INVIVO ANTIOXIDANT POTENTIAL ANALYTES: PROTEINS, VITAMIN C, and GSH OF ALSTONIA BOONEI DE WILD ROOT EXTRACT

BY

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A STUDY PRESENTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN.

IN PARTIAL FULFILLMENT OF THE AWARD OF BACHELOR OF SCIENCE (B.Sc) IN BIOCHEMISTRY.

FEBRUARY, 2025

CERTIFICATION

I, hereby certify that this project was carried out by MONDAY BLESSING EMEN, with matriculation number LSC2006812, in the department of Biochemistry, faculty of Life Sciences, University of Benin, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) Honors degree.

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DEDICATION

To God almighty, who in His awesome faithfulness grants me grace to complete whatever I start, and also to my late mum Monday Sylvia Uduak may her sweet and humble soul continue to rest in perfect peace.

ACKNOWLEDGEMENT

My infinite gratitude goes to the LORD ALMIGHTY for His faithfulness and benevolence towards my academic pursuit.

To my distinguished supervisor PROF. E.C. ONYENEKE for him patience, guidance, moral and academic support during the course of this research work.

My greatest gratitude to my father (Monday Harry Johnson) for his financial support, and also to my sweet sister Deborah Grace for for her support and guidelines may the Almighty God continue to bless them.

I want to give special thanks to all the lecturers in the department of biochemistry for impacting me with their knowledge and making it possible for me to come this far, may God richly bless them.

Special thanks to the Head of Department, Prof. E.C. Onyeneke, for his fatherly concerns, guidance and diligence towards seeing us attain value in life.

Special thanks to Finian for his care and support.

To everyone else who has in one way or another influenced the success of this study, I am eternally grateful.

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ABSTRACT

This study evaluates the antioxidant and toxicity profiles of *Alstonia boonei* aqueous root extract in Wistar rats, a plant widely used in African traditional medicine for its therapeutic properties. Despite its extensive traditional use, limited scientific data exist on its biochemical effects and potential toxicity. This study aims to assess the antioxidant activity and safety profile of *Alstonia boonei* to validate its pharmacological relevance.

Fourteen Wistar rats were assigned to three groups : a control group and four treatment groups receiving varying doses (100, 250, and 500 mg/kg) of *Alstonia boonei* root extract for 21 days. Biochemical assays assessed glutathione (GSH), vitamin C levels, total protein concentration, and organ weights. Acute and subacute toxicity studies were conducted, and statistical significance was determined using one-way ANOVA ($p \leq 0.05$).

No mortality or observable toxicity symptoms were recorded, confirming the extract's safety at doses up to 5000 mg/kg. A dose-dependent increase in total protein concentration was observed in the liver, reaching 2.60 ± 0.13 g/dL in the 100 mg/kg group ($p \leq 0.05$), suggesting enhanced protein synthesis. However, GSH levels declined significantly in the liver from 124.59 ± 2.62 µg/mL (control) to 23.77 ± 0.82 µg/mL (100 mg/kg), indicating a potential transient depletion of antioxidant reserves. Vitamin C levels showed a compensatory increase, peaking at 76.62 ± 27.27 µg/mL in the liver of the 500 mg/kg group.

The findings suggest that *Alstonia boonei* root extract exhibits strong antioxidant potential while maintaining a favorable safety profile. However, the observed depletion of GSH highlights the

need for caution in prolonged use. Future research should explore long-term effects, optimal dosage, and molecular mechanisms to ensure its safe application in medicine.

CHAPTER ONE

Introduction And Literature Review

1.1 Introduction

Medicinal plants play a crucial role in healthcare systems worldwide, particularly in developing countries where traditional medicine forms a significant part of primary healthcare. The World Health Organization (WHO) estimates that approximately 80% of the global population relies on traditional medicine for primary healthcare needs (WHO, 2013). Among these medicinal plants, *Alstonia boonei* has gained significant attention due to its diverse therapeutic applications in traditional medicine. Native to West Africa, this tree, often referred to as "God’s tree," is renowned for its efficacy in treating malaria, fever, rheumatism, and other ailments (Adeoye *et al*., 2015). Its therapeutic properties are attributed to its rich phytochemical composition, including alkaloids, flavonoids, saponins, and tannins, which exhibit pharmacological activities such as antimalarial, anti-inflammatory, and antioxidant effects (Ajaiyeoba *et al.,* 2012; Atanu *et al*., 2021).

Phytochemicals, the bioactive compounds produced by plants, serve as natural defenses against pathogens and environmental stressors(Akinmoladun *et al*., 2018). These compounds, which include alkaloids, flavonoids, tannins, and terpenoids, are increasingly recognized for their pharmacological potential in addressing contemporary health challenges such as antimicrobial resistance, chronic diseases, and oxidative stress (Nair *et al*., 2013; Mollica *et al*., 2022). Research into the phytochemical properties of *Alstonia boonei* has revealed its promising applications in treating diseases like malaria, degenerative disorders, and chronic inflammatory conditions(Adeoye *et al.,* 2015).. For instance, its antioxidant activity plays a crucial role in combating oxidative stress, a key factor in the development of chronic and degenerative diseases (Mollica *et al.*, 2022; Omoya and Oyebola, 2019).

Despite its widespread use in traditional medicine, the phytochemical profile and mechanisms of action of Alstonia boonei remain underexplored. Advanced analytical techniques, such as gas chromatography-mass spectrometry (GC-MS), provide opportunities to identify and characterize the plant's bioactive compounds, facilitating the development of novel therapeutic agents (Imam *et al.,* 2017). This study aims to bridge this knowledge gap by conducting a comprehensive in vivo antioxidant potential **o**f *Alstonia boonei* aqueous root extract by analyzing its effects on oxidative stress markers, including vitamin C, glutathione (GSH), and total protein concentration**,** in Wistar rats*,* thereby contributing to the global effort to harness medicinal plants for therapeutic purposes.

Alstonia boonei contributes to biodiversity in tropical forests. Its tall, deciduous structure provides habitat and food for various wildlife species. The flowers attract pollinators such as bees, while the seeds serve as a food source for birds and small mammals (Adeoye *et al.,* 2015).

Like many large tropical trees, *A. boonei* plays a role in carbon sequestration, helping mitigate climate change. Studies have shown that matur*e A. boonei* trees can store significant amounts of carbon in their biomass, highlighting their ecological importance (Akinmoladun *et al*., 2018).

1.1.1 Background of the Study

Medicinal plants have long been recognized as essential components of traditional medicine and have served as the foundation for modern pharmacology. The World Health Organization (WHO) highlights that approximately 80% of the global population, especially in developing regions, depends on traditional medicine for primary healthcare needs (WHO, 2013). This reliance underscores the critical role of medicinal plants in addressing global health challenges, including infectious diseases, chronic illnesses, and the growing issue of antimicrobial resistance. Among the array of medicinal plants used in traditional African medicine, *Alstonia boonei* is particularly noteworthy for its extensive therapeutic applications and cultural significance. Known locally as "God’s tree," this plant is an invaluable resource in the treatment of malaria, fever, rheumatism, and other ailments (Adeoye *et al*., 2015; Atanu *et al*., 2021).

