

**THE EFFECT OF *Acanthus montanus* (Nees) T. Anderson LEAF
AQUEOUS EXTRACT ON LIPID PROFILE IN MALE WISTAR RATS.**

BY

Oghene-Oruese Celine AVWENAYERI

SR/2270/RPR/25/12

UNIVERSITY OF BENIN

BENIN CITY

OCTOBER, 2025

**THE EFFECT OF *Acanthus montanus* (Nees) T. Anderson LEAF
AQUEOUS EXTRACT ON LIPID PROFILE IN MALE WISTAR RATS.**

BY

Oghene-Oruese Celine AVWENAYERI

SR/2270/RPR/25/12

**DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY,
FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, IN
PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE
AWARD OF THE DEGREE OF BACHELOR OF SCIENCE OF THE
UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.**

OCTOBER, 2025.

AUTHOR'S STATEMENT

I hereby grant the University of Benin, through the University of Benin Library, a non-exclusive, worldwide right to reproduce and distribute my thesis and abstract (hereinafter "the Work"), in whole or in part, through any media, in its present form or any translated version for preservation and accessibility, provided such translation does not alter its content. This grant is royalty-free, and I retain the right to publish the Work in its current or future versions elsewhere.

Warranties

I further affirm that:

1. I am the sole author of the Work and grant the University of Benin the right to make available four (4) years after the award of my degree, in compliance with the University of Benin Senate regulations.
2. The Work does not contain confidential information requiring third-party consent for disclosure.
3. I have exercised due diligence to ensure that the Work is original and does not breach any Nigerian law or infringe upon any third party's copyright or other Intellectual Property Rights, to the best of my knowledge.
4. Where the Work includes copyrighted material not owned by me, I have obtained unrestricted permission from the copyright holder to grant this license to the University of Benin Library. Such third-party materials are clearly identified and acknowledged within the Work.
5. In the event of any copyright dispute concerning the Work, I agree to indemnify and hold harmless the University of Benin, its officers, employees, and agents from any liability arising from the material authorized under this agreement.

6. The University of Benin is under no obligation whatsoever to take legal action on my behalf as the Depositor in the event of an intellectual property rights infringement or any other related dispute in the material deposited.

Author`s Name	Signature/Date	Email
----------------------	-----------------------	--------------

Supervisor's Name	Signature/Date	Email
--------------------------	-----------------------	--------------

Head of Department	Signature/Date	Email
---------------------------	-----------------------	--------------

CERTIFICATION

We certified that this project work was carried out by Oghene-Oruese Celine AVWENAYERI in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo state. Nigeria.

.....

Dr Joseph O. Erhabor
(Project Supervisor)

.....

Date

.....

Prof. B. Ikhajiagbe
(Head of Department)

.....

Date

DEDICATION

This project work is dedicated to JEHOVAH God for his unwavering support, divine guidance and endless mercy throughout this project work.

ACKNOWLEDGEMENTS

I am genuinely grateful to JEHOVAH GOD for His loving support and divine guidance in the course of this program.

I wish to convey my sincere and heartfelt appreciation to my supervisor, Dr. J. O. Erhabor for his valuable insights, and continuous support throughout the project period. I am truly grateful to Dr. Benjamin O. Gabriel for his expert guidance and support during the course of this program.

Special thanks to the Head of Department, Prof. B. Ikhajiagbe.

My deepest appreciation goes to my parents, Mr and Mrs Okevwie Sunday and younger brother, Ochuko for their consistent support, care, words of encouragement and financial assistance. I am extremely grateful.

A warm appreciation is given to my friends: Eunice, Arike, Wale, Divine and Chexi for their support and motivation and to my project mates for their assistance during the various stages of this work.

TABLE OF CONTENTS

TITLE PAGE	i
AUTHOR'S STATEMENT	ii
CERTIFICATION.....	iv

DEDICATION.....	v
LIST OF TABLES	ix
LIST OF FIGURES	xi
ABSTRACT	xiii
CHAPTER ONE	1
1.0 INTRODUCTION	1
1.1 General Overview	1
1.2 <i>Acanthus montanus</i> (Nees) T. Anderson	3
1.3 Botanical Description of <i>Acanthus montanus</i> (Nees) T. Anderson	3
1.4 Taxonomy of <i>Acanthus montanus</i> (Nees) T. Anderson	4
1.5 Ethno-medicinal importance of <i>Acanthus montanus</i> (Nees) T. Anderson	4
1.6 Biological impacts of <i>Acanthus montanus</i> (Nees) T. Anderson	4
1.7 Aim and Objectives	6
CHAPTER TWO	7
2.0 MATERIALS AND METHOD	7
2.1 Plant Collection	7
2.2 Preparation of Sample	7
2.3 Experimental Animal	7
2.4 Experimental Design	8
2.5 Lipid Profile Analysis	8
2.6.Total Cholesterol	9
2.7 Testosterone Evaluation	11

2.8 Data Analysis	12
CHAPTER THREE	13
3.0 RESULTS	13
3.1 Lipid Profile	13
3.1.1: Effect of <i>Acanthus montanus</i> leaf aqueous extracts on Cholesterol level	14
3.1.2: Effect of <i>Acanthus montanus</i> leaf aqueous extract on triglyceride level.	14
3.1.3: Effect of <i>Acanthus montanus</i> leaf aqueous extract on High-Density Lipoprotein level.	16
CHAPTER FOUR	19
4.0 DISCUSSION	19
CONCLUSION	20
REFERENCES	22

LIST OF TABLES

Table 1: Effect of *Acanthus montanus* leaf aqueous extract on Cholesterol levels in Male Wistar Rats.

Table 2: Effect of *Acanthus montanus* leaf aqueous extract on High-Density Lipoprotein levels in Male Wistar Rats.

Table 3: Effect of *Acanthus montanus* leaf aqueous extract on Testosterone levels in Male Wistar Rats.

LIST OF FIGURES

Figure 1: The effect of *Acanthus montanus* leaf extract on Triglycerides level in Male Wistar Rats.

Figure 2: Effect of *Acanthus montanus* leaf aqueous extract on Low-Density Lipoprotein levels in Male Wistar Rats.

