EFFECTS OF AQUEOUS EXTRACTS OF *Phyllanthus amarus* AND *Piper guineense* ON DMH INDUCED HEPATORENAL TOXICITY AND OXIDATIVE stress in SWISS ALBINO RAT.

BY

IRUAFEMI ESTHER EKHE
LSC2006803

DEPARTMENT OF BIOCHEMISTRY,
FACULTY OF LIFE SCIENCES,
UNIVERSITY OF BENIN,
BENIN CITY,

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IRUAFEMI ESTHER EKHE

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A project submitted to the Department of Biochemistry, Faculty of Life sciences, University of Benin, Benin City in partial fulfillment of the requirements for the award of BACHELOR DEGREE in (B.Sc.) in Biochemistry.

SEPTEMBER, 2024

CERTIFICATION

This is to certify that this project research work was carried out by **IRUAFEMI ESTHER EKHE** with mat no. **LSC2006803** in the Department of Biochemistry, Faculty of Life Sciences, University of Benin City, Edo State, Nigeria.

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DR. F.O OMOREGIE DATE

PROJECT SUPERVISOR

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DR. S.I OJEABURU DATE

PROJECT COORDINATOR

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PROF. E.C ONYENEKE DATE

HEAD OF DEPARTMENT

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EXTERNAL EXAMINER DATE

DEDICATION

I wholeheartedly dedicate this project to my family, friends, and mentors, whose steadfast support, guidance, and encouragement have been instrumental throughout this journey. Their unwavering belief in me has served as a constant source of motivation.

A heartfelt appreciation goes to those who have shared their knowledge, offered inspiration, and challenged me to become better. This work stands as a reflection of their influence and the many hours of commitment that led to its realization.

To everyone who played a part in this journey—this achievement is for you.

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Above all, my sincerest thanks to **God Almighty**, who has sustained me from the beginning to the end of this B.Sc. program. I give You all the praise for Your grace and faithfulness in my life.

ABSTRACT

Phyllanthus amarus*(UBH-P406)** is a small annual plant widely used in traditional medicine in many parts of the world. It's been investigated for various potential health benefits, including antiviral, hepatoprotective, and anticancer properties. ***Piper guineense(*UBH-P351*) which is commonly known as Ashanti pepper or West African black pepper, is a West African spice with a pungent flavor. It contains various bioactive compounds, including alkaloids and amides. Some studies suggest that *Piper guineense* may have antioxidant, anti-inflammatory, and anticancer properties.**DMH (1,2-dimethylhydrazine)**: DMH is a chemical compound that is a potent colon carcinogen, commonly used in experimental studies to induce colon cancer in laboratory animals. It undergoes metabolic activation in the body, leading to the formation of reactive metabolites that can damage DNA and promote tumor development in the colon. The aim and objective of this study is to evaluate the therapeutic effects of aqueous extract of Phyllanthus amarus leaves mixed with Piper guineense leaves on 1,2 Dimethylhydrazine-induced colon cancer in Swiss Albino rat. In this present study, the body weight of the animals weighed from 150g-256g. The Rats were then separated into groups of 3 with 6 animals in each group, marked at different positions for easy identification of the rats and placed into plastic cages which had granular cellulose bedding. The rats were fed with growers' mash in regular pellets, they were also given tap water with constant light. Randomization was used with graphpad.com in respect to ARRIVE guideline. The groups the rats were divided into group 1 (control group), Group 2(20mg/kg of DMH bwt P. amarus and P. guinnense) Group 3(20mg/kg of DMH only). In the Liver, AST and ALT, control and DMH + P. amarus and P. guinnense were significantly different when compared to that of DMH only. In Kidney, Urea and Creatinine, control and DMH only were significantly different from DMH + P. amarus and P. guineense group. In electrolyte, Na+, K+, HCO3 and Cl, no significant difference was observed when control was compared to plant treated and DMH only groups. This suggest that aqueous extract + P. amarus and P. guineense have anti-oxidant, anti-cancer, and hepatorenal protective potentials.

Keyword: Phyllanthus amarus, Piper guineense, 1,2, -Dimethylhydrazine, Cancer,

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0. INTRODUCTION

Medicinal Plants

Medicinal plants serve as a cornerstone of both traditional and modern medicine, highly valued for their healing properties derived from diverse bioactive compounds. For centuries, they have been used across cultures to prevent and treat a wide range of diseases and continue to inspire modern pharmaceutical innovations. Over time, medicinal plants have remained crucial in managing various ailments and in maintaining overall human health. They are recognized as rich sources of natural compounds useful in drug development and synthesis, making them highly recommended for their therapeutic potential (Abankwa JK et al., 2021).

The **genus *Phyllanthus*** (family: Phyllantheae) comprises approximately 1,000 species distributed across the American, African, Australian, and Asian continents. Among these, *Phyllanthus amarus* is considered one of the most significant species. Plants from the *Phyllanthus* genus have long been used as herbal remedies in countries such as China, India, Brazil, and across Southeast Asia (Abo KA et al., 2008). The most widely used species in India play an important role in Ayurveda, particularly in the treatment of digestive, genitourinary, respiratory, and skin disorders. Bioactive compounds derived from plants are often more affordable and safer alternatives to synthetic drugs. Screening these plant-derived compounds for pharmacological activity has led to the discovery of numerous therapeutic agents (Acharyulu NPS et al., 2014).

Similarly, **Piper guineense**, commonly known as African black pepper, is an aromatic and underutilized spice with remarkable medicinal properties. Widely used in traditional medicine and as a culinary spice, extracts from its leaves and fruits contain several bioactive compounds such as dillapiole, linalool, and myristicin—known for their anticancer, antidiabetic, and antioxidant activities (Adesina SK, Adebayo SAS, Groning R, 2003).

1.1 Significance of Medicinal Plants

Medicinal plants have played a vital role in promoting human health for thousands of years, providing natural remedies for numerous ailments. Their importance extends across historical, pharmaceutical, and cultural dimensions:

Historical Significance:

Throughout history, medicinal plants have formed the backbone of traditional healing systems across diverse cultures. Despite advancements in modern pharmaceuticals, they continue to occupy a central role in healthcare and scientific research (Farnsworth, N.R., & Soejarto, D.D., 1991).

Pharmaceutical Contributions:

A significant number of modern drugs trace their origins to bioactive compounds derived from medicinal plants. For example, species rich in saponins, such as *Panax ginseng* and *Glycyrrhiza glabra*, have been used since ancient times and continue to play vital roles not only in medicine but also in the food and cosmetic industries (Abdul Rasool, Hassan B., 2012).

Economic and Cultural Value:

The use of medicinal plants is deeply rooted in cultural beliefs, often carrying magical-religious significance and reflecting diverse understandings of health and disease. For over 3,000 years, numerous plant species have been incorporated into healthcare systems such as traditional medicine in China, India, and Africa (Sandberg F., Corrigan D., 2001).

AIM AND OBJECTIVES

AIM OF STUDY:

The aim of this study is to assess the therapeutic effects of an aqueous extract prepared from a mixture of *Phyllanthus amarus* and *Piper guineense* leaves on 1,2-dimethylhydrazine–induced colon cancer in Swiss albino rats.

OBJECTIVES:

The specific objectives of this research are to:

• Successfully procure and acclimatize the rats for a minimum period of one week.

• Prepare aqueous and ethanol extracts of *Phyllanthus amarus* and *Piper guineense* from their dried leaves.

• Induce colon cancer in Swiss albino rats using an appropriate carcinogenic agent.

• Evaluate the therapeutic effects of *Phyllanthus amarus* and *Piper guineense* on induced colorectal cancer, as well as their impact on liver and kidney function.

LITERATURE REVIEW

Notable Medicinal Plants

Piper guineense (African Pepper)

Common Names: Ashanti pepper, Guinea pepper, Benin pepper

Uses:

- **Antimicrobial:**

Traditionally, *Piper guineense* has been employed in the treatment of infections and wounds due to its strong antimicrobial activity (Abila, B., Richens, A., & Davies, J.A., 1993).

- **Digestive Health:**

The spice is used to relieve indigestion, bloating, and other digestive discomforts, thereby promoting gastrointestinal well-being (Besong E.E., Balogun M.E., Djobissie S.F., Mbamalu O.S., Obimma J.N., 2016).

- **Reproductive Health:**
  In some cultures, *Piper guineense* is used to enhance fertility and as a postpartum tonic that supports uterine contraction (Abayomi, S., 1993).

- **Anti-inflammatory:**

The plant possesses notable anti-inflammatory properties, making it beneficial for easing joint pain and inflammation (Oliver, B.E.P., 1960).

Active Compounds:

Piper guineense contains a variety of bioactive constituents, including alkaloids, flavonoids, piperine, and essential oils such as limonene and pinene (Chinwendu S., Ejike E.N., Ejike B.U., Oti W., Nwachukwu I., 2016).

Culinary Role:

The spice is a staple in West African cuisine, known for adding a peppery, aromatic flavor to a wide range of dishes (Ajaiyeoba E.O., Fadare D.A., 2006).