The diverse pharmacological activities of *Alstonia boonei* are attributed to its phytochemical composition. Alkaloids, flavonoids, tannins, saponins, and terpenoids are some of the bioactive compounds found in the plant, each contributing to its therapeutic effects. These phytochemicals serve as the plant’s natural defense mechanisms against environmental stressors and pathogens, while also exhibiting significant pharmacological potential in humans (Mollica et al., 2022; Imam *et al*., 2017). Alkaloids, for instance, are well-documented for their antimalarial properties, which include inhibiting the folate biosynthesis pathway in Plasmodium parasites and inducing mitochondrial dysfunction in infected cells (Olanlokun *et al.,* 2021). Flavonoids, on the other hand, are potent antioxidants, mitigating oxidative stress—a major contributor to chronic and degenerative diseases such as cardiovascular conditions, diabetes, and neurodegenerative disorders (Mollica *et al.,* 2022).

The plant’s antioxidant properties play a critical role in neutralizing free radicals, thereby protecting cells and tissues from oxidative damage. This is particularly important in the management of chronic diseases such as cancer, diabetes, and cardiovascular disorders, where oxidative stress is a key pathological factor. Studies have shown that the stem bark and leaves of Alstonia boonei possess significant radical scavenging activity, further emphasizing its potential in combating oxidative stress and related health challenges (Mollica *et al.*, 2022; Orumwensodia and Uadia, 2023). Additionally, tannins and saponins in the plant exhibit strong anti-inflammatory and antimicrobial activities, making it a valuable resource in addressing inflammatory diseases and antibiotic resistance (Nair *et al.,* 2013).

Beyond its antioxidant and anti-inflammatory effects, *Alstonia boonei* has demonstrated notable antimalarial activity (Otuu *et al.*, 2023; Omoya and Oyebola, 2019). Malaria remains a significant public health challenge, particularly in sub-Saharan Africa, where *Plasmodium* species continue to exact a heavy toll on human health (WHO, 2013). The alkaloids in *Alstonia boonei* have been found to effectively combat *Plasmodium berghei* infections in vivo, highlighting the plant's potential as a natural antimalarial agent (Babamale *et al.*, 2017; Otuu *et al.*, 2023). Moreover, betulinic acid, a compound isolated from the plant, has been shown to enhance mitochondrial pore opening and inhibit folate biosynthesis in malarial parasites, providing a mechanistic basis for its antimalarial efficacy (Olanlokun *et al.*, 2021). These findings not only validate the traditional use of *Alstonia boonei* in treating malaria but also open new avenues for the development of plant-based antimalarial drugs (Orumwensodia and Uadia, 2023).

In addition to its antimalarial properties, *Alstonia boonei* exhibits analgesic and antipyretic effects, which are largely attributed to its flavonoid content (Nair *et al.*, 2013). These properties make the plant useful in the management of pain and fever, common symptoms associated with many infectious and inflammatory conditions (Fasola *et al.*, 2013). The plant also shows promise in managing degenerative diseases such as Alzheimer's and Parkinson's, as its antioxidant and anti-inflammatory properties may help mitigate the neuroinflammation and oxidative stress that characterize these conditions (Mollica *et al.*, 2022; Nair *et al.*, 2013). Studies have demonstrated that the plant's bioactive compounds can reduce oxidative damage in neuronal tissues, thereby slowing the progression of neurodegenerative disorders (Mollica *et al.*, 2022).

Toxicological studies have assessed the safety of *Alstonia boonei* root extracts, revealing that the plant is generally safe for therapeutic use at appropriate doses (Olanlokun and Olorunsogo, 2018). However, high concentrations may pose risks, emphasizing the importance of standardizing dosage and formulations for clinical applications (Omoregie and Folashade, 2015). The presence of bioactive alkaloids and flavonoids necessitates careful evaluation of potential side effects and toxicity profiles (Owolabi *et al.*, 2016). This underscores the need for further research to optimize the therapeutic potential of the plant while ensuring its safety.

Despite its extensive use in traditional medicine and the promising findings from preliminary studies, significant gaps remain in the scientific understanding of *Alstonia boonei*'s phytochemical profile and mechanisms of action (Omoregie and Folashade, 2015). Traditional practitioners have relied on the plant for centuries, yet modern research has only begun to explore its full potential (WHO, 2013). Advanced analytical techniques, such as gas chromatography-mass spectrometry (GC-MS), provide a valuable opportunity to identify and characterize the plant's bioactive compounds, paving the way for the development of novel therapeutic agents (Imam *et al.*, 2017). Understanding the specific bioactive components and their pharmacokinetics could enhance the efficacy and safety of *Alstonia boonei*-based treatments (Omoya and Oyebola, 2019).

This study aims to bridge the knowledge gap by conducting a comprehensive phytochemical analysis of *Alstonia boonei*, focusing on its antioxidant, antimalarial, and other pharmacological properties. The findings are expected to contribute to the global effort to harness medicinal plants as sources of safe, effective, and accessible therapeutic agents (WHO, 2013). By uncovering the phytochemical composition and mechanisms underlying its therapeutic effects, this research seeks to validate the traditional uses of *Alstonia boonei* while exploring its potential in addressing contemporary healthcare challenges such as chronic diseases, degenerative conditions, and antimicrobial resistance (Mollica *et al.*, 2022; Otuu *et al.*, 2023).

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1.1.2 Aim and Objectives of Study

1.1.2.1 Aim of the Study

This study aims to evaluate the in vivo antioxidant potential of *Alstonia boonei* aqueous root extract by analyzing its effects on oxidative stress markers, including vitamin C, glutathione (GSH), and total protein concentration**,** in Wistar rats.

1.1.2.2 Objectives of the Study

The specific objectives of this research are:

1. To assess the acute toxicity profile of *Alstonia boonei* root extract in Wistar rats to determine its safety and tolerability.
2. To evaluate the effects of *Alstonia boonei* root extract on vitamin C levels in different organ tissues as an indicator of antioxidant potential.
3. To determine the impact of *Alstonia boonei* root extract on glutathione (GSH) concentration as a measure of cellular oxidative stress modulation.
4. To investigate the total protein concentration in treated and untreated groups to assess potential alterations in protein synthesis and metabolic activity.

5. To compare antioxidant responses across different extract doses (100 mg/kg, 250 mg/kg, and 500 mg/kg) to establish dose-dependent variations.
6. To analyze statistical correlations between vitamin C, GSH, and protein levels to determine their interrelationships in oxidative stress management.

1.2 Literature Review

1.2.1 Botanical Description of *Alstonia boonei*

Alstonia boonei is a tall, deciduous tree belonging to the *Apocynaceae* family. It typically grows to a height of 30-40 meters with a diameter of approximately 60-120 centimeters (Asase & Peterson, 2019). The tree is characterized by its straight trunk, which is often fluted at the base, and a dense crown with horizontal branches. The bark is greyish or pale brown and exudes a sticky latex when incised, a feature common to members of the *Apocynaceae* family (Mollica *et al*., 2022).

The leaves of *A. boonei* are simple, oblong, and arranged in whorls of 3-10 at each nodeas seen in plate 1.1. They are leathery with a glossy surface and measure between 6-18 cm in length and 3-7 cm in width. The plant produces small, fragrant, greenish-white flowers borne in terminal cymes. Its fruits are paired follicles containing numerous seeds that are equipped with tufts of silky hairs, aiding in wind dispersal (Orumwensodia & Uadia, 2023).

