EVALUATION OF THE POLY HERBAL AQUEOUS LEAF EXTRACT ON BACTERIA INDUCED DIARRHEA AND PERIPHERAL BLOOD SMEAR IN RATS

BY

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THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

SEPTEMBER, 2023

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE. IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (HONOURS) DEGREE (B. Sc), SCIENCE LABORATORY TECHNOLOGY (MICROBIOLOGY TECHNIQUES)

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CERTIFICATION

This is to certify that the project work was carried out by Blossom ODIBO with the Matriculation Number LSC1706089 of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo state, Nigeria.

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DEDICATION

This project work is dedicated to God Almighty, my lord and savior and I am grateful for his protection and guidance throughout this project work. It is through His divine guidance that I have been able to navigate challenges, find inspiration, and seek knowledge.

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ABSTRACT

Since the beginning of time, people and animals have used plants as a source of medicine. Diarrhea is a common gastrointestinal disorder characterized by loose, watery stools occurring more frequently than usual. This work aimed at evaluating the anti-diarrhoeal property of the polyherbal aqueous extract on bacterial induced diarrhea in Wistar rat. Diarrhea was induced in twenty-five (25) male Wistar rats, orally administered with *Shigella dysenteriae* and *Escherichia coli bacteria* isolated at 1 ml. Graded doses (25, 50 and 100 mg/kg) of the polyherbal plant extract reduced the effect of the bacteria induced diarrheoal. The results showed a slight reduction in the number of wet stools, weight of stools and total number of stools when compared with the control. The peripheral blood smear showed no deteriorative effect on the blood cells, with no significant difference across the treated groups when compared with the control. In conclusion, the antidiarrhoeal activities of the polyherbal aqueous extract effect could be implicated due to the presence of the phytoconstituents in the plants. Thus, this finding supports the claimed ethnomedicinal use of the polyherbal plant extract for the management of diarrhea.

CHAPTER ONE

INTRODUCTION

Diarrhea is a common gastrointestinal disorder characterized by loose, watery stools occurring more frequently than usual. It is a prevalent condition that affects people of all ages worldwide and can have varying degrees of severity, while most cases of diarrhea are acute and short-lived, some instances can be chronic, requiring medical attention and management. For infants and young children, the usual water content in feces is roughly 10 ml/kg/day; for teenagers and adults, it is 200 g/day. Due to an imbalance in the healthy operation of the small and large intestine's physiological processes that are responsible for the absorption of various ions, other substrates, and ultimately water, diarrhea is the increase in the amount of water in stools. Numerous pathogens, including bacteria, viruses, and protozoa, can cause diarrhea (Ahns *et al*., 2010). The rotavirus, which is the primary cause of acute diarrhea and accounts for 40% of all hospital admissions for diarrhea among children under five worldwide, is one of the most dangerous viral and bacterial pathogens affecting people in developing countries (Parashar *et al*.,2006). E. coli, Salmonella, Campylobacter, and Shigella are the main bacterial pathogens (Ahns *et al*., 2010). The first three of these pathogens are currently posing a hazard to our food supply and are transferred to humans through animal reservoirs. (DuPont, 2007) infants have the highest prevalence of campylobacter and salmonella infections, perhaps as a result of household cross-contamination and the fact that infants have a lower threshold for clinical infection than older children and adults do. Diarrhea accounts for 9% of all under-five fatalities worldwide, making it the second-leading cause of death, according to estimates, 1.8 million people per year in underdeveloped countries pass away from diarrheal diseases, and more than 80 % of these deaths are in children under the age of five (Bakir *et al.,* 2017; Hodge *et al*., 2016). Children with diarrhea will experience a wide range of issues, such as appetite loss, electrolyte

imbalance, malnutrition, an increased risk of contracting other infectious diseases, and slowed physical and mental development. Diarrhea is linked to a number of issues due to its detrimental effects on physical and cognitive development. It results in 72.8 million years of incapacity and lost productivity, worsens family finances, and puts a strain on the healthcare system (Aikins *et al*., 2010). The prevalence and management of diarrheal illnesses are influenced by the socioeconomic status of the household. The welfare of the home in relation to its socioeconomic level is significant in influencing the treatment interventions provided during pediatric sickness since the family is a sick child's initial point of contact (Lwin *et al*., 2020) Lowering the morbidity and mortality related to diarrhea requires early diagnosis and proper treatment (Lwin *et al*., 2020), diarrheal illnesses are self-limiting, and nearly half cases of diarrheal illness last less than one day (Herikstad *et al*., 2002). Since the beginning of time, people and animals have used plants as a source of medicine in simple forms such decoctions, syrups, powders, infusions, and ointments (Ghorbani, 2014). Natural substances derived from plants called "herbal medicines" are used to cure ailments in traditional local or regional medicine. These substances are intricate blends of organic compounds that can originate from any unprocessed or refined portion of a plant. Both developed and developing nations continue to practice primary healthcare using herbal remedies (Sahoo *et al*., 2010). The main treatment for diseases like diarrhea is antibiotics, yet a large rise in antibiotic resistance has been seen in common human pathogens around the world (Christina *et al*., 2013). Similar to this, Oral Rehydration Therapy (ORT) has been generally recognized as a significant contributor to the decrease in infant mortality from diarrhea (Juliane *et al*., 2014) However, the disease's attack rate has not altered, and this treatment frequently fails when a patient has a high stool production. In many African nations, using natural medicines to cure diarrhea is a common practice. These plants, which are abundant in nature and are widely accepted by the populace as less expensive alternatives to traditional treatments. Herbs are a prevalent component in folk

healing practices all around the world. Antidiarrheal and antidysenteric properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and reducing sugars (Longanga *et al*., 2000).

1.1 Aim and objectives of this study

The aim of this study, is to evaluate the anti-diarrheal property of the polyherbal aqueous extract on bacterial induced diarrhea in wistar rat

1.2 Objectives

The following are the objectives of this study, which includes to;

1. Investigate the effect of the polyherbal extract on bacterial induced diarrhea in wistar rats
2. Evaluate the peripheral blood smear on polyherbal extract in wistar rats.
3. Study the histopathological effect of the extract on the heart cells of wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Andrographis paniculata* (Burm. f.) Nees,

The herbaceous plant also referred to as "King of Bitters" because the taste of the entire plant is exceedingly bitter. It belongs to the Acanthaceae family. It is commonly grown in southern Asia. Over the years, Asia and Europe have mostly employed the leaves and roots for various therapeutic purposes either as a folk treatment for a variety of ailments or as a herbal supplement for health promotion. Bangladesh, China, Hong Kong, India, Pakistan, Philippines, Malaysia, Indonesia, and Thailand all employ *A. paniculata* as a traditional herbal remedy (Mishra *et al.*, 2007; Khare, 2007). Researchers have done a number of experiments and published publications concerning the herb's therapeutic potential. According to the phytochemical investigations, *A. paniculata* includes a variety of substances, including flavonoids, diterpenoid lactones and other substances. *A. paniculata* has a variety of pharmacological effects as a result of its existence (Akbar, 2011; Kabir *et al*., 2014). The medicinal plant *A. paniculata* has been used for generations to successfully cure a variety of conditions, including respiratory illnesses, skin infections, herpes, dysentery, fever, sore throat, lower urinary tract infections, inflammation reduction, and diarrhoea (Jarukamjorn and Nemoto, 2008). The most widely utilized section of *A. paniculata* is the aerial portion, and its extracts contain lactones, flavonoids, diterpenoids, and flavonoid glycosides. This plant's significance in the world of medicinal plants has been greatly increased by the distinctive secondary metabolites found in it.

Figure 2.1; *Andrographis paniculata* young leaves

2.1.1 Botanical description

In wet, shady areas, the annual herbaceous plant *Andrographis paniculata* grows upright to a height of 30-110 cm. The stem is abruptly quadrangular, heavily branching, and brittle in texture, making it easy to break. Simple, opposite, glabrous, lanceolate, 1-3 cm wide, and with an acute whole border are the characteristics of the leaves. A panicle's terminal and axillary inflorescence measures 10 to 30 mm in length and has a tiny bract and a short pedicel. The flowers have a calyx with five tiny, linear sepals. The lower tips of the corolla tubes are broadly cuneate, 3-lobed, white with violet markings, while the upper lip is rectangular, about 6 mm long, and white with a yellowish top. Two throat-inserted stamens with basally bearded anthers. 2-celled, superior ovary with extruded style. The herb's capsule is upright, linear-oblong, 1-2 cm long, compressed, longitudinally ruffled on broad faces, and covered in fine glandular hairs. Seeds are quite tiny (Niranjan *et al.,* 2010).

