EVALUATION OF ANTIMICROBIAL ACTIVITY, CYTOTOXICITY AND PHYTOCHEMICAL COMPOSITION OF *Ocimum americanum* L. (LAMIACEAE)

Hashim Mohamed Ali
(BPharm)
J56/34824/2019

A thesis submitted in partial fulfilment of requirements for Master of Science of University of Nairobi (Pharmacology and Toxicology)

Department of Public Health, Pharmacology and Toxicology

Faculty of Veterinary Medicine

University of Nairobi.

2022

DECLARATION

This thesis is my original work and has not been presented for a degree award in any other University.

Hashim Mohamed Ali
(J56/34824/2019)

Signature  Date: 01/11/2022

This thesis has been submitted for examination with our approval as University Supervisors

1. Dr. Joseph Mwanzia Nguta, BVM, MSc, PhD.
   Department of Public Health, Pharmacology & Toxicology,
   Faculty of Veterinary Medicine,
   University of Nairobi.

Signature  Date: 01/11/2022

2. Dr. Isaac Ole Mapenay, BVM, MSc, PhD.
   Department of Public Health, Pharmacology & Toxicology,
   Faculty of Veterinary Medicine,
   University of Nairobi.

Signature  Date: 02/11/2022

DEDICATION

I dedicate this research work with all my love and respect to my beloved wife and children; UmmulKheir Maryam, MwanaFahari Raheel, MwanaKuwoka Amira and MwanaMkuu Rayan who made a lot of sacrifice during the entire duration of the course. May Allah the almighty provides them with strength and understanding to give me much more conducive environment for studies.

ACKNOWLEDGEMENTS

The author first and foremost thanks the Almighty Allah (ﷲ) for best owing him with the opportunity, strength and will power to undertake this research project and I extend my sincere appreciation to Dr. Joseph Mwanzia Nguta for invaluable guidance and supervision throughout the project. Also, I extend my appreciation to Dr. Isaac Ole Mapeney as the second supervisor. I am also grateful to Dr. Fredrick Mutie Musila of Technical University of Kenya for his prompt data analysis and interpretation thereof. Similarly, I am indebted to Dr. Michell Okumu for Probit analysis as well as data presentation. Moreover, I thank my colleagues Dr. Dorine Nyak Matara and Dr. Sylvia Maveke for technical assistance, not forgetting the staff of Public Health, Pharmacology & Toxicology Department, who were of great support throughout the antimicrobial, toxicological and phytochemical studies. I commend their assistance, particularly Dr. Nduhiu, Mr. Bett, Mr. Maloba, Mr. Nderitu and Mrs. Pacho.

I would also like to appreciate Mr. Mbale of Botany Department of National Museums of Kenya for the plant botanical identification.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

AMDR	Antimicrobial Drugs Resistance
AMR	Antimicrobial Resistance
ANOVA	Analysis of Variance
ATM	African Traditional Medicine
ATCC	American Type Culture Collection
BACUC	Biosafety, Animal Care and Use Committee
DMSO	Dimethyl Sulfoxide
EDTA	Ethylenediaminetetraacetic Acid
FVM	Faculty of Veterinary Medicine,
GBD	Global Burden of Disease
GIT	Gastrointestinal Tract
INT	*p*-iodonitrotetrazolium violet
LD_{50}	Median Lethal Dose
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
MIC	Minimum Inhibitory Concentration
NACOSTI	National Council for Science and Technology
UNESCO	United Nations Educational, Scientific and Cultural Organization
UTI	Urinary Tract Infections
RTIs	Respiratory Tract Infections
rDNA	Recombinant Deoxyribonucleic Acid
SEM	Standard Error of the Means
TM	Traditional Medicine
WHO	World Health Organization

ABSTRACT

Estimates by the World Health Organization indicates that 80 % of the global population use herbal medicines for prophylaxis and curative purposes. In rural Kenya, Traditional Medicine is the primary source of healthcare, and most often the only source of healthcare service. This is due to its ease of accessibility, affordability and trust by millions of people. The cost factor of most phytomedicines makes them all the more agreeable at a time of spiralling healthcare expenses and nearly widespread austerity. Even though conventional medicine exists concomitantly with Traditional Medicine, phytomedicines have often been popular for cultural and historical reasons. Herbal medicinal products have turned out to be commercially widely available, particularly in industrialised nations. The rationale for the use of *Ocimum americanum* L. (Lamiaceae) in antimicrobial phytotherapy is largely based on the long-term experience of traditional medicine practitioners. This study aimed to investigate the antimicrobial activity and cytotoxicity of crude extracts and their fractions in a microbial and brine shrimp model. Several solvents were selected, their crude extracts and fractions evaluated for their antimicrobial activity, cytotoxicity and phytochemical composition. These included: aqueous, acetone, 70 % hydroethanolic, chloroform and ethyl acetate. Standard bacterial strains of *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25925), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603) and one fungal strain, *Candida albicans* were used to assess the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the *Ocimum americanum* L. sample extracts and their fractions via standard antimicrobial procedures at the microbiology laboratory, Department of Public Health, Pharmacology and Toxicology University of Nairobi. The data was evaluated for susceptibility of bacterial species and considered significant at 95 % confidence interval. Cytotoxicity of the crude samples and fractions were analysed using brine shrimp lethality test with ten-fold dilutions of 1000 µg/mL, 100 µg/mL and l0 µg/mL. Median Lethal Concentration at $p < 0.05$ confidence intervals was determined using Probit analysis. Established

phytochemical screening tests were performed to show the presence or absence of secondary metabolites. Cardiac glycosides, flavonoids, tannins, phenolics and reducing sugars were present in all sample extracts and fractions while polyuronides were absent. Two crude extracts and their fractions exhibited activity against the tested microorganisms. The hydroethanolic extract and its fractions were most active against the tested microbes. There was no significance difference ($p > 0.05$) in antibacterial activity between the acetonic and hydroethanolic extracts and their fractions. Gram-positive bacteria were more susceptible to the extracts/fractions than Gram-negative microbes. *Bacillus cereus* was most susceptible while *Escherichia coli* exhibited the highest resistance. All the sample extracts had statistically significant ($p < 0.05$) cytotoxicity at $LC_{50} < 1000$ µg/mL. Amongst all the samples the fractions of aqueous alcohol crude samples were more prospective thus good candidate for further research. Chloroform fraction of hydroethanolic sample extract was highly toxic with LC_{50} value of 0.59 µg/mL. The ethyl acetate sample fractions of aqueous alcohol crude sample have demonstrated promising antimicrobic effects against the Gram-positive microorganism *Bacillus cereus*. The fractions of hydroethanolic crude samples have potential bioactive molecules which are accountable for antimicrobial and cytotoxicity properties respectively. Results from the present research will provide a groundwork for finding an innovative natural phytomedicine. However extensive study is needed to quantify, isolate and characterise the phytocompounds.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Traditional Medicinal Practice (TMP) originated since time immemorial in different parts of the world (Tyler, 2000). African herbal medicinal practice have proceeded via descendants by verbal transmission with little written records or no records at all (Okigbo & Mmeka, 2006). Phytomedicines and folklore practices of established safety and effectiveness endow the primary health care objectives and assures accessibility to the whole society. According to the estimates by World Health Organization for millions of people worldwide, TMP is the primary provenance of healthcare, and most commonly the solitary fount of primary care provision. This is owing to its affordability, ease of accessibility and confidence by millions of individuals. The financial aspect of most herbal medicines render them all the more acceptable at a time of soaring medical care expenditures and virtually rife austerity (WHO, 2013). Although orthodox medicine coexists concurrently with TMP, traditional herbal medicines have commonly been prevalent for historical and traditional motives. Herbal medicinal products have turned out to be commercially widely available, particularly in industrialised nations (Schulz *et al.*, 2001). Estimates by the WHO indicates that 80 % of the worldwide populace consume phytomedicines for prophylaxis and curative purposes, and in Africa the practice is much higher (WHO, 2013).

The benefit of using phytomedicines over xenobiotic alternative is that they are comparatively safer, conferring effective prophylactic or curative therapy as well as affordability. The use of herbal medicines in emerging nations as a cultural basis for healthcare has been extensively observed (UNESCO, 1996). Additionally, the increasing dependence on the use of herbal medicines in the developed countries has been as a result of the progress of several crude extracts from medicinal herbs as well as from folkloric use of countryside therapies (UNESCO, 1998).

Continental Africa is inherently gifted with cornucopia of vegetation, approximated to thousands of types. Phytologists estimate that about 10% of flora in Africa is of therapeutic importance and some of the medicinal herbs have been systematically evaluated and their traditional role established (Gurib-Fakim & Mahomoodally, 2013). The medicinal herb *Ocimum americanum* L. (Lamiaceae) is native to subtropics and tropical parts of the globe including Indian subcontinent and continental Africa (Ali *et al.,* 2022). The species belong to the genus *Ocimum* which contains more than 60 species of aromatic herbs and shrubs (Simon *et al.,* 1999; Zengin *et al.,* 2019). The Lamiaceae species are example of herbal plants that are problematic to differentiate on the basis of just leaf morphology, due to the varied shapes of leaf within the species (Vieira *et al.,* 2003). O*cimum americanum* L. is a small erect branched, annual perennial aromatic herb which grows up to 1 m high. Stems are somewhat rounded or quadrangular, woody close to the base, hirsute and adpressed. Leaves are scarcely elliptical, typically hairless up to 25 mm in length (Sarma & Babu, 2011). The aromatic medicinal shrub has a widespread topographical dispersal in East Africa, making it the most common phyto-botanical flora in the area (Kokwaro, 2009). In Kenya, it is extensively dispersed in the jungle boundaries, secondary woodland and prairie, riparian spots and in arid zones, mostly in the mounds (Beentje *et al.,* 1994).

In Eastern Africa depending with the local tongue variation, the Swahili people refer to *Ocimum* (basil) as *Kivumbani/Mvumbani/Mrihani* (Hiltunen & Holm, 1999). Some genus from the family Lamiaceae has important medicinal properties that have high bioprospecting potential. Numerous species indigenous in East Africa are utilised in traditional therapeutic practices and some of their biophysiological effects have been appraised. African (hoary) basil is utilised for non-medicinal and medicinal objectives in diverse local traditions in East Africa (Hiltunen & Holm, 1999; Paton *et al.,* 1999). The main important property of the Lamiaceae (mint) family is associated with its constituent of essential oils. Also known to as volatile oils due to their characteristic high volatility, they are a rich mix with wide spectrum of

biophysiological properties. The essential oils obtained from the sweet-smelling herbal plants are a natural blend of phytocompounds with strong fragrance; they are by-products of secondary metabolism (Morsy & Hammad, 2021; Shadia *et al.,* 2007; Sutili *et al.,* 2016). *Ocimum americanum* L. has a wide range of bioactive compounds in the form of volatile oils. These include camphor, eugenol, methyl eugenol, methyl chavicol, farnesene, linalool, limonene and terpineol (Matasyoh *et al.,* 2006; Paton *et al.,* 1999; Shadia *et al.,* 2007; Sutili *et al.,* 2016).

1.2 Statement of the problem

All antimicrobials drugs that are launched into the marketplace have restricted therapeutic use owing to their intrinsic or acquired mechanism of microbial resistance (Walsh, 2003). Antimicrobial Resistance (AMR) has developed due to the undiscerning use of antibiotic feed additives in animal farming. Conversely humans have intensified the AMR by misuse and noncompliance of their prescribed antimicrobial drugs regimens (Runyoro *et al.,* 2006; Walsh, 2003). Moreover re-emergence of infectious diseases and the inflated rate of antimicrobics have been a key factor exacerbating to the vain control of infectious diseases in the developing nations such as Kenya (Runyoro *et al.,* 2006). Consequently, TM has been an alternative and affordable primary healthcare substitute. *Ocimum americanum* L. aerial parts are among some of purportedly effective ethnobotanical remedies currently available at a relatively low cost (Malik *et al.,* 2018). The plant materials are mostly sold in raw form and literature on the most efficient standard method of extraction is scanty.

1.3 Justification

Natural products of medicinal plants may provide a potential source of antimicrobial bioactive molecule conceivably with a novel mechanism of action. *Ocimum americanum* L. (Lamiaceae) is traditionally utilised against diarrhoea and dysentery (Runyoro *et al.*, 2006; Vidhya *et al.*, 2020). Although literature on antimicrobial activity of *Ocimum americanum* L. plant extracts has been reported, data on the antimicrobial activity and safety of derived fractions is scanty.

This study seeks to bridge these gaps in order to guarantee the safety and efficacy of traditional botanical used *Ocimum americanum* L. The research thus evaluated the antimicrobial activity, cytotoxicity and phytochemical composition of *Ocimum americanum* L. crude extracts and fractions. Findings from the present research would provide a potential foundation for finding of a new natural product derived molecule. Cytotoxicity studies are central in hazard assessment and safety evaluation phase of plant extracts. Therefore, this study is highly appropriate because it comes at a time when the use of herbal medicines in the treatment of many bacterial infections has taken a centre stage.

1.4 Study hypothesis

It was hypothesised that *Ocimum americanum* L. (Lamiaceae) crude extracts and fractions had antimicrobial activity, were cytotoxic and contained significant phytocompounds.

1.5 Objectives

1.5.1 General objective:

To investigate the antimicrobial activity, cytotoxicity and phytochemical composition of *Ocimum americanum* L. (Lamiaceae).

1.5.2 Specific Objectives:

1. Investigate the antimicrobial activity of *Ocimum americanum* L. (Lamiaceae) crude extracts and fractions using microbial organisms.
2. Evaluate the cytotoxicity of *Ocimum americanum* L. (Lamiaceae) crude extracts and fractions using *Artemia salina* nauplii.
3. Determine the phytochemical composition of *Ocimum americanum* L. (Lamiaceae) crude extracts and fractions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Many African aromatic and medicinal plants contain bioactive compounds which have curative properties. The phytochemical (bioactive) compounds are by-products of the plants usual catabolic-metabolic reactions. These compounds are known as secondary products of which there are several classes such as: alkaloids, coumarins, flavonoids, glycosides, tannins and terpenoids (Harborne, 1984; Ramawat *et al.,* 2009). In addition to these compounds, plants contain other chemical substances that avert unwanted adverse effects of the major bioactive metabolites or help in the absorption of the major bioactive compounds. For example opium poppy extracts from *Papaver Somniferum,* comprises of other compounds apart from morphine and the results demonstrate less unwanted adverse reactions in contrast to synthetic morphine (Okigbo *et al.,* 2009). Compared to synthetic medicines, many phytomedicines modulate their effects through synergistic action of numerous ligands interacting at solitary or multiple receptors linked with a certain biological process (Kaufman *et al.,* 2006). As reported by Tyler (2000), these synergistic pharmacodynamic actions can be beneficial in minimizing the adverse effects correlated with the predominance of a synthetic ligand in the body. Kaufaman *et al.,* (2006) reported how synergistic interactions improve the effectiveness of a number of phytotherapies.

2.2 Infectious diseases

Infectious diseases also referred to as communicable or transmittable diseases are conditions brought about by an infectious microbial agent such as bacteria, fungi, viruses and parasites. Usually they are transmitted from one person to another directly such as eating/drinking contaminated food, or indirectly such as a bite from a vector (WHO, 2018). Infections are a primary source of illnesses and mortality worldwide. The infections may be caused by both pathogenic and non-pathogenic microbes also referred to as opportunistic microorganisms.

Opportunistic infections are a leading cause of morbidity in immunocompromised and/or immunosuppressed patients. Examples of opportunistic pathogens includes; *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Cinti *et al.*, 2008; Faria *et al.*, 2015).

2.2.1 Bacterial pathogens

Pathogenic bacteria globally cause significant morbidity such as respiratory tract infections (RTIs) which are caused by *Pseudomonas* and *Streptococcus* bacteria and foodborne infections caused by *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* and *Shigella* bacteria. Similarly, high infants mortality in the third world countries is contributed by the pathogenic bacteria (Cinti e*t al.*, 2008). *Staphylococcus* and *Streptococcus* are part of a healthy skin microbiota and usually commensal on the normal skin and nasopharyngeal sections. Under altered conditions where they may find entry via a skin lesion or through sexual activity the microbes can possibly initiate skin infections or potentially cause meningitis, pneumonia or sepsis. These infections can become extremely debilitating resulting into a systemic inflammatory response hence causing massive vasodilation, shock and ultimately death (Fish, 2002).

2.2.1.1 Bacillus cereus

Bacillus cereus is a rod-shaped, motile, toxin-producing, facultative anaerobic and spore-forming Gram-positive bacterium that is ubiquitously distributed in the environment (McDowell *et al.*, 2021). They are micro cells, usually in chains and form elliptical spores, which are resistant to moderate sterilisation such as cooking. *B. cereus* are decidedly motile and beta-hemolytic on blood agar (Bottone, 2010). The pathogen is mostly associated with food poisoning, and has been reported to cause potentially serious non-intestinal infections. It usually causes gastrointestinal tract (GIT) illness which is characterised by abdominal discomfort, nausea, emesis and diarrhoea (McDowell *et al.*, 2021). However it has been linked to severe illness in immunosuppressed/compromised patient with serious infections of bacteremia, brain abscess, septicemia, endophthalmitis, empyema, fulminant sepsis, meningitis, osteomyelitis, and urinary tract infection (UTI) (Bottone, 2010; McDowell *et al.*,

2021). Its pathogenicity of GIT or non-intestinal is connected with the secretion of cell-disastrous exoenzymes. Amongst these emitted toxins is a vomiting-inducing toxin, proteases, three distinct phospholipases and four haemolysis toxins. *Bacillus cereus* secretes a potent β-lactamase type I which have shown a marked resistance to susceptible antimicrobials (Bottone, 2010).

2.2.1.2 Staphylococcus aureus

Staphylococcus aureus is a spherical shaped, facultative anaerobic and Gram-positive bacterium commensal of the human microbiota. It is an opportunistic pathogen, usually found on the skin, mammary glands, mouth, mucus membranes, upper respiratory tract, gastrointestinal tract and genitourinary tract (Taylor & Unakal, 2021). *Staphylococci aureus* are microcellular, non-spores forming, non-motile and beta-haemolytic on blood agar. Their colonies are smooth, entire, creamy-yellowish in colour, somewhat raised and luminous (Tong *et al.,* 2015).

Staphylococcus aureus is a major human bacterial pathogen that infects its host with wide range of pathophysiology. Infections are generally acquired in the community or nosocomial settings and it can precipitate multiple illness. As a hospital-acquired pathogen it is a main cause of illness and death (Tong *et al.,* 2015). Most infections of *S. aureus* are severe and pyogenic including dermal and soft tissue conditions such as carbuncles, cellulitis, folliculitis, furuncles, impetigo and scaled skin syndrome. Other infections are acute endocarditis, bacteremia, cerebritis, myocarditis, pericarditis, pneumonia, food poisoning, meningitis, osteomyelitis, toxic shock syndrome, septic arthritis, gastroenteritis and urinary tract infections (Taylor & Unakal, 2021). Chemotherapeutic management remains a challenge owing to the advent of multi-drug resistant strains such as Methicillin Resistant *Staphylococcus aureus* (MRSA) (Taylor & Unakal, 2021; Tong *et al.,* 2015).

2.2.1.3 Escherichia coli

Escherichia coli is a rod shaped, coliform, facultative anaerobic and Gram-negative bacterium which is usually resident in the lower GIT of homeothermic animals. As a resident microbiota it lives symbiotically with hosts and rarely cause infection (Allocati *et al.,* 2013). However it is one of the most common animal and human opportunistic pathogen which is accountable for a wide variety of infections (Kaper *et al.,* 2004; Nataro & Kaper, 1998). The innocuous strains are commensal in the GIT and can benefit their hosts by producing vitamin K and prevent colonisation of the gut by pathogenic microbes and relate mutualistically with the host (Gomes *et al.,* 2016). The unusual features of *E. coli* are characterised by its capability to grow with or without oxygen, easy to culture and availability of the complete genome makes it a significant workhorse in biotechnology. It is utilised in a wide spectrum of application in both medical and industrial arena and it is the most utilised organism in the field of rDNA technology (Allocati *et al.,* 2013; Yoon *et al.,* 2009).

