PHYTOCHEMICAL ANALYSIS, *IN-VITRO* INHIBITORY ACTIVITIES, AND *IN-VIVO* ACUTE TOXICOLOGY STUDIES OF SEVEN KENYAN MEDICINAL PLANTS AGAINST SELECTED BACTERIA AND FUNGUS

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SCIENCES OF KENYATTA UNIVERSITY.

SEPTEMBER 2025

DECLARATION

I, Nguimbous Simone Pierrette, declare that the work presented in this thesis is my original work and has not been submitted for a degree or examination in any other university or for the obtention of any other award.

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DEDICATION

This thesis is dedicated to the entire family of NYEMB NGUIMBOUS for their love, prayers, moral and financial support throughout my educational journey thus far.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AMR	Antimicrobial Resistant
BIH	Brain Infusion Heart
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
DMSO	Dimethyl Sulfoxide
DRC	Democratic Republic of Congo
GARP	Global Antibiotic Resistance Partnership
GoK	Government of Kenya
HIV	Human Immunodeficiency Virus
LC_{50}	Lethal Concentration 50
MBC	Minimum Bactericidal Concentration
MDR	Multidrug Resistant
MFC	Minimal Fungicidal Concentration
MH	Mueller-Hinton
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-Resistant *Staphylococcus aureus*
Mt.	Mount
SDA	Sabouraud Dextrose Agar
Spp.	Species
SPSS	Statistical Packages for Social Sciences
STIs	Sexually Transmissible Infections
UTIs	Urinary Tract Infections
WHO	World Health Organization
XDR	Extra-Drug Resistant

ABSTRACT

Globally, and particularly in less-developed countries, one of the principal factors associated with morbidity and mortality is infectious diseases. Over the years, the abuse and misuse of pharmaceutical products have caused an increase in resistant microbes. Today, the rate of infectious disease cases continues to increase to dangerously high levels as most pharmaceutical products have lost their efficacy. It is worth noting that close to 80% of the African population utilizes medicinal plants, and more than 70% of Kenyans rely on traditional remedies as a primary source of curatives. However, issues such as scarcity of information concerning their active compounds and pharmacological and toxicological properties considerably affect their usage in modern medicine. Therefore, this study assessed the presence or absence of major phytochemicals in stem bark and/or roots of *Carissa edulis, Acanthus ebracteatus, Albizia gummifera, Prunus africana, Combretum molle, Warbugia ugandensis,* and *Cuscuta* spp., evaluated their in vitro inhibitory activities against *C. albicans* (ATTC 10231), *E. coli* (ATTC 25922), and *S. aureus* (ATCC 25923) and tested for possible toxic effects in Swiss albino mice (only for highly potent extracts). Selected plants were collected from the Mt. Kenya and Elgon regions. Crude extracts were made by macerating powdery plant samples in methanol. Each plant was then screened for major phytoconstituents using standard methods. Polar and nonpolar extracts were obtained via sequential solvent–solvent partitioning using hexane, dichloromethane, ethyl acetate, and methanol at room temperature. Sterile dimethyl sulfoxide solution (DMSO; 5% in water) was used to dissolve the extracts, and each was tested for inhibitory activity in vitro using the agar disk diffusion (Kirby-Bauer) method. MICs were determined for active plant extracts by means of 96-well microtiter plates (broth microdilution method). MBCs and MFCs were obtained by subculturing the contents of the last wells. Extracts with significant bactericidal/fungicidal activities at concentrations $\geq$ 250 mg/ml were further tested for toxicity using a total of 50 Swiss albino mice. Acute toxicity was investigated for a period of 14 days at concentrations of 500, 866, and 1500 mg/kg body weight. Mice were kept under careful observation throughout the study and were euthanized on the 15th day. Blood collected was used for biochemical and hematological tests. The data obtained were analyzed using SPSS software and ANOVA ($p>0.05$). Phytochemical screening revealed that each tested plant contained a range of different secondary metabolites. Preliminary assessment using the Kirby-Bauer method showed that the *W. ugadensis* DCM extract had the highest activity against *C. albicans, E. coli,* and *S. aureus*, with mean inhibition zones of 21.00 ± 0.58, 10.00 ± 0.57, and 15.67 ± 0.33 mm, respectively. MIC testing demonstrated that *E. coli* had the lowest susceptibility, whereas *S. aureus* had the highest susceptibility to the various extracts. Findings from the lethality assay demonstrated that selected plant extracts (*A. gummifera* ethyl acetate, *P. africana* methanol, and *W. ugadensis* DCM) did not cause significant alterations in the mean body weights, relative organ weights, or behavioral, hematological and biochemical parameters of mice at the tested concentrations. Although mice treated with *W. ugadensis* DCM at 866 and 1500 mg/kg were observed to have lose weight during the first week after intake of the extract, these 3 extracts were deemed antimicrobials and safe for administration, with LD50 >1500 mg/kg. Nonetheless, additional research to confirm their safety, particularly in situations of repeated and prolonged therapy, is needed. Also, there is a need to identify specific bioactive compound(s) responsible for their antibacterial and antifungal effects.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Infectious diseases are among the major threats to human health (Weinstein, 2001). Over the years, the abuse and misuse of pharmaceutical products have caused an increase in the number of microbes that are resistant to antimicrobials. Estimates show that by 2030, AMR, MDR and XDR microorganisms will be the leading source of infectious illnesses around the globe (WHO, 2019). From the CDC's report on antibiotic/antimicrobial resistance threats, MRSA, drug-resistant *Candida* and carbapenem-resistant Enterobacterales, such as *E. coli*, are classified among microorganisms of serious and urgent threat to human health (CDC, 2019).

In many traditional cultures, medicinal plants play crucial roles in relieving health challenges. This is particularly eminent on the African continent, where approximately eighty percent of inhabitants utilize medicinal plants to cure illnesses and to sustain good health (WHO, 2012). For example, more than seventy percent of the Kenyan population depends on folklore medicine as the main source of curative substances, while a greater percentage (approximately 90%) of the population utilizes medicinal plants at one moment or another (Kipkore *et al.,* 2014). Availability, efficacy and affordability are factors that contribute to the partiality toward traditional medicines. However, in vivo studies using animal models on the therapeutic effects of medicinal plant extracts revealed that most of these plants possess bioactive components that at high concentrations, simultaneous consumption with other drugs, and usage for long periods may have toxic effects (Akwa

and Nguimbous, 2021). Culturally, the use of traditional medicines is also more approved (Kiringe, 2005), although people have little or no irrefutable scientific data to support the use of most plants in preventing and treating ailments. Phytochemical screening is essential to identify the active constituents that may be responsible for antimicrobial activity. Concurrently, in-vitro assays will evaluate the efficacy of plant extracts against clinically relevant bacteria and fungi, offering insight into their therapeutic potential. Furthermore, in-vivo acute toxicity studies in animal models are necessary to determine safety margins and potential health risks associated with the use of these plant-based remedies.

By bridging the gap between traditional medicine and modern pharmacological research, this study aimed to contribute to the development of evidence-based natural therapeutics. The study examined the phytochemical constituents of seven medicinal plants (*Carissa edulis, Acanthus ebracteatus, Albizia gummifera, Prunus africana, Combretum molle, Warbugia ugandensis,* and *Cuscuta* spp.) sourced from the Mt. Kenya and Mt. Elgon regions, evaluated their *in vitro* inhibitory activities against selected bacteria and fungus and tested for their toxicities in vivo using Swiss albino mouse models.

1.2 Statement of the problem

Globally, and particularly in less-developed countries, one of the main causes contributing to morbidity and mortality is infectious diseases. They account for over 15 million deaths annually across the globe, with about 80% of those fatalities occurring in Africa (Boutayeb, 2010). One of the principal health problems in Kenya, for example, includes the elevated load of infectious diseases within the population (CDC, 2017).

Despite the wide range of antimicrobial products now available, the rate of infectious disease cases is increasing to dangerously high levels (Brown and Wright, 2016). The

ability to treat common infectious illnesses is now menaced due to the development and rapid spread of new resistance mechanisms within microbial populations (WHO, 2020). Medicinal plants may possess components with very useful properties that are effective in the management of diseases. However, issues such as scarcity of information concerning their active compounds and pharmacological and toxicological properties considerably affect their usage in modern medicine (Njume and Goduka, 2012). Additionally, the disappearance of these potential curatives is alarming. Unsustainable collection and destruction of plants' natural environment have led to the rapid decrease in medicinal plant resources in Kenya (Fabricant and Farnsworth, 2001).

1.3 Justification of the research

Modern medicine and the successful management of infectious diseases are now in danger due to the emergence of antimicrobial resistance (AMR) (Golkar *et al.,* 2014). AMR can result from natural genetic changes in microorganisms over time, while other contributing factors include misuse, improper disposal and counterfeiting of antimicrobials. This growing resistance has intensified the search for alternative therapeutic agents, particularly from natural sources. A number of plants commonly available in nature are utilized as substitutes for chemical drugs and act as therapeutics for various ailments. This therefore supports their usage as key ingredients for the development of novel pharmaceutical products. To date, various studies have identified compounds present in medicinal plants that have effective antimicrobial properties (Afolayan, 2003). Phytochemical and antimicrobial screenings have increasingly demonstrated that numerous higher plants contain secondary metabolites (such as alkaloids, flavonoids, tannins, and terpenoids), with significant antibacterial and antifungal activities. Between 1981 and 2014, it was observed

that approximately fiftyone percent of new pharmaceutical products were naturally made from plants and were proven via scientific experiments to have antagonistic activities against various infectious microbes and cancer cells (Newman and Cragg, 2016). This underscores the importance of systematic phytochemical screening as a foundational step in drug discovery and development. Therefore, plants can be regarded as potential raw materials for the manufacturing of new pharmaceutical products. Conservation and afforestation of medicinal plants will be encouraged if credible scientific data can support their usage as antimicrobial agents in folk medicine. More research on the bioactive compounds and antimicrobial and toxic properties of these plants is thus indispensable.

1.4 Hypotheses

i) There are no phytochemicals in extracts of stem barks and/or roots of *C. edulis, A. ebracteatus, A. gummifera, P. africana, C. molle, W. ugandensis,* and *Cuscuta* spp. ii) Polar and nonpolar extracts of selected medicinal plants possess no significant antifungal and antibacterial capabilities *in-vitro* against *C. albicans, E. coli* and *S. aureus* iii) Active polar or nonpolar extracts of selected medicinal plants do not exhibit any toxic effects in Swiss albino mice.

1.5 Objectives

1.5.1 General Objective:

To screen for phytochemicals in extracts of seven medicinal plants harvested from Mt. Elgon and Mt. Kenya regions, and to evaluate their antimicrobial and toxic properties.

1.5.2 Specific Objectives:

i) To determine the presence of phytochemicals in stem bark and/or roots of *C. edulis, A. ebracteatus, A. gummifera, P. africana, C. molle, W. ugandensis,* and *Cuscuta* spp. collected from Mt. Elgon and Mt. Kenya regions.

ii) To determine the *in-vitro* antibacterial and antifungal properties of methanolic, ethyl acetate, dichloromethane, and hexane extracts of *C. edulis, A. ebracteatus, A. gummifera, P. africana, C. molle, W. ugandensis,* and *Cuscuta* spp. against *C. albicans, E. coli* and *S. aureus*. iii) To evaluate the acute toxicity of selected active medicinal plant extracts using Swiss albino mouse models.

1.6 Significance of the study

The significance of this study lies in its comprehensive evaluation of seven medicinal plants traditionally used in Kenya, addressing their phytochemical constituents, antimicrobial efficacy, and acute toxicity. Through a combination of in vitro and in vivo approaches, the study offers credible scientific data that supports and validates the ethnomedicinal claims associated with these species.

In the context of rising antimicrobial resistance (AMR), the identification of plant-based compounds with antibacterial and antifungal properties contributes to the ongoing global search for alternative therapeutic agents that are both affordable and effective. The use of Swiss albino mice for toxicity assessment also enhances the safety profile of the extracts, addressing a common limitation in traditional medicine use—lack of toxicity data.

Furthermore, the research holds practical value for public health, pharmacological development, and biodiversity conservation. It provides a scientific basis for further preclinical studies, informs safe traditional use, and supports the conservation and sustainable utilization of medicinal plant resources. The outcomes of this work may guide future drug development efforts and policy decisions related to herbal medicine and natural product research in Kenya and beyond.

1.7 Scope and Limitations

This study only involved the aforementioned plant species, plant part(s), and test microorganisms. The age and developmental stage of the plants were not accounted for in this study. Phytochemical screening and lethality assays were limited to qualitative analysis and acute toxicity testing, respectively. Finally, environmental factors and climatic and soil conditions were not considered in the study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Infectious diseases

Bacteria, fungi, parasites, and viruses are the causative agents of disorders commonly referred to as infectious diseases. Despite technological advancements, sanitization improvements, and increased access to healthcare, outbreaks of infectious diseases have caused a significant global impact throughout the past 20 years. A series of serious infectious disease outbreaks have been observed such as the COVID-19 pandemic, the Zika virus disease epidemic in 2015, the Ebola virus disease epidemic from 2013–2016 in West Africa, the Middle East respiratory syndrome coronavirus outbreak in 2012, the swine

flu pandemic in 2009, and the severe acute respiratory syndrome coronavirus outbreak in 2003, which all crossed boundaries, infected individuals worldwide, and significantly increased mortality and morbidity (Baker *et al.,* 2022).

The rising incidence of infectious diseases persists, especially in developing nations. Annually, around 15 million people worldwide die from infectious diseases, with more than 80% of those fatalities occurring in Africa (Boutayeb, 2010). Infectious diseases have a detrimental effect on African nations that extends beyond the healthcare industry and poses a threat to various other industries. It is worth noting from the UNDP report in 2006 (UNDP, 2006) that infectious diseases in African nations are affecting all aspects of human development (HD). A large part of most African countries including Kenya have lost their healthcare workforce due to several infectious illnesses (UN, 2004). Increased levels of development in developing nations, alongside an increase in the number of people living in cramped, subpar housing, gave rise to novel possibilities for the appearance and spread of infectious illnesses.

2.2 Antimicrobial resistance

Antimicrobial agents are synthetic, semisynthetic, or natural substances used to inhibit the development or completely kill microorganisms including bacteria, fungi, viruses, and parasites. When organisms such as parasites, fungi, viruses, and bacteria become resistant to the medications typically used to treat them, this is known as antimicrobial resistance (AMR). One of the most pressing health concerns of this current century is AMR, which poses a danger to the efficient avoidance and management of microbial
infections.

Antimicrobials, which in the past assisted in the treatment of millions of patients worldwide, are as a result losing their effectiveness at a startling speed. The occurrence of infections caused by drug-resistant fungal pathogens is rising and worsening the already complicated treatment options. A large number of fungal infections have ongoing treatment challenges like toxicity, particularly for patients with compromised immunity, including those living with chronic respiratory disease, HIV/AIDS, cancer, organ transplants, and post-primary tuberculosis infection (WHO, 2022). Globally, increased rates of resistance to antibiotics frequently employed for managing common bacterial illnesses, including UTIs, STIs, sepsis, and several types of diarrheas, have also been observed. This indicates that effective antibiotics are going out of stock, causing more difficult to treat infections and eventually more deaths. To date, a censorious gap is left in research and development, especially for antibacterial agents against gram-negative carbapenem resistant bacteria (WHO, 2021). The speedy worldwide progression of panresistant alongside multi-resistant bacteria, so-called "superbugs," which cause diseases that cannot be treated with existing antimicrobial products including antibiotics is of particular concern. (WHO, 2021).

AMR naturally occurs with time, usually as a result of genetic changes. However, factors such as misuse, improper disposal and counterfeiting of antimicrobials; poor disease/infection prevention and management in farms and healthcare facilities; substandard quality, cheap medicines, diagnostics and vaccines; lack of legislation enforcement; limited provision of clean water, hygiene and sanitation (WASH) both for animals and humans; and paucity of knowledge and awareness, have all helped to speed up the emergence and spread of antimicrobial resistance in pathogenic microbes (WHO, 2021). The WHO urged prompt action and coordinated efforts to lessen this hazard in 2016,

which if not sufficiently reduced, was estimated as having caused roughly 700 000 fatalities in 2014 and anticipated to result in the deaths of 10 million people in 2050. (WHO, 2016). In the United States, antibiotic resistance is responsible for about 23,000 deaths and greater than 2 million infections each year, comparable to a 20-billion-dollar total expenditure and an additional 35-billion-dollar productivity loss. (CDC, 2013). Antimicrobial resistance is a disturbing trend that is reaching alarmingly high proportions in underdeveloped nations such as Kenya. (Ayukekbong *et al.,* 2017). For instance, the 2011 GARP – Report of the Kenya Working Group highlighted resistance to antibacterial drugs as a major problem in the country and offered suggestions to stop its propagation.
(GARP, 2011). Antimicrobial resistance has wide-ranging effects in addition to health issues. It threatens the accomplishment of the Sustainable Development Goals and affects the productivity of patients and/or that of their keepers through extended stays in the hospital and the need for intensive care and more expensive medicinal drugs (WHO, 2021). It increases morbidity and mortality, poses an imminent danger to food and health security and has a detrimental effect on trade and economies. Globally, AMR is recognized as a major public health concern. The WHO has declared it one of the top ten global health threats, warning that without urgent action, common infections and minor injuries may once again become fatal (WHO, 2020). The search for new antimicrobial agents, including those derived from medicinal plants, is therefore vital in addressing the
AMR crisis.

