PHYTOCHEMICAL COMPOSITION, TOXIC EFFECTS, AND ANTIMICROBIAL ACTIVITY STUDIES OF *DIGITARIA ABYSSINICA* (A. RICH.) STAPF (POACEAE) RHIZOME EXTRACTS AGAINST SELECTED UROPATHOGENIC MICROORGANISMS

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J56/34157/2019

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF SCIENCE DEGREE IN PHARMACOLOGY AND TOXICOLOGY

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

FACULTY OF VETERINARY MEDICINE

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DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any university.

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DEDICATION

I dedicate this thesis to my father Mr. Simon Sapunyo, my mother Mary Sapunyo, my siblings Mercy, Grace and Moses for their immeasurable support, guidance and sacrifice towards my education.

ACKNOWLEDGEMENT

First, I would like to acknowledge the Almighty God for His knowledge, strength, guidance and good health throughout the entire study. Second, I owe a debt of gratitude to Prof. James Mbaria and Dr. Laetitia Kanja, my supervisors, for their direction, inspiration, and assistance in completing my research. I'd like to thank Mr. Ken Maloba, Mr. Charles Asava, Mr. Nderitu, Mr. Bett and Ms. Leah of the University of Nairobi's PHPT Department for their technical assistance during this research.

I would also like to express my gratitude to Chairperson Dr. Marianne Mureithi and the entire staff of the University of Nairobi, Medical Microbiology Department for allowing and supporting me when conducting the antimicrobial susceptibility testing. My special heartfelt gratitude to Mr. Meshack Juma Chief Laboratory technologist at the Obstetrics and Gynecology Department- University of Nairobi, for his time, support and expert knowledge during the antimicrobial activity research.

Special appreciation to my dear friend Brian Muyukani for all the consistent support, encouragement, fruitful views and discussions.

My special thanks to Mr. Musembi Kimeu and Mrs. Carol Kyalo from the University of Nairobi, Department of Land Resource Management and Agricultural Technology (LARMAT) Department for their help on plant collection and voucher specimen identification. I would also like to express my sincere gratitude to Dr. Jared Onyancha for sharing his in-depth knowledge in the field of complementary medicine. I value Dr. Mitch Okumu for his great research ideas throughout this project period.

Last but not least, I want to express my sincere gratitude to the entire Sapunyo’s family for their unwavering support, inspiration, and prayers during my academic career. May God richly bless you all.

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LIST OF ACRONYMS AND ABBREVIATIONS

AIDS	**Acquired Immunodeficiency Syndrome**
AMR	**Antimicrobial Resistance**
ANOVA	**Analysis of Variance**
ATCC	**American Type Culture Collection**
BAUEC	**Biosafety, Animal Use and Ethics Committee**
CABI	**Centre for Agriculture and Biosciences International**
CLSI	**Clinical and Laboratory Standards Institute**
DCM	**Dichloromethane**
DMSO	**Dimethyl sulfoxide**
DNA	**Deoxyribonucleic acid**
FVM	**Faculty of Veterinary Medicine**
GCMS	**Gas Chromatography Mass Spectrometry**
HIV	**Human Immunodeficiency Virus**
HSV	**herpes simplex virus**
LARMAT	**Land Resource Management and Agricultural Technology**
LD_{50}	**Lethal Dose 50**

MBC	**Minimum Bactericidal Concentration**
MFC	**Minimum Fungicidal Concentration**
MIC	**Minimum Inhibitory Concentration**
NACOSTI	**National Commission for Science, Technology and Innovation**
NMR	**Nuclear Magnetic Resonance**
OECD	**Organization for Economic Co-operation and Development**
PHPT	**Public Health Pharmacology and Toxicology**
SEM	**Standard error of the mean**
STD	**Sexually Transmitted Disease**
STI	**Sexually Transmitted Infection**
Tukey HSD	**Tukey honest significant difference**
UDP	**Up-and-Down Procedure**
UPEC	**Uropathogenic *Escherichia coli***
UTI	**Urinary Tract Infection**
WHO	**World Health Organization**

ABSTRACT

Urinary tract infections (UTIs) are among the most severe public health problems that affect over one hundred and fifty million people worldwide annually. They are caused by a wide range of microorganisms where *Escherichia coli* is known to be the main causative pathogen. Medicinal plants are used in traditional Kenya setup for treatment and in the most recent as alternative source of treatment of UTIs due to increased cost of treatment and many challenges experienced with antibiotic therapy. The current study was designed to investigate the phytochemical composition, acute oral toxicity and antimicrobial activity of *Digitaria abyssinica* rhizome extracts against *Staphylococcus aureus, Escherichia coli, Neisseria gonorrhoeae* and *Candida albicans*. The rhizomes of *Digitaria abyssinica* were obtained, dried, ground and extracted using water and organic solvents (methanol and dichloromethane). Phytochemical assay was carried out using standard phytochemical screening methods. The Organization for Economic Co-operation and Development (OECD) guidelines for testing of chemicals document 425 for 2008 was used to assess the toxic effects on female Wistar rats. Disk diffusion method was used to obtain the zones of inhibition while microbroth dilution techniques were used to obtain the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) for the antimicrobial assay. The data that was obtained from phytochemical screening was entered into a Word sheet and presented in a table. The weights of the Wistar rats from day 0, day 7 and day 14 following oral admission of the *Digitaria abyssinica* rhizome extracts were entered in an Excel sheet. All experiments in the antimicrobial activity testing were performed in triplicates for verification and validation of data. GraphPad Prism software version 9.0.0 was used to compute mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) and post hoc ANOVA at 95% confidence level and statistical significancy was taken at $p < 0.05$. Results revealed that saponins, phenolics, alkaloids, cardiac glycosides, tannins, flavonoids, steroids, and terpenes were present in the powder, aqueous, methanol, and

dichloromethane:methanol extracts. All the extracts had an LD_{50} of above 2,000 mg/kg of body weight and there was no observation of behavioural changes. The extracts revealed antifungal activity against the *Candida albicans*. However, there was no antibacterial activity at a concentration of 500 mg/ml on *Staphylococcus aureus*, *Escherichia coli* and *Neisseria gonorrhoeae.* The highest antifungal activity was demonstrated by aqueous extract which MIC of 31.25 mg/ml, MFC 62.5 mg/ml and a zone of inhibition of 16.33 ± 0.82 mm at a concentration of 500 mg/ml. The current study reports that *Digitaria abyssinica* rhizome extracts had antifungal activity, thus confirming its traditional use to treat candidiasis. This research recommends that other solvents could be used, which could lead to improved yield and biological activity. Comprehensive research of the antimicrobial efficacy of the studied plant extracts on other microbial strains of clinical significance are encouraged. It is necessary to do additional research to determine the precise mode(s) by which the plant extracts under study exert their antifungal activity. To determine the safety of long-term usage of *Digitaria abyssinica* rhizome extracts, extensive sub-acute and chronic investigations should be conducted.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Urinary tract infections (UTIs) are termed as one of the most severe public health problems and affect over one hundred and fifty million people worldwide per year (Sihra *et al.,* 2018). UTIs affect both genders but females are more susceptible due to the differences in urogenital and reproductive anatomy, physiology and lifestyle. UTIs are known to affect more than half of women at least once in their lives and reinfection is reported to be more recurrent in young women (Tache *et al.,* 2022). Urinogenital infections are caused by bacteria and fungi, the most causative agent is *Escherichia coli* usually called uropathogenic *Escherichia coli* (UPEC) (Ana *et al.,* 2016). Other pathogenic microorganisms are *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Staphylococcus saprophyticus*, *Staphylococcus aureus* and Candida species (Ronald, 2003).

Currently, the conventional treatment of uropathogenic infections experience great challenges including the increasing antimicrobial resistance, high recurrence rates and side effects caused by antibiotic therapy that are of great threat to the economic burden (Cock *et al.,* 2021).

Despite the numerous successes of conventional medicines, there has still been drawbacks and challenges associated with its use. These are but not limited to their high cost and unavailability of conventional medicine. Conventional medicine is expensive has also been associated with numerous side effects. As a result of its accessibility, cost, perceived effectiveness, and safety, the use of herbal medicines in the therapy of a variety of disorders continues to gain traction in a number of cultures (Munguti, 1997).

The WHO estimates that more than 80% of the global population, particularly in low-income nations, relies on traditional medicine to cover their primary healthcare needs (Agidew, 2022). Although the efficacy of many herbal products has been proved, therapies utilizing these compounds have showed promising potential. However, many of these drugs remain untested, and their usage is either inadequately or not at all regulated. As a result, it is difficult to advocate the safe and sensible use of these agents because little information is available about their mode of action, potential adverse responses, contraindications, and interactions with already available orthodox medications and functional foods. Since safety is still a major concern when using herbal remedies, it is crucial that the proper regulatory agencies put in place the necessary safeguards to protect public health by ensuring that all herbal medications are secure and of high suitable quality (Ekor, 2014).

Digitaria is a genus of plants in the grass family Poaceae. The Poaceae includes cereals, grasses, bamboos and the grasses of the natural grasslands (Peterson *et al*., 2020). Some of them are currently in use include *Vetiveria nigritana* (black vertiver grass), which is used to treat HIV/AIDS, *Cymbopogon citratus* (lemon grass), which is used to cure malaria. Bahama grass (*Cynodon dactylon*) is used to stop vomiting. Cocograss (*Cyperus rotundus*) is used to treat malaria and stomachaches. *Oryza sativa* (rice) is used to treat herpes labialis. *Sorghum bicolor* (guinea corn) is used to treat infertility (Oseghale *et al*., 2017).

Digitaria abyssinica (Poaceae) is commonly referred to as East African couch grass (Baguma *et al*., 1995). It is a common weed that is used as food for livestock characterized by low nutritional value (Heuzé *et al*., 2019; Nyabundi and Kimemia, 1998). Different parts of the plant are used traditionally used to treat flu and diarrhea (Tugume *et al*., 2016), liver diseases (Mukazayire *et al*., 2011), hernia (Kibuuka & Anywar, 2015) (Kibuuka & Anywar, 2015), malaria, yellow fever, and wound healing (Namukobe *et al*., 2011; Tugume *et al*., 2016). In Kenya, the *Digitaria abyssinica* rhizome's decoction has been traditionally used to treat urinary infections mainly gonorrhea and candidiasis (Kamau *et al*., 2016). Although it's

known to be used, there is limited scientific data on biological activity, phytochemistry, or safety of this plant and therefore, the current study of *Digitaria abyssinica* aimed at developing scientific data on the phytochemical composition, toxic effects and antimicrobial activities of *Digitaria abyssinica* rhizome in order to validate the available ethnobotanical information of use of the plant.

1.2 Statement of the problem

Urinary tract infections (UTIs) are significant general medical challenges in Kenya and are associated with broad wellbeing, societal and monetary outcomes. UTIs significantly affect general wellbeing in view of their possible intricacies, extent, as well as their cooperation with HIV/AIDS. They influence the wellbeing and societal prosperity of women, as well as other need populaces lopsidedly by critically affecting their sexual and conceptive wellbeing. UTIs cause medical issues and complexities, necessitating consideration (NASCOP, 2018). Gonorrhea is the second most frequently reported notifiable disease worldwide (Dos Santos *et al*., 2022). It is caused by gonococcal *Neisseria* or gonococcal, or GC, a Gram-negative diplococcal. Gonorrhea is a transitional infection of columnar epithelium. It is estimated to have affected 87 million people (between the ages 15 and 49) worldwide, 11.4 million in the African region in 2016. In Kenya, it follows *Chlamydia trachomatis*. In Kenya, a study was conducted in 2012, four clinics found in Kisumu, Nairobi, Mombasa and Kilifi showed 53.2% prevalence of fluoroquinolone resistant gonorrhea (Lagace-Wiens *et al*., 2012).

Around the world, gonococcal diseases are currently a dire issue since *Neisseria gonorrhoeae* quickly develops resistance from numerous antibiotics thus is the most significant challenge to controlling gonorrhea (Merrick *et al*., 2022). Over the long run, *Neisseria gonorrhoeae* has gotten less susceptible to various antibiotics, including penicillin, sulfonamides, tetracyclines and fluoroquinolones (Merrick *et al*., 2022). Also, *Candida albicans* remains the most frequently isolated Candida species in the clinical setting. In some countries, a marked shift towards species of Candida that have increased resistance to azoles such

as fluconazole, the standard antifungal drug of choice in many countries, and to the recently introduced antifungals known as echinocandins, is reported (Chowdhary *et al*., 2017).

1.3 Justification of the study

The current treatment against a majority of the bacterial infections has faced enormous challenges following the resistance to antibiotics. *Neisseria gonorrhoeae*, is a perfect example, the bacteria that causes the STD called gonorrhea. This has resulted in the rise of bacterial infections and the limited treatment options for diseases. As such, ethnomedicine forms an alternative therapy for such infections. Most of the local people rely on traditional medicine as a source of disease treatment. Furthermore, traditional medicine has been regarded as a cheaper way and user-friendly way of treating bacterial infections.

Ethnobotanical studies done on *Digitaria abyssinica* rhizome has established that the plant traditionally applies in the treatment of STDs such as gonorrhea and candidiasis (Kamau, 2018). With *Digitaria abyssinica* being a common grass plant in Kenya, very little study has been conducted scientifically on its phytochemicals and antibacterial medicinal uses of the plant in treatment of gonorrhea. This research will also add to the pool of knowledge the efficacy and safety levels of *Digitaria abyssinica* rhizome extract.

1.4 Objectives

1.4.1 General objective

To investigate phytochemical composition, acute toxicity and antimicrobial potency of *Digitaria abyssinica* rhizome extract.

1.4.2 Specific objectives

i. To screen for phytochemical constituents of *Digitaria abyssinica* rhizome extracts

ii. To determine acute toxic effects of *Digitaria abyssinica* rhizome extracts on Wistar rats

iii. To evaluate antimicrobial activities of *Digitaria abyssinica* rhizome extracts

1.5 Hypothesis

i. The extracts of *Digitaria abyssinica* rhizome do not have phytochemicals

ii. The extracts of *Digitaria abyssinica* rhizome do not exhibit toxicity effects in female Wistar rats

iii. The extracts of *Digitaria abyssinica* rhizome do not exhibit antimicrobial activity

CHAPTER TWO

LITERATURE REVIEW

2.1 Microbial infections

Microbial infections are caused by the entry of the host body tissue by microbes which include bacteria, viruses, fungi and parasites. These infections can be grouped according to the invading microorganisms, the symptoms of disease and the duration of infection (Kamanja, 2014). Microbial infections are treated using antimicrobial agent that include antibacterial, antivirals, antifungals and antiprotozoal drugs while prevention may be achieved through proper hygiene, vaccinations and breaking the life cycle of parasites (Danquah *et al*., 2022). Microbial infections are among conditions treated utilizing ethnomedicine in Kenya (Njoroge & Bussmann, 2007). Microbial contaminations majorly affect human wellbeing, particularly in African rural areas due to challenges in availability, accessibility, and excessive costs of antibiotics (Eloff & Mcgaw, 2014).

2.2 Urinary tract infections (UTIs)

In the many regions of the urinary system, including the kidney, ureter, bladder, and urethra, urinary tract infections (UTIs) brought on by different pathogenic microorganisms are common (Li *et al*., 2020). Urinary tract infections (UTIs) are a serious clinical issue that frequently affect 150 million people worldwide per year (Sihra *et al*., 2018). It has been estimated that up to 50% of women encounter a UTI at least once in their lives (Tache *et al*., 2022).

In Africa, related studies among adults have also revealed novel evidence that proves UTIs are an actual health burden (Bankole *et al*., 2011; Kabugo *et al*., 2016; Seifu & Gebissa, 2018).

In Kenya, the total prevalence rate of UTIs was 27.6%, with women having a much higher prevalence rate than men (19.2% vs. 80.7%). UTI prevalence was 34% in pregnant women, which was higher than it was for other participant groups. The percentage of UTI cases was higher in women who rarely changed their underwear each day, at 34.8%. With 38.5%, 21%, and 19.3%, respectively, *E. coli*, *S. aureus*, and *K. pneumoniae* were the most prevalent bacterial pathogens (Wanja *et al*., 2021).

UTIs can be classified as uncomplicated or complicated clinically. People who are otherwise healthy and have no structural or neurological abnormalities of the urinary tract are most commonly affected by uncomplicated UTIs. There are two types of these infections: lower UTIs (cystitis) and higher UTIs (pyelonephritis). The term "complicated UTI" refers to UTIs that are linked to conditions that weaken the host's ability to protect the urinary tract, such as urinary obstruction, urinary retention brought on by neurological disorders, immunosuppression, renal failure, renal transplantation, pregnancy, and the presence of foreign objects like calculi, indwelling catheters, or other drainage device (Ana *et al*., 2016).

Both Gram-positive and Gram-negative bacteria, as well as some fungi can cause UTIs. UPEC is the most typical causal agent for both simple and complex UTIs. In terms of the bacteria that cause uncomplicated UTIs, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and Candida spp. follow UPEC in prevalence. *Enterococcus* spp., *K. pneumoniae*, Candida spp., *S. aureus, P. mirabilis*, *P. aeruginosa*, and GBS are the most prevalent causal agents causing complicated UTIs, with UPEC being the second-most frequent (Ana *et al*., 2016).

In Kenya, Trimethoprim (64%), Sulfamethoxazole (57%), Nalidixic acid (54%) Ciprofloxacin (26%), Cefuroxime (13%), Amoxicillin-clavulanic acid (10%), Nitrofurantoin (9%), and Cefixime (7%), were the medications with the most prevalent resistance profiles. There was 15%, 14%, and 11%, respectively,

resistance to broad-spectrum antibiotics such ceftazidime, gentamycin and ceftriaxone. Also, 66% of the microorganisms were multidrug resistant (MDR) (Kiiru *et al*., 2022).

2.2.1 *Klebsiella pneumoniae*

A Gram-negative, encapsulated, non-motile bacterium known as *Klebsiella pneumoniae* is present in the environment and has been linked to pneumonia in patients with diabetes mellitus or alcohol use disorder. The bacterium typically colonizes the oropharynx and gastrointestinal (GI) tract mucosa of humans. The bacterium can exhibit significant levels of pathogenicity and antibiotic resistance once it has entered the host. The bacterium is responsible for 3% to 8% of all nosocomial bacterial infections and is currently thought to be the most common cause of hospital-acquired pneumonia in the United States (Ashurst & Dawson, 2022).

Given the low prevalence of *K. pneumoniae* in the local population, standard recommendations for antibiotic therapy should be followed in the treatment of pneumonia. Once *K. pneumoniae* infection is suspected or proven, antibiotic therapy should be customized to the region's specific antibiotic sensitivity. Current treatment plans for community-acquired *K. pneumoniae* pneumonia include a 14-day course of either a respiratory quinolone or a third- or fourth-generation cephalosporin as monotherapy, or either of the aforementioned plans in combination with an aminoglycoside. A course of aztreonam or a respiratory quinolone should be started if the patient has a penicillin allergy. Until sensitivities are discovered, a carbapenem can be used as monotherapy for nosocomial infections (Ashurst & Dawson, 2022).

2.2.2 *Staphylococcus saprophyticus*

One significant type of Gram-positive, coagulase-negative bacteria that can cause urinary tract infections is *Staphylococcus saprophyticus*. *S. saprophyticus* which is found in the periurethral area, skin, and mucosal environment of the genitourinary tract, is the second most common cause of UTI, accounting for 40% of cases, particularly in women.(Souza *et al*., 2019).

Treatment of patients infected with this infection is complicated by *S. saprophyticus*' resistance to antibiotics routinely used in the empirical treatment of cystitis, such as cefixime and Fosfomycin trometamol (Pailhoriès *et al*., 2017).

