ANTI-INFLAMMATORY, ANTIMICROBIAL, ANTIDIARRHEA AND TOXIC EFFECTS OF THE AQUEOUS AND METHANOLIC LEAF AND FRUIT EXTRACTS OF CUCUMIS DIPSACEUS IN WINSTAR RATS AND NEW ZEALAND WHITE RABBITS

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DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

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DECLARATION

This thesis is my original work and has not been submitted for award of a degree in the University or any other Institution.

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DEDICATION

I dedicate this thesis to my loving family, my husband Joachim Nyamwaro, and my children Valerie, Sienna, and Ava, from whom I derive immense inspiration.

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of variance
AQF	Aqueous Fruit Extract
AQL	Aqueous Leaf Extract
BAUEC	Biosafety, Animal use and Ethics Committee
BW	Body weight
CDC	Centre for Disease control
CFU	Colony Forming Unit
CGR	Control Group of Rats
CLSI	Clinical and Laboratory Standard Institute
DMSO	Dimethyl sulphoxide
DPHPT UoN	Department of Public Health, Pharmacology and Toxicology, University of Nairobi
EGR	Experimental Group of Rats
GPS	Global positioning System
HPLC	High Performance Liquid Chromatography
Hrs	Hours
Kg	Kilogram
LD_{50}	Median Lethal dose
MDR	Multi Drug Resistant
MEOHF	Methanolic Fruit Extract
MEOHL	Methanolic Leaf Extract
MHA	Mueller Hinton Agar
MICs	Minimum Inhibitory Concentrations
MSDS	Material Safety and Data Sheets
N	Sample size
NACOSTI	National Commission for Science, Technology and Innovation

NSAIDs	Non- Steroidal anti-inflammatory drugs
OECD	Organisation for Economic Co-operation Development
SDA	Sabouraud Dextrose Agar
SEM	Standard Error of the Mean
UDP	Up -Down-Procedure
WHO	World Health Organisation

ABSTRACT

Inflammation, diarrhea, and microbial infections produce high morbidity and mortality globally, contributing to a high burden of disease in developing countries. Despite the utilisation of conventional drugs to treat inflammation, diarrhea, and microbial infections, their inaccessibility, unaffordability and their side effects hinder the successful treatment and alleviation of disease. The emergence of antimicrobial-resistant bacteria and fungi strains have compromised the efficiency of antimicrobial chemotherapy, which leads to undesirable sequalae. There is an urgent need to search for alternative therapies, which are efficacious, affordable, accessible and safe in order to alleviate human suffering and promote the quality of life. Medicinal plants present a feasible alternative source of potent pharmacological molecules, which are affordable, easily accessible and safe due to the many biologically active phytochemicals they have. Although medicinal plants have been used by humans for ages as medicines and food, only a few have been empirically investigated. *Cucumis dipsaceus* has a long history of ethnomedicinal usage in treating inflammation, diarrhea, and microbial infections, in Kenya and other countries. However, there is scanty empirical data to validate its efficacy and safety. The anti-inflammatory antidiarrhea, antimicrobial, and toxic effects of methanolic and aqueous leaf and fruit (methanolic and aqueous leaf and fruit) extracts of *C. dipsaceus* were investigated. The *in vivo* anti-inflammatory activities of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* were studied using the formalin-induced paw oedema technique in Wistar rats. The castor oil-induced diarrhea method was used to determine the antidiarrhea activity of the methanolic and aqueous leaf and fruit extracts of the studied plant in Wistar rats. Isolated rabbit ileum was used to determine the effects of the *C. dipsaceus* extracts on the gastrointestinal motility. Antimicrobial activity of the methanolic and aqueous leaf and fruit extracts was determined using the disk diffusion and broth microdilution techniques described previously. The acute oral and dermal toxicity effects of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* were studied in rabbits and rats, respectively, using appropriate guidelines. Data was analysed using GraphPad Prism statistical software version 9.1. The results indicated significant reductions in formalin-induced paw oedema in the Wistar rats by methanolic and aqueous leaf and fruit extracts of *C. dipsaceus*, in a dose- and time-dependent manner ($P<0.05$), with percentage inhibition of between 1.56% and 97.59%. Methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* significantly ($P<0.05$) inhibited diarrhea and intestinal motility in Wistar rats and rabbits respectively, in a dose-dependent manner, thereby depicting their antidiarrhea effects. Furthermore, the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* significantly inhibited the growth of *P. aeruginosa, S. enteriditis, E. coli, C. albicans,* and *B. subtilis* in varying degrees as depicted by the different growth inhibition zones of between 6 mm and 20 mm and the Minimum Inhibitory Concentrations of 3.125 µg/ml. The observed anti-inflammatory, antidiarrhea, and antimicrobial activities of the plant extracts were attributed to various phytochemicals extractable by water and methanol, which exerted pharmacological efficacy via various mechanisms. Moreover, aqueous and the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* showed no observable acute dermal toxicity effects on the abraded and intact skins of New Zealand White rabbits. Similarly, the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* showed no observable acute oral toxicity during the 14-day experimental period. In conclusion, the extracts were considered safe according to the OECD Guidelines. Further pharmacological and toxicological investigations of the tested plant extracts using more advanced techniques should be done in order to elucidate and optimise bioactive molecules for treating inflammation, diarrhea, and microbial infections.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Traditional medicine has been practiced for ages, even before the emergence of modern medicine (Sofowora *et al.*, 2013). Previously, herbalism was based on crude preparations in order to manage various diseases, however, with the advent of modern technology, various plant-derived drugs have been developed to present-day drugs such as creams, ointments, injections, capsules, and Tablets (Ozioma and Chinwe, 2019; Mahomoodally, 2013). Although conventional drugs were developed for various diseases, the usage of medicinal plants in order to treat various diseases is still dominant, due to their easy availability, accessibility, and relative affordability, especially in sub-Saharan Africa (James *et al.*, 2018). The World Health Organization (WHO) reported that more than 80% of people, especially in the low and medium income countries, depend on traditional medicine with over 85 % of the medicines being derived from plant extracts (WHO, 2018). Traditional medicine is very common in Kenya, with various ethnic communities utilising plant-based remedies in order to prevent and treat diseases (Kigen *et al*., 2016, 2019; Kipkore *et al*., 2014). Herbal medicines are relatively cheap, accessible and cause minimal side effects (Nasri and Shirzad, 2013).Despite the prominence of herbal medicine, documented empirical data on their efficacy, phytochemical composition, mechanisms of action, and toxicity profile is still scarce.

Despite the extensive uses of medicinal plants for the alleviation of pain and inflammation management, they are excluded from conventional healthcare plans (Alemu *et al.*, 2018). Phytochemicals can offer novel compounds of therapeutic value; hence, there is need for their empirical characterisation.

Inflammation is an immune response, which is often non-specific, and is evoked after injury, pathogenic invasion, chemical irritation, or allergy (Ptaschinski and Lukacs, 2018). The process involves recognition of injury or pathogen, activation of mediators, recruitment of defence cells, breakdown of diseased tissues, and promotion of tissue repair. Sometimes, inflammation persists due to defects in the body's defence system, thus exacerbating the associated syndrome's sequelae (Chen *et al*., 2018; Hunter, 2012).

Antimicrobial molecules are crucial in lowering the burden of communicable diseases affecting the world population (Abbafati *et al.*, 2020a; Christou, 2011). However, availability of fewer, or the lack of efficacious antimicrobial agents which can thwart resistant pathogenic strains poses a great public health problem worldwide (Dadgostar, 2019; Frieri *et al.*, 2017). In the view of rapidly emerging isolates of resistant strains, development of effective agents is critical. Notably, the history of new antimicrobials is tainted with rapid emergence of resistance, further dimming the hope for long term effectiveness of the current therapeutic agents (Ayukekbong *et al.*, 2017; Manandhar *et al*., 2019). Scientists need to continuously explore the derivates of medicinal plants that contain useful secondary metabolites such as alkaloids, tannins, flavonoids and phenolic compounds that contain anti-inflammatory, antioxidant and antimicrobial properties (Kathare *et al*., 2021a; Nyarang and Bonareri, 2021; Obey *et al*., 2016; Scalbert, 1991).

Cucumis dipsaceus has been used widely to manage inflammation and pain, and in the treatment of bacterial infections, among other functions, including consumption as food (Kipkore *et al*., 2014; Mutie *et al*., 2020; Tefera and Kim, 2019). Previous research indicate that leaf extracts of *C. dipsaceus* have antimicrobial activity (Shivakoti *et al*., 2015), and various parts including fruits possess anti-inflammatory, analgesic, diuretic, anti-dysentery, among other properties (Kaur and Lata, 2019). In Kenya, a concoction of boiled roots is used to treat abdominal pains among the Keiyo residents (Kigen *et al*., 2014). The present study

explored anti-inflammatory, antimicrobial, antidiarrhea, and toxic effects of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus*.

1.2 Problem statement and justification

Inflammatory diseases cause debilitating physical, psychological, and economic effects, to the affected patients, globally, with the highest burden lying in the less developed Nations, especially in sub-Saharan Africa (Abbafati *et al*., 2020a, 2020b; Alema *et al*., 2020; Henschke *et al*., 2015; Jairath and Feagan, 2020). Microbial infections of the gastrointestinal tract, cause gastric irritation, inflammation, and impaired gastric functioning, manifesting in abdominal discomfort and diarrhea (CDC, 2015; Christou, 2011; Troeger *et al*., 2017). Diarrhea is among the leading causes of morbidity and mortality worldwide especially in the children, elderly, and immunocompromised persons (CDC, 2015).

Despite the availability of conventional anti-inflammatory, antimicrobial, and antidiarrhea drugs, inflammatory disorders, microbial infections, and diarrhea still continues to pose global Public health problem (Alatab *et al.*, 2020; Centers for Disease Control and Prevention, 2015; Jairath and Feagan, 2020; Mathers, 2017). The available anti-inflammatory drugs are associated with adverse effects, following chronic use (Monteiro and Steagall, 2019). For instance, the commonest anti-inflammatories cause gastrointestinal bleeding, intestinal perforations, indigestion, constipation, hepatotoxicity, nephrotoxicity, cardiotoxicity, among other side effects (Felson, 2016; Harirforoosh *et al.*, 2013; Moriasi *et al.*, 2021).

The emergence of antimicrobial resistant strains of bacteria and fungi, has complicated successful use of chemotherapy (Ayukekbong *et al*., 2017).The anti-inflammatory agents used now, cause undesirable effects, including constipation, gastric irritation, cardiotoxicity, hepatotoxicity, nephrotoxicity, low efficacy among others (Caldwell and Cluff, 1974; Dadgostar, 2019; Fernebro, 2011; Mahmoud abd El-Baky, 2016; Mohsen *et al.*, 2020;

Shehab *et al.,* 2016). Antidiarrhea drugs are associated with constipation, impaired gastric motility, among other life-threatening effects (WHO, 1990; Dosso *et al., 2012*; Niemegeers *et al*., 1981; Sisay *et al.*, 2017).

Conventional anti-inflammatory, antimicrobial, and antidiarrhea drugs are costly, inaccessible, and unavailable, especially in rural areas of developing Countries, where over 80 % of the global burden of diseases lies, due to inadequate, and insufficient healthcare systems, and resources (Mathers, 2017; Murray and Lopez, 1996; WHO, 2020). Therefore, alternative drug agents, which are safe, efficacious, affordable, easily accessible are required to avert human suffering, are required especially in the developing Nations.

Medicinal plants present a viable source of potent therapies against inflammation, microbial infections, and diarrhea, among other diseases, due to their rich ethnomedical history of application, and the diverse array of bioactive principles they contain (Kathare *et al.,* 2021; Moriasi *et al.,* 2021). However, only few medicinal plants have been empirically investigated and validated for use against the ailments they are traditionally used to treat. Moreover, herbal medicines are preferred because of their affordability, availability, ease of usage, ability to treat multiple conditions, and their minimal adverse effects.

Despite the widespread applications of plant-based ethnomedicines to treat diseases, various safety concerns have been raised (George, 2011). This is due to the lack of standard preparation guidelines, specific dose regimens for each disease, appropriate labelling and marketing, empirical Pharmacological, Toxicological and safety profile data, and regulations governing the practice (Abdullahi, 2011; George, 2011; Kaur *et al.,* 2013; Zhang *et al.*, 2012). Therefore, empirical pharmacologic investigations and safety evaluations of herbals may foster the elucidation of efficacious and safe therapies, against various diseases, especially those which are associated with inflammation, microbial infections, and diarrhea, which negatively impact human health.

Despite the utilisation of *C. dipsaceus* in traditional medicine in order to manage inflammatory, microbial, and diarrhea associated diseases (Kareru *et al*., 2007; Kigen *et al*., 2014; Mutie *et al.*, 2020; Njoroge and Newton, 1994), there is scanty empirical evidence to back the claimed Pharmacologic efficacy and safety. This study investigated the anti-inflammatory, antimicrobial, antidiarrhea and toxic effects of methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* to lay a framework towards validation of their usage, and the development of potent, safe, accessible, and affordable therapies.

1.3 Study objectives

1.3.1 General objective

i. The main objective of the study was to investigate the anti-inflammatory, antimicrobial, antidiarrhea, and toxic effects of the aqueous and methanolic leaf and fruit extracts of *Cucumis dipsaceus* Wistar rats and New Zealand White rabbits.

1.3.2 Specific objectives

The following were the specific objectives of the study:

i. To determine the anti-inflammatory activity of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* in formalin-induced oedema in Wistar rat model.
ii. To investigate the antidiarrhea effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* in castor oil-induced diarrhea in Wistar rats and New Zealand White rabbits.
iii. To determine the antimicrobial effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on selected enteric bacteria and *candida albicans*.

iv. To investigate the acute oral and dermal toxicity effects of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* using Wistar rats and New Zealand White rabbits.

1.4 Research questions

This study was guided by the following research questions:

i. Do the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* have anti-inflammatory activity in formalin-induced oedema in Wistar rat model?

ii. Do the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* have antidiarrhea activity in castor oil-induced diarrhea in Wistar rat and New Zealand White rabbit models?

iii. Do the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* have anti-microbial activity against select enteric bacteria and *candida albican* strains?

iv. Do the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* cause acute oral and dermal toxicity effects in Wistar rats and New Zealand White rabbits?

CHAPTER TWO

LITERATURE REVIEW

2.1 Inflammation, microbial infections and diarrhea

2.1.1 Inflammation

Inflammation is a biological mechanism used by the body to protect against tissue injury, and can be induced by endogenous and exogenous factors (Lordan *et al.*, 2019; Sahlmann and Ströbel, 2016). The process is initiated as a way of eliminating the injurious stimuli, and to promote healing of the damaged tissue (Medzhitov, 2008).

The inflammation cascade begins when host cells recognise inflammatory triggers, via their transmembrane Toll-like receptors (TLRs) and NOD-like Receptors (NLRs), distinguish self from non-self by recognising specific damage- and pathogen associated molecular patterns (DAMPs and PAMPs) (Chen *et al*., 2018; Lordan *et al*., 2019). These triggers downstream signal transduction, leading to the activation of various cells and molecules, including cytokines such as interleukin-six (IL-6), interleukin 1-beta (IL-1β) and TNF-α to spur an inflammatory response.

Inflammatory mediators such as prostaglandins, serotonin, and histamines, are activated, and work in conjunction with cytokines to produce the classic signs of inflammation (Chen *et al.*, 2018; Lordan *et al.*, 2019). Inflammation is beneficial to the organism as its primary role is to avert tissue damage, remove injurious stimuli, and promote tissue repair; however, defects in its regulation often leads to persistent inflammation which leads to degenerative diseases such as diabetes, rheumatoid arthritis, autoimmune diseases, neurodegenerative disorders, cardiovascular diseases, and cancer, among others (Ptaschinski and Lukacs, 2018).

Inflammation is the hallmark of infectious disease, which is commonly characterised by allergic responses, such as those observed in hypersensitivity pneumonitis, among other manifestations (Hakansson and Molin, 2011; Staudacher and Stevens, 2019). Various

microbes, such as *Staphylococcus* and *Streptococcus* pyogenic bacterial species, and fungal pathogens like *Candida albicans* are associated with neutrophilic inflammatory responses, which may later evolve into granulomatous responses (Procop and Wilson, 2001). The incidence of microbial-associated inflammatory conditions is increased significantly in immunocompromised persons, and, in particular, in resource-limited remote areas of the world (Jairath and Feagan, 2020).

2.1.2 Microbial infections

Microbial infections are the commonest aetiologic drivers of human diseases (Christou, 2011). They are key contributors to high burden of diseases worldwide, especially in vulnerable persons in the less developed countries of Africa and Asia, thereby posing a global public health risk (CDC, 2015; Christou, 2011). The common symptoms of microbial infections are fever, chills, body aches, abscesses, septicaemia, and diarrhea. Some symptoms may be specific to certain microbes, which can be of diagnostic value (Elston *et al*., 2015; Fernebro, 2011). Microbial culture is a common practice, which is used to identify the specific strain responsible for a particular disease.

Pathogenic bacteria are responsible for most infections, affecting humans and animals (Kathare *et al*., 2021b). They exert their pathogenicity by either secreting toxins, which alter the normal cell physiology of the host, or by evoking the host's immune response via their products (Kaper *et al.*, 2004; Monack *et al*., 2004). For instance, *E. coli* produces a toxin (Shiga toxin), which disrupt protein synthesis by the host cells, leading to local cell death (Liu, 2019; Poolman, 2016). The colonisation of the gastrointestinal tract by the *E. coli* alters the structure of intestinal villi and microvilli, which in turn, impairs proper absorption of water, minerals and nutrients (Liu, 2019). As a result, diarrhea and toxic shock are the common consequences of *E. coli* infection, which may be life-threatening if not managed adequately (Operario and Houpt, 2012).

2.1.3 Diarrhea

Diarrhea which is associated with microbial infections is the leading cause of preventable deaths which affect infants and children in the less developed world (Pires *et al*., 2015). The WHO and United Nations Children's Fund (UNICEF) estimates that annually, there are 2.5 billion diarrhea cases, of which over 1.9 billion cases comprise of children aged 5 years and below who die globally (WHO, 2008). Approximately 78 % of all children who succumb to diarrhea are from Africa and Southeast Asia, where the burden and morbidity of diarrhea are heavy with over 7 episodes per child compared with one or two cases per child annually in the developed Countries (Alkizim *et al*., 2011; Pires *et al*., 2015; WHO, 2008).

In most cases, diarrhea is caused by diarrheagenic bacteria like *E. coli, Shigella, Salmonella* among others, protozoa like *Entamoeba histolytica* and *Giardia lamblia*, and viruses including rotavirus and adenoviruses (Kelly *et al*., 2018; Sjöling *et al*., 2015). Bacterial toxins like the Shiga toxin and *Clostridium difficile* are key drivers of diarrhea (Kelly *et al*., 2018). *Staphylococcus aureus* are drivers in diarrhea resulting from food poisoning which mostly occur within six hours. Parasitic helminths, malabsorption and inflammatory bowel syndromes like Crohn's disease have also been implicated in diarrhea disease (Alatab *et al*., 2020; Operario and Houpt, 2012).

The major categories of diarrhea, which are encountered commonly, are secretory diarrhea, osmotic diarrhea, and motor diarrhea, and are characterised by a decrease in the diameter of the digestive tract, malabsorption of water, and augmented intestinal peristalsis (Fernández-Bañares *et al*., 2016). Diarrhea can be acute (≤ 2 weeks), persistent (2-4 weeks), or chronic persisting for more than four weeks (Fernández-Bañares *et al*., 2016).

2.2 Conventional management of inflammation, microbial infections, and diarrhea

Currently, various treatments are adopted for the mitigation of different inflammatory disorders (Monteiro and Steagall, 2019). For instance, 5-aminosalicylates are the first-line

choice for remission of ulcerative colitis in affected patients; however, this class of drugs has demonstrated low efficacy in managing Crohn's disease (Su *et al.*, 2019). Corticosteroids like prednisolone are effective in remission of inflammatory bowel disease associated with diarrhea. Immunomodulators including thiopurine and methotrexate are used for Crohn's disease and ulcerative colitis remission in affected patients. Immune suppressants like oral tacrolimus and biologic immune system inhibitors like tofacitinib and adalimumab have been used to treat inflammatory disorders (Damião *et al.*, 2019).

NSAIDs such as ibuprofen, indomethacin, acetylsalicylic acid, and diclofenac are the bastion in managing pain and inflammation (Fokunang, 2018). They primarily confer their pharmacological efficacy by blocking the activity of the Cyclooxygenase enzyme (COX) 1 and/or 2, which catalyses the synthesis of prostaglandins from arachidonic acid derived from membrane phospholipids (Bacchi *et al*., 2012; Fitzpatrick, 2004; Lucas, 2016). Unfortunately, the inhibition of the COX enzyme causes various side effects, such as gastrointestinal bleeding, constipation, gastric ulcers, among other associated effects. NSAID therapy is associated with hepatotoxicity, cardiotoxicity, dependence, nephrotoxicity, among other life-threatening symptoms (Gan, 2010; Harirforoosh *et al*., 2013; Lucas, 2016).