2.3 Microorganisms involved in the study

Antibiotic-resistant "priority pathogens" were listed by the WHO in 2017 in an attempt to guide and advance new antibiotic research and development (R&D), as well as to foster

public health action toward the fight against the growing resistance of microorganisms to antimicrobial products worldwide. According to this report, the most critical group includes Enterobacteriaceae like *Serratia, Klebsiella, Proteus,* and *E. coli.* The second high priority category includes various growing drug-resistant bacteria like *Staphylococcus aureus, Enterococcus faecium* and others, that are responsible for more common diseases (WHO, 2017). In October 2022, the WHO made the first global attempt to methodically prioritize fungal pathogens of public health importance and created a list of priority pathogenic fungi (WHO FPPL). From this list, the 4 fungal pathogens that pose a "critical threat" are *Candida albicans, Candida auris, Cryptococcus neoformans,* and *Aspergillus fumigatus* (WHO, 2022). In line with the aforementioned priority pathogens, this study involved the use of 3 test microorganisms: *C. albicans, S. aureus,* and *E. coli*

2.3.1 *Candida albicans*

Candida albicans is a dimorphic yeast fungus that naturally inhabits the gastrointestinal tract, vagina, and mucosal cavities of the body (Shao *et al.,* 2007). It is progressively becoming a major human pathogen particularly in people with immunocompromised status due to factors including pregnancy, advanced age, diabetes mellitus, prolonged antimicrobial and aggressive cancer chemotherapy, organ transplantation, and invasive surgical procedures (Keleta, 2016). Currently, the number of existing drug products against fungal infections is limited; therefore, the spread of antifungal resistance genes critically restricts treatment options. A rising cause of health concern, fungal pathogen resistance, is particularly alarming in those with invasive fungal infections, serious infections affecting the heart, blood, eyes, brain, or other body parts (CDC, 2022). For the past years in developing countries, medicinal plant extracts have been utilized to treat infectious diseases

like candidiasis. Recently, an increase in resistance by *C. albicans* to routinely used antifungals has been observed; thus, the need to search for new antifungal agents is imperative (Sardi *et al.,* 2011).

2.3.2 *Staphylococcus aureus*

Gram-positive *S. aureus* bacteria are a typical component of the skin flora. They are frequently associated with infections acquired both in health care settings and in the community. They are the cause of various infectious diseases including lethal pneumonia, endocarditis, skin infections, bacteremia, soft tissue infections, osteomyelitis, as well as toxic shock syndrome (Guo *et al.,* 2020). In Africa, infections caused by *Staphylococcus* spp. and associated AMR have been seen, although noticeable differences between and within countries have been observed (Wangai *et al.,* 2019). Nonetheless, from the global AMR surveillance report by the WHO, the absence of sufficient information in Africa has been observed to be the main issue in the assessment of the burden of antimicrobial resistance in African countries including Kenya (WHO, 2014). For the past decades, a wide range of antibiotics were administered against infections caused by *S. aureus*, but eventually, there was an emergence of multi-drug resistant *S. aureus*. Today, AMR in *S. aureus* is widely spread and is a major public health concern globally, with resistance to methicillin as one of the most common examples observed in *S. aureus* (Mukherjee *et al.,* 2021). It has been shown that people with drug-sensitive infections are 64% less likely to die compared to individuals who have infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (WHO, 2021).

2.3.3 *Escherichia coli*

The facultative anaerobic gram-negative bacteria *E.* coli typically inhabits warm-blooded animals and humans' guts, constituting the main facultative flora in the gastrointestinal tract (Sahoo, 2012). Ordinarily, *E. coli* is nonpathogenic; however, when found in surplus, they are a common cause of various frequent infections, such as deadly bloodstream and UTIs (Kennedy *et al.,* 2008). Antibiotic resistance has been observed at high rates in the bacteria *E. coli* and is spreading rapidly. This is particularly true for third generation cephalosporins, fourth-generation cephalosporins, and fluoroquinolones
(Peter, 2009). Strains of *E. coli* resistant to fluoroquinolones (an antibiotic class routinely used for managing UTIs) are now extensively spread around the world (WHO, 2021). One of the few effective treatments for deadly infections due to carbapenem-resistant Enterobacteriaceae is colistin (WHO, 2021). Yet, in many regions and countries, resistance to colistin in some bacteria has already been observed. Consequently, they cause infections for which available antibiotics are ineffective. Thus, as noted by the WHO as priority pathogen list, the need to search for new, effective, and non-toxic antimicrobial products that can treat infections brought on by gram-negative microorganisms that are resistant to standard therapy becomes crucial (WHO, 2021). Interestingly, some medicinal plant extracts have proven to possess antimicrobial potential against enteric pathogens such as *E. coli* present in food products (Fullerton *et al.,* 2011).

2.4 Medicinal plants

2.4.1 General Introduction

Herbal remedies have been utilized in traditional medicine to prevent and manage various types of ailments since the dawn of time, as well as to extract various important bioactive

compounds (Rasool, 2012). Medicinal plants are extensively used in Africa mainly for the fact that they are easily available and less costly compared to most pharmaceutical drugs. With more than 7000 different plant species, the diversified flora of Kenya is one of the most abundant in East Africa (MEWNR, 2015), resulting in the discovery of various medicinal plants in the region. Despite the network established by the World Health Organization to foster the safe and responsible use of medicinal plants, there is still a paucity of regulation of traditional medicine and scientific research proofs to support the claimed medicinal potential of some plant extracts (Ahn, 2017). Additionally, the fate of therapeutic plants is endangered from both general and specific threats, including but not limited to habitat destruction, climate change, and over-collection to attain market demands. A number of ethnopharmacological and ethnobotanical research studies on Kenya's medicinal plants have been made and documented. However, many indigenous communities, areas and counties still need to be explored.

2.4.2 *Warbugia ugadensis*

The plant *Warbugia ugadensis* is an East African-native species of evergreen tree that is 70 cm in diameter and 4.5-30 m tall (figure 2.1), It belongs to the family Canellaceae. It is well regarded because of its valuable medicinal qualities and is considered the secondmost important species of medicinal plant in Kenya for in-depth research (Wamalwa *et al.,* 2006). In nations including Kenya, Tanzania, Uganda, Swaziland, Malawi, South Africa, Ethiopia, and DRC, *W. ugandensis* is widely dispersed (Orwa *et al.,* 2009). The species has been referred to by a variety of names, including "Mukuzanume" or

“Muwiya” in Luganda and “East African greenheart”, “East African green wood”, and

"pepper-bark tree" in English (ICRAF, 1992). Locally, it is known as "Muthiga" by the Kikuyu community in Kenya. Sexually transmitted illnesses, stomach aches, chest infections, coughs, internal ulcers, and leishmaniasis can all be treated using the plant's stem bark and leaves (Were *et al.,* 2010; Githinji *et al.,* 2010). In the Kisii area of southwest Kenya, the plant is said to be used as a remedy for diabetes, pneumonia, as well as malaria (Maobe and Nyarango, 2013). Cardiac glycosides, flavonoids, alkaloids, terpenoids, polyphenols, and terpenes, among other metabolites of therapeutic significance, have reportedly been found in the plant extract (Maobe and Nyarango,

2013). A study revealed that *W. ugandensis* collected in various communities around the Kenyan Rift Valley demonstrated antimicrobial efficacy toward *Candida albicans* along with *Staphylococcus aureus,* yet ineffective over *E. coli* (Abuto *et al.,* 2016).

Figure 2.1: ***W. ugadensis***

(Source : https://www.flickr.com/photos/icraf/27381539294)

2.4.3 *Carissa edulis*

The Apocynaceae family includes *Carissa edulis*, a 5-meter-tall spiky bush plant that grows natively in several parts of Kenya, including the Mt. Kenya region in the counties of Meru

and Embu (figure 2.2). It is extensively dispersed across tropical Africa, reaching as far south as Zimbabwe and Zambia. Additionally, the plant can be observed in Arabia, Madagascar, Indochina, and India (Hutchinson and Dalziel, 1963; Sofowora, 1980; Burkill, 1985). The Hausa word "cizaki" is often used in Nigeria to refer to this plant (Gbile, 1980). It goes by the names "Mkokolo" and “Mpambala” in Malawi

(Sofowora, 1980), and in Kenya, “Olamuriaki” and “Mukawa” are its local names by the Maasai and Kikuyu communities, respectively. *C. edulis* has a wide variety of traditional uses and is rich in secondary metabolites. The fruits and leaves were reported to contain carbohydrates, cardiac glycosides, flavonoids, steroids, saponins, tannins, as well as terpenes (Ibrahim *et al.,* 2005). For millennia, it has been used by many Kenyan communities to treat illnesses like headaches, epilepsy, coughs, toothaches, chest problems, sickle cell anaemia, fevers, and sexually transmitted diseases like rabies and syphilis (Kiringe, 2006). Its pungent roots are used as a diuretic and as a treatment for gonorrhoea, syphilis, and rabies in Ethiopia (Addis *et al.,* 2001).

Figure 2.2: ***C. edulis***

(Source : http://www.africanplants.senckenberg.de/root/index.php?page_id=78&id=277)

2.4.4 *Acanthus ebracteatus*

The Acanthaceae family includes *Acanthus ebracteatus* vahl (figure 2.3), a medicinal mangrove plant with multiple uses. It is found everywhere from tropical Australia to the western Pacific islands and Southeast Asia (Ragavan *et al.,* 2015). Sea holly and holly mangrove are examples of commonly used names of the plant. Even though there isn't much data on the phytochemical diversity of *A. ebracteatus*, earlier research has found that it contains aliphatic alcohol, megastigmane glycosides, aliphatic glycosides, lignan glycosides, phenolic glycosides, flavonoids, and terpenes (Prasansuklab and Tencomnao, 2018; Li*et al.,* 2009). According to pharmacological studies of the plant, *A. ebracteatus* possesses anti-inflammatory, wound-healing, and neuroprotective properties (Prasansuklab and Tencomnao, 2018; Pratoomsoot *et al.,* 2020; Wisuitiprot *et al.,* 2022). According to Hokputsa *et al.,* (2004), skin conditions, cough, hepatitis, boils, hepatosplenomegaly, joint inflammation, lymphoma and asthma are among the ailments for which its seeds, roots, stems, and leaves can be employed as a treatment.

Figure 2.3: ***A. ebracteatus***

(Source: Kwan Han. http://www.natureloveyou.sg/Acanthus%20ebracteatus/Main1.html)

2.4.5 *Albizia gummifera*

The Fabaceae family includes the native African tree species *Albizia gummifera* (Orwa *et al.,* 2009) (figure 2.4). It is known as “Seet” by the Nandi community in Kenya and is used to cure a variety of illnesses. The tree's pod extract is used to treat stomach illnesses, its root is ground into a paste to treat skin conditions, and to cure malaria, a decoction made from its bark is utilized. (Ofulla *et al.,* 1996). Previous investigations have demonstrated that extracts from several *A. gummifera* sections have antibacterial properties (Mbosso *et al.,* 2010; Mmushi *et al.,* 2010). Spermine alkaloids, oleanane saponins, and triterpenes are associated with *A. gummifera's* anticancer, antibacterial, antiplasmodial, and antitrypanosomal characteristics (Tefera *et al.,* 2010; Rukunga *et al.,* 2007).

Figure 2.4: ***A. gummifera***

(http://www.westafricanplants.senckenberg.de/root/index.php?page_id=14&id=4034)

2.4.6 *Prunus africana*

A member of the Rosaceae family, *Prunus africana* is popularly known by the term pygeum or African cherry (Hills and Cheek, 2021). It can be found in West Africa, Comoros, Madagascar, and central Africa (Katanga, Congo), and is indigenous to the highland

tropical woods that are 900 to 3400 meters above sea level in Madagascar and Sub-Saharan Africa (figure 2.5). It is widely spread throughout many Kenyan regions, especially in the Mount Kenya forest, and can be found throughout the mountainous forests of Africa and underlying islands in 22 countries. (Hall *et al.,* 2000). Its indigenous names are "Muiri" and "Orkujuk" in the Kikuyu and Maasai communities of Kenya respectively. Prostafx, tadenan, as well as pygenil are a few natural product formulations from *P. africana* that are offered for sale in markets. Extracts from the roots and stem barks contain compounds that have characteristics that fight cancer, viruses, and inflammation (Kadu *et al.,* 2012). Kenyan folk medicine employs *P. africana* for managing chest pain, fever, as well as malaria (Kokwaro, 1993). Allergies, kidney problems, prostate gland illness, and diarrhoea are some additional traditional applications (Iwu, 1993). Findings from a research indicated that flavonoids and terpenes were the main secondary metabolites found in the stem barks of this plant (Bii *et al.,* 2010).

Figure 2.5: *P. africana*

(Sources: https://pza.sanbi.org/prunus-africana)

2.4.7 *Cuscuta* spp.

The Convolvulaceae family is home to the genus *Cuscuta*, often known as dodder (figure 2.6). The world's temperate and tropical regions both contain members of this genus. Dodder is widely spread in Kenya's Coast, Western, Nyanza, Central, and Rift Valley Counties. Traditional healthcare has long employed this genus for various applications, and many phytochemicals with medicinal value were extracted and characterized. (Jafari *et al.,* 2015). The idea that this genus can have a substantial impact on the search for novel and more effective therapeutic agents is encouraged by the presence of a large number of valuable bioactive compounds, a broad spectrum of biological characteristics, as well as their curative significance in traditional medicine. For stomach aches, the Embu people of Kenya consume a decoction of the entire plant (Kareru *et al.,* 2006). To cure ear infections, the stem's sap is used to make ear drops (Njoroge and Bussmann 2006).

Figure 2.6: *Cuscuta* spp.

(Source: https://www.flickr.com/photos/56047685@N02/36756047653)

2.4.8 *Combretum molle*

The plant *Combretum molle* is a member of the Combretaceae family. It differs from various species of *Combretum* by having a bigger, straighter trunk, dense crown, and

rougher bark (figure 2.7). It can be found in places with a predominance of forests and densely forested prairies on the peninsula of Arabia as well as in tropical Africa, frequently creating sole tufts on hillsides (Keay, 1989). “Muama” and “Kiama” are some of its indigenous names by the Kamba community in Kenya. In Africa, many illnesses, like HIV and malaria, are often treated with *C. molle.* (Regassa and Mengistu, 2012). It is used in Kenya by the Kamba community to alleviate dysentery and stomach-aches (Kokwaro, 2009). Secondary metabolites like flavonoids, steroids, alkaloids, essential oils, coumarins, and terpenoids are abundant in various parts of the *C. molle* plant (Batta, 2016; Fankam *et al.,* 2015).

Figure 2.7: *C. molle*

(Source: plantbook.co.za/combretum-molle/)

2.5 Extraction of natural products derived from plant sources

Medically, the word *extraction* may refer to the separation of pharmaceutically active components from plants with the help of particular solvents using standard methods. The extraction of natural products originates from ancient Egyptian and Mesopotamian times, where the manufacture of waxes and medically-active oils or perfumes was a main source

of income (Azwanida, 2015). Today, several methods are employed to extract bioactive compounds from medicinal plants, each selected based on the chemical nature of the target compounds and the type of plant material. Traditional techniques such as maceration, which involves soaking plant materials in solvents at room temperature, are simple and suitable for thermolabile compounds, though they are time-intensive and may yield low concentrations (Azwanida, 2015). Boiling or decoction is another traditional approach, commonly used for water-soluble phytochemicals in herbal medicine, but it poses a risk of degrading heat-sensitive compounds (Cowan, 1999). More efficient techniques like percolation allow for continuous solvent passage through the plant matrix, improving extraction efficiency (Tiwari *et al.,* 2011). Soxhlet extraction is a widely used hot continuous method ideal for both polar and non-polar compounds; however, it may not be suitable for volatile or thermolabile substances (Handique *et al.,* 2016). Advanced techniques such as microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE) have gained prominence due to their speed, efficiency, and low solvent consumption. MAE uses microwave energy to rapidly heat the solvent and plant matrix, enhancing the release of bioactives (Mandal *et al.,* 2007), while UAE employs ultrasound waves to rupture plant cell walls and facilitate compound release with minimal thermal degradation (Vinatoru, 2001). These modern methods are increasingly favored in pharmacognosy and natural product research for their high yield and preservation of compound integrity.

2.6 Qualitative Phytochemical Screening

Phytochemicals are chemical substances produced by plants via secondary or primary metabolism that typically have biological functions in the plant host, such as imparting,

scent, colour, and flavour; contribute to the growth of the plant; and aid in defence against rivals, pathogens like fungi, bacteria, and plant viruses; as well as against predators like insects (Molyneux *et al.,* 2007). Plants produce a wide array of chemical compounds broadly classified into primary and secondary metabolites. While primary metabolites such as carbohydrates, amino acids, and nucleotides are essential for growth and development, secondary metabolites are not directly involved in growth but play vital roles in plant defense, signaling, and ecological interactions (Bourgaud *et al.,* 2001). These compounds include alkaloids, phenolics, flavonoids, terpenoids, tannins, saponins, and glycosides, many of which possess pharmacological properties and are widely studied for their therapeutic potential. The scientific procedure of studying, scrutinizing, extracting, experimenting, and subsequently identifying various classes of phytoconstituents found in various portions of a plant is known as phytochemical screening. Generally, examination that focuses on determining whether a particular phytochemical is present or absent is referred to as qualitative phytochemical screening. Bioactive substances found in medicinal herbs that are most frequently detected are flavonoids, phenolic compounds, tannins, and alkaloids. Nonetheless, a variety of phytoconstituents with known antimicrobial activities that fall under the molecular categories of lectins, terpenoids, polyacetylenes, phenolics, and polypeptides have also been identified (Hill, 1952).

