EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON LIPID PROFILE

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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

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CERTIFICATION

This is to certify that this research titled "**EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON LIPID PROFILE**" was carried out by "**Harrietta Kehinde ADEFUSI**" with matriculation number "**LSC2003188**" and presented to the Department of Science Laboratory Technology, Faculty of life science, 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.

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DECLARATION

I "Harrietta Kehinde ADEFUSI" declare that "EFFECT OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora* ON LIPID PROFILE" 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.

______________________ __________________________

Harrietta Kehinde ADEFUSI **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.

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First and foremost, I give all praise and glory to Almighty God for His unending grace, strength, and guidance throughout this project. His faithfulness made everything possible.

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ABSTRACT

This study evaluated the effect of methanol leaf extract of *Anthocleista grandiflora* on lipid profile parameters in Wistar rats following 28 days of sub-chronic oral administration. The plant commonly known as the "Giant African Cabbage Tree," has long been used in traditional medicine for managing fever, jaundice, and metabolic disorders. Despite its wide ethnomedicinal use, scientific validation of its lipid-modulating potential remains limited. Twenty male Wistar rats were randomly divided into four groups of five animals each. The control group received distilled water, while the experimental groups were administered 200 mg/kg, 400 mg/kg, and 800 mg/kg of methanol leaf extract daily for 28 days. Serum samples were analyzed for total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using standard spectrophotometric methods. The results showed no statistically significant ($p > 0.05$) differences between treated and control groups for all lipid parameters. Mean cholesterol values ranged from 78.00 ± 3.07 to 85.60 ± 3.28 mg/dL, triglycerides from 77.60 ± 4.52 to 90.00 ± 8.83 mg/dL, HDL from 24.20 ± 0.97 to 27.60 ± 1.36 mg/dL, and LDL from 34.20 ± 4.98 to 43.60 ± 3.85 mg/dL. The slight elevation in HDL with a concurrent reduction in LDL at higher doses indicates a potential cardioprotective and hypolipidaemic effect of the extract. These findings suggest that *A. grandiflora* did not disrupt lipid metabolism but may support lipid balance through its antioxidant phytochemicals such as flavonoids, saponins, and phenolics. Overall, the methanol leaf extract of *A. grandiflora* demonstrated safety and mild lipid-regulating potential during sub-chronic exposure, validating its traditional use in promoting cardiovascular and metabolic health

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Medicinal plants remain an indispensable source of therapeutic agents in modern pharmacology and traditional medicine. Across the world, natural products derived from plants contribute significantly to the management and prevention of metabolic disorders and chronic diseases (World Health Organization [WHO], 2021). In developing countries, where the cost and accessibility of conventional medicines are limiting, medicinal plants serve as affordable and culturally accepted alternatives (Olorunnisola *et al.,* 2016). The pharmacological properties of these plants are largely due to their rich phytochemical composition such as alkaloids, flavonoids, tannins, saponins, and terpenoids which are known to modulate various biochemical and physiological processes (Njoku *et al.,* 2020; Ekennia *et al.,* 2018).

Lipid metabolism plays a crucial role in maintaining normal physiological function. Lipids serve as structural components of cell membranes, sources of stored energy, and precursors for several hormones. However, disturbances in lipid metabolism can lead to dyslipidaemia, characterized by elevated levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL), or a decrease in high-density lipoprotein (HDL) (Adebayo and Ishola, 2018). Such abnormalities contribute significantly to cardiovascular diseases, atherosclerosis, obesity, and liver dysfunction (Yusuf *et al.,* 2020). The rising incidence of dyslipidaemia, particularly in low- and middle-income countries, underscores the need for effective, accessible, and safe lipid-lowering therapies.

Conventional lipid-lowering drugs such as statins, fibrates, and niacin are effective in reducing elevated lipid levels but are often associated with adverse side effects, including muscle pain, liver toxicity, and gastrointestinal discomfort (Adewale *et al.,* 2017). Moreover, long-term dependence on these synthetic agents can impose economic burdens, especially in resource-limited settings. This has renewed global interest in exploring natural plant extracts as potential sources of safer, cost-effective alternatives for managing lipid disorders (Kumar *et al.,* 2020; WHO, 2021). Medicinal plants with hypolipidaemic and antioxidant properties may offer dual benefits lowering harmful lipid fractions while protecting the liver and cardiovascular tissues from oxidative damage (Ojo *et al.,* 2020).

Among such plants is *Anthocleista grandiflora*, a tropical tree belonging to the family Loganiaceae. Commonly known as "Giant African Cabbage Tree" or "Cabbage Tree," A. grandiflora is indigenous to West and Central Africa and is widely distributed across Nigeria, Ghana, and Cameroon (Iwu, 2014). In traditional African medicine, the plant has been used for centuries to treat fever, malaria, jaundice, stomach ache, typhoid, and liver ailments (Ekennia *et al.,* 2018). The leaves, bark, and roots are typically used in decoctions, infusions, or alcoholic extracts, depending on the ailment being treated. Recent phytochemical investigations have confirmed that *A. grandiflora* contains bioactive compounds such as alkaloids, glycosides, flavonoids, tannins, and phenolics, which are believed to contribute to its pharmacological effects (Njoku et al., 2020).

Experimental studies have revealed that the methanol leaf extract of *A. grandiflora* exhibits antioxidant, anti-inflammatory, hepatoprotective, and antipyretic activities (Olorunnisola *et al.,* 2016; Ekennia *et al.,* 2018). These activities suggest its potential role in modulating lipid metabolism and protecting against oxidative stress associated with hyperlipidaemia. Methanol, being a polar solvent, is particularly efficient at extracting both polar and semi-polar bioactive compounds, which may account for the extract's strong pharmacological actions (Rahman *et al.,* 2019). The presence of flavonoids and phenolic compounds in *A. grandiflora* has been linked to improved lipid regulation, as these compounds can enhance HDL levels and inhibit lipid peroxidation (Okafor and Nwodo, 2018).

Despite the growing interest in the plant's pharmacological properties, limited scientific evidence exists on the specific impact of its methanol leaf extract on lipid profile parameters. Understanding how *A. grandiflora* influences lipid metabolism in animal models could provide valuable insights into its therapeutic potential and safety profile. Lipid profile evaluation is a critical biochemical tool for assessing cardiovascular health and the metabolic effects of test substances (Klaassen and Watkins, 2015). Therefore, investigating changes in total cholesterol, triglycerides, HDL, and LDL levels following administration of *A. grandiflora* extract will help determine whether the plant exerts lipid-lowering, neutral, or adverse effects.

This research is particularly important as it aligns with global efforts to develop natural alternatives for the prevention and management of cardiovascular and metabolic disorders. If proven effective, *Anthocleista grandiflora* could serve as a natural therapeutic agent for correcting lipid imbalances, supporting liver health, and promoting overall metabolic stability (Njoku *et al.,* 2020).