Microbial infections are treated generally using antimicrobial drugs, which either stop the growth of microbes, or kill them (Ullah and Ali, 2017). Antibacterial agents are the most prescribed in the treatment of diarrheagenic bacterial infections, among other associated infections, which are the common causes of high morbidity, especially in children and the immunocompromised subjects (Alkizim *et al*., 2011; WHO, 2008).

Bacteriostatic agents inhibit bacterial growth by primarily interfering with protein synthesis or other metabolic pathways in the cell. They include sulphonamides, amphenicols, spectinomycin, trimethoprim, tigecycline (glycylcycline), macrolides, and tetracyclines (Ullah and Ali, 2017).

Bactericidal agents work by disrupting the cell wall or cell membrane, leading to cell death due to leakage of cell contents or influx of unsolicited materials, or by impairing DNA replication and protein synthesis. Bactericidal antibacterial drugs include Penicillins, Carbapenems, Aminoglycosides, Quinolones, Glycopeptides, and Polymyxins (Silva *et al*., 2011). Penicillin include methicillin, penicillin, cloxacillin, among others, carbapenems like aztreonam, and Imipenem, Polymyxins like polymyxin B and colistin, and glycopeptides like Vancomycin inhibit cell wall and cell membrane synthesis (Keren *et al*., 2013).

Despite the crucial role of antimicrobial chemotherapy in reducing the burden of microbial infections and associated sequelae, most of these drugs have low efficacies and cause serious undesirable effects, including immunosuppression, allergy, hypersensitivity, hepatotoxicity, nephrotoxicity, cardiotoxicity, among others (Mandell *et al*., 2001; Purssell, 2020).These therapies are usually, inaccessible, unaffordable, and unavailable to people of low income, especially in the developing world.

The emergence of antimicrobial resistant strains due to antibiotic overuse, non-compliance to treatment schedule, rampant usage of antibiotics in livestock, have rendered previously efficacious antibiotics useless (Cheng *et al*., 2016; Marshall *et al*., 2020). This has complicated the quest to curb microbial infections, especially those linked to rates of ill health and death, in the world (Purssell, 2020). Therefore, there is an urgent need to search for alternative stratagems that can thwart antimicrobial resistance and alleviate human suffering (CDC, 2020; WHO, 2015).

Antidiarrhea therapy involves the administration of synthetic drugs such as loperamide, atropine maleate, oral rehydration solutions (ORS), diphenoxylate, kaolin, pectin, and antibiotics (Awouters *et al*., 1983; Niemegeers *et al*., 1981).antidiarrhea agents like Loperamide, anticholinergic mediators, and opiates work by decreasing gastrointestinal secretions and reducing motility (Awouters *et al*., 1983; Niemegeers *et al*., 1981). However,

potential adverse effects associated with antidiarrhea drugs, such as bloating, weakened gastrointestinal system, among others limit their use (Alkizim *et al.,* 2011; Guo *et al.,* 2014; Teferi *et al.,* 2019; WHO, 2008).

Another approach for diarrhea disease treatment involves the enteric administration of food to restore the body's catabolic state, replacement of lost fluids and electrolytes, and to foster enterocyte regeneration ((WHO), 1990). Oral Rehydration Therapy (ORT) using rehydration salts is the most affordable intervention aimed at restoring the lost electrolytes through diarrhea (Alkizim *et al.*, 2011). Zinc supplements are given to promote cell growth, and to restore electrolyte balance in the gastrointestinal tract, thus improving prognosis.

Antimicrobial drugs like Cotrimoxazole and Nalidixic acid are used to fight diarrheagenic enteropathogens like *Vibrio cholerae, Giardia lamblia,* and *Salmonella spp.* Eradicating these infections and their associated toxins helps to curb arrest diarrhea and restore health (Alkizim *et al.,* 2011). The use of antiemetics, probiotics and proper diet regimen also plays a pivotal role in reducing the severity and consequences of diarrhea, especially in children (WHO/UNICEF, 2004).Antidiarrhea therapy is commonly associated with numerous problems including inaccessibility, lack of affordability, and the multi-drug resistance of the microbes (Mekonnen *et al*., 2018; Rahman *et al*., 2019), which call for alternative therapies and strategies.

2.3 Herbal Management of Inflammation, Microbial infections, and Diarrhea

For many years, humans have used plant products to manage different conditions, like inflammation, microbial infections and diarrhea (Che *et al*., 2017; James *et al*., 2018; Leonti and Verpoorte, 2017; Othman and Farooqui, 2015). The relatively fewer adverse effects, low cost, availability, and accessibility give the medicinal plants and their products an advantage over conventional medicines, especially in rural communities (Troeger *et al*., 2017). Herbal medicines are relied on by 80% of the world's populace, particularly in resource-limited

settings, for primary healthcare, according to a World Health Organisations report (WHO, 2013; WHO, 2018).

A Variety of medicinal plants are used as remedies for inflammation, microbial infections and diarrhea in traditional medicine and have been applied across time. Various ethnic groups around the world use various plant-based remedies in order to manage and treat infectious diseases, diarrhea, and associated symptoms (Semenya and Maroyi, 2012; Kigen *et al.,* 2014; Ghasemian *et al*., 2016; Oguntibeju, 2018; Sen *et al.,* 2018; Nyamwamu and Bonareri, 2020). Moreover, various scholars have investigated the antibacterial andantidiarrhea activities of various traditionally used medicinal plants in order to validate their healing claims and to foster the development of alternative, accessible, affordable, efficacious and safer therapies (Mathabe *et al*., 2006; Mekonnen *et al*., 2018; Nyang'au *et al*., 2017; Sisay *et al*., 2017; Talreja, 2010).

2.4 *Cucumis dipsaceus*

2.4.1 Botanical classification

Cucumis dipsaceus Ehrenb. Ex Spach, is a flowering plant of Cucurbitaceae family (CABI, n.d.). The Cucurbitaceae family includes other nutritionally and medically important plants such as cucumber, musk melon, gourds, watermelon, and pumpkin.

2.4.2 Description and distribution

Cucumis dipsaceus is a short-lived annual climbing herb, with weak stems, often growing along paths and fences. Its leaves are ovate and shallowly trilobed, with dense hairs on the upper and lower surfaces. The plant is native to East Africa, especially in Ethiopia, Kenya and Somalia, and is well distributed in Tropical and Subtropical regions of the world (CABI, n.d.).

2.4.3 Ethno-medicinal uses of *C. dipsaceus*

Leaf poultices of *C. dipsaceus* are used traditionally to treat wounds, and fruit juice is taken with milk as an antidote against poisoning. Leaf and fruit preparations are widely used to treat diarrhea, diabetic complications, tuberculosis, snakebite envenomation, stomach pains, among other uses, in traditional medicine (Kareru *et al*., 2007; Kaur and Lata, 2019; Lulekal *et al*., 2008; Zenebe *et al.*, 2012), and as food (Mutie *et al.*, 2020).

2.4.4 Phytochemistry and biological activity of *C. dipsaceus*

Proximate analysis of fruit extracts has shown the presence of carbohydrates, fatty acids, mucins, and essential amino acids, such as alanine, valine, isoleucine, methionine (Kaur and Lata, 2019). Other phytochemicals contained by this plant, such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids, and glycosides have been documented (Kaur and Lata, 2019).

Fruit extracts of *C. dipsaceus* have antioxidant activity, high concentrations of antioxidant - associated phytochemicals (phenolics, flavonoids and tannins) (Nivedhini *et al*., 2014), and its aerial extracts have demonstrated cytotoxic properties against K652 and Hep2 tumour cell lines (Lata and Mittal, 2015). Leaf extracts of *C. dipsaceus* have antibacterial activity against *E. coli, S. aureus, P. aeruginosa, P. vulgaris* and *B. substilis* (Gauniyal and Teotia, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and preparation of plant materials

Leaves and fruits of *Cucumis dipsaceus* were harvested from cultivated regions of Nkando sublocation, Mbaaria location, Kiirua division, Buuri sub-county in Meru County of Kenya in February 2020 (hot and dry season).

The plant was locally identified by the help of a herbalist as '*Kagerema'* and was selected for the current study based on its ethnomedical information. Voucher specimens were prepared, taxonomically authenticated (NMK/BOT/CTX1/8) and duplicates were archived for future reference.

The gathered leaves and mature fruits were washed gently to remove dirt and spread on a bench to dry for four weeks at room temperature (25^{o}C) in order to dry. An electric plant mill (Laboratory mill serial no. 17180, Christy Hunt Engineering Ltd. England) was used to crash the dried leaves and fruits separately into coarse powders, which were weighed and packaged separately in labelled khaki envelopes while awaiting extraction (Nostro *et al*., 2000). Plates 1a-1d shows the photographs of *C. dipsaceus* captured *in situ.*

Plate 1a: A photograph showing a maturing fruit of ***Cucumis dipsaceus***

Plate 1b: A photograph showing a flower and climbing tendrils of ***Cucumis dipsaceus***

Plate 1c: A photograph showing leaves of ***Cucumis dipsaceus***

Plate 1d: A photograph of the Researcher (Dr. Purity Kimathi) and Herbalist harvesting leaves and fruits of ***Cucumis dipsaceus***

3.2 Extraction methods

The cold and hot maceration methods were employed using HPLC grade methanol and distilled water respectively, in order to obtain the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus*, respectively, following standard procedures described by Harborne (1998) and modified by Moriasi *et al*. (2020).

3.2.1 Methanolic extraction procedure

The cold maceration procedure was adopted. Briefly, 400 g of the dry leaf and fruit powders of *C. dipsaceus* were macerated separately in 1000 ml of pure methanol and shaken gently and allowed to extract for 48 hrs with regular shaking. The mixtures were filtered through cotton wool rolls into clean conical flasks. The resultant filtrates were filtered again through Whatman papers (No. 1) into round bottomed flasks and concentrated *in vacuo* at 50 ºC. The resultant extracts were poured into labelled pre- weighed sample bottles and dried completely at 35 ^{o}C in a hot-air oven. Then, the percentage yields of the methanolic leaf and fruit extracts were calculated using the formulae of Truong *et al*. (2019) as follows:

$$\% \text{ Yield} = \frac{(\text{Weight of sampe bottle+extract}) - \text{Weight of sample bottle}}{\text{Weight of macerated powder}} \times 100$$

3.2.2 Aqueous extraction procedure

The hot maceration method was used for aqueous extraction. Briefly, 400 grams of the dried fruit and leaf powders of *C. dipsaceus* were soaked in 2.75 litres of distilled water and heated in a water bath at 60^{o}C for two hours. The mixtures were first filtered, as described in section 3.2.1, and transferred into 200 ml freeze-drying flasks and lyophilised for 48 hours. The lyophilised extracts were transferred into clean pre-weighed sample bottles and weighed, and the percentage yields determined using the formula in section 3.2.1.

3.3 Experimental animals

In this study, adult Wistar rats, aged 8-12 weeks and weighing 110 grams to 120 grams were acquired from the animal breeding facility of the Department of Public Health,

Pharmacology and Toxicology (PHPT), University of Nairobi (UoN). The animals were used as models, to investigate the anti-inflammatory, antidiarrhea and acute oral toxicity properties of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus.* They were housed in polypropylene cages supplemented with bedding material from softwood shavings and offered clean drinking water and standard rodent pellets *ad-libitum.* White female adult New Zealand rabbits aged 12 weeks and weighing 2.8-3.0 Kg were used for acute dermal irritation test. All the experimental animals were acclimatised to laboratory conditions (Temperature: 25±2 ºC; Relative humidity: 55-61%; 12 hours of dark and 12 hrs of light cycle) for 7 days before experimentation. The animals were handled humanely and manipulated according to the guidelines described by the OECD (2008; 2017) and the Biosafety, Animal Use and Ethics Committee (BAUEC) of the University of Nairobi.

3.3.1 Preparation of experimental dosages

Following pilot studies, three dose levels of 10 mg/Kg BW, 50 mg/Kg BW, and 250 mg/Kg BW, and, 100 mg/Kg BW, 200 mg/Kg BW, and 400 mg/Kg BW were selected for the determination of anti-inflammatory and antidiarrhea activities, respectively, of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus*. The extract doses for acute oral and dermal toxicity assays were prepared following the OECD procedures (OECD, 2008; 2017).The OECD/OCDE dosage preparation guidelines (OECD, 2008) illustrated by Erhierhie *et al*. (2014) were followed in preparing stock solutions, which were serial diluted in order to achieve the required doses for administration. The following formula was used to prepare stock doses.

$$\text{Animal equivalent dose} = \frac{\text{Weight of experimental animal}}{1000\ \text{g}} \times \text{Selected dose (mg)}$$

3.4 Investigation of acute toxicity effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus*

3.4.1 Acute oral toxicity effects of the studied plant extracts in Wistar rats

The Up-and-Down-Procedure (UDP) for acute oral toxicity study described by the OECD/OCDE (OECD, 2008) was followed. Briefly, six experimental rats were selected randomly and divided into two groups, each consisting of three rats (control group and experimental group) for each studied plant extract. The rats were marked on their tails using a red permanent marker pen for easy identification and fasted for four hours before dosing. Doses of 175 mg/kg BW, 550 mg/kg BW and 2000 mg/Kg BW of each study extract was orally given to the experimental groups of rats, while 10 ml/Kg BW of normal saline was administered into the control groups of rats orally. Thereafter, the experimental animals were individually monitored for signs of toxicity by observing the appearance of mucous membrane, body weight, sleep, eyes, diarrhea, skin fur, salivation, tremors, convulsions, coma, lethargy and mortality after 30 minutes, 1 hour, 2 hours, 4 hours, 24 hours, 48 hours, 7 days and 14 days, respectively, as per the OECD Guidelines (OECD, 2008), and recorded.

3.4.2 Acute dermal irritation /corrosion test on New Zealand White rabbits

The acute dermal irritation/corrosion test described by the OECD (2017) was adopted in order to investigate the acute dermal toxicity of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* on intact and abraded skins of five (5) healthy New Zealand White rabbits. Briefly, four healthy rabbits, with intact skins, were randomly selected for each studied extract. The rabbit fur on the dorsal area of the trunk was carefully clipped, approximately twenty-four hours prior to the test, and only the rabbits with intact skin were used.

Two patches were created on each rabbit for intact skin test and abraded skin test. For the abraded skin test, minor abrasions were carefully made on the skin using the tip of the needle through the stratum corneum (not sufficiently deep to reach the dermis to avoid bleeding).

After that, 0.5 ml of 50 % w/v of the plant extracts under study at a dose of 2000 mg/Kg BW were moistened and applied (applied area=6 cm^2), covered with a gauze patch, and fixed with a non-irritating tape. The untreated skin areas of the test animals served as the control and were applied topical glycerol. Ingestion or inhalation of the extracts by the rabbits was avoided. The intensity of irritation and/corrosion was observed and scored after thirty minutes, one hour, two hours, four hours, six hours, twenty-four hours, forty-eight hours, and seventy-two hours, and recorded. Any residue of the applied extract was removed carefully using distilled water without interfering with epidermis integrity.

3.5 Anti-inflammatory activity of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* in Wistar rats

The formalin-induced paw-oedema technique described by Hunskaar and Hole, (1987) and modified by Shojaii *et al*. (2015) was used to investigate the anti-inflammatory activities of methanolic and aqueous leaf and fruit extracts in Wistar rats according to the experimental design summarised in Table 3.1. The experimental rats were divided randomly into 5 groups, each comprising of five rats (n = 5). The first, second, and third groups orally received the plant extracts at doses of 10, 50 and 250 mg/Kg BW, respectively. The fourth group of rats were given diclofenac (4 mg/Kg BW) orally and they served as positive control group. The fifth group of rats were orally administered with 10 ml/Kg BW of normal saline and they served as negative control group.

The right hind paw diameters of all experimental rats were measured using digital vernier calliper and recorded as the baseline. After 30 minutes, 0.1 mL of 2.5 % formalin was injected in the sub-plantar area to evoke oedema (inflammation). The diameter of the formalin-injected paw was measured using a digital vernier callipers (Ugo Basile, Italy) hourly for five hours. The percentage inhibition/suppression of formalin-induced inflammation (oedema) was calculated according to the following formula.

$$\%\ Inhibition\ of\ inflammation = \frac{PSD1 - PSD0}{PSD0} \times 100$$

Where, *PSD*1= Paw size diameter after induction of oedema; and *PSD*0= Paw size diameter before induction of oedema.

Table 3.1: Experimental design for the investigation of the anti-inflammatory activity of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* in Wistar rats

Group	Status	Treatment
I	Experimental-1	10 mg/Kg BW extract + 100 µl of 2.5 % of formalin
II	Experimental-2	50 mg/Kg BW extract + 100 µl of 2.5 % of formalin
III	Experimental-3	250 mg/Kg BW extract + 100 µl of 2.5 % of formalin
IV	Positive control	4 mg/Kg BW Diclofenac + 100µl of 2.5 % of formalin
V	Negative Control	10 ml/Kg BW normal saline + 100 µl ml of 2.5% of formalin

Extract: Aqueous/Methanolic leaf/fruit extract of *C. dipsaceus;* n= 5 rats per group.

3.6 Determination of the effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on gastrointestinal motility

3.6.1 Determination of antidiarrhea activity of the studied plant extracts in Wistar rats

The castor-oil induced diarrhea method of Rahman *et al*. (2019) was adopted in this study. Briefly, experimental rats were fasted for 18 hrs and divided randomly into 5 groups (n=5). Groups I-III were orally administered with the plant extracts at doses of 100, 200, and 400 mg/Kg BW, respectively. Group IV rats were treated with 3 mg/Kg BW of Loperamide and were the positive control. Group V rats were given 10 ml/Kg BW of normal saline and represented the negative control of the experiment. After one hour, all the experimental rats were orally treated with 1 ml of castor oil and kept in separate metabolic cages lined with adsorbent paper.

The average number of dry faeces, and wet diarrhea droppings were recorded and were used to determine the percentage inhibition of diarrhea in experimental rats, as a measure of antidiarrhea activity. Table 3.2 presents the experimental design.

Table 3.2: Experimental design for the investigation of the antidiarrhea activity of the aqueous and methanolic leaf and fruit extracts of C. dipsaceus in Wistar rats

Group	Status	Treatment
I	Experimental-1	100 mg/Kg BW extract + 100 µl of castor oil
II	Experimental-2	200 mg/Kg BW extract + 100 µl of castor oil
III	Experimental-3	400 mg/Kg BW extract + 100 µl of castor oil
IV	Positive control	3 mg/Kg BW Loperamide + 100 µl of castor oil
V	Negative Control	10 ml/Kg BW normal saline + 100 µl of castor oil

Extract: Aqueous/Methanolic leaf/fruit extract of *C. dipsaceus;* n= 5 rats per group.

3.6.2 Determination of the effects of the plant extracts on intestinal contraction of isolated rabbit ileum

The method of Rahman *et al*. (2019) was adopted in this study. The experimental rabbits were fasted for 18 hours prior to the study, after which, they were sacrificed, and the ileum dissected out and washed off mesenteries. The isolated ileum was sectioned into segments measuring 2.5 cm long, which were hung onto an organ bath containing Tyrode solution. The temperature of the organ bath was maintained at 37^{o}C, with continuous supply of carbogen. Tension (1 g) was applied, and the tissues were allowed to stabilize for 45 minutes. They were then treated with acetylcholine, which was supplied at intervals of 3 minutes. Then, 100, 200, and 400 mg/Kg BW of methanolic and aqueous leaf and fruit extract were applied, and their motility determined as the percentage of ileum contractions obtained immediately before and after application. The responses were recorded with transducers which were coupled to a data recorder.

3.7 Determination of the antimicrobial activity of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus*

3.7.1 Microbial strains

The antimicrobial activity of the studied plant extracts was investigated using *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 25922), *Salmonella enteriditis* (ATCC 13076), Bacillus *subtilis (*ATCC 6051), and *Candida albicans* (AT 10231), which were selected based on their clinical relevance. They were were retrieved from the microbiology laboratory of the PHPT UoN.

3.7.2 Preparation and standardisation of microbial inocula

The bacterial strains used in this study (*E. coli*, *P. aeruginosa, S. enteriditis* and, *B. subtilis*) were cultured in Mueller-Hinton for 24 hours according to the procedures recommended by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2014). After that, the bacterial colonies

were isolated and standardized using normal saline in order to achieve a turbidity corresponding to 0.5 McFarland scale of about 1-2×10^8 colony forming units (cfu) per milliliter (cfu/ml) of solution of the inocula, to get absorbance of 0.10-0.15 at wavelength of 530 nm, using a spectrophotometer (CLSI, 2014). The obtained inocula were used to inoculate the discs in the disc diffusion technique and in the broth microdilution assay for determining the Minimum Inhibitory Concentrations (MICs), accordingly.