2.6.1 Alkaloids

A number of nitrogen atoms (in some cases amino or amido) are found in the structures of a vast family of chemical compounds called alkaloids, which are found in nature. They originate from amino acids and can be produced by plants and some animals as secondary metabolites (Kurek, 2019). Alkaloids are found in copious amounts in therapeutic herbs

and are often produced by many kinds of plants, primarily by blooming plants as well as from a few animals in some instances. They are kept at various levels in a plant's stem, leaves, fruits, and roots (Kurek, 2019). Alkaloids are used in traditional and modern medicine in quantities ranging from 25 to 75 percent, which highlights their enormous pharmacological importance and significant therapeutic value (Khan, 2016b; Pervez *et al.,* 2016). Alkaloids have demonstrated potent biological effects on both human and animal cells at extremely low doses, including antifungal, antibacterial, antiinflammatory, analgesic, anti-cancer, pain relief, local anaesthetic, neuropharmacological, and many more properties (Kurek, 2019). According to a study by Garba and Okeniyi (2012), alkaloids derived from various medicinal herbs in Nigeria demonstrated strong antifungal alongside antibacterial actions against gram-negative and grampositive microorganisms. Quinine, morphine, caffeine, strychnine, atropine, nicotine and ephedrine are examples of well-known alkaloids (Chisholm, 2015).

2.6.2 Tannins

Tannins, also known as tannoids or tannic acid, are a group of naturally occurring, astringent polyphenolic macromolecules in plants that attach to proteins and precipitate them, as well as to many additional organic substances including amino acids and alkaloids. They are present in a vast variety of plant species from all climatic regions, all over the world. The bark, leaves, wood, roots, buds, fruits, stems, seeds, and plant galls—pathological growth brought on by insect attacks—all contain tannins that can drain out of the plants (USDA, 2022). The tannins found in all of these plant parts may aid in controlling

plant development as well as protecting the plant itself from predators by serving as insecticides (Ferrell and Thorington, 2006).

Tannins that accumulate in tree bark shield the tree from bacterial or fungal infection. They also have an impact on human health and medicine. Tonsillitis, skin eruptions, haemorrhoids, and pharyngitis have all been treated with tannins owing to its astringent and styptic qualities. Internally, it has also been employed as an antidote for glycosidic, metallic, and alkaloidal poisons because it makes insoluble precipitates with these poisons (Britannica, 2022). Tannins have been discovered to have substantial significance as cytotoxic and anticancer agents in addition to being efficient against microbes like viruses, bacteria, yeasts, and fungi (Joshi *et al.,* 2013). By preventing the bacteria *E. coli* from attaching to the cells lining the urinary tract, tannins in *Vaccinium macrocarpon* (cranberries) have been scientifically shown to aid in preventing urinary tract infections in women (USDA, 2022).

2.6.3 Flavonoids

As a group of polyphenolic bioactive compounds found in plants, flavonoids are a significant class of naturally occurring low molecular weight chemicals that are widely consumed in human diets (Delage, 2015). They are chemical compounds that come from plants and are produced naturally. They can be found in different plant parts. More than 5000 naturally produced flavonoids from different plants were successfully discovered and classified on the basis of their chemical makeup (Ververidis *et al.,* 2007). Due to their significant presence in many different kinds of plant-based drinks and foods like fruits,

wine, vegetables, chocolate, and tea, they are sometimes referred to as dietary flavonoids. A wide range of health-promoting properties are attributed to flavonoids, which are crucial ingredients in many pharmaceutical, nutraceutical, cosmetic, and medical preparations. This is because they have the ability to influence important cellular enzyme processes and have antioxidative, anticarcinogenic, antimutagenic, and anti-inflammatory qualities (Panche *et al.,* 2016). Additionally, they participate in a range of biological processes in both animals and plants and have been demonstrated to possess insecticidal, antifungal, and antibacterial properties. (Abdel *et al.,* 2013). Furthermore, some flavonoids have suppressive activities against pathogens such as *Fusarium oxysporum* that affect plants (Galeotti *et al.,* 2008).

2.6.4 Saponins

Higher plants, microorganisms, and marine organisms all naturally contain saponins, which also sometimes called triterpene glycosides. Saponins are a complex and diverse class of structurally and chemically varied phytochemicals, due to their capacity of generating firm, soap-like bubbles in water-based environments. Since
saponins are fat and water soluble, when agitated in aqueous solutions, they generate foams that give them their helpful soapy features. They are employed in the synthesis of steroids and the production of soap, medicines, fire extinguishers, artificial dietary supplements, and carbonated drinks.

Saponins have been found to exhibit numerous physiological effects, like antiinflammatory, antifungal, antibacterial, antiviral, anti-cancer, anti-ulcer, haemolytic, hepatoprotective, cardioprotective, and immunoregulatory qualities (Zhang and Hu,

1985; Okubo *et al.,* 1994; Just *et al.,* 1998; Woldemichael and Wink, 2001; Cheng *et al.,* 2016; Song *et al.,* 2016). They are advertised commercially as food additives and are employed in ethnomedicine. Studies have demonstrated that saponin-rich fractions of medicinal plant extracts are efficient against a variety of bacteria, including *C. albicans, Salmonella typhi, E. coli,* and *Aeromonas hydrophila* (Hassan *et al.,* 2010; Deboshree and Roymon 2012).

2.6.5 Terpenoids

The most ubiquitous and varied in structure natural compounds present throughout numerous plants are terpenoids, sometimes referred to as isoprenoids. It has been confirmed by numerous *in-vitro*, preclinical, and clinical studies that this chemical family exhibits an extensive spectrum of crucial medicinal characteristics. Terpenoids enhance transdermal absorption; have anti-inflammatory, anti-bacterial, antiviral, antimalarial, and anticancer properties; prevent and treat cardiovascular disorders; and have hypoglycemic properties (Yang *et al.,* 2020). Terpenoids also offer a wide range of potential uses, including insect resistance, immune modulation, antioxidation, antiaging, and neuroprotection, according to earlier research (Yang *et al.,* 2020).

2.6.6 Glycosides

Glycosides are organic substances that come from plants or animals. Secondary metabolites from medicinal plants are abundant and have diverse pharmacological and biological functions. Glycosides are naturally occurring compounds among these metabolites, and they have exceptional medicinal value as well as applications (Khan *et al.,* 2019).

According to Khan *et al.* (2018), glycosides discovered in several plants showed strong cytotoxic effects on a variety of cancerous cell lines in early preclinical experiments. A 1970 investigation by Soulef *et al.* on the antimicrobial capabilities of glycoside concentrates showed potent inhibitory effects on various bacterial strains (Soulef *et al.,* 1970). This study showed that the antibacterial properties of glycoside extracts vary from one microbial species to another.

2.7 *In-vitro* antimicrobial testing

Antimicrobial susceptibility testing is a procedure used to specifically determine which type of antimicrobial product(s) will be effective against a particular microbe. It can be used to predict therapeutic outcomes, for drug discovery and epidemiology. Various laboratory techniques are available to assess an extract's or fraction's *in-vitro* antibacterial or antifungal effectiveness. The three categories of screening techniques now used to identify the antimicrobial activity of natural materials are dilution, diffusion, and bioautographic techniques. Diffusion and bioautographic methods are referred to as qualitative approaches since they can only provide an approximate indication of whether the compounds present have antimicrobial effects. Conversely, since they establish the minimal inhibitory concentration, dilution procedures are regarded as quantitative assays (Vanden and Vlietinck, 1991). The basic and most well-known antimicrobial susceptibility testing procedures include agar disk diffusion and broth dilution techniques.

2.7.1 Agar disk-diffusion technique

This technique is used in several clinical microbiology facilities for regular antibiotic susceptibility testing, and it's a 1940 invention (Heatley, 1994). To provide qualitative findings, the antibiogram here categorizes microorganisms into either resistant, intermediate, or susceptible (Jorgensen and Ferraro, 2009). However, due to the fact that a microbe's growth suppression does not always imply its death, this method fails to distinguish whether actions are bacteriostatic or bactericidal. Nonetheless, compared to other methods, this assay has lots of benefits, notably its ease of use, inexpensiveness, ability to screen many different types of bacteria and therapeutic medicines, as well as the straightforwardness of results in terms of interpretation and understanding (Balouiri *et al.,* 2016).

2.7.2 Broth dilution technique

The best techniques for determining minimum inhibitory concentration values are dilution procedures due to the fact that they give a means to roughly calculate the concentration of therapeutic substance that is under test. One of the simplest techniques for determining an antimicrobial's effectiveness is by using the broth micro- or macro dilution methods. The microdilution technique, however, has more benefits over the macro-dilution including its reproducibility, reduction in reagents and working area, which are all brought about by the downsizing of the test (Balouiri *et al.,* 2016). Other techniques include the time-kill test, ATP bioluminescence assay, antimicrobial gradient method (Etest), and flow cytofluorometric techniques. (Balouiri *et al.,* 2016). For an in-depth assessment of the

antimicrobial potency of a substance, time-kill tests and flow cytofluorometric techniques would be ideal because they show what kind of
action (bacteriostatic vs bactericidal) the substance exhibits, in addition to if the effect is dependent on time or dependent on concentration and finally, the extent to which destruction of cells in the test microorganism occurred (Balouiri *et al.,* 2016).

2.8 Toxicity testing

The term "toxicity" may refer to the extent to which a compound or chemical may adversely affect humans or animals. According to the International Union of Pure and Applied Chemistry (IUPAC), acute toxicity is the term used to describe the negative effects of a chemical that occur following only one dosage or after numerous dosages in a short period of time, typically 14 days post intake of the product (IUPAC, 2006). Since it is unethical to use human subjects for acute (or chronic) toxicity studies, presently there are mainly three types of toxicity models used in preclinical studies to evaluate a substance's toxicity. These include *in vitro*, *in silico,* and *in vivo* models.

2.8.1 *In vitro* toxicity models

These models utilize particular component(s) from a living system such as one cell, an organ, or a tissue and examine it in a controlled setting with little to no interference from the external or internal environment (Summerfield and Dong 2013). These models provide the benefits of reduced time and expense, but since they are simplified, one or more essential traits of the target organism are omitted, and the models do not adequately depict the organism (Madorran *et al.,* 2020).

2.8.2 *In silico* toxicity models

In silico, which is linked to the more well-known words *In vivo* and *In vitro* research, originally meant "performed on computer or through computer simulation," is a term used to describe experimental approaches carried out by computers in contemporary scientific research (Moore 2021). The main benefits of these toxicity models are their quick testing times and lower testing costs (Summerfield and Dong 2013). Again, *in silico* toxicity models do not give a thorough understanding of the biochemical and physiological functions occurring in the human body, thus making it difficult to create efficient algorithms to forecast a drug's toxicity (Jeong *et al.,* 2000).

2.8.3 *In vivo* toxicity models

In vivo toxicity examines the effects of various medications and substances on the health and behaviour of living organisms. According to Madorran *et al.* (2020), the *in vivo* toxicity model is the only one that takes into account an entire organism, with all biochemical and physiological reactions, and as such, it is the only one that is capable of offering information on how the drug is distributed within the body, in addition to potential reactions between medicinal products and organs other than the one being targeted. These models help to investigate the relationship between exposure to a certain substance or drug and toxic effect(s) in animal models, to explain potential toxic target organs and adverse reactions, to forecast the safety of humans and to provide credible toxicokinetic proof for subsequent clinical studies of the drug in humans (Creative Bioarray, 2023). It is considered that if adequately evaluated, the impact of any substance seen in laboratory animals also

applies to humans (Klaassen and Amdur 2013). Therefore, despite various drawbacks to today's *in vivo* toxicity experiments, particularly in light of the substantial biological variations between humans and other animals (Darwin and Wallace, 1998), *in vivo* studies probably offer the most accurate models for evaluating the toxic effects of drugs.

This study, therefore, employed in vivo models using Swiss albino mice to assess the acute toxicity profiles of selected herbal extracts. The primary objective was to determine the immediate toxicological effects following single-dose oral administration, including observations of behavioral changes, physiological responses, and mortality over a 14-day period. Parameters such as body weight, food and water intake, and organ appearance (gross pathology) were also monitored to evaluate potential adverse effects, thereby providing preliminary safety data essential for future therapeutic development. This study differed from prior work by offering a multifaceted evaluation of seven medicinal plants traditionally used in Kenya, combining phytochemical screening, *in vitro* antimicrobial testing, and *in vivo* acute toxicity assessment—an approach not often integrated in a single study. Unlike earlier research that often focused on individual aspects or isolated species, this study provided a broader and more comprehensive analysis of therapeutic potential and safety. While this study did not assess environmental or soil conditions, collecting plants from two ecologically distinct regions—Mt. Kenya and Mt. Elgon—added valuable context. Geographic variation is known to influence phytochemical content, so this distinction provided a valuable basis for comparing results with similar studies conducted in other regions and supported the need to consider geographical origin when validating or standardizing plant-based therapeutics.

Finally, this research contributed novel toxicity data for several understudied plants using Swiss albino mice, helping to address primary critical safety gaps in the traditional use of herbal remedies involving these plants.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Medicinal plant sampling was conducted in two ecologically distinct regions of Kenya: around Mount Elgon (western highlands) in the county of Bungoma, and Mount Kenya (central highlands) located within Meru and Tharaka Nithi Counties. These areas were selected based on ethnobotanical evidence indicating rich medicinal plant usage by local communities.

3.1.1 Position and location of Mount Elgon in Bungoma County

The County Bungoma is located within latitude 0° 28’ and latitude 1° 30’ north of the equator, and longitude 34° 20’ and 35° 30’ east of the Greenwich Meridian. It spans an area of 3032.4 km^2 (GoK, 2013b). The county shares frontiers with the following: Busia County to the west and southwest, the County Trans Nzoia to the northeast, Uganda to the northwest, and the County Kakamega, located toward the east and southeast (Figure 3.1a). There are two wet seasons: a brief rainy season from August to October and a lengthy rainy period from March to July. Due to different altitude levels, yearly temperatures range between 0°C and 32°C, with the lowest temperature (slightly less than 0°C) recorded at the highest peak of Mt. Elgon. The county’s altitude varies from about 1200 m above sea level in low zones to around 4,321 m at mountain borders (GoK, 2013b).

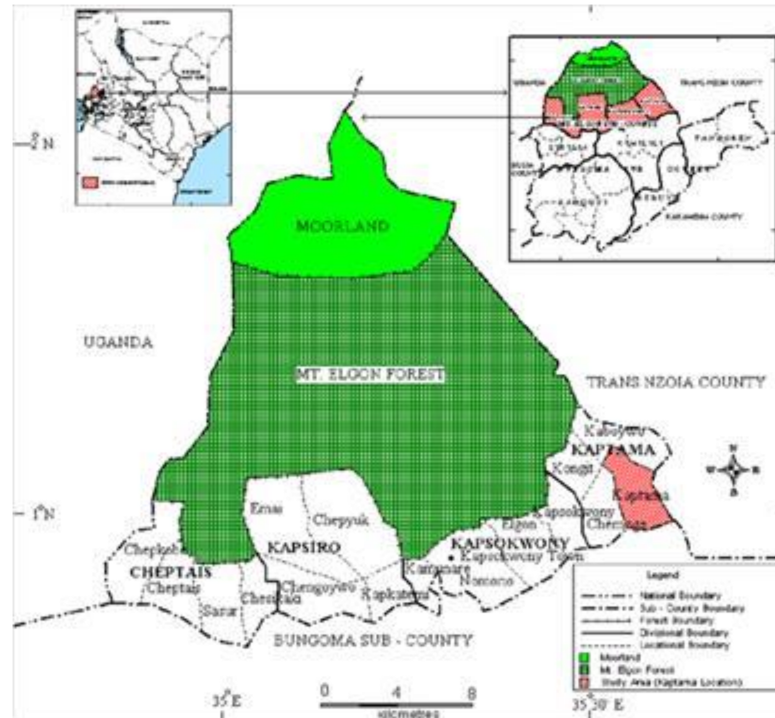

Figure 3.1a: Location of Mt. Elgon Subcounty in Bungoma County

3.1.2 Position and location of Mount Kenya in the Counties of Tharaka Nithi and Meru

Mount Kenya is found in Central and Eastern parts of Kenya, as shown in Figure 3.1b. It lies roughly 150 kilometers (90 miles) north-northeast of Nairobi, the country's capital, and 90 kilometers (55+7/8 mi) north of the equator (Map, 2006). There are two distinctive dry and rainy seasons each year, which are similar to the climatic conditions in the lowlands (Thompson, 1966). The weather along the mountain changes widely, ranging from 1,374 to 5,199 m (4,508 to 17,057 ft.) in height, and it has various affected areas. The Indian Ocean's impact causes the low, southeastern sides of the mountain to get the most humid weather. Frequent rainfall on these slopes favors the presence of a dense mountain forest (Spink, Lieut., 1945).

Figure 3.1b: Mount Kenya Region

3.2 Study design

This study employed a Completely Randomized Design for laboratory experiments to reduce bias and ensure uniform treatment allocation and also incorporated cross-sectional elements for comparative analysis of the different plant species. The study was structured in three sequential phases: phytochemical analysis, in-vitro antimicrobial assays, and invivo acute toxicity testing using Swiss albino mice.

3.3 Sampling of plant materials

A purposive sampling method was used, guided by traditional healers and local herbalists to identify and collect plant species commonly used in Folk medicine to treat microbial infections. The study involved the stem bark and/or roots of eight plant samples, comprising of seven different medicinal plant species. The stem barks and/or roots of *Carissa edulis, Acanthus ebracteatus, Albizia gummifera, Prunus africana, Combretum molle, Warbugia*

ugandensis, and *Cuscuta* spp. were randomly collected in dense areas of Mt. Elgon and Mt. Kenya regions. A plant taxonomist from the National Museum of Kenya, Nairobi, together with the local herbalists, helped in the identification of collected plant species. Voucher samples kept were at Kenyatta University's Department of Plant
Sciences herbarium.

