EVALUATION OF THE BIOCHEMICAL EFFECTS OF *Terminalia mantaly* H. PERRIER (COMBRETACEAE) LEAF EXTRACT

BY

DANIEL TEGA EDUWA

(PHA1908479)

DEPARTMENT OF PHARMACOGNOSY

FACULTY OF PHARMACY

UNIVERSITY OF BENIN

BENIN CITY

EDO STATE

NOVEMBER, 2025

EVALUATION OF THE BIOCHEMICAL EFFECTS OF *Terminalia mantaly* H. PERRIER (COMBRETACEAE) LEAF EXTRACT

BY

DANIEL TEGA EDUWA

(PHA1908479)

DEPARTMENT OF PHARMACOGNOSY

FACULTY OF PHARMACY

UNIVERSITY OF BENIN

BENIN CITY

EDO STATE

NOVEMBER, 2025

EVALUATION OF THE BIOCHEMICAL EFFECTS OF THE METHANOL LEAF EXTRACT OF *Terminalia mantaly* H. PERRIER (COMBRETACEAE)

BY

DANIEL TEGA EDUWA

(PHA1908479)

A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHARMACOGNOSY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

DEPARTMENT OF PHARMACOGNOSY

FACULTY OF PHARMACY

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

CERTIFICATION

This is to certify that this research project was carried out by DANIEL TEGA EDUWA, with matriculation number, PHA1908479, in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin city, Edo State, Nigeria.

_____ _____

Dr. Osamuyi H. Uwumarongie Date

(Project Supervisor)

_____ _____

Dr. Osamuyi H. Uwumarongie Date

(Head Of Department, Pharmacognosy)

DEDICATION

This work is dedicated to the Almighty God, who has brought me thus far throughout my years of Academic pursuit, He alone deserves all adoration.

ACKNOWLEDGEMENT

I want to appreciate Almighty God for his keeping and protecting me even up to this final phase of my academic study in this prestigious school and for providing me with the resources and finances I needed at each point in time during the course of this research project. May his name alone be praised.

My sincere gratitude also goes to my Project Supervisor Dr. Osamuyi H. Uwumarongie for his words of advice and close guidance throughout this research project, God bless you immensely Sir.

I also want to appreciate Dr. (Mrs) L.A. Emokpae of Medical Laboratory Science, School of basic medical sciences, University of Benin, for her support and contribution to the success of this project.

My sincere appreciation also goes to my Awesome parents Rev. and Engr. Phillip Eduwa for their love, financial support and motivation.

To my fellow project colleagues, I appreciate you all for patiently working with me, motivating me and contributing to the success of this project.

I also want to appreciate the staff of the Department of Pharmacognosy, including both Academic and Non-academic staff for their assistance all throughout this project period.

And lastly, in general, I would also like to express my gratitude to everyone who has contributed to the success of this project one way or another, including those who guided, supported and encouraged me. I thank you all from the bottom of my heart.

TABLE OF CONTENTS

LIST OF TABLES

TABLES PAGE

LIST OF FIGURES

FIGURES PAGE

ABSTRACT

The increasing global reliance on medicinal plants for therapeutic purposes especially in developing countries has intensified the need for scientific validation of their biochemical activities and safety. *Terminalia mantaly* H. Perrier, a member of the Combretaceae family is traditionally used in various African communities for the treatment of wounds, gastrointestinal issues, and inflammatory conditions. This study investigated the effects of the methanol leaf extract of *T. mantaly* on various biochemical parameters of female *Wistar* rats, when administered for 28 days.

Fresh leaves of *T. mantaly* were harvested, authenticated, shade-dried and then oven-dried, before milling into powdered form using an electric miller. The resulting powdered leaf was subjected to methanol (100%) extraction with the aid of a Soxhlet apparatus, concentrated with a rotary evaporator, and reduced to dryness on a thermostatically controlled water bath (65℃). The extract was administered orally to four groups of female *Wistar* rats containing 5 rats each, at concentrations of 200, 400 and 800 mg/kg respectively, for 28 days. The liver, renal and lipid parameters were assayed using standard methods.

The results obtained showed that only aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly reduced ($p < 0.01, 0.05$) when compared with the control, for the liver function parameters. All the renal function parameters assayed were not significantly different from the control; while low-density lipoprotein (LDL) was found to be significantly different ($p < 0.01$) from the control, for the assayed lipid profile. LDL was significantly elevated with increased dose while high-density lipoprotein (HDL) showed reduction which was not significantly different from the control.

In conclusion, the extract could be said to be relatively safe at the low dose 200mg/kg. Caution is advised when the extract is used at high doses and for longer periods, as elevated levels of

LDL can increase the risk of developing atherosclerosis, cardiovascular diseases and coronary artery disease.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study:

Medicinal plants have long served as a cornerstone of traditional healthcare systems, particularly in developing countries where access to modern pharmaceuticals is often limited.

In modern medical practice, plants are at the centre of the preparation of some important drugs, for the treatment of many diseases (Khan *et al*., 2020). Approximately 80% of the global population relies on plant-based remedies for primary healthcare needs, underscoring the importance of validating their therapeutic claims through scientific research (World Health Organization, 2002). Plants synthesize a variety of metabolites which may be beneficial or potentially toxic to mankind (Chaachouay and Zidane, 2024). To ensure safety, there must be studies to show the safety profiles of herbs claimed to be beneficial to humans and animals before deciding to use them. (Moreira *et al*., 2018). One of such plants that is beneficial to humans and animals is *Terminalia mantaly.*

Terminalia mantaly H. Perrier, a member of the Combretaceae family, is a tropical tree widely distributed across West and Central Africa. Traditionally, various parts of the plant, especially its leaves, have been used in ethnomedicine to treat wound infections, gastrointestinal disturbances, and inflammatory conditions (Das *et al*., 2020). Despite its widespread use, empirical data supporting its pharmacological properties and toxicological safety remain limited, necessitating further investigation. Of the different solvents available for the extraction of plant materials for use in the treatment of varying ailments, methanol extraction is a widely accepted method for extracting bioactive compounds from plant materials, due to its efficiency in dissolving both polar and non-polar constituents. Studies on *Terminalia species* including *T. mantaly* have revealed the presence of secondary metabolites such as flavonoids, tannins,

alkaloids, saponins, and phenolic compounds known for their antioxidant, antimicrobial, and anti-inflammatory activities. (Yunusa *et al*., 2024). However, specific studies on *T. mantaly* are relatively scarce, particularly those involving controlled animal models. Hence this study was designed to evaluate the safety potentials of *T. mantaly.* By analyzing key physiological parameters such as liver function, renal function, and lipid profile, this research seeks to provide scientific insight into the plant's therapeutic potential and safety profile. The findings will contribute to the growing body of knowledge on medicinal plants and may support the development of novel phytotherapeutic agents using *T. mantaly*.

1.2 Taxonomy of *T. mantaly*:

The Taxonomy of *T. mantaly* is shown below (The Useful Tropical Plants database, n.d.)

Kingdom: Plantae

Sub kingdom: Angiosperms

Division: Magnoliophyta

Class: Magnoliopsida

Order: Myrtales

Family: Combretaceae

Genus: *Terminalia*

Species: *mantaly*

Botanical name: *Terminalia mantaly* H. Perrier. (Combretaceae)

1.3 Description of *T. mantaly*

Terminalia mantaly is a medium to large deciduous or semi-evergreen tree commonly known as the brown ivory tree, Umbrella tree or Madagascar almond; often reaching 10 – 30 meters in height and with a characteristic layered, horizontal branches forming a flat spreading crown. Its trunk is usually straight, with a diameter that can expand up to 1 meter. Its leaves are simple, with a shiny dark green surface that measures about 8 to 16 centimetres in length. The flowers are small, inconspicuous, fragrant, yellowish-white and appear in clusters at the leaf axils. *T. mantaly* produces small, brown, ellipsoid, woody fruits resembling almonds, surrounded by a hard shell and falls to the ground when mature hence its name Madagascar almond. The bark is fibrous and has been used locally for dyes and tanning (The Useful Tropical Plants database; Joujeh and Joujeh, 2023).

T. mantaly is indigenous to Africa, particularly prevalent in countries like Tanzania, Nigeria, Madagascar, Zambia, and Zimbabwe; and can be cultivated in a well-drained soil, partial shade and under tropical conditions. The common habitats in which *T. mantaly* could be found include Rainforests, woodland savannas, riverine areas (African plant database, 2020).

T. mantaly flourishes in tropical climates, requiring warm temperatures and ample sunlight. It prefers well drained soils ranging from sandy to loamy, with a slightly acidic to neutral pH. The tree is also notable for its tolerance to drought, making it an excellent specimen for arid tropical regions (Botanical realm, 2024).

T. mantaly exhibits a steady growth rate, typically reaching maturity within 10 to 15 years. During the flowering season which generally occurs in the late spring to early summer, pollination primarily occurs through insects. The formation of seeds happens soon after flowers bloom, with woody capsules developing that eventually disperse the seeds across the landscape, facilitating natural regeneration (Botanical realm, 2024).

Figure 1: *T. mantaly* tree growing in its cultivated habitat at The Faculty of Pharmacy, University of Benin, Benin City, Edo state.