LIST OF PLATE

Plate 1: *Acanthus montanus* (Nees) T. Anderson (Anonymous, 2007).

ABSTRACT

Medicinal plants are reliable and effective in treating various health issues, ranging from minor to major health issues. This study examined the effect of *Acanthus montanus* leaf aqueous extract on the lipid profile of male Wistar Rats. Various standard methods were used to determine the effect of the aqueous extract of *A. montanus* leaf on lipid levels. The aqueous extract of the plant was administered orally at different dosages of 25, 50, and 100 mg/kg to the animals. The 100 mg/kg of Sildenafil Citrate was the positive control, while 1ml of distilled water was administered as the negative control. Treatments were administered for 14 days. The results obtained from this experiment showed that the plant extract can slightly reduce cholesterol levels (130-140 mg/dl), triglyceride levels (52-96 mg/dl), high-density (39.5-44.5 mg/dl) and low-density lipoprotein (77-88 mg/dl), and balance testosterone levels (3.15-3.70 mg/ml). This research supports the potential use of the extract as a therapeutic or preventive agent for hypertriglyceridemia while preserving lipoprotein balance and reproductive hormone homeostasis.

CHAPTER ONE

1.0

INTRODUCTION

1.1 General Overview

Medicinal plants are invaluable natural resources, bridging the past and present by linking traditional healing systems with modern pharmacology. A medicinal plant is any plant that contains active compounds used for therapeutic purposes, such as treating ailments or synthesising useful drugs. These plants or parts of plants, including roots, leaves, stems, and barks, can be used directly for healing or as the basis for modern pharmaceuticals (Soforowa *et al.*, 2013). From ancient times to today, plants have been central to human health, forming the backbone of traditional medicine across cultures. From India's Ayurveda to traditional Chinese (ICM), African ethno-medicine and the healing traditions of indigenous people in the Americas, medicinal plants have long supported human well-being. Medicinal plants provide a key source of inspiration and raw materials for modern pharmaceutical drug development (Amnei *et al.*, 2019).

Traditional medicine has a long history of contributing to conventional medicine and continues to hold promise. For centuries, across countries, people have turned to traditional healers, home remedies and ancient medicinal knowledge to address their health and wellbeing. Various systems of traditional medicine are being used worldwide, including acupuncture, herbal medicine, indigenous traditional medicine, naturopathy, chiropractic, osteopathy, and ayurvedic and unani medicine (WHO, 2019). Traditional medicine is sometimes seen as prescientific; its practices and treatments are to be replaced by modern medicine. Around 40% of pharmaceutical products are derived from nature and traditional knowledge, including landmark drugs: Aspirin and Artemisinin for treating malaria, Vincristine and Vinblastine, from *Catharanthus roseus* are used in treating cancer therapy, and Digoxin from *Digitalis purpurea* is crucial for treating heart conditions. A closer look at

these drugs reveals that the scientists behind them built on traditional knowledge to achieve breakthrough discoveries. Across cultures, medicinal plants have been deeply intertwined with spiritual and cultural practices. In traditional African medicine, plants like the African baobab (*Adansonia digitata*) are used not only for their physical healing properties but also in rituals to promote spiritual well-being (Wickens and Lowe, 2008). Indigenous communities have employed plants like Sage (*Salvia officinalis*) for both medicinal and ceremonial purposes (Moerman, 1998). Others include Willow bark (containing salicin), which brought about the development of Aspirin. Artemisinin, from *Artemisia annus*, is one of the most effective treatments for Malaria today (Nobel Prize, 2015). These medicinal plants serve medicinal purposes and as food, shelter, raw materials and other basic necessities. They help man in all aspects, whether for providing food, shelter, firewood, fodder, cultural or spiritual values or as remedies for illnesses and cures for diseases (Gregory, 1997; Adodo, 2010; Giday *et al.*, 2009).

Despite the benefits of these plants, medicinal plants face hurdles including adulteration, inconsistent quality and potential toxicity. Misidentification of these plants can lead to adverse effects, as seen in contaminated herbal products. Unsuitable harvesting practices, economic pressure such as overharvesting for commercial demand, exacerbate biodiversity loss (Bhat *et al.*, 2021). Climate change can pose a profound threat altering habitats and reducing yield of vulnerable specie and phytochemicals, variability (Anthony, 2023). Habitat loss, fragmentation and land use change can cause deforestation, urbanization and agricultural expansion reduces available wild habitat for medicinal plants (Amanda, 2023). These plants can be conserved in various ways such as in-Situ conservation, ex-situ conservation, community-based conservation, domestication and cultivation.

1.2 *Acanthus montanus* (Nees) T. Anderson

Acanthus montanus plant, commonly called False Thistle, Bear Breeches or Mountain Thistle, is native to tropical West Africa, the Mediterranean region and Asia. It thrives in mountainous regions, forested areas and also in tropical and subtropical climates; in temperate areas (MacDonald et al., 2022). It may die back in winter and regrow in spring. It is grown as an ornamental plant because of its attractive foliage and striking flowers. It grows from about 1-1.8m in height. It prefers growing in shade to growing in direct sunlight. Its foliage does better in shade, and its flowers best grow in the sun. This plant grows in well-drained, fertile, moderately moist soils. It requires regular watering, though it is known to tolerate some drought once established.



Plate 1: *Acanthus montanus* (Nees) T. Anderson (Anonymous, 2007).

1.3 Botanical Description of *Acanthus montanus* (Nees) T. Anderson

This is a tropical perennial, evergreen shrubby herb that may grow up to 1-2 meters in height. It is robust and semi-woody at the base, with a tendency to form clumps. The stems are erect and branched, slightly woody at the base and often covered with short hairs or spines along

the ridges. The leaves are large in size, about 30-60 cm long having an opposite and simple arrangement. Its upper surface has a dark-green glossy colour, shiny above and paler below (Iwu, 2014). It produces flowers that are elongated, having upright spikes that are pinkish to reddish-hued in colour. The fruits it produces is a capsule, ovoid and explosively dehiscent when mature, containing four (4) large black, hard seeds. It has a thickened rootstocks with a fibrous root system.

