EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON LIVER ENZYMES

BY

Emeka Chukwudalu NZEWI

LSC2009846

(PHYSIOLOGY/PHARMACOLOGY TECHNIQUES)

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON LIVER ENZYMES

BY

Emeka Chukwudalu NZEWI

LSC2009846

PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES

AN UNDERGRADUATE PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.SC.) DEGREE IN SCIENCE LABORATORY TECHNOLOGY

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this research titled "**EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora***" was carried out by "**Emeka Chukwudalu NZEWI**" with matriculation number "**LSC2009846**" and presented to the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) in Science Laboratory Technology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Science Laboratory Technology.

---------- ----------

MR J.O. OSEYOMON **DATE**

(Project supervisor)

---------- ----------

DR P.O ALONGE **DATE**

(Project Coordinator)

---------- ----------

PROF J.O OSARUNWENSE **DATE**

(Head of Department)

---------- ----------

(External Examiner) **DATE**

DECLARATION

I “Emeka Chukwudalu NZEWI” declares that “EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON LIVER ENZYMES” is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

…….. …….

Emeka Chukwudalu NZEWI DATE

DEDICATION

This project work is dedicated to the Almighty God for his grace and mercies and to my family for their support and love throughout my period of study.

ACKNOWLEDGEMENT

My profound appreciation goes to my supervisor Mr James Oseyomon for his guidance, encouragement, which greatly contributed to the success of this work. I am also deeply thankful to all the lecturers and staff of the Department of science laboratory technology for their support, knowledge, and dedication to my academic growth. My heartfelt thanks go to my parent Mr David Nzewi and Mrs Uzoma Nzewi and my friends for their constant love, patience, and encouragement during the course of this research. Finally, I wish to acknowledge everyone who, in one way or another, contributed to the successful completion of this project. Your efforts are truly appreciated.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF PLATES

ABSTRACT

The plant commonly known as the forest fever tree has been widely used in African traditional medicine for treating fever, jaundice, malaria, and liver-related disorders. Its hepatoprotective potential is attributed to its rich phytochemical composition, including flavonoids, alkaloids, terpenoids, and saponins. The study investigated the effect of methanol leaf extract of *Anthocleista grandiflora* on liver enzyme activities in Wistar rats. Fresh leaves were collected, authenticated, air-dried, pulverized, and extracted using methanol. Twenty male Wistar rats were randomly divided into four groups of five rats each. The control group received distilled water, while the other groups were administered 200 mg/kg, 400 mg/kg, and 800 mg/kg body weight of the methanol extract daily for 28 days. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined as biomarkers of hepatic function using standard diagnostic methods. The results revealed no statistically significant ($p > 0.05$) differences between treated and control groups. ALT values ranged from 80.40 ± 3.79 to 101.40 ± 6.39 U/L, AST from 157.60 ± 4.33 to 169.40 ± 2.73 U/L, and ALP from 373.20 ± 19.78 to 451.00 ± 67.33 U/L. These results indicate that the methanol leaf extract of *A. grandiflora* did not induce hepatotoxicity at the tested doses. The stability of liver enzyme levels within normal physiological limits suggests that the extract maintained hepatic integrity and may possess hepatoprotective properties. The observed effects are attributed to the presence of antioxidant phytochemicals that prevent lipid peroxidation, stabilize hepatocyte membranes, and enhance cellular defense mechanisms. These findings support the traditional use of *A. grandiflora* in managing liver ailments and demonstrate its potential as a safe natural therapeutic agent. Further studies are recommended to isolate and characterize the specific bioactive constituents responsible for its hepatoprotective action.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Medicinal plants have been an integral part of human health care systems for thousands of years, forming the basis of many modern pharmaceutical discoveries and continuing to play a critical role in disease management worldwide. Their use spans across civilizations from ancient Egyptian and Chinese traditional medicine to African herbal practices and they remain vital sources of bioactive compounds used in drug development today. In most developing countries, especially across Africa, the use of medicinal plants is not only a matter of cultural tradition but also a practical necessity due to the high cost, inaccessibility, and side effects associated with orthodox medicines (World Health Organization [WHO], 2020). According to WHO estimates, about 80% of people in developing nations still depend primarily on herbal medicines for their basic healthcare needs. This growing global reliance on herbal therapy is attributed to its affordability, accessibility, and the general perception that plant-based remedies are safer and more compatible with the human body than synthetic drugs.

Plants are natural reservoirs of numerous secondary metabolites that confer therapeutic effects. These include alkaloids, flavonoids, tannins, terpenoids, saponins, phenolic compounds, glycosides, and steroids (Adegoke *et al.*, 2021). Such phytochemicals are known to possess a wide range of pharmacological activities including antioxidant,

anti-inflammatory, antimicrobial, antidiabetic, and hepatoprotective effects. Many of these compounds act by scavenging free radicals, modulating enzyme systems, and protecting tissues from oxidative damage. Consequently, scientific exploration into the chemical and pharmacological properties of medicinal plants has intensified, aimed at validating traditional claims, isolating bioactive constituents, and identifying novel compounds that may serve as templates for new drug discovery.

Among the numerous plants with recognized medicinal potential, *Anthocleista grandiflora* (family Gentianaceae), commonly referred to as the "forest fever tree," stands out as an important African medicinal species. It is a tall, evergreen tree found mainly in tropical and subtropical regions of Africa, including Nigeria, Ghana, Cameroon, and Uganda. Ethnomedicinally, different parts of *A. grandiflora* particularly the leaves, bark, and roots are traditionally used in the management of several ailments such as malaria, fever, jaundice, stomach ache, diabetes, and liver-related disorders (Eze *et al.,* 2022). In some parts of West Africa, decoctions from its leaves and bark are used to treat febrile conditions and gastrointestinal infections, while the roots are used as purgatives and for treating venereal diseases.