2.1.2 Taxonomy of *Andrographis paniculata*

Kingdom: Plantae,

Division: Angiosperma,

Class: Dicotyledonae,

Order: Personales,

Family: Acanthaceae,

Genus: Andrographis,

Species: *Andrographis paniculata* (Mishra *et al.*, 2007)

2.1.3 Ethnomedicinal uses of *Andrographis paniculata*

Since ancient times, both Asia and Europe have employed the leaves and roots of *Andrographis paniculata* to treat a wide range of medical conditions. But there are some restricted uses for the entire plant as well. It is advised to use this remedy to remove toxins from the body and to reduce body heat in cases of fever due to its "cold property" activity. Due to their powerful "blood purifying" capabilities, the herbs are also advised for usage in cases of leprosy, gonorrhoea, scabies, boils, skin eruptions, and chronic and seasonal fever (Akbar, 2011) The conventional medicine likewise makes extensive use of it for medical purposes (Kabir *et al*.,2014). The components of *A. paniculata* have been used as a traditional medicine in Asia for centuries to cure a wide range of conditions, including stomach aches, inflammation, pyrexia, and intermittent fevers. The entire plant of *A. paniculata* has been used as a remedy for toxic bug stings and snake bites. In addition, dyspepsia, influenza, dysentery, malaria, and respiratory infections are all treated with it (Jarukamjorn *et al.*, 2010). It has been utilized as a herb with anti-cold properties to reduce body heat and fever. Additionally, it aids in the

removal of pollutants from the body. The tribal people of Tamilnadu in India used this herb to treat a wide range of conditions, including dysmenorrhoea, leucorrhea, pre- and post-natal care, malaria, jaundice, gonorrhoea, wounds, cuts, boils, and skin illnesses (Panossian *et al.*, 2002). Through aqua puncture, or the injection of an infusion into acupuncture points, *Andrographis paniculata* has more recently been employed in the treatment of chronic bronchitis (Mills and Bone, 2000).

2.1.4 Reported photochemistry of *Andrographis paniculata*

the leaves, stems, roots, and whole plants of *Andrographis paniculata* have been documented for phytochemicals with pharmacological properties. The aerial parts of *A. paniculata* have been described for its multiple uses in the extraction of phytoconstituents. In terms of the section used, geographic location, season, and time of harvest, phytochemical compositions vary greatly (Phosphane *et al*., 2004; Li and Fitzloff, 2004). The aerial parts of the *A. paniculata* contain a variety of chemical components, including andrographolide, a diterpene lactone that is colorless, crystalline, and bitter to the tongue (Abhishek *et al.*, 2020) Other compounds include 14-deoxy-11-oxoandrographolide, didehydro andrographolide andrographlide D, 14deoxyandrographolide, non-bitter compound is neo andrographolide, homoandrographolide, andrographosterin, andrograpanin, α- sitosterol, stigmasterol. Apigenin-7, 4-dio-methyl ether, 5- hydroxy 7,8,2, 3-tetramethoxy flavones, monohydroxy trimethyl flavones, andrographin, dihydroxy di-methoxy flavoue, panicolin, andrographoneo, andrographoside, andropani-culoside A (3,7,8) andrograpanin, Isoandrographolide and skollcaflavone (912). Six entlabdane diterpenoids i.e. 3-o-beta-Dglucopyranosyl-14, 19- dideoxyandrographolide, 14-deox, 17- hydroxyandrographolide, 19-o-[beta-D-apiofuranosy 1-2beta-D-glucopyranoyl]-3, 14-dideoxyandiographolide, 3-obeta-D-glucopyranosyl-andro-grapholide, 12S-hydroxy andrographolide and andrographatoside. Several bacterial and fungal strains were inhibited by these substances. Four xanthones, 1,

8-dihydroxy-3,7-dimethoxyxanthone, 4,8-di-hydroxy-2,7-dimethoxyxanthone, 1,2-dihydroxy-6, 8-dimethoxyxanthone and 3,7,8-trimethoxy-1-hydroxyxanthone from the roots were reported by Dua *et al*., 2004.

2.1.5 Phamarcological study of *Andrographis paniculata*

Anti-microbial activity

Aqueous extract, andrographolides, and arabinogalactan proteins extracted from *A. paniculata* dry herb were tested for antimicrobial activity. The aqueous extract and arabinogalactan proteins were found to have antibacterial action against *Bacillus subtilis*, Escherichia coli, and Pseudomonas aeruginosa, but andrographolide was found to be active solely against B. subtilis. All three have been shown to have antifungal efficacy against *Candida albicans* (Singh *et al*., 2003). Anti-bacterial activity against *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *B. subtilis* was tested in five unusual noriridoides, andrographidoides A-E. None of the substances inhibited activity (MIC>100 g/mL). Positive controls included gentamycin, chloramphenicol, and ciprofloxacin (Xu *et al*., 2012).

Anti-inflammatory/anti-allergic activity

The aqueous extract combined with the methanol extract of the leaves showed significant alleviation of lipopolysaccharide induced release of pro-inflammatory mediators (NO, IL-1β and IL-6), inflammatory mediators (PGE2 and TXB2) and allergic mediators (LTB4) but no inhibition was observed against histamine release (Chandrasekaran *et al*., 2010). Seven photochemicals, namely, andrographolide, neoandrographolide, isoandrographolide, andrograpanin, 7-O-methylwogonin, 14-deoxy-11,12- didehydroandrographolide and skullcapflavone isolated from *A. paniculata* leaves were screened for in vitro anti-inflammatory and anti-allergic potential. The results showed that andrographolide,

isoandrographolide, 7-O-methylwogonin and skullcapflavone-1 significantly inhibited inflammatory mediators NO and PGE2 release from lipopolysacharide (LPS) stimulated cultured macrophages. Whereas, IL-1β production in LPS stimulated macrophages was inhibited by andrographolide, isoandrographolide and 7-O-methylwogonin. Also, IL-6 production from LPS induced macrophages was significantly ($P<0.01$) inhibited by andrographolide, isoandrographolide and skullcapflavone-1 in a concentration dependent manner. The results also showed that andrographolide, isoandrographolide and skullcapflavone-1 significantly suppressed TXB4 released in A23187 activated HL-60 promyelocytic leukemia cells. Furthermore, the anti-allergic properties of the phytoconstituents was investigated on A23187 induced LTB4 production. The result showed 30.5% and 19.6% inhibition of LTB4 production in A23187 induced HL-60 promyeolocytic leukemia cells at concentrations of 63 µmol/L and 33.5 µmol/L for skullcapflavone and 7-O-methylwogonin respectively. The IC50 value for the reference standard captopril was 48 µmol/L. 7-O-methylwogonin was the only phytoconstituent that potently inhibited A23187 induced histamine release in RBL- 2H3 rat basophil leukemic cells in a dose dependent manner (Chandrasekaran *et al*.,2011).

Anti-oxidant activity

Andrographolide and aqueous extract of *A. paniculata* herbs were tested for anti-oxidant activity on nicotine-induced oxidative stress in male wistar rats' liver, kidney, heart, lungs, and spleen, and the results showed that intraperitoneal administration of andro (25 mg/kg) and Aphanamixis polystachya (25 mg/kg) for 7 days significantly (P0.05) reduced levels of lipid peroxidation and increased the anti-oxidant enzymes status in the five organs screened compared to nicotine and vehicle only treated group(Neogy *et al.*, 2008).

Antidiabetic activity

Andrographolide and 14-deoxy-11,12-didehydroandrographolide, isolated from an alcoholic extract of the aerial parts of *A. paniculata,* reduced phenotypes associated with diabetic nephropathy in MES-13 cells, including secretion of the extracellular matrix protein fibronectin, the cytokine TGF-, oxidative stress, and the apoptosis marker caspase-3. 14-deoxy-11,12-didehydroandrographolide inhibited apoptosis marker caspase-3, fibrosis marker cytokine TGF-, and plasminogen activator inhibitor-1 more effectively than andrographolide. In the MES-13 cells, both chemicals reduced reactive oxygen species (Lee *et al*., 2004) In streptozocin-induced hyperglycemic rats, the aqueous extract (50 mg/kg) of *A. paniculata* raw material resulted in a substantial (P0.05) reduction (52.9%) in blood glucose level. However, freeze dried *A. paniculata* material (6.25 mg/kg body weight) exhibited a more significant (P<0.001) drop (61.81%) in blood glucose level. Furthermore, the aqueous extract of *A. paniculata* did not result in a substantial reduction in blood glucose levels in normoglycemic rats (Husen *et al*., 2004).

Antihyperglycemic activity

Inhibiting -glycosidase and -amylase activity, as well as increasing insulin sensitivity, are thought to be useful techniques for lowering postprandial blood glucose levels. These enzymes are involved in carbohydrate digestion and absorption, resulting in a postprandial increase in blood glucose (Kajaria *et al*., 2013). Insulin resistance is characterized by hyperinsulinemia and high blood glucose levels, and it is linked to a variety of metabolic hormonal abnormalities, including dyslipidemia, abnormal uric acid metabolism, increased ovarian testosterone secretion, endothelial dysfunction, elevated procoagulant factors, and elevated inflammatory markers (Reaven, 2004). *Andrographis peniculata* extracts and andro- grapholide efficiently demonstrated antihyperglycemic effects by (a) reducing blood glucose levels by inhibition of -glycosidase and -amylase (Subramanian and Asmawi, 2006; Xu *et al*., 2007) and (b) enhancing insulin sensitivity, hence boosting glucose uptake and oxidation by peripheral tissues.

