PHYTOCHEMICAL COMPOSITION, ANTIDIARRHEAL ACTIVITY AND ANTIBACTERIAL EFFECT OF AQUEOUS LEAF EXTRACT OF *Plectranthus barbatus* (Andrews)

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156/CE/20960/2020

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (BIOCHEMISTRY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

MAY, 2024

DECLARATION

I, Ajwang Emmah Clarice, duly declare that this thesis is my original work and has not been presented for a degree or any other award in any university

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DEDICATION

To my family who have been so supportive of this worthy cause. Mr. Moses Agumba, my dear husband, for your tremendous support, words of encouragement, and push to complete this work. Kibali, Wema, and Taraji, your presence in my life inspired me to work harder. My heartfelt thanks go to my dear mother, Mrs. Pamela Adede, for her emotional and spiritual support. Your prayers have enabled me to accomplish this.

ACKNOWLEDGMENTS

I thank God for the gift of wisdom, good health, and the means to complete my dissertation.

Dr. Mathew Ngugi Piero and Dr. George Omwenga Isanda, your invaluable input in my research work has helped shape my research potential. God bless you for your esteemed scholarly guidance, assistance, and knowledge.

Thank you to the Department of Microbiology, Biochemistry, and Biotechnology, technicians especially Ms. Ann and Mr. Daniel, for their willingness to provide needed technical assistance. Thank you for your inspiration and assistance, Dr. James Kimani, Mr. Paul Nyalo, Mr. Moriasi Gervason, Dr. Phillip Ogolla, and Dr. Kenneth Osano.

Dr. Carolyn Muruthi, your dedication and mentorship have made all of this possible. Best wishes from God.

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ABBREVIATIONS AND ACRONYMS

AAD	Antibiotic Associated Diarrhea
ADI	Antidiarrheal Index
ANOVA	Analysis of Variance
CLSI	Clinical and Laboratory Standards Institute
DD	Disc Diffusion
dH_2O	Distilled Water
DHIS	Division of Health Informatics and Surveillance
ENS	Enteric Nervous System
HIV	Human Immunodeficiency Virus
KDHS	Kenya Demographic and Health Survey
LMICs	Low- And Middle-Income Communities
LC-MS	Liquid Chromatography- Mass Spectrometry
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
NENT	National Committee for Research Ethics in Science and Technology
OECD	Organization for Economic Cooperation and Development
ORT	Oral Rehydration Therapy
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization

ABSTRACT

Diarrhea is defined as the movement of unformed or watery stool more than three times a day. Globally, diarrheal infections remain a public health problem, especially in children. With nearly 1.7 billion episodes and 1.3 million fatalities reported annually. Developing countries bear 78% of diarrhea burden worldwide. A range of viral, bacterial, and parasitic species induce diarrhea in humans, including rotavirus and *Escherichia coli*. The mainstays of pharmacological therapy for infectious diarrhea include probiotics, antibacterials, antiviral drugs, and intestinal adsorbents. However, these clinical treatments are not devoid of shortcomings, including prohibitive costs, drug-drug interactions, and adverse effects such as lethargy, constipation, respiratory depression, and coma. Medicinal plants, including *Plectranthus barbatus* have folkloric remedies against diarrhea. However, there is a paucity of knowledge to scientifically validate the efficacy of the leaves of *P. barbatus* on diarrheal infections. The study, therefore, was undertaken to ascertain the antidiarrheal efficacy, antibacterial activity, bioactive composition, and toxicity profiles of *P. barbatus* aqueous leaf extracts. Antidiarrheal activity and acute toxicity were carried out on mice. Using Swiss albino mice, castor oil-induced diarrhea, charcoal meal-based gastrointestinal motility, and castor oil-induced secretion models were employed to assess antidiarrheal activity. In all of the test models, animals were randomly assigned into six groups consisting of six animals in each. Group I received distilled water, group II received 10 ml/kgbw of the vehicle (distilled water), while group III was treated with standard drug (3 mg/kgbw loperamide) in the respective models, whereas groups IV to VI received 100, 200, and 400 mg/kgbw of the aqueous leaf extracts of *Plectranthus barbatus*. Antibacterial activity was carried out on selected bacterial pathogens. Quantitative phytocompound analysis was evaluated using liquid chromatography-mass spectrometry. Data were analysed using one-way analysis of variance followed by Tukey's test, and $p< 0.05$ was considered statistically significant at 95% confidence of interval.The study results indicated that *P. barbatus* extract has antidiarrheal activity. The plant extract prolonged the onset of diarrhea, caused a significant decline in the occurrence of wet feces and intestinal transit. Additionally, the extract elicited a reduction in the accumulation of intraluminal fluid, resulting in a decrease in distension, intestinal overload, and water content in the fecal drops. Loperamide showed a statistically similar antidiarrheal effect with the extract at a dosage of 200mg/kgbw suggesting a probable effective dosage of the extract. This study demonstrated that the aqueous leaf extracts of *P. barbatus* exhibited diarrheal inhibition activity. The percentage inhibition was dose dependent with 100, 200 and 400mg/kgbw showing 49.98±1.61, 66.12±2.17 and 75.80±2.16% inhibition of diarrheal output respectively ($p<0.05$). Further, *P. barbatus* demonstrated antibacterial activity against pathogens associated with diarrheal diseases. The extract had varying Mean Zones of Inhibition (MZI), 7.33±0.33 to 17.17±0.73mm, against the bacterial pathogens, with higher effects observed against *P. aeruginosa* and *B. subtilis.* Acute toxicity assays on mice showed that *P. barbatus* extract was non-toxic at the dosage level of 2000mg/kgbw. LC-MS analysis detected the presence of phytocompounds associated with antidiarrheal and antibacterial effects. Findings from this study offer scientific validation for the folkloric utilization of *P. barbatus* in the management of diarrhea. However, further studies should be conducted to explore the mechanistic approach to the reduction of diarrhea and the comprehensive chronic toxicological effects on biochemical and hematological parameters.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Diarrhea is defined as the movement of unformed or watery stool more than three times a day (Ayalew *et al*., 2022). It is characterized by elevated gastrointestinal secretion and motility as well as a reduction in the absorption of fluid. The severity and frequency of diarrheal infections are aggravated by poor sanitation, hygiene practices, and inaccessibility to clean water (Abdul- Mutalib *et al*., 2015).

Globally, diarrheal infections remain a public health problem, especially in children. With nearly 1.7 billion episodes and 1.3 million fatalities reported annually, diarrhea is one of the leading causes of death in children under the age of five (WHO, 2018; Ayalew *et al*., 2022). Furthermore, estimates reveal that diarrhea accounts for 15% of all child mortality that occur each day (Zhang *et al*., 2016).

Developing countries, especially those in Africa and Asia, bear 78% of the diarrheal burden worldwide (WHO, 2018). For instance, African countries in 2015, reported 30 million cases of diarrhea and approximately 340, 000 diarrhea-related deaths (Manetu *et al.,* 2021). Further, in these countries, diarrhea accounts for more than four-fifths of under-five deaths (WHO, 2018). The discrepancy is due to deprived sanitary and hygienic conditions and deficit of unsafe drinking water. In regard to this, 780 million people in these nations lack access to safe water, and 3 billion reside in poor sanitary conditions (WHO, 2019).

In Kenya, despite the remarkable strides made in the reduction of diarrhea-related deaths, diarrheal infections remain a serious public health issue (KDHS, 2014). According to the District Health Information System (DHIS, 2019), estimates show that in 2018, there were approximately 2 million documented incidences of diarrheal episodes in children. Previous research has also revealed that children get diarrhea twice every two weeks (Guillaume *et al.,* 2020).

Additionally, the mortality rate of children below five years was reported to be at 16%, surpassing deaths related to malaria and HIV combined (Manetu *et al*., 2021). Similar to other countries, diarrhea-related incidences and deaths in Kenya are associated with poor sanitation and hygiene practices.

A range of viral, bacterial, and parasitic species induce diarrhea in humans. The disease can spread through the fecal-oral route, tainted food and water, and person-to-person transmission (Ashbolt, 2015). According to Breurec *et al*. (2016), the most frequent etiological causes of diarrhea in children under five years of age include rotavirus, adenovirus, and *Escherichia coli* (*E. coli*). The pathogens induce a variety of diarrheal episodes, including acute watery diarrhea, which can lead to varying degrees of dehydration, and chronic diarrhea, which can cause nutritional losses, malabsorption, bloody diarrhea, and even mortality (WHO, 2019).

Pathogens release toxins that cause the ion channels of the epithelial cells of the intestine to open, allowing electrolytes and water to enter the digestive tract, leading to episodes of

diarrhea. Entry of electrolytes and water leads to the alteration of electrolyte assimilation. This causes the accumulation of non-absorbable chemicals in the intestines (Zhu *et al*., 2022). Furthermore, the toxins alter the expression of genes in the gastrointestinal tract, causing them to produce intimin, a protein that allows the bacteria to connect to the intestinal wall, resulting in severe diarrhea (Zhu *et al*., 2022).

The mainstays of pharmacological therapy for infectious diarrhea include probiotics, antibacterials, antiviral drugs, and intestinal adsorbents (Li *et al*., 2021). Nevertheless, these clinical treatments are not devoid of shortcomings, including prohibitive costs, drug-drug interactions, and adverse effects such as lethargy, constipation, respiratory depression, and coma. Furthermore, some bacteria causing diarrhea, including *E. coli*, are gaining resistance to conventional antibiotic drugs (Silverman *et al.*, 2017). In view of the complexity associated with conventional treatments, there is a need to explore affordable and efficacious antidiarrheal drugs (Sisay *et al*., 2017).

Medicinal plants are considered an alternative medication in the treatment and management of diarrhea. The anti-diarrheal efficacy of herbal plants is attributable to the diversity of their chemical properties and minimal toxicity (Omwenga *et al*., 2014). Moreover, they are arguably available and affordable, and a large population rely on the medicinal plants, especially in developing countries (Ferede *et al*., 2021). Several studies depict that in Sub-Saharan Africa, communities rely on the therapeutic benefits of medicinal plants as a remedy for infectious diarrhea. These include *Lantana camara*,

Moringa stenopetala, *Casuarina equisetifolia,* and *Lepidium sativamum* (Tadesse *et al*., 2017; Ferede *et al*., 2021).

Plectranthus barbatus, which belongs to the family Lamiaceae, has a wide variety of traditional medicinal uses among African communities. Traditionally, this plant is used to manage different ailments, including respiratory disorders, epilepsy, heart diseases, constipation, and stomach aches (Alasbahi and Melzig, 2010). Moreover, in Kenya, among the Luo community, based on folkloric practices, the leaves are claimed to have antidiarrheal effects (Lukhoba *et al*., 2006). Nonetheless, there is a scarcity of knowledge to scientifically validate the efficacy of the leaves of *P. barbatus* against diarrheal infections. Therefore, this study sought to determine the antidiarrheal efficacy, antibacterial properties, phytochemical composition, and safety profile of *P. barbatus* aqueous leaf extracts.

1.2 Problem statement

Globally, despite efforts to reduce diarrheal infections, the prevalence and mortality of diarrhea continue to rise. Estimates show that 1.7 billion cases are reported each year in children below five years old (WHO, 2018). Additionally, statistics reveal that 15% of children worldwide die from diarrheal infections each day (UNICEF, 2016). Developing nations endure a disproportionate share of the burden, with estimates revealing that 78% of the diarrheal burden occurs in these countries.

Diarrheal infections are still a significant concern in Kenya, with a large number of diarrheal cases recorded each year (DHIS, 2019). Furthermore, the mortality rate of

children below five years has been reported to be 16%, surpassing deaths related to malaria and HIV combined (Manetu *et al*., 2021).

Further, despite the obvious milestones achieved in curbing diarrheal diseases with the use of conventional drugs, they are not devoid of drawbacks. These include adverse effects, among which are constipation, dizziness, abdominal pain, respiratory depression, and coma. Furthermore, diarrheal pathogens are acquiring antibiotic resistance, posing a barrier to diarrhea therapy. These conventional drugs also constitute the cost burden associated with the treatment of diarrheal diseases (Li *et al*., 2021).

In view of the challenges associated with conventional drugs, medicinal plants, including *Plectranthus barbatus*, are traditionally used among some Kenyan communities to manage diarrheal diseases. However, there is scanty information to scientifically validate their medicinal potential for diarrheal diseases. Therefore, *Plectranthus barbatus* has not yet been integrated for alternative or complementary use in diarrhea treatment.

1.3 Justification

Diarrheal infections continue to be the major cause of child mortality. Increased prevalence of diarrheal diseases is an indication that the current measures to control diarrhea are limited and overwhelmed by the infections (Manetu *et al*., 2021). Therefore, it is critical to investigate potential ways of combating diarrheal infections. The prohibitive cost and severe adverse effects associated with the current treatment regimen necessitate the need to search for efficacious and affordable antidiarrheal drugs.

Due to their cultural acceptance, affordability, and availability, medicinal plants are considered a substitute in the management of diarrheal infections. *Plectranthus barbatus* has traditionally been used to treat diarrheal infections. Nevertheless, there is scanty information to scientifically validate their medicinal potential for diarrhea infections. Hence, the current study sought to explore the antidiarrheal properties, antibacterial, phytochemical profile, and safety of *Plectranthus barbatus* leaf extract, leading to its validation and informed usage.