Plate 1.1: The leaves of *Alstonia boonei* (Ajaiyeoba *et al*., 2012).

1.2.2 Origin and Distribution

Alstonia boonei, known by various indigenous names across West Africa, is a significant tree species indigenous to the tropical regions of the continent. In Nigeria, it is locally called "*Egbu*" among the Igbo people, while in other regions, it may be referred to as "Stool wood" or "Pattern wood" due to its utility in furniture making (Olanlokun *et al*., 2018). The tree is widely distributed across countries including Nigeria, Ghana, Côte d'Ivoire, and Cameroon, with a particular prevalence in the ecological zones of tropical West Africa (Asase and Peterson, 2019).

Nigerian names include:

Yoruba: *Ahun*

Igbo: *Egbu*

Hausa: *Ukpokpo*

International names include:

Arabic *Quasabhuva*

Assamese *Chiorta*, *Kalmegh*

Azerbaijani *Acılar Şahı, Acılar Xanı* (*khan*ı)

Bengali *Kalmegh*

Burmese *Se-ga-gyi*

Chinese *Chuan Xin Lian*

English The Creat, King of Bitters

French *Chirette verte, Roi des amers*

Gujarati *Kariyatu*

Hindi *Kirayat*, *Kalpanath*,

Indonesian *Sambiroto*, *Sambiloto*

Japanese *Senshinren*

Kannada *Nelaberu*

Lao *La-Sa-Bee*

Malay *Hempedu Bumi*, *Sambiloto*

Malayalam *Nelavepu, Kiriyattu* (Afolabi and Adekemi, 2020).

The species thrives in diverse forest ecosystems, particularly flourishing in lowland rainforests and moist, deciduous forests. These habitats are characterized by specific environmental conditions, including an annual rainfall range of 1,000-3,000 mm and temperatures consistently between 24°C to 30°C, which provide optimal growth conditions for *A. boonei* (Afolabi and Adekemi, 2020). Its adaptability to these tropical environments has contributed to its widespread distribution and ecological significance in the region.

Globally, A. boonei holds considerable economic and medicinal importance. The tree's lightweight, versatile wood is prized in furniture manufacturing, while its bark has been a cornerstone of traditional medicine practices across West Africa. Local communities have long utilized various parts of the tree for treating multiple health conditions, particularly malaria and related ailments (Orumwensodia and Uadia, 2023). However, this extensive harvesting has raised significant conservation concerns, highlighting the critical need for sustainable management and preservation strategies to protect this valuable species (Imam *et al*., 2017).

The conservation challenges are further compounded by the tree's cultural and economic significance. Its multiple uses in traditional medicine, timber production, and local industries make it a crucial resource for many West African communities. Researchers and environmental experts are increasingly advocating for comprehensive conservation approaches that balance human needs with ecological preservation (Atanu *et al*., 2021).

1.2.3 Habitat and Cultivation

Alstonia boonei demonstrates remarkable ecological adaptability, thriving in diverse tropical forest ecosystems that range from wet to dry environments. The tree exhibits exceptional resilience across various soil compositions, successfully establishing itself in loamy, sandy, and clay soil types, with a critical requirement of adequate drainage (Asase and Peterson, 2019). While demonstrating notable drought tolerance, A. boonei reaches its optimal growth potential in regions characterized by high humidity and consistent rainfall patterns, typically found in tropical West African landscapes (Afolabi and Adekemi, 2020).

The propagation of *Alstonia boonei* involves several sophisticated strategies that reflect both traditional agricultural practices and modern conservation approaches. Seed-based reproduction remains the primary method, with seeds demanding specific germination conditions—specifically, a warm and consistently moist environment that mimics the tree's natural tropical habitat (Mollica *et al*., 2022). Botanical researchers have also developed vegetative propagation techniques using stem cuttings, a method that ensures genetic consistency and allows for more controlled reproduction of desirable tree characteristics (Imam *et al.,* 2017).

Recognizing the potential risks of overharvesting, agricultural scientists and environmental experts have proposed innovative agroforestry strategies to preserve and sustainably manage *A. boonei* populations. These initiatives involve integrating the species with complementary crop systems, an approach designed to optimize land utilization while simultaneously maintaining critical biodiversity in tropical forest ecosystems (Orumwensodia and Uadia, 2023). Such comprehensive conservation strategies not only protect the species but also provide economic opportunities for local communities dependent on forest resources.

The tree's ecological versatility extends beyond its immediate habitat, making it a valuable species for sustainable agricultural and forestry practices. Its ability to adapt to varying environmental conditions while providing multiple economic and medicinal benefits positions A.

boonei as a critically important plant in tropical West African ecological systems (Atanu *et al*., 2021).

1.2.4 Taxonomy

The taxonomy of *Alstonia boonei* is as follows:

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Gentianales*

Family: *Apocynaceae*

Genus: *Alstonia*

Species: *Alstonia boonei* (Orumwensodia and Uadia, 2023).

1.2.5 Traditional and Medicinal Uses of *Alstonia boonei*

In the rich tapestry of traditional African medicine, *Alstonia boonei* emerges as a remarkably versatile therapeutic plant, deeply embedded in the healing practices of West African communities. The bark of this tree has long been revered as a potent medicinal resource, with a wide range of therapeutic applications that address numerous health conditions (Orumwensodia and Uadia, 2023). Traditionally, practitioners have utilized bark-based preparations as comprehensive treatments for various ailments, including fever, malaria, and rheumatism.

The medicinal versatility of *A. boonei* extends far beyond these primary applications. Practitioners prepare decoctions through intricate traditional methods, administering them orally to address complex health challenges such as diarrhea, hypertension, and diabetes (Atanu *et al*., 2021). Topical treatments are equally significant, with bark-derived poultices carefully applied to wounds and boils, demonstrating the plant's multifaceted healing potential.

Alstonia boonei is commonly prepared as a decoction or infusion. The bark is boiled in water for several hours to extract its bioactive compounds, which are then administered orally or applied topically (Owolabi *et al*., 2016). In some cases, the latex is used directly on wounds or mixed with other herbal remedies to enhance its efficacy.

The tree's therapeutic arsenal is not limited to its bark. Its latex, extracted through traditional methods, serves as a powerful anthelmintic agent and an effective treatment for various skin infections (Olanlokun *et al.,* 2021). The leaves, too, play a crucial role in traditional medicine, particularly in managing respiratory conditions. Practitioners have long used leaf-based preparations to alleviate symptoms of asthma and bronchitis, highlighting the holistic approach to healing embedded in traditional African medicinal practices (Afolabi and Adekemi, 2020).

Modern scientific research has begun to validate many of these traditional uses, transforming A. boonei from a locally used medicinal plant to an object of significant pharmacological interest. Researchers have been particularly intrigued by the plant's potential antimalarial properties, conducting extensive studies to understand its therapeutic mechanisms (Imam *et al*., 2017). The convergence of traditional knowledge and contemporary scientific investigation underscores the immense potential of Alstonia boonei as a valuable medicinal resource.

This comprehensive approach to healing reflects the deep ecological and cultural knowledge of traditional African medical practices. The multiple applications of A. boonei demonstrate a sophisticated understanding of plant-based medicine that goes far beyond simple symptom treatment, representing a holistic approach to health and wellness (Mollica *et al*., 2022).