1.4 Taxonomy of *Acanthus montanus* (Nees) T. Anderson

Acanthus montanus (Nees) T. Anderson is a member of the kingdom Plantae, including all living green plants. It is under the division Tracheophyta, meaning it is a vascular plants and has tissues designed to conduct water and nutrients. It is classified under the class Magnoliopsida, order Lamiales. It is a member of the Acanthaceae family, Genus - *Acanthus* and species, *Acanthus montanus* (Nees) T. Anderson.

1.5 Ethno-medicinal importance of *Acanthus montanus* (Nees) T. Anderson

Acanthus montanus holds significant cultural, medicinal and scientific importance. It is widely used in African traditional medicine, ranging from respiratory conditions to reproductive health and wound healing properties, serving as an accessible remedy for common ailments. Its pharmacological activities underscore its potential for drug development, particularly for anti-infective, anti-pyretic, anti-inflammatory and anti-diabetic, hepato-protective, anti-microbial, antioxidant agents (Patricia *et al.*, 2022).

1.6 Biological impacts of *Acanthus montanus* (Nees) T. Anderson

The biological effects of activities of *Acanthus montanus* plant is of great significance. Series of researches and experiments have been done using this plant in order to understand its therapeutic activities. These activities includes anti-inflammatory, analgesic, anti- respiratory, antipyretic, antimicrobial, anti-cancer, anti-convulsant and so on.

1.6.1 Anti-inflammatory and Analgesic properties

The methanolic extracts significantly suppress swelling and inflammation in experimental modules (Olajide *et al.*, 2003). Its anti-inflammatory properties was described through various in-vitro studies. The plant reduces edema, inhabits nitric oxide production, stabilizes membranes and provides pain relief in models of acute and chronic inflammatory, treating pains, boils and arthritis. In southeastern Nigeria, the root is popular and acclaimed highly effective in the treatment of furuncles. Studies has shown that the root extracts has a great effect on inflammations (Asongalem *et al.*, 2004). They are used for bathing to relieve aches and pains such as backaches, rheumatism, chest pain.

1.6.2 Anti-respiratory and Antipyretic disorder

Leaf infusions and decoctions are commonly used to alleviate coughs, asthma, chest complaints and pharyngitis. In Nigeria and Cameroon, *Acanthus montanus* plant has proven effective in treating cough in women and children (Burkill, 1985). The leaf aqueous extracts are also administered orally to reduce fever

1.6.3 Anti-microbial and Anti-infective activity

Ethanollic extracts of the roots of *Acanthus montanus* show moderate inhibition against pathogens like staphylococcus aureus, Pseudomonas aeruginosa and mycobacteria, supporting its use for treating infections and wounds (Godofred *et al.*, 2018). Experimental studies have shown that it is able to shorten bleeding and clotting times in rats with effects comparable to Vitamin K when given at a higher dose (Uchenna *et a.*, 2024). Phytochemicals in the roots of *the Acanthus montanus plant are responsible for* suppressing microbial infections (Okoli *et al.*, 2008).

1.6.4 Antidiabetics and Hepatoprotective Properties

The leaf aqueous extract of *the Acanthus montanus plant is known to lower blood sugar in diabetic models and protect* against liver damage from toxins like acetaminophen and carbon tetrachloride in rats (Godofred, 2018).

1.6.5 Urogenital and Reproductive Health Disorders

The methanolic leaf extracts of *Acanthus montanus* helps reduce uterine contractions induced by oxytocin and acetylcholine, to prevent uterine pain, threatened abortions and miscarriages (Ugwu *et al.*, 2016) It is also used to treat urogenital infections like endometritis, gonorrhoea, leucorrhoea, cystitis, urethral pain and syphilis. In the Democratic Republic of Congo, pounded leaves are combined with other plants like *Ananas comosus* and *Costus* spp. to treat such infections (Okoli *et al.*, 2008).

1.6.6 Other activities

The *Acanthus montanus* plant is useful in almost all aspects. A widely and commonly used plant that helps in other areas such as anticancer, anticonvulsant, sedative, mosquitocidal, anthelmintic, immunomodulatory, nervous and musculoskeletal conditions.

1.7 Aim and Objectives

1.7.1 Aim of Study

This study aims to evaluate the effect of *Acanthus montanus* leaf aqueous extract on lipid profile and testosterone production in Male Wistar Rats.

1.7.1 Objectives of Study

The objective of this study includes:

- Evaluating the effect of *Acanthus montanus* leaf aqueous extract on Cholesterol in male Wistar Rats.
- Determining the effect of *Acanthus montanus* leaf aqueous extract on Triglycerides in male Wistar Rats.
- Investigating the effect of *Acanthus montanus* leaf aqueous extract on Low-Density Lipoprotein and High-Density Lipoprotein in male Wistar Rats.
- Determining the effect of *Acanthus montanus* leaf aqueous extract on Testosterone Production in male Wistar Rats.

CHAPTER TWO

2.0

MATERIALS AND METHOD

2.1 Plant Collection

Fresh leaves of *Acanthus montanus* were collected from the Bolorunduro community, Akure, Ondo State, Nigeria. It was identified and authenticated by Prof. H.A. Akinnibosun in the Herbarium Unit of the Department of Plant Biology and Biotechnology with Voucher number UBH-A45.

2.2 Preparation of Sample

Fresh leaf samples of *Acanthus montanus* were rinsed, sliced into tiny pieces and then air-dried for four (4) weeks. After air-drying, they were milled or blended into a finely particulate powder using a grinder. The powdered sample was weighed for extraction. 1700g of the leaf powder was homogenised with 300 ml of distilled water. The leaf powder was steeped in distilled water for 72 hours to allow for the extraction of the bioactive compounds. The steeped mixture was sieved, the residue discarded, while the filtrate was concentrated into a semi-solid with a regulated HH-S water bath, with a temperature of about 45°C.

2.3 Experimental Animal

Fifteen (15) adult male Wistar Rats weighing 126-159g were obtained from the Department of Anatomy, University of Benin.. These species of rats were used because of their ease of breeding and availability. The animals were sheltered in a plastic, suitable and conducive clean cage in the Phytomedicine Unit animal shelter of the Department of Plant Biology and Biotechnology, University of Benin. They were acclimatised to their new home and to feed pellets suitable for laboratory animals. They were handled in line with the guide for the care and use of Laboratory animals (National Research Council, 2011).