1.3 Research questions

i. What are the antidiarrheal properties of the aqueous leaf extract of *Plectranthus barbatus*?
ii. What is the antibacterial activity of the aqueous extract from *Plectranthus barbatus* leaves against selected bacterial pathogens?
iii. What are the phytochemicals in the aqueous extract of *Plectranthus barbatus* leaves correlated with antidiarrheal and antibacterial effects?
iv. What are the acute toxicity effects of the aqueous extract from *Plectranthus barbatus* leaves extract in mice?

1.4 Study objectives

1.4.1 Main objective

To investigate the antidiarrheal and gastrointestinal effects, antibacterial efficacy, quantitative phytochemical profile, and acute toxicity of aqueous *Plectranthus barbatus* leaf extracts.

1.4.2 Specific objectives

i. To investigate the efficacy of the aqueous leaf extract of *Plectranthus barbatus* in alleviating diarrhea symptoms through in vivo experimentation, focusing on its antidiarrheal properties.

ii. To assess the antibacterial efficacy of the aqueous extract of *Plectranthus barbatus* leaves against selected bacterial pathogens to determine its potential as a natural antimicrobial agent.

iii. To identify and characterize the phytochemical compounds present in the aqueous extract of *Plectranthus barbatus* leaves and assess their potential correlation with antidiarrheal and antibacterial activities.

iv. To determine the acute toxicity profile of the aqueous extract of *Plectranthus barbatus* leaves in mice.

1.4.3 Significance of the study

Diarrhea remains to be a leading health issue worldwide, and finding effective treatments is crucial. Investigating the antidiarrheal properties of *P. barbatus* can lead to the development of natural remedies or pharmaceuticals to alleviate diarrhea symptoms. With the rise of antibiotic resistance, exploring alternative antibacterial agents can contribute to the discovery of new treatments for bacterial infections. Understanding the phytochemicals present in *P. barbatus* can also provide insights into its potential medicinal properties and hence the bioactive compounds that contribute to its therapeutic effects.

This study will be able to shed light on the traditional uses of P. barbatus in herbal medicine and validate its efficacy through scientific research leading to its incorporation into mainstream medicine or serve as a basis for further pharmacological investigations. If proven effective and non-toxic, this extract could offer a low-cost and accessible treatment option for diarrhea and bacterial infections, especially in regions where access to conventional medications is limited. Additionally, understanding the medicinal properties of the extract can also raise awareness about its conservation and sustainable harvesting practices to ensure its availability for future generations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diarrheal ailments

Introduction

Diarrhea is a gastrointestinal ailment characterized by an increase in the frequency of liquid or semisolid stools passing through the gastrointestinal tract (GIT), as well as an increase in secretions, motility, and fluid absorption (Abdul- Mutalib *et al*., 2015; Kirk *et al*., 2017). These illnesses are caused by bacteria, parasites, and viruses, and they spread through contaminated food, water, or from person to person as a result of unhygienic conditions (Degu *et al*., 2020). Infection-related diarrhea has spread throughout developing countries due to deteriorating sanitation and insufficient access to safe drinking water. Nearly 780 million individuals all over the world have limited access to clean water, and about 2.5 billion are forced to make do with inadequate sanitation (WHO, 2019).

2.1.2 Epidemiology of diarrheal diseases

After acute respiratory infections, congenital anomalies, preterm birth complications, and intrapartum-related complications, diarrhea is the fifth cause of mortality, accounting for 15% of all child fatalities globally in children under five (Woldeyohannes *et al*., 2022). In 2015, there were 526,000 mortalities in children under the age of five due to diarrheal diseases worldwide, with 295,000 occurring in Sub-Saharan Africa (UNICEF, 2016). Nearly 78% of deaths of children below the age of 5 as a result of diarrheal ailments have been reported in Southeast Asia and Africa, with only 15 countries accounting for nearly 75% of all deaths from childhood diarrhea (Mokomane *et al*., 2018; Manetu *et al*., 2021).

The District Health Information Software (DHIS) documented over 1.6 million incidences of diarrhea among young children in Kenya (DHIS, 2019). Furthermore, the death rate from diarrheal ailments among children is extremely high, roughly 16% higher than ailments from malaria and HIV combined (Guillaume *et al*., 2020).

2.2 Aetiology of diarrheal ailments

Several studies carried out have revealed that the most common diarrhea-associated agents in children below five are viruses: human adenovirus (HAdV), rotavirus (RV), astrovirus, and norovirus; bacteria, including *Shigella spp* and *Escherichia coli*; and the protozoa *Entamoeba histolytica* (Breurec *et al.*, 2016; Kirk *et al*., 2017).

2.2.1 Adenovirus

The virus falls under the genus *Mastadenovirus*. HAdV consists of seven species and about 70 serotypes and is the causative agent of several of diseases, such as nephritis and pancreatitis. Nonetheless, HAdV is primarily linked with gastroenteritis or diarrheal illnesses in young children. Different genotypes of HAdV cause varying clinical features. For instance, adenoviruses serotypes 40 and 41 affect the gut, causing gastroenteritis and, therefore, being referred to as enteric adenoviruses (Qiu *et al*., 2018).

The enteric adenovirus is known to account for 20% of diarrhea cases among children. However, previous studies have reported the existence of non-enteric adenoviruses in children, including HAdV-E. According to Jalal *et al*. (2005), their roles in inducing diarrhea are not clear, which could be attributed to a lack of healthy control. However, in their study, Qiu *et al*. (2018) reported that HAdVs present among healthy and children with could be implicated in causing diarrhea. Similarly, Afrad *et al*. (2018) demonstrated

the genetic diversity of HAdVs, including non-enteric types, among diarrhea patients, suggesting the need to clarify their role in diarrheal disease. Additionally, it has been reported that there are different adenoviruses between hospital- and community-acquired diarrhea among children (Jalal *et al*., 2005).

2.2.2 Rotavirus

Rotaviruses, non-enveloped RNA viruses, are the leading etiologic agents of infantile gastroenteritis. They are ubiquitous and are reported to infect almost all children between the ages of 3 and 5. The virus is associated with fatalities in children under five years old. Estimates show that 50% of hospitalized children are as a result of rotavirus infection (Breurec *et al*., 2016).

The virus infects enterocytes, thereby inducing diarrhea via the destruction of the absorptive intestines resulting in malabsorption. Notably, rotavirus-associated diarrhea is characterized by a limited inflammatory response and non-bloody stools and lasts for a short duration (Wiegering *et al*., 2011). Further, it has two proposed mechanisms: osmotic diarrhea, which is associated with malabsorption, and secretory diarrhea due to the induction of ENS (Mohammed, 2013; Colombara *et al*., 2016). It has also been demonstrated that rotavirus can result in antigenemia and viremia which are associated with severe symptoms of acute gastroenteritis (Silverman *et al*., 2017). Apart from diarrhea, rotaviruses induce vomiting, fever, and malaise. Vomiting leads to dehydration and hampers the effectiveness of treatment interventions, including oral dehydration therapy (Breurec *et al*., 2016).

Rotaviruses are classified according to their antigenicity and basis of sequence differences, with species A being the most rampant in children. Previous studies have reported that species A is geographically distributed, with disease-causing strains highest in developing countries (Bialowas *et al*., 2016). Rotavirus is shed in feces during diarrhea associated with rotavirus. It is transmitted primarily via the fecal-oral route. Additionally, transmission via airborne droplets, though not proven, is used to clarify acquisition of the respective antibody after 3 years (Crawford *et al*., 2017). In recent times, published research has demonstrate that introduction of vaccination has led to a reduction in rotavirus disease (Tate and Parashar, 2014).

2.2.3 Astrovirus

Astroviruses, the only genus in the family of *astroviridae*, have been observed to cause enteritis and diarrhea in humans (Moser and Schultz-Cherry, 2008). The RNA viruses have been described as the leading agents of infantile viral gastroenteritis in the world, infecting children under 2 years old (Koci *et al*., 2003). According to Moser and Schultz-Cherry (2008), in most cases, the virus is the second rotavirus to be detected in young children.

Notably, the infections are identified in nearly 2% of asymptomatic people since their infections are mild in humans. The viruses were first detected in the stools of children with diarrhea. However, host-specific agents have also been associated with various avian and mammalian species. Infection with an astrovirus is clinically presented as gastroenteritis. Patients with astrovirus-infected diarrhea present symptoms including

nausea, headache, malaise, vomiting, fever, abdominal discomfort, and anxiety (Shen *et al*., 2016).

Astroviruses are spread via fecal-oral routes and contaminated meals and water. They are extremely stable in harsh environmental conditions. Moser and Schultz-Cherry (2008) reported that they are resistant to inactivation by some detergents, bleach, alcohols, heat, and ultra violet treatment. Additionally, they have the ability to survive in tap and marine water for up to 3 months, with colder temperatures increasing their survival potential.

2.2.4 Norovirus

Noroviruses, RNA viruses, are a diverse group belonging to the family *Caliciviridaei* (Wiegering *et al*., 2011). It has been described as causing gastroenteritis, with the highest incidence rates among children. The viruses are classified into genogroups ranging from I to VII. Primarily, it has been observed that GI, GII, and GIV are agents of human diseases (Siebenga *et al*., 2009).

Gastroenteritis caused by noroviruses presents in the form of acute vomiting and diarrhea. The latter is accompanied by fever, nausea, abdominal cramps, and non-bloody, watery stool. Norovirus is contagious and the principal route of spread is through fecal-oral means. Other routes of transmission include contaminated environments. Previous studies report that norovirus is the chief cause of outbreaks and sporadic cases of foodborne disease (Glass *et al*., 2009).

2.2.5 Bacterial Diarrhea

Bacterial infection is considered the leading cause of diarrhea. Research done by Shen *et al*. (2016) demonstrated that bacterial infection was dominant at 32%, with *Salmonella sp.* and *C. jejuni* being the leading causes of bacterial gastroenteritis. Moreover, diarrhea caused by bacteria has been reported to be associated with severe symptoms.

Bacteria cause diarrhea via various mechanisms, including mucosal invasion, toxin production, and adherence. Attachment of the pathogens to the epithelium induces the production of toxins, which increase adenosine monophosphate production and eventually compromise the functions of enterocytes (Zhou *et al*., 2018; Florez *et al*., 2020). The induction of cytokines may also be provoked by the toxins, resulting in the induction of prostaglandins, thereby interfering with intraluminal water balance. These mechanisms comprise the functions of the small intestine, including the absorption of fluids, which leads to watery stools (Troeger *et al*., 2017; Fine and Schiller, 2022).

The size of the bacteria is an important element in causing the pathology. For example, in *E. coli* and *Shigella*, a minimum of 10 to 100 bacteria can lead to infection (Gasparinho *et al*., 2015). On the other hand, 100-1,000,000 *V. cholera* bacteria result in infection (Fine and Schiller, 2022). Moreover, it has been reported that each bacterium is associated with varying symptoms and causes. For example, salmonella infection presents as feverish diseases with rose spots, abdominal discomfort, bradycardia, and leucopenia, among others. It is associated with milk, poultry, and eggs (Shen *et al*., 2016; Sattar and Singh, 2022).

2.2.6 Non-infectious Diarrhea

Non-infectious diarrhea is commonly caused by drugs, toxins, food additives, medications, irritable bowel disorder, malabsorption, and inflammatory bowel syndrome (Khan *et al*., 2023). Diarrhea has been reported to occur due to the intake of some drugs such as Digoxin which is usually prescribed for the treatment of chronic heart disorders. Antibiotics are prescribed for the treatment of various ailments including diarrhea, but often these same agents can induce diarrhea (Akram *et al*., 2020).

Antibiotics alter the microbial balance commonly resulting in antibiotic-associated diarrhea (AAD). It is defined as diarrhea that occurs in the long-term use of antimicrobial drugs leading to dysbiosis of the intestinal flora (Sathiyasekaran *et al*., 2023). With the increasing degree of intestinal dysbiosis, the clinical manifestations of AAD can progress from mild diarrhea to acute and severe disease such as pseudomembranous colitis or toxic megacolon (seen in Clostridium difficile infection) (Zheng *et al*., 2021). The incidence and severity of clinical manifestations of AAD are related to the type of antibiotic, duration of use, patient health status, and the type of pathogen to which the patient is exposed (Yang *et al*., 2023). Diverticulitis, irritable bowel syndrome, and bowel infarction or gangrenous bowel are some common causes of non-infectious diarrhea (Li *et al*., 2021).

2.3 Treatment of diarrheal ailments

2.3.1 Therapies using conventional medications

During diarrhea, infants experience severe dehydration and loss of electrolytes very quickly (Marbaniang, 2020). Implementing prompt and effective management and prevention strategies has the potential to decrease morbidity and mortality associated with diarrhea (Isran and Fatimah, 2023). A variety of drugs with various mechanisms of action are used in the treatment and management of diarrheal diseases. They work to inhibit secretion, rehydrate the buccal cavity, and fight viruses and bacteria that cause diarrhea. As a result, they treat and manage bowel looseness (Li *et al*., 2021). Oral rehydration therapy has also been used to prevent and treat dehydration associated with diarrhea in developing nations (Crawford *et al*., 2017). Oral rehydration solutions induce the absorption of glucose, sodium, and water. The therapy is described as the cornerstone of the treatment of diarrhea in children with repeated episodes of diarrhea and vomiting. In extreme cases intravenous fluids are used in cases of hyperemesis and severe dehydration associated with electrolyte imbalance (Fine and Schiller, 2022).