The ethnopharmacological knowledge surrounding *Alstonia boonei* provides a foundation for scientific exploration. In many West African communities, traditional healers use the plant to address a wide range of ailments, including fever, malaria, and gastrointestinal disorders.

Alstonia boonei is increasingly being integrated into Complementary and Alternative Medicine (CAM) practices. Herbal preparations derived from the plant are used alongside conventional treatments for conditions such as diabetes, hypertension, and infections. This integration reflects a growing recognition of the value of traditional medicine in holistic healthcare (Ajaiyeoba *et al*., 2012).

Despite its potential, several challenges hinder the mainstream adoption of Alstonia boonei. These include the lack of standardized formulations, limited clinical data, and regulatory hurdles. Addressing these challenges requires coordinated efforts between researchers, policymakers, and healthcare providers (Adeoye *et al.,* 2015).

1.2.6 Chemical and Bioactive Components of *Alstonia boonei*

1.2.6.1 Chemistry of *Alstonia boonei*

The phytochemical composition of *Alstonia boonei* represents a complex and fascinating array of bioactive compounds that underpin its remarkable medicinal properties. Beyond the primary secondary metabolites previously identified, recent comprehensive analyses have revealed an even more intricate chemical profile that demonstrates the plant's extraordinary therapeutic potential (Mollica *et a*l., 2022).

Alkaloids remain a cornerstone of *A. boonei*'s phytochemical signature, with echitamine and boonein standing out as particularly significant compounds. These alkaloids are primarily responsible for the plant's renowned antimalarial and analgesic effects, providing a scientific basis for its traditional medicinal applications (Olanlokun *et al.,* 2021). The research by Atanu *et al.* (2021) has further elucidated the complex mechanisms through which these alkaloids interact with biological systems.

Flavonoids continue to play a crucial role, demonstrating potent antioxidant and anti-inflammatory properties that contribute to the plant's therapeutic potential. Tannins maintain their significance, providing astringent and antimicrobial activities that have long been valued in traditional medicine (Orumwensodia and Uadia, 2023).

An expanded analysis reveals an even more sophisticated phytochemical profile. Additional compounds of significant interest include:

- Xanthones: Demonstrated remarkable antioxidant and potential anti-inflammatory properties

- Stigmasterol: A plant sterol with potential cholesterol-lowering effects

- Vitamin E: A powerful antioxidant supporting cellular health

- Phytol: A diterpene alcohol with potential antimicrobial characteristics

- Squalene: A triterpene with noted antioxidant and potential anti-cancer properties

- Sitosterol: A plant sterol with potential cardiovascular benefits

- Saponins: Continuing to show immunomodulatory potential

- Terpenoids: Maintaining their association with antiprotozoan activities; as illustrated in Figure 1.1 (Imam *et al.,* 2017)

The synergistic interaction of these compounds provides a comprehensive explanation for the diverse therapeutic applications of *Alstonia boonei*. Each phytochemical contributes unique biological activities, creating a complex molecular network that explains the plant's effectiveness in traditional and potential modern medical applications (Afolabi and Adekemi, 2020).

Scientific investigations continue to unravel the intricate mechanisms of these phytochemicals, bridging the gap between traditional knowledge and modern pharmacological understanding. The multifaceted nature of *A. boonei's* chemical composition underscores its potential as a valuable resource in drug discovery and development, particularly in addressing tropical diseases and inflammatory conditions (Atanu *et al.*, 2021).

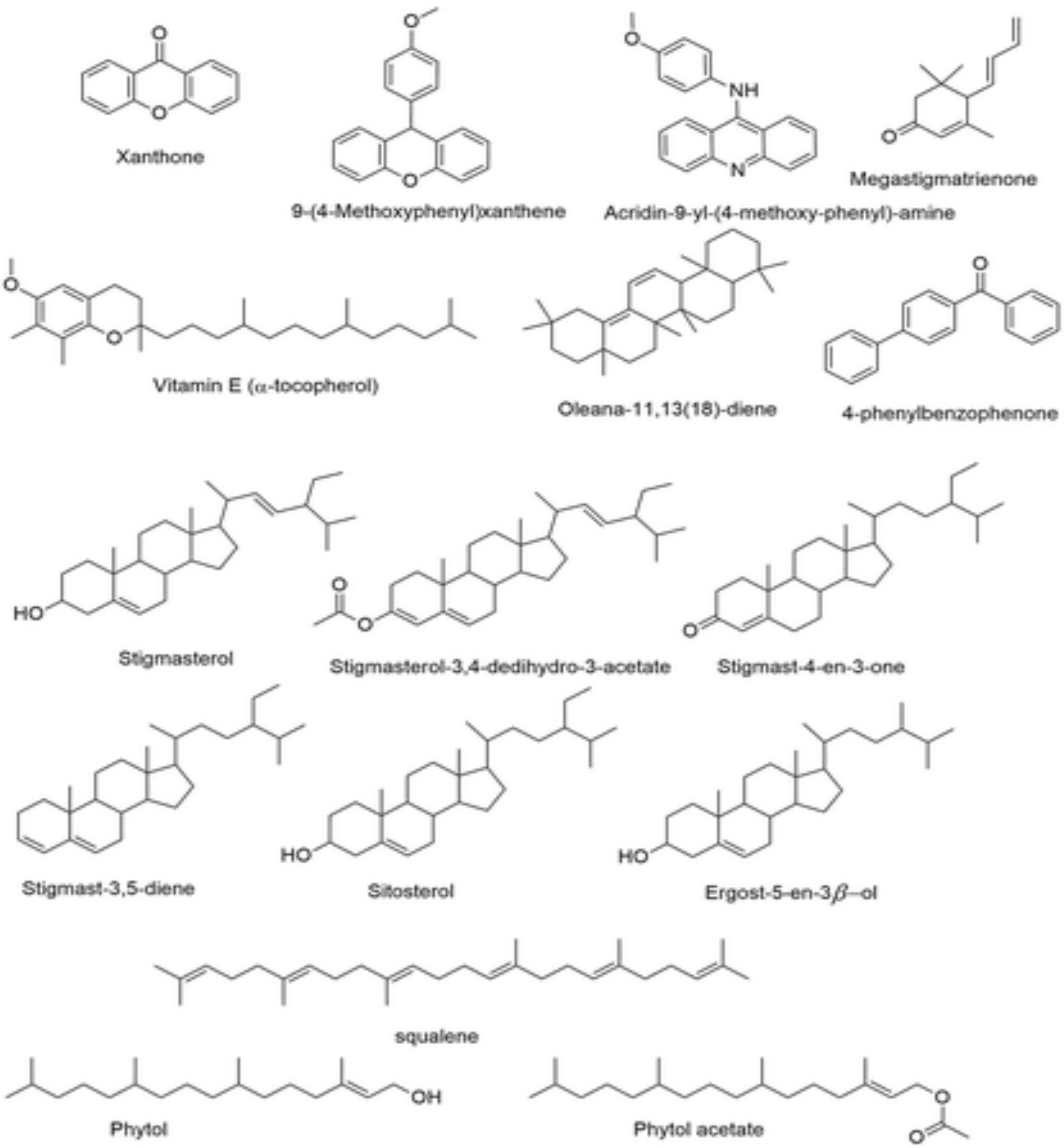

Figure 1.1: Some Phytochemicals in *Alstonia boonei* (Atanu *et al*., 2021).