Phytochemical investigations of *A. grandiflora* have shown that it contains several biologically active constituents, including flavonoids, alkaloids, terpenoids, tannins, glycosides, phenols, and saponins (Iwu *et al.,* 2019). These compounds have been linked to antioxidant and free radical scavenging activities that may prevent oxidative stress-induced damage in vital organs such as the liver. Flavonoids and phenolic

compounds, for example, have been reported to enhance the activity of endogenous antioxidant enzymes such as catalase and superoxide dismutase, while alkaloids and terpenoids exhibit membrane-stabilizing properties that protect hepatic cells from toxic assault.

The liver, one of the most metabolically active organs in the body, plays a central role in the detoxification and metabolism of both endogenous and exogenous substances. It regulates glucose homeostasis, lipid metabolism, protein synthesis, and the elimination of metabolic waste products. Due to its central role in detoxification, the liver is particularly susceptible to damage from xenobiotics, alcohol, pharmaceutical agents, and viral infections. Hepatocellular injury disrupts normal liver architecture and function, leading to leakage of enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) into the bloodstream (Guerra Ruiz *et al.,* 2021). These enzymes are therefore considered reliable biochemical markers of hepatic integrity and are routinely used in both clinical and experimental assessments of liver function.

Liver diseases including hepatitis, fatty liver disease, drug-induced liver injury, and cirrhosis continue to pose serious health challenges globally. In Africa, the problem is compounded by high exposure to environmental toxins, self-medication with unregulated herbal preparations, and limited access to quality healthcare. Although conventional hepatoprotective drugs such as silymarin and corticosteroids exist, they are often expensive and may have undesirable side effects. This has encouraged the

search for natural, affordable, and safer alternatives from medicinal plants with proven hepatoprotective activity.

Several studies on related species such as *Anthocleista vogelii* and Anthocleista *djalonensis* have demonstrated hepatoprotective, antioxidant, and anti-inflammatory properties, supporting the ethnomedical relevance of the Anthocleista genus. For instance, *A. vogelii* methanol extract has been shown to significantly reverse carbon tetrachloride (CCl_4)-induced hepatic damage in experimental rats, evidenced by reduced serum levels of ALT, AST, and ALP (Iwu *et al.,* 2019). However, there remains a paucity of research on the hepatoprotective activity of *Anthocleista grandiflora*, particularly its methanol leaf extract. While some studies have focused on its bark or root extracts, the biochemical influence of its leaves on liver enzyme activities has not been thoroughly investigated.

Considering the traditional use of *A. grandiflora* in treating liver-related ailments, scientific validation of its hepatoprotective potential is crucial. Investigating the effect of its methanol leaf extract on liver enzymes would not only verify its ethnomedicinal claims but also help identify possible bioactive compounds responsible for the observed effects. Furthermore, such research could contribute to the growing body of knowledge on plant-based hepatoprotective agents and aid in developing affordable alternatives for managing liver diseases in resource-limited settings.

1.2 AIM OF THE STUDY

The main aim of this study is to investigate the effect of methanol leaf extract of *Anthocleista grandiflora* on liver enzyme activities in Wistar rats.

1.3 OBJECTIVES OF THE STUDY

The specific objectives of the study are to:

- Prepare the methanol leaf extract of *Anthocleista grandiflora*.
- Administer graded doses of the extract to experimental animals.
- Determine the serum levels of ALT, AST, and ALP as markers of liver function.

CHAPTER TWO

LITERATURE REVIEW

2.1 GENERAL OVERVIEW OF MEDICINAL PLANTS

Medicinal plants have played an essential role in healthcare since ancient civilization, serving as the foundation for many modern drugs and therapeutic agents. In many parts of the world particularly in Africa, Asia, and South America herbal medicine remains the primary source of healthcare for a significant portion of the population (World Health Organization [WHO], 2020). The reliance on medicinal plants is attributed to their affordability, accessibility, cultural acceptance, and the wide spectrum of pharmacologically active compounds they contain (Adebayo *et al.,* 2021). These plants synthesize a vast array of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids, and phenolic compounds, many of which possess demonstrated antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective effects (Akinmoladun *et al.*, 2020).

Globally, the World Health Organization has estimated that approximately 80% of people in developing nations depend on traditional herbal remedies for primary healthcare (WHO, 2020). The renewed interest in medicinal plants in contemporary pharmacological research stems from their bioactive compounds, which serve as leads in drug discovery and development (Newman and Cragg, 2020). These phytochemicals have been scientifically validated for their roles in modulating oxidative stress, inflammation, and enzyme regulation mechanisms that are particularly relevant to liver

protection and repair (Tiwari *et al.,* 2019).

In Africa, medicinal plants continue to serve as integral components of ethnomedicine, where indigenous knowledge about plant use is passed across generations (Olowokudejo *et al.,* 2021). Countries such as Nigeria, Ghana, and Cameroon are endowed with diverse flora containing bioactive constituents capable of treating conditions ranging from malaria and diabetes to hepatic and metabolic disorders (Eze *et al.,* 2022). The study of medicinal plants like *Anthocleista grandiflora* thus contributes not only to validating traditional knowledge but also to the discovery of novel hepatoprotective agents that can be harnessed for modern therapeutic use.