1.4 Traditional uses of *T. mantaly*

In West and Central African ethnomedicine, leaves, bark and other parts of *T. mantaly* are used for wound healing and as antiseptic through poultices made from crushed leaves or bark which are then applied to wounds to promote healing and prevent infection. *T. mantaly* is also used in the treatment of gastrointestinal complaints such as diarrhoea, dysentery, and stomach discomfort, the bark's astringent properties help reduce intestinal inflammation and fluid loss (Das *et al*., 2020). The leaves of *T. mantaly* are also applied topically to help in the relief of inflammatory conditions and as an analgesic to relieve pain. (Joujeh and Joujeh, 2023). Ethnobotanical surveys and local medicinal plant compendia document these uses and the plant's place in community healthcare practices (Joujeh and Joujeh, 2023; The Useful Tropical Plants database).

1.5 Medical uses of *T. mantaly*

T. mantaly have the following class of compounds: flavonoids, tannins, terpenoids and phenolics that are associated with antioxidant, antimicrobial and anti-inflammatory effects; genus-level reviews and species-specific reports note similar constituents in *T. mantaly* and related taxa used for preclinical evaluations (Das, Shin, and Lee, 2020; Joujeh and Joujeh, 2023).

Antioxidants are vital in protecting the body from oxidative stress, a factor associated with numerous chronic illnesses, including cancer, cardiovascular diseases, and neurodegenerative disorders (Lobo *et al*., 2010). The antioxidant activity of *T. mantaly* is likely due to its flavonoid and phenolic content, which can neutralize free radicals and mitigate oxidative damage to cells and tissues. Additionally, *T. mantaly* has demonstrated antibacterial properties against various pathogens. These effects may be attributed to the presence of compounds like tannins, saponins, and alkaloids, known for their antimicrobial properties (Jiofack *et al*., 2009). The

antibacterial properties of *T. mantaly* could be beneficial in treating bacterial infections. Given its phytochemical composition, *T. mantaly* leaf extract exhibits significant antioxidant and antibacterial activities, highlighting its potential for therapeutic applications. Further research is necessary to isolate and identify the specific compounds responsible for these effects and to assess their efficacy and safety for medical use.

1.6 Other uses of *T. mantaly*

Beyond medicinal applications, *T. mantaly* is widely planted as an ornamental and shade tree in urban landscaping, used in reforestation and agroforestry projects, and locally exploited for tannins, dyes, fuelwood and small-scale craft materials (The Useful Tropical Plants database; Joujeh and Joujeh, 2023).

The ecological importance of *T. mantaly* goes beyond its visual appeal. This tree also plays a crucial role in its native habitat by stabilizing the soil with its extensive root system. It helps prevent erosion, particularly in areas prone to landslides and heavy rainfall. Also, its flowers provide a rich nectar source for bees and other pollinators, thereby enhancing local biodiversity. (Botanical realm, 2024).

1.7 Scientific investigations of *T. mantaly*

Phytochemical and pharmacological studies of *Terminalia species* report classes of bioactive compounds such as flavonoids, tannins, terpenoids and phenolics. (Joujeh and Joujeh, 2023).

Several studies have focused on the methanol and ethanol extracts of *T. mantaly* leaves, revealing significant antioxidant, antimicrobial, and phytochemical properties. For instance, (Yunusa *et al*., 2024) conducted a phytochemical screening and antioxidant assay of the methanol leaf extract identifying various bioactive compounds, including alcohols, phenols, carboxylic acids, aldehydes, ketones, and amines through FTIR analysis of the extract. The

extract demonstrated notable antibacterial effects against bacterial strains such as *Salmonella typhi*, *S. aureus* and *E. coli* supporting its traditional use in treating infections.

In another study, (Eze *et al*., 2021) evaluated the ethanol leaf extract for antimicrobial activity and found that it inhibited the growth of several pathogenic bacteria and fungi, including *Candida albicans*, further validating its ethnomedicinal applications.

Similarly, (Okpara and Akporhuarhoo, 2018) compared the nutritive and anti-nutritional profiles of fresh, air-dried, and sun-dried leaves of *T. mantaly*, confirming the presence of bioactive compounds and suggesting its potential as a dietary supplement in rural communities.

Research on the stem and bark of *T. mantaly* has focused on essential oil composition and broader pharmacological profiling. (Joujeh and Joujeh, 2023) reported the presence of volatile compounds such as caryophyllene, nerolidol, humulene, and ionones in the essential oils extracted from the bark and stem. These compounds are known for their anti-inflammatory, antimicrobial, and analgesic properties.

A broader review by (Das *et al*., 2020) on the genus *Terminalia* highlighted the therapeutic relevance of bark extracts, noting their use in wound healing, gastrointestinal disorders, and oxidative stress-related conditions. Although species-specific data on *T. mantaly* bark is limited, its chemical similarity to other *Terminalia species* suggests comparable pharmacological potential.

Also, research by (Mariscal *et al*., 2020) on the In Vivo Anti-plasmodial Activity of the stem of *Terminalia mantaly* provide evidence that the aqueous extract from the stem bark of *T. mantaly* is relatively safe and highly potent on P.berghei-infected mice and thereby validate the use of *T. mantaly* in folk medicine to treat malaria and related symptoms.

1.8 Significance of the study

Evaluating the biochemical effects and safety of the methanol leaf extract of *T. mantaly* provides empirical data to support or qualify traditional claims, informs potential phytopharmaceutical development, and addresses public health concerns about safety and standardization of herbal remedies (Builders, 2019; Jitareanu *et al*., 2023).

1.9 Scope and limitations

The study focuses on biochemical assessment of liver, renal and lipid parameters in female *Wistar* rats following 28 days of oral administration of methanol leaf extract of *T. mantaly* and includes subacute safety context. Limitations include species differences that restrict direct extrapolation to humans, phytochemical variability from geographical and seasonal factors, and limited prior species-specific toxicological data for direct comparison (Jitareanu *et al*., 2023; Builders, 2019).

1.10 Benefits of herbal medicines

Herbal medicines increase healthcare access where conventional drugs are scarce or costly, provide culturally accepted therapeutic options and serve as sources of novel bioactive compounds; documented benefits include antioxidant, antimicrobial and anti-inflammatory activities attributable to plant secondary metabolites (Das *et al*., 2020; Builders, 2019).

it is critically important to understand the ethnopharmacological uses of various medicinal plants, as it provides reliable information on the evaluation of natural products existing in those medicinal plants (Buenz *et al*., 2018). Although the development of modern medicines is quickly growing, there is still a large amount of population preferring herbal medicines than the conventional system of medicines due to their effectiveness, lack of medical alternatives, enhancing cost of modern medicines, and cultural preferences (Heinrich, 2000; Tabuti *et al*., 2003; Amalraj and Gopi, 2017). Based on the data from WHO, about 80% of the global

population depends on traditional medicine, and 60% of the Indian population in rural areas use herbal medicines (Amalraj and Gopi., 2017). These natural medicines are generally easy to access, safe, cost-effective, and efficient (Amalraj and Gopi, 2017). Except for the medical values, various plants are also widely used as food (Konczak *et al*., 2014), health care products (Kim and Song, 2013), veterinary medicine (Upadhyay *et al*., 2011), possessing extensive impacts on daily life.

1.11 Safety and Toxicity of Herbal medicines

Natural origin does not guarantee safety; herbal products may pose risks from intrinsic phytotoxins, contamination, adulteration, lack of standardization and herb–drug interactions. Systematic toxicological evaluation using acute, subacute, subchronic and chronic study designs is essential to establish safety margins and identify target-organ effects (Jitareanu *et al*., 2023; Builders, 2019).

1.11.1 Science of Toxicity

Toxicology examines dose-response relationships, exposure routes, target-organ identification and mechanistic endpoints to determine risk and safe exposure levels for bioactive substances (Jitareanu *et al*., 2023).

Toxicity is the quality of being toxic or poisonous, it indicates the state of adverse effects caused by the interaction between toxicants and cells. The toxic effects may take place prior to the binding of the toxicants to the vital organs such as liver and kidneys. Hence evaluating the toxic properties of a substance is crucial when considering the substance for public health consumption. Some herbal products can produce toxicity risk, including hepatotoxicity which can lead to liver damage. Others can be nephrotoxic leading to severe kidney damage. Some have also been linked to carcinogenicity (IARC, 2019). Other Herbal medicines like St. John's Wort can be neurotoxic, causing neurological adverse effects. (NCCIH,2020).

Liver and kidneys are the primary organs affected by metabolic reactions of toxicants (Dybing *et al*., 2002; Saad *et al*., 2006) and are useful in predicting toxicity effects of phyto-therapeutic products or drugs (Bello *et al*., 2016). The liver is the main target for toxic compounds because of its prior exposure to foreign substances absorbed in the intestine before reaching into the blood circulation (first pass effect), (Samuel *et al*., 2012). Although toxins may harm the liver, the liver helps detoxifies toxins (Ravikumar *et al*., 2012). Thus, in experimental animals, liver function tests would allow to understand the toxic effects, which can be extrapolated for safety, if used in humans. Toxic effects are produced when drugs or plant extracts are administered acutely, sub-acutely, sub-chronically or chronically. First, the toxicant is delivered to its target, interacts with endogenous target molecules or alters the environment, triggering perturbations in cell function and/or structure, which initiate repair mechanisms at the molecular, cellular and/or tissue levels. (Berhan, 2025)

Evaluation of toxicity in clinical context typically includes acute, sub-acute, sub-chronic, chronic, carcinogenic and reproductive effects. This study was designed to investigate the possible toxicological effects of *T. mantaly* extract on various biochemical parameters of female *Wistar* rats.