2.4 Experimental Design

The fifteen animals were divided into five (5) groups of three (3) animals each. The five (5) groups received different treatments of the extract. Below are the various amounts of treatments they were given:

Group 1: Received 25 mg/kg of aqueous extract of *Acanthus montanus*.

Group 2: Received 50 mg/kg of aqueous extract of *Acanthus montanus*.

Group 3: Received 100 mg/kg of aqueous extract of *Acanthus montanus*.

Group 4: (Positive control) received 100 mg/kg of Sildenafil citrate.

Group 5: (Negative control) Received 1 ml of distilled water.

The extracts were all given to the animals through oral administration, for a time period of two weeks (14) days.

2.5 Lipid Profile Analysis

The lipid profile analysis was done as part of an **Aphrodisiac study** that was carried out.

2.5.1 Blood Collection

The Blood collection process was done by first exposing the animals to chloroform for about a minute. The chloroform was used as an anaesthetic. This ensured that the rats did not feel pain during blood collection procedures. After injection, the animal was placed on its back and was given a cardiac puncture. Cardiac puncture is a terminal blood collection method used in male Wistar rats. It is commonly used when large volumes are needed for tests (The Laboratory Rat, 2000). The chest was disinfected with ethanol, and a syringe was inserted between the 3rd – 5th intercostal space, slightly left of the sternum at an angle of about 10-30 degrees, in order to draw out the blood from the animal.

2.5.2 Blood Preparation for Lipid Profile Analysis

The blood collected from the animal and transferred into an EDTA vacutainer tube was allowed to clot for 30-45 minutes at room temperature, centrifuged at 2000-3000g for 10-15 minutes at 4°C, and then stored for analysis.

2.6.Total Cholesterol

The total cholesterol concentration was established using diagnostic kits for wet reagents. This uses a modified method of Trinder (1969). An enzymatic colourimeter method for determining total cholesterol, using one reagent and an endpoint protocol. It is safer, no corrosive chemicals are used, more specific and automation-friendly. Materials used for this analysis were distilled water, plasma sample, a standard reaction, a reagent and three (3) test tubes, which were labeled A, B and C. 1000μ of the reagent was distributed into three (3) test tubes provided - test tube A, B, and C. 10μ of the standard solution, distilled water and the plasma sample were added to the already provided test tube containing 1000μ of the reagent. The mixture was thoroughly shaken for some time to allow a comprehensive mix. They were allowed to stand in a room with a suitable temperature for about 10 minutes, in order to notice the change in colour. After this, the standard and sample absorbance were measured with the UV spectrophotometer at 500nm. The formula below was used to calculate the concentration of cholesterol present:

Cholesterol (mg/dl) = Absorbance of sample/ Absorbance of standard × Concentration of standard (mg/dl).

2.6.1 Total Triglycerides

The total triglycerides from this experiment was obtained using the enzymatic method with wet reagent diagnostic kits. This analysis uses the method of Tietz (1995). Three (3) different test tubes, test tube A, B and C, a standard solution and a plasma sample were used. 1000μ of the reagent was pipetted into the three (3) test tubes. Test tubes A contained only the reagent

known as (Blank reagent). Test tubes B and C contained 10 μ standard solution and plasma sample. They were mixed thoroughly and allowed to stand for 10 minutes in a room to allow for colour change. With the aid of a spectrophotometer, at 500nm, the blank sample and standard absorbance rate were taken. This procedure was repeated for each of the sample left. Concentration of triglycerides of the sample was calculated using the formula below:

Total Triglycerides (mg/dl) = Absorbance of sample / Absorbance of standard \times Concentration of standard (mg/dl).

2.6.2 High-Density Lipoprotein and Low-Density Lipoprotein

The High-Density Lipoprotein (HDL) is also known as "good cholesterol". This is because it helps to transport excess cholesterol from the bloodstream and tissues back to the liver, where it can be processed and removed from the body, thereby reducing the risk of plaque buildup in arteries (Jomard *et al.*, 2020). The Low-Density Lipoprotein (LDL) is also known as "bad cholesterol". This is because it can lead to cholesterol buildup in the walls of arteries, forming plaques that narrow or block blood vessels. This increases the risk of atherosclerosis, heart attack, and stroke. The (HDL) was determined using the enzymatic method. A precipitating agent made up of 0.55 mmd / l Phosphotungstic acid, 25 mmd/l Magnesium chloride (Friedwald *et al.*, 1972), a reagent, plasma sample, a standard solution and 2 test tubes were used. 500 μ of the reagent was pipetted into two (2) separate test tubes. A standard solution was added to test tube A, while a plasma sample for 200 μ was added for test tube B. Each sample were thoroughly mixed and allowed to stand for 10 minutes in a room to notice the changes in colour. A spectrophotometer was used to measure the absorbance of the sample and the absorbance of the standard. It was measured at 500nm. To calculate the amount of HDL present, the formula below was used:

HDL (mg/dl) = Absorbance of sample / Absorbance of standard \times Concentration of standard (mg/dl).

For Low-Density Lipoprotein (LDL), $LDL \text{ (mg/dl)} = \text{Total Cholesterol} - \text{Triglycerides} / 5$.

2.7 Testosterone Evaluation

The level of testosterone in the animals' plasma was assessed using an established procedure, such as the manufacturer's manual, which was used as a guide (Gauthaman and Adaiken, 2008; Neychev and Mitev, 2016). This method depends on the viable interactions between the testosterone reference standard and the test sample. 10 μ of testosterone in different concentrations, such as 0, 0.1, 0.5, 2.0, 6.0, and 18.0 ng/ml, were apportioned. The test sample was distributed into the Goat Anti-Rabbit IgG - Coated microtitre wells. 100 μ of testosterone conjugate reagent and 50 μ of rabbit anti-testosterone reagent were added. They were mixed thoroughly for about half a minute and then incubated at 37°C for an hour and thirty minutes. The HRP-labelled testosterone competed with the inherent testosterone found in the standard and sample during incubation for the binding site on a specific testosterone antibody. In order to remove the liberated testosterone peroxide conjugate, the Goat Rabbit IgG-Coated microtitre wells were washed about five 5 times with distilled water. 100 μ of TMB reagent was added to each of the wells, and the solution was thoroughly stirred for five (5) seconds. Incubation of the solution was done immediately at room temperature for 20 minutes, so as to ensure the resulting blue colour was obtained. Afterwards, 100 μ μ L of stop solution was added to each well to stop the colour change. It later changed from Blue to Yellow. Using a microtitre well reader, the absorbance of the samples was measured within 15 minutes at 450nm. It was observed that the intensity of the colour was proportional to the amount of enzymes available and inversely proportional to the liberated testosterone in the sample.