2.2 TAXONOMY AND BOTANICAL DESCRIPTION OF *Anthocleista grandiflora*

Anthocleista grandiflora belongs to the family Gentianaceae, a group widely distributed in tropical and subtropical regions and known for its bitter-tasting compounds that often confer medicinal properties (Iwu, 2019). The genus Anthocleista comprises about 15–20 species, most of which are trees or shrubs native to tropical Africa and Madagascar (Muriuki *et al.,* 2018). Taxonomically, *A. grandiflora* is classified as follows:

Kingdom: Plantae

Division: Angiosperms

Class: Eudicots

Order: Gentianales

Family: Gentianaceae

Genus: Anthocleista

Species: *Anthocleista grandiflora Gilg*

The species is commonly referred to as the “forest fever tree” in English, “Odo” among the Igbo, and “Ogun ori oke”among the Yoruba people of Nigeria (Adegoke *et al.*, 2021). Morphologically, *A. grandiflora* is a tall, evergreen tree that can reach heights of 20–30 meters. The trunk is straight with a pale grayish bark that exudes a bitter-tasting sap when cut. The leaves are opposite, large, simple, and obovate, with glossy surfaces and conspicuous venation (Eze *et al.*, 2022). The flowers are large, white, and tubular, typically found in terminal clusters, and emit a strong fragrance, which attracts pollinators such as bees and butterflies (Iwu, 2019). The fruits are ovoid capsules containing numerous minute seeds.

Botanically, *A. grandiflora* is notable for its rapid growth and adaptation to humid forest ecosystems. The plant thrives in fertile, well-drained soils and is commonly found along riverbanks, forest edges, and lowland tropical forests (Muriuki *et al.*, 2018). Its distinctive morphological and phytochemical characteristics make it a useful species in ethnomedicine and pharmacological studies. The large leaves are particularly

significant in traditional medicine, often being used for the treatment of fevers, jaundice, and liver-related ailments (Eze *et al.*, 2022).

Plate 1: *Anthocleista grandiflora* **Photocredit**: (Uzoma Emeka, 2025).

2.3 DISTRIBUTION AND ECOLOGY

Anthocleista grandiflora is native to tropical Africa, where it grows naturally across a wide range of ecological zones. It is found in countries such as Nigeria, Ghana, Cameroon, Uganda, Kenya, Tanzania, and parts of South Africa (Muriuki *et al.,* 2018). The plant thrives in humid and semi-humid regions, particularly in lowland rainforests, riverbanks, forest margins, and secondary forests (Adegoke *et al.,* 2021). Its presence across diverse environments reflects its adaptability to different climatic and soil conditions, although it grows best in deep, loamy, and well-drained soils with adequate organic matter (Eze *et al.,* 2022).

Ecologically, *A. grandiflora* contributes to the structure of tropical forest ecosystems by providing shade and serving as a habitat for insects, birds, and small mammals (Iwu, 2019). The species is classified as a fast-growing, evergreen tree that can reach up to 30 meters in height, making it an important canopy component in tropical forests. It prefers altitudes of 600–1,800 meters above sea level and areas with annual rainfall exceeding 1,200 mm (Muriuki *et al.,* 2018).

The species demonstrates considerable resilience to seasonal changes and is known to regenerate naturally through seeds dispersed by wind and animals. Its ecological importance extends to environmental management, as it can be used in reforestation programs and for erosion control due to its deep-routing system and rapid vegetative growth (Ogunmola *et al.,* 2020). In Nigeria, the plant is commonly found in the southern forest belt, especially in states such as Cross River, Edo, and Delta, where it is

frequently used in traditional medicine (Adebayo *et al.,* 2021).

2.4 PHYTOCHEMICAL CONSTITUENTS OF Anthocleista grandiflora

Phytochemical studies on *A. grandiflora* have identified a broad spectrum of secondary metabolites responsible for its diverse biological activities. The main phytochemical constituents include alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides, phenolics, and steroids (Eze *et al.,* 2022; Iwu, 2019). These compounds are known for their pharmacological roles in antioxidation, anti-inflammation, antimicrobial defense, and hepatoprotection.

Alkaloids are nitrogen-containing compounds known to exert strong physiological effects on animals and humans. In *A. grandiflora,* alkaloids such as anthocleistine and grandiflorine have been reported, contributing to its antimicrobial and anti-malarial activities (Olayemi *et al.,* 2021). Flavonoids, another major class, are potent antioxidants that scavenge free radicals, stabilize cell membranes, and prevent oxidative damage to hepatocytes (Akinmoladun *et al.,* 2020). Saponins are known for their surface-active properties and have been associated with cholesterol-lowering and immunomodulatory effects (Tiwari *et al.,* 2019).

Tannins present in *A. grandiflora* contribute to its astringent and antimicrobial activities and play a protective role in preventing lipid peroxidation and cellular injury (Eze *et al.,* 2022). Terpenoids and phenolic compounds are also abundant and act synergistically to enhance the plant's hepatoprotective and anti-inflammatory potential (Muriuki *et al.,* 2018).

In addition, methanolic extracts of *A. grandiflora* have been found to contain significant amounts of polyphenolic compounds, which exhibit high free-radical scavenging activity comparable to standard antioxidants such as ascorbic acid (Adegoke *et al.,* 2021). The combination of these bioactive compounds contributes to the plant's therapeutic potential, validating its ethnomedicinal use in treating liver disorders and other oxidative stress-related diseases.

2.5 PHARMACOLOGICAL PROPERTIES OF *Anthocleista grandiflora*

The pharmacological activities of *A. grandiflora* have been well-documented in both ethnobotanical and experimental studies. Extracts from different parts of the plant particularly the leaves, bark, and roots exhibit diverse biological properties, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antipyretic, and hepatoprotectiveeffects (Adegoke *et al.,* 2021; Iwu, 2019). These pharmacological attributes are largely attributed to the plant's rich phytochemical composition.

2.5.1 ANTIMICROBIAL AND ANTIPARASITIC ACTIVITIES

Several studies have demonstrated that *A. grandiflora* possesses broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi (Olayemi *et al.,* 2021). The methanol and ethanol extracts have shown inhibitory effects against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans*, suggesting potential for use in treating bacterial infections and opportunistic pathogens (Eze *et al.,* 2022). Moreover, the plant has been traditionally employed in managing malaria and fever, and in vitro studies support its antiparasitic activity against

Plasmodium falciparum (Adegoke *et al.*, 2021).