Hepatoprotective activity

Andrographis paniculata has long been utilized as a hepatoprotective agent and a stimulant for a variety of liver enzymes. In Ayurvedic and Unani medicine, it is also utilized as a component in polyherbal formulations for the treatment of liver diseases (Akbar, 2011). Along with various AP extracts, andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and 14-deoxy-11,12-didehydroandrographolide compounds have been shown to have hepatoprotective effects (Akowuah *et al.*,2009; Roy *et al*., 2010) The leaf extract and andrographolide were tested in a comparative research against carbon tetrachloride- (CCl4-) induced hepatic microsomal lipid peroxidation. Only the leaf extract, not andrographolide, totally prevented the high concentration CCl4-induced microsomal lipid peroxidation in vitro, indicating that the hepatoprotective action is not primarily related to the presence of andrographolide.

Anticancer activity

Anticancer Activity. Andrographolide inhibited cancer cell growth, cell-cycle arrests, or cell differentiation, while also strengthening the body's own immune system against cancer cells and inducing apoptosis and necrosis (Vojdani and Eder, 2006). The dichloromethane fraction of methanol extract suppressed the growth of HT-29 colon cancer cells considerably. Andrographolide, the primary bioactive constituent of AP extracted from dichloromethane, suppressed the growth of a varied cancer cell representing various types of human tumors (Ajaya *et al.,* 2004). Aditya et al., (2014) recently showed that methanol extract of AP was shown to be much less effective against both MCF-7 breast and HT-29 colon cancer cell lines. This poor activity could be attributed to the active ingredients' low penetrating power.

2.2 *Annona muricata*

Annona muricata is a lowland tropical fruit-bearing Annonaceae tree. *Annona muricata* is also known as graviola, soursop, durian belanda, and guanabana in Malaysia. The genus name Annona may be derived from the Latin word anon, which means "yearly produce." It could be further defined as "the fruit production habits of the many species in this genus." Britton and Wilson (1924), on the other hand, proposed that this genus name was derived from 'Hanon,' which implies "the aboriginal name of a tropical America tree, most likely Santo Domingo." Soursop gets its name from the fruit's acidic and sweet characteristics (Moghadamtousi *et al*., 2015). Many research has revealed anti-tumor, anti-helminth, anti-fungal, anti-bacterial, hypotensive, anti-viral, and anti-inflammatory properties of *A. muricata* (Syed *et al*., 2016; Miranda *et al*., 2021). *Annona muricata* leaves and bark, for example, have been utilized for therapeutic purposes. Over 200 chemical substances, including phenolics, acetogenins, and alkaloids, have been identified and isolated (Coria-Téllez *et al*., 2018). This plant is regarded a promising alternative treatment for diabetic mellitus (DM), hypertension, cancer, and bacterial infections due to its medicinal and pharmacological activities (Chukwunonso et al., 2019; Sokpe *et al*., 2020). Furthermore, it is less expensive, more easily accessible, and more environmentally friendly than currently sold pharmaceuticals, making it a good package to consider for new prospective medications (Opara *et al*., 2021). Sirsak, graviola, paw-paw, soursop, 'durian belanda,' and guanabana are all names given to *A. muricata*. It has been documented to be cultivated in the warm lowlands of Eastern and Western Africa, Australia, North America, temperate and tropical Asia, the Caribbean, Mesoamerica, and the south-central Pacific Islands. *Annona muricata* is a member of the custard-apple genus, which encompasses roughly 125 species. When compared to other Annonaceae family species, this one is the most often grown. *A. muricata* is widely planted for its edible fruits, and it has become naturalized outside of its native regions in tropical America and Africa (Leonti *et al*., 2018).

Figure 2.2: *Annona muricata*, whole plant part

2.2.1 Botanical description of *Annona muricata*

Annona muricata is a little evergreen tree. It can be thin and upright, or low spreading and bushy, with a straggly appearance as it ages. It stands 5 to 10 m tall, has low branches, and is 15 to 83 cm in diameter. The hermaphrodite blooms, which have a distinct aroma, grow singly or in small clusters on ancient wood. The blossoming phase begins between the ages of three and four, though this can vary depending on the surroundings. Depending on how it is pollinated, the fruit can range from heart-shaped to oval. When pollination is insufficient and unfertilized ovules fail to mature, the fruit becomes deformed, irregular in shape, and small. The skin has many recurved soft spines that are spaced 0.5 to 1.3 cm apart (Leonti *et al*., 2018). The flesh of the fruit consists of an edible white pulp, fiber, and a core of indigestible black seeds. The pulp is also used to flavour confections such as sweets, sorbets, and ice cream, as well as fruit nectar,

smoothies, and fruit-juice beverages. Unless a blender is employed, the seeds are normally left in the preparation and removed before eating.

2.2.2 Taxonomy of *Zingiber officinale*

Kingdom: Plantae

Class: Tracheophyte

Division: Angiosperm

Order: Magnolialis

Family: Annonaeae

Genus: Annona

Specie: *Annona muricata*

2.2.3 Ethnomedicinal uses of *Annona muricata*

The entire *Annona muricata* plant has been used for ages to treat a wide range of diseases and injuries. Direct intake, topical treatments, decoctions, and juicing are some of the preparation techniques. *A. muricata* has been used for a long time to treat a number of ailments, including cancer, diabetes, hypertension, respiratory disorders, fever, and bacterial infections, according to a review of its pharmacological activities. In South and North America as well as West Africa, this plant is frequently used in traditional medicine to cure a variety of ailments (Adewole and Ojewole, 2009). *Annona muricata*'s bark, root, seed, and leaf are frequently decocted for therapeutic and medical purposes. Soursop leaves are soaked in water to treat skin conditions in Indonesia and other South Pacific nations. In particular, in Mexico and Brazil, the leaves are used to treat discomfort such asthma, colds, and flu. According to a review

(Gavamukulya *et al*., 2017) other nations utilize it for the same reason as well, like Martinique and Nicaragua. In addition, locals in New Guinea and Ecuador use these leaves externally to ease discomfort. The leaves are used in Malaysia to treat malaria and internal and external parasite illnesses. Diabetes, sleeplessness, migraines, and cystitis can all be treated with the leaves (Mutakin *et al.*, 2022). The leaves of *A. muricata*, which is found in Eastern Africa's Tanzania, are among the most popular plants used to treat diabetes. Patients can obtain this drug locally at markets, from neighbors, or from vendors of traditional medicines. Some participants also mentioned that they had grown healing plants close to their homes. Local herbalists claim that they made the diabetes treatments themselves using their experience and locally sourced components. Low doses of its decoction are taken to reduce blood glucose levels. The interview underlined that patients must adhere to a set dosage because it is quite potent and may cause negative effects or an excessive fall in glucose level. Additionally, its decoction is applied topically to treat pain and abscesses while also having anti-rheumatic and anti-nerve impact (Solanki *et al*., 2020) All portions of *A. muricata* are used to cure cancer, diabetes, parasite infections, and stomach aches in various tropical Sub-Saharan African nations, including Uganda. On the skin, bio insecticides, bio pesticides, and insect repellents are also applied to the roots, leaves, seeds, and unripe fruit. In India, *A. muricata* is used to treat catarrh and is said to contain antiphlogistic and anthelmintic properties in its roots, bark, and leaves. *A. muricata* aqueous extract is also used to control insects including thrips, aphids, and lepidopteran larvae (Leonti *et al*., 2018). Other plant species have been utilized in conjunction with *A. muricata*. Juice produced from a combination of *A. muricata, Hibiscus rosa-sinensis L*., and *A. squamosa* leaves is applied to the head in Malaysia to prevent fainting (Leonti *et al*., 2018).