2.2.3 *Enterococcus faecalis*

Enterococcus faecalis is a Gram-positive, catalase-negative, fermentative, non-spore-forming, facultatively anaerobic bacteria. Their ovoid cells have a diameter of between 0.5 and 1 μm. The majority of strains are nonhemolytic and nonmotile and can be found alone, in pairs, or in short chains (Colaco, 2018). Treatment of faecalis endocarditis with ampicillin and ceftriaxone is efficient and safe (Gavaldà *et al*., 2007).

Ampicillin for *E. faecalis* infections and ampicillin in combination with aminoglycosides or cephalosporins for *E. faecalis* endocarditis are currently the most widely used treatments for enterococcal infections, which are treated while catheters are changed or removed. Alternative treatments include quinipristin-dalfoprisin, daptomycin, or linezolid if the infectious species is resistant to vancomycin, aminoglycosides, and/or ampicillin, as is frequently the case with *E. faecium* (van Harten *et al*., 2017).

2.2.4 Group B Streptococcus (GBS)

The Group B Streptococcus (GBS), also known as *Streptococcus agalactiae*, is a Gram-positive, encapsulated, beta-hemolytic, facultative anaerobe that is a component of the commensal microbiome of humans (Hanson *et al*., 2022). Penicillin continues to be the first-choice antibiotic to prevent GBS and cure GBS disease because *Streptococcus agalactiae* is still fully susceptible to penicillin and to most lactams. But over the past 20 years, resistance to macrolides and clindamycin in invasive GBS isolates has gone from 5% to a typical 20 to 30% (Dadi *et al*., 2022).

2.2.5 *Proteus mirabilis*

Proteus mirabilis is a member of the Enterobacteriaceae family, an important opportunistic and food-borne pathogen, and the second most common cause of urinary tract infections after UPEC. *Proteus mirabilis* can live in a variety of places, including the intestines of humans, wild animals, and domestic animals. It can infect immunosuppressed hosts and result in infections that cause diarrhea, urinary tract infections (UTI), and keratitis (Gong *et al*., 2019).

It is significant to note that *P. mirabilis* now poses a significant diagnosis and treatment challenge due to its antimicrobial resistance, including recorded cases of resistance to colistin, nitrofurans, tigecycline, tetracycline, and -lactams (Qu *et al*., 2022).

2.2.6 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a monoflagellated, rod-shaped, Gram-negative bacteria. It looks pearlescent and smells like grapes or tortilla chips. *P. aeruginosa* grows well between 25 and 37 °C and its capacity to grow at 42 °C helps set it apart from many other Pseudomonas species (Wu *et al*., 2014).

Due to *P. aeruginosa* high level of antibiotic resistance, infections are exceedingly challenging to treat. infections that are caused by both innate and acquired resistance. While the latter mostly relates to the acquisition of resistance genes on mobile genetic components, intrinsic resistance mostly refers to resistance mechanisms that are encoded in the chromosome (Breidenstein *et al*., 2011).

Aminoglycosides, quinolones, and β-lactams are just a few of the antibiotics that *P. aeruginosa* has shown resistance to (Pang *et al*., 2019).

2.2.7 *Staphylococcus aureus*

Staphylococcus aureus is a member of genus Staphylococcus. They are anaerobic Gram-positive cocci. They are large cells usually 6 to 8 μm in diameter and are not motile. They do not form spores and are hemolytic on blood agar. The colonies are pigmented and have a cream-yellow colour. They are smooth,

entire, slightly raised and translucent. *S. aureus* is a human opportunistic pathogen and is found on skin, skin glands, mouth, intestinal tract, genitor-urinary tract, upper respiratory tract, mammary glands and mucus membranes. As a nosocomial pathogen, it is a major cause of mortality and morbidity. Most of the infections due to *S. aureus* are acute and pyogenic and include skin infections such as furuncles, cellulitis, impetigo and post-operative wound infections (Musau, 2011).

Other infections are bacteremia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, cerebritis, meningitis, scalded skin syndrome, food poisoning, toxic shock syndrome and chorioamnionitis (Murray *et al*., 2003). *S. aureus*, which people typically carry on their skin or mucus membranes, often causes skin and soft tissue infections, but also spreads readily throughout the body via the bloodstream and can cause infection of the lungs, abdomen, heart valves, and almost any other site (Doron & Gorbach, 2008). *S. aureus* isolates were found to be highly resistant to tetracycline, ciprofloxacin, erythromycin, cefoxitin, norfloxacin and oxacillin but very sensitive to trimethoprim, rifampicin, chloramphenicol, moxifloxacin, gentamicin, ofloxacin, vancomycin, and clindamycin, according to resistance patterns (Shittu & Lin, 2006).

2.2.8 Candidiasis

Candidiasis, which includes both superficial infections and invasive disease is the most common cause of fungal infection worldwide. In their capacity to colonize the urinary tract and produce an invasive illness, Candida species seem to be exceptional. Candida bloodstream infections (BSI) cause significant mortality and elicit a major threat to intensive care unit (ICU) patients (Chowdhary *et al*., 2017). *Candida albicans* remains the most frequently isolated Candida species in the clinical setting, in some countries, a marked shift towards species of Candida that have increased resistance to azoles such as fluconazole (FLU), the standard antifungal drug of choice in many countries, and to the recently introduced antifungals known as echinocandins, is reported (Chowdhary *et al*., 2017).

2.2.9 Herpes simplex virus infection

The herpes simplex virus (HSV) is a very common illness around the world, with the most common kind affecting over 3.7 billion persons under the age of 50. There are two forms of HSV; HSV-1 and HSV-2. HSV-1 is primarily spread through oral-to-oral contact and causes oral herpes (including cold sores), but it can also cause genital herpes. HSV-2 is a sexually transmitted herpes virus that causes genital herpes (Looker *et al*., 2008). Oral and genital herpes are usually asymptomatic or go unnoticed, but they can cause painful blisters or ulcers at the infection site, which can range from mild to severe. The infection lasts a lifetime, and symptoms might return for years. Some treatments can help to lessen the severity and frequency of symptoms, but they won't be able to cure the infection. The most effective treatments for persons infected with HSV are antiviral medications including acyclovir, famciclovir, and valacyclovir. These medications can help to lessen the severity and frequency of symptoms, but they will not cure the illness. Recurrences of aciclovir-resistant HSV infections are more frequently documented in immunocompromised individuals, particularly in HSCT recipients, as a result of protracted viral shedding and aciclovir exposure (Muller *et al*., 2022; Serris *et al*., 2022).

2.2.10 Syphilis

Syphilis also known as endemic syphilis and yaws are all caused by the spirochaete bacterium known as *Treponema pallidum*. Syphilis is a chronic, multi-stage infectious disease that is typically spread sexually by coming into contact with an active lesion of a partner or congenitally from an infected pregnant woman to her fetus (Stamm, 2015). Benzyl penicillin, (Penicillin G), the first-line treatment for all stages of syphilis, is primarily used to identify and treat infected people and their contacts because there is no vaccination to prevent syphilis. With the A2058G mutation, macrolide-resistant *T. pallidum* is currently widespread throughout the USA, Canada, Europe, and China. It has also recently been discovered in

Sydney, Australia. The increasing incidence of macrolide-resistant *T. pallidum* is highly correlated with recent macrolide use for unrelated diseases (such as skin, vaginal, or oral infections) (Stamm, 2015).

In another recent research, macrolides should not be used in syphilis treatment recommendations. According to this recent study on 25 *T. pallidum* DNA samples from active syphilis patients that revealed an 88% incidence of macrolide-resistance mutations but no resistance to tetracycline (Scurtu *et al*., 2022). According to estimates, syphilis infection more than triples the chance of HIV transmission (WHO, 2008).

2.2.11 *Chlamydia trachomatis* infection

The most prevalent bacterial STI is chlamydia. *Chlamydia trachomatis* is a Gram-negative bacterium that infects the columnar epithelium of the cervix, urethra, and rectum, as well as non-genital sites such as the lungs and eyes. Gonorrhea is usually co-infected in those who have this infection. Chlamydia symptoms include discharge and a burning sensation while urinating, but the majority of infected individuals experience no symptoms at all. Chlamydia can harm the reproductive system even when there are no symptoms present (World Health Organization, 2016). The first-line therapy recommended by the 2015 Treatment Recommendations for Uncomplicated Urogenital *Chlamydia trachomatis* Infections is a 7-day course of doxycycline (100 mg per os (PO) twice a day) or azithromycin (1g orally once). The drugs erythromycin, levofloxacin, ofloxacin, josamycin, and rifampicin are used as second or third-line antibiotics (Scurtu *et al*., 2022). Utilizing azithromycin and clarithromycin, the antimicrobial resistance was identified in 2.0 and 2.4 %, respectively (Suzuki *et al*., 2022).

2.2.12 *Trichomonas vaginalis* infections

The most prevalent parasite that causes the STD trich, also known as trichomoniasis, is the flagellated protozoan known as *Trichomonas vaginalis*. *T. vaginalis* is the most common, non-viral sexually transmitted infection (STI) worldwide. According to estimates from the World Health Organization, 170

million infections occur annually across the globe. Urethritis, prostatitis, and vaginitis are the three primary clinical symptoms of *T. vaginalis* infections. Additionally, trichomonas infection can raise the risk of tubal infertility and pelvic inflammatory illness (Meri *et al*., 2000). Nitroimidazoles (metronidazole and tinidazole) are the only recommended drugs for treating *T. vaginalis* infection. But for years, it has been noticed that some strains of *T. vaginalis* are resistant to nitroimidazoles (Kirkcaldy *et al*., 2012; Meri *et al*., 2000). There was resistance of *T. vaginalis* to tinidazole and metronidazole in 17/178 (9.6%) and 1/178 (0.56%) of the strains, respectively (Schwebke & Barrientes, 2006). Recently, in 5 to 10% of cases, resistance has been noted; however, this number may be increasing (Graves *et al*., 2022; Mabaso *et al*., 2021).

2.2.13 Gonorrhea

The bacterium *Neisseria gonorrheae* causes gonorrhea. The Gram-negative coccus affects the mucous membranes and contributes to a muco-purulent discharge. Primarily, the pathogen affects the genital areas, oropharynx, anorectum, as well as the ocular membrane in young adults. In most patients, the prominent methods of gonorrhea transmission include anally, orally, or vaginal sex (Munyuli, 2019). In 2016, there were 86.9 million new cases worldwide, with the incidence rate, a measure of new infections or diagnoses in a certain time period, being 20 per 1000 women and 26 per 1000 men. (Kirkcaldy *et al*., 2019). Drug-resistant *N. gonorrhoeae* was categorized as a "superbug" by the Centre for Disease Control (CDC) in 2012 and as a "High Priority" disease by the World Health Organization (WHO) in 2017's "WHO Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics." (Scurtu *et al*., 2022)

The high prevalence of *N. gonorrhoeae* resistance to nearly all relevant antimicrobials that have been and are still widely used for treatment, including sulphonamides, penicillins, tetracyclines, quinolones, early

generation macrolides, and cephalosporins, jeopardizes the achievement of these targets and highlights the urgent need for new antimicrobial agents with activity against *N. gonorrhoeae* (Iwuji *et al*., 2022).

2.3 Conventional treatment of microbial infections

Microbial infections are treated using antimicrobial agent that include antibacterial, antivirals, antifungals and antiprotozoal drugs while prevention may be achieved through proper hygiene, vaccinations and breaking the life cycle of parasites (Kamanja, 2014). Conventional drugs usually provide effective antibiotic therapy for microbial infections but there is an increasing challenge of antibiotic resistance, (Martin & Ernst, 2003). In addition to this conventional medicine are expensive and inadequate for the treatment of diseases but are also often with adulterations are side effects (Vaghasiya & Chanda, 2007).

Despite the huge success of conventional medicines in treating diseases and infections, there are still numerous challenges associated with this therapy (White, 2019). One of the greatest challenges is antimicrobial resistance (AMR). AMR occurs when bacteria, viruses, fungi and parasites change over time and no longer respond to medicines making infections harder to treat and increasing the risk of disease spread, severe illness and death (WHO, 2022). Therefore, this is need to search for new antimicrobial agents to fight the ever-increasing diseases and infections. Herbal medicine forms a very promising solution. However, there is need for research to determine the safety doses for such medicines (Ekor, 2014; Moreira *et al*., 2014).

2.4 Conventional treatment of UTIs

Antimicrobial drugs are used to treat UTIs. Conventional treatment options include patient-tailored medication based on urinary cultures and susceptibility to potential antibiotics, as well as the use of broad-spectrum antimicrobial medicines for empirical or preventive therapy. Multi-drug resistance infections are still becoming more prevalent, which has caused concerns about regularly given antimicrobial medications like those used to treat UTIs (Ventola, 2015).

2.4.1 Antibacterial drugs against UTIs

For otherwise healthy adult non-pregnant females with acute uncomplicated bacterial cystitis, nitrofurantoin, a 3-g single dose of Fosfomycin tromethamine (Monurol), or pivmecillinam are the first-line empiric antibiotic treatments that are advised. In many communities, trimethoprim-sulfamethoxazole (Bactrim, Septra) and ciprofloxacin (cipro) are ineffective as an empiric treatment for UTIs due to high rates of resistance, especially in patients who have recently been exposed to them or in patients who are at risk of infections with Enterobacteriales that produce extended-spectrum -lactamases (ESBLs) (Bader *et al*., 2020).

Oral cephalosporins like cephalexin (Keflex) and cefixime, fluoroquinolones, and lactams like amoxicillin-potassium clavulanate (Augmentin) are among the second-line treatment alternatives. Multidrug resistance (MDR)-*Pseudomonas spp*.-related UTIs can be treated with fluoroquinolones, ceftazidime, cefepime, piperacillin-tazobactam, carbapenems such as imipenem-cilastatin/relebactam, meropenem, and fosfomycin, ceftolozane-tazobactam (Bader *et al*., 2020).

World Health Organization (WHO), Ministry of Health (MOH) Kenya recommends amoxicillin or cotrimoxazole for 7 to 14 days; nitrofurantoin can also be used (WHO, 2009).

2.4.2 Antifungal drugs against UTIs

While fungus-related urinary tract infections (UTIs) are uncommon in healthy individuals, they can arise to up to 40% of hospitalized patients or patients with other concomitant conditions (Thomas & Tracy, 2015). The most prevalent Candida species, especially *Candida albicans,* are known to cause urinary tract infections in humans. *Candida glabrata* and *Candida tropicalis* are thought to be responsible for 10 to 35% of Candida-related UTIs. Less frequently seen in urine are other Candida species such *Candida krusei* and *Candida parapsilosis* (de Andrade Monteiro & Ribeiro Alves dos Santos, 2020).

A class of drugs known as azole antifungals include an azole ring and inhibit a variety of fungus from growing. Those with two nitrogens in the azole ring (the imidazoles; examples include clotrimazole, econazole, ketoconazole, miconazole, and tioconazole) and those with three nitrogens in the azole ring are divided into two categories (the triazoles; examples include fluconazole, itraconazole, posaconazole, and voriconazole). Lanosterol 14-alpha-demethylase, a cytochrome P450-dependent enzyme that converts lanosterol into ergosterol, the primary sterol in the fungal cell membrane, is inhibited by azole antifungals. Ergosterol depletion disrupts the cell membrane, which leads to cell death (Drugs.com, 2022).

The antifungal drug of preference is fluconazole, which, in its oral formulation produces high urine concentrations and has low cost and toxicity. Moreover, it effective against a variety of Candida species, including *Candida albicans* (Pappas *et al*., 2015).

Fluconazole is ineffective against *Candida krusei* and a lot of isolates of *C. glabrata* have fluconazole resistance. Flucytosine is ineffective against *C. krusei* hence amphotericin B deoxycholate is the therapy of choice for patients infected with this organism. Treatment with amphotericin B deoxycholate and/or flucytosine is advised for patients with fluconazole-resistant *C. glabrata*. Flucytosine may result in bone marrow toxicity, but amphotericin B is frequently linked to nephrotoxicity. Both flucytosine and amphotericin B have considerable toxicities (Kauffman, 2014).

Echinocandins, which include caspofungin, micafungin, and anidulafungin, are very effective against most species of Candida, including *C. glabrata* and *C. krusei*. These medications are frequently used as first-line options for candidemia and invasive candidiasis due to their wide-spectrum action against Candida species and low side effects. Echinocandins do not reach considerable amounts in urine, hence current recommendations do not support their usage for Candida UTIs (Pappas *et al*., 2015).

2.5 Herbal medicine for microbial infections

Traditional medicine is widely practiced in Kenya, where this has been documented by ethnobotanical surveys (Jacob *et al*., 2004; Kareru *et al*., 2007; Mokua *et al*., 2021; Njoroge *et al*., 2015). The high cost of imported conventional drugs and/or inaccessibility to western health care facilities has led to overreliance on traditional medicine since it is affordable and available to rural people. Traditional medicine, on the other hand, is regarded from a cultural standpoint as an effective and acceptable approach even when access to western healthcare facilities is present (Jacob *et al*., 2004; Munguti, 1997).

A large number of plants overall are utilized in ethnomedicine to treat bacterial diseases. *Amaranthus hybridus* from the family Caesalpiniaceae is used in treating urinary tract infection (UTI), kidney and stomach ailments (Wagate *et al*., 2008). *Harrisonia abyssinica* from the Simaroubaceae family is used in the treatment of, syphilis, malaria, infertility, eye ointment, pneumonia and stomach (Cyrus *et al*., 2008). *Schkuhria pinnata* from the Compositae family is used in the treatment of malaria, joint pains and diabetes (Wagate *et al*., 2008). *Ziziphus abyssinica* from the Rhamnaceae family has been used in ethnomedicine in several African countries as analgesics (Boakye-Gyasi *et al*., 2017). *Coriandrum sativum* from the Apiaceae family is used in the treatment of gastrointestinal diseases in India. *Brassica alba* and *Lepidium sativum* from the Brassicaceae family is used in alleviating viral infections in India. *Terminalia bellirica* (Gaern.) from the Combretaceae family applies in addressing cough, fever, diarrhea, dysentery in India. *Hyptis suaveolens* from the Lamiaceae family is used to treat fever, gonorrhea and headache in Bangladesh. *Osmium basilicum* is used in the treatment of cough or bacterial and viral infection in India. *Vetiveria zizanioides* is used to treat bacterial infection and fever in India. The fruits of *Piper nigrum* from the Piperaceae family is used to treat fever, coughs, diarrhea and diabetes. The roots of *Curcuma caesia* from the Zingiberaceae family is used to treat tonsilitis in India. The rhizomes of *Curcuma pseudomontana* are used to treat cold (Hossan *et al*., 2018).

2.6 African plants with antimicrobial activities

Several African plants have shown antimicrobial properties. These include however not restricted to the following plants. *Ajuga remota* is conventionally utilized as a natural solution effective in fever, infections, and is endorsed for malaria by about 66% of the Kenyan herbalist (Cocquyt *et al*., 2011). *Terminalia kilimandscharica* from the Combretaceae family is utilized to STDs and cough (Cyrus *et al*., 2008). *Entada leptostachya* is a climber that is broadly appropriated in parched and semi-dry territories of Mbeere, Embu, Kamba and Tharaka people group of Kenya. Its rhizome is utilized along with the *Hamsonia abyssinica* rhizome, resulting into a blend utilized to treat tuberculosis, as well as to alleviate chest pains (Mutembei *et al*., 2018).

Croton macrostachyus from the Euphorbiaceae family is found through tropical Africa, including Madagascar. All through the region where it's found, an infusion, a decoction, or maceration of leaves, stem bark or rhizome bark are taken as a laxative and vermifuge. Leaf sap is utilized correspondingly. The seed oil is an incredible laxative. In Kenya, the Luhya public lick the ashes of consumed leaves as a cough cure. A leaf decoction is likewise consumed in treating cough and stomach issues. A rhizome decoction is used for the treatment of indigestion. Among the Kikuyu community, its used to treat malaria. Leaf juice is put on injuries to improve blood thickening, and furthermore to treat warts, bruises, ringworms and sores. In Kenya and Tanzania, a rhizome infusion is taken orally to control intestinal parasites (Mutembei *et al*., 2018). *Acacia nilotica* is accounted as having antibacterial properties effective against pathogenic microorganisms like *S. aureus, E. coli, P. aeruginosa*, as well as *M. tuberculosis.* In a comparative antimicrobial assessment among acacia species *Acacia nilotica* displayed significant antimicrobial properties against, *S. typhi*, *E. coli* and *S. aureus* (Saini *et al*., 2008).