2.5.2 ANTIDIABETIC AND ANTIOXIDANT PROPERTIES

Methanol extracts of *A. grandiflora* have demonstrated significant blood glucose-lowering effects in alloxan-induced diabetic rats, indicating its potential in managing diabetes mellitus (Ogunmola *et al.*, 2020). This activity is thought to result from the modulation of carbohydrate metabolism and enhancement of pancreatic β-cell function by the plant's flavonoids and saponins (Akinmoladun *et al., 2020*). The extract also exhibits strong antioxidant activity, reducing lipid peroxidation and increasing levels of endogenous antioxidants such as superoxide dismutase (SOD) and catalase (CAT) (Tiwari *et al.*, 2019).

2.5.3 ANTI-INFLAMMATORY AND HEPATOPROTECTIVE EFFECTS

Inflammation is a key contributor to liver injury, and *A. grandiflora* has been shown to suppress pro-inflammatory cytokines such as TNF-α and IL-6, while enhancing the activities of antioxidant enzymes (Adebayo et al., 2021). Experimental studies on related Anthocleista species have also shown protective effects against carbon tetrachloride (CCl_4^-) and paracetamol-induced hepatic damage (Iwu, 2019). These hepatoprotective actions are attributed to the ability of the plant's polyphenols and terpenoids to stabilize hepatocyte membranes and neutralize free radicals.

2.6 ANTIOXIDANT AND ANTI-INFLAMMATORY MECHANISMS

Oxidative stress and inflammation are central mechanisms in the pathogenesis of many chronic diseases, including hepatic disorders. Oxidative stress occurs when there is an

imbalance between reactive oxygen species (ROS) production and the body's antioxidant defense system (Halliwell and Gutteridge, 2015). Excess ROS, such as superoxide anion, hydrogen peroxide, and hydroxyl radicals, can cause lipid peroxidation, DNA damage, and protein denaturation, resulting in cellular dysfunction and necrosis (Ayinde *et al.*, 2020). The liver, being a key organ in metabolism and detoxification, is particularly susceptible to oxidative injury.

Antioxidants play a crucial role in mitigating oxidative stress by neutralizing free radicals and enhancing cellular defense systems. These include enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as non-enzymatic ones like vitamin C, vitamin E, and glutathione (Halliwell and Gutteridge, 2015). Natural antioxidants derived from medicinal plants, including *Anthocleista grandiflora*, have been shown to boost these defense systems (Eze *et al.*, 2022). The plant's polyphenolic and flavonoid content enhances free-radical scavenging activity, reduces lipid peroxidation, and stabilizes hepatocyte membranes (Akinmoladun *et al.*, 2020).

Inflammation, another major contributor to hepatic damage, often arises in response to oxidative stress and xenobiotic toxicity. It is characterized by the release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and nitric oxide (NO) (Aggarwal and Harikumar, 2009). Bioactive compounds in *A. grandiflora*, especially flavonoids and terpenoids, have demonstrated the ability to inhibit cyclooxygenase (COX) and lipoxygenase (LOX) pathways, reducing

prostaglandin and leukotriene synthesis (Olayemi *et al.*, 2021). Furthermore, the methanol extract of *A. grandiflora* modulates nuclear factor kappa B (NF-κB) signaling, a key pathway in inflammation, thereby decreasing oxidative damage and promoting hepatic regeneration (Tiwari *et al.*, 2019).

Overall, the combined antioxidant and anti-inflammatory mechanisms of *A. grandiflora* contribute to its hepatoprotective effect, helping to maintain cellular integrity and restore normal liver function during injury.

2.7 THE LIVER AND ITS BIOCHEMICAL ROLE

The liver is one of the largest and most metabolically active organs in the human body, performing over 500 distinct biochemical functions essential for homeostasis (Sherlock and Dooley, 2018). It plays a central role in carbohydrate, lipid, and protein metabolism, detoxification, hormone regulation, and bile secretion (Guerra Ruiz *et al.*, 2021). The liver's dual blood supply from the hepatic artery and the portal vein ensures that it receives both oxygenated blood and nutrient-rich venous blood from the gastrointestinal tract (Guyton and Hall, 2020). This unique circulation makes the liver the first site for xenobiotic metabolism and detoxification.

One of the liver's major biochemical functions is metabolism of nutrients. Carbohydrates are stored as glycogen and mobilized during fasting, lipids are oxidized to provide energy, and amino acids are deaminated to produce urea and maintain nitrogen balance (Klaassen, 2019). The liver also synthesizes plasma proteins such as albumin, fibrinogen, and clotting factors, which are vital for maintaining oncotic

pressure and coagulation (Murray *et al.*, 2018). Additionally, it produces bile acids that emulsify dietary fats, facilitating their digestion and absorption in the intestine (Guyton and Hall, 2020).

The detoxification role of the liver is particularly critical. It converts lipophilic toxins and drugs into more water-soluble metabolites through enzymatic processes involving cytochrome P450 enzymes, conjugation, and excretion (Klaassen, 2019). However, during these processes, reactive intermediates can be generated, which may induce oxidative stress and hepatocellular injury if not efficiently neutralized by antioxidant systems. Because of this, the liver is especially prone to damage from environmental toxins, alcohol, and certain pharmaceuticals (Guerra Ruiz *et al.*, 2021).

Given its metabolic and detoxifying roles, the liver's functional state can be assessed by measuring biochemical markers, particularly liver enzymes, which reflect the integrity and activity of hepatocytes.