E. coli lives mutualistically in the large intestines and rarely infects its host. However, numerous pathogenic strains can cause intestinal and extraintestinal illness in healthy and immunosuppressed host (Allocati *et al.,* 2013; Nataro & Kaper, 1998). The pathogen is well known to cause bacteraemia, diarrhoea, enteritis, septicaemia, meningitis and UTIs (Kaper *et al.,* 2004). The diarrhoeal disease is an acute public health problem and a primary cause of illness and deaths in infants and paediatric patient especially in lower income countries (Allocati *et al.,* 2013; Gomes *et al.,* 2016).

2.2.1.4 Klebsiella pneumoniae

Klebsiella pneumoniae is a rod shaped, encapsulated, facultative anaerobic, non-motile and Gram-negative bacterium of the family *Enterobacteriaceae*. It commonly occurs as normal microbiota of the mouth, gut, skin and is also found naturally in the soil (Effah *et al.,* 2020). *Klebsiella pneumoniae* is an opportunistic bacterium which is ubiquitous in nature such that it is very important clinically and have received much public health anxiety. It causes a wide

spectra of infections and has exhibited increased antimicrobial resistance (Xu *et al.,* 2017). This microorganism is responsible for 33.33% of all Gram-negative infections such as cystitis, endocarditis, pneumonia, septicaemia and surgical wound infections. Consequently, prolonged morbidity and high rates of mortality coupled with high cost of medical care are often characteristic of illness caused by this pathogen (Effah *et al.,* 2020; Shiri *et al.,* 2017)**.**

The majority of cases of *K. pneumoniae* infections are nosocomial-acquired, while the clinicians are well aware of community-acquired bacterial pneumonia found predominantly in alcoholics (Heidary *et al.,* 2018). The hospital-acquired infections are due to the opportunistic nature of the pathogen which primarily infect the immunocompromised patient who are hospitalised and suffer from acute conditions (Podschun & Ullmann, 1998). The substantial increase in the prevalence of multidrug resistant and extremely drug resistant microorganism belonging to the *Enterobacteriaceae* family is a major economic burden as these microbes are natural microbiota of animal and human microbiome (Effah *et al.,* 2020; Heidary *et al.,* 2018).

2.2.2 Fungal pathogens

Pathogenic fungi are opportunistic microorganisms which can infect animals, plants and humans. However more fungi are known to cause infections to the plantae kingdom than to the animalia kingdom (Drummond *et al.,* 2014). The genus *Candida* includes several species which maybe pathogenic to immunosuppressed/compromised patients. *Candida albicans* species is a natural microflora in the human microbiome and it is an opportunistic pathogen in animals including human (Kabir *et al.,* 2012; Kadosh, 2019).

2.2.2.1 Candida albicans

The fungus species *Candida albicans* is a commensal microorganism that lives on the gut and genitourinary tract as a normal microbiota of mammalian animals including humans (Kabir *et al.,* 2012). Usually it is an opportunistic pathogen that infect immunocompromised patients (Limon *et al.,* 2017). It is commonly known as dimorphic microbe since it is a kind of diploid and grows both as filamentous as well as yeast (Kadosh, 2019). *Candida albicans* is the most

common fungus species isolated from biofilms either formed on human tissue or permanent medical implement (Nobile & Johnson, 2015). The organism can easily be subcultured with general media in the laboratory and readily studied both *in vitro* and *in vivo* (Gow & Yadav, 2017; Limon *et al.*, 2017). Consequently, the species *Candida albicans* is generally utilized as a model organism for pathogenic fungus (Kabir *et al.*, 2012).

Candida albicans is the cause of wide spectra of systemic and mucosal infections particularly to the immunocompromised/suppressed patients (Kadosh, 2019). Candidiasis, an overgrowth of the fungal microorganism is a human infection that is caused by one of the few species of the genera *Candida* (Martins *et al.*, 2014). Candidiasis can be categorised into two groups depending on the seriousness of the infection. In the first group is the mucosal infections which include mouth and vaginal thrush which is characterised by white spots on affected mucus membrane. While the second group is the systemic infection which is referred to as candidemia or bloodstream infection (Kabir *et al.*, 2012; Kadosh, 2019).

2.3 Ethnomedicine

The use of medicinal herbal plants as a natural source of ethnomedicine is based on the extensive experience of numerous generations of herbalist and traditional medicines practitioners spread all over ethnic communities. Throughout the world each culture has accumulated a body of herbal medicinal knowledge as part of its folk custom, but most of them remained oral tradition being passed by word of mouth from one generation to the next(Abbasi *et al.*, 2015). The earliest known herbal medicinal record is a 4000-year-old clay tablet written by Sumerians documenting herbal preparations with their various illness. During the time of ancient Egypt, a vast knowledge of herbal medicinal plant existed which was preserved in the Ebers papyrus along with other hundreds of therapies. Moreover, ancient China and India had their own traditional systems well established. Chinese medicine had a pharmacopoeia with thousands of medicinal plants remedies which was published in 1600, while Ayurvedic medicine has its *Materia medica* comprising of hundreds of plants which is still in use at

present. Hippocrates the father of western medicine believed that diseases had natural causes and he used medicinal herbal plants for his therapy (Suvajdžić *et al.*, 2016). In the Middle Ages western medicine came to a standstill but in the Islamic sphere progress was being made. The most outstanding of the eleventh century was Ibnu Sina (Avicenna) who compiled the "Canon of Medicine" a voluminous work which included new information on ethnomedicine (Abbasi et al., 2015). Currently there is a renewed interest in studying herbal plants for bioactive useful compounds, with some of the top research institutes and leading pharmaceutical companies involved in ethnopharmacological research (Heinrich, 2010).

2.3.1 Ethnopharmacology

Ethnopharmacology is a multidisciplinary approach to the study of physiological and toxicological properties of natural preparation used for animals including humans whose effect is either beneficial or toxic (Heinrich, 2014). Consequently, ethnopharmacology is a highly innovative technique in drug development which leads to the discovery of new nutraceuticals and pharmaceuticals (Abbasi *et al*., 2015). The natural molecules continue to be one of the most significant sources of a novel bioactive leads compound. According to Chin *et al.,* (2006) more than 50 % of new pharmaceutical/nutraceutical launched in the market are of natural source, their analogous or derivatives (Chin *et al.,* 2006; Heinrich, 2010). The focus of ethnopharmacology today has certainly progressed to understanding the benefits and risks of generally used local medicinal plants with the objective of contributing to effective and safer utilization of natural resources. Moreover the many studies in ethnopharmacological research gives a testimony to the flourishing research interest in how we use medicinal herbal plants as either food, medicine or poisons in animals (Heinrich *et al.,* 2009).

2.3.2 Description of the herbal plant *Ocimum americanum* L. (Lamiaceae)

Ocimum americanum L. (Lamiaceae) is a small erect branched, sweet-smelling yearly herbaceous plant that raises upto 1 m tall (Figure 2.1). The shrub has a long taproot that runs deep into the soil. Stems are somewhat rounded or quadrangular, adpressed and woody near

the base with retrorse hairs. Leaves are slander and lanceolate up to 25 mm long, margin entire or sparsely serrate with acute apex and cuneate base, pubescent with short antrorse and long patent hairs on midrib and lateral veins beneath. Flowers are small in spiciform inflorescence of closely set whorls in purple, pinkish or white colour. Seeds are nutlets black, narrowly ellipsoidal, minutely punctulate (Ali *et al.*, 2021; Sarma & Babu, 2011). The whole shrub is highly aromatic, with aroma akin to citrus. The ethnobotanical plant is highly variable and polymorphic with numerous types, some of which have previously been thought as different subspecies or species. Common chemotypes includes; camphoraceous, floral-lemony and spicy (Paton *et al.*, 1999). The tropical medicinal plant from the mint (Lamiaceae) family is usually known as American basil, hoary basil or mosquito plant (Shadia *et al.*, 2007). Hoary basil is a widely dispersed species in the subtropics and tropical areas of the globe. In Kenya it is extensively dispersed in grassland, secondary bushland, forest margins, dry and riverine sites as well as in hills (Githinji & Kokwaro, 1993; Kokwaro, 2009).

Taxonomical tree

Domain: *Eukaryotae*

Kingdom: *Plantae*

Phylum: *Tracheophyta*

Subphylum: *Angiospermae*

Clade: *Eudicots*

Class: *Asteridae*

Order: *Lamiales*

Family: *Lamiaceae*

Genus: *Ocimum*

Species*:* *Ocimum americanum* L.

Figure 2.1: *Ocimum americanum* L. (Lamiaceae) aerial parts sourced from Pate Island, Lamu County.

2.3.3 Traditional uses of *Ocimum americanum* L. (Lamiaceae)

Ocimum americanum L. is utilised for curative and non-curative objectives in divergent traditional societies in East Africa. The herbal shrub is used for flavouring of tobacco, peppermint in tea and as body cologne. Its leaves and branches are usually used as insect repellents or insecticides against flies, mosquitoes, bees, and other pests. The leaves and/or branches are placed on the rooftops or burned to provide the repulsive effects (Ali *et al.*, 2021). Leaves of the aromatic herbal plant are either squashed between the palms of the hands and snuffled, or warm water vapor are puffed to clear the anterior nares as well as to loosen bronchial phlegm (Pattnaik & Chand, 1996). The Swahili people of Lamu County of Kenya use aerial parts of the medicinal herb for acute management of elevated blood pressure (*bokhari*), to cure abdominal cramp and as peppermint in black tea (Ali *et al.*, 2021; Watt *et al.*, 1962). Decoctions from the medicinal herb are used to cure coughs, tuberculosis, stomach ache, piles (haemorrhoids), eye and ear grievances (Vidhya *et al.*, 2020). Concoctions are utilised to cure peptic ulcers and also as anti-purgative remedy in Eastern Africa. In India (Tamil Nadu) the herbal plant is known locally as *Nai thulasi* and its leaf decoction is customarily employed to treat diabetes, dysentery, constipation, diarrhoea, and haemorrhoids (Bassole *et al*., 2005; Vidhya *et al.*, 2020).

2.4 Phytochemicals from *O. americanum* L. and their pharmacological properties

The primary significant activities of the Labiatae family are bound to its content of essential (volatile) oils and crude extracts. The volatile oils have a varied range of biological and pharmacological activities (Githinji & Kokwaro, 1993). In recent years, the plant extracts and their volatile oils from various aromatic medicinal herbs have fascinated a vast research devotion due to their prospects as a source of bioactive compounds and natural insecticides (Runyoro *et al.,* 2010). Particularly, the antimicrobial action of volatile oils is the basis of numerous uses including; alternative medicine and phytotherapy, preservation of fresh and processed food, as well as pharmaceuticals (Runyoro *et al.,* 2010).

Ocimum americanum L. has a variety of bioactive substances in the form of volatile oils. These include amongst others; eugenol, methyl eugenol, linalool and trans-caryophyllene. Methyl eugenol constitutes the highest percentage (up to 71 %) of the active compounds (Shadia *et al.*, 2007). Eugenol a bioactive compound has a range of properties including: antimicrobial activity (Runyoro *et al.,* 2010), cytotoxic activity (Tajo & Thoppil, 1998), as well as being an effective mosquito repellent (Chokechaijaroenporn *et al.,* 1994). Antioxidant properties of eugenol are not well cited in the literature. The scavenging property of the phenolic bioactive compounds have been documented on numerous species of the genus *Ocimum* (Aluko *et al.,* 2013; Javanmardi *et al.*, 2003; Zengin *et al.*, 2019). *Ocimum americanum* L. is reported to have varying biological activities including; antimicrobial (Carović-Stanko *et al.,* 2010; Vidhya *et al.,* 2020), antifungal (Vieira *et al.*, 2003), gastric cytoprotective antiulcer as well as acute gastric ulcer (Sutili *et al.,* 2016) and larvicidal activity (Madhiyazhagan *et al.,* 2014).

2.4.1 Alkaloids

Alkaloids are a group of organic bioactive substances that comprise at least one N-atom in their heterocyclic ring, usually alkaline in nature and most have a bitter taste (Kurek, 2019; Pengelly, 2021). In addition to the nitrogen atom the heterocyclic hydrocarbon compounds may also encompass oxygen, sulphur and infrequently, other atoms such as bromine, phosphorus and chlorine on their ring structure (Kurek, 2019). Alkaloids occur naturally in plants, animals and microorganisms. The alkaloids content of plants is typically small within a few percentages (10 to 25% in higher plants) and is heterogenous over the plant parenchyma. Depending on the nature of plants, higher concentration is detected in the bark (cinchona), seeds (Strychnine tree), leaves (black henbane) and root (*Rauvolfia serpentina*) respectively. Moreover, different parts of the plants may contain diverse alkaloids (Kukula-Koch & Widelski, 2017).

Alkaloids have a varied array of biophysiological activities (Table 2.1) including: analgesic (morphine), antiasthma (ephedrine), antiarrhythmic (quinidine), antimalarial (quinine), anticancer (homoharringtonine), antihyperglycemic (piperine), antibacterial (chelerythrine),

cholinomimetic (galantamine) and vasodilatory (vincamine) activities (Harborne, 1984; Kurek, 2019). Moreover, some alkaloids can precipitate toxicity such as atropine, hyoscyamine and tubocurarine. Additionally many alkaloids are popular as stimulant (caffeine, nicotine, theobromine and cocaine), and psychotropic activities (psilocin) and have been used in entheogenic rituals, while others found use in traditional medicine, recreational drugs as precursor for drug development (Kukula-Koch & Widelski, 2017; Kurek, 2019).

Table 2.1: **Major alkaloids classes with examples and pharmacological uses, adapted from Pengelly, 2021.**

Class	Alkaloid	Pharmacological action
Alkaloid amines	Colchicine	Uric acid solvent
	Ephedrine	Bronchodilator
Purine alkaloids	Caffeine	CNS stimulant
	Theobromine	Heart stimulant, vasodilator and a diuretic
	Theophylline	Antiasthma drug
Imidazole	Pilocarpine	Miotic, cholinergic
Tropane	Atropine	Antidote of cholinesterase inhibitors
	Scopolamine	Anticholinergic and CNS depressant
	Hyoscyamine	Anticholinergic, mydriatic effect
Isoquinoline	Emetine	Antiprotozoal agent, emesis
	Morphine	Analgesic
	Tubocurarine	Muscle relaxant
Pyrrolizidine	Senecionine	Hepatotoxin, DNA damage
	Symphytine	Hepatotoxin
Pyridine/piperidine	Lobeline	Expectorant, bronchodilator
	Nicotine	Stimulant, nicotinic acetylcholine receptor agonist
Quinoline	Glaucine	Antitussive
	Quinidine	Antiarrhythmic
	Quinine	Antipyretic, antimalarial
Indole	Ajmaline	Antiarrhythmic
	Reserpine	Antihypertensive
	Yohimbine	Stimulant, aphrodisiac
	Physostigmine	Inhibitor of acetylcholinesterase
Quinolizidine	Sparteine	Antiarrhythmic, diuretic, oxytocic

Vinca alkaloids	Vinblastine, vincristine	Antitumor
	Vincamine	vasodilating, antihypertensive

2.4.2 Anthraquinones

Anthraquinone is an aromatic organic natural compound with a chemical formula $C_{14}H_8O_2$, also known as anthracenedione or dioxoanthracene. The polycyclic structure consist of tricyclic rings with two keto groups attached to the central ring (Deitersen *et al.*, 2019). They are an important group of colourful, bioactive and abundant secondary metabolites found in bacteria, fungi and plants. Anthraquinones comprise a class of natural and synthetic compounds with a varied array of uses (Malik & Müller, 2016). In addition to their application as dyes anthraquinone derivatives have been utilized for therapeutic purposes as anti-inflammatory, antimicrobial and as a laxative agent (Martorell *et al.*, 2021). Furthermore a bioactive anthraquinone derivative Reactive Blue 2 (RB-2) is an important pharmacological tool for biochemical and drug research, also may serve as a bioprospecting lead structure for the development of subsequent pharmaceutical (Malik & Müller, 2016; Martorell *et al.*, 2021).

Current medical application includes; antidiabetic, antiarthritic, anti-inflammatory, antibacterial, antifungal, antiviral, anticancer and laxative (Malik & Müller, 2016). In particular, anthraquinone laxatives have been used for centuries and widely studied anthranoid derivative. The compound occurs in plants mainly in their glycosidic form. When taken orally the laxative bypasses hydrolysis in the stomach and small intestines due to the β-glycosidic linkage between aromatic ring and sugar moiety. Consequently, it transits unchanged to the large intestines where bacterial β-glucosidases and reductase cleaves the glycosylated sugar moiety into a bioactive aglycones. The aglycones have two distinct cellular mechanisms, an accelerated transit due to colonic motility and alterations in colonic absorption and secretion. The absorption and secretion activity are primarily brought by a direct contact between the purgatives and the epithelial cells, while colonic motion is due to indirect epithelial cell impairment that causes loose diarrhoea. The absorbed laxative that finds its way to the blood

circulation induces a rapid extracellular potassium ions (K^+) depletion through gastrointestinal routes leading to loose watery stool (Malik & Müller, 2016; Martorell *et al.,* 2021; Shukla *et al.,* 2017; Van Gorkom *et al.,* 1999).

A review by Shukla *et al.,* (2017) reported potential toxicity of plants extracts with anthraquinone compounds. The biomolecule contains the quinone moiety that is a potential genotoxic and have the ability to change the redox system thus disrupting mitochondrial activity or by nucleophilic addition reaction with biomolecules including proteins and DNA. The mechanism of toxicity follows anthranoid derivatives producing a Reactive Oxygen Species (ROS) surplus which forms complexes with iron ion (Fe^{2+}) that undertake a redox cycling and oxygen radical generation (Martorell *et al.,* 2021; Shukla *et al.,* 2017).

2.4.3 Cardiac glycosides

Glycosides are a class of organic bioactive compounds categorized by the fact that structurally they comprise of an aglycone (active moiety) and glycone (sugar moiety) attached to one or more aglycone by a special bond. Biochemically they are hydroxyls of a sugar that can be easily hydrolysed with enzymes to form ethers with other alcohols (Pengelly, 2021). Glycosides are classified based on the characteristics of the aglycone. In spite of their extensive distribution in nature some classes are often found in the same botanic families consistently with the same aglycone type e.g., Asteraceae – flavonoids, Brassicaceae – glucosinolate, Rosaceae – cyanogenic and Scrophulariaceae – cardiac. Glycosides are widely distributed throughout the plantae kingdom and some maybe highly toxic particularly cardiac and cyanogenic glycosides. They are mainly soluble in aqueous and organic solvents, however boiling usually hydrolyse them rendering them non-toxic and the aglycones are somehow less soluble (Evans, 2002; Pengelly, 2021).

Cardiac glycosides are bioactive organic compounds found in plants and toad (*Rhinella marina*). They are widespread in nature with potent cardiovascular activities (Botelho *et al.,* 2019). The cardiotonic steroids have cyclic carboxylic esters (lactone rings) linked in the β-site

at Carbon-17. The glycones are glycosidically linked via Carbon-3-hydroxyl group of the steroid active moiety. The active moiety has a polycyclic steroidal nucleus (gonane) with hydroxyl (OH^{-}) groups at position C-3 and C-14. These compounds are subclassified according to their steroid moiety; the penta-lactone ring are known as cardenolides while the hexa-lactone ring are referred to as bufadienolides (Pengelly, 2021). Cardiac glycosides have both inotropic (force) and chronotropic (rate) influence on the cardiac muscles. They block heart sodium-potassium ATPase which leads to increased intracellular calcium ions resulting in an increased contraction of myocardium. In cardiac failure they cause a complete evacuation of blood from the heart ventricles and shortens the span of systole, thus myocardium has additional period to relax in between the contractions. Elevated output lowers the cardiac rate and improves kidney function (Evans, 2002; Pengelly, 2021).

Cardioactive steroids have a very low therapeutic index/ratio (TI/TR), implying the toxic dose (TD) is not much lower than the therapeutic effective dose (ED). Cardiac glycosides poisoning precipitates several dysfunctions in the human systems, including gastrointestinal, neurological (drowsiness, headache, neuralgia) and the most life-threating cardiovascular toxicity such as arrhythmias and deteriorating cardiac failure (Shukla *et al.,* 2017).