Probiotics have also been employed in the management of acute diarrhea when administered in sufficient quantities, including *Lactobacillus sp*. and *Saccharomyces boulardii*. The probiotics have been shown to reduce diarrhea in 2 days (Cremon *et al*., 2018). Use of probiotics alone or probiotics combined with conventional western medical treatment has not only effectively prevented the incidence of AAD, but also improved the overall efficiency and clinical cure rate, shortened the duration of diarrhea, the mean frequency of diarrhea, the average hospitalization time and antidiarrheal time.

Additionally, the incidence of adverse effects is low revealing the safety of probiotics (Yang *et al*., 2023). Several mechanisms have been postulated, including reduction of pro-inflammatory cytokines, induction of enterocyte migration and/or proliferation, and modulation of antigen-presenting cells and T cells (Crawford *et al.*, 2017; Sattar and Singh, 2022). Crawford *et al*. (2017) point out that some probiotic strains may be beneficial, while others may not.

Dietary management has also been considered an effective therapy to address acute diarrhea in children. This includes continuous breastfeeding for toddlers. Additionally, food withdrawal is also encouraged for about four to six hours after starting rehydration (Crawford *et al.*, 2017). Zinc supplementation has been shown to reduce the severity and duration of diarrhea. As a micronutrient, it is required for numerous metabolic processes in the body. Zinc primarily reduces episodes related to diarrhea and their occurrence for about 3 months, according to previous studies (Lazzerini and Wanzira, 2016). Despite the unclear mode of action of zinc, animal studies have shown that it is endowed with antisecretory and anti-inflammatory effects (Crawford *et al*., 2017).

Conventional drugs used in the treatment of diarrhea include loperamide and racecadotril. As a synthetic opiate agonist, loperamide inhibits secretion and causes a reduction in peristaltic activity, resulting in decrease in the loss of electrolytes and fluids and increase in the consistency of stool achieved via prostaglandin synthesis inhibition (Kirk *et al*., 2017). Notably, loperamide is not administered to infants, that is, children under the age of two. Its use in children raises serious concerns due to several adverse effects reported,

including abdominal distension, cardiac adverse reactions, and fatalities (Iijima *et al*., 2017; Florez *et al*., 2020).

As a drug inhibitor, racecadotril inhibits secretion, hence inhibiting enkephalinase selectively (Gordon and Akobeng, 2016; Eberlin *et al*., 2018). On the other hand, smectite, a medicinal clay, is used to reduce stool output, with evidence suggesting that it reduces the duration of diarrhea by about 24 hours (Das *et al*., 2015).

Intestinal adsorbents such as kaolin, activated charcoal, attapulgite, bismuth subsalicylate and polycarbophil have been incorporated in the treatment of diarrhea. They primarily work by physically binding to the harmful substances in the intestines, preventing their absorption and facilitating their excretion from the body (Riddle *et al*., 2016). They can also help to soothe intestinal inflammation and discomfort associated with diarrhea (Sokic-Milutivovic *et al*., 2021). Kaolin for example, is a natural clay mineral that binds to toxins and bacteria hence reduce their absorption in the intestines. Bismuth subsalicylate on the other hand forms a protective coating on the intestinal lining and has antimicrobial properties, reducing diarrhea and its associated symptoms (Brum *et al*., 2020). These clinical options, however, are not without drawbacks.

Serious invasive diarrhea or complications from diarrhea episodes may necessitate the use of antimicrobial drugs (Iijima *et al*., 2017). Infections such as salmonellosis, shigellosis, and cholera are treated with antibiotics (Bauza *et al*., 2017). Quinolones and macrolides are some of the best options for empirical treatment of microorganisms

associated with diarrhea, but ciprofloxacin should be used with caution because a patient is susceptible to arthropathy (Tian *et al*., 2016).

2.3.2 Alternative therapies

In many nations around the world, it is customary practice to cure diarrhea with medicinal plants. These customs practices were empirically transmitted from one generation to the next without knowledge of the potential mechanisms, safety, or efficacy of herbal remedies (Ahmadu *et al*., 2007; Degu *et al*., 2020). Herbal medicine is essential in the development of powerful restorative agents, as the majority of individuals in underdeveloped nations depend on them for necessary preventive medicine, including the cure for diarrhea (Tadesse *et al*., 2017; Kifle *et al*., 2021). More than 70% of Kenyans rely on herbal plants as a first line of defense, while more than 90% use herbs from time to time to treat a variety of ailments (Mbuni *et al*., 2020). In isolated rural areas in LMICs, plant species with therapeutic value are commonly used since they are affordable, are believed to have few negative effects, and are accessible (Ahmad *et al*., 2021).

A variety of secondary metabolites found in herbs have been linked to antidiarrheal properties (Musila *et al*., 2017). *P. barbatus'* antidiarrheal activity is due to the presence of alkaloids (Agidew, 2022). Previous research has shown that aqueous and organic extracts of *P. barbatus* have antibacterial effects, which are associated with the presence of phenolics, which are known to interfere with the bacteria's various cellular activities (Rodrigues *et al*., 2016; Borges *et al*., 2020). *Plectranthus* contains various species with bioactive compounds of medical significance, laying the groundwork for natural product

research. A large number of diseases managed using this species demonstrates the medicinal wealth of this genus, providing limitless potential for drug development (Cordeiro *et al*., 2022).

2.4 Plant under investigation

Plectranthus barbatus (Andrews) is a branched plant with curative value that is long-lasting. *Plectranthus barbatus* belongs to the family Lamiaceae and the genus plectranthus. This genus is native to subtropical regions of Africa, Asia, and India. It thrives in warm climates with well-drained soil and prefers partial shade to full sun. It can tolerate various soil types but grows best in fertile, loamy soil. In its natural habitat, it can be found growing on hillsides, in rocky areas, and along stream banks. It can withstand short periods of drought once established (Lukhoba *et al*., 2006, Alasbahi and Melzig, 2010, Kigen *et al*., 2016).It contains approximately 300 species, 45 of which are used traditionally on the African continent (Musila *et al*., 2017). It has 1-2-foot-tall stems and inflorescent flowers. Research by Alasbahi and Melzig (2010) and Nguta (2019) showed that its roots are thick, succulent, and fasciculate, containing forskolin, which has adenylyl cyclase activation properties and is attributed to *P. barbatus's* wide range of pharmacological properties and ethnomedicinal uses.

Plectranthus barbatus is used to treat spasms caused by infections of the gastrointestinal tract (Kapewangolo *et al*., 2013). It is utilized to manage stomach aches and as a purgative, as well as to treat ringworms and wounds, shrink swelling on bruises, and bathe measles-infected children in Kenya (Lukhoba *et al*., 2006). Numerous studies have

demonstrated that the entire plant has antiviral, antioxidant, antifungal, antibacterial, and antiprotozoal activity (Porfírio *et al*., 2010; Cordeiro *et al*., 2022).

Plate 2.1: A picture of *Plectranthus barbatus* (Andrews) (Source author)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Sample Collection

With the assistance of a local herbalist, fresh leaves of *P. barbatus* were collected from Seme Sub-County in Kisumu County, Kenya. During the collection process, ethnobotanical information was recorded, and the samples transported to Kenyatta University laboratories in the department of Biochemistry, Microbiology, and Biotechnology, where an accredited taxonomist from Kenyatta University authenticated the specimen. Kenyatta University's Department of Plant Sciences' herbarium received the sample. For future reference, a specimen voucher number of ECI/2021 was issued.

3.2 Plant Material, Preparation and Extraction

Materials were washed under running water before being dried four weeks at ambient temperature and under shade. Pulverization of the dried plant samples was performed using a Gibson electrical miller. In 1000mL of double distilled water, 200g of fine powder plant materials were soaked. For 120 minutes, the extraction was performed in a water bath at 60°C for 120 minutes, followed by decantation, and re-soaking for a further 2 hours. A muslin cloth and Whatman filter paper No. 1 were then used to sieve the solution, and resulting supernatant freeze dried, (using a freeze drier) and kept at 4°C awaiting use. Lyophilization was chosen for the extraction of aqueous leaf extract of *P. barbatus* due to its ability to preserve the integrity and activity of the delicate active compounds, retain the aroma and flavor of the extract by minimizing exposure to high temperatures, and minimize solvent residues making it a suitable method for obtaining

high quality extracts for various applications. Percentage yield of the extract was established using a formula previously described by Senguttuvan *et al*. (2014);

$$\text{Percentage Yield} = \frac{\text{Dry weight of Extract}}{\text{Dry weight of Plant Material}} \times 100$$

3.3 Determination of In vivo Antidiarrheal Activity

3.3.1 Laboratory Animals

Swiss Albino mice weighing approximately 25g and aged 6 weeks were used in this study. The animals were bred at Kenyatta University's Department of Microbiology, Biochemistry, and Biotechnology's Animal Breeding and Experimentation Facility. They were housed in polypropylene cages with access to food and water *ad libitum* in a twelve-hour dark and light cycles. They were given one week to acclimate before the experiment began. In the selection and care of laboratory animals, the 2018 National Committee for Research Ethics in Science and Technology (NENT) guidelines were followed (NENT, 2018).

3.3.2 Grouping and dosing

Mice of either sex were randomly assigned into 6 groups of 3 extracts treated and 3 control groups with 6 mice per group. All groups received their respective treatments using an oral gavage. The first group was assigned as the normal control and received distilled water, the second group was assigned as negative control and received 10ml/kgbw of distilled water, the fourth, fifth, and sixth groups received 100, 200, and 400 mg/kgbw of extract, respectively, whereas the third group was assigned as positive control and received 3 mg/kgbw of loperamide.

The doses of aqueous extract of *P. barbatus* were determined as 100, 200, and 400 mg/kgbw based on acute toxicity test. That is, 1/ 10th of 2000 mg/kgbw of the dose used in acute toxicity test is used to determine the middle dose, and one-half of and 2 times the middle dose was used to determine the lowest and highest doses, respectively. The mice were euthanized using ketamine (50mg/mL) and later incinerated.

3.3.3 Induction of Diarrhea

An aliquot of 0.5mL castor oil (Faholo Chemical LTD, Kenya) was orally administered to the mice to induce diarrhea. Diarrhea was determined to have been induced in an animal by the animal passing watery stool. Castor oil is a potent laxative and is used to induce diarrhea in mice as a standardized method that provides a consistent and reproducible model for testing the efficacy of potential antidiarrheal agents in preclinical studies.

3.3.4 Determination of Antidiarrheal Activity

Thirty-six mice of either sex were chosen at random and grouped into 6 groups, each having 6 animals. Normal control (Group I) was administered dH_2O (vehicle). Group II (negative control) animals obtained distilled water and induced with diarrhea one hour later. The positive control (Group III) animals were given the Loperamide (3mg/kgbw) (Imodium ® Janssen-Cilag, Val-de-Reuil, France) and then induced with diarrhea one hour later. The test groups, groups IV, V and VI, received orally, *P. barbatus* extract at varying doses of 100, 200 and 400 mg/kgbw, respectively, diarrhea was then induced one hour later. Table 3.1 shows the summarized design.

Table 3.1: Experimental Protocol for Determination of Antidiarrheal Activity

Animal Group	Treatment
I (Normal Control)	Distilled water
II (Negative Control)	Distilled water and induced with diarrhea one hour later
III (Positive Control)	Loperamide (3mg/kgbw) and induced with diarrhea one hour later
IV	*P. barbatus* extract(100mg/kgbw) and induced with diarrhea one hour later
V	*P. barbatus* extract(200mg/kgbw) and induced with diarrhea one hour later
VI	*P. barbatus* extract(400mg/kgbw) and induced with diarrhea one hour later

The mice were individually housed in polypropylene cages lined with permeable papers on the floor. These white papers were changed hourly until the fourth hour. Within the observations period, the start of defecation, stool regularity and the mass of the paper for every animal were recorded. The mice were euthanized using ketamine(50mg/mL) and later desiccated in an incinerator. After a 24-hour storage period, each adsorbent paper was air-dried. The difference in weight of the white paper and that which contained dried stool was obtained. (Abdela, 2019; Ayalew *et al*., 2022). Inhibition of diarrheal and fecal output weights were computed by a formulae expressed by Teferi *et al*. (2019);

$$\%\ \text{inhibition} = \frac{\text{Average number of WFC} - \text{Average number of WFT}}{\text{Average number of WFC}} \times 100$$

Where;

WFC represents wet stool in the control group

WFT represents wet stool in the experimental group

$$\text{Wet fecal output (\%)} = \frac{\text{Mean weight of wet feces of each group}}{\text{Mean weight of wet feces of control}} \times 100$$

$$\text{\% of total fecal output} = \frac{\text{Mean weight of total feces of each group}}{\text{Mean weight of total feces of control}} \times 100$$

3.3.5 Determination of Gastrointestinal Motility

To ascertain the effects of the extract on gastrointestinal tract, thirty-six animals were randomly sorted into 6 groups (6 mice each); and fasted for eighteen hours, though water was made available. Group I animals were given dH_2O orally (vehicle) only. Group II animals were given dH_2O orally (10mL/kgbw), and induced with diarrhea after one hour and orally given 1mL of 5% activated charcoal suspension after one hour. Group III (Positive control) animals were given the reference drug, Loperamide, (3mg/kgbw). One hour later, diarrhea was induced, and 1mL activated charcoal suspension was administered orally. Groups IV- VI animals were orally given 100-400 mg/kgbw doses of the extract respectively, followed by induction of diarrhea after an hour. The animals were then orally administered with 1mL of 5% activated charcoal suspension (marker) (ULAX®, West-Coast Pharmaceutical Works Ltd, Gujarat) after one hour. Table 3.2 summarizes the design.