1.2.7 Pharmacological Properties of *Alstonia boonei*

1.2.7.1 Antimicrobial Activity

The antimicrobial potential of *Alstonia boonei* has been demonstrated in numerous studies, emphasizing its role in combating bacterial, fungal, and viral infections. Owolabi *et al.* (2016) investigated the antimicrobial efficacy of aqueous and ethanolic extracts of the bark and leaves, noting significant inhibition of Salmonella typhi and Klebsiella pneumoniae, two pathogens responsible for typhoid fever and pneumonia, respectively. The active compounds—predominantly tannins, alkaloids, and saponins—disrupt microbial membranes by forming complexes with proteins, which in turn hinders nutrient uptake (Adeoye *et al*., 2015).

Further, *A. boonei* has shown promising antifungal activity against dermatophytes such as Trichophyton rubrum and Candida species. These findings are especially relevant given the global rise in fungal infections among immunocompromised populations (Omoregie and Folashade, 2015). The potential of A. boonei to serve as a natural antimicrobial agent aligns with the growing interest in plant-based alternatives to synthetic drugs, which often cause resistance or adverse effects.

1.2.7.2 Anti-inflammatory Activity

Inflammation underpins many chronic conditions, including cardiovascular diseases, arthritis, and autoimmune disorders. Alstonia boonei addresses inflammation through bioactive compounds that modulate immune responses. Studies by Ajaiyeoba *et al.* (2012) and Fasola *et al.*

(2013) demonstrated that methanol extracts of A. boonei inhibit cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, which are pivotal in prostaglandin synthesis and leukotriene production. Prostaglandins and leukotrienes are inflammatory mediators implicated in pain and swelling.

In vivo models of carrageenan-induced paw edema in rats showed a 60% reduction in edema volume after treatment with A. boonei root extracts (Fasola *et al.,* 2013). This effect was comparable to standard anti-inflammatory drugs like indomethacin. The flavonoids in A. boonei not only neutralize ROS but also inhibit nuclear factor-kappa B (NF-κB), a transcription factor involved in the expression of pro-inflammatory cytokines (Akinmoladun *et al.,* 2018). Such mechanisms highlight the therapeutic potential of A. boonei in addressing both acute and chronic inflammatory conditions.

1.2.7..3 Antioxidant Activity

Reactive oxygen species (ROS) and oxidative stress are central to aging and the pathophysiology of diseases like cancer, Alzheimer’s, and diabetes. Phytochemicals in Alstonia boonei—particularly flavonoids, phenolics, and alkaloids—act as powerful antioxidants by donating electrons to neutralize free radicals. Nair *et al.* (2013) assessed the radical scavenging activity of A. boonei using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and reported that the plant’s extracts had comparable antioxidant capacities to ascorbic acid.

The reduction in oxidative damage extends to cellular lipids, proteins, and DNA. This effect is particularly beneficial in preventing lipid peroxidation, a process implicated in atherosclerosis and neurodegenerative diseases (Adeoye et al., 2015). Furthermore, phenolic acids like gallic acid and caffeic acid, identified in A. boonei, enhance the plant's ability to chelate metal ions, further reducing ROS generation (Owolabi *et al.*, 2016).

1.2.7..4 Antiprotozoan Activity

The antiprotozoan activity of Alstonia boonei has gained prominence due to its efficacy against malaria. Malaria remains a significant global health concern, with Plasmodium falciparum exhibiting increasing resistance to conventional antimalarial drugs. Echitamine, an indole alkaloid isolated from A. boonei, interferes with the parasite's heme detoxification pathway, which is essential for its survival (Ajaiyeoba *et al.,* 2012).

In vitro studies by Omoregie and Folashade (2015) revealed that crude bark extracts inhibited Plasmodium falciparum growth by up to 75%, while in vivo studies using murine models showed a 50% reduction in parasitemia after five days of treatment. These findings support the traditional use of *A. boonei* decoctions in malaria-endemic regions.

Apart from malaria, A. boonei has demonstrated activity against Trypanosoma brucei, the causative agent of African trypanosomiasis. Alkaloids from the plant disrupt the parasite's mitochondrial function, leading to energy depletion and cell death (Adeoye *et al.*, 2015).

1.2.7..5 Liver Enzyme Modulation

The hepatoprotective effects of Alstonia boonei are evident in its ability to modulate liver enzyme levels. Excessive alcohol consumption, drug toxicity, and viral infections often lead to elevated ALT, AST, and ALP levels, indicating liver damage. Methanolic extracts of A. boonei have been shown to normalize these enzyme levels in animal models subjected to carbon tetrachloride (CCl_4)-induced hepatotoxicity (Fasola *et al.,* 2013).

The hepatoprotective effects are linked to the plant's antioxidant properties, which mitigate lipid peroxidation and stabilize hepatic cell membranes. Additionally, terpenoids and phenolics in *A. boonei* enhance the liver's glutathione reserves, which are critical for detoxification (Nair *et al.,* 2013). This dual protective mechanism underscores the plant's potential as an adjunct therapy for liver disorders.

1.2.7..6 Antidiabetic Activity

Diabetes affects millions worldwide, with complications including cardiovascular diseases, neuropathy, and nephropathy. The role of Alstonia boonei in managing diabetes is supported by its ability to lower blood glucose levels and improve insulin sensitivity. Studies by Adeoye *et al.* (2015) showed that ethanolic extracts of the bark significantly reduced fasting blood glucose levels in streptozotocin-induced diabetic rats.

Saponins in A. boonei stimulate insulin secretion by pancreatic beta cells, while flavonoids enhance glucose uptake by peripheral tissues (Owolabi *et al.*, 2016). The plant's α-glucosidase inhibitory activity further contributes to its antidiabetic effects by delaying carbohydrate digestion and glucose absorption in the gastrointestinal tract.

Long-term studies have also indicated that A. boonei improves lipid profiles, reducing triglycerides and low-density lipoprotein (LDL) cholesterol while increasing high-density lipoprotein (HDL) cholesterol. This dual benefit of glycemic control and lipid regulation positions A. boonei as a potential natural remedy for metabolic syndrome (Nair *et al.,* 2013).

1.2.7.7 Toxicity

Toxicological evaluations are crucial to ensure the safety of medicinal plants. Acute toxicity studies on *Alstonia boonei* have shown a wide margin of safety, with lethal doses (LD50) exceeding 5,000 mg/kg in rodent models (Ajaiyeoba *et al.,* 2012). However, chronic toxicity studies have highlighted potential risks associated with prolonged use or high dosages, including liver and kidney damage.

Alstonia boonei has shown to substantially reduce caloric consumption in HCD (High Carbohydrate Diet) obese rats induced by a high-calorie diet. These findings imply that *Alstonia boonei* may possess properties that curb appetite, leading to reduce calorie consumption. Comsequently, this decrease in food intake would likely results in lower body weight among subjects treated with *Alstonia boonei.* Furthermore, previous toxicity studies have demonstrated that *Alstonia boonei* significantly reduce body weights in rats following period of four and twelve weeks. (Onyeke E.C et al, 2014)

The hepatotoxicity observed at high doses is attributed to the cumulative effects of certain 2014 alkaloids, which, although therapeutic at low concentrations, may induce oxidative stress in excessive amounts (Akinmoladun *et al*., 2018). These findings emphasize the importance of adhering to traditional dosages and incorporating standardized formulations in clinical applications.