2.2.4 Reported phytochemistry of *Annona muricata*

There have reportedly been 218 bioactive chemicals discovered in *A. muricata*. Acetogenins are the most common substances, followed by alkaloids, phenols, and other substances. The primary plant organs being investigated are leaves and seeds, likely because they have been in use the longest. Although aqueous extracts have recently received attention, organic extracts still account for the majority of discovered phytochemicals. Alkaloids; they are organic substances that are found in nature and include simple nitrogen atoms. Reticuline and coreximine are the most prevalent in *A. muricata*, and leaves have the highest concentration of alkaloids (Fofana *et al*., 2011; Matsushige *et al*., 2011), although they have also been discovered in roots, stems, and fruit. According to Mohanty *et al*. (2008), the isoquinoline, aporphine, and protoberberine types of alkaloids make up the majority of those found in A. muricata. Acetogenins; *A. muricata* various organs and tissues, including the leaves, stems, bark, seeds (Chang *et al*., 2003; Li *et al*., 2001; Liaw *et al*., 2002), pulp (Ragasa *et al*., 2012), and fruit peel (Jaramillo *et al*., 2000), have been found to contain more than 120 acetogenins. Acetogenins have a long, aliphatic chain of 35 to 38 carbons attached to a c-lactone ring, terminally substituted by b-unsaturated methyl (occasionally a ketolactone), one or two tetrahydrofurans (THF) located along the hydrocarbon chain, and a specific number of oxygen groups (hydroxyl, acetoxyls, ketones, epoxy). Phenolic compounds; *A. muricata* has been shown to contain 37 phenolic compounds, according to reports. Quercetin and gallic acid are two significant phenolic compounds discovered in *A. muricata* leaves (Nawwar *et al*., 2012; Correa-Gordillo *et al*., 2012). The pulp has been shown to contain flavonoids and lipophilic antioxidant chemicals such tocopherols and tocotrienols (Correa-Gordillo *et al*., 2012). *A. muricata* has also been shown to contain other substances, including vitamins, carotenoids, amides, cyclopeptides, and megastigmanes.

2.2.5 Pharmacological study of *Annona muricata*

Cytotoxic activity

According to ethnobotanical sources, *A. muricata* is increasingly used as a cancer treatment, which may be connected to studies showing that it has selective cytotoxic effect (George *et al*., 2012). Because several of the extracts studied in vitro were found to be more harmful to cancer cell lines than to normal cells, this bioactivity is thought to be selective (Dai *et al.,* 2011; George *et al*., 2012; Gavamukulya *et al*., 2014). According to Nawwar *et al*. (2012), 100 lg/ml had no effect on the viability of non-cancerous cells, whereas 1.6 and 50 lg/ml of the hydroalcoholic extract of *A. muricata* leaves did. Additionally, it has been claimed that this selective activity promotes healing. Healing takes longer in tumor cells (Torres).

Antioxidant activity

Annona muricata has been the subject of several antioxidant assays. Studies on the antioxidant activity of *A. muricata* were collated by Correa-Gordillo *et al*. (2012) taking into account various assays, various plant sections, and various solvents employed. The DPPH and $ABTS^{+}$ tests, the ORAC assay for measuring oxygen radicals, the FRAP assay for measuring reduction power, and bleaching of b-carotene were some of the techniques used to calculate the total antioxidant capacity. Juice, fresh or dry leaves, fresh or frozen pulp, and frozen or fresh leaves have all been tested for antioxidant activity. According to ABTS, FRAP, and ORAC measurements of pulp antioxidant activity, the antioxidant chemicals from *A. muricata* are primarily lipophilic, and their mechanism of action is hydrogen donation. (Correa-Gordillo *et al*., 2012).

Insecticidal, larvicidal and repellent activity

Seed, leaves, bark, stems, roots, flowers, and seeds of *A. muricata* all exhibited insecticidal action (Bobadilla *et al*., 2005; Predes *et al*., 2011). The larvae of Aedes aegypti, Anopheles albimanus, Spodoptera litura, Callosobruchus maculatus, and Plutella xylostella were all inhibited by ethanol extracts (Bobadilla *et al*., 2005; Morales *et al*., 2004; Sanabria *et al*., 2009;

Morales *et al*., 2004). According to several studies (Bobadilla *et al*., 2005; Morales *et al*., 2004; Sanabria *et al*., 2009), *A. muricata* seed extracts had the strongest insecticidal effects. This is likely because the extracts contain chemicals such acetogenins, fatty acids, and alkaloids.

Hypoglycemic activity

In murine models, *A. muricata* leaf extracts shown hypoglycemic action (Adewole and Caxton-Martins, 2006). In these investigations, the histology and biochemistry of the pancreas were examined along with the effect of aqueous and methanol extracts of A. muricata leaves on lowering the concentration of blood glucose in rats with diabetes induced by streptozotocin (STZ). Rats given extracts of *A. muricata* did not exhibit the changes often seen in diabetic rats in their pancreatic b-cells. There has been a documented rise in insulin concentration and antioxidant enzyme activity in pancreatic serum. After a month of daily use, near-normal blood glucose levels, body weight, food and water intake, lipid profile, and oxidative defense were achieved. *A. muricata* extract may be able to counteract the harmful effects of STZ by acting as an antioxidant and protecting pancreatic b-cells (Florence *et al*., 2014).

Anti-cancer activity

Rats with colonic aberrant crypt foci caused by azoxymethane responded favorably to an extract of *A. muricata* leaves made of ethyl acetate (Moghadamtousi *et al*., 2015). The extract acts as aceto-genins, which up-regulate the Bax protein, down-regulate PCNA and Bcl-2 proteins, and restore the levels of antioxidant enzymes. Malondialdehyde (MDA), a lipid radical produced as a result of excessive ROS generation, was found to be present in higher concentrations in individuals with colorectal cancer (Moghadamtousi *et al*., 2015). Treatment

with *A. muricata* extract decreased MDA production in colon tissue, demonstrating its antioxidant protection.

Anti-protozoal activity

Annona muricata extracts and some of their isolated compounds have shown effectiveness against protozoans responsible for human diseases, as is the case of the genera Plasmodium (Boyom *et al*., 2011), Leishmania (Osorio *et al*., 2007), Biomphalaria (Luna *et al*., 2005), responsible for malaria, leishmaniasis and schistosomiasis respectively. Due to the need for antimalarial medications in tropical regions, the anti-plasmodic action is of special importance. Although less effective than the over-the-counter medications chloroquine and artemisinin, methanol extract of this species has demonstrated to suppress this parasite in vitro (Boyom *et al*., 2011).

Hypotensive activity

In normotensive rats, a dose-dependent reduction in mean arterial pressure (MAP) was induced by a leaf extract of *A. muricata* (Nwokocha *et al*., 2012). It was hypothesized that the aqueous extract of *A. muricata* did not exert its hypotensive effects via endothelium or nitric oxide-dependent mechanisms. According to studies, plant extracts lower blood pressure by blocking the calcium ion channel, and their capacity to relax high K+-induced contractions further supports their Ca+ antagonistic effects (Nwokocha *et al*., 2012). Alkaloid substances such reticulin, anomurine, and coreximine, as well as some components of essential oils like b-caryophyllene, have been linked to the hypotensive impact (Nwokocha *et al*., 2012).

2.3 *Zingiber officinale*

The Zingiberaceae family includes ginger (*Zingiber officinale*) Rhizomatous in nature, it is cultivated all throughout Africa, South-Eastern Asia, China, as well as in some regions of

Japan, Austria, and Latin America (Sasidharan and Menon, 2010). Since ancient times, ginger has been used as a medicine to cure a variety of ailments, including fever, indigestion, hypertension, sprains, sore throats, cramps, and rheumatism (Ali, 2008). An array of biological properties, including antibacterial activity (Azu and Onyeagba, 2007), antifungal activity (Singh *et al.*, 2005), anti-inflammatory activity (Kumar *et al*., 2013), and an anti-coagulant impact (Nurtjahja-Tjendraputra *et al*., 2003), have been demonstrated by pharmacological studies of ginger. Studies on *Z. officinale's* phytochemical and antibacterial capabilities from several Kaduna State locations were conducted (Idris, Tijja, 2016; Suleiman *et al*., 2019). *Zingiber officinale* (Zingiberaceae) is a significant plant with numerous ethnomedical and nutritional benefits; as a result, it is widely utilized as a spice, flavoring ingredient, and herbal treatment around the world. *Z. officinale* has historically been used to treat a wide range of illnesses, including nausea, vomiting, asthma, coughing, palpitations, inflammation, dyspepsia, loss of appetite, constipation, indigestion, and pain in Ayurveda, Siddha, Chinese, Arabian, African, and Caribbean medical systems (Grzanna *et al*., 2005). *Z. officinale* has been widely researched for its therapeutic potential using cutting-edge scientific methods over the past few decades. A number of bioactive chemicals have been extracted from the plant's various sections and analyzed pharmacologically. The plant has been shown to have larvicidal efficacy, analgesic activity, anti-inflammatory activity, antioxidant activity, anti-cancer activity, nephroprotective activity and hepatoprotective activity (Omoya and Akharaiyi, 2004; Ayse *et al*., 2008; Al-Tahtawy *et al*., 2011).

Figure 2.3: *Zingiber officinale* leaves, stem and rhizome.