Table 3.2: Experimental Design for Determination of Gastrointestinal Motility

Animal Group	Treatment
I (Normal Control)	Distilled water and 1mL activated charcoal suspension
II (Negative Control)	Distilled water (10mL/kgbw) and induced with diarrhea one hour later after which 1mL of activated charcoal suspension was orally administered one hour later
III (Positive Control)	Loperamide (3mg/kgbw) and induced with diarrhea one hour later after which 1mL of activated charcoal suspension was orally administered one hour later
IV	*P. barbatus* extract (100mg/kgbw) and induced with diarrhea one hour later after which 1mL of activated charcoal suspension was orally administered one hour later
V	*P. barbatus* extract (200mg/kgbw) and induced with diarrhea one hour later after which 1mL of activated charcoal suspension was orally administered one hour later
VI	*P. barbatus* extract (400mg/kgbw) and induced with diarrhea one hour later after which 1mL of activated charcoal suspension was orally administered one hour later

The mice were euthanized using ketamine (50mg/mL) after half one hour, dissected whereby the small bowel, was cut up from pylorus to cecum and the distance covered by the charcoal measured (Teferi *et al*., 2019).The formula described by Abdela (2019) was used in calculating percentage inhibition;

$$\text{Peristaltic index} = \frac{\text{Distance travelled by charcoal meal}}{\text{The whole length of small intestine}} \times 100$$

$$\%\ \text{inhibition of Gastrointestinal Motility} = \frac{(Dc - Dt)}{Dc} \times 100$$

Where;

Dc represents the average distance covered by the marker in negative control group.

Dt represents the average distance covered by the marker in the experimental group.

3.3.6 Determination of Enteropooling Activity

Thirty-six animals were randomly sorted into 6 groups (6 mice each); and fasted for eighteen hours, though they had free access to H_2O. Group I received dH_2O orally. Mice in Group II (Negative control) received distilled water, followed by induction of diarrhea one hour later. Group III (Positive control) animals were given the reference drug, Loperamide, (3mg/kgbw) and induced with diarrhea, after one hour. Mice in the test groups received the extract orally at different concentrations and then induced with diarrhea one hour later. This design is summarized in table 3.3.

Table 3.3: Experimental Design for Determination of Enteropooling Activity

Animal Group	Treatment
I (Normal Control)	Distilled water
II (Negative Control)	Distilled water(10mL/kgbw) and induced with diarrhea one hour later
III (Positive Control)	Loperamide(3mg/kgbw) and induced with diarrhea one hour later
IV	*P. barbatus* extract(100mg/kgbw) and induced with diarrhea one hour later
V	*P. barbatus* extract(200mg/kgbw) and induced with diarrhea one hour later
VI	*P. barbatus* extract(400mg/kgbw) and induced with diarrhea one hour later

The animals were euthanized using ketamine (50mg/mL) after one hour, and dissected, the ileum taken out and weighed. The intestines ‘contents were then unloaded into test tubes, and the volume taken. The mass of the ileum after milking the contents was recorded. Using the formula depicted by Tadesse *et al.* (2017), the difference in weight of the intestines before and after milking their contents was determined in order to calculate the percentage inhibition.

$$\% \text{ inhibition} = \frac{(\text{MVICC} - \text{MVICT})}{\text{MVICC}} \times 100$$

Where;

MVICC is the mean capacity of intestinal contents in group II
MVICT is the average capacity of intestinal content in III, IV, V and VI groups

3.3.7 Antidiarrheal Index (ADI) Calculation

ADI of the extract was carried out using the formula by Ferede *et al*. (2021) using a combination of the variables obtained in the previous sections (anti-diarrheal activity, gastrointestinal motility, and enteropooling activity).

$$\text{Anti-diarrheal index} = \sqrt{\text{Dfreq} \times \text{Gmeq} \times \text{Pfreq}}$$

Where;

Pfreq- is the number of wet stool reduction (in group II)
Dfreq - delay in the onset of defecation (in group II)
Gmeq –is the reduction of gut meal travel (in group II)

$$\text{Dfreq} = \frac{\text{Mean onset in the test group} - \text{Mean onset of diarrhea in control group}}{\text{Mean onset of diarrhea in the controlgroup}} \times 100$$

3.4 Determination of Antibacterial Activity

3.4.1 Preparation of the Extract Solution

A mass of 0.2g of the *P. barbatus* extract was dissolved in 100µL of 5% dimethyl sulfoxide. Distilled water was added to top up the volume to 1000µL to make a 0.2 g/mL, w/v stock solution, which was then refrigerated. The stock solution (200mg/mL) was serially diluted to achieve a concentration of 20mg/mL for antimicrobial sensitivity testing.

3.4.2 Mueller Hinton Agar (MHA) Preparation

In 1l of distilled water, 38.0g of MHA was dispersed. To completely dissolve the agar, it was heated to 121°C. The medium was then sterilized in an autoclave set to 15lbs (249.8^{o}F) pressure for quarter an hour, cooled and dispersed into sterile petri dishes.

3.4.3 Microbial strains

The microbial standard strains were sourced from the Kenyatta University Microbiology Laboratory. They included *Escherichia coli* ATCC 25921, *Bacillus subtilis* ATCC 21333, *Salmonella typhi* ATCC 1410, *Staphylococcus aureus* ATCC 25823, *Pseudomonas aeruginosa* ATCC 1508 and *Shigella flexneri* ATCC 28125.

3.4.4 Agar Disc Diffusion (DD) Method

The bacteria were grown on MHA for 18 hours at 37°C according to Clinical and Laboratory Standards Institute protocol (CLSI, 2020). Before inoculating the plates, the bacterial suspension was done in saline conditions and the turbidity was modified to 0.5 on the Mac Farland scale. Six mm diameter Whatman diffusion discs were soaked in the extract. Each MHA plate received five discs, three of which were soaked with the extract at different concentrations, one in DMSO, and the other soaked in ciprofloxacin, the reference drug.

Cotton swabs were used to evenly inoculate the standardized bacterial suspensions in the agar plates, left to stand for 18h at 37°C. This was followed measuring the widths of the inhibition zones. This procedure was performed in triplicate. Antibacterial potential of the studied extract was determined as follows: values less than 7mm were considered

inactive, values between 8-11mm were considered active, and zones greater than 11mm were considered very active (Ikram *et al*., 2021).

3.4.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was evaluated using the Nikolic *et al*. (2014) protocol. In sterile 96-well plates containing MH Broth, *P. barbatus* extract was double-diluted to varying concentrations (200 mg/mL to 1.5625mg/mL). This was accomplished by mixing 100mL of extracts with 100mL of Mueller Hinton Broth. Following dilution, each well received 20µL of each test bacteria, and turbidity was modified to the 0.5 McFarland scale. The plates were then left to stand for 24 hours at 37°C. To serve as an indicator, a 50µL solution of 1% resazurin was topped up to each well and re-incubated. MIC was describe as the least concentration at which resazurin's color changed from blue to pink (Nikolić *et al*., 2014).

To determine MBC, a sterile cotton swab was used to disperse an aliquot of 10µL of the samples from every well with concentrations below and above the MIC of the antibacterial agent under test onto the MHA plate. The plates were then left to stand for a day at 37°C. MBC was defined after 24 hours of incubation at which no growth was detected on the plates. Bacteriostatic effects were determined in terms of the growth of bacteria on MHA plates, whereas bactericidal effects were computed in terms of bacterial growth absence on MHA plates.

3.5 Determination of Quantitative Phytochemical Profile

This was done using Liquid Chromatography-Mass Spectrometry technique as follows; one gram of the extract was weighed with Sartorius analytical balance and reconstituted using 1mL of LC-MS grade water and subjected to centrifugation at 15,000 revolutions per minute for 300 secs. Using a glass pipette, the reconstituted extract was transferred into small clean 1.5mL vials. Centrifugation was undertaken to remove all particulates from the extracts, thus preventing the possibility of column blockages. From the extract, stock solution was prepared of 10mg/10mL and 100µL of the stock solution was placed into an HPLC vial (Pyrex) and 20 µL was injected into LC-MS –ESI vial.

The analysis was done using C18 column (Agilent 6475 model, Torrance, CA). The commencing temperature was 45^{o}C. Mobile phase was composed of Water and Acetonitrile with a flow rate of 0.4mL/min. The systematic change in solvent composition over time during the chromatographic separation started at 5%, whereas the final gradient program was 100% for 25 min. The mass spectrometer parameter included dry gas flow rate of 400L/h with desolvation temperature at 380^{o}C, and the spectra was achieved in positive mode with a range of 100 to 1500 m/z. Analyte peaks were all quantified using the same method, with perfect spectrum-intensity vs chemical formula based on the existing National Institute of Standards and Technology (NIST) chemical library.

3.6 Determination of Acute Oral Toxicity

The Organization for Economic Cooperation and Development (OECD) guidelines 425 were followed when testing the acute oral toxicity of *P. barbatus* (OECD, 2008).

2000mg/kgbw of the extract was administered orally to 5 healthy female Swiss albino mice in the test group while the control group received the vehicle (distilled water) to compare against the test group. One mouse was subjected to fasting for 4h with provision of water before receiving the extract. It was then fasted for a further 2 hours while strict observations were made for the basic signs of toxicity for 24 hours. If no death was observed within the set time, the other four mice were subjected to the same procedure and closely monitored for four hours at half-hour intervals for signs of toxicity as described by Ferede *et al*. (2021). The animals were then placed in cages for observation for 14 days, with their weights taken on days 7 and 14. After 14 days of treatment, the mice were euthanized with ketamine (50mg/mL) for organ harvesting. They were then dissected, and the liver, heart, spleen, kidney, and brain were removed and weighed. Organ weights were normalized to the body weight of the respective animal to calculate relative organ weights. This is typically done by dividing the absolute organ weight of the animal and multiplying by 100 to express the result as a percentage.

3.7 Ethical approval

Approval for the study was obtained from the Kenyatta University Animal Care and Use Committee (approval number: PKUA/2707/11831). Additionally, license number 402104 was granted from the National Commission for Science, Technology, and Innovation Research (NACOSTI).

3.8 Data Management and Statistical Analysis.

The data was inventoried using a Microsoft Excel spreadsheet and then analyzed using Minitab software version 19. The data were descriptively analyzed and expressed as Means standard error of the mean (SEM). To determine statistical significance, the

inferential statistic, One-Way Analysis of Variance (ANOVA), and Tukey's post hoc test were performed for pairwise comparison of means. Unpaired T-test was done to compare organ weights, normal and treated. The significance level was set at 95%. Tables and graphs were used to present the data.

CHAPTER FOUR

RESULTS

4.1 Percentage Extract Yield

The percentage yield of the aqueous leaf extract of *P. barbatus* was 6 %. The extract was a dark green solid residue in appearance.

4.2 Effects of Aqueous Leaf Extract of *P. barbatus* on Castor Oil-Induced Diarrhea in Mice

The current study evaluated the effects of *P. barbatus* leaf extract in mice induced with diarrhea. As shown in table 4.1 mice treated with different dosage levels of *P. barbatus* extracts significantly prolonged the commencement of diarrhea in a dose dependent trend ($p< 0.05$). The longest delay in initiation of diarrhea was observed upon administration of the extract at 400mg/kgbw (139.33±1.96), which was significantly similar to the activity of the loperamide ($p>0.05$; Table 4.1). On the contrary, the mice in the negative control group had a significantly lower latency time of diarrheal onset as compared with that receiving the test sample ($p < 0.05$; Table 4.1).

From the study, *P. barbatus* extracts showed potent efficacy on the incidences of defecation in mice induced with diarrhea. Further, it was observed that there was a significant decline in the frequency of defecation in mice compared to the negative control group (Table 4.1; $p<0.05$). The highest decrease in wet fecal output was observed at the dose of 400mg/kgbw (27.451±0.78%), followed by the doses of 200 (32.52±1.28%) and 100mg/kgbw (46.41±0.84). In addition, the total fecal output in animals treated with aqueous leaf extract of *P. barbatus* was significantly lower compared to that in the untreated group ($p<0.05$: Table 4.1). Nevertheless, the potency of

the extract dose of 200mg/kgbw on the wet and total fecal output were similar to those of loperamide ($p>0.05$; Figure 4.1). The effect of the *P. barbatus* extract dose of 100mg/kgbw on the total fecal weight was statistically lower than other dosages ($p<0.05$; Table 4.1). The study revealed further that *P. barbatus* extract exhibited diarrheal inhibition activity. The percentage inhibition was dose dependent with extract doses of 100, 200, and 400mg/kgbw showing percentage inhibition of 49.98±1.61, 66.12±2.17, and 75.80±2.16% respectively. Furthermore, as shown in table 4.1, there was no significant variation in percentage inhibition of diarrhea at the dose of 200mg/kgbw and the positive control ($p<0.05$).