With the aid of the calibration curve, the testosterone levels were determined. The calibration curve plot the concentration of the standard against absorbance.

The formula for Testosterone concentration (ng / ml) = $C_s \times F$

Cs = Corresponding testosterone concentration from calibration curve.

F = Dilution factor.

2.8 Data Analysis

The results obtained were evaluated using SPSS version 26. The data were presented as mean \pm SEM, and the statistical significance was calculated using a one-way ANOVA, followed by Dunnett's test, where $p < 0.05$ was considered statistically significant.

CHAPTER THREE

3.0

RESULTS

3.1 Lipid Profile

The results of the lipid profile analysis of the male Wistar rats include cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein, and testosterone concentration.

The **Tables** and **figures** below show the result of the effect of the leaf aqueous extract of *Acanthus montanus* on lipid levels, including the steroid hormone (testosterone) in Male Wistar Rats, compared to the negative control group (which was treated with Distilled water) and the positive control group (which was treated with Sildenafil citrate).

3.1.1: Effect of *Acanthus montanus* leaf aqueous extracts on Cholesterol level

The results in Table 1 show the effect of the different treatments on cholesterol levels. The two (2) doses 50 and 100 mg/kg) of the leaf extract of *Acanthus montanus* and the positive control were able to lower the cholesterol levels compared to the negative control group. The 50 mg/kg dosage of the plant extracts had the best effect in lowering the total cholesterol level of the animals.

Table 1: Effect of *Acanthus montanus* leaf aqueous extract on Cholesterol levels in Male Wistar rats.

Groups	Doses (mg/kg)	Cholesterol (mg/dl)
<i>Acanthus montanus</i>	25	140.0 ± 1.0 ^a
<i>Acanthus montanus</i>	50	130.0 ± 3.0 ^a
<i>Acanthus montanus</i>	100	136.5 ± 5.5 ^a
(Sildenafil Citrate)	100	130.0 ± 2.0 ^a
Negative Control	1ml	139.5 ± 3.5 ^a

Values are expressed as Mean ± SEM (n=3). Superscripts with the same letters are not significantly different from each other (p > 0.05). Negative control-distilled water; Sildenafil citrate- positive control.

3.1.2: Effect of *Acanthus montanus* leaf aqueous extract on triglyceride level.

The figure below illustrates the effect of *Acanthus montanus* leaf aqueous extract and the different treatments on Triglyceride levels. The effects of the aqueous extract of *A. montanus* administered at doses of 25, 50, and 100 mg/kg were evaluated. 100 mg/kg of the plant extract caused an increase in triglyceride levels, while the other doses produced a significant reduction in triglyceride levels. The negative control group revealed that the animals naturally possess a high triglyceride level. In contrast, the positive control group, which

received 100 mg/kg of Sildenafil Citrate, exhibited the lowest level of triglyceride. Among the various treatments administered using the plant extract, the 50 mg/kg dosage was the most effective in reducing triglyceride levels.

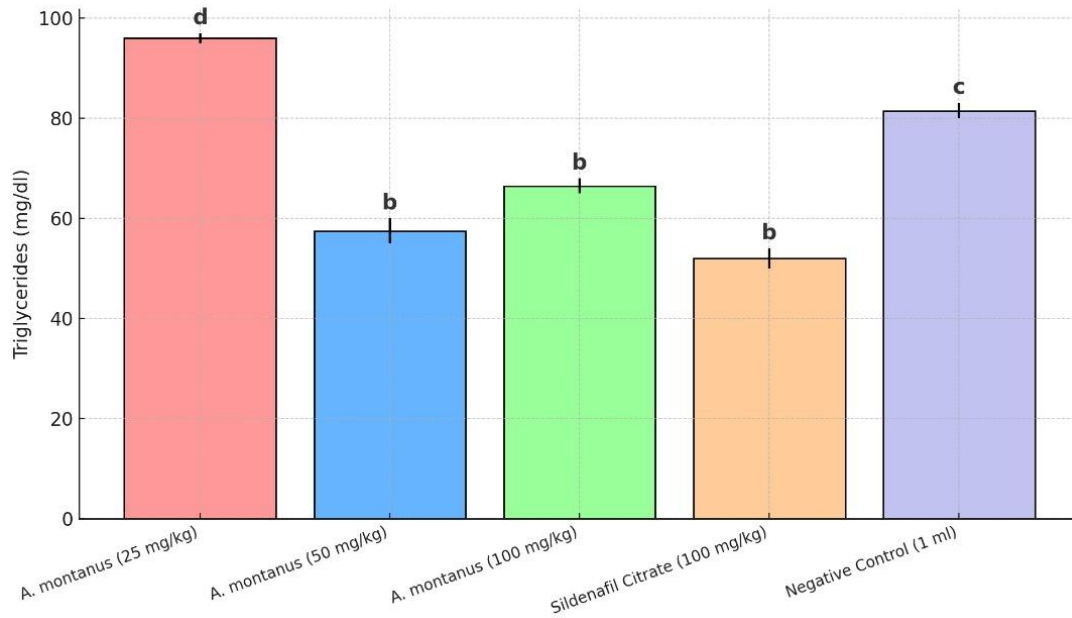


Figure 1: The effect of *Acanthus montanus* leaf extract on Triglycerides levels in Male Wistar Rats. Values are expressed as Mean \pm SEM (n=3). Values with different superscript letters differ significantly from each other ($p > 0.05$). Negative control-distilled water; Sildenafil citrate- positive control.

3.1.3: Effect of *Acanthus montanus* leaf aqueous extract on High-Density Lipoprotein level.