Similarly, the CLSI procedure for culturing and standardization of fungal microbes was followed in preparing *C. albicans* for experimentation (CLSI, 2014). Briefly, the stock of *C. albicans* was retrieved and cultured in Sabouraud dextrose agar (SDA; Oxoid) for 24 hours. Then, the colonies were isolated and standardized using normal saline to a 0.5 McFarland standard (1-5×10^6 cfu/ml), with absorbance of 0.11-0.14 at a wavelength of 530 nm using a spectrophotometer (CLSI, 2014). The prepared inoculum was used for the disc diffusion and broth microdilution experiments accordingly.

3.7.3 Preparation of extracts

The methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* were weighed accurately (1 mg) and suspended in 10 ml of 1.4 % of Dimethyl sulphoxide (DMSO), in clean labelled 15 ml Eppendorf tubes. The respective tubes were then vortexed vigorously for 30 minutes to ensure complete dissolution. The resulting stock solutions had 100 µg/ml of the respective extracts. The stock solutions were then diluted two-fold by applying the serial dilution technique in order to obtain lower concentrations of 50, 25, 12.5, 6.25 and 3.125 µg/ml, respectively.

3.7.4 The disc diffusion assay

Circular disks measuring six millimeters in diameter were made by poking Whatman filter papers (No.1), and sterilizing in an autoclave at 121 ^{o}C, for 15 minutes. The discs were then arranged appropriately on the solidified media in petri dishes (5 per plate). Markings were made at the bottom of each plate to identify the respective discs and type of treatment applied, and the strain of microbe under study. Then, using a sterile micropipette, 20 µl of each studied plant extracts, at respective concentrations, were aspirated and dispensed carefully onto respective discs. The discs were then gently but firmly pressed onto the media inoculated with 1 ml of the respective microbial strains, used in this study,in order/ to ensure proper contact.

The experiments were set up in triplicate, with Dimethyl sulphoxide (DMSO) as negative control, and Nystatin (for the fungal strain) and Ciprofloxacin (for bacterial strains) as positive controls. The plates were transferred into an incubator set at 37^{o}C and incubated for 24 hours. After incubation, the plates were examined, and a digital zone reader was used to measure the inhibition zones of microbial growth in millimeters (mm).

3.7.5 Determination of Minimum Inhibitory Concentration

The Clinical and Laboratory Standard Institute method (CLSI, 2014) which was modified by Golus *et al*. (2016) was used to determine the MIC of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus,* following the disc diffusion assay. Briefly 10 µl of the respective plant extracts, which were prepared previously (section 3.7.3) were transferred into clean and labeled Eppendorf tubes with 90 µl of molten Mueller-Hinton agar in triplicate, and vortexed gently. 200 µl of the molten agar containing the studied plant extracts, were aspirated using a micropipette and dispensed into sterile 96-U-shaped multiwell plates, and micro-diluted serially in two-fold, at 100 µl volumes.

The respective positive (Ciprofloxacin/ Nystatin) and negative controls were also included in each micro-titre plate using the same procedure. The plates were left undisturbed at ambient temperature (25 ^{o}C) for 30 minutes to facilitate solidification of the agar. Afterwards, 2 µl of respective inocula containing 10^4 cfu, were carefully dispensed into each well with a multichannel micropipette and allowed to mix with the treated media for 10 minutes at 25 ^{o}C. Sterile water was added to the wells around the multiwell plates to moisturize the plates and avoid dehydration during incubation. The plates were then wrapped in nylon bags and kept at 37^{o}C for 18 hours. After that, 2 µl of freshly prepared resazurin dye was added into each well, and plates were further incubated for 45 minutes at 37^{o}C in an incubator. The plates were carefully examined visually based on the resazurin color changes, and the lowest concentration of the studied extracts and positive control drugs, that completely inhibited microbial growth, was considered as the MIC according to the CLSI Guidelines (CLSI, 2014).

3.8 Statistical management and data analysis

Quantitative data, which was obtained from the anti-inflammatory and gastrointestinal motility experiments, were recorded in a Microsoft spreadsheet and then exported to GraphPad Prism statistical software version 9.1 for analysis. Descriptive statistics were performed and mean ± standard error of the mean (SEM) ($\bar{x} \pm SEM$) of replicate experiments was calculated. One-way or Two-way ANOVA were performed as appropriate, to determine whether there were significant differences among means. This was followed by Tukey's *post hoc* test for pairwise comparisons and separations of means.

Un-paired student *t*-test statistic was used to compare the differences between two independent variables (intestinal contraction frequency before vs after treatment). During data analysis, the significance levels of $P<0.05$ and $P< 0.0001$ were considered. Acute dermal and acute oral toxicity data were tabulated and the respective median lethal

concentrations of the studied plant extracts (LD_{50} values), were determined and interpreted according to the OECD Guidelines (OECD, 2008; 2017).

3.9 Ethical considerations

This research was performed after ethical clearance by the University of Nairobi, Biosafety, Animal Use, and Ethics Committee (FVM BAUEC/2020/264). Furthermore, a research license was obtained from the National Commission for Science, Technology, and Innovation (NACOSTI) (NACOSTI/P/21/11835). The experimental animals were cared for, handled, manipulated, and disposed of as per the guidelines and procedures set out by the University of Nairobi Biosafety, Animal Use and Ethics Committee, NACOSTI and the OECD (2008; OECD 2017). All the chemicals, which were used, were disposed according to the manufacture's guidelines and the respective Material Safety and Data Sheets (MSDS).

CHAPTER FOUR

RESULTS

4.1 Anti-inflammatory activity of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* in Wistar rats

In this study, aqueous fruit extracts of *C. dipsaceus* exhibited a significant dose-dependent inhibition of inflammation in each hour up to the fourth hour ($P<0.05$ Table 4.1). However, the percentage inhibitions of inflammation at extract doses of 50 mg/Kg BW and 250 mg/Kg BW, in Wistar rats were not significantly different ($P>0.05$; Table 4.1).

A significant dose-dependent increase in the percentage of inhibition of inflammation was observed in rats which received the aqueous leaf extract of *C. dipsaceus* in the first and fifth hour ($P<0.05$; Table 4.1). In the first and second hour, respectively, no significant differences in the percentage inhibition of inflammation were observed in Wistar rats, which were treated with the aqueous leaf extracts of *C. dipsaceus* in the second and third hour, respectively ($P>0.05$; Table 4.1). The percentage of inhibition of inflammation rats administered with the 50 mg/Kg BW and 250 mg/Kg BW of the aqueous leaf extract of *C. dipsaceus*, were comparable in the fourth hour ($P>0.05$; Table 4.1).

At dose levels of 10 mg/Kg BW and 50 mg/Kg BW, the methanolic fruit extract of *C. dipsaceus* showed no significant difference in the percentage inhibition of inflammation in the treated rats in the first and fifth hours, respectively ($P>0.05$; Table 4.1). However, at a dose of 250 mg/Kg BW, this extract exhibited a significantly higher percentage inhibition of inflammation compared with those recorded at the two lower dose levels ($P<0.05$; Table 4.1).

In the second hour, no significant differences between the percentage inhibitions of inflammation were recorded in rats which received the methanolic fruit extracts of *C. dipsaceus* at doses of 50 mg/Kg BW and 250 mg/Kg BW ($P>0.05$). However, these

percentages of inhibition of inflammation were significantly higher than those recorded at a dose of 10 mg/Kg BW, in the second hour ($P<0.05$; Table 4.1). Significant dose-dependent increases in the percentage inhibitions of inflammation were observed in rats which received the methanolic fruit extracts of *C. dipsaceus* in the third and fifth hours ($P<0.05$; Table 4.1).

The anti-inflammatory effects of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* were also compared across the five-hour experimental period in this study. The aqueous leaf extracts (AQL) of the studied plant showed a significantly higher anti-inflammatory activity in the second hour, at all dose levels, and significantly decreased with time towards the fifth hour ($P<0.05$; Table 4.1). At dose levels of 10 mg/Kg BW and 250 mg/Kg BW, respectively, the aqueous fruit extracts of *C. dipsaceus* exhibited significantly higher anti-inflammatory effects in the fourth hour. In the fifth hour, the aqueous fruit extracts of *C. dipsaceus* exhibited significantly higher anti-inflammatory effects in Wistar rats at a dose of 50 mg/Kg BW ($P<0.05$; Table 4.1).

On the other hand, all the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus*, except the methanolic leaf extract at a dose of 10 mg/Kg BW, showed significantly higher anti-inflammatory activity in the third hour than the other hours ($P<0.05$; Table 4.1). The positive control drug (4 mg/Kg BW Diclofenac) recorded a significant time-dependent increase in anti-inflammatory activity across the experimental period ($P<0.05$; Table 4.1). The anti-inflammatory effects of the studied plant extracts were significantly influenced by time, type of extract, and dose level, in this study (Table 4.1).

Table 4.: Anti-inflammatory activity of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* in Wistar rats

Treatment Extract (mg/Kg BW)	% Inhibition of inflammation				
	1st Hr.	2nd Hr.	3rd Hr.	4th Hr.	5th Hr.
AQF 10	$55.88\pm0.36^{c}_{c}$	$70.26\pm0.40^{c}_{b}$	$73.54\pm0.95^{c}_{ab}$	$74.15\pm0.82^{c}_{a}$	$73.90\pm0.81^{c}_{ab}$
AQF 50	$66.86\pm0.69^{b}_{d}$	$79.93\pm0.34^{b}_{c}$	$80.69\pm0.29^{b}_{c}$	$86.59\pm0.96^{b}_{b}$	$92.95\pm0.95^{b}_{a}$
AQF 250	$78.34\pm0.32^{a}_{d}$	$90.92\pm3.35^{a}_{c}$	$95.47\pm0.63^{a}_{ab}$	$97.59\pm0.82^{a}_{a}$	$93.94\pm0.24^{b}_{b}$
AQL 10	$17.06\pm0.27^{f}_{cd}$	$51.90\pm0.66^{d}_{a}$	$43.87\pm0.40^{f}_{b}$	$12.61\pm0.71^{i}_{e}$	$13.79\pm0.97^{g}_{de}$
AQL 50	$29.14\pm0.36^{e}_{c}$	$51.82\pm0.37^{d}_{a}$	$39.22\pm0.40^{f}_{b}$	$36.58\pm0.43^{g}_{b}$	$25.03\pm0.41^{f}_{d}$
AQL 250	$47.02\pm1.09^{d}_{b}$	$51.41\pm0.91^{d}_{a}$	$44.93\pm0.53^{f}_{b}$	$35.66\pm0.82^{g}_{c}$	$32.22\pm0.63^{e}_{c}$
MEoHF 10	$11.03\pm0.62^{g}_{d}$	$17.57\pm0.43^{g}_{c}$	$29.45\pm0.72^{g}_{a}$	$26.12\pm0.21^{h}_{b}$	$27.58\pm0.44^{f}_{b}$
MEoHF 50	$12.62\pm0.27^{fg}_{d}$	$29.06\pm1.26^{f}_{c}$	$39.60\pm0.21^{f}_{a}$	$30.02\pm0.40^{h}_{b}$	$32.73\pm0.41^{e}_{b}$
MEoHF 250	$27.06\pm0.71^{e}_{c}$	$33.51\pm0.25^{f}_{b}$	$62.20\pm0.47^{d}_{a}$	$46.01\pm0.34^{f}_{b}$	$43.50\pm0.84^{d}_{b}$
MEoHL 10	$1.56\pm0.27^{h}_{d}$	$6.85\pm0.80^{h}_{c}$	$27.85\pm0.76^{g}_{a}$	$30.50\pm0.97^{h}_{a}$	$13.37\pm0.39^{g}_{b}$
MEoHL 50	$17.22\pm0.41^{f}_{d}$	$26.59\pm0.34^{f}_{c}$	$54.60\pm0.43^{e}_{a}$	$50.86\pm0.78^{e}_{bc}$	$46.09\pm0.68^{d}_{b}$
MEoHL 250	$24.16\pm1.37^{e}_{d}$	$40.86\pm4.88^{e}_{c}$	$62.17\pm1.31^{d}_{a}$	$58.76\pm0.94^{d}_{a}$	$50.52\pm0.70^{d}_{b}$
Diclofenac 4	$75.90\pm1.19^{a}_{c}$	$94.50\pm0.39^{a}_{b}$	$97.59\pm0.58^{a}_{ab}$	$98.75\pm0.30^{a}_{a}$	$98.97\pm0.29^{a}_{a}$

Values are presented as $\bar{x} \pm SEM$; Values with different lowercase superscript alphabets within the same column, and those with different subscript lowercase alphabets in the same row are significantly different (P<0.05; Two-Way ANOVA with Tukey's *post hoc* test). AQL: Aqueous leaf extract of *C. dipsaceus*; AQF: Aqueous fruit extract of *C. dipsaceus*; MEoHF: Methanolic fruit extract of *C. dipsaceus*; MEoHL: Methanolic leaf extract of *C. dipsaceus*.

4.2 Antimicrobial effects of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on selected bacteria and *candida albicans*

The antimicrobial efficacy of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* on *S. enteriditis, P. Aeruginosa, E. coli, B. subtilis,* and *C. albicans* were investigated. The results revealed that the growth inhibition zones of *S. enteriditis*, produced by the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* at concentrations of

3.125 µg/ml, 6.25 µg/ml, and 12.5 µg/ml were not significantly different ($p>0.05$; Table 4.2). However, these inhibition zones were significantly smaller than those obtained at concentrations of 25 µg/ml, 50 µg/ml, and 100 µg/ml, indicating concentration dependent increase in antibacterial activity ($P<0.05$; Table 4.2).

At a concentration of 100 µg/ml, the aqueous leaf extract of *C. dipsaceus* produced a significantly bigger inhibition zone on *S. enteriditis* than the other extracts ($P<0.05$; Table 4.2). Notably, the standard drug (10 µg/ml Ciprofloxacin) produced significantly higher inhibition zones of *S. enteriditis* growth than all the tested plant extracts ($P<0.05$; Table 4.2).

Table 4.: Antimicrobial effects of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on *S. enteriditis*

Treatment	Concentration (µg/ml)	Inhibition zone of *S. enteriditis* growth in mm			
		AQL	AQF	MEoHL	MEoHF
C. dipsaceus	3.125	$6.00\pm0.00^{e}_{a}$	$6.00\pm0.00^{d}_{a}$	$6.00\pm0.00^{d}_{a}$	$6.00\pm0.00^{d}_{a}$
	6.25	$6.00\pm0.00^{e}_{a}$	$6.00\pm0.00^{d}_{a}$	$6.00\pm0.00^{d}_{a}$	$6.00\pm0.00^{d}_{a}$
	12.5	$6.00\pm0.00^{e}_{a}$	$6.00\pm0.00^{d}_{a}$	$6.00\pm0.00^{d}_{a}$	$6.00\pm0.00^{d}_{a}$
	25	$9.00\pm0.00^{d}_{a}$	$9.33\pm0.33^{c}_{a}$	$10.33\pm0.33^{c}_{a}$	$10.00\pm0.58^{c}_{a}$
	50	$11.33\pm1.33^{c}_{a}$	$10.67\pm0.33^{c}_{a}$	$11.67\pm0.33^{c}_{a}$	$11.33\pm0.33^{c}_{a}$
	100	$15.67\pm0.33^{b}_{a}$	$13.33\pm0.33^{b}_{b}$	$14.67\pm0.33^{b}_{ab}$	$13.33\pm0.33^{b}_{b}$
Ciprofloxacin	10	35.67 ± 0.33^{a}	35.67 ± 0.33^{a}	35.67 ± 0.33^{a}	35.67 ± 0.33^{a}

Values are expressed as $\bar{X} \pm SEM$; Means with different superscript alphabets within the same column, and those with different subscript alphabets within the same row are significantly different ($P< 0.05$; Two-Way ANOVA with Tukey's post *hoc test*). AQL: Aqueous leaf extract of *C. dipsaceus*; AQF: Aqueous fruit extract of *C. dipsaceus*; MEoHF: Methanolic fruit extract of *C. dipsaceus*; MEoHL: Methanolic leaf extract of *C. dipsaceus*.

The *E. coli* growth inhibition zones produced by the aqueous leaf and fruit, and methanolic leaf and fruit extracts of *C. dipsaceus*, at concentrations of 3.125 µg/ml, 6.25 µg/ml and 12.5 µg/ml, were not significantly different (P>0.05; Table 4.3). Generally, a significant concentration-dependent increase in *E. coli* growth inhibition by all the studied plant extracts was observed in this study (p<0.05; Table 4.3). In addition, the methanolic fruit extracts of *C. dipsaceus* produced significantly bigger *E. coli* growth inhibition zones, at all the tested concentrations except at 10 µg/ml, compared with the other extracts (P<0.05; Table 4.3). Ciprofloxacin produced significantly bigger *E. coli* growth inhibition zones than all the tested plant extracts (P<0.05; Table 4.3).

Table 4.3: Antimicrobial effects of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on *E. coli*

Treatment	Concentration (µg/ml)	Inhibition zone of *E. coli* growth in mm			
		AQL	AQF	MEoHL	MEoHF
C. dipsaceus	3.125	$6.00\pm0.00^{e}_{a}$	$6.00\pm0.00^{c}_{a}$	$6.00\pm0.00^{c}_{a}$	$6.00\pm0.00^{e}_{a}$
	6.25	$6.00\pm0.00^{e}_{b}$	$6.00\pm0.00^{c}_{b}$	$6.00\pm0.00^{c}_{b}$	$7.33\pm0.33^{d}_{a}$
	12.5	$6.00\pm0.00^{e}_{b}$	$6.00\pm0.00^{c}_{b}$	$6.00\pm0.00^{c}_{b}$	$9.00\pm0.00^{c}_{a}$
	25	$9.00\pm0.00^{d}_{a}$	$6.00\pm0.00^{c}_{c}$	$7.33\pm0.33^{c}_{b}$	$9.67\pm0.33^{c}_{a}$
	50	$10.33\pm0.33^{c}_{b}$	$6.00\pm0.00^{c}_{d}$	$8.33\pm0.33^{c}_{c}$	$12.33\pm0.33^{b}_{a}$
	100	$12.33\pm0.33^{b}_{b}$	$7.33\pm0.33^{b}_{d}$	$9.67\pm0.33^{b}_{c}$	$13.33\pm0.33^{b}_{a}$
Ciprofloxacin	10	40.00 ± 0.00^{a}	40.00 ± 0.00^{a}	40.67 ± 0.67^{a}	40.67 ± 0.67^{a}

Values are expressed as $\bar{X} \pm SEM$; Means with different superscript alphabets within the same column, and those with different subscript alphabets within the same row are significantly different (P<0.05; Two-Way ANOVA with Tukey's post *hoc test*). AQL: Aqueous leaf extract of *C. dipsaceus*; AQF: Aqueous fruit extract of *C. dipsaceus*; MEoHF: Methanolic fruit extract of *C. dipsaceus*; MEoHL: Methanolic leaf extract of *C. dipsaceus*.

There were no significant differences between the *P. aeruginosa* growth inhibition zones produced by the aqueous and methanolic leaf extracts of *C. dipsaceus*, at concentrations of 3.125 µg/ml and 6.25 µg/ml (P>0.05; Table 4.4). Overall, each of the plant extracts exhibited a significant concentration-dependent inhibition of *P. aeruginosa* growth, as evidenced by the increasing zones of inhibition (P<0.05; Table 4.4).

Notably, the methanolic fruit extract of *C. dipsaceus* produced significantly bigger inhibitions of *P. aeruginosa* growth, at all concentrations, compared with the zones produced by all the other extracts (P<0.05; Table 4.4). Additionally, Ciprofloxacin exhibited significantly bigger inhibition zones on *P. aeruginosa* than all the extracts of *C. dipsaceus* (P<0.05; Table 4.4).

Table 4.4: Antimicrobial effects of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on *P. aeruginosa*

Treatment	Concentration (µg/ml)	Inhibition zone of *P. aeruginosa* growth in mm			
		AQL	AQF	MEoHL	MEoHF
C. dipsaceus	3.125	$6.00\pm0.00^{f}_{c}$	$7.00\pm0.00^{f}_{b}$	$6.00\pm0.00^{e}_{c}$	$10.00\pm0.00^{g}_{a}$
	6.25	$6.00\pm0.00^{f}_{c}$	$7.00\pm0.00^{f}_{b}$	$6.00\pm0.00^{e}_{c}$	$12.33\pm0.33^{f}_{a}$
	12.5	$7.00\pm0.00^{e}_{c}$	$8.00\pm0.00^{e}_{b}$	$6.00\pm0.00^{e}_{d}$	$15.33\pm0.33^{e}_{a}$
	25	$8.00\pm0.00^{d}_{c}$	$9.33\pm0.33^{d}_{b}$	$8.33\pm0.33^{d}_{c}$	$16.67\pm0.33^{d}_{a}$
	50	$9.00\pm0.00^{c}_{d}$	$11.67\pm0.33^{c}_{b}$	$10.00\pm0.00^{c}_{c}$	$18.00\pm0.00^{c}_{a}$
	100	$11.67\pm0.33^{b}_{d}$	$14.67\pm0.33^{b}_{b}$	$13.67\pm0.33^{b}_{c}$	$20.33\pm0.33^{b}_{a}$
Ciprofloxacin	10	$32.67\pm0.33^{a}_{a}$	$33.00\pm0.00^{a}_{a}$	$32.33\pm0.33^{a}_{a}$	$32.67\pm0.33^{a}_{a}$

Values are expressed as $\bar{X} \pm SEM$; Means with different superscript alphabets within the same column, and those with different subscript alphabets within the same row are significantly different (P<0.05; Two-Way ANOVA with Tukey's post *hoc test*). AQL: Aqueous leaf extract of *C. dipsaceus*; AQF: Aqueous fruit extract of *C. dipsaceus*; MEoHF: Methanolic fruit extract of *C. dipsaceus*; MEoHL: Methanolic leaf extract of *C. dipsaceus*.