2.3.1Botanical description of *Zingiber officinale*

Zingiber officinale is a herbaceous perennial with fibrous roots, aerial branches, and a rhizome. The aerial shoots, which can grow to a height of 1.5 meters, are pseudo stems made up of several narrow leaves carried on short petioles with overlapping sheaths at the bases. At the intersection of the leaves and sheaths, ligules are present on leaf blades that are 5–30 cm long and 8–20 mm wide. The leaves have a noticeable midrib and are lance-shaped, distichous, and glabrous. The fleshy, golden interior of the underground stem, also known as the rhizome, contains secretory cells and ethereal oils. Along the stem, there are nodes that, with the exception of the first few, contain axillary buds that grow, enabling the rhizome to enlarge in accordance with a sympodial development pattern (Ravindrun *et al*., 2005). There are both fibrous and fleshy roots; the fibrous roots, which have root hairs, aid in nutrient and water absorption, while the thicker, fleshier roots also aid in stability. The rhizome-born flowering spikes, or scapes, are composed of overlapping bracts, each of which has a solitary axillary flower surrounded by a dangerous bracteole. When flowering does occur, the insect-pollinated

inflorescence is transitory and is likely influenced by climate and cultural variables. Shortly after being planted, the ginger in Reading's tropical glasshouse blossomed for more than six months. The labellum, or petaloid stamen, of the ginger blossom is colorful. The inferior ovary is globose, the tubular corolla is divided into three lobes at the top, the style is long, and the lone remaining anther is free and fertile (Ravindran *et al.*, 2005). The fruit is a fleshy capsule with a characteristic white aril within that loculicidally dehisces (Ravindrun *et al*., 2005).

2.3.2 Taxonomy of *Zingiber officinale*

Kingdom: Plantae

Class: Tracheophytes

Division: Monocots

Order: Zingiberales

Family: Zingiberaceae

Genus: Zingiber

Specie: *Zingiber officinale*

2.3.3 Ethnomedicnal uses of *Zingiber officinale*

According to ancient Chinese and Indian scriptures, ginger has been grown and used medicinally since prehistoric times. It was a crucial component of herbal remedies for rheumatism, constipation, vomiting, and other digestive diseases as well as for catarrh. It is unknown if the species *Zingiber officinale* exists in the wild. It is believed that south-east Asia

is where it first appeared (Afzal *et al*. 2001). The ginger rhizome is used in ayurvedic medicine, which was first documented in Sanskrit scriptures from 2000 BC, as a carminative, digestive aid, anti-colic, and a therapy for piles. Additionally, for headaches and toothaches, a ginger paste was administered as a topical stimulant and rubefacient. Ginger is regarded as a warming treatment that relieves external problems in traditional Chinese medicine. Fresh ginger is used to treat nausea, vomiting, coughing, and abdominal distension. It can also be used to encourage sweating and lessen the harmful effects of other herbs. The Pharmacopoeia of the People's Republic of China (2005) contains monographs for both fresh and dry ginger rhizomes.

2.3.4 Reported photochemistry *Zingiber officinale*

69 volatile chemicals make up 97 % of the *Zingiber officinale* rhizome's entire makeup in essential oils. Zingiberene (28,62%), Camphene (9,32%), Ar-curcumene (9,09%), Phellandrene (7,97%), E-farnesene (5,52%), Bisabolene (5,40%), and Pinene (2,57%) are the compounds with the highest concentrations (Asadi-Samani *et al*., 2013). Their biological qualities, including their antibacterial, antioxidant, cytotoxic, insecticidal, and anti-inflammatory activities, as well as their value in maintaining food attributes, have been well-documented (An *et al*., 2016). The primary source of bioactive components in the rhizome of *Zingiber officinale* are non-volatile substances (oleoresins). 34 oleoresins have currently been identified, accounting for 88.6% of the overall content (Asadi-Samani *et al*., 2013). The most significant subgroups are the gingerols (1-(4-hydroxy-3-methoxyphenyl)-5-hydroxyalcan-3-one), shogaols (1-(4-hydroxy-3-methoxyphenyl)-4-decen-3-one), and paradols. While gingerols are mostly present in fresh ginger rhizomes, shogaols are more prevalent in dried ginger rhizomes (Kiran *et al*., 2013).

2.3.5 Pharmacological study of *Zingiber officinale*

Anti-inflammatory activity

Ginger is said to have a variety of health benefits, including the reduction of pain, inflammation, and edema. Each of the dried ginger extracts, dry gingerol-enriched extracts, and [6]-gingerol were reported to have analgesic and powerful anti-inflammatory effects by (Young *et al*., 2005), (Minghetti *et al*., 2007). According to Rinster *et al*. (2000), ginger may be helpful for treating rheumatism, osteoarthritis, and inflammation. However, there is controversy around the efficacy and safety of ginger for the treatment of arthritis due to discrepancies in clinical research (Marcus and Suarez-Almazor 2001). In a previous study, Sharma, Srivastava, and Gan found that ginger oil (33 mg/kg) given orally to rats for 26 days significantly reduced the edema of the paws and joints connected to severe chronic adjuvant arthritis. More recently, in the streptococcal cell wall-induced arthritic animal model of rheumatoid arthritis, the efficiency of a crude ginger extract and a fraction containing solely gingerols and derivatives to reduce joint swelling were examined (Funk *et al*., 2009). The findings showed that although both extracts could stop joint inflammation, the crude dichloromethane extract was superior (when normalized to gingerol content) in stopping joint inflammation and destruction (Funk *et al*., 2009). This is because it also contained essential oils and more polar compounds. One human trial found no difference between ginger and a placebo in people with hip or knee osteoarthritis (Bliddal *et al*., 2000). As opposed to the control group, patients with osteoarthritis of the knee consistently responded better to therapy with ginger extract (Altman and Marcussen, 2001).

Antidiabetic activity

It has been hypothesized that ginger contains anti-diabetic properties. Ginger consumption improved glucose tolerance and raised serum insulin levels in the streptozotocin-induced diabetic rat model, suggesting that it may help regulate blood sugar levels (Islam and Choi 2008). Treatment with a ginger extract resulted in a considerable decrease in the insulin resistance caused by fructose's rise of lipid levels, body weight, hyperglycemia, and hyperinsulinemia (Kadnur and Goyal, 2005). In streptozotocin-induced diabetic rats, an

aqueous extract of raw ginger (administered daily, 500 mg/kg intraperitoneal) decreased serum glucose, cholesterol, and triacylglycerol levels; decreased urine protein levels; decreased water intake; and decreased urine output; and prevented the weight loss associated with diabetes in this model. Additionally, [6]-gingerol has been shown to improve 3T3-L1 preadipocyte development and insulin-sensitive glucose absorption (Sekiya *et al*., 2004). The downregulation of adiponectin expression caused by TNF- in 3T3-L1 adipocytes was considerably reduced by [6]-shogaol or [6]-gingerol, according to a later investigation (Isa et al. 2008). While [6]-gingerol operated via inhibiting TNF-induced JNKs signaling, [6]-shogaol appeared to behave as a PPAR (peroxisome proliferator-activated receptor) agonist (Isa *et al*., 2008). These findings imply that ginger may be helpful in reducing the negative consequences of diabetes in people.

Antinausea activity

Ginger has probably been used to treat nausea and vomiting symptoms the most frequently and consistently throughout history. The advantages and risks of using herbal remedies to treat liver and gastrointestinal distress have been reviewed (Langmead and Rampton 2001), and a number of studies have found that ginger is generally effective as an antiemetic (Ernst and Pittler, 2000; Langmead and Rampton (2001). Dupuis and Nathan (2003); Boone and Shields (2005); Borrelli *et al*., (2005); Bryer (2005) Mahesh Ginger's ability to break up and evacuate intestinal gas through its carminative impact has been linked to its usefulness as an antiemetic. According to the findings of a randomized, double-blind research involving healthy volunteers, ginger significantly sped up stomach emptying and induced antral contractions (Wu *et al*., 2008). In the past, [6]-gingesulfonic acid, derived from ginger root, was demonstrated to be successful in treating rat stomach ulcers brought on by HCl/ethanol. Compared to [6]-gingerol or [6]-shogaol, this substance displayed stronger antiulcer efficacy but less potent pungency.

Anticarcinogenic activity

The potential cancer therapeutic uses of ginger and its different components are currently the subject of intense interest from several research groups, including our own. In previous reviews (Shukla and Singh 2007; Aggarwal *et al*., 2008), several elements of the chemopreventive effects of different phytochemical foods and medicines, including ginger, were discussed. Numerous forms of ginger, including a crude or imperfectly purified extract, gingerols, including [6]-gingerol, shogaols, particularly [6]-shogaol, and zerumbone, a sesquiterpene molecule generated from ginger as well as a number of other components and metabolites, were the focus of studies on their anticancer properties. Numerous cancer types, including lymphoma, hepatoma, colorectal cancer, breast cancer, skin cancer, liver cancer, and bladder cancer, have been studied to see whether ginger is useful in preventing or restraining the growth of cancer. Antioxidant properties, the potential to induce apoptosis, reduce proliferation, cause cell-cycle arrest, and suppress activator protein 1 (AP-1) and NF-B/COX-2 signaling pathway are some of the explanations put out to explain ginger's anticancer properties.