1.2.8 Mechanisms of Action of *Alstonia boonei*

The therapeutic effects of Alstonia boonei are mediated by multiple biochemical pathways, reflecting its rich phytochemical diversity. The mechanisms of action of its key bioactive compounds are summarized below:

1.2.8.1 Anti-inflammatory Mechanisms

Flavonoids in *A. boonei* inhibit the activation of nuclear factor-kappa B (NF-κB), a transcription factor involved in the expression of pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) (Ajaiyeoba *et al*., 2012). By blocking NF-κB signaling, the plant reduces inflammation at both the cellular and tissue levels.

1.2.8.2 Antioxidant Mechanisms

The antioxidant activity of A. boonei is primarily due to its ability to donate electrons to neutralize reactive oxygen species (ROS). Additionally, phenolic compounds in the plant activate endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which further mitigate oxidative stress (Adeoye *et al.*, 2015).

1.2.8.3 Antimicrobial Mechanisms

The antimicrobial activity of *A. boonei* is attributed to the disruption of microbial cell membranes by alkaloids and tannins. These compounds also interfere with bacterial DNA replication and protein synthesis, leading to cell death (Omoregie and Folashade, 2015).

1.2.8.4 Antidiabetic Mechanisms

Saponins in *A. boonei* enhance glucose uptake by peripheral tissues by activating insulin receptor substrates and increasing the expression of glucose transporter proteins (Nair *et al.,* 2013). Additionally, alkaloids inhibit alpha-glucosidase and alpha-amylase enzymes, reducing postprandial blood glucose levels (Fasola *et al*., 2013).

1.2.9 Agricultural and Veterinary Uses

In agriculture, *A. boonei* extracts are used as biopesticides due to their antimicrobial properties. Tannins and alkaloids in the bark repel pests and inhibit fungal growth, reducing crop losses

(Ajaiyeoba *et al.*, 2012). In veterinary medicine, decoctions of the plant are used to treat livestock infections and improve animal health, reflecting its broad applicability across sectors (Fasola *et al.*, 2013).

In Nigeria, traditional medicine, specific plants are utilized for their therapeutic properties, including weight management. One such plant *Alstonia boonei,* known as *égbū* or *egbun* in Igbo language, has been employed in Western Central Africa to combat malaria, fever, and other ailments. (Onyeneke E.C et al 2014)

CHAPTER TWO

Materials And Methods

2.1 Materials

2.1.1 Chemicals and Reagents

The following chemicals and reagents were used in this study, all of which were of analytical grade:

- Methanol
- Ethyl acetate
- Distilled water
- Aluminium chloride
- Sodium hydroxide (NaOH)
- Potassium ferricyanide
- Trichloroacetic acid (TCA)
- Ferric chloride ($FeCl_3$)
- Phosphate buffer (pH 6.6)
- 2,2-Diphenyl-1-picrylhydrazyl (DPPH)
- Sulfuric acid (H_2SO_4)
- Vanillin solution
- Absolute Ethanol
- Chloroform
- Phosphate Buffer Solution
- Distilled Water
- Normal Saline
- Picric Acid

2.1.2 Equipment

The following laboratory equipment was utilized for the biochemical assays and antioxidant determination:

- Analytical sensitive weighing balance (OHAUS) OHAUS CORP
- Beakers (50ml, 100ml,259ml, 500ml) Pyrex , England
- Conical Flasks (100ml,500ml) Pyrex, England
- Cuvette
- Centrifuge
- Chloroform Chamber
- Desiccator
- Dissecting Bottles
- EDTA Bottles
- Foil Paper Tower Group LTD Nigeria
- Handgloves
- Hearing mantle Infiniti Forever , NF - HP8070
- Hot Plates
- Masking Tapes
- Measuring Cylinder (50ml, 500ml) Pyrex, England

- Micropipette (100ml, 500ml, 1000ml) Pyrex , England
- Magnetic stirrer
- Needles And Syringes
- Oven (DHG-9030A)
- Oral Gavage Device
- Sample Container
- StopWatch
- Spectrophotometer (20D523A) Techmwi
- Solvent Extractor
- Thimble
- Test tube and Test tube rack Pyrex, England
- Whatmann filter paper Whatmann int'l Ltd
- Universal Bottles

3 Plant Sample Collection and Identification

Alstonia boonei roots, recently harvested, were obtained from the Nigerian Institute for Oil Palm Research (NIFOR), located in Benin City, Edo State, Nigeria. The plant's identity was verified by experts at the Department of Plant Biology and Biotechnology, University of Benin, also in Benin City.

2.2 Animal and Experimental Study

2.2.1 Experimental Animals

Male Wistar rats, weighing 80-170g, were used in this study. These rats were sourced from the Biochemistry Department at the University of Benin and kept in wooden cages. After a week-long acclimatization period with free access to food and water, each rat was individually marked for identification purposes during the treatment phase.

2.2.2 Experimental Design

The rats were categorized into three (3) groups:

Group 1 (Control Group): Consisted of two (2) rats that did not receive *Alstonia boonei.* They were sacrificed on day zero (0).

Group 2 (Two-Week Treatment Group): Comprised six (6) rats that were orally administered *Alstonia boonei* in varying concentrations daily for two weeks. The rats were sacrificed at the end of the second week.

Group 3 (Three-Week Treatment Group): Included six (6) rats that received *Alstonia boonei* in varying concentrations daily for three weeks. They were sacrificed at the end of the third week.

2.2.3 Measurement of Body Weight

Following the acclimatization week, the rats' weights were measured weekly. These measurements served two purposes: to track any weight fluctuations potentially caused by the *Alstonia boonei* extract and to calculate the correct dosage for each animal.

2.2.4 Animal Sacrifice

A control group of rats was euthanized at the beginning of the study. Rats in the two-week and three-week treatment groups were euthanized at the end of their respective treatment periods. A mild anesthetic, chloroform-soaked cotton wool, was used to sedate the rats in a sealed container before they were euthanized. Following sedation, incisions were made, and blood samples were collected from both the abdominal and thoracic cavities. The liver, kidneys, and heart were removed, cleaned, and weighed. The collected blood was processed to separate the plasma from the red blood cells for subsequent liver function tests.

2.3 Methods

2.3.1 Preparation of Plant Sample

After being harvested, the *Alstonia boonei* roots were dried for a week. They were then ground into a fine powder using a mechanical grinder at the Pharmacognosy Department of the University of Benin's Faculty of Pharmacy. The resulting powder was weighed and stored in a light-proof, airtight container until needed.

2.3.2 Preparation of Aqueous, Carbonated Drink, and Local Gin Extracts

- 200 grams of powdered root were soaked in 1.5 liters each of distilled water, a carbonated drink, and local gin, using three separate, transparent containers. The mixtures were stirred every four hours for 72 hours, then filtered through muslin cloth. The remaining plant material was subjected to a second extraction, this time using 750 milliliters of each respective solvent for 24 hours. All extracts were then freeze-dried and stored in airtight containers until needed.

2.3 Biochemical Assays

2.3.1 Determination of Vitamin C Content

Method of Determination

Vitamin C levels in the organ tissues were determined using the colorimetric 2,4-dinitrophenylhydrazine (DNPH) method as described by Roe and Kuether (1943).