1.11.2 Factors affecting toxicity

Factors which affect toxicity includes the following:

1. Dosage and Concentration:

The amount of a substance administered is one of the most critical determinants of toxicity. Even beneficial compounds can become harmful at high doses. As Paracelsus famously stated, “The dose makes the poison.” Herbal extracts may contain potent bioactive compounds that, in excess, can overwhelm metabolic pathways or cause organ damage (Mensah *et al*., 2019).

2. Duration and Frequency of Exposure:

Repeated or prolonged exposure to herbal compounds can lead to accumulation in tissues, potentially resulting in subacute or chronic toxicity. For example, long-term use of certain alkaloid-containing plants has been linked to hepatotoxicity and nephrotoxicity (Jităreanu *et al*., 2023).

3. Route of Administration:

The method by which a substance enters the body i.e. oral, topical, intravenous routes etc. affects its absorption and distribution. Oral administration, common with herbal remedies, involves first-pass metabolism in the liver, which can either detoxify or activate compounds (Mensah *et al*., 2019).

4. Individual Variability:

Genetic makeup, age, sex, nutritional status, and underlying health conditions all influence how an individual responds to a substance. For instance, children and the elderly may be more susceptible to toxic effects due to immature or weakened metabolic systems (Springer Nature, 2023).

5. Interactions with Other Substances:

Herbal medicines can interact with conventional drugs, leading to enhanced toxicity or reduced efficacy. For example, St. John's Wort is known to interfere with cytochrome P450 enzymes, altering the metabolism of many pharmaceuticals (Jităreanu *et al*., 2023).

6. Quality and Purity of Herbal Products:

Contamination with heavy metals, pesticides, or adulterants can significantly increase toxicity. Poor harvesting, processing, or storage practices may also degrade active compounds or introduce harmful substances (Mensah *et al*., 2019).

7. Phytochemical Composition:

The specific chemical constituents of a plant determine its pharmacological and toxicological profile. Plants rich in alkaloids, glycosides, or saponins may have narrow therapeutic windows and require careful dosing (Jităreanu *et al*., 2023).

1.11.3 Acute Toxicity of Medicinal plants

Acute toxicity studies (single-dose assays following OECD guidelines) establish approximate lethal doses (LD_{50}) and immediate adverse effects that follows exposure to high dose of a substance in a short period. It informs dose selection for repeated-dose studies and initial safety characterization (Jitareanu *et al*., 2023).

These tests provide a screening method for toxicity evaluation particularly for new unclassified substances. The LD_{50} (lethal dose 50% i.e. dose that is lethal to 50% of test subjects) is the quantifiable indicator of the capacity of an agent to cause harm. Other decisive factors such as therapeutic dose and therapeutic index of test compounds can be derived from these tests (Akhila *et al*., 2007). The Information obtained from acute toxicity studies provide a basis for classification and hazard assessment of new chemical compounds thus providing the information used for cautionary statements on labels of potentially poisonous chemical substances (OECD, 2001; Damalas and Koutroubas, 2016).

The rapid increase in reliance in herbal medicine because of its use as anxiolytics, laxatives, pain relief, weight loss etc, leads to indiscriminate consumption with the potential to cause adverse effects, as the quantity consumed is largely unrestricted.

Acute intake especially at high dose, may be characterized by adverse effects (Ekor, 2014). Acute and sub-acute toxicity tests are routinely performed for the investigation of natural products or drugs. Acute toxicity test is the first step to determine the adverse effects of substances within 14 days of administration of single dose (Rhiouani *et al*., 2008). It is usually

administered via oral routes to determine the median lethal dose (LD_{50}) for a particular toxic substance in testing animals (usually rats or mice) (Gandhare *et al*., 2011).

1.11.4 Subacute Toxicity of Medicinal plants

Subacute studies (typically 14–28 days) evaluate effects of repeated exposure on clinical signs, body weight, food intake, haematology, biochemical processes and organ histology to detect early and potentially reversible toxicities (Jitareanu *et al*., 2023; Builders, 2019).

1.11.5 Subchronic and Chronic Toxicity of Medicinal plants

Subchronic toxicity refers to the evaluation of adverse effects arising from repeated exposure to a substance over a moderate period (around 60 days) while chronic toxicity refers to adverse health effects that arise from prolonged or repeated exposure to a substance over an extended period (≥3 months) These studies assess cumulative, delayed and progressive toxicities, as well as carcinogenic potential and reproductive/developmental toxicity where relevant for long-term use (Jitareanu *et al*., 2023).

1.11.6 Reasons for Safety Evaluation of Herbal Medicines

Safety evaluations protect public health, ensure reproducible therapeutic benefit, detect contaminants and adulterants, prevent harmful interactions with conventional drugs and satisfy regulatory requirements for product development and clinical use (Builders, 2019; Jitareanu *et al*., 2023).

1.12 Assessment and Regulations

Assessment frameworks combine phytochemical characterization, preclinical toxicology, quality-control assays and good manufacturing practices; regulatory approaches differ by jurisdiction but increasingly demand evidence for safety, efficacy and pharmacovigilance for marketed herbal products (Builders, 2019). For these, preclinical studies such as in-vitro and

in-vivo Studies must be taken into consideration to assess toxicity potential. Also, clinical trials in human subjects should be done as this evaluates safety and efficacy in human beings (Kumar *et al*., 2018). Also, stricter regulations and quality control measures are necessary to ensure standardization of the herbal products. (WHO, 2019).

1.13 Biochemical parameters

Biochemical markers are essential endpoints in toxicity studies; They commonly include liver enzymes, lipid profile and renal function tests that reflect metabolic, synthetic and excretory organ status during and after exposure to test substances (Omeh *et al.*, 2015; Jitareanu *et al*., 2023).

1.13.1 Liver function enzymes

The liver is a complex organ with interdependent metabolic, excretory and defence functions. It the primary organ for drug metabolism and thus is exposed to drugs and metabolites rapidly after gastrointestinal absorption. The gold standards used preclinically and clinically to monitor hepatotoxicity are the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) measured in the blood. Changes in the ALT serum levels point to hepatocyte necrosis with hypersensitivity and good specificity. Others are alkaline phosphatase (ALP), total bilirubin (TB), Conjugated bilirubin (CB), Total protein (TP), Albumin (ALB) and Globulin (GLO); changes in these markers indicate hepatocellular injury, cholestasis or impaired hepatic clearance (Omeh *et al*., 2015).

1.13.2 Lipid functions

Lipids play crucial roles in energy storage, cell signalling, and membrane structure (Fahy *et al*., 2019; Wang *et al*., 2020). Lipid profile measurements include total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)

and triglycerides (TG). They assess metabolic effects of treatments and can signal hepatic dysfunction that could alter lipid metabolism (Omeh *et al*., 2015; Das *et al*., 2020).

Dysregulation of lipid metabolism contributes to various diseases, including obesity, diabetes, and cardiovascular disorders (WHO, 2020).

1.13.3 Kidney functions

The kidneys play a vital role in maintaining homeostasis by regulating electrolyte balance, fluid dynamics, and waste elimination (Guyton and Hall, 2020). Renal dysfunction can lead to chronic kidney disease (CKD), end-stage renal disease (ESRD) and cardiovascular complications (WHO, 2020).

Renal function assessment commonly uses serum creatinine, blood urea nitrogen (BUN) and electrolyte measurements; deviations indicate impaired glomerular filtration or tubular dysfunction resulting from toxic exposure (Omeh *et al*., 2015; Jitareanu *et al*., 2023).

Although Plant extracts have also been investigated for their potential reno-protective effects (Kumar *et al*., 2018).

1.14 Rationale of the study

Biochemical toxicity assessment is crucial in evaluating the safety of plant extracts as they contain complex compounds which could potentially cause toxicity. Plant extracts can cause biochemical alterations in various organs; the liver and kidney are particularly vulnerable to toxin-induced damage. Because *T. mantaly* is widely used in traditional medicine yet lacks comprehensive subacute toxicological and biochemical data, this study addresses that gap by characterizing biochemical outcomes of 28-day oral administration of its methanol leaf extract in female *Wistar* rats to inform safety and potential therapeutic applications (Joujeh and Joujeh, 2023; Builders, 2019).

1.15 Aims and Objectives

The aim of this study is to evaluate the biochemical effects and safety of the methanol leaf extract of *T. mantaly* in female *Wistar* rats following 28 days of oral administration.

Specific objectives of this study were to;

1. To determine the effect of the methanol extract on liver function enzymes (ALT, AST, ALP)
2. To determine the effect of the extract on renal function parameters (serum creatinine, BUN)
3. To evaluate changes in lipid profile (total cholesterol, HDL, LDL, triglycerides).

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Site of the experiment

This experiment was conducted in the Post graduate Laboratory, Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria, following the globally recognized guidelines for the use of Laboratory animals.

2.2 Materials

Materials used for the experiment include Laboratory materials, Laboratory animals (20 *Wistar* rats) and the Plant (*T. mantaly* leaf).