Cardiovascular disease-preventive effects

In addition to its effects on cancer, some research suggests that ginger may also have preventative effects on cardiovascular health and a number of other chronic problems. According to a review of in vitro and animal research, ginger has been shown to have anti-inflammatory, antioxidant, antiplatelet, hypotensive, and hypolipidemic properties that may be used to treat many aspects of cardiovascular disease (Nicoll and Henein, 2009). More research is necessary because human studies are less conclusive. Because of an apparent correlation between ginger and reported incidences of increased risk of bleeding after surgery (Chang and Whitaker, 2001; Pribitkin and Boger, 2001) or if taken with anticoagulant medications like warfarin (Heck *et al*., 2000), caution has been advised when taking ginger and

other herbal extracts. The information is inconclusive, but (Vaes and Chyka, 2000). According to at least one study (Weidner and Sigwart, 2000), ginger has no impact on blood pressure, heart rate, or coagulation markers and does not interact with anticoagulant medications like warfarin. A further investigation that found ginger had no impact on the coagulation status, pharmacokinetics, or pharmacodynamics of warfarin in healthy people corroborated these findings (Jiang, Williams *et al*., 2005) In a number of animal models, an aqueous ginger extract was found to cause a dose-dependent reduction in arterial blood pressure (Ghayur and Gilani, 2005).

2.4 Diarrhea

An estimated nine million children worldwide, most of them under five, pass away from diarrhea each year (De Wet *et al*., 2010). The majority of deaths occur in rural African areas where access to clean, safe water is a major barrier to the spread of diarrheal illnesses and where health care facilities are limited (Forsberg *et al*., 2009; Mwambete and Joseph, 2010). Mwambete and Joseph (2010) also estimated that in the world, diarrhea kills more young children than all forms of malaria, AIDS, and tuberculosis combined. In some rural areas of the developing world, mothers have limited knowledge of the elements that predispose to diarrhea, and sometimes the frequent incidence of children diarrhea is mistakenly seen as a child's developmental stage, which can sometimes almost always result in mortality (Mwambete and Joseph, 2010). Sarmin *et al.* (2021) defined invasive diarrhea as having mucoid, small-volume, with or without visible blood stools that are accompanied by fever and intense stomach pain. A further indicator of invasive diarrhea is the presence of pus cells >20/HPF and RBC (any quantity) in the stool with an alkaline pH. Acute watery diarrhea (AWD) is defined as having three or more watery stools per day. Patients with AWD may have mild, moderate, or severe dehydration at presentation. We defined dehydrating diarrhea as mild to severe dehydration (Sarmin *et al*., 2021). Additionally, small intestinal diarrhea is typically severe, watery, and

accompanied by bloating, pain, and abdominal discomfort. Less severe and accompanied by unpleasant bowel motions and little abdominal discomfort, large intestinal diarrhea is less profuse. *Salmonella spp., Vibrio cholerae, Clostridium perfringens*, and *E. coli* (toxicogenic - STEC) affect the small bowel, while *Campylobacter, Shigella*, *Yersinia*, and *E. coli* (enteroinvasive) mostly impact the colon (Riddle *et al*., 2017; DuPont, 2016).

2.5 Peripheral blood smear

The peripheral blood film is a fundamental and very useful haematological technique that clinicians can use for screening, diagnosis, and monitoring the course of the disease and the effectiveness of treatment. A small layer of blood is smeared on a glass microscope slide and then dyed to enable a microscopic examination of the different blood cells. This is known as a blood smear, peripheral blood smear, or blood film. Blood smears are commonly used to look for blood parasites such those that cause malaria and filariasis and are studied in the investigation of hematological (blood) disorders. The purpose of a blood smear examination is to explore anomalous results or to validate data that the automated analyzer has indicated as unreliable. This procedure is typically done in conjunction with a full blood count (Gulati *et al*., 2013). A peripheral blood film is frequently requested clinically by the attending clinician due to a clinical suspicion, or less frequently, it is started by the laboratory (Bain, 2005). Based on abnormal results from an automated count or patient clinical data whose diagnosis may be confirmed by a peripheral blood film, the laboratory may order a peripheral blood film. The latter is controlled by particular laboratory policies or regional regulatory standards (Bain, 2005). Anemia can be diagnosed by a microscopic study of the size, shape, and color of red blood cells. Characteristic anomalies on the blood film are a result of diseases such iron deficiency anemia, sickle cell disease, megaloblastic anemia, and microangiopathic haemolytic anemia. (Bain, 2005). The blood smear can be used to estimate the proportions of various white blood cell types. A manual white blood cell differential is what this is. The presence of aberrant

cells, such as the circulating blast cells found in acute leukemia, as well as abnormalities in the proportions of white blood cell types, such as neutrophilia and eosinophilia, can be detected using the white blood cell differential (Choladda, 2015). The preferred diagnostic technique for some parasitic illnesses, including malaria and babesiosis, is a blood smear test (Jon, 2009). Rarely, in patients with severe sepsis, bacteria may be seen on the blood smear (Gerard *et al*., 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Collection and Identification

The three selected plants, *Andrographis* paniculata, *Annona muricata* and *Zingiber officinale* was obtained from Benin City, Edo state. It was authenticated by a plant taxonomist (Dr. O. Timothy) in the Department of Plant Biology and Biotechnology. The voucher numbers issued were *Andrographis paniculata* (UBH-A411), *Annona muricata* (UBH-A419) and *Zingiber officinale* (UBH-Z094).

3.2 Preparation of Sample

Fresh leaves of *Andrographis paniculata, Annona muricata* and *Zingiber officinale* were cleaned in clean water, cut into pieces, and shade-dried for fourteen (14) days. Additional oven drying took place for 24 hours at 45 °C. To generate a polyherbal mixture, the powdered leaf samples were individually weighed using different weight units. Distilled water was subsequently utilized to extract the mixture for 72 hours while intermittently swirling and shaking. It was filtered, and the filtrate was then concentrated into a semi-solid using crucibles over a water bath. The formula below was used to calculate the percentage yield.

$$\% \text{ yield of extract} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.3 Experimental animals

For the experimental study, twenty-five (25) male Wistar rats weighing 190–220 g were obtained from the animal house of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo state with ethical number (LS21511) Five groups (n=3) were formed by random selection. Prior to the trial, the animals spent fourteen (14) days becoming acclimated to the laboratory environment and were given unlimited access to grower pellet food and water. Prior to each trial, animals were fasted for a whole night with unrestricted access to water. Throughout the duration of this work, the ethical committee for the use of animals' guild was followed.

3.4 Microbial inducement

Twenty-five (25) rats were split into five groups (n=5). *Shigella dysenteriae* and *Escherichia coli* bacteria isolates comprising of 1 ml each, were mixed and given orally to each rat via an orogastric tube. The rats were examined for symptoms of disease after twenty-four (24) hours.

3.5 Experimental protocol

To make it easier to identify the rats, distinctive marks were made on their bodies using ink. Additionally, the body weights of each rat was determined using weighing scales. The dosage of the medication and the polyherbal extract given to the rats was determined as follows:

$$\text{Stock} = \frac{\text{Dose x highest body weight}}{1000}$$

To calculate the dosage of amoxicillin and the polyherbal extract to be given to each rat, the stock was divided by the rat's body weight. 0.32 g of the polyherbal extract was weighed and dissolved in 18.5 ml of distilled water before orally given to the rats for 3 days. Group 1 animals received distilled water as a control, while group 2 animals received 100 mg/kg of amoxicillin. The polyherbal plant extract was administered to Groups 3, 4 and 5 at doses of 25, 50, and 100 mg/kg, which are the groups that would get the plant extract treatment.

3.6 Peripheral Blood Smear

The rats were sacrificed by exposure to chloroform for sixty (60) seconds. Blood samples were taken using a sterile syringe and placed in sterile bottles containing Ethylenediaminetetraacetic acid (EDTA) It inhibits clotting and it does not distort blood cells (Rod and Hangerman, 2000). Morphologic abnormalities of peripheral blood cells are discovered by microscopic examination with the oil immersion lens of well-prepared films of peripheral blood stained with Wright's stain. One should focus on sections of the slide where the red cells appear singly and have a core pallor in order to properly assess the erythrocyte shape. While erythrocytes in dense portions of the slide have their shape altered

by cell contact, erythrocytes far out on the feathery edge show no signs of central pallor. On peripheral blood films, erythrocytes frequently undergo fictitious alterations. Red cell cytoplasmic vacuolization is an artifact. Echinocytes (crenated red cells) are frequently brought on by the staining solution's hypertonicity or alkalinity. When the staining solution is overly acidic, stomatocytes may develop. Target cells are artifacts when they appear in one region of the image but not another because naturally occurring target cells will be dispersed equally throughout the slide. Monocytes and neutrophils frequently assemble towards the blood film's feathery end and its edges. Granulocytes of blood that have had their clotting time reduced by EDTA may develop cytoplasmic vacuoles. Amorphous nuclear material (also known as "basket" cells) is left behind when leukocytes break during the creation of the blood film. Chronic lymphocytic leukemia's lymphocytes are more prone to rupture. Although platelets often appear alone on the blood film, there can occasionally be significant platelet aggregation, which makes it more challenging to estimate the amount of platelets.