3.4 Pretreatment of plant materials and crude extract preparations

Selected plant samples were brought to the microbiology laboratory at Kenyatta University, and each part was thoroughly washed under running water, had a distilled water rinse, and exposed to two to three weeks of air drying in the shade. Each sample was then cut into small pieces (figure 3.2) and finally ground into coarse powder using a grinding mill machine (Appendix I, picture 1). For approximately 48 hours, each powdery sample (300 g) was fully immersed in 1.5 L of laboratory methanol at ambient temperature (Appendix I, picture 2), with occasional swirling. The filtrates were separated from residues using Whatman № 1 filter papers and a vacuum pump. Liquids obtained were boiled down in a rotary evaporator at temperatures of 64–65°C and 120 rpm (Appendix I, picture 3), after which they were left to air dry at ambient temperature. Weights of obtained dry methanolic crude extracts were taken and then placed in storage at low temperatures (~5°C) for future use in the study (Gwee *et al.,* 2013).

Figure 3.2: Collected Plant Samples Cut into Small Pieces

Key: A= *Acanthus ebracteatus* (roots), **B**= *Acanthus ebracteatus* (stem bark), **C**= *Albizia gummifera*, **D**= *Carissa edulis*, **E**= *Combretum molle*, **F**= *Cuscuta* spp., **G**= *Prunus africana*, **H**= *W. ugadensis*

3.5 Polar and nonpolar extract preparations

Nonpolar solvents as well as polar solvents can be used to extract various plant-based chemicals having numerous pharmacological applications. Nonpolar alongside polar extracts of *C. edulis, A. ebracteatus, A. gummifera, P. africana, C. molle, W. ugandensis,* and *Cuscuta* spp. were obtained via sequential solvent–solvent partitioning of crude extracts at room temperature using separating funnels. Four different polarity solvents AR grade (Analytical Research Grade) chemicals were used; hexane, dichloromethane, ethyl acetate, and methanol. Previously obtained dried extracts were solubilized using 50 mL of distilled water, and poured into separating funnels. Equal volumes of hexane were then added into the funnels, and the mixtures were vigorously shaken. Upon standing, two distinct layers were formed (Appendix I, picture 4). The hexane layer for each extract was then carefully separated from the mixture and put into a beaker. After separation of the

hexane portion, 50 mL of DCM was sequentially poured into the water and then left to sit for some time. Later, the DCM portion became apparent and got removed, and the same procedure was performed using ethyl acetate and finally methanol. Each obtained polar and nonpolar extract was concentrated using a rotary evaporator, after which they were let to air dry at ambient temperature. A total of 32 dried polar and nonpolar extracts were obtained, and put into sterile Bijou bottles (Appendix I, picture 5) (Gwee *et al.,* 2013).

3.6 Culture media preparation

Mueller-Hinton Agar (MHA) was used for bacterial cultures, and Potato Dextrose Agar (PDA) for fungal cultures. For MHA, 38 g of dehydrated powder was dissolved in 1 L of distilled water, heated until clear, and autoclaved at 121°C for 15 minutes. After cooling to 45–50°C, the medium was poured into sterile Petri dishes (CLSI, 2020).

Similarly, 39 g of PDA was prepared in 1 L of distilled water, sterilized, and poured into plates under aseptic conditions (Atlas, 2010; Cappuccino & Welsh, 2019). All plates were stored at 4°C and checked for sterility before use.

3.7 Susceptibility test disc preparation

Whatman N°.1 filter papers were punched and used to make discs of diameter 6mm.

Resulting disc papers were put into universal bottles and sterilized by autoclaving at 121°C for 15 to 20 mins (Appendix I, picture 6) (CLSI, 2020). The resulting sterile discs were then impregnated with prepared 500 mg/ml stock solutions of *Carissa edulis, Acanthus ebracteatus, Albizia gummifera, Prunus africana, Combretum molle, Warbugia ugandensis,* and *Cuscuta* spp. by gradually infusing 20 µl of each extract into the discs with

the help of a micropipette (Balouiri *et al.,* 2016). Each extract was given to the discs time to absorb completely and was later placed in sterile petri dishes for them to dry over about 30 minutes (figure 3.3). These were aseptically done in a laminar flow cabinet to limit contamination. The dried impregnated discs were later used to test for antibacterial effectiveness on *E. coli* and *S. aureus*, as well as for potential antifungal efficacy against *C. albicans*.

Figure 3.3: Paper Punches Impregnated with Plant Extracts

3.8 Test microorganisms

Two standard bacterial strains and one standard fungal strain were used in this research: one gram positive strain (*S. aureus* ATCC 25923), one gram negative strain (*E. coli* ATTC 25922) and a yeast fungus (*C. albicans* ATTC 10231). Kenyatta University's microbiology labotarory provided all of the test microbes.

3.9 Qualitative phytochemical screening

This was done to find out whether major phytoconstituents, including alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids and phenols, were present in each sample. Crude

extracts previously obtained were utilized to determine the chemical compounds found within stem barks and/or roots of selected medicinal plants using standard methods with some modifications.

3.9.1 Testing for Alkaloids

Approximately 0.05 g of methanolic crude extract was put in 1 mL of 1% HCl, gently swirled, and warmed using a hot water bath. Two to three drops of Mayer's reagent (mercuric chloride mixed with potassium iodide dissolved in water) was then added into the mixture and observed. Alkaloids were detected by the occurrence of a cream-colored precipitate (Evans and Trease, 2009; Savithrama *et al.,* 2012).

3.9.2 Testing for Flavonoids

Screening flavonoids in each extract was done using the Shinoda test. In 1 mL of the solvent methanol, 0.05 g of extract was disintegrated and the mixture was warmed in a hot water bath. To this mixture, 2 mL of 1% HCl was poured, after which 3 pieces of magnesium ribbon were added. Flavonoids were present in some extracts, as evidenced by the development of a pink/red color (Trease and Evans, 2002).

3.9.3 Testing for Tannins

One millilitre of distilled water was added to 0.05 g of each extract. The combination was then given some drips of ferric chloride solution (1%), and observations were made. Colours that suggested tannins were present were either blue, blue-green, blue-black or green. (Trease and Evans, 2002).

3.9.4 Testing for Saponins

About 0.05 g of each plant's methanolic crude extract was combined with 2 mL of distilled water, warmed over a hot water bath and then allowed to cool. The resulting mixture was then shaken vigorously. The development of a steady foam served as proof that saponins were present (Evans and Trease, 2009; Savithrama *et al.,* 2012).

3.9.5 Testing for Glycosides

In a test tube, approximately 0.5 ml of each extract was mixed with 2 ml of chloroform and shaken. Concentrated sulphuric acid (a few drops) was added into the mixture and observed. Appearance of a reddish-brown steroid ring confirmed glycoside's presence (Usman *et al.,* 2017).

3.9.6 Testing for Terpenoids

To detect the presence or absence of terpenoids, Salkowski test was employed. Approximately 5 mL of each extract was mixed with 2 mL of chloroform and then 3 mL of concentrated sulphuric acid. Formation of a reddish-brown coloration at the interface of the formed layer was indicative of the presence of terpenoids (Harborne, 1998; Siddiqui *et al.,* 2009).

3.9.7 Testing for Phenols

About 0.05 g of each plant extract was dissolved in 1 mL of methanol. A few drops of 10% lead acetate solution was then added to the mixture and observed. Appearance of white precipitates was evidence of the presence of phenolic compounds (Kokate, 2005).

3.10 Standard inocula preparation

Few distinct colonies of microbes under investigation were picked with the help of an inoculating loop (sterile). In a test tube, each microorganism was thoroughly suspended in 2 mL of sterile 0.9% saline solution. Each suspension's turbidity was then regulated up to a 0.5 McFarland standard (this corresponds to a bacterial concentration of about 10^8 CFU/mL and 10^7 CFU/mL for yeasts) (Jan, 2009)

3.11 Animal experimental model preparation

A total of 50 healthy young adult nulliparous, nonpregnant mice of the same sex (females) were used. Test animal models were all aged between 8 and 12 weeks old, with an average weight per group of about 22-26 g at the commencement of the study. The mice were provided by Kenya Medical Research Institute (KEMRI), Kenya. Prior to dosing, test animals are randomly selected, marked using a marker pen and picric acid to allow independent identification of each mouse/group of mice, and kept in cages for 7 days to allow for acclimatization to their new environment. The animal models were kept in a laboratory room having a temperature of about 22ºC (+ 3ºC) and an artificial light source to provide 12 h of darkness and 12 h of lighting per day (OECD, 2001). The mice were fed with conventional laboratory diets and provided with an unlimited supply of drinking water in mouse water bottles.

The approval of all experimental methods was granted by the Kenyatta University Animal Use Research Ethics Committee, Kenya. Selected plant extracts were assessed for their acute toxicities in female Swiss albino mice as per the Organization for Economic Cooperation and Development (OECD) guidelines number 423 with few minor changes.

3.12 Antimicrobial susceptibility testing

3.12.1 Kirby-Bauer disc diffusion method

About 0.5 g of each extract was mixed with 1000 μL sterile dimethyl sulfoxide solution (DMSO; 5% in water) to prepare a stock solution with 500 mg/mL as the final concentration. A few dried extract-impregnated filter paper discs (polar and nonpolar extracts) were aseptically placed on the surface of Mueller-Hinton plates that had already been loaded with each bacterial (*E. coli* (ATTC 25922), and *S. aureus* (ATCC 25923)) inoculum and on PDA plates that had been loaded with the *C. albicans* (ATTC 10231) inoculum. Diameters of zones of inhibition were measured after 24 h of incubation and noted in millimeters (Appendix I, picture 7). Each extract was tested in triplicate. Positive controls used for this experiment were ciprofloxacin for bacterial pathogens and fluconazole for the fungal microbes. Dried paper discs impregnated with sterile 5% DMSO solution served as negative controls. Effectiveness was conferred only to extracts that inhibited microbial growth with a mean zone of inhibition equal to or greater than 10 mm (Ajaiyeoba and Sama, 2006).

3.12.2 Minimum inhibitory concentrations (MICs)

The smallest dose of an antimicrobial product that is capable of preventing the growth of a microbe was determined to find the lowest concentration of each extract that was capable of impeding the growth of *E. coli*, *S. aureus*, and *C. albicans*. Determination of MICs was done only for extracts that produced a mean zone of inhibition of at least 10 mm from the disc diffusion assay. This was achieved by the broth microdilution technique utilizing 96-

well microtiter plates. Prior to the bioassay, each extract was solubilized using 5% DMSO solution. Two hundred microliters (200 µl) of each crude extract at a concentration 500 mg/ml was dispensed in the first wells using a micropipette. One hundred microliters (100 µl) of 5% DMSO solution was then poured into all the other wells of the 96-well microtiter plates. A twofold serial dilution was then made up to the 8th wells as described in the modified procedure of Wiegand and the CLSI guidelines (Wikler *et al.,* 2011). Finally, 100 µl of the 8th wells' (last wells) content was drawn and discarded. The 9th wells served as growth control wells, in which no extract was added. Each well thus had a uniform volume of 100 µl, with concentrations ranging from 500 mg/ml to 3.91 mg/ml. Previously sterilized paper discs, 6 mm in diameter were then impregnated with 20 µl of each dilution. A 0.5 McFarland broth inoculum was prepared and inoculated onto sterile media (MHA and PDA for bacteria and *Candida* respectively). The discs were then inoculated on the surface of petri dishes containing the pure fungal/bacterial lawn, and incubated for 24 hours at 37ºC for bacteria and 24-72 hours at 37ºC for *Candida*. Each test was performed in triplicate. The antimicrobial activity of each concentration was demonstrated by the formation of clear zones of inhibition (ZOIs) around the discs following incubation. Diameters of zones of inhibition were measured in millimeters (mm) using a transparent ruler and noted. MIC values were then obtained by matching the minimum diameter of the zone of inhibition with the lowest concentration of the extracts at which microbial growth was suppressed (Abuto *et al.,* 2016).

3.12.3 Minimum bactericidal/fungicidal concentrations

To find out if growth suppression was reversible and which concentration of extract totally killed the microbes, MBCs and MFCs were researched. Determination of the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) aimed at obtaining the smallest dose of each extract that was capable of completely killing the test bacteria and fungus respectively. MBC and MFC values were obtained using data from the MIC assay. A 0.5 McFarland broth inoculum was prepared and inoculated onto sterile media (MHA for bacteria and PDA for *C. albicans*). Contents of the last wells (impregnated on sterile paper discs) that produced observable diameters of inhibition zones similar to those of negative growth control wells were aseptically put on culture plates containing *C. albicans* and test bacteria inoculum. The concentration of extracts that gave no observable growth after incubation for 24 h at 37°C was noted as
MBC or MFC (Irkin and Korukluaglu, 2006).

3.13 Toxicity testing

3.13.1 Acute oral toxicity

This refers to unfavorable reactions that happen after taking a substance orally either once or several times within a short period. A toxicity assay was only carried out for plant extracts that showed significant bactericidal/fungicidal activities at concentrations ≥ 250 mg/ml. A total of 3 extracts (*W. ugadensis* DCM, *A. gummifera* ethyl acetate, and *P. africana* methanol) were tested for toxicity *in vivo* using mouse models. This test was performed using the Acute Toxic Class (ATC) method which involved using healthy female, nulliparous and nonpregnant mice as described by the OECD guidelines 423 (OECD, 2001). For each extract, Swiss albino mice were randomly selected, labeled

(Appendix I, picture 9), and grouped into three groups comprising 5 mice each (individual groups corresponding to a particular extract concentration) and then kept in their cage for 7 days prior to dosing to allow acclimatization to the laboratory conditions (Appendix I, picture 8). All groups of mice were kept on fasting overnight prior to administration of the extracts. Following the fasting period, the mice were all weighed (Appendix I, picture 9), and doses were calculated based on their body weight. Extracts were then prepared using 5% DMSO solution. Using a gavage tube, 0.1 mL of each test extract was administered orally at concentrations of 500 mg/kg (group I), 866 mg/kg (group II), and 1500 mg/kg (group III) body weight (Appendix I, picture 9). The control group (group IV) was treated with 0.1 mL of 5% DMSO solution only. Following extract administration, the mice were kept under careful surveillance for 1 h and occasionally for 4 h. They were then observed every 24 hours for the subsequent 14 days. Weights were taken every week, and clinical observations were made throughout the study period, including death and physical and behavioral changes. Finally, on the last day, the mice were again fasted, their final weight recorded, and macroscopic physical examinations were carried out (Million *et al.,* 2019). They were later anesthetized using diethyl ether impregnated on a cotton ball within a conical tube, such that the mice did not come into direct contact with the cotton. The induction process took about 5 to 10 minutes, and the animals were euthanized. Blood samples were obtained via the cardiac puncture method (Appendix I, picture 10). The collected blood was partitioned into two parts: one part was put in red serum vials for biochemical tests, while the other part was poured in EDTA tubes for hematology tests. A sharp scalpel was then used to remove about 2 mm of tissue from the mouse tail tips, and by gently pressing the tails, blood was collected directly on glucose test strips, and their blood glucose values were recorded (Appendix I, picture 11). Their organs (liver, kidney,

spleen, lungs, and heart) were then removed and weighed to estimate their relative organ weights. Their bodies were later incinerated in an incinerator.

3.14 Data analysis

Tests were performed in triplicate, and the data collected were transferred to Microsoft Excel sheets. SPSS software, version 22, was used to analyze diameter readings of zones of inhibition and concentration values, where descriptive statistics were carried out to obtain their mean values and results were given with regards to mean and standard error of mean (mean ± SEM). One-way ANOVA was then utilized to compare the mean MICs of each extract against selected test microorganisms. Significant differences between the concentration values and mean MICs of various plant extracts were ascertained using post-hoc analysis (Tukey's HSD test) (Kebede *et al.,* 2021). For the toxicity assay, oneway ANOVA with Tukey's HSD test was applied to assess mean parameter values and significant differences between groups. Figures obtained are displayed as the mean ± standard error of the mean (SEM). P values < 0.05 were considered significant (Yeo *et al.,*2012).

CHAPTER FOUR

RESULTS

4.1 Qualitative phytochemical screening

Each plant sample was tested for the presence or absence of alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids and phenols using standard methods with some modifications. The results obtained were then recorded as shown in Table 4.1. *A. gummifera* and *Cuscuta* spp. revealed the presence of all tested bioactive compounds. *W. ugadensis* and *P. africana* also indicated the presence of almost all tested phytochemicals with the exception of flavonoids and glycosides respectively (Table 4.1). No alkaloids were detected in the stem bark and roots of *A. ebracteatus*, however, the stem bark also lacked flavonoids (Table 4.1). Both alkaloids and glycosides were absent in *C. molle*, and in *C. edulis* extract, saponins, flavonoids, and alkaloids were also not detected (Table 4.1).

A notable limitation of using methanol for obtaining crude extracts was that methanol is less effective at extracting certain compounds, such as glycosides, which are often sparingly soluble in it. This likely explains the absence of glycosides in the *P. africana* extract, despite literature reports confirming their presence in the species. Thus, while methanol is suitable for general screening, it may not yield a complete phytochemical profile.

Table 4.1: Qualitative phytochemical screening of medicinal plant samples

Phytoconstituents	**Plant Samples**							
	W. ugadensis	***A. ebracteatus* (SB)**	***A. ebracteatus* (R)**	*C. edulis*	***P. africana***	***C. molle***	***A. gummifera***	***Cuscuta* spp.**
Saponins	+	+	+	-	+	+	+	+
Phenols	+	+	+	+	+	+	+	+
Flavonoids	-	-	+	-	+	+	+	+

Terpenoids	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	-	-	+	+
Alkaloids	+	-	-	-	+	-	+	+
Tannins	+	+	+	+	+	+	+	+

Key: (+) = detected, (-) = Not detected, SB= Stem bark, R= Roots

4.2 Antimicrobial screening

A total of 32 plant extracts at a concentration 500 mg/ml impregnated on sterile paper discs were tested against standard strains of selected microorganisms; *Escherichia coli, Staphylococcus aureus,* and *Candida albicans*. Bacterial and fungal suspensions of turbidity 0.5 McFarland standard were used, and each test was performed in triplicate.