There were no significant differences between the zones of inhibition produced by *B. subtilis* at concentrations of 3.125 µg/ml and 6.25 µg/ml of each studied extracts (P<0.05; Table 4.5). The methanolic fruit extracts of *C. dipsaceus* (50 µg/ml and 100 µg/ml) produced significantly bigger *B. subtilis* growth inhibition zones, compared with all the other extracts of similar concentrations in this study (P<0.05; Table 4.5). Similarly, aqueous fruit extracts of *C. dipsaceus* (12.5 µg/ml and 25 µg/ml) showed significantly higher inhibitions of *B. subtilis* growth than other extracts (P<0.05; Table 4.5). Significantly bigger inhibition zones were produced by Ciprofloxacin compared to all the other plant extracts (P<0.05; Table 4.5).

Table 4.5: Antimicrobial effects of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on *B. subtilis*

Treatment	**Concentration (µg/ml)**	**Inhibition zone of *B. subtilis* growth in mm**			
		AQL	**AQF**	**MEoHL**	**MEoHF**
C. dipsaceus	3.125	$6.00\pm0.00^{f}_{a}$	$7.00\pm0.00^{g}_{a}$	$6.00\pm0.00^{g}_{a}$	$6.00\pm0.00^{f}_{a}$
	6.25	$6.00\pm0.00^{f}_{b}$	$8.00\pm0.00^{fg}_{a}$	$6.33\pm0.33^{fg}_{b}$	$6.67\pm0.33^{f}_{b}$
	12.5	$7.00\pm0.00^{ef}_{b}$	$9.33\pm0.33^{ef}_{a}$	$7.67\pm0.33^{ef}_{b}$	$8.00\pm0.00^{e}_{b}$
	25	$8.00\pm0.00^{de}_{c}$	$11.00\pm0.58^{d}_{a}$	$9.33\pm0.33^{d}_{b}$	$9.33\pm0.67^{de}_{b}$
	50	$9.00\pm0.00^{cd}_{c}$	$13.67\pm0.33^{c}_{a}$	$10.67\pm0.33^{cd}_{b}$	$14.67\pm0.33^{c}_{a}$
	100	$13.33\pm0.33^{b}_{c}$	$15.33\pm0.33^{b}_{b}$	$12.33\pm0.33^{b}_{c}$	$17.33\pm0.33^{b}_{a}$
Ciprofloxacin	10	$21.33\pm0.33^{a}_{a}$	$22.00\pm0.58^{a}_{a}$	$22.00\pm0.58^{a}_{a}$	$22.33\pm0.33^{a}_{a}$

Values are expressed as $\bar{X} \pm SEM$; Means with different superscript alphabets within the same column, and those with different subscript alphabets within the same row are significantly different (P<0.05; Two-Way ANOVA with Tukey's post *hoc test*). AQL: Aqueous leaf extract of *C. dipsaceus*; AQF: Aqueous fruit extract of *C. dipsaceus*; MEoHF: Methanolic fruit extract of *C. dipsaceus*; MEoHL: Methanolic leaf extract of *C. dipsaceus*.

The antifungal effects of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* on *C. albicans* were also investigated in this study. The results showed a significant concentration-dependent increase in *C. albicans'* growth inhibition by the aqueous leaf extract of *C. dipsaceus* (P<0.05; Table 4.6). The *C. albicans* growth inhibition by the aqueous fruit, and methanolic leaf and fruit extracts of *C. dipsaceus* was insignificant (P>0.05; Table

4.6). Notably, the *C. albicans'* growth inhibition zone caused by 100 µg/ml of the aqueous leaf extracts of *C. dipsaceus* was similar to that of Nystatin in the study (P>0.05; Table 4.6).

Table 4.6: Antimicrobial effects of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on *C. albicans*

Treatment	Concentration (µg/ml)	Inhibition zone of *C. albicans* growth in mm			
		AQL	AQF	MEoHL	MEoHF
C. dipsaceus	3.125	$7.67\pm0.33^{e}_{a}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$
	6.25	$8.00\pm0.00^{e}_{b}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$
	12.5	$10.00\pm0.00^{d}_{a}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$
	25	$12.00\pm0.00^{c}_{b}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$
	50	$13.33\pm0.33^{b}_{a}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$
	100	$15.00\pm0.58^{a}_{a}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$	$6.00\pm0.00^{b}_{b}$
Nystatin	10	$15.00\pm0.00^{a}_{a}$	$15.00\pm0.00^{a}_{a}$	$15.67\pm0.33^{a}_{a}$	$15.33\pm0.33^{a}_{a}$

Values are expressed as $\bar{X} \pm SEM$; Means with different superscript alphabets within the same column, and those with different subscript alphabets within the same row are significantly different (P<0.05; Two-Way ANOVA with Tukey's post *hoc test*). AQL: Aqueous leaf extract of *C. dipsaceus*; AQF: Aqueous fruit extract of *C. dipsaceus*; MEoHF: Methanolic fruit extract of *C. dipsaceus*; MEoHL: Methanolic leaf extract of *C. dipsaceus*.

The minimum concentrations of the methanolic and aqueous leaf and fruit extracts and standard drugs that could inhibit growth of the selected microbial strains (Minimum Inhibitory Concentration (MIC) were determined in the study (Table 4.7). The aqueous fruit extracts of *C. dipsaceus* showed the lowest MIC of 1.563 µg/ml against *P. aeruginosa* compared with other extracts. On the other hand, aqueous leaf extracts of *C, dipsaceus* showed the lowest MIC of 3.125 µg/ml against the fungal strain (*C. albicans*), while the other extracts were inactive (Table 4.7). In addition, the methanolic leaf extracts of *C. dipsaceus* showed a low MIC value of 6.25 µg/ml against *E. coli*, whereas the methanolic fruit extracts showed lower MIC values (3.125 µg/ml) against *S. enteriditis* and *E. coli* (Table 4.7).

Table 4.7: Minimum Inhibitory Concentrations of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus*

Treatment	Minimum Inhibitory Concentration (MIC) (µg/ml)				
	S. enteriditis	***E. coli***	***P. aeruginosa***	***B. subtilis***	***C. albicans***
AQL	25	25	2.25	6.25	3.125
AQF	12.50	28	1.563	3.125	ND
MEoHL	12.5	6.25	12.5	12.5	ND
MEoHF	3.125	3.125	3.125	25	ND
Standard	0.65	0.32	0.61	0.67	1.5

AQL: Aqueous leaf extract of *C. dipsaceus*; AQF: Aqueous fruit extract of *C. dipsaceus*; MEoHF: Methanolic fruit extract of *C. dipsaceus*; MEoHL: Methanolic leaf extract of *C. dipsaceus*; Standard: for bacterial strains it was Ciprofloxacin (10 µg/ml), while for the fungal strain, it was Nystatin (10 µg/ml); ND: Not Determined.

4.3 Effects of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on gastrointestinal motility in Wistar rats

4.3.1 Inhibition of diarrhea in castor oil-induced diarrhea in Wistar rats

The results showed that, the aqueous fruit extracts of *C. dipsaceus* significantly inhibited diarrhea in castor oil-induced diarrhea in rats, in a positive dose-dependent manner ($P<0.0001$; Figure 4.1). Notably, at a dose of 400 mg/Kg BW of the aqueous fruit extracts of *C. dipsaceus*, the percentage inhibition of diarrhea was like that produced by Loperamide (Figure 4.1).

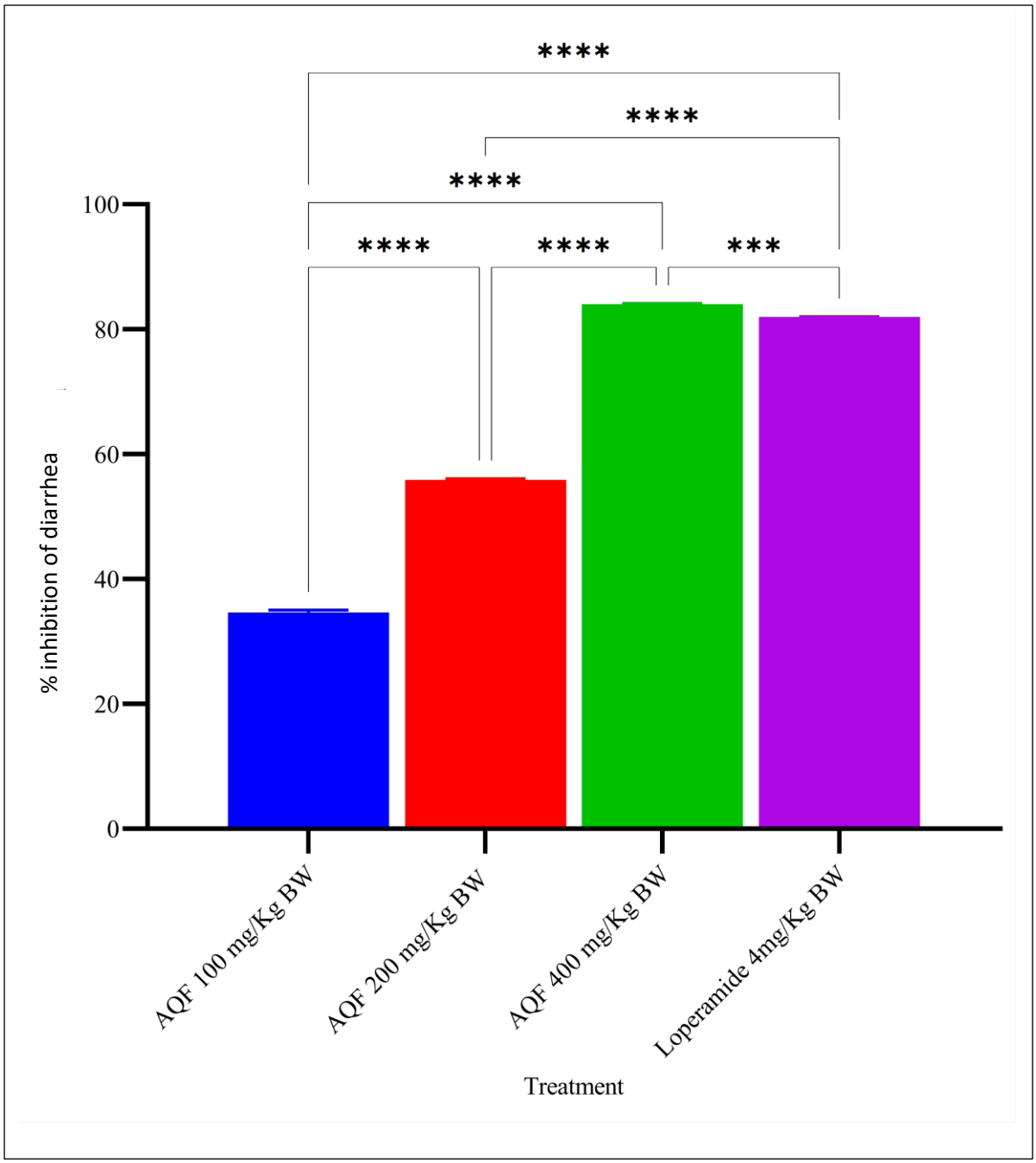

Figure 4.1: Percentage inhibition of diarrhea by the aqueous fruit extracts of *C. dipsaceus* in castor oil-induced diarrhea in Wistar rats

Bars are plotted as $\bar{x} \pm SEM$; **** indicates P<0.0001; *** indicates P=0.0005 (One-Way ANOVA with Tukey's *post hoc* test). AQF: Aqueous fruit extract of *C. dipsaceus.*

Similarly, the aqueous leaf extracts of *C. dipsaceus* significantly inhibited castor oil-induced diarrhea in Wistar rats in a positive dose dependent manner (P<0.0001; Figure 4.2). Loperamide (Positive control drug) produced a significantly higher percentage inhibition of

castor oil-induced diarrhea in Wistar rats than the aqueous leaf extracts of *C. dipsaceus* (P<0.0001; Figure 4.2).

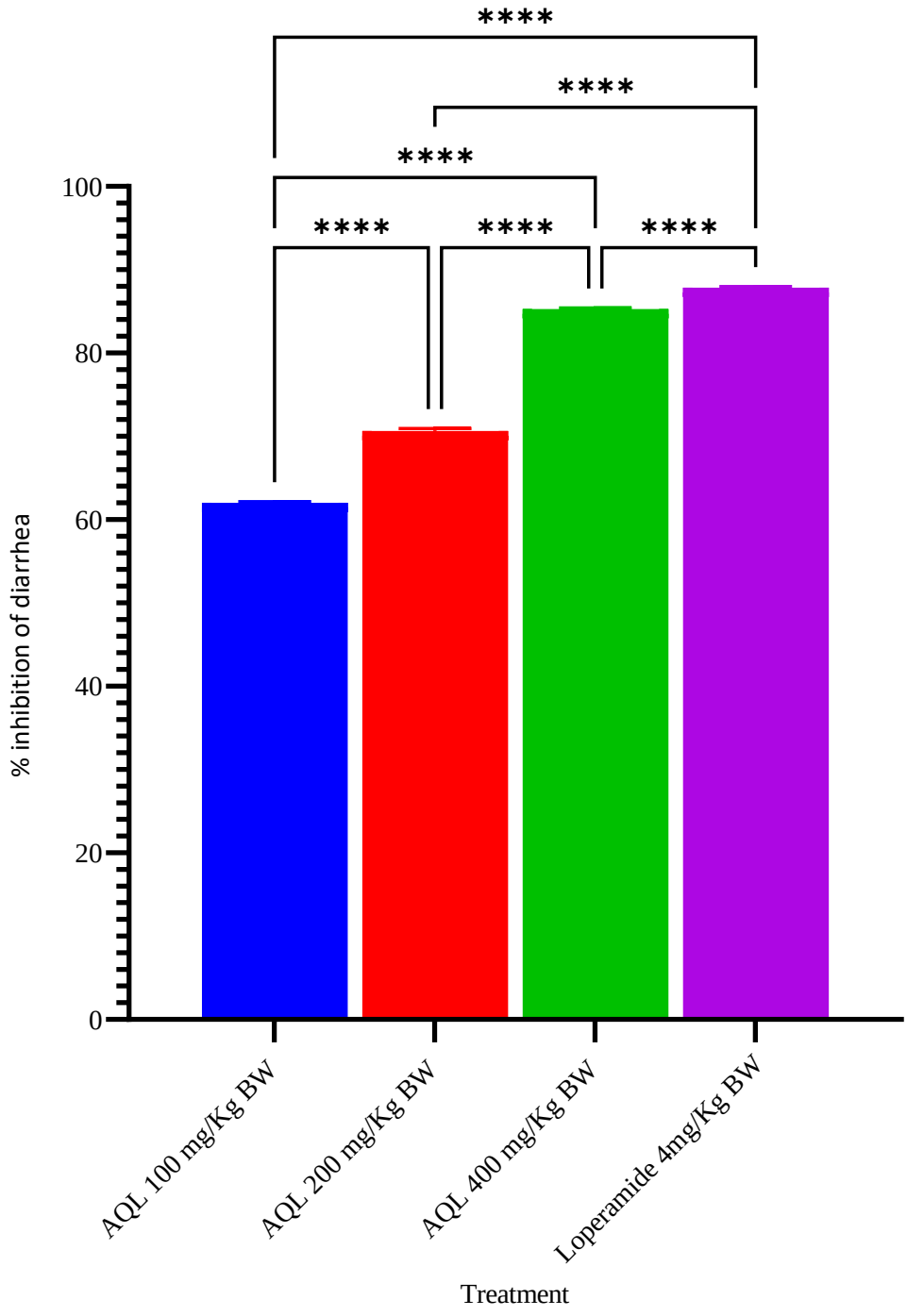

Figure 4.2: Percentage inhibition of diarrhea by the aqueous leaf extracts of *C. dipsaceus* in castor oil-induced diarrhea in Wistar rats

Bars are plotted as $\bar{x} \pm SEM$; **** indicates P<0.0001 (One-Way ANOVA with Tukey's *post hoc* test). AQL: Aqueous leaf extract of *C. dipsaceus.*

The results further revealed significant percentage inhibitions of castor oil-induced diarrhea in Wistar rats by the methanolic fruit extracts of *C. dipsaceus* in a dose-dependent manner (P<0.0001; Figure 4.3). However, the positive control drug (Loperamide) showed significantly higher percentage inhibition of castor oil-induced diarrhea compared with the inhibitions caused by the methanolic fruit extracts of *C. dipsaceus* (P<0.0001; Figure 4.3).

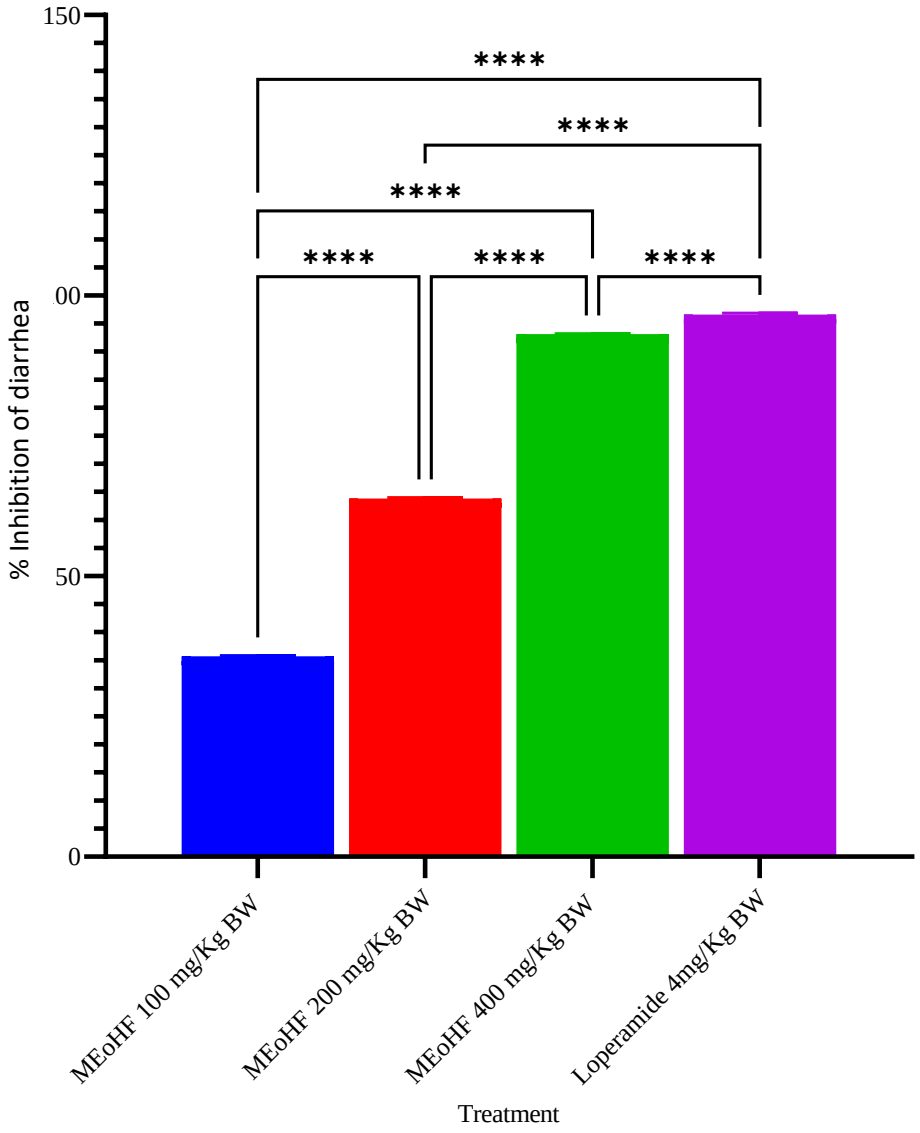

Figure 4.3: Percentage inhibition of diarrhea by the methanolic fruit extract of *C. dipsaceus* in castor oil-induced diarrhea in Wistar rats
Bars are plotted as $\bar{x} \pm SEM$; **** indicates P<0.0001 (One-Way ANOVA with Tukey's *post hoc* test). MEoHF: Methanolic fruit extract of *C. dipsaceus.*

It was also observed that the methanolic leaf extracts of *C. dipsaceus* significantly inhibited castor oil-induced defaection in rats, in a dose-dependent fashion (P<0.0001; Figure 4.4). The percentage inhibition of diarrhea exhibited by Loperamide was significantly higher than the inhibition of the methanolic leaf extracts of *C. dipsaceus* at all the tested dose levels tested (P<0.0001; Figure 4.4).