3.7 Histopathological analysis

the animals were sacrificed, and the abdomen was cut open, using a sterile scissor from a dissecting kit and the heart was collected. The heart was submerged in neutral buffered formalin, which is a common fixing agent. With the help of this method, specimens can be preserved for a longer period of time while maintaining their structural integrity, enabling microscopic examination for further research analysis (Likhithaswamy *et al*., 2022). The organs were thoroughly dehydrated with 99.9%, 70%, and 96% ethanol before being rinsed with distilled water. A critical step for classifying various tissue types and evaluating morphological

changes was the cutting and staining of sections with a thickness of around 4 m (Fischer *et al.,* 2008). Under an x 400 magnification optical photomicroscope, stained tissues were examined.

3.8 Data Analysis

Results were analyzed with Graph pad prism version 6. Data was presented as Mean ± S.E.M, and statistical significance were calculated using One-way ANOVA, followed by Dunnett's test where $P<0.05$ were considered statistically significant.

CHAPTER FOUR

RESULTS

The results obtained from this present study had no significant difference across graded doses of the extract when compared with the untreated control in dry stool, diarrhea stool, weight of the stool and total number of stool as shown in Table 4.1

Table 4.1: Effects of polyherbal aqueous extract on baseline diarrhoea level in rats.

Parameters	Doses mg/kg	Dry stool	Diarrhoea stool	Weight of stool	Total number of stool
Control	0.2 ml	21.00±5.69[a]	1.33±0.67[a]	4.50±0.22[a]	20.67±5.21[a]
Amoxycilliin	100	18.00±4.16[a]	1.00±0.58[a]	2.23±0.59[a]	19.00±1.00[a]
PAE	25	10.00±2.89[a]	1.00±0.58[a]	2.87±0.47[a]	10.67±0.67[a]
PAE	50	10.33±2.91[a]	1.33±0.88[a]	2.00±0.00[a]	11.67±1.67[a]
PAE	100	7.67±3.71[a]	0.67±0.33[a]	1.40±0.33[a]	8.33±0.67[a]

Values were expressed in mean±SEM and the level of significance were determined. All values with same letters are not statistically significant

The results obtained from this present study had no significant difference across graded doses of the extract when compared with the untreated control in dry stool, diarrhea stool, weight of the stool and total number of stool as shown in Table 4.2

Table 4.2: Effects of polyherbal aqueous extract after induction of diarrhoea level in rats.

Parameters	Doses mg/kg	Dry stool	Diarrhoea stool	Weight of stool	Total number of stool

Control	**0.2 ml**	4.00±0.58[a]	5.33±0.33[a]	5.33±1.33[a]	17.33±0.88[a]
Amoxycilliin	**100**	4.33±0.88[a]	5.33±0.20[a]	4.67±0.45[a]	13.00±1.53[a]
PAE	**25**	3.67±0.88[a]	6.33±0.20[b]	5.00±1.00[a]	16.67±0.67[a]
PAE	**50**	3.00±1.16[a]	5.33±0.20[a]	4.33±0.88[a]	17.00±1.16[a]
PAE	**100**	4.67±0.76[a]	5.67±0.88[a]	5.33±1.67[a]	17.00±1.53[a]

Values were expressed in mean±SEM and the level of significance were determined. All values with same letters are not statistically significant

Report from this present study showed the effect of the polyherbal extract with significant reduction across graded doses of the dry stool, diarrhea stool, weight of stool and total number of stool when compared with the untreated control, except for amoxicillin (100 mg/kg) that showed a slight increase in the total number of stool (Table 4.3)

Table 4.3: Effects of polyherbal aqueous extract after treatment of diarrhea level in rats.

Values were expressed in mean±SEM and the level of significance were determined. All values with same letters are not statistically significant

Parameters	Doses mg/kg	Dry stool	Diarrhoea stool	Weight of stool	Total number of stool
Control	**0.2 ml**	17.33±3.71[a]	7.33±1.45[a]	1.60±0.40[a]	20.67±5.21[a]
Amoxycilliin	**100**	11.33±1.18[a]	1.00±0.58[b]	0.77±0.23[b]	19.00±1.00[a]
PAE	**25**	15.00±1.73[a]	1.33±0.03[b]	0.70±0.65[b]	10.67±0.67[b]
PAE	**50**	17.00±2.00[a]	0.33±0.03[c]	0.63±0.19[b]	11.67±1.67[b]
PAE	**100**	15.67±2.96[a]	0.00±0.00[c]	0.47±0.03[c]	8.33±1.67[b]

The result obtained from the peripheral blood smear of white blood components exhibited the presence of granulocyte, small lymphocyte and lymphoblast with no significant deteriorative effect when compared with the control groups, except at 100 mg/kg of granulocyte level that had a significant reduction while, large lymphocyte, lymphoblast, monocyte, eosinophil and neutrophil they showed varied responses as showed in Table 4.4.

Table 4.4: Effects of polyherbal aqueous extract on white blood cell peripheral blood smear in rats

Parameters	Doses mg/kg	Granulocyte	Small Lymphocyte	Large Lymphocyte	Lymphoblast	Monocyte	Eosinophil	Neutrophil
Control	**0.2 ml**	0.00 ± 0.00^{a}	2.67 ± 0.33^{a}	2.33 ± 0.33^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.0 ± 0.0^{a}
Amoxycilliin	**100**	0.00 ± 0.00^{a}	1.33 ± 0.07^{a}	1.67 ± 0.09^{a}	0.33 ± 0.03^{b}	0.33 ± 0.03^{b}	0.33 ± 0.03^{a}	0.33 ± 0.03^{a}
PAE	**25**	0.00 ± 0.00^{a}	2.00 ± 0.00^{a}	1.67 ± 0.03^{a}	0.33 ± 0.03^{b}	0.33 ± 0.03^{b}	1.33 ± 0.03^{b}	0.33 ± 0.03^{a}
PAE	**50**	0.00 ± 0.00^{a}	2.33 ± 0.33^{a}	1.67 ± 0.03^{a}	0.67 ± 0.03^{b}	0.00 ± 0.00^{a}	0.67 ± 0.03^{b}	1.00 ± 0.00^{b}
PAE	**100**	0.33 ± 0.03^{b}	2.33 ± 0.33^{a}	2.00 ± 0.58^{a}	0.33 ± 0.03^{b}	0.00 ± 0.00^{a}	1.00 ± 0.06^{b}	0.67 ± 0.07^{b}

Values were expressed in mean±SEM and the level of significance were determined. All values with same letters are not statistically significant

According to Table 4.5, the result showed a varied response across the graded doses of the extract of the indices except for nomochromic and polychromatic cells that had no significant differences, although with slight reduction when compared with the untreated control (Table 4.5)

Table 4.5: Effects of polyherbal aqueous extract on red blood cell peripheral blood smear in rats.

Parameters	Doses mg/kg	Macrocytic cells	Nomocytic cells	Nomochromic cells	Polychromatic Cells	Target cells	Crenatal cells	Stomatocytes
Control	0.2 ml	0.33±0.03[a]	2.00±0.58[a]	2.67±0.33[a]	2.00±0.00[a]	0.67±0.03[a]	1.00±0.06[a]	0.00±0.00[a]
Amoxycilliin	100	0.33±0.03[a]	2.33±0.67[a]	2.00±0.58[a]	1.67±0.33[a]	1.67±0.33[b]	1.33±0.07[a]	0.33±0.03[b]
PAE	25	0.33±0.03[a]	2.67±0.33[b]	2.67±0.03[a]	1.67±0.33[a]	1.67±0.33[b]	1.00±0.06[b]	0.00±0.00[a]
PAE	50	1.00±0.00[b]	2.00±0.00[a]	2.00±0.00[a]	1.67±0.33[a]	1.67±0.33[b]	1.67±0.09[b]	0.33±0.03[b]
PAE	100	1.00±0.06[b]	2.67±0.33[b]	2.67±0.33[a]	1.33±0.33[a]	2.00±0.57[b]	0.67±0.07[a]	0.33±0.03[b]

Values were expressed in mean±SEM and the level of significance were determined. All values with same letters are not statistically significant

The evaluation of the extract carried out elicited no conspicuous histopathological defect depicted across graded doses of the extract when compared with the control groups. Rather a normal morphological display of the architecture frame work of cardiac cells, showed normal structure across the doses (Figure 4.1).