4.2.1 Efficacy testing using Kirby-Bauer disc diffusion method

All the tested extracts exhibited varied diameters of zones of inhibition against selected bacteria and fungus, corresponding to different antimicrobial effects of the extracts. Compared to all other extracts, *W. ugadensis* DCM showed the largest average zones of inhibition against all tested microorganisms; *S. aureus* (15.67±0.33 mm), *E. coli* (10.00±0.57 mm) and *C. albicans* (21.00±0.58 mm) (table 4.2).

4.2.1.1 Efficacy testing using Kirby-Bauer disc diffusion method against *S. aureus*

W. ugadensis DCM and *P. africana* ethyl acetate and methanolic extracts showed significantly larger zones of inhibition compared to all other extracts against *S. aureus* ($p<0.05$; Table 4.2). The average zones of inhibition produced by *W. ugadensis* methanol and hexane, *C. molle* methanol, and *A. gummifera* ethyl acetate and methanolic extracts

against *S. aureus* were all significantly similar (p>0.05; Table 4.2). On the other hand, the antibacterial activity exhibited by the *A. gummifera* hexane extract was comparable to those of both *A. gummifera* and *P. africana* ethyl acetate extracts (p>0.05; Table 4.2). The positive control (ciprofloxacin) exhibited a significantly higher antibacterial activity of 32.33±0.33 mm against *S. aureus* compared to all tested extracts while the negative control had no activity (p<0.05; Table 4.2). Extracts with zones of inhibition significantly similar to that of the negative control were disregarded in subsequent tests.

4.2.1.2 Efficacy testing using Kirby-Bauer disc diffusion method against *E. coli*

Compared to all extracts tested against *E. coli*, *W. ugadensis* DCM was the only extract that showed a significant antibacterial activity of 10.00±0.57 mm (p<0.05; Table 4.2). Again, the positive control (ciprofloxacin) had a significantly higher activity, as demonstrated by a larger zone of inhibition of 31.00±0.58 mm compared to the other tested extracts (p<0.05; Table 4.2). All the remaining extracts, however, were noted to have diameters of growth inhibition significantly similar to that of the negative control
(DMSO) (p>0.05; Table 4.2) and thus were ignored in subsequent tests.

4.2.1.3 Efficacy testing using Kirby-Bauer disc diffusion method against *C. albicans*

When tested against *C. albicans*, *W. ugadensis* DCM again showed the highest antifungal activity compared to other extracts with an inhibition zone of 21.00±0.58 mm (p<0.05; Table 4.2), which was significantly similar to that demonstrated by the positive control fluconazole (p>0.05; Table 4.2). The hexane extract of *W. ugadensis* also exhibited moderate activity with a zone of inhibition of 11.33±0.33 mm. The remaining extracts,

however, did not show any antifungal effect against *C. albicans* and as such were not considered in subsequent tests. They all had an average zone of inhibition significantly similar to that of the negative control ($p>0.05$: Table 4.2).

Table 4.2: Average diameter of zones of inhibition in millimeter of selected plant extracts against *S. aureus, E. coli,* and *C. albicans*

Treatments (Plant Extracts)	**Inhibition/mm ± SE Mean**		
	S. aureus	*E. coli*	*C. albicans*
A. ebracteatus (R) DCM	8.33 ± 0.33^{f}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
A. ebracteatus (R) EA	7.67 ± 0.33^{fg}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
A. ebracteatus (R) Hex	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
A. ebracteatus (R) MeOH	7.00 ± 0.00^{fgh}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
A. ebracteatus (SB) DCM	7.00 ± 0.00^{fgh}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
A. ebracteatus (SB) EA	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
A. ebracteatus (SB) Hex	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
A. ebracteatus (SB) MeOH	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
A. gummifera DCM	6.33 ± 0.33^{gh}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
A. gummifera EA	$\mathbf{12.33 \pm 0.33^{de}}$	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
A. gummifera Hex	$\mathbf{13.33 \pm 0.33^{cd}}$	6.67 ± 0.00^{c}	6.00 ± 0.00^{c}
A. gummifera MeOH	$\mathbf{11.67 \pm 0.33^{e}}$	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
C. edulis DCM	7.00 ± 0.00^{fgh}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
C. edulis EA	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
C. edulis Hex	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
C. edulis MeOH	7.00 ± 0.00^{fgh}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
C. molle DCM	7.67 ± 0.33^{fg}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
C. molle EA	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
C. molle Hex	6.33 ± 0.33^{gh}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}

C. molle MeOH	**11.67 ± 0.33^{e}**	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
Cuscuta spp DCM	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
Cuscuta spp EA	7.00 ± 0.00^{fgh}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
Cuscuta spp Hex	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
Cuscuta spp MeOH	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
P. africana DCM	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
P. africana EA	**14.67 ± 0.33^{bc}**	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
P. africana Hex	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
P. africana MeOH	**15.33 ± 0.33^{b}**	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
W. ugadensis DCM	**15.67 ± 0.33^{b}**	**10.00 ± 0.57^{b}**	**21.00 ± 0.58^{a}**
W. ugadensis EA	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
W. ugadensis Hex	**12.00 ± 1.00^{e}**	7.00 ± 0.00^{c}	**11.33 ± 0.33^{b}**
W. ugadensis MeOH	**11.00 ± 0.58^{e}**	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
Negative Control	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
Positive control	32.33±0.33^{a}	31.00±0.58^{a}	22.33±0.33^{a}

Key: R= roots, SB= stem bark, DCM= dichloromethane, EA= ethyl acetate, Hex= hexane, MeOH= methanol, mm= millimeter, SE Mean= standard error of mean, Superscripts= Grouping Information using the Tukey`s HSD test and 95% Confidence.

Values with similar lowercase superscript letters are not significantly different column-wise using one-way ANOVA and Tukey's multiple comparison ($p>0.05$)

4.2.2 Efficacy testing using minimum inhibitory concentrations

As the extracts did not follow standardized dosing or pharmacokinetics, susceptibility classifications (e.g., resistant, intermediate, susceptible) based on CLSI breakpoints were not applied. Instead, extracts that showed antimicrobial activities (Zone of inhibition ≥ 10 mm) from the disk diffusion assay were considered indicative of significant antimicrobial activity, consistent with previous phytochemical screening studies. These extracts were further tested to determine MICs values using the broth dilution method. A total of 9 plant extracts were tested against *S. aureus*, 1 extract against *E. coli*, and 2 extracts against *C. albicans*. For each extract concentration, tests were performed in triplicate alongside paper punches/discs (6 mm in diameter) pre-soaked with 5% DMSO solution which served as

negative controls. Ciprofloxacin (30 mcg) and fluconazole (25 mcg) served as positive controls for bacteria and fungus respectively.

4.2.2.1 Minimum inhibitory concentration of plant extracts against *S. aureus*

All tested plant extracts exhibited antibacterial activity against *S. aureus,* with varied zones of inhibition (Table 4.3) (Plate 4.1). *W. ugadensis* DCM exhibited inhibitory activity up to the fourth dilution (31.25 mg/ml), wherein at concentrations of 500, 250, and 125 mg/ml the effects were significantly higher compared to those at subsequent dilutions (62.5, 31.25, 15.62, 7.82, and 3.91 mg/ml) ($p<0.05$; Table 4.3). The reference drug ciprofloxacin (30 mcg) caused a significantly larger zone of inhibition and thus had higher antibacterial activity against *S. aureus* compared to *W. ugadensis* DCM extract at all tested concentrations. The zone of inhibition exhibited by the negative control (DMSO) was similar to those of the extract at concentrations of 15.62, 7.81 and 3.91 mg/ml ($p>0.05$; Table 4.3).

Table 4.3: Minimum inhibitory concentration average diameter of zones of inhibition against *S. aureus*

Concentration/ mg/ml	**Inhibition/mm ± SE Mean**								
	W.u DCM	*W.u* MeOH	*W.u* Hex	*P.a* MeOH	*P.a* EA	*A.g* MeOH	*A.g* Hex	*A.g* EA	*C.m* MeOH
500	$16.33±0.33^{b}$	$9.67 ±0.33^{b}$	$\mathbf{11.00±0.58^{b}}$	$12.33±0.33^{b}$	$11.67±0.33^{b}$	$12.33±0.33^{b}$	$12.33±0.33^{b}$	$12.67±0.33^{b}$	$10.67±0.33^{b}$
250	$15.67±0.33^{bc}$	$\mathbf{8.33± 0.33^{c}}$	$7.33±0.33^{c}$	$12.33±0.33^{b}$	$10.33±0.33^{c}$	$11.67±0.33^{bc}$	$11.00±0.58^{bc}$	$11.67±0.33^{bc}$	$\mathbf{9.33±0.33^{c}}$
125	$15.33±0.33^{bc}$	$6.00± 0.00^{d}$	$7.00±0.00^{c}$	$10.33±0.33^{c}$	$\mathbf{8.33±0.33^{d}}$	$10.67±0.33^{c}$	$10.67±0.33^{cd}$	$10.67±0.33^{c}$	$6.67±0.33^{d}$
62.5	$14.33±0.67^{c}$	$6.00 ±0.00^{d}$	$7.00±0.00^{c}$	$9.33±0.33^{c}$	$6.67±0.33^{e}$	$9.33±0.33^{d}$	$9.33±0.33^{de}$	$10.33±0.33^{c}$	$6.00±0.00^{d}$
31.25	$\mathbf{12.33±0.67^{d}}$	$6.00± 0.00^{d}$	$6.33±0.33^{c}$	$\mathbf{8.00±0.00^{d}}$	$6.00±0.00^{e}$	$\mathbf{8.00±0.00^{e}}$	$\mathbf{8.67±0.33^{ef}}$	$\mathbf{8.33±0.33^{d}}$	$6.00±0.00^{d}$
15.62	$7.00 ± 0.00^{e}$	$6.00 ±0.00^{d}$	$6.33±0.33^{c}$	$6.00±0.00^{e}$	$6.00±0.00^{e}$	$7.00±0.00^{ef}$	$7.33±0.33^{fg}$	$6.33±0.33^{e}$	$6.00±0.00^{d}$
7.81	$7.00 ± 0.00^{e}$	$6.00± 0.00^{d}$	$6.00±0.00^{c}$	$6.00±0.00^{e}$	$6.00±0.00^{e}$	$6.33±0.33^{f}$	$6.00±0.00^{g}$	$6.00±0.00^{e}$	$6.00±0.00^{d}$
3.91	$6.00 ± 0.00^{e}$	$6.00±0.00^{d}$	$6.00±0.00^{c}$	$6.00±0.00^{e}$	$6.00±0.00^{e}$	$6.00±0.00^{f}$	$6.00±0.00^{g}$	$6.00±0.00^{e}$	$6.00±0.00^{d}$
Negative Control	$6.00 ± 0.00^{e}$	$6.00±0.00^{d}$	$6.00±0.00^{c}$	$6.00±0.00^{e}$	$6.00±0.00^{e}$	$6.00±0.00^{f}$	$6.00±0.00^{g}$	$6.00±0.00^{e}$	$6.00±0.00^{d}$
Positive Control	$32.33±0.33^{a}$	$32.33±0.33^{a}$	$32.33±0.33^{a}$	$32.33±0.33^{a}$	$32.33±0.33^{a}$	$32.33±0.33^{a}$	$32.33±0.33^{a}$	$32.33±0.33^{a}$	$32.33±0.33^{a}$

Values with similar lowercase superscript letter are not significantly different column-wise using one way ANOVA and Tukey's multiple comparison ($p>0.05$).

Key: *W.u= W. ugadensis, P.a= P. africana, A.g= A. gummifera, C.m= C. molle,* DCM= dichloromethane, EA= ethyl acetate, Hex= hexane, MeOH= methanol, mm= millimetre, SE Mean= standard error of mean, Superscripts= Grouping Information using the Tukey Method and 95% Confidence.

W. ugadensis methanolic extract only exhibited antibacterial activity up to a concentration of 250 mg/ml, with the highest effect observed at a concentration of 500 mg/ml ($p<0.05$; Table 4.3). The zone of inhibition of the negative control was significantly comparable to that of the extract at concentrations of 125, 62.5,31.25,15.62, 7.81, and 3.91 mg/ml. On the other hand, the effect of the positive control (ciprofloxacin) was significantly higher compared to all tested concentrations of *W. ugadensis* methanolic extract

At a concentration of 500 mg/ml, *W. ugadensis* hexane showed antibacterial activity against *S. aureus* with an average inhibition zone of 11.00±0.58 mm, but at higher dilutions, no antimicrobial effect was observed ($p<0.05$; Table 4.3). Again, the positive control had significantly higher activity than that of the extract at all tested concentrations. The negative control's effect was observed to be significantly similar to that of the hexane extract of *W. ugadensis* at concentrations of 250, 125, 62.5, 31.25,

15.62, 7.81, and 3.91 mg/ml ($p>0.05$; Table 4.3).

The antibacterial activity of the *P. africana* methanolic extract against *S. aureus* at a concentration of 500 mg/ml was significantly similar to that observed at a concentration of 250 mg/ml ($p>0.05$; Table 4.3), which in turn was significantly higher than those of subsequent dilutions. At concentrations of 125 and 62.5 mg/ml, the extract was noted to have a significantly commensurate inhibitory ability against *S. aureus*. Once more, the positive control (ciprofloxacin) caused a significantly larger zone of inhibition in comparison with all tested concentrations of *P. africana* methanolic extract, whereas the negative control (DMSO) exhibited an inhibitory action significantly comparable to that of the extract at concentrations of 15.62, 7.81, and 3.91mg/ml ($p>0.05$; Table 4.3). *P. africana* ethyl acetate extract showed antibacterial activity against *S. aureus* up to a concentration of 125 mg/ml, with a larger zone of inhibition of 11.67±0.33 noted at 500 mg/ml ($p<0.05$;

Table 4.3). At concentrations of 62.5, 31.25, 15.62, 7.81, and 3.91 mg/ml, the extract demonstrated no antibacterial potential, with zones of inhibition significantly similar to that of the negative control. Compared to the positive control (ciprofloxacin), the effect of all tested concentrations of *P. africana* ethyl acetate against *S. aureus* was significantly lower. The antibacterial activity exhibited by the *A. gummifera* methanolic extract against *S. aureus* was higher at concentrations of 500 and 250 mg/ml, with both exhibiting significantly similar zones of inhibition. However, the highest inhibitory effect was caused by the positive control (ciprofloxacin), with an average zone of inhibition of 32.33±0.33 mm. The negative control (DMSO) had no activity against *S. aureus* and exhibited a zone of inhibition significantly comparable to that of *A. gummifera* methanolic extract at concentrations of 15.62, 7.81, and 3.91 mg/ml ($p>0.05$; Table 4.3). At concentrations of both 500 and 250 mg/ml, the hexane extract of *A. gummifera* exhibited significantly similar activity against *S. aureus*. The zones of inhibition produced by the extract at concentrations of 125 and 62.5 mg/ml were also significantly the same. However, compared to all tested concentrations, the positive control (ciprofloxacin) exhibited a significantly higher antimicrobial activity. Extract concentrations of 15.62, 7.81, and 3.91 mg/ml had no effect against *S. aureus* and exhibited zones of inhibition significantly comparable to that of the negative control
(DMSO) ($p>0.05$; Table 4.3).

Comparing all tested dilutions of *A. gummifera* ethyl acetate extract, higher antibacterial potential against *S. aureus* was achieved at a concentration of 500mg/ml, which was significantly similar to the effect observed at 250 mg/ml ($p>0.05$; Table 4.3). Zones of inhibition recorded at concentrations of 250, 125, and 62.5 mg/ml were all significantly

comparable to one another ($p>0.05$; Table 4.3). Again, all tested concentrations of *A. gummifera* ethyl acetate demonstrated a significantly lower activity compared to the positive control (ciprofloxacin) ($p<0.05$; Table 4.3). The negative control (DMSO) on the other hand, had an average zone of inhibition significantly similar to those of the extract at concentrations of 15.62, 7.81, and 3.91 mg/ml ($p>0.05$; Table 4.3).

The *C. molle* methanolic extract only showed activity against *S. aureus* up to the first dilution (250 mg/ml), with a higher antibacterial effect observed at a concentration of 500 mg/ml. The reference drug ciprofloxacin (30 mcg) produced the highest inhibitory activity compared to those of all extract concentrations ($p<0.05$; Table 4.3). Dilutions with concentrations of 125, 62.5, 31.25, 15.62, 7.81, and 3.91 mg/ml showed no effect against *S. aureus* and exhibited zones of inhibition significantly similar to that of the negative control (DMSO).