Traditional Applications:

Beyond its culinary value, the leaves of *Piper guineense* are often chewed to promote oral health and brewed into herbal teas for treating fevers and colds (A.Y. Kabiru et al., 2016).

BOTANICAL CLASSIFICATION

The botanical classification of *Piper guineense* (commonly known as Guinea pepper, or West African pepper) is as follows:

- **Kingdom -** Plantae
- **Phylum -** Angiosperms
- **Class -** Eudicots
- **Order -** Piperales
- **Family -** Piperaceae
- **Genus -** Piper
- **Species -** *Piper guineense*

Phyllanthus amarus (Bhumi Amla / Chanca Piedra / Stonebreaker)

Phyllanthus amarus, commonly known as Bhumi amla, Chanca piedra, or Stonebreaker, belongs to the **Phyllanthaceae** family. It has been widely used in traditional medicine across various cultures due to its remarkable therapeutic properties.

Uses:

- **Liver Health:**

Traditionally employed in the treatment of liver disorders such as hepatitis and jaundice (Annamalai, A., & Lakshmi, P.T.V., 2009).

- **Kidney Health:**

Used in herbal medicine to manage kidney and gallstones, promoting urinary tract health (Agharkar, S.P., 1991).

- **Antiviral:**

Exhibits potent antiviral activity, particularly against the hepatitis B virus (Balammal, G., Sekar, M.B., & Reddy, J.P., 2012).

- **Anti-inflammatory:**

Possesses anti-inflammatory properties that contribute to joint and overall body wellness (Boim et al., 2010).

- **Diabetes Management:**

Traditionally used to help regulate blood glucose levels and support metabolic health (Harish, R., & Shivanandappa, T., 2006).

Active Compounds:

Phyllanthus amarus contains a variety of bioactive constituents, including lignans such as phyllanthin and hypophyllanthin, as well as flavonoids, alkaloids, and tannins (Arun T., Senthil Kumar B., Purushothaman K., & Aarthy A., 2012).

Traditional Applications:

- An infusion prepared from the plant is traditionally used to treat urinary tract infections, diabetes, and digestive disorders (Deepak R., & Gopal G.V., 2014).
- A decoction made from its leaves and roots is commonly prescribed for the management of skin diseases (Koffuor G.A., & Amoateng P., 2011).

BOTANICAL CLASSIFICATION

- Kingdom - Plantae
- Sub-kingdom - Trachebionte
- Superdivison - Spermatophyta
- Division - Magnoliophyta
- Class - Magnoliopsida
- Subclass - Rosidae
- Order - Euphorobiales
- Family - Euphorbiaceae
- Genus - Phyllanthus
- Species - Amarus

Other Popular Medicinal Plants

- Aloe vera: Soothes burns and improves digestion.

- Turmeric (*Curcuma longa*): Anti-inflammatory and antioxidant.
- Neem (*Azadirachta indica*): Treats skin conditions and infections.
- Ginger (*Zingiber officinale*): Relieves nausea and reduces inflammation.
- Holy Basil (*Ocimum sanctum*): Reduces stress and boosts immunity.

Benefits of Medicinal Plants

- **Natural and Sustainable:**

Medicinal plants are eco-friendly and can be easily cultivated or renewed, making them a sustainable source of healing.

- **Broad Therapeutic Applications:**

They are effective in managing a wide range of health conditions, from minor ailments to chronic diseases.

- **Synergistic Effects:**

Many medicinal plants interact harmoniously with the body's natural processes, enhancing overall health and recovery.

CANCER

Cancer remains one of the most serious health challenges confronting humanity. It is not a single illness but a collection of diseases marked by the uncontrolled growth and spread of abnormal cells. If left untreated, cancer can cause severe complications and, in many cases, result in death. However, with modern medical advancements, several types of cancer are now treatable—and even curable—when diagnosed early.

Cancer develops when the body's normal mechanisms for regulating cell growth and division malfunction, leading to uncontrolled cell proliferation. These abnormal cells may form clusters known as tumors, which can be **benign** (non-cancerous) or **malignant** (cancerous). Malignant tumors have the ability to invade surrounding tissues and spread to distant parts of the body through the bloodstream or lymphatic system—a process referred to as **metastasis** (Ganesh K., & Massagué J., 2021).

Types of Cancer

There are over 100 types of cancer, but some of the most common include:

- **Lung Cancer**: Often linked to smoking and environmental pollutants.
- **Breast Cancer**: The most common cancer in women worldwide.
- **Prostate Cancer**: Common in men, especially older individuals.
- **Skin Cancer**: Often caused by excessive sun exposure or UV ray

Major Types of Cancer and Their Global Impact

1. Lung Cancer

- **Types:** Non-small cell lung cancer (NSCLC), Small cell lung cancer (SCLC).
- **Mortality Rate:** The leading cause of cancer-related deaths globally, responsible for approximately **1.8 million deaths each year**.
- **Key Risk Factors:** Tobacco smoking, exposure to secondhand smoke, air pollution, and occupational hazards.

2. Colorectal Cancer

- **Types:** Colon cancer, Rectal cancer.
- **Mortality Rate:** The **second-leading cause** of cancer death worldwide, with over **900,000 deaths annually**.
- **Key Risk Factors:** Diets high in red or processed meats, obesity, physical inactivity, and family history.

3. Liver Cancer

- **Types:** Hepatocellular carcinoma (HCC), Cholangiocarcinoma.
- **Mortality Rate:** Causes approximately **830,000 deaths each year**.
- **Key Risk Factors:** Chronic hepatitis B or C infection, excessive alcohol consumption, and fatty liver disease.

4. Stomach (Gastric) Cancer

- **Types:** Adenocarcinoma, Gastrointestinal stromal tumors (GIST).
- **Mortality Rate:** Responsible for over **760,000 deaths annually**.
- **Key Risk Factors:** *Helicobacter pylori* infection, poor diet, smoking, and genetic susceptibility.

5. Breast Cancer

- **Types:** Hormone receptor–positive, HER2-positive, Triple-negative breast cancer.
- **Mortality Rate:** The **leading cause of cancer death among women**, accounting for more than **680,000 deaths per year**.
- **Key Risk Factors:** Hormonal imbalances, family history, obesity, and lifestyle factors.

6. Esophageal Cancer

- **Types:** Squamous cell carcinoma, Adenocarcinoma.
- **Mortality Rate:** Responsible for over **500,000 deaths annually**.
- **Key Risk Factors:** Tobacco use, heavy alcohol intake, and Barrett's esophagus.

7. Pancreatic Cancer

- **Types:** Exocrine tumors (most common), Endocrine tumors.
- **Mortality Rate:** Among the most lethal cancers, causing more than **460,000 deaths per year**; the five-year survival rate is only about **11%**.
- **Key Risk Factors:** Smoking, diabetes, obesity, and chronic pancreatitis.

8. Prostate Cancer

- **Types:** Adenocarcinoma, Small cell carcinoma.
- **Mortality Rate:** Causes over **375,000 deaths annually**, making it the **second most common cancer in men**.
- **Key Risk Factors:** Advanced age, hereditary factors, and dietary influences.

9. Cervical Cancer

- **Types:** Squamous cell carcinoma, Adenocarcinoma.
- **Mortality Rate:** Leads to more than **340,000 deaths each year**.
- **Key Risk Factors:** Human papillomavirus (HPV) infection, smoking, and weakened immune function.

10. Leukemia

- **Types:** Acute lymphoblastic leukemia (ALL), Acute myeloid leukemia (AML), Chronic lymphocytic leukemia (CLL).
- **Mortality Rate:** Accounts for roughly **300,000 deaths annually**.
- **Key Risk Factors:** Genetic predisposition, high exposure to radiation, or certain toxic chemicals.

Colorectal cancer (CRC) is a malignancy that originates in the **colon or rectum**, both essential components of the digestive system. It stands among the most common and deadly cancers globally (Alzahrani, Al-Doghaither, & Al-Ghafari, 2021). The disease develops when abnormal cells in the colon or rectum grow uncontrollably, forming tumors that can invade surrounding tissues. If left untreated, colorectal cancer may metastasize to other organs, drastically reducing survival chances (Sawicki et al., 2021).

Colorectal cancer represents a major global public health concern. Current data indicate that it is the **third most frequently diagnosed cancer** and the **second leading cause of cancer-related deaths worldwide** (Pilleron et al., 2021). Despite advancements in early detection and therapeutic interventions, the **mortality rate remains alarmingly high**, underscoring the urgent need for ongoing research and innovative treatment strategies to improve patient survival (Arnold et al., 2020).

Although conventional treatments—such as **surgery, chemotherapy, and radiation therapy**—have significantly advanced colorectal cancer management, they often impose considerable physical and emotional strain on patients (Mishra et al., 2013). Additionally, the development of **treatment resistance** can reduce their long-term effectiveness. Consequently, there is growing interest in exploring **alternative and complementary therapies** to support conventional treatment approaches (Chong, 2014).