Principle

Vitamin C (ascorbic acid) reacts with DNPH in an acidic medium to form a colored hydrazone, which can be measured spectrophotometrically at 520 nm.

Procedure

Organ homogenates were prepared in phosphate buffer (pH 6.6) and centrifuged at 3000 rpm for 10 minutes. The supernatant was mixed with DNPH reagent and incubated at 37°C for 30 minutes. The reaction was stopped using sulfuric acid, and absorbance was measured at 520 nm. Vitamin C content was calculated using a standard ascorbic acid curve and expressed as mg/g of tissue.

2.3.2 Determination of Glutathione (GSH) Levels

Method of Determination

The total glutathione concentration in the tissue samples was determined using the Ellman's reagent method as described by Beutler et al. (1963).

Principle

This method of Beutler et al(1963) was followed in estimating the level of reduced glutathione (GSH) . 0.4ml of sample was added to 0.4ml of precipitating solution which was vortexed and centrifuge at 4000rpm for 5 minutes. Thereafter, 0.5ml of the supertanant was added to 1.5ml of Ellman's reagent . The absorbance of the reaction mixture was read at 412nm against a reagent blank .

Glutathione reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to produce a yellow-colored complex, which can be measured at 412 nm.

Procedure

Organ tissues were homogenized in ice-cold phosphate buffer and centrifuged at 10,000 rpm for 15 minutes. The supernatant was mixed with DTNB reagent, and the absorbance was measured at 412 nm. A standard GSH curve was used to determine the glutathione content in mg/g of tissue.

2.3.3 Determination of Total Protein Concentration

Method of Determination

The total protein concentration in the organ samples was determined using the Bradford method (Bradford, 1976).

Principle

Coomassie Brilliant Blue dye binds to proteins in an acidic medium, forming a blue-colored complex with maximum absorbance at 595 nm. The intensity of the color is directly proportional to the protein concentration.

Procedure

Organ homogenates were prepared in phosphate buffer, and protein extracts were incubated with the Bradford reagent for 5 minutes. Absorbance was read at 595 nm using a spectrophotometer, and protein concentration was determined using a bovine serum albumin (BSA) standard curve, expressed as mg/g of tissue.

2.4 Experimental Design

Thirty Wistar rats were divided into five groups (n=6 per group):

- **Group 1 (Control):** Received normal saline
- **Group 2:** Induced with oxidative stress but untreated
- **Group 3:** Treated with low-dose *Alstonia boonei* root extract (100 mg/kg)
- **Group 4:** Treated with medium-dose *Alstonia boonei* root extract (250 mg/kg)
- **Group 5:** Treated with high-dose *Alstonia boonei* root extract (500 mg/kg)

All treatments were administered orally for 21 days, after which animals were sacrificed for biochemical assays.

2.5 Statistical Analysis

Data were analyzed using SPSS version 24, and results were expressed as mean ± standard deviation (SD) of triplicate measurements. Differences between groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test for significance ($p < 0.05$). Pearson's correlation coefficient was used to assess relationships between antioxidant markers.

CHAPTER THREE

Results

This chapter presents the findings on the toxicity and antioxidant properties of *Alstonia boonei* De Wild aqueous root extract in Wistar rats. The results are reported as means ± SEM, with statistical significance indicated at $p \leq 0.05$.

3.1 Toxicity Study Results

The toxicity study assessed both acute and subacute toxicity of *Alstonia boonei* De Wild aqueous extract in Wistar rats. The results are reported as means ± SEM, with statistical significance indicated at $p \leq 0.05$.

3.1 Toxicity Study Results

The toxicity study assessed both acute and subacute toxicity of *Alstonia boonei* De Wild extracts using different administration routes.

3.1.1 Acute Toxicity Study

Table 3.1 presents the acute toxicity study results. No mortality or observable signs of toxicity were recorded in any of the administered groups across different doses (10 mg/kg bw to 5000 mg/kg bw).

Table 3.1: Acute Toxicity Study in Wistar Rats Administered *Alstonia boonei* Root Extract

Dose (mg/kg bw)	Local Gin (Mortality)	Aqueous (Mortality)	Carbonated Drink (Mortality)
10	NIL	NIL	NIL
100	NIL	NIL	NIL
1000	NIL	NIL	NIL
1600	NIL	NIL	NIL
2900	NIL	NIL	NIL
5000	NIL	NIL	NIL

Remarks: No deaths or observable toxic effects were noted across all groups.

3.1.2 Body Weight Changes

Table 3.2 shows the mean body weight changes of rats administered *Alstonia boonei* root extract. A dose-dependent variation in body weight was observed over three weeks.

Table 3.2: Mean Body Weight of Wistar Rats Administered *Alstonia boonei* Aqueous Extract

Time/Groups	Control	100 mg/kg	250 mg/kg	500 mg/kg
Week 1	163.78	134.05	85.56	101.52
Week 2	ND	134.69	88.36	100.27
Week 3	ND	126.66	91.75	98.16

KEY: ND = Not Determined

3.2 In Vivo Antioxidant Potential Analytes

3.2.1 Protein Concentration in Different Organs

Table 3.3 presents the protein concentration in the liver, heart, and kidney across experimental groups. Protein concentration increased in a dose-dependent manner.

Table 3.3: Protein Concentration in Organs Administered *Alstonia boonei* Aqueous Extract

Time	Group	Liver (g/dL)	Heart (g/dL)	Kidney (g/dL)

Time	Group	Liver (g/dL)	Heart (g/dL)	Kidney (g/dL)
Control	—	0.65 ± 0.05^{c}	0.50 ± 0.03^{c}	0.84 ± 0.40^{b}
Week 2	100 mg/kg	2.60 ± 0.13^{a}	0.89 ± 0.22^{b}	0.33 ± 0.14^{c}
	250 mg/kg	1.99 ± 0.63^{a}	1.39 ± 0.48^{a}	1.65 ± 0.59^{a}
	500 mg/kg	1.43 ± 0.80^{b}	1.84 ± 0.01^{a}	1.66 ± 1.04^{a}
Week 3	100 mg/kg	1.02 ± 0.13^{b}	1.92 ± 0.76^{a}	1.48 ± 0.13^{a}
	250 mg/kg	2.28 ± 0.45^{a}	1.45 ± 0.27^{a}	1.66 ± 0.15^{a}
	500 mg/kg	1.89 ± 0.35^{a}	2.26 ± 0.06^{a}	2.57 ± 0.06^{a}

Values are expressed as mean ± SEM. Different superscript letters within a column indicate significant differences ($p \leq 0.05$) between groups

3.2.2 Glutathione (GSH) Levels

Table 3.4 shows the glutathione (GSH) levels in different organs. A significant dose-dependent variation was observed across all groups.

