AKENTEN APPIAH-MENKA UNIVERSITY OF SKILLS TRAINING AND ENTREPRENEURIAL DEVELOPMENT

HEPATOPROTECTIVE EFFICACY OF COMBINED ETHANOLIC LEAF EXTRACT OF *VERNONIA AMYGDALINA* AND *MOMORDICA CHARANTIA* AGAINST ACETAMINOPHEN INDUCED HEPATOTOXICITY IN WISTAR RATS

DENNIS KWABENA FRIMPONG

JANUARY, 2024

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BY

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A thesis submitted to the School of Graduate Studies, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development in partial fulfillment of the requirements for the award of a Master of Philosophy degree in Biology

JANUARY, 2024

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

Dennis Kwabena Frimpong

Signature: ……. Date: …..……

Supervisors' Declaration

I hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development.

Dr. Addison Duodu (Principal Supervisor)

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ABSTRACT

Vernonia amygdalina and *Momordica charantia* are utilized in ethnomedical practices for the treatment of liver disorders due to their significant phytochemical content. This study aimed to examin the potential hepatoprotective effect of a combined ethanolic leaf extract of *Vernonia amygdalina* and *Momordica charantia* against acetaminophen-induced hepatotoxicity in male Wistar rats. The research was conducted at the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Asante Mampong Campus. Wistar rats for the study were divided into six groups of five rats each. Group 1 served as the normal control, receiving normal saline daily for 28 days. Hepatotoxicity was induced in groups 2, 3, 4, 5 and 6 by administering 3000 mg/kg of acetaminophen daily for 14 days. Groups 2, 3, 4, 5, and 6 received daily oral doses of silymarin (140mg/kg), *Vernonia amygdalina* extract (100 mg/kg), *Momordica charantia* extract (100 mg/kg), and the combined *Vernonia amygdalina* and *Momordica charantia* extracts (100 mg/kg each), respectively, for 14 days. Group 3, the acetaminophen control, were left untreated. The qualitative phytochemical screening of *Vernonia amygdalina*, *Momordica charantia*, and the combined *Vernonia amygdalina* and *Momordica charantia* extract revealed diverse phytochemicals. Haematological and biochemical indices were evaluated with automated analyzers, and histostructure was assessed using light microscopy. Data analysis was performed with Minitab statistical software version 20.3, using one-way ANOVA and Tukey's test for comparative analysis. The qualitative phytochemical screening indicated that anthraquinones were absent in all *Vernonia amygdalina* and *Momordica charantia* extracts. Tannins were present in *Vernonia amygdalina* and *Momordica charantia* but not in the combined *Vernonia amygdalina* and *Momordica charantia*. Steroids were found in *Vernonia amygdalina* and the combined *Vernonia amygdalina* and *Momordica*

charantia extract but not in *Momordica charantia*. Acetaminophen at a dose of 3000mg/kg significantly increased WBC count. *Vernonia amygdalina*, *Momordica charantia*, and the combined *Vernonia amygdalina* and *Momordica charantia* combined extract similarly improved liver biochemical indices, despite acetaminophen significantly raising ALT levels, confirming hepatotoxicity. The study revealed that, *Vernonia amygdalina*, *Momordica charantia*, and the combined *Vernonia amygdalina* and *Momordica charantia* extract exhibited similar hepatoprotective effects.

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Additionally, I posthumously acknowledge my late parents, whose unwavering love, encouragement, and sacrifices have been the cornerstone of my aspirations. I am also thankful to all my brothers and all those who rendered support in diverse ways to me during the course of my study.

DEDICATION

This work is dedicated to God Almighty whose faithfulness has brought me this far. Also, to my late father, Mr. Sylvester Kwadwo. Yeboah (Boaso) and late mother, Madam Esther Abena Afriyie.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

ABBREVIATIONS

VA	-	Vernonia amygdalina
MC	-	Momordica charantia
NAFLD	-	Non-alcoholic fatty liver disease
ANOVA	-	Analysis of variance
WHO	-	World Health Organization
PSC	-	primary sclerosis cholangitis
PBC	-	primary biliary cirrhosis
HBV	-	hepatitis B Virus
HCV	-	hepatitis C virus
HCC	-	hepatocellular carcinoma
BW	-	body weight
NAPQI	-	N-accytyl-p-benzoquinone imine
EDTA	-	Ethylenediaminetetraacetic acid
H and E	-	hematoxylin and eosin
W.H.O	–	World Health Organization

CHAPTER ONE

INTRODUCTION

1.0 Background to the Study

The liver is the largest internal organ found in vertebrates which performs several important biological functions such as detoxification, elimination of endogenous compounds, protein, and biochemical synthesis required for digestion and growth. The liver is one of the body's line of defense due to its crucial involvement in the detoxification of toxins and xenobiotics, as well as the regulation of various metabolic processes such as glycogen storage, red blood cell breakdown, and hormone production (Martini *et al.,* 2023). The liver's pivotal function in biotransformation, drug clearance, and its potential to influence other vital organs directly and indirectly, makes it very sensitive to damage (Reddy, 2019).

Liver diseases frequently advance from subclinical icteric hepatitis to necroinflammatory hepatitis, fibrosis, cirrhosis, and hepatocellular cancer (Johnson *et al.,* 2015). Liver diseases ranked as the eleventh most prevalent cause of mortality and the fifteenth most significant cause of morbidity in the year 2016. These diseases accounted for 2.2% of global deaths and 1.5% of disability-adjusted life years (Cheemerla & Balakrishnan, 2021). In 2017, liver disorders claimed the lives of 1.32 million people globally, with males accounting for two-thirds of the deaths and women accounting for one-third (Sepanlou, *et al.,* 2020).

Liver cancer accounts for 7.8 and 8.1% of global cases and deaths respectively according to El-Kassas & Elbadry, (2022). Cirrhosis-related mortality in Sub-Saharan

Africa recorded more than doubled between 1980 and 2010, with the Central African Republic, Gabon, Malawi, Uganda, and Cote d'Ivoire in the top 10% nations in 2010 affected with liver cirrhosis (Vento *et al.,* 2018). More than 50% of African liver disease patients admitted to hospitals are at the end-stage due to poverty, lack of trust in Western medicine, distance from healthcare facilities, and mortality is high (Myer *et al.,* 2013). Approximately 70 million individuals in Africa are affected by chronic viral hepatitis, with approximately 60 million cases attributed to hepatitis B and 10 million cases attributed to hepatitis C (Obeagu *et al*., 2016). Ghana is no exception when it comes to liver disorders. In 2020, W.H.O reported 6,053 mortality rates associated with liver diseases in Ghana, accounting for 3.46% of total deaths.

Ghana ranks thirty-fifth in the world with age-adjusted death rate of 36.74 per 100,000 (WHO, 2020). Hepatic tissue damage from viral hepatitis infections, excessive alcohol use, non-alcoholic fatty liver disease (NAFLD), and pollution are the main causes of liver diseases (Park *et al.,* 2017). Environmental contaminants, including physical, chemical, biological, and radioactive toxins, and contaminated food consumption harm human health (Elbakry *et al.,* 2022). Extended exposure to these factors causes 24% of worldwide sickness and 23% of fatalities (Xu *et al.,* 2022).

Acetaminophen is a popular analgesic (pain reliever) and antipyretic (fever reducer) medication. Acetaminophen is a popular drug among Ghanaians, particularly those living in rural areas that have limited access to healthcare services, due to its affordability and accessibility (Alhusaini *et al.,* 2022). In spite of its great safety record, acetaminophen toxicity represents a highly widespread kind of pharmaceutical toxicity on a global scale (Alhusaini *et al.,* 2022).

If untreated, acetaminophen overdose can cause acute hepatotoxicity, which is characterized by lethal massive hepatocyte necrosis (Wang, 2022). Hepatic damage can also develop as a result of inappropriate drug doses, chronic drug usage, and drug use in conjunction with other medications, resulting in acute, chronic, or irreversible liver disease (Church *et al*. 2018).

Medical plants have been the foundation of health care from ancient times and are now important in developing and developed countries for chemotherapeutic purposes (Farombi, 2003). Herbal medicine is essential in health, particularly in remote parts of impoverished countries with few healthcare facilities. W.H.O estimates that over three-quarters of the global population uses herbs to cure a variety of disorders, including liver problems (Daniyal *et al.,* 2019). Ghanaians have used herbs to treat several maladies for generations. Traditional herbal therapy is still widely used in Ghana, especially in rural areas without access to mainstream medicine (Peprah *et al.,* 2019).

Many medicinal plants are used in traditional medicine to treat liver diseases (Arman *et al.,* 2022). *Vernonia amygdalina* is a common African shrub in Asia, it is commonly found in drainage lines, natural forests, and commercial plantations (Kadiri & Olawoye, 2016). *Vernonia amygdalina* is in the Asteraceae family and commonly called 'African bitter leaf'. *Vernonia amygdalina* leaf is used in African cuisine to flavor soups and stews. It is used in herbal medicine for liver detoxification, immune system stimulation, and reducing inflammation (Oleka-Ariwodo, 2021). Bitter leaf is used for nutrition and therapy. *Vernonia amygdalina* is rich in vitamins and minerals, including vitamin C, calcium, and iron, and is a good source of dietary fibre (Saraphanchotiwitthaya & Sripalakit, 2015). Mweenda, (2017) found that *Vernonia*

amygdalina plant medicinal properties is due to the presence of compounds like vernolide, vernodalol, phenolics, flavonoids, hydroxycinnamic acid, steroidal saponins, tannins, and alkaloids found in the plant, this makes it effective in treating many diseases. *Momordica charantia* (MC) or bitter melon, has long been recognized for its medical values (Mukherje *et al.,* 2023). It is a tropical and subtropical vine of the *Cucurbitaceae* family that is widely planted for its edible fruit throughout Asia, Africa, and the Caribbean. Its various kinds vary significantly in terms of fruit form and bitterness. According to studies, *Momordica charantia* has been shown to exhibit anti-diabetic, anti-viral, anticancer, anti-leukemic, anti-bacterial, anthelmintic, anti-mutagenic, anti-mycobacterial, anti-oxidant, antiulcer, anti-inflammatory, hypo-cholesterolemic, hypotriglyceridemic, hypotensive, immunostimulant, and insecticidal properties (Jia *et al.,* 2017), these properties are as a result of its bioactive composition.

Mormodica charantia and *Vernonia amygdalina* have been shown to have significant hepatoprotective effects in animal studies. The two plants have been traditionally used singly in many African countries for the treatment of various diseases, and recent studies has confirmed their potential in the management and treatment of liver diseases (Aydin *et al.,* 2020). Synergistically, the two plants may work through different pathways to provide more comprehensive protection for the liver. However, further studies are needed to elucidate the combined potency of *Momordica charantia* and *Vernonia amygdalina* in treating liver diseases.

1.2 Problem Statement

The global impact of hepatic disorders on public health has become a matter of great concern (Asrani *et al.,* 2019). According to Asrani *et al*. (2019), liver diseases are ranked as the tenth leading cause of death globally, resulting in over 2 million deaths annually. In 2016, hepatic diseases accounted for 2.2% of deaths and 1.5% of disability-adjusted life years globally, ranking them eleventh and fifteenth in terms of morbidity and mortality, respectively (Cheemerla & Balakrishnan, 2021). Viral hepatitis is a major cause of liver disease in Ghana, with hepatitis B being the most common kind: It is projected that around 12% of Ghanaians have hepatitis B, making Ghana one of the countries with the highest hepatitis B prevalence in the world (Abesig *et al.,* 2022). Also, around 6% of the Ghanaian population is afflicted with hepatitis C (Agyeman *et al.,* 2016). Another major cause of liver disease in Ghana is excessive alcohol intake (Duah *et al.,* 2021), 13% of Ghanaians over the age of 15 frequently use alcohol, according to the World Health Organization (Hormenu *et al.,* 2018). Hepatotoxicity from consuming excessive alcohol can develop into liver cirrhosis (Hyun *et al.,* 2021). Non-alcoholic fatty liver disease (NAFLD) is also becoming extremely pervasive in Ghana (Ssentongo *et al*, 2022), a disorder in which fat builds up in the liver.

The country's NAFLD prevalence is believed to be over 30%, with obesity and diabetes being major risk factors (Van Dijk *et al*, 2022). According to W.H.O data from 2020, the death toll from liver disease in Ghana reached 6,053, accounting for 3.46% of all deaths, with an age-adjusted mortality rate of 36.74 per 100,000 people ranking Ghana thirty-fifth in the world. The prevalence of liver cirrhosis in Ghana has doubled in the last decade with Hepatitis B and C remaining the principal causes of liver diseases in the country, with prevalence rates of 12.3% and 2.4%, respectively. (Duah, *et al.,* 2020).

Despite significant breakthroughs in modern medicine, the cost of treatment for liver diseases, such as drug induced liver disorder, viral hepatitis, alcoholic and non-alcoholic fatty liver diseases, and liver cancer, are prohibitively expensive, making it difficult for many people to access conventional medication and treatment (Northup *et al.,* 2021). Diverse medicinal plants and their preparations are used to treat liver disorders in ethnomedical practices and traditional medicine in low and middle-income countries (Garedew *et al.,* 2018). The vast majority of herbal remedies expedite the liver's innate healing mechanism.

Most developing continents such as Africa and Asia have turned to traditional herbal remedies in the treatment of a variety of illnesses, including liver ailments because treating liver disorders with Western medicine is expensive (Singh *et al*, 2020). The availability and lower cost of herbal treatments have made traditional herbal usage in treating liver disorders a popular treatment option across the African continent (Peprah *et al.,* 2019).

The utilization of *Vernonia amygdalina* and *Momordica charantia* in the treatment of various ailments has been attributed to their significant phytochemical content, as documented by Ogbonna *et al*. (2017).

Research conducted in Nigeria on *Vernonia amygdalina* and *Momordica charantia* has examined the efficacy of traditional remedies in the treatment of several health conditions, including malaria, diabetes, anemia, and liver illness (Iyiol *et al.*, 2024). The hepatoprotective activity of *Vernonia amygdalina* extract has been shown to be highly promising, as indicated by several studies conducted by Tokofai *et al*. (2021),

Tilaye *et al*. (2018), Sitorus and Nerdy (2018), and Johnson *et al*. (2015). Similarly, a study conducted by Moharir *et al*. (2019), Abiola (2017), and Mada *et al*. (2014) collectively showed that the leaves of *Mormodica charantia* had therapeutic properties that can mitigate liver damage. This beneficial impact can be attributed to the presence of specific phytochemical constituents within the plant, which exhibit antioxidant activity. However, the existing body of literature on the combined effectiveness of *Vernonia amygdalina* and *Mormodica charantia* in the treatment of hepatic disease is limited. Consequently, the primary objective of this study is to examine the hepatoprotective effectiveness of a combined leaf extract of *Momordica charantia* and *Vernonia amygdalina* against acetaminophen induced hepatotoxicity in male Wistar rats.

1.3 Research Questions

The research questions that were addressed included:

i. what are the phytochemical compositions of *Vernonia amygdalina* and *Momordica charantia* and their combined ethanolic extract?
ii. to what extent could the *Vernonia amygdalina* and *Momordica charantia* extract produce hepatoprotection?
iii. to what extent could the *Vernonia amygdalina* and *Momordica charantia* extract affect blood parameters (WBCs, RBCs, and HB, HCT, MCV, MCH, MCHC)?
iv. to what extent could the *Vernonia amygdalina* and *Momordica charantia* extract affect liver biochemical parameters?
v. to what extent could the *Vernonia amygdalina* and *Momordica charantia* extract affect liver tissues?

1.4 Main Objectives of the Study

The main objective of this study was to investigate the hepatoprotective efficacy of combined *Momordica charantia* and *Vernonia amygdalina* leaf extract on acetaminophen-induced hepatotoxicity in Male Wistar rats.

1.4.1 Specific Research Objectives

The specific Objectives of the Research were to:

1. investigate the phytochemical compositions of *Vernonia amygdalina*, *Momordica charantia* and their combined leaf extract.
2. assess the effect of the *Vernonia amygdalina* and *Momordica charantia* and their combined leaf extract on blood parameters (WBCs, RBCs, and HB, HCT, MCV, MCH, MCHC.).
3. investigate the effect of the *Vernonia amygdalina*, *Momordica charantia* and their combined leaf extract on liver biochemical parameters.
4. ascertain the extent to which the *Vernonia amygdalina*, *Momordica charantia* and their combined leaf extract will affect liver tissue (histopathological studies).

1.5 Justification

Globally, the prevalence of disorders related to hepatotoxic agents that causes liver inflammation is on the rise (Reddy, 2019; Church *et al.,* 2018). As a result, there is an urgent need for developing countries such as Ghana to have an effective treatment that would help prevent and minimize the prevalence of liver diseases such as drug induced liver disorders and hepatitis at a cheaper cost and readily accessible. According to Mweenda (2017), *Vernonia amygdalina* plant has several medicinal properties that

have been linked to sesquiterpene lactones-like-vernolize and vernodalol, as well as phytochemicals such as phenolics, flavonoids, hydroxycinnamic acid, steroidal saponins, tannins, and alkaloids.

Mormodica charantia, on the other hand, possesses bioactive substances such as alkaloids, phenolics, saponins, tannins, flavonoids, carbohydrates, amino acids, terpenoids, steroids, and glycosides, making it particularly helpful in treating ailments such as diabetes and liver disorders (Karale *et al.,* 2022; Sheikh *et al.,* 2017; Mada *et al.,* 2014). A lot of research has been done on employing herbal treatments, particularly *Vernonia amygdalina* and *Momordica charantia*, in treating hepatic diseases. Therefore, it would be prudent to investigate the synergistic effects of *Vernonia amygdalina* and *Momordica charantia* on hepatotoxicity, which has not yet been documented in literature.

1.6 Significance of the Study

The importance of the liver in the regular functioning of the body systems cannot be overstated. Several biological functions are disrupted when the liver is impaired. Given the abundance of disorders linked with the liver, as well as the chronic nature of these diseases, the economic costs of their management are fairly large on individuals and society as a whole. This is especially true in Africa's poor emerging countries. As a result, cheaper natural sources, such as medicinal plants, could bring economic assistance and minimize out-of-pocket expenses for many patients.