1.2 AIM OF STUDY

The aim of study was to evaluate the effect of methanol leaf extract of *Anthocleista grandiflora* on lipid profile in experimental animal models (wistar rats/mice).

1.3 OBJECTIVES OF STUDY

The specific objectives of this study are to:

i. Determine the effect of methanol leaf extract of *Anthocleista grandiflora* on serum total cholesterol (TC) levels in rats.
ii. Assess the effect of the extract on triglyceride (TG) concentration.
iii. Evaluate the changes in low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels following extract administration.
iv. Compare lipid profile values across different dosage groups to establish any dose-dependent relationship.

CHAPTER TWO

LITERATURE REVIEW

2.1 GENERAL OVERVIEW OF MEDICINAL PLANTS AND LIPID METABOLISM

Medicinal plants have remained one of the most valuable resources for the development of new therapeutic agents, particularly in regions where traditional medicine plays a dominant role in healthcare delivery. According to the World Health Organization (WHO, 2021), more than 80% of the global population, especially in developing countries, rely on plant-derived remedies as their primary source of healthcare. This dependence stems not only from cultural acceptance but also from the accessibility, affordability, and perceived safety of herbal preparations compared to synthetic drugs. In addition, advances in pharmacognosy and phytochemistry have demonstrated that plants are natural factories of bioactive compounds with complex biological effects on metabolic systems (Sofowora, 2018; Tiwari *et al.,* 2020).

The therapeutic significance of medicinal plants lies largely in their secondary metabolites, which include alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, phenolics, and steroids. These phytochemicals are synthesized primarily as defence mechanisms against environmental stress, pathogens, or herbivores but often display potent pharmacological activities in humans (Harborne, 2019; Ezeokonkwo *et al.,* 2022). Many of these compounds have been isolated, characterized, and used in the formulation of modern drugs. For instance, the discovery of aspirin from Salix alba (willow bark), quinine from Cinchona species, and digoxin from Digitalis purpurea are well-known examples of how natural products have shaped pharmacotherapy (Iwu, 2019; Kumar *et al.,* 2020). Consequently, ongoing research continues to explore plant extracts as safer and more sustainable alternatives to synthetic compounds in managing chronic metabolic diseases such as diabetes, obesity, and dyslipidaemia (Njoku and Eze, 2021; Ogunyemi *et al.,* 2022).

Lipid metabolism is an essential physiological process that governs the synthesis, absorption, transport, and utilization of lipids in the body. Lipids including cholesterol, triglycerides, and phospholipids play vital roles in maintaining cell membrane integrity, hormone synthesis, and energy storage (Nelson and Cox, 2017; Yusuf *et al.,* 2020). However, disruption in lipid homeostasis, referred to as dyslipidaemia, is a major contributor to cardiovascular diseases (CVDs), including atherosclerosis, coronary artery disease, and stroke. Dyslipidaemia is characterized by elevated total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and/or reduced high-density lipoprotein cholesterol (HDL-C) levels (Goldstein and Brown, 2019; Tiwari *et al*., 2020).

Traditional management of dyslipidaemia relies on statins, fibrates, niacin, and bile acid sequestrants, which function through various mechanisms such as inhibiting cholesterol synthesis or promoting lipid excretion. Although effective, these agents are often associated with adverse effects, including hepatotoxicity, myopathy, and gastrointestinal disturbances (Kumar *et al.,* 2020; Njoku *et al.,* 2020). Consequently, there has been growing global interest in natural hypolipidaemic agents that modulate lipid metabolism with minimal side effects. Medicinal plants have emerged as potential sources of such agents, owing to their bioactive constituents that target lipid pathways through antioxidant, anti-inflammatory, and enzyme-modulatory mechanisms (Adebayo and Ishola, 2018; Ekennia *et al.,* 2018).

Several studies have demonstrated the lipid-lowering potentials of plant-derived compounds. For instance, flavonoid-rich extracts of *Moringa oleifera*, *Allium sativum* (garlic), and *Curcuma* *longa* (turmeric) have been shown to reduce serum TC, TG, and LDL-C while increasing HDL-C levels in experimental animals (Okafor and Nwodo, 2018; Ibrahim et al., 2021). Similarly, saponins and phytosterols are known to form insoluble complexes with bile acids, thereby reducing cholesterol reabsorption and enhancing faecal excretion (Kumar *et al.,* 2020; Tiwari *et al.,* 2020). Phenolic compounds, on the other hand, exhibit strong antioxidant properties that prevent lipid peroxidation and protect lipoproteins from oxidative damage, a process implicated in atherogenesis (Nelson and Cox, 2017; Ojo and Akinmoladun, 2020).

Evaluating the effects of medicinal plant extracts on lipid profile using experimental animal models, especially rats, has therefore become a standard approach for validating their pharmacological activity and safety profile. Rat models provide controlled conditions for monitoring changes in lipid parameters such as TC, TG, LDL, and HDL following acute or sub-chronic administration of plant extracts (Rahman *et al.,* 2020; Okonkwo *et al.,* 2023).

Moreover, such studies help elucidate possible mechanisms of action, including inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, stimulation of bile acid synthesis, or enhancement of lipoprotein lipase activity (Goldstein and Brown, 2019; Kumar *et al.,* 2020).

Recent pharmacological evidence suggests that several African medicinal plants, including *Anthocleista grandiflora*, possess bioactive components capable of influencing lipid metabolism and improving cardiovascular function (Njoku *et al.,* 2020; Olorunnisola *et al.,* 2020). The hypolipidaemic effect of *A. grandiflora* is believed to be associated with the synergistic action of flavonoids, alkaloids, saponins, and tannins that modulate lipid biosynthesis and promote the excretion of excess cholesterol (Ibrahim *et al.,* 2021; Okeke *et al.,* 2022). Understanding how these bioactive compounds affect lipid homeostasis in controlled sub-chronic studies provides a vital step toward developing standardized, safe, and effective plant-based therapies for dyslipidaemia and related metabolic disorders.

2.2 TAXONOMY OF *Anthocleista grandiflora*

Anthocleista grandiflora is a member of the genus Anthocleista, belonging to the family Gentianaceae a family well-known for its species rich in iridoid compounds, alkaloids, and other pharmacologically active metabolites (Olorunnisola *et al.,* 2020; Ibrahim *et al.,* 2021). The genus comprises about 15–20 species distributed across tropical Africa, Madagascar, and parts of Southeast Asia. Among these, A. grandiflora is one of the largest species, often recognised for its majestic height and striking foliage (Njoku et al., 2020).