2.4.4 Cyanogenetic glycosides

Cyanogenic glycosides are natural phytotoxin which occurs in various edible and herbal plants. The secondary metabolic product usually yields cyanide following enzymatic hydrolysis. Consumption of herbal plant with cyanogenic glycosides may precipitate acute cyanide toxicity which is characterized by central nervous system (CNS) damage and growth retardation (Bolarinwa *et al.,* 2016). However, processing techniques tend to detoxify the glycosides and decrease the hazard of cyanide exposure. Cyanogenic glycosides are a class of nitrile with structural variation and a number of amino acids as the precursor of the glycosides (Breyer-Brandwijk, 1962).

Amygdalin and prunasin are amongst common cyanogenic glycosides of plants. Laetrile a drug containing amygdalin has been used as antitumor medication, however its use is now limited. These glycosides in small doses do exhibit digestive, expectorant and sedative properties (Pengelly, 2021). Prussic (hydrocyanic) acid which occurs in the form of hydrogen cyanide is one of the most noxious of all phytotoxins. The toxicity of the prussic acid includes deactivation of respirational enzymes, which leads to vertigo and face flushing. In large dosages complete shutdown of CNS occurs and ultimately death follows. However, consumption of high doses of the crude plants (> 3.5 mg/Kg) is needed for lethal result to ensue. Human body is capable of detoxifying the nitriles by changing them to thiocynates which are excreted in the excreta (Bruneton, 1995).

2.4.5 Flavonoids

Flavonoids are phytocompounds which occur in aglycone, glycosidic form and free state. These compounds with chemical structure of C_6-C_3-C_6 occur as white or yellow pigment in plants. Flavonoids are phenolic water-soluble compounds which change colour when treated with alkaline solution or ammonia, hence are easily identified in solution or on chromatograms. The glycosidic form commonly occurs in plant bound to sugar moiety and any of flavonoid aglycone, thus may be present in a single plant in various glycosidic combination (Harborne, 1984). Flavonoids are ubiquitous in the plantae kingdom, usually products of both acetate and shikimic acid processes. Mostly all plants have a sequence of closely related flavonoids with varying degree of hydroxylation and/or oxidation forms. Consequently, numerous structural groups occur, the most common being flavonols (quercetin) and flavones (apigenin). Flavonoids are the most important component of the human diet and are found universally in plants, normal consumption is approximated at 1 g flavonoid/day/person (Pengelly, 2021).

Therapeutically flavonoids are referred to as ‘biophysiological stress modifiers’, since they affect the cardiovascular system and strengthen the blood capillaries hence serve as a defence against environmental stressors (Middleton, 1988; Middleton & Kandaswami, 1992).

Flavonoids as a dietary constituent are reported to have numerous phytopharmacological properties including; antioxidant, antimicrobial, anti-inflammatory, antitumor, enzymatic modulator and are responsible for metabolic syndromes such as cardiovascular diseases (Kumar & Pandey, 2013). Their properties are structural dependent. Functional groups in flavonoids facilitate their scavenging activity by complexing metal ions and/or oxidizing free radicals. The metal ions chelation is very critical in the inhibition of free radicals generation which damages targeted bioactive molecules (Kumar & Pandey, 2013; Pengelly, 2021). The enzymatic modulation of flavonoids is due to the presence of functional hydroxyl group which is capable of alternatively stimulating or inhibiting particular enzymes such as aldose reductase – causes diabetic cataracts which is inhibited by quercetrin; xanthine oxidase – causes hyperuricaemia which is blocked by free hydroxyl groups (Pengelly, 2021).

2.4.6 Phenols

Phenols are unique class of monocyclic phytoconstituent within vegetable, fruit and plant fibre. Phytophenols are universally found as secondary metabolites in plants, as a result they are consumed in high amounts. They are highly diverse and multifunctional bioactive compounds with considerable health potential and several studies reported that increasing consumption of plant fibre minimizes the incidence of metabolic syndromes (Kling *et al.,* 2016). The compound is characterized by a hydroxyl functional group attached to aromatic benzene ring with a structural formula of C_6H_5OH. The aromatic polycyclic structure may accept additional alternatives particularly CH_3 - (methyl) group. Simple phenolic compounds comprise of a benzene nucleus in which hydroxyl group has substituted a hydrogen atom. Phenols are tertiary alcohols which are weakly acid and widely distributed amongst all families of plants (Pengelly, 2021).

These phytocompounds act as natural antimicrobial and provide antioxidant, anti-inflammatory and antitumor activities. Phenol reduce oxidative stress and eventually circumvent the destructive effects of reactive oxygen species elicited on the cellular systems (Kling *et al.,*

2016). Simplest phenols consist of an arene with a benzene nucleus bearing hydroxyl (OH^-) groups, these includes benzenetriol (pyrogallol) and hydroquinone. Addition into the phenol structure of a carboxyl group produces C_6C_1 compounds which are widely distributed in nature and have significant therapeutic properties. The most notable of this group are salicylic acid and gallic acid. Phenol and its derivatives are crucial for production of numerous pharmaceuticals and herbicides. Phenol itself is a typical bacteriostatic agent and plays a major role as an antiseptic agent (Pengelly, 2021).

2.4.7 Phytosterols/triterpenes

Triterpenes are large and diverse class of phytocompounds made up of several subclasses including phytosterols. Triterpenoid are derivative of a C_{30} precursor squalene, which have analogous structure to phytosteroids obtained in florae whose C_{27} skeleton is similarly derivative of $(C_5H_8)_6$ squalene (Pengelly, 2021).

Phytosterol are structural constituent of cell membrane of plants, as thus they consist of both plant stanols and sterols. The most abundant source of naturally occurring phytosterol are vegetable oils and its products. Phytosterols can be found as conjugate of glycolipids or esters as well as free form. The compound is composed of a tetracyclic structure and is linked by a hydroxyl bound at C3 and an additional ethyl or methyl group in the side chain which is not existing in animal steroids. Additionally, the compounds can be classified by the number of methyl groups in C4, namely 4-monomethyl-, desmethyl-, or 4,4-dimethyl steroids. Phytosterols such as sitosterol and stigmasterol are central constituent of plant biomembranes, and they are crucial starting material in the manufacturing of steroidal medicines (Moreau *et al.,* 2018).

Therapeutically phytosterols are useful components of the human nutrition since they play a role in blood cholesterol regulation and blood sugar reduction (Salehi-Sahlabadi, Kord Varkaneh, *et al.,* 2020). In addition to low density lipoprotein (LDL) reduction, phytosterol possess antioxidant, anti-inflammatory, anti-neoplastic and anti-atherogenicity properties

which are of clinical significance even for people without high blood LDL cholesterol (Berger *et al.,* 2004). However, several studies have reported that phytosterols decreases the absorption and plasma levels of various antioxidants and lipid-soluble vitamins. Moreover, a rare genetic disease referred as Sitosterolemia – accumulation of phytosterols in the human body can lead to untimely death. The defect in the genes (ABCG5 and ABCG8) involves the transfer of phytosterols out of the liver and intestine, subsequently the phytosterols accumulate in the blood and tissues (Moreau *et al.,* 2018).

2.4.8 Tannins

Tannins are large phytocompounds derived from phenolic acids. They are grouped as polyphenolic compounds, which are widespread in the back of trees, stems, leaves, fruits and insect galls throughout the world. They are complex molecules that readily bind with cellulose, mineral, proteins and starches, resulting to insoluble compounds resistant to decomposition (Mustafa & El-kamali, 2019). Tannins have a high molecular weight (500 - 5000 Da) comprising sufficient phenolic hydroxy moiety to allow for stable cross links with proteins, as a result the covalent formation may inhibit enzymatic degradation. Tannins can be classified into two categories; nonhydrolysable (condensed) and hydrolysable tannins (Pengelly, 2021). Nonhydrolysable tannins have a more complex structure than hydrolysable tannins, however their complex structures are yet to be determined. Condensed tannins are widespread in vegetables, grains, fruits and legume. Hydrolysable tannins are derivative of simple phenolics, mainly 3,4,5-trihydroxybenzoic (gallic) acid which is bounded to a glucose molecule by esterification. During hydrolysis tannins breakdown to give gallotannins (glucose and gallic acid) or ellagitannins (glucose and ellagic acid) which are readily soluble in aqueous and alcohol (Chung *et al.,* 1998; Pengelly, 2021).

Nutritionally tannins are often considered undesirable due to their ability to form stable bonds with starch, proteins and digestive enzymes thus decreasing their nutritional values as a functional food (Chung *et al.,* 1998). Studies have reported numerous pharmacological

properties of tannins including; antimicrobial, antioxidants, anti-inflammatory, antidiabetic, anti-obesity and antitumor (Si *et al.,* 2021). Moreover, tannins are used for their astringent action – cause contraction of tissue, diminishes exudations, as well as blanching and wrinkling of mucous membranes. When used to dress wounds they form a thin protective layer thus prevent exudates (Pengelly, 2021; Si *et al.,* 2021).

2.4.9 Terpenoids

Terpenoids (terpenes) are the biggest and most varied class of phytocompounds derived from isoprene - C_5 unit compound and its polymers are known as terpenes. Terpenoids are transformed group of terpenes with additional functional groups and oxidized methyl group removed or moved at several positions. Terpenoids are further subclassified and the number of units designated in the specific terpene serves as a basis of classification i.e. monoterpenes (C_{10} – 2 units), sesquiterpenes (C_{15} – 3 units), diterpenes (C_{20} – 4 units), sesterpenes (C_{25} – 5 units), triterpenes (C_{30} – 6 units), tetraterpenes (C_{40} – 8 isoprene units), and polyterpenes $(C_5H_8)n$ (Pengelly, 2021; Perveen, 2018).

Terpenoids play a significant role in traditional culinary - used for their aroma, taste and colouring of food. Terpenoids are responsible for the flavours of cloves, cinnamon and ginger, the fragrance of eucalyptus, the red colour in tomatoes and the yellow pigment in sunflowers (Cox-Georgian et al., 2019). Terpenoids the largest and most diverse class of plant secondary metabolites, have considerable pharmacological properties and consequently account for substantial research interest and approximately 60% of natural products in the market. Due to their structural variations bioactive terpenoids are used universally for diverse roles in the fields of cosmetics, food and pharmaceutical industries. Taxol a diterpene and its derivatives inhibits proliferation of different neoplastic cells thus used to managed different types of cancers (Perveen, 2018). Artemisinin, a sesquiterpene and its derivatives have atypical endoperoxide bridge which is responsible for antimalarial and anthelmintic activity. They have the benefit over other medication in having the ability to kill rapidly and arrest all the stages of the life

cycle of the parasites (Wang *et al.*, 2019). Moreover studies have reported several other pharmacological activities of terpenoids including; antimicrobial, antiviral, anti-inflammatory, antidiabetic and antidepressants (Pengelly, 2021; Perveen, 2018).

2.4.10 Saponins

Saponins are naturally occurring bitter tasting steroidal glycosides that foam when shaken in water. Also referred to as triterpene glycosides, they are structurally a combination of lipophilic sapogenins (aglycone) and hydrophilic sugars (glycone) which give them the characteristic soapy like effect or detergency and the ability as a surfactant - decrease surface tension (Mustafa & El-kamali, 2019). Saponins are both lipid and water soluble which renders them the valuable detergent properties (Harborne & Baxter, 1993). Saponins are traditionally subdivided into steroid and triterpenoid glycosides and further classified based on their aglycone (sapogenins) chemical structure. Oleanolic acid a triterpenoid saponin is the most common aglycone widely distributed in the plantae kingdom. Steroidal saponins comprise of steroid aglycone on their structures which are usually arranged into hexa-rings. They have C_{27} skeleton which are used as primers for cortisone, sex hormones and vitamin D. e.g. hecogenin and diosgenin (Kregiel *et al.*, 2017; Pengelly, 2021).

Saponins are widely employed in the manufacture of dietary supplements, drugs, soaps, fire extinguishers, carbonated beverages and cosmetics (Guclu-Ustundag & Mazza, 2007). Pharmacological properties of saponins include; lowering of LDL cholesterol, cytotoxic activity on tumor cells via induction of apoptosis, antidiabetic effect, antimicrobial, antiviral, anthelmintic and insecticidal activities (Kregiel *et al.*, 2017; Mustafa & El-kamali, 2019). Traditionally many saponins rich herbs are used for wound healing due to their significant local effects on skin. Similarly their local effect on the digestive system assist digestion and accelerate and increases GIT ability to absorb important active components from foods e.g. calcium (Pengelly, 2021). Some saponin compounds have a similar chemical structures to that of human steroidal hormone i.e. sex and stress hormones from the gonad and adrenal, thus this

forms the phytopharmacological basis for the traditional herbal adaptogens (Harborne & Baxter, 1993).

2.4.11 Reducing sugars

Sugars constitute the principal source of energy and structural substance for defence mechanisms in plants. Similarly sugars play a crucial role as nutritional and central regulatory substance that control expression of genes related to metabolism, disease resistance, stress response, growth and development of plants (Morkunas & Ratajczak, 2014). Reducing and nonreducing sugars play crucial role in the processes of anabolic and catabolic pathways and support in the manufacture of secondary metabolites that characterize the medicinal properties of herbal plants (Khatri & Chhetri, 2020). In diseases resistance sugars augment oxidative eruption at an initial phase of infection, increases lignification of cell membranes, induce the production of flavonoids and stimulate certain pathogenic-related proteins. Moreover certain sugars act as priming mediator which stimulate higher plant resistance to pathogenic microbial agents (Morkunas & Ratajczak, 2014).

2.5 Antimicrobial assays

Antimicrobial susceptibility analysis is generally used for prediction of treatment outcome, epidemiology and drug discovery. A number of techniques can be utilised to screen or evaluate the *in vitro* antimicrobial properties of a plant based crude extract. The primary methods include; agar well diffusion and broth dilution methods (Balouiri *et al.,* 2016). The diffusion technique is commonly known as qualitative method since it can only reveal the presence or absence of crude extract antimicrobial activity. However dilution techniques are referred to as quantitative bioassay because they determine the Minimum Inhibitory Concentrations (MIC) (Valgas *et al.,* 2007).

2.5.1 Agar well diffusion technique

Agar well diffusion bioassay is extensively utilised to screen and evaluate the antimicrobial properties of plant crude extracts. On agar plate the inoculated media is bored with a sterilized

cork borer to give small well of 6-8 mm diameter. An aliquot of 100 µl of the crude extract at specific concentrations is pipetted into the agar well and allowed to diffuse into the media. The agar dishes are then brooded in an oven at 37^0 C on upright position for 24 hours. The inhibition zone around each pit is measured and the antimicrobial activity is determined on the zone of inhibition diameter (Balouiri *et al.*, 2016; Njue *et al.*, 2014; Valgas *et al.*, 2007).

2.5.2 Broth dilution technique

Broth macrodilution or microdilution is primary antimicrobial analysis method. The macrodilution technique involves preparation of two-fold serial dilutions of the crude extract in the liquid media dispensed in test tubes containing 2 mL volume of media. Then each test tube is inoculated with the prepared standard microbial suspension adjusted to 0.5 McFarland Unit. The well mixed test tubes are then incubated for 24 h at 37^0 C for bacterial and room temperature for fungal microorganism. After 24 h the test tubes are inspected for turbidity to ensure microbial growth. A variation of the macrodilution is the microdilution technique which utilises smaller volumes and 96-well microtiter plate. After incubation microorganism growth is indicated by a suitable dye. Consequently the least concentration of the antimicrobial crude sample that inhibits proliferation of the microbial agent is documented as the MIC (Balouiri *et al.*, 2016; Eloff, 1998; Ramadwa *et al.*, 2019).

Dilution technique is utilised to quantitatively evaluate the *in vitro* antimicrobial effects against bacterial and fungus microorganisms. The method determines the MICs values of antimicrobial crude extract from the broth medium. MIC is described as the least concentration of antimicrobic solution that inhibits the visual proliferation of test organism and it is commonly expressed in mg/L or µg/mL. In addition, evaluation of Minimum Bactericidal Concentration (MBC) or Minimum Fungicidal Concentration is the most usual approximation of bactericidal or fungicidal properties. MBC/MFC is usually determined after broth dilution by subculturing a sample which did not show microbial growth. Pour plate media which is a nonselective is

used to evaluate the number of surviving microbes after 24 h incubation (Balouiri *et al.*, 2016; Valgas *et al.*, 2007).

2.6 Cytotoxicity

The fundamental theory of toxicology is the fact that toxicity is dose-related; even salt can lead to poisoning when consumed in large amounts, while for highly toxic compound such as botulinum toxin there is a dosage beneath which there is no noticeable adverse reactions (Grandjean, 2016). Toxicity is species-determined, making cross-species evaluation difficult. However, due to the limitations of exposure-response theory, a new Drug Toxicity Index theory (DTI) has been put forward lately. DTI describes dose toxicity, recognizes hepatotoxic substances, gives mechanism understandings, evaluates medical results and has potential for use as a screening tool (Dixit, 2019).

Ancient reports of the poisonous herbal plants were documented by Galen, a Greek physician and pharmacist. He demonstrated that herbal plants contain both medicinal compounds and toxic constituents (Cheng & Zhen, 2004). The theory that folkloric use of herbs perhaps for thousands of years confirms its efficacy and safety does not essentially hold factual (De Smet, 1995). This is because, the chronic and subtle forms of lethality, such as hepatotoxicity and carcinogenicity might well have been unnoticed by preceding researchers and it is these kinds of noxiousness that are of utmost apprehension when evaluating the security of phytomedicines (Shaw *et al.*, 1997). The primary aim of toxicological evaluation of any extracts is to identify side effects and to determine the limits such as the median lethal dose (LD_{50}) of exposure at which toxic effects manifest (Sims *et al.*, 2010).

Volatile oils of *Ocimum americanum* L. which has abundant phenolics bioactive compounds have been shown to disturb biophysiological membranes, resulting to breakup and seepage of protoplasmic contents. Therefore, these crude metabolites show dual activities, both protective

and disruptive on the plasma cells contingent on their precise biochemical characteristic and the level of distress the microorganism is experiencing (Sutili *et al.,* 2016).

2.6.1 Brine shrimp lethality bioassay

The brine Shrimp Lethality Assay (BSLA) is a simple and rapid bioassay technique used for analysing the cytotoxic properties of phytochemical present in medicinal plant crude extracts. It is a general and low-cost bioassay which is capable of detecting wide range of bioactive compounds in plant crude samples (Meyer *et al.,* 1982; Waghulde *et al.,* 2019). Brine shrimp nauplii have been previously used for numerous bioassay systems including analyses of; anaethetics, mycotoxins, dinoflagellate toxins, pesticide residues, stream pollutants, morphine like molecules, oil dispersants toxicity, marine toxicants and cocarcinogenicity of phorbol esters (Meyer *et al.,* 1982).

The data from BLSA generates median lethal concentrations (LC_{50} in µg/mL) by plotting percentage mortality against the logarithm of the crude extract concentration (Hamidi *et al.,* 2014). LC_{50} values are calculated using Finney's Probit model and contrasted to Clarkson's or Meyer's lethality scales. For Meyer index of LC_{50} < 1000 µg/mL is regarded as noxious and LC_{50} > 1000 µg/mL innocuous. While for Clarkson criteria values between 0 - 100 µg/mL are considered as highly noxious, values of 101 - 500 µg/mL are regarded as moderately noxious, values of 501 - 1000 µg/mL slightly toxic and amounts > 1000 µg/mL are considered innocuous (Clarkson *et al.,* 2004; Meyer *et al.,* 1982; Hamidi *et al.,* 2014).

The BSLA essentially detect compounds that are sufficiently toxic to kill brine shrimp nauplii on exposure to the crude extract solution. A compound is considered to be toxic if it causes dysfunction in a health organism or inhibits crucial metabolic pathways resulting in deviation of behaviour or ultimately death (Ameen *et al.,* 2011). Additionally, several reports have confirmed a positive correlation between the results of LC_{50} and Median Lethal Dose (LD_{50}) as obtained from BSLA and Acute Oral Toxicity Assay in mice respectively (Hamidi *et al.,*

2014; Parra *et al.*, 2001). Similarly McLaughlin (1991) reported a good correlation between brine shrimp cytotoxicity of and antitumor activity in human (McLaughlin, 1991).