Garlic (*Allium sativum*) is a consumable plant which has produced a great deal of interest all through mankind's set of experiences as a medicinal plant. Garlic oil showed significant antibacterial properties,

especially against methicillin-resistant *S. aureus* (Viswanathan *et al*., 2014). The leaves and bark of *Warburgia ugandensis* is used to treat asthma in the Mbeere and Embu communities of Kenya. The rhizomes *of Plectranthus barbatus* and the inner fresh *of Engleromyces goetzei* are also used to treat asthma among the same communities (Kareru *et al*., 2007).

Oxygonum sinuatum has been used by Embu and Mbeere communities as a treatment for typhoid (Kareru *et al*., 2007). It has also been reported to be used as a treatment for stomach ache and urinary complications (Njoroge *et al*., 2015). *Oxygonum sinuatum* has also been reported to be used in wound healing in Uganda (Tugume *et al*., 2016).

2.7 Herbal plants used to treat UTIs

The roots of *Acacia ataxacantha* and *Plectranthus barbatus* are used as a treatment for gonorrhea. *Aloe kendongensis* leaves also apply in treating gonorrhea (Kareru *et al*., 2007). (Kamau, 2018) reported that *Digitaria abyssinica* rhizome from the Poaceae family is traditionally used to treat gonorrhea and candidiasis.

2.8 Poaceae

2.8.1 Classification and distribution of Poaceae plants

There are around 12,000 grass species in around 771 genera that are grouped into 12 subfamilies. Following the Asteraceae, Orchidaceae, Fabaceae and Rubiaceae, Poaceae are the fifth most significant plant family. The grass family is estimated to be the most abundantly and widely distributed species. Grasses grow on most landmasses, only missing from Antarctica (Brown *et al*., 1895).

2.8.2 Phytochemistry of Poaceae plants

Phytochemicals are biologically active substances that occur naturally in plants. They serve as a natural defense system for plants and give smell, color and flavor. These are non-nutritive chemicals in plant that have defensive or disease preventive activities (Babu & Savithramma, 2013). Qualitative phytochemical

research has uncovered that the plant's aqueous extract from the Poaceae family contains phytoconstituents like terpenoids, tannins, flavonoids, lignin, volatile oils, fatty acids, glycosides, quinones, saponins, phenols, steroids, coumarins and alkaloids (Babu & Savithramma, 2013).

2.8.3 Biological activities of Poaceae plants

The family Poaceae is of incredible monetary and therapeutic significance as it incorporates all bamboos, cereals and sugarcanes. Terpenoids, unsaturated fats flavonoids, phenolic acids, steroids, hydroxamic acids, as well as alkaloids are the primary bioactive class mixtures from Poaceae. The therapeutic potential of plant types of Gramineae are precise and include antimicrobial, antioxidant, antifungal, anticancer and cytotoxic properties. Additionally, some grass species demonstrate antioxidant activities and are significantly effective in treating inflammations. A few species have shown antiparasitic properties, antimalarial and anthelmintic, hepatoprotective, antihyperglycemic. Mitigating antipyretic, inflammation and pain-relieving exercises are likewise among the diverse revealed biological activities of family Poaceae (Abdelkader *et al*., 2016).

2.8.4 Ethnomedicinal uses of Poaceae plants

Aside from the fact that plants are rich in nutrients and minerals fundamental for normal body metabolism, they are additionally referred to contain auxiliary metabolites like steroids, glycosides, tannins, cardiovascular, alkaloids, flavonoids, phenols, saponins, and numerous different phytochemicals which have been found to have medicinal activities when utilized enough (Oseghale *et al*., 2017). Ethnomedicine is a common practice in Kenya as indicated by ethnobotanical reviews (Kareru *et al*., 2007; Njoroge & Bussmann, 2007). The significant expense of imported conventional medicines as well as detachment to western medical drugs has prompted overreliance on ethnomedicine since it is moderate and accessible to the rural communities. Then again, in any event, when western medicine is made available, ethnomedicine

is seen as an effective and an adequate framework from a social point of view (Wagate *et al*., 2008). Additionally, fodder grasses comprise numerous phenols and flavonoids, alongside other secondary metabolites. Subsequently, such species have a critical therapeutic relevance (Fatima *et al*., 2018). Some family Poaceae plants are utilized as a diuretic, anti-inflammatory, antidiabetic, hypertension, anthelmintic, antiulcer as well as possess antioxidant properties (Fatima *et al*., 2018).

2.8.5 Use of Poaceae to treat UTIs

Poaceae or Gramineae is a huge and considerably pervasive category of monocotyledonous blooming grasses. Poaceae incorporates the grasses, bamboos, cereals and normal field grasses, as well as developed yards (turf) and fodder (Brown *et al*., 1895). The grass family (Gramineae or Poaceae) is naturally and economically of the utmost significance in the flowering plants groups; individuals from the family such as maize, wheat, and rice, which give a considerable portion of the caloric utilization for humankind. Numerous grasses additionally utilize high-efficiency (C4) photosynthesis, a pathway that has started ordinarily autonomously within the family (Kellogg, 2013). Individuals from the Poaceae family have been seen throughout the years to be valuable in the management of various diseases (Oseghale *et al*., 2017).

2.9 *Digitaria abyssinica*

Digitaria abyssinica is commonly referred to as East African couch grass. *Digitaria abyssinica* is a creeping, perennial grass featuring long, thin, fanning rhizomes, which structure a thick tangle underneath the soil surface. Culms decumbent close to base, getting erect, around 50 cm, once in a while up to 1 m high (Baguma *et al*., 1995). The *Digitaria abyssinica* rhizome from the Poaceae family has shown pharmacological activity against some STDs such as gonorrhea and candidiasis (Kamau, 2018).

Figure 2.1: *Digitaria abyssinica*. (Forest and Kim Starr).

2.9.1 Classification and distribution of *Digitaria abyssinica*

Table 2.1. Taxonomic tree of *Digitaria abyssinica*

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Monocotyledonae
Order	Cyperales
Family	Poaceae
Genus	Digitaria
Species	*Digitaria abyssinica*

Digitaria abyssinica is a member of the grass family of Poaceae. It is especially basic as a weed in East Africa. The distribution of *D. abyssinica* is from Nigeria to Arabia, and southwards to South Africa. It has also been recorded in Madagascar, India and Sri Lanka. *D. abyssinica* is broadly dispersed in East Africa from ocean level to 2,900 m, especially in moister areas. It happens as a weed in a wide scope of yields and soils, including those where least tillage is done. It is a typical part of characteristic fields at higher elevations in East Africa. *D. abyssinica* is used as a fodder for cattle although it has low nutritional value (CABI, 2023).

2.9.2 Phytochemistry of *Digitaria abyssinica*

Qualitative phytochemical studies of the family Poaceae has shown that the aqueous extract of plant species contained phytoconstituents like terpenoids, flavonoids, tannins, glycosides, lignin, quinones, saponins, phenols, steroids, coumarins and alkaloids.(Babu & Savithramma, 2013). However, from the literature review, phytochemical studies of *Digitaria abyssinica* rhizome has not yet been done. One of the objectives for the project proposal will be to analyze the phytochemistry of *Digitaria abyssinica* rhizome.

2.9.3 Biological assays of *Digitaria abyssinica*

Antimicrobial activity testing can be performed by any of the following methods: broth dilution, disk diffusion and agar well diffusion test. Disk diffusion and broth dilution methods were used in the study.

2.9.4 Ethnomedicinal uses of *Digitaria abyssinica*

The *Digitaria abyssinica* rhizome has been used traditionally to treat wounds in Uganda (Tugume *et al*., 2016). It has also been reported to be used to control convulsions in Uganda (Tugume *et al*., 2016). It has also been reported to be used traditionally to treat gonorrhea and candidiasis (Kamau, 2018).

2.10 Acute Toxicity Tests

The acute toxicity test was done as described by OECD 2008, document 425. The test procedure is of value because it aims to minimize the number of animals required to determine the acute oral toxicity of a given test compound of interest. In addition to the estimation of LD_{50} and confidence intervals, the procedure allows the observation of signs of toxicity. The procedure describes that either the main or the limit test can be conducted. The limit test is a sequential test that uses a maximum of 5 animals. The limit test is used in situation where the experimenter has information indicating that the test material is likely to be non-toxic. The limit test of 2,000 mg/kg was used since (Kamau, 2018) reported the use of *Digitaria abyssinica* decoction for treatment of STDs (gonorrhea and candidiasis). Female Wistar rats were used as

recommended by the test procedure, OECD 2008, Document 425, since they are generally more sensitive than males.

2.11 Antimicrobial susceptibility tests

2.11.1 Broth dilution method

Serial two-fold dilutions of the antimicrobial agent to be tested are prepared and placed in sterile tubes with appropriate broth that will allow for growth of the test microorganisms. Control tubes which don't contain any antimicrobial agent are also set. After 24-hour incubation, the tubes are observed for turbidity which is a show of microbial growth. The microorganisms will grow in the control tubes and any other tube without sufficient concentration of antimicrobial agent to inhibit growth. A variation of the broth dilution is the micro broth dilution method which uses volumes in pi and test samples in pg. After incubation, microbial growth is indicated by turbidity or use of a suitable dye. In broth dilution methods, the lowest concentration of the antimicrobial agent that inhibits growth of the microorganisms is regarded as the minimum inhibitory concentration (Moshi *et al*., 2009; Mothana *et al*., 2008; Suffredini *et al*., 2006; Wagate *et al*., 2008).

2.11.2 Disk diffusion assay

Disk diffusion assay employs the use of cellulose disks. The disks were impregnated with the antimicrobial agent and then placed equidistantly onto the inoculated plates. The plates were incubated for a stipulated 24 to 48 hours' time to allow microbial growth. Antimicrobial activity was determined by measuring the diameters of zones of inhibition of the *D. abyssinica* rhizome extracts against the each of the four microorganisms (Kisangau *et al*., 2007; Moshi *et al*., 2009; Mothana *et al*., 2008; Parekh & Chanda, 2009).

2.11.3 Well diffusion assay

Small circular wells were punched in the inoculated agar media. These wells were then filled with the antimicrobial agent of interest. The solution was allowed to diffuse into the media and then incubated. The antimicrobial activity was determined as a measure of the zone of inhibition around each well (Kisangau *et al*., 2007; Wagate *et al*., 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The *Digitaria abyssinica* rhizome was obtained from Mua, Kyaani, Machakos county. The county borders Nairobi and Kiambu counties to the west, Embu to the north, Kitui to the east, Makueni to the south, Kajiado to the south west, and Murang'a and Kirinyaga to the north west. The area is located 1.3932° South, 37.2062° East. Kyaani lies on 1612m/5288.71 ft m above sea level. It is located between longitudes 37° 15' 48.294" E and latitudes 1° 31' 3.6624" S. The county's lowest minimum elevation is 692m and the maximum elevation is 3,868 m with an average elevation of 1,394 m. The county covers an area of 6,043 square Kilometres. The region is characterized by tropical dry Savanna climate. Additionally, the study region classified as arid and semi-arid characterized by dry and wet seasons. Moreover, the annual precipitation in the area is approximated at 500 mm, with a distribution of short rains in September to December, as well as long rains from March to May. The temperatures are consistent and range from highs of around 35℃, and to lows averaging at 16℃. The long periods of January to March experience the highest temperatures and the least in the period of July (Wikiwand, 2023).

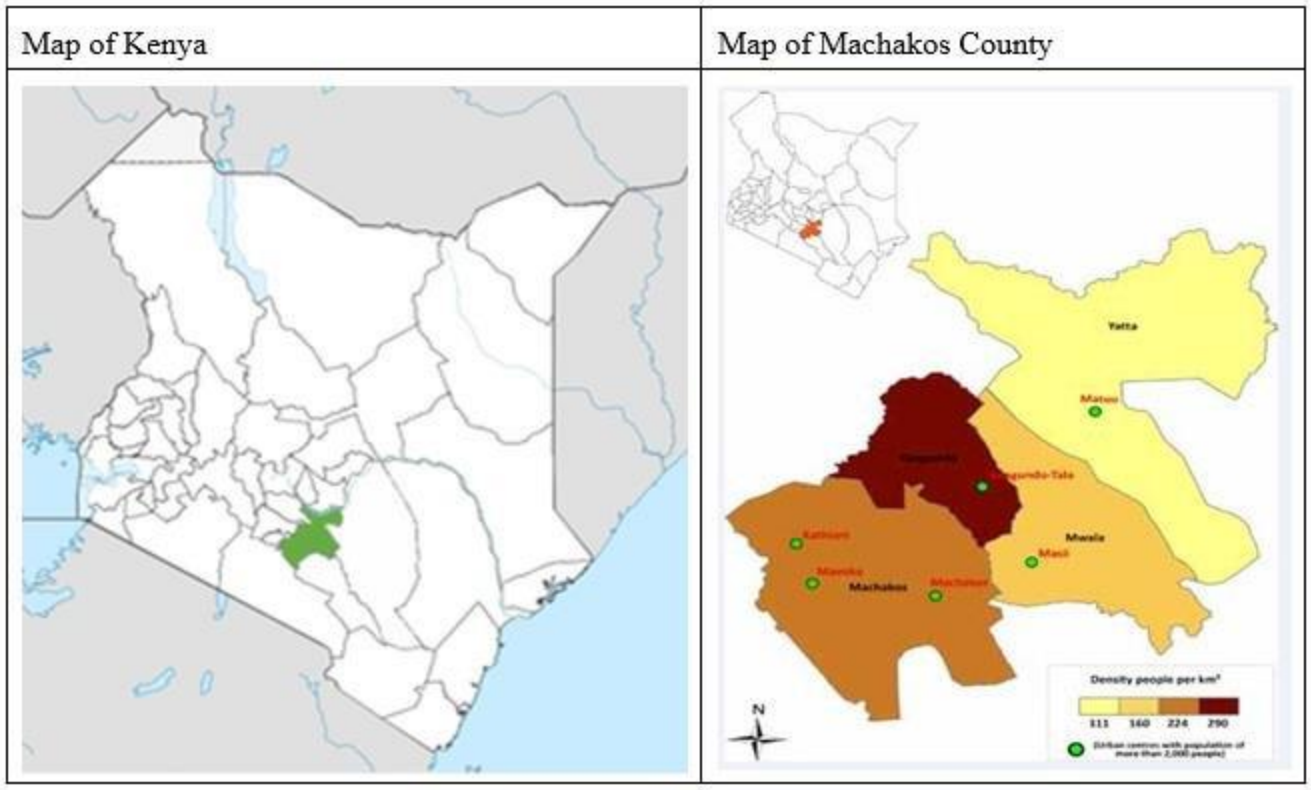

Figure 3.2: A map of Machakos county in Kenya. (Nyambok, (2014)

3.2 Collection and identification of the plant material

Ten (10) kg of uprooted *Digitaria abyssinica* rhizomes were collected from Mua, Kyaani, Machakos county with the help of a reputable plant taxonomist. A taxonomist at the University of Nairobi, Land Resource Management and Agricultural Technology (LARMAT) herbarium identified the plant and a specimen allocated a reference number LARMAT/Herb/Da was then deposited for ease of reference.

Figure 3.2: A photo of *Digitaria abyssinica* taken in Mua, Kyaani Machakos County, the study area.

3.3 Preparation of the plant material

Ten kilograms of fresh rhizomes of *Digitaria abyssinica* were cleaned properly with tap water, then air-dried in well-ventilated, insect, rodent and dust free room at the University of Nairobi, Public Health Pharmacology and Toxicology (PHPT) Department for 10 days. Ten kilograms of dried samples were then pulverized into powder using a laboratory milling machine at the LARMAT Department. Ten kilograms of powder was then packed in sterile and clean airtight polythene bag, labelled, kept away from light, heat and moisture awaiting extraction.

3.4 Extraction of the plant materials

The procedure illustrated by (Harborne, 1998) and later improved by (Bibi *et al*., 2012) was used to prepare the aqueous, methanol and a mixture of dichloromethane and methanol (1:1) extracts from the dried powder of *Digitaria abyssinica* rhizomes. All the chemicals and reagents used were of analytical reagent grade.

3.4.1 Aqueous Extract

Cold maceration procedure involved accurately measuring 300 grams of the dried plant powder using an analytical balance. The plant powder was then soaked in 2.5 litres of distilled water and stirred continuously for 48 hours. The resultant mixture was then decanted and filtered through cotton gauze. The resultant filtrate was filtered through a Whatman No. 1 filter paper. The filtrate was collected into light-resistant bottles and stored at 4°C. After 24 hours, the mouth of the bottles was covered with muslin cloth and attached to a freeze drier (Virtis, New York). The setup was left overnight to obtain a freeze-dried product. The obtained freeze-dried product was weighed using an analytical balance and recorded before it was stored in tightly closed, light-resistant bottles at 4°C in a refrigerator awaiting bioassay. The percentage yield of the freeze-dried product was then calculated as percentage weight by weight (%w/w).

Calculation of percentage yield;

% Of crude extract yield= (M1-M2/M3) ×100,

Where;

M1 = mass of container + extract

M2 = mass of empty container

M3 = mass of the rhizome powder sample

3.4.2 Methanol Extract

Two hundred (200) grams of the powdered sample were accurately weighed on an analytical balance and poured into a conical flask wrapped in aluminum foil. 1 litre of analytical grade methanol were then gradually added to the powder. The contents were shaken until a slurry of uniform consistency was formed. Phytochemicals present in the rhizome powder were extracted using maceration with constant shaking for 48 hours. This process was repeated for another batch of 200 grams of the rhizome powder. The resultant mixture was then decanted and filtered through cotton gauze. The resultant filtrate was then filtered through a Whatman No. 1 filter paper.

The resultant filtrates were combined and reduced in vacuo at 50°C using a rotary evaporator to get rid of the excess organic solvent and concentrate the extract. Further solvent removal and concentration of the extract was done in a sand bath set at 35°C for 5 days. The percentage yield of the dried product was then calculated as percentage weight by weight (%w/w).

Calculation of percentage yield;

% Of crude extract yield= (M3-M2/M1) ×100,

Where;

M3 = mass of container + extract

M2 = mass of empty container

M1 = mass of the initial rhizome powder sample

The resultant extract was then stored under refrigeration at 4°C in well-closed, light-resistant amber bottles awaiting analysis.

3.4.3 Dichloromethane and methanol (1:1) Extract

Two hundred (200) grams of the rhizome powder were accurately weighed on an electronic analytical balance and poured into a 1.8 litre conical flask wrapped in aluminum foil. 500 ml of analytical grade dichloromethane was mixed with 500 ml of analytical grade methanol to make a solution of 1 litre. The mixture was then gradually added to the powder. The contents were shaken until a slurry of uniform consistency was formed. Phytochemicals present in the rhizome powder were extracted using cold maceration with constant shaking for 48 hours. This process was repeated for another batch of 200 grams of the rhizome powder. The resultant mixture was then decanted and filtered through cotton gauze. The resultant filtrate was then filtered through a Whatman No. 1 filter paper.

The resultant filtrates were combined and reduced in vacuo at 50°C using a rotary evaporator to get rid of the excess organic solvent and concentrate the extract. Further solvent removal and concentration of the

extract was done in a sand bath set at 35°C for 5 days. The percentage yield of the dried product was then calculated as percentage weight by weight (%w/w).

Calculation of percentage yield;

% Of crude extract yield= (M3-M2/M1) ×100,

Where;

M3=mass of container + extract

M2=mass of empty container

M1=mass of the initial rhizome powder sample

The dried plant extract was then stored under refrigeration at 4°C in well-closed, light-resistant bottles awaiting analysis.