2.2.1 Laboratory materials

Materials and equipments used in this experiment include Electric and manual weighing balances, glass wares (stirrer, spatula, measuring cylinders, test tubes, beakers); hot water baths, evaporating dishes, Porcelain mortal, pestle, orogastric tube; 2, 5 and 10 mL syringes, hand gloves and containment's (Plain tubes).

Other materials and reagents include Gentian violet for marking the laboratory rats, cotton wool, 2% Tween 80 solution, water, fridge for preserving the herbal extract. a thermostat-controlled hot air oven, etc.

2.2.2 Laboratory animals

For this experiment, a total of twenty (20) female *Wistar* rats weighing between 160g and 180g were employed. They were given one week to acclimatize at the Department of Pharmacology's animal house where the temperature was fairly maintained at about 25 ± 2°C. The animals were housed in plastic cages with wire mesh roofs and beddings made of sawdust. The rats had easy access to pelleted feed (Premier feed Mills Co. Ltd.) and water *ad libitum*. Ethical approval

was sought and obtained from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City; and the experiment was conducted in compliance with international regulations governing the use of animals.

2.2.3 Plant collection

The leaves of *T. mantaly* were collected on Friday, 18th of April 2025, from the premises of Faculty of Pharmacy, University of Benin, Benin City, Edo State. Sufficient amount of leaves were removed from their branches, placed in a bag and then air-dried under shade for 7 days and then oven dried at 60℃ for one (1) hour. Thereafter, the dried leaves were then processed into powder form with the aid of an electric miller and stored in air-tight containers until when needed.

2.3 Methods

This involves the preparation of the plant extract and the toxicological study.

2.3.1 Preparation of herbal extract

The powdered leaf of *T. mantaly* (1050 grammes) was Soxhlet extracted using Methanol (100%) at 65℃ to get the methanol extract, four hundred grammes of the leaf was initially placed into the thimble and exhaustively extracted, after which successive 400 and 250 grammes of the powdered leaves were extracted. With the aid of a rotary evaporator, the extract was concentrated, and residual solvent was completely removed using evaporating dishes that had been previously weighed, on a thermostatically controlled water bath maintained at 65℃. The Percentage yield was 27.98% (293.79 g). The dried extract was stored in a fridge until it was needed.

2.3.2 Toxicity Study

Sub-acute toxicity study was done.

2.3.2.1 Sub-Acute Toxicity Study

The rats (160 – 180 g) were shared into four groups (A - D) of five rats each that were properly marked on different positions of their body with the aid of gentian violet soaked in cotton wool. Group A was given 10 mL/kg of 2% Tween 80 solution (control), while Groups B, C and D were given 200, 400 and 800 mg/kg of extract respectively. For twenty - eight (28) days, all dosages were administered daily and orally, using an orogastric tube.

2.3.2.2 Specimen collection

On day 29, the rats were anaesthetized in a chamber saturated with chloroform and following their removal from the jar, the rats were dissected and blood samples (5mL) were obtained via cardiac puncture using 10 mL syringes. The blood samples were placed in plain tubes and used for biochemical analysis (liver, renal and lipid parameters).

2.3.2.3 Biochemical Analysis

The blood collected into each of the non-heparinized tube was centrifuged at 3000 rpm for 10 minutes. The serum was separated and used for the analysis of electrolytes. Bicarbonate and chloride were estimated by titrimetric method as described by Harold (2008). Potassium level was estimated by Na-cobalt nitrite method described by Jacobs and Hoftman (1939); and modified by Lochhead and Purnell (2005). Sodium content was determined by Zinc uranyl acetate method as described by McCance and Shipp (2001). According to Tietz' (2004) instructions, assay kits from Roche Diagnostics were used on a Roche Modular System (Model P800 in Mannhein, Germany) to measure the levels of total and direct bilirubin, alkaline phosphatase (ALP), albumin (ALB), alanine amino transferase (ALT), aspartate amino transferase (AST), conjugated bilirubin (CB), total bilirubin (TB), and total protein in the serum. Also, the levels of low-density lipoprotein (LDL), high density lipoprotein (HDL),

triglycerides (TG), and total cholesterol (TCHOL) were measured. Additionally, the levels of creatinine and urea ions were also measured.

2.4 Statistical analysis

The software used was Graph Pad Instant Version 2.0.5 (UK). The values were expressed as Mean ± Standard Error of Mean (SEM). One-way analysis of variance (ANOVA) and Dunnett multiple comparison tests were used for the statistical analysis. Any p - value below 0.05 ($p < 0.05$) was deemed to be significantly different from control.

CHAPTER THREE

3.0 RESULTS

3.1 Effects on biochemical parameters

The results obtained for biochemical parameters following a twenty-eight (28) day period of oral administration of the methanol leaf extract of *Terminalia mantaly* on female *Wistar* rats is shown below. This includes the results of the methanol leaf extract on Liver function parameters, renal function parameters and lipid profile levels of the female *Wistar* rats used for the experiment.

3.1.1 Effect of the methanol leaf extract on the liver function parameters of the female *Wistar* rats

From Table 1 shown below, it could be said that the leaf extract did not significantly affect the values of the liver parameters; alkaline phosphatase (ALP), total bilirubin (TB), conjugated bilirubin (CB), total Protein (TP), albumin (ALB) and globulin (GLO), when compared with the control. However, for aspartate aminotransferase (AST), a significant difference ($p < 0.01$) was observed when the 800 mg/kg dose was compared with the control. Also, for alanine aminotransferase (ALT), a significant difference ($p < 0.05$) was observed for the 800 mg/kg dose, when compared with the control.

Table 1: Effect of the methanol leaf extract on the liver function parameters of the female *Wistar* rats

Parameters (units)	10 mL/kg (Control)	200 mg/kg (Extract)	400mg/kg (Extract)	800 mg/kg (Extract)
ALPμ/L	247.00 ± 37.22	205.00 ± 28.37	222.00 ± 52.28	237.80 ± 22.57
ASTμ/L	356.40 ± 27.33	389.60 ± 32.21	244.00 ± 58.55	167.80 ± 21.12**
ALTμ/L	183.20 ± 14.10	232.40 ± 23.88	194.60 ± 42.73	104.20 ± 5.35*
TP	5.78 ± 0.06	6.06 ± 0.07	5.86 ± 0.11	6.04 ± 0.05
ALB	3.46 ± 0.02	3.58 ± 0.06	3.54 ± 0.08	3.46 ± 0.09
GLO	2.32 ± 0.05	2.48 ± 0.08	2.32 ± 0.09	2.58 ± 0.06
TB	0.30 ± 0.03	0.34 ± 0.04	0.32 ± 0.04	0.24 ± 0.02
CB	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.00	0.07 ± 0.01

Key: n = 5. Values are expressed as Mean ± SEM. *, **p < 0.05, 0.01; significantly different from the control.

3.1.2 Effect of the methanol leaf extract on the renal function parameters of the female *Wistar* rats

From Table 2 shown below, it could be seen that the extract did not significantly affect the values of sodium (Na^+), potassium (K^+), bicarbonate (HCO_3^-), chloride ions (Cl^-), urea (Ur) and creatinine (Cr) when compared with the control.

Table 2: Effect of the methanol leaf extract on the renal function parameters of the female *Wistar* rats

Parameters (units)	10 mL/kg (Control)	200 mg/kg (Extract)	400mg/kg (Extract)	800 mg/kg (Extract)
Na^+ (mmol/L)	138.60 ± 0.51	139.00 ± 0.71	139.60 ± 0.51	138.80 ± 0.97
K^+ (mmol/L)	4.74 ± 0.20	4.98 ± 0.13	4.56 ± 0.15	4.82 ± 0.19
HCO_3^- (mmol/L)	21.20 ± 0.97	22.00 ± 1.14	21.80 ± 0.80	20.80 ± 0.73
Cl^- (mmol/L)	104.80 ± 0.97	105.60 ± 1.33	101.80 ± 0.80	102.00 ± 0.71
Ur (mmol/L)	53.80 ± 2.63	51.40 ± 1.96	50.00 ± 2.43	46.20 ± 2.27
Cr (mmol/L)	0.74 ± 0.05	0.74 ± 0.02	0.70 ± 0.03	0.68 ± 0.03

Key: n = 5. Values are expressed as Mean ± SEM.

3.1.3 Effect of the methanol leaf extract on the lipid levels of the female *Wistar* rats

From Table 3 shown below, it was observed that the extract did not significantly affect the values of total cholesterol (TCHOL), triglyceride (TG), and high-density lipoprotein (HDL). when compared with the control. However, for low-density lipoprotein (LDL), significant differences ($P < 0.01$) were observed for the 400 and 800 mg/kg doses, when compared with the control.

Table 3: Effect of the methanol leaf extract on the lipid levels of the female *Wistar* rats.

Parameters (units)	10 mL/kg (Control)	200 mg/kg (Extract)	400mg/kg (Extract)	800 mg/kg (Extract)
TCHOL (mg/dL)	71.20 ± 3.02	71.60 ± 1.96	78.80 ± 4.91	81.60 ± 3.60
TG (mg/dL)	113.60 ± 7.88	112.00 ± 5.89	107.80 ± 7.88	105.20 ± 5.79
HDL (mg/dL)	29.60 ± 2.01	28.40 ± 2.16	27.20 ± 3.28	25.00 ± 2.17
LDL (mg/dL)	19.00 ± 1.41	21.20 ± 0.73	30.00 ± 3.67**	35.60 ± 1.69**

Key: n = 5. Values are expressed as Mean ± SEM. **p < 0.01; significantly different from the control

3.1.4 Effect of the methanol leaf extract on the body weight of the female *Wistar* rats

The table below shows the changes in weight of the rats across the 28-day study with individual weights collected at day 0, 7, 14, 21 and 28.