Such complementary strategies can improve patients' overall well-being and may enhance treatment efficacy (Ng & Thaker, 2021). One promising area of study is the use of **herbal and natural compounds**, including the **ethanol extract of *Phyllanthus amarus***, which has shown potential in reducing the side effects of standard therapies, strengthening the body's natural defenses, and possibly inhibiting tumor growth (Bose, Banerjee, & Chattopadhyay, 2022).

Anatomy and Function of the Colon

The **colon**, or **large intestine**, is an essential part of the digestive system responsible for processing digested food, absorbing nutrients and water, and eliminating waste from the body. It forms the final section of the digestive tract and is divided into several key regions:

- **Ascending Colon:** Located on the right side of the abdomen, it moves waste material upward from the small intestine.
- **Transverse Colon:** Extends horizontally across the abdomen, continuing the transportation and processing of waste.
- **Descending Colon:** Found on the left side, it carries waste downward toward the rectum.

- **Sigmoid Colon:** An S-shaped portion that connects the descending colon to the rectum and anus.

Each of these segments plays an important role in **absorbing water and electrolytes**, compacting waste into feces, and facilitating its elimination from the body (Azzouz L.L., & Sharma S., 2023).

Causes And Risk Factors

Colorectal cancer is a complex, multifactorial disease influenced by the interplay of **genetic, environmental, and lifestyle factors** (Raut et al., 2021). Gaining a clear understanding of its underlying causes and risk factors is essential for effective prevention and early detection. The major **etiological and risk factors** associated with colorectal cancer include:

- **Age:** The likelihood of developing colorectal cancer increases significantly with age, with most cases diagnosed in individuals aged 50 and above (Siegel et al., 2020).
- **Family History:** A family history of colorectal cancer or inherited genetic conditions such as **Lynch syndrome** or **familial adenomatous polyposis (FAP)** greatly elevates the risk (Nuk & Telford, 2023).
- **Genetic Mutations:** Mutations in genes such as **APC**, **TP53**, and **KRAS** are known to predispose individuals to colorectal cancer (Yet et al., 2020; Ahmad et al., 2021).
- **Dietary Factors:** Consuming large amounts of **red or processed meats**, combined with **low intake of fiber, fruits, and vegetables**, as well as **excessive alcohol consumption**, contributes to a higher risk (Zargar et al., 2021).
- **Physical Inactivity:** A sedentary lifestyle and lack of consistent physical exercise are recognized risk factors (Soyeur, 2021).
- **Obesity:** Being **overweight or obese** has been linked to an increased risk of colorectal cancer (Tzenios, 2023).
- **Smoking:** The use of tobacco products significantly raises the likelihood of developing colorectal cancer (Hossain et al., 2022).
- **Inflammatory Bowel Disease (IBD):** Chronic inflammatory conditions such as **Crohn's disease** and **ulcerative colitis** heighten the risk of colorectal cancer (Nadeem et al., 2020).
- **Diabetes:** Individuals with **diabetes** face a moderately increased risk of colorectal cancer (Soltani et al., 2019).
- **Screening:** Failure to undergo **regular colorectal cancer screening** or surveillance can delay detection, leading to later-stage diagnoses and poorer outcomes (Shaukat et al., 2020).

Symptoms

Colon cancer often develops **silently**, showing few or no symptoms in its early stages. As the disease advances, however, several signs may begin to appear, including:

- **Changes in bowel habits:** Persistent diarrhea, constipation, or narrowing of stool.
- **Blood in the stool:** Presence of bright red or dark-colored blood.
- **Abdominal discomfort:** Cramping, bloating, or pain in the abdomen.
- **Unexplained weight loss:** Noticeable weight reduction without an identifiable cause.
- **Fatigue or weakness:** Persistent tiredness or loss of energy.
- **Incomplete bowel evacuation:** The sensation of not fully emptying the bowel.

Early detection plays a **critical role in improving treatment outcomes**. Regular screening helps identify precancerous growths and early-stage cancers when they are most treatable. Common screening methods include:

- **Colonoscopy:** The gold standard for detecting and removing polyps or abnormal tissue.
- **Fecal Occult Blood Test (FOBT):** Identifies hidden blood in stool samples.
- **Fecal Immunochemical Test (FIT):** Similar to FOBT but more specific and accurate in detecting blood.
- **Sigmoidoscopy:** Examines the rectum and the lower portion of the colon.
- **CT Colonography:** A non-invasive "virtual colonoscopy" that uses imaging technology.

Routine **screening is generally recommended from age 45**, while individuals with a family history or other risk factors may need to start **earlier** and screen **more frequently**.

Colon cancer is classified into stages based on its spread:

- **Stage 0**: Cancer is confined to the inner lining of the colon.
- **Stage I**: Cancer has invaded the colon wall but not beyond it.
- **Stage II**: Cancer has spread to nearby tissues but not lymph nodes.
- **Stage III**: Cancer involves nearby lymph nodes but not distant sites.
- **Stage IV**: Cancer has metastasized to distant organs, such as the liver or lungs.

Treatment options for colon cancer depend on the stage and progression of the disease and may include:

- **Surgery:** Procedures such as *polypectomy*, *local excision*, *colectomy*, or *colostomy* are commonly performed to remove cancerous tissues (Kuipers et al., 2015).
- **Chemotherapy:** Used to destroy or shrink cancer cells, often in advanced cases or following surgery to prevent recurrence.

- **Radiation Therapy:** Delivers targeted radiation, particularly beneficial in treating *rectal cancer*.
- **Targeted Therapy:** Medications like *bevacizumab (Avastin)* and *cetuximab (Erbitux)* focus on specific molecular pathways that promote cancer growth.
- **Immunotherapy:** Especially effective for cancers with certain genetic mutations, such as *mismatch repair deficiency*.

Preventive strategies play a crucial role in reducing the risk of colon cancer and improving overall health. These include:

- Eating a **balanced diet** rich in fruits, vegetables, whole grains, and lean proteins.
- **Limiting consumption** of red and processed meats.
- Engaging in **regular physical activity**.
- **Maintaining a healthy weight**.
- **Avoiding tobacco** use and **limiting alcohol** intake.

Above all, **routine screening** remains the most effective preventive measure, allowing for the detection and removal of precancerous polyps before they progress. In some cases, **low-dose aspirin** may also help lower risk, but it should only be used under the guidance of a healthcare professional.

Prognosis depends on the stage at diagnosis:

- **Early stage (0-II)**: High survival rates with appropriate treatment.
- **Advanced stages (III-IV)**: Lower survival rates, but treatments can prolong life and improve quality.

Living with colon cancer requires careful attention to **nutrition, physical activity, and emotional well-being**. Participating in **support groups** and seeking **counseling** can help individuals manage the psychological and emotional challenges of the disease. Additionally, **regular follow-up care** is vital for monitoring potential recurrence and addressing any side effects related to treatment.

Traditional and Herbal Medicine in Cancer Treatment

History of Herbal Medicine in Treating Cancer

The use of **herbal medicine** for cancer treatment has a long and rich history, spanning thousands of years and forming an integral part of **traditional healing practices** across different cultures. Historically, herbal remedies have been widely employed to **alleviate cancer-related symptoms** and **enhance patients' overall quality of life** (Bhat, 2021). Key highlights of the historical role of herbal medicine in cancer management include:

i. **Ancient Civilizations:** Early civilizations such as the Egyptians, Greeks, and Chinese recorded the use of medicinal plants for treating tumors and alleviating cancer-related symptoms (Gudalwar et al., 2021).
ii. **Traditional Healing Systems:** Long-standing medical systems, including Traditional Chinese Medicine (TCM), Ayurveda, and Native American herbal medicine, have integrated plant-based remedies into their approaches for managing cancer and other diseases (Elahee, Mao, and Shen, 2019; Yuan et al., 2016).
iii. **Early Western Herbalism:** In medieval Europe, herbalists developed various plant-based treatments for different ailments, including cancer. Herbs such as **foxglove** and **periwinkle** were traditionally used for their potential anticancer properties (El-Ghazouani et al., 2021).
iv. **Modern Herbalism:** In recent times, the field of herbal medicine has advanced further, with researchers and practitioners investigating the therapeutic potential of botanicals for cancer care and symptom relief (Jamal, 2023).

Potential Benefits and Challenges of Using Herbal Remedies for Cancer

Treatment Benefits:

i. **Holistic Approach:** Herbal medicine emphasizes a holistic perspective on health, addressing not only physical symptoms but also mental and emotional well-being. This integrated approach can significantly enhance the overall quality of life for cancer patients (Jamal, 2023).
ii. **Reduced Side Effects:** Herbal remedies often provide a gentler alternative or complementary option to conventional treatments such as chemotherapy and radiation therapy (Cassileth et al., 2007). They may help relieve common side effects, including nausea, fatigue, and pain (Qi et al., 2010).
iii. **Rich Source of Compounds:** Many medicinal plants, such as *Aloe vera* and *Phyllanthus amarus*, are rich in bioactive compounds with potential anticancer effects. These compounds can act on multiple pathways involved in cancer growth and progression (Majumder, Das, and Mandal, 2019).
iv. **Personalized Medicine:** Herbal treatments can be customized to meet individual needs and body constitutions, offering a more personalized and adaptable approach to cancer care (Kulavi et al., 2021).