3.5 Phytochemical screening of aqueous, methanol and DCM/methanol (1:1) rhizome extracts of *Digitaria abyssinica*

Phytochemical screening of the phytoconstituents in the aqueous, methanol and dichlomethane methanol (1:1) rhizome extracts of *Digitaria abyssinica* was be done using standard methods of analysis with slight modification as described by (Houghton & Raman, 1998) and (Harborne, 1998). The phytochemicals of interest that were tested for include; saponins, phenolics, alkaloids, cardiac glycosides, tannins, coumarins, flavonoids, steroids and terpenoids.

3.5.1 Saponins (Froth test)

About 0.1 g of the water, methanol, and dichloromethane: methanol (1:1) extracts of *D. abyssinica* rhizome were added to 10 ml of distilled water in separate test tubes, respectively. The mixtures were

boiled for 10 minutes, and they were filtered using Whatman filter paper No.1. A mixture of3 ml distilled water and 5 ml of the filtrate was agitated vigorously for 15 seconds and left to stand for 10 minutes. Frothing which persisted for about 3 minutes was an indication of saponins (Evans, 2009).

3.5.2 Phenolics

Approximately, 0.1 g of the water, methanol, and dichloromethane: methanol (1:1) extracts of *D. abyssinica* rhizome were measured and put into separate test tubes and 10 ml of 70% ethanol were added. The mixtures were boiled using water for five minutes. The extracts were then cooled, and they were filtered through Whatman filter paper No.1. Five drops of 5% of ferric chloride were added into 2 ml of each respective extract. The formation of a green precipitate indicates the presence of phenols (Evans, 2009).

3.5.3 Test for Alkaloids

Two tests, namely, Mayer's and Dragendorff's tests, were done to detect alkaloids in the extracts.

3.5.3.1 Mayer's Test.

Approximately, 0.1 g of water, methanol, and dichloromethane: methanol (1:1) extracts of *D. abyssinica* rhizome were mixed with 5 ml of 1% HCl in separate test tubes, respectively; each mixture was warmed and then filtered through Whatman filter paper No.1. Two drops of Mayer's reagent (mercuric potassium iodide) were added to 2 ml of water, methanol, and dichloromethane: methanol (1:1) extracts. The appearance of a cream-colored precipitate indicates the presence of alkaloids (Moriasi *et al*., 2020; Muthee *et al*., 2022).

3.5.3.2 Dragendorff's Test

The test was carried out by adding two drops of Dragendorff's reagent (potassium bismuth iodide solution) to 2 ml of the filtered water, methanol, and dichloromethane: methanol (1:1) extracts in separate test tubes. A reddish-brown precipitate indicates the presence of alkaloids (Moriasi *et al*., 2020; Muthee *et al*., 2022).

3.5.4 Glycosides

3.5.4.1 Keller–Killiani Test.

Glacial acetic acid (4.0 ml) solution with 1 drop of 2.0% FeCl3 mixture was added to the 10 ml water, methanol, and dichloromethane: methanol (1:1) extracts of *D. abyssinica* rhizome in separate test tubes. One milliliter of concentrated sulphuric acid was added to the mixture and a reddish- brown ring formed between the layers which progressively turned blue indicating the presence of steroidal glycosides with deoxy sugars (Evans, 2009).

3.5.4.2 Kedde Test.

One milliliter of 2% solution of 3,5-dinitrobenzoic acid in 95% alcohol was added to the 2 ml of the water, methanol, and dichloromethane: methanol (1: 1) extracts of *D. abyssinica* rhizome. The solution was made alkaline with 5% sodium hydroxide. The appearance of a purple-blue color indicates the presence of an unsaturated lactone ring in cardenolides (Evans, 2009).

3.5.5 Tannins

About 0.5 g of the sample was boiled in 20 ml of water and filtered. 0.1% of ferric chloride was added to the filtrate. The formation of a brownish-green or blue-black color was an indication of the presence of tannins (Kamau, 2018).

3.5.6 Coumarins

Approximately, 0.5 g of the extracts and powder of *D. abyssinica* were added into separate test tubes. The test tubes were covered with 4lter paper which was moistened with 1N NaOH. The tubes were warmed in a hot water bath and then allowed to cool. Yellow fluorescent color was an indication of coumarins (Kamau, 2018).

3.5.7 Flavonoids (Sodium Hydroxide Reagent Test).

Approximately, 0.1 g of the water, methanol, and dichloromethane: methanol (1: 1) extracts of *D. abyssinica* rhizome were warmed in 10 ml of 70% ethanol and thereafter hydrolyzed with 10% hydrochloric acid. Sodium hydroxide (10%; 1ml) was added to the mixture and the appearance of yellow color was a positive test for the presence of flavonoids (Onyancha *et al*., 2015; Ushie *et al*., 2017).

3.5.8 Steroids

3.5.8.1 Salkowski's Test.

Approximately, 2 mg of the water, methanol, and dichloromethane: methanol (1:1) extracts of *D. abyssinica* rhizome were dissolved in 1 ml of chloroform and then shaken gently. Five drops of concentrated sulphuric acid were added along the side of the test tube. A reddish-brown color that was formed at the interface indicated steroids (Mujeeb *et al*., 2014; Onyancha *et al*., 2015).

3.5.8.2 Liebermann–Burchard Test.

About 2ml of acetic acid was added to 1mL of the water, methanol, and dichloromethane: methanol (1:1) extracts. After cooling the solution in an ice bath, concentrated sulphuric was added carefully. The development of violet to blue or bluish-green color confirms the test for steroids (Alabri *et al*., 2014; Shaikh *et al*., 2021).

3.5.9 Terpenoids (Salkowski's Test).

Approximately, 2 mg of the water, methanol, and dichloromethane: methanol (1:1) extracts of *D. abyssinica* rhizome were dissolved in 2mL chloroform along with concentrated sulphuric acid. The red-brown color at the interface indicates terpenoids (Muthee *et al*., 2022; Shaikh *et al*., 2021).

3.6 Acute oral toxicity testing

3.6.1 Experimental animals

Fifteen 8-10-week, female *Wistar* albino rats weighing 125 ± 45 grams were used to assess the acute oral toxicity *Digitaria abyssinica* rhizome extracts. These animals were purchased from the Kabete Vet Lab animal house. They were transported to the Public Health, Pharmacology and Toxicology (PHPT) Department animal house where they were housed for 5 days to acclimatize following Biosafety, Animal Use and Ethical Committee (BAUEC) guidelines.

All the experimental animals were nulliparous and non-pregnant. They were housed at a temperature of 25 ± 3°C and 56-60% relative humidity. A 12-hour day and night cycle were maintained and the animals were fed on standard rat pellets from a commercial feed supplier (Unga Feeds). Water was provided ad libitum.

3.6.2 Preparation of doses

Normal saline was used as the vehicle. For the aqueous extract dosage preparation, an analytical balance was used to accurately measure 2,000 mg of the aqueous extract which was dissolved in 10ml of normal saline.

For the methanol extract dosage preparation, an analytical balance was used to accurately measure 2,000mg of the methanol extract which was dissolved in 10ml of normal saline.

For the DCM/methanol (1:1) extract dosage preparation, an analytical balance was used to accurately measure 2,000mg of the DCM/methanol (1:1) extract which was dissolved in 10ml of normal saline.

The following formula was used to calculate the individual animal dose administration.

$$\text{Animal dose (mg/kg bw)} = \frac{\text{body weight of the animal (g)}}{100} \times \text{selected dose}$$

3.6.3 Acute oral toxicity study of aqueous and methanol rhizome extracts of *Digitaria abyssinica*

The Up-and-Down procedure described by OECD (2008, Document No. 425) was used to evaluate the acute oral toxicity of the aqueous, methanol and DCM/methanol extracts of *Digitaria abyssinica*. The Wistar rats were randomly assigned to three groups of five (5) rats each. Each animal was individually weighed and labeled with a permanent marker on its tail to enable identification. They were fasted overnight before commencement of the study.

Group 1 was administered orally with 2,000mg/kg bw aqueous rhizome extract of *Digitaria abyssinica* whereas Group 2 was given 2,000mg/kg bw methanol rhizome extract and Group 3 was given 2,000 mg/kg/bw DCM/Methanol (1:1) rhizome extract of *Digitaria abyssinica* orally. Thereafter, wellness parameters such as lethargy, salivation, mucous membrane appearance, skin, hair, diarrhea, unconsciousness, changes in body weight, mortality as well as sleep were observed and documented in 30 minutes, 1 hour, 4 hours, 24 hours, 48 hours, 7 days, and 14 days correspondingly.

3.7 Antimicrobial studies

3.7.1 Test micro-organisms

A fungal micro-organism and three bacterial strains were obtained from the stock cultures from the University of Nairobi, Medical Microbiology Department.

Table 3.1: Microbes used in the antimicrobial studies.

Name of the micro-organism	Microbe type	Gram stain	Strain type
Staphylococcus aureus	Bacteria	Positive	ATCC 29213
Escherichia coli	Bacteria	Negative	ATCC 25922
Neisseria gonorrhoeae	Bacteria	Negative	Clinical isolate
Candida albicans	Fungus	-	Clinical isolate

3.7.2. Biosafety procedures observed during the research.

Only authorized persons were allowed to enter the laboratory working areas. Laboratory doors were kept closed. Lab coats and gloves were worn all the time when working in the laboratory. Hand washing was observed following removal of the gloves and immediately following contact with the infectious materials and animals. The laboratory was kept neat, clean and free of materials that are not pertinent to the work. A biosafety level II cabinet was used when handling the infectious materials. Work surfaces were decontaminated before and after use and immediately after spills. All contaminated materials, specimens and cultures were decontaminated before disposal by autoclaving.

3.7.3 Preparation of Inoculums

In order to prepare the stock cultures, Clinical and Laboratory Standards Institute (CLSI) guidelines and procedures were used. The method as described by (Mailu *et al*., 2021) was used with slight modifications. Briefly, bacterial stock cultures were sub cultured on respective media and incubated at 37 °C for 24 hours to obtain young growing culture. *N. gonorrhoeae* was cultured on Thayer Martin agar while isolates of *E. coli* and *S. aureus* were cultured on MacConkey and Sheep Blood Agar respectively. *Candida albicans*

was sub cultured in Sabouraud Dextrose Agar (SDA) at 37°C for 48 hours. The test strains were suspended in Mueller-Hinton broth (MHB) to give a final density of 1.5 x 10^6 of bacteria colony forming units and 1.5 x10^5 fungal colony forming units.

3.7.4 Preparation of the stock solutions

Six different concentrations of 15.625 mg/ml, 31.25 mg/ml, 62.50 mg/ml, 125 mg/ml, 250 mg/ml and 500 mg/ml of each of the three extracts (aqueous, methanol, DCM/methanol (1:1) were prepared for susceptibility testing using 1% DMSO as a diluent. A vortex mixer was used to facilitate dissolving of extracts into the 1% DMSO. A constant volume 20 µl of each of the individual stock solution was pipetted using micro titer-pipette onto sterile filter paper disks measuring 6 mm to prepare the respective concentrations of the plant extracts. 20 µl of 1% DMSO was impregnated onto sterile filter paper disks which was used as the negative control for the experiment.

3.7.5 Disk diffusion method

Antimicrobial activity was evaluated using the disk diffusion method as described by (Balouiri *et al*., 2016) with slight modifications. Appropriate Agar plates were inoculated with respective isolates of the test micro-organism. Sterile filter paper disks (6 mm in diameter) containing each of the 3 extracts at a desired concentration of either (15.625, 31.25, 62.50, 125, 250, 500) mg/ml were placed on the surface of the agar, using sterile forceps. 1% dimethyl sulfoxide (DMSO) was used as the negative control. Cephalexin (30 µg), Ciprofloxacin (5 µg) and Tetracycline (30 µg) sensitivity disks were used as control against *S. aureus*; Cephalexin (30 µg), Ciprofloxacin (5 µg) and Tetracycline (30 µg) sensitivity disks were used as control against *E. Coli*, Cephalexin (30 µg) and Azithromycin (15 µg) sensitivity disks control susceptibility of *N. gonorheae* while Clotrimazole (50 µg), Econazole (50 µg), Ketoconazole (50 µg), Miconazole (50 µg) and Nystatin (100 µg) - disks were used as control of the extract against *C. albicans.*

The Mueller Hinton agar plates inoculated with *Staphylococcus aureus* and those inoculated with *Escherichia Coli* were incubated at 37 °C for 24 hours; Thayer Martin Media inoculated with *N. gonorrhea* incubated at 5% CO_2 for 48 hours while Sabouraud Dextrose Agar inoculated with *C. albicans* was incubated at 37°C for 24 hours. Generally, the antimicrobial agent diffuses into the agar and inhibits the germination and growth of the test micro-organism. After the incubation period, the diameters of the inhibition zones were measured in millimeters using a transparent ruler. All the tests were done in triplicates and the means calculated as the final results.

3.7.6 Broth micro dilution technique

The broth micro dilution procedure as described by Mailu *et al*. (2021) with modification was used to determine minimum inhibitory concentration for the active crude extracts against the test microorganisms. Six culture tubes with 2 ml sterile Mueller Hilton broth were prepared. From the stock solution, two-fold serial dilutions were prepared. 0.1 ml of each microorganism was inoculated into each tube of diluted plant extract using a micro-pipette. The bacterial organisms and the fungal organism were then incubated for 24 hours at 37°C. The extract's minimum inhibitory concentration (MIC) value was determined by observing the lowest concentration of plant extracts that prevented visible growth of microorganisms resulting into no visible growth (turbidity).

To determine minimum bactericidal concentration (MBC), all broth in tubes with no visible bacterial growth were aseptically cultivated in sterile agar using the pour plate method and incubated in appropriate temperature and conditions. The MIC value is the lowest concentration of plant extract that demonstrates no visible bacterial growth. All tubes with no visible fungal growth were aseptically cultured in sterile molten agar and incubated using striking method to determine minimum bactericidal concentration (MBC). The minimal fungicidal concentration (MFC) value was defined as the lowest plant extract's concentration that shows no visible fungal growth. Tubes that were just inoculated with microorganisms

and tubes that were only inoculated with media served as controls. All the experiments were carried out in triplicate and the results recorded in a table.

3.8 Ethical approval

Before commencement of the study, ethical approval was obtained from the Faculty of Veterinary Medicine Biosafety, Animal Use and Ethics Committee (BAUEC) of the University of Nairobi and a reference number assigned **FVM BAUEC/2021/290.**

The research license for the study was obtained from the National Commission for Science, Technology, and Innovation (NACOSTI) and a license number assigned **NACOSTI/P/21/11253**.

3.9 Data Analysis

All experiments were performed in triplicates for verification and validation of data. Data was analyzed by GraphPad Prism version 9.0.0 and the results are provided as mean SEM. ANOVA and post hoc ANOVA using the Tukey's HSD test with 95% confidence level was used to compare the differences in means among and between the groups, respectively. Differences (among and between groups) were considered to be statistically significant at $p < 0.05$. A p-value of less than 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

4.1: Percentage yield

The given quantities of *Digitaria abyssinica* was found to yield the amounts shown in the table.

Table 4.1: Yields of the *Digitaria abyssinica* rhizome extracts

Plant extract	**Weight of the powder (gm) in a solvent (litres)**	**Weight of the extract (gm)**	**% Yield % w/w**
Aqueous extract	300 gm in 2.5 litres distilled water	21	7
Methanol extract	400 gm in 2 litres	9.32	2.33
DCM/methanol (1:1)	400 gm in 1 litre of DCM and 1 litre methanol	7.56	1.89

The percentage yield of the dried whole rhizome of *D. abyssinica* ranged from 7% to 1.89%. The highest yield was obtained from the aqueous extract at 7.0%, which was followed by methanol at 2.33%, and the lowest yield was extracted from dichloromethane/methanol at 1.89%.

4.2 Phytochemical composition of *Digitaria abyssinica* rhizomes obtained from Mua, Kyaani

Phenolics, cardiac glycosides, tannins, and flavonoids were present in the *D. abyssinica* aqueous extract, but saponins, alkaloids, terpenoids, and steroids were not. The *D. abyssinica* methanol extract contained saponins, phenolics, alkaloids, cardiac glycosides, tannins, and flavonoids but devoid of terpenoids and steroids. The *D. abyssinica* dichloromethane/methanol (1:1) extract included saponins, phenolics, cardiac glycosides, tannins, and flavonoids but neither alkaloid or terpenoids. The phytochemicals present or absent in *D. abyssinica* rhizome extracts are shown in table 4.2 below.

Table 4.2: Phytochemical composition of *D. abyssinica* rhizome extract

Phytochemicals	Method/reagent	Powder	Aqueous	Methanol	Dichloromethane: methanol (1:1)
Saponins	Foam test	+	-	+	+
Phenolics	Ferric chloride	+	+	+	+
Alkaloids	Mayer's	-	-	-	-
	Drangendorff's	+	+	+	+
Cardiac glycosides	Keller Killiani	+	+	+	+
	Keddie	+	+	+	+
Tannins	Ferric chloride	+	+	+	+
Coumarins	Ferric chloride	+	+	+	+
Flavonoids	NaOH	+	+	+	+
Steroids	Liebermann-Burchard	+	+	+	+
Terpenoids	Salkowski	+	+	-+	+

Key (+): present, (-): absent

4.3 Acute oral toxicity testing of aqueous, methanol and DCM/methanol (1:1) rhizome extracts of *Digitaria abyssinica* on Wistar rats

There were no physical changes in the skin, fur, eyes and the mucous membranes after oral administration of the aqueous, methanol and dichlomethane/methanol rhizome extracts of *D. abyssinica* at a concentration of 2,000mg/kg. Furthermore, there was so significant changes in salivation, lacrimation,

sweat, urine incontinence and defecation. During handling, the animals showed no evidence of aggressiveness.

Table 4.3 is a summary of the mean weight gain in animals treated with the aqueous, methanol and DCM/methanol extracts of *D. abyssinica* over a period of 14 days.

Table 4.3: Effect of a 2,000mg/kg dose of aqueous, methanol and DCM/methanol extracts of *Digitaria abyssinica* on the weight of Wistar rats.

Treatment	Mean weight gain
2,000 mg/kg Aqueous (Day 7)	31.92±2.12[a]
2,000 mg/kg methanol (Day 7)	34.91±5.72[a]
2,000 mg/kg DCM/methanol (1:1) (Day 7)	26.11±11.93[a]
2,000 mg/kg Aqueous (Day 14)	38.02±7.79[a]
2,000 mg/kg methanol (Day 14)	47.70±13.93[a]
2,000 mg/kg DCM/methanol (1:1) (Day 14)	44.88±8.68[a]

Values are presented as Mean ± standard error of the mean (SEM). Means with different superscript letters are significantly different (One-way ANOVA and Tukey's test; $p< 0.05$).

In the acute toxicity study, 5 female Wistar rats given a 2000 mg/kg dose of aqueous, 5 female Wistar rats were given methanol, and 5 female Wistar rats were given dichloromethane-methanol *D. abyssinica* extracts showed no signs of toxicity or mortality after 24 hours and 14 days. The LD_{50} is greater than 2000 mg/kg, according to the results. Furthermore, there was no significant difference in percentage weight

gain between aqueous extract-treated rats and methanol extract-treated rats during the first week of treatment *(p=0.8974)*.

In the first week of treatment, there was no significant difference in percentage weight gain between aqueous extract-treated rats and dichloromethane-methanol extract-treated rats *(p=0.8628)*. In the second week of treatment, there was no significant difference in percentage weight gain between *D. abyssinica* aqueous extract-treated rats and *D. abyssinica* methanol extract-treated rats *(p=0.3818)*.

In the second week of treatment, the percentage weight gains in rats treated with dichloromethane-methanol extract was significantly higher *(p=0.0202)* than the percentage weight gain in rats treated with aqueous extract. In the second week of treatment, there was no significant difference in percentage weight gain between methanol extract-treated rats and dichloromethane-methanol extract-treated rats *(p=0.6566)*.