This study seeks to demonstrate the ameliorative effect of a combined extract of *Vernonia amygdalina* and *Momordica charantia,* plants used singly in folk medicine,

in the attenuation of liver diseases induced by toxic substances. Hence justifying *Vernonia amygdalina* and *Momordica charantia* widespread use in African traditional medicine, as well as providing a less expensive, more readily available, and effective source of liver disorder treatment (Iyamah *et al.,* 2014). The findings of this study could lay the groundwork for the development of an effective plant-based supplement or drug to treat hepatic diseases, as well as bridge the knowledge gap about whether combined *Momordica charantia* and *Vernonia amygdalina* can offer effective hepatoprotection.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Liver and Its Functions

The liver is a large and complex organ located in the upper right quadrant of the abdomen, just below the diaphragm. The liver has a reddish-brown hue and possesses an average weight of roughly 1.5 kg in the adult human body. The organ in question is anatomically partitioned into two distinct lobes, namely the comparatively bigger right lobe and the relatively smaller left lobe. The aforementioned lobes are further subdivided into smaller segments, whereby each segment is endowed with its distinct blood supply, lymphatic drainage, and bile drainage. The liver is enveloped by a delicate layer of connective tissue known as a capsule, which serves to preserve its form and organization. The hepatic parenchyma, located underneath the capsule, consists of liver lobules, which are histologically constituted of polygonal columnar units of 0.5-2.0 mm. These liver lobules serve as the functional tissue of the liver (Juza & Pauli, 2014).

The liver lobules consist of hepatocytes, which are the principal cellular components of the liver, along with additional cell types including Kupffer cells, stellate cells, and endothelial cells (Elobu *et al.,* 2018). The liver encompasses several significant anatomical components, as elucidated by Elobu *et al.,* (2018), such as the hepatic portal vein, the hepatic artery, the common bile duct, the gallbladder, and the hepatic veins. The hepatic portal vein is responsible for transporting blood from the gastrointestinal system, pancreas, and spleen to the liver, while the hepatic artery is responsible for

delivering oxygenated blood to the liver. The common bile duct is responsible for transporting bile from the liver and gallbladder to the small intestine.

The liver exhibits a significant level of vascularity due to its reception of a dual blood supply derived from both the hepatic artery and the portal vein (Haobam, 2018). This unique vascular arrangement contributes to the liver's exceptional efficiency in executing its many physiological activities. Metabolism is considered to be one of the key physiological processes performed by the liver (Wang & Tontonoz, 2018). The liver assumes the role of metabolizing nutrients derived from meals into bioavailable forms that may be effectively used by the body for energy production. The process of metabolism involves the conversion of carbohydrates, lipids, and proteins into glucose, fatty acids, and amino acids, respectively. Moreover, the liver functions as a reservoir for glucose by storing it in the form of glycogen, then releasing it into the circulation to meet the body's energy demands. The liver also performs a crucial function in the process of detoxification. The process involves the removal of poisons and hazardous chemicals, including drugs, alcohol, and other contaminants, from the bloodstream (Tekade *et al*., 2023).

The liver employs enzymatic processes to metabolize these poisons into less deleterious compounds, facilitating their excretion from the body through urine or faeces (Gracia-Lor *et al.,* 2017). In addition to its primary functions, the liver is responsible for the synthesis and secretion of bile, a substance that plays a crucial role in the digestion and absorption of dietary fats as well as fat-soluble vitamins. The gallbladder serves as a reservoir for bile, which is then discharged into the small intestine as required. The liver plays a significant role in the synthesis of many proteins, such as albumin and clotting

factors (Ehlting *et al.,* 2021). Albumin plays a crucial role in the regulation of fluid equilibrium inside the human body, while clotting factors are required for the processes of homeostasis and tissue repair. The liver's diverse range of tasks renders it susceptible to many disorders, consequently impeding its normal physiological processes.

2.2 Liver Disorders

The liver's multifaceted role inside the human body renders it susceptible to several disorders (Nnamudi *et al.,* 2020). In the study conducted by Nnamudi *et al*. (2020), it was found that many risk variables have been identified as potential contributors to the increased susceptibility to hepatic disorders. These risk factors include high alcohol intake, obesity, familial predisposition, as well as exposure to toxins and chemicals. According to Asrani *et al*. (2019), Liver disease may manifest due to several factors, including drug induction, viral infections, metabolic disorders such as autoimmune deficiency, and genetic factors. For instance, hepatitis B virus, hemochromatosis, and type 2 diabetes are examples of conditions associated with liver illness (Chowdhury & Mehta, 2022). Liver disorders are often categorized as acute and chronic infections (Gustot & Jalan, 2019). According to Sowunmi & Gonzo, (2023), acute liver infections encompass a range of conditions such as hepatitis, hepatosis, liver cirrhosis, liver injury, acute hepatitis, and chronic active hepatitis B.

On the other hand, chronic infections comprise primary sclerosis cholangitis (PSC), primary biliary cirrhosis (PBC), alcoholic fibrosis, and alcoholic hepatitis (Rouf *et al.,* 2021; Kaushik & Lebwohl, 2019). Liver damage may lead to liver failure and can result in mortality if it is not appropriately treated and managed (Li, Sun & Liu, 2019). The symptoms associated with liver disorder might exhibit variability contingent upon the

gravity and fundamental etiology of the condition (Singal *et al.,* 2018). Several typical symptoms of liver disorder are weariness, weakness, jaundice, stomach discomfort, edema in the legs and abdomen, and pruritus.

Nevertheless, many liver illnesses may exhibit no symptoms and are only identified by regular blood tests or imaging examinations (Ofosu *et al.,* 2018).

2.3 Prevalence of Liver Diseases

Liver diseases are a leading cause of mortality and morbidity globally, accounting for two million fatalities annually (3.5 percent of all deaths) (Asrani *et al.,* 2019). According to Cheemerla and Balakrishnan, (2021), liver diseases ranked as the eleventh most significant contributor to mortality and the fifteenth major cause of morbidity in the year 2016. These conditions accounted for 2.2% of global deaths and 1.5% of disability-adjusted life years. According to a study conducted by Sepanlou *et a*l. (2020), it was found that liver problems were responsible for causing the deaths of around 1.32 million individuals worldwide in the year 2017. The study further revealed that males constituted two-thirds of the total fatalities, while females accounted for the remaining one-third. Based on the latest Global Cancer Statistics (Deo *et al.,* 2020), the prevalence and fatality rate of liver cancer instances in Africa constitute 7.8 and 8.1% of worldwide occurrences, correspondingly.

The death rate associated with cirrhosis in Sub-Saharan Africa had a significant increase of over 100% between 1980 and 2010. Notably, the Central African Republic, Gabon, Malawi, Uganda, and Cote d'Ivoire were in the top 10% of countries with the highest cirrhosis-related mortality rates in 2010 (Vento *et al.,* 2018). Asrani *et al*.

(2019), reported that a significant number of individuals in Africa, about 70 million, are affected by chronic viral hepatitis, this includes approximately 60 million individuals with hepatitis B and 10 million individuals with hepatitis C. Schmelzer *et al.* (2020), indicated that, the prevalence of Hepatitis A infection is high in low- and middle-income nations characterized by inadequate sanitary conditions and hygienic practices.

It has been shown that a significant proportion of children (90%) acquire the hepatitis A virus before reaching the age of 10, often without exhibiting any symptoms. Studies by Breakwell *et al*. (2017) indicated that, most African nations have a high prevalence of hepatitis B. However, the absence of adequate sanitation facilities and limited access to clean drinking water in many African countries has also contributed to the occurrence of hepatitis A (Van Abel & Taylor, 2018). According to a study conducted by Patterson *et al*. (2019), the epidemiology of hepatitis A in Africa indicates that the continent should not be classified as an area with high endemicity of hepatitis A virus (HAV), based on the available seroprevalence data. Cirrhosis and hepatocellular carcinoma are the main outcomes of persistent infection, HBV also causes acute and chronic liver injury. Transmission occurs mostly through contaminated blood or body fluid and this may happen via unprotected sexual contact, drug injectors sharing needles, and healthcare needlestick injuries. According to a report by Cao *et al*. (2022) about 296 million individuals worldwide have chronic hepatitis B.

Approximately 1.5 million new HBV infections occurred in 2019, with 820,000 fatalities from HBV-related cirrhosis or hepatocellular cancer. The prevalence of HBV infection exhibits significant variation among different areas designated by the World

Health Organization (WHO). Notably, the African and Western Pacific regions are particularly burdened, with corresponding prevalence rates of 6.1% and 6.2% in the general population (Mahamat *et al.,* 2021). Based on the 2022 study by the World Health Organization (W.H.O), it is estimated that the Hepatitis B virus, known for its high fatality rate, affects a population of about 91 million individuals in Africa. The World Health Organization's Western Pacific Region, including nations such as China, Mongolia, and the Pacific Islands, has the most significant incidence of hepatitis B (Spearman *et a*l., 2022).

According to Shaltiel *et al.* (2021), report, the global prevalence of HCV infection exceeds 200 million individuals, primarily concentrated in Asia and Northern Africa. Of those infected, approximately 80% progress to chronic HCV infection, with nearly 20% experiencing liver complications such as fibrosis, hepatocellular carcinoma, cirrhosis, and end-stage liver disease. In a study conducted by Tada *et al.* (2019), it was shown that a significant proportion exceeding 50% of hepatitis C virus (HCV) patients do not exhibit any noticeable symptoms during the early stage of infection. Consequently, these cases often go undiagnosed until the occurrence of advanced hepatic fibrosis.

Additionally, there are 3-4 million new infections and an annual mortality rate of 350,000 to 500,000 associated with this disease. The prevalence of Hepatitis C Virus (HCV) in the general population in Africa varies between 0.1% and 17.5%, with the specific rate being contingent upon the nation in question (Grace-Ifechukwudebelu *et al.,* 2017). According to Karoney & Siika, (2013), Egypt exhibits the greatest prevalence rate of 17.5%, followed by Cameroon with a prevalence rate of 13.8%, and

Burundi with a prevalence rate of 11.3%. According to Feutseu *et al.* (2023), the estimated prevalence of HCV in sub-Saharan Africa is around 2.9%. Despite the high incidence and infectious nature of the Hepatitis C Virus (HCV), its diagnosis and reporting across Africa, except Egypt, remain little addressed (Chaabna *et al.,* 2018).

The global incidence of mortality and morbidity associated with alcohol use, particularly in relation to liver problems, is substantial and has shown a notable upward trend (Mellinger, 2019). Alcohol-related liver disease is a widespread form of chronic liver disease on a global scale, including around 30% of hepatocellular carcinoma (HCC) cases and HCC-related mortality (Ganne-carrié *et al.,* 2021). Pimpin *et al*. (2018), indicated that, there is significant heterogeneity in the prevalence of primary liver cancer attributable to alcohol use across different nations and regions worldwide. According to an article by Liu *et al*. (2021) titled "Alcohol-Related Liver Disease" alcohol is responsible for about one-third of incidence instances of primary liver cancer on a global scale. The chronic use of alcohol induces changes in the structure and impairs the functional capabilities of the liver via the initiation of steatosis, steatohepatitis, and cirrhosis (Osna *et al.,* 2023). According to Mitra & Chowdhury, (2020), the global alcohol-attributable mortality rate in 2016 was recorded at 38.8 per 100,000 individuals, accompanied by a corresponding figure of 1759 disability-adjusted life-years (DALYs) per 100,000 individuals.

In contrast, Non-alcoholic fatty liver disease (NAFLD) is a significant hepatic illness characterized by a substantial prevalence rate. According to the article by Mitra *et al*. (2020) on the epidemiology of non-alcoholic and alcoholic fatty liver disorders, it was shown that the global prevalence of NAFLD is 25.24%, exhibiting significant regional disparities worldwide. The Middle East and South American nations have reported the

highest prevalence rates, mostly based on ultrasound data, at around 30%. Conversely, studies conducted in Africa, albeit limited in number, have revealed a much lower prevalence rate of 13% (Mitra *et al*., 2020).

2.4 Prevalence of Liver Diseases in Ghana

Liver diseases pose a substantial health burden in Ghana, akin to several regions around the globe. Ghana has seen a gradual rise in the prevalence rates of liver illnesses, such as hepatitis B and C, alcoholic liver disease, and non-alcoholic fatty liver disease (NAFLD), as reported by Nartey *et al*. (2022). Liver disease in Ghana has been a subject of scholarly investigation since the 1950s, with notable contributions from authors such as G.M. Edington. In his publication in 1957, Edington observed a prevalent occurrence of liver enlargement among the population, along with a high incidence of liver cirrhosis and liver cancer cases (Edington, (1957); Nartey, (2022). Consequently, liver disease has persisted as a substantial concern in Ghana, both historically and in the present day. Liver illness may manifest as either acute or chronic. Chronic liver disease is characterized by ongoing and progressive damage to the liver that persists for a duration beyond six months (Ofori-asenso & Agyeman, 2016). Agbozo *et al*. (2022), reported that, hepatitis B is a prevalent liver disease in Ghana. As per the classification provided by the World Health Organization (WHO), Ghana is designated as a region characterized by a substantial prevalence of hepatitis B infection, with an estimated proportion of 12% to 15% of the population being infected.

Senoo-Dogbey & Wuaku, (2022), indicated that, Ghana is among the nations that bear a significant burden of hepatitis B on a worldwide scale. Hepatitis C, albeit exhibiting a decreased prevalence in comparison to hepatitis B (Guure *et al.,* 2022), remains a

significant problem. According to the findings of Aboagye *et al*. (2021), in their study titled "Alcohol consumption among tertiary students in the Hohoe municipality, Ghana: analysis of prevalence, effects, and associated factors from a cross-sectional study," it was observed that the consumption of alcohol in Ghana has led to a notable concern, specifically in relation to the occurrence of alcoholic liver disease. Excessive alcohol intake has been shown to be associated with the development of liver inflammation, cirrhosis, and several other disorders affecting the liver (Liu *et al.,* 2021). According to Awimba, (2018), In his article titled " factors contributing to alcohol use among the youth aged (16-35)– a case study in Bolgatanga municipality, Ghana," showed that the incidence of alcoholic liver disease in Ghana is influenced by the cultural and societal acceptability of alcohol. Agyei-Nkansah (2017) stated that the incidence of non-alcoholic fatty liver disease (NAFLD) is on the rise in Ghana, posing a significant health concern. Non-alcoholic fatty liver disease (NAFLD) has a strong association with lifestyle-related variables, including obesity, sedentary behaviour, and bad dietary patterns (Valenzuela-Vallejo *et al.,* 2023). With the ongoing epidemiological change in Ghana, characterized by a growing prevalence of obesity and urbanization, it is anticipated that the incidence of non-alcoholic fatty liver disease (NAFLD) would also escalate. Based on the statistics provided by the World Health Organization (WHO) in 2020, the number of fatalities caused by liver disease in Ghana amounted to 6,053, accounting for about 3.46% of the total number of deaths. Ghana ranks thirty-fifth globally in terms of its age-adjusted death rate, which is at 36.74 per 100,000 inhabitants.

2.5 Treatment of Liver Disorders

The growing number of people suffering from liver dysfunction as a result of excessive consumption of alcohol and drugs, as well as other chemicals, has paved the way for researchers into herbal treatment (Maharaja *et al.,* 2020). Given the exorbitant expenses and restricted availability of conventional medications, the demand for alternative liver treatments is of utmost importance (Andrade *et al*., 2019). Medicinal plants provide a cost-effective and readily available option for liver treatment, extending its benefits to a wider community (Ahmed *et al*., 2023). Herbal medication has been utilized for many years to ease disorders related to the liver and other internal organs, and it has now become a favorable therapy internationally for pathological liver conditions (Xiong & Guan, 2017). Gupta *et al.* (2017), reported that, in recent years, researchers have employed scientific methods to examine the benefits of plants for the treatment of liver illnesses, albeit the mechanisms and modes of action of these plants, as well as their therapeutic efficacy, have not been validated in many cases.

Several hundred plants have been explored to yet, but only a few have been properly studied. Many people have indicated about a collection of herbal remedies used to preserve the liver, including Liv-52, Camellia sinensis (green tea), Glycyrrhizaglabra (licorice), Silymarin (milk thistle), and FuzhengHuayu (Jeslyne, 2017). According to Di Paolo *et al*. (2019), the growing popularity of herbal medications reflects their perceived efficacy in the treatment and prevention of disease, as well as the assumption that these treatments are safe since they are natural. For diverse liver disorders such as hepatitis, cirrhosis, and fatty liver disease, Western medicine provides a variety of pharmaceutical therapies and interventions. However, the prohibitive cost of obtaining

Western medicine for treating liver problems forces many developing countries to rely on herbal remedies (Mensah, *et al.,* 2019).

2.6 Vernonia amygdalina (VA)

Vernonia amygdalina, often known as bitter leaf, is a verdant leafy botanical specimen that has a distinctive smell and a pronounced bitter flavor. The plant's nomenclature, *Vernonia amygdalina,* was derived from the name of the 17th-century botanist, William Vernon, as documented by Asuzu (2018). *Vernonia amygdalina* is a woody shrub with a height ranging from 2 to 10 meters, displaying a quick regenerative capacity upon planting. The plant in question is a seedless species that is reproduced by the method of stem cutting. The Vegetative Apical Meristem (VAM) has an elliptical morphology in terms of leaf form, with a maximum length of around 20 centimeters. *Vernonia amygdalina* belongs to the plant group known as angiosperms, specifically classified under the order Asterales, family Asteraceae, genus Vernonia, and species VA.