BSLA is an inexpensive technique which uses very small quantity of the crude extract. The assay is a suitable technique for screening pharmacological properties of several plant species, as such it generates groundwork results that can be supported up by more precise methods once the bioactive molecules have been identified and isolated. Since its inception this *in vivo* assay has been continuously utilised for bioassay-guided fractionation of cytotoxic and antineoplastic medication (Hamidi *et al.*, 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant material collection and identification

Ocimum americanum L. (Lamiaceae) was sourced from Lamu County - Pate Island (- 2.13885 S; 41.00249), in Coast province of Kenya. The plant materials were packed carefully and transferred to the Department of Public Health Pharmacology and Toxicology, University of Nairobi. The medicinal herb identification and authentication was undertaken at the National Museums of Kenya (East African Herbarium) by Mr. M. Mbale. Voucher sample was placed and reference code NMK/BOT/CTX/5/1/3 was granted for the plant specimen.

3.2 Preparation and extraction of plant material

3.2.1 Aqueous extracts

The plant foliage was cleaned with tap water and sluiced with distilled water, air-sere at atmospheric temperature to a constant mass. The dried-up plants matrices were ground to a uniform powder by the use of a power grinder. The milled powder (500 g) was soaked in 1 L of cold distilled water and stirred three times a day for 48 hours at room temperature (Seo *et al.,* 2014). The extract was then sieved using Whatman filter paper No. 1 and the filtrate (crude extract) frozen at -80^0 C for 2 hours. The frozen extract was lyophilised to dry granulate which was stowed in an airtight container at 4^0 C, until use. The procedure was repeated several times to rise the yield. Percentage ratio of the crude sample was calculated on w/w basis.

3.2.2 Hydroethanolic extracts

The milled plant material 500 g, was cold macerated in 70 % aqueous-ethanolic solution under constant stirring for 48 hours at room temperature to yield the hydroethanolic extract (Azwanida, 2015). The macerated mixture was first filtered through cotton wool and then filter paper (Whatman No. 1). Subsequently the filtrate was put under reduced pressure (600 mmHg) to evaporate the solvent at 40^0 C in a rotary evaporator, and the vestigial solvent was removed in a flow-stream of hot air oven at 40 ± 1^0 C. The extract was then lyophilized to acquire dry

crude extract which was measured, vialed and stored in a refrigerator at 4 ± 1^0 C, awaiting use. Percentage ratio of the crude sample was calculated on w/w basis

3.2.3 Acetonic extracts

The pulverized plant material (500 g) was cold macerated in an extraction jar, by adding 1000 mL of analytical grade acetone, stirred three times a day for 72 hours at atmospheric temperature. The crude extract was then sieved using cotton wool and Whatman filter paper No. 1 and the extract placed into a round-bottom flask and connected to rotary evaporator. The crude sample was further dried on a hot (40^0 C) sand bath till constant weight. The crude sample was weighed and placed in labelled airtight container and stowed at 4^0 C, pending usage. Percentage ratio was computed on w/w basis.

3.3 Liquid-liquid fractionation (LLF)

The solvent-solvent partitioning was done using the method explained by Kuo *et al.,* (2004) with adjustments. The crude samples acquired using aqueous, hydroethanolic and acetone solvents were fractionated in ethyl acetate and chloroform using solvent-solvent partitioning. The extracting fraction was collected in round bottom flask and attached to a vacuum evaporator at 40^0 C to eliminate the solvent. The residual solvent was desiccated on a sand bath at 40^0 C overnight. The dried-up organic portions were weighed to determine their yields. All dried extracts of ethyl acetate and chloroform were vialed in amber bottle and chilled at 4^0 C waiting analysis.

3.4 Antimicrobial assays

3.4.1 Test microorganisms

The microbial pathogens utilised in this study were standard of the American Type Cell Collection (ATCC). The preference of the sample microorganisms was grounded on the commendation of Clinical Laboratory Standards Institutes (CLSI) that appraised test microbes be utilised as clinical standard. They were Gram-negative strains; *Klebsiella pneumoniae* (ATCC 700603) and *Escherichia coli* (ATCC 25922), Gram-positive strains; *Staphylococcus*

aureus (ATCC 25925) and *Bacillus cereus* (ATCC 11778) and one fungal strain *Candida albicans* (ATCC 10231). They were made available by the Microbiology section of the Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi.

3.4.2 Microbial cultures

The standard microorganisms were obtained from stock cultures and subcultured on a blood agar. The subcultured microbes were preserved at 4^0 C while awaiting use. A working sample of the microorganisms was prepared by appending a colony of the microbial culture onto 10 mL of sterilized physiological saline solution to produce a concentration of 0.5 MacFarland.

3.4.3 Preparation of test extracts

Preparation of standard stock solutions of 500 mg/mL was carried out by dissolving 2000 mg of the sample fraction or extract in 1mL of 1 % dimethyl sulfoxide (DMSO) and then triturating with 3 mL of sterile broth to makeup to 4 mL. On a series of six test tubes 2 mL of sterile Muller Hinton Broth (MHB) was dispensed on each. Two-fold sequential dilution of 250, 125, 62.5, 31.25, 15.625 and 7.8125 mg/mL was prepared by dispensing 2 mL of the stock solution in the first test tube and serially diluting by mixing and removing 2 mL down the series till the last test tube where 2 mL was discarded to maintain uniformity. Stock solutions of positive control (Ceftriaxone, Terbinafine), negative control DMSO were prepared as test extracts and serially diluted similar to the test extracts. All the preparations were done in triplicate.

3.4.4 Agar well diffusion method

Agar well method as explained by Njue *et al.,* (2014) was utilised to assess the inhibition zone diameter of the six fractions and three crude extracts of the ethnobotanical plant American basil. Triplicate wells were bored aseptically at equidistance on an inoculated media (Muller Hinton Agar) with a sterile cork borer. A volume of 100 µL of each fraction and crude extract prepared at specific concentration were micropipetted into the wells and allowed to permeate into the media. The bacterial microorganisms were incubated at 37^0 C for 24 h and the fungal

pathogen at room temperature 72 h. The zone of inhibition about each agar well was measured in mm and the antimicrobial activity was determined on the diameter of the inhibition zone (Balouiri *et al.,* 2016; Njue *et al.,* 2014; Valgas *et al.,* 2007). Ceftriaxone and Terbinafine were used as positives reference drugs and DMSO was used as negative control respectively. Triplicate experiments were carried out to guarantee representative results. The MICs, MBC or MFC were computed as described by Bloomfield with modification. By plotting in x-axis natural logarithm of concentration (In_C) versus squared zone of inhibition (Z^2) in y-axis, the number that intersect X = 0 will be Mt. Additionally MIC was then computed as 0.25 x Mt and MBC or MFC equals MIC x 4 (Bloomfield, 1991; Parhusip & Sitanggang, 2011).

3.4.5 Broth dilution technique

A sequential dilution technique as a microorganism proliferation marker was utilised to determine the MIC according to Balouiri *et al.,* (2016) and Ramadwa *et al.,* (2019) with modifications. Two-fold serial dilutions were made as follows: using a dispenser a 2 mL of broth were placed into a series of test tubes. A volume of 2 mL of the stock solution (500 mg/mL) of the specific fraction or extract was placed onto the first test tube on the series and serially diluted by mixing and removing 2 mL. The same procedure was repeated down the line to give concentrations of 250, 125, 62.5, 31.25, 15.625 and 7.8125 mg/mL respectively. To ensure consistency in volumes of fractions/extracts 2 mL from the last sample tubes were discarded. A volume of 100 µL of the subcultured standard microorganisms was micropipetted to each test tube. The bacterial test tubes were incubated at 37^0 C in a humidified oven for 24 h while the fungal pathogen was incubated at room temperature for 72 h. The minimum inhibitory concentration was reported as highest dilution or lowest concentration of the fraction/crude extract that inhibits microorganism proliferation analysed on turbidness. Moreover, for the sample tubes which exhibited no discernable microbial proliferation, agar plate technique was utilised to evaluate MBC/MFC – defined as the lowest concentration at which no microbial growth is visually noticeable after subculturing into a fresh agar plate.

Ceftriaxone and Terbinafine were utilised as positives references drugs while DMSO as a negative control respectively. Triplicate experiments were carried out on all the samples to ensure representative and eligible data.

3.5 Brine shrimp cytotoxicity

To assess the lethality of the six fractions and three crude extracts of *Ocimum americanum* L., brine bioassay according to Meyer *et al.,* (1982) was employed to evaluate their cytotoxicity.

3.5.1 Preparation of artificial marine solution

Using an electronic balance 16 g of the marine salt (Sera GmbH Germany) was weighed and transferred into a 125 mL beaker. Distilled water was added and stirred to dissolve the marine salt. Quantitatively transferred the contents of the beaker into a 500 mL volumetric flask and placed it on a Sonicator (Sonic405 – model LUC – 405, Korea). The sonicated marine solution was filtered by Whatman filter paper number 1 and made upto 500 mL mark with distilled water.

3.5.2 Incubating and harvesting of *Artemia salina* nauplii

Artemia salina eggs (Dohse Aquaristik GmbH & Co. KG Germany) were hatched in a low surface plate with a barrier and several 2 mm holes creating binary nonequal compartments. The hatching container was filled-up with the brine solution which was made with sea salt and 50 mg yeast sprinkled into the darken segment. The smaller compartment was illuminated by incandescent light and gently exposed with fresh air. After 24 h, the hatched Brine shrimp's nauplii were transferred to fresh brine solution and incubated for an extra 24 h under illumination. The brine shrimp nauplii were picked-up with a pipette from the illumined section.

3.5.3 Preparation of crude extracts and fractions

Using analytical balance 50 mg of crude extract/fraction were weighed into a labelled and transferred into a glass vial. The content of the vial was dissolved by adding 1 mL of 1% DMSO

and placing the vial in a Sonicator. After dissolving 4 mL of marine solution was added to makeup to 50 mg/5 mL = 10 mg/mL = 10,000 µg/mL.

3.5.4 Brine shrimp bioassay

Ten (10) brine shrimp nauplii were macroscopically counted in a Pasteur pipette. The nauplii were moved into sample test tubes containing concentrations of 1000 µg/mL, 100 µg/mL and 10 µg/mL and made them to 5 mL with artificial saline water. A sprinkle of active yeast was supplemented as nutrition to all test tubes. DMSO was used as a negative control and Vincristine as a positive standard reference drug. The entire test sample was illuminated under florescent tube light. With the assistance of a magnifying lens (power x3) the surviving *Artemia salina* nauplii were counted after 24 h. The fatality proportion at the three dose levels and standards were estimated as follows; Mortality % = [(Dead larvae number/Total larvae number) x100]. The subsisting *Artemia salina* nauplii were terminated by adding of 150 µL of 5 % Carbolic acid (hydroxybenzene) to all test sample. All the tests were conducted in quintet.

3.6 Phytochemical screening

3.6.1 Alkaloids screening (Dragendorff's/Mayer's test)

Approximately 500 mg of the sample was dissolved in 10 mL of ethanol solution, simmered and sieved. Dilute aqueous ammonia (2 mL) was added to 5 mL of the sample filtrate. To extricate the basic alkaloids, 5 mL of trichloromethane (chloroform) was added to the filtrate and shaken. Acetic acid, 10 mL was added to extract the chloroform layer. The test solution was divided into two aliquots; Dragendorff's solution was added to the first aliquot and Mayer's solution was added to the second portion. The development of a creamy precipitate (Mayer's reagent) or a reddish brown precipitate (Dragendorff's reagent) was acknowledge as affirmative test for the occurrence of alkaloids in the sample (Abubakar & Haque, 2020; Evans, 2002).

3.6.2 Anthraquinones screening (Borntrager's test)

Approximately 500 mg of the sample was simmered with 10 mL of aqueous H_2SO_4 acid and sieved while hot and left to cool. Added 5 mL of trichloromethane to the filtrate and shaken, the formed chloroform layer was transferred into another test tube. Aqueous ammonia (1mL) was added into the sample filtrate and the subsequent mixture was examined for colour change. Appearance of yellow colouration was indicative of the anthraquinones presence (Evans, 2002; Mustafa & El-kamali, 2019).

3.6.3 Cardiac glycosides screening (Keller-Killiani test)

Approximately 500 mg of the sample was dissolved in 5 mL distilled water. Added 2 mL of anhydrous ethanoic (acetic) acid containing a droplet of ferric chloride ($FeCl_3$) solution. Concentrated sulfuric (H_2SO_4) acid (1 mL) was added to test sample and observed. The development of brownish loop at the boundary of the two tiers with the acetic acid strata appearing blueish-greenish upon standing indicated a positive test for cardiac glycosides (Abubakar & Haque, 2020; Harborne, 1998).

3.6.4 Cyanogenetic glycosides

Approximately weighed 500 mg of crude extract/fraction and triturated with distilled water. About 2 mL of the extract aqueous sample was mixed with 2 mL chloroform in a test-tube. A picrate paper was suspended inside the test-tube. The test-tube was then corked firmly and heated in a steam bath for upto 10 min. The conversion of the yellow picrate paper into reddish colouration by the cyanide gas indicated the presence of cyanogenetic glycosides (Harborne, 1998).

3.6.5 Flavonoids screening (Ammonia test)

Approximately 500 mg of *Ocimum americanum* L. crude extract or fraction was weighed and transferred into a test tube, dissolved the sample and filtered. Dilute ammonia (5ml) was added to the test sample followed by 1 mL of Concentrated H_2SO_4 acid. The disappearance of yellow

colouration upon standing confirms positive test for flavonoids (BaoDuy *et al.*, 2015; Harborne, 1998).

3.6.6 Phenolics screening (Ferric chloride test)

Approximately 0.5 g of *Ocimum americanum* L. crude extract or fraction was weighed and transferred into a sample tube and dissolved with distilled water. Aqueous or alcoholic ferric chloride 5 % (2 mL) was added to the test solution. The development of blue, dark green, purple or black colours confirmed positive test for phenolics compounds (Abubakar & Haque, 2020; Evans, 2002; Harborne, 1998).

3.6.7 Terpenoids screening (Salkowski test)

Approximately 500 mg of *Ocimum americanum* L. crude sample or fraction was weighed and placed into a sample tube. Trichloromethane (2 mL) was added to the test sample. 3 mL of Conc sulfuric acid was carefully added to the test sample which formed a layer. Development of reddish brown colour on the boundary confirmed positive test for terpenoids (Abubakar & Haque, 2020; Evans, 2002; Pengelly, 2021).

3.6.8 Saponins screening (Frothing test)

Approximately weighed 0.5 g of *Ocimum americanum* L. crude extract or fraction and placed into a sample tube. Distilled water (5 mL) was added and mixed briefly then shaken vigorously. Persistence of formed foam/froth for upto 15 minutes was indicative of the presence of saponins (Harborne, 1998).

3.6.9 Reducing sugars screening (Benedicts' test)

Approximately weighed 500 mg of *Ocimum americanum* L. crude sample or fraction and placed into a sample tube. Distilled water was added and the admixture heated on a steam bath for 10 minutes. Some drops of Benedict's solution were added and cooled the test sample. Appearance of colour change from green, yellow, orange, brick red coloration/ precipitation was indicative of presence of reducing sugars (Harborne, 1998).

3.6.10 Polyuronides (gums, mucilage, pectin)

Approximately 500 mg of *Ocimum americanum* L. sample was weighed and dissolved in distilled water. Briefly, 2 mL of an aqueous sample extract/fraction was mixed with acetone (10 mL) in a sample tube. Appearance of precipitation indicated the presence of polyuronides (Evans, 2002).

3.6.11 Tannins screening (Ferric chloride test)

Approximately 500 mg of the sample was simmered in a 10 mL distilled water in a sample tube. About 2 mL of the mixture was then transferred into another sample, and ferric chloride solution was added 2-3 drops. Appearance of blackish green or blackish blue coloration confirmed presence of tannin (Evans, 2002).

3.6.12 Phytosterols screening (Lieberman-Burchard's test)

Approximately 500 mg of *Ocimum americanum* L. sample was weighed and dissolved in distilled water. About 10 mL of an aqueous sample was extracted with 10 mL of diethyl ether in a separating funnel. A portion of diethyl ether extract was evaporated to dryness in a crucible. Equal volumes of acetic anhydride and chloroform were then added to the dried diethyl ether extract, mixed thoroughly, and divided into two equal portions (one to serve as a negative control). Conc sulfuric acid, one drop was added to the test solution and observed. Development of greenish colouration was indicative of phytosterol presence, while reddish brown colour was indicative of triterpene presence (BaoDuy *et al.*, 2015; Evans, 2002).

CHAPTER FOUR

4.0 RESULTS

4.1 Extraction yield of different solvents

The percentage extraction yield is as shown in the Table 4.1.

Table 4.1: **Percentage yield of the *O. americanum* L. crude extracts with different solvents**

Crude Extract	Weight of Extract	Percentage Yield
Acetonic	5.77 g/500 g	1.154 %
70 % Hydroethanolic	13 g/500 g	2.6 %
Aqueous	22.59 g/500 g	4.518 %

The acetonic crude extract was a dark greenish powder with characteristic aromatic scent which was stable at room temperature. The percentage yield (1.154 %) was the lowest of the three solvent and the powder was sparingly soluble in water but readily soluble in acetone and DMSO. The hydroethanolic crude extract was a dark brown powder with no characteristic aromatic scent which was stable at room temperature. The powder was soluble in water, ethanol and readily soluble in DMSO. The aqueous crude extract was a dark brown powder with no characteristic aromatic scent which was stable at ambient condition. The aqueous sample percentage yield (4.518%) was the highest amongst the three solvents. The powder was freely soluble in water and DMSO.

4.2 Antimicrobial sensitivity testing of *Ocimum americanum* L.

4.2.1 Agar well diffusion bioassay

4.2.1.1 Acetone crude extract

The acetonic crude extract of *Ocimum americanum* L. zone of inhibition against the five pathogenic microorganisms is shown in the Table 4.2 and Figure 4.1.

Table 4.2: Inhibition of microbial growth by acetone crude extract of *O. americanum* L.

Conc (mg/mL)	Zone of Inhibition (mm)				
	B. cereus	***E. coli***	***S. aureus***	***K. pneumoniae***	***C. albicans***

500	15.67 ± 0.33	0.00 ± 0.00	14.50 ± 0.29	0.00 ± 0.00	15.00 ± 0.58
250	14.33 ± 0.67	0.00 ± 0.00	14.66 ± 0.33	0.00 ± 0.00	14.33 ± 0.33
125	13.33 ± 0.33	0.00 ± 0.00	14.00 ± 0.58	0.00 ± 0.00	12.33 ± 0.33
62.5	13.83 ± 0.17	0.00 ± 0.00	12.00 ± 0.58	0.00 ± 0.00	11.17 ± 0.17
31.25	12.00 ± 0.58	0.00 ± 0.00	11.66 ± 0.88	0.00 ± 0.00	9.83 ± 0.44
15.62	11.50 ± 0.29	0.00 ± 0.00	11.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7.81	10.67 ± 0.33	0.00 ± 0.00	9.67 ± 0.33	0.00 ± 0.00	0.00 ± 0.00
Cef/Ter	30.00 ± 1.16	45.00 ± 0.58	50.00 ± 1.15	50.00 ± 1.73	45.00 ± 1.73
DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	00.00 ± 0.00

Key: Zone of Inhibition as a mean ± SEM of the triplicate tests

mm – Zone of inhibition SI unit millimeter

Conc – Concentration in milligram per liter

0.00 - means there was no observed inhibition

Cef – The positive control for bacterial microorganisms Ceftriaxone

Ter – The positive control for the fungal microorganism Terbinafine

DMSO – The negative control Dimethyl sulfoxide

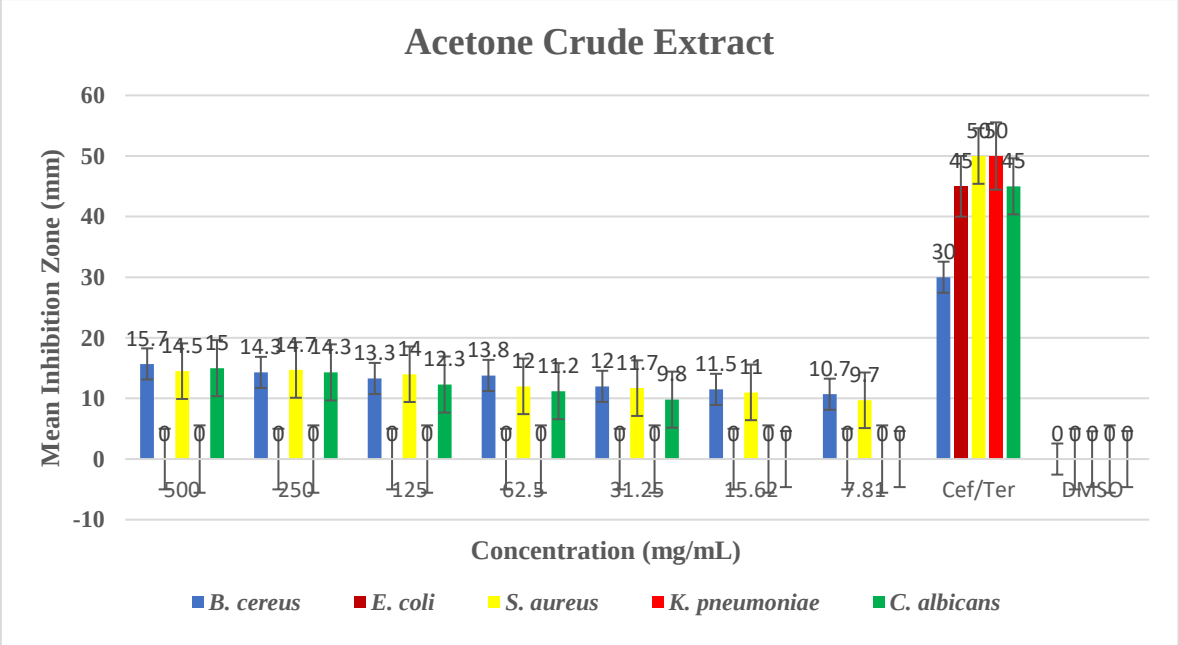


Figure 4.1: Microbial growth inhibition zones against various concentrations of acetone crude extract of *O. americanum* L.