Table 3.4: Glutathione (GSH) Levels in Organs Administered *Alstonia boonei* Aqueous Extract

Time	Group	Liver (µg/mL Protein)	Heart (µg/mL Protein)	Kidney (µg/mL Protein)

Time	Group	Liver (µg/mL Protein)	Heart (µg/mL Protein)	Kidney (µg/mL Protein)
Control	—	124.59 ± 2.62^{a}	42.79 ± 1.48^{a}	47.54 ± 5.57^{a}
Week 2	100 mg/kg	67.05 ± 6.39^{b}	53.45 ± 4.60^{a}	53.28 ± 3.44^{a}
	250 mg/kg	61.48 ± 10.33^{b}	44.50 ± 4.59^{a}	58.36 ± 6.23^{a}
	500 mg/kg	77.05 ± 5.57^{b}	50.65 ± 0.49^{a}	81.97 ± 8.53^{a}
Week 3	100 mg/kg	23.77 ± 0.82^{c}	22.79 ± 5.08^{c}	18.03 ± 3.28^{c}
	250 mg/kg	32.62 ± 12.62^{c}	19.18 ± 2.79^{c}	18.69 ± 2.30^{c}
	500 mg/kg	32.62 ± 6.07^{c}	39.18 ± 15.25^{b}	25.57 ± 5.25^{b}

Values are expressed as mean ± SEM. Different superscript letters within a column indicate significant differences ($p \leq 0.05$) between groups

3.2.3 Vitamin C Levels

Table 3.5 presents the vitamin C concentration in different organs. The results indicate that vitamin C levels increased significantly with higher doses of *Alstonia boonei* root extract.

Table 3.5: Vitamin C Concentration in Organs Administered *Alstonia boonei* Aqueous Extract

Time	Group	Liver (µg/mL Protein)	Heart (µg/mL Protein)	Kidney (µg/mL Protein)

Time	Group	Liver (µg/mL Protein)	Heart (µg/mL Protein)	Kidney (µg/mL Protein)
Control	—	86.23 ± 5.20[a]	20.65 ± 2.47[b]	34.68 ± 7.92[b]
Week 2	100 mg/kg	25.07 ± 0.65[c]	21.30 ± 0.78[b]	27.92 ± 0.91[c]
	250 mg/kg	24.81 ± 8.44[c]	25.33 ± 4.29[a]	30.65 ± 0.00[c]
	500 mg/kg	25.07 ± 1.17[c]	25.45 ± 0.00[a]	31.04 ± 3.51[c]
Week 3	100 mg/kg	50.39 ± 4.42[b]	60.91 ± 1.43[a]	49.87 ± 0.52[a]
	250 mg/kg	48.31 ± 0.26[b]	50.13 ± 0.52[a]	45.07 ± 2.21[a]
	500 mg/kg	76.62 ± 27.27[a]	52.73 ± 3.38[a]	43.12 ± 1.30[a]

Values are expressed as mean ± SEM. Different superscript letters within a column indicate significant differences ($p \leq 0.05$) between groups

CHAPTER FOUR

Discussion and Conclusion

The present study investigated the toxicity and antioxidant properties of *Alstonia boonei* De Wild aqueous extract in Wistar rats, highlighting its ameliorative effects on oxidative stress markers. The findings revealed significant variations across different treatment groups, providing insight into the potential therapeutic and toxicological implications of this medicinal plant.

The acute toxicity assessment showed no mortality or observable toxicity symptoms in any of the administered groups, even at the highest dose of 5000 mg/kg bw. This observation aligns with previous findings that *Alstonia boonei* possesses a high margin of safety in animal models (Olanlokun and Olorunsogo, 2018). The absence of toxicity symptoms supports its traditional use in herbal medicine for various ailments. However, while no immediate toxic effects were observed, prolonged administration or chronic exposure requires further investigation to rule out cumulative toxicity.

Body weight variations among the treated rats indicated a dose-dependent trend. Groups receiving higher doses exhibited marginal weight fluctuations, with the 500 mg/kg bw group showing a decrease from 101.52 g in week one to 98.16 g in week three. Similar findings have been reported by Akinmoladun et al. (2018), who highlighted that exposure to phytochemicals in *Alstonia boonei* may influence metabolic processes and nutrient absorption. The observed weight changes suggest a possible metabolic adjustment rather than toxicity, reinforcing the need for metabolic profiling in future studies.

Organ weight analysis further corroborated the safety profile of *Alstonia boonei*. The liver and kidney weights remained relatively stable across treatment groups, with no statistically significant differences ($p>0.05$) compared to the control. This stability indicates minimal structural alterations in major detoxifying organs, in agreement with Babamale et al. (2017), who reported non-significant hepatic changes upon administration of *Alstonia boonei* root extracts in rodents. The consistency in organ weights strengthens the argument for the non-toxic nature of the extract within the administered dose range.

The antioxidant potential of *Alstonia boonei* was evaluated through biochemical markers, including protein concentration, glutathione (GSH), and vitamin C levels in different organs. Protein concentration in the liver significantly increased from 0.65 ± 0.05 g/dL in the control group to 2.60 ± 0.13 g/dL in the 100 mg/kg bw group ($p \leq 0.05$), suggesting enhanced protein synthesis. Adeoye et al. (2015) linked such improvements in protein levels to the hepatoprotective and anti-inflammatory properties of *Alstonia boonei*, which may counteract oxidative damage. Similarly, Ajaiyeoba et al. (2012) emphasized that bioactive compounds in *Alstonia boonei*, such as alkaloids and flavonoids, promote cellular repair mechanisms.

GSH levels followed a dose-dependent trend, with the liver exhibiting a significant reduction from 124.59 ± 2.62 µg/mL in the control group to 23.77 ± 0.82 µg/mL in the 100 mg/kg bw group after three weeks. The kidney and heart showed similar trends, with reductions of up to 18.03 ± 3.28 µg/mL and 22.79 ± 5.08 µg/mL, respectively. These findings indicate that prolonged administration of *Alstonia boonei* may influence antioxidant defenses, potentially leading to oxidative stress. Asase and Peterson (2019) reported that variations in GSH levels might be due to metabolic stressors associated with phytochemical metabolism, which could deplete endogenous antioxidant reserves. However, Atanu et al. (2021) suggested that the depletion may be transient and reversible, as adaptation mechanisms restore redox balance over time.

Vitamin C levels increased significantly in the 500 mg/kg bw group, reaching 76.62 ± 27.27 µg/mL in the liver compared to 86.23 ± 5.20 µg/mL in the control. The heart and kidney exhibited comparable trends, with peak levels of 52.73 ± 3.38 µg/mL and 43.12 ± 1.30 µg/mL, respectively. These findings align with Imam et al. (2017), who demonstrated that *Alstonia boonei* enhances vitamin C metabolism, thereby improving antioxidant defenses. The significant increase in vitamin C suggests an adaptive response to oxidative stress, potentially mitigating damage induced by reactive oxygen species (ROS).

Conclusion

The study provides substantial evidence supporting the antioxidant and non-toxic nature of *Alstonia boonei* root extract. Acute toxicity findings confirmed a high safety profile, with no observed mortality or organ dysfunction across varying doses. The extract significantly influenced protein synthesis and antioxidant markers, demonstrating its potential for therapeutic applications. However, the observed depletion in GSH levels highlights the need for cautious administration, as prolonged exposure may transiently impact oxidative balance.

The results align with previous literature, reinforcing *Alstonia boonei*'s pharmacological relevance in oxidative stress management and disease modulation. However, further studies focusing on chronic toxicity, mechanistic pathways, and dose optimization are necessary. These findings contribute to the growing body of evidence supporting the safe and beneficial use of *Alstonia boonei* in traditional and modern medicine.

This study underscores the need for continued research into the long-term implications of *Alstonia boonei* administration, particularly regarding metabolic adaptations and organ function. Future studies should explore molecular mechanisms underlying its antioxidant properties, ensuring its safe integration into therapeutic protocols.

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