the aqueous root extract of *Anthocleista djalonensis* using liver homogenates from Wistar rats. The experimental outcomes revealed that the extract significantly reduced the level of malondialdehyde (MDA), a marker of lipid peroxidation, while exhibiting no significant changes in catalase (CAT), superoxide dismutase (SOD), and liver-to-body weight ratio. These results suggest that the extract exerts its antioxidant action primarily through non-enzymatic mechanisms, possibly due to its phytochemical constituents.

The reduction in MDA concentration is a key finding, indicating the plant's ability to suppress lipid peroxidation—a process that leads to membrane damage and cellular dysfunction. MDA is one of the most common indicators of oxidative stress and lipid degradation in biological systems (Ayala *et al.,* 2014). The reduction observed in treated groups suggests that *A. djalonensis* rootextract effectively scavenged reactive oxygen species (ROS), thereby preventing peroxidative injury. This aligns with the findings of Awah *et al*. (2010), who reported that extracts of *A. djalonensis* possess potent antioxidant activity, attributed to the high content of flavonoids and phenolic compounds.

Lipid peroxidation, is viewed traditionally as an effect of free radical activity, which has been liked to various cellular damages. Initially explored in the context of of fat and oil rancidity.It,s role expanded to encompass a broad range of cellular injuries like; cell death, inflammation and disruption of cellular function (Pablo, 2017). Free radicals not only induces lipid peroxidation but also have the potential to lead to a fatty liver which can cause chronic liver injury such as non-alcoholic fatty liver disease (NAFLD), which has been observed to be an important medical

problem worldwide (Morita *et al.,* 2012). Lipid peroxidation can also lead to effects on the liver due to it also being an effect of free radicals, example of such effects are; fibrosis, impaired liver function, cirrhosis and also non-fatty liver disease (NAFLD) (Sen *et al.,* 2013). The reduction in malondialdehyde (MDA) activity reduces the potential of lipid peroxidation.

The non-significant variations in SOD and CAT activities further support the notion that the extract's antioxidant effect is not necessarily due to enzymatic modulation but rather through direct scavenging of free radicals. According to Gulcin (2025) and Nimse (2015), plant-based antioxidants can function either by enhancing endogenous enzyme activities or by directly neutralizing reactive oxygen species (ROS). The stable enzyme levels observed in this study indicate that the extract maintains physiological antioxidant homeostasis without overstimulation, a desirable property for therapeutic agents.

The phytochemical composition of *A. djalonensis* plays a vital role in its bioactivity. Compounds such as flavonoids, tannins, alkaloids, saponins, and terpenoids are well-known for their antioxidant and hepatoprotective effects (Nsofor, 2024; Monon *et al.,* 2022). The synergistic interaction of these phytochemicals likely contributes to the root's observed efficacy.

Another significant finding is the unchanged liver-to-body weight ratio, which indicates that the aqueous root extract did not exert any hepatotoxic or hypertrophic effects on the liver. This observation is consistent with the study of Enoghase and Innih (2025), who reported that *A. djalonensis* extracts have low cytotoxicity and can protect hepatocytes from oxidative damage. The absence of adverse morphological changes highlights the safety of the extract, further validating its traditional use for treating liver-related (like hepatitis, cirrhosis, jaundice) and inflammatory conditions (Enoghase and Innih 2025).

In the context of oxidative stress, maintaining a balance between free radicals and antioxidants is essential for cellular health. An imbalance results in oxidative stress, which contributes to the onset of several degenerative diseases such as diabetes, cardiovascular disorders, and cancer (Goodarzi, 2018; Meydani, 2001). The results of this study demonstrate that *A. djalonensis* could serve as a natural antioxidant source to mitigate such conditions by maintaining redox equilibrium.

The results from this investigation provides support to the ethnomedicinal claims regarding *Anthocleista djalonensis*. The extract demonstrated the ability to protect liver tissues against oxidative stress by suppressing lipid peroxidation and stabilizing antioxidant enzyme activities. These outcomes align with previous studies (Okoli and Iroegbu, 2004; Oyinlade *et al.,* 2017; Igbinaduwa and Benjamin, 2023) and reinforce the growing recognition of *A. djalonensis* as a potent natural antioxidant with therapeutic potential in oxidative stress-related pathologies.

5.2 CONCLUSION

The results of this study revealed that the aqueous root extract of *Anthocleista djalonensis* significantly reduced malondialdehyde (MDA) levels in liver homogenates. These findings provide scientific validation for the traditional medicinal use of *A. djalonensis* and support its potential for development into standardized herbal formulations targeting oxidative stress and liver disorders like cirrhosis and hepatitis through direct free radical scavenging.

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