The results obtained from Table 2, show the level of High-Density Lipoprotein across the different groups. The negative control group exhibited the highest HDL level, in contrast to the positive control group, which recorded the lowest average HDL level. The group treated with *Acanthus montanus* leaf aqueous extract showed a slight reduction in HDL level, when compared with the negative control.

Table 2: Effect of *Acanthus montanus* leaf aqueous extracts on High-Density Lipoprotein levels in Male Wistar Rats.

Groups	Doses (mg/kg)	High-Density Lipoprotein (mg/dl)
<i>Acanthus montanus</i>	25	43.5 ± 1.5 ^a
<i>Acanthus montanus</i>	50	41.5 ± 1.5 ^a
<i>Acanthus montanus</i>	100	40.5 ± 1.5 ^a
Sildenafil Citrate	100	39.5 ± 1.5 ^a
Negative Control	1ml	44.5 ± 2.5 ^a

Values are expressed as Mean ± SEM (n=3). Superscripts with the same letters are not significantly different from each other (p > 0.05). Negative control-distilled water; Sildenafil citrate- positive control.

3.1.4: Effect of *Acanthus montanus* leaf aqueous extract on Low-Density Lipoprotein level.

The results in **Figure 2** demonstrate the effects of different treatments on the Low-Density Lipoprotein (LDL) level of the animals. The administration of the aqueous leaf extracts of *Acanthus montanus* at 50 mg/kg produced a slight reduction in LDL level. At 25 mg/kg and 100 mg/kg, the extract did not lower LDL levels as compared with the other groups. The 50 mg/kg dosage was therefore the most effective in reducing LDL. Unexpectedly, the treatment with Sildenafil Citrate resulted in a higher LDL level.

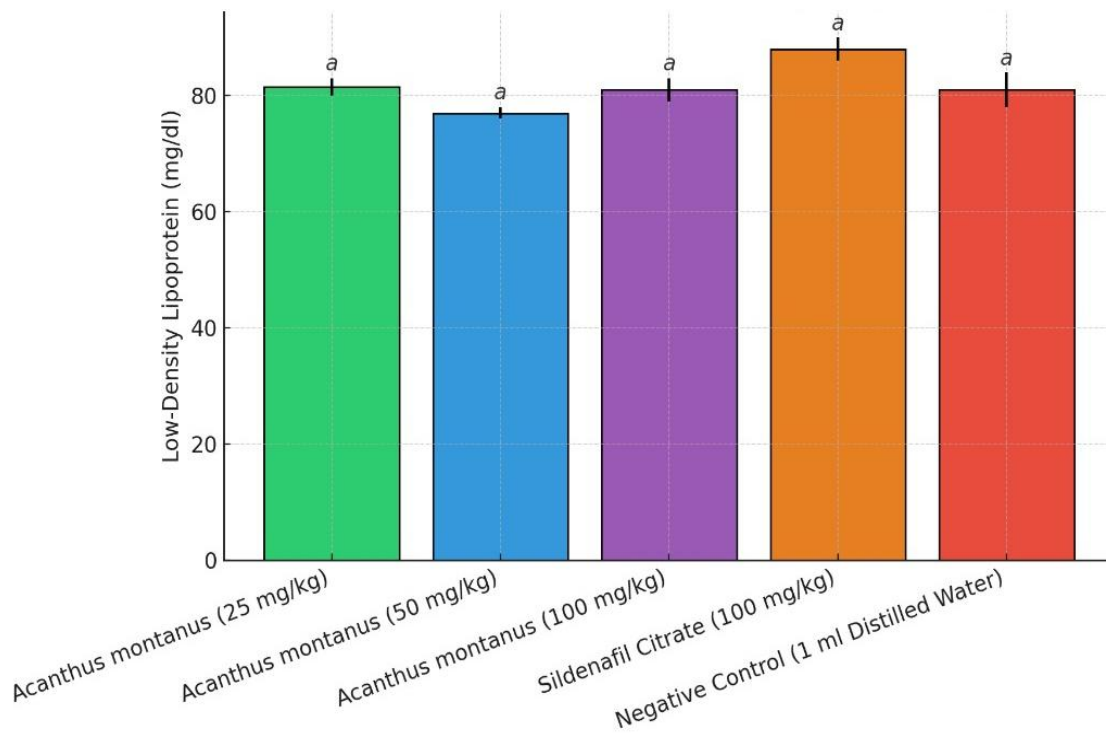


Figure 2: The effect of *Acanthus montanus* leaf extract on Low-Density Lipoprotein level in Male Wistar Rats. Values are expressed as Mean \pm SEM (n=3). Superscripts with the same letters are not significantly different from each other ($p > 0.05$). Negative control-distilled water; Sildenafil citrate- positive control

3.1.5: Effect of *Acanthus montanus* leaf aqueous extract on Testosterone level.

The results in **Table 3** show the evaluation of testosterone levels in the animals. The values remained stable with slight variations across all the groups, indicating that the aqueous leaf extract of *Acanthus montanus* does not affect testosterone production but helps maintain it within a normal range. Among the treatment doses, 50 mg/kg produced the highest testosterone level when compared with the other aqueous extracts. The 25 mg/kg and 100 mg/kg doses reduced the testosterone level below the control values.

Table 3: Effect of *Acanthus montanus* leaf aqueous extracts on Testosterone levels in Male Wistar Rats.

Groups	Doses (mg/kg)	Testosterone (mg/ml)
<i>Acanthus montanus</i>	25	3.25 ± 0.15 ^a
<i>Acanthus montanus</i>	50	3.55 ± 0.15 ^a
<i>Acanthus montanus</i>	100	3.15 ± 0.15 ^a
Sildenafil Citrate	100	3.70 ± 3.70 ^a
Negative Control	1ml	3.50 ± 0.20 ^a

Values are expressed as Mean ± SEM (n=3). Superscripts with the same letters are not significantly different from each other (p > 0.05). Negative control-distilled water; Sildenafil citrate- positive control

CHAPTER FOUR

4.0

DISCUSSION

The *Acanthus montanus* plant possesses several therapeutic properties, and it is known to be effective in treating different ailments such as coughs, asthma, diabetes, urogenital disorders, hyperlipidemia (Ojaiko *et al.*, 2013; Onuoha *et al.*, 2018), wound infections, microbial and fungal infections, and dyslipidemia (Omeh *et al.*, 2012; Ojaiko *et al.*, 2015; Jellinger *et al.*, 2017; Nwafor *et al.*, 2022). This plant's roots, leaves, stems and seeds are widely used in traditional medicine across regions like Nigeria, Cameroon and other parts of West Africa (Okoli *et al.*, 2008).