Table 4.1: Effect of Aqueous Leaf Extract of *P. barbatus* on Castor Oil Induced Diarrhea in Mice

Treatment	Onset of diarrhea (min)	No. of wet feces (%)	Total no. of feces (%)	Wt. of wet feces (g)	Wt. of total feces (g)	Inhibition of defecation (%)
Distilled water	0.00	0.00	0.00	0.00	0.00	100.00
Distilled Water	046.17±1.64^{d}	100.00±0.00^{a}	100.00±0.00^{a}	0.51±0.01^{a}	0.22±0.01^{a}	00.00±0.00^{d}
Loperamide (3mgkgbw)	132.33±2.01^{a}	033.91±1.43^{c}	042.23±1.79^{c}	0.17±0.01^{c}	0.09±0.00^{c}	67.73±2.04^{b}
Extract (100mg/kgbw)	088.50±1.80^{c}	046.41±0.84^{b}	052.65±1.54^{b}	0.24±0.00^{b}	0.12±0.00^{b}	49.98±1.61^{c}
Extract (200mg/kgbw)	111.00±2.30^{b}	032.52±1.28^{c}	044.70±1.46^{c}	0.17±0.01^{c}	0.09±0.00^{c}	66.12±2.17^{b}
Extract (400mg/kgbw)	139.33±1.96^{a}	027.45±0.78^{d}	030.11±1.94^{d}	0.14±0.00^{d}	0.06±0.00^{d}	75.80±2.16^{a}

Values expressed as mean ± SEM (n=6). Means with different superscripts within the columns are significantly different ($p>0.05$) by one way ANOVA and Tukey's post hoc test. Min-minutes, No-number, Wt.-weight

4.3 Effect of Aqueous Leaf Extract of *P. barbatus* on Gastrointestinal Motility in Mice Induced with Diarrhea

Finding from this study revealed that *P. barbatus* extract elicited a reduction in gastrointestinal motility in mice induced with diarrhea. At all doses, it was evident that the extract conferred a significant decrease in transit of charcoal meal in the gastrointestinal compared with the negative control (figure 4.1; $p<0.05$). Moreover, as shown in figure 4.1, it was observed that reduction in intestinal transit of the charcoal meal by the extract dose of 200mg/kgbw and loperamide (positive control) were statistically comparable ($p>0.05$). Further, the dose of 400mg/kgbw prevented the passage of the meal through the bowel and significantly ($p<0.05$) reduced the peristaltic index at 11.46±0.53 in comparison to the negative control (50.46±2.07).

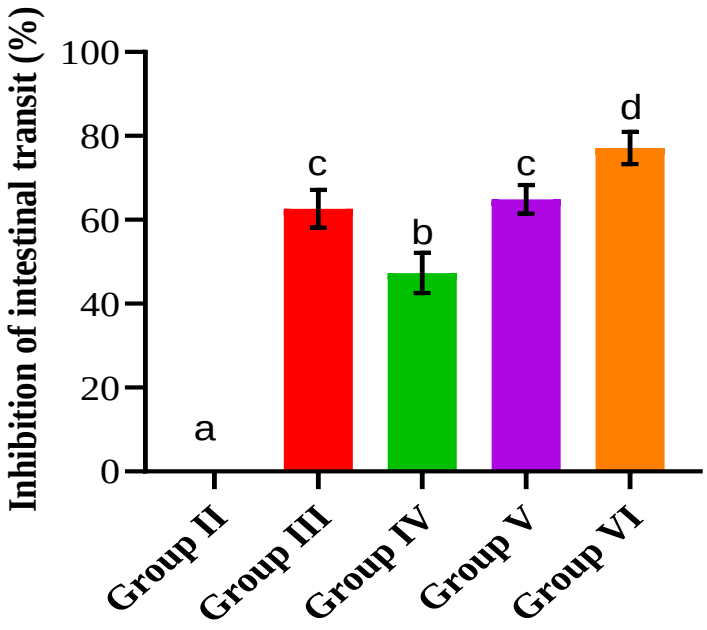

Figure 4.1: Effect of Aqueous Leaf Extract of *P. barbatus* on Inhibition of Gastrointestinal Motility.
Bars with different lowercase letter vary significantly ($p<0.05$) using one-way ANOVA followed by Tukey's multiple comparison.

4.4 Effect of the Aqueous Leaf Extract of *P. barbatus on* Enteropooling in Mice Induced with Diarrhea

The plant extract hindered the buildup of castor oil-induced gastrointestinal fluid. The highest extract dosage (400mg/kgbw) produced the highest percentage reduction However, as depicted in figure 4.2, it was evident that the effects of the extract dose of 200mg/kgbw was significantly similar to that of loperamide ($p>0.05$; Figure 4.2). A reduction in the mean weight and mean volume of the intestinal contents was observed in the experimental groups as compared with that of the negative control group (Appendix II).

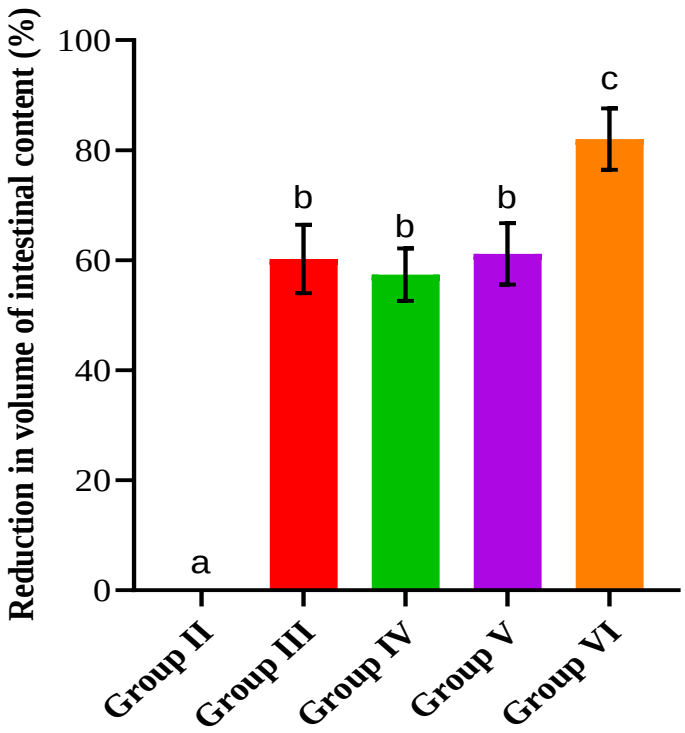

Figure 4.2: Effect of Aqueous Leaf Extract of *P. barbatus* on Enteropooling in Mice Induced with Diarrhea.
Bars with different lowercase letter vary significantly ($p <0.05$) using one-way ANOVA followed by Tukey's multiple comparison.

4.5 Anti-Diarrheal Index (ADI) of *Plectranthus barbatus* leaf extract

Anti-diarrheal effects of *P. barbatus* extract were also quantified in terms of anti-diarrheal index (ADI). *In vivo* ADI parameters included delay in defecation, percentage inhibition of wet fecal, and reduction of ileum contents. The extract demonstrated a remarkable dose dependent increase in the ADI value of aqueous leaf extract of *P. barbatus* with maximum effect being detected at the highest concentration of the extract ($p<0.05$; Figure 4.3). The higher the ADI, reflects how effectual the sample is at exerting antidiarrheal effects.

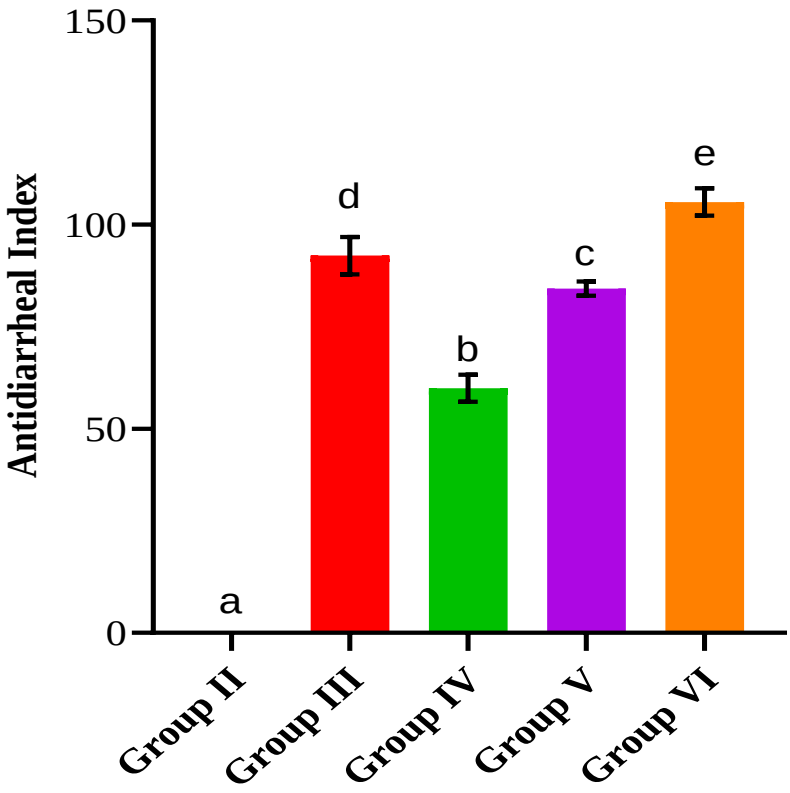

Figure 4.3: In vivo Anti-diarrheal index of aqueous leaf extract of *P. barbatus*
Bars with different lowercase letter vary significantly ($p < 0.05$) using one-way ANOVA followed by Tukey's multiple comparison.

4.6 Antibacterial Activity of Aqueous Leaf Extract of *P. barbatus*

P. barbatus extract exhibited varying level of antibacterial activity on selected bacterial pathogens (Table 4.3). As shown in Picture 4.1, the visible growth inhibitory zones surrounding the paper discs infused with various dilutions of the extract demonstrated this.

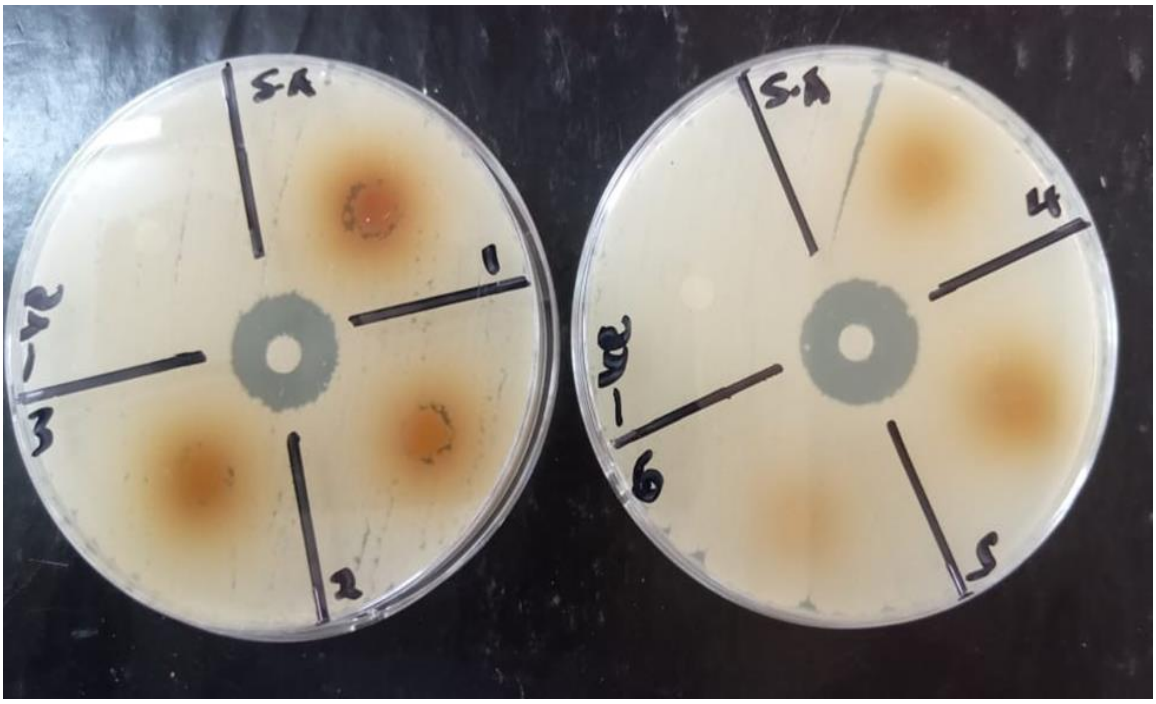

Plate 4.1: Antibacterial sensitivity of *P. barbatus* leaf extract against *Staphylococcus aureus*
1=200, 2=175,3=150,4=125,5=100 and 6=75mg/mL, -ve=DMSO (Dimethyl sulfoxide), middle=positive control (ciprofloxacin).

Table 4.2 shows that the tested extract concentration ranges of 75-150mg/mL showed no discernible zone of inhibition against *E. coli*, *S. typhi*, Shigella, and *S. aureus*. At extract concentrations of 75 and 100mg/mL, a similar observation was made on *B. subtilis*. *Plectranthus barbatus* extract concentrations of 175 and 200mg/mL, nevertheless, exhibited antibacterial properties against the tested bacterial pathogens. *Plectranthus barbatus* leaf extract revealed dose-dependent antibacterial potential against *P.*

aeruginosa. The effect of extract concentration of 150mg/mL, conversely, was statistically similar from the activity of extract dose of 175mg/mL ($p>0.05$; Table 4.2). Ciprofloxacin, the positive control, displayed significantly higher effects on the tested pathogens than *P. barbatus* extract ($p<0.05$; Table 4.2).