The taxonomic hierarchy of *Anthocleista grandiflora* according to the Angiosperm Phylogeny Group (APG IV, 2016) classification system is as follows:

Kingdom: Plantae
Subkingdom: Tracheobionta (Vascular plants)
Division: Magnoliophyta (Angiosperms)
Class: Magnoliopsida (Dicotyledons)
Order: Gentianales
Family: Gentianaceae
Genus: Anthocleista
Species: *Anthocleista grandiflora Gilg*

The genus name Anthocleista originates from the Greek words anthos (flower) and kleistos (closed), possibly referring to the flower morphology of some species within the genus. The species epithet grandiflora translates to “large-flowered,” describing its distinctive white blossoms that make it easily identifiable in forest landscapes (Njoku *et al.,* 2020; Iwu, 2019).

Phylogenetically, *Anthocleista grandiflora* is closely related to other Gentianaceae species such as *Gentiana lutea* and *Swertia chirayita*, both known for their strong bitter principles and medicinal applications (Adebayo *et al.,* 2018; Kumar *et al.,* 2020). Members of this family are typically characterised by opposite leaves, tubular flowers, and capsules containing numerous small seeds.

These morphological traits, along with their unique secondary metabolite profiles, have made them valuable both in traditional and modern medicine (Ogunyemi *et al.,* 2022).

Taxonomic studies further show that *A. grandiflora* shares several features with other African species like *Anthocleista vogelii* and *Anthocleista djalonensis,* although it can be distinguished by its taller growth (up to 30 metres), larger leaves, and broader ecological distribution (Okeke *et al.,* 2022). The bark and leaves are often aromatic and bitter, characteristics commonly associated with plants of the Gentianaceae family, due to the presence of iridoid glycosides and alkaloid constituents (Olorunnisola *et al.,* 2020).

Plate 1: *Anthocleista grandiflora* **Photocredit:** (Adefusi Harrietta, 2025).

2.3 BOTANICAL DESCRIPTION OF *Anthocleista grandiflora*

Anthocleista grandiflora Gilg is a tall, tropical tree that belongs to the family Gentianaceae. It is one of the largest and most conspicuous members of its genus, easily recognised in the forest by its towering height and large, glossy leaves. The species is commonly referred to as the "Cabbage Tree" or "Giant African Cabbage Tree" due to the arrangement and texture of its broad leaves (Njoku *et al.,* 2020; Olorunnisola *et al.,* 2020).

2.3.1. MORPHOLOGICAL DESCRIPTION

Habit and Size: *A. grandiflora* is a deciduous to semi-evergreen tree that typically grows between 10 and 30 meters in height, with a straight, cylindrical trunk that can reach up to 1 meter in diameter (Iwu, 2019; Ogunyemi *et al.,* 2022). The bark is greyish-brown to dark brown, rough in older trees, and smooth in young plants. When incised, the bark exudes a bitter, yellowish sap with a characteristic aromatic odor (Okonkwo *et al.,* 2023).

i. Leaves: The leaves are simple, opposite, and clustered near the tips of young branches, forming a pseudo-rosette pattern. They are broadly elliptic to obovate, measuring 15–45 cm in length and 10–25 cm in width, with entire margins and a glossy green surface. The leaf base is cuneate to rounded, and the apex is abruptly acuminate (Njoku *et al.,* 2020). The petioles are short, typically 1–3 cm long. The venation is distinctly pinnate with a prominent midrib, contributing to the cabbage-like appearance (Olorunnisola *et al.,* 2020; Ibrahim *et al.,* 2021).

ii. Stem and Bark: The stem of *A. grandiflora* is woody, erect, and cylindrical with a brown to grey bark that becomes fissured as the plant matures. The inner bark is yellowish and bitter due to the presence of alkaloids and iridoid glycosides (Ekennia *et al.,* 2018). The wood is relatively soft and light, often used in local craft and fuelwood production

(Okeke *et al.,* 2022).

iii. Flowers: The plant produces large, fragrant and white to creamy flowers arranged in terminal panicles. Each flower measures approximately 6–12 cm in diameter, with a tubular corolla and four to eight lobes that are slightly reflexed (Njoku *et al.,* 2020). The calyx is short, green, and persistent. The stamens are inserted near the base of the corolla tube, and the ovary is superior with two locules. Flowering generally occurs between October and February, depending on the regional climate (Iwu, 2019; Ogunyemi *et al.,* 2022).

iv. Fruits and Seeds: The fruit is a large, ellipsoidal berry measuring 5–10 cm in length and 4–7 cm in diameter, turning from green to orange or yellow upon ripening (Olorunnisola et al., 2020). The pericarp is fleshy and contains numerous small, flattened seeds embedded in pulp. The seeds are brown, oblong, and surrounded by mucilaginous material, which aids in seed dispersal by animals and water (Njoku *et al.,* 2020).

v. Roots: The root system is extensive and fibrous, providing strong anchorage and supporting the tree's height. The roots are known to contain high concentrations of secondary metabolites such as alkaloids, tannins, and saponins, which are believed to contribute to its medicinal uses (Adebayo *et al.,* 2018; Ibrahim *et al.,* 2021).

2.3.2 ANATOMICAL AND MICROSCOPIC CHARACTERISTICS

Microscopic examination of *A. grandiflora* leaf and stem reveals parenchymatous tissues with abundant starch granules, calcium oxalate crystals, and oil glands (Ogunyemi et al., 2022). The vascular bundles are collateral and well developed, with distinct xylem and phloem zones. Secretory canals are observed within the cortex, and the presence of trichomes on the lower epidermis indicates adaptive features for moisture conservation (Njoku *et al.,* 2020; Okeke *et al.,* 2022).

2.3.3 CHEMICAL AND MORPHOLOGICAL CORRELATION

The bitter taste and aromatic smell of the leaves and bark are attributed to the presence of iridoid glycosides, alkaloids, and phenolic compounds, which are common in Gentianaceae plants (Olorunnisola *et al.,* 2020; Kumar *et al.,* 2020). These compounds not only account for the plant's pharmacological effects such as hepatoprotection, anti-inflammatory, and antioxidant activity but also serve as chemotaxonomic markers for species identification (Njoku *et al.,* 2020).

2.3.4 ECOLOGICAL ADAPTATION

A. grandiflora exhibits remarkable ecological adaptability. It thrives in tropical lowland and montane forests, often found along riverbanks and forest edges where soil moisture is high (Ogunyemi *et al.,* 2022). Its large leaves allow for efficient light capture in shaded environments, while its extensive root system ensures stability in waterlogged or loose soils. The plant's flowering and fruiting patterns are closely linked to rainfall, with seed germination enhanced in moist environments (Okonkwo *et al.,* 2023).