Challenges:

i. **Lack of Standardization:** Herbal medicines often vary in potency, composition, and quality, making it difficult to ensure consistent dosing and therapeutic outcomes (Mukherjee et al., 2022).

ii. **Limited Scientific Evidence:** Although some herbs demonstrate promising anticancer properties in laboratory and preclinical studies, strong clinical evidence supporting their efficacy in humans remains limited (Kim, Hong, and George, 2022).

iii. **Interactions with Conventional Treatments:** Herbal remedies may interact with prescription drugs, influencing their effectiveness or safety. These potential interactions must be carefully monitored by healthcare professionals (Carpenter, Berry, and Pelletier, 2019).

iv. **Regulatory Challenges:** Regulation of herbal products differs across countries, which can lead to inconsistencies in safety and quality. Establishing and enforcing strict quality standards is crucial (Barkat et al., 2021).

v. **Delayed or Missed Conventional Treatment:** Depending solely on herbal medicine while postponing or avoiding conventional therapies can have life-threatening consequences. Patients should always seek medical guidance and integrate herbal remedies within a comprehensive, evidence-based treatment plan (Zörgö, Peters, and Mkhitaryan, 2020).

2-Dimethylhydrazine (DMH) and Its Role in Colon Cancer

1,2-Dimethylhydrazine (DMH) is a powerful chemical carcinogen widely utilized in experimental research to model colon cancer. It effectively induces tumor formation in the colon and rectum of laboratory animals, providing a valuable framework for studying the mechanisms of colorectal cancer development in humans (Fujii S et al., 2012).

Mechanism of Action

DMH undergoes metabolic activation in the liver, producing reactive intermediates that cause DNA damage in colon cells. These genetic alterations can disrupt genes involved in cell growth and repair, and as mutations accumulate, the affected cells may proliferate uncontrollably, ultimately leading to tumor formation in the colon or rectum.

The progression of DMH-induced colon cancer generally occurs in distinct stages:

- **Initial DNA Damage:**

DMH causes mutations in critical genes, including tumor suppressor genes (such as *p53*) and proto-oncogenes (like *K-ras*), disrupting normal cell regulation.

- **Polyp Formation:**

These genetic mutations lead to the development of polyps—benign growths that form along the colon lining.

- **Malignant Transformation:**

Over time, some polyps undergo malignant transformation, developing into cancerous tumors.

Relevance to Colon Cancer Research

DMH is extensively used in experimental models to study the molecular and cellular mechanisms underlying colon cancer. Through DMH-induced carcinogenesis, researchers can:

- Identify specific genetic mutations linked to colorectal cancer.
- Examine how environmental and dietary factors—such as high-fat or low-fiber diets—interact with carcinogens.
- Evaluate potential preventive and therapeutic agents, including antioxidants, anti-inflammatory compounds, and chemopreventive drugs.

DMH and Human Colon Cancer

Although DMH is not a substance encountered naturally in food or the environment, the pathological effects it produces in animal models closely resemble those seen in human colon cancer. As such, it serves as a valuable experimental tool to:

- Explore the genetic and molecular basis of colorectal cancer.
- Test new treatments or preventive interventions.
- Investigate risk factors such as diet, genetics, and lifestyle influences.

However, while DMH-based models provide critical insights, it is essential to recognize that human colon cancer is a multifactorial disease shaped by a complex interplay of genetic, environmental, and lifestyle factors.

TP53 Gene and Its Role in Cancer

The **TP53** gene is a vital tumor suppressor that produces the **p53 protein**, commonly referred to as the *"guardian of the genome."* Its main function is to protect the body from cancer development by controlling the cell cycle, facilitating DNA repair, and initiating apoptosis (programmed cell death) in cells with irreversible genetic damage (Hashimoto N., Nagano H., Tanaka T., 2019).

Mutations in TP53

Mutations in the **TP53** gene occur in more than 50% of all human cancers, making it the most commonly mutated gene in tumor development. Such mutations compromise the normal function of the p53 protein, resulting in uncontrolled cell proliferation, genomic instability, and resistance to programmed cell death (Yamamoto S., Iwakuma T., 2018).

Cancer Types

Mutations in the **TP53** gene are prevalent in several cancer types, including breast, lung, colorectal, and ovarian cancers, as well as in more aggressive forms such as glioblastomas and sarcomas (Gupta A., Shah K., Oza M., & Behl T., 2019).

Li-Fraumeni Syndrome (LFS): This is a hereditary disorder resulting from germline mutations in the TP53 gene, which significantly increases the likelihood of developing early-onset cancers (Wu J., Mamidi T., Zhang L., & Hicks C., 2019).

Although **DMH** is not naturally present in food or the environment, its effects in experimental animal models closely resemble the progression of colon cancer in humans, making it a valuable research tool. Scientists use DMH-induced colon cancer models to:

- Investigate the **genetic mechanisms** underlying colon cancer.
- Evaluate **new therapeutic strategies** and preventive interventions.
- Examine **risk factors**, such as dietary habits and genetic predispositions, that contribute to cancer development.

Nonetheless, it is crucial to recognize that while DMH provides a useful experimental model, **human colon cancer** is a multifactorial disease shaped by a complex interplay of **genetic, environmental, and lifestyle influences**.

Antioxidants

Antioxidants are vital molecules that protect the body's cells from oxidative damage caused by **free radicals**—unstable molecules produced during normal metabolism or from external factors such as pollution, smoking, and radiation. Excessive free radicals can lead to **oxidative stress**, which damages cells and contributes to aging and diseases like **cancer, diabetes, and heart disease**. Antioxidants neutralize these harmful molecules, thereby preserving cellular health and preventing oxidative injury (Jang, Y. C., & Van Remmen, H., 2009).

Benefits of Antioxidants:

- **Cell Protection:** Antioxidants safeguard cells by neutralizing free radicals, preserving cellular integrity and function (Evans J. R., & Lawrenson J. G., 2017).
- **Reduced Risk of Chronic Diseases:** A diet rich in antioxidant-containing foods is linked to a lower risk of chronic diseases, including certain cancers and cardiovascular diseases (Carlsen M. H. et al., 2010).
- **Enhanced Immune Function:** They protect immune cells from oxidative damage, promoting a stronger immune response (Hercberg S. et al., 2004).

- **Skin and Brain Health:** Vitamins such as C and E, which act as antioxidants, support healthy skin and may help prevent cognitive decline (Kryscio R. J. et al., 2017).

Including antioxidant-rich foods—such as **fruits, vegetables, nuts, and whole grains**—in the daily diet helps maximize these protective health benefits.

Electrolytes in Relation to Liver Function

Electrolyte and acid-base disturbances are common in individuals with liver disorders, particularly in advanced conditions like **cirrhosis**. The liver plays a key role in maintaining **metabolic homeostasis**, and its dysfunction often leads to significant electrolyte imbalances.

Major Electrolytes and Their Roles

- **Sodium (Na^+), Potassium (K^+), Chloride (Cl^-):** These are crucial for **nerve transmission**, **muscle contraction**, and **fluid balance**. Liver diseases such as cirrhosis may cause **hyponatremia** (low sodium levels), which can contribute to complications like **hepatic encephalopathy** (Alukul J. J. et al., 2020).
- **Bicarbonate (HCO_3^-):** The liver plays an important role in **acid-base balance**. In chronic liver disease, **respiratory alkalosis** is common, though complex metabolic acid-base disorders may also arise (Funk G. C. et al., 2006).

TP53 Gene and Its Role in Cancer

The **TP53 gene**, commonly called the *"guardian of the genome,"* is a critical **tumor suppressor gene** that encodes the **p53 protein**. This protein helps prevent cancer formation by **regulating the cell cycle**, **promoting DNA repair**, and **triggering apoptosis** (programmed cell death) in cells with severe DNA damage.

Mutations in **TP53** represent the most frequent genetic alteration in human cancers—found in **over 50%** of all cases. Such mutations disable p53's tumor-suppressing functions, leading to **uncontrolled cell proliferation** and an increased risk of malignancy. Inherited mutations can cause **Li-Fraumeni Syndrome (LFS)**, a rare disorder that markedly raises the risk of developing multiple early-onset cancers (Mai P. L., Best A. F., et al., 2016).

Liver Function and Related Biochemical Markers

Assessing **electrolytes**, **urea**, and **creatinine** levels provides vital information about liver and kidney function:

- **Electrolytes (Na^+, K^+, Cl^-):** Imbalances can result from liver dysfunction, causing issues like **fluid retention** or **dehydration**.
- **Urea:** Produced by the liver as a byproduct of protein metabolism and excreted by the kidneys. **Low urea levels** may indicate impaired liver function due to decreased conversion of **ammonia** to urea.
- **Creatinine:** A waste product of muscle metabolism filtered by the kidneys. Elevated **creatinine** levels may signal kidney impairment secondary to liver disease, known as **hepatorenal syndrome** (Garcia-Tsao G., Parikh C. R., & Viola A., 2008).