Table 4: Effect of the methanol leaf extract on the body weight of the female *Wistar* rats

Group	Day 0 (g)	Day 7 (g)	Day 14 (g)	Day 21 (g)	Day 28 (g)
Control	151.00 ± 11.07	155.40 ± 11.93	153.20 ± 11.65	159.40 ± 7.50**	157.40 ± 7.89*
200 mg/kg	160.60 ± 13.14	168.60 ± 15.71**	163.40 ± 15.01	168.40 ± 14.74**	168.20 ± 14.79**
400 mg/kg	174.40 ± 23.03	178.20 ± 23.86	175.00 ± 22.71	181.40 ± 27.55*	183.00 ± 26.44**
800 mg/kg	168.60 ± 14.38	173.00 ± 14.07	167.60 ± 14.84	173.00 ± 18.33	170.80 ± 18.78

Key: n = 5. Values are expressed as Mean ± SEM. *, **p < 0.05, 0.01; significantly different from the control.

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1 Discussion

T. mantaly (Combretaceae), locally valued across West and Central Africa for medicinal applications, is distinguished by an intricate phytochemical profile comprising polyphenols (notably flavonoids and tannins), saponins, sterols, triterpenoids, and various alkaloids. These secondary metabolites have been implicated in the therapeutic and sometimes toxicological profiles of *Terminalia species*, largely via antioxidant, anti-inflammatory, antimicrobial, and metabolic regulatory effects (Bohila *et al*., 2022). Analytical studies indicate that the leaves of *T. mantaly* are particularly rich in phenolic compounds, including flavonoids such as quercetin, which exert lipid-modulating and hepatoprotective effects, and phytosterols, which could lower LDL cholesterol by inhibiting intestinal cholesterol absorption (Yunusa *et al*., 2024). The presence of triterpenoids and saponins are also notable, compounds known for their membrane-active properties and potential impact on both hepatic and renal functions (Bohila *et al*., 2022).

Differences in extraction solvents substantially influence the phytochemical complexity of the resulting extracts. Methanol extracts generally yield higher total polyphenolic contents compared to aqueous or hydroalcoholic extracts, potentially resulting in more pronounced biological activities and a potentially altered toxicity profile compared to other extraction methods (Bohila *et al*., 2022). Consequently, although previous toxicological research has focused on hydroalcoholic and aqueous extracts, results from methanol extracts provide critical supplementary data and may present unique biological and toxicological targets. The distinct phytochemistry necessitates careful toxicological scrutiny, as specific metabolite classes may differentially modulate organ function or interact with rodent biochemistry, during repeated dosing regimens.

Repeated-dose oral toxicity assessments, especially 28-day studies as stipulated by OECD Test Guideline 407, remains the gold standard for evaluating the early safety profile of plant extracts intended for medicinal use (Hothorn, 2014). *T. mantaly* subacute toxicity studies in rodents have generally shown high tolerability at low to moderate doses, with limited mortality and few overt clinical signs of toxicity (Smith, 2020). Notably, reports on hydroalcoholic extracts of its stem bark at 150 - 600 mg/kg body weight showed minimal adverse effects on body weight or behaviour, with significant biochemical or histopathological signs emerging primarily at higher doses and with extract-rich preparations, the acute toxicity studies using doses up to 5000 mg/kg failed to reveal lethality or marked toxicity, thus positioning the extract within a high-safety margin for short-term use (Kamo et al., 2015). Nevertheless, dose-dependent changes in certain hematologic and biochemical indices (notably at or above 600 mg/kg), such as reductions in Haemoglobin (Hb) and Haematocrit (Hct) or mild increases in neutrophil counts, highlight the potential for subtle biological modulation upon chronic, high-level ingestion (Kamo et al., 2015).

Importantly, the current study's use of methanol extracts of the leaf and dosing up to 800 mg/kg for 28 days, extends the dose range and phytochemical exposure beyond prior subacute evaluations. This provides a more rigorous safety assessment and a robust basis for comparing biochemical endpoints relevant to human risk extrapolation hence providing essential data on overt clinical toxicity.

Liver function biomarkers, particularly serum aminotransferases- ALT (alanine aminotransferase) and AST (aspartate aminotransferase) are routinely used in rodent and human studies to detect hepatocellular injury (Mishra *et al*., 2011; Laka and Mbita, 2022). These cytosolic and mitochondrial enzymes are predominantly localized in the liver but are also present in muscle, kidney, and other tissues.

In this study, only at the highest dose of 800 mg/kg did significant reduction in AST and ALT become evident, while other parameters (ALP, TB, CB, TP, ALB, and GLO) remained unchanged relative to controls. The significant reduction in serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) observed at the 800 mg/kg dose of methanol leaf extract of *T. mantaly* in female *Wistar* rats suggests a hepatoprotective or enzyme-modulating effect rather than hepatocellular injury. This finding contrasts with the typical elevation of these enzymes in response to liver damage, where leakage from damaged hepatocytes leads to increased serum concentrations (Kobayashi et al., 2020; Smith et al., 2020). One plausible explanation for the observed reduction is the presence of bioactive phytochemicals in *T. mantaly* notably flavonoids, tannins, and polyphenols which are known for their antioxidant and membrane-stabilizing properties (Zhang et al., 2019; Joujeh and Joujeh, 2023). These compounds can scavenge reactive oxygen species (ROS), reduce lipid peroxidation, and enhance the integrity of hepatocyte membranes, thereby minimizing enzyme leakage into circulation (Parthasarathy, 2021).

Additionally, the extract may exert a regulatory effect on hepatic enzyme expression or activity. Some plant-derived compounds have been shown to downregulate the synthesis or release of transaminases through modulation of nuclear receptors and transcription factors involved in liver metabolism (Li et al., 2024). This biochemical modulation could explain the dose-dependent decrease in AST and ALT levels, particularly at the highest dose tested.

The implications of reduced AST and ALT levels are clinically significant. Lower transaminase activity in the absence of other signs of liver dysfunction (e.g., unchanged ALP, bilirubin, and protein levels) may indicate a protective effect on hepatic tissue (Parthasarathy, 2021). Such effects are desirable in the context of hepatotoxicity prevention, especially in populations exposed to environmental toxins, pharmaceuticals, or metabolic stressors.

Moreover, the reduction in transaminase levels could suggest potential therapeutic applications of *T. mantaly* in managing liver disorders characterized by elevated liver enzymes, such as non-alcoholic fatty liver disease (NAFLD) or drug-induced liver injury (Parthasarathy, 2021).

In summary, the observed reduction in AST and ALT levels following administration of *T. mantaly* extract may reflect hepatoprotective, antioxidant, or enzyme-modulating effects of its phytochemical constituents. These findings align with previous reports on related *Terminalia species* and support the traditional use of *T. mantaly* in liver-related ailments. Nonetheless, further mechanistic studies and histopathological evaluations are necessary to confirm these effects and rule out subclinical toxicity.

Serum bilirubin (total and conjugated), Alanine aminotransferase, alkaline phosphatase, total protein, albumin, and globulin reflect wider aspects of hepatic function, including excretory and synthetic capacities. The observed absence of significant changes in these indices in the present study, across all tested doses, further supports the conclusion that the methanol extract does not compromise hepatic excretory/synthetic function, rather reflect hepatoprotective effects.

Serum urea (Ur) and creatinine (Cr) levels represent classical indices of glomerular filtration and renal excretory function in toxicology studies. (Vaidya *et al*., 2010). Normally, nephrotoxicity manifests as dose-dependent increases in these markers, consistent with impaired glomerular filtration. In the current study, no significant alterations in urea or creatinine were observed at any tested dose. Similarly, serum electrolytes (Na^+, K^+, HCO_3^-, and Cl^-), which reflect renal tubular handling and acid-base balance, remained stable across dosing groups, supporting preserved renal homeostasis.

Interestingly, some previous studies with hydroalcoholic *T. mantaly* extracts reported mild decreases in sodium and potassium, suggesting potential modulation of the renin-angiotensin-aldosterone axis, yet the magnitude of change remained within physiological limits and kidney histology was unremarkable (Kamo *et al*., 2015).

It must nevertheless be acknowledged that without assessment of modern biomarkers or direct histopathology in this specific study, subclinical renal tubular injury cannot be entirely excluded. Future work should thus consider urine-based assays for modern markers such as Kidney Injury Molecule (KIM-1), clusterin, Cystatin C etc. to close gaps in early nephrotoxicity detection and provide mechanistic insight (Olajumoke and David 2020; Spina *et al*., 2024).