2.4 GEOGRAPHICAL DISTRIBUTION AND HABITAT OF *Anthocleista grandiflora*

Anthocleista grandiflora is a tropical tree species native to sub-Saharan Africa, particularly abundant in West, Central, and East Africa (Njoku *et al.,* 2020; Olorunnisola *et al.,* 2020). It is widely distributed across Nigeria, Ghana, Cameroon, Sierra Leone, Uganda, Kenya, Tanzania, Zimbabwe, and South Africa, where it occupies diverse ecological niches ranging from lowland rainforests to montane forest regions (Ogunyemi *et al.,* 2022; Okonkwo *et al.,* 2023). In Nigeria, the plant is commonly found in the rainforest and Guinea savanna zones, particularly in states such as Cross River, Edo, Delta, Imo, Enugu, and Ogun (Iwu, 2019; Adebayo *et al.,* 2018).

The natural habitat of *A. grandiflora* consists primarily of moist, well-drained soils along riverbanks, forest margins, and valleys, where it benefits from consistent soil moisture and partial shade (Njoku *et al.,* 2020). The species is typically found at altitudes ranging from 500 to 1800 meters above sea level, depending on regional topography (Olorunnisola *et al.,* 2020). It thrives in humid environments with an annual rainfall of 1200–2000 mm and mean temperatures between 25 °C and 32 °C (Ogunyemi *et al.,* 2022). These environmental conditions contribute to its vigorous growth and large leaf development, which are adaptive features for efficient photosynthesis and transpiration control in tropical ecosystems.

In West Africa, *Anthocleista grandiflora* is most common in Nigeria, Ghana, and Cameroon. In Nigeria, it occurs in dense rainforest belts of the South-South and South-East regions, where it grows both as a wild and cultivated medicinal species (Njoku *et al.,* 2020). Local herbalists often harvest the leaves, roots, and bark directly from the forest for medicinal preparations (Ogunyemi *et al.,* 2022). In Ghana, the species locally known as "Wuruduro" or "Abako"is cultivated for its medicinal and ornamental value (Iwu, 2019). Its natural occurrence extends through the Volta basin, where it is frequently associated with riverine vegetation (Olorunnisola *et al.,* 2020).

In Central Africa, particularly in Cameroon, Gabon, and the Democratic Republic of Congo, *A. grandiflora* forms part of the understory vegetation of humid equatorial forests (Okonkwo *et al.,* 2023). The tree's large canopy contributes to the structural diversity of tropical forest ecosystems, providing shelter and shade for smaller plants and fauna. In Southern Africa, including Zimbabwe and South Africa, it is found in montane forests and along stream valleys, particularly in the eastern parts of KwaZulu-Natal and Mpumalanga (Ibrahim *et al.,* 2021).

Ecologically, *A. grandiflora* demonstrates high adaptability to varying soil and climatic conditions. Its fibrous root system helps in soil stabilization and water absorption, making it vital for preventing erosion in sloped or riverine areas (Olorunnisola *et al.,* 2020). The plant's large, glossy leaves maximize photosynthetic efficiency even under shaded conditions, allowing it to thrive as an understory species (Njoku *et al.,* 2020). Additionally, its flowers are highly attractive to pollinators such as bees, butterflies, and birds, while its fleshy fruits are consumed and dispersed by bats and monkeys (Ogunyemi *et al.,* 2022).

The species also plays a modest but significant role in forest regeneration, since its seeds germinate easily and help in recolonizing disturbed forest areas (Okonkwo *et al.,* 2023). Because of its medicinal importance, the plant has been subject to selective harvesting, particularly for roots and bark, which has raised concerns about sustainability. Conservationists have recommended community-based replanting and sustainable harvesting programs to prevent local depletion (Adebayo *et al.,* 2018).

Beyond its ecological presence, *A. grandiflora* holds cultural and ethnomedicinal importance among several African communities. Its availability across different regions has contributed to its widespread use in traditional medicine. The species' adaptability has also made it a marker of ecological health, as its occurrence indicates the presence of nutrient-rich, moist soils typical of regenerating forests (Olorunnisola *et al.,* 2020).

2.5 ETHNOMEDICINAL AND TRADITIONAL USES OF *Anthocleista grandiflora*

The use of *Anthocleista grandiflora* in traditional medicine is well established across sub-Saharan Africa, where various parts of the plant such as leaves, bark, roots, and fruitsare used in the treatment of numerous ailments. The plant is highly regarded among local herbal practitioners for its wide pharmacological spectrum, which includes applications in managing fever, malaria, jaundice, diabetes, rheumatism, and gastrointestinal disorders (Njoku *et al.,* 2020; Olorunnisola *et al.,* 2020). Its consistent inclusion in ethnomedicinal preparations across different cultural regions demonstrates its therapeutic importance and long-standing reliability in indigenous medicine (Iwu, 2019).

2.5.1 TRADITIONAL APPLICATIONS

In Nigeria, the leaves and stem bark of *A. grandiflora* are widely used in decoctions to treat malaria, typhoid fever, and jaundice (Adebayo *et al.,* 2018). The fresh leaves are boiled in water, and the resulting extract is consumed orally, often mixed with honey or lime juice to reduce bitterness. The root bark is also ground and combined with palm wine as a tonic to stimulate appetite and relieve general body weakness (Ogunyemi *et al.,* 2022). In some regions of southeastern Nigeria, the plant is used as a febrifuge and anthelmintic, particularly in children's preparations (Ekennia *et al.,* 2018).

Among the Akan of Ghana, *A. grandiflora* locally known as "Wuruduro"is employed to treat liver-related disorders, stomach pain, and dysentery (Olorunnisola *et al.,* 2020). Decoctions of the bark are used as a blood purifier and detoxifying agent. In addition, the plant's extract is consumed postpartum to restore vitality and prevent infections in women (Njoku *et al.,* 2020). Similarly, in Cameroon and other Central African countries, the roots are used as a remedy for fever, joint pain, and gonorrhea, while the bark is chewed as a stimulant and aphrodisiac (Okonkwo *et al.,* 2023).

2.5.2 MEDICINAL PREPARATION METHODS

Traditional healers employ various methods to extract the plant's medicinal constituents. The aqueous decoction method, which involves boiling plant material in water, is most common and aligns with the ethnomedical practice of using water as a solvent to extract bioactive compounds (Njoku *et al.,* 2020). Other preparation methods include infusions, macerations, and alcoholic tinctures, depending on the desired potency and shelf life of the extract (Ogunyemi *et al.,* 2022). The methanol leaf extract, used in modern pharmacological studies, mimics the solubility profile of traditional preparations while offering better extraction efficiency for both polar and non-polar phytochemicals (Ekennia *et al.,* 2018).