4.4 Antimicrobial susceptibility testing of *Digitaria abyssinica*

4.4.1 Disk diffusion method

4.4.1.1 Aqueous extract

The zone of inhibition of the aqueous extract of *D. abyssinica* against the four microbes is shown in the table and figure below where the *C. albicans* recorded the highest zone of inhibition. The aqueous extract was not active against the two Gram-negative bacteria (*E. coli* and *N. gonorrheae*) and the Gram-positive (*S. aureus*) at a strength of 500 mg/ml.

Table 4.4: Zones of inhibition (ZI) of aqueous rhizome extract of *Digitaria abyssinica*

Concentration (mg/ml)	Zones of inhibition (mm)			
	Test microorganisms			
	Candida albicans	***Escherichia coli***	***Neisseria gonorrhoeae***	***Staphylococcus aureus***
500	16.33±0.82 (++)	0.00 (-)	0.00 (-)	0.00 (-)
250	15.67±0.55 (++)	0.00 (-)	0.00 (-)	0.00 (-)
125	15.50±0.55 (++)	0.00 (-)	0.00 (-)	0.00 (-)
62.5	9.67±0.52 (+)	0.00 (-)	0.00 (-)	0.00 (-)
31.25	7.83±0.75 (-)	0.00 (-)	0.00 (-)	0.00 (-)
15.625	6.50±0.55 (-)	0.00 (-)	0.00 (-)	0.00 (-)
Negative control (DMSO)	0.00	0.00	0.00	0.00
Positive controls CTR	27.00±0.89	NT	NT	NT
EC	26.67±0.52	NT	NT	NT

KET	34.17±0.75	NT	NT	NT
MCZ	24.83±0.75	NT	NT	NT
NY	7.17±0.41 (-)	NT	NT	NT
AZM	NT	6.00±0.00 (-)	21.00±0.71 (+++)	NT
CRO	NT	29.00±0.89 (+++)	28.50±4.23 (+++)	NT
CL	NT	12.33±0.52 (+)	NT	15.50±0.55 (++)
CIP	NT	30.33±0.82 (+++)	NT	22.17±0.75 (+++)
TE	NT	18.67±0.52 (++)	NT	11.33±0.52 (+)

KEY: Zones of inhibition were indicated as mean ± SEM of the triplicate experiments

0.0 Meant that there was no observed inhibition

NT; Not tested

DMSO Dimethyl sulfoxide

CTR Clotrimazole, EC Econazole, KET Ketoconazole, MCZ Miconazole and NY Nystatin were the positive controls for the fungal organisms.

AZM Azithromycin, CRO ceftriaxone, CL Cephalexin, CIP Ciprofloxacin, and TE Tetracycline were the positive controls for the bacterial organisms.

From the data, there were notable statistically significant differences in the growth inhibitions of the varying strengths of the aqueous extract against the human pathogens used. Measurable significance differences ($p<0.05$) were observed between the growth inhibitions caused by the aqueous extract and the reference drugs. All concentrations did not inhibit *E. coli*, *S. aureus* and *N. gonorrhoeae*. The diameter of the zone of inhibition was zero for all the concentrations, thus all obtained p values were 1.00. Hence, there was no significant differences between any two concentrations.

The aqueous extract of *D. abyssinica* rhizome demonstrated moderate to high antimicrobial effects against *C. albicans* in a dose dependent manner. Measurable statistical significance differences ($p<0.05$) were observed in the zones of inhibition from a dose of 15.625 mg/ml to 500 mg/ml. The mean zone of inhibition produced by a 15.625 mg/ml concentration of the *D. abyssinica* aqueous extract was significantly lower ($p<0.05$) than the mean zone of inhibition produced by 31.25 mg/ml to 500 mg/ml concentrations of the extract. The mean zone of inhibition produced by a 31.25 mg/ml concentration of the *D. abyssinica* aqueous extract was significantly lower ($p<0.05$) than the mean zone of inhibition produced by 62.50 mg/ml to 500 mg/ml concentrations of the extract. There was no significant difference ($p>0.05$) in the mean zone inhibition produced by 125 mg/ml, 250 mg/ml, and 500 mg/ml concentrations of *D. abyssinica* aqueous extract. The mean zone of inhibition produced by 62.50 mg/ml to 500 mg/ml concentrations of the aqueous extract was significantly higher ($p<0.05$) than the mean zone of inhibition produced by Nystatin. All extract concentrations had a significantly lower mean zone of inhibition than the standards: clotrimazole, econazole, ketoconazole and miconazole.

4.4.1.2 Methanol Extract

The methanol extract showed inhibition against *C. albicans*, the fungal organisms. However, the methanol extract was not active against *E. coli, N. gonorrhoeae* and *S. aureus* even at a concentration of 500mg/ml.

Table 4.5: Diameter of zones of inhibition (ZI) of methanol rhizome extract of *Digitaria abyssinica*

Concentration (mg/ml)	**Diameter of zones of inhibition (mm)**			
	Test microorganisms			
	Candida albicans	***Escherichia coli***	***Neisseria gonorrhoeae***	***Staphylococcus aureus***
500	15.17±0.75	0.00	0.00	0.00
250	11.50±0.55	0.00	0.00	0.00
125	11.67±0.52	0.00	0.00	0.00
62.5	8.83±0.75	0.00	0.00	0.00
31.25	8.33±0.52	0.00	0.00	0.00
15.625	6.83±0.41	0.00	0.00	0.00
Negative control (DMSO)	0.00	0.00	0.00	0.00
Positive controls CTR	27.00±0.89	NT	NT	NT
EC	26.67±0.52	NT	NT	NT

KET	34.17±0.75	NT	NT	NT
MCZ	24.83±0.75	NT	NT	NT
NY	7.17±0.41	NT	NT	NT
AZM	NT	6.00±0.00	21.00±0.71	NT
CRO	NT	29.00±0.89	28.50±4.23	NT
CL	NT	12.33±0.52	NT	15.50±0.55
CIP	NT	30.33±0.82	NT	22.17±0.75
TE	NT	18.67±0.52	NT	11.33±0.52

KEY: Zones of inhibition were indicated as mean ± SEM of the triplicate experiments

1.0 Meant that there was no observed inhibition

NT - Not tested

DMSO - Dimethyl sulfoxide

CTR - Clotrimazole, EC - Econazole, KET - Ketoconazole, MCZ - Miconazole and NY - Nystatin were the positive controls for the fungal organisms.

AZM - Azithromycin, CRO - Ceftriaxone, CL - Cephalexin, CIP - Ciprofloxacin, and TE - Tetracycline were the positive controls for the bacterial organisms.

The various concentrations of the methanol extract had statistically significant differences in terms of the growth inhibitions of each microbe investigated. There was a statistically significance difference ($p>0.05$) between inhibitions caused by the methanol extract and the reference drugs. However, there was no significance differences in the growth inhibitions caused by the 3 crude extracts. All the concentrations did not inhibit *E. coli, N. gonorrhoeae* and *S. aureus*. The diameter of the zones of inhibition was zero for all the concentrations thus obtained p values of 1.00. Hence, there was no significance differences between any two concentrations.

The *D. abyssinica* methanol extract exhibited moderate to high antifungal activity in a dose-response relationship against the fungal microbe *C. albicans.* The lowest mean zone of inhibition of 6.83 ± 0.4 produced a concentration of 15.625 mg/ml. There was no significant difference ($p>0.05$) in the mean zone of inhibition produced by 31.25 mg/mL and 62.5 mg/mL concentrations of *D. abyssinica* rhizome methanol extract. Also, there was no significant difference *($p>0.05$)* in the mean zone of inhibition produced by 62.5 mg/mL and 125 mg/mL concentrations of *D. abyssinica* rhizome methanol extract which was higher than the previous concentrations. The highest mean zone of inhibition of 15.17 ± 0.75 was produced by 500 mg/ml of the *D. abyssinica* methanol extract. However, all the methanol extract concentrations had a significantly lower mean zone of inhibition than clotrimazole, econazole, ketoconazole, and miconazole. *D. abyssinica* rhizome methanol extract did not inhibit Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *N. gonorrhoeae*) bacteria.

4.4.1.3 Dichlomethane/methanol (1:1) extract

C. albicans recorded the highest diameter of inhibitions while the DCM/methanol (1:1) was not active against *E. coli, N. gonorrhoeae* and *S. aureus* at a strength of 500mg/ml.

Table 4.6: Zones of inhibition (ZI) of DCM/methanol (1:1) rhizome extract of *Digitaria abyssinica*

Conc (mg/ml)	Zones of inhibition (mm)			
	Test microorganisms			
	Candida albicans	***Escherichia coli***	***Neisseria gonorrhoeae***	***Staphylococcus aureus***
500	10.50±0.55	0.00	0.00	0.00
250	9.50±0.55	0.00	0.00	0.00
125	9.50±0.55	0.00	0.00	0.00
62.5	8.17±0.41	0.00	0.00	0.00
31.25	6.83±0.41	0.00	0.00	0.00
15.625	0.00	0.00	0.00	0.00
Negative controls (DMSO)	0.00	0.00	0.00	0.00
CTR	27.00±0.89	NT	NT	NT
EC	26.67±0.52	NT	NT	NT
KET	34.17±0.75	NT	NT	NT
MCZ	24.83±0.75	NT	NT	NT

NY	7.17±0.41	NT	NT	NT
AZM	NT	6.00±0.00	21.00±0.71	NT
CRO	NT	29.00±0.89	28.50±4.23	NT
CL	NT	12.33±0.52	NT	15.50±0.55
CIP	NT	30.33±0.82	NT	22.17±0.75
TE	NT	18.67±0.52	NT	11.33±0.52

KEY: Zones of inhibition were indicated as mean ± SEM of the triplicate experiment

0.0 Meant that there was no observed inhibition

NT - Not tested

DMSO Dimethyl sulfoxide

CTR Clotrimazole, EC Econazole, KET Ketoconazole, MCZ Miconazole and NY Nystatin were the positive controls for the fungal organisms.

AZM Azithromycin, CRO ceftriaxone, CL Cephalexin, CIP Ciprofloxacin, and TE Tetracycline were the positive controls for the bacterial organisms.

The various concentrations showed statistically significant differences in terms of growth inhibitions among the various concentrations of DCM/methanol extract under each microbe investigated. There was a statistically significant difference seen among the growth inhibitions caused by the DCM/methanol and the control drugs. All concentrations did not inhibit *E. coli, N. gonorrhoeae* and *S. aureus.* Therefore, their

diameters of zones of inhibition were zero for all concentrations thus all obtained p values were 1.00. Hence, there was no significant differences between any two concentrations of the DCM/methanol (1:1) extract.

The *D. abyssinica* rhizome DCM/ methanol (1:1) extract demonstrated a slight to moderate antimicrobial effect in a dose-response relationship against *C. albicans*. There was no antifungal activity at the lowest concentration of 15.625 mg/ml. A mean zone of inhibition of 6.83±0.41 was observed at a dose of 31.25mg/ml. The mean zone of inhibition produced by a 62.5 mg/ml concentration of the *D. abyssinica* DCM/methanol (1:1) extract was significantly lower ($p<0.05$) than the mean zone of inhibition produced by 125 mg/ml and 250 mg/ml concentrations of the extract. There was a significant difference ($p>0.05$) in the mean zone of inhibition of the 500mg/ml and the previous lower doses of the *D. abyssinica* DCM/methanol extract. Clotrimazole, econazole, ketoconazole, and miconazole all demonstrated a much higher mean zone of inhibition than DCM/methanol extract doses. Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *N. gonorrhea*) bacteria were not inhibited by *D. abyssinica* rhizome DCM/methanol extract.

Of the three extracts (aqueous, methanol and DCM/methanol (1:1) *D. abyssinica* aqueous rhizome extract was the most efficacious. The highest antifungal activity with a mean inhibition zone value of 16.33±0.82 mm was observed in aqueous extracts of *D. abyssinica* in disk diffusion at 500 mg/ml with a MIC value of 31.25 mg/ml against *C. albicans*. *D. abyssinica* aqueous rhizome extract performed better than Nystatin which had a mean inhibition zone of 7.17 ± 0.41. However, *D. abyssinica* aqueous rhizome extract mean zone of inhibition had a significant difference ($p>0.05$) than the standard reference drugs: clotrimazole, econazole, ketoconazole and miconazole.

Table 4.7: Summary of the zones of inhibition of *Digitaria abyssinica* crude rhizome extracts against four microbes at various concentration.

Zone of inhibition (mm)						
Pathogen	**Concentration (mg/mL)**	**Aqueous extract**	**Methanol Extract**	**Dichloromethane-methanol extract**	**Negative control**	**Positive Control**
Candida albicans	15.625	$6.50^{a}\pm0.55$	$6.83^{a}\pm0.41$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	Clotrimazole:$27.00^{f}\pm0.89$
	31.25	$7.83^{b}\pm0.75$	$8.33^{b}\pm0.52$	$6.83^{b}\pm0.41$		Econazole:$26.67^{f}\pm0.52$
	62.50	$9.67^{c}\pm0.52$	$8.83^{b}\pm0.75$	$8.17^{c}\pm0.41$		Ketoconazole:$34.17^{g}\pm0.75$
	125.00	$15.50^{d}\pm0.55$	$11.67^{c}\pm0.52$	$9.50^{d}\pm0.55$		Miconazole:$24.83^{e}\pm0.75$
	250.00	$15.67^{d}\pm0.52$	$11.50^{c}\pm0.55$	$9.50^{d}\pm0.55$		Nystatin: $7.17^{ab}\pm0.41$
	500.00	$16.33^{d}\pm0.82$	$15.17^{d}\pm0.75$	$10.50^{e}\pm0.55$		
Escherichia coli	15.625	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	Azithromycin: $6.00^{b}\pm0.00$
	31.25	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$		Ceftriaxone:$29.00^{e}\pm0.89$
	62.50	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$		Cephalexin:$12.33^{c}\pm0.52$
	125.00	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$		Ciprofloxacin:$30.33^{f}\pm0.82$
	250.00	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$		Tetracycline: $18.67^{d}\pm0.52$
	500.00	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$	$0.00^{a}\pm0.00$		

Neisseria	15.625	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	Azithromycin: 21.00^{b}±0.71
gonorrhoeae	31.25	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00		Ceftriaxone: 28.50^{c}±4.23
	62.50	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00		
	125.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00		
	250.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00		
	500.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00		
Staphylococcus	15.625	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	Cephalexin: 15.50^{c}±0.55
aureus	31.25	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00		Ciprofloxacin:22.17^{d}±0.75
	62.50	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00		Tetracycline:11.33^{b}±0.52
	125.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00		
	500.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00		

Values are expressed as mean ± SD of three separate determinations. Means with different superscripts along the column are significantly different at $p<0.05$.

4.4.2 Microbroth dilution technique

Table 4.8: The minimum inhibitory concentration of the crude rhizome extracts of *Digitaria abyssinica*

	Minimum inhibitory conc. (MIC) (mg/ml)			
Extracts	**Test organisms**			
	Candida albicans	***Escherichia coli***	***Neisseria gonorrhoeae***	***Staphylococcus aureus***
Aqueous	31.25	> 250	> 250	> 250
Methanol	31.25	> 250	> 250	> 250
DCM/methanol	62.5	> 250	> 250	> 250

Table 4.9: The minimum bactericidal concentration/minimum fungicidal concentration of the crude rhizome extracts of *Digitaria abyssinica*

	MBC/MFC (mg/ml)			
Extracts	**Test organisms**			
	Candida albicans	***Escherichia coli***	***Neisseria gonorrhoeae***	***Staphylococcus aureus***
Aqueous	62.5	> 250	> 250	> 250
Methanol	62.5	> 250	> 250	> 250
DCM/methanol	125	> 250	> 250	> 250

Table 4.10: Average MIC and MBC/MFC (mg/ml) of aqueous, methanol and DCM/methanol of *Digitaria abyssinica* against the 4 test organisms.

	Extracts					
Test organism	Aqueous		Methanol		DCM/methanol	
	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC
Candida albicans	31.25	62.5	31.25	62.5	62.5	125
Escherichia coli	> 250	> 250	> 250	> 250	> 250	> 250
Neisseria gonorrhoeae	> 250	> 250	> 250	> 250	> 250	> 250
Staphylococcus aureus	> 250	> 250	> 250	> 250	> 250	> 250

KEY

MIC – Minimum inhibitory concentration

MBC – Minimum bactericidal concentration

MFC – Minimum fungicidal concentration

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

Plant-based antimicrobials have enormous potential to treat viral, protozoal, fungal, and bacterial illnesses without causing any known negative side effects (Chandra *et al*., 2017). The findings of the current study indicate that *D. abyssinica* rhizome extracts have antifungal activity against *C. albicans*. Previous research has demonstrated that plant extracts with an inhibition zone larger than 6 mm against specific microbial strains have antimicrobial activity according to (Atef *et al*., 2019; Kathare *et al*., 2021; Mwitari *et al*., 2013; Nanasombat *et al*., 2018).

Water is the preferred solvent for extraction since the aqueous extract had the highest yield of 7% and its extract elicited the highest antifungal activity against *C. albicans*. This may be as result of the cold maceration technique used for extraction, which preserved the phytochemicals hence were not denatured since the extraction was carried out at room temperature. Some Digitaria species were proven to possess antimicrobial activities. This study was in agreement with another member of the same species *Digitaria sanguinalis* whose *p*-Coumaric acid exhibited highest antifungal activity against *Aspergillus fumigates* and *C. albicans* (Ibrahim *et al*., 2019).

Using the Up-and-Down approach proposed by the (OECD, 2008) Document 425, the acute oral toxicity effects of both the aqueous, methanol and dichlomethane/methanol (1:1) were investigated in this research. This procedure has also been used in previous studies by (Das *et al*., 2019; Olela *et al*., 2020; Wangusi *et al*., 2021). In the experimental rats, there were no evidence of toxicity. In conclusion, oral administration of the *D. abyssinica* rhizome aqueous, methanol and dichlomethane/methanol (1:1) extract's LD_{50} is greater than 2,000

mg/kg, and hence the extracts under research can be classified as practically non-toxic and hence the drug is found to be safe.

The current study serves as the first report on the phytochemical constitution of *D. abyssinica* rhizome extracts. The phytochemicals present in a plant extract determine its pharmacological activity. For many decades, it has been recognized that these phytochemicals work either solely or in synergy with others to elicit pharmacological action, including but not limited to the following; antibacterial, antifungal, analgesic, antiviral, anti- inflammatory, antitumor, anthelmintic action and effects on the central nervous system.

Saponins are naturally occurring surface-active glycosides that have foaming (soap) characteristics when dissolved in water. Because they can create stable, soap-like foams in aqueous solutions, saponins get their name. Aglycone (sterol or common triterpene) units are joined to one or more carbohydrate chains to form saponins which are glycosides. The interaction of saponins with membrane sterols has been proposed as the main mechanism underlying their antifungal effect (Arif *et al*., 2009; Francis *et al*., 2002). Saponins were present on the *D. abyssinica* methanol and dichlomethane/methanol (1:1) but absent or present in below detection levels on the aqueous extract.

Surprisingly, *D. abyssinica* rhizome aqueous extract possessed the highest antifungal activity with a mean zone of inhibition of 16.33 ± 0.82 at a concentration of 500mg/ml. Therefore, this suggests that the observed antifungal activity could be due to the presence of other classes of phytochemicals in the aqueous extract. Saponins have been shown to possess antimicrobial activity. Saponins are thought to act by damaging the cell membrane allowing cellular components including proteins and certain enzymes to leak out and ultimately resulting in cell death (Murugan *et al*., 2013).