According to Egharevba (2014), Vernonia is a genus including over 1,000 species of forbs and shrubs, with VA being the most notable species within this genus. According to the study conducted by Unegbu *et al*. (2020) titled "Phytochemical and Antibacterial Activities of Vernonia Amygdalina Leaves (Bitter Leaf) on two Drug-Resistant Bacteria," indicated that, bitter taste of Vernonia Amygdalina (VA) may be attributed to its bioactive ingredients, including alkaloids, saponins, tannins, and glycosides. The leaves of VA plants are used as a kind of green leafy vegetable and may be ingested in either their whole form or as an aqueous extract. These extracts are often employed as tonics in the treatment of different ailments (Unegbu *et al*. (2020)). The tender and newly sprouted foliage of the plant is mostly favoured for the management of several

health conditions, including diabetes, malaria, fever, constipation, high blood pressure, and as a laxative, among the indigenous communities residing in the southern region of Ghana (Asante *et al.,* 2017).

Plate 2.1*: Vernonia amygdalina* **(**Edamisan, 2019)

Table 2.1: Botanical classification of *Vernonia amygdalina*

Subdivision	Name
Kingdom	Plantae
Division	*Angiosperm*
Genus	*Vernonia*
Family	*Asteracea*
Species	*V. amygdalina*
Class	*Dycotyledone*
Order	*Asterales*
Botanical Name	*Vernonia amygdalina*
Common Names:	English- bitter leave Twi – Onwono French – Ndole Hausa- chusar-doki Yoruba - ewuro

As adapted from *(*Kaur *et al.,* 2019*,* Danladi *et al*., 2018, Sitanimezi, 2017).

2.7 Ecological Distribution of *Vernonia amygdalina*

Vernonia amygdalina is widespread throughout tropical Africa, including Nigeria, Ghana, Cameroon, and the Democratic Republic of the Congo (Oyeyemi *et al.,* 2018). Olivier *et al*. (2016), reported that this plant is widespread in Asia, Mexico, Central and South America, and North America. It can be found in lowland rainforests, savannahs, and even disturbed areas such as farmlands and roadsides (Danladi *et al.,* 2018). This extensive distribution demonstrates the plant's adaptability to various environmental conditions. According to Sitanimezi (2017), the genus Vernonia consists of more than 1,000 species, 500 of which are found in Africa and Asia, 300 in Mexico, central and south America, and 16 in North America.

Ikhajiagbe (2017) indicated that *Vernonia amygdalina's* tolerance to a variety of soil types is one of its most important adaptations. It can thrive in both sand and clay soils with good drainage. This adaptability enables the species to colonize a variety of

habitats and to expand its ecological range. Additionally, it has been observed that the plant thrives in areas with high rainfall, but it can also sustain dry periods, making it resistant to precipitation fluctuations. In many African societies, the ecological distribution of Vernonia amygdalina intersects with cultural practices (Gumisiriza, *et al.,* 2021). In some communities, *Vernonia amygdalina* is regarded as a valuable crop and is utilized as a verdant vegetable in a variety of traditional dishes (Mahmud, 2019).

2.8 Nutritional Values of *Vernonia amygdalina*

Research has indicated that *Vernonia amygdalina,* including its leaves, stems, and root, contains a range of essential nutrients such as proteins, fats, fibers, amino acids, minerals, vitamins, and carbohydrates (Alara *et al.,* 2017; Ojimelukwe & Amaechi, 2019). Previous research findings indicate that *V. amygdalina* leaves contain various components, including carbohydrates (4.31 mg/100 g), proteins (20.2 mg/100 g), lipids (15.0 mg/100 g), acids (10.26 mg/100 g), iodine (35.82 mg/100 g), hydrocyanic acid (0.46 mg/100 g), total oxalate (0.62 mg/100 g), and amino acids such as thiamine (170 mg/100 g), pyridoxine (2.6 mg/100 g), ascorbic acid (20.49 mg/100 g), glycine (4.63 mg/100 g), cysteine (1.84 mg/100 g), casein hydrolysate (96.99 mg/100 g), and nicotinamide (1.65 mg/100 g) (Alara *et al.,* 2017). Deguenon *et al*. (2020) reported that the primary minerals found in the leaves of *Vernonia amygdalina* are magnesium (Mg), calcium (Ca), sodium (Na), and potassium (K), whereas the secondary minerals present are copper (Cu), manganese (Mn), zinc (Zn), iron (Fe), and phosphorus (P).

2.9 Phytochemical Constituents of *Vernonia amygdalina*

Numerous researches have been conducted to isolate and characterize various bioactive components derived from *Vernonia amygdalina*. The phytochemical investigations

yielded the identification of many compounds including flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and multiple categories of sesquiterpene lactones (Alara *et al.,* 2017; Asante *et al.,* 2017).

2.9.1 Flavonoids

Flavonoids are a class of naturally occurring chemical compounds that are present in plants and have diverse phenolic structures. In a study conducted by Karak (2019), it was observed that polyphenolic chemicals are present in vascular plants in the form of aglycones, glucosides, and methylated derivatives. Flavonoids can be categorized into two distinct groups, namely flavone, and isoflavone, based on the specific arrangement of the benzenoid substituent. They are ubiquitously found in all regions of *Vernonia amygdalina* (Kaur *et al.,* 2019). Flavonoids play a crucial role in various aspects, including sensory perception, pigmentation, preservation of essential nutrients and biological catalysts, as well as inhibition of lipid oxidation (Speisky et al., 2022)

Studies conducted by Fawwaz *et al*. (2023) and Alara *et a*l. (2017) have demonstrated the presence of flavonoids in Vernonia amygdalina. In the review of the Phytochemical and Pharmacological Properties of *Vernonia amygdalina*, Alara *et al*. (2017) highlighted the significance of flavonoids in pharmacology. They emphasized that flavonoids play a crucial role in various physiological activities within the human body, such as antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anticancer, and antiviral functions.

2.9.2 Saponins

Saponins are a type of bioactive chemical found across the plant kingdom (Nguyen *et al.,* 2020). According to Abera *et al*. (2023) Saponins are natural chemicals that, when mixed with water, generate a soapy lather. Based on their chemical structure, they are classed as triterpenoids or steroidal saponins. According to Kaur *et al*. (2019), saponins are found in many plants and are thought to have a variety of activities, including defense against herbivores, disease resistance, and plant growth stimulation. *Vernonia amygdalina* saponins, such as vernoniosides, vernodalol, vernodalin, vernogenin, and vernolepin, have been found and isolated in studies by Wang *et al*. (2021). According to Danladi *et al*. (2018), these saponins have a wide range of biological activities and have been linked to a variety of health advantages.

Scaria *et al*. (2020) discovered that saponins have anti-mutagenic and anticancer properties and that they can reduce the risk of human cancers by preventing cancer cells from developing. According to *Rai et al*. (2021), one of the significant qualities of saponins is their ability to behave as natural surfactants, which allows them to interact with cell membranes and disturb their structure. *Vernonia amygdalina* saponins exhibit anti-inflammatory and antioxidant properties (Kaur *et al.,* 2019). Saponins have been shown in studies by Yi (2019), to reduce the production of inflammatory markers and oxidative stress in animal models, implying that saponins from bitter leaf may have therapeutic potential in conditions associated with chronic inflammation and oxidative damage, such as cardiovascular disease and neurodegenerative disorders.

2.9.3 Alkaloids

Alkaloids represent a category of fundamental, naturally existing organic compounds characterized by the presence of at least one nitrogen atom. The term "alkaloid" was initially assigned due to their propensity to undergo reactions with acids, resulting in the formation of salts. Alkaloids exhibit a wide range of significant physiological impacts on both humans and other animal species (Bhattacharya & Naitam, 2019; Pandrangi *et al.,* 2022). Alkaloids, in their unadulterated form, typically exhibit characteristics such as colorlessness, nonvolatility, crystalline solid state, and a propensity for possessing a bitter taste. Several alkaloids, including morphine, strychnine, quinine, ephedrine, and nicotine, have been identified in *Vernonia amygdalina* according to a study conducted by Gavhale *et al*. (2023) and Kandé *et al*. (2022). Alkaloids have a wide range of biological activities, including emetic, anticholinergic, anticancer, diuretic, sympathomimetic, antiviral, antihypertensive, analgesic, depressive, muscle relaxant, anti-inflammatory, antibacterial, and antiulcer properties (Asiedu, 2017). The authors Ojimelukwe and Amaechi, (2019), have demonstrated the presence of antioxidant properties in alkaloids using several experimental models and pathological contexts. Esther (2023), asserts that alkaloids possess sedative, antimalarial, and anticancer activities.

2.9.4 Tannins

According to a study by Oko *et al*. 2018, *Vernonia amygdalina* has four types of tannins. The research findings indicate that the percentage composition of tannic acid and acertannin is higher in both the leaves and stem of *Vernonia amygdalina* compared to hamamelitannin and leucopetunidin-3-glucoside. Tannins have the ability to bind to and cause the precipitation of proteins, as well as numerous other organic substances

such as amino acids and alkaloids (Okey *et al.,* 2023). In addition to its culinary uses, tannins have found application in many sectors, including leather tanning, ink manufacturing, and water treatment (Zhang *et al.,* 2023). *Vernonia amygdalina* possesses antioxidant qualities and has been the subject of research into its possible health benefits, such as its anti-inflammatory and anti-cancer activities, which are attributed to its composition of tannins (Ojimelukwe & Amaechi, 2019; Kaur *et al.,* 2019).

2.9.5 Phenolics

Various studies have provided evidence of the existence of phenolic compounds in *Vernonia amygdalina* (Thanh & Tran, 2021; Erukainure *et al*. 2019; Kaur *et al.,* 2019). Phenolic compounds are aromatic hydrocarbon groups that possess hydroxyl groups (-OH) inside their structure. The three primary dietary phenolic components of significant importance are flavonoids, phenolic acids, and polyphenols. Research has demonstrated that phenolics possess a diverse array of antioxidant capabilities, rendering them valuable as preventive agents against disease processes mediated by free radicals (Airaodion *et al.,* 2019). There are several different biological activities of phenolic acids, such as antioxidant, anti-ulcer, anti-inflammatory, anti-tumour, anti-spasmodic, anti-depressant, and cytotoxic effects (Fawwaz *et al.,* 2023). Based on the findings of Lutz *et al*. (2019), it has been observed that phenolic compounds possess protective properties against oxidative damage, hence mitigating the risk of developing degenerative diseases such as cardiovascular diseases, inflammation, and cancer.

2.9.6 Triterpenoids

Triterpenoids exhibit a broad distribution among several medicinal and food plant species. Triterpenoids have a wide range of structural diversity and are classified as natural compounds that possess a fundamental steroidal framework that undergoes numerous modifications (Dinday, & Ghosh, 2023). According to Wang *et al*. (2015), this phytochemical group is being examined for potential usage in new functional foods, cosmetics, foods, and healthcare items.

According to a study conducted by Sureda, *et al*. (2021), it has been found that certain triterpenoids, including ursolic acid, oleanolic acid, betulinic acid, and lupeol, exhibit significant anticancer effects. Nwozo, *et al.* (2023), have reported the isolation of triterpenoids from various plant parts of *Vernonia amygdalina*, including the leaf, root, and stem. *Vernonia amygdalina*, often known as bitter leaf, has a wide range of pharmacological activities, such as anticancer, anti-inflammatory, hepatoprotective, antioxidant, antibacterial, antileukemia, analgesic, and anti-nociceptive effects (Alara *et al.,* 2017; Boadu *et al.,* 2019; Jayaweera, 2022). Several isolated triterpenes include thiamine, ascorbic acid, pyridoxine, glycine, cysteine, casein hydrolysate, eucalyptol, beta piene, myrtenal, and alpha-muurolol.

2.10 Pharmacological Effects of *Vernonia amygdalina*

According to literature, *Vernonia amygdalina* has been used safely as a nutrition and medicine (Adedapo *et al.,* 2014; Ojimelukwe & Amaechi, 2019). Several studies have been conducted to demonstrate *Vernonia amygdalina* varied pharmacological effects.

Table 2.2: Potential Health Benefits of Components of *Vernonia Amygdalina*

Potential health benefits	Nature of nutrients and phytochemicals involved	Possible mode of action	References
Anti-malaria	vernodalol, vernoleptin Vernodalin, vernolin,	It inhibits plasmodium parasite growth	Abay *et al.,* 2015; Alara *et al.,* 2017; Kaur *et al.,* 2019; Ojimelukwe & Amaechi, 2019; Ejiofor *et al.,* 2020
Cancer prevention and management	Sesquiterpene lactones	Vernodalinol suppresses cancer cells proliferation	Johnson *et al.,* 2017; Asiedu, 2017; Yedjou *et al.,* 2018; Hasibuan *et al.,* 2020; Burhan *et al.,* 2022; Nerdy *et al.,* 2022
Diabetes prevention and management	Not yet known	Vernonia amygdalina stimulates glucose absorption and utilization in muscle and liver cells. It boosts glucose tolerance and postprandial blood glucose levels while also ensuring pancreatic beta cell regeneration.	Efiong *et al.,* 2013; Asante *et al.,* 2017; Erukainure *et al.,* 2019; Jayaweera, 2022; Esther, 2023
Antioxidant activity	Polyphenols, tannins, saponins, flavonoids	These phytochemicals mitigate the effects of stress, infections and radiations	Qing *et al.,* 2014; Asiedu, 2017; Alara *et al.,* 2018 Adeoye *et al.,* 2018; ; Raimi *et al.,* 2020; Akoto *et al.,* 2021; Esther, 2023;
Hepatoprotection	flavonoids	Reducing oxidative stress	M. Johnson *et al.,* 2015; Usunomena *et al.,* 2016; Tilaye *et al.,* 2018; Kaur *et al.,* 2019; Johnson *et al.,* 2021
Antimicrobial property	Sesquiterpene lactones (Vernodalol, vernolide)		Oboh & Masodje, 2009; Anibijuwon *et al.,* 2012; Alara *et al.,* 2017; Tunasamy *et al.,* 2019; Ali *et al.,* 2019; Akoto *et al.,* 2021; Hassan *et al.,* 2022;

As adapted from (Ojimelukwe & Amaechi, 2019).

2.11 Documented Effects of *Vernonia amygdalina on* Haematological Parameters

Haematological markers play a crucial role in evaluating the physiological condition of animals subjected to environmental and physiological variations (Ikwuka *et al.,* 2021). Numerous research investigations have been conducted to examine the impact of *Vernonia amygdalina* on haematological parameters. The findings of Johnson *et al*. (2021), demonstrated that methanolic leaf extract of *Vernonia amygdalina* exhibited a statistically significant decrease in white blood cell (WBC) counts in Wistar rats. However, there were no significant effects observed on red blood cell (RBC) counts and related indices (haemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC)when compared to the control group. This observation suggests that there was no occurrence of red blood cell (RBC) destruction and no alteration in the rate of RBC creation, as reported by Johnson *et al*. (2021).

In contrast, a study conducted by Airaodion *et al*. (2019) showed that administration of *V. amygdalina* leaf extract to Wistar rats resulted in a reduction in erythrocyte parameters in the blood. According to a study conducted by Oyedeji *et al.* (2013), it was proposed that the leaves of *Vernonia amygdalina* may possess the ability to hinder the release of erythropoietin from the kidneys. Erythropoietin serves as a humoral regulator for the production of red blood cells (RBCs) and has an impact on the blood's capacity to carry oxygen, as well as the quantity of oxygen transported to the tissues.
A study by Chike *et al*. (2018), demonstrated that the administration of ethanolic leaf extract of *Vernonia amygdalina* for 14 days did not result in a significant alteration in the erythrocyte parameters, including red cell count, haemoglobin concentration,

packed cell volume, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). However, prolonged administration of an ethanolic leaf extract of *Vernonia amygdalina* resulted in a noteworthy decrease in the blood concentrations of erythrocyte parameters, particularly for red blood cell (RBC) count, haemoglobin (Hb) level, and packed cell volume (PCV). This observation suggests that *V. amygdalina* may possess the ability to inhibit the release of erythropoietin from the kidneys, which serves as the hormonal regulator of red blood cell production.

The study conducted by Ikwuka *et al*. (2021), investigated the impact of *Vernonia amygdalina* on haematological indices and kidney function in rats subjected to stress. The findings revealed a statistically significant, albeit modest, elevation in white blood cell counts following the administration of *Vernonia amygdalina* leaf extract. The study conducted by Ikwuka *et al.* (2021), observed a considerable rise in haemoglobin concentration levels. This finding suggests that *Vernonia amygdalina* may possess the capacity to enhance the release of erythropoietin from the kidneys, which acts as the humoral regulator of erythrocyte synthesis.

Oyedeji *et al.* (2013), reported no significant effects on red blood cell (RBC) counts and indices (Hb, PCV, MCV, MCH, and MCHC) of Wistar rats treated with *Vernonia amygdalina* leaves extract. This indicates that there was no red blood cell breakdown or change in the rate of RBC formation (erythropoiesis). The treatment of *Vernonia amygdalina* leaf extract resulted in an insignificant rise in TWBC count. Furthermore, this study found that *Vernonia amygdalina* extract had no effect on platelet count,

which could indicate that Vernonia amygdalina does not have the potential to increase thrombopoietin production.

2.12 Effects of *Vernonia amygdalina* on liver Biochemistry

According to Uchendu, (2018), research findings, the administration of *Vernonia amygdalina* leaves extract resulted in a considerable reduction in the levels of AST, ALT, and ALP following the administration of acetaminophen, which had initially caused an increase in the levels of these enzymes. Uchendu, (2018), showed that the extract of *Vernonia amygdalina* had a significant impact on reducing the levels of serum total bilirubin and serum conjugated bilirubin in Wistar rats. This suggests that *Vernonia amygdalina* effectively mitigated the hepatotoxic effects caused by acetaminophen. Adegboye *et al.* (2017) published an article on "Hepatoprotective Effect of Methanolic Leaf Extract of *Vernonia Amygdalina* against Acetaminophen-Induced Hepatotoxicity in Wistar Albino Rats," revealed that administration of *Vernonia amygdalina* significantly decreased serum activities of ALT, AST, and GGT, which were increased in acetaminophen -treated rats.