2.5.3 REPORTED PHARMACOLOGICAL CORRELATIONS

Modern pharmacological studies have validated many of the traditional claims associated with *A. grandiflora*. For instance, its hepatoprotective activity supports its ethnomedicinal use in managing jaundice and liver diseases (Njoku *et al.,* 2020). The antipyretic and anti-inflammatory properties observed in experimental models correspond to its traditional application in fever and rheumatism (Adebayo *et al.,* 2018). Moreover, its hypoglycemic and hypolipidemic activities justify its use in managing diabetes and metabolic disorders (Olorunnisola *et al.,* 2020).

Additionally, the antimicrobial properties of the plant provide a scientific basis for its use in treating gastrointestinal infections and wounds (Okonkwo *et al.,* 2023). Extracts from the leaves and bark have demonstrated inhibitory effects against *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*, which are common pathogens implicated in diarrhoea and skin infections (Ogunyemi *et al.,* 2022).

2.5.4 REGIONAL ETHNOMEDICINAL DIVERSITY

The versatility of *A. grandiflora* in ethnomedicine reflects its broad distribution and adaptability across different ecological zones. In East Africa, particularly in Kenya and Tanzania, traditional healers employ the bark decoction to manage hypertension, chest pain, and respiratory infections (Ibrahim *et al.,* 2021). In South Africa, it is used in traditional Zulu medicine as a blood cleanser and treatment for sores, where it is locally referred to as "Mutowo"(Olorunnisola *et al.,* 2020).

2.5.5 SOCIOECONOMIC IMPORTANCE

The ethnomedicinal significance of *A. grandiflora* extends to its economic value. The plant contributes to rural livelihoods through the sale of herbal preparations in local markets, especially in Nigeria and Ghana (Adebayo *et al.,* 2018). Its bark and roots are often traded as crude drug materials in traditional medicine markets, reflecting its sustained demand and commercial viability (Ogunyemi *et al.,* 2022).

2.6 PHYTOCHEMICAL COMPOSITION OF *Anthocleista grandiflora*

The pharmacological significance of *Anthocleista grandiflora* is closely associated with its complex phytochemical composition. Plants in the genus Anthocleista are known for their high content of bioactive secondary metabolites, which contribute to their therapeutic effects and traditional applications (Njoku *et al.,* 2020; Olorunnisola *et al.,* 2020). Phytochemical investigations of the methanol, ethanol, and aqueous extracts of *A. grandiflora* leaves, bark, and roots have revealed the presence of various classes of compounds, including alkaloids, flavonoids, saponins, tannins, terpenoids, cardiac glycosides, steroids, phenols, and anthraquinones (Ekennia *et al.,* 2018; Ogunyemi *et al.,* 2022).

2.6.1 ALKALOIDS

Alkaloids are nitrogen-containing compounds with diverse pharmacological actions such as anti-inflammatory, antimicrobial, and analgesic effects. Studies by Njoku *et al.* (2020) and Ibrahim *et al.* (2021) reported that alkaloids in A. grandiflora exhibit hepatoprotective and hypolipidemic properties through modulation of hepatic enzymes involved in cholesterol biosynthesis. These compounds inhibit HMG-CoA reductase, the key enzyme responsible for endogenous cholesterol formation, thereby contributing to lipid-lowering effects (Okonkwo *et al.,* 2023). Additionally, alkaloids have been shown to enhance bile secretion and promote the excretion of bile acids, further aiding in cholesterol clearance (Olorunnisola *et al.,* 2020).

2.6.2 FLAVONOIDS

Flavonoids are one of the most abundant secondary metabolites in *A. grandiflora*. They are well known for their antioxidant, anti-inflammatory, and vascular-protective roles (Adebayo *et al.,* 2018). These compounds scavenge reactive oxygen species (ROS) and inhibit lipid peroxidation, protecting cellular membranes and lipoproteins from oxidative damage (Njoku *et al.,* 2020; Ibrahim *et al.,* 2021). In addition, flavonoids improve high-density lipoprotein (HDL) synthesis and reduce low-density lipoprotein (LDL) oxidation, both of which are crucial for maintaining a healthy lipid profile (Goldstein and Brown, 2019). The antioxidant potential of these flavonoids supports the plant's traditional use in the management of metabolic and cardiovascular disorders.

2.6.3 SAPONINS

Saponins are glycosidic compounds that possess strong surface-active properties. Their ability to form insoluble complexes with cholesterol and bile acids makes them important agents in lipid regulation (Kumar *et al.,* 2020). Studies have shown that saponins in *A. grandiflora* promote the excretion of cholesterol and triglycerides through the gastrointestinal tract (Olorunnisola *et al.,* 2020; Ogunyemi *et al.,* 2022). Moreover, they exhibit antihyperlipidemic and antioxidant properties, which together contribute to improved lipid metabolism and cardiovascular protection.

2.6.4 TANNINS

Tannins are polyphenolic compounds responsible for the astringent taste of many plant extracts. They possess antimicrobial, antioxidant, and anti-inflammatory effects (Njoku *et al.,* 2020). In *A. grandiflora*, tannins contribute to liver protection and aid in the prevention of lipid peroxidation in hepatocytes (Ekennia *et al.,* 2018). Their astringent properties also account for the plant's traditional use in treating diarrhoea and wounds.

2.6.5 TERPENOIDS

Terpenoids are another important group of compounds found in *A. grandiflora*. These molecules play a vital role in regulating lipid synthesis and metabolism by influencing key hepatic enzymes, including acetyl-CoA carboxylase and fatty acid synthase (Ibrahim *et al.,* 2021). Terpenoids also exhibit anti-inflammatory and antioxidant properties, thereby protecting tissues from oxidative and inflammatory damage (Adebayo *et al.,* 2018). Their contribution to the modulation of lipid metabolism further validates the plant's hypolipidemic potential.

2.6.6 CARDIAC GLYCOSIDES AND STEROIDS

Cardiac glycosides are known for their role in regulating heart function by modulating Na^+/K^+-ATPase activity (Okonkwo *et al.,* 2023). The presence of these compounds in A. grandiflora suggests potential cardiovascular benefits, including improved myocardial contractility and circulatory efficiency. Steroidal compounds, on the other hand, serve as precursors for hormones involved in metabolism and also exhibit anti-inflammatory effects (Ogunyemi *et al.,* 2022).

2.6.7 PHENOLIC COMPOUNDS AND ANTHRAQUINONES

Phenolic compounds are strong antioxidants that neutralize free radicals and inhibit oxidative stress-related damage (Ibrahim *et al.,* 2021). Anthraquinones, though present in smaller quantities, possess mild laxative and antimicrobial properties that aid in detoxification and intestinal cleansing (Ekennia *et al.,* 2018). Together, these compounds contribute to the overall detoxifying and restorative capacity of the plant.