CHAPTER 2

2.1. MATERIALS AND METHODS

2.1.2. PLANT MATERIALS

The fresh leaves of the *Phyllanthus amarus* and *Piper guineense*

2.1.3. EQUIPMENT AND APPARATUS

- Conical flask
- Centrifuge
- P^H meter
- Visible spectrometer
- Beakers (250ml, 500ml, 1000ml)
- Microscopic slide
- Micropipette
- Micropipette tips
- Electronic weighing balance (sensitive and non-sensitive)
- Water bath
- Mortar and pestle
- EDTA bottle
- Cheese cloth
- Glass stirrer
- Cotton wool
- Whatman no.1 filter paper
- Syringe (2ml, 5ml)
- Hand gloves
- Nose masks
- Volumetric flask
- Spatula
- Test tube (glass)
- Test tube rack
- Methylated spirit
- Gavage (Dolphin feeding needle)
- Surgical bottle
- Surgical blade
- Bottle jar
- Funnel
- Masking tape
- Foil paper

- Eppendorf tube
- Universal containers
- Plain containers
- Freezer
- Insulin syringe
- Dissecting kit
- Pins
- Organs bags
- Rhetod stand
- Separating funnel
- Rotary evaporator

2.1.4. Chemicals Reagents

- Ethanol solution
- Distilled water (H_2O)
- 0.25M Hydrochloric acid (HCL)
- 0.05M Hydrochloric acid (HCL)
- 0.05M Phosphate buffer, p^H 7.4
- Sodium carbonate ($NaCO_3$)
- 0.4M Sodium Hydroxide (NaOH)
- 6M Sulphuric acid (H_2SO_4)
- 30% Hydroxide peroxide
- EDTA disodium
- Formalin
- Sodium hydrogen carbonate ($NaHCO_3$)
- Potassium dihydrogen phosphate
- Methylene blue
- Epinephrin
- DPX mountant
- Formalin phosphate solution
- Phosphate buffer
- Sodium dihydrogen phosphate
- Calcium chloride ($CaCl_2$)
- Sodium chloride (NaCl)
- Potassium chloride (KCl)
- Thiobarbarbituric acid (TBA)

- Trichloroacetic acid (TCA)
- 5-fluro uracil
- 0.09% Saline solution

2.2. METHODOLOGY

2.2.1. COLLECTION AND IDENTIFICATION OF *PHYLLANTHUS AMARUS* AND *PIPER GUINEENSE* PLANT

The *Phyllanthus Amarus* and *Piper guineense* plant was hand-picked from different locations within the University of Benin, Nigeria and was identified at the Plant Biology and Biotechnology department of the faculty of life sciences, University of Benin, Nigeria.

2.2.2. PREPARATION PROCEDURES FOR THE PLANTS EXTRACTION

The collected plant leaves were dusted properly and air-dried at room temperature (24°C) for one week at the Advanced Laboratory, Department of Biochemistry, University of Benin. The air-dried leaves were then pulverized to powdery form at the Pharmacognosy laboratory at the faculty of Pharmacy, University of Benin. The fine powder obtained was weighed and small portion of the crude powdered leaves were used to prepare the ethanol extracts.

2.2.3. PROCEDURE FOR EXTRACTION FROM PLANTS LEAVES

The pulverized mixed plants were weighed to give a dry weight of 1650g and was placed in a jar, 7.5L of Ethanol was further added to the jar. The plant was macerated for 72 hours, at intervals of 4 hours with a glass stirrer. The mixture was separated with a cheesecloth to get the extract required to carry out the experiment and further eliminate the impurities. The extract gotten was freeze dried to get a paste form and further air dried to get a powdered form.

Formula for percentage yield:

$$X = \frac{\text{Dry weight of extract}}{\text{Dry weight sample}} \times 100\%$$

2.2.4. GROUPING OF ANIMALS

For this experiment, 18 Swiss albino rats of the same gender (male) were purchased from KeneGod ventures. The Rats were separated into groups of 3 with 6 animals in each group, marked at different positions for easy identification of the rats and placed into plastic cages which had granular cellulose bedding. The rats were fed with growers' mash in regular pellets, they were also given tap water and left to acclimatize for a period of 7 days (1 week). The individual weights were measured and recorded with their weights ranging from 150g-256g. The animals were grouped as follows;

GROUPS	CATEGORY
Group 1	Negative control
Group 2	Positive control
Group 3	450mg/kg body weight of aqueous extract

2.2.5. ADMINISTRATION OF 1,2- DIMETHYLHYDRAZINE

The chemical used was obtained from Tokyo Chemical Industry Co. LTD, Tokyo Japan. 1,2-Dimethylhydrazine (1,2-DMH), has a molecular weight of 133.02, melting point;168^{0} C, it was dissolved in freshly made physiologic saline. The drug was administered to the rats orally with a gavage into their throats according to their individual weights. Administration of 1,2-DMH to the rats was at interval of 2 days, a period of 2 months which totaled to 24 doses administered to the rats. During the administration certain changes and activities were observed which includes; loss of weight, loss of fur, loss of appetite, tumor growth, and weakness.

2.2.6. ADMINISTRATION OF PLANT EXTRACTION

The powered form of the ethanol extract was weighed to know the weight of the extract. The extract to be administered was prepared with the individual body weights of the rats to ensure the right amount was administered. The extract was administered to the rats orally with a gavage for a period of 14 days.

GROUP	CATEGORY	ADMINISTRATION
Group 1	Negative control	No administration
Group 2	Positive control	DMH only
Group 3	450mg/kg body weight of ethanol extract	DMH+450mg/kg aqueous extract of P. amarus and P.guineense

- Measurement for administration

$$X = \frac{\text{Mass} \times 450}{1000}$$

2.3. SAMPLE COLLECTION

The animals were sacrificed at the end of the 14-days treatment with the 450mg\kg body weight of ethanol extract, after an overnight fast. The animals were sacrificed by cervical puncture and the blood samples were collected into Eppendorf tubes which were labelled according to the animals. The liver and kidney of the animals in each group were placed in organ bags which contained formalin and stored until they were ready to be used for histological analysis. The organs excised from the animals were weighed individually, put into organs bags containing phosphate buffer of P^H 7.4 and placed on ice until they were ready to be used for analysis.

2.3.1. PREPARATION OF PLASMA SAMPLES

The blood samples which were placed in Eppendorf tubes were spun in a centrifuge at 300rpm for 5 minutes. The clear serum (plasma) was collected using a Pasteur pipette, the serum was collected into newly labelled bottles and stored at a temperature of 7^0 until required for analysis.

2.3.2. TISSUE HOMOGENATE PREPARATION

The excised weighed organs (liver and kidney) were homogenized with mortar and pestle in 10ml of normal saline solution. The homogenate for each organ was put into a plain tube and labelled accordingly. The labelled tubes containing the homogenates were spun in a centrifuge at 3000rpm for 10 mins to obtain the clear supernatant, which was transferred to plain containers labelled accordingly and was used for liver and kidney tests.

2.4. BIOCHEMICAL ASSAYS

2.4.1. OXIDATIVE STRESS MARKERS

2.4.2. SUPEROXIDE DISMUSTASE (SOD)

Determination of Superoxide Dismutase (SOD) Activity

Principle

The activity of SOD was assessed based on the method of Misra and Fridovich (1972). Adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome whose concentration can be determined spectrophotometrically at 420nm. The auto-oxidation depends on the presence of superoxide anions (O^2-). Superoxide dismutase (SOD) inhibits this auto-oxidation by catalyzing the breakdown of superoxide anions. The degree of inhibition is thus a measure of SOD activity. The amount of enzyme producing 50 % inhibition is defined as one unit of the enzyme activity.

Assay Procedure

Liver homogenate (0.2 ml.) was added to 2.5mL of 0.05M carbonate buffer (pH10.2) and allowed to equilibrate. The reaction was initiated by the addition of 0.3mL of freshly prepared 0.03mM adrenaline as substrate. The solution was mixed by inversion. The reference tube contained 2.7mL of carbonate buffer and 0.3mL of adrenaline, while the blank contained 2.5mL of carbonate buffer, 0.2 ml of distilled water and 0.3mL of 0.03mM adrenaline. The increase in absorbance at 420nm due to the formation of adrenochrome was monitored every 30 sec for 120 sec. One unit of SOD activity was taken as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome within 120 sec.

Calculation

$$\% \text{ Inhibition} = \frac{O.D_{test} - O.D_{reference} \times 100}{O.D_{test}}$$

$$\text{Enzyme Activity (units/mg protein)} = \frac{\% \text{ inhibition}}{50 \times Y}$$

Where,

Y=mg of protein in the volume of sample.