Phenolics are a group of resins that are commonly created by the reaction of formaldehyde and phenol (carbolic acid), which is catalyzed by an acid or base. There is evidence that increasing hydroxylation causes increased toxicity, and that the site(s) and amount of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms (Arif *et al*., 2009). Phenolics were present in all the three extracts (aqueous, methanol and DCM/methanol (1:1). Phenolics have been shown to possess antimicrobial activity. The ability of antifungal agents to bind to membrane ergosterol is one of the suggested mechanisms of antimicrobial activity of phenols. This could explain why *D. abyssinica* rhizome aqueous, methanolic, and DCM/methanol (1:1) extracts have fungicidal effects on *C. albicans*.

Alkaloids have been shown to be responsible for antimicrobial activity (Elgharbawy *et al*., 2020). Alkaloids were only present only in the *D. abyssinica* methanol extract but absent in the methanol and dichlomethane/methanol (1:1) extracts. There are multiple instances of alkaloids serving as antifungals. Their antifungal effect is typically pleiotropic, inhibiting protein synthesis and intercalating fungal deoxyribonucleic acid (DNA) or by promoting the production of fungus inhibitors. The first alkaloid that was beneficial for medicine was morphine, which was discovered in 1805 in the opium plant *Papaver somniferum* (Elgharbawy *et al*., 2020).

Secondary metabolites known as flavonoids are vast classes of polyphenols that are mostly synthesized in plants in response to microbial infection (Murugan *et al*., 2013). Their wide structure of flavonoids is constituted of two phenyl rings joined together by a 3-carbonated heterocyclic ring (C6-C3-C6) and in total has a 15-C skeleton (Aboody & Mickymaray, 2020). Phytochemical analysis showed that all the three extracts of *D. abyssinica* (aqueous, methanol and dichloromethane/methanol (1:1) possessed flavonoids. According to several in vivo and clinical studies, flavonoids have a variety of pharmacological effects,

including anti-oxidant, antidiabetic, anti-obesity, anti-hyperlipidemic, anti-inflammatory, anti-osteoporotic effect, anti-allergic and antithrombotic, hepatoprotective, neuroprotective, reno-protective, chemo-preventive and anticarcinogenic, anti-bacterial, antifungal, and antiviral activities. When used against a variety of pathogenic organisms, including *C. albicans*, flavonoids have been demonstrated to be effective antifungal agents. Several different mechanisms, including disruption of the plasma membrane, the development of mitochondrial dysfunction, the inhibition of cell wall formation, cell division, RNA and protein synthesis, and the efflux-mediated pumping system, are used by flavonoids to suppress fungal growth (Aboody & Mickymaray, 2020). Therefore, this suggest that antifungal activity possessed by *D. abyssinica* aqueous, methanol and dichlomethane/methanol (1:1) extracts could be due to the presence of high amounts of flavonoids in the extracts.

Tannins were present in all the three extracts (aqueous, methanol and DCM/methanol (1:1). Tannins have been shown to possess antifungal activity against *C. albicans* (Monteiro & Santos, 2016). Tannins bind to proline-rich proteins and inhibit the synthesis of new proteins (Murugan *et al*., 2013). Terpenoids though not present in all the extracts, such as triterpenoids, diterpenoids, and sesquiterpenoids have been employed in the pharmaceutical sector as pesticides, antibiotics, and anthelmintics (Matara *et al*., 2021). Terpenoids have qualities that are anti-parasitic, anti-allergic, anti-inflammatory, immunomodulatory, anti- microbial, anti-viral, anti-fungal, and anti-hyperglycemic (Elgharbawy *et al*., 2020). Thymol and carvacrol are the two most important phytochemicals for figuring out how responsive terpenoids are. Thymol often induces morphological changes in the *C. albicans* envelope and inhibits the production and viability of hyphae. (Elgharbawy *et al*., 2020).

5.2 CONCLUSION

The following conclusions were made.

i. *Digitaria abyssinica* aqueous extract yielded the highest yield (7%) when compared to methanol and dichloromethane/methanol (1:1) 2.33% and 1.89% respectively.
ii. The aqueous, methanol and dichlomethane/methanol (1:1) *D. abyssinica* rhizome extracts show varying antifungal activity on *C. albicans*.
iii. The aqueous extract had the best antifungal activity against *C. albicans* compared to dichloromethane/methanol (1:1) and methanol.
iv. The aqueous, methanol and dichlomethane/methanol (1:1) *D. abyssinica* rhizome extracts did not exhibit antibacterial activity against *E. coli*, *N. gonorrhoeae* and *S. aureus*.
v. The *Digitaria abyssinica* rhizome aqueous, methanol and dichlomethane/methanol (1:1) extracts had an LD_{50} of greater than 2000mg/kg/bw (1:1) since no observable toxic effects were reported in experimental rats.

5. 3 RECOMMENDATIONS

From this study, the following recommendations were made:

i. Other solvents could be used, which could lead to improved yield and biological activity.
ii. Furthermore, investigation of the antimicrobial efficacy of the studied plant extracts on other microbial strains of clinical significance are encouraged.
iii. It is necessary to conduct additional research to determine the precise mode(s) by which the plant extracts under study exert their antifungal activity.
iv. Comprehensive research can be conducted to elucidate the structures of chemical constituents, such as flavonoids, poly-phenols, essential oils and polysaccharides from *D. abyssinica* using gas chromatography mass spectrometry (GC-MS), 1D and 2D nuclear magnetic resonance (NMR), and infrared spectrometry (IR).
v. Extensive sub-acute and chronic studies should be done on *D. abyssinica* rhizome extracts.
vi. Future research should also include quantitative phytochemical evaluation, isolation, characterization, and development of antimicrobial compounds from *D. abyssinica* rhizome extracts.

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APPENDICES
Appendix 1: NACOSTI Research permit

REPUBLIC OF KENYA

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Date of Issue: **17/June/2021**

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Appendix 2: Biosafety, Animal Use and Ethics Committee approval

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

P.O. Box 30197,
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REF: FVM BAUEC/2021/290

William Lemayan Sapunyo,
University of Nairobi
Dept. of PHP & Toxicology,
08/03/2021

Dear Sapunyo,

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

Anti-bacterial, Acute oral toxicity and Phytochemistry studies of Digitaria abyssinica.

William Lemayan J56/34157/2019

We refer to your MSc. proposal submitted to our committee for review and your application letter dated 1st March 2021. We have reviewed your application for ethical clearance for the study.

The animal husbandry, acute oral toxicity and anti-bacterial protocol and number of mice to be used in the study meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

We have also noted that KVB registered veterinary surgeons will supervise the study.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D

Chairperson, Biosafety, Animal Use and Ethics Committee,

Faculty of Veterinary Medicine,

University of Nairobi

Appendix 3: Data on antimicrobial activity analysis.

Raw data of the zones of inhibitions of the four human pathogens.

Staphylococcus. Aureus										
15.625mg/ml	31.25mg/ml	62.5mg/ml	125mg/ml	250mg/ml	500mg/ml		Ciprofloxacin (CIP)	Tetracycline (TE)	Cephalexin (CL)	
0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0		22,23	11,11	15,15	
0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	6,6 ; 6,6 ; 7,7	6,6 ; 6,6 ; 7,7	6,6 ; 7,7 ; 7,7		23,22	12,12	16,16	
0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	6,6 ; 6,7 ; 7,7	7,7; 7,7; 7,7		22,21	11,11	15,16	
Escherichia coli										
15.625mg/ml	31.25mg/ml	62.5mg/ml	125mg/ml	250mg/ml	500mg/ml		Ciprofloxacin	Azithromycin	Tetracycline	Ceftri
0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0		31,29	6,6	19,19	30,30
0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0		30,31	6,6	18,19	28,29
0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0		31,30	6,6	19,18	29,28
Candida albicans		Ketoconazole (KET)	Miconazole(MCZ)	Econazole (EC)	Nystatin (NY)	Clotrimazole (CTR)				
15.625mg/ml	31.25mg/ml	62.5mg/ml	125mg/ml	250mg/ml	500ml/ml		34,35	24,24	26,27	7,7
6,7; 7,6; 6,7	7,8; 8,9; 8,7	10,9; 9,10; 10,10	16,16; 15,15; 16,15	16,15; 16,16; 15,16	17,17; 16,17; 15,16		33,34	26,25	27,27	7,8
7,7; 7,7; 6,7	8,8; 8,9; 9,8	10;9; 8,8; 9;9	11,12; 12;12; 12,11	12,11; 12,12; 11,11	15,14; 16;15; 15,16		35,34	25,25	27,26	7,7
0,0 ; 0,0 ; 0,0	7,7; 7,6; 7,7	8,8; 8,9; 8,8	9,9; 10,10; 10,9	9,9;	11,10;					

				10,10; 9,10	11,10; 10,11					
Neisseria gonorrhoeae										
15.625mg/ml	31.25mg/ml	62.5mg/ml	125mg/ml	250mg/ml	500mg/ml		Azithromycin (AZM)	Ceftriaxone (CRO)		
0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0		22,21	29,30		
0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0		20,21	30,31		
0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0	0,0 ; 0,0 ; 0,0		21,20	31,30		

Zones of inhibition of *Candida albicans*

Concentration	AQ_CA	MEOH_CA	DCMM_CA
15.63	6	7	0
15.63	7	7	0
15.63	7	7	0
15.63	6	7	0
15.63	6	6	0
15.63	7	7	0
31.25	7	8	7
31.25	8	8	7
31.25	8	8	7
31.25	9	9	6
31.25	8	9	7
31.25	7	8	7
62.5	10	10	8
62.5	9	9	8
62.5	9	8	8
62.5	10	8	9
62.5	10	9	8
62.5	10	9	8
125	16	11	9
125	16	12	9
125	15	12	10
125	15	12	10
125	16	12	10
125	15	11	9
250	16	12	9
250	15	11	9
250	16	12	10
250	16	12	10
250	15	11	9
250	16	11	10
500	17	15	11
500	17	14	10
500	16	16	11
500	17	15	10
500	15	15	10
500	16	16	11
Clotrimazole	27		
Clotrimazole	26		
Clotrimazole	26		
Clotrimazole	27		
Clotrimazole	28		
Clotrimazole	28		
Econazole	26		
Econazole	27		
Econazole	27		

Econazole	27
Econazole	27
Econazole	26
Ketoconazole	34
Ketoconazole	35
Ketoconazole	33
Ketoconazole	34
Ketoconazole	35
Ketoconazole	34
Miconazole	24
Miconazole	24
Miconazole	26
Miconazole	25
Miconazole	25
Miconazole	25
Nystatin	7
Nystatin	7
Nystatin	7
Nystatin	8
Nystatin	7
Nystatin	7

Zones of inhibition of *Escherichia coli*

Concentration	AQ_EC	MEOH_EC	DCMM_EC
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
125	0	0	0
125	0	0	0
125	0	0	0
125	0	0	0

125	0	0	0
125	0	0	0
250	0	0	0
250	0	0	0
250	0	0	0
250	0	0	0
250	0	0	0
250	0	0	0
500	0	0	0
500	0	0	0
500	0	0	0
500	0	0	0
500	0	0	0
500	0	0	0

Azithromycin	6
Azithromycin	6
Azithromycin	6
Azithromycin	6
Azithromycin	6
Azithromycin	6
Ceftriaxone	30
Ceftriaxone	30
Ceftriaxone	28
Ceftriaxone	29
Ceftriaxone	29
Ceftriaxone	28
Cephalexin	12
Cephalexin	13
Cephalexin	12
Cephalexin	12
Cephalexin	13
Cephalexin	12
Ciprofloxacin	31
Ciprofloxacin	29
Ciprofloxacin	30
Ciprofloxacin	31
Ciprofloxacin	31
Ciprofloxacin	30
Tetracycline	19
Tetracycline	19
Tetracycline	18
Tetracycline	19
Tetracycline	19
Tetracycline	18

Zones of inhibition of *Neisseria gonorrheae*

Concentration	AQ_NG	MEOH_NG	DCMM_NG
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
125	0	0	0
125	0	0	0
125	0	0	0
125	0	0	0
125	0	0	0
125	0	0	0
250	0	0	0
250	0	0	0
250	0	0	0
250	0	0	0
250	0	0	0
250	0	0	0
500	0	0	0
500	0	0	0
500	0	0	0
500	0	0	0
500	0	0	0
500	0	0	0
Azithromycin	22		
Azithromycin	21		
Azithromycin	20		
Azithromycin	21		
Azithromycin	21		
Ceftriaxone	20		
Ceftriaxone	29		
Ceftriaxone	30		
Ceftriaxone	30		

Ceftriaxone	31
Ceftriaxone	31

Zones of inhibition of *Staphylococcus aureus*

Concentration	AQ_SA	MEOH_SA	DCMM_SA
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
15.63	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
31.25	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
62.5	0	0	0
125	0	6	0
125	0	6	0
125	0	6	0
125	0	6	0
125	0	7	0
125	0	7	0
250	0	6	6
250	0	6	6
250	0	6	6
250	0	6	7
250	0	7	7
250	0	7	7
500	0	6	7
500	0	6	7
500	0	7	7
500	0	7	7
500	0	7	7
500	0	7	7
Cephalexin	15		
Cephalexin	15		
Cephalexin	16		
Cephalexin	16		
Cephalexin	15		
Cephalexin	16		
Ciprofloxacin	22		

Ciprofloxacin	23
Ciprofloxacin	23
Ciprofloxacin	22
Ciprofloxacin	22
Ciprofloxacin	21
Tetracycline	11
Tetracycline	11
Tetracycline	12
Tetracycline	12
Tetracycline	11
Tetracycline	11

Appendix 4: Data on acute oral toxicity testing

Raw data from acute oral toxicity testing

AQEOUS EXTRACT																		
RAT	**WEIGHT**	**30 MINUTES**	**1 HOUR**	**4 HOURS**	**24 HOURS**	**48 HOURS**	**3 DAYS**	**4 DAYS**	**5 DAYS**	**6 DAYS**	**7 DAYS**	**8 DAYS**	**9 DAYS**	**10 DAYS**	**11 DAYS**	**12 DAYS**	**13 DAYS**	**14 DAYS**
1	171.86	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 201.93g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 201.81g
2	172.97	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 203.39g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 209.15g
3	165.75	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 196.47g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 201.68g
4	170.19	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 203.93g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 207.20g
5	160.92	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 195.58g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 211.93g

METHANOL EXTRACT																		
RAT	**WEIGHT**	**30 MINUTES**	**1 HOUR**	**4 HOURS**	**24 HOURS**	**48 HOURS**	**3 DAYS**	**4 DAYS**	**5 DAYS**	**6 DAYS**	**7 DAYS**	**8 DAYS**	**9 DAYS**	**10 DAYS**	**11 DAYS**	**12 DAYS**	**13 DAYS**	**14 DAYS**
1	142.37	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 179.27g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 194.48g
2	140.82	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 183.54g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 197.71g
3	138.02	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 164.93g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 173.68g
4	138.75	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 173.06g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 190.75g
5	133.99	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 167.72g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 175.85g

DCM/METHANOL (50:50) EXTRACT																		
RAT	**WEIGHT**	**30 MINUTES**	**1 HOUR**	**4 HOURS**	**24 HOURS**	**48 HOURS**	**3 DAYS**	**4 DAYS**	**5 DAYS**	**6 DAYS**	**7 DAYS**	**8 DAYS**	**9 DAYS**	**10 DAYS**	**11 DAYS**	**12 DAYS**	**13 DAYS**	**14 DAYS**
1	126.39	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 166.01g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 190.22g
2	129.02	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 140.47g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 160.02g
3	114.51	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 130.57g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 147.13g
4	87.43	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 121.21g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 140.84g
5	83.82	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 113.44g	NOO	NOO	NOO	NOO	NOO	NOO	NOO. WEIGHT: 127.38g

KEY: NOO-No observable change

COMMENTS:
No mortality
Normal weight gain
No observable lethal effects

Week 1 weight gain data analysis output

GenStat Release 15.1 (PC/Unknown) 05 August 2021 13:16:56

Registered to: ICRISAT

GenStat Fifteenth Edition
GenStat Procedure Library Release PL23.1

```
  1  SET [WORKINGDIRECTORY='C:/Users/O/Documents']
  2  "Data taken from file: 'C:/Users/O/Desktop/Dr_William_PHPT_1.xlsx'"
  3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
  4  READ [PRINT=*; SETNVALUES=yes] _stitle_
  8  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Excel file: C:\Users\O\Desktop\Dr_William_PHPT_1.xlsx
on: 5-Aug-2021 13:19:25
taken from sheet "WG_wk1", cells A2:B16

```
  9  DELETE [REDEFINE=yes] Treatment_week_1,Weight_gain
 10  UNITS [NVALUES=*]
 11  FACTOR [MODIFY=no; NVALUES=15; LEVELS=3; LABELS=!t('AQ','DCM_MEOH','MEOH')\
 12  ; REFERENCE=1] Treatment_week_1
 13  READ Treatment_week_1; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment_week_1	15	0	3

```
 15  VARIATE [NVALUES=15] Weight_gain
 16  READ Weight_gain
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Weight_gain	11.45	30.98	42.72	15	0

```
 19
 20  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
 21  "One-way design"
 22  DELETE [REDEFINE=yes] _ibalance
 23  A2WAY [PRINT=aovtable,information,effects,means; TREATMENTS=Treatment_week_1;
FPROB=yes;\
 24   PSE=diff; PLOT=*; EXIT=_ibalance] Weight_gain; SAVE=_a2save
```

Analysis of variance

Variate: Weight_gain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment_week_1	2	200.60	100.30	1.68	0.228
Residual	12	718.23	59.85		
Total	14	918.83			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

units 12 -14.7 s.e. 6.9

Tables of effects

Variate: Weight_gain

Treatment_week_1 effects, e.s.e. 3.46, rep. 5

Treatment_week_1	AQ	DCM_MEOH	MEOH
	0.9	-4.9	3.9

Tables of means

Variate: Weight_gain

Grand mean 31.0

Treatment_week_1	AQ	DCM_MEOH	MEOH
	31.9	26.1	34.9

Standard errors of differences of means

Table	Treatment_week_1
rep.	5
d.f.	12
s.e.d.	4.89

```
  25  IF _ibalance.eq.0 .OR. _ibalance.eq.1
  26    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
  27    AKEEP [SAVE=_a2save[2]] Treatment_week_1; MEAN=_mean; REP=_rep;
VARIANCE=_var; RTERM=_resid
  28    AKEEP [SAVE=_a2save[2]] #_resid; DF=_rdf
  29    AMCOMPARISON [PRINT=comparison,description,letter; METHOD=tukey;
DIRECTION=ascending;\
  30   PROB=0.05; SAVE=_a2save[2]] Treatment_week_1
```

Tukey's 95% confidence intervals

Treatment_week_1

Comparison	Difference	Lower 95%	Upper 95%	Significant
DCM_MEOH vs AQ	-5.816	-18.87	7.238	no
DCM_MEOH vs MEOH	-8.808	-21.86	4.246	no
AQ vs MEOH	-2.992	-16.05	10.062	no

	Mean	
DCM_MEOH	26.11	a
AQ	31.92	a
MEOH	34.91	a

```
  31  ENDIF
  32  SET [IN=*]
  38  A2DISPLAY [PRINT=aovtable,information,effects,mean; FPROB=yes; PSE=diff]
_a2save
```

Analysis of variance

Variate: Weight_gain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment_week_1	2	200.60	100.30	1.68	0.228
Residual	12	718.23	59.85		
Total	14	918.83			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

units 12 -14.7 s.e. 6.9

Tables of effects

Variate: Weight_gain

Treatment_week_1 effects, e.s.e. 3.46, rep. 5

Treatment_week_1	AQ	DCM_MEOH	MEOH
	0.9	-4.9	3.9

Tables of means

Variate: Weight_gain

Grand mean 31.0

Treatment_week_1	AQ	DCM_MEOH	MEOH
	31.9	26.1	34.9

Standard errors of differences of means

Table	Treatment_week_1
rep.	5
d.f.	12
s.e.d.	4.89