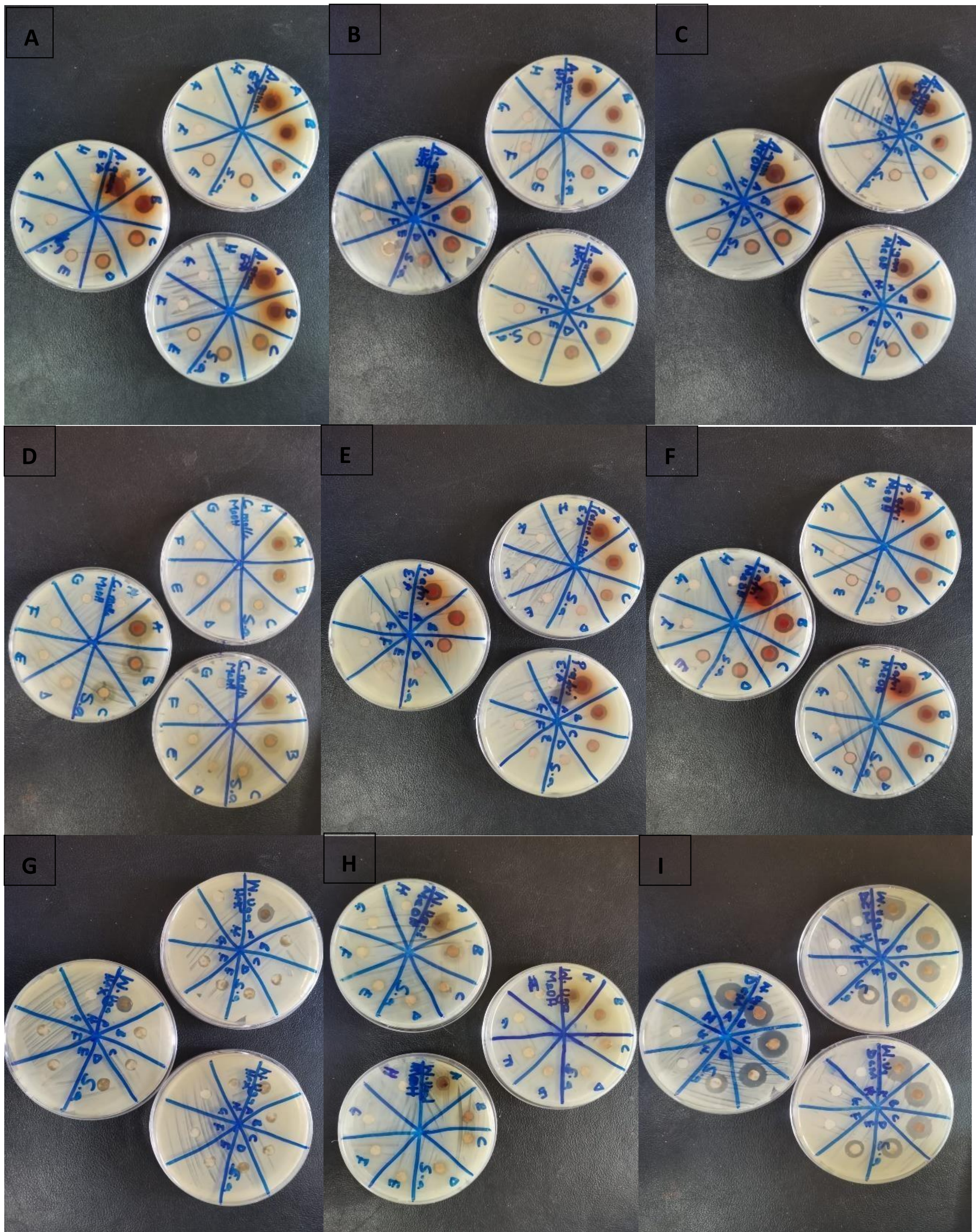

Plate 4.1: MIC results of selected plant extracts against *S. aureus*.

Key: A= *A. gummifera* ethyl acetate extract, **B**= *A. gummifera* hexane extract, **C**= *A. gummifera* methanol extract, **D**= *C. molle* methanol extract, **E**= *P. africana* ethyl acetate extract, **F**= *P. africana* methanol extract, **G**= *W. ugadensis* hexane extract, **H**= *W. ugadensis* methanol extract, **I**= *W. ugadensis* DCM extract.

Letters A-H refer to the different concentrations of the extracts in descending order

4.2.2.2 Minimum inhibitory concentration of plant extracts against *E. coli*

W. ugadensis DCM was the only extract tested for MIC against *E. coli* (Plate 4.2). The extract only inhibited the growth of *E. coli* up to a concentration of 250 mg/ml. Higher activity was observed at a concentration of 500 mg/ml with an average zone of inhibition of 10.33±0.33 mm, which was significantly larger than those observed at other concentrations ($p<0.05$, Table 4.4). At concentrations of 125, 62.5, 31.25, 15.62, 7.81, and 3.91 mg/ml the extract showed no inhibitory activity against *E. coli* and exhibited zones of inhibition significantly comparable to that of the negative control (DMSO) ($P>0.05$, table 4.4). The positive control ciprofloxacin (30 mcg) however, demonstrated the highest antimicrobial effect compared to all tested concentrations of *W. ugadensis* DCM, with an average zone of inhibition of 31.00±0.58 mm ($p<0.05$, table 4.4)

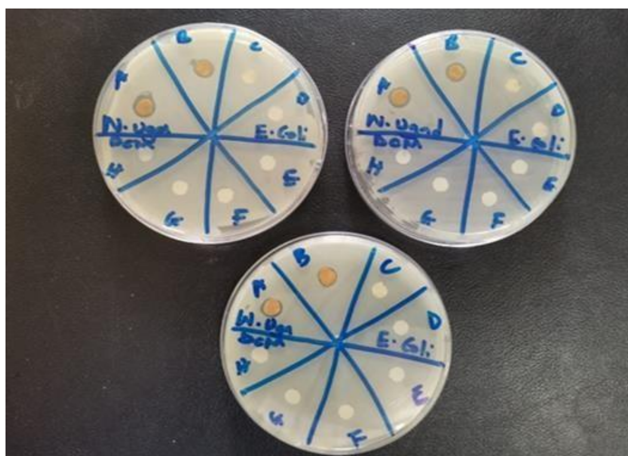

Plate 4.2: MIC results of *W. ugadensis* DCM extract against *E. coli*

Letters A-H refer to the different concentrations of the extracts in descending order

4.2.2.3 Minimum inhibitory concentration of plant extracts against *C. albicans*

Both *W. ugadensis* DCM and hexane extracts were tested for MICs against *C. albicans* (Plate 4.3). *W. ugadensis* DCM showed very good antifungal activity against *C. albicans* up to the second dilution (125 mg/ml). The highest inhibitory effect was observed at a concentration of 500 mg/ml, with an average zone of inhibition significantly larger than those of subsequent concentrations ($p<0.05$, Table 4.5). At this concentration (500 mg/ml), the extract was interestingly observed to have an antifungal action significantly similar to that of the positive control fluconazole (25 mcg). Extract concentrations below 125 mg/ml showed no antifungal activity against *C. albicans* and exhibited zones of inhibition significantly comparable to that of the negative control ($p>0.05$, Table 4.5).

At 500 mg/ml, the *W. ugadensis* hexane extract produced an average zone of inhibition of 10.00±0.58 mm against *C. albicans,* which was significantly larger than those exhibited by the following concentrations ($p<0.05$, Table 4.5). The extract, however, did not show further antifungal activity at subsequent dilutions and produced zones of inhibition significantly similar to that of the negative control (DMSO) ($p>0.05$, Table 4.5). The positive control (fluconazole) on the other hand, with an average zone of inhibition of 22.33±0.33 mm demonstrated the highest antifungal activity compared to all tested concentrations of *W. ugadensis* hexane extract against *C. albicans* ($p<0.05$, Table 4.5).

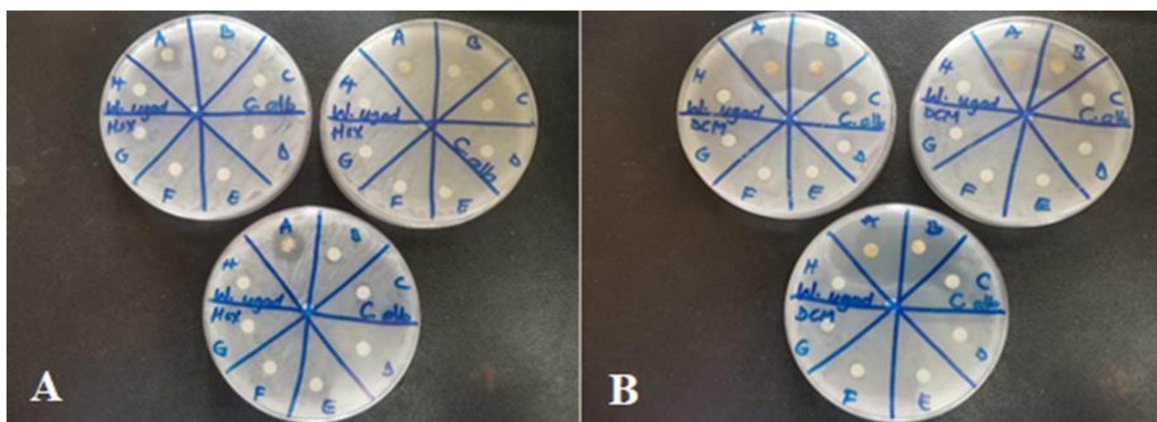

Plate 4.3: MIC results of selected plant extracts against *C. albicans*

Key: **A**= *W. ugadensis* Hexane extract, **B**= *W. ugadensis* DCM extract

Letters A-H refer to the different concentrations of the extracts in descending order

Table 4.4: MIC average diameter of zones of inhibition against *E. coli*

Concentration	Inhibition/mm ± SE Mean
	W. ugadensis DCM
500 mg/ml	10.33±0.33^{b}
250 mg/ml	**8.67±0.33^{c}**
125 mg/ml	6.00±0.00^{d}
62.5 mg/ml	6.00±0.00^{d}
31.25 mg/ml	6.00±0.00^{d}
15.62 mg/ml	6.00±0.00^{d}
7.81 mg/ml	6.00±0.00^{d}
3.91 mg/ml	6.00±0.00^{d}
Negative Control	6.00 ± 0.00^{d}
Positive Control	31.00±0.58^{a}

Key: DCM= dichloromethane, mm= millimeter, SE Mean= standard error of mean, Superscripts= Grouping Information using the Tukey Method and 95% Confidence

Table 4.5: MIC average diameter of zones of inhibition against *C. albicans*

Concentration	Inhibition/mm ± SE Mean	
	W. ugadensis DCM	*W. ugadensis* Hexane
500 mg/ml	21.00±0.58^{a}	**10.00±0.58^{b}**

250 mg/ml	18.00±1.00[b]	6.33±0.33[c]
125 mg/ml	**14.67±1.45[c]**	6.00±0.33[c]
62.5 mg/ml	6.67±0.33[d]	6.00±0.00[c]
31.25 mg/ml	6.33±0.33[d]	6.00±0.00[c]
15.62 mg/ml	6.00±0.00[d]	6.00±0.00[c]
7.81 mg/ml	6.00±0.00[d]	6.00±0.00[c]
3.91 mg/ml	6.00±0.00[d]	6.00±0.00[c]
Negative Control	6.00±0.00[d]	6.00±0.00[c]
Positive Control	22.33±0.33[a]	22.33±0.33[a]

Key: DCM= dichloromethane, mm= millimeter, SE Mean= standard error of mean, Superscripts= Grouping Information using the Tukey Method and 95% Confidence.

4.2.3 Minimum bactericidal/fungicidal concentration in milligrams per milliliter (mg/ml) of selected plant extracts against test microorganisms

W. ugadensis DCM showed the highest bactericidal and fungicidal effects against S. *aureus, E. coli,* and *C. albicans*. The antimicrobial potential of the *W. ugadensis* DCM extract was highest against *S. aureus*, demonstrated by a bactericidal effect achieved at a lower concentration (62.5 mg/ml) compared to all other test microorganisms (table 4.6). The extract was also observed to be more fungicidal than bactericidal against *C. albicans* and *E. coli*. This was evidenced by a fungicidal action attained at a lower concentration (250 mg/ml) than that required for bactericidal activity (500 mg/ml) (Table 4.6). The methanolic extract of *W. ugadensis* had no antibacterial effect against *S. aureus* as revealed by an unmet bactericidal effect at all tested concentrations (Table 4.6). The extract *W. ugadensis* hexane also exhibited no fungicidal action against *C. albicans;* nonetheless, it completely killed *S. aureus* at a concentration of 500 mg/ml (Table 4.6). Against *S. aureus*, *A. gummifera* ethyl acetate and *P. africana* methanolic extracts both showed bactericidal activity at a concentration of 125 mg/ml (Table 4.6). On the other hand, the hexane and methanolic

extracts of *A. gummifera* both demonstrated bactericidal effects at 250 mg/ml. At an initial concentration of 500 mg/ml, extracts of *P. africana* ethyl acetate and *C. molle* methanol all exhibited complete death of *S. aureus* (Table 4.6).

Table 4.6: MBCs/MFCs of selected plant extracts against *S. aureus, E. coli*, and *C. albicans*

Plant Samples	Plant Extracts	MBC/MFC (mg/ml)		
		S. aureus	***E. coli***	***C. albicans***
W. ugadensis	DCM	62.5	500	250
	Methanol	>500	NA	NA
	Hexane	500	NA	>500
P. africana	Methanol	125	NA	NA
	Ethyl acetate	500	NA	NA
A. gummifera	Methanol	250	NA	NA
	Hexane	250	NA	NA
	Ethyl acetate	125	NA	NA
C. molle	Methanol	500	NA	NA

Key: DCM= dichloromethane, NA= Not applicable (Extract not considered for the test)

4.3 Toxicity assay

4.3.1 Acute oral toxicity

The acute toxicity of selected plant extracts was obtained using OECD guideline 423, wherein doses of 500 mg/kg, 866 mg/kg, and 1500 mg/kg body weight were used. After a single-dose administration of each plant extract at various concentrations, the mice were kept under careful surveillance for 1 h and occasionally for the next 4 hours. Thereafter, they were observed every 24 hours during the remaining period of the study for any sign of noxiousness. Following oral administration of the test plant extracts at various doses, symptoms such as abnormal respiration, changes in water and food intake, wheezing,

declined or increased animal movements (depression/anxiety), piloerection, diarrhea or vomiting were observed in the extract-treated groups. However, no visible adverse or lethal effects associated to the treatments were noted, and the hematological and biochemical parameters in each treated group were significantly similar to those of the control group. Thus, the tested extracts were considered nontoxic at doses of 500 mg/kg, 866 mg/kg, and 1500 mg/kg body weight, with LD_{50} values greater than 1500 mg/kg body weight.

4.3.1.1 Effects of selected extracts on relative organ and mean body weights

The body weight of each mouse, including those of the control group, was taken on the initial day of the extract administration (day 0), on day 7, day 14, and finally after starvation on the euthanizing day (day 15) (Figure 4.1). The single-dose administration of *A. gummifera* ethyl acetate and *P. africana* methanol extracts at concentrations 500 mg/kg, 866 mg/kg, and 1500 mg/kg body weight did not impact significant changes in the mean body weight of mice. Throughout the study, the mice were noted to progressively gain weight likewise those in the control group ($p>0.05$, Figure 4.1). Mouse groups administered with *W. ugadensis* DCM extract at concentrations of 866 and 1500 mg/kg body weight however, experienced a decrease in body weight during the first few days of the study. Nevertheless, these 2 groups gradually regained weight and assumed weight changes similar to those of the control and other test groups until the end of the study. Following final starvation, all groups of mice including the control were observed to have lost weight as noted in Figure 4.1. Single dose administration of the test extracts for 14 days did not bring about any significant changes in the relative organ weights of the extract-treated groups apropos the control group (Table 4.7). These findings disclosed that vital organs, including heart, liver, kidneys, lungs, and spleen were not negatively affected throughout

the entire study period. Relative organ weights from all treated groups were significantly similar to those of the control ($p>0.05$, Table 4.7).

Relative organ weight = [organ weight (g) ÷ body weight (g)] × 100.

Figure 4.1: Effects of selected extracts on mean body weight of mice with time

Table 4.7: Effects of selected extracts on relative organ weights of mice

Plant Extracts	Concentrations (mg/kg)	Relative organ weight ± SEM				
		Heart	**Liver**	**Kidneys**	**Lungs**	**Spleen**
A. gummifera	500	0.58±0.056[a]	4.96±0.149[a]	1.36±0.041[a]	0.78±0.063[a]	0.35±0.013[a]
	866	0.59±0.062[a]	5.02±0.087[a]	1.36±0.043[a]	0.77±0.060[a]	0.35±0.015[a]
	1500	0.58±0.035[a]	4.87±0.132[a]	1.36±0.047[a]	0.78±0.064[a]	0.35±0.017[a]
P. africana	500	0.57±0.046[a]	5.08±0.050[a]	1.39±0.026[a]	0.76±0.041[a]	0.36±0.016[a]
	866	0.54±0.038[a]	4.98±0.085[a]	1.31±0.043[a]	0.77±0.083[a]	0.35±0.019[a]

	1500	0.56±0.041[a]	4.74±0.183[a]	1.39±0.046[a]	0.74±0.076[a]	0.36±0.013[a]
W. ugadensis DCM	500	0.58±0.031[a]	4.83±0.138[a]	1.35±0.041[a]	0.75±0.054[a]	0.36±0.013[a]
	866	0.60±0.013[a]	4.81±0.133[a]	1.37±0.050[a]	0.74±0.056[a]	0.34±0.015[a]
	1500	0.58±0.034[a]	4.88±0.146[a]	1.37±0.047[a]	0.78±0.083[a]	0.36±0.022[a]
Control	5% DMSO	0.58±0.037[a]	4.96±0.128[a]	1.38±0.040[a]	0.77±0.065[a]	0.36±0.015[a]

Key: SEM= Standard error of mean, mg/kg= Milligrams per kilogram

4.3.1.2 Effects of selected extracts on mouse blood glucose levels (after 14 days)

Blood glucose levels were obtained at the end of the study (day 15) with the help of a glucometer. Results demonstrated that there were no significant differences in the blood glucose levels of the treated mouse groups and the control group ($p>0.05$, Table 4.8).