Adegboye *et al*. (2017) found that *Vernonia amygdalina* leaf extract reduced lipid peroxidation, indicating the extract's antioxidant and anti-lipid peroxidative properties as well as its hepatoprotective effects (by lowering hepatic marker enzymes and restoring the level of proteins). Uchendu (2018), published article on Hepatoprotective Activity of *Vernonia Amygdalina* Leaf Ethanolic Extract in White Rats Induced by Acetaminophen showed that free radicals derived from acetaminophen oxidation by the liver caused integrity disruption of hepatocyte membrane, causing the release of various enzymes from hepatocytes, including Serum Glutamic Oxaloacetic Transaminase

(SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT). When these enzymes from the hepatocytes rise in the blood, it is a sign of liver damage. However, the results of the study by Hussain *et al*. (2021) showed that SGOT and SGPT levels of rats treated with *Vernonia amygdalina* leaf dropped significantly. This showed that the administration of *Vernonia amygdalina* leaf ethanolic extract protected the livers of male rats given acetaminophen

(Hussain *et al*., 2021)

2.13 Toxicology of *Vernonia amygdalina*

A study conducted by Alara *et al*. (2017), examined the toxicological properties of *V. amygdalina* leaf extracts on different rat species. The findings indicated that treated groups did not exhibit any clinical indications of toxicity or adverse toxicological effects, with the exception of a notable reduction in red blood cell count and a dose-dependent elevation in serum bilirubin levels. A study by Bolajoko *et al*. (2019), examined the metal contents and acute toxicity of a combination of *Vernonia amygdalina* leaves and Garcinia kola seeds. The findings of the study indicated that the extract of *Vernonia amygdalina* leaves did not exhibit toxicity towards rats, as evidenced by the absence of death in the acute oral toxicity trials. The histology examination conducted in this study demonstrated that there were no significant alterations observed in the kidney and liver of the rats which received treatment.

According to a study conducted by Juwita *et al*. (2021), the quantity of erythrocytes, leukocytes, haemoglobin, and hematocrit were not affected by variations in the dose and time of administration of the water fraction of *Vernonia amygdalina.* The findings of this study suggest that Vernonia amygdalina did not exhibit any harmful effects when

administered at doses of 10 mg/kg BW, 20 mg/kg BW, and 40 mg/kg BW in male white mice. Similarly, Imaga and Bamigbetan, (2013) found no significant variation in the glucose level, haematological profile, liver, and kidney function of the experimental rats following the administration of the extracts. According to the findings of Egharevba *et al.* (2014), the observed toxicity limit of *Vernonia amygdalina* leaf extracts was found to be negligible in comparison to compounds with high toxicity levels, which exhibit toxicity at levels below 1 mg/kg.

2.15 Momordica charantia

Momordica charantia (MC), commonly referred to as bitter melon, is a plant in the Cucurbitaceae family. It grows throughout the Amazon, Asia, South America, India, East Africa, and the Caribbean, and has traditionally been used as both food and medicine (Ahmad *et al.,* 2016). Bitter melon is an annual plant that is scandent and monoecious.

The puberulent tendrils are 20cm long, while the petioles are 4-6cm long and covered in white pubescent hairs. MC is a climber with a twining growth form that climbs using tendrils. Simple, palmately veined leaves with a lobed and crenate border are alternately placed along the stem. According to Tonu & Dutta, (2020), the leaves estimated diameter is 4-12cm, and its length is 4-12cm, membranous. The leaves have 5-7 partite lobes and are puberulent on the veins. The plant produces solitary yellow flowers. MC produces elongated fruits that look like warty gourds or cucumbers. Bitter gourd is monoecious, which means that both male and female flowers are produced on the same plant (Khan *et al.,* 2021). The unripe fruit is white or green and tastes harsh. *Momordica*

charantia grows well in tropical and subtropical environments, but it may also thrive in a variety of other environments (Uranw *et al.,* 2022).

(https://doi.org/10.1079/cabicompendium.34678)

Plate 2.2: *Momordica charantia* leaves

Table 2.3: Classification of *Momordica charantia*

Subdivision	**Name**
Kingdom	Plantae
Phylum	*Angiosperms*
Class	*Eudicots*
Order	*Cucurbitales*
Family	*Cucurbitaceae*
Genus	Momordica
Species	M. charantia
Botanical Name	*Momordica charantia*

As adapted from Khalid *et al.,* 2021.

2.16 Ecological Distribution of *Momordica charantia*

Momordica charantia occurs naturally in a variety of forms that can be identified by the size of the fruits. According to Şesan, (2020), there are 12 species in Asia and Australia and 47 species in Africa (24 dioecious and 23 monoecious).

Bitter gourds prefer quite high temperate climates with temperatures of 25°C and above (Bisbis *et al.,* 2018). The common cucurbit, *Momordica charantia* is found throughout Africa's wild flora, occurring almost everywhere in tropical Africa, according to research by Schaefer & Renner, (2010). Shamsi & Miya, (2020), stated that *Momordica charantia* is sporadically grown in East Africa, primarily by people of Asian descent using Asian cultivars. According to Shamsi and Miya, (2020), MC is a significant market vegetable in southern and eastern Asia, including southern China, India, Sri Lanka, Vietnam, Thailand, Malaysia, and the Philippines.

Table 2.4: Major bioactive and Nutritional components of *M. charantia*

Bioactive components	Distribution	References
Terpenoids	leaves, fruit, Stem	Sur & Ray, 2021; Oyelere *et al.,* 2022; Masithoh *et al.,* 2019; Daniel *et al.,* 2014
Phenolics	Fruit, pericarp, seed	Khalid *et al.,* 2021; Valyaie *et al.,* 2021;Sur & Ray, 2021; Torre *et al.,* 2020; Lopes *et al.,* 2020;
Sterols	Pericarp, fruit	Tanwar & Khatri, 2023; Nuchtavorn *et al.,* 2023; Sur & Ray, 2021; Shivanagoudra *et al.,* 2019; Mohammed & Sevindik, 2023
alkaloids momordicin	leaves and fruits	Singh *et al.,* 2023; Satya Vani Chekka & Naresh Kumar Mantipelly, 2020; Saeed *et al.,* 2018; Chanda *et al.,* 2019; Ahmad *et al.,* 2016;
Saponins	Fruit, root, seed	Melo *et al.,* 2022; Luis *et al.,* 2019; Jia *et al.,* 2017; Daniel *et al.,* 2014;
Charantin	leaves, fruits, Stem	Altinterim, 2012; Nagappan *et al.,* 2018; Amarowicz & Manea, 2021
Peptides and proteins	Seed	Melo *et al.,* 2022; Saeed *et al.,* 2018; Tufail *et al.,* 2018
Polysaccharides	Various parts of plants Seed	Saeed *et al.,* 2018; Deng *et al.,* 2014; Fan *et al.,* 2019; Saeed *et al.,* 2018; Sahu
Lipids	Seed, flesh	Rishabh Kumar, Jain Ashish & Bansal, 2011

As adapted from Jia *et al.,* 2017

2.17 Pharmacology of *Momordica charantia*

Momordica charantia, commonly known as bitter melon, is a highly esteemed vegetable in various culinary traditions. Studies have provided evidence indicating a significant association between a substantial consumption of fruits and vegetables of *Momordica charantia*, and a decreased likelihood of acquiring both acute and chronic diseases (Demmers *et al.,* 2022). These diseases include but are not limited to cardiovascular disease, cancer, diabetes, and hepatotoxicity (Singh *et al.,* 2023). According to Talebi, (2020), *Momordica charantia* demonstrates utility in a wide range

of metabolic and physiological processes within the human body. Khalid *et al*. (2021) have reported that *Momordica charantia* exhibits various pharmacological actions.

Table 2.5: Some documented potential health benefits of *Momordica charantia*

Potential health benefits	Nature of nutrients and phytochemicals involved	Possible mode of action	References
Anti-Oxidant Properties	Phenolics	collapses cell membranes to inhibit pathological conditions	Chahar & Sharma, 2017; Satya Vani Chekka & Naresh Kumar Mantipelly, 2020; Khalid *et al.,* 2021; Alper & Özay, 2022;
Anti–Diabetic Properties	Steroids	lowering blood glucose levels by ensuring increase glucose utilization by the liver	Sahu Rishabh Kumar, Jain Ashish & Bansal, 2011; Altinterim, 2012; Joseph & Jini, 2013; Panday *et al.,* 2014; Xu *et al.,* 2015; Chanda *et al.,* 2019; Parra *et al.,* 2021
Antimicrobial Property	steroids, flavonoids, alkaloids, and tannins	Significant inhibitory effect on microbes	Mwambete, 2009; Mada *et al.,* 2013; Leelaprakash *et al.,* 2014; Masithoh *et al.,* 2019; Satya Vani Chekka & Naresh Kumar Mantipelly, 2020;
Anticancer Property	triterpenes, steroids	Suppress the development of abnormal cells.	Li *et al.,* 2012; Upadhyay *et al.,* 2015; Singh *et al.,* 2016; Jia *et al.,* 2017; Bortolotti *et al.,* 2019
Hepatoprotective properties	Flavonoids, Saponins	Reducing oxidative stress	Saboo *et al.,* 2013; S. Mada, 2014; Salem *et al.,* 2019; Singh & Maheshwari, 2023; Aydin & Kaya, 2020;
Antimalarial activity	alkaloids momordicin	Inhibits growth of plasmodium parasite	Upadhyay *et al.,* 2015; Ahmad *et al.,* 2016b; Akanji *et al.,* 2016; Gandhi *et al.,* 2018;

As adapted from Mohammed & Sevindik, 2023*; J*ia et al., 2017.

2.18 Effects of *Momordica charantia* on Haematological Parameters

According to research conducted by Obiandu *et al*. (2020), administration of *Momordica charantia* leaf extract at doses of 200 mg/kg bw and 400 mg/kg bw had no significant effect on the growth and differentiation of the red blood cell series in the bone marrow, nor did the extract of *Momordica charantia* result in a significant change in the concentration of haemoglobin. Additionally, packed cell volume (PCV) was not significantly altered. According to Obiandu *et al*. (2020), the 200 mg/kg dose of *Momordica charantia* extract led to an increase in the WBC count in the bodies of treated rodents. However, the increased dose had no discernible effect on the WBC count. The number of platelets increased after the administration *of Momordica charantia* extracts. Umar (2017), published article on Effects of Oral Administration of *Momordica charantia* on haematological Parameters of adult Albino Rats indicated that oral administration of *Momordica charantia* resulted in the elevation of WBC count.

However, there was no statistically significant change in RBC count between the experimental and control groups. *Momordica charantia* increased lymphocyte, neutrophil, and eosinophil counts after oral treatment (Umar, 2017). Red blood cell (RBC) count, haemoglobin (Hb), and packed cell volume (PCV) counts indicated changes in the rate of RBCs formation, as reported by Husna *et al*. (2013) after administration of *Momordica charantia* leaves extract. White blood cell count, mean corpuscular volume and mean corpuscular haemoglobin concentration levels were not significantly different after *Momordica charantia* leaf extract administration. Husna *et al*. (2013) found that administration of *Momordica charantia* leaves extract resulted in a correlation decrease in red blood cell (RBC) count, haemoglobin (Hb), and packed cell volume (PCV) counts, indicating changes in the pace of RBC synthesis. In addition,

there were no significant differences in white blood cell count (WBC), mean corpuscular volume (MCV), or mean corpuscular haemoglobin concentration (MCHC) levels after administration of *Momordica charantia* leaves extract.

2.19 Documented Findings on the Effects of *Momordica charantia* on Liver Biochemistry

The study conducted by Oyesola *et al*. (2021) investigated the impact of administering Leaf extract of *Momordica charantia* on liver enzymes, duodenum antioxidant enzymes, and disaccharidases, as well as the histology of the liver and duodenum. The results indicated that the administration of leaf extract of *Momordica charantia* at doses of 100 mg/kg body weight and 400 mg/kg body weight led to a significant reduction in total protein (TP), albumin (Alb), aspartate aminotransferase (AST), and alanine transaminase (ALT) levels in Wistar rats. However, the administration of Leaf extract of *Momordica charantia* at doses of 100 mg/kg body weight and 400 mg/kg body weight resulted in a substantial increase in ALP levels. Studies by Cyril-olnta and Monkhua(2016), showed that, the administration of leaf extract of *Momordica charantia* did not have any significant effect on liver ALT nor AST.

Serum and liver total proteins, serum albumins, and globulins were also not significantly altered by *Momordica charantia* administration. According to Cyril-olnta *et al*. (2016), the administration of M charantia at doses of 100 and 200 mg/kg body weight for a duration of 28 days did not result in any detrimental effects on the hepatic integrity or synthetic capacity of the liver in normal mice. The research conducted by Salem *et al*. (2019) demonstrated that the leaf extracts of *Momordica* charantia exhibited notable effects on liver enzymes, specifically AST, ALT, and ALP, by

dramatically lowering their levels. Salem *et al*. (2019) reported that the administration of MC fruit and leaf extract resulted in decreased levels of ALT, AST, and ALP in the blood. This observation suggests that the extract may have a protective effect on the plasma membrane, mitigating the damage caused by acetaminophen. According to Salem *et al.* (2019), the hepatoprotective function of MC extracts appears to be attributed to the presence of flavonoids, as well as other constituents like saponins, tannins, and alkaloids. These compounds possess the capacity to effectively scavenge free radicals, hence enhancing the activity of antioxidant enzymes.

2.21 Toxicology of *Momordica charantia*

Husna *et al*. (2013), reported in their article on Acute Oral Toxicity Effects of *Momordica charantia* in Sprague Dawley Rats that no death occurred among the experimental animals following the administration of 300mg/kg and 2000mg/kg dosages of *Momordica Charantia* leaf extracts, but the rats did experience depressive disorders and dizziness for the first 30 minutes after feeding. According to Husna *et al.* (2013), the lethal dose (LD50) of *Momordica charantia* ethanolic extract is regarded safe to consume at less than 2000 mg/kg. However, the study reveals that the maximum dose may have harmful consequences to the blood, tissue, and important organs, particularly the liver. Lalèyè *et al*. (2016) reported that a dose of 2000 mg/kg of ethanolic and aqueous extracts had no adverse effect on rats.

Their result revealed that this plant has a non-destructive effect on the liver and kidneys, which was validated by histological investigation. According to Patil & Patil, (2011), in vivo clinical trials have shown that all portions of the bitter melon plant including it leaves are generally low in toxicity when consumed orally. According to Patil and Patil

(2011), when extracts are given intravenously, however, toxicity and even death have been documented in laboratory animals. Other research has found that extracts of the fruit and leaf (taken orally) are safe during pregnancy (Patil & Patil, 2011).

2.22 Acetaminophen

Acetaminophen (N-acetyl-p-aminophenol) is a frequently prescribed analgesic and antipyretic (Freo *et al.,* 2021). Acetaminophen is a commonly used over-the-counter medication, and Tylenol and Panadol are well-known brand names. It is a non-opioid analgesic and antipyretic used to treat fever, common cold, headache, mild to moderate muscle pain, arthritis, neuralgia and many others (Petrovic, 2023). Due to its availability and low cost, acetaminophen is utilized in numerous pharmaceutical products. Some historical records indicate that acetaminophen has existed since 1852 (Toussaint *et al.,* 2010). Acetaminophen is available in several pharmaceutical formulations, including chewable tablets, soluble or effervescent tablets, suppositories, capsules, syrup/liquid, and intravascular injections (intravenous) (Saccomano, 2019).

A publication by Simkin *et al*. (2012) provided indications that the prevalence of acetaminophen can be attributed to its widespread availability on the market, as well as its production and distribution by numerous pharmaceutical companies under various brand names, including but not limited to Panadol, Entamol, and Novadol. It is readily available for purchase without a prescription from both chemical shops and pharmacies, therefore obviating the necessity for medical authorization. Acetaminophen is widely utilized as a pharmaceutical intervention for the management of pain and fever in various regions, including the United States, Europe, and Asia (Kholili *et al.,* 2023). Acetaminophen has emerged as a widely misused analgesic in Ghana, with individuals

frequently consuming this prescription in response to minor discomfort or headaches, sometimes without adequate awareness of its potential adverse effects on crucial internal organs, such as the liver (Debrah, 2020).

2.23 Acetaminophen Metabolism and Toxicity

The liver is the primary site of acetaminophen metabolism, which involves two major pathways: glucuronidation and sulfation. According to the glucuronidation pathway, the association of acetaminophen and glucuronic acid results in the formation of acetaminophen glucuronide. The glucuronide of acetaminophen is then eliminated in the urine. The sulfation pathway, on the other hand, begins with the conjugation of acetaminophen with sulfate to produce acetaminophen sulfate, which is excreted via urine due to its water solubility. A small amount of acetaminophen is also metabolized by the cytochrome P450 enzyme (CYP2E1) to yield N-accytyl-p-benzoquinone imine (NAPQI) (Salem *et al.,* 2019). This substance is detoxicated by combining it with glutathione to produce mercapturic acid. Excessive NAPQI production may result in liver detoxification.

According to a study conducted by Salem *et al.* (2019), the administration of acetaminophen has been found to induce liver toxicity in animal model experiments, resulting in damage to hepatocytes. Hepatocyte damage of the liver occurs due to the generation of reactive oxygen radicals resulting from the biotransformation of acetaminophen into NAPQI, a highly reactive radical. This process can lead to hepatocyte damage and a decrease in the levels of antioxidant enzymes, particularly GPx. According to Abiola *et al*. (2017), the administration of acetaminophen at high dosages has been found to cause hepatotoxicity, mostly attributed to the excessive

generation of its intermediate oxidative metabolite, N-acetyl-p-benzoquinone imine (NAPQI), resulting in the development of lipid peroxides.

Rotundo & Pyrsopoulos, (2020), showed that, dosage and timing of acetaminophen ingestion in relation to the administration of N-acetylcysteine (NAC) treatment are significant determinants in the development and intensity of acetaminophen-induced liver damage. Acute liver injury may manifest when the utilization of a medication is within or below the prescribed daily maximum dosage of 4000mg. Acetaminophen poisoning commonly arises from the consumption of acetaminophen above the recommended maximum dosage (Rotundo & Pyrsopoulos, 2020).