Table 4.2: Antibacterial effects of aqueous leaf extract of *P. barbatus*

	Zones of inhibition (mm)					
Treatment	***E. coli***	***S. typhi***	***Shigella***	***S. aureus***	***P. aeruginosa***	***B. subtilis***
DMSO	6.00±0.00^{d}	6.00±0.00^{d}	6.00±0.00^{d}	6.00±0.00^{d}	6.00±0.00^{f}	6.00±0.00^{e}
Ciprofloxacin	16.83±0.44^{a}	17.17±0.44^{a}	20.17±0.44^{a}	22.50±0.29^{a}	23.67±0.33^{a}	15.00±0.58^{a}
Extract (75mg/mL)	6.00±0.00^{d}	6.00±0.00^{d}	6.00±0.00^{d}	6.00±0.00^{d}	7.33±0.33^{f}	6.00±0.00^{e}
Extract (100mg/mL)	6.00±0.00^{d}	6.00±0.00^{d}	6.00±0.00^{d}	6.00±0.00^{d}	9.67±0.33^{e}	6.33±0.33^{e}
Extract (125mg/mL)	6.00±0.00^{d}	6.00±0.00^{d}	6.00±0.00^{d}	6.33±0.33^{d}	11.83±0.17^{d}	8.83±0.44^{d}
Extract (150mg/mL)	6.33±0.33^{d}	6.50±0.29^{d}	6.67±0.33^{d}	11.50±0.50^{c}	14.00±0.58^{c}	10.67±0.33^{c}
Extract (175mg/mL)	11.00±0.57^{c}	11.17±0.44^{c}	12.67±0.33^{c}	12.67±0.33^{c}	15.83±0.44^{bc}	13.00±0.57^{b}
Extract (200mg/mL)	13.17±0.60^{b}	14.17±0.73^{b}	14.83±0.73^{b}	14.67±0.33^{b}	17.17±0.73^{b}	15.83±0.17^{a}

Results are mean ± SEM of triplicate measures. Values with different lowercase superscript letter along the column are statistically similar ($p<0.05$) by one way ANOVA followed by Tukey's multiple comparison.
Minimum Zone of Inhibition values< 7 indicate no activity, between 8-11 indicate activity and >12 highly active.

4.7 Minimum Inhibitory and Bactericidal Concentrations of Aqueous Leaf Extract of *P. barbatus*

Microbial sensitivity to *P. barbatus* extract represented by mean MIC values ranged from 75 to 175mg/mL (Table 4.3). *P. barbatus* extracts inhibited *S. aureus*, *E. coli*, *S. typhi*, and Shigella in statistically similar ways ($p>0.05$). However, the extract inhibited *P. aeruginosa* and *B. subtills* significantly ($p<0.05$; Table 4.2).

Generally, *P. barbatus* extract displayed varying MBC values as shown in Table 4.3. Bactericidal activities against *S. typhi*, *Shigella*, *S. aureus*, and *B. subtilis* were comparable ($p>0.05$). Furthermore, as shown in Table 4.3, a significantly lower dose of *P. barbatus* extract was required to induce bactericidal effects against *P. aeruginosa* as compared to the other microbes ($p<0.05$).

Table 4.3: MIC and MBC of Aqueous Leaf Extract of *P. barbatus*

Bacteria strain	MIC (mg/mL)	MBC (mg/mL)
E. coli	175.00 ± 0.00^{a}	>200
S. typhi	166.67 ± 8.33^{a}	200.00 ± 0.00^{a}
Shigella	158.33 ± 8.33^{a}	191.67 ± 8.33^{a}
S. aureus	141.67 ± 8.33^{a}	183.33 ± 8.33^{a}
P. aeruginosa	75.00 ± 0.00^{c}	100.00 ± 0.00^{b}
B. subtilis	108.33 ± 8.33^{b}	183.33 ± 8.33^{a}

Values expressed as mean ± SEM based on seven concentrations, three replicates (n=3). Means with different lowercase superscript letter along the column are significantly different by one-way ANOVA ($p<0.05$) followed by Tukey's multiple comparison. MIC-Minimum inhibitory concentration, MBC-Minimum bactericidal concentration.

4.8 Quantitative Phytochemical Profile of Aqueous Leaf Extract of *Plectranthus barbatus*

In this study, LC-MS analysis of the aqueous *P. barbatus* leaf extract identified 23 phytocompounds viz flavonoids, phenolics, and diterpenes (Table 4.4). The most abundant flavonoid was Methoxy-kaempferol-3-glucuronide with a concentration of 1.34µg/mg, followed by Luteolin-O-glucuronide (0.71µg/mg), Methoxy-kaempferol-3-glucuronide (0.51 µg/mg), and 4′-Methoxy-quercetin-3-O-glucuronide (0.31µg/mg). Vicenin-2 was the least abundant compound with a concentration of 0.16 µg/mg.

The most abundant phenolic was Rosmarinic acid (0.45µg/mg), followed by gallic acid with concentration of 0.36 µg/mg (Table 4.3). Several diterpenes were detected including 3,6,12-Trihydroxy-2-acetyl-8,12-abietadien-7,11,14-trione (1.70µg/mg), 3,6,11,12,14-Pentahydroxy-2-acetyl-5,7,11,13-abietatetraen-7-one (0.48µg/mg), 3,6,7,12,16-Pentahydroxy-2-acetyl-5,8,12-abietatrien-11,14-dione (0.43µg/mg) and Forskolin (0.37µg/mg). The LC-MS spectrum of *P. barbatus* extract revealed detected phytocompounds with varying retention time is depicted in Figure 4.4.

Table 4.4: Quantitative Phytochemical Profile of Aqueous Leaf Extract of *P. barbatus*

Class of compound	Compound Name	Molecular Formula	Retention Time (min)	Conc. (µg/mg)
Flavonoids	Vicenin-2	$C_{27}H_{30}O_{15}$	25.69	0.16
	4′-Methoxy-quercetin-3-*O*-glucuronide	$C_{22}H_{20}0_{13}$	26.74	0.31
	Methoxy-kaempferol-3-glucuronide	$C_{22}H_{20}O_{12}$	28.74	1.34
	Methoxy-kaempferol-7-glucuronide	$C_{22}H_{20}O_{13}$	28.84	0.51
	3′,4′-Dimethoxy-luteolin-7-glucuronide	$C_{23}H_{22}O_{12}$	29.15	0.39
	Luteolin-O-glucuronide	$C_{21}H_{18}O_{12}$	29.67	0.71
Phenolics	Rosmarinic acid	$C_{18}H_{16}0_{8}$	26.54	0.45
	Gallic acid	$C_6H_2(OH)_3CO_2H$	5.47	0.36
Terpenoids	3,6,7,12,16-Pentahydroxy-2-acetyl-5,8,12-abietatrien-11,14-dione	$C_{22}H_{30}O_9$	25.29	0.43
	3,6,11,12,14-Pentahydroxy-2-acetyl-5,7,11,13-abietatetraen-7-one	$C_{24}H_{32}O_{10}$	28.34	0.48
	3,6,12-Trihydroxy-2-acetyl-8,12-abietadien-7,11,14-trione	$C_{22}H_{28}O_8$	29.81	1.7
	Forskolin	$C_{39}H_{64}O_{13}$	25.80	0.37

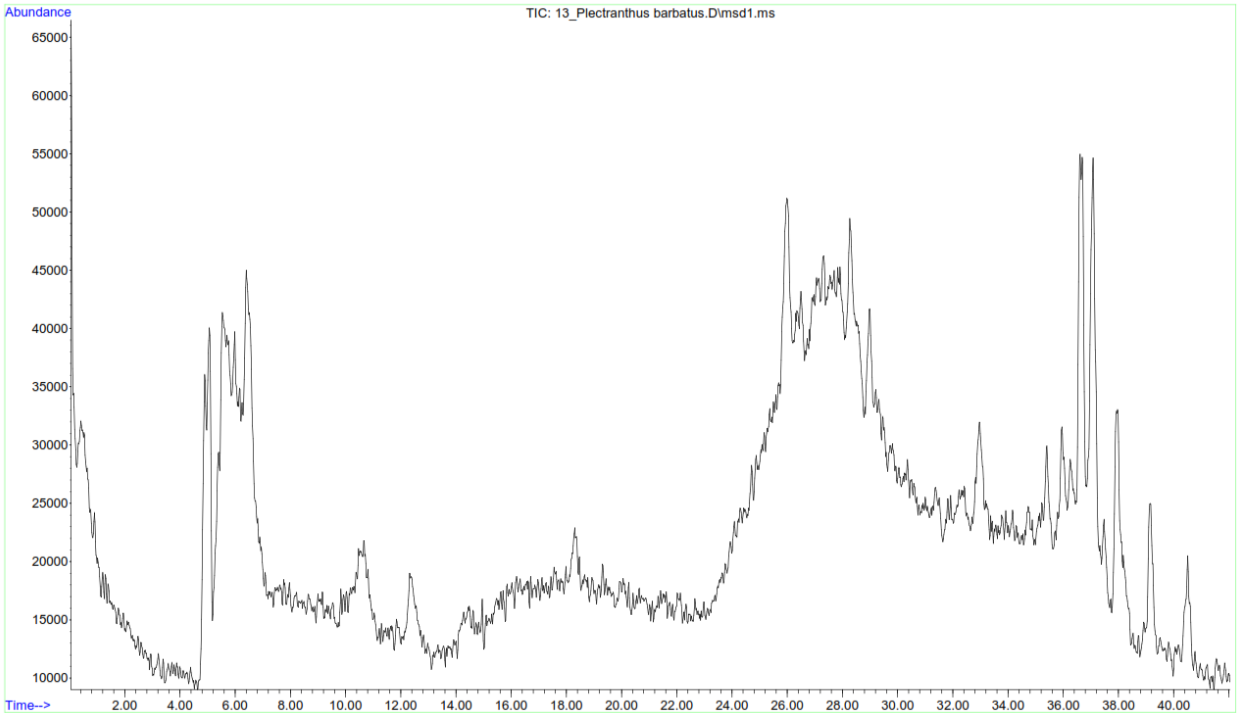

Figure 4.4: LC-MS chromatogram of aqueous extract of *P. barbatus*
X-axis presents retention time whereas Y-axis presents the percentage abundance.Retention times of the phytoconstituents are shown above peaks.

4.9 Acute Oral Toxicity

The study included an acute oral toxicity test. No signs of evident toxicity or fatalities after 14 days of oral administration of *P. barbatus* extract. There were no changes in some of the observed behavioral activities of mice such as, movement frequency and nature, autonomic, neurological, or physical aspects. There were no observable signs of anxiety, coma, polyuria, seizures, skin and hair changes, ocular and mucous membrane alterations, salivation, piloerection, bradycardia, or tachycardia. Furthermore, there were no symptoms of vasodilation in the animals, such as redness of the skin, tongue, ear, or foot pad. The mean absolute organ weight and organ: body weight ratios for the treatment group (2000mg/kgbw) and the normal control were calculated and compared. Orally administered *P. barbatus* aqueous leaf extracts at a dose of 2000mg/kgbw did not reveal

significant activity on the changes of body weight changes or organs to body weights compared to normal control mice as shown in figure 4.5.

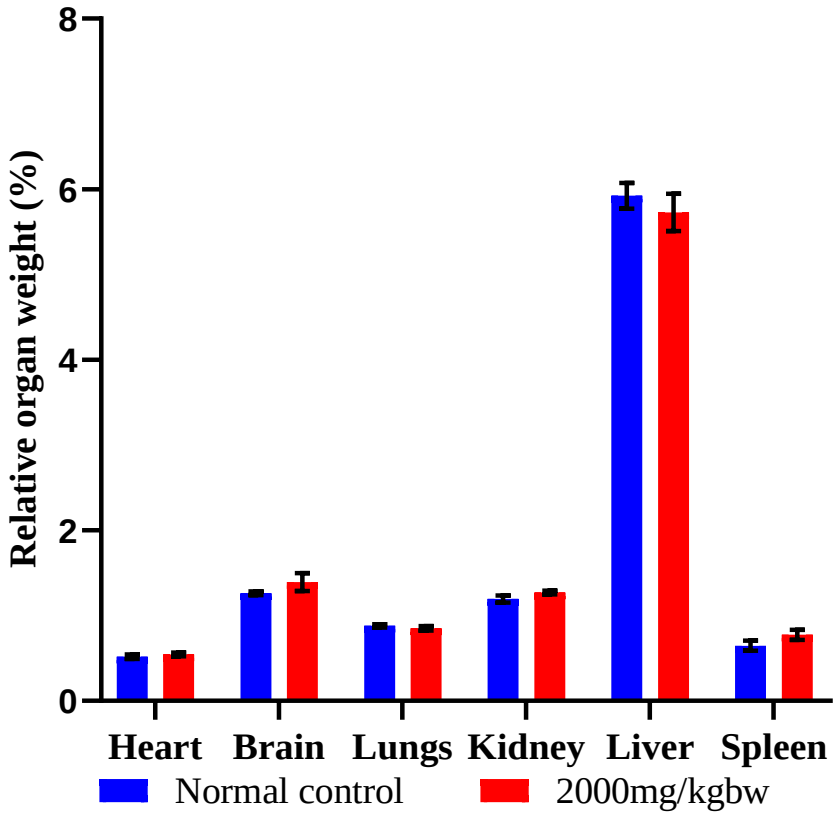

Figure 4.5: Acute toxicity effects of the aqueous leaf extract of *P. barbatus* on the organ weight.
Bars at the same organ are insignificant using independent t-test ($p > 0.05$).