Table 4.8: Effect of selected plant extracts on mouse blood glucose levels (after 14 days)

Plant Extract	Concentration (mg/kg)	Blood Glucose Level/mmol/L ± SEM
A. gummifera	500	7.46±0.169[a]
	866	7.84±0.206[a]
	1500	8.12±0.218[a]
P. africana	500	7.94±0.420[a]
	866	8.14±0.075[a]
	1500	7.34±0.108[a]
W. ugadensis	500	7.50±0.170[a]
	866	8.12±0.414[a]
	1500	7.60±0.089[a]
Control	5% DMSO	7.84±0.275[a]

Key: mmol/L= Millimole per liter, SEM= Standard error of mean, mg/kg= Milligram per kilogram

4.3.1.3 Effects of selected extracts on hematological parameters

Tables 4.9, 4.10, and 4.11 give a summary of the results obtained from hematology blood tests performed on the experimental mouse groups. From the results, no significant differences were noted in the levels of WBC, Neu, Lym, Mon, Eos, Bas, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, PLT, MPV, PDW, and PCT in all treated groups in comparison to the control group ($p>0.05$).

Table 4.9: Effects of selected plant extracts on mouse white blood cells

Plant Extracts	Concentration (mg/kg)	White Blood Cells Parameters (10^3 µL) ± SEM					
		WBC	Neu	Lym	Mon	Eos	Bas
W. ugadensis	5% DMSO (Control)	7.474±0.489^{a}	0.682±0.094^{a}	6.730±0.523^{a}	0.022±0.008^{a}	0.012±0.002^{a}	0.028±0.006^{a}
	500	7.916±0.581^{a}	0.786±0.079^{a}	6.986±0.559^{a}	0.088±0.013^{a}	0.016±0.004^{a}	0.040±0.010^{a}
	866	8.754±0.369^{a}	0.604±0.119^{a}	7.988±0.420^{a}	0.096±0.038^{a}	0.012±0.002^{a}	0.054±0.009^{a}
	1500	8.894±0.067^{a}	0.944±0.039^{a}	7.034±0.051^{a}	0.070±0.007^{a}	0.020±0.004^{a}	0.036±0.004^{a}
P. africana	5% DMSO (Control)	7.474±0.489^{a}	0.682±0.094^{a}	6.730±0.523^{a}	0.022±0.008^{a}	0.012±0.002^{a}	0.028±0.006^{a}
	500	7.674±0.364^{a}	0.718±0.0835^{a}	6.858±0.403^{a}	0.044±0.008^{a}	0.016±0.004^{a}	0.036±0.005^{a}
	866	7.576±0.251^{a}	0.944±0.039^{a}	6.560±0.252^{a}	0.022±0.006^{a}	0.016±0.004^{a}	0.034±0.005^{a}
	1500	7.680±0.178^{a}	0.788±0.057^{a}	6.824±0.221^{a}	0.026±0.0051^{a}	0.018±0.004^{a}	0.026±0.007^{a}
A. gummifera	5% DMSO (Control)	7.474±0.489^{a}	0.682±0.094^{a}	6.730±0.523^{a}	0.022±0.008^{a}	0.012±0.002^{a}	0.028±0.006^{a}
	500	7.990±0.269^{a}	0.688±0.0552^{a}	7.250±0.238^{a}	0.018±0.004^{a}	0.012±0.002^{a}	0.022±0.005^{a}
	866	8.028±0.211^{a}	0.706±0.0452^{a}	7.264±0.240^{a}	0.020±0.005^{a}	0.014±0.002^{a}	0.024±0.006^{a}

	1500	8.308±0.257[a]	0.814±0.034[a]	7.426±0.243[a]	0.026±0.002[a]	0.016±0.004[a]	0.026±0.008[a]

Key: WBC= White Blood Cells, Neu= Neutrophils, Lym= Lymphocytes, Mon= Monocytes, Eos= Eosinophils, Bas= Basophils, SE Mean= standard error of mean,

Superscripts= Grouping Information using the Tukey Method and 95% Confidence.

Values with similar lowercase superscript letters are not significantly different column-wise using one way ANOVA and Tukey's multiple comparison ($p>0.05$)

Table 4.10: Effects of selected plant extracts on mouse red blood cells

Plant Extracts	Concentration (mg/kg)	Red Blood Cells Parameters ± SEM						
		RBC (10^6 µL)	HGB(g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC(g/dL)	RDW-CV (%)
W. ugadensis	5% DMSO (Control)	8.70±0.088[a]	14.83±0.078[a]	45.26±0.479[a]	52.05±0.927[a]	17.05±0.143[a]	32.78±0.322[a]	24.57±0.257[a]
	500	8.61±0.087[a]	14.50±0.122[a]	45.84±0.501[a]	53.22±0.434[a]	16.86±0.060[a]	31.66±0.271[a]	24.84±0.125[a]
	866	8.42 ±0.181[a]	14.32±0.282[a]	43.08±1.05[a]	51.20±0.989[a]	16.79±0.095[a]	33.12±0.198[a]	24.12±0.132[a]
	1500	8.55 ±0.166[a]	14.34±0.204[a]	43.92±1.06[a]	51.30±1.560[a]	16.61±0.436[a]	32.85±0.713[a]	24.04±0.248[a]
P. africana	5% DMSO (Control)	8.70±0.088[a]	14.83±0.0785[a]	45.26±0.479[a]	52.05±0.927[a]	17.05±0.143[a]	32.78±0.322[a]	24.57±0.257[a]
	500	8.76±0.261[a]	15.11±0.189[a]	45.54±0.753[a]	52.04±0.958[a]	17.27±0.351[a]	33.21±0.303[a]	24.84±0.392[a]
	866	8.64±0.178[a]	14.92±0.306[a]	44.54±0.920[a]	51.54±0.693[a]	17.26±0.220[a]	33.47±0.037[a]	24.48±0.549[a]
	1500	8.75±0.272[a]	15.32±0.425[a]	45.14±1.340[a]	51.68±1.330[a]	17.48±0.150[a]	33.96±0.772[a]	24.36±0.769[a]
A. gummifera	5% DMSO (Control)	8.70±0.0879[a]	14.83±0.078[a]	45.26±0.479[a]	52.05±0.927[a]	17.05±0.143[a]	32.78±0.322[a]	24.57±0.257[a]

	500	8.55±0.108[a]	14.60±0.205[a]	44.77±0.698[a]	52.36±0.640[a]	17.07±0.161[a]	32.64±0.507[a]	24.60±0.207[a]
	866	8.60±0.084[a]	14.60±0.263[a]	45.28±0.457[a]	52.66±0.685[a]	16.96±0.154[a]	32.22±0.658[a]	24.44±0.248[a]
	1500	8.68±0.062[a]	15.18±0.110[a]	45.33±0.307[a]	52.25±0.431[a]	17.46±0.157[a]	33.47±0.019[a]	24.49±0.222[a]

Key: RBC= Red Blood Cells, HGB= Hemoglobin, HCT= Hematocrit, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Hemoglobin, MCHC= Mean corpuscular Hemoglobin Concentration, RDW-CV= Red Cell Distribution Width, SE Mean= standard error of mean, Superscripts= Grouping Information using the Tukey Method and 95% Confidence.

Table 4.11: Effects of selected plant extracts on mouse blood platelets

Plant Extracts	Concentration (mg/kg)	Platelets Parameters ± SEM			
		PLT (10^3/uL)	MPV (fL)	PDW (fL)	PCT (%)
W. ugadensis	5% DMSO (Control)	902.6±29.2[a]	6.776±0.066[a]	7.120±0.193[a]	0.612±0.023[a]
	500	906.2±33.2[a]	6.798±0.165[a]	7.080±0.229[a]	0.612±0.009[a]
	866	916.0±34.1[a]	6.544±0.068[a]	6.800±0.084[a]	0.601±0.018[a]
	1500	910.8±30.4[a]	6.642±0.121[a]	7.040±0.129[a]	0.605±0.021[a]
P. africana	5% DMSO (Control)	902.6±29.2[a]	6.776±0.066[a]	7.120±0.193[a]	0.612±0.023[a]
	500	906.6±31.2[a]	6.822±0.096[a]	7.098±0.159[a]	0.621±0.0297[a]
	866	915.2±29.8[a]	6.762±0.149[a]	7.080±0.215[a]	0.619±0.018[a]
	1500	901.4±30.7[a]	6.700±0.130[a]	6.920±0.136[a]	0.603±0.0157[a]
A. gummifera	5% DMSO (Control)	902.6±29.2[a]	6.776±0.066[a]	7.120±0.193[a]	0.612±0.023[a]
	500	915.8±23.6[a]	6.780±0.107[a]	6.980±0.116[a]	0.624±0.016[a]
	866	916.0±25.6[a]	6.820±0.0583[a]	6.980±0.086[a]	0.626±0.0183[a]
	1500	920.2±24.3[a]	6.900±0.126[a]	7.100±0.141[a]	0.634±0.0130[a]

Key: PLT= Platelets, MPV= mean platelet volume, PDW= platelet distribution width, PCT= procalcitonin, SE mean= standard error of mean, Superscripts= grouping information using the Tukey method and 95% confidence.

Values with similar lowercase superscript letters are not significantly different column-wise using one way ANOVA and Tukey's multiple comparison ($p>0.05$)

4.3.1.4 Effects of selected extracts on mouse biochemical parameters

Data obtained from various biochemical tests against each treatment concentration and the control are shown in Table 4.12,4.14, and 4.15. The *A. gummifera* ethyl acetate, *P. africana* methanol, and *W. ugadensis* DCM treatments exhibited no significant alteration in the levels of T-bil, D-bil, ALT, AST, ALP, TP, IBil, and ALB at all tested concentrations in comparison with the effects observed in the control group ($p>0.05$, Table 4.12). That is, the levels of T-bil, D-bil, ALT, AST, ALP, TP, IBil, and ALB in all tested groups were significantly similar to values obtained in the control (normal) group. On a similar note, no significant differences were observed in the levels of CREA, UREA, sodium, potassium, and chloride in all treated groups compared to the levels noted in the control ($p>0.05$, Table 4.13).

Table 4.12: Effects of selected plant extracts on mouse liver parameters

Plant Extracts	Conc. (mg/kg)	Liver Function Test Parameters ± SEM							
		T-bil	D-bil	ALT	AST	ALP	TP	IBil	ALB
W. ugadensis	1500	1.18±0.052[a]	0.86±0.056[a]	84.14±0.749[a]	292.06±9.09[a]	198.56±3.14[a]	59.50±1.050[a]	0.32±0.020[a]	31.36±0.154[a]
	866	1.17±0.043[a]	0.85±0.023[a]	84.34±0.705[a]	290.8±11.1[a]	197.60±2.14[a]	59.50±0.541[a]	0.32±0.037[a]	31.20±0.100[a]
	500	1.17±0.030[a]	0.85±0.053[a]	83.52±0.635[a]	282.7±15.6[a]	195.90±3.76[a]	59.30±0.987[a]	0.32±0.037[a]	31.32±0.124[a]
	5% DMSO (Control)	1.176±0.06[a]	0.87±0.053[a]	83.96±0.522[a]	285.0±15.3[a]	199.60±1.90[a]	59.32±0.353[a]	0.30±0.031[a]	31.18±0.373[a]
P. africana	1500	1.18±0.061[a]	0.90±0.046[a]	83.38±0.278[a]	286.1±5.61[a]	199.34±0.99[a]	59.20±0.307[a]	0.28±0.020[a]	30.84±0.37[a]
	866	1.14±0.057[a]	0.86±0.047[a]	83.60±0.326[a]	287.52±7.13[a]	198.2±0.956[a]	59.12±0.260[a]	0.28±0.020[a]	30.86±0.51[a]
	500	1.14±0.048[a]	0.86±0.047[a]	82.88±0.563[a]	285.26±3.79[a]	198.90±0.58[a]	58.34±0.417[a]	0.28±0.020[a]	31.14±0.28[a]
	5% DMSO (Control)	1.17±0.059[a]	0.87±0.053[a]	83.96±0.522[a]	285.0±15.3[a]	199.60±1.9[a]	59.32±0.353[a]	0.30±0.0316[a]	31.18±0.37[a]
A. gummifera	1500	1.20±0.028[a]	0.90±0.056[a]	83.08±0.692[a]	288.14±8.86[a]	198.58±2.26[a]	59.14±0.367[a]	0.30±0.044[a]	31.22±0.39[a]
	866	1.17±0.045[a]	0.89±0.049[a]	83.80±0.919[a]	285.6±13.2[a]	197.62±1.96[a]	59.32±0.606[a]	0.28±0.049[a]	30.60±0.56[a]
	500	1.14±0.068[a]	0.88±0.053[a]	83.90±0.582[a]	285.4±11.5[a]	198.5±0.95[a]	58.46±1.070[a]	0.26±0.024[a]	30.88±0.45[a]

	5% DMSO (Control)	1.176±0.06[a]	0.87±0.053[a]	83.96±0.522[a]	285.0±15.3[a]	199.60±1.90[a]	59.32±0.353[a]	0.30±0.031[a]	31.18±0.37[a]

Key: T-bil= Total bilirubin, D-bil= Direct bilirubin, ALT= Alanine transaminase, AST= Aspartate aminotransferase, ALP= Alkaline phosphatase, TP=Total protein, IBil= Indirect bilirubin, ALB= Albumin, SE Mean= standard error of mean, Superscripts= Grouping Information using the Tukey Method and 95% Confidence

Table 4.13: Effects of selected plant extracts on mouse kidney parameters

Plant Extracts	**Concentration(mg/kg)**	**Kidney Function Test Parameters ± SEM**				
		CREA	**UREA**	**Sodium**	**Potassium**	**Chloride**
W. ugadensis	1500	37.48±0.929[a]	9.38±0.334[a]	157.50±1.58[a]	6.39±0.164[a]	113.74±0.622[a]
	866	37.46±0.828[a]	9.39±0.258[a]	156.66±1.44[a]	6.34±0.148[a]	113.92±0.648[a]
	500	36.66±1.60[a]	9.37±0.224[a]	156.36±2.45[a]	6.39±0.147[a]	113.80±0.772[a]
	5% DMSO (Control)	36.02±1.41[a]	9.23±0.254[a]	155.62±2.12[a]	6.20±0.063[a]	113.92±0.873[a]
P. africana	1500	36.00±0.874[a]	9.19±0.191[a]	155.46±2.17[a]	6.36±0.192[a]	114.34±0.690[a]
	866	36.10±1.43[a]	9.008±0.166[a]	155.76±1.24[a]	6.314±0.20[a]	113.92±0.700[a]
	500	36.04±1.15[a]	9.062±0.253[a]	155.68±1.13[a]	6.14±0.132[a]	113.98±0.673[a]
	5% DMSO (Control)	36.02±1.41[a]	9.23±0.254[a]	155.62±2.12[a]	6.204±0.063[a]	113.92±0.873[a]

A. gummifera	1500	36.20±1.29^{a}	9.186±0.257^{a}	155.54±1.10^{a}	6.238±0.085^{a}	114.18±0.793^{a}
	866	36.16±0.763^{a}	9.106±0.241^{a}	155.32±1.17^{a}	6.16±0.092^{a}	114.02±0.805^{a}
	500	36.06±0.77^{a}	9.182±0.236^{a}	155.92±1.09^{a}	6.056±0.078^{a}	113.98±0.56^{a}
	5% DMSO (Control)	36.02±1.41^{a}	9.23±0.254^{a}	155.62±2.12^{a}	6.204±0.063^{a}	113.92±0.873^{a}

Key: CREA= Creatinine, SE Mean= standard error of mean, Superscripts= Grouping Information using the Tukey Method and 95% Confidence

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 DISCUSSION

The rapid spread of resistance genes among different microbial populations and the global rise in antimicrobial resistance of commonly used and available pharmaceutical products has led to an imperative need for new, effective, and nontoxic drugs. Fortunately, medicinal plants, which for decades have been used in folk medicine to treat and manage various ailments, are now being looked upon and stand as potential sources for the development of new drugs. It is impossible to overstate the significance of traditional medicines, as they are utilized extensively not just in Kenya but also around the world for a wide range of applications (Lukhoba *et al.,* 2006). The plants involved in this study are believed to have antimicrobial properties, and literature support their usage in traditional medicine as antimicrobial agents. However, the paucity of information on these plants to scientifically back up these beliefs is of concern. Furthermore, there is a scarcity of research evaluating the inhibitory effects of these plants using different solvents, and their possible toxic effects in animal models. This study thus examined the phytochemical constituents (qualitatively) of these plants and the antibacterial and antifungal properties of their extracts against standard strains of *Escherichia coli, Staphylococcus aureus*, and *Candida albicans.* Further tests were also carried out to determine whether the active extracts of these plants were potentially safe in animal models (mice).