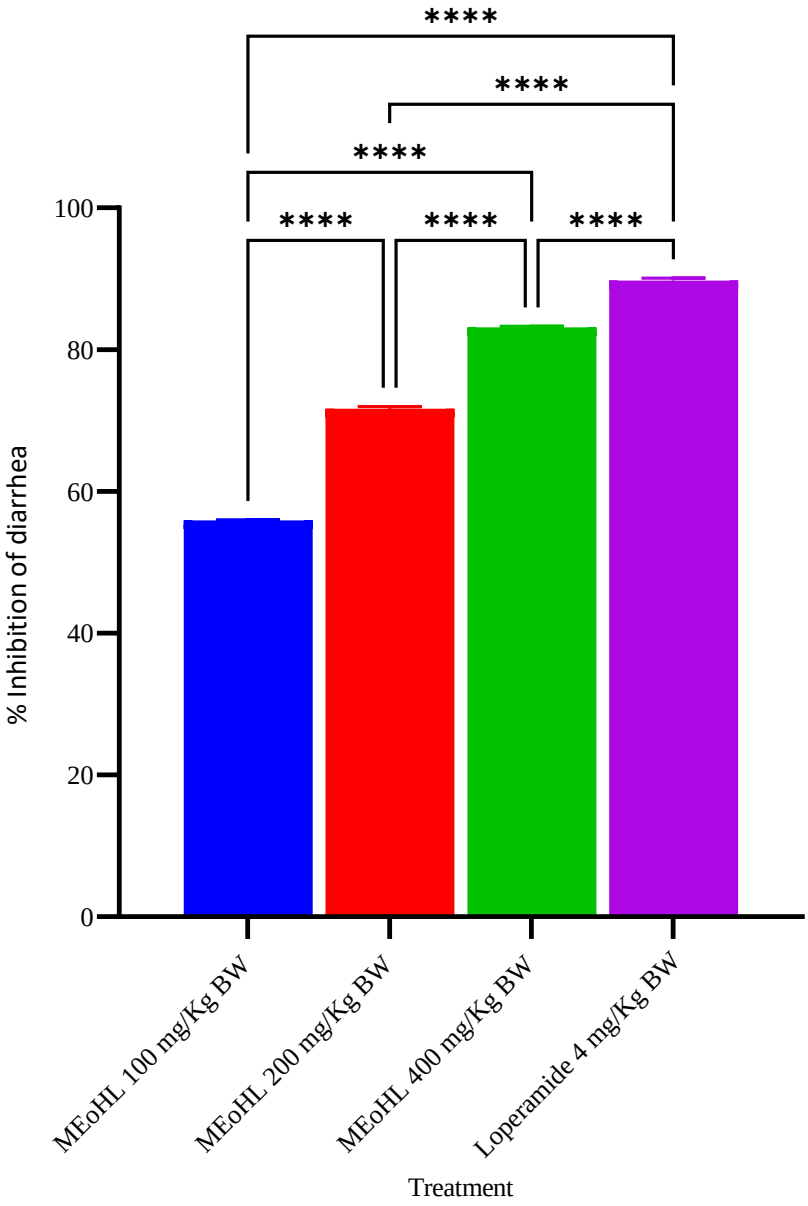

Figure 4.4: Percentage inhibition of diarrhea by the methanolic fruit extract of *C. dipsaceus* in castor oil-induced diarrhea in Wistar rats
Bars are plotted as $\bar{x} \pm SEM$; **** indicates P<0.0001 (One-Way ANOVA with Tukey's *post hoc* test). MEoHF: Methanolic fruit extract of *C. dipsaceus.*

In this study, the percentage inhibitions of diarrhea were compared among the plant extracts. The results showed that at a dose level of 100 mg/Kg BW, the aqueous leaf extract of *C. dipsaceus* exhibited a significantly higher percentage inhibition of diarrhea than all the other extracts ($P<0.05$; Figure 4.5). There was no significant difference between the percentage inhibitions caused by aqueous fruit and methanolic fruit extracts of *C. dipsaceus* ($P>0.05$); However, these inhibitions were significantly lower than those exhibited by the aqueous and methanolic leaf extracts of *C. dipsaceus* ($P>0.05$; Figure 4.5).

There was no significant difference between the percentage inhibitions of diarrhea between aqueous and methanolic leaf extracts of *C. dipsaceus* at a dose of 200 mg/Kg BW ($P>0.05$; Figure 5.4). However, the percentage inhibitions of diarrhea showed that the aqueous and methanolic leaf extracts of *C. dipsaceus*, at a dose level of 200 mg/Kg BW, were significantly higher than those of the aqueous and methanolic fruit extracts at the same dose ($P<0.05$; Figure 4.5). The aqueous fruit extract of the tested plant extracts caused significantly lower percentage inhibitions of diarrhea than all the other extracts, at a dose level of 200 mg/Kg BW ($P<0.05$; Figure 4.5).

At a dose level of 400 mg/Kg BW, the percentage inhibitions of diarrhea exhibited by aqueous fruit and methanolic leaf extracts of *C. dipsaceus* were not significantly different ($P>0.05$; Figure 4.5); However, they were significantly lower than those produced by the other extracts ($P<0.05$; Figure 4.5). The methanolic fruit extracts of *C. dipsaceus* produced a significantly higher inhibition of castor oil-induced diarrhea at a dose level of 400 mg/Kg BW, than all the other tested plant extracts ($P<0.05$; Figure 4.5).

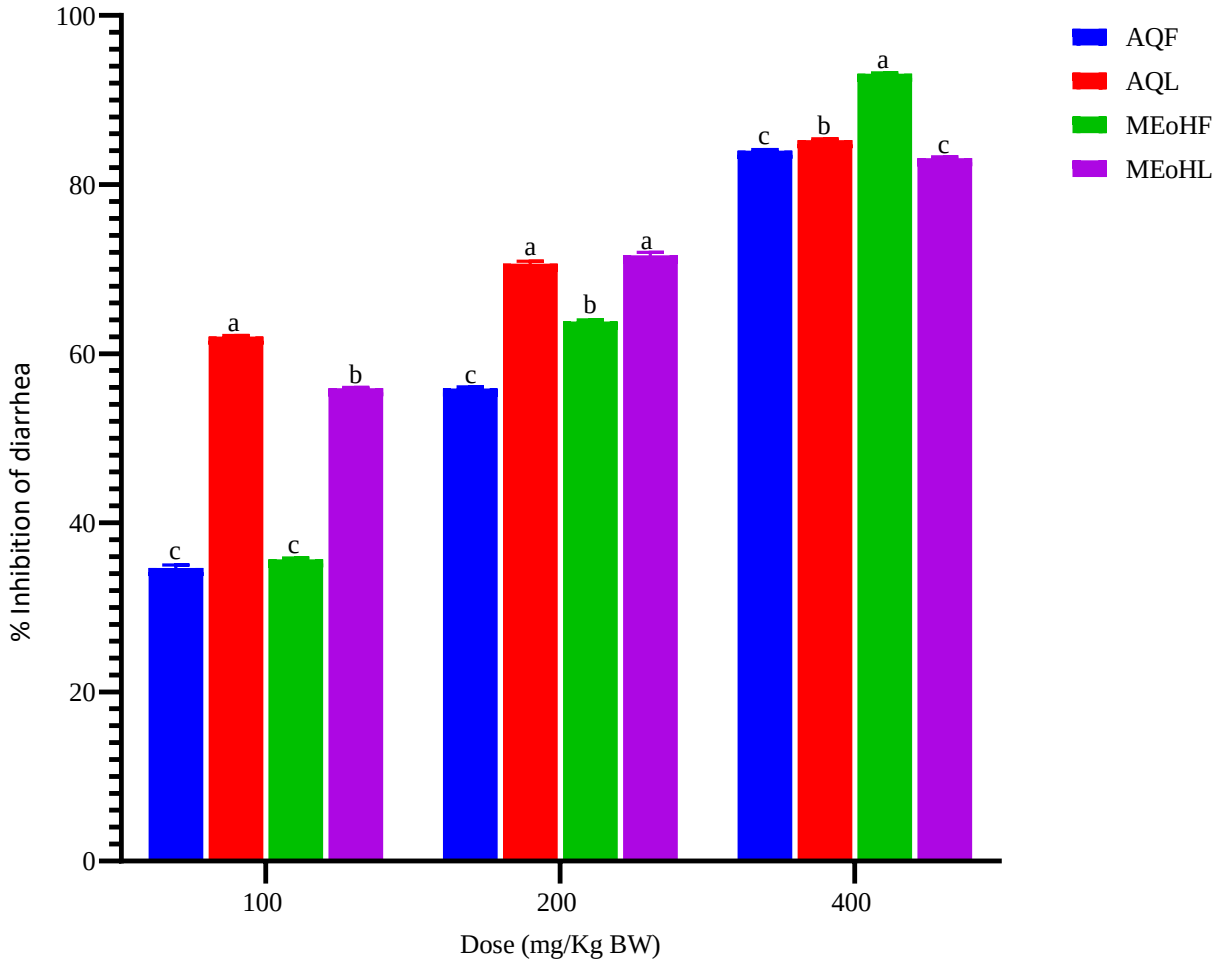

Figure 4.5: Comparison among the percentage inhibition of diarrhea exhibited by the aqueous and methanolic extracts of *C. dipsaceus* in castor oil-induced diarrhea in Wistar rats.

Bars are plotted as $\bar{x} \pm SEM$; Bars denoted by different letters within the same dose level are significantly different (P<0.05; One-Way ANOVA with Tukey's *post hoc* test). AQL: Aqueous leaf extract of *C. dipsaceus*; AQF: Aqueous fruit extract of *C. dipsaceus*; MEoHF: Methanolic fruit extract of *C. dipsaceus*; MEoHL: Methanolic leaf extract of *C. dipsaceus*.

4.3.2 Effects of the methanolic leaf and fruit extracts of *C. dipsaceus* on gastrointestinal contraction of isolated rabbit ileum

Table 4.8 presents the gastrointestinal frequencies, which were recorded before and after the administration of the methanolic leaf and fruit extracts of *C. dipsaceus* isolated rabbit ileum. The frequencies of gastrointestinal contractions produced by the methanolic leaf extracts (200 mg/Kg BW and 400 mg/Kg BW) and the methanolic fruit extracts (400 mg/Kg BW) were

significantly lower than that caused by the standard drug (Loperamide 4 mg/Kg BW) after treatment (P<0.05; Table 4.8).

Table 4.8: Effects of methanolic leaf and fruit extracts of *C. dipsaceus* on gastrointestinal contraction frequency of isolated rabbit ileum

Treatment	Gastrointestinal contraction frequency	
Extract (mg/Kg BW)	**Before administration**	**After administration**
MEoHL 100	$23.67\pm0.33^{A}_{bc}$	$13.83\pm0.17^{B}_{b}$
MEoHL 200	$17.33\pm0.03^{A}_{d}$	$9.23\pm0.23^{B}_{c}$
MEoHL 400	$14.00\pm0.00^{A}_{e}$	$6.17\pm0.03^{B}_{d}$
MEoHF 100	$27.67\pm0.33^{A}_{a}$	$19.33\pm0.33^{B}_{a}$
MEoHF 200	$21.00\pm0.58^{A}_{c}$	$13.97\pm0.03^{B}_{b}$
MEoHF 400	$21.67\pm0.88^{A}_{c}$	$8.33\pm0.88^{B}_{c}$
Loperamide 4	$22.00\pm0.58^{A}_{c}$	$15.00\pm0.58^{B}_{b}$

The values are expressed as $\bar{x} \pm SEM$; Means with different lowercase subscript letters within the same column are significantly different (P<0.05; One-Way ANOVA followed by Tukey's test). Means having different uppercase superscript letters within the same row are significantly different (P<0.05; Un-paired student t-test). MEoHL: Methanolic leaf extract of *C. dipsaceus;* MEoHF: Methanolic fruit extract of *C. dipsaceus*.

4.4 Acute toxicity effects of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* in Wistar rats and New Zealand White rabbits

4.4.1 Acute dermal toxicity effects of the tested plant extracts on New Zealand White rabbits

The results showed that the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* did not cause any signs of acute dermal toxicity effects in experimental rabbits when applied on intact and abraded skin at a dose level of 2000 mg/Kg BW. Hence, the median lethal dose (LD_{50}) was shown to be >2000 mg/Kg BW for extracts (Table 4.9)

Table 4.9: Acute dermal toxicity effects of aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on New Zealand White rabbits

Time	Intact skin							Abraded skin						
	Irritation	Scars	Pain	Erythema	Ulceration	Oedema	Fur	Irritation	Scars	Pain	Erythema	Ulceration	Oedema	Fur
30 Min.	Absent	Absent	Absent	Absent	Absent	Absent	Normal	Absent	Absent	Absent	Absent	Absent	Absent	Normal
1 Hr.	Absent	Absent	Absent	Absent	Absent	Absent	Normal	Absent	Absent	Absent	Absent	Absent	Absent	Normal
2 Hrs.	Absent	Absent	Absent	Absent	Absent	Absent	Normal	Absent	Absent	Absent	Absent	Absent	Absent	Normal
4 Hrs.	Absent	Absent	Absent	Absent	Absent	Absent	Normal	Absent	Absent	Absent	Absent	Absent	Absent	Normal
6 Hrs.	Absent	Absent	Absent	Absent	Absent	Absent	Normal	Absent	Absent	Absent	Absent	Absent	Absent	Normal
24 Hrs.	Absent	Absent	Absent	Absent	Absent	Mild	Normal	Absent	Absent	Absent	Absent	Absent	mild	Normal
48 Hrs.	Absent	Absent	Absent	Absent	Absent	Absent	Normal	Absent	Absent	Absent	Absent	Absent	Absent	Normal
72 Hrs.	Absent	Absent	Absent	Absent	Absent	Absent	Normal	Absent	Absent	Absent	Absent	Absent	Absent	Normal

Experimental rabbits were given 2000 mg/Kg BW of aqueous/methanolic leaf/fruit extracts of *C. dipsaceus* topically. Control rabbits were given glycerol topically; n= 4 Rabbits per treatment

4.4.2 Acute oral toxicity effects of the plant extracts in Wistar rats

The methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* did not cause acute oral toxicity signs in Wistar rats even at a dose of 2000 mg/Kg BW. Therefore, LD_{50} values for each of the investigated plant extracts were above 2000 mg/Kg BW. Table 4.10 shows the results of acute oral toxicity study.

Table 4.10: Acute oral toxicity effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* in Wistar rats

Wellness parameter	Observation											
	30 min- 2 Hrs.		4 Hrs.		24 Hrs.		48 Hrs.		7 days		14 days	
	EGR	CGR	EGR	CGR	EGR	CGR	EGR	CGR	EGR	CGR	EGR	CGR
Skin and Fur appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Faecal matter consistency	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Urination and urine appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Itching	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Convulsions and tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Breathing	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Somatomotor activity	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Aggression	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Grooming	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Teeth	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mortality/Death	None	None	None	None	None	None	None	None	None	None	None	None

EGR: Experimental Group Rats (were given 175 mg/Kg BW/ 550 mg/Kg BW/ 2000 mg/Kg BW of the aqueous/methanolic leaf/fruit extracts of *C. dipsaceus*) orally; CGR: Control group Rats (were given 10 ml/Kg BW of normal saline only); n=3 animals per step.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Persistent inflammation and diseases are the leading causes of physical debility, psychosocial suffering, morbidity and death globally (Abbafati *et al*., 2020; World Health Organization (WHO), 2020). Additionally, inflammation is a symptom which characterise diseases such as inflammatory bowel syndrome, arthritis, diabetes mellitus, neurodegenerative disorders, and organ failures, (Sommer *et al*., 2018). Previous studies indicate that over 50 % of patients receive inadequate treatment for inflammation and associated syndromes, which adversely affect their quality of life (Furman *et al*., 2019; Jairath and Feagan, 2020; Moriasi *et al*., 2021).

The most prescribed steroidal and non-steroidal anti-inflammatory drugs relief inflammation and pain but they evoke adverse effects with life-threatening complications (Felson, 2016; Harirforoosh *et al*., 2013; Monteiro and Steagall, 2019). The use of NSAIDs therapy is implicated in liver damage, renal failure, oedema, gastrointestinal perforations and bleeding, bronchospasms, cardiovascular disorders, among other side effects (Felson, 2016; Harirforoosh *et al*., 2013). Besides, steroidal anti-inflammatories are associated with dizziness, manic depression, erectile dysfunction, immunosuppression, hypertension, among other adverse effects (Monteiro and Steagall, 2019). Social abuse, constipation, addiction, and respiratory disorders, are associated with opioid analgesic therapy (Köhler *et al*., 2014). Owing to the therapeutic shortfalls of conventional anti-inflammatory, antidiarrhea, and antimicrobial agents, their inaccessibility, unavailability, and high costs, especially in the Sub-Saharan Africa, there is a renewed interest in search of affordable, potent, accessible, and safe alternative therapies, especially from natural sources.

Empirical investigations of medicinal plants based on their ethnomedical information presents a feasible strategy for elucidating novel therapeutic molecules against inflammation, diarrhea, and associated ailments, which are safe, efficacious, and affordable (Bauer *et al*., 2016; Hassan and Bhardwaj, 2018; James *et al*., 2018; Jima and Megersa, 2018; Liu *et al*., 2016; Tugume and Nyakoojo, 2019; WHO, 2018). Therefore, owing to the inadequacies of current therapies for inflammation and diarrhea, this research work was carried out to seek evidence of anti-diarrhea, anti-inflammatory, anti-bacterial and anti- fungal effects *C. dipsaceus*, based on its ethnomedical usage in the treatment of diarrhea and inflammation, and associated diseases.

In the present research activity, oedema was induced using formalin on hind paw as described by Hunskaar and Hole, (1987) and modified by Shojaii *et al*. (2015) was used. In this method, when 2.5 % formalin is injected into the intraplantar region of the hind limb of experimental rodents, it evokes an immunologic response, which is characterised by oedema (Bauer *et al*., 2016; Hassan and Bhardwaj, 2018; James *et al*., 2018; Jima and Megersa, 2018; Liu *et al*., 2016; Tugume and Nyakoojo, 2019; WHO, 2018). The increased secretion of pro-inflammatory mediators, like histamine, kinins, and serotonin, is responsible for the observed oedema. Therefore, a drug agent capable of averting formalin-induced oedema is a potential anti-inflammatory therapy.

The findings of this study show that the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus*, significantly inhibited formalin-induced inflammation in experimental rats, subject to concentration- and duration-dependence. The ability to counter inflammation of the studied plant concentrates may be attributed to the differences in extraction solvents used (water and methanol), which may have extracted various anti-inflammatory phytochemicals, in varying concentrations (Dhanani *et al*., 2017; Kurmukov, 2013). Also, the fruit and leaf extracts of *Cucumis dipsaceus*, possibly contain varied concentrations of various phytochemicals, hence

contributing to the observed differences in anti-inflammatory effects (Riaz *et al*., 2018). Therefore, it is postulated that the aqueous fruit extracts of *C.dipsaceus* may be containing higher amounts of anti-inflammatory-associated phytochemicals which are responsible for the higher anti-inflammatory effects than the methanolic fruit extracts, methanolic leaf extracts and aqueous leaf extracts.

Previous studies showed that water and methanol, extract polar chemical substance such as phenols, flavonoids, coumarins, among others which exhibit anti-inflammatory efficacy as documented in literature. (Moriasi *et al*., 2021; Olela *et al*., 2020). Phytochemicals alter the inflammatory cascade by reducing the secretion of proinflammatory mediators, averting oxidative stress, and promoting cellular recovery (Howes, 2017; Kong *et al*., 2021; Zhu *et al*., 2018). Consequently, the efficacy of the plant concentrates may be accredited to the presence of bioactive amalgams, which, either individually, or synergistically, inhibit inflammation by affecting various pathways. Nevertheless, specific analysis should be performed in order to establish the anti-inflammatory molecules and their specific modes of action.

Diarrhea is the most common ailment and the leading hygienics and sanitation worry affecting equatorial and sub-equatorial Nations, with high morbidity and deaths, especially in infants, under five year olds ,the elderly, and immunosuppressed patients (Murray and Lopez, 1996; Pires *et al*., 2015).

Microbial-induced diarrhea is responsible for over 8 million deaths of infants and children aged below five years annually (Abbafati *et al*., 2020a; Pires *et al*., 2015; Troeger *et al*., 2017, 2018). The centre for disease control (CDC) indicates that 1 out of 9 children die of diarrhea, with a cumulative average of 801,000 deaths every year in Africa (Centres for Disease Control and Prevention, 2015). Due to the low hygiene standards, especially in the rural areas of the less developed Countries, the unaffordability and inaccessibility of quality healthcare, the inhabitants largely utilise medicinal plants to either prevent or treat diarrhea

diseases. Indeed, the WHO reports that a big population of individuals use herbal remedies in order to manage various diseases including inflammation and diarrhea, and associated diseases, due to their supposed potency, safety, accessibility and affordability (WHO, 2013; 2018).