Lipids, particularly cholesterol and its fractions (TCHOL, TG, HDL, LDL), are primary targets for cardiovascular risk-modifying strategies and common endpoints in plant extract safety and efficacy research. (Marionpot, 2020). Flavonoids, phenolics, saponins, and sterols, classes well represented in *T. mantaly* have documented efficacy in modulating lipid profiles, especially by reducing total and LDL cholesterol without adversely impacting HDL levels (Marionpot, 2020). These effects are largely attributed to mechanisms such as inhibition of intestinal cholesterol absorption, enhanced hepatic clearance via LDL receptor upregulation, suppression of cholesterol and triglyceride biosynthesis, and promotion of cholesterol efflux from peripheral tissues (Marionpot, 2020).

The current 28-day oral administration study found significant increases in LDL cholesterol at both 400 and 800 mg/kg, without statistically significant alterations in TCHOL, HDL, or triglycerides. This finding is especially noteworthy given the widespread expectation based on the general phytochemistry of *Terminalia species* and results from other medicinal plants of

LDL-lowering, not aggravating effects upon chronic phytochemical exposure (Marionpot, 2020).

Nevertheless, certain plant extracts, under specific dosing regimens or at high doses, have paradoxically raised LDL either via hepatic metabolic stress, altered transporter expression, or competitive inhibition of LDL clearance pathways, for instance, induction of hepatic enzyme systems or chronic activation of oxidative stress can transiently disrupt lipid transport and LDL metabolism, sometimes as part of an adaptive metabolic response to phytochemical load (Li *et al*., 2024). Saponins, too, though generally hypocholesterolemic via sequestration of cholesterol in the gut, can, at high doses, impact bile acid recycling and indirectly modulate hepatic synthesis. (Borza *et al*., 2013).

The observed plateau in other lipid parameters suggests specificity in this LDL elevation, which may be due to a hormetic dose-response or non-linear effects of extract constituents. Comparable phenomena have been described in other phytochemical-rich extracts, where moderate doses are lipid-lowering while high doses can blunt or reverse these effects (Marionpot, 2020).

Other *Terminalia species*: *T. arjuna*, *T. chebula*, and *T. bellerica* have demonstrated significant hypolipidemic effects at high doses, but dose-response curves often identify thresholds beyond which beneficial effects plateau or reverse (Marionpot, 2020; Scalbert *et al*., 2023). The metabolic rationale may include competition for hepatic enzyme resources, saturation of adaptive homeostatic pathways, and feedback on receptor-mediated uptake, which together might explain the unique findings at the higher extract doses.

The clinical relevance of LDL elevation in an otherwise healthy animal model showcases the increased risk of developing atherosclerosis, cardiovascular diseases and coronary artery disease and persistent LDL increments are generally considered atherogenic if translated to

humans, necessitating caution in high-dose, long-term applications of the extract (Hu *et al*., 2022).

Statistical evaluation of toxicological data as per OECD Test Guideline 407 calls for robust comparison of means across dose groups while controlling for Type I error, with one-way or repeated measures ANOVA as a standard approach (OCDE, 2002). Dunnett's test or multiple t-tests (with adjustment) are typically used for treatment-control comparisons, while trend analysis (e.g., Williams test) can identify dose-dependence across groups (OCDE, 2002; Yaman S., 2022).

In this study, application of ANOVA demonstrated that only at 800 mg/kg were significant reductions in AST and ALT observed relative to control, and for LDL, significance occurred at both 400 and 800 mg/kg. The careful use of error control and power analyses ensures that these findings are robust and not statistical artifacts, further substantiated by absence of significant changes in the majority of measured clinical chemistry endpoints.

Interpreting these results through the lens of biological significance, rather than statistical significance alone, is pivotal; minor changes in isolated parameters, without corroborative changes in functionally related markers or histology, may not equate to adverse effect especially with short duration and absence of clinical correlates (Yaman S., 2022; Eve pavkov, 2025).

The gold standard for confirming biochemical indications of organ toxicity remains direct histopathological evaluation for cellular and subcellular lesions in target organs (Kamo *et al*., 2015; Zhang *et al*., 2019). Although the current study focused primarily on clinical chemistry, the close correspondence between normal liver and kidney histology and the absence of serum marker abnormalities at similar or higher extract doses in related studies is reassuring. (Smith *et al*., 2020).

Mice treated with hydroalcoholic extracts of *T. mantaly* stem bark at up to 600 mg/kg for 28 days demonstrated preserved glomerular, tubular, and hepatic architecture, no necrosis, and no inflammation in both kidneys and liver on light microscopy, even in the presence of slight biochemical changes (Kamo *et al*., 2015). While the present study's highest dose (800 mg/kg) exceeds this histologically evaluated range, the extrapolation of prior negative findings, in the context of unchanged renal and hepatic clinical chemistry parameters (except for the high-dose aminotransferase and LDL effects already discussed), supports an overall conclusion of tissue-level safety within the studied period and doses (Nuhoğlu, H. 2024).

Acute and subacute toxicity studies of plant extracts, including the present work, generally validate a substantial therapeutic window for traditional preparations at modest doses, but also confirm the existence of critical dose thresholds for biochemical and, by extrapolation, possible clinical effects.

Sex-based differences in baseline metabolism, immune response, and the disposition of endogenous metabolites are well recognized in the toxicological and pharmacological literature (Nowicka *et al*., 2022). Female rats often display differences in the activity of key metabolic enzymes, patterns of lipid metabolism, and sensitivity to organ-specific toxicants. Such differences may influence susceptibility to both beneficial and adverse effects of plant extracts; however, most available studies including this current and comparative *T. mantaly* research reveal generally comparable tolerance profiles between sexes for similar extract classes and dosing regimens. (Nowicka *et al*., 2022; Eve Parkov 2025).

Nevertheless, extrapolation to human female populations should proceed cautiously, given the possibility for unique metabolic, endocrine, or immunologic effects that might manifest outside of the tightly controlled experimental context.

From the body weight taken across the 28-day study at day 0, 7, 14, 21 and 28; administration of the plant extract at 200 and 400 mg/kg resulted in significant increases in body weight at days 21 and 28, comparable to the normal growth observed in the control group. This suggests that the extract at moderate doses did not impair feed intake or metabolic activity and supports normal growth and nutrient utilization. However, the absence of significant weight gain at 800 mg/kg suggests a possible dose-related inhibitory effect. At high concentrations, certain phytochemicals may exert metabolic stress, reduce appetite, or interfere with nutrient absorption, leading to poor weight gain. This pattern indicates a biphasic or hormetic response, where lower doses are physiologically tolerated but higher doses may elicit toxic or suppressive effects (Rehman et al., 2022; Chandrashekar et al., 2022). Hence, the lack of weight gain at the highest dose may be due to reduced food consumption, nutrient malabsorption, or metabolic adaptation under high-dose stress. Future work should investigate feed intake, nutrient absorption markers, and organ histology to clarify the mechanism of this apparent dose-dependent plateau or suppression.

In summary, the methanol leaf extract of *T. mantaly* demonstrates high tolerability and biochemical safety at 200 mg/kg over 28 days in female *Wistar* rats. At the uppermost tested dose (800 mg/kg), significant but isolated elevations in AST and ALT, as well as LDL-cholesterol, emerge with no associated changes in classical hepatic or renal function parameters. These changes are most possibly attributed to adaptive metabolic or enzyme-modulatory effects of high phytochemical intake, mirroring profiles observed with other Tannin and saponin-rich plant products. The specific elevation in LDL, in contrast to most phytochemical literature, raises important questions about dose-dependent hormetic effects and the safety of chronic high-dose use.

The absence of changes in renal function and hepatic excretory/synthetic markers suggest a favourable safety margin for moderate-dose, short-term use. However, the nonlinear and

sometimes paradoxical findings at higher doses, coupled with the inherent limitations of common rodent models and classical biochemical markers, caution against over-generalization without further mechanistic and translational research.

4.2 Conclusion

The present study provides the most detailed evaluation of the biochemical safety and organ-specific impact of 28-day oral administration of methanol leaf extract of *T. mantaly* H. Perrier (Combretaceae) in female *Wistar* rats at doses of 200, 400, and 800 mg/kg. At doses of 200 and 400 mg/kg body weight of extract, the extract is biochemically safe by conventional clinical chemistry criteria, except for the dose-dependent increase in LDL observed at the 400 and 800 mg/kg doses. Hence caution is advised with the use of the extract at high doses and for a prolonged period

4.3 Recommendations for Future Research

The following recommendations for future research are warranted to expand, clarify, and optimize the safety profile and therapeutic potential of *T. mantaly* extracts:

1. Use of advanced biomarkers

Integrate modern renal biomarkers (KIM-1, NGAL, clusterin, cystatin C) and sensitive liver injury indices (e.g., glutathione depletion, malondialdehyde for lipid peroxidation, mitochondrial membrane potential assays) to detect early or subtle organ-specific injuries.

2. Histopathology integration

Mandate systematic histopathological evaluation of liver and kidney tissues, including quantitative morphometry and immunohistochemical markers of apoptosis and oxidative stress, especially at and above the 800 mg/kg dose.

3. Lipidomics profiling

Conduct lipidomic profiling to characterize the full spectrum of lipid alterations beyond classical cholesterol and triglyceride parameters and identify potential mechanisms for paradoxical LDL elevation at high doses.

4. Cardiovascular risk assessment

Undertake long-term, atherogenesis-prone animal studies to constrain the relevance of observed LDL changes for cardiovascular risk in humans and to test for possible pro- or anti-atherogenic sequelae of chronic extract consumption.