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, RECOMMENDATIONS

5.1 Discussion

Findings from the study revealed that *P. barbatus* leaf extract exhibited remarkable antidiarrheal effects and inhibitory activities on selected bacterial pathogens associated with diarrhea. Additionally, the extract was found to be safe upon acute oral toxicity test. Antidiarrheal and antibacterial activity of plant extracts have extensively been studied in previous studies, for instance, extracts of *Sapium ellipticum, Plectranthus aegypticus, P. otostegiodes, P. lanuginose, Clutia abyssinica* and *Clerodendrum myricoides* have been documented to have potent antidiarrheal and antibacterial activities (Musila *et al*., 2017; Degu *et al*., 2020; Zayede *et al*., 2020; Desta *et al*., 2021).

Additionally, preceding studies have established antidiarrheal and antibacterial potency of aqueous plant extracts. For example, *Lantana camara* displayed antidiarrheal potential in castor oil induced diarrhea in mice (Tadesse *et al*., 2017). Antidiarrheal efficacy of stem bark extract of *Sapium ellipticum* extract has also been reported (Wansi *et al*., 2014). Additionally, aqueous leaf extracts of *Labisia pumila* demonstrated growth inhibitory activity against selected microorganisms such as *P. aeruginosa* and *E. coli* (Karimi *et al*., 2015). Further, *Hibiscus sabdariffa* and *Rosamarinus officinalis* showed potent bactericidal effect on *B. subtilis*, *S. aureus* and *E. coli* (Gonelimali *et al*., 2018).

The antidiarrheal effect of *P. barbatus* was assessed through castor oil induced diarrheal model. Utilization of castor oil as a diarrheagenic agent has been documented (Ferede *et*

al., 2021; Zhu *et al.,* 2022). Castor oil induction of diarrhea has been shown to mimic the natural pathophysiology of diarrhea due to its laxative effect (Desta *et al*., 2022). The effect is mediated by ricinoleic acid, a metabolite secreted by intestinal lipase. Additionally, the metabolite causes local inflammation and irritation of the stomach lining, which lead to the release of diarrheagenic mediators including prostaglandins. Prostaglandins reduce intestinal fluid absorption by inhibiting Na+/K+-ATPase activity. It also increases net secretion of intestinal electrolytes and water, gastrointestinal motility, and nitric oxide activation (Sisay *et al*., 2017; Usman *et al*., 2021). Loperamide, the positive control used in this study antagonizes the effects of ricinoleic acid, thereby causing reduction of diarrhea (Ferede *et al*., 2021).

In vivo antidiarrheal assay was assessed through different parameters including commencement of diarrhea, frequency, and mass of wet feces in addition to the mass of total feces. Previous studies have validated the use of these parameters (Desta *et al*., 2021; Ferede *et al*., 2021). Findings showed that an aqueous extract of *P. barbatus* delayed the onset of diarrhea, elicited a decrease in the rate of recurrence and mass of wet, and total fecal output in a concentration-dependent manner. Dose dependency of these parameters suggest that high doses of the extract are required to effectively inhibit diarrhea (Sisay *et al*., 2017). This study's findings are consistent with those previously reported on the methanol and aqueous leaf extracts of *Cordia africana* and *Lantana camara* Linn, respectively (Tadesse *et al*., 2017; Ferede *et al*., 2021). Preceding researches have reported that medicinal plants with antidiarrheal effect prolonged diarrhea onset and cause the reduction of frequency and mass of wet feces (Desta *et al*.,

2022). Root extracts of *Clutia abyssinia* and leaf extracts of *Berssama abyssinica* and *Bixa Orellana* have been reported to have a remarkable activity on the impediment of onset of diarrhea and reduction of the frequency. In addition, the extracts reduced mass and frequency of wet feces (Tagne *et al*., 2019, Zayede *et al*., 2020 Ayalew *et al*., 2022).

P. barbatus extract was subjected to enteropooling and motility tests to further ascertain the mode of action (Mekonnen *et al*., 2018). The *P. barbatus* extract was found to reduce accumulation of intraluminal fluid in the enteropooling assay. This could reduce distension, intestinal overload, and water content in fecal drops, resulting in less frequent defecation and diarrheal drops (Kifle *et al*., 2021). This could be attributed to ricinoleic acid's inhibition of prostaglandin E2 receptors, a mechanism consistent with loperamide's action (Mekonnen *et al*., 2017). Therefore, *P. barbatus* extract could have inhibited gastrointestinal enteropooling and hypersecretion by increasing facilitated electrolyte and intestinal water absorption (Degu *et al*., 2020).

Furthermore, *P. barbatus* extract inhibited propulsion of the charcoal meal, thereby reducing intestinal transit and, eventually, diarrhea. The study’s finding implies that the *P. barbatus* extract can affect peristaltic movement in the lumen, indicating an antimotility effect (Amabeoku, 2009; Ferede *et al*., 2021). Protracted transit through the intestines also increases fluid absorption from feces. This causes the stool to become dry as it moves through the intestines (Asrie *et al*., 2016). In addition, antimotility activities of *P. barbatus* extract could be related to mechanisms that influence inhibition of

serotonergic and cholinergic activities, which are associated with gut motility (Kifle *et al*., 2021).

Previous studies have reported antidiarrheal activities of plants extracts through the aforementioned mechanisms. Methanol extracts of *Clutia abyssinica* and *Myrtus communis* have been shown to have induced antidiarrheal effect in Swiss Albino mice by reducing gastrointestinal motility and stimulation of reabsorption of intestinal water and electrolytes (Degu *et al*., 2020). Similarly, antimotility and anti-enteropooling activities of *Hagenia abyssinica*, *Withania somnifera* and *Cordia africana* have been reported (Ferede *et al*., 2021; Kifle *et al*., 2021; Desta *et al*., 2022). This study further revealed a high antidiarrheal index of the extract, an indication of its effectiveness to treat diarrhea (Degu *et al*., 2020; Woldeyohannes *et al*., 2022). The findings are in concordance with those of Mekonnen *et al*. (2017), Tadesse *et al*. (2017), Degu *et al*. (2020) and Desta *et al*. (2022).

The study revealed that *P. barbatus* extract exhibited antibacterial activity on pathogens associated with diarrheal diseases. The extract had varying Mean Zones of Inhibition (MZI) against the bacterial pathogens tested. The variation could be due to genetic differences among the pathogens (Kerner *et al*., 2023). The MZI ranged from 7.33±0.33 to 17.17±0.73mm with higher effects observed against *P. aeruginosa* and *B. subtilis.* Against these pathogens, the extract elicited dose dependent growth inhibitory activities. However, *P. barbatus* extract revealed active inhibitory activity (8-11mm) against *E. coli*, *S. typhi*, *Shigella* and *S. aureus* at the highest concentrations (175 and 200mg/ml).

This could be taken to suggest that high doses of *P. barbatus* extracts are needed to induce growth inhibitory effects against the tested pathogens (Abdulaziz, 2021).

The effects of the *P. barbatus* leaf extract against selected pathogens, corroborate earlier studies of Viji *et al* (2010), where *Cardiospermum helicacabum* extract showed concentration dependent growth inhibitory effect on the studied pathogens including *B. subtilis* and *S. typhi*. Additionally, *Coleus forskholii* demonstrated antimicrobial effects against *S. aureus* and *P. aeruginosa* at high concentrations of 100, 150 and 200mg/ml (Abdulaziz, 2021).

The antidiarrheal activities of *P. barbatus* can be ascribed to a range of phytocompound in the extract. In this study, flavonoids including 4-methoxy-quercetin-3-O-glucuronide, methoxy-kaempferol-3-glucuronide,3,4-dimethoxy-luteolin-7-glucuronide, and luteolin-O- glucuronide were detected in the extract. Quercetin as well as its derivatives have been reported to reduce capillary permeability in abdominal cavity and reduce intestinal movement (Xiao *et al*., 2011). Additionally, quercetin inhibits growth of pathogens that affect the gastrointestinal system including *E. coli*. The bioactivity was achieved through various mechanisms including disruption of the integrity of bacterial cell membrane (Nguyen & Bhattacharya, 2022). The antidiarrheic effect of quercetin could also be attributed to its antihistamine activities that are related to inhibition of prostaglandin biosynthesis (Yadav & Tangpu, 2008).

Additionally, methoxy-kaempferol-3-glucuronide and methoxy-kaempferol-7-glucuronide have antimicrobial, anti-inflammatory, and antioxidant properties, which are associated with antidiarrheal effects (Mbaveng *et al*., 2014). Through their ability to complex with bacterial cell walls, as well as soluble and extracellular proteins, these compounds have been shown to interfere with the growth of diarrhea-causing agents including *S. aureus* (Konaté *et al*., 2015).

The anti-inflammatory activities of the aforementioned compounds aid in the inhibition of cyclooxygenase enzymes, which are required in the synthesis prostaglandins (El Sayed *et al*., 2020; Tutunchi *et al*., 2020). Additionally, previous studies show that flavonoids including vicenin-2 reduce *COX-2* expression by hindering the NF-$_K$B pathway. These activities stimulate protein kinase (AMPK), which suppresses pro-inflammatory signaling pathways, thereby increasing absorption of electrolytes (Tutunchi *et al*., 2020). Moreover, flavonoids have been shown to confer antidiarrheal activities through their ability to hinder intestinal mucosa and hydro electrolytic secretions (Xiao *et al.*, 2011; Alexander *et al*., 2016).

Phenolic compounds, including rosmarinic acid and gallic acid exert anti-diarrheal activities through different mechanisms. The compounds have been demonstrated to reduce transit and intestinal secretion (Sisay *et al*., 2017). The compound are also endowed with anti-inflammatory and analgesic properties associated with antidiarrheal activities (Pei *et al*., 2016).

As effective antimicrobial agents, phenolics inhibit growth of pathogens associated with diarrheal diseases including *E. coli* (Su *et al*., 2010). Phenolics, including gallic acid have been demonstrated to exert their bactericidal activities by interrupting the integrity of the cell membrane (Sobolewska *et al*., 2016). Moreover, the compounds bind to bacterial DNA, subsequently inhibiting cellular functions such as replication, transcription, and expression, ultimately causing cell death. Their antioxidant activities have been demonstrated to protect the intestinal mucosa against castor oil (Lou *et al*., 2012). In addition, antioxidant properties are arguably responsible for the observed inhibitory activities.

Rosmarinic acid exerts its antibacterial by interfering with the membrane of the cytoplasm thereby coagulating the cell contents and as a result compromise active transport. Ahmad *et al.* (2015) also suggested that these phenolic compounds can act with interacting proteins therefore generating a redox imbalance.

Diterpenoids including forskolin isolated from *P. barbatus* have been demonstrated to exhibit strong antibacterial and anti-spasmodic properties, pointing to their potential application in the care of digestive problems (Patel, 2010). Previous studies have shown that forskolin stimulates adenylate cyclase, which increases the amount of the second messenger cyclic AMP in cells and has a number of biochemical and physiological effects, including reducing histamine release, which in turn reduces inflammation and, ultimately, diarrheal episodes. Additionally, forskolin's antibacterial action was previously shown to be

demonstrated by its inhibitory effects on the bacterial galactose-H^+ transport protein which interfered with the survival and pathogenicity of *E. coli* (Salehi *et al*., 2019).

In this study, *P. barbatus* extract was found to be safe upon acute oral toxicity test. Findings revealed no changes in the mice's behavioral activities and movement frequency. In addition, there were no clinical signs of anxiety, coma, polyuria, seizures, skin and hair changes, ocular and mucous membrane alterations, salivation and piloerection. Moreover, oral administration of *P. barbatus* to mice had no effect on weights of the body and organs. The present findings agree with that of Ezeonwumelu *et al*. (2019) who revealed that the aqueous extracts of *P. barbatus* were safe up to a dosage level of 10,000mg/kg. Similarly, Mwitari *et al*. (2013) and Cordeiro *et al*. (2022) demonstrated that *P. barbatus* extracts exhibited no toxicity to normal cells. The findings illustrate the reason as to why the leaves of *P. barbatus* are used in treating diarrhea by the local communities as it has been demonstrated that it has the ability to inhibit diarrhea as well as act against various bacteria that cause diarrhea, with no toxic effects.

5.2 Conclusions

In conclusion, this study established that;

i. *Plectranthus barbatus* leaf extract has exhibited remarkable antidiarrheal effect in mice induced with diarrhea.
ii. *Plectranthus barbatus* aqueous leaf extract has potent antibacterial effects against *E. coli*, *S. typhi*, Shigella, *S. aureus*, and *P. aeruginosa*.
iii. In mice, *Plectranthus barbatus* extract did not cause observable toxic effects.

iv. *Plectranthus barbatus* aqueous leaf extract contains phytochemicals with antidiarrheal and antibacterial properties.