Diarrhea is a common manifestation of inflammation and microbial infection in the gastrointestinal tract (Camilleri *et al*., 2017; Troeger *et al*., 2017). It causes dehydration due to excessive fluid loss, digestion and absorption disorders, nutrient loss, among other agonising and life-threatening effects in the affected individuals, if not managed adequately (Camilleri *et al*., 2017; Kelly *et al*., 2018). Present antidiarrhea drugs are associated with undesirable effects, and are not universally accessible and affordable, especially in low income earning Countries, like Kenya (Awouters *et al*., 1983; Heel *et al*., 1978; Murek *et al*., 2010; Niemegeers *et al*., 1981). Due to the devastating sequelae of diarrhea caused by bowel inflammation and microbial infections and the poignancies of the current therapies, there is an urgent need to search for better and safe alternative drugs to maintain good standard of health and save lives. Accordingly, the antidiarrhea effects of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* were investigated in the study.

The standard castor oil-induced diarrhea method modified by Rahman *et al*. (2019) to determine the antidiarrhea effects of the studied plant extracts was adopted. When castor oil administered into an animal model, it induces irritation and inflammation of the intestinal mucosa (Rahman *et al*., 2019). This is achieved through its major ingredient, ricinoleic acid, which evokes prostaglandin secretion, as an immunological response, thereby stimulating and upregulating intestinal motility and secretions which are characteristic in diarrhea. In addition, castor oil inhibits sodium and potassium ion absorption, while reducing ATPase in the small intestines, and the colon, which results in reduced transit times, due to high peristaltic activity (Mekonnen *et al*., 2018; Rahman *et al*., 2019; Sisay *et al*., 2017).

Therefore, a drug agent that inhibits, or reverses the castor oil-induced diarrhea, is a potential antidiarrhea agent.

The methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* significantly inhibited the castor oil-induced diarrhea and intestinal contraction frequency, indicating their antidiarrhea efficacy. These findings suggest that the investigated plant extracts may have exerted anti-inflammatory effects by inhibiting prostaglandins synthesis which ameliorated the castor-oil induced irritation and inflammation of the intestinal mucosa (Mekonnen *et al*., 2018). Perhaps, the studied plant extracts may have also maintained or restored the intestinal electrolyte homeostasis in order to prevent diarrhea (Mekonnen *et al*., 2018; Rahman *et al*., 2019; Teferi *et al*., 2019).

The positive control drug used in this study, Loperamide, exerts its antidiarrhea efficacy by regulating electrolyte balance and secretion in the gastrointestinal tract (Heel *et al*., 1978; Murek *et al*., 2010). Perhaps, the mechanism of antidiarrhea activity of the methanolic and aqueous leaf and fruit of *C. dipsaceus* may be similar to that of Loperamide. The studied plant extracts may be possessing anticholinergic effects which are associated with reduced gastric motility and secretions, through a mechanism like that of atropine, which antagonises the muscarinic acetylcholine receptors of the gastrointestinal tract (Bardal *et al*., 2011).

Various phytochemicals, such as phenols, flavonoids, saponins, glycosides, among others exhibit antidiarrhea effects by reducing gastric motility frequency and secretions (Dosso *et al*., 2012; Mekonnen *et al*., 2018). It is possible that the phytochemicals present in the studied plant extracts were responsible for relieving the symptoms of diarrhea of the plant extracts, which were observed in the current study. Nevertheless, specific antidiarrhea components in the plant extracts and mode(s) of pharmacologic action(s) require to be further investigated through focused empirical investigations.

Microbial infections are key etiologic agents for inflammation (Chauhan and Saha, 2018; Staudacher and Stevens, 2019). Various pathogenic microbial species produce metabolites and other components, including the lipopolysaccharide, endotoxins, and cell capsule carbohydrates which damage the integrity of the gut and evoke immunological responses with devastating sequelae (Chauhan and Saha, 2018). Balanced gut microbiota and normal flora modulate immunity and promote gut health (Fan and Pedersen, 2021; Sekirov *et al*., 2010). However, colonisation by pathogenic microbes deters normal gut microbiota population and functioning resulting in gastrointestinal dysfunction, manifesting in indigestion, diarrhea, among other distressing symptoms (Fan and Pedersen, 2021).

Most antimicrobial drugs, which are used in clinical practice are ineffective, unaffordable, inaccessible, and cause adverse effects, coupled with the emergence of resistant strains pose a major challenge in antimicrobial therapy (Ayukekbong *et al*., 2017; Mahmoud abd El-Baky, 2016; Mandal *et al*., 2014). As a result, there is a need for alternative medicines which can be used to treat bacterial and fungal associated diseases. Hence, this study investigated the antimicrobial effects of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* against some microbial strains.

The antimicrobial effects of the studied plant extracts on *E. coli, S. enteriditis, B, subtilis, P. aeruginosa,* and *C. albicans* were determined using the broth micro dilution techniques and disk diffusion method stipulated in the CLSI Guidelines (CLSI, 2014). The obtained zones of microbial growth inhibition from the disk diffusion assay, and the Minimum Inhibitory Concentrations (MIC) from the broth microdilution assay were considered indicators of antimicrobial efficacy.

Available data indicate that the plant extracts which produce microbial growth inhibition zones of 6-9 mm possesses a weak antimicrobial activity; those with inhibition zones of 9-12

mm possesses moderate activity; those exhibiting inhibition zones of 13-16 mm have high antimicrobial activity; those with inhibition zones of 16-19 mm have very high antimicrobial activity and those producing inhibition zones measuring >20 mm have remarkable antibiotic activity (Mwitari *et al*., 2013; Nanasombat *et al*., 2018; Saquib *et al*., 2019). Based on this appraisal criteria, the studied plant extracts demonstrated mixed antimicrobial effects against selected microbial strains, in a concentration and extract-type dependent manner.

The four plant extracts which were studied were inactive at concentrations of < 12.5 µg/ml, and moderately active at concentrations of 25-100 µg/ml against *S. enteriditis*, in this study. Similarly, the aqueous leaf and fruit, and methanolic leaf extracts of *C. dipsaceus* were not active against *E. coli* at a concentration of < 12.5 µg/ml. Weak to moderate antimicrobial effects against *E. coli* were recorded at concentrations of 25-100 µg/ml. However, the methanolic fruit extracts of *C. dipsaceus* exhibited weak to high antimicrobial activity against *E. coli* and demonstrated superior efficacy than the other extracts. Besides, the aqueous leaf extracts of *C. dipsaceus* showed weak to moderate activity against *P. aeruginosa*.

The aqueous fruit and methanolic leaf extracts of *C. dipsaceus* demonstrated weak to high antimicrobial activities against *P. aeruginosa.* Moderate to very high antimicrobial activity against *P. aeruginosa* was exhibited by the methanolic fruit extracts of *C. dipsaceus*, which also proved to be more potent than the other extracts. When *B. subtilis* was exposed to the test plant extracts, the aqueous and methanolic leaf extracts of *C. dipsaceus* exerted weak to high antimicrobial effects, while the aqueous and methanolic fruit extracts showed weak to very high antimicrobial efficacy, in a concentration dependent fashion.

In all the experimented bacterial strains, the positive control drug (Ciprofloxacin) showed remarkable efficacy, as demonstrated by its highest (>20 mm) microbial growth inhibition zones. Only the aqueous leaf extract of *C. dipsaceus* demonstrated antifungal activity against *C. albicans* (weak to very high), in this study, Nystatin (the positive control drug) had very

high activity. The varied antimicrobial activity of the studied plant extracts may be due to the presence of various antimicrobial phytochemicals, in varying concentrations.

The Minimum Inhibitory Concentrations (MIC) of each of the studied plant extract, against the selected microbial strains, was determined by using broth microdilution assay technique described by the CLSI (CLSI, 2014). MIC values are used to determine the degree of antimicrobial agent's efficacy. Published data shows that plant extracts and chemical substances whose MIC values are below 1000 µg/ml are potential sources of efficacious antibiotics (Anyanwu and Okoye, 2017; Ezeja *et al*., 2012; Kathare *et al*., 2021).

In this study, the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* showed very low MIC values against the selected bacterial strains, indicating that they have high antimicrobial efficacy and potency. In addition, the low MIC value recorded for aqueous leaf extracts of *C. dipsaceus* against *C. albicans* indicates it has a high antifungal efficacy. The antimicrobial effects of the investigated plant extracts of *C. dipsaceus* reported in this study are attributable to the presence of various antimicrobial-associated bioactive secondary metabolites.

Previous studies indicate that various phytochemical compounds which are present in the aerial parts of *C. dipsaceus,* especially flavonoids, tannins, terpenoids, phenols, among others, have antimicrobial effects (Eldahshan and Singab, 2013; Kurmukov, 2013; Molyneux *et al*., 2007). Therefore, it is suggested that similar phytochemicals were responsible for the antimicrobial efficacy of the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* in this study. Furthermore, the differences in antimicrobial effects against various strains of bacteria, could be due to the variances in concentration and phytochemicals present in the extracts, which may have different modes of bioactivity against various microbes (Al-snafi, 2015; Kuglerova *et al*., 2011).

Medicinal plants have been utilised historically due to their affordability, easy accessibility and presumed safety and potency (Olela *et al*., 2020; Moriasi *et al*., 2021). However, because of inadequate empirical data, various safety concerns and pharmacologic efficacy have been raised (George, 2011). For instance, there is no specific dosages or guidelines for herbal preparations, storage, labelling, marketing and specific indications, like those of pharmaceutical drugs (Gakuya *et al*., 2020; Moreira *et al*., 2014). In addition, there is scanty empirical information on herbal drug-herbal drug, and herbal drug-conventional drug interactions and associated effects, which raise safety concerns (Aydin *et al*., 2016; Nasri and Shirzad, 2013). Therefore, it is important to study the safety and toxicity profiles of ethnomedicinally used plants to appraise their safety.

Despite the long-lasting utilisation of *C. dipsaceus* to manage various diseases in the Kenyan traditional medicine, no sufficient data on its safety and toxicity profile was available. This study investigated the acute dermal and oral toxic effects of *C. dipsaceus*. The OECD guidelines for acute oral toxicity (OECD, 2008) and acute dermal toxicity (OECD, 2017) were adopted in this study. The methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* did not elicit any observable acute oral toxic effects and hence they were deemed to be safe according to the OECD Guidelines (OECD, 2008). Similarly, the tested plant extracts did not cause any symptom associated with acute dermal toxicity in experimental animals and were therefore considered to be safe as per the OECD recommendations (OECD, 2017).

In Kenya, the leaves of *C. dipsaceus* are consumed as vegetables, and as medicines (Njoroge and Newton, 1994). The fruits and leaves are utilised in managing wounds, stomach-aches, diarrhea, poisoning, snakebite envenomation, diabetes mellitus among other diseases and as food in Kenya (Kipkore *et al*., 2014b; Kokwaro, 2009; Mutie *et al*., 2020). It is therefore

suggested that the diverse applications of *C dipsaceus* are due to its safety, efficacy, and health benefits.

Based on the toxicity results reported in this study, the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* demonstrated a higher prospect of offering efficacious and safe anti-inflammatory, antimicrobial and antidiarrhea therapies upon further experimentation. Therefore, extensive *in vivo* studies including clinical setups should be done to establish conclusive safety with sub -acute and chronic toxicity profiles. Nevertheless, this study partly confirms the ethnomedical application of *C. dipsaceus* for managing inflammation, microbial infections and diarrhea in humans and animals.

5.2 Conclusion

The following conclusions were made from the findings of the study:

i. The methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* have appreciable activity on formalin-induced oedema in Wistar rats.
ii. The methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* have significant antidiarrhea activities in castor oil-induced diarrhea in Wistar rats.
iii. The methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* have varied degrees of antimicrobial activity against the selected bacterial and fungal strains.
iv. The methanolic and aqueous leaf and fruit extracts of *C. dipsaceus* do not cause adverse effects when administered orally in Wistar rats, or when applied topically onto New Zealand White rabbits.

5.3 Recommendations

The following recommendations were made from the study:

Further empirical studies should be done to determine the specific phytochemical compounds in methanolic and aqueous leaf and fruit of the studied plant parts, with the aim of exploring

details their anti-inflammatory, antidiarrhea, and antimicrobial effects, observed in the present study and their mode(s) of bioactivity. It is also recommended that extensive bioscreening, and toxicological investigations of the extracts under study to be done using other alternative technologies to establish their Pharmacological activities and safety profiles.

REFERENCES

Abbafati, C., Machado, D. B., Cislaghi, B., Salman, O. M., Karanikolos, M., McKee, M., Abbas, K. M., Brady, O. J., Larson, H. J., Trias-Llimós, S., Cummins, S., Langan, S. M., Sartorius, B., Hafiz, A., Jenabi, E., Mohammad Gholi Mezerji, N., Borzouei, S., Azarian, G., Khazaei, S., Zhu, C. (2020a). Global age-sex-specific fertility, mortality, healthy life expectancy (HALE), and population estimates in 204 countries and territories, 1950–2019: a comprehensive demographic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *396*(10258), 1160–1203. https://doi.org/10.1016/S0140-6736(20)30977-6.

Abbafati, Cristiana, Abbas, K. M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., Abdollahpour, I., Abegaz, K. H., Abolhassani, H., Aboyans, V., Abreu, L. G., Abrigo, M. R. M., Abualhasan, A., Abu-Raddad, L. J., Abushouk, A. I., Adabi, M., Adekanmbi, V., Adeoye, A. M., Adetokunboh, O. O., … Murray, C. J. L. (2020b). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *396*(10258), 1204–1222. https://doi.org/10.1016/S0140-6736(20)30925-9.

Abdullahi, A. A. (2011). Trends and challenges of traditional medicine in Africa. *African Journal of Traditional, Complementary and Alternative Medicines*. https://doi.org/10.4314/ajtcam.v8i5S.5.

Al-snafi, A. E. (2015). *Pharmacology and Toxicology THERAPEUTIC PROPERTIES OF MEDICINAL PLANTS : A REVIEW*. *5*(3), 177–192. Accessed on 12th November 2019

Alatab, S., Sepanlou, S. G., Ikuta, K., Vahedi, H., Bisignano, C., Safiri, S., Sadeghi, A., Nixon, M. R., Abdoli, A., Abolhassani, H., Alipour, V., Almadi, M. A. H., Almasi-Hashiani, A., Anushiravani, A., Arabloo, J., Atique, S., Awasthi, A., Badawi, A.,

Alema, N. M., Periasamy, G., Sibhat, G. G., Tekulu, G. H., and Hiben, M. G. (2020). Antidiabetic activity of extracts of terminalia brownii fresen. Stem bark in mice. *Journal of Experimental Pharmacology*, *12*, 61–71. https://doi.org/10.2147/JEP.S240266.

Alemu, A., Tamiru, W., Nedi, T., and Shibeshi, W. (2018). Analgesic and Anti-Inflammatory Effects of 80% Methanol Extract of Leonotis ocymifolia (Burm.f.) Iwarsson Leaves in Rodent Models. *Evidence-Based Complementary and Alternative Medicine*, *2018*, 1–8. https://doi.org/10.1155/2018/1614793.

Alkizim, F., Matheka, D., and Muriithi, A. (2011). Childhood diarrhea: Failing conventional measures, what next? *Pan African Medical Journal*, *8*, 1–9. https://doi.org/10.4314/pamj.v8i1.71164.

Anyanwu, M. U., and Okoye, R. C. (2017). Antimicrobial activity of Nigerian medicinal plants. *Journal of Intercultural Ethnopharmacology*, *6*(2), 240–259. https://doi.org/10.5455/jice.20170106073231.

Awouters, F., Niemegeers, C. J. E., and Janssen, P. A. J. (1983). Pharmacology ofantidiarrhea drugs. *Annual Review of Pharmacology and Toxicology*, *Vol. 23*(113), 279–301. https://doi.org/10.1146/annurev.pa.23.040183.001431.

Aydin, A., Aktay, G., and Yesilada, E. (2016). A guidance manual for the toxicity assessment of traditional herbal medicines. *Natural Product Communications*, *11*(11), 1763–1773. https://doi.org/10.1177/1934578x1601101131.

Ayukekbong, J. A., Ntemgwa, M., and Atabe, A. N. (2017). The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrobial Resistance and Infection Control*, *6*(1), 1–8. https://doi.org/10.1186/s13756-017-0208-x.

Bacchi, S., Palumbo, P., Sponta, A., and Coppolino, M. F. (2012). Clinical Pharmacology of Non-Steroidal Anti-Inflammatory Drugs: A Review. *Anti-Inflammatory and Anti-Allergy Agents in Medicinal Chemistry*. https://doi.org/10.2174/187152312803476255.

Bardal, S., Waechter, J., and Martin, D. (2011). *Applied Pharmacology*.

Bauer, B. A., Tilburt, J. C., Sood, A., Li, G. xi, and Wang, S. han. (2016). Complementary and alternative medicine therapies for chronic pain. *Chinese Journal of Integrative Medicine*, *22*(6), 403–411. https://doi.org/10.1007/s11655-016-2258-y.

Ben Olela James Mbaria Timothy Wachira, and Moriasi, G. (2020). Acute Oral Toxicity and Anti-inflammatory and Analgesic Effects of Aqueous and Methanolic Stem Bark Extracts of Piliostigma thonningii (Schumach.). *Evidence-Based Complementary and Alternative Medicine*, *2020*(2020), 10.

CABI. (n.d.). *Cucumis dipsaceus (hedgehog gourd)*. Retrieved August 4, 2021, from https://www.cabi.org/isc/datasheet/31694#40ac02b9-8aae-4672-aa7a-c6747e364dc7.

Caldwell, J. R., and Cluff, L. E. (1974). Adverse Reactions to Antimicrobial Agents. *JAMA: The Journal of the American Medical Association*, *230*(1), 77–80. https://doi.org/10.1001/jama.1974.03240010045029.

Camilleri, M., Sellin, J. H., and Barrett, K. E. (2017). Pathophysiology, Evaluation, and Management of Chronic Watery Diarrhea. *Gastroenterology*, *152*(3), 515-532.e2. https://doi.org/10.1053/j.gastro.2016.10.014.

Centers for Disease Control and Prevention (CDC). (2020). *Antibiotic-Resistant Infections Threaten Modern Medicine*. 2. https://www.cdc.gov/drugresistance/pdf/threats-report/Threat-Modern-Medicine-508.pdf.

Centers for Disease Control and Prevention. (2015). Global Diarrhea Burden Diarrhea: Common Illness, Global Killer. In *U.S. Department of Health and Human Services*. https://www.cdc.gov/healthywater/global/diarrhea-burden.html.

Chauhan, P., and Saha, B. (2018). Metabolic regulation of infection and inflammation. *Cytokine*, *112*(November), 1–11. https://doi.org/10.1016/j.cyto.2018.11.016.

Che, C. T., George, V., Ijinu, T. P., Pushpangadan, P., and Andrae-Marobela, K. (2017). Traditional Medicine. In *Pharmacognosy: Fundamentals, Applications and Strategy*. https://doi.org/10.1016/B978-0-12-802104-0.00002-0.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., and Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, *9*(6), 7204–7218. https://doi.org/10.18632/oncotarget.23208.

Cheng, G., Dai, M., Ahmed, S., Hao, H., Wang, X., and Yuan, Z. (2016). Antimicrobial drugs in fighting against antimicrobial resistance. *Frontiers in Microbiology*, *7*(APR), 1–11. https://doi.org/10.3389/fmicb.2016.00470.

Christou, L. (2011). The global burden of bacterial and viral zoonotic infections. *Clinical Microbiology and Infection*, *17*(3), 326–330. https://doi.org/10.1111/j.1469-0691.2010.03441.x.

Clinical Laboratory Standards Institute (CLSI). (2014). *M100-S24 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement* (Issue January).

Dadgostar, P. (2019). Antimicrobial resistance: implications and costs. *Infection and Drug Resistance*, *12*, 3903–3910. https://doi.org/10.2147/IDR.S234610.

Damião, A. O. M. C., De Azevedo, M. F. C., De Sousa Carlos, A., Wada, M. Y., Silva, T. V. M., and De Castro Feitosa, F. (2019). Conventional therapy for moderate to severe inflammatory bowel disease: A systematic literature review. *World Journal of Gastroenterology*, *25*(9), 1142–1157. https://doi.org/10.3748/wjg.v25.i9.1142.

Dhanani, T., Shah, S., Gajbhiye, N. A., and Kumar, S. (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. *Arabian Journal of Chemistry*, *10*, S1193–S1199. https://doi.org/10.1016/j.arabjc.2013.02.015.

Dosso, K., N'guessan, B. B., Bidie, A. P., Gnangoran, B. N., Méité, S., N'guessan, D., Yapo, A. P., and Ehilé, E. E. (2012).antidiarrhea activity of an ethanol extract of the stem bark of Piliostigma reticulatum (caesalpiniaceae) in rats. *African Journal of Traditional, Complementary and Alternative Medicines*, *9*(2), 242–249. https://doi.org/10.4314/ajtcam.v9i2.9.