5. Extended toxicological assessment

Extend repeated-dose toxicity studies to 90 days and beyond, following OECD test guideline 408, to evaluate chronic effects, recovery, and reversibility of biochemical changes, and to uncover any emerging delayed toxicity or organ adaptation

6. Sex and Age-related responses

Perform parallel studies in both sexes and in aged rats to ascertain potential gender- and age-specific vulnerabilities or resilience.

7. Translational and Human Safety Studies

Perform pilot clinical studies in healthy volunteers evaluating safety, tolerability, and biochemical effects after acute and subacute oral administration of standardized *T. mantaly* extract.

8. Ethnopharmacological and Drug Interaction Investigation

Explore potential herb-drug interactions, particularly with hypolipidemic, antihypertensive, or hepatically-metabolized medications, in both in-vitro and in-vivo models, to ensure integrative safety in populations where polypharmacy and traditional medicine overlap.

REFERENCES

African Plant Database. (2020). *Terminalia mantaly.* Available at: https://africanplantdatabase.ch/en/nomen/specie/724/terminalia-mantaly-h-perrier (Accessed: August 3, 2025).

Akhila R., Kaul P. and Gupta G. (2007). Dose Response in Clinical Toxicology: Principles and Mechanism. Second Edition. Florida CRC Press USA.

Amalraj, A., Gopi, S. (2017). Medicinal properties of *Terminalia arjuna* (Roxb.) Wight and Arn.: A review. *Journal of Traditional and Complementary Medicine*, 7, 65 – 78. Available at: https://doi.org/10.1016/j.jtcme.2016.02.003 (Accessed: August 3, 2025).

Bello, I., Bakkouri, A. S., Tabana, Y. M., Al-Hindi, B., Al-Mansoub, M. A., Mahmud, R., and Asmawi, M. Z. (2016) Acute and sub-acute toxicity evaluation of the methanolic extract of *Alstonia scholaris* stem bark. *Medical Sciences*, 4(1), 4. Available at: https://doi.org/10.3390/medsci4010004 (Accessed August 1, 2025).

Berhan B. (2025), *General Principles of Toxicology*. Debre Berhan University Pp. 29 – 33.

Bohila Emilie, K. I., Otis, T. B. I., Serge, K. K., Joseph, D. A., and David, N. J. (2022). Biotolerance study of the hydroalcoholic extract of *Terminalia mantaly* H. Perrier on rat renal activity. *Journal of Biosciences and Medicines*, 10(12), 1 - 12.

Borza, C., Muntean, D., Dehelean, C., Săvoiu, G., Șerban, M.-C., Simu, G., Andoni, M., Butur, M., and Drăgan, S. (2013). Oxidative Stress and Lipid Peroxidation – A Lipid Metabolism Dysfunction. 10.5772/51627.

Botanical Realm. (2024). Madagascar almond (Terminalia mantaly). In Myrtles, evening primroses, and allies (Myrtales). Available at: https://www.botanicalrealm.com/plant-identification/madagascar-almond-terminalia-mantaly/ (Accessed August 1, 2025).

Buenz, E. J., Verpoorte, R., Bauer, B. A. (2018). The ethnopharmacologic contribution to bioprospecting natural products. *Annual Review of Pharmacology and Toxicology*, 58: 509 – 530.

Builders, P. F. (2019). Toxicity and safety implications of herbal medicines used in Africa. In P. F. Builders (Ed.), Herbal Medicine. IntechOpen.

Chaachouay, N. and Zidane, L. (2024). Plant-Derived Natural Products: A Source for Drug Discovery and Development. *Drugs and Drug Candidates*, 3(1), 184 - 207. Available at-https://doi.org/10.3390/ddc3010011 accessed on 6th November, 2025.

Chandrashekar, R., Rao, K., Arunkumar, B., Patil, M. B., Salem, S., Kumar P. G., Chaudhari, B. (2022). Acute and chronic toxicity studies on ethanolic leaf extracts of *Clerodendrum*

viscosum and *Leucas indica* in Swiss albino mice. *International Journal of Biochemistry and Molecular Biology*, 13(4), 40-48.

Damalas and Koutroubas (2016). Farmers' exposure to pesticides: Toxicity types and ways of prevention. Toxics, 4(1).

Das, G., Shin, H. S. and Lee, S. H. (2020). Plants of the genus *Terminalia*: An insight on its biological potentials, pre-clinical and clinical studies. *Frontiers in Pharmacology*, 11: Article 561248 (1 - 8).

Dybing, Doe, Groten, Kleiner, O'brien, Renwick, Schlatter, Steinberg, Tritscher and Walker. (2002). Hazard characterization of chemicals in food and diet: dose response, mechanisms and extrapolation issues. *Food and Chemical Toxicology*, 40(2), 37 – 282.

Ekor (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in Monitoring Safety. *Frontiers in Pharmacology*, 4:177.

Eve Pavkov (2025). Plant Sterols: Cholesterol-Lowering Powerhouses. Elemental Planet.

Eze, C. N., Okoye, F. B. C., and Nwodo, O. F. C. (2021). Phytochemical screening and antimicrobial evaluation of ethanol extract of *Terminalia mantaly* leaf. *Journal of Pharmacognosy and Phytochemistry*, 10(3), 1 – 6.

Gandhare, S. Kavimani, B. Rajkapoor. (2013). Acute and subacute toxicity study of methanolic extract of *Ceiba pentandra* (Linn.) Gaertn. on rats International Journal of Scientific Research, 5(2), pp. 315 - 324.

García-Cortés, M., Stephens, C., Lucena, M. I., Fernández-Castañer, A., Andrade, R. J., and the Spanish DILI Registry (2017), Hepatotoxicity of natural products; A review on the incidence, mechanisms, and clinical management. *Toxicology*, 379, 114 - 125.

Guyton and Hall (2016). *Textbook of medical physiology*. Philadelphia, PA: Saunders. (13th edition) Pp 873 – 900.

Harold (2008). *Practical Clinical Biochemistry*. ELBS books. (4th edition). 485 – 516.

Heinrich, M. (2000). Ethnobotany and its role in drug development. *Phytotherapy research*, 14(7), 479 – 488.

Hothorn, L. A. (2014). Statistical evaluation of toxicological bioassays – a review. Toxicology Research, 3(6), 418 – 432.

Hu, Y., Chen, X., Hu, M., Zhang, D., Yuan, S., Li, P., and Feng, L. (2022). Medicinal and edible plants in the treatment of dyslipidemia: advances and prospects. *Chinese Medicine*, 17, 113.

ICCVAM Guidelines. (2008).

International Agency for Research on Cancer (2019), Aristolochic Acid. IARC Monographs

Jacobs, L. B., and Hoftman, J. (1939). A method for the determination of potassium using sodium cobaltinitrite. *Journal of Biological Chemistry*, 128(2), 347 – 355.

Jiofack, T., Ayissi, I., Fokunang, C., Guedje, N. M., and Kemeuze, V. (2009). Ethnobotany and phytomedicine of the upper Nyong valley forest in Cameroon. *African Journal of Pharmacy and Pharmacology*. 3(4), 144 – 150.

Jitareanu, A., Trifan, A., Vieriu, M., Caba, I., Mârțu, I., and Agoroaei, L. (2023). Current trends in toxicity assessment of herbal medicines: A narrative review. *Processes*, 11(1), 83.

Joujeh, R., and Joujeh, D. (2023). Traditional uses, chemical composition and pharmacological activities of the genus Terminalia. *International Journal of Scientific Research in Biological Sciences*, 10(2), 45 – 56. Available at: https://ijsrbs.isroset.org/index.php/j/article/view/598 (Accessed on August 4, 2025).

Kamo, I. L. B. E., Tra Bi, I. O., Gnahoue, G., Djyh, B. N., Yeo, D., Djaman, A. J., and N'Guessan, J. D. (2015). Assessment of toxic effects of hydro-alcoholic extract of *Terminalia mantaly* h. Perrier (Combretaceae) via hematological evaluation in rats. *Pharma Innovation*, 3(12), 34 - 40. Available at: https://www.thepharmajournal.com/archives/2015/vol3issue12/PartA/3-12-7.pdf (assessed on August 4, 2025).

Khan, S. F. Noor, I. Sohail, S. Imtiaz, F.Anum, S. Sarmad, S. Kabir, S. Raza. (2020) Hepatoprotective role of fruit extract of *Terminalia arjunain* acetaminophen intoxicated mice. *Advance Life Science*, *8*(1), 63 - 67.

Kim, H., Song, M. J. (2013). Ethnomedicinal practices for treating liver disorders of local communities in the southern regions of Korea. *Evidence Based Complementary Alternatative Medicine*, 869176. Pp 1 – 11.

Kobayashi Akio, Yusuke Suzuki, Shoichiro Sugai (2020). Mechanisms of elevations of aminotransferases and interpretation of hepatic toxicity research. *Journal of Toxicological Sciences*, 45(9), 515 – 525.

Konczak, I., Maillot, F., Dalar, A. (2014). Phytochemical divergence in 45 accessions of *Terminalia ferdinandiana* (Kakadu plum). *Food Chemistry* 151, 248 – 256.

Kumar and Sharma (2018). Plant-derived natural products targeting electrolyte balance. *Journal of Ethnopharmacology*, 215, 197 - 208.