The study investigated the effects of the plant extract on serum lipid parameters—total cholesterol, triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and testosterone in rats at varying doses (25, 50, and 100 mg/kg), alongside positive and negative controls. Evaluation of total cholesterol levels revealed no statistically significant differences among the treatment groups (Table 1). The 25 mg/kg dose and the negative control (distilled water) had similar effects on cholesterol levels (140.0 and 139.5 mg/dl) while the 50 mg/kg dose and the positive control displayed relatively close effects (130.0 and 130.0 mg/dl). The 100 mg/kg dose produced intermediate cholesterol levels. This outcome indicates that the extract does not markedly alter cholesterol metabolism under the conditions tested. Comparable findings have been reported in studies where certain plant extracts or dietary fibres influenced triglycerides more strongly than total cholesterol (Öztürk *et al.*, 2021). Triglyceride (TG) levels showed significant variation between groups (Figure 2). The 25 mg/kg dose caused a slight elevation above normal ranges, while 50 mg/kg and 100 mg/kg of the extract significantly reduced TG levels compared to controls. Results revealed that animals in the negative control group naturally maintained high triglyceride levels, whereas the positive control group receiving 100 mg/kg of Sildenafil Citrate showed a

significant decrease, presenting the lowest triglyceride levels. Elevated TG is a well-established risk factor for fatty liver disease, pancreatitis, metabolic syndrome, type 2 diabetes, cardiovascular disease and atherosclerosis (Andallu *et al.*, 2009; Hirano, 2013). The reduction in TG observed in this study suggests that the plant extract may have a protective effect against hypertriglyceridemia, consistent with reports of hypolipidemic effects from other plant-based interventions in rodent models (Öztürk *et al.*, 2021; Albandari *et al.*, 2023). HDL (“good cholesterol”) and LDL (“bad cholesterol”) levels did not differ significantly across groups (Tables 2 and Figure 2). A slight decrease was observed in both parameters, but the changes were neither statistically significant nor detrimental to lipid balance. Maintenance of HDL is desirable due to its role in reverse cholesterol transport, while stable LDL levels reduce the risk of pro-atherogenic effects. Similar neutral outcomes on lipoproteins have been observed in other studies where triglycerides were reduced without marked alterations in HDL or LDL (Öztürk *et al.*, 2021). No significant differences in testosterone levels were observed between treatment and control groups (Table 3). This suggests the plant extract does not interfere with testicular steroidogenesis at the administered doses. Since cholesterol and lipoprotein carriers serve as substrates for testosterone biosynthesis in Leydig cells (Travert *et al.*, 2000), stability in LDL and HDL levels may account for the absence of changes in testosterone levels. Moreover, modest reductions in TG and LDL support, rather than impair, steroid hormone production (Triash *et al.*, 2009).

CONCLUSION

This analysis indicates that the *Acanthus montanus* leaf aqueous extract exerts a selective effect on serum lipids, significantly reducing triglycerides at medium and high doses, without adversely altering total cholesterol, HDL, LDL, or testosterone levels. This profile is favourable, as it suggests potential use of the extract as a therapeutic or preventive agent for

hypertriglyceridemia while preserving lipoprotein balance and reproductive hormone homeostasis.

REFERENCES

- Adodo, A. (2010). Medicinal Plants of Nigeria. *An Ethnobotanical Survey and Plant Album*. Pp. 10–12.
- Akobumdu, I. O. and Agyakwa, C. W. (1987). A Handbook of West African Weeds. *Nigeria International Institute of Tropical Agriculture*. Pp. 126–127.
- Albandari, A. A., Waheeba E. A., Raya, A., Raghad, M. A., Mona, S. A., Sami, A. A., Ard, E. M. E. and Hassan M. M. (2023). Hypolipidemic, Hypoglycemia and Ameliorative Effects of Boiled Parsley (*Petroselinum crispum*) and Mallow (*Corchorus olitorius*) Leaf Extract in High-Fat Diet Rats. *Foods*, **12(23)**: 4074.
- Amanda, C. (2023). The Impact of Deforestation on Medicinal Plant Species in Africa. *Africa Up Close/Wilson Centre*. Pp 1–5.
- Amnei, M., Josè, L. M., and Mahendra, R. (2019). Ethnobotany: Application of Medicinal Plants. *United Kingdom: CRC Press/Taylor and Francis Group*.
- Andallu, B., Suryakantham, V., Lakshmi B. V. and Reddy, G. K. (2009). Effect of mulberry (*Morus indica* L.) therapy on plasma and erythrocyte lipids in patients with type 2 diabetes. *Clinica Chimica Acta*, **314(1)**, 47–53.
- Anonymous (2007). *Acanthus montanus*. Shinjuku Gyoen National Garden. [Available at:<http://tropicalflowers.la.coocan.jp/Acanthaceae/Acanthus%20montanus/Acanthus%20montanus.htm>] [Accessed: 11/10/25].
- Anthony, S. (2023). Medicinal Plant Conservation: Preserving Nature's Healing Treasures. *Medicinal and Aromatic Plants*. **12**: 457.
- Asongalem, E. A., Foyet, H. S., Ekobo, S., Dimo, T., Kamtchouing, P. (2004). Anti-inflammatory, analgesic and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. *Journal of Ethnopharmacology*. **95(1)**: 63–68.