The acetonic crude extract did not show any effect against the two Gram-negative bacterial pathogens *K. pneumoniae* and *E. coli*. However, the crude extract was effective against the two Gram-positive microbe's *B. cereus* and *S. aureus* as well as the fungal pathogens *C. albicans*.

Bacillus cereus bacteria had the highest inhibition zone of 15.67 ± 0.33 mm at the highest concentration of 500 mg/mL, compared to *S. aureus* (14.50 ± 0.29) and *C. albicans* (15.00 ± 0.58). While the standard drug ceftriaxone/terbinafine recorded an inhibition zone of 30.00 ± 1.16 mm, 50.00 ± 1.15 mm and 45.00 ± 1.73 mm against *B. cereus*, *S. aureus* and *C. albicans* respectively. The negative control DMSO had no effects on all the tested microbes.

4.2.1.2 Chloroform fraction of the acetonic crude extract

The chloroformic fraction zone of inhibition against the five pathogenic microorganisms is shown in the Table 4.3 and Figure 4.2.

Table 4.3: Inhibition of microbial growth by chloroform fraction of the acetonic extract

Conc (mg/mL)	Zone of Inhibition (mm)				
	B. cereus	***E. coli***	***S. aureus***	***K. pneumoniae***	***C. albicans***
500	15.50 ± 0.50	0.00 ± 0.00	12.00 ± 0.58	0.00 ± 0.00	16.83 ± 0.17
250	14.33 ± 0.33	0.00 ± 0.00	10.67 ± 0.33	0.00 ± 0.00	14.67 ± 0.33
125	13.50 ± 0.29	0.00 ± 0.00	9.67 ± 0.33	0.00 ± 0.00	12.00 ± 0.58
62.5	12.00 ± 0.58	0.00 ± 0.00	9.33 ± 0.33	0.00 ± 0.00	10.00 ± 0.58
31.25	11.67 ± 0.33	0.00 ± 0.00	9.67 ± 0.33	0.00 ± 0.00	9.17 ± 0.17
15.62	11.33 ± 0.33	0.00 ± 0.00	9.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7.81	9.50 ± 0.29	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cef/Ter	30.00 ± 1.16	45.00 ± 0.58	50.00 ± 1.15	50.00 ± 1.73	45.00 ± 1.73
DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Key: Zone of Inhibition as a mean ± SEM of the triplicate tests

mm – Zone of inhibition SI unit millimeter

Conc – Concentration in milligram per liter

0.00 - means there was no observed inhibition

Cef – The positive control for bacterial microorganisms Ceftriaxone

Ter – The positive control for the fungal microorganism Terbinafine

DMSO – The negative control Dimethyl sulfoxide

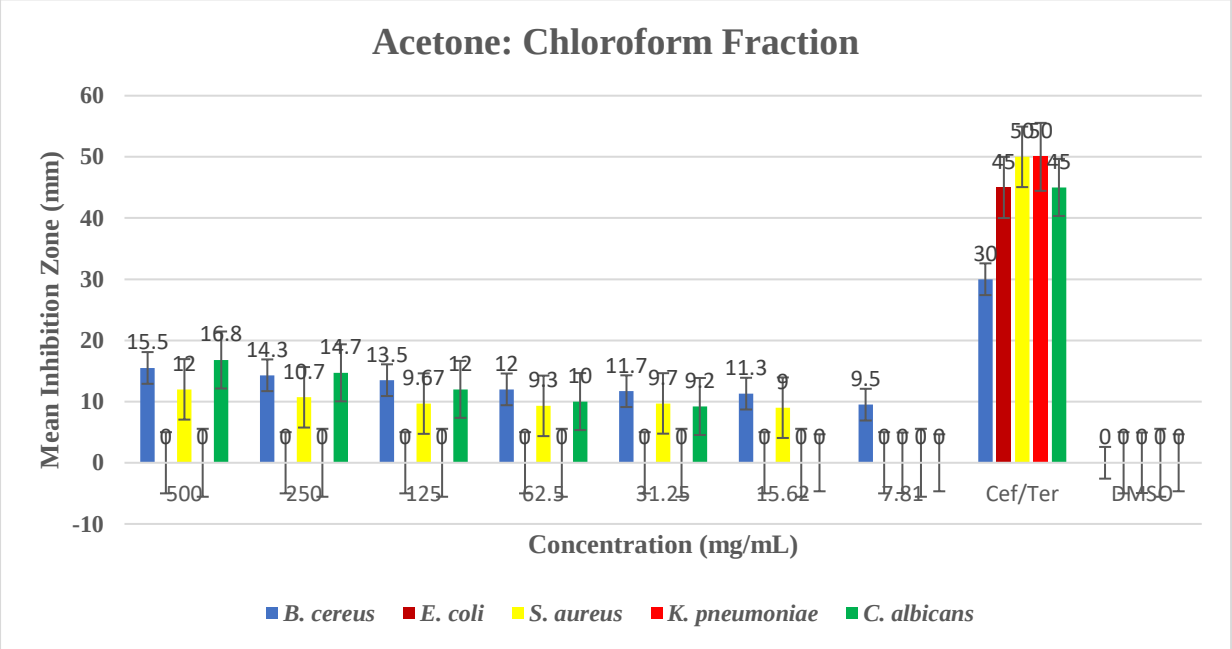


Figure 4.2: Microbial growth inhibition against various concentrations of chloroformic fraction of the acetone crude extract of *O. americanum* L.

The chloroform fraction of acetonic crude extract was not effective against the two Gram-negative bacterial pathogens *K. pneumoniae* and *E. coli*. However, the fraction did show some effect on the two Gram-positive microorganisms and the fungus pathogens. The fungus *C. albicans* had the highest inhibition zone of 16.83 ± 0.17 mm, at the highest concentration of 500 mg/mL, compared with *B. cereus* (15.50 ± 0.50 mm) and *S. aureus* (12.00 ± 0.58 mm). While the positive control drug terbinafine/ceftriaxone recorded an inhibition zone of 45.00 ± 1.73 mm, 30.00 ± 1.16 mm and 50.00 ± 1.15 mm against *C. albicans*, *B. cereus* and *S. aureus* respectively. The negative control DMSO had no effects on all the tested microorganisms.

4.2.1.3 Ethyl acetate fraction of the acetonic crude extract

The ethyl acetate fraction zone of inhibition against the five pathogenic microbes is presented in the Table 4.4 and Figure 4.3.

Table 4.4: Inhibition of microbial growth by ethyl acetate fraction of the acetone extract

Conc (mg/mL)	Zone of Inhibition (mm)				
	B. cereus	***E. coli***	***S. aureus***	***K. pneumoniae***	***C. albicans***
500	14.00 ± 0.58	0.00 ± 0.00	11.17 ± 0.44	0.00 ± 0.00	15.50 ± 0.29
250	13.00 ± 0.58	0.00 ± 0.00	10.00 ± 0.29	0.00 ± 0.00	9.00 ± 4.51

125	13.00 ± 0.00	0.00 ± 0.00	9.17 ± 0.17	0.00 ± 0.00	12.50 ± 0.29
62.5	11.50 ± 0.29	0.00 ± 0.00	9.33 ± 0.33	0.00 ± 0.00	10.67 ± 0.58
31.25	11.83 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.33 ± 3.18
15.62	10.67 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.17 ± 3.17
7.81	10.00 ± 0.29	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cef/Ter	30.00 ± 1.16	45.00 ± 0.58	50.00 ± 1.15	50.00 ± 1.73	45.00 ± 1.73
DMSO	0.00 ± 0.00	0.00 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Key: Zone of Inhibition as a mean ± SEM of the triplicate tests

mm – Zone of inhibition SI unit millimeter

Conc – Concentration in milligram per liter

0.00 - means there was no observed inhibition

Cef – The positive control for bacterial microorganisms Ceftriaxone

Ter – The positive control for the fungal microorganism Terbinafine

DMSO – The negative control Dimethyl sulfoxide

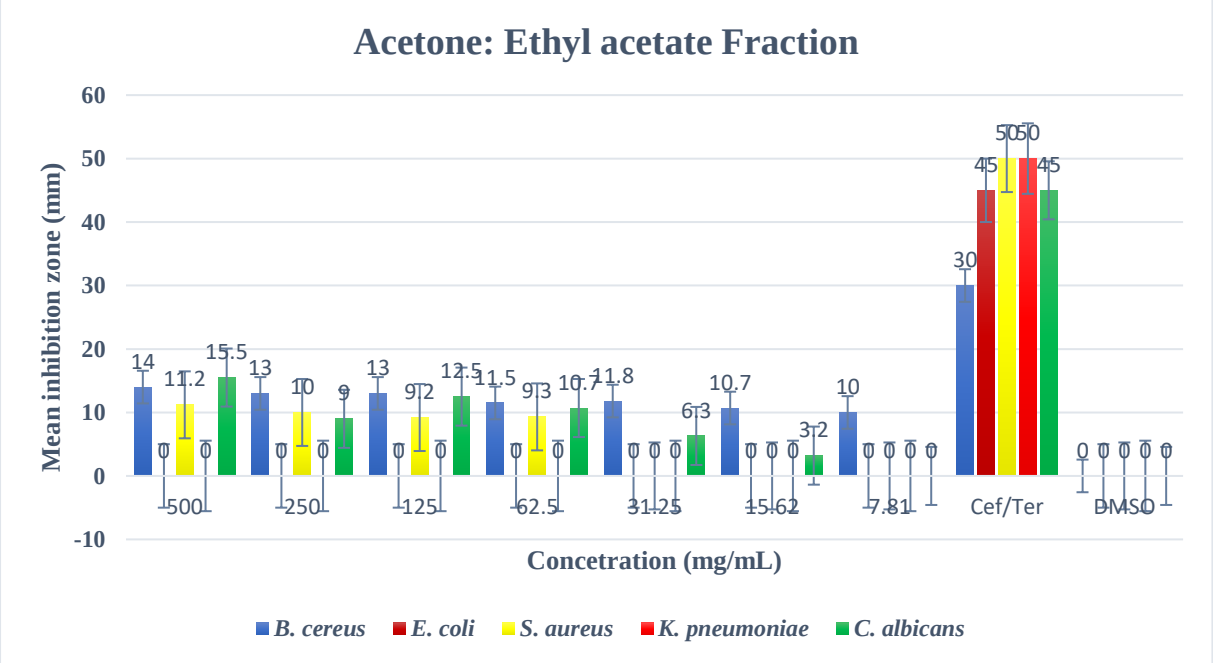


Figure 4.3: Microbial growth inhibition against various concentrations of ethyl acetate fraction of the acetonic crude extract of *O. americanum* L.

The ethyl acetate fraction of acetonic crude extracts was not effective against the two Gram-negative microorganisms *K. pneumoniae* and *E. coli*. However, the fraction was active against the two Gram-positive bacteria and the fungal pathogens. The fungus *C. albicans* had the highest inhibition zone of 15.50 ± 0.29 mm, at the highest concentration 500 mg/mL compared

to *B. cereus* (14.00 ± 0.58 mm) and *S. aureus* (11.17 ± 0.44 mm). While the standard drug terbinafine/ceftriaxone recorded an inhibition zone of 45.00 ± 1.73 mm, 30.00 ± 1.16 mm and 50.00 ± 1.15 mm against *C. albicans, B. cereus* and *S. aureus* respectively. The negative control DMSO had no effects on all the tested microbial pathogens.

4.2.1.4 Hydroethanol crude extract

The hydroethanol crude extract zone of inhibition against the five pathogenic microbes is shown in the Table 4.5 and Figure 4.4.

Table 4.5: Inhibition of microbial growth by hydroethanol extract of *O. americanum* L.

Conc (mg/mL)	Zone of Inhibition (mm)				
	B. cereus	***E. coli***	***S. aureus***	***K. pneumoniae***	***C. albicans***
500	16.33 ± 0.67	0.00 ± 0.00	17.17 ± 0.44	0.00 ± 0.00	0.00 ± 0.00
250	13.17 ± 0.17	0.00 ± 0.00	13.33 ± 0.33	0.00 ± 0.00	0.00 ± 0.00
125	10.83 ± 0.44	0.00 ± 0.00	10.50 ± 0.29	0.00 ± 0.00	0.00 ± 0.00
62.5	11.00 ± 0.00	0.00 ± 0.00	9.83 ± 0.17	0.00 ± 0.00	0.00 ± 0.00
31.25	9.83 ± 0.44	0.00 ± 0.00	6.17 ± 3.09	0.00 ± 0.00	0.00 ± 0.00
15.62	9.67 ± 0.33	0.00 ± 0.00	3.00 ± 3.00	0.00 ± 0.00	0.00 ± 0.00
7.81	9.50 ± 0.29	0.00 ± 0.00	3.00 ± 3.00	0.00 ± 0.00	0.00 ± 0.00
Cef/Ter	30.00 ± 1.16	45.00 ± 0.58	50.00 ± 1.15	50.00 ± 1.73	45.00 ± 1.73
DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Key: Zone of Inhibition as a mean ± SEM of the triplicate tests

mm – Zone of inhibition SI unit millimeter

Conc – Concentration in milligram per liter

0.00 - means there was no observed inhibition

Cef – The positive control for bacterial microorganisms Ceftriaxone

Ter – The positive control for the fungal microorganism Terbinafine

DMSO – The negative control Dimethyl sulfoxide

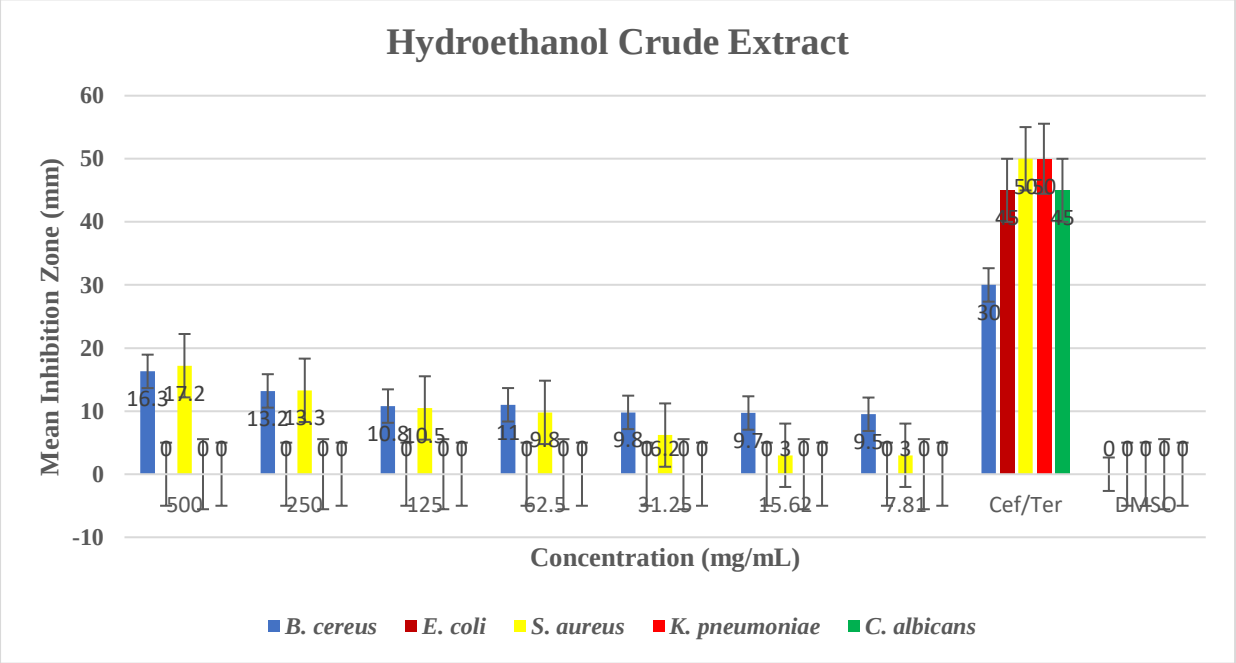


Figure 4.4: Microbial growth inhibition against varoius concentrations of the hydroethanol crude extract of *O. americanum* L.

The hydroethanolic crude extract did not show any effect against the two Gram-negative bacterial and the fungal pathogen *C. albicans*. However, the crude extract did show some activity on the two Gram-positive microorganisms. *S. aureus* had the highest inhibition zone of 17.17 ± 0.44 mm, at the highest concentration of 500 mg/mL compared to B. cereus (16.33 ± 0.67 mm). While the positive control drug ceftriaxone documented an inhibition zone of 30.00 ± 1.16 mm and 50.00 ± 1.15 mm against *S. aureus* and *B. cereus* respectively. The negative control DMSO had no effects on all the tested microorganisms.

4.2.1.5 Chloroform fraction of hydroethanolic crude extract

The chloroformic fraction zone of inhibition against the five pathogenic microorganisms is presented in the Table 4.6 and Figure 4.5.