5.3 Recommendations

5.3.1 Recommendations from the study

The aqueous extract *Plectranthus barbatus* can be utilized in the development of anti-diarrheal agents. The aqueous extract of *Plectranthus barbatus* can be used in the development of antibiotic agents against *E. coli*, *S. typhi*, *Shigella*, *S. aureus*, *P. aeruginosa* and *B. subtilis*, and the aqueous extract *Plectranthus barbatus* has phytocompounds that can be harnessed in treating diarrhea and bacteria.

5.3.2 Limitations of the study

Acute toxicity studies provide limited insight into the potential long-term or chronic effects of repeated exposure to the extract and the observation period was short and may not capture delayed toxic effects that manifest beyond the study's time frame. Factors such as stress, fasting state, or variation in gastrointestinal transit time can influence the response to castor oil-induced diarrhea and confound the interpretation of the results. Relying solely on castor oil as the inducer of diarrhea may overlook other important etiological factors contributing to diarrhea, such as infectious agents, dietary intolerances, or inflammatory conditions.

5.4.3 Recommendations for further studies

Conduct further studies to establish mechanism of antidiarrheal and antibacterial actions of *Plectranthus barbatus* extract. Evaluate antidiarrheal effect of other parts of the plants. Isolation and bioassay of phytocompounds in *Plectranthus barbatus.* Comprehensive chronic toxicity studies to establish safety profiles of the plant extract. Use of individual

bacterial isolates to induce diarrhea and further assess the antidiarrheal activity of the extract.

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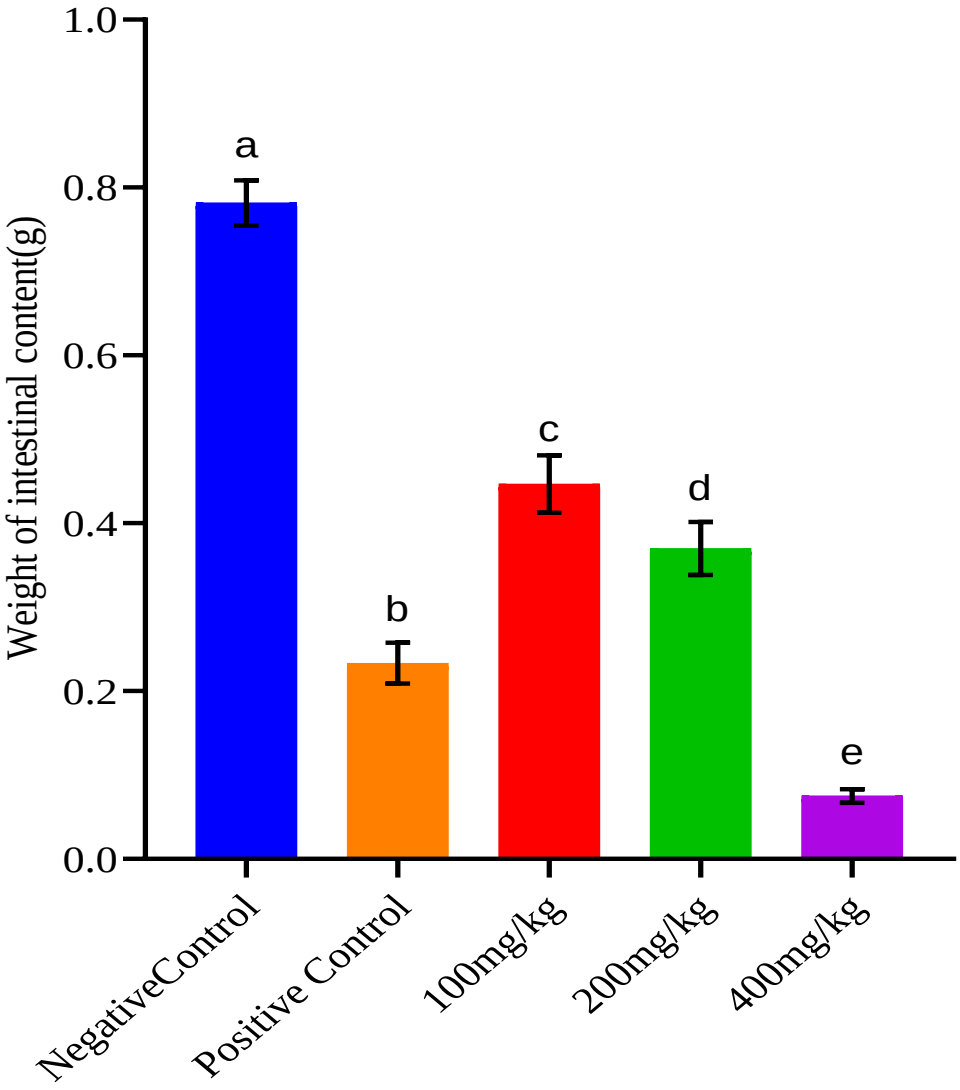
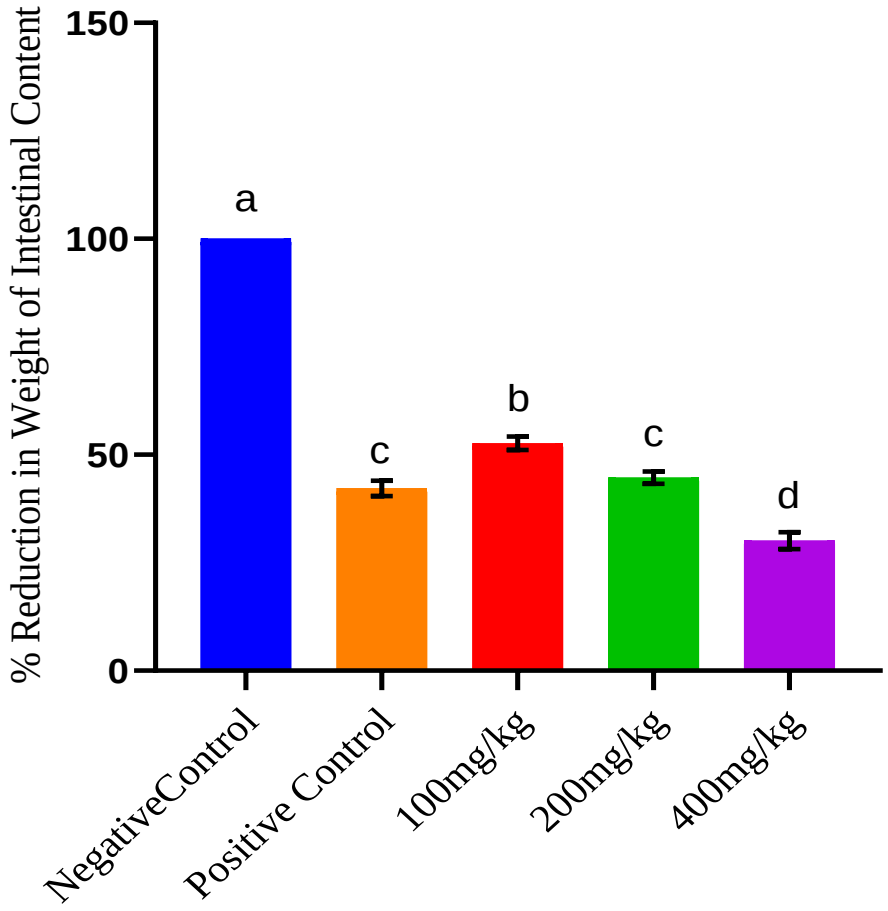
APPENDICES

Appendix I: Effect of Aqueous Leaf Extract of P. barbatus on Enteropooling in Mice Induced with Diarrhea



Bars with different lowercase letter vary significantly ($p < 0.05$) using one-way ANOVA followed by Tukey's multiple comparison.

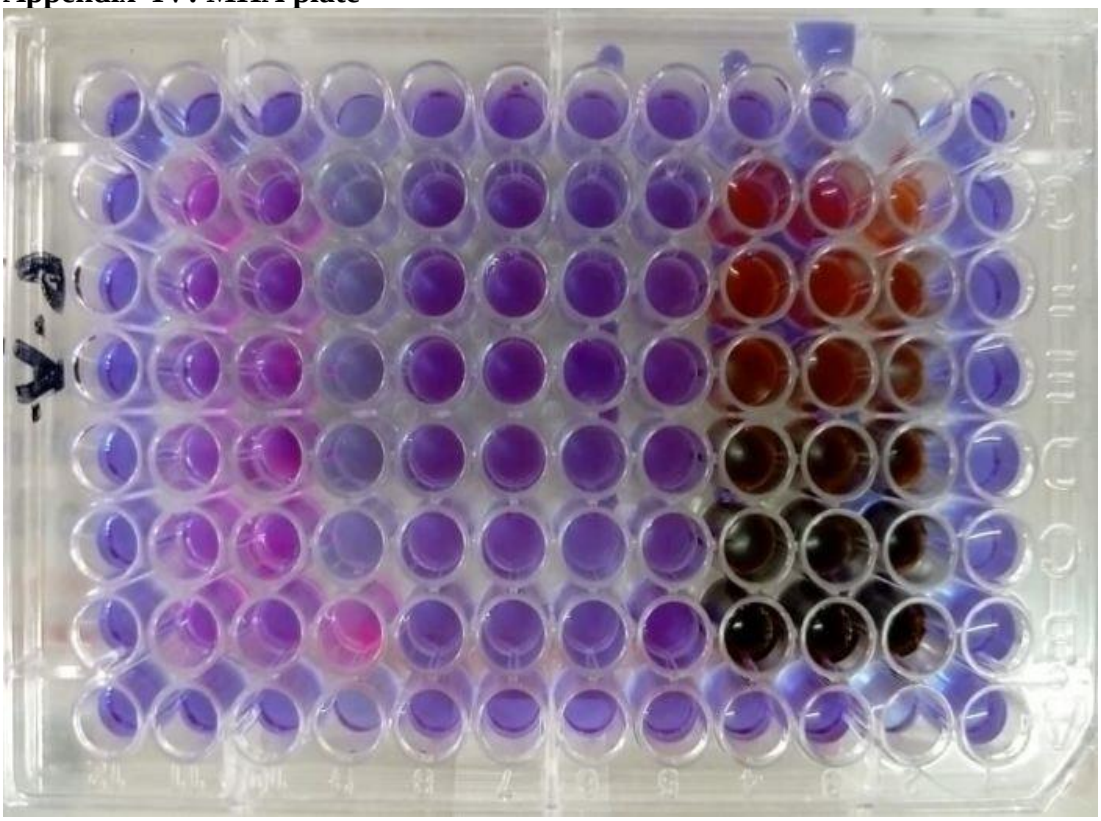
Appendix II: Effect of Aqueous Leaf Extract of *P. barbatus* on Enteropooling in Mice Induced with Diarrhea



Bars with different lowercase letter vary significantly ($p < 0.05$) using one-way ANOVA followed by Tukey's multiple comparison.

Appendix III: MBC plates

Appendix IV: MHA plate



Appendix V: *In vivo* Antidiarrheal Indices of Aqueous Leaf Extract of *P. Barbatus*

Treatment	Dose(mg/kg)	Delay in diarrhea onset (Dfreq)	Charcoal meal travel reduction (Gmeq)	Reduction in number of wet stools (Pfreq)	*In Vivo* ADI
Water	010 mL/kg	000.00	00.00	00.00	00.00
Loperamide	003mg/kg	182.62±4.36	62.59±1.84	67.73±2.04	092.40±1.86[b]
***P. barbatus* extract**	100mg/kg	091.68±3.90	47.32±1.96	49.98±1.61	059.91±1.34[d]
***P. barbatus* extract**	200mg/kg	140.42±4.40	64.88±1.41	66.12±2.16	084.29±0.71[c]
***P. barbatus* extract**	400mg/kg	201.78±4.25	77.10±1.56	75.80±2.16	105.55±1.38[a]

Values expressed as mean ± SEM (n=6). Means with different superscripts within the columns are significantly different ($p>0.05$) by one way ANOVA and Tukey's multiple comparison.

Appendix VI: Research Authorization

KENYATTA UNIVERSITY
OFFICE OF THE EXECUTIVE DEAN GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Our Ref: I56/CE/20960/2020 **DATE:** 22nd March 2023

Director General,
National Commission for Science, Technology and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MS. AJWANG EMMAH CLARICE – REG. NO. I56/CE/20960/2020

I write to introduce Ms. Ajwang Emmah Clarice who is a Postgraduate Student of this University. She is registered for M.Sc. degree programme in the **Department of Department of Biochemistry, Microbiology and Biotechnology.**

Ms Ajwang Emmah Clarice intends to conduct research for a M.Sc. Thesis Proposal entitled, ***"Phytochemical Composition, Antidiarrheal Activity and Antibacterial Effect of Aqueous Leaf Extract of Pleacranthus Barbatus (Andrews)".***

Any assistance given will be highly appreciated.

Yours faithfully,

KENYATTA UNIVERSITY OFFICE OF EXECUTIVE DEAN 13 JUN 2023 GRADUATE SCHOOL P.O. Box 43844-00100 NAIROBI

PROF. ELISHIBA KIMANI
EXECUTIVE DEAN, GRADUATE SCHOOL

Appendix VII: Nacosti Authorization

REPUBLIC OF KENYA