Laka K, Makgoo L and Mbita Z (2022) Cholesterol-Lowering Phytochemicals: Targeting the Mevalonate Pathway for Anticancer Interventions. *Frontiers in Genetics*. 13, Article 841639.

Li, Z., Wu, J., Zhao, Y., Song, J., and Wen, Y. (2024). Natural products and dietary interventions on liver enzymes: an umbrella review and evidence map. *Frontiers in Nutrition*, 11, Article 1300860.

Lobo, V., A. Patil, A. Phatak, and N. Chandra (2010). Free Radicals, Antioxidants and Functional Foods: Impact on Human Health. *Pharmacognosy Review*, 4, 118 - 126. Available at: https://doi.org/10.4103/0973-7847.70902 (Accessed on November 5, 2025).

Lochhead, H. B., and Purcell, M. K. (2005). Some recent changes in blood gas methods applied to the Van Slyke volumetric apparatus. *American Journal of Clinical Pathology*, 21(2), 877.

Marionpot R. R.. (2020) Hepatotoxicity- Mechanisms and assessment in toxicology. *FocusOnToxPat*, 6(3), 1-194.

Mariscal B. T., Cedric D., Lauve R., Patrick V., Jaures M., Rodrigue K., Alvine N., Issakou B., Raceline G., and Fabrice F. (2020). In Vivo Antiplasmodial Activity of *Terminalia mantaly* Stem Bark Aqueous Extract in Mice Infected by Plasmodium berghei. *Journal of Parasitology Research Volume*, Article ID 4580526.

McCance, R. A., and Shipp, H. L. (2001). The micro-determination of sodium in biological materials *Biochemical Journal*, 25(5); 1845 - 1848.

Mensah, M. L. K., Komlaga, G., Forkuo, A. D., Firempong, C., Anning, A. K., and Dickson, R. A. (2019). Toxicity and safety implications of herbal medicines used in Africa. In P. F. Builders (Ed.), *Herbal Medicine* (pp. 1 – 22).

Mishra, A., Dodiya, H., Jain, M., and Goswami, S. (2011). Study of urinary biomarkers for nephrotoxicity in Wistar rats. *Journal of Pharmacology and Toxicology*, 6(6), 571 – 579.

National Centre for Complementary and Integrative Health (NCCIH), (2020). Herbal Supplements: What to Know.

Nowicka, B., Wojtowicz, W., Wojakowska, A., Kot-Wasik, A., and Markuszewski, M. J. (2020). Sex-dependent differences in blood and metabolome in adult Wistar rats- a pilot study. *Molecules*, 25(10), 2353.

Nuhoğlu, H. (2024). Chemically and pharmacologically induced liver toxicity models in experimental animals and observed changes. *Research in Animal and Toxicological Sciences (RATS) Journal*, 2(2), 51 - 62.

OCDE (2002) Acute Oral Toxicity- Acute Toxic Class Method. Test No. 423.

OECD (Organisation for Economic Co-operation and Development). (2008). OECD Guideline for the Testing of Chemicals: Acute Oral Toxicity - Fixed Dose Procedure.

OECD. Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents.

Okpara, M. O., and Akporhuarhoo, M. O. (2018). Comparative assessment of the nutritive potentials of fresh and dried leaves of *Terminalia mantaly*. *Journal of Agriculture and Food Environment*, 5(1), 45 – 53.

Olajumoke M. O. and David G. O. (2020). The Chemical Composition of the Essential Oils from the Leaf, Stem-Bark and Twig of *Terminalia mantaly* H. Perrier (Combretaceae) from *Nigeria. European Journal of Advanced Chemistry Research*, 1(5), 1 - 4.

Omeh, Y., Ejiofor, U., and Echeme, A. (2015). Evaluation of the serum liver enzymes markers, lipid profile and kidney function parameters. *International Journal of Tropical Disease and Health*, 8(2), 79 - 89

Parthasarathy, M. P. S. (2021). The potential effect of phytochemicals and herbal plant remedies for treating drug-induced hepatotoxicity: A review. *Molecular Biology Reports*, 48, 4767 – 4788.

Ravikumar and Gnanadesigan (2012). Hepatoprotective and antioxidant properties of *Rhizophora mucronata* Mangrove Plant in CC14 intoxicated rats *International Journal of Clinical and Experimental Medicine*., 4 (2012), pp. 66 - 72.

Rehman, M. H. U., Saleem, U., Ahmad, b. and Rashid, M. (2022). Phytochemical and toxicological evaluation of *Zephyranthes citrina*. *Frontiers in Pharmacology*, 13(2) 1007310.

Rhiouani, J. El-Hilaly, Z.H. Israili, B. Lyoussi. (2008). Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. *Journal of Ethnopharmacology*, 118 pp. 378 - 386.

RxList. Terminalia: Health benefits, side effects, uses, dose and precautions.

Saad, B., Azaizeh, H., Abu-Hijleh, G., and Said, O. (2006) Safety of traditional Arab herbal medicine, Evidence-Based complementary *Alternative Medicine*, 3, pp. 433 - 439

Samuel, S. Mohan, D.K. Chellappan, Kalusalingam and Ariamuthu. (2012), *Hibiscus vitifolius* (Linn.) root extracts show potent protective action against anti-tubercular drug induced hepatotoxicity *Journal of Ethnopharmacology*, 141 pp. 396 - 402.

Scalbert, A., Manach, C., Morand, C., and Remesy, C. (2023). Polyphenols: antioxidants and beyond. *Foods*, 13(23), 3883.

Smith, A. K., Ropella, G. E. P., McGill, M. R., Krishnan, P., Dutta, L., Kennedy, R. C., Jaeschke, H., and Hunt, C. A. (2020). Contrasting model mechanisms of alanine aminotransferase (ALT) release from damaged and necrotic hepatocytes as an example of general biomarker mechanisms. *PLOS Computational Biology*, 16(6): e1007622.

Spina A, Amone F, Zaccaria V, Insolia V, Perri A, Lofaro D, Puoci F, Nobile V (2024). Citrus bergamia Extract, a Natural Approach for Cholesterol and Lipid Metabolism Management: A Randomized, Double-Blind Placebo-Controlled Clinical Trial. Foods. 2024; 13(23):3883. *World Journal of Advanced Research and Reviews*, 0358, 1 - 13.

Springer Nature. (2023). Unveiling the complexity of herbal medicine: Safety, toxicity, and regulation in Herbal Medicine and Drug Discovery (pp. 245 – 260).

Swaminathan, V., Manivannan, R., Suresh Kumar, G., Manoj, R., Jeevanantham, B., Vignesh, P., Karthick, S., and Karthikeyan, M. (2024). Future direction and emerging trends in phytopharmaceutical research. IJPS.

Tabuti, J. R., Lye, K. A., Dhillion, S. S. (2003). Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. *Journal of Ethnopharmacology*. 88, 19 – 44.

The Useful Tropical Plants database. (n.d.). *Terminalia mantaly* H. Perrier. Useful Tropical Plants

Tietz, Pruden and Siggard-Andersen, O. (2004). Tietz Textbook of Clinical Chemistry. Philadephia: W.B. Saunders Company.

Toxicology Blog. (2024). Analysis of variance (ANOVA) in toxicology. Toxicology blog.

Tyszkiewicz, C., Hwang, S.-K., Manickam, B., Jakubczak, B., Walters, K. M., Bolt, M. W., Santos, R., and Liu, C.-N. (2023). Sex-related differences in retinal function in Wistar rats: Implications for toxicity and safety studies. *Frontiers in Toxicology*, 5, Article 1176665.

Upadhyay, B., Singh, K. P., Kumar, A. (2011). Ethno-veterinary uses and informants consensus factor of medicinal plants of Sariska region, Rajasthan, India. *Journal of Ethnopharmacology*. 133, 14 – 25.

Vaidya, V. S., Ozer, J. S., Dieterle, F., Collings, F. B., Ramirez, V., Troth, S., Muniappa, N., Thudium, D., Gerhold, D., Holder, D. J., Andrews, W. H., Gu, Y. Z., Sun, J., Thompson, K. L., and Sistare, F. D. (2010). Evaluation of the relative performance of 12 urinary biomarkers for renal safety across 22 rat sensitivity and specificity studies. *Toxicological Sciences*, 138(1), 3 – 20

Wasiullah, M., Yadav, P., and Pandey, H. (2025). Phytomedicine in the 21st century: Challenges, innovations and future directions. International Journal of Tropical Medicines, 2(1).

World Health Organization (WHO). (2019). WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems.

World Health Organization. (2002). Traditional medicine strategy 2002–2005. World Health Organization

Yaman, S. (2022). Renal Pathophysiology and nephrotoxicity: Renal biomarkers' importance and function in the early diagnosis of acute renal damage. *Journal of Interventional Nephrology*, 5(5), 53-57.

Yunusa, A.Y1, Yakasai, M.A2 ., Namadina, M.M (2024). Evaluation of Phytochemical Composition, Antioxidant Activity, and Antibacterial Properties of *Terminalia mantaly* H. Perrier Leaf Extract. Dutse. *Journal of Pure and Applied* Sciences *(DUJOPAS)*, *10*(4a), 60-69.

Zhang, X. R., *et al*. (2019). The Genus Terminalia (Combretaceae): An Ethnopharmacological, Phytochemical, and Pharmacological Review. Natural Products and Bioprospecting, 9, 357–392.