2.24 Effects of Acetaminophen on Haematological and Biochemical Parameters

According to Juma *et al*. (2015), administration of Acetaminophen at a dosage of 2 g/kg bw to male rats resulted in a significant decrease in RBCs and haemoglobin (Hb) concentration, but not in WBCs count. Rahman *et al.* (2022), showed that oral administration of acetaminophen at a dose of 200 mg/kg resulted in a significant decreased in total neutrophils, monocytes, eosinophils, and platelets. Studies by Payasi *et al*. (2010), showed that, no significant changes were observed in haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelet counts, and erythrocyte sedimentation rate (ESR) in Wistar rats treated with acetaminophen Infusion while increasing leucocyte and lymphocyte counts in rats.

Research conducted by Wadekar *et al.* (2014), demonstrated that the administration of acetaminophen at a dosage of 2kg/mg led to a significant increase in the concentrations

of ALT, ALP, and bilirubin in rats. This finding demonstrates that excessive doses of acetaminophen result in hepatotoxicity, as evidenced by elevated levels of ALP, ALT, and bilirubin. Omeodu *et al*. (2022) demonstrated that the oral administration of 10mL/kg of acetaminophen for 10 days to Wistar rats led to a notable elevation in the levels of AST, ALT, ALP, and bilirubin. Conversely, there was a decrease in the levels of serum albumin and globulin. A study conducted by Ekam and Udosen, (2012) demonstrated a notable decrease in the total protein and globulin levels in rats subjected to acetaminophen challenge. However, there was no significant decline observed in the albumin levels. Work done Omeodu *et al.* (2022), reported that, treatment of acetaminophen at 10 mL/kg per day for 10 days to Wistar rats resulted in a significant increase in AST, ALT, ALP, and bilirubin levels while serum albumin and globulin levels were lowered. Ekam & Udosen (2012), discovered a substantial drop in liver total protein and globulin levels in acetaminophen-treated rats, but no significant decrease in albumin levels. Rahimi *et al.* (2022), study on Hepatorenal Protective Effects of Hydroalcoholic Extract of Solidago canadensis L. against Acetaminophen-Induced Toxicity in Mice, indicated that, acetaminophen administration resulted in a significant reduction in levels of, ALP, ALT, AST, bilirubin in mice. Rahimi *et al*. (2022) also reported that acetaminophen-induced mice displayed a substantial increase in serum total protein and albumin levels, although total bilirubin levels were found to have not considerably changed, direct bilirubin levels were found to have increased significantly.

2.25 Acetaminophen and Oxidative Stress

Oxidative stress arises from a disparity between the generation and buildup of oxygen reactive species (ROS) in cells and tissues, and the ability of a biological system to

eliminate these reactive substances (Afzal *et al*., 2023). Overdoses of drugs have also been linked to oxidative stress. Acetaminophen overdoses have been shown to induce oxidative stress by inducing hepatotoxicity (Sitanimezi, 2017). In cases of acetaminophen toxicity, saturation of glucanosyltransferases and sulfotransferases occurs, which redirects the drug's metabolism to cytochrome P450, resulting in the production of NAPQI in quantities that deplete glutathione (Arconzo *et al*., 2023). NAPQI accumulates in hepatocytes when glutathione levels are low, forming covalent bonds with cellular proteins and altering their structure and function (Chun, 2009). With the resulting cellular disruption, the activity of calcium ATPase decreases and cytosolic calcium levels rise (Saab, 2013). The abnormal cellular calcium homeostasis results in altered hepatic cell permeability, with the formation of blebs in the cell membrane and loss of its structural integrity (Celes, 2013).

2.26 Silymarin

Silymarin is a standardized flavonolignan extract produced from the seeds of the milk thistle Silybum marianum (Adetuyi *et al.,* 2021). The main components of silymarin are silybin, silidianin, silychristin, and isosilybin with silychristin, and isosilybin being major components of silymarin (Mihailović *et al.,* 2023). In terms of antioxidant and anti-inflammatory action, silybin is regarded to be the most physiologically active component of silymarin extract. Silymarin has been used for over 2000 years as a natural medicine for treating hepatitis and cirrhosis, as well as to protect the liver from toxic substances (Hamza *et al.,* 2016). Silymarin acts by anti-oxidative, immunomodulatory, and liver regenerating mechanisms in experimental liver diseases (Hamza *et al.,* 2016).

Silymarin also has an impact on the production of RNA and DNA. Silymarin protects the hepatocyte membrane and prevents harmful or xenobiotic chemicals from entering (Abd Eldaim *et al.,* 2021). Its phenolic composition allows it to donate electrons to stabilize FR and reactive oxygen species (ROS). Silymarin also affects intracellular glutathione, which prevents membrane lipoperoxidation (Abd Eldaim *et al.,* 2021). According to Abd Eldaim *et al.* (2021), silymarin plays a role in preserving the structural integrity of the hepatocyte membrane and inhibiting the infiltration of harmful chemicals or xenobiotics. By virtue of its phenolic properties, it exhibits the ability to donate electrons for the purpose of stabilizing free radicals (FR) and reactive oxygen species (ROS). According to Haddadi *et al*. (2020), the impact of silymarin extends to intracellular glutathione, hence inhibiting the process of lipoperoxidation in cellular membranes.

CHATPER THREE

MATERIALS AND METHODS

3.1 Study Location and Duration

The study was carried out in Asante Mampong in the Asante Region of Ghana at the Animal Science Department of the College of Agriculture Education of the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (Mampong-Asante Campus). Asante Mampong constitutes one of the six municipality within the Asante Region of Ghana, which has a total of twenty-seven districts. According to the Draft Strategic Environmental Assessment Report (2010), Asante Mampong is located approximately 57 kilometers away from Kumasi, which is the regional capital of Asante (Frimpong, 2015). The geographical location of the area under consideration is situated in the northern region of the Asante Region. It is next to the Sekyere East, Afigya-Sekyere, and Ejura Sekyeredumasi districts in the north, east, south, and west directions respectively, as reported by Yar *et al.* (2023).

The Asante Mampong Municipality is situated between the longitudes of 0.05 and the latitudes of 6.55°N and 7.30°N. According to Yar *et al.* (2023), the municipality experiences an ambient temperature of 28 °C and a relative humidity of 63%. According to Frimpong (2015), the Municipality experiences an average annual precipitation of 1270 mm, characterized by two distinct rainy seasons. The primary rainy season commences in March and reaches its peak in May. Mampong-Asante is situated within the transitional vegetation zone of Ghana, characterized by a semi-deciduous forest ecosystem interspersed with solitary trees and shrubs. This plant type supports a dense understory of grass cover, as reported by the Meteorological station

(MSD) in 2008. Agriculture serves as the primary economic sector within the municipality, engaging approximately 67.30% of the total labor force (Yar *et al.,* 2023). Additionally, the commerce industry also contributes to employment opportunities for a portion of the working population. The municipal district encompasses a comprehensive network of markets, financial institutions, wholesalers/retailers, transportation enterprises, hotels, and restaurants. The study was carried out within a period of eight months commencing from 1st February, 2023 – 30th August, 2023. However, animal experimentations took a period of four (4) weeks beginning from June 17th and to July 14th, 2023.

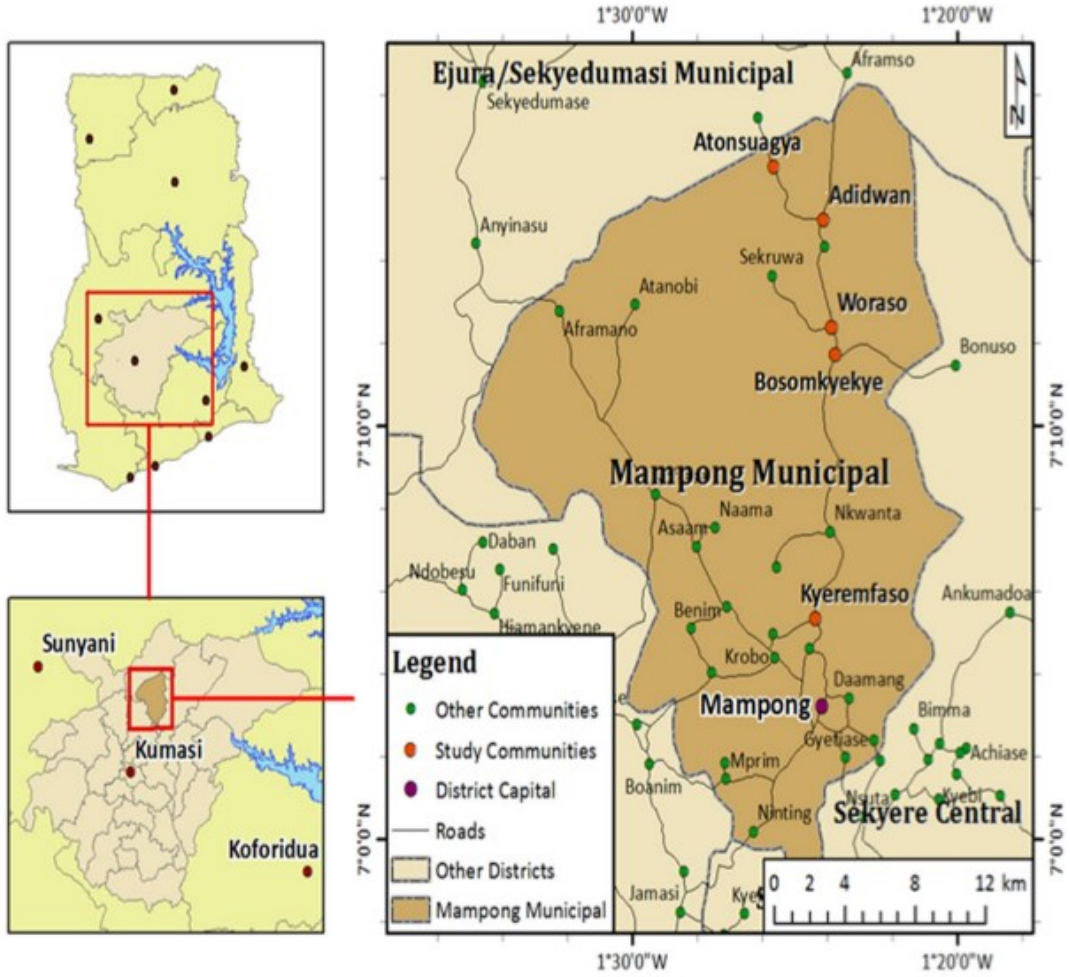

Plate 3.1 Map of study area. The image was adapted from (Forkuoh *et al.*, 2018).

3.2 Experimental Design

The study employed a completely randomized design (CRD). A total of thirty (30) male Wistar rats, with weights ranging from 120g to 160g, were acquired and accommodated in the Animal House facility at Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Asante, Mampong Campus, for the purpose of this investigation. The animals were provided access to food and water *ad libitum* and were given a period of 14 days to acclimatize to their environment prior to the start of the experiment. The Wistar rats were assigned to six groups via randomization, with each group consisting of five males.

Group 1: (normal control) received 1ml of 9% NaCl (Normal Saline) for 28 days

Group2: (positive control) received 3000mg/kg acetaminophen +140 mg/kg silymarin for 28days

Group3: (negative control) received 3000mg/kg acetaminophen and left for 28days

Group4: received 3000mg/kg acetaminophen + 100mg/kg *Vernonia amygdalina* extract for 28days

Group5: received 3000mg/kg acetaminophen + 100mg/kg *Momordica charantia extract* for 28days

Group6: 3000mg/kg acetaminophen + 200mg/kg *Vernonia amygdalina* extract + *Momordica charantia (bitter melon*) for 28days

3.3 Maintenance of Experimental Animals

3.3.1 Housing

The Wistar rats used for the study were accommodated in galvanized metal cages with a 2mm wire mesh. The cages were divided into six compartments, each measuring

80cm x 70cm x 50cm, and were furnished with soft wood shavings as bedding material. The soft wood shavings were regularly replaced to maintain a clean environment. Each individual compartment was equipped with an opening located at its uppermost section, facilitating entry into the interior of the enclosure. The ambient temperature within the room was consistently regulated at 26 ± 3°C, with a 12-hour light-dark cycle. Test materials were administered orally using an oral gavage. The animals were identified using fur dye and picric acid. The study adhered to the National Institute of Health Guidelines for Care and Use of Laboratory Animals while conducting all animal studies, procedures, and techniques.

Plate 3.2: Experimental animals (Wistar rats) in cage units

3.3.2 Feeding

Experimental animals were fed food and water a*d libitum*. They were provided with commercially formulated diet obtained from Galdus Ghana Limited. The feed consisted of a combination of the following components: maize, soybean meal, nutricell, wheat, grits lecithin, feed-phosphate, lysine, sulfate, fishmeal, limefine, sunflower oil, mould inhibitor, sodium chloride (salt), oat, premix, liquid choline chloride, xylanase, phytase, high-protein sunflower, Vitamin E, wheat peas, corn gluten, and rapeseed meal. The manufacturer provided the calculated composition of the feed, which is expressed as a percentage (%) of metabolizable energy (ME) at 3150 Kcal/kg. The composition of the feed includes the following components: crude protein at a level of 22.00%, crude fat at a level of 7.50%, lysine at a level of 2.50%, methionine at a level of 1.30%, methionine plus cysteine at a level of 0.60%, calcium at a level of 0.95%, sodium at a level of 0.95%, phosphorus at a level of 0.60%. Additionally, the feed contains the antioxidant E321, enzymes 4a11/4a24, a mould inhibitor, and added vitamins. (Source: feed Label, Koudjis Animal Nutrition).

3.4 Plant Collection

The leaves of *Momordica charantia* and *Vernonia amygdalina* were taken from Hwediem, a town located within the Mampong municipality. The process of plant authentication was done utilizing the online identification software known as Pl@ntNet, which may be accessed at the web address: https://identify.plantnet.org.

3.5 Plant Extraction Procedure

The plant extraction was conducted with the Mother Tincture process developed by Jean Michael in 1994 as described by Effah-Yeboah *et al*, (2021). The leaflets of

Momordica charantia and *Vernonia amygdalina* were isolated from the stem and subjected to a thorough cleaning process using distilled water to eliminate any potential contaminants. The leaves were subjected to a two-week air-drying process at room temperature (26±1°C) within the Science laboratory of the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development. The dried leaves were pulverized into a fine powder using an electric blender, subsequently sifted to get a refined consistency, and finally kept in sealed zip bags. In order to achieve sufficient dilution and extraction, a quantity of 50 grams of powdered leaves was immersed in 500 milliliters of ethanol (90%) for a duration of three days. This process was conducted in sterile plastic containers, with intermittent agitation, at a constant room temperature of 26°C. The extract was filtered using a 1.5-micron Whatman filter paper obtained from Sigma Aldrich in the United States. Subsequently, the extract was concentrated to a semi-solid form in a Clifton water bath maintained at a temperature of 42 degrees Celsius. This process was carried out in order to mitigate the risk of denaturation of the phytoconstituents present in the extract.

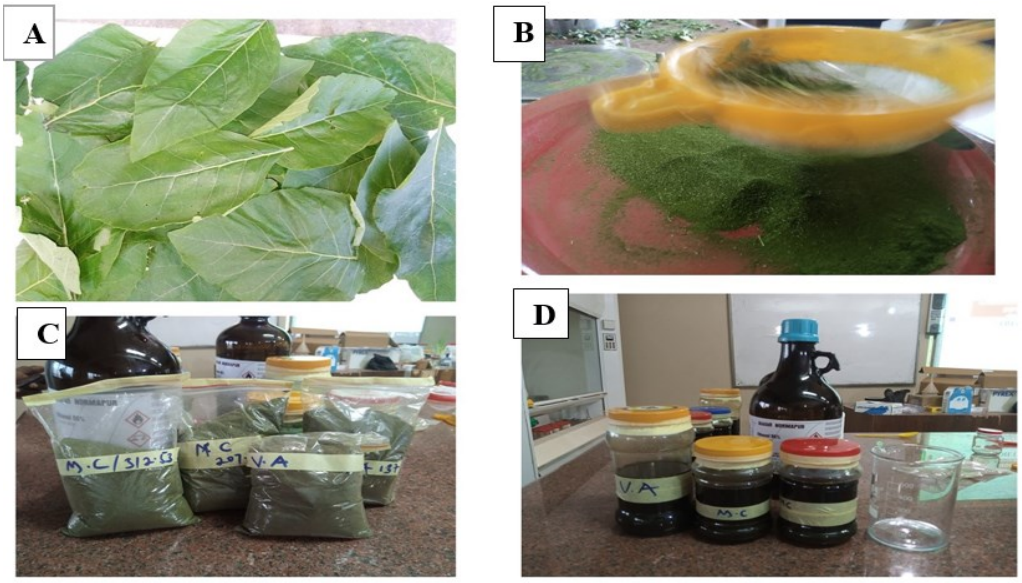

Plate 3.3: [A] Air drying of leaves, [B] blending and sieving, [C] powdered leaves stored in zip bags, [D] soaked powdered leaves in ethanol

3.6 Dose Calculation and Administration of Treatments

The mean bodyweight (BW) of the experimental animals in grams(g) for each treatment group was converted to kilograms. The resulting mean bodyweight in kilograms was multiplied by the dosage to be given to each treatment per kilogram of their bodyweight. The answer was divided by the concentration of the treatments.

Calculations

A group with a body weight average of 188.0g and receiving 100mg/kg/BW

= 188.0g/1000 = 0.188kg

0.188kg x 100mg/kg/BW = 18.8mg (0.0188g)

Using the weighed *Vernonia amygdalina* (VA) extract obtained at 16.8g, the extract was then dissolved in100mL of sterile distilled water to obtain concentration of the *Vernonia amygdalina*.

VA extract $= \frac{16.8g}{100ml} = 0.168g/ml$,

Extract to be administered as obtained by $\frac{0.0188g}{0.168g/ml} = 0.11190ml$

= 111.90 microliters

The same procedure was followed to determine the dose calculation for the groups that were administered with 100mg/kg/bw of *Momordica charantia*, and 3000mg/kg/bw acetaminophen and 200mg/kg/bw standard liver drug (silymarin). The dose used in this study for acetaminophen administration was adapted from the study by Raimi *et al* .(2020); Johnson *et al*. (2015), who indicated, oral administration of 3000mg /kg body weight of acetaminophen once a day for a period of 14 days resulted liver damage in rats.