The therapeutic effects of *A. grandiflora* cannot be attributed to a single constituent but rather to the synergistic interaction among its various phytochemicals. Flavonoids and phenols, for instance, enhance antioxidant defences; alkaloids and saponins regulate lipid metabolism; while tannins and terpenoids promote organ protection and healing (Njoku *et al.,* 2020; Ibrahim *et al.,* 2021). This synergism accounts for the plant's multiple pharmacological actions including hepatoprotective, hypolipidemic, antioxidant, and anti-inflammatory effects (Adebayo *et al.,* 2018; Olorunnisola *et al.,* 2020).

2.7 PHARMACOLOGICAL ACTIVITIES OF *anthocleista grandiflora*

Over the past decade, numerous studies have explored the pharmacological potential of *Anthocleista grandiflora,* confirming its wide spectrum of biological activities. The plant exhibits antioxidant, anti-inflammatory, hepatoprotective, antidiabetic, antimicrobial, and hypolipidemic properties (Ekennia *et al.,* 2018; Njoku *et al.,* 2020; Ogunyemi *et al.,* 2022). These effects validate its extensive use in traditional medicine and provide a scientific foundation for its possible development as a phytotherapeutic agent.

2.7.1 ANTIOXIDANT ACTIVITY

Antioxidant mechanisms are central to the biological effects of *A. grandiflora*. Reactive oxygen species (ROS) and free radicals are known contributors to cellular damage, lipid peroxidation, and chronic diseases such as cardiovascular disorders and diabetes (Goldstein and Brown, 2019). Methanol and ethanol extracts of *A. grandiflora* leaves have demonstrated significant free-radical-scavenging capacity using assays such as DPPH and FRAP (Njoku *et al.,* 2020; Ibrahim *et al.,* 2021). The high antioxidant potential has been attributed to the presence of phenolic compounds, flavonoids, and terpenoids, which neutralize free radicals and enhance endogenous enzymatic defences such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Olorunnisola *et al.,* 2020).

2.7.2 ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

The anti-inflammatory activity of *A. grandiflora* is linked to its ability to modulate inflammatory mediators including prostaglandins and cytokines. Ekennia *et al.* (2018) observed that methanol leaf extract significantly inhibited carrageenan-induced paw oedema and acetic-acid-induced writhing in mice, showing dose-dependent suppression of pain and inflammation comparable to indomethacin. The anti-inflammatory properties are largely associated with flavonoids, alkaloids, and terpenoids, which inhibit cyclooxygenase (COX) and lipoxygenase (LOX) pathways (Adebayo *et al.,* 2018). These findings support the ethnomedicinal use of the plant in the management of arthritis, fever, and rheumatic pain.

2.7.3 HEPATOPROTECTIVE ACTIVITY

The hepatoprotective potential of *A. grandiflora* has been confirmed in several animal studies. Njoku *et al*. (2020) reported that methanol leaf extract administered to rats with carbon-tetrachloride (CCl_4)–induced hepatotoxicity resulted in a significant reduction in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels compared to untreated controls. Histopathological analyses revealed restoration of hepatic architecture and decreased necrotic lesions. The hepatoprotection was attributed to antioxidant flavonoids and phenolic compounds, which prevent lipid peroxidation and stabilize hepatocellular membranes (Ogunyemi *et al.,* 2022). This hepatoprotective action is particularly relevant because the liver plays a central role in lipid synthesis and metabolism, suggesting indirect benefits for lipid profile regulation.

2.7.4 ANTIMICROBIAL AND ANTIPARASITIC ACTIVITY

Methanol and ethanol extracts of *A. grandiflora* have shown broad-spectrum antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Candida albicans* (Ibrahim *et al.,* 2021). The antimicrobial effects are linked to the presence of alkaloids, tannins, and saponins, which disrupt microbial cell membranes and inhibit DNA replication (Olorunnisola *et al.,* 2020). In addition, the plant demonstrates antiplasmodial effects against *Plasmodium falciparum*, supporting its traditional use in treating malaria and febrile illnesses

(Adebayo *et al.,* 2018).

2.7.5 ANTIDIABETIC AND METABOLIC MODULATION

Several studies have reported the hypoglycaemic and insulin-sensitising effects of *A. grandiflora.* Ekennia *et al.* (2018) demonstrated that methanol extract significantly lowered fasting blood glucose in streptozotocin-induced diabetic rats. The extract enhanced pancreatic β-cell function and increased hepatic glycogen storage. These actions were attributed to flavonoids and alkaloids that improve insulin sensitivity and reduce oxidative stress in pancreatic tissues (Njoku *et al.,* 2020). Since diabetes and dyslipidaemia frequently coexist, these antidiabetic effects may contribute indirectly to lipid profile improvement.

2.7.6 HYPOLIPIDEMIC ACTIVITY

The hypolipidemic potential of *A. grandiflora* is of particular importance in the context of cardiovascular health. Methanol extract has been shown to significantly reduce total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) levels while increasing high-density lipoprotein (HDL) concentrations in experimental animals (Ogunyemi *et al.,* 2022). The lipid-modulating effects are mediated through inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis, as well as through enhanced conversion of cholesterol into bile acids (Goldstein and Brown, 2019). Saponins and flavonoids in the extract also promote cholesterol excretion and prevent LDL oxidation, thereby reducing atherogenic risk (Kumar *et al.,* 2020).

These results suggest that *A. grandiflora* possesses potent lipid-regulating properties comparable to standard hypolipidemic drugs, though with potentially fewer side effects. Moreover, the plant's antioxidant activity complements its hypolipidemic effect by protecting plasma lipoproteins from oxidative damage, a crucial step in the prevention of atherosclerosis.

Additional studies have indicated that *A. grandiflora* exhibits antipyretic, diuretic, antimalarial, and wound-healing activities (Njoku *et al.,* 2020; Ibrahim *et al.,* 2021). These therapeutic actions enhance its versatility in traditional medicine. Its diuretic activity supports detoxification and helps regulate blood pressure, while its wound-healing properties stem from tannins and flavonoids that promote collagen synthesis and tissue repair (Olorunnisola *et al.,* 2020).

Taken together, the broad pharmacological profile of *Anthocleista grandiflora* reflects the synergistic action of its diverse phytochemicals. The plant's methanol leaf extract not only modulates lipid and glucose metabolism but also strengthens antioxidant defences and organ protection systems, supporting its traditional use and underscoring its potential as a natural therapeutic agent for metabolic and cardiovascular diseases.

2.8 POSSIBLE MECHANISMS OF ACTION OF *Anthocleista grandiflora*

The pharmacological actions of *Anthocleista grandiflora*, especially its hypolipidemic and hepatoprotective effects, are mediated through a combination of biochemical, physiological, and molecular mechanisms. These mechanisms work synergistically to regulate lipid metabolism, enhance antioxidant defences, and maintain cellular integrity (Ogunyemi *et al.,* 2022; Njoku *et al.,* 2020).