Eldahshan, O. A., and Singab, A. N. B. (2013). Journal of Pharmacognosy and Phytochemistry. *Journal of Pharmacognosy and Phytochemistry Carotenoids*, *2*(1), 225–234.

Elston, D. M., Gibson, L. E., and Kutzner, H. (2015). Infectious Diseases. In *Handbook of Practical Immunohistochemistry* (pp. 641–663). Springer New York. https://doi.org/10.1007/978-1-4939-1578-1_32.

Erhierhie, Oghenesuvwe, E., Ekene, N. E., and Daniel Lotanna, A. (2014). Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. *Journal of Natural Sciences Research Www*, *4*(18), 2225–2921.

Ezeja, I. M., Ezeigbo, I. I., Madubuike, K. G., Udeh, N. E., Ukweni, I. A., Akomas, S. C., and Ifenkwe, D. C. (2012).antidiarrhea activity of Pterocarpus erinaceus methanol leaf extract in experimentally-induced diarrhea. *Asian Pacific Journal of Tropical Medicine*, *5*(2), 147–150. https://doi.org/10.1016/S1995-7645(12)60014-5.

Fan, Y., and Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. *Nature Reviews Microbiology*, *19*(1), 55–71. https://doi.org/10.1038/s41579-020-0433-9.

Felson, D. T. (2016). Safety of Nonsteroidal Antiinflammatory Drugs. *New England Journal of Medicine*, *375*(26), 2595–2596. https://doi.org/10.1056/NEJMe1614257.

Fernández-Bañares, F., Accarino, A., Balboa, A., Domènech, E., Esteve, M., Garcia-Planella, E., Guardiola, J., Molero, X., Rodríguez-Luna, A., Ruiz-Cerulla, A., Santos, J., and Vaquero, E. (2016). Chronic diarrhea: Definition, classification and diagnosis. *Gastroenterología y Hepatología (English Edition)*, *39*(8), 535–559. https://doi.org/10.1016/j.gastre.2016.07.008.

Fernebro, J. (2011). Fighting bacterial infections - Future treatment options. *Drug Resistance Updates*, *14*(2), 125–139. https://doi.org/10.1016/j.drup.2011.02.001.

Fitzpatrick, F. (2004). Cyclooxygenase Enzymes: Regulation and Function. *Current Pharmaceutical Design*, *10*(6), 577–588. https://doi.org/10.2174/1381612043453144.

Fokunang, C. (2018). Overview of non-steroidal anti-inflammatory drugs (nsaids) in resource limited countries. *MOJ Toxicology*, *4*(1), 5–13. https://doi.org/10.15406/mojt.2018.04.00081.

Frieri, M., Kumar, K., and Boutin, A. (2017). Antibiotic resistance. *Journal of Infection and Public Health*, *10*(4), 369–378. https://doi.org/10.1016/j.jiph.2016.08.007.

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., and Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nature Medicine*, *25*(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0.

Gakuya, D. W., Okumu, M. O., Kiama, S. G., Mbaria, J. M., Gathumbi, P. K., Mathiu, P. M., and Nguta, J. M. (2020). Traditional medicine in Kenya: Past and current status, challenges, and the way forward. *Scientific African*, *8*, e00360. https://doi.org/10.1016/j.sciaf.2020.e00360.

Gan, T. J. (2010). Diclofenac: An update on its mechanism of action and safety profile. *Current Medical Research and Opinion*, *26*(7), 1715–1731. https://doi.org/10.1185/03007995.2010.486301.

Garre, M., Garo, B., and Hutin, P. (1996). Antibacterial agents. *Medecine et Maladies Infectieuses*, *26*(1), 22–25. https://doi.org/10.1016/S0399-077X(96)80260-5.

Gauniyal, P., and Teotia, S. (2015). Evaluation of Antimicrobial Activity and Phytochemical Screening of Twenty Medicinal Plants against Oral Microbes. *Literati Journal of Pharmaceutical Drug Delivery Technologies*, *1*(1), 1–4.

George, P. (2011). Concerns regarding the safety and toxicity of medicinal plants - An overview. *Journal of Applied Pharmaceutical Science*, *1*(6), 40–44.

Ghasemian, M., Owlia, S., and Owlia, M. B. (2016). Review of Anti-Inflammatory Herbal Medicines. In *Advances in Pharmacological Sciences* (Vol. 2016). https://doi.org/10.1155/2016/9130979.

Golus, J., Sawicki, R., Widelski, J., and Ginalska, G. (2016). The agar microdilution method – a new method for antimicrobial susceptibility testing for essential oils and plant extracts. *Journal of Applied Microbiology*, *121*(5), 1291–1299. https://doi.org/10.1111/jam.13253.

Guo, H., Zhang, J., Gao, W., Qu, Z., and Liu, C. (2014). Gastrointestinal effect of methanol extract of Radix Aucklandiae and selected active substances on the transit activity of rat isolated intestinal strips. *Pharmaceutical Biology*, *52*(9), 1141–1149. https://doi.org/10.3109/13880209.2013.879601.

Hakansson, A., and Molin, G. (2011). Gut microbiota and inflammation. *Nutrients*, *3*(6), 637–687. https://doi.org/10.3390/nu3060637.

Harborne, J. B. (1998). Phytochemical Methods A Guide To Modern Tecniques Of Plant Analysis, Third Edition. In *Chapman and Hall*.

https://doi.org/10.1017/CBO9781107415324.004;

Harirforoosh, S., Asghar, W., and Jamali, F. (2013). Adverse effects of nonsteroidal antiinflammatory drugs: An update of gastrointestinal, cardiovascular and renal complications. *Journal of Pharmacy and Pharmaceutical Sciences*, *16*(5), 821–847. https://doi.org/10.18433/j3vw2f.

Hassan, S., and Bhardwaj, S. (2018). Complementary and alternative medicine. *Alternative and Complementary Therapies*, *24*(6), 294–295. https://doi.org/10.1089/act.2018.29196.cru. A

Heel, R. C., Brogden, R. N., Speight, T. M., and Avery, G. S. (1978). Loperamide. *Drugs*, *15*(1), 33–52. https://doi.org/10.2165/00003495-197815010-00003.

Henschke, N., Kamper, S. J., and Maher, C. G. (2015). The epidemiology and economic consequences of pain. *Mayo Clinic Proceedings*, *90*(1), 139–147. https://doi.org/10.1016/j.mayocp.2014.09.010.

Howes, M. J. R. (2017). Phytochemicals as anti-inflammatory nutraceuticals and phytopharmaceuticals. In *Immunity and Inflammation in Health and Disease: Emerging Roles of Nutraceuticals and Functional Foods in Immune Support*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-805417-8.00028-7.

Hunskaar, S., and Hole, K. (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain*, *30*(1), 103–114. https://doi.org/10.1016/0304-3959(87)90088-1.

Hunter, P. (2012). The inflammation theory of disease. the growing realization that chronic inflammation is crucial in many diseases opens new avenues for treatment. *EMBO Reports*, *13*(11), 968–970. https://doi.org/10.1038/embor.2012.142.

Jairath, V., and Feagan, B. G. (2020). Global burden of inflammatory bowel disease. *The Lancet Gastroenterology and Hepatology*, *5*(1), 2–3. https://doi.org/10.1016/S2468-

1253(19)30358-9.

James, P. B., Wardle, J., Steel, A., and Adams, J. (2018). Traditional, complementary and alternative medicine use in Sub-Saharan Africa: a systematic review. *BMJ Global Health*, *3*(5), e000895. https://doi.org/10.1136/bmjgh-2018-000895.

Jima, T. T., and Megersa, M. (2018). Ethnobotanical Study of Medicinal Plants Used to Treat Human Diseases in Berbere District, Bale Zone of Oromia Regional State, South East Ethiopia. *Evidence-Based Complementary and Alternative Medicine*, *2018*, 1–16. https://doi.org/10.1155/2018/8602945.

***Josephine Ozioma, E.-O., and Antoinette Nwamaka Chinwe, O. (*2019)**. Herbal Medicines in African Traditional Medicine. *Herbal Medicine*. https://doi.org/10.5772/intechopen.80348.

Kaper, J. B., Nataro, J. P., and Mobley, H. L. T. (2004). Pathogenic Escherichia coli. In *Nature Reviews Microbiology*. https://doi.org/10.1038/nrmicro818.

Kareru, P. G., Kenji, G. M., Gachanja, A. N., Keriko, J. M., and Mungai, G. (2007). Traditional medicines among the Embu and Mbeere peoples of Kenya. *African Journal of Traditional, Complementary and Alternative Medicines*, *4*(1), 75–86.

Kathare, J. M., Mbaria, J. M., Nguta, J. M., and Moriasi, G. A. (2021a). Antimicrobial , Cytotoxicity , Acute Oral Toxicity , and Qualitative Phytochemical Screening of the Aqueous and Methanolic Stem-Bark Extracts of *Croton megalocarpu*s Hutch . (Euphorbiaceae). *The Journal of Phytopharmacology*, *10*(2), 117–125. https://doi.org/10.31254/phyto.2021.10208.

Kathare, J. M., Mbaria, J. M., Nguta, J. M., and Moriasi, G. A. (2021b). Antimicrobial, Cytotoxicity, Acute Oral Toxicity and Qualitative Phytochemical Screening of the Aqueous and Methanolic Extracts of *Physalis peruviana* L (Solanaceae). *Applied Microbiology Open Access*, *7*(2), 1–10. https://www.longdom.org/open-

access/antimicrobial-cytotoxicity-acute-oral-toxicity-and-qualitative-phytochemical-screening-of-the-aqueous-and-methanolic-ext.pdf.

Kaur, J,. Kaur, S., Mahajan, A. (2013). Herbal Medicines: Possible Risks and Benefits. *American Journal of Phytomedicine and Clinical Therapeutics*, *141104*, 226–239. http://ajpct.org/PA-400145-%5B12%5D.pdf.

Kaur, G., and Lata, S. (2019). A Review on: Phytochemical and Pharmacological Study of Annona Squamosa. *PharmaTutor*, *8*(8), 149–154. http://www.pharmatutorjournal.com/index.php/pt/article/view/a-reveiw-on-phytochemical-and-pharmacological-study-of-annona-squamosa.

Kelly, L., Jenkins, H., and Whyte, L. (2018). Pathophysiology of diarrhea. *Paediatrics and Child Health (United Kingdom)*, *28*(11), 520–526. https://doi.org/10.1016/j.paed.2018.09.002.

Keren, I., Wu, Y., Inocencio, J., Mulcahy, L. R., and Lewis, K. (2013). Killing by Bactericidal Antibiotics Does Not Depend on Reactive Oxygen Species. *Science*, *339*(6124), 1213–1216. https://doi.org/10.1126/science.1232688.

Kigen, G., Kamuren, Z., Njiru, E., Wanjohi, B., and Kipkore, W. (2019). Ethnomedical Survey of the Plants Used by Traditional Healers in Narok County, Kenya. *Evidence-Based Complementary and Alternative Medicine*, *2019*. https://doi.org/10.1155/2019/8976937.

Kigen, G., Maritim, A., Some, F., Kibosia, J., Rono, H., Chepkwony, S., Kipkore, W., and Wanjoh, B. (2016). Ethnopharmacological survey of the medicinal plants used in Tindiret, Nandi county, Kenya. *African Journal of Traditional, Complementary and Alternative Medicines*, *13*(3), 156–168. https://doi.org/10.4314/ajtcam.v13i3.19.

Kigen, G., Some, F., Kibosia, J., Rono, H., Kiprop, E., Wanjohi, B., Kigen, P., and Kipkore, W. (2014). Ethnomedicinal Plants Traditionally Used by the Keiyo Community in Elgeyo Marakwet County, Kenya. *Journal of Biodiversity, Bioprospecting and Development*, *01*(03), 1–11. https://doi.org/10.4172/2376-0214.1000132.

Kipkore, W., Wanjohi, B., Rono, H., and Kigen, G. (2014). A study of the medicinal plants used by the Marakwet Community in Kenya. *Journal of Ethnobiology and Ethnomedicine*, *10*(1), 1–22. https://doi.org/10.1186/1746-4269-10-24.

Köhler, O., E. Benros, M., Nordentoft, M., Farkouh, M. E., Iyengar, R. L., Mors, O., and Krogh, J. (2014). Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects a systematic review and meta-analysis of randomized clinical trials. *JAMA Psychiatry*, *71*(12), 1381–1391. https://doi.org/10.1001/jamapsychiatry.2014.1611.

Kokwaro, J. . (2009). *Medicinal Plants of East Africa* (Third edit). University of Nairobi Press. www.uonbi.ac.ke/press.

Kong, M., Xie, K., Lv, M., Li, J., Yao, J., Yan, K., Wu, X., Xu, Y., and Ye, D. (2021). Anti-inflammatory phytochemicals for the treatment of diabetes and its complications: Lessons learned and future promise. *Biomedicine and Pharmacotherapy*, *133*(September 2020), 110975. https://doi.org/10.1016/j.biopha.2020.110975.

Kuglerova, M., Tesarova, H., Grade, J. T., Halamova, K., Wanyana, O., Damme, P. Van, and Kokoska, L. (2011). Antimicrobial and antioxidative effects of Ugandan medicinal barks. *African Journal of Biotechnology*, *10*(18), 3628–3632. https://doi.org/10.5897/AJB09.1815.

Kurmukov, A. G. (2013). Phytochemistry of medicinal plants. *Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan*, *1*(6), 13–14. https://doi.org/10.1007/978-1-4614-

3912-7_4.

Lata, S., and Mittal, S. K. (2015). Pharmacognosy, phytochemistry and pharmacology of cucumis dipsaceus ehrenb. *International Journal of Pharmacognosy and Phytochemical Research*, *7*(3), 446–449.

Leonti, M., and Verpoorte, R. (2017). Traditional Mediterranean and European herbal medicines. *Journal of Ethnopharmacology*. https://doi.org/10.1016/j.jep.2017.01.052.

Liu, D. (2019). Escherichia coli. In *Encyclopedia of Microbiology*. https://doi.org/10.1016/B978-0-12-801238-3.02291-1.

Liu, X., Lin, Y. J., and Cheng, Y. (2016). Complementary and Alternative Therapies for Inflammatory Diseases. *Evidence-Based Complementary and Alternative Medicine*, *2016*. https://doi.org/10.1155/2016/8324815.

Lordan, R., Tsoupras, A., and Zabetakis, I. (2019). Inflammation. In *The Impact of Nutrition and Statins on Cardiovascular Diseases* (pp. 23–51). Elsevier. https://doi.org/10.1016/B978-0-12-813792-5.00002-1.

Lucas, S. (2016). The Pharmacology of Indomethacin. *Headache*, *56*(2), 436–446. https://doi.org/10.1111/head.12769. 0

Lulekal, E., Kelbessa, E., Bekele, T., and Yineger, H. (2008). An ethnobotanical study of medicinal plants in Mana Angetu District, southeastern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, *4*. https://doi.org/10.1186/1746-4269-4-10.

Mahmoud abd El-Baky, R. (2016). The Future Challenges Facing Antimicrobial Therapy: Resistance and Persistence. *American Journal of Microbiological Research*, *4*(1), 1–15. https://doi.org/10.12691/ajmr-4-1-1.

Mahomoodally, M. F. (2013). Traditional medicines in Africa: An appraisal of ten potent African medicinal plants. In *Evidence-based Complementary and Alternative Medicine*. https://doi.org/10.1155/2013/617459.

Manandhar, S., Luitel, S., and Dahal, R. K. (2019). In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. *Journal of Tropical Medicine*, *2019*. https://doi.org/10.1155/2019/1895340.

Mandal, S. M., Roy, A., Ghosh, A. K., Hazra, T. K., Basak, A., and Franco, O. L. (2014). Challenges and future prospects of antibiotic therapy: From peptides to phages utilization. *Frontiers in Pharmacology*, *5 MAY*(May), 1–12. https://doi.org/10.3389/fphar.2014.00105.

Mandell, L. A., Ball, P., and Tillotson, G. (2001). Antimicrobial safety and tolerability: Differences and dilemmas. *Clinical Infectious Diseases*, *32*(Suppl 1), S72–S79. https://doi.org/10.1086/319379.

Marshall, R. L., Lloyd, G. S., Lawler, A. J., Element, S. J., Kaur, J., Ciusa, M. L., Ricci, V., Tschumi, A., Kühne, H., Alderwick, L. J., and Piddock, L. J. V. (2020). New multidrug efflux inhibitors for gram-negative bacteria. *MBio*, *11*(4), 1–19. https://doi.org/10.1128/mBio.01340-20.

Mathabe, M. C., Nikolova, R. V, Lall, N., and Nyazema, N. Z. (2006). *Antibacterial activities of medicinal plants used for the treatment of diarrhea in Limpopo Province , South Africa*. *105*, 286–293. https://doi.org/10.1016/j.jep.2006.01.029.

Mathers, C. (2017). Global Burden of Disease. In *International Encyclopedia of Public Health* (pp. 256–267). Elsevier. https://doi.org/10.1016/B978-0-12-803678-5.00175-2.

Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature*, *454*(7203), 428–435. https://doi.org/10.1038/nature07201.

Mekonnen, B., Asrie, A. B., and Wubneh, Z. B. (2018).antidiarrhea Activity of 80% Methanolic Leaf Extract of Justicia schimperiana. *Evidence-Based Complementary and Alternative Medicine*, *2018*. https://doi.org/10.1155/2018/3037120.

Mohsen, S., Dickinson, J. A., and Somayaji, R. (2020). Update on the adverse effects of antimicrobial therapies in community practice. *Canadian Family Physician*, *66*(9), 651–659.

Molina-Santiago, C., and Ramos, J. L. (2014). Bactericidal and bacteriostatic antibiotics and the Fenton reaction. *Microbial Biotechnology*, *7*(3), 194–195. https://doi.org/10.1111/1751-7915.12120.

Molyneux, R. J., Lee, S. T., Gardner, D. R., Panter, K. E., and James, L. F. (2007). Phytochemicals: The good, the bad and the ugly? *Phytochemistry*, *68*(22–24), 2973–2985. https://doi.org/10.1016/j.phytochem.2007.09.004. Accessed 16th November 2019

Monack, D. M., Mueller, A., and Falkow, S. (2004). Persistent bacterial infections: The interface of the pathogen and the host immune system. In *Nature Reviews Microbiology*. https://doi.org/10.1038/nrmicro955.

Monteiro, B., and Steagall, P. V. (2019). Antiinflammatory Drugs. *Veterinary Clinics of North America: Small Animal Practice*, *49*(6), 993–1011. https://doi.org/10.1016/j.cvsm.2019.07.009.

Moreira, D. de L., Teixeira, S. S., Monteiro, M. H. D., De-Oliveira, A. C. A. X., and Paumgartten, F. J. R. (2014). Traditional use and safety of herbal medicines. *Brazilian Journal of Pharmacognosy*. https://doi.org/10.1016/j.bjp.2014.03.006.

Moriasi, G. A., Ireri, A. M., Nelson, E. M., and Ngugi, M. P. (2021a). *In vivo* anti-inflammatory, anti-nociceptive, and in vitro antioxidant efficacy, and acute oral toxicity effects of the aqueous and methanolic stem bark extracts of *Lonchocarpus eriocalyx* (Harms.). *Heliyon*, *7*(5), e07145. https://doi.org/10.1016/j.heliyon.2021.e07145.

Moriasi, G., Ireri, A., and Ngugi, M. (2020). Cognitive-Enhancing, Ex Vivo Antilipid Peroxidation and Qualitative Phytochemical Evaluation of the Aqueous and Methanolic Stem Bark Extracts of Lonchocarpus eriocalyx (Harms.). *Biochemistry Research*

International, *2020*, 1–16. https://doi.org/10.1155/2020/8819045.

Moriasi, G., Kibiti, C., and Ngugi, M. (2021). In Vivo Antidiabetic Efficacy, Mineral Element Composition, and Qualitative Phytochemistry of the Aqueous Leaf Extracts of Pentas zanzibarica (Klotzsch.) Vatke and Olea europaea subspecies africana (Mill.). *Journal of Advanced Biotechnology and Experimental Therapeutics*, *4*(3), 334. https://doi.org/10.5455/jabet.2021.d134.

Murek, M., Kopic, S., and Geibel, J. (2010). Evidence for intestinal chloride secretion. *Experimental Physiology*, *95*(4), 471–478. https://doi.org/10.1113/expphysiol.2009.049445.

Murray, C. J. ., and Lopez, A. D. (1996). Global Burden of Disease and Injury Series the Global Burden of Disease. *Oms*, 1–46.

Mutie, F. M., Rono, P. C., Kathambi, V., Hu, G. W., and Wang, Q. F. (2020). Conservation of wild food plants and their potential for combatting food insecurity in kenya as exemplified by the drylands of Kitui county. *Plants*, *9*(8), 1–25. https://doi.org/10.3390/plants9081017.