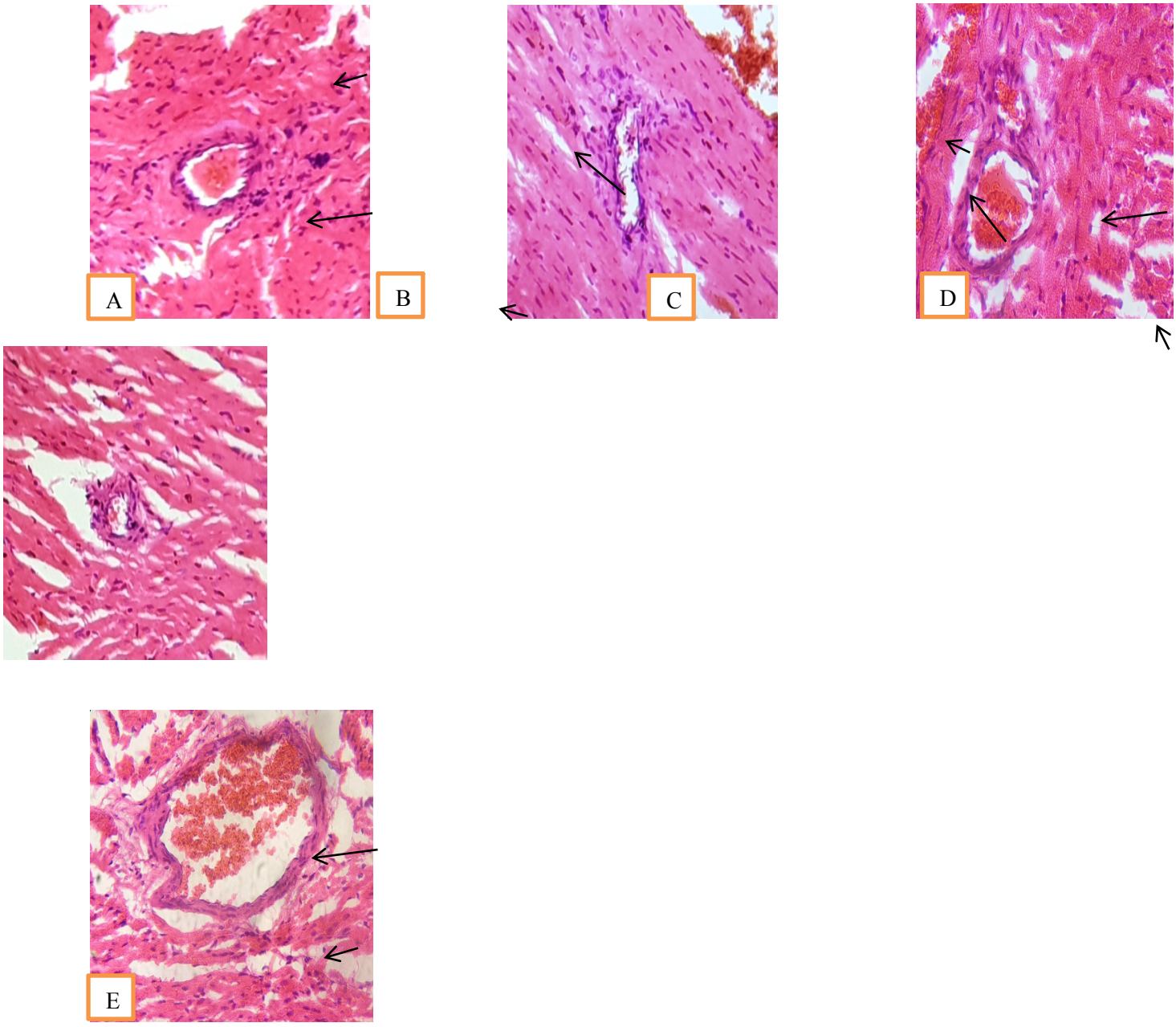

Plate 4.4: Effect of *the* polyherbal aqueous extract on cardiac cells

A. Control Heart composed of bundles of myocardial fibres (short arrow), interstitial space and visible coronary artery (long arrow)
B. 100 mg/kg Amoxycillin*;* Heart composed of bundles of myocardial fibres (short arrow), interstitial space and visible atrophied coronary artery (long arrow)

C. 25 mg/kg Polyherbal aqueous extract*;* Heart composed of bundles of myocardial fibres (short arrow), interstitial space and visible congested coronary artery (long arrow)

D. 50 mg/kg Polyherbal aqueous extract*;* Heart composed of bundles of myocardial fibres (short arrow), interstitial space and visible coronary artery (long arrow).

E. 100 mg/kg Polyherbal aqueous extract*;* Heart composed of bundles of myocardial fibres (short arrow), interstitial space and visible congested dilated coronary artery (long arrow) X 40 magnification. Eosin and myosin stain.

CHAPTER FIVE

5.1 Discussion

Diarrhoeal can be defined as an increase in the fluidity, volume, and frequency of bowel movements, as well as an increase in fluid secretion and a decrease in fluid absorption. As a result, the body loses water and electrolytes (Awe *et al*., 2011). Studies on plant-based medications have been conducted in an effort to find novel antidiarrhoeal agents with improved efficacy and safety *in vivo* models were used to evaluate the effects of these medicinal herbs on electrolyte secretion, water secretion, and gastrointestinal transit (Oghenesuvwe *et al*., 2018). The results obtained from this present study of the polyherbal aqueous extract on baseline diarrhoeal level across graded doses showed the proliferation of bacterial induced diarrhoeal with no significant difference across the groups on the dry stool, diarrhea stool, weight of the stool and total number of stool as shown in Table 4.1.

The results obtained from the polyherbal aqueous extract revealed a significant difference in dose-dependent manner, elicited an inhibitory effect across graded doses of the extract when compared to the control group across the diarrhoeal parameters (dry stool, diarrhea stool, weight of stools and total number of stools) as shown in Table 4.3. This outcome corresponded with other studies in respect of antidiarrheal herbs as reported on *Saussurea lappa* by Negi *et al*. (2013), *Urena lobata* by Yadav and V. Tangpu (2007) and it also corresponded with the research done on *Lithocarpus dealbata*, and *Pterocarpus erinaceus* by Ezeja *et al*. (2012), which claims that antidiarrheal plants are known to reduce number of wet stools, consistency of fecal droppings as well as delay in the onset of diarrhea. These could be attributed to the

presence of numerous phytochemicals across the treatment groups when Table 4.4 showed the result of the polyherbal aqueous extract on white blood cell peripheral blood smear, across the granulocyte had no significant difference across the polyherbal plant extract except for granulocyte at 100 mg/kg which showed a significant increase when compared with the control groups (Bowman and Rand, 1980; Synder *et al*., 1977; Ajagbonn *et al*., 1999; Dioka *et al*., 2002). Ingestion of some plant materials (either in the raw form or as extracts) has been reported to cause anemia, which may result from splenic sequestration of red bloods, impaired red blood cell production or primary bone marrow dysfunction (Muller and Tobin, 1980). The current study showed that the polyherbal aqueous extract caused a significant increase in the neutrophils, and the associated concomitant significant decreases in leukocytes values such as the small lymphocytes and large lymphocytes as shown in the current study (Table 4.4), which could be explained by the polyherbal aqueous extract had a stimulatory effect on adrenocortical function in animals (Ahmed and ElQirbi, 1993). It is well reported that glucocorticoids increase the concentration of neutrophils and decreases the concentration of lymphocytes, monocytes, eosinophils and basophils (Dallak *et al*., 2010). The decrease in the number of lymphocytes in the peripheral blood of the polyherbal aqueous extract treated rats is possibly due to the fact that these cells migrate to the affected damaged tissue after the polyherbal aqueous extract intoxication as reported in some previous studies (Dallak *et al*., 2010; Al-Hashem *et al*., 2011). Al-Hashem *et al*. (2011) reported that the reduction in circulating lymphocytes is the result of their movements from the vascular beds to lymphoid tissues. The study also showed that there was a significant increase in the neutrophils level as indicated in Table 4.4. Beside the effect of glucocorticoids, the increase in the neutrophils could be possibly due to the increase in flow of blood from bone marrow and decrease migration from blood vessels. This study is not in agreement with the study of Dallak *et al*. (2010) who reported that administration of *C. edulis* to rabbits resulted in a significant decrease in red blood cells as observed in Table 4.5. However, this variation with our results could be due to the type of extract used in their study and possibly due to the dose of *C. edulis* used. Due to no reactions in the heart histology, it had a normal architectural structure across graded doses of the extract.

5.2 Conclusion

The findings of this study demonstrated that the polyherbal aqueous extract possessed a significant antidiarrheal activity. The antidiarrheal activities of the polyherbal aqueous extract

could probably be attributed to the presence of the phytochemicals found in the plants. Thus, this finding supported the claimed ethnomedicinal use of the polyherbal plant extract for the management of diarrhoeal.

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