2.8.1 ANTIOXIDANT MECHANISMS

The antioxidant mechanism of *A. grandiflora* plays a central role in its overall pharmacological profile. Reactive oxygen species (ROS) and free radicals are known to initiate lipid peroxidation and damage biological membranes, particularly in hepatic tissues (Goldstein & Brown, 2019). Flavonoids, phenolic compounds, and terpenoids present in the methanol extract act as potent antioxidants by neutralising free radicals and enhancing the activities of endogenous enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Olorunnisola *et al.,* 2020).

By inhibiting lipid peroxidation, these compounds prevent the oxidative degradation of lipids and maintain membrane fluidity. This not only protects hepatic and cardiac tissues but also prevents oxidative modification of low-density lipoprotein (LDL), a crucial step in the pathogenesis of atherosclerosis (Kumar *et al.,* 2020).

2.8.2 REGULATION OF LIPID METABOLISM

The hypolipidemic effect of *A. grandiflora* is primarily associated with its influence on lipid biosynthesis and catabolism. The methanol leaf extract inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme responsible for cholesterol synthesis in the liver (Ogunyemi *et al.,* 2022). This inhibition reduces the endogenous production of cholesterol, leading to decreased plasma levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C).

In addition, saponins and tannins in the extract promote bile acid excretion by forming insoluble complexes with cholesterol in the intestine (Njoku *et al.,* 2020). This mechanism not only enhances the removal of cholesterol from the body but also stimulates the conversion of hepatic cholesterol into bile acids, thus maintaining a healthy lipid balance.

Flavonoids also contribute to lipid regulation by increasing high-density lipoprotein cholesterol (HDL-C) synthesis, which facilitates reverse cholesterol transport — the process by which excess cholesterol is transported from peripheral tissues back to the liver for excretion (Ibrahim *et al.,* 2021). The overall result is an improved HDL/LDL ratio, which is beneficial for cardiovascular health.

2.8.3 HEPATOPROTECTIVE MECHANISMS

The liver is the central organ responsible for lipid metabolism, and maintaining its functional integrity is essential for normal lipid homeostasis. Methanol extracts of *A. grandiflora* protect hepatic tissues from toxins such as carbon tetrachloride (CCl_4) and paracetamol by reducing oxidative stress and stabilising cell membranes (Njoku *et al.,* 2020). Flavonoids and phenolic compounds enhance membrane stability and prevent the leakage of liver enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Olorunnisola *et al.,* 2020).

This hepatoprotective mechanism ensures efficient lipid and carbohydrate metabolism, prevents fatty liver accumulation, and enhances detoxification processes. The restoration of normal hepatic enzyme activity observed in treated rats supports this conclusion (Ibrahim *et al.,* 2021).

2.8.4 ANTI-INFLAMMATORY MECHANISMS

Inflammation plays a significant role in lipid metabolism disorders and the progression of cardiovascular diseases. The anti-inflammatory activity of *A. grandiflora* involves the suppression of inflammatory mediators such as prostaglandins, tumour necrosis factor-alpha (TNF-α), and interleukins (IL-1β and IL-6) (Adebayo *et al.,* 2018). Flavonoids and alkaloids in the extract inhibit cyclooxygenase (COX) and lipoxygenase (LOX) pathways, thereby reducing the production of pro-inflammatory eicosanoids (Ekennia *et al.,* 2018).

This anti-inflammatory response protects blood vessels and hepatic tissues from inflammatory damage and supports lipid homeostasis by preventing oxidative and cytokine-induced disruption of lipid metabolism (Ogunyemi *et al.,* 2022).

2.8.5 ANTIHYPERGLYCAEMIC AND ENDOCRINE MODULATION

The hypoglycaemic activity of *A. grandiflora* also contributes indirectly to lipid regulation. By enhancing insulin sensitivity and reducing hyperglycaemia, the plant extract minimises lipid accumulation in the liver and adipose tissues (Njoku *et al.,* 2020). Improved glucose utilisation reduces the conversion of excess glucose into triglycerides, thereby decreasing plasma triglyceride levels (Olorunnisola *et al.,* 2020).

Furthermore, alkaloids and flavonoids modulate the endocrine system by stimulating pancreatic β-cell function and improving insulin secretion, which helps maintain both glucose and lipid equilibrium (Ibrahim *et al.*, 2021).

2.8.6 MEMBRANE STABILISATION AND CELLULAR PROTECTION

The membrane-stabilising properties of *A. grandiflora* are largely attributed to the actions of polyphenols and glycosides. These compounds protect the structural integrity of cell membranes against oxidative, inflammatory, and toxic insults (Olorunnisola *et al.,* 2020). By preserving cellular homeostasis, they ensure normal lipid transport and metabolism within tissues. The plant's stabilising effect on erythrocyte and hepatocyte membranes further suggests its potential in reducing haemolysis and liver injury, which are common complications of hyperlipidaemia and metabolic stress (Njoku *et al.,* 2020).

2.8.7 SYNERGISTIC MECHANISMS

The diverse pharmacological effects of *A. grandiflora* result from synergistic interactions among its phytochemical constituents. For example, flavonoids and phenols exhibit combined antioxidant effects, while saponins and alkaloids complement each other in regulating lipid synthesis and cholesterol excretion (Ogunyemi *et al.,* 2022). Terpenoids enhance hepatic enzymatic activity, which further promotes lipid breakdown.

This synergy ensures that the plant's extract acts on multiple biological targets simultaneously, providing a holistic regulation of lipid and oxidative balance. The combination of antioxidant, anti-inflammatory, hepatoprotective, and hypolipidemic actions positions *A. grandiflora* as a promising phytotherapeutic agent for managing dyslipidaemia and associated metabolic disorders.

CHAPTER THREE

MATERIALS AND METHODS

3.1 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*

3.2 COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

Fresh leaves of *Anthocleista grandiflora* were collected from Igbanke village in Orhionwon Local Government Area, Edo State, Nigeria. The plant was identified and authenticated by a taxonomist at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, where a voucher specimen (UBH-W44) was deposited for reference.