Also, the dose (140mg/kg) of Silymarin was adapted from Sule *et al*. (2021) while the doses of *V. amygdalina* and *M. charantia* was adapted from work done by Si torus & Nerdy, (2018) and Ogungbe, (2017).

3.7. Chemicals and Drugs

Acetaminophen (500 mg) used to induce liver damage and Silymarin (140 mg) used as the standard liver drug for the study was purchased from a local pharmacy (Lansah Chemist-Kumasi).

3.9 Induction of Hepatocellular Acute Toxicity

After two weeks of the acclimatization period, the experimental rats in groups 2,3,4,5, and 6 were induced with 3000mg/kg body weight of acetaminophen for 14 days. The doses and duration of induction were chosen based on a previous study by Raimi (2020), who demonstrated that, oral administration of acetaminophen for a period of 14 days resulted in liver damage. A study by Johnson *et al.* (2015) showed, oral administration of 2000mg /kg body weight of acetaminophen once a day for a period of 14 days showed liver damage in rats.

3.10 Determination of Phytochemical Constituents of *Momordica charantia* and *Vernonia amygdalina*

The ethanolic leaf extract of *Momordica charantia* and *Vernonia amygdalina* were analyzed for the presence of the following phytochemicals: alkaloids, phenolic acids, saponins, tannins, steroids, flavonoids, cardiac glycoside, anthraquinones, and cyanogenic glycosides. The procedures employed in the phytochemical screening process have been described by Harborne (1970) and Effah -Yeboah *et al.* (2021). Prior

to the test, 50 grams of the powdered leaves were soaked for three days in 500 milliliters of 90% ethanol in sterile plastic containers at 26 °C with intermittent shaking to ensure adequate dilution and extraction. The extract was filtered using Whatman filter paper (1.5 Sigma Aldrich, USA) and then concentrated to a semi-solid state at 42°C using a Clifton water bath (to prevent denaturation of the active constituents). The extracts were subsequently dissolved in specific volumes of distilled water for qualitative phytoconstituent analysis.

3.10.1 Test for Alkaloids

A portion of the *Vernonia amygdalina* and *Momordica charantia* extract was mixed with a few droplets of 35% dilute hydrochloric acid and 0.5 ml Wagner's reagent. A flocculent brown precipitate was indicative of the presence of alkaloid.

3.10.2 Test for Phenolic Compounds

A portion of the *Vernonia amygdalina* and *Momordica charantia* extract was mixed into few drops of diluted Folin Ciocalteu reagent and aqueous sodium carbonate solutions. The mixture was allowed to stand for 10minutes and the formation of grey colour indicated the presence of Phenolic groups.

3.10.3 Test for Saponins

About 0.5 ml of the *Vernonia amygdalina* and *Momordica charantia* extract was dissolved in 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth with a honeycomb structure indicating the presence of saponins.

3.10.4 Test for Tannins

A portion of the *Vernonia amygdalina* and *Momordica charantia* extract was mixed with a few drops of 0.1% Ferric chloride and observed for brownish green colouration indicating the presence of tannins

3.10.5 Test for Steroids and Triterpenoid

Salkowski test was used to determine the presence of steroids and triterpenoids. *Vernonia amygdalina* and *Momordica charantia* extracts were treated in chloroform with a few drops of strong sulfuric acid, agitated briskly, and left to stand. the emergence of red colour at the lower layer indicated the presence of steroids, whilst triterpenoids were detected by the production of yellow colour at the lower layer.

3.10.6 Test for Flavonoids

The presence of terpenoids was confirmed by observing a colour change from red to purple when chloroform soluble fraction of the extract was mixed with an equal volume of 98% concentrated sulphuric acid (H_2SO_4)

3.10.7 Test for Anthraquinones

Born tranger's test was used to test for the presence of anthraquinones. Ethanolic extract of *Momordica charantia* and *Vernonia amygdalina* were heated at 270° C for 10 minutes in weak sulphuric acid. After that, it was cooled and filtered. The filtrate was then extracted with chloroform and treated with dilute ammonia. The presence of anthraquinones derivatives were shown when the colour of the layer changed from pink to red.

3.10.8 Test for Terpenoids

The presence of terpenoids was shown by the production of a red to purple hue when the chloroform soluble fraction of the extract was treated with an equal volume of 98% concentrated H_2SO_4.

3.10.9 Cyanogenic Glycosides

About 250 µl of the *Momordica charantia* and *Vernonia amygdalina* extract were added to an equal volume of cold concentrated sulphuric acid. Formation of intense green/blue/black/red colour indicated the presence of glycosides.

3.11 Procedure for Dissection

After the duration of twenty-eight days of treatment administering, the Wistar rats were fasted overnight prior to being sacrificed. Subsequently, the experimental animals were subjected to macroscopic examination, followed by weighing and sacrificed while under the influence of light chloroform anesthesia. A midline abdominal incision was performed on the ventral surface of the rats using sterilized surgical instruments, including blade, scissors, pins, and forceps. This procedure was carried out after the rats had been anesthetized for around two (2) minutes. Subsequently, the rats were positioned in a supine orientation on a dissection board and securely fastened with pins to ensure minimal movement throughout the dissection process. Subsequently, the rats underwent surgical incisions to reveal their interior organs. The dissection of experimental rats was conducted at the Biology laboratory of the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong Campus.

Plate 3.4: Researcher dissecting experimental animals

3.12 Sample Collection

Blood samples were obtained through cardiac puncture using a sterile syringe and needle. These samples were then transferred into ethylenediaminetetraacetic acid (EDTA) tubes for haematological analysis and gel and clot tubes for serum biochemical analysis. Additionally, the livers of the rats were collected and preserved in a solution of 10% formalin for subsequent histopathological examination.

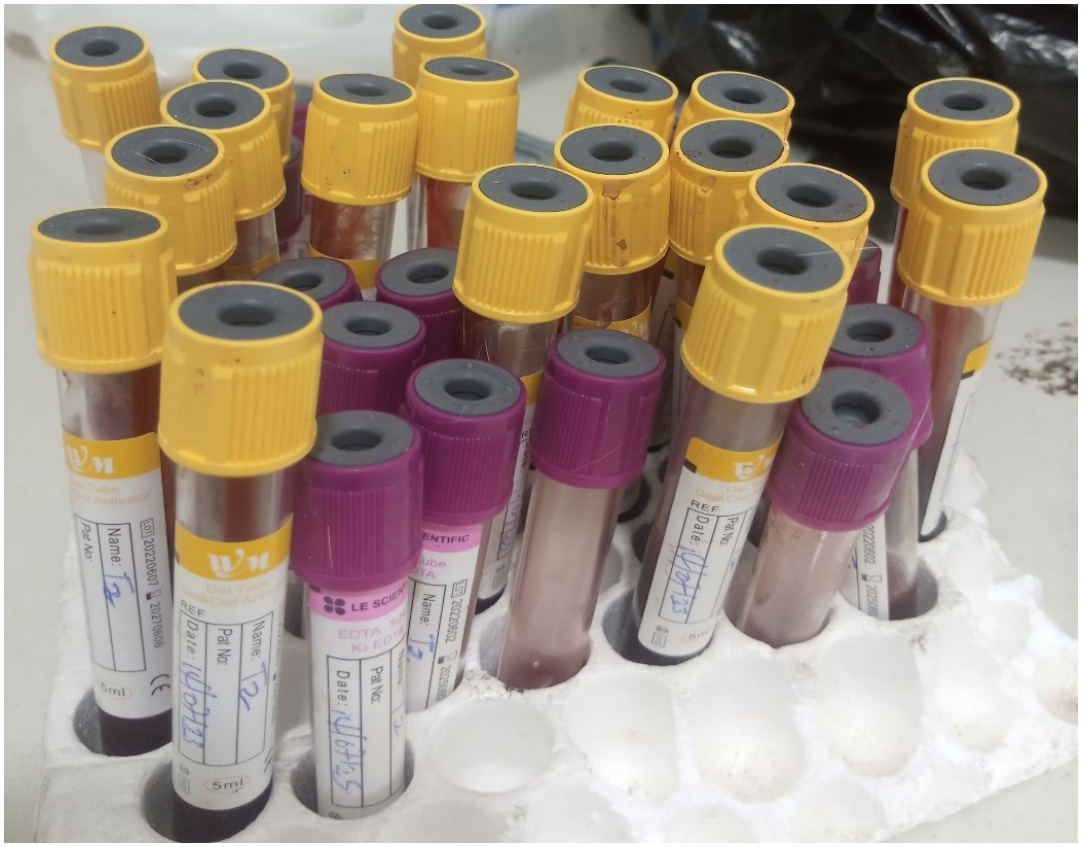

Plate 3.5: Blood samples collected through cardiac puncture into EDTA and gel & clot activator tubes.

3.13 Parameters Measured

3.13.1 Haematological Parameter

The blood samples collected in EDTA tubes were subsequently subjected to analysis using a haematology auto-analyzer, specifically the Automatic Haematology Analyzer with model number Rayto RT-7600s, was produced in China, in Guangzhou. The analysis focused on various haematological indices, including red blood cells (RBCs), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), monocytes (MON), neutrophils (NEU), and lymphocytes (LYM). The analysis was undertaken at the Asante Mampong Maternity Medical Center.

3.13.2 Determination of Liver Biochemical Indices

An additional aliquot of blood was collected into sterile vacutainer tubes, which were subsequently subjected to centrifugation at a speed of 13000 revolutions per minute for a duration of five minutes. The serum was subsequently aspirated, and biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), and bilirubin in serum were analyzed using commercially accessible kits and a standard BS-120 Mindray Chemistry Analyzer (Effah-Yeboah *et al.,* 2021).

3.13.3 Histology of Liver

Histopathological examinations were conducted on the liver specimens obtained from both untreated and treated Wistar rats, using the protocols outlined by Effah-yeboah *et al*. (2021), Mezban and Hussein (2015), and Rao *et al*. (2006). A solution containing a concentration of 10% formalin was utilized for the purpose of tissue fixation. The tissues were further dehydrated using ethanol with a concentration ranging from 50% to 100%. Following the initial step, the specimens underwent a rinsing process utilizing xylene in order to effectively remove the solvent, specifically ethanol. Finally, the tissues were immersed in paraffin wax, a process employed to facilitate dissection by enhancing their structural integrity. Additionally, the tissues were subjected to a de-paraffinization process using xylene prior to sectioning. Prior to water rehydration, the specimens were subjected to a series of cleaning procedures using ethanol of decreasing concentrations ranging from 100% to 50%. The sections that had been deparaffinized were subjected to staining using the hematoxylin and eosin (H&E) procedure, as described by Andrés-Manzano *et al*. (2015). The tissues were subsequently subjected

to examination under a light microscope, and photomicrographs of the relevant sections were captured for subsequent analysis.

3.14 Data Analysis

The results of the study were analyzed with Minitab statistical software, version 20.0, and presented as the mean value together with the standard error of the mean (SEM). Comparisons between the groups' parameters were made using a one-way analysis of variance (ANOVA), followed by a post-hoc Tukey-HSD test. All of the data was evaluated using a confidence range of 95%, and the findings were deemed statistically significant when the p-value was less than 0.05. ($p < 0.05$).

3.15 Ethical Clearance

Ethical clearance was sort from the Committee for Human Research and Ethics at UENR with reference number CHRE/AP/216/024.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Screening of Ethanolic Leaf Extracts of *Vernonia amygdalina* and *Momordica charantia* and the Combined *Vernonia amygdalina* and *Momordica charantia*

Table 4.1 shows the phytochemical composition of the ethanolic leaf extract *Vernonia amygdalina*, *Momordica charantia*, and the combined *Vernonia amygdalina* and *Momordica charantia*. This study's qualitative phytochemical examination revealed that *Vernonia amygdalina* leaves are exceptionally rich in flavonoids, alkaloids, phenolic compounds, saponins, tannins, terpenoids, steroids, triterpenoid, and cyanogenic glycosides. However, anthraquinones were not detected from the *Vernonia amygdalina* ethanolic leaf extract.

Table 4.1: Phytochemical screening

Phytochemical Constituents	VA	MC	VA+MC
Tannins	+ + +	+ + +	- - -
Alkaloids	+ + +	+ + +	+ + +
Cyanogenic glycosides	+ + +	+ + +	+ + +
Phenolic compound	+ + +	+ + +	+ + +
Saponins	+ + +	+ + +	+ + +
Flavonoid	+ + +	+ + +	+ + +
Triterpenoids	+ + +	+ + +	+ + +
Anthraquinones	- - -	- - -	- - -
Steroids	+ + +	- - -	+ + +
Terpenoids	+ + +	+ + +	+ + +

+++ detected. - - - not detected; VA = Vernonia amygdalina; MC = Momordica charantia

The analysis of phytochemicals in *Momordica charantia* revealed the existence of several bioactive compounds, including tannins, alkaloids, cyanogenic glycosides, phenolic compounds, saponins, flavonoids, triterpenoids, and terpenoids. anthraquinones and steroids were not detected from the ethanolic leaf extract of Momordica *charantia.*

The phytochemical analysis conducted on the ethanolic leaf extract of the combined *Vernonia amygdalina* and *Momordica charantia* demonstrated the presence of many bioactive components, including alkaloids, saponins, cyanogenic glycosides, terpenoids, phenolic compounds, flavonoids, triterpenoids, and steroids. The analysis of the combined extract indicated the absence of anthraquinones, a finding that was not observed in the screening of the individual plant extract. However, steroid which was only present in *Vernonia amygdalina* ethanolic leaf extract was detected from the combined extract. Tannins were present in both *Vernonia amygdalina* and *Momordica charantia* extracts but were not detected in the combined extract of *Vernonia amygdalina* and *Momordica charantia* extract.

4.2 Effects of Treatments on Haematological Parameters of Acetaminophen Intoxicated Rats

The current study showed insignificant ($P > 0.05$) effect among experimental rats treated with silymarin, *Vernonia amygdalina*, *Momordica charantia* and the combined *Vernonia amygdalina* and *Momordica charantia* leaf extract of both plants on red blood cells (RBCs). The current study showed that rats induced with acetaminophen at dosage of 3000 mg/kg/bw (negative control) did not exhibit any statistically significant effects on red blood cells (RBCs). There was a slight, non-significant decrease in RBCs in the

negative control group (7.247±0.271 L) compared to the normal control (7.607±0.304 L). The study found a statistically significant increase in white blood cell (WBC) count in acetaminophen-induced rats (negative control) compared to the positive control ($p < 0.05$). Silymarin, *Vernonia amygdalina* and *Momordica charantia* did not significantly affect WBC counts compared to the normal control. No significant differences in hemoglobin (HGB) or hematocrit (HCT) were observed among the positive control, acetaminophen-induced, and extract-treated rats compared to the normal control.

The study observed a slight but statistically insignificant increase in hematocrit count in the group treated with *Vernonia amygdalina* extract compared to the normal control group. Invariably, the combined extract groups exhibited a decreased in HCT count compared to the extracts in their uncombined forms. In the study, there were no significant differences in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) between the positive control, acetaminophen-induced, and extract-treated rat groups compared to the normal control group. However, rats treated with *Vernonia amygdalina* showed a slight reduction in MCHC compared to the positive control group.

Table 4.2: Haematological parameters of acetaminophen intoxicated rats treated with silymarin, *Vernonia amygdalina*, *Momordica charantia*, and combined extracts of *Vernonia amygdalina* and *Momordica charantia*.

Parameter	Normal Control	Positive Control	Negative control	Ac+VA	Ac+Mc	Ac+VA+MC	Reference Range	pValue
RBC (10^12/L)	7.607 ± 0.304	7.500 ± 0.467	7.247 ± 0.271	7.8933± 0.0260	8.033 ± 0.124	7.7200±0.077	8.20–9.50*	0.378
WBC (10^9/L)	5.140±0.106^{b}	5.480±0.235^{b}	6.840±0.372^{a}	5.567±0.234^{b}	5.493±0.199^{b}	4.983±0.228^{b}	2.47–14.42*	0.002
HGB (g/dL)	15.067±0.801	15.067±0.406	14.233± 0.285	15.733 ± 0.203	15.633± 0.186	16.100± 0.351	91–103*	0.098
HCT%	39.13±4.34	40.73±2.53	40.13 ±2.43	43.800± 0.513	43.567±0.484	41.867± 0.133	42–48*	0.652
MCV (fL)	55.87±1.43	54.30 ±0.603	55.27±1.56	55.500± 0.802	54.767±0.835	54.233±0.410	48–54*	0.828
MCH (pg)	21.200±0.416	20.167±0.817	20.533± 0.546	19.933±0.240	19.633± 0.517	20.933± 0.578	16.10–19.30*	0.365
MCHC(g/dL)	37.97±1.59	37.13± 1.36	37.20± 1.08	34.87± 1.54	35.833±0.418	38.500± 0.917	340–361*	0.357
PLT (10^9/L)	766.3± 61.7	716.7± 33.8	650.7±59.0	741.7± 47.3	724.3± 83.7	775 ±102	573–998*	0.822

*Values are expressed as mean ± SEM; $p < 0.05$ was considered statistically significant. Values in the same row followed by the same superscript are not significantly different. Red blood cells (RBC); White blood cells (WBC); haemoglobin (HGB); haematocrit (HCT); mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration, (MCHC); Platelets (PLT), Acetaminophen (Ac); Vernonia amygdalina (VA); Momordica charantia (MC), Reference range with * was adapted from, Augustine et al., (2020)*

The results revealed that, treatments administration exhibited no statistically significant ($p > 0.05$) differences in mean values of platelets count compared to the to the mean value of the normal control. Rats that were induced with acetaminophen and untreated (negative control) however had a mean value slightly reduced (650.7±59.0L) compared to the normal control. In this current study, administration of silymarin, acetaminophen, *Vernonia amygdalina* and *Momordica charantia* and the combined *Vernonia amygdalina* and *Momordica charantia* extract exhibited no statistically significant ($p > 0.05$) differences in mean values of platelets count compared to the to the mean value of the normal control. Rats induced with acetaminophen and untreated (negative control) had a slightly reduced mean value compared to the normal control, with results showing no significant difference ($p > 0.05$).