5.1.1 Qualitative phytochemical screening

Selected plants were tested for the presence or absence of some major phytochemicals in an attempt to unravel the source of their numerous medicinal properties. Screening of the

stem bark and/or roots of *C. edulis, A. ebracteatus, A. gummifera, P. africana, C. molle, W. ugandensis,* and *Cuscuta* spp. revealed the type of bioactive compounds present in these plant parts as shown in Table 4.1. Just as studies from the past demonstrate that secondary metabolites from plants are a significant source of microbicides, insecticides, and several pharmaceuticals (Kolapo *et al.,* 2007), findings from this study demonstrated that each tested plant contains a range of different secondary metabolites, which probably play a role in their antimicrobial effects. All crude extracts indicated the presence of phenols, terpenoids, and tannins (Table 4.1). Tannins in particular, are a class of specific phytochemicals having a wide range of medicinal uses, including anti-inflammatory, antiviral, antiulcer, and antiparasitic applications (Akiyama *et al.,* 2001; Lu *et al.,* 2004; Kolodziej and Kiderlen, 2005). Interestingly, tannins combine with proline-rich proteins to form irreversible complexes that prevent cells from producing protein (Dharmananda, 2003). According to Soine (1964), they are recognized to have antibacterial properties and have been shown to be effective against microorganisms that cause diarrhea (Choi *et al.,* 2009). Moreover, terpenoids found in plant materials are known to have antimicrobial effects (Xu *et al.,* 2009). Numerous naturally occurring triterpenoids, which have been isolated from various plant sections, have been found to possess fungicidal, bactericidal, anticancer, antiviral, cytotoxic, anti-inflammatory, analgesic, and antiallergic properties (Patocka, 2003). Alkaloids, on the other hand, have been proven to have antibacterial, antimalarial, analgesic, and antiseptic properties, whereas the majority of biological impacts on cell development and division that occur in humans are caused by saponins, which also have an inhibitory influence on inflammation (Koevi *et al.,* 2015). Looking at the 3 medicinal plants that demonstrated the greatest effectiveness in the study, *A. gummifera* typically contained all screened phytochemicals, which are similar to those

found in leaf extracts of *A. gummifera* in a study conducted by Oloruntola *et al.* (2021). *W. ugadensis* on the other hand, had all tested bioactive compounds with the exception of flavonoids, which, according to Chhabra *et al.* (1984), have been found to have cytotoxic, anti-inflammatory, and antiviral properties. This is in accordance with findings from a study carried out by Oloya *et al.* (2022), wherein no flavonoids were detected in both aqueous and organic extracts of the plant stem bark. Similarly, *P. africana* was observed to contain all screened metabolites except glycosides (Table 4.1). This is supported by previous studies that demonstrated the absence of this type of compound in *P. africana* stem bark (Mutuma *et al.,* 2020).

5.1.2 Antimicrobial activity of plant extracts

Out of 32 extracts tested against *E. coli* (ATTC 25922), *S. aureus* (ATCC 25923), and *C. albicans* (ATTC 10231), only 9 extracts showed significant antibacterial/antifungal activity against test microorganisms. The data obtained revealed that *E. coli* had the lowest susceptibility among the three tested microorganisms, whereas *S. aureus* had the highest susceptibility to the various extracts, with the exception of *W. uagadensis* DCM, which uniquely exhibited greater activity against *C. albicans*. This observation was in conformity with studies on medicinal plants conducted by Cheruiyot *et al.* (2009) and Yibeltal *et al.* (2013), who reported that when compared to *E. coli*, *S. aureus* and *C. albicans* were the most sensitive to plant extracts in spite of plant parts, extraction method, and solvent used. In general, due to the morphological differences between gram-positive and gram-negative microorganisms, plant extracts are more efficient against gram-positive (*S. aureus*) than gram-negative (*E. coli*) bacteria, which may account for the variability in the antibacterial activity of the extracts in this investigation (Suffredini *et al.,* 2006). From this research, *W.*

ugadensis DCM extract exhibited the highest antibacterial and antifungal effects compared to all other tested extracts against *E. coli*, *S. aureus*, and *C. albicans* (Table 4.2). This was further confirmed via the microdilution method, where *W. ugadensis* DCM exhibited microbial activity at minimum concentrations of 31.25 mg/ml (mean zone of inhibition 12.33±0.67 mm), 250 mg/ml (mean zone of inhibition 8.67±0.33 mm), and 125 mg/ml (mean zone of inhibition 14.67±1.45 mm) against *S. aureus, E. coli*, and *C. albicans,* respectively. These findings agree with observations made from an earlier investigation that stated that compared to methanolic extracts, DCM solvent extracts had the strongest antimicrobial effects (Abuto *et al.,* 2016). Another study done by Mwitari *et al.* (2013) demonstrated that the dichloromethane part of *W. ugadensis* has moderate to very high activity against *C. albicans*. This observation could be explained by the fact that depending on the degree of solubility or polarity, different solvents have the ability to extract different molecules (Saggoo *et al.,* 2010). Therefore, one can say that in comparison to other extracts in this study (hexane, ethyl acetate, and methanol), chemical constituents accountable for antimicrobial action were more soluble in the DCM solvent extract of *W. ugadensis*. The methanolic extract of all medicinal plants involved in this study did not show any activity against *E. coli* and *C. albicans*. Among the four methanolic extracts that showed activity against *S. aureus*, the most effective were those of *P. africana* and *A. gummifera,* which exhibited similar effects. They were followed by *C. molle* and *W. ugadensis* both with comparable MIC values. These findings correlate with those of Bii *et al.* (2010) in a study on the possible uses of *P. africana*, which demonstrated the strong efficacy of *P. africana* methanol extracts against bacterial strains.

P. africana ethyl acetate extract was less effective against *S. aureus* compared to its methanolic counterpart. In a study conducted by Mwitari *et al.* (2013), similar observations

were made, whereby whilst the ethyl acetate fraction of *P. africana* demonstrated only modest efficacy against *S. aureus*, the methanol extract had good activity. Against *E. coli* and *C. albicans*, all tested ethyl acetate plant extracts were ineffective.

The hexane extract of *W. ugadensis* exhibited weak antimicrobial effects against both *S. aureus* and *C. albicans,* yet the extract's efficacy was observed to be slightly higher against *S. aureus* than *C. albicans*. These results highlighted the greater vulnerability to microbial products usually observed in bacteria compared to fungi. Similar findings by Makopa *et al.* (2020) in an investigation of the antibacterial and antifungal effects of leaf extracts from *Persea americana* suggested that compared to fungi, bacteria were more vulnerable to extracts. In another experiment testing the ability of five medicinal extracts to combat multidrug-resistant bacterial and fungal strains, similar outcomes were found with *Eucalyptus globulus* and *Terminalia arjuna*, which were found to be more active against bacteria than fungi (Khan *et al.,* 2009). Assuming that the bioactive compounds target cell wall synthesis, the most likely reason might be the chemical makeup of their outer cell walls, whereby fungi and bacteria have different cell walls made of chitin and peptidoglycan, respectively (Lenardon *et al.,* 2010; Epand *et al.,* 2016). These variations in how microorganisms respond to different extracts, however, further raise the question of how these bioactive extracts work.

5.1.3 Toxicity assay

A. gummifera, P. africana, and *W. ugadensis* are well-known medicinal plants that are widely used in folk medicine for the prevention or management of various ailments (Mbosso *et al.,* 2010; Kadu *et al.,* 2012; Were *et al.,* 2010). The indiscriminate use of medicinal plants notably in rural areas and underdeveloped countries on the basis of their

natural nature and effectiveness has long been a misconception, as associated side/toxic effects are ignored. Previous *in vivo* studies using animal models on the therapeutic effects of some medicinal plant extracts revealed that most of them possess bioactive components that at high concentrations, simultaneous consumption with other drugs, and usage for long periods may have toxic consequences (Akwa and Nguimbous, 2021). Therefore, to ascertain the safety of *A. gummifera, P. africana,* and *W. ugadensis*, toxicological evaluation is essential. Because acute toxicity studies are required to determine a more appropriate dose range for managing possible side effects of medicinal products, this study was conducted to assess the acute toxicity of selected plant extracts that had previously shown antimicrobial properties against *E. coli, S. aureus*, and *C. albicans*. The experiment was performed using Swiss albino mice models, according to the OECD guidelines 423.

5.1.3.1 Acute toxicity of *W. ugadensis* DCM extract

Following a single dose administration of *W. ugadensis* DCM extract at various concentrations (500, 866, and 1500 mg/kg body weight), neither death nor alterations in behavior were observed. At concentrations of 866 and 1500 mg/kg however, a slight decrease in body weight compared to that of the control and 500 mg/kg treated groups was noted in the first few days after ingestion of the extract. Nonetheless, as the study progressed, the mice were found to progressively regain their weights at a significantly comparable rate to those of the control and 500mg/kg treated groups. These observations were somewhat different from those of Karani *et al.* (2013), who experienced a progressive increase in the mean weights of experimental mice significantly comparable to those of controls all through the study time. It is worth noting that numerous variables, such as

stress, motivation, general health, level of activity, metabolism, and feeding habits, can all influence weight increase or reduction. Therefore, since all blood parameters and relative organ weights in mice treated with 866 and 1500 mg/kg body weight of *W. ugadensis* DCM were all significantly similar to those of the control and other treated groups, weight changes experienced in the course of the study could possibly be associated with differences in the animals' metabolisms. An article by Harvard Health Publishing (2021) on the role of metabolism in weight loss suggested that genetics do play a role in metabolism, which is quite significant when it comes to weight. It states that animals with high/fast metabolism expend more calories both at rest and when active (HHP, 2021). This could potentially explain the changes in body weight observed in some treated mouse groups following initial starvation.

Hematological parameters are highly accurate indicators of the physiological alterations that occur in response to any harmful chemicals, and usually, extrapolated data obtained from animal research have a high predictive score for toxicity to humans (Jain *et al.,* 2009). A number of harmful substances build up in the liver, where they are detoxified; thus, liver function tests are essential to identify and keep track of any liver injury or disease possibly associated with medicinal extracts. Kidneys, on the other hand, are frequently considered toxicity targets because they receive around 25% of the cardiac blood flow and are therefore exposed to every drug that enters systemic circulation (Wolfgang and Spiros, 1996). From this study, there were no significant alterations in liver and kidney function test parameters in all treatment groups in comparison to values noted in the control group. These results are in conformity with hematological and biochemical findings from a study on *W. ugadensis* safety in the treatment of asthma conducted by Karani *et al.* (2013), wherein experimental mice were not harmed when given test doses of *W. ugandensis* stem

bark water extracts, and from that study, the LD_{50} value was estimated to be > 5000 mg/kg body weight.

Additionally, glucose levels recorded at the end of the study in mouse groups treated with varied concentrations of *W. ugadensis* DCM extract were significantly similar to those of the control group. This indicated that, within the range of tested concentrations, the extract did not cause any detectable changes in the mice's blood sugar levels.

This finding therefore supports the continued use of *W. ugadensis* in traditional medicine and provides a scientific basis for potential therapeutic application

5.1.3.2 Acute toxicity of *P. africana* methanol extract

After administration of *P. africana* methanolic extract (500, 866, and 1500 mg/kg body weight) in a single dose, no behavioral changes or mortality occurred even at the highest tested concentration of the extract. In addition, the mean weights of the treated mouse groups gradually rose throughout the study and did not differ significantly from the control group's mean weights. This is in conformity with findings from a study on the safety of *P. africana* in asthma treatment by Karani *et al.* (2013), wherein low doses (less than 1581.6 mg/kg body weight) of *P. africana* aqueous extracts exhibited no adverse consequences in the tested mice. Other studies conducted by Andro and Riffaud (1995) and Murray (1995) also reported that at dosages of 100 mg/kg body weight, *P. africana* had no discernible negative effects, thus agreeing with these results.

Sensitive indicators of the alterations in physiology brought about by any hazardous chemical are hematologic parameters (Jain *et al.,* 2009). Results suggested that *P. africana* methanolic extract exhibited negligible changes in these parameters, as demonstrated by values in all treated groups that were significantly comparable to those of the control group.

Damage to liver cells may make them more permeable, subsequently causing aminotransferases to be released into the bloodstream (Ogunlana *et al.,* 2013). Thus, elevated levels of total proteins, ALT, and AST may arise as a result of hepatotoxic druginduced liver damage (Ramaiah, 2011). Urea, on the other hand, is an indicator of acute renal failure and is the primary acute marker after renal damage (Olayod *et al.,* 2020). Blood urea and creatinine levels are both typically used to evaluate renal function; therefore, increased blood creatinine and urea levels indicate damage (Travlos *et al.,* 1996). Since there were no significant changes in the serum levels of these markers of liver and kidney function, it was concluded that the livers and kidneys of the treated mice did not experience any tissue alterations. The study's findings thus imply that administration of selected plant extracts does not result in liver or kidney damage at the tested concentrations. Again, data obtained from the glucose test revealed that at the tested doses, administering *P. africana* methanolic extract had no adverse effects on the blood sugar levels. The values noted in all treated groups were significantly similar to those of the control (Table 4.8).

5.1.3.3 Acute toxicity of *A. gummifera* ethyl acetate extract

A. gummifera ethyl acetate did not cause any behavioral abnormalities or death in Swiss albino mice after being administered in single doses at 500, 866, and 1500 mg/kg body weight. Additionally, throughout the course of the test, their average weights increased gradually and did not significantly differ from those of the control group. This implied that the mice were still able to properly process all of the food nutrients, including proteins, lipids, and carbs, as these nutrients are crucial for carrying out many physiological processes (Stevens and Mylecraine, 1994).

At the tested concentrations of *A. gummifera* ethyl acetate, no signs of toxicity or abnormalities in the levels of blood glucose, hematologic and biochemical parameters, or relative organ weights were observed in comparison with the levels noted in the control group.

5.2 CONCLUSIONS

1. Phytochemical screening revealed that the selected medicinal plants involved in this study abound in bioactive compounds. Various phytochemicals present can have antibacterial/antifungal activities against a range of microorganisms and could serve in the development of new pharmaceutical products, especially in this era where a good number of microbes have already developed resistance to most available drugs.
2. This research also demonstrated the antimicrobial potential of methanolic extracts of *W. ugadensis, P. africana, A. gummifera,* and *C. molle*; hexane extracts of *W. ugadensis* and *A. gummifera*; DCM extract of *W. ugadensis*; and ethyl acetate extracts of *P. africana* and *A. gummifera* against *C. albicans, S. aureus*, and *E. coli*. Out of all tested extracts, the DCM part of *W. ugadensis* showed the highest antibacterial and antifungal effects against all tested microorganisms. *S. aureus* demonstrated the highest susceptibility to the tested extracts, while *E. coli* had the lowest susceptibility. This work thus promotes the sustainable use and conservation of all aforementioned plant species because of their affective bioactivity.
3. The study assessed the safety and antimicrobial potential of seven Kenyan medicinal plants collected from Mt. Kenya and Mt. Elgon. Acute toxicity assays in Swiss albino mice showed no adverse effects across all groups, with LD_{50} values >1500 mg/kg, confirming the safety of A. gummifera (ethyl acetate extract), P. africana (methanol extract), and W.

ugadensis (DCM extract). These solvents were identified as the most suitable for the respective plants. The research is among the first to provide toxicological data on these species, validating traditional medicinal claims by demonstrating antimicrobial activity and establishing a scientific foundation for future bioactive compound isolation. Overall, the findings support safe traditional use, development of natural antimicrobial agents, and conservation of Kenya's medicinal flora.

5.3 RECOMMENDATIONS

1. Data obtained from this study highlights the need to identify specific bioactive compound(s) responsible for the observed antibacterial and antifungal activities of the plant extracts involved in this study, as well as investigate their possible mechanisms of action.
2. These findings also lay a foundation for future tests to validate and develop these extracts as potential sources or substitute treatments in the management of diseases caused by *C. albicans, S. aureus*, and *E. coli*.
3. My capacity to compare outcomes has been constrained by a paucity of pertinent literature on the selected medicinal plant extracts. However, to confirm that these extracts are not toxic, it will be important to consider the impact of a number of variables, including the plants' parts (stem barks, leaves, flowers, seeds, and roots), their developmental stages and maturation, conditions of storage, changes in seasons, and the environment in which they are grown. Also, despite the medical benefits and trends in the utilization of *A. gummifera, P. africana* and *W. ugadensis* extracts, emphasis is needed for additional research to confirm their hematological and biochemical impacts, particularly in situations where therapy is repeated and last for longer periods than mentioned in this study.

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APPENDIX I

Picture 1: Grinding Plant Samples using a Grinding Mill Machine (Kenyatta University)

Figure 2: Collected Plant Samples macerating in Laboratory grade methanol (Chemistry lab, Kenyatta University)

Picture 3: Concentrating Plant Extracts using a Rotary Evaporator (Chemistry Lab, Kenyatta University)

Figure 4: Solvent-Solvent partitioning of plant extracts using Hexane, Dichloromethane, Ethyl acetate, and Methanol (Chemistry lab, Kenyatta University)

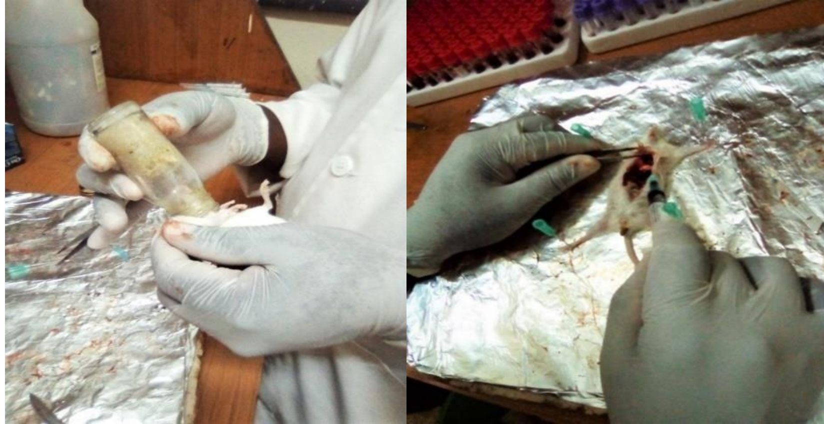
Figure 5: Polar and Non-polar dried extracts placed in sterile Bijou bottles

Figure 6: Paper punches (6mm in diameter)

Picture 7: Measuring Diameters of Zones of Inhibition (Microbiology Lab, Kenyatta University)

Figure 8: Grouping and Caging of Swiss albino mice (Animal Lab, Kenyatta University)

Picture 9: Weighing, Labelling and Administration of Plant Extracts in Swiss albino Mice (Animal Lab, Kenyatta University)



Picture 10: Anaesthetizing Swiss albino mice using Chloroform and Blood collection using Cardiac Puncture Method (Animal Laboratory, Kenyatta University)

Picture 11: Transferring blood Tubes and Measuring Blood Glucose Levels (Animal Lab, Kenyatta University)