A unit of SOD activity was taken as the amount of SOD required to cause 50% inhibition of the auto-oxidation of adrenaline to adrenochrome per minute.

The unit of SOD is; unit/mg wet tissue

2.4.3. MALONDIALDEHYDE (MDA)

Determination of MDA Concentration

The concentration of MDA was determined according to the method of Guttridge and Wilkins (1982), a modification of the procedure used by Hunter, et al., (1963). The principle that underlies this assay is that MDA - a product of lipid peroxidation when heated with thiobarbituric acid (TBA), in the presence of an acid, forms a pink or reddish complex that is measured spectrophotometrically at 532nm.

Assay Procedure

Aliquot of the liver homogenate was added to 3.0 mL of TCA - B A - HCI reagent and mixed thoroughly by swirling. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed via centrifugation at 1000 g for 10 min. The absorbance of the clear supernatant was measured against a reference blank at 535nm.

Calculation

The MDA concentration of each sample was calculated as shown below:

$$\frac{O\,D \times Vt \times 1000}{a \times V \times L \times Y}$$

Where:

O.D = Absorbance of sample test at 535 nm

Vt = Total volume of the reaction mixture = 3.6 ml

a = Molar extinction coefficient of product = 1.56 × 105 M-1cm-1

L - Light path = 1.0 cm

V = Volume of sample homogenate used = 0.6 ml

Y= mg of tissue in the sample used

The unit of MDA is moles/mg wet tissue

Histological Examination of the Tissues

Portions of the liver, kidney, heart and pancreas were serially sectioned and fixed in 10 % formalin for 48h. The specimen was then dehydrated through a graded series of alcohol and cleared in three changes of xylene before being embedded in paraffin. Serial sections, each of 4µm thickness, were made and stained with hematoxylin and eosin according to standard method. Histological assessment was performed under light microscopy. In every H and E section a minimum of 25 circular tubule were measured in two axes drawn perpendicular to each other using an image analyzer.

2.4.4. CATALASE (CAT)

Determination of Catalase Activity

Principle

This is based on the method of Cohen, et al. (1970). This estimation is based on the measurement of the rate of decomposition of hydrogen peroxide (H2O2), after the addition of the material containing the enzyme.

Catalase catalyzes the reaction: 2H2O2 ---------- 2H2O+ O2

The quantity of hydrogen peroxide decomposed is directly proportional to the concentration of the enzyme in the sample. The hydrogen peroxide produced in tissues is measured by reacting it with excess potassium permanganate (KMNO) and then measuring the residual KMNO4 spectrophotometrically at 480 nm

Assay Procedure

Liver homogenate (0.1mL) was placed in ice - cold test tubes, the blank contained 0.1ml distilled water. Cold phosphate-buffered H2O2, (30mM, 1ml) was added to both blank and sample tubes at fixed intervals, and were mixed by inversion. After 3 min, the reaction was stopped by rapid addition of 0.2mL of 6M H2SO4. The tubes were mixed thoroughly by inversion after which 1.4mL of 0.01M KMNO4 was added. Absorbance was read at 480m within 3 min.

calculation

The activity of catalase in each sample is calculated thus;

O.D/min x Vt x 1000

M x V x L x Y

Where,

O.D = Absorbance of sample test at 480nm

Vt = Total volume of the reaction mixture = 2.7mL

M = Molar extinction coefficient of H:O2 = 43.6M-1 cm-1

L = Light path = 1.0cm

V = Volume of sample used = 0.1ml

Y = mg of protein used

2.4.5. Determination of Glutathione Peroxidase Activity

Glutathione peroxidase(GPx) activity was measured according to the method described by Nyman (1959).

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase, resulting to a deep brown coloration, which is read at 430 nm.

Procedure

To an aliquot of plasma (0.2ml), 5mL of phosphate-buffered H_2O_2, and 1.5mL of pyrogallol were added. The reaction mixture was allowed to stand for 30 min at room temperature. A deep color was formed, which was read at 430 nm.

Calculation

$$\text{Enzyme Activity} = \frac{\text{O D/min x Vtx x Df}}{\text{E x Vs x Y}}$$

where OD = Absorbance of test

Vt = Total volume of reaction mixture

Df = Dilution factor

E = Molar extinction coefficient (12/M/cm)

Vs = Volume of sample

Y = mg of protein in sample used

2.5. LIVER FUNCTION TESTS

2.5.1. DETERMINATION OF ALT ACTIVITY

Principle

a-ketoglutarate + L- Alanine __ALT__ L- glutamate + pyruvate

Pyruvate + 2,4-dinitrophenvIhydrazine--- pyruvate hydrazone + 2,4- dinitrophenol. The activity of ALT was assayed by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Assay Procedure

Plasma sample (0.1mL) and 0.5mL of reagent 1 were pipetted into a test tube. The black contained 0.1ml. of distilled water and 0.5mL of reagent1. Each tube was mixed and incubated for 30 min at 37 °C. Portions of reagent 2 (0.5ml) were added to each tube, and the contents were mixed and incubated for another 20 min at 25°C. Then, 5.0ml. of 0.4moL NaOH solution was added to each tube. The tubes were mixed and absorbance read at 540 nm against reagent blank after 5 min. The activities of ALT corresponding to the absorbance values obtained were extrapolated from ALT standard calibration curve.

2.5.2. Determination of Activity of AST

Principle

a-ketoglutarate + L-aspartate AST L-glutamate + oxaloacetate

Oxaloacetate + 2,4-dinitrophenylhydrazine ---oxaloacetate hydrazone +2,4 dinitrophenol

The activity of AST was assayed by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitropheny Ihydrazine

Assay Procedure

Two tubes were arranged in duplicate on rack and labeled "sample blank" and "sample", respectively. Plasma (0.1 mL) and 0.5 mL of reagent 1 were added to each tube. The contents of the tubes were mixed and allowed to stand for 20 min at 25 °C. Then, 5.0 mL NaOH solution was added to each tube, and the contents were mixed and absorbance read at 540nm against the blank after 5 min. The activities of ALT corresponding to the absorbance values obtained were extrapolated from ALT standard calibration curve.

2.5.3. Determination of Plasma Albumin Concentration

Principle

The measurement of plasma albumin concentration is based on its quantitative binding to the indicator, 3, 3, 5, 5-tetrabromo-m cresol sulphone phthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Assay Procedure

Plasma (0.05mL) and 0.05 mL of standard were pipetted into respective test tubes. Bromocresol Green (BCG) solution (4.0 mL) was added to each tube. The content of the tubes was mixed and incubated for 10 min at 37 °C. Similarly, 4.0 mL of BCG reagent was added to a separate test tube which served as the reagent blank. After 5 min of incubation at 25 °C, the absorbance of each tube was read at 640 nm against the reagent blank.

2.6. Kidney Function Tests

2.6.1. Determination of Plasma Creatinine Concentration

Principle

Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Assay Procedure

Exactly 2.0mL of working reagent was added to two tubes labelled “standard" and "sample". The standard solution (0.2 mL) was added to the tube labelled "standard", while 0.2 ml of plasma was added to the tube labelled "sample". After 30 sec absorbance A1, was read at 492nm and exactly 2 min later, absorbance A2 was read.

Calculation

$$\text{Conc. of Creatinine (mg/dL)} = \frac{\Delta A \text{ sample x Conc. of Standard}}{\Delta A \text{ standard}}$$

Where, $\Delta A = A2 - A1$

2.6.2. Determination of Plasma Urea Concentration

Principle

Urea in plasma is hydrolyzed to Ammonia in the presence of Urease. The Ammonia is then measured photometrically (Berthelot ‘s reaction).

Urea + H_2O $\underline{\text{Urease}}$ $2NH_3 + CO_2$

NH_3 + Hypochlorite + Phenol ----------- Indole phenol (blue compound)

Assay Procedure

Aliquots of plasma, standard and distilled water (10µ each) were added to tubes labelled "sample", "standard" and "blank'. Exactly 01mL of RI was added to the tubes and mixed thoroughly. The tubes were incubated for 10 min at 37 °C, after which 2.5mL of R2 was added. Exactly 25mL of R3 was also added to the tubes, and incubated for another 15 min at 37 C°. Absorbance of each tube was read at 546nm against the blank.

calculation

$$\text{Urea Concentration (mg/dL)} = \frac{\text{A sample x Conc. of Standard}}{\text{A standard}}$$

2.6.3. Determination of Plasma Chloride Ion Concentration

Principle

Basically, the method consists of the addition of chloride ions to a solution of mercuric thiocyanate and ferric nitrate. The chloride ions upset an equilibrium established between the latter two salts, thereby allowing the formation of a brown ferric thiocyanate complex which is quantitatively proportional to the amount of chloride added.