4.4 Effects of Treatments on Liver Biochemical Parameters of Acetaminophen Intoxicated Rats

The results of this study showed statistically significant ($P < 0.05$) increase in mean value of serum ALT of rats treated with 3000 mg/kg/bw of acetaminophen for 14 days (negative control) compared to the positive control rats. The results of this study also revealed a statistically significant decrease ($P<0.05$) in ALT levels among rats treated with Silymarin, *Vernonia amygdalina, Momordica charantia* and the combined *Vernonia amygdalina* and *Momordica charantia* extracts compared to ALT levels of rats in the acetaminophen induced group (negative control**).** The study showed no statistically significant ($p > 0.05$) differences of mean values in levels of serum AST, ALP and GGT following the administration of silymarin, acetaminophen, *Vernonia amygdalina, Momordica charantia* and the combined extracts compared to the mean values of serum AST, ALP and GGT of rats in the control groups.

However, Wistar rats within the acetaminophen induced and untreated group (negative control) exhibited a slight elevation though insignificant ($p > 0.05$) mean values of serum AST, ALP and GGT compared to rats of the normal control.

The current study also exhibited no significant ($p > 0.05$) differences in mean values of total protein (TP), albumin (ALB) and globulin (GLB) following the administration of silymarin, acetaminophen, *Vernonia amygdalina, Momordica charantia* and the combined leaf extracts of *Vernonia amygdalina* and *Momordica charantia*. However, results of the serum biochemistry of this study showed a moderate insignificant ($p > 0.05$) reduction in mean values of total protein and albumin among rats induced with acetaminophen compared to the normal control total protein and albumin respectively. Again, this present study showed that, administration of silymarin, *Vernonia amygdalina, Momordica charantia* and the combined leaf extracts of *Vernonia amygdalina* and *Momordica charantia* showed a slight increase though insignificant ($p > 0.05$) in total protein albumin compared to the acetaminophen induced group

Table 4.3: Liver Serum biochemical parameters of acetaminophen intoxicated rats treated with silymarin, *V. amygdalina*, *Momordica charantia*, and combined extracts of *V. amygdalina* and *Momordica charantia*,

parameters	Normal control	Positive control	Negative control	Ac+VA	Ac + MC	Ac +VA + MC	Reference Range	pValue
ALT (U/l)	109.47±7.31[b]	103.47±6.99[b]	280.6±46.8[a]	131.0± 44.9 [b]	138.1±31.2[b]	125.5±10.6[b]	35 – 80 **	0.011
AST (U/l)	321.2±15.5	324.7±15.8	391.8± 23.4	337.9±17.2	342.47±8.01	329.7±25.4	60–139*	0.153
ALP (U/l)	438.7±41.9	459.8± 41.9	539 ±131	407.3± 43.3	335.0±31.4	449.9±52.6	50– 150***	0.451
GGT(U/L)	10.367± 0.0882	10.100±0.603	11.567±0.745	10.433±0.167	11.433±0.841	10.467±0.240	-	0.322
TP (g/l)	75.43±1.39	75.93±3.04	71.60±1.11	75.83±3.15	78.03±2.50	77.80±2.07	40–60*	0.471
ALB(g/l)	33.20±1.55	36.63±2.47	31.267±0.418	32.033±0.867	38.67±2.57	38.30±2.15	44.40–58.40*	0.051
GBN (g/l)	42.233± 0.684	39.300±0.800	40.30±1.44	43.80±2.34	39.37±1.69	39.533± 0.296	1.5 – 2.5 *[+]	0.191
BlT(µmol/L)	3.100±0.451	6.10±1.36	8.70±2.76	7.60±2.14	4.87±1.78	5.333±0.669	0.2 - 0.5**	0.194
BID (µmol/L)	1.200±0.173	1.267±0.467	1.533±0.546	2.333±0.694	1.533±0.533	1.633± 0.371	0.03 – 0.05*[+]	0.649
BI-IN (µmol/L)	1.967±0.353	4.83±1.24	5.80± 3.61	5.27±1.46	3.33±1.25	3.700±0.321	0.01- 0.012	0.666

*Values are expressed as mean ± SEM; $p < 0.05$ was considered statistically significant. Values in the same row followed by the same superscript are not significantly different. Aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase (ALP); gamma-glutamyltransferase (GGT); total protein (TP); albumin (ALB); globulin (GBN); bilirubin total (BIT); bilirubin direct (BID), bilirubin indirect (BI-IN). Reference range was adapted from : * (He et al., 2017); **(Augustine et al., 2020); *** (Mahmudul et al., 2018); *[+] (Giknis et al., 2008)*

The levels of bilirubin were additionally determined in this study. Bilirubin is an orange-red compound that is made in animals as part of the normal process of breaking down haem. This process of catabolism is needed for the body to get rid of waste products that are made when old or damaged red blood cells are broken down. Bilirubin is an important maker of liver and blood. Bilirubin is produced by cells of the reticuloendothelial system, including phagocytes, Kupffer cells of the liver, and cells of the spleen and bone marrow, through a two-stage reaction. This study ascertained the levels of total bilirubin, direct or conjugated bilirubin and indirect bilirubin or unconjugated bilirubin. The findings of this study revealed statistically insignificant ($p > 0.05$) difference in mean values of total bilirubin, direct or conjugated bilirubin (portion of bilirubin processed by the liver and ready for excretion) and indirect bilirubin or unconjugated bilirubin (portion of bilirubin that has not been processed by the liver and circulating in the bloodstream) following administration of acetaminophen, silymarin and the extracts compared to the normal control.

4.5 Effects of Treatments on Liver Histology of Acetaminophen Intoxicated Rats

Wistar rats that received Normal saline (Normal control). showed no histopathological alteration and normal histological structure of the sinusoids, portal vein Kupfer cells, central vein and surrounding hepatocytes in the parenchyma. Wister rats that received 140kg/mg silymarin (Positive control), showed moderate histopathological alteration of central vein in the parenchyma, dilated sinusoids, normal hepatocytes. of Wister rats induced with 3000kg/mg acetaminophen (negative control), showed severe histopathological alteration with severe dilation of sinusoids associated with

inflammatory cells infiltration and oedema in the periductal tissue surrounding the cystic dilated bile ducts with swelling around central vein region.

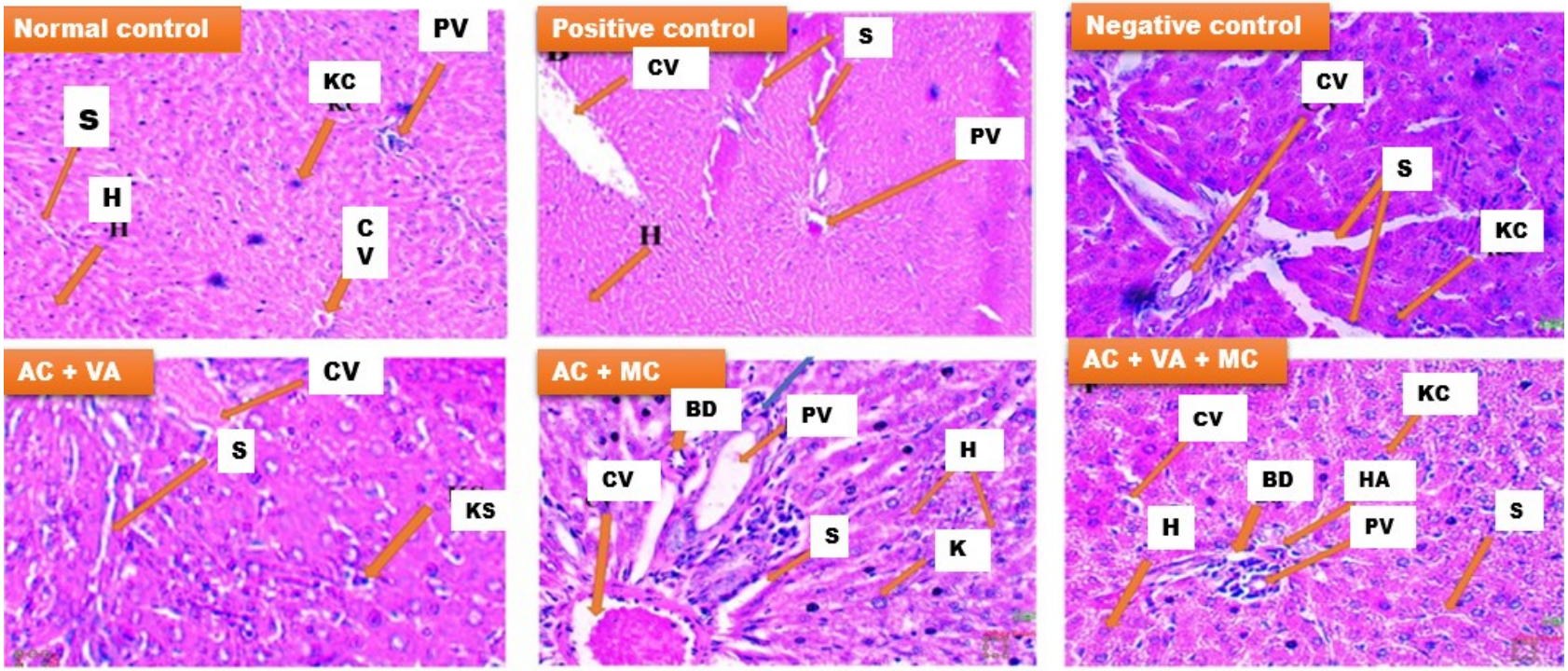

Plate 4.1: Photomicrographs of liver sections stained with hematoxylin and eosin (H&E). The slides were examined at a magnification of x10 andx40 under a light microscope. The slides show the effect of the extracts on liver architecture in acetaminophen-induced hepatotoxicity in male Wistar rats. (A) Normal control,), (B) acetaminophen + silymarin, (C) acetaminophen control (negative control, (D) acetaminophen + VA (100mg/Kg B.W), (E) acetaminophen + MC (100mg/Kg B.W), (F) acetaminophen + VA+MC (100mg/Kg B.W)

Wister rats induced with 3000kg/mg acetaminophen and treated with 100mg/kg *Vernonia amygdalina* showed moderate sinusoids dilation, mild swelling of central vein with no histopathological alteration in hepatocytes and Kupfer cells. Wister rats induced with 3000kg/mg acetaminophen and treated with 100mg/kg *Momordica charantia* showed moderate sinusoids dilation, moderate dilation and congestion of the central vein. Photomicrograph of liver section of Wister rats induced with 3000kg/mg acetaminophen and treated with combined 100mg/kg *Vernonia amygdalina* and 100mg/kg *Momordica charantia* leaf extract showed moderate sinusoids dilation, no histopathological alteration in hepatocytes and Kupfer cells, moderate swelling and congestion around portal vein.

CHAPTER FIVE

DISCUSSIONS

5.1 Phytochemical Constituents of Ethanolic Leaf Extracts of *Vernonia amygdalina*

The remarkable abundance of flavonoids, alkaloids, phenolic compounds, saponins, tannins, terpenoids, steroids, triterpenoids, and cyanogenic glycosides in *Vernonia amygdalina* observed in this study aligns with the findings of Asante *et al.* (2017), who identified these phytochemical classes in the ethanolic leaf extract of *Vernonia amygdalina*. However, this study did not detect anthraquinones in the extract, which contrasts with the findings of Olusola-Makinde *et al*. (2023). Previous studies by Ghasemzadeh *et al*. (2018) suggested that variations in phytochemical content within the same plant species can be attributed to different environmental factors. Alara *et al*. (2017) identified and reported the presence of flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, and triterpenoids in *Vernonia amygdalina* in their earlier phytochemical studies, which is consistent with the findings of the current study.

Hassan *et al*. (2022) revealed the absence of alkaloids in methanolic leaf extract of *Vernonia amygdalina*, but other phytochemicals such as terpenoid, saponin, tannins, and flavonoids were discovered by qualitative phytochemical screening. Through qualitative analysis, Johnson *et al*. (2015) indicated the presence of tannin, alkaloids, glycosides, anthraquinone, flavonoids, phlobatanin, and saponin in methanolic leaf extract of *Vernonia amygdalina*. However, the existence of phlobatanin in their findings contradicts the findings of the current study. Previous research by Si torus &

Nerdy, (2018) found that the presence of phytonutrients, flavonoids, and phenol in *V. amygdalina* confers it hepatoprotective effects. According to Si torus & Nerdy, (2018), the hepatoprotective action of *Vernonia amygdalina* ethanolic leaf extract may be due to antioxidant activity in the plant. The presence of phenol and flavonoids in *Vernonia amygdalina* may explain the antioxidant activity. Flavonoids are hypothesized to limit liver damage by attaching to free radicals, reducing their influence on the liver (Vargas-Mendoza *et al.,* 2014). Previously, Ojimelukwe & Amaechi (2019) reported that flavonoids have two major modes of action: direct scavenging of free radicals or interfering with specific enzyme activities such as nitric oxide synthase activity or xanthine oxidase activity. Furthermore, flavonoids and terpenoids are anticancer, antioxidant, antibacterial, anti-diabetic, and hepatoprotective (Atangwho *et al.,* 2012; Ojimelukwe & Amaechi, 2019; Kaur *et al.,* 2019). In addition to its hepatoprotective qualities due to the presence of flavonoids, *Vernonia amygdalina* other phytochemicals endow it with a plethora of pharmacological activities, as evidenced by numerous prior studies. Ali *et al*. (2019), showed that, alkaloids have metabolic roles and govern development in biological systems by interfering with cell division; thus, the presence of alkaloids in *V. amygdalina* may explain for their usage as antimicrobial agents. Similarly, the other phytochemical contained in *V. amygdalina* contributes to its pharmacological qualities such as anti-inflammatory, immunomodulatory, anti-cancer, anti-malaria, anti-diabetic. (Ojimelukwe and Amaechi, 2019; Karfi *et al.,* 2021).

5.2 Phytochemical Constituents of Ethanol Leaf Extracts of *Momordica charantia*

The observed phytochemicals found in the ethanolic leaf extract of *Momordica charantia* is consistent with the findings of Chinonye & Chijioke-okere, (2019), who

reported the existence of alkaloids, saponins, flavonoids, phenols, glycosides, and tannins. A study by Oliveira *et al*. (2018), on the phytochemical profile and biological activity of *Momordica charantia* successfully identified compounds, including alkaloids, tannins, saponins, flavonoids, cardiac glycosides, and steroids. Nevertheless, the inclusion of steroids in their study is contrary to finding of this study. In a study conducted by Mada *et al*. (2013), showed that, the qualitative analysis and screening of phytochemicals in their ethanol leaf extract of *Momordica charantia* revealed the presence of alkaloids, tannins, saponins, flavonoids, and cardiac glycosides. These findings align with the results obtained in the present study. Salem *et al*. (2019), showed that the hepatoprotective effects of MC extracts can be attributed to the presence of flavonoids, as well as other constituents like saponins, tannins, and alkaloids. These compounds possess the capability to effectively scavenge free radicals, hence enhancing the activity of antioxidant enzymes. According to Adi, (2017), their review of phytochemicals highlighted the abundance of various bioactive compounds in *Momordica charantia*. These compounds, such as triterpenes, proteins, steroids, alkaloids, and phenolics, are associated with the plant's diverse biological and pharmacological properties.

Notably, *Vernonia amygdalina* is known to exhibits anti-diabetic, antioxidant, anti-cancer, anti-tumor, antimicrobial, anti-fertility, anti-viral, anti-helminthic, antimalarial, anti-ulcerative, and immunomodulatory activities (Jarmai *et al*., 2022). In addition, a study conducted by Karale *et al*. (2022), it was demonstrated that *Momordica charantia* contains antioxidant components, including phenolics, flavonoids, and polyphenols. These compounds have the potential to effectively scavenge free radicals, hence inhibiting oxidative mechanisms. Semiz and Sen (2007) revealed that the

hepatoprotective effect of *Momordica charantia* extract in rats appears to be attributed to the augmentation of antioxidant enzymes. Hence, it may be said that *Momordica charantia* possesses significant efficacy in addressing hepatic diseases owing to its antioxidant attributes.

5.3 Phytochemical Constituents of Ethanolic Leaf Extracts of Combined *Vernonia amygdalina* and *Momordica charantia*

An intriguing finding in this study was the absence of tannins observed during the qualitative screening of the combined extract. The absence of tannins in the combined extract of *Vernonia amygdalina* and *Momordica charantia* may be attributed to a potential chemical interaction between the two components, resulting in the inactivation of tannins derived from both plants, so leaving them undetectable. According to a study conducted by Liu *et al*. (2019), it was found that the consumption of high amounts of tannins may lead to liver damage due to their antioxidant properties, which have the capacity to interfere with the regular functioning of the liver. According to Proietti *et al*. (2015), tannins possess the capacity to interfere with the assimilation of essential nutrients, thereby jeopardizing the overall functionality of the liver. The present investigation also demonstrated the presence of steroids upon combining *Vernonia amygdalina* and *Momordica charantia* extracts. However, steroids were not identified through the qualitative screening of *Momordica charantia*, whereas they were present in the extract of *Vernonia amygdalina*. Previous research conducted by Rebolledo *et al*. (2015), has demonstrated that steroids exhibit anti-inflammatory effects.

5.5 Haematological Parameters

The present study also aimed to examine the impact of silymarin, ethanolic extract of *Vernonia amygdalina*, *Momordica charantia*, and a combined *Vernonia amygdalina* and *Momordica charantia* leaf extract on haematological indicators in male Wistar rats induced with acetaminophen.