Table 4.6: Inhibition of microbial growth by chloroform fraction of the hydroethanolic crude extract

Conc (mg/mL)	Zone of Inhibition (mm)				
	B. cereus	***E. coli***	***S. aureus***	***K. pneumoniae***	***C. albicans***
500	20.00 ± 0.58	0.00 ± 0.00	18.50 ± 0.29	0.00 ± 0.00	0.00 ± 0.00

250	18.17 ± 0.60	0.00 ± 0.00	15.67 ± 0.44	0.00 ± 0.00	0.00 ± 0.00
125	14.83 ± 0.44	0.00 ± 0.00	12.83 ± 0.44	0.00 ± 0.00	0.00 ± 0.00
62.5	9.83 ± 4.94	0.00 ± 0.00	9.67 ± 0.17	0.00 ± 0.00	0.00 ± 0.00
31.25	13.00 ± 0.58	0.00 ± 0.00	9.17 ± 0.17	0.00 ± 0.00	0.00 ± 0.00
15.62	9.33 ± 0.33	0.00 ± 0.00	6.00 ± 3.00	0.00 ± 0.00	0.00 ± 0.00
7.81	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cef/Ter	30.00 ± 1.16	45.00 ± 0.58	50.00 ± 1.15	50.00 ± 1.73	45.00 ± 1.73
DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Key: Zone of Inhibition as a mean ± SEM of the triplicate tests

mm – Zone of inhibition SI unit millimeter

Conc – Concentration in milligram per liter

0.00 - means there was no observed inhibition

Cef – The positive control for bacterial microorganisms Ceftriaxone

Ter – The positive control for the fungal microorganism Terbinafine

DMSO – The negative control Dimethyl sulfoxide

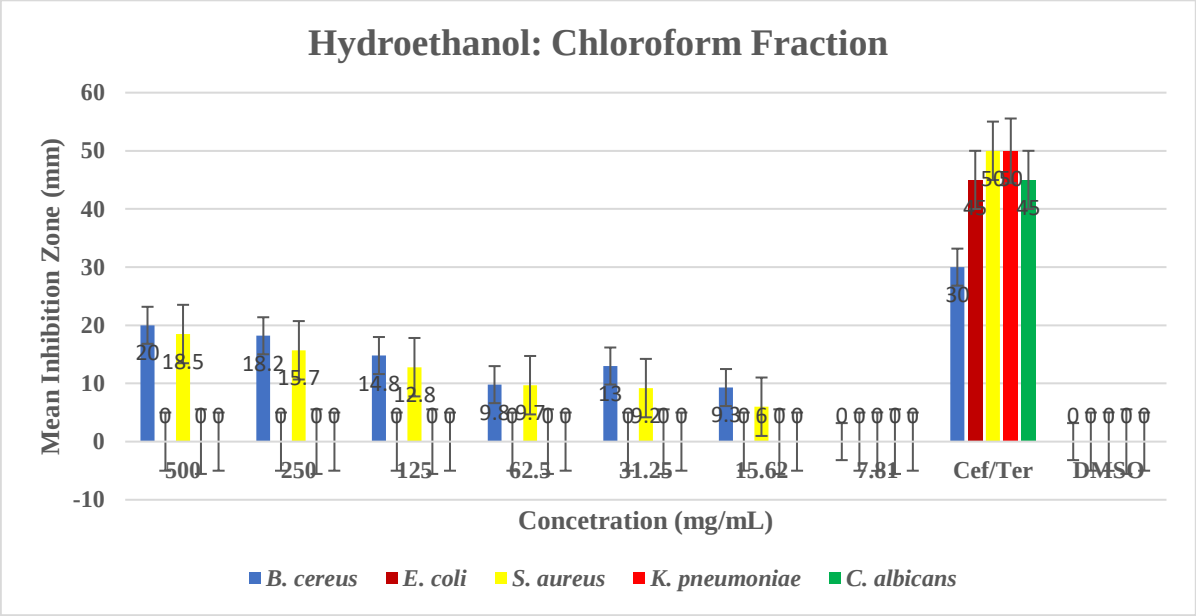


Figure 4.5: Microbial growth inhibition against various concentrations of chloroformic fraction of the hydroethanolic crude extract of *O. americanum* L.

The chloroformic fraction of hydroethanolic crude extract was not active against the two Gram-negative microbes and the fungus *C. albicans*. However, the fraction was active against the two Gram-positive bacterial pathogens. *Bacillus cereus* had the highest inhibition zone of 20.00 ± 0.58 mm, at the highest concentration of 500 mg/mL compared to S. aureus (18.50 ± 0.29

mm). While the standard drug Ceftriaxone recorded an inhibition zone of 30.00 ± 1.16 mm and 50.00 ± 1.15 mm against *B. cereus* and *S. aureus* respectively. The negative control DMSO had no effects on all the tested pathogens.

4.2.1.6 Ethyl acetate fraction of hydroethanolic crude extract

The zone of inhibition of ethyl acetate fraction against the five pathogenic microbes is shown in the Table 4.7 and Figure 4.6.

Table 4.7: Inhibition of microbial growth by ethyl acetate fraction of hydroethanolic crude extract

Conc (mg/mL)	**Zone of Inhibition (mm)**				
	B. cereus	***E. coli***	***S. aureus***	***K. pneumoniae***	***C. albicans***
500	26.50 ± 0.29	0.00 ± 0.00	16.83 ± 0.60	0.00 ± 0.00	0.00 ± 0.00
250	26.00 ± 0.00	0.00 ± 0.00	16.17 ± 0.17	0.00 ± 0.00	0.00 ± 0.00
125	23.83 ± 0.60	0.00 ± 0.00	11.83 ± 0.17	0.00 ± 0.00	0.00 ± 0.00
62.5	20.00 ± 0.58	0.00 ± 0.00	9.83 ± 0.44	0.00 ± 0.00	0.00 ± 0.00
31.25	17.50 ± 0.29	0.00 ± 0.00	6.17 ± 3.09	0.00 ± 0.00	0.00 ± 0.00
15.62	14.33 ± 0.44	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
7.81	13.17 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cef/Ter	30.00 ± 1.16	45.00 ± 0.58	50.00 ± 1.15	50.00 ± 1.73	45.00 ± 1.73
DMSO	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Key: Zone of Inhibition as a mean ± SEM of the triplicate tests

mm – Zone of inhibition SI unit millimeter

Conc – Concentration in milligram per liter

0.00 - means there was no observed inhibition

Cef – The positive control for bacterial microorganisms Ceftriaxone

Ter – The positive control for the fungal microorganism Terbinafine

DMSO – The negative control Dimethyl sulfoxide

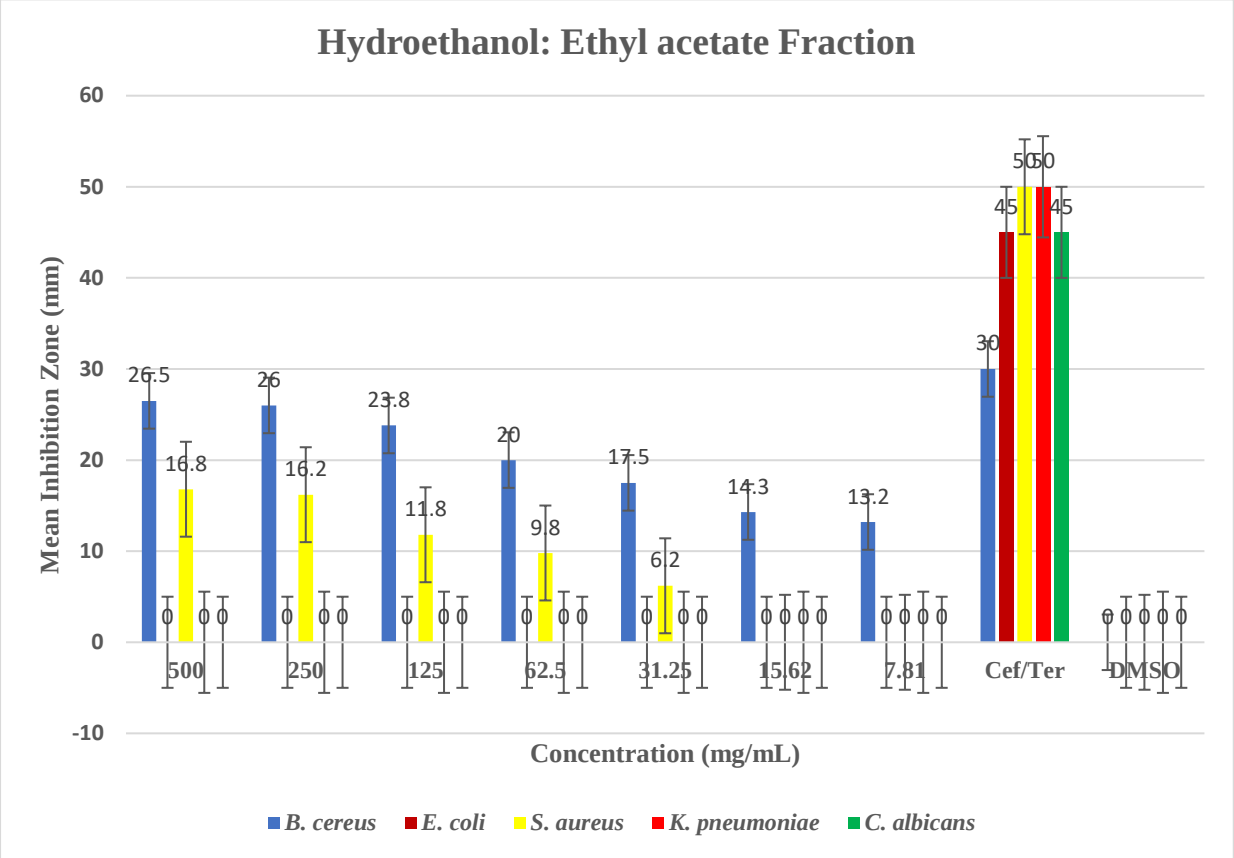


Figure 4.6: Microbial growth inhibition against various concentrations of ethyl acetate fraction of the hydroethanolic crude extract of *O. americanum* L.

The ethyl acetate fraction of hydroethanolic crude extract was not active against *K. pneumoniae* and *E. coli* as well as the fungus *C. albicans*. However, the fraction was effective against the two Gram-positive bacterial pathogens. *Bacillus cereus* had the highest inhibition zone of 26.50 ± 0.29 mm, at the highest concentration of 500 mg/mL compared to *S. aureus* (16.83 ± 0.60 mm). While the positive reference drug Ceftriaxone recorded an inhibition zone of 30.00 ± 1.16 mm and 50.00 ± 1.15 mm against *B. cereus* and *S. aureus* respectively. The negative control DMSO had no effects on all the tested microorganisms.

4.2.1.7 Summarised results of ANOVA, TUKEY and Dunnett test

The summarised results of ANOVA, TUKEY and Dunnett test using letters in superscript are presented in Table 4.8 below.

Table 4.8: Summary of microbial growth inhibition by acetone and hydroethanolic crude extracts and their fractions against test pathogens

Microorganism	Conc mg/ml	Zone of Inhibition (mm)						Positive control	Negative control
		Acetone extract	Chloroform fraction	Ethyl acetate fraction	Hydroethanol extract	Chloroform fraction	Ethyl acetate fraction		
Bacillus cereus (Gram +ve)	500	$15.67^{b} \pm 0.33$	$15.50^{b} \pm 0.50$	$14.00^{b} \pm 0.58$	$16.33^{b} \pm 0.67$	$20.00^{b} \pm 0.58$	$26.50^{ab} \pm 0.29$	$30.00^{a} \pm 1.16$	$0.00^{d} \pm 0.00$
	250	$14.33^{b} \pm 0.67$	$14.33^{b} \pm 0.33$	$13.00^{b} \pm 0.58$	$13.17^{c} \pm 0.17$	$18.17^{b} \pm 0.60$	$26.00^{ab} \pm 0.00$		
	125	$13.33^{b} \pm 0.33$	$13.50^{b} \pm 0.29$	$13.00^{b} \pm 0.00$	$10.83^{d} \pm 0.44$	$14.83^{c} \pm 0.44$	$23.83^{c} \pm 0.60$		
	62.5	$13.83^{b} \pm 0.17$	$12.00^{c} \pm 0.58$	$11.50^{c} \pm 0.29$	$11.00^{d} \pm 0.00$	$9.83^{c} \pm 4.94$	$20.00^{d} \pm 0.58$		
	31.25	$12.00^{c} \pm 0.58$	$11.67^{c} \pm 0.33$	$11.83^{c} \pm 0.17$	$9.83^{d} \pm 0.44$	$13.00^{c} \pm 0.58$	$17.50^{e} \pm 0.29$		
	15.62	$11.50^{c} \pm 0.29$	$11.33^{c} \pm 0.33$	$10.67^{c} \pm 0.17$	$9.67^{d} \pm 0.33$	$9.33^{c} \pm 0.33$	$14.33^{f} \pm 0.44$		
	7.81	$10.67^{c} \pm 0.33$	$9.50^{e} \pm 0.29$	$10.00^{c} \pm 0.29$	$9.50^{d} \pm 0.29$	$0.00^{g} \pm 0.00$	$13.17^{f} \pm 0.17$		
Escherichia coli	500	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	$45.00^{a} \pm 0.58$	$0.00^{d} \pm 0.00$
Staphylococcus aureus (Gram +ve)	500	$14.50^{b} \pm 0.29$	$12.00^{b} \pm 0.58$	$11.17^{b} \pm 0.44$	$17.17^{b} \pm 0.44$	$18.50^{b} \pm 0.29$	$16.83^{b} \pm 0.60$	$50.00^{a} \pm 1.15$	$0.00^{d} \pm 0.00$
	250	$14.66^{b} \pm 0.33$	$10.67^{b} \pm 0.33$	$10.00^{b} \pm 0.29$	$13.33^{c} \pm 0.33$	$15.67^{c} \pm 0.44$	$16.17^{b} \pm 0.17$		
	125	$14.00^{b} \pm 0.58$	$9.67^{c} \pm 0.33$	$9.17^{b} \pm 0.17$	$10.50^{d} \pm 0.29$	$12.83^{d} \pm 0.44$	$11.83^{b} \pm 0.17$		
	62.5	$12.00^{c} \pm 0.58$	$9.33^{c} \pm 0.33$	$9.33^{b} \pm 0.33$	$9.83^{d} \pm 0.17$	$9.67^{e} \pm 0.17$	$9.83^{c} \pm 0.44$		
	31.25	$11.66^{c} \pm 0.88$	$9.67^{c} \pm 0.33$	$0.00^{d} \pm 0.00$	$6.17^{d} \pm 3.09$	$9.17^{e} \pm 0.17$	$6.17^{c} \pm 3.09$		
	15.62	$11.00^{c} \pm 0.00$	$9.00^{c} \pm 0.00$	$0.00^{d} \pm 0.00$	$3.00^{e} \pm 3.00$	$6.00^{e} \pm 3.00$	$0.00^{f} \pm 0.00$		
	7.81	$9.67^{c} \pm 0.33$	$0.00^{d} \pm 0.00$	$0.00^{d} \pm 0.00$	$3.00^{e} \pm 3.00$	$0.00^{f} \pm 0.00$	$0.00^{f} \pm 0.00$		
Klebsiella pneumoniae	500	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$50.00^{a} \pm 1.73$	$0.00^{b} \pm 0.00$
Candida albicans (Fungus)	500	$15.00^{b} \pm 0.58$	$16.83^{b} \pm 0.17$	$15.50^{b} \pm 0.29$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$45.00^{a} \pm 1.73$	$0.00^{d} \pm 0.00$
	250	$14.33^{b} \pm 0.33$	$14.67^{c} \pm 0.33$	$9.00^{b} \pm 4.51$					
	125	$12.33^{c} \pm 0.33$	$12.00^{d} \pm 0.58$	$12.50^{b} \pm 0.29$					
	62.5	$11.17^{c} \pm 0.17$	$10.00^{e} \pm 0.58$	$10.67^{b} \pm 0.58$					
	31.25	$9.83^{d} \pm 0.44$	$9.17^{e} \pm 0.27$	$6.33^{b} \pm 3.18$					
	15.62	$0.00^{e} \pm 0.00$	$0.00^{g} \pm 0.00$	$3.17^{c} \pm 3.17$					
	7.81	$0.00^{e} \pm 0.00$	$0.00^{g} \pm 0.00$	$0.00^{c} \pm 0.00$					

Key: Zone of Inhibition as a mean ± SEM of the triplicate tests

mm – Zone of inhibition SI unit millimeter

Conc – Concentration in milligram per liter

0.00 - means there was no observed inhibition

Positive control – Ceftriaxone/Terbinafine for bacterial microorganisms

Negative control - (DMSO) dimethyl sulfoxide for all the tested microbes

Preliminary ANOVA detects significant differences among groups and post hoc is done if differences among means are detected by preliminary ANOVA. Tukey HSD test compares two groups at a go, while Dunnett test compares each mean with the control mean. During the ANOVA analysis the set *P* value (probability/significance level) was 0.05. Means tagged with the identical superscript alphabet within the considered clique i.e., inhibition zone is not statistical different ($p > 0.05$). Positive standard mean is tagged with '**a**', negative standard with '**d**', 500 mg/mL, 250 mg/mL and 125 mg/mL with '**b**' indicating they are significantly comparable/they had the same efficacy against *Staphylococcus aureus*, while 62.5 mg/mL, 31.25 mg/mL, 15.62 mg/mL and 7.81 mg/mL have been tagged with '**c**', because they also had significantly comparable inhibitory effect against Gram-positive bacteria *S. aureus* i.e., their differences are probably significant ($p < 0.05$).

Comparisons whose differences are statistically significant have obtained *P* value/significant level of < 0.05 and as a comparison of growth inhibitory of 500 mg/mL with growth inhibition of either 7.81 mg/mL, 15.62 mg/mL, 31.25 mg/mL or 62.5 mg/mL gave a significance level (*P* value) of 0.000, 0.03, 0.017 and 0.04 respectively. These values are < 0.05, thus the difference between 500 mg/mL growth inhibitory and either 7.81 mg/mL, 15.62 mg/mL, 31.25 mg/mL or 62.5 mg/mL growth inhibitions are big enough such that the mean growth inhibitions by 500 mg/mL cannot lie within the same 95 % confidence interval with mean growth inhibitions of 7.81 mg/mL, 15.62 mg/mL, 31.25 mg/mL and 62.5 mg/mL hence they are statistically different ($p < 0.05$).

The data presented on the summary Table 4.8 above shows significant differences in growth inhibition amongst the various concentrations of acetonic crude extract and its fractions against the test pathogenic microbes. There were no significant differences ($p > 0.05$) amongst the larger means as indicated by superscript letter 'b' in the Table 4.8. Similarly, there was no significance difference ($p > 0.05$) among the smaller means as indicated by superscript letter 'c'. However, there was a statistical difference ($p < 0.05$) between the larger means and smaller means. Conversely there was statistical difference ($p < 0.05$) between the acetone crude extract and its fractions with the reference drugs and negative controls. The acetonic extract and fractions were not active against the Gram-negative bacterial pathogens *K. pneumoniae* and *E. coli.*

The various concentrations of hydroethanol crude extract reported statistically significant differences in growth inhibition against the test pathogenic microorganisms. There was no statistical difference ($p > 0.05$) amongst the smaller means as indicated by the superscript 'd'. However, amongst the larger means there was statistical significance ($p < 0.05$) as indicated by the superscript 'b' and 'c'. Equally there was a statistical difference ($p < 0.05$) between the smaller means and larger means. Similarly, there was statistical difference ($p < 0.05$) between hydroethanol crude extract and the reference drugs. The hydroethanol extract did not show any activity against the fungus and the Gram-negative pathogens.

The various concentrations of chloroform fraction of hydroethanolic extract reported statistically significant differences in growth inhibition against the test microbial organisms. There was no statistical difference ($p > 0.05$) amongst the larger means denoted by superscript 'b'. Similarly, there was no statistical difference ($p > 0.05$) among the smaller means denoted by superscript 'c', while the lowest concentration denoted by 'g' was ineffective. However, there was statistical significance ($p < 0.05$) amongst the smaller and larger means. Conversely there was statistical significance ($p < 0.05$) between the chloroformic fraction and the standard

drugs. The chloroformic fraction did not show any efficacy against the fungus pathogen *C. albicans* and the Gram-negative microbes *K. pneumoniae* and *E. coli*.

The ethyl acetate fraction of hydroethanolic crude extract reported excellent growth inhibition against the test microorganisms. There was no statistical significance ($p > 0.05$) among the smallest means denoted by 'f'. However, there was statistical difference ($p < 0.05$) amongst the larger, medium and smaller means. There was no statistical significance ($p > 0.05$) amongst the two larger means and the positive control drug ceftriaxone denoted by 'ab'. Equally there was statistical significance ($p < 0.05$) between the medium, smaller means and the reference drug. The ethyl acetate fraction did not show any bioactivity against the Gram-negative microorganisms as well as the fungus *C. albicans*.

4.2.2 Broth dilution bioassay

The minimum bactericidal/fungicidal concentrations of *Ocimum americanum* L. crude extracts and fractions against the five test microbes are shown in the Table 4.9.