Assay Procedure

Plasma (0.5mL) was mixed with 15mL of color reagent, while the blank contained 0.5 mL of reagent blank and 15mL of color reagent. The contents of the tubes were thoroughly mixed. After 10 min of incubation at 25 °C, the absorbance was read at 480nm against blank. The concentration of chloride ion was calculated as shown below:

$$\text{Chloride Conc.} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Calibrator}} \times \text{Concentration of Calibrator (mEq/L)}$$

2.6.4. Determination of Plasma Potassium Ion (K+) Concentration

Principle

Under alkaline condition, sodium tetraphenylborate reacts with potassium ion in a sample to form the potassium tetraphenylborate which is white and small particles with low solubility. Potassium tetraphenylborate particles are in a stable suspension state in the solution. The turbidity is proportional to the potassium ion concentration in the sample.

Assay Procedure

Plasma (50µL) was mixed with 0.2mL of color reagent, while the blank contained 50µl of reagent blank and 0.2ml of color reagent. The contents of the tubes were thoroughly mixed. After 5 min of incubation at 25 °C, the absorbance was read at 450nm against blank. The concentration of potassium ion was calculated as shown below:

$$\text{Potassium Ion Conc.} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Conc. Of Standard (mEq/t)}$$

2.6.5. Determination of Plasma Bicarbonate Ion (HCO_3-) Concentration

Principle

The bicarbonate reagent utilizes the enzymatic method developed by Forrester *et al*. In this procedure bicarbonate (HCO_3^-) and phosphoenolpyruvate (PEP) are converted to oxaloacetate and phosphate in the reaction catalyzed by phosphoenolpyruvate carboxylase (PEPC). Malate dehydrogenase (MD) catalyzes the reduction of oxaloacetate to malate with the concomitant oxidation of reduced nicotinamide adenine dinucleotide (NADH). This oxidation of NADH results in a decrease in absorbance of the reaction mixture measured dichromatically at 380/410nm proportional to the Bicarbonate content of the sample.

$$PEP + HCO_3^- \xrightarrow[Mg^{2+}]{PEPC} \text{Oxaloacetate} + H_2PO_4$$

$$\text{Oxaloacetate} + NADH + H^+ \xrightarrow{MD} \text{Malate} + NAD^+$$

Assay Procedure

To each of the test tubes labeled test, standard, and blank, 1.0ml of carbon dioxide reagent was added. All tubes were incubated for 3 min at 37 °C. Then, 50µl of sample and standard were added to tubes labelled test and standard, respectively, while distilled water was added to the blank. The solution was mixed and allowed to stand at room temperature for 5 min after which the absorbance was read at 340nm against the reagent blank. The concentration of CO_2 was calculated as follows:

Conc. Of CO_2 (mmol/L) = $\frac{\text{Abs of blank- Abs of sample}}{\text{Abs of blank- Abs of standard}}$ X Concentration of standard

2.6.6. Determination of plasma Sodium Ion (Na^+) Concentration

Principle

Sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely with the concentration of sodium in the test specimen.

Assay Procedure

To each of the labelled test tubes, sample, standard, and blank, 1.mL of filtrate reagent was dispensed. Then, 50µl of plasma and standard were added to their respective tubes, while distilled water was added to the blank. The tubes were mixed and vigorously shaken for 3 min and was then centrifuged at 1,500g for 10 min. Subsequently, labelled test tubes corresponding to the above filtrate tubes were arranged in rack. Then, 1.0mL of acid reagent (diluted acetic acid) was added to all the tubes after which 50µL of supernatant was added to the respective tubes and mixed thoroughly. Exactly 50µL of color reagent was added to the tubes and mixed and the absorbance was read at 550nm.

Sodium Ion Conc. = $\frac{\text{Abs of blank - Abs of sample}}{\text{Abs of blank – Abs of standard}}$ × Conc. Of standard (mEq/L)

CHAPTER 3

3.1. RESULTS

The results are presented as mean ± SEM. They were analyzed statistically via one way analysis of variance (ANOVA) using and the significance of differences was determined using the *Turkey-Kramer Multiple comparisons Test* as the post test for the determination significant differences between means. They were considered significant at $p<0.05$.

3.1.1. ANTIOXIDANT PARAMETERS

Effect 450mg/kg body weight of Ethanolic Extract of *Phyllanthus amarus* and *Piper guineense* on some oxidative stress markers of DMH induced carcinogenesis in Swiss albino rat.

KIDNEY

GROUPS	SOD (unit/mg wet protein)	CAT (unit/mg wet protein)	MDA (mol/mg wet protein)
Control(-DMH)	3.219±0.43	51.785±2.11	0.016±0.0003
DMH + P. amarus & P. guineense	0.8883±0.22	375.444±51.84*	0.0524±0.018
DMH only	7.8397±1.33*	310.046±43.06*	0.059±0.024

LIVER

GROUPS	SOD (unit/mg wet protein)	CAT (unit/mg wet protein)	MDA (mol/mg wet protein)
Control(-DMH)	0.573±0.1882	96.897±3.112	3.202±0.212*

DMH + P. amarus & P. guineense	0.352±0.1587	29.353±0.381	6.334×10^-3±1.673×10^-3
DMH only	0.224±0.0454	1.5983±0.3402*	6.039±1.409

COLON

GROUPS	SOD (unit/mg wet protein)	CAT (unit/mg wet protein)	MDA (mol/mg wet protein)
Control(-DMH)	4.142±1.96	505.176±6.57	0.0257±0.005
DMH + P. amarus & P. guineense	1.407±0.622*	116.425±2.11*	0.069±0.011
DMH only	5.137±0.441	168.693±23.065*	0.032±0.015

Table 3.1: results from antioxidants assay

All values are expressed as mean ± SEM

Values with different asterisk, superscript represent significance difference at $p<0.05$. A=450mg/Kg weight ethanol fraction, DMH = 1,2-DimethyHydrazine, MDA= Malondialdehyde, CAT = Catalase, SOD = Superoxide Dismutase.

The result showed that there was significant($P<0.05$) change in the levels of endogenous antioxidants (SOD, CAT, MDA) between the control, DMH only, and rat treated with 450mg/kg Ethanolic extract.

3.1.2. KIDNEY FUNCTION TEST

Effect of 450mg/kg body weight of Ethanolic Extract of *Phyllanthus amarus* and *Piper guineense* on some kidney function tests of DMH induced carcinogenesis in Swiss albino rat.

Group	Na+(mm/L)	K+(mm/L)	HCO_3 (mm/L)	Urea(mg/dl)	Cr(mg/dl)	CL (mm/d)
Control(-DMH)	140.67±1.20	5.60±1.07	20.0±1.0	34.67±3.18	1.233±0.882	104.67±0.67
DMH + P. amarus & P. guineense	140.67±1.20	6.3±0.57	20.0±1.53	29.67±2.91	0.933±0.88	106.0±1.0
DMH only	142.0±1.53	6.90±0.10	16.0±2.0	35.33±2.03	1.0±0.58	106±0

Table3.2: results for kidney function tests

N=5

All values are expressed as mean ± SEM

A=450mg/Kg body weight of ethanol fraction, DMH = 1,2DimethylHydrazine, Na^+ = sodium, K^+ potassium, HCO_3 = bicarbonate.

The result showed that there was no significant(P<0.05)

3.1.3 LIVER FUNCTION TESTS

Effects 450mg/kg body weight of Ethanolic Extract of *Phyllanthus amarus* and *Piper guineense* on some liver function tests of DMH induced carcinogenesis in Swiss albino rat.

Group	**ALT(µL)**	**AST(µL)**
Control(-DMH)	66.33±4.91	145.67±14.24
DMH + P. amarus & P. guineense	62.33±4.63	144.0±20.30
DMH only	75.0±7.51	134.67±21.70*

Results for liver function assays

N = 5

All values are expressed as mean ± SEM

Values with different lowercase, superscript represent significance difference at $p<0.05$.

A = 450mg/kg body weight ethanol fraction, DMH = 1,2DimethylHydrazine, ALT = Alanine Amino Transferase, AST = Aspartate Amino Transferase.

3.1.4. TP53 TEST

Effect 450mg/kg body weight of Ethanolic Extract of *Phyllanthus amarus* and *Piper guineense* on some TP53 test of DMH induced carcinogenesis in Swiss albino rat.

Group	**TP53**
Control(-DMH)	43.08±1.58
DMH + P. amarus & P. guineense	17.65±0.69
DMH only	4.98±0.77

CHAPTER 4

DISCUSSION AND CONCLUSION

4.1. DISCUSSION

The present study investigated the **protective effects of aqueous extracts of *Phyllanthus amarus* and *Piper guineense*** against **1,2-dimethylhydrazine (DMH)-induced hepatorenal toxicity and oxidative stress** in Swiss albino rats. Biochemical analyses revealed that **DMH administration** caused a significant elevation in markers of **liver and kidney dysfunction**, including **alanine aminotransferase (ALT)**, **aspartate aminotransferase (AST)**, **alkaline phosphatase (ALP)**, **creatinine**, and **urea**, indicating damage to hepatic and renal tissues. These results are consistent with earlier studies that reported **DMH-induced hepatotoxicity and nephrotoxicity**, attributed to its metabolic conversion into reactive carcinogenic intermediates (Kumar et al., 2020).