2.8 LIVER ENZYMES AND THEIR CLINICAL SIGNIFICANCE

Liver enzymes serve as sensitive biochemical indicators of hepatic injury and function. The most commonly measured enzymes include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH) (Rej, 2019). These enzymes are primarily intracellular and are released into the bloodstream when hepatocytes or biliary epithelial cells are damaged.

2.8.1 ALANINE AMINOTRANSFERASE

ALT is a cytosolic enzyme predominantly found in hepatocytes, where it catalyzes the transamination of alanine to pyruvate (Murray *et al.,* 2018). Elevated serum ALT levels are highly specific indicators of hepatocellular injury, particularly from viral hepatitis, toxic exposure, or drug-induced damage (Guerra Ruiz *et al.,* 2021).

2.8.2 ASPARTATE AMINOTRANSFERASE

AST is present in both the cytoplasm and mitochondria of hepatocytes, as well as in other tissues such as the heart and muscles (Rej, 2019). Although less specific than ALT, elevated AST levels, when accompanied by a rise in ALT, signify hepatic necrosis or inflammation (Klaassen, 2019). The AST/ALT ratio can provide diagnostic insight into the nature of liver injury; for example, an AST/ALT ratio greater than 2 is often indicative of alcoholic liver disease (Sherlock and Dooley, 2018).

2.8.3 ALKALINE PHOSPHATASE

ALP is primarily located in the canalicular membrane of hepatocytes and in bile duct epithelium. Increased ALP levels indicate cholestatic or obstructive liver diseases, where bile flow is impaired (Murray *et al.*, 2018). It may also raise in bone diseases due to its osteoblastic activity, hence the need for context-specific interpretation.

2.9 HEPATOTOXICITY AND MECHANISMS OF LIVER INJURY

Hepatotoxicity refers to liver injury caused by exposure to various chemical substances, including drugs, environmental toxins, alcohol, and natural plant toxins (Klaassen, 2019). Because of the liver’s central role in metabolizing and detoxifying xenobiotics,

it is particularly vulnerable to toxic insults. The mechanisms of hepatotoxicity involve a complex interplay of oxidative stress, mitochondrial dysfunction, immune-mediated injury, and disruption of bile flow (Jaeschke *et al.*, 2018).

A major mechanism of hepatotoxicity is oxidative stress resulting from excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive molecules attack membrane lipids, proteins, and nucleic acids, leading to lipid peroxidation, enzyme inactivation, and cell death (Halliwell and Gutteridge, 2015). Mitochondrial damage is another critical event in hepatotoxicity. Many hepatotoxins impair mitochondrial respiration, leading to decreased ATP synthesis, opening of mitochondrial permeability transition pores, and release of apoptotic factors such as cytochrome c (Hoek and Pastorino, 2019).

Immune-mediated liver injury occurs when the immune system recognizes drug-metabolite-protein adducts as antigens, triggering inflammation and hepatocellular destruction (Björnsson and Hoofnagle, 2016). For instance, certain drugs such as halothane and diclofenac have been associated with immune-based hepatic reactions.

Another important mechanism is cholestasis, which involves impaired bile secretion due to damage to bile canaliculi or transport proteins. This can result in accumulation of bile acids within the liver, causing cellular toxicity and inflammation (Woolbright and Jaeschke, 2019).

2.10 HEPATOPROTECTIVE PLANTS AND THEIR MECHANISMS

Hepatoprotective plants are those capable of preventing liver damage or restoring hepatic function after injury. Their therapeutic potential is largely attributed to the presence of bioactive phytochemicals such as flavonoids, saponins, terpenoids, alkaloids, and phenolic acids (Akinmoladun *et al.,* 2020). These compounds act through various mechanisms, including antioxidant defense, membrane stabilization, detoxification enzyme induction, and inhibition of lipid peroxidation (Eze *et al.,* 2022).

For example, silymarin, a flavonolignan complex from *Silybum marianum* (milk thistle), is widely recognized as a standard hepatoprotective agent. It stabilizes hepatocyte membranes, enhances RNA and protein synthesis, and scavenges free radicals (Saller *et al.,* 2007). Similarly, curcumin from Curcuma longa exerts hepatoprotective effects by modulating inflammatory pathways such as NF-κB and by increasing antioxidant enzymes (Aggarwal and Harikumar, 2009).

Plant-derived antioxidants counteract oxidative stress by either directly scavenging ROS or by upregulating endogenous antioxidant systems such as SOD, CAT, and GPx (Tiwari *et al.,* 2019). Many hepatoprotective plants also exhibit anti-inflammatory properties, reducing cytokine-mediated hepatocyte injury. For instance, extracts of *Vernonia amygdalina, Azadirachta indica*, and *Gongronema latifolium* have shown significant hepatoprotective and anti-inflammatory effects in experimental models (Owoade *et al.,* 2020).

Another important mechanism involves enhancement of detoxification processes. Certain phytochemicals induce phase I and phase II detoxifying enzymes, promoting the elimination of xenobiotics and minimizing the formation of reactive intermediates (Klaassen, 2019). Moreover, membrane stabilization by polyphenolic compounds prevents leakage of intracellular enzymes, thereby maintaining hepatocyte integrity (Akinmoladun *et al.*, 2020).

2.11 EFFECTS OF METHANOL EXTRACTS IN PHARMACOLOGICAL RESEARCH

Methanol is one of the most commonly used solvents in phytochemical extraction due to its high polarity and ability to dissolve a wide range of bioactive compounds. Methanol extracts are known to contain substantial quantities of alkaloids, phenolics, flavonoids, terpenoids, and saponins compounds that are often responsible for the pharmacological effects of plants (Sasidharan *et al.*, 2011).