3.3 PREPARATION OF EXTRACT

The leaves were separated from the stems, rinsed with clean water to remove dust and debris, and air-dried at room temperature until constant weight was achieved. The dried leaves were pulverized into fine powder using a mechanical grinder and stored in an airtight amber container. Eight kilograms (8 kg) of powdered material were soaked in 3 L of 99.9% methanol for 72 hours with intermittent shaking. The mixture was filtered using a porous sieve lined with wire gauze. The filtrate was concentrated using a water bath at 40 °C to obtain a brownish-green, jelly-like crude extract, which was stored in a sterile glass container under refrigeration until required. The final yield was weighed and the percentage recovery was determined

3.4 EXPERIMENTAL ANIMALS

The experiment was done using twenty (20) male Wister rats weighing between 159g to 230g. 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 allowed one week of acclimatization before they were randomly grouped. They were housed in standard plastic cages and allowed access to rat pellets (Pelletized grower feed, Vital Feed Ltd, Jos, Nigeria) and tap water *ad-libitum*. All experimental animals were handled according to institutional and international guidelines for the use of experimental animals (Ozolua*et al*., 2009).

3.5 EXPERIMENTAL DESIGN

From the outcome of the acute toxicity test, where no death was observed at 5000 mg/Kg, three doses of the extract, 200, 400, and 800 mg/kg were selected for the test. These doses of the extract were chosen based on the method of Bautista *et al*., 2004; Ozolua and Uwaya, 2013, and Prasanth*et al*., 2015. Twenty male rats were randomly divided into four groups (n=5) with their initial body weight measured. Group I served as the control and the rats were fed with normal rat feed, and with distilled water (as the vehicle for the extract). Groups II, III, and IV rats were given 200, 400, and 800 mg/Kg of *Anthocleista grandiflora* leaf extract respectively. The vehicle and extract were given orally once daily using an orogastric tube (oral gavage) for 28 days. At the end of the experiment, the final body weights, and biochemical assays were determined.

3.6 BLOOD SAMPLE COLLECTION

At the end of the treatment period of 28 days, the animals were anesthetized by placing them in a closed jar containing cotton wool 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 tabletop 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 clearly labeled plain bottles that were used for the lipid profile assay.

3.7 ASSAY OF LIPID PROFILE

Analysis of lipid profile was performed by use of a biochemical analyzer machine. SELECTRA PRO-S (model S-series, ELITechGroup Clinical Chemistry) was the biochemical auto-analyser machine that was used for all the assays; total cholesterol, triglycerides, HDL, and LDL. The assay kits for cholesterol, triacylglycerols, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were obtained from RANDOX Laboratories Ltd.

3.8 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 4.1: Biochemical indices following 28 days daily oral administration of methanol plant extract of *Anthocleista grandiflora Gilg.*

Parameter	Control	200 mg/kg	400 mg/kg	800 mg/kg
Cholesterol	78.00 ± 3.07	85.60 ± 3.28	78.80 ± 4.33	83.60 ± 3.08
Triglycerides	86.80 ± 4.14	87.20 ± 12.87	77.60 ± 4.52	90.00 ± 8.83
HDL	24.20 ± 0.97	24.60 ± 1.03	25.60 ± 1.03	27.60 ± 1.36
LDL	36.40 ± 3.14	43.60 ± 3.85	37.60 ± 5.46	34.20 ± 4.98

Key: HDL = High- density lipoprotein; LDL = Lower-density lipoprotein; Mean ± SEM (n = 5).

Table 4.1presents the biochemical parameters of rats following 28 days of treatment with methanol leaf extract of *Anthocleista grandiflora*. The parameters evaluated include Cholesterol, Triglycerides, High- density lipoprotein (HDL) and Lower-density lipoprotein (LDL). Results are expressed as Mean ± SEM for each treatment group (Control, 200 mg/kg, 400 mg/kg, and 800 mg/kg).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Table 4.1 presents the lipid profile of rats following 28 days of daily oral administration of methanol leaf extract of *Anthocleista grandiflora* at doses of 200, 400, and 800 mg/kg body weight. The parameters evaluated include total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), which serve as key indicators of lipid metabolism and cardiovascular risk.

There were no statistically significant ($p > 0.05$) differences in serum total cholesterol and triglyceride levels among the treated groups compared to the control. Cholesterol concentrations ranged between 78.00 ± 3.07 mg/dL and 85.60 ± 3.28 mg/dL, while triglyceride levels varied between 77.60 ± 4.52 mg/dL and 90.00 ± 8.83 mg/dL. The absence of a dose-dependent trend or significant elevation suggests that the methanol extract of *A. grandiflora* did not disrupt lipid biosynthesis or metabolism during sub-chronic administration. Maintenance of normal cholesterol and triglyceride levels implies that the extract neither induced hyperlipidemia nor exerted adverse hepatic effects that could alter lipid regulation. This finding aligns with earlier reports by Olorunnisola et al. (2012) and Ezeonwumelu et al. (2011), which observed that extracts from *Anthocleista* species do not significantly affect serum lipid concentrations in rats following prolonged exposure.

Serum HDL levels showed a mild, dose-dependent increase from 24.20 ± 0.97 mg/dL in the control group to 27.60 ± 1.36 mg/dL at the highest dose (800 mg/kg). HDL is known as "good cholesterol" due to its role in reverse cholesterol transport, where it facilitates the removal of cholesterol from peripheral tissues to the liver for excretion (Guyton & Hall, 2011). The observed increase in HDL at higher doses may reflect a cardioprotective potential of *A. grandiflora* extract, possibly due to its flavonoid and phenolic constituents, which have been reported to enhance HDL synthesis and antioxidant defense (Bulus et al., 2011; Olorunnisola et al., 2012). Similar HDL-elevating effects have been reported in rats treated with extracts of *A. vogelii* and *A. djalonensis* (Ezeonwumelu et al., 2011), both of which share phytochemical similarities with *A. grandiflora*.

LDL levels fluctuated across the treatment groups but remained within physiological limits (34.20 ± 4.98 to 43.60 ± 3.85 mg/dL). While a slight increase was recorded at 200 mg/kg, higher doses (400 and 800 mg/kg) produced values comparable to or slightly lower than control. This pattern suggests that the extract may have a mild lipid-lowering or regulatory effect at higher doses, preventing LDL accumulation.

Since elevated LDL is a known risk factor for atherosclerosis and cardiovascular diseases, the observed LDL stability across treatment groups indicates that *A. grandiflora* may help maintain lipid balance without promoting lipid peroxidation or oxidative stress (Kumar *et al.,* 2011; Hall, 2011).

5.2 CONCLUSION

Overall, the lipid profile results indicate that 28-day oral administration of methanol leaf extract of *A. grandiflora* did not induce dyslipidemia or alter lipid homeostasis in rats. The stable levels of total cholesterol, triglycerides, HDL, and LDL across all groups suggest that the extract is safe on lipid metabolism and may even possess mild cardioprotective properties at higher doses.

The maintenance of normal lipid indices may be attributed to the presence of bioactive compounds such as alkaloids, flavonoids, and terpenoids, which have been reported to exhibit antioxidant and lipid-normalizing effects in various *Anthocleista* species (Bulus *et al.,* 2011; Olorunnisola *et al.,* 2012).

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