- Bhat, J.A., Kumar, M., Negi, A.K., (2021). Impact of Climate Change on Medicinal Plants; A Review. *Environmental Challenges*, **3**, 1000.
- Ugwu, M. C., Ezea, C. J., Akpa, P. A. and Obiekwe, J. O., (2016). Effect of *Acanthus montanus* leaf extract on uterine contractions. *Journal of Chemical Society of Nigeria*, **41**(2): 1-10.
- El-Mehi, A. E. and Faried, H. R. (2010). Protective role of curcumin on streptozotocin-induced diabetes in Wistar rats. *International Journal of Diabetes in Developing Countries*, **30**(4), 167–173.
- Friedwald, W. T., Levy, R. I. and Fredrickson, D. S. (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, without the Use of the Preparative Ultracentrifuge. *Clinical Chemistry*, **18**(6), 499 – 502.
- Gauthaman, K. and Adaikan, P. G. (2008). The Hormonal Effects of *Tribulus terrestris* and its Role in the Management of Erectile Dysfunction – An Evaluation using Primates; Rabbits and Rats. *Phytomedicine*, **15**(1 – 2): 44 – 54.
- Giday, M., Asfaw, Z., Elmyvist, T. and Woldu, Z. (2009). An Ethnobotanical Study of Medicinal Plants used by the Zay People in Ethiopia. *Journal of Ethno pharmacology*, **85** (1): 43-52.
- Hirano, T. (2013). Pathophysiology of diabetic dyslipidemia. *Journal of Atherosclerosis and Thrombosis*, **25**(9), 771–782.
- Iwu, M. M. (2014) Handbook of African Medicinal Plants. *United States: CRC Press, Taylor and Francis Group*. Pp 8.
- Jangpangi, D., Patni, B., Chandola, V. and Chandra, S. (2025). Medicinal Plants in a Changing Climate: Understanding the links between environmental Stress and Secondary Metabolite Synthesis. *Frontiers In Plant Science*, **16**, 1587337.

- Jellinger, P. S., Handelsman, Y., Rosenblit, P. D., Bloomgarden, Z. T., Fonseca, V. A., Garber, A. J., Grunberger, G., Guerin, C. K., Bell, D. S. H., Mechanick, J. I., Pessah-Pollack, R., Wyne, K., Smith, D., Brinton, E. A., Fazio, S. and Davidson, M. (2017). American Association of Clinical Endocrinologist and American College of Endocrinology Guidelines for Management of Dyslipidemia And Prevention of Cardiovascular Disease. *Endocrine Practice : Official Journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists*, **23**(2): 1–87.
- Jomard, A. and Osto, E. (2020). High Density Lipoproteins: Metabolism, Function, and Therapeutic Potential. *Frontiers in cardiovascular medicine*.
- Mac Donald, I., Paul, Ojoba. and Benjamin, G. (2022). Acanthus montanus (News) T. Anderson: Review. *Asian Journal of Pharmacognosy*.
- National Research Council (US) Committee for the Update of the Guidelines for the Care and Use of Laboratory Animals. (2011). *Guide for the care and use of laboratory animals* (8th ed.). National Academies Press.
- Nechyev, V. and Mitev, V. I. (2005). The Aphrodisiac Herb *Tribulus terrestris* does not Influence the Androgen Production in Young Men. *Journal of Ethnopharmacology*, **101**: 319 – 323.
- Ojiako, A. O., Chikezie P. C. and Zedech, U. C. (2013). Serum Lipid Profile of Hyperlipidemic Rabbits (*Lepus townsendii*) Treated with Leaf Extracts of *Hibiscus rosasinensis*, *Emilia coccinea*, *Acanthus montanus* and *Asystasia gangetica*. *Asian Pacific Journal of Tropical Biomedicine*, **4**(2), 113–120.
- Ojaiko, O. A., Chikezie, P. C. and Ogbuji, A. C. (2015). Blood glucose level and lipid profile of alloxan-induced hyperglycemic rats treated with single and combinatorial herbal formulations. *Journal of traditional and complementary medicine*, **6**(2), 184–192.

- Okoli, C. O., Akah, P. A., Onuoha, N. J., Okoye, T. C., Nwoye, A. C. and Nworu, C. S. (2008). *Acanthus montanus*: an experimental evaluation of the antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles. *BMC complementary and alternative medicine*, **8**, 27.
- Olajide, O. A., Makinde, J. M. and Adeyemi, O. O. (2003). Anti-inflammatory properties of *Acanthus montanus* leaf extract. *West African Journal of Pharmacology and Drug Research*, **(2)**1, 45-54.
- Olajide, O. A., Adegoroye, O. A. and Adewunmi, C. O. (2004). Analgesic and antipyretic effects of *Acanthus montanus* leaf extract. *Physiotherapy Research*, **18**(12), 955-958.
- Onuoha, O. U., Osuocha, K. U. and Chukwu, E. C. (2018). Phytochemical Profiling, Hypolipidemic, Haematological and Body Weight Effects of *Acanthus Montanus* Leaf Extracts in Male and Female Albino Rats. *European Journal of Experimental Biology*, **8**(1), 1–8.
- Öztürk, M., et al. (2021). Hematological and biochemical reference intervals in Wistar rats. *Eurasian Journal of Veterinary Sciences*, **37**(4), 267–273.
- Pandeam, S. and Sukarya, P. T., (2013). 3500 Plant Species of the Botanic Garden of Indonesia. Publisher: *PT. Sukarya and Sukarya Pandetama*.
- Tietz, N. W. (1995). Clinical Guide to Laboratory Tests. *W.B. Saunders Company, Philadelphia*, (3rd ed.), pp. 518 – 519.
- Tilford, G. L. (1997). Edible and Medicinal Plants of the West. *United States: Mountain Press Publishers*.
- Travert, C., Fofana, M., Carreau, S. and Le Goff, D. (2000). Serum lipoproteins increase testosterone production in hCG-desensitized Leydig cells. *Molecular and Cellular Biochemistry*, **202**(1-2), 93–100.

- Triash, K., (2009). Relationship between lipid metabolism and testosterone biosynthesis. *Endocrine Journal*, **56**(5), 657–663.
- Trinder, P. (1969). Determination of Glucose in Blood Using Glucose Oxidase with Alternative Oxygen Receptor. *Annals of Clinical Biochemistry*, **6**: 24 – 27.
- WHO (2019). WHO Global Report on Traditional and Complementary Medicine. *World Health Organization*.
- Wickers, G. E and Lowe, P. (2008). The Baobab's; Pachycauls of Africa, Madagascar and Australia. *Springer, Berlin*.