```
  39    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
  40    AKEEP [SAVE=_a2save[2]] Treatment_week_1; MEAN=_mean; REP=_rep;
VARIANCE=_var; RTERM=_resid
  41    AKEEP [SAVE=_a2save[2]] #_resid; DF=_rdf
  42    AMCOMPARISON [PRINT=comparison,description,letter; METHOD=tukey;
DIRECTION=ascending;\
  43   PROB=0.05; SAVE=_a2save[2]] Treatment_week_1
```

Tukey's 95% confidence intervals

Treatment_week_1

Comparison	Difference	Lower 95%	Upper 95%	Significant
DCM_MEOH vs AQ	-5.816	-18.87	7.238	no
DCM_MEOH vs MEOH	-8.808	-21.86	4.246	no
AQ vs MEOH	-2.992	-16.05	10.062	no

	Mean	
DCM_MEOH	26.11	a
AQ	31.92	a
MEOH	34.91	a

```
  44  DESCRIBE [SELECTION=nval,mean,median,range,sd,sem; GROUPS=Treatment_week_1]
Weight_gain
```

Summary statistics for Weight_gain: Treatment_week_1 AQ

Number of values =	5
Mean =	31.92
Median =	30.72
Range =	4.59
Standard deviation =	2.117
Standard error of mean =	0.947

Summary statistics for Weight_gain: Treatment_week_1 DCM_MEOH

Number of values =	5
Mean =	26.11
Median =	29.62
Range =	28.17
Standard deviation =	11.93
Standard error of mean =	5.337

Summary statistics for Weight_gain: Treatment_week_1 MEOH

Number of values =	5

Mean =	34.91
Median =	34.31
Range =	15.81
Standard deviation =	5.716
Standard error of mean =	2.556

GenStat Fifteenth Edition
GenStat Procedure Library Release PL23.1

```
 1  SET [WORKINGDIRECTORY='C:/Users/O/Documents']
 2  "Data taken from file: 'C:/Users/O/Desktop/Dr_William_PHPT_1.xlsx'"
 3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
 4  READ [PRINT=*; SETNVALUES=yes] _stitle_
 8  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Excel file: C:\Users\O\Desktop\Dr_William_PHPT_1.xlsx
on: 5-Aug-2021 13:28:23
taken from sheet "Week 2_raw data", cells A2:D16

```
 9  DELETE [REDEFINE=yes] Treatment_week_2,Weight_day_0,Weight_day_7,Weight_gain
10  UNITS [NVALUES=*]
11  FACTOR [MODIFY=no; NVALUES=15; LEVELS=3; LABELS=!t('AQ','DCM_MEOH','MEOH')\
12  ; REFERENCE=1] Treatment_week_2
13  READ Treatment_week_2; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment_week_2	15	0	3

```
15  VARIATE [NVALUES=15] Weight_day_0
16  READ Weight_day_0
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Weight_day_0	83.82	138.5	173.0	15	0

```
19  VARIATE [NVALUES=15] Weight_day_7
20  READ Weight_day_7
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Weight_day_7	127.4	182.0	211.9	15	0

```
23  VARIATE [NVALUES=15] Weight_gain
24  READ Weight_gain
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Weight_gain	29.95	43.53	63.83	15	0

```
27
28  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
29  "Data taken from file: 'C:/Users/O/Desktop/Dr_William_PHPT_1.xlsx'"
30  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
31  READ [PRINT=*; SETNVALUES=yes] _stitle_
35  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Excel file: C:\Users\O\Desktop\Dr_William_PHPT_1.xlsx
on: 5-Aug-2021 13:28:53

taken from sheet "WG_wk2", cells A2:B16

```
  36  UNITS [NVALUES=*]
  37  FACTOR [MODIFY=yes; NVALUES=15; LEVELS=3; LABELS=!t('AQ','DCM_MEOH','MEOH')\
  38  ; REFERENCE=1] Treatment_week_2
  39  READ Treatment_week_2; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment_week_2	15	0	3

```
  41  VARIATE [NVALUES=15] Weight_gain
  42  READ Weight_gain
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Weight_gain	29.95	43.53	63.83	15	0

```
  45
  46  %PostMessage 1129; 0; 100001 "Sheet Update Completed"
  47  "One-way design"
  48  DELETE [REDEFINE=yes] _ibalance
  49  A2WAY [PRINT=aovtable,information,effects,means; TREATMENTS=Treatment_week_2;
FPROB=yes;\
  50   PSE=diff; PLOT=*; EXIT=_ibalance] Weight_gain; SAVE=_a2save
```

Analysis of variance

Variate: Weight_gain

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment_week_2	2	248.3	124.1	1.13	0.356
Residual	12	1320.7	110.1		
Total	14	1569.0			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

units 11 18.9 s.e. 9.4

Tables of effects

Variate: Weight_gain

Treatment_week_2 effects, e.s.e. 4.69, rep. 5

Treatment_week_2	AQ	DCM_MEOH	MEOH
	-5.5	1.3	4.2

Tables of means

Variate: Weight_gain

Grand mean 43.5

Treatment_week_2	AQ	DCM_MEOH	MEOH
	38.0	44.9	47.7

Standard errors of differences of means

Table	Treatment_week_2
rep.	5
d.f.	12
s.e.d.	6.63

```
  51  IF _ibalance.eq.0 .OR. _ibalance.eq.1
  52    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
  53    AKEEP [SAVE=_a2save[2]] Treatment_week_2; MEAN=_mean; REP=_rep;
VARIANCE=_var; RTERM=_resid
  54    AKEEP [SAVE=_a2save[2]] #_resid; DF=_rdf
  55    AMCOMPARISON [PRINT=comparison,description,letter; METHOD=tukey;
DIRECTION=ascending;\
  56   PROB=0.05; SAVE=_a2save[2]] Treatment_week_2
```

Tukey's 95% confidence intervals

Treatment_week_2

Comparison	Difference	Lower 95%	Upper 95%	Significant
AQ vs DCM_MEOH	-6.868	-24.57	10.83	no
AQ vs MEOH	-9.688	-27.39	8.01	no
DCM_MEOH vs MEOH	-2.820	-20.52	14.88	no

	Mean	
AQ	38.02	a
DCM_MEOH	44.88	a
MEOH	47.70	a