Table 4.9: **The MBC/MFC of *Ocimum americanum* L. crude extracts and fractions against test microbes**

Extract/ Fraction	**MBC/MFC (mg/mL)**				
	B. cereus	***E. coli***	***S. aureus***	***K. pneumoniae***	***C. albicans***
Acetone	208.33±72.17	166.76±72.17	52.08±18.04	145.83±95.47	250.00± 0.00
Chloroform*	166.67±72.17	83.33 ± 36.08	104.17±36.08	250.00± 0.00	166.76±72.17
Ethyl acetate*	166.67±72.17	166.76±72.17	166.76±72.17	83.33±36.08	166.76±72.17
Hydroethanol	208.33±72.17	83.33 ± 36.08	208.33±72.17	333.33±36.08	**IE**
Chloroform*	83.33±36.08	20.83 ± 9.02	83.33±36.08	208.33±72.17	**IE**
Ethyl acetate*	166.67±72.17	41.67 ± 18.04	104.17±36.08	208.33±72.17	**IE**

Key: MBC - Minimum Bactericidal Concentration in milligram per millilitre

MFC - Minimum Fungicidal Concentration in milligram per millilitre

IE – Ineffective against the tested microbial pathogen

Extract – Crude extract of *Ocimum americanum* L. (Lamiaceae)

Fraction – of *Ocimum americanum* L. (Lamiaceae) crude extract indicated by (*)

The water crude extracts and fractions were not active against all the tested microorganisms thus determination of MIC, MBC/MFC was not possible. Acetonic crude extracts and fractions did show some activity against all the tested microbial pathogens. On the other hand, hydroethanolic sample was active to all bacterial pathogens but was ineffective to the fungal pathogen *C. albicans.*

4.2.2.1 Summarised results of agar well diffusion and broth microdilution

The summarised results of MIC, MBC/MFC and the ratio of MBC/MIC are shown in Table 4.10 below.

Table 4.10: Summary of MIC, MBC, MFC of *Ocimum americanum* L. crude extract and their fractions of various solvents against test microbials

		Macro dilution (mg/mL)			Agar well diffusion (mg/mL)		
Pathogen	**Sample**	**MIC (mg/mL)**	**MBC (mg/mL)**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**
Bacillus cereus (Gram +ve)	**Acetone crude extract**	ND	208.33±72.17	_	1.93±0.14	7.74±0.55	4.01
	*Chloroform fraction	ND	166.67±72.17	_	1.38±0.34	5.50±1.37	3.99
	*Ethyl acetate fraction	ND	166.67±72.17	_	1.91±0.15	7.64±0.61	4.00
	70% Hydro ethanolic crude extract	ND	208.33±72.17	_	1.54±0.09	6.15±0.37	3.99
	*Chloroform fraction	ND	83.33±36.08	_	2.70±0.81	10.81±3.26	4.00
	*Ethyl acetate fraction	ND	166.67±72.17		3.43±0.23	13.71±0.93	4.00
Staphylococcus aureus (Gram +ve)	**Acetone crude extract**	ND	52.08±18.04	_	1.49±0.62	5.95±2.50	3.99
	*Chloroform fraction	ND	104.17±36.08	_	0.72±0.53	2.90±2.11	4.03
	*Ethyl acetate fraction	ND	166.67±72.17	_	2.26±0.06	9.01±0.22	3.99
	70% Hydroethanolic crude extract	ND	208.33±72.17	_	1.84±0.78	7.34±3.12	3.99
	*Chloroform fraction	ND	83.33±36.08	_	2.87±0.21	11.46±0.84	3.99
	*Ethyl acetate fraction	ND	104.17±36.08		3.34±0.14	13.37±0.55	4.00
Escherichia coli (Gram –ve)	**Acetone crude extract**	ND	166.67±72.17	_	IE	IE	_
	*Chloroform fraction	ND	83.33±36.08	_	IE	IE	_
	*Ethyl acetate fraction	ND	166.67±72.17	_	IE	IE	_
	70% Hydroethanolic crude extract	ND	83.33±36.08	_	IE	IE	_
	*Chloroform fraction	ND	20.83±9.02	_	IE	IE	_
	*Ethyl acetate fraction	ND	41.67±18.04		IE	IE	_
Klebsiella pneumoniae (Gram-ve)	**Acetone crude extract**	ND	145.83±95.47	_	IE	IE	_
	*Chloroform fraction	ND	250.00±0.00	_	IE	IE	_
	*Ethyl acetate fraction	ND	83.33±36.08	_	IE	IE	_
	70% Hydroethanolic crude extract	ND	333.33±144.33	_	IE	IE	_
	*Chloroform fraction	ND	208.33±72.17	_	IE	IE	_
	*Ethyl acetate fraction	ND	208.33±72.17	_	IE	IE	_
Candida albicans (Fungi)	**Acetone crude extract**	ND	250.00±0.00	_	2.87±0.18	11.46±0.74	3.99
	*Chloroform fraction	ND	166.67±72.17	_	2.88±0.31	11.52±1.24	4.00
	*Ethyl acetate fraction	ND	166.67±72.17	_	2.65±0.58	10.58±2.30	3.99
	70% Hydroethanolic crude extract	ND	IE	_	IE	IE	_
	*Chloroform fraction	ND	IE	_	IE	IE	_
	*Ethyl acetate fraction	ND	IE		IE	IE	_

Key: MIC - Minimum Inhibitory Concentration in milligram per millilitre

MBC - Minimum Bactericidal Concentration in milligram per millilitre

MFC - Minimum Fungicidal Concentration in milligram per millilitre

IE – Ineffective against the tested microbial pathogen

ND - Not determined (concentration could not be determined due to opaque)

Fraction – of *Ocimum americanum* L. (Lamiaceae) crude extract indicated by (*)

MBC/MIC - Ratio of Minimum Inhibitory Concentration to Minimum Bactericidal Concentration

From the microbroth dilution bioassay MIC could not be determined due to the intense colour of the crude samples and their fractions hence turbidity was not visualizable. Consequently, the ratio of MBC/MIC could not be determined. This is a limitation of microbroth dilution bioassay on this particular plant's crude extracts and fractions.

4.2.3 Brine shrimp bioassay

The lethality of *Ocimum americanum* L crude samples and their fractions against brine shrimp larvae are shown in the Table 4.11 and Figure 4.7.

Table 4.11: Summary of cytotoxicity of *Ocimum americanum* L. crude extracts and fractions against *Artemia salina* larvae (brine shrimp nauplii)

Sample	Mortality per test dose			LC_{50} µg/mL	95 % CI	Clarkson's scale
	10 µg/mL	100 µg/mL	1000 µg/mL			
Vincristine	19	50	50	11.83	-	Highly toxic
Aqueous crude extract	0	3	35	559.71	397.09-811.09	Slightly toxic
Chloroform fraction of the aqueous extract	0	0	50	303.39	198.42-488.86	Moderately toxic
Ethyl acetate fraction of the aqueous extract	0	0	50	303.39	198.42-488.86	Moderately toxic
Hydroethanol crude extract	0	0	49	347.80	217.06-498.10	Moderately toxic
Chloroform fraction of the hydroethanolic crude extract	49	50	50	0.59	-	Highly toxic
Ethyl acetate fraction of the hydroethanolic crude extract	0	48	50	44.65	4.65-65.86	Highly toxic
Acetone crude extract	0	0	50	303.39	198.42-488.86	Moderately toxic

Chloroform fraction of the acetonic crude extract	0	0	50	303.39	198.42-488.86	Moderately toxic
Ethyl acetate fraction of the acetonic crude extract	0	0	50	303.39	198.42-488.86	Moderately toxic

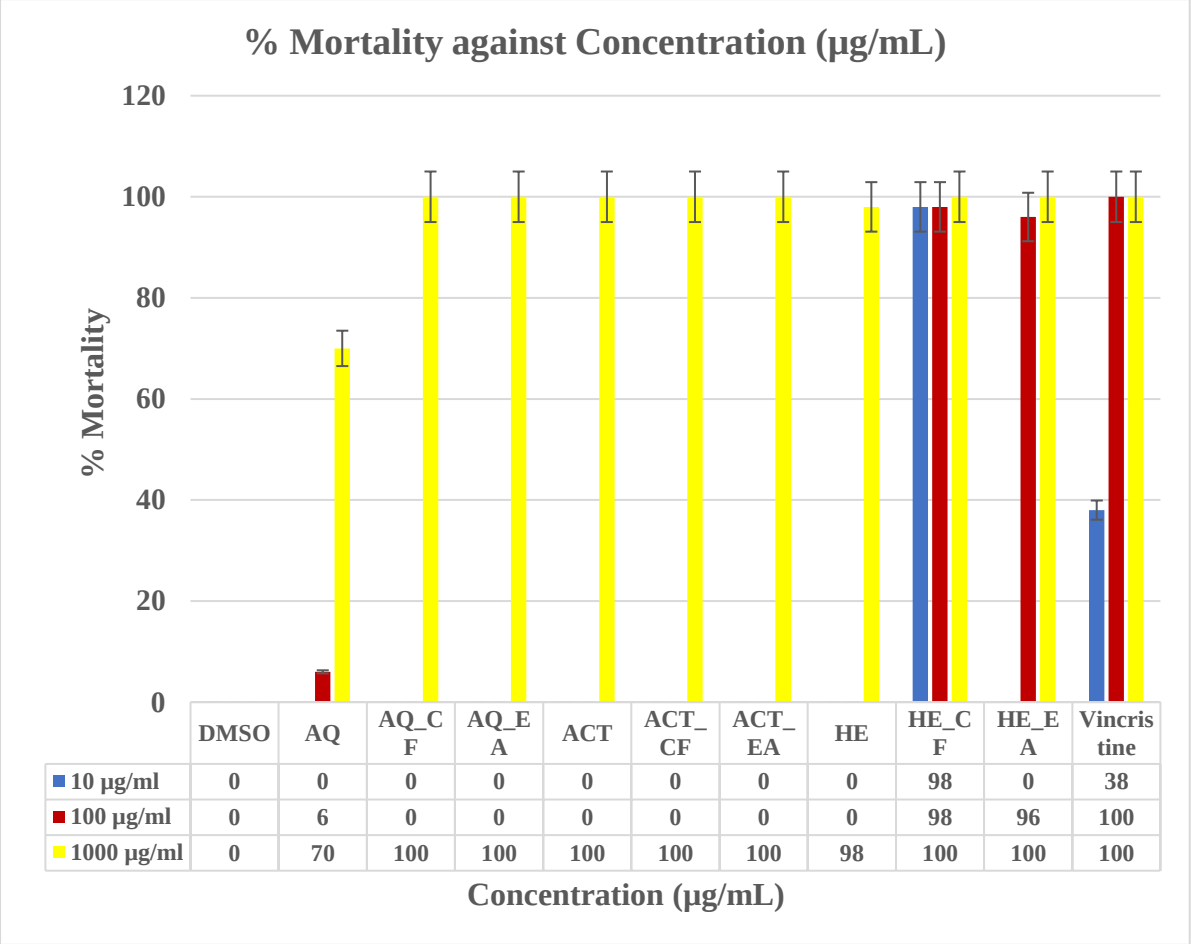
Key: Vincristine – Positive control drug

CI – Confidence interval

Sample – crude extracts and fractions of *Ocimum americanum* L.

LC_{50} – Median Lethal Concentration in microgram per litre

Mortality – Number of dead nauplii per dose in microgram per litre



Key: AQ – Aqueous crude extract; **AQ_CF** – Chloroformic fraction of aqueous extract; **AQ_EA** – Ethyl acetate fraction of aqueous extract; **ACT** – Acetone crude extract; **ACT_CF** – Chloroformic fraction of acetone extract; **ACT_EA** – Ethyl acetate fraction of acetonic extract; **HE** – Hydroethanolic crude extract; **HE_CF** – Chloroformic fraction of hydroethanolic

extract; **HE_EA** – Ethyl acetate fraction of hydroethanolic extract; DMSO – Dimethyl Sulfoxide the negative control; Vincristine – the positive control.

Figure 4.7: Brine shrimp % mortality against various concentrations of *O. americanum* L. crude extract and their fractions.

All the tested crude extracts and fractions of *Ocimum americanum* L. (Lamiaceae) were cytotoxic against the *Artemia salina* larvae according to Clarkson's toxicity criteria. Majority of the tested samples were moderately cytotoxic against the brine shrimp nauplii. The least cytotoxic sample was the aqueous crude extract with a Median Lethal Concertation (LC_{50}) of 559.71 µg/mL. While the most cytotoxic sample was the Chloroformic fraction of hydroethanolic extract which reported a median toxicity of 0.59 µg/mL against the *Artemia salina* nauplii. The positive control Vincristine had LC_{50} of 11.83 µg/mL which is extremely cytotoxic according to Clarkson's toxicity index but was less cytotoxic against the brine shrimp nauplii compared to the Chloroform fraction.

4.2.4 Phytochemical profile

The phytochemical composition of *Ocimum americanum* L. (Lamiaceae) crude samples and their fractions is shown in the Table 4.12.

Table 4.12: Phytochemical profile of *Ocimum americanum* L. crude extracts and their fractions

Phytochemical Profile									
Phytochemical	**AQ**	**CF_AQ**	**EA_AQ**	**HE**	**CF_HE**	**EA_HE**	**ACT**	**CF_ACT**	**EA_ACT**
Alkaloids	+	+	+	+	—	—	—	—	—
Flavonoids	+	+	+	+	+	+	+	+	+
Phenolics	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Reducing sugars	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+
Cyanogenetic glycosides	+	—	—	+	—	—	+	—	+
Anthraquinones	—	—	—	—	—	—	+	+	—
Terpenoids	—	+	+	—	+	+	+	+	+

Saponins	+	+	—	+	+	+	—	—	—
Phytosterols	—	+	+	—	+	+	+	+	+
Polyuronides	—	—	—	—	—	—	—	—	—

Key: Absence (-) Presence (+)

AQ: Aqueous crude extract;

CF_AQ: Chloroformic fraction of aqueous extract;

EA_AQ: Ethyl acetate fraction of aqueous extract;

HE: Hydroethanol crude extract;

CF_HE: Chloroformic fraction of hydroethanolic extract;

EA_HE: Ethyl acetate fraction of hydroethanolic extract;

ACT: Acetone crude extract;

CF_ACT: Chloroformic fraction of acetonic extract;

EA_ACT: Ethyl acetate fraction of acetonic extract;

In the current report the following phytocompounds were found in the herbal plant *Ocimum americanum* L. (Lamiaceae) crude extracts and fractions; anthraquinones, alkaloids, flavonoids, phenolics, saponins, cyanogenetic glycosides, tannins, cardiac glycosides, terpenoids, reducing sugars, and phytosterols. From the results phenolics, flavonoids, tannins, cardiac glycosides and reducing sugars were detected in all the crude samples and their fractions, however polyuronides were not detected in all the samples. The aqueous and hydroethanolic crude extracts had alkaloids, phenolics, flavonoids, cardiac glycosides, cyanogenic glycosides, saponins, tannins and reducing sugars. Similarly, acetonic crude extract had most of the screened phytochemicals except alkaloids, saponins and polyuronides.

Figure 4.8: Positive frothing test for saponins showing aqueous and hydroethanol persistence frothing.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Antimicrobial resistance (AMR) is a universal public health and development menace (Mailu *et al.,* 2021; WHO, 2021). Generally AMR develops naturally through genetic mutations over time and resistant pathogens are found ubiquitously in the ecosystems (WHO, 2021). They can spread amongst animals in their ecologies parallelly or vertically including from the source of their food chain (Prestinaci *et al.,* 2015; WHO, 2021). The emergency of antimicrobial drug resistance is due to irresponsible overuse of the antibiotic feed additives in animals farming. Equally, humans have escalated the AMR by misuse and non-compliance to their prescribed antimicrobial treatments (Ali *et al.,* 2022; Pandey *et al.,* 2014; Runyoro *et al.,* 2006; Walsh, 2003). Subsequently, alternative approaches are required to counter antimicrobial resistance and phytocompounds are the natural resources at our disposition (Ali *et al.,* 2022; Vidhya *et al.,* 2020). Numerous studies have reported their findings that, medicinal plant essential oils and extracts have excellent anti-inflammatory, antioxidants and anti-infective properties due to their phytoconstituents (Krua *et al.,* 2015). Additionally several species of basil (*Ocimum*) are well known in ethnomedicine and studies have reported to demonstrate; antifungal (Vieira *et al.,* 2003), antimicrobial (Ali *et al.,* 2022; Carović-Stanko *et al.,* 2010; Vidhya *et al.,* 2020), anthelminthics (Aderibigbe & Idowu, 2020), gastric cytoprotective antiulcer (Sutili *et al.,* 2016), nematocidal (Pandey *et al.,* 2014) and larvicidal (Madhiyazhagan *et al.,* 2014; Nguta *et al.,* 2010) properties.

Several extraction techniques such as Soxhlet, maceration, subcritical water, supercritical fluid and ultrasound assisted method are utilised to extract secondary metabolites from the plants matrices (Abubakar & Haque, 2020). However yield of extraction is dependent not only on the extraction technique but also dependent on the solvent choice used for extraction (Dirar *et al.,* 2019; Do *et al.,* 2014; Zengin *et al.,* 2019). The data presented in the current study of *Ocimum*

americanum L. (Lamiaceae) aqueous, hydroethanolic (aqueous alcohol) and acetone (dimethyl ketone/propanone) crude extracts and their fractions contains various classes of phytocompounds. The phytochemicals are credited to be accountable for the perceived ethnopharmacological efficacy (Zengin *et al.,* 2019). The results have also demonstrated the influence of solvent selection on the percentage yield of the crude extracts from the medicinal plant matrices (Ali *et al.,* 2022). The aqueous crude sample had the highest yield proportion of 4.52 %, while 70 % aqueous alcohol crude sample reported a percentage yield of 2.60 %, and the least proportional yield was seen in the acetonic crude sample at 1.15 %. It was observed that extraction yield increased with increased polarity of the solvent utilised. This is because alteration of solvents polarity tends to influence the efficiency of crude extract extraction (Zengin *et al.,* 2019). This is in agreement with an earlier reports that acetonic extraction yields are low (Ali *et al.,* 2022; Do *et al.,* 2014; Mailu, 2021; Truong *et al.,* 2019). Plant materials have varied phytoconstituents with different solubilities in various extraction solvents (Abubakar & Haque, 2020; Truong *et al.,* 2019). The ideal extraction solvent depends on that specific plant matrices and the bioactive compounds that are to be extracted (Zengin *et al.,* 2019). Hence selection of an ideal solvent of extraction for different plant biomass is normally difficult (Truong *et al.,* 2019). However, the most suitable solvent systems are mixtures of aqueous containing organic solvent such as acetone, ethanol or ethyl acetate (Do *et al.,* 2014). This is in accordance with the findings in that the ethyl acetate and chloroformic fractions of hydroethanolic crude sample were the most active in both growth inhibitions against *B. cereus* and lethality against the *Artemia salina* larvae.

On the antimicrobial activity of phytocompounds, the hydrophobic character of their carbon backbone and the polar character of their functional groups are the most important (Vieira *et al.,* 2014). Phytocompounds have intricate molecular structures, with semi-rigid cyclic skeleton. Their carbon chain has greater than five spinnable C-C bonds with several H-bond donors and acceptors. Also the compounds contain numerous chiral centres, with extensive polar surface

area and a molecular weight > 500 Da (Jacob, 2009; Lahlou, 2013). These characteristic are crucial in their suitability as medicinal compounds because they feature in absorption and transport of the phytocompounds to the molecular target sites (Karau *et al.,* 2015). The hierarchy of activity from lowest to highest of the phytochemicals is as follows; hydrocarbons < ethers < alcohols < ketones < aldehydes < phenolics (Vieira *et al.,* 2014). Therefore the extracts with rich phenolics components demonstrate the broadest spectrum and highest activity against the microbial pathogens (El-Shiekh *et al.,* 2012; Vieira *et al.,* 2014).