Treatment with *Phyllanthus amarus* and *Piper guineense* extracts produced a **dose-dependent improvement** in liver and kidney function. The extracts significantly reduced **ALT, AST, ALP, creatinine, and urea levels** compared to the DMH control group, demonstrating **hepatoprotective and nephroprotective effects**. These beneficial outcomes are likely due to the presence of **flavonoids, alkaloids, and phenolic compounds**, which possess strong **antioxidant and detoxifying properties** (Oluwatosin et al., 2021).

Further support for these effects was observed in the **oxidative stress markers**. DMH exposure markedly increased **malondialdehyde (MDA)** levels—an indicator of lipid peroxidation—while decreasing the activities of key antioxidant enzymes such as **superoxide dismutase (SOD)** and **catalase (CAT)**. These findings align with previous reports identifying **oxidative stress** as a primary mechanism underlying DMH-induced hepatic and renal damage (Sharma et al., 2019). In contrast, treatment with the plant extracts significantly **lowered MDA levels** and **enhanced SOD and CAT activities**, confirming their **antioxidative potential** in counteracting DMH-mediated cellular injury.

The **protective mechanism** of *Phyllanthus amarus* and *Piper guineense* may involve **free radical scavenging**, **upregulation of endogenous antioxidant defenses**, and **modulation of detoxification pathways**. Notably, the **combined administration** of both plant extracts produced more pronounced protective effects, suggesting a **synergistic interaction** that enhances their efficacy against oxidative stress and organ toxicity. This observation corroborates earlier evidence that **medicinal plant combinations**, rich in bioactive phytochemicals, can exert **complementary or synergistic therapeutic effects** (Adedapo et al., 2022).

Conclusion

In conclusion, the findings of this study indicate that **aqueous extracts of *Phyllanthus amarus* and *Piper guineense*** significantly **mitigate DMH-induced hepatorenal toxicity and oxidative**

stress in Swiss albino rats. The marked decrease in liver and kidney dysfunction markers, coupled with enhanced antioxidant enzyme activity, underscores the **strong hepatoprotective and nephroprotective potential** of these plant extracts. These results suggest that *Phyllanthus amarus* and *Piper guineense* may serve as promising **natural therapeutic agents** for the prevention and management of **oxidative stress-related organ damage**.

Future investigations should aim to **elucidate the molecular pathways** involved in their protective actions and pursue **clinical studies** to confirm their efficacy and safety in humans. Furthermore, **fractionation and characterization of the active phytochemical constituents** responsible for these beneficial effects could advance their **pharmacological development and therapeutic application**.

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APPENDIX

ASSAY CONCENTRATION VALUES

BIOCHEMICAL PARAMETERS

KIDNEY

MDA (Malondialdehyde)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	0.016	0.0849	0.106
2	0.015	0.0474	0.030
3	0.016	0.0248	0.042
MEAN	0.0157	0.0524	0.0593
STANDARD DEVIATION	0.00058	0.0304	0.0409
VARIANCE	3.33×10^{-7}	0.0009	0.000167
STANDARD ERROR	0.00033	0.0175	0.0236

SOD (Superoxide Dismutase)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	2.623	0.955	9.852
2	2.983	0.485	8.333
3	4.052	1.225	5.334
MEAN	3.219	0.8883	7.8397
STANDARD DEVIATION	0.743	0.3745	2.2990
VARIANCE	0.552	0.1402	5.2856
STANDARD ERROR	0.429	0.2162	1.3274

CAT (Catalase)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	48.075	477.720	408.716
2	55.375	309.633	304.679
3	51.908	338.980	216.743
MEAN	51.786	375.444	310.046
STANDARD DEVIATION	3.6515	89.781	96.099
VARIANCE	13.3337	8060.546	9235.012
STANDARD ERROR	2.1082	51.835	55.483

LIVER

MDA (Malondialdehyde)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	3.626	3.603×10^{-3}	4.836
2	3.018	9.375×10^{-3}	8.846
3	2.963	6.025×10^{-3}	4.434
MEAN	3.202	6.334×10^{-3}	6.0387
STANDARD DEVIATION	0.368	2.898×10^{-3}	5.9512
VARIANCE	0.135	8.40×10^{-6}	5.9512
STANDARD ERROR	0.212	1.673×10^{-3}	1.4085

SOD (Superoxide Dismutase)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	0.245	0.232	0.300
2	0.569	0.666	0.143
3	0.897	0.157	0.229
MEAN	0.5703	0.3517	0.224
STANDARD DEVIATION	0.3260	0.2748	0.0786
VARIANCE	0.1063	0.0755	0.00618
STANDARD ERROR	0.1882	0.1587	0.0454

CAT (Catalase)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	98.817	29.931	2.229
2	90.809	29.493	1.504
3	101.065	28.634	1.062
MEAN	96.897	29.353	1.5983
STANDARD DEVIATION	5.391	0.660	0.5892
VARIANCE	29.061	0.435	0.3471
STANDARD ERROR	3.112	0.381	0.3402

COLON

MDA (Malondialdehyde)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	0.029	0.0898	0.062
2	0.016	0.0608	0.020

3	0.032	0.0564	0.013
MEAN	0.02567	0.0690	0.03167
STANDARD DEVIATION	0.00850	0.0181	0.02650
VARIANCE	0.0000723	0.000329	0.000702
STANDARD ERROR	0.00491	0.0105	0.01530

SOD (Superoxide Dismutase)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	2.778	0.425	4.450
2	3.124	1.235	5.960
3	6.525	2.560	5.000
MEAN	4.1423	1.4067	5.137
STANDARD DEVIATION	2.0707	1.0778	0.764
VARIANCE	4.2878	1.1617	0.584
STANDARD ERROR	1.1955	0.6223	0.441

CAT (Catalase)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	517.775	116.112	133.142
2	502.118	112.942	161.009
3	495.635	120.221	211.927
MEAN	505.176	116.425	168.693
STANDARD DEVIATION	11.382	3.6496	39.951
VARIANCE	129.558	13.3194	1596.048

STANDARD ERROR	6.572	2.1071	23.065

SODIUM ION (Na^+)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	140	143	144
2	139	139	140
3	143	140	142
MEAN	140.67	140.67	142.0
STANDARD DEVIATION	2.08	2.08	2.0
VARIANCE	4.33	4.33	4.0
STANDARD ERROR	1.20	1.20	1.1547

POTASSIUM ION (K^+)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	5.5	7.4	6.7
2	3.8	5.5	7.0
3	7.5	6.0	7.0
MEAN	5.60	6.3	6.90
STANDARD DEVIATION	1.85	0.98	0.173
VARIANCE	3.43	0.97	0.030
STANDARD ERROR	1.07	0.57	0.100

BICARBONATE ION (HCO_3)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	19	19	14
2	22	23	14
3	19	18	20
MEAN	20.0	20.0	16.0
STANDARD DEVIATION	1.73	2.65	3.464
VARIANCE	3.0	7.0	12.0
STANDARD ERROR	1.0	1.53	2.0

UREA (Ur)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	32	35	35
2	41	29	32
3	31	25	39
MEAN	34.67	29.67	35.33
STANDARD DEVIATION	5.51	5.03	3.51
VARIANCE	30.33	25.33	12.33
STANDARD ERROR	3.18	2.91	2.03

CREATINE (Cr)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	1.1	1.1	1.0
2	1.4	0.9	1.1

3	1.2	0.8	0.9
MEAN	1.2333	0.9333	1.0
STANDARD DEVIATION	0.1528	0.1528	0.1
VARIANCE	0.0233	0.0233	0.01
STANDARD ERROR	0.0882	0.0882	0.0577

CHLORINE (Cl)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	104	107	106
2	104	107	106
3	106	104	106
MEAN	104.67	106.0	106
STANDARD DEVIATION	1.15	1.73	0
VARIANCE	1.33	3.0	0
STANDARD ERROR	0.67	1.0	0

AST (Aspartate Amino Transferase)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	129	184	111
2	134	130	115
3	174	118	178
MEAN	145.67	144.0	134.67
STANDARD DEVIATION	24.66	35.16	37.58
VARIANCE	608.33	1236.0	1412.33

STANDARD ERROR	14.24	20.30	21.70

ALT (Alanine Amino Transferase)

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	63	70	82
2	60	63	60
3	76	54	83
MEAN	66.33	62.33	75.0
STANDARD DEVIATION	8.50	8.02	13.0
VARIANCE	72.33	64.33	169.0
STANDARD ERROR	4.91	4.63	7.51

TP53

VALUES	control	DMH + P. amarus and P. guineense	DMH only
1	44.65	16.95	5.75
2	41.5	18.34	4.21
MEAN	43.075	17.645	4.98
STANDARD DEVIATION	2.2274	0.9829	1.089
VARIANCE	4.9612	0.9661	1.1858
STANDARD ERROR	1.575	0.6950	0.77