Mwitari, P. G., Ayeka, P. A., Ondicho, J., Matu, E. N., and Bii, C. C. (2013). Antimicrobial Activity and Probable Mechanisms of Action of Medicinal Plants of Kenya: Withania somnifera, Warbugia ugandensis, Prunus africana and Plectrunthus barbatus. *PLoS ONE*, *8*(6), 4–12. https://doi.org/10.1371/journal.pone.0065619.

Nanasombat, S., Kuncharoen, N., Ritcharoon, B., and Sukcharoen, P. (2018). Antibacterial activity of thai medicinal plant extracts against oral and gastrointestinal pathogenic bacteria and prebiotic effect on the growth of lactobacillus acidophilus. *Chiang Mai Journal of Science*, *45*(1), 33–44.

Nasri, H., and Shirzad, H. (2013). Toxicity and safety of medicinal plants. *Journal of HerbMed Pharmacology*, *2*(2), 21–22.

Niemegeers, C. J. E., Colpaert, F. C., and Awouters, F. H. L. (1981). Pharmacology andantidiarrhea effect of Loperamide. *Drug Development Research*, *1*(1), 1–20. https://doi.org/10.1002/ddr.430010102.

Nivedhini, V., Chandran, R., and Parimelazhagan, T. (2014). Chemical composition and antioxidant activity of Cucumis dipsaceus Ehrenb. Ex Spach fruit. *International Food Research Journal*, *21*(4), 1465–1472.

Njoroge, G. N., and Newton, L. E. (1994). Edible and Poisonous Species of Cucurbitaceae in the Central Highlands of Kenya. *Journal of East African Natural History*, *83*(2), 101–115. https://doi.org/10.2982/0012-8317(1994)83[101:eapsoc]2.0.co;2.

Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA(2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol. 30(5):379-84. doi: 10.1046/j.1472-765x.2000.00731.x. PMID: 10792667.

Nyamwamu, N., and Bonareri, N. L. (2020). Ethnobotanical survey and documentation of medicinal plants used by abagusii traditional healers in the treatment of diarrhea and other common ailments in Kenya. *International Journal of Applied Research*, *6*(7), 182–185.

Nyang'au, H. O., Maingi, J., and Kebira, A. (2017). The efficacy of some medicinal plants used locally within Transmara west, Narok County, Kenya against selected Enterobacteria and Candida. *IOSR Journal of Pharmacy and Biological Sciences*, *12*(01), 115–122. https://doi.org/10.9790/3008-120101115122.

Nyarang, N., and Bonareri, N. L. (2021). *Antimicrobial and phytochemical screening of Euphorbia hirta used by Abagusii Mothers in treatment of infectious diseases among young children Antimicrobial and phytochemical screening of Euphorbia hirta used by Abagusii Mothers in treatment of infectious*.

Obey, J. K., von Wright, A., Orjala, J., Kauhanen, J., and Tikkanen-Kaukanen, C. (2016). Antimicrobial Activity of Croton macrostachyus Stem Bark Extracts against Several Human Pathogenic Bacteria *Journal of Pathogens*, *2016*(Mic), 1–5. https://doi.org/10.1155/2016/1453428.

Organisation for Economic Co-operation and Development (OECD). (2008). Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. In *Test* (Issue October). OECD. https://doi.org/10.1787/9789264071049-en.

Organisation for Economic Cco-operation and Development (OECD). (2017). *Test No. 402: Acute Dermal Toxicity* (Issue October). OECD. https://doi.org/10.1787/9789264070585-en.

Oguntibeju, O. O. (2018). Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. *Journal of Inflammation Research*, *11*, 307–317.

Olela, B., Mbaria, J., Wachira, T., and Moriasi, G. (2020). Acute Oral Toxicity and Anti-inflammatory and Analgesic Effects of Aqueous and Methanolic Stem Bark Extracts of Piliostigma thonningii (Schumach.). *Evidence-Based Complementary and Alternative Medicine*, *2020*, 1–10. https://doi.org/10.1155/2020/5651390.

Operario, D., and Houpt, E. (2012). Defining the Etiology of Diarrhea: Novel Approaches. *National Institutes of Health*, *24*(5), 464–471. https://doi.org/10.1097/QCO.0b013e32834aa13a.

Othman, C. N., and Farooqui, M. (2015). Traditional and Complementary Medicine. *Procedia - Social and Behavioral Sciences*. https://doi.org/10.1016/j.sbspro.2015.01.036.

Pires, S. M., Fischer-Walker, C. L., Lanata, C. F., Devleesschauwer, B., Hall, A. J., Kirk, M. D., Duarte, A. S. R., Black, R. E., and Angulo, F. J. (2015). Aetiology-Specific Estimates of the Global and Regional Incidence and Mortality of Diarrhea

Diseases Commonly Transmitted through Food. *PLOS ONE*, *10*(12), e0142927. https://doi.org/10.1371/journal.pone.0142927.

Poolman, J. T. (2016). Escherichia coli. In *International Encyclopedia of Public Health*. https://doi.org/10.1016/B978-0-12-803678-5.00504-X.

Procop, G. W., and Wilson, M. (2001). Infectious Disease Pathology. *Clinical Infectious Diseases*, *32*(11), 1589–1601. https://doi.org/10.1086/320537.

Ptaschinski, C., and Lukacs, N. W. (2018). Acute and chronic inflammation induces disease pathogenesis. In *Molecular Pathology: The Molecular Basis of Human Disease* (Second Edi, pp. 25–43). Elsevier Inc. https://doi.org/10.1016/B978-0-12-802761-5.00002-X.

Purssell, E. (2020). Antimicrobials. In P. Hood and E. Khan (Eds.), *Understanding Pharmacology in Nursing Practice*. Springer International Publishing. https://doi.org/10.1007/978-3-030-32004-1.

Rahman, S. M. M., Islam, N., and Ahammad, F. (2019).antidiarrhea *activities of methanol and ethyl acetate extract of Hemigraphis alternata leaves in mice.*

Riaz, B., Zahoor, M. K., Zahoor, M. A., Majeed, H. N., Javed, I., Ahmad, A., Jabeen, F., Zulhussnain, M., and Sultana, K. (2018). Toxicity, Phytochemical Composition, and Enzyme Inhibitory Activities of Some Indigenous Weed Plant Extracts in Fruit Fly, Drosophila melanogaster. *Evidence-Based Complementary and Alternative Medicine*, *2018*. https://doi.org/10.1155/2018/2325659.

Saga, T., and Yamaguchi, K. (2009). History of antimicrobial agents and resistant bacteria. *Japan Medical Association Journal*, *52*(2), 103–108.

Sahlmann, C. O., and Ströbel, P. (2016). Pathophysiology of inflammation. In *NuklearMedizin*. https://doi.org/10.1055/s-0037-1616468.

Saquib, S. A., Alqahtani, N. A., Ahmad, I., Kader, M. A., Al Shahrani, S. S., and Asiri, E. A. (2019). Evaluation and comparison of antibacterial efficacy of herbal extracts in combination with antibiotics on periodontal pathobionts: An in vitro microbiological study. *Antibiotics*, *8*(3), 1–12. https://doi.org/10.3390/antibiotics8030089.

Scalbert, A. (1991). Antimicrobial properties of tannins. In *Phytochemistry*. https://doi.org/10.1016/0031-9422(91)83426-L.

Sekirov, I., Russell, S. L., Caetano M Antunes, L., and Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological Reviews*, *90*(3), 859–904. https://doi.org/10.1152/physrev.00045.2009.

Semenya, S. S., and Maroyi, A. (2012). Medicinal plants used by the Bapedi traditional healers to treat diarrhea in the Limpopo Province , South Africa. *Journal of Ethnopharmacology*, *144*(2), 395–401. https://doi.org/10.1016/j.jep.2012.09.027.

Sen A, Capelli V, Husain M(2018). Cognition and dementia in older patients with epilepsy Brain. 141(6):1592-1608. doi: 10.1093/brain/awy022. PMID: 29506031; PMCID: PMC5972564.

Shehab, N., Lovegrove, M. C., Geller, A. I., Rose, K. O., Weidle, N. J., and Budnitz, D. S. (2016). US emergency department visits for outpatient adverse drug events, 2013-2014. *JAMA - Journal of the American Medical Association*, *316*(20), 2115–2125. https://doi.org/10.1001/jama.2016.16201.

Shivakoti C.H, B.Swetha, Satya, B. L., SVVNSM.Laxmi, and K.Ramanjaneyelu. (2015). Evaluation of Antimicrobial Activity and Phytochemical Screening of Cucumis Evaluation of Antimicrobial Activity and Phytochemical Screening of Cucumis Dipsaceus Ehrenb . Leaves. *Literati Journal of Pharmaceutical Drug Delivery Technologies*, *01*(01), 1–4.

Shojaii, A., Motaghinejad, M., Norouzi, S., and Motevalian, M. (2015). Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of Astragalus hamosus in animal models. *Iranian Journal of Pharmaceutical Research, 14*(1), 263–269. https://doi.org/10.22037/ijpr.2015.1620.

Silva, F., Lourenço, O., Queiroz, J. A., and Domingues, F. C. (2011). Bacteriostatic versus bactericidal activity of Ciprofloxacin in Escherichia coli assessed by flow cytometry using a novel far-red dye. *Journal of Antibiotics*, *64*(4), 321–325. https://doi.org/10.1038/ja.2011.5.

Sisay, M., Engidawork, E., and Shibeshi, W. (2017). Evaluation of theantidiarrhea activity of the leaf extracts of Myrtus communis Linn (Myrtaceae) in mice model. *BMC Complementary and Alternative Medicine*, *17*(1), 1–11. https://doi.org/10.1186/s12906-017-1625-3.

Sjöling, Å., Sadeghipoorjahromi, L., Novak, D., and Tobias, J. (2015). Detection of major diarrheagenic bacterial pathogens by multiplex PCR panels. *Microbiological Research*, *172*, 34–40. https://doi.org/10.1016/j.micres.2014.12.003.

Sofowora, A., Ogunbodede, E., and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary, and Alternative Medicines : AJTCAM / African Networks on Ethnomedicines*, *10*(5), 210–229. https://doi.org/10.4314/ajtcam.v10i5.2.

Sommer, C., Leinders, M., and Üçeyler, N. (2018). Inflammation in the pathophysiology of neuropathic pain. *Pain*, *159*(3), 595–602. https://doi.org/10.1097/j.pain.

Staudacher, A. G., and Stevens, W. W. (2019). Sinus Infections, Inflammation, and Asthma. *Immunology and Allergy Clinics of North America*, *39*(3), 403–415. https://doi.org/10.1016/j.iac.2019.03.008.

Su, H. J., Chiu, Y. T., Chiu, C. T., Lin, Y. C., Wang, C. Y., Hsieh, J. Y., and Wei, S. C. (2019). Inflammatory bowel disease and its treatment in 2018: Global and Taiwanese status updates. *Journal of the Formosan Medical Association*, *118*(7), 1083–1092. https://doi.org/10.1016/j.jfma.2018.07.005.

Talreja, T. (2010). Screening of Crude Extract of Flavonoids of Moringa oleifera against Bacteria and Fungal Pathogen. *Journal of Phytology*, *2*(11), 31–35.

Tefera, B. N., and Kim, Y. D. (2019). Ethnobotanical study of medicinal plants in the Hawassa Zuria District, Sidama zone, Southern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, *15*(1), 1–21. https://doi.org/10.1186/s13002-019-0302-7.

Teferi, M. Y., Abdulwuhab, M., and Yesuf, J. S. (2019). Evaluation of In Vivoantidiarrhea Activity of 80% Methanolic Leaf Extract of Osyris quadripartita Decne (Santalaceae) in Swiss Albino Mice. *Journal of Evidence-Based Integrative Medicine*, *24*, 1–9. https://doi.org/10.1177/2515690X19833340.

Troeger, C., Blacker, B. F., Khalil, I. A., Rao, P. C., Cao, S., Zimsen, S. R., Albertson, S. B., Stanaway, J. D., Deshpande, A., Abebe, Z., Alvis-Guzman, N., Amare, A. T., Asgedom, S. W., Anteneh, Z. A., Antonio, C. A. T., Aremu, O., Asfaw, E. T., Atey, T. M., Atique, S., … Reiner, R. C. (2018). Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Infectious Diseases*, *18*(11), 1211–1228. https://doi.org/10.1016/S1473-3099(18)30362-1.

Troeger, C., Forouzanfar, M., Rao, P. C., Khalil, I., Brown, A., Reiner, R. C., Fullman, N., Thompson, R. L., Abajobir, A., Ahmed, M., Alemayohu, M. A., Alvis-Guzman, N., Amare, A. T., Antonio, C. A., Asayesh, H., Avokpaho, E., Awasthi, A., Bacha, U., Barac, A., … Mokdad, A. H. (2017). Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhea diseases: a systematic analysis for the

Global Burden of Disease Study 2015. *The Lancet Infectious Diseases*, *17*(9), 909–948. https://doi.org/10.1016/S1473-3099(17)30276-1. A

Truong, D.-H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., and Nguyen, H. C. (2019). Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and In Vitro Anti-Inflammatory Activities of Severinia buxifolia. *Journal of Food Quality*, *2019*, 1–9. https://doi.org/10.1155/2019/8178294.

Tugume, P., and Nyakoojo, C. (2019). Ethno-pharmacological survey of herbal remedies used in the treatment of paediatric diseases in Buhunga parish, Rukungiri District, Uganda. *BMC Complementary and Alternative Medicine*, *19*(1), 1–10. https://doi.org/10.1186/s12906-019-2763-6.

Ullah, H., and Ali, S. (2017). Classification of Anti-Bacterial Agents and Their Functions. *Antibacterial Agents*. https://doi.org/10.5772/intechopen.68695;

World Health Oorganisation (WHO) /Uited Nations Children's Fund (UNICEF). (2004). *Clinical Management of Leading Cause of Child*. 2–4.

World Health Orhanisation (WHO). (2008). World HealthOrganization. Diarrhea : Why children are still dying and what can be done. In *Vasa*.

World Health Orghanisation (WHO). (1990). *The rational use of drugs in the management of acute diarrhea in children*.

World Health Organization. (2018). *Traditional and complementary medicine in primary health care*.

World Health Organization (WHO). (2013). WHO Traditional Medicine Strategy 2014-2023. In *World Health Organization (WHO)*. https://doi.org/2013.

World Health Organization (WHO). (2015). Global Action Plan on Antimicrobial Resistance. In *Microbe Magazine* (Vol. 10, Issue 9). https://doi.org/10.1128/microbe.10.354.1.

World Health Organization (WHO). (2020). Global report on the epidemiology and burden of sepsis: current evidence, identifying gaps and future directions. In *Global report on the epidemiology and burden of sepsis: current evidence, identifying gaps and future directions*. http://apps.who.int/bookorders.%0Ahttps://apps.who.int/iris/bitstream/handle/10665/334216/9789240010789-eng.pdf.

Zenebe, G., Zerihun, M., and Solomon, Z. (2012). An ethnobotanical study of medicinal plants in Asgede Tsimbila District, Northwestern Tigray, Northern Ethiopia. *Ethnobotany Research and Applications*, *10*, 305–320. https://doi.org/10.17348/era.10.0.305-320.

Zhang, J., Wider, B., Shang, H., Li, X., and Ernst, E. (2012). Quality of herbal medicines: Challenges and solutions. In *Complementary Therapies in Medicine*. https://doi.org/10.1016/j.ctim.2011.09.004.

Zhu, F., Du, B., and Xu, B. (2018). Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. *Critical Reviews in Food Science and Nutrition*, *58*(8), 1260–1270. https://doi.org/10.1080/10408398.2016.1251390.

APPENDICES

Appendix I: Ethical Approval by the Faculty Biosafety, Animal Use and Ethics Committee

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2020/264

Dr. Purity Kanana Kimathi,
University of Nairobi
Dept. PHP & Toxicology
24/02/2020

Dear Dr. Kimathi,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

Anti-Inflammatory, Anti-Bacterial, Gastro intestinal motility and toxic effects of aqueous and methanolic leaf and fruit extracts of *Cucumis dipsaceus*.

Dr. Purity Kanana Kimathi J56/11743/2018

We refer to your MSc. proposal submitted to our committee for review and your application letter dated February 2020. We have reviewed your application for ethical clearance for the study.
The number of rats and rabbits to be used in the study meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines. The study protocols are adequate and concise.
We have also noted that registered veterinary surgeons will supervise the work.
We hereby give approval for you to proceed with the project as outlined in the submitted proposal.
Yours sincerely,

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix II: Botanical identification of the study plant by the botany Department of National Museums of Kenya

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

REF: NMK/BOT/CTX/1/8

03/02/2020

Purity Kanana
Tel.-0722-291185
NAIROBI.

Dear Ms. Kanana,

PLANT IDENTIFICATION

The plant specimen you brought to us for identification has been determined as follows:

01 Cucumis dipsaceus Ehrenb. Ex Spach. **(Family: Cucurbitaceae)**

Thank you for consulting the EAH for plant identification and confirmation.

Yours Sincerely

NATIONAL MUSEUMS OF KENYA 10 FEB 2020 HERBARIUM NAIROBI

Mathias Mbale
For: Head, Botany Department.

Address P.O. Box 40658 00100 GPO Nairobi Telephone 254 20 4448930/3 . 254 20 3742161/4 . 254 20 3742131/4 Fax 254 20 3741424 Website www.museums.or.ke

Appendix III: Research permit granted by the National Commission for Science, Technology and Innovation

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **192543**

Date of Issue: **21/July/2021**

RESEARCH LICENSE

This is to Certify that Dr.. Purity KANANA Kimathi of University of Nairobi, has been licensed to conduct research in Meru on the topic: ANTI-INFLAMMATORY, ANTIBACTERIAL, GASTRO-INTESTINAL MOTILITY AND TOXIC EFFECTS OF AQUEOUS AND METHANOLIC LEAF AND FRUIT EXTRACTS OF CUCUMIS DIPSACEUS for the period ending : 21/July/2022.

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Appendix IV: Plagiarism test report

Anti-Inflammatory, Antimicrobial, Antidiarrhoeal, and Toxic Effects of The Aqueous and Methanolic Leaf and Fruit Extracts of Cucumis dipsaceus (Ehrenb. Ex Spach.)

ORIGINALITY REPORT

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Appendix V: Published Research article based on this thesis

J Herbmed Pharmacol. 2022; 11(2): 213-225.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2022.26

Journal of Herbmed Pharmacology

Antidiarrheal, antimicrobial, and toxic effects of the aqueous and methanolic leaf and fruit extracts of *Cucumis dipsaceus* (Ehrenb. Ex Spach.)

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ABSTRACT

Introduction: *Cucumis dipsaceus* is used to treat diarrhoea, microbial infections, among other diseases across the world; however, there is insufficient empirical data to validate its efficacy, toxicity, and safety. Accordingly, we investigated the antidiarrheal, antimicrobial, and toxic effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus*.

Methods: Antidiarrheal activities of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* were investigated using the castor oil-induced diarrhoea technique in a Wistar rat model. The disk diffusion and broth microdilution methods were adopted to determine the antimicrobial activities of the studied plant extracts. The acute oral toxicity effects of the studied plant extracts were investigated in Wistar rats according to the Organisation for Economic Co-operation and Development (OECD) guidelines.

Results: The aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* significantly ($P<0.05$) inhibited diarrhoea in a dose-dependent manner in experimental rats. Besides, the studied extracts significantly ($P<0.05$) inhibited the growth of *Salmonella enteritidis*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans* in varying degrees, as depicted by their growth inhibition zones (>6.00 mm) and minimum inhibitory concentrations (MICs <1000 µg/mL). Moreover, the studied extracts did not cause any observable acute oral toxicity effects in the experimental rats across the 14-day experimental period.

Conclusion: The aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* present a potential source of safe and efficacious lead compounds for developing antidiarrheal and antimicrobial therapies.

Implication for health policy/practice/research/medical education:
This research article valorises *Cucumis dipsaceus* as a potential source of efficacious and safe antidiarrheal and antimicrobial lead compounds.

Please cite this paper as: Kimathi PK, Maitho TE, Mbaria JM, Moriasi AG. Antidiarrheal, antimicrobial, and toxic effects of the aqueous and methanolic leaf and fruit extracts of *Cucumis dipsaceus* (Ehrenb. Ex Spach.). J Herbmed Pharmacol. 2022;11(2):213-225. doi: 10.34172/jhp.2022.26.

Introduction

Microbial infections of the gastrointestinal tract cause gastric irritation, inflammation, and impaired gastric functioning, manifesting in abdominal discomfort and diarrhoea (1–3). Diarrhoea is among the leading causes of morbidity and mortality worldwide, especially in children, the elderly, and immunocompromised persons (4). Despite the availability of conventional antimicrobial and antidiarrheal drugs, the global public health burden of microbial infections is still high (4). The emergence of antimicrobial-resistant strains of bacteria and fungi has further complicated the successful use of chemotherapy (5).

The presently used antimicrobial agents cause undesirable effects, including constipation, gastric irritation, cardiotoxicity, hepatotoxicity, nephrotoxicity, low efficacy, among other adverse effects (6). Besides, the conventional antidiarrheal drugs are associated with

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