```
  57  ENDIF
  58  SET [IN=*]
  64  A2DISPLAY [PRINT=aovtable,information,effects,mean; FPROB=yes; PSE=diff]
_a2save
```

Summary statistics for Weight_gain: Treatment_week_2 AQ

Number of values =	5
Mean =	38.02
Median =	36.18
Range =	21.06
Standard deviation =	7.788
Standard error of mean =	3.483
Variance =	60.66

Summary statistics for Weight_gain: Treatment_week_2 DCM_MEOH

Number of values =	5
Mean =	44.88
Median =	43.56
Range =	32.83
Standard deviation =	13.93
Standard error of mean =	6.231
Variance =	194.1

Summary statistics for Weight_gain: Treatment_week_2 MEOH

Number of values =	5
Mean =	47.70
Median =	52
Range =	21.23
Standard deviation =	8.681
Standard error of mean =	3.882
Variance =	75.37

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2023, Article ID 4552095, 11 pages https://doi.org/10.1155/2023/4552095

Research Article

Phytochemical Screening, Toxic Effects, and Antimicrobial Activity Studies of *Digitariaabyssinica* (Hochst. ex A.Rich.) Stapf (Poaceae) Rhizome Extracts against Selected Uropathogenic Microorganisms

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Received 11 September 2022; Revised 22 December 2022; Accepted 29 December 2022; Published 5 January 2023

Academic Editor: Daniela Rigano

In Kenya, the *D. abyssinica* rhizome's decoction is traditionally used to treat urinary tract infections (UTIs), mainly gonorrhea and candidiasis. UTIs are the most severe public health problems that affect over one hundred and fifty million people worldwide annually. They are caused by a wide range of microorganisms where *Escherichia coli* is known to be the main causative pathogen. Medicinal plants are used in traditional Kenya set up for treatment and most recently as an alternative source of treatment for UTIs due to the increased cost of treatment and many challenges experienced with antibiotic therapy. The current study is designed to investigate the phytochemical composition, acute oral toxicity, and antimicrobial activity of *Digitaria abyssinica* rhizome extracts against *Staphylococcus aureus*, *Escherichia coli*, *Neisseria gonorrhea*, and *Candida albicans*. The rhizomes of *D. abyssinica* were obtained, dried, ground, and extracted using water and organic solvents. The phytochemical assay was carried out using standard phytochemical screening methods. Single-dose toxicity studies were done to determine LD_{50} while disk diffusion and microbroth dilution techniques were used to determine antimicrobial activity. Results revealed that saponins, phenolics, alkaloids, cardiac glycosides, tannins, flavonoids, steroids, and terpenes were present in the powder, aqueous, methanol, and dichloromethane : methanol extracts. All the extracts had an LD_{50} of above 2,000 mg/kg of body weight and there was no observation of behavioral changes. Also, the aqueous and methanol extracts revealed antifungal activity against *Candida albicans* with the lowest average minimum zone of inhibition at MIC of 31.25 mg/ml. The study did not reveal antibacterial activity for any extract against the studied uropathogenic bacteria, *Staphylococcus aureus*, *Escherichia coli*, and *Neisseria gonorrhoeae.* The results from the current study suggested that *D. abyssinica* rhizome aqueous and methanol extracts have potential antifungal activity against *C. albicans*, thus validating the folklore of its use to treat candidiasis.

1. Introduction

Urinary tract infections (UTIs) are termed severe public health problems and affect over one hundred and fifty million people worldwide per year [1]. UTIs are known to affect more than half of women at least once in their lives and reinfection is reported to be more recurrent in

young women [2]. Urinogenital infections are caused by bacteria and fungi, the most causative agent is *Escherichia coli* usually called uropathogenic *Escherichia coli* (UPEC) [3]. Other pathogenic microorganisms are *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Staphylococcus saprophyticus*, *Staphylococcus aureus*, and *Candida* species [4]. The most recommended antibiotics for the treatment of UTIs are trimethoprim, sulfamethoxazole, ciprofloxacin, and ampicillin. Nevertheless, increasing rates of antibiotic resistance and high recurrent rates and the spread of multidrug resistance (MDR) threaten to greatly enhance the burden that these common infections place on society [5]. There is a continuous effort toward the development of alternative therapies that can be used to manage drug resistance [3, 6]. A large population of over 80% in lowincome countries uses medicinal plants as an alternative source of antibiotics and other conditions for primary health care [7–9]. The World Health Organization advocates the use of medicinal and aromatic plants as an alternative source of drugs that can be potentially effective in the treatment of uropathogens [10, 11]. Some plants have demonstrated antimicrobial activities [8, 10–14].

Scientific literature has been published about the plant extracts of the Poaceae family that have chemicals with antimicrobial activity [15–18]. Some of the Poaceae plants that have demonstrated *in vitro* antibacterial activities are *Cynodon dactylon*, *Cymbopogon citratus*, *Triticum aestivum*, *Bambusa vulgaris*, *Dichanthium annulatum*, *Dactyloctenium aegyptium*, *Imperata cylindrica*, *Eleusine indica*, *Saccharum spontaneum*, and *Vetiveria zizanioides* [19–21]. In addition, *Panicum maximum* and *Cymbopogon citratus* have that they comprise secondary metabolites that are active against fungal strains [22, 23]. The activity of Zea mays silk extracts is also known for the treatment of uropathogenic microorganisms [24]. The current study focuses on *D. abyssinica* (Poaceae) referred to as East African couch grass [25]. It is a common weed that is used as food for livestock characterized by low nutritional value [26, 27]. Different parts of the plant are used traditionally to treat flu and diarrhea [28], liver diseases [29], hernia [30], malaria, yellow fever, and wound healing [28, 31]. In Kenya, the *D. abyssinica* rhizome's decoction has been traditionally used to treat urinary tract infections, mainly gonorrhea and candidiasis [32]. There is limited scientific evidence on the biological activities of *D. abyssinica*. Based on the ethnomedicinal claims of *D. abyssinica*, the current study seeks to evaluate the safety, phytochemistry, and antimicrobial activity of *D. abyssinica* rhizome extracts against the selected uropathogenic microorganisms, *Staphylococcus aureus*, *Escherichia coli*, *Neisseria gonorrhea*, and *Candida albicans*. The study aims to validate the ethnomedicinal claims of using the extracts of *D.* abyssinica rhizome by traditional medicine practitioners and herbalists.

2. Materials and Methods

2.1. Chemicals, Reagents, and Drugs. The chemicals for extraction and preparation of extractions, absolute ethanol (AR), absolute methanol (AR), and dimethyl sulfoxide (DMSO) were from Loba Chemie Pvt., India. Mueller Hinton agar, Mueller Hinton broth, and GC agar media were supplied by HiMedia Laboratories Pvt. Ltd, India. While VCNT and vitox were from Oxoid Limited. Antibacterial sensitivity

disks ciprofloxacin, cephalexin, azithromycin, tetracycline, and ceftriaxone sensitivity disks (Liofilchem) and antifungal sensitivity disks: clotrimazole, econazole, ketoconazole, miconazole, and nystatin (Sanofi Diagnostics Pasteur) were used in this study. The chemicals and reagents were of analytical grade.

2.2. Collection of Plant Materials. The fresh rhizomes of *D. abyssinica* were collected from Mua, Kyaani, in Machakos county in January 2021 with the help of Mr. Musembi Kimeu, a plant taxonomist at the University of Nairobi, Land

Resource Management and Agricultural Technology (LARMAT). The plant materials were transported to the Department of Public Health, Pharmacology, and Toxicology (PHPT), University of Nairobi and were identified and authenticated by Ms. Carol Kyalo of the University of Nairobi, Land Resource Management and Agricultural Technology (LARMAT). A voucher specimen number LARMAT/Herb/Da was assigned and deposited in the LARMAT herbarium for ease of reference.

2.3. Extraction of the Plant Material. The fresh rhizome of *D. abyssinica* was cleaned with clean water, then air-dried in a well-ventilated, insect- and rodent-free at room temperature. The rhizomes were pulverized into powder by use of an electric mill. The resulting powder was kept in a welllabeled manilla sack and kept in a cool and nonhumid place awaiting extraction.

Aqueous extraction was prepared by cold maceration by soaking 300 g of ground powder in 2.5 liters of distilled water. The extraction mixture was stirred continuously and allowed to macerate for 48 hours and then filtered through a Whatman No. 1 filter paper. The resultant filtrate was then lyophilized using a freeze dryer. The obtained freeze-dried product was weighed and stored in airtight plastic vials at 4°C in a refrigerator awaiting further analysis.

The methanolic extract was prepared by measuring 200 g of *D. abyssinica* rhizome extract into an extraction jar, adding 1 liter of analytical methanol gradually and then shaking vigorously for 48 hours to macerate. The process was repeated for another batch of 200 g of rhizome powder. The resultant mixture was then decanted and filtered through cotton gauze to remove course residues. The resultant filtrate was then filtered through a Whatman No. 1 filter paper. The resultant filtrate was then combined and reduced in a vacuum at 50°C using a rotary evaporator. To further remove the solvent and concentrate the extract, the extract was placed in a clean, dry, and light-resistant bottle and placed in a sand bath set at 35°C. Finally, it was weighed using an analytical balance, and the percentage yield of the extract was calculated and stored at 4°C in a refrigerator awaiting further analysis.

The dichloromethane : methanol (1 :1) extract was prepared by measuring 200 g of rhizome powder into an extraction jar. An equal amount of dichloromethane and methanol was mixed to make a solution of 1 liter. The mixture was then gradually added to the powder. The mixture was shaken vigorously for 48 hours and allowed to macerate. The abovementioned process was then repeated for another batch of 200 g of the rhizome powder. The resultant mixture was then decanted and then filtered through gauze. The resultant mixture was then filtered through a Whatman No.1 filter paper. The resultant mixture from the first and the second batch was combined and reduced in a vacuum (in vacuo) at 50°C using

a rotary evaporator. To further remove the solvent and concentrate the extract, the extract was placed in a clean, dry, and light-resistant bottle and placed in a sand bath set at 35°C. Finally, it was weighed using an analytical balance and stored at 4°C in a refrigerator until use.

2.4. Experimental Animals. Fifteen 8–10-week, female *Wistar* albino rats weighing 125 ± 45 grams were used to assess the acute oral toxicity of *Digitaria abyssinica* rhizome extracts. These animals were purchased from the Kabete Vet Lab animal house. They were transported to the Public Health, Pharmacology, and Toxicology (PHPT) Department animal house where they were housed for 5 days to acclimatize following Biosafety, Animal Use and Ethical Committee (BAUEC) guidelines. All the experimental animals were nulliparous and nonpregnant. They were housed at a temperature of 25 ± 3°C and 56–60% relative humidity. A 12-hour day and night cycle were maintained, and the animals were fed on standard rat pellets from a commercial feed supplier (Unga Feeds). Water was provided ad libitum.

2.5. Phytochemical Screening. The analysis of the phytochemical groups of compounds, namely, plant flavonoids, tannins, saponins, phenols, coumarins, steroids, terpenoids, glycosides and alkaloids of water, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizomes were done using standard phytochemical screening procedures described by [33, 34] as modified by [35]. The tests were performed in triplicates to ensure the results' accuracy and were examined by visual observations.

2.5.1. Test for Saponins (Froth Test). About 0.1 g of the water, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizome were added to 10 ml of distilled water in separate test tubes, respectively. The mixtures were boiled for 10 minutes, and they were filtered using Whatman filter paper No.1. A mixture of 3 ml distilled water and 5 ml of the filtrate was agitated vigorously for 15 seconds and left to stand for 10 minutes. Frothing which persisted for about 3 minutes was an indication of saponins [36].

2.5.2. Test for Alkaloids. Two tests, namely, Mayer's and Dragendorff's tests, were done to detect alkaloids in the extracts.

(1) *Mayer's Test*. Approximately, 0.1 g of water, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizome were mixed with 5 ml of 1% HCl in separate test tubes, respectively; each mixture was warmed and then filtered through Whatman filter paper No.1. Two drops of Mayer's reagent (mercuric potassium iodide) were added to 2 ml of water, methanol, and dichloromethane : methanol (1 :1) extracts. The appearance of a cream-colored precipitate indicates the presence of alkaloids [37, 38].

(2) *Dragendorff's Test*. The test was carried out by adding two drops of Dragendorff's reagent (potassium bismuth iodide solution) to 2 ml of the filtered water, methanol, and dichloromethane : methanol (1 :1) extracts in separate test tubes. A reddish-brown precipitate indicates the presence of alkaloids [37, 38].

2.5.3. Test for Flavonoids (Sodium Hydroxide Reagent Test). Approximately, 0.1 g of the water, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizome were warmed in 10 ml of 70% ethanol and thereafter hydrolyzed with 10% hydrochloric acid. Sodium hydroxide

(10%; 1 ml) was added to the mixture and the appearance of yellow color was a positive test for the presence of flavonoids [39, 40].

2.5.4. Test for Phenolics. Approximately, 0.1 g of the water, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizome were measured and put into separate test tubes and 10 ml of 70% ethanol were added. The mixtures were boiled using water for five minutes. The extracts were then cooled, and they were filtered through Whatman filter paper No.1. Five drops of 5% of ferric chloride were added into 2 ml of each respective extract. The formation of a green precipitate indicates the presence of phenols [36].

2.5.5. Test for Glycosides. (1) *Keller–Killiani Test*. Glacial acetic acid (4.0 ml) solution with 1 drop of 2.0% $FeCl_3$ mixture was added to the 10 ml water, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizome in separate test tubes. One milliliter of concentrated sulphuric acid was added to the mixture and a reddishbrown ring formed between the layers which progressively turned blue indicating the presence of steroidal glycosides with deoxy sugars [36].

(2) *Kedde Test*. One milliliter of 2% solution of 3,5dinitrobenzoic acid in 95% alcohol was added to the 2 ml of the water, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizome. The solution was made alkaline with 5% sodium hydroxide. The appearance of a purple-blue color indicates the presence of an unsaturated lactone ring in cardenolides [36].

2.5.6. Test for Steroids. (1) *Salkowski's Test*. Approximately, 2 mg of the water, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizome were dissolved in 1 ml of chloroform and then shaken gently. Five drops of concentrated sulphuric acid were added along the side of the test tube. A reddish-brown color that was formed at the interface indicated steroids [39, 41].

(2) *Liebermann–Burchard Test*. About 2 mL of acetic acid was added to 1 mL of the water, methanol, and dichloromethane : methanol (1 :1) extracts. After cooling the solution in an ice bath, concentrated sulphuric was added carefully. The development of violet to blue or bluish-green color confirms the test for steroids [42, 43].

2.5.7. Test for Terpenoids (Salkowski's Test). Approximately, 2 mg of the water, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizome were dissolved in 2 mL chloroform along with concentrated sulphuric acid. The red-brown color at the interface indicates terpenoids [37, 42].

2.5.8. Test for Coumarins. Approximately, 0.5 g of the extracts and powder of *D. abyssinica* were added into separate test tubes. The test tubes were covered with filter paper which was moistened with 1 N NaOH. The tubes were warmed in a hot water bath and then allowed to cool. Yellow fluorescent color was an indication of coumarins [44].

2.5.9. Test for Tannins. About 0.5 g of the sample was boiled in 20 ml of water and filtered. 0.1% of ferric chloride was added to the filtrate. The formation of a brownish-green or blue-black color was an indication of the presence of tannins [44].

2. 6.Single-DoseToxicityStudy. The up-and-down procedure for acute oral toxicity described by the Organization for Economic Cooperation and Development (OECD), [45] Document No. 425 was used to determine the safety of the aqueous, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizome. Five female Wistar rats were used to perform the limit tests for each of the aqueous extracts. A dose of 2000 mg/kg body weight of each extract was given orally to one female rat, and fatalities were not observed; thereafter, four additional animals were dosed sequentially. In addition, the rats were observed for wellness parameters that included the skin and fur appearance, fecal matter consistency, urination and urine appearance, itching, salivation, convulsions, tremors, breathing, coma somatomotor activity, aggression, grooming, eyes, and unconsciousness or death. The obsevations were made at time intervals of 30 minutes, 4 hours, 24 hours, 48 hours, 7 days, and 14 days. The procedure was repeated to evaluate the methanol and dichloromethane : methanol (1 :1) extracts too.

2.7. Antimicrobial Studies

2.7.1. Test Microorganisms. A fungal microorganism and 3 bacterial strains were obtained from the stock cultures from the University of Nairobi, Medical Microbiology Department Laboratories. The standard bacterial strains of *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and clinical isolates of *Neisseria gonorrhea* and *Candida albicans* were used in this study.

2.7.2. Preparation of Inoculums. To prepare the stock cultures, CLSI guidelines and procedures were used. The method as described by [46] was used with modifications. In brief, bacterial stock cultures were subcultured on respective media and incubated at 37°C for 24 hours. To obtain young growing culture, Neisseria gonorrhea isolates were cutured on Thayer Martin agar while isolates of *Escherichia coli* and *Staphylococcus aureus* were cultured on MacConkey and sheep blood agar, respectively. *Candida albicans* were subcultured in Sabouraud Dextrose Agar (SDA) at 37°C for 48 hours. The test strains were suspended in Mueller–Hinton broth (MHB) to give a final density of 1.5×10^6 bacteria colony-forming units and 1.5×10^5 fungal colonyforming units.

2.7.3. Preparation of the Stock Solutions. Six different concentrations of 15.625 mg/ml, 31.25 mg/ml, 62.50 mg/ ml, 125 mg/ml, 250 mg/ml, and 500 mg/ml of each of the three extracts (aqueous, methanol, and dichloromethane : methanol (1 :1) were prepared for susceptibility testing using 1% DMSO as a diluent. A vortex mixer was used to facilitate the dissolving of extracts into the 1% DMSO. A constant volume of 20 μl of each of the individual stock solutions was pipetted using a micro titer-pipette onto sterile filter paper disks measuring 6 mm to prepare the respective concentrations of the plant extracts. 20 μl of 1% DMSO was impregnated onto sterile filter paper disks which were used as the negative control for the experiment.

2.7.4. Disk Diffusion Method. Antimicrobial activity was evaluated using the disk diffusion

Table 1: Phytochemical composition of *D. abyssinica* rhizome powder and extracts.

Phytochemicals	Method/reagent	Powder	Aqueous	Methanol	Dichloromethane : methanol (1 :1)
Saponins	Foam test	+	–	+	+
Phenolics	Ferric chloride	+	+	+	+
Alkaloids	Mayer's	–	–	–	–
	Drangendorff's	+	+	+	+
Cardiac glycosides	Keller–Killiani	+	+	+	+
	Keddie	+	+	+	+
Tannins	Ferric chloride	+	+	+	+
Coumarins	Ferric chloride	+	+	+	+
Flavonoids	NaOH	+	+	+	+
Steroids	Liebermann–Burchard	+	+	+	+
Terpenoids	Salkowski	+	+	–+	+

Key: (+)—present; (−)—absent.

method as described by [47] with modifications. Appropriate agar plates were inoculated with respective isolates of the test microorganism. Sterile filter paper disks (6 mm in diameter) containing each of the 3 extracts at the desired concentration of (15.625, 31.25, 62.50, 125, 250, and 500) mg/ml were placed on the surface of the agar, using sterile forceps. 1% dimethyl sulfoxide (DMSO) was used as the negative control. Cephalexin (30 μg), ciprofloxacin (5 μg), and tetracycline (30 μg) sensitivity disks were used as a control against *Staphylococcus aureus*; cephalexin (30 μg), ciprofloxacin (5 μg), and tetracycline (30 μg) sensitivity disks were used as a control against *Escherichia Coli*, cephalexin (30 μg), and azithromycin (15 μg) sensitivity disks control susceptibility of *Neisseria gonorrhoeae* while clotrimazole (50 μg), econazole (50 μg), ketoconazole (50 μg), miconazole (50 μg), and nystatin (100 μg) disks were used as control of the extract against *Candida albicans.*

The Mueller–Hinton agar plates inoculated with *S. aureus* and those inoculated with *E. coli* were incubated at 37°C for 24 hours. Thayer Martin Media inoculated with *Neisseria gonorrhoeae* was incubated at 5% CO_2 for 48 hours while Sabouraud Dextrose Agar inoculated with *Candida albicans* was incubated at 37°C for 24 hours. Generally, the antimicrobial agent diffuses into the agar and inhibits the germination and growth of the test microorganism.

After the incubation period, the diameters of the inhibition zones were measured in millimeters using a transparent ruler. All the tests were done in triplicates and the means were calculated as the results.

2.7.5. Broth Macrodilution Technique. The broth macrodilution procedure as described in 2021 by Mailu et al. [46] with modification was used to determine the minimum inhibitory concentration for the active crude extracts against the test microorganisms. Six culture tubes with 2 ml sterile Mueller–Hilton broth were prepared. From the stock solution, two-fold serial dilutions were prepared. 0.1 ml of each microorganism was inoculated into each tube of diluted plant extract using a micropipette. The bacterial organisms and the

fungal organism were then incubated for 24 hours at 37°C. The extract's minimum inhibitory concentration (MIC) value was determined by observing the lowest concentration of plant extracts that prevented the visible growth of microorganisms resulting in no visible growth (turbidity).

To determine MBC, all broth in tubes with no visible bacterial growth was aseptically cultivated in sterile agar using the streak-plate method and incubated at appropriate temperatures and conditions. The MIC value is the lowest concentration of the plant extract that demonstrates no visible bacterial growth. All tubes with no visible fungal growth were aseptically cultured in sterile molten agar and incubated using the streak-plate method to determine the minimum bactericidal concentration (MBC). The minimal fungicidal concentration (MFC) value was defined as the lowest plant extract concentration that shows no visible fungal growth. Tubes that were just inoculated with microorganisms and tubes that were only inoculated with media served as controls. All the experiments were carried out in triplicate, and the results were recorded in a table.

2.8. Statistical Analysis. All experiments were performed in triplicates. Data were analyzed by GraphPad Prism version 9.0.0 and the results are provided as mean ± SEM. One-way analysis of variance (ANOVA) and post hoc ANOVA using Tukey's HSD test with a 95% confidence level was used to compare the differences in the mean zone of inhibitions among and between the groups, respectively. Differences among groups were statistically significant at $p < 0.05$.

2.9. Ethical Approval. The study was performed after obtaining institutional ethical approval from the Faculty of Veterinary Medicine Biosafety; Animal Use and Ethics Committee (BAUEC) of the University of Nairobi ethical approval, reference number FVM BAUEC/2021/290; and a research permit from the National Commission for Science, Technology, and Innovation (NACOSTI), license number NACOSTI/P/21/11253.

3. Results

3.1. Phytochemical Composition. Aqueous extracts had the highest yield value (7%), followed by methanol with 2.3% and the mixture of dichloromethane : methanol (1 :1) recorded the least (1.89% yield value). The three extracts and the powder of *D. abyssinica* rhizome possessed secondary metabolites (Table 1). Except for the aqueous extract, saponins were detected in the powder and all other extracts. Alkaloids, glycosides, phenolics, coumarins, tannins, flavonoids, steroids, and terpenoids were present in the powder and all other extracts (aqueous dichloromethane : methanol (1 :1) and methanol) from *D. abyssinica* rhizome. Mayer's test for alkaloids was negative while Dragendorff's test was positive for all the samples.

3.2. Effects of Single-Dose Toxicity. The study of the toxic effects of *D. abyssinica* aqueous, methanol, and dichloromethane : methanol extracts after oral single-dose administration revealed no signs of unwellness (Table 2). In addition, there wasn't any rat mortality that was observed at a single dose of 2,000 mg/kg body weight (bw). Therefore, the extracts were classified as nontoxic according to the OECD 425 guidelines. The LD_{50} of the extracts was found to be more than 2000 mg/kg. To the best of our

knowledge, this was the first time the toxicity profile of *D. abyssinica* is being reported.

3.3. Antimicrobial Activity. The crude extracts of *D. abyssinica* rhizome were tested for antimicrobial efficacy against four pathogenic microorganisms utilizing disk diffusion and microdilution techniques. The study revealed that the extracts had antifungal activity and did not demonstrate antibacterial activity against the studied microorganisms. Tables 3 and 4 summarize that the aqueous, methanol, and

Table 2: Showing effects of *D. abyssinica* extracts on female Wistar rats wellness parameters and LD_{50} at 2000 mg/kg oral single-dose.

Wellness parameter	Observation after: 30 minutes		4 hours		24 hours		48 hours		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
Fecal 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
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
Mortality/death	None	None	None	None	None	None	None	None	None	None	None	None

Key: EGR—experimental group of female Wistar rats (administered with the studied plant extracts); CGR—control group of female Wistar rats (administered with physiological saline only).

Table 3: Mean diameter zone of inhibition of *Digitaria abyssinica* rhizome extracts and controls using disk diffusion method.

Pathogen	Concentration (mg/mL)	Zone of inhibition (mm)				
		Aqueous extract	Methanol extract	Dichloromethane : methanol extract	Negative control	Positive control
Candida albicans	15.625	$6.50^{a} \pm 0.55$	$6.83^{a} \pm 0.41$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	Clotrimazole: $27.00^{f} \pm 0.89$
	31.25	$7.83^{b} \pm 0.75$	$8.33^{b} \pm 0.52$	$6.83^{b} \pm 0.41$		Econazole: $26.67^{f} \pm 0.52$
	62.50	$9.67^{c} \pm 0.52$	$8.83^{b} \pm 0.75$	$8.17^{c} \pm 0.41$		Ketoconazole: $34.17^{g} \pm 0.75$
	125.00	$15.50^{d} \pm 0.55$	$11.67^{c} \pm 0.52$	$9.50^{d} \pm 0.55$		Miconazole: $24.83^{e} \pm 0.75$
	250.00	$15.67^{d} \pm 0.52$	$11.50^{c} \pm 0.55$	$9.50^{d} \pm 0.55$		Nystatin: $7.17^{ab} \pm 0.41$
	500.00	$16.33^{d} \pm 0.82$	$15.17^{d} \pm 0.75$	$10.50^{e} \pm 0.55$		
Escherichia coli	15.625	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	Azithromycin: $6.00^{b} \pm 0.00$
	31.25	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		Ceftriaxone: $29.00^{e} \pm 0.89$
	62.50	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		Cephalexin: $12.33^{c} \pm 0.52$
	125.00	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		Ciprofloxacin: $30.33^{f} \pm 0.82$
	250.00	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		Tetracycline: $18.67^{d} \pm 0.52$
	500.00	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		
Neisseria gonorrhoeae	15.625	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	Azithromycin: $21.00^{b} \pm 0.71$
	31.25	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		
	62.50	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		Ceftriaxone: $28.50^{c} \pm 4.23$
	125.00	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		
	250.00	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		
	500.00	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		
Staphylococcus aureus	15.625	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	Cephalexin: $15.50^{c} \pm 0.55$
	31.25	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		Ciprofloxacin: $22.17^{d} \pm 0.75$
	62.50	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		
	125.00	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		Tetracycline: $11.33^{b} \pm 0.52$
	500.00	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$		

Values are expressed as mean ± SD of three separate determinations. Means with different superscripts along the column are significantly different at $p < 0.05$.

Table 4: Average MIC and MBC or MFC (mg/ml) of *D. abyssinica* extracts against selected uropathogenic microorganisms.

Test organism	Aqueous extract		Methanol extract		Dichloromethane : methanol extract	
	MIC	MBC or MFC	MIC	MBC or MFC	MIC	MBC or MFC
Candida. albicans	31.25	62.5^{MBC}	31.25	62.5^{MBC}	62.5	125_{MBC}
Escherichia coli	>250	$>250^{MBC}$	>250	$>250^{MBC}$	>250	$>250^{MBC}$
Neisseria gonorrhoeae	>250	$>250^{MBC}$	>250	$>250^{MBC}$	>250	$>250^{MBC}$
Staphylococcus aureus	>250	$>250^{MFC}$	>250	$>250^{MFC}$	>250	$>250^{MFC}$

Key: MIC—minimum inhibitory concentration; MBC—minimum bactericidal concentration; MFC—minimum fungicidal concentration.

dichloromethane : methanol (1 :1) extracts revealed antifungal activity against *C. albicans*. The highest antifungal activity was demonstrated by aqueous extract which had a zone of inhibition of 16.33 ± 0.82 (Table 3) at a concentration of 500 mg/ml and a MIC of 31.25 mg/ml (Table 4). All three extracts (aqueous, methanol, and dichloromethane : methanol (1 :1) were inactive against the bacterial micro-

organisms of *E. coli*, *N. gonorrhea*, and *S. aureus.*

4. Discussion

Medicinal plants are endowed with secondary metabolites. These plants continue to provide solutions to human and livestock ailments in traditional medicine systems for primary health care. In the current study, chemical compounds known to have antimicrobial activities were detected in the rhizome extracts of *D. abyssinica*. Phenols, tannins, flavonoids, terpenoids, saponins, alkaloids, and coumarins which were positively indicated in the aqueous, methanol, and dichloromethane : methanol (1 :1) extracts are known to have antimicrobial activities and may be explored to develop botanicals that can be used to combat antimicrobial resistance [48]. To the best of our knowledge, this is the first published report on the qualitative phytochemical composition of the powder and extracts from *D abyssinica* rhizome. Also, toxic effects and antimicrobial activity studies have not been reported in the literature. However, reports exist for several plants from the *Digitaria* genus that possesses active phytochemicals with antimicrobial activities and other varied biological activities including analgesic, antiviral, anti-inflammatory, antitumor, anthelmintic action, and effects on the central nervous system [49, 50].

All female Wistar rats in this study, survived after administration of a single dose of 2000 mg/kg of the aqueous, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* to the Wistar rats orally. Therefore, the dose of the *D. abyssinica* rhizome extracts that can kill half of the tested animals was more than 2000 mg/kg. According to the Organization for Economic Cooperation and Development (OECD) test no 425, the findings in this study indicate that the extracts are not toxic to the female Wistar rats as in many other acute toxicity studies of herbal medicines [45, 51, 52]. The current research indicates that *D. abyssinica* rhizome extracts have antifungal activity against *C. albicans* and that the strength of the activity was directly proportional to the concentration of the extract loaded to the disk. The size of the zones of inhibition of the aqueous extract was more than 15 mm at concentrations 125 mg/ml, 250 mg/ml, and 500 mg/ml (Table 3) which is interpreted as potential antifungal activity [53, 54]. There was no statistical difference in activity at concentrations more than 125 mg/ml at $p < 0.05$ (Table 3). Though there was noted low activity at 15.625 mg/ml, 31.25 mg/ml, and 62.50 mg/ml of zones of inhibition between 6.5 mm and 9.67 mm, there were statistical differences in activity at $p< 0.05$.

The methanol extract also had high antifungal activity (15.17 ± 0.75 mm) at a concentration of 500 mg/ml and is statistically different from the activity of 11.67 ± 0.52 mm exerted by the extract at a concentration of 250 mg/ml at p

< 0.05. Subsequently, zones of inhibition of 11.50 ± 0.55, 8.83 ± 0.75, 8.33 ± 0.52, and 6.83 ± 0.41 mm at concentrations of 125, 62.50, 31.25, and 15.625 mg/ml, respectively. There were notable statistical differences between the antifungal activities of the methanol extract at concentrations between 15.625 and

31.25 mg/ml ($p< 0.05$) and between 62.50 and 125 mg/ml. However, there were no statistical differences in activities between concentrations 31.25 and 62.50 mg/ml and between concentrations 125 and 250 mg/ml at $p< 0.05$. The zones of inhibition that revealed the activity of the mixture of dichloromethane : methanol *D. abyssinica* rhizome extract similarly reflected proportionality to extract concentrations (Table 3). The aqueous, methanol, and dichloromethane : methanol (1 :1) extracts of *D. abyssinica* did not show any zone of inhibition against the bacterial strains, *E. coli*, *N. gonorrhoeae*, and *S. aureus*, in this study.

5. Conclusion and Recommendation

Based on the current study, it was concluded that the aqueous, methanol, and mixture of dichloromethane : methanol (1 :1) extracts of *D. abyssinica* rhizome possess phytochemicals compounds with anticandidal activity. The extracts can be classified as nontoxic and therefore safe for female Wistar rats when administered orally at a single dose. It was further deduced that the extracts had low antifungal effects. Nevertheless, herbalists and traditional medicine practitioners have used the plant material in wide cultural setups. Given that herbalist frequently uses aqueous extracts in their practice, these study findings provide scientific evidence to validate the use of the extracts in the management of candidiasis. Bioassay-guided fractionation was recommended to isolate anticandidal compounds from *D. abyssinica* rhizome extracts. In addition, quantitative phytochemical studies and subchronic and chronic toxicity assays were recommended.

Data Availability

All data underlying the results are available as part of the article and no additional source data are required.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

Authors' Contributions

The research concept was developed by Mr. William Lemayian and Dr. Jared Onyancha. William Lemayian performed the experiments, analyzed the data, and drafted the manuscript. Prof. James Mbaria and Laetitia Kanja supervised the entire study. Mr. Meshack Juma helped in the design and improvement of the methodology. All authors reviewed and approved the final manuscript for publication.

Acknowledgments

The authors would like to acknowledge the Department of Public Health, Pharmacology, and Toxicology of the University of Nairobi for providing the laboratory facility, model animals, reagents, and equipment for this study. The authors appreciate Mr. Maloba and Mr. Asava of the Department of Public Health, Pharmacology, and Toxicology (UoN) for their technical assistance. The authors would also like to acknowledge the Department of Medical Microbiology of the University of Nairobi for providing the microbes, reagents, and equipment for this study. Dr. Marianne Mureithi, Mrs. Felistas Wayua, Dr. Moses

Victor Musyoki, Dr. Magdaline Burugu, and Mr. Jonathan Oloo are highly appreciated for their technical assistance. This research and publication was funded by William Lemayian Sapunyo's personal savings.

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