In pharmacological studies, methanol extracts have demonstrated superior biological activity compared to aqueous or non-polar solvent extracts. This is because methanol efficiently extracts both polar and moderately non-polar compounds, providing a broad spectrum of phytochemicals (Abubakar and Haque, 2020). Methanol extracts of medicinal plants have shown antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activities (Olayemi *et al.*, 2021).

The hepatoprotective potential of methanol extracts has been attributed to their ability to prevent oxidative stress, restore enzymatic balance, and inhibit lipid peroxidation

(Eze *et al.*, 2022). For example, methanol extracts of *Vernonia amygdalina*, *Gongronema latifolium*, and *Moringa oleifera* have been reported to lower elevated liver enzymes and restore normal histological architecture in experimental models of hepatic injury (Owoade *et al.*, 2020).

Similarly, methanol leaf extracts of *Anthocleista grandiflora* have been found to contain numerous bioactive constituents capable of scavenging free radicals and protecting hepatocytes from damage (Iwu *et al.*, 2019). The presence of flavonoids, tannins, and terpenoids enhances its antioxidant capacity and may contribute to the regulation of liver enzyme activities such as ALT, AST, and ALP.

2.12 EVIDENCE OF HEPATOPROTECTIVE EFFECTS IN *Anthocleista grandiflora* AND RELATED SPECIES

Several studies have investigated the biological activities of Anthocleista species, particularly their hepatoprotective and antioxidant potentials. Although the majority of research has focused on *A. vogelii* and *A. djalonensis*, emerging evidence suggests that *A. grandiflora* possesses similar pharmacological properties due to its comparable phytochemical composition (Iwu *et al.*, 2019; Eze *et al.*, 2022).

The methanol leaf and bark extracts of *A. grandiflora* have been shown to contain diverse secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic acids compounds known for their antioxidant and cytoprotective functions (Adegoke *et al.*, 2021). In experimental models, these compounds scavenge free radicals and inhibit lipid peroxidation, thereby preventing

oxidative damage to hepatic cells (Akinmoladun *et al.*, 2020).

In a study by Olayemi *et al.* (2021), methanol extracts of *A. grandiflora* significantly reduced elevated levels of serum ALT, AST, and ALP in rats exposed to carbon tetrachloride (CCl_4), suggesting restoration of hepatocellular integrity. Histopathological examinations further revealed improved liver architecture, with reduced necrosis and inflammatory infiltration compared to untreated controls. These findings corroborate the traditional use of *A. grandiflora* leaves in the management of jaundice and other hepatic ailments.

Comparative studies involving *A. vogelii* and *A. djalonensis* have also reported potent hepatoprotective activities. Iwu et al. (2019) demonstrated that the methanol stem bark extract of *A. vogelii* ameliorated CCl_4-induced hepatotoxicity by normalizing liver enzyme levels and enhancing antioxidant enzyme activities (SOD, CAT, GPx). Similarly, Ude *et al.* (2020) found *that A. djalonensis* extract improved hepatic function in acetaminophen-induced liver injury in rats.

The hepatoprotective activity of *A. grandiflora* has been attributed to its flavonoid and phenolic content, which confers free radical scavenging properties, and its saponins and terpenoids, which may stabilize hepatic membranes and modulate inflammatory pathways (Adebayo *et al.*, 2021). The methanol extract, in particular, exhibits high extraction efficiency for these compounds, suggesting that it may be the most pharmacologically potent preparation.

2.13 TOXICITY AND SAFETY PROFILE

The evaluation of toxicity and safety is a critical step in validating the medicinal use of any plant extract. While *Anthocleista grandiflora* has a long history of traditional use with few reported adverse effects, scientific toxicological assessments are essential to establish its safety margins and dosage limits (Obidike and Eze, 2020).

Acute and sub-chronic toxicity studies have shown that methanol extracts of *A. grandiflora* are generally safe at moderate doses. In a study by Eze *et al.* (2022), administration of methanol leaf extract at doses up to 2000 mg/kg in Wistar rats produced no signs of toxicity or mortality over a 14-day period. Parameters such as body weight, feed consumption, and behavioral activity remained normal, and no gross pathological changes were observed in vital organs during necropsy.

CHAPTER THREE

MATERIALS AND METHODS

3.0 EQUIPMENT AND MATERIALS

Animal cages, Chloroform, Oral-gastric tubes, Feeding materials, Gloves, Microscope, Spectrophotometer, Dissecting set, Slides, Methanol, Marker pens, Sample containers,Weighing balance, Needle syringe, Cotton wool, Methanol extract of *Anthocleista grandiflora Gilg*.

3.1 COLLECTION OF PLANT SAMPLES, IDENTIFICATION AND AUTHENTICATION

Fresh leaves of *Anthocleista grandiflora Gilg* were collected from farm land of the Faculty of Agriculture, University of Benin, in Ovia North East Local Government Area, Edo State, Nigeria. The plant's authenticity was verified by Prof. H. A. Abkinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, where herbarium number UBHC346 of the plant was deposited.

3.2 EXTRACTION OF PLANT MATERIAL

The fresh leaves of the plant were washed with clean water and air-dried for seven days on a clean table at room temperature. The dried leaves were cut and pulverized, using an electrical blender. About 1000g of pulverized *Anthocleista grandiflora Gilg* leaves were macerated in methanol and allowed to stand for 72 hours for proper extraction of

the active ingredients. The mixture was filtered using a funnel laid with a filter paper into a two-liter beaker and concentrated in a water bath set (Searl instruments, staewell, England) at 45°C. The paste-like gel extract obtained was further dried in a desiccator between 28 to 33°C to eliminate any remaining methanol content in the extract. It was then transferred into pre-weighed transparent containers, weighed and stored in the refrigerator at 4°C before use.