The insignificant red blood cell (RBC) levels observed in this study align with the findings of Johnson *et al*. (2021) and Obiandu *et al*. (2020), who reported that ethanolic leaf extracts of *Vernonia amygdalina* and *Momordica charantia* had no significant effect on RBCs. Similarly, normal saline, silymarin, and the combined *Vernonia amygdalina* and *Momordica charantia* treatments showed no impact on RBC levels, with all treated groups falling within the normal range of 8.20 – 9.50 x 10^{12}/L, as noted by Johnson *et al*. (2021). These results suggest that there was no RBC hemolysis or alteration in erythropoiesis following the administration of Vernonia amygdalina and Momordica charantia (Johnson et al., 2021).

In contrast, a study conducted by Airaodion *et al*. (2019) demonstrated that the administration of leaf extract of *Vernonia amygdalina* to Wistar rats led to a decrease in the red blood cell (RBC) count. The slight decrease in red blood cells among rats induced with acetaminophen and left untreated could imply that, acetaminophen could have caused destruction of matured RBC and reduction in the rate of erythropoiesis, this occurs when oxygen-carrying capacity of the RBCs decreases and amount of oxygen reaching the cells also decreases, potentially causing anaemia and impairing body functions. Previous studies conducted by Latif *et al*. (2021) and Okerulu &

Onwuka, (2022), suggested that acetaminophen induces erythrocyte depletion through altering red blood cell count.

White blood cells (WBCs) of all treated groups and the normal control of the current study were within the normal range of 2.47 – 14.42. However, the significant increase in WBCs in the acetaminophen-treated group of this current study could be due to acetaminophen's action causing infection, trauma, inflammation, as well as other disorders. The current findings are congruent with Khayyat (2021), who indicated a significant increase in WBC cell counts following acetaminophen administration in adult male rats. The insignificant effects of *Vernonia amygdalina* on WBCs validates a study by Oyedeji *et al*. (2013), who reported that treatment of rats with *Vernonia amygdalina* leaf extract exhibited no effects on total white blood cells count. Al-Hizab *et al*. (2013), reported that *Momordica charantia* leaf extract had no significant effect on WBCs which agrees with the finding of this study. Also, the combined extract of *Vernonia amygdalina* and *Momordica charantia* had no effects on WBCs count compared to the normal control. The insignificant effect on white blood cell count following the administration of the combined extract of *Vernonia amygdalina* and *Momordica charantia* is likely due to its non-immunomodulatory properties, which help preserve immunological stability without inducing an inflammatory reaction. silymarin administration had no effect on WBCs count, this finding is in agreement with of that of Mahmoud *et al*, (2020), who showed that administration of silymarin exhibited no changes in WBCs count. Vona *et al*. (2021) indicated that, the selective action of silymarin on liver cells and its antioxidant properties largely target hepatic function without significantly affecting immune cell populations or their activities, which suggests that it may have no impact on white blood cells (WBCs).

The observed insignificant effects of haemoglobin (HGB) and hematocrit (HCT) following treatments administration may suggest that, the treatments may not disrupt the stability of red blood cells or erythropoiesis. Treatments may predominantly target other physiological pathways without affecting the production or volume of red blood cells (Okpara et al., 2023). This study's finding is in concordance with that of Chike *et al*. (2018) who showed that, administration of ethanolic leaf extract of *Vernonia amygdalina* for 14 days had no significant effect on HGB and HCT count. The present study findings however contradict with that of Oyedeji *et al*, (2013), who reported a significant decreased in HGB and HCT following treatment of rats with 7.5mg/kg BW of *Vernonia amygdalina* and *Momordica charantia* leaf extract for 42 days. Haemoglobin counts of all treated groups were below the normal reference range of 91 – 103 dL, while hematocrit counts remained within the normal range of 48 – 42%, however, the Slightly increased in hematocrits count by *Vernonia amygdalina* extract compared to the normal control as observed by the current study may suggests that, *Vernonia amygdalina* may be able to increase the release of erythropoietin from the kidneys, which works as a humoral regulator of erythrocyte synthesis, resulting in the production of hematocrit.

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were not affected following treatment administration. This could imply that individual red blood cells' size, haemoglobin content, or haemoglobin concentration were not interfered with treatments. This current study finding is in agreement with Johnson *et al*. (2021), who indicated that methanolic leaf extract of *V. amygdalina* exhibited insignificant effects on (haemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin

(MCH), and mean corpuscular haemoglobin concentration (MCHC). Salem *et al*. (2020) reported that, the phytochemical characteristics of *Vernonia amygdalina* and *Momordica charantia* are well-known to affect metabolic and immunological pathways mostly without changing erythropoiesis or red blood cell shape. Likewise, not changing their production or structural properties. These treatments probably preserve normal ranges of erythrocyte indices, suggesting their selective biological effects.

In this current study, treatments administration exhibited no significant differences in mean values of platelets count compared to the mean value of the normal control. Platelets count of all treatment groups and the normal control were within the normal reference range of 573 – 998 L. This suggests that, the treatments nor the normal saline had no effects on the platelets count of the experimental animals. However, the slight reduction in platelets count among rats induced with acetaminophen (acetaminophen) and untreated (negative control) compared to the normal reference range of 573 – 998 L, indicated that acetaminophen could impede platelet formation, leading to thrombocytopenia, a medical disorder characterized by an insufficient platelet count in the bloodstream. Zubairu *et al*. (2021), indicated that, administration of 2000 mg/kg of acetaminophen to albino rats resulted a reduction in platelets count which contradicts with the finding of this study. According to Carpenter (2020), the liver's ability to metabolize acetaminophen can become overwhelmed, resulting in the generation of a poisonous metabolite known as N-acetyl-p-benzoquinone imine (NAPQI). According to Massart (2017), the presence of NAPQI has the potency to induce significant hepatic injury, hence potentially impacting the hematopoietic function of the bone marrow in platelet production.

5.6 Liver Serum Biochemistry

The results of the serum biochemistry are shown in Table 4.4 above. Liver Serum biochemistry examinations are a series of diagnostic tests that provide insightful information about the health and performance of the liver. These tests encompass a range of parameters that assess various aspects of liver functions, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein (TP), albumin (ALB), globulin (GBN), bilirubin total (BIT), bilirubin direct (BID) and bilirubin indirect (BI-IN).

Since hepatocytes are the primary component that regulates the metabolic activities of the liver, they are more vulnerable to necrosis, which results in the release of liver enzymes such as AST, ALT, and ALP into the bloodstream (Salem *et al.,* 2019). The current study revealed a significant increase in alanine aminotransferase (ALT) levels among rats treated with 3000 mg/kg acetaminophen compared to those treated with normal saline. The elevated serum ALT levels in rats treated with 3000 mg/kg body weight of acetaminophen for 14 days suggest that acetaminophen administration may cause liver injury, leading to the release of ALT from hepatocytes into the bloodstream. The increase in mean values of serum ALT of rats treated with 3000mg/kg/Bw of acetaminophen for 14 days is in concordance with studies conducted by Naz *et al.* (2023), who demonstrated that, the administration of acetaminophen at a dosage of 2kg/mg led to a significant increase in the concentrations of ALT. Oxidation of acetaminophen by the liver results in the generation of free radicals, which subsequently leads to the breakdown of the integrity of the hepatocyte membrane (Koç et al. 2023), this disruption ultimately causes the release of a variety of enzymes from the

hepatocytes. In contrast to the significant increase in serum ALT levels in rats treated with 3000 mg/kg acetaminophen for 14 days, Rahimi *et al*. (2022) reported that the administration of acetaminophen does not have any significant impact on ALT levels.

The significant decrease in ALT levels among rats treated with Silymarin, *Vernonia. amygdalina, Momordica charantia* and the combined *Vernonia. amygdalina* and *Momordica charantia* extracts compared to ALT levels of rats in the acetaminophen induced group (negative control**)** suggests that administration of silymarin, *Vernonia. amygdalina, Momordica charantia and the combined Vernonia. amygdalina* and *Momordica charantia extracts* demonstrated a significant inhibition of acetaminophen-induced liver damage, as indicated by a notable reduction in the plasma activity of ALT. According to Gillessen and Schmidt (2020), silymarin's hepatoprotective effectiveness stems from its ability to serve as a free radical scavenger and modulate enzymes involved in the development of cellular damage, fibrosis, inflammation, and cirrhosis. Salem *et al.* (2019) reported that, the hepatoprotective activity shown by *Vernonia amygdalina* and *Momordica charantia* ethanolic leaf extract may be caused by antioxidant activity in the plant. The presence of phenol and flavonoids in *Vernonia amygdalina and Momordica charantia* may be responsible for the antioxidant activity. Flavonoids are hypothesized to reduce liver damage by binding to free radicals, reducing their influence on the liver (Xu *et al*., 2022). The present study revealed that, the combined *Vernonia amygdalina* and *Momordica charantia* extract brought the level of ALT to near normal comparing the individual extract treated groups to the normal reference range of 35 – 80 UI as reported by Augustine *et al*. (2020) and normal control group. The aforementioned observation of this study could potentially be attributed to the absence of tannins in the combined extracts of *Vernonia amygdalina and*

Momordica charantia. According to Sruthi and Indira (2016), tannins have been found to have several physiological effects, including the acceleration of blood coagulation, reduction of blood pressure, decrease in serum cholesterol levels, induction of liver necrosis, and modulation of immunoresponses. Additionally, the presence of steroids in the *Vernonia amygdalina and Momordica charantia* could potentially serve as an anti-inflammatory agent counteracting the effects of acetaminophen.

The insignificant differences of mean values in levels of serum AST, ALP and GGT following the administration of silymarin, acetaminophen, *V. Amygdalina, M. charantia* and the combined *Vernonia amygdalina* and *Momordica charantia* extracts compared to the mean values of serum AST, ALP and GGT of rats in the control groups concurs to the findings of Asuk & Ugwu, (2018), Cyril-olnta and Monkhua &Akanbi (2016). However, AST and ALP levels were high above the normal reference range of 60–139 UI and 50– 150 respectively among Wistar rats induced with 3000 mg/kg acetaminophen. The increase in levels of AST and ALP of rats in the acetaminophen-induced untreated group (negative control) maybe an indication of liver damage induced by the acetaminophen (Johnson *et al.,* 2015). The significant increase in liver biomarker enzyme ALT which is specific to the liver and the slight increase though insignificant in (AST, ALP and GGT) in the plasma of Wistar rats induced maybe an indication of the hepatotoxicity caused by acetaminophen. Johnson *et al*. (2015), reported that, elevated levels of transaminases, specifically aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are indicative of their release from the cytoplasm of hepatocytes and these enzymes serve as sensitive indicators for necrotic lesions inside the liver. Therefore, the significant release of transaminases into the bloodstream is suggestive of extensive damage to the membranes of hepatic tissue

caused of acetaminophen intoxication. Alkaline phosphatase (ALP) is a membrane bound enzyme and its elevations in plasma indicate membrane disruption in the organ (Mada, 2014). Alkaline phosphatases, although not a liver specific enzyme, but the liver's major source of this enzyme increases during cholestasis (Pollock & Minuk, 2017). GGT on the other hand is also not liver specific but biliary system specific. However, GGT elevation is not specific for cholestatic liver disease.

Kwo *et al.* (2017), reported that, GGT can be elevated in patients with pancreatic disease, myocardial infarction, renal failure, emphysema, diabetes, and in patients taking certain medications such as phenytoin and barbiturates. The current study exhibited no significant differences in mean values of total protein (TP), albumin (ALB) and globulin (GLB) following the administration of silymarin, acetaminophen, *Vernonia amygdalina, Momordica charantia* and the combined leaf extracts of *Vernonia amygdalina* and *Momordica charantia*. However, TP and globulin levels of all treated groups and normal control group were high above the normal reference range of 40–60 g/l and 1.5 – 2.5 g/l as reported by Giknis *et al*. 2008.

However, results of the serum biochemistry (Table 4.4) of this study showed a moderate reduction in mean values of total protein and albumin among rats induced with acetaminophen compared to the normal controls total protein and albumin. The moderate insignificant reduction in mean values of total protein and albumin among rats induced with acetaminophen are in harmony with Cyril-olnta , monkhua &Akanbi, (2016), who showed that, the administration of Leaf extract of *Momordica charantia* had no significant effect on total proteins, serum albumins, and globulins. The slight decreased in serum albumin and total proteins fractions maybe evident of chronic

hepatic necrosis caused by the toxic effect N-acetyl-p-benzoquinone imine (NAPQI) of acetaminophen. Acetaminophen is oxidized by cytochrome p-450 and produces reactive intermediate metabolite NAPQI which in overdose causes severe liver damage (Saikrithika & Senthil ,2023).

In contrast to the findings of the current study, Rahimi *et al*. (2022), revealed that administration of acetaminophen resulted in a significant increase in albumin level and total proteins. Albumin is a protein that is made in the liver and gets into the bloodstream to help keep fluid from leaking out of blood vessels. Low albumin levels (hypoalbuminemia) is an indication of the liver impaired ability to make enough albumin.

The insignificant difference in mean values of total bilirubin, direct or conjugated bilirubin (portion of bilirubin processed by the liver and ready for excretion) and indirect bilirubin or unconjugated bilirubin (portion of bilirubin that has not been processed by the liver and circulating in the bloodstream) following administration of acetaminophen, silymarin and the extracts compared to the normal control concurs with the findings of Kandé *et al*. (2022), Cyril-olnta , monkhua &Akanbi (2016), Rahimi *et al*. (2022) and Vidimce *et al*.(2021)**,** who indicated no significant effects on level of bilirubin following administration of silymarin, acetaminophen *Vernonia amygdalina, Momordica charantia* and the combined leaf extracts of *Vernonia amygdalina* and *Momordica charantia*. However, all treated groups exhibited elevated levels of total bilirubin, bilirubin direct, and bilirubin indirect compared to the normal reference range of 0.2 - 0.5 µmol/L, 0.03 – 0.05 µmol/L and 0.01- 0.012 µmol/L as reported by Augustine *et al.* (2020). However, the acetaminophen induced rats showed a slight

increase in total bilirubin (and bilirubin indirect (compared to the control's total bilirubin) and bilirubin indirect respectively. Increment in levels of bilirubin release maybe attribute to liver damage caused by acetaminophen.

5.7 Liver Histopathology

The histological examinations revealed that the administration of acetaminophen at a dosage of 3000 mg/kg bw resulted in pronounced histopathological damage in the liver. This damage was characterized by notable cellular degeneration, moderate to severe hepatocyte necrosis, and swelling in the immediate area of the central vein region. Additionally, a mild infiltration of inflammatory cells was observed. The majority of the hepatic lobule suffered damage, with significant loss of its typical pattern following administration of 3000mg/kg acetaminophen. These alterations were shown to be strongly linked with the observed increases in transaminase activity in acetaminophen-induced hepatotoxicity. These observations concur to a study by Raimi *et al.,* (2020). Hepatic tissue from silymarin (100 mg/kg) treated rats (positive control) demonstrated significant liver protection against acetaminophen-induced liver damage, as evidenced by the presence of mild to moderate hepatocyte necrosis, milder dilation of sinusoids and the central vein region, and a few to moderate inflammatory cell infiltration. This observation agrees with the finding of Ahmad *et al.,* (2019).

Wistar rats induced with 3000 kg/mg acetaminophen and treated with 100mg/kg of *Vernonia amygdalina* ethanolic leaf extract showed moderate sinusoids dilation, mild swelling of central vein with no histopathological alteration in hepatocytes and Kupfer cells whiles rats induced with 3000kg/mg acetaminophen and treated with 100mg/kg *Momordica charantia* showed sinusoids dilation with congestions in dilation of central

vein, Photomicrograph of liver section of Wister rats induced with 3000 mg/kg acetaminophen and treated with combined 100 mg/kg *Vernonia amygdalina* and 100 mg/kg *Momordica charantia* leaf extract showed improved generation of hepatic cells, moderate sinusoids dilation, no histopathological alteration in hepatocytes and Kupfer cells, moderate swelling and congestion around portal vein. Combined *Vernonia amygdalina* and *Momordica charantia* extract treatment, ameliorated hepatic damage caused by acetaminophen. This can be attributed to the presence of diverse bioactive components acting as antioxidant in combating free radicals responsible for causing liver damage.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the results of this study demonstrated that:

i. Anthraquinones were not detected in *Vernonia amygdalina*, *Momordica charantia*, or the combined *Vernonia amygdalina* and *Momordica charantia* ethanolic extracts.

ii. Tannins were found in *Vernonia amygdalina* and *Momordica charantia* but were absent in the combined *Vernonia amygdalina* and *Momordica charantia* extract.

iii. Steroids were present in *Vernonia amygdalina* and the combined *Vernonia amygdalina* and *Momordica charantia* extracts but not detected in *Momordica charantia*.

iv. Acetaminophen at a dose of 3000mg/kg significantly increased WBCs count in experimental rats whiles the other treatments did not significantly affect haematological indices.

v. Administration of *Vernonia amygdalina*, *Momordica charantia* and the combined *Vernonia amygdalina* and *Momordica charantia* had a similar treatment effects on liver biochemical indices however, acetaminophen induction caused a significant increase in ALT enzyme indicating hepatotoxicity, this was confirmed by the histological studies.

vi. Findings from this study suggest that, *Vernonia amygdalina*, *Momordica charantia* and the combined *Vernonia amygdalina* and *Momordica charantia* had a similar hepatoprotective effects

6.2 Recommendations

i. It is recommended that further studies should be conducted to identify the specific active constituents that contribute to the synergistic impact shown in the amelioration of liver disease/damage by *Vernonia amygdalina* and *Momordica charantia*.

ii. It is recommended to conduct the same investigation employing female Wistar rats in order to consider potential physiological changes based on sex, thereby assuring a thorough comprehension and relevance of the results to both genders.

iii. It is recommended that further studies should be carried out to ascertain the exact chemical reactions responsible in eliminating/rendering inactive of some phytoconstituents of the combined *Vernonia amygdalina* and *Momordica charantia.*

iv. It is recommended that another study be conducted to assess and determine the quantities of other biologically active components of *Vernonia amygdalina* and *Momordica charantia* in their combined and individual forms, as this will provide more comprehensive information on the medicinal properties of these plants.

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