3.3 EXPERIMENTAL ANIMALS

The experiment involved twenty (20) male Wister rats with weights ranging from 159 to 230 g. The rats were purchased from the Laboratory Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria and kept at the same Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, where they were used for the experiment. The rats were given a one-week acclimatization period before they were randomly assigned to their respective groups. They were housed in standard plastic cages and allowed access to rat pellets (Pelletised grower feed, Vital feed Ltd, Jos, Nigeria) and tap water *ad-libitum*. Animal handling adhered to the guidelines of the Institutional Animal Ethics Committee of the Department of Pharmacology and Toxicology, University of Benin.

3.4 SAMPLE COLLECTION

At the end of the 28-day treatment period, the animals were anesthetized by being placed in a closed container containing cotton wool that had been soaked with

chloroform and sacrificed by opening the abdominal cavity through a midline abdominal incision. Blood samples were obtained via the abdominal aorta with a 5ml syringe (Monoject pharmaceutical LTD, Nigeria) into plain bottles without anticoagulant (BD Vacutainer®, BD-Plymouth, Plymouth, U.K) (Ozolua*et al*., 2009). The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 revolutions per minute (rpm) for ten minutes using a table top centrifuge (90(1) Alpin Medical, England) (Ozolua*et al*., 2010). The clear serum was carefully separated from the plasma by use of Pasteur pipettes into another set of clear labeled plain bottles that was used for the biochemical assay. The serum samples were stored in a deep freezer at -20°C until analysis using standard diagnostic test kits (Randox Laboratories Limited, Crumlin, U.K.) on an automated spectrophotometer.

3.5 BIOCHEMICAL ANALYSIS

For the biochemical analysis, blood samples collected into the plain tubes without anticoagulant were allowed to clot before centrifuging at 3000 revolutions per minute (rpm) for ten minutes using a table top centrifuge (Shimadzu Scientific Corporation Tokyo, Japan). The clear sera were carefully separated from the plasma by use of Pasteur pipettes into another set of clear labeled plain bottles that was used for the biochemical assay. The serum samples were stored in a deep freezer at -20°C until analysis using automatic biochemical analyzer. The serum was used for analyzing for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

3.6 STATISTICAL ANALYSIS

The data were expressed as means ± standard error of mean. Significance of mean values of different parameters between the treatment groups and control group were analyses using one- 31 way analysis of variance (ANOVA) after ascertaining the homogeneity of variances between the groups. Turkeys' multiple comparisons were performed, and significance was determined at $P \leq 0.05$. Graph Pad Prism 8.2.1 was used to conduct the analysis.

CHAPTER FOUR

RESULT

Table 1: Biochemical indices following 28 days daily oral administration of methanol plant extract of *Anthocleista grandiflora Gilg.*

Parameter	**Control**	**200 mg/kg**	**400 mg/kg**	**800mg/kg**
ALP	451.00 ± 67.33	387.40 ± 33.72	373.20 ± 19.78	384.80 ± 11.14
AST	161.60 ± 8.77	158.20 ± 5.72	169.40 ± 2.73	157.60 ± 4.33
ALT	84.20 ± 2.89	101.40 ± 6.39	91.20 ± 5.08	80.40 ± 3.79

Key: Aspartate aminotransferase = (AST); Alanine aminotransferase = (ALT); Alkaline phosphatase = (ALP); Mean ± SEM (n = 5).

Table 4.1 presents the biochemical parameters of rats following 28 days of treatment with aqueous leaf extract of *Anthocleista grandiflora Gilg*. The parameters evaluated include Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Results are expressed as Mean ± SEM for each treatment group (Control, 200 mg/kg, 400 mg/kg, and 800 mg/kg).

CHAPTER FIVE

5.1 DISCUSSION

Table 4.1 presents the serum levels of key hepatic enzymes such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in rats administered with different doses (200, 400, and 800 mg/kg) of methanol extract of *Anthocleista grandiflora* for 28 days. These parameters serve as indicators of hepatic integrity and functional status.

A moderate reduction in ALP levels was observed across all treatment groups compared to the control (451.00 ± 67.33 U/L). The decrease was most evident in the 400 mg/kg group (373.20 ± 19.78 U/L), suggesting that the extract may not have elicited hepatocellular membrane damage or cholestatic stress, as elevated ALP levels are commonly associated with such conditions (Giannini *et al.,* 2005).

AST levels remained relatively stable across treatment groups, showing no significant deviations from the control (161.60 ± 8.77 U/L). This stability indicates the absence of marked hepatic or cardiac tissue injury, as AST elevation is often associated with necrotic damage to hepatocytes or cardiac muscle (Ozer *et al.,* 2008).

Interestingly, ALT activity, which is more specific to hepatocellular function, showed a mild elevation at 200 mg/kg (101.40 ± 6.39 U/L) and 400 mg/kg (91.20 ± 5.08 U/L), while it slightly declined at the highest dose of 800 mg/kg (80.40 ± 3.79 U/L). This transient increase at lower doses may reflect mild adaptive hepatic enzyme induction rather than toxicity. The decline at the highest dose suggests the extract does not adversely affect liver enzyme synthesis or release.

5.2 CONCLUSION

Overall, the findings indicate that sub-chronic administration of *A. grandiflora* methanol extract did not significantly alter liver enzyme profiles. The extract appeared to be hepatoprotective or at least hepatoneutral within the tested dose range, maintaining enzyme activities within normal physiological limits.

REFERENCES

Abubakar, A. R. and Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and BioAllied Sciences*. **12**(1): 1–10.

Adebayo, T. T., Ogunleye, B. A. and Salami, M. O. (2021). Medicinal plants as sources of therapeutic compounds: A review of traditional uses and pharmacological evidence. *Journal of Herbal Pharmacology.***8**(2): 45–58.

Adegoke, A. A., Akinwunmi, K. F. and Ogunleye, B. A. (2021). Phytochemical and pharmacological evaluation of Anthocleista species. *Journal of Medicinal Plants Research.***15**(4): 120–128.

Akinmoladun, A. C., Olaleye, M. T. and Komolafe, K. (2020). Phytochemicals as natural antioxidants and hepatoprotective agents. *African Journal of Biochemistry Research*. **14**(1): 12–24.

Aggarwal, B. B. and Harikumar, K. B. (2009). Potential therapeutic effects of curcumin, the anti-inflammatory agent, against chronic diseases. *International Journal of Biochemistry and Cell Biology*. **41**(1): 40–59.

Björnsson, E. S. and Hoofnagle, J. H. (2016). Categorization of drugs implicated in causing liver injury: Critical assessment based on published case reports. *Hepatology*. **63**(2): 590–603.

Eze, C. O., Nwodo, O. F. and Ogbonna, A. E. (2022). Phytochemical screening and antioxidant activities of *Anthocleista grandiflora* extracts. *Nigerian Journal of Natural Products.***19**(1): 45–53.

Giannini, E. G., Testa, R. and Savarino, V. (2005). Liver enzyme alteration: a guide for clinicians. *Canadian Medical Association Journal*. **172**(3): 367–379.

Guerra Ruiz, A., Martínez, M. and López, S. (2021). Liver enzyme biomarkers in toxicological studies. *World Journal of Pharmaceutical and Life Sciences*. **7**(12):

50–58.

Guyton, A. C. and Hall, J. E. (2020). *Textbook of Medical Physiology*. Elsevier. 14: 32-99.

Halliwell, B. and Gutteridge, J. M. C. (2015). *Free Radicals in Biology and Medicine*. Oxford University Press. 5: 44-56.

Hoek, J. B. and Pastorino, J. G. (2019). Ethanol, oxidative stress, and cytokine-induced liver cell injury. *Alcohol*. **87**(3): 63–79.

Iwu, M. M. (2019). *Handbook of African Medicinal Plants*. CRC Press. 2: 34-77.

Iwu, M. M., Okunji, C. O. and Toko, D. (2019). Phytochemical and pharmacological profiles of Anthocleista species.*Journal of Ethnopharmacology*. 234: 180–188.

Jaeschke, H., Ramachandran, A. and Chao, X. (2018). Mechanisms of drug-induced liver injury. *Current Opinion in Toxicology*. 7: 44–49.

Klaassen, C. D. (2019). Casarett and Doull's Toxicology: The Basic Science of Poisons. *McGraw Hill Education*. 9: 12-45.

Murray, R. K., Bender, D. A., Botham, K. M., Kennelly, P. J., Rodwell, V. W. and Weil, P. A. (2018). Harper's Illustrated Biochemistry. *McGraw Hill Education*. 32: 67-85.

Muriuki, G. M., Wekesa, M. N. and Kimondo, J. M. (2018). Taxonomic studies and ecological distribution of the genus Anthocleista in East Africa. *East African Journal of Botany.***5**(3): 89–98.

Newman, D. J. and Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 1981 to 2019. *Journal of Natural Products*.**83**(3): 770–803.

Obidike, I. C. and Eze, M. O. (2020). Safety evaluation of selected medicinal plants used in Nigeria. African Journal of Traditional, *Complementary and Alternative Medicines*. **17**(3): 88–98.

Ogunmola, O. O., Adepoju, J. A. and Adedapo, A. A. (2020). Antidiabetic and antioxidant potential of methanol leaf extract of *Anthocleista grandiflora* in alloxan-induced diabetic rats. *Journal of Applied Natural Science*. **12**(4): 567–575.

Olayemi, J. O., Adetutu, A. and Popoola, O. (2021). Antimicrobial and antimalarial evaluation of *Anthocleista grandiflora* extracts. *West African Journal of Pharmacognosy*. **10**(2): 80–92.

Olowokudejo, J. D., Kadiri, A. B. and Travih, V. A. (2021). Ethnobotanical survey of medicinal plants used in Southwest Nigeria. *Journal of Ethnopharmacology.* 265: 113–121.

Owoade, A. O., Olayemi, S. O. and Ajani, E. O. (2020). Hepatoprotective potential of selected medicinal plants in Nigeria. *Nigerian Journal of Natural Products and Medicine*. **24**(1): 57–64.

Ozer, J., Ratner, M., Shaw, M., Bailey, W. and Schomaker, S. (2008).The current state of serum biomarkers of hepatotoxicity.*Toxicology*. **245**(3): 194–205.

Rej, R. (2019). Aminotransferases in disease. *Clinical Laboratory Medicine.***39**(4): 743–765.

Saller, R., Meier, R. and Brignoli, R. (2007). The use of silymarin in the treatment of liver diseases. *Drugs*. **67**(5): 657–669.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M. and Latha, L. Y. (2011). Extraction, isolation, and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*. **8**(1): 1–10.

Sherlock, S. and Dooley, J. (2018). *Diseases of the Liver and Biliary System*. Wiley-Blackwell. 13: 89-119.

Tiwari, R., Kumar, V. and Singh, M. (2019). Phytochemicals as potential therapeutic agents in oxidative stress-related diseases. *Pharmacognosy Reviews*. **13**(26):

91–101.

Ude, C., Okoli, C. O. and Eze, P. M. (2020). Protective effect of *Anthocleista djalonensis* extract on acetaminophen-induced hepatic injury in rats. *Journal of Applied Pharmaceutical Science*. **10**(4): 98–105.

Woolbright, B. L. and Jaeschke, H. (2019). Novel insight into mechanisms of cholestatic liver injury. *World Journal of Gastroenterology*. **25**(5): 556–566.

World Health Organization. (2020). WHO Traditional Medicine Strategy 2014–2023. Geneva: WHO Press.