The inhibitory effects of *Ocimum americanum* L. fractions and crude samples proved active against Gram-positive microorganisms *Staphylococcus aureus*, *Bacillus cereus* and the fungal pathogen *Candida albicans*. However, the crude samples and fractions of *Ocimum americanum* L. were ineffective against Gram-negative microbial pathogens *Escherichia coli* and *Klebsiella pneumoniae*. The aqua fractions and crude samples did not demonstrate any antimicrobial activities against all the test microbes which conform with an earlier report by Vidhya *et al* (2020). Although Gram-positive microorganism possess broad plasma membrane, their murein sacculus is readily porous to particular biomolecules compared to that of Gram-negative bacterium. This can be explained by the absence of the outer lipopolysaccharide membrane (Matara *et al.,* 2021; Parhusip *et al.,* 2020; Vidhya *et al.,* 2020). Conversely the ineffectiveness of the water samples is owing to dipole polarisation, the apolar external (endotoxin) layer and lipid bilayer form a aquaphobic (water repellent) barricade thus repelling/inhibiting entrance of aqueous biomolecules into the cytoplasm (Casem, 2016). The most active sample was that of hydroethanolic crude extract fraction, ethyl acetate which recorded the highest zone of inhibition of (26.50 ± 0.29) and (26.00 ± 0.00) at 500 mg/mL and 250 mg/mL respectively against the two Gram-positive bacteria. These were not statistically significant ($p > 0.05$) from the inhibitory effect of the reference drug Ceftriaxone (30.00 ± 1.16). Potential antimicrobial bioactive compounds are projected depending on the inhibition zone diameter obtained using agar diffusion bioassay (Thaweboon & Thaweboon, 2009). Zone of Inhibition Diameter (ZID)

is classified into four categories as; very strong ZID > 20 mm; strong ZID 10 - 20 mm; medium ZID 5 – 10 mm and weak ZID < 5 mm (Davis & Stout, 1971; Ouchari *et al.,* 2019; Parhusip *et al.,* 2020). Most of acetonic and hydroethanolic crude samples and their fractions reported medium to strong inhibitory effects on the Gram-positive microbes *S. aureus* and *B. cereus*. Only acetonic extract and its fractions demonstrated medium to strong inhibition against the fungus *C. albicans*. The antibacterial effects may be as a results of phytocompounds such as flavonoids and phenolics which contains reactive hydroxyl group in its structure (Parhusip *et al.,* 2020; Vidhya *et al.,* 2020). In the present study, the hydroethanolic crude sample fraction ethyl acetate demonstrated excellent antibacterial effect against the Gram-positive bacterium *Bacillus cereus*. This is in agreement with previous studies that reported similar results for ethyl acetate sample excellent inhibitory activity as well as highly cytotoxic against *Artemia salina* nauplii (Ali *et al.,* 2022; Vidhya *et al.,* 2020; Zengin *et al.,* 2019).

Acetonic and aqueous alcohol samples of *Ocimum americanum* L. (Lamiaceae) were sensitive against the tested microbials pathogens as indicated by MICs, but the tested microorganisms were insensitive to all of the aliquots of water crude samples and their fractionation portions. The aqueous alcohol and acetonic crude samples and their fractions were sensitive against the Gram-positive microorganisms *Staphylococcus aureus* and *Bacillus cereus* whereas only acetonic and its fractions were sensitive to the fungal microorganism *Candida albicans*. MBC/MFC of American basil crude samples and fractions were sensitive against all test microbials excluding the water crude sample and their fractions, while aqueous alcohol crude sample and their fractions were insensitive to the fungal pathogen *Candida albicans*. Bioactive molecules that exhibit antimicrobic activities are classified as bacteriostatic, when the ratio MBC/MIC is greater than 4 and bactericidal when the ratio of MBC/MIC is equal to or less than 4 (Ali et *al.,* 2022; Hossan *et al.,* 2018; Muia *et al.,* 2020). Using the previous statement as a reference, we report that the crude extract of dimethyl ketone (ratio = 4.01) is a bacteriostatic in disposition, whereas the chloroformic and ethyl acetate fractions are

bactericidal against the *Staphylococcus, Bacillus* and *Candid* microorganisms. Equally the fractions and crude sample of aqueous alcohol are bacteriocidic against the Gram-positive microbes but not the fungal pathogen. However, caution need to be taken in elucidation of the results, given that the larger the value of MIC, it is more likely that the computed figures fail to find clinical relevance (Ali *et al.,* 2022; Muia *et al.,* 2020). Moreover, several studies have reported lower MIC figures against the test microbial of the Gram-positive bacteria. It is proposed that the data variations of the MIC presented and the reported from previous studies may be due to the differences in the presence of phytocompounds among the same species as well as the bioassay used (Ali *et al.,* 2022; Vidhya *et al.,* 2020; Zengin *et al.,* 2019). The present study reported smaller antimicrobic activity in-reference to the standard drugs at the equivalent (250 mg/mL) concentration. In the current study it is reported that there is no statistical difference ($p > 0.05$) in the average inhibitory diameter of the acetonic and hydroethanolic fractions and crude samples. On the other side, there was a significant difference ($p < 0.05$) amongst the means of inhibition for the two crude samples and their fractions contrasted to the reference drugs under varied strengths and dilutions.

The toxicologic bioassay composed of brine shrimp larvae, is commonly utilised to screen for a variety of toxicologic and biophysiological properties of medicinal crude samples and is considered predictive of toxicity (Ali *et al.,* 2022; Zengin *et al.,* 2019). The model demonstrated a potential lethality of all *Ocimum americanum* L. fractions and crude samples against the brine shrimp larvae. The median lethal concentration (LC_{50}) data was elucidated by the usage of Clarkson's toxicity index and compared to Meyer's toxicity scale. On a Meyer's index LC_{50} of < 1000 µg/mL are toxic and LC_{50} of > 1000 µg/mL are nontoxic (Meyer *et al.,* 1982). While on the Clarkson's scale the LC_{50} of between 0 – 100 µg/mL are considered highly cytotoxic, LC_{50} between 101 – 500 µg/mL are regarded as moderately cytotoxic, LC_{50} of between 501 – 1000 µg/mL are deemed slightly cytotoxic and LC_{50} of > 1000 µg/mL are regarded as nontoxic (Clarkson *et al.,* 2004; Hamidi *et al.,* 2014). The analysed data gave very low LC_{50} values on

Clarkson's index with high percentage mortality comparable to the reference drug. Ethyl acetate and chloroform fractions of hydroethanolic extract recorded the highest lethality against *Artemia salina* nauplii and this is the first time being documented. The chloroformic sample fraction had the least LC_{50} of 0.59 µg/mL which is lower than that of the positive control drug Vincristine (LC_{50} 11.83 µg/mL). As a result, the chloroformic sample demonstrated an excellent potential for bioprospecting of a novel biomolecule which may be developed and used to further better therapeutic outcomes. Conversely the ethyl acetate fraction (LC_{50} 44.65 µg/mL) was highly cytotoxic contrasted to the Clarkson's scale. Zengin *et al.,* (2019) demonstrated best toxicological profile with ethyl acetate extract of *Ocimum americanum* L. which corroborates with our findings (Ali *et al.,* 2022).

The distilled water crude extract was slightly toxic with LC_{50} of 559.71 µg/mL and this clarifies the perceived outcomes that the medicinal herb decoction/concoction for short term therapeutic use do not exhibit any indication of adverse events in the clients. The fractions of acetone and aqueous as well as crude samples of acetone had moderate cytotoxicity (LC_{50} of 303.39 µg/mL) against the brine shrimp larvae. This demonstrated that the partition coefficient (K_D) of the test solvents was small i.e. the solvents have weak fractionation and extraction power thus similar LC_{50} for several test solvents (Ali *et al.,* 2022; Zhang *et al.,* 2018). The discrepancy in the cytotoxicity amongst the fractions and crude samples is as a results of wide dispersal of the phytocompounds on the samples (Matara *et al.,* 2021; Nguta *et al.,* 2012).

Secondary metabolites obtained from plant matrices are either byproduct of plant metabolic process or synthesized for plant protection. However these bioactive compounds can either be beneficial or lethal to human physiology (Mustafa & El-kamali, 2019; Trease & Evans, 1989). The phytocompounds screening of *Ocimum americanum* L. (Lamiaceae) fractions and crude samples in the present study reports the presence of anthraquinones (anthracenedione or dioxoanthracene), alkaloids, cyanogenetic glycosides, cardiac glycosides, phenols (phenolics), saponins (triterpene glycosides), terpenoids (isoprenoids), tannins, triterpenes and reducing

sugars whereas polyuronides were not detected in all fractions and crude samples. Similarly, Karau *et al.,* (2015) and Vidhya *et al.,* (2020) documented the presence of alkaloids, phenols, glycosides, steroids, tannins, isoprenoids, saponins, flavonoids and reducing sugars in the aqueous, acetonic and ethyl acetate extract of *Ocimum americanum* L. which is in agreement with the current study (Karau *et al.,* 2015; Vidhya *et al.,* 2020). Generally the activity of phytochemicals is as a results of their collective action of both the active and minor components, thus numerous active compounds might have synergistic effects while the inactive components may influence absorption, bioavailability and the rate of reactions (Carović-Stanko *et al.,* 2010; Pandey *et al.,* 2014).

The alkaloids were existent in aqueous alcohol and aqueous crude samples although absent in the acetonic crude sample. Equally the alkaloids were present in all the fractions of water extract but absent from the fractions of acetone and hydroethanolic crude extracts. Alkaloids generally have strong biophysiological activities in humans, although some with partial effects (Bribi, 2018). They obstruct the cyclooxygenase cascade, as a result prevent chemokines and interleukin(s)-1 (IL-1, IL-12 and IL-18) that precipitate nociception during inflammatory process. Studies have also reported that alkaloids have antispasmodic, antimicrobic, and antimalarial properties (Bribi, 2018; Matara *et al.,* 2021). The anthraquinones were absent in all the crude samples except that of acetone crude sample and its chloroformic fractionation portion. All the test samples of the study plant had detected cardiac glycosides. Normally found as a phytocompound in numerous plants, these secondary metabolites possess a diverse range of physiological properties on the myocardial activities and have similarly been recommended to be used in cancer therapy (Riganti *et al.,* 2011). The cyanogenetic glycosides were only extant in the dimethyl ketone, aqueous alcohol and water crude extracts as well as the fraction of acetonic crude sample, ethyl acetate. The tannins, phenolics, flavonoids and reducing sugars were detected in all the crude extracts and fractions. Phenolics and flavonoids have excellent antioxidants, anti-inflammatory, antimutagenic and anticarcinogenic properties coupled with

their ability to regulate vital molecular enzymatic function (Lin *et al.,* 2016; Panche *et al.,* 2016). Tannins are bitter, astringent polyphenolic phytocompound that readily bind, shrink and precipitate various organic substances. They also have strong antioxidative activities, due to the reduction potential property in them (Mustafa & El-kamali, 2019). The *Ocimum americanum* L. (Lamiaceae) fractions and crude samples which was found to contain tannins could be beneficial for therapeutic purpose. High content of tannins has been found useful due to their capacity as antimicrobial, anticancer, antiseptic and wound healing properties (Matara *et al.,* 2021; Pengelly, 2021; Si *et al.,* 2021).

Saponins (triterpene glycosides) phytocompounds were detected in the aqueous crude extracts and factions as well as in aqueous alcohol crude sample though absent in the acetonic samples. The triterpene glycosides are a subcategory of isoprenoids, with varied properties, both useful and noxious. They are naturally bitter tasting, toxic phytocompounds that are foamy when shaken in aqueous solution (Mustafa & El-kamali, 2019). Research findings indicate that saponins are toxic to rapid growing tumor cell via initiation of programmed cell death and immunomodulate against malignant proliferation, lowers blood lipid and decreases blood sugar level (Kregiel *et al.,* 2017; Mustafa & El-kamali, 2019; Shi *et al.,* 2004). The phytosterols (phytosteroids or sterols) were detected in the organic extractant i.e., in the acetonic fractions and crude samples. Phytosteroids are mostly triterpenes which are essential structural constituent of plant cell lipid bilayer, while free sterols are implicated in stabilising the cell membranes (Karau *et al.,* 2015). Phytosterols rich supplements have been reported to decrease LDL cholesterol after long term consumption (Salehi-Sahlabadi, Varkaneh, *et al.,* 2020). Sterols are chemo-preventive bioactive compounds with antineoplastic properties via several mechanisms (Salehi *et al.,* 2021). McCann *et al* (2003) reported that nutritional sterols intake decreases the danger of various forms of tumors including tumors of the ovaries (Bradford & Awad, 2007; McCann *et al.,* 2003).

5.2 Conclusions and recommendations

This research work pursued to validate the medicinal properties and guarantee the safety and effectiveness of the folkloric utilised *Ocimum americanum* L. (Lamiaceae). The project investigated the antimicrobic properties, toxicity and phytocompounds constituents of fractions and crude samples of *Ocimum americanum* L. (Lamiaceae). The bioassay results for the present study, support that the medicinal plant contains numerous phytochemical compounds which are liable for the antimicrobic activities and toxicological properties. The data also features the effects of extractant selection on the yield of extraction of the crude sample of the medicinal herb matrices. Amongst all the samples the fractions of aqueous alcohol crude samples were more prospective thus good candidate for further research. The ethyl acetate sample fractions of aqueous alcohol crude sample have demonstrated promising antimicrobic effects against the Gram-positive microorganism *Bacillus cereus*. Conversely the chloroform fraction of hydroethanolic extract recorded high toxicity against the brine shrimp larvae thus potential compound for bioprospecting. The fractions of hydroethanolic crude samples have potential bioactive molecules which are accountable for antimicrobial and cytotoxicity properties respectively. Results from the present research will provide groundwork for finding an innovative natural phytomedicine. However extensive study is needed to quantify the phytocompounds, bioactive compound isolation and characterization, *in vivo* chronic toxicity and clinical studies profiling.

REFERENCES

Abbasi, A. M., Shah, M. H., & Khan, M. A. (2015). Ethnobotany and Ethnomedicine. In *Wild Edible Vegetables of Lesser Himalayas: Ethnobotanical and Nutraceutical Aspects, Volume 1* (pp. 19–29). Springer International Publishing.

Abubakar, A. R., & Haque, M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmacy & Bioallied Sciences*, ***12*(1)**, 1.

Aderibigbe, S. A., & Idowu, S. O. (2020). Anthelmintic activity of Ocimum gratissimum and Cymbopogon citratus leaf extracts against Haemonchus place adult worm. *Journal of Pharmacy & Bioresources*, ***17*(1).**

Ali, H. M., Nguta, J. M., Musila, F. M., Omambia, V. M., Matara, D. N., Mapenay, I. O., & Mokoro Omambia, V. (2021). Ethnopharmacological uses, biological activities, chemistry and toxicological aspects of Ocimum americanum var. americanum (Lamiaceae). *The Journal of Phytopharmacology*, ***10*(1)**, 56–60.

Ali, H., Nguta, J., Musila, F., Ole-Mapenay, I., Matara, D., & Mailu, J. (2022). Evaluation of Antimicrobial Activity, Cytotoxicity, and Phytochemical Composition of Ocimum americanum L. (Lamiaceae). *Evidence-Based Complementary and Alternative Medicine*, *2022*, 1–11.

Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). Escherichia coli in Europe: An Overview. *International Journal of Environmental Research and Public Health 2013, Vol. 10, Pages 6235-6254*, ***10*(12),** 6235–6254.

Aluko, B. T., Oloyede, O. I., & Afolayan, A. J. (2013). Polyphenolic contents and free radical scavenging potential of extracts from leaves of Ocimum americanum L. *Pakistan Journal of Biological Sciences: Pjbs*, ***16*(1)**, 22–30.

Ameen, O. M., Usman, L. A., Oladosu, I. A., Olawore, N. O., & Ogunw, I. A. (2011). Bioactivity of rhizome essential oils from two varieties of Cyperus articulatus (L.) grown in Nigeria, using brine shrimp (Artemia salina) lethality tests. *Journal of Medicinal Plants Research*, ***5*(6),** 1031–1033.

Azwanida, N. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med Aromat Plants*, ***4*(3)**, 196. -

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. In *Journal of Pharmaceutical Analysis* (Vol. 6, Issue 2, pp. 71–79). Xi'an Jiaotong University.

BaoDuy, N. Le, Trang, D., & Manag, N. T. (2015). Preliminary phytochemical analysis of leaf extracts ofthuja orientalis (l.) Endl. *Ijrsm.Com*.

Bassole, I. H. N., Nebie, R., Savadogo, A., Ouattara, C. T., Barro, N., & Traore, S. A. (2005). Composition and antimicrobial activities of the leaf and flower essential oils of Lippia chevalieri and Ocimum canum from Burkina Faso. *African Journal of Biotechnology*, ***4*(10).**

Beentje, H. ., Adamson, J., & Bhanderi, D. (1994). *Kenya trees, shrubs, and lianas* (1st Edition). National Museums of Kenya.

Berger, A., Jones, P. J. H., & Abumweis, S. S. (2004). Plant sterols: Factors affecting their efficacy and safety as functional food ingredients. *Lipids in Health and Disease*, *3*.

Bloomfield, S. F. (1991). Methods for assessing antimicrobial activity. *Mechanisms of Action of Chemical Biocides. WB Blackwell Scientific Publications, London*, 1–22.

Bolarinwa, I. F., Oke, M. O., Olaniyan, S. A., & Ajala, A. S. (2016). A Review of Cyanogenic Glycosides in Edible Plants. In *Toxicology - New Aspects to This Scientific Conundrum*. IntechOpen.

Botelho, A. F. M., Pierezan, F., Soto-Blanco, B., & Melo, M. M. (2019). A review of cardiac glycosides: Structure, toxicokinetics, clinical signs, diagnosis and antineoplastic potential. In *Toxicon* (Vol. 158, pp. 63–68). Toxicon.

Bottone, E. J. (2010). Bacillus cereus, a Volatile Human Pathogen. *CLINICAL MICROBIOLOGY REVIEWS*, ***23*****(2),** 382–398.

Bozin, B., Mimica-Dukic, N., Simin, N., & Anackov, G. (2006). Characterization of the volatile composition of essential oils of some lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *Journal of Agricultural and Food Chemistry*, ***54*****(5),** 1822–1828.

Bradford, P. G., & Awad, A. B. (2007). Phytosterols as anticancer compounds. *Molecular Nutrition & Food Research*, ***51*****(2),** 161–170.

Breyer-Brandwijk, M. G. (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. *The Medicinal and Poisonous Plants of Southern and Eastern Africa Being an Account of Their Medicinal and Other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal., Edn 2*.

Bribi, N. (2018). Pharmacological activity of Alkaloids*: A Review*. *1*.

Bruneton, J. (1995). Pharmacognosy, phytochemistry, medicinal plants. Lavoisier publishing.

Carović-Stanko, K., Orlić, S., Politeo, O., Strikić, F., Kolak, I., Milos, M., & Satovic, Z. (2010). Composition and antibacterial activities of essential oils of seven Ocimum taxa. *Food Chemistry*, ***119*****(1),** 196–201.

Casem, M. L. (2016). Chapter 5 - Membranes and Membrane Transport. In M. L. B. T.-C. S. in C. B. Casem (Ed.), *Problem Sets in Biological and Biomedical Sciences* (pp. 105–125).

Academic Press.

Cheng, Z., & C Zhen. (2004). The Cheng Zhi-Fan collectanea of medical history. *Peking University Medical Press*.

Chin, Y.-W., Balunas, M. J., Chai, H. B., & Kinghorn, A. D. (2006). Drug discovery from natural sources. *The AAPS Journal*, ***8*(2),** E239–E253.

Chogo, J. B., & Crank, G. (1981). Chemical composition and biological activity of the tanzanian plant ocimum suave. *Journal of Natural Products*, ***44*(3),** 308–311.

Chokechaijaroenporn, O., Bunyapraphatsara, N., & Kongchuensin, S. (1994). Mosquito repellent activities of Ocimum volatile oils. *Phytomedicine*, ***1*(2)**, 135–139.

Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition*, ***38*(6),** 421–464.

Cinti, S., Malani, A., & Riddell, J. (2008). *Chapter 11 - Infectious Diseases* (J. J. B. T.-C. M. H. Heidelbaugh (ed.); pp. 182–206). W.B. Saunders.

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P. J., & Folb, P. I. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of Ethnopharmacology*, ***92*(2–3)**, 177–191.

Cox-Georgian, D., Ramadoss, N., Dona, C., & Basu, C. (2019). Therapeutic and Medicinal Uses of Terpenes. *Medicinal Plants*, 333.

Davis, W. W., & Stout, T. R. (1971). Disc Plate Method of Microbiological Antibiotic Assay I. Factors Influencing Variability and Error1. *APPLIED MICROBIOLOGY*, 659–665.

De Smet, P. A. G. M. (1995). Health Risks of Herbal Remedies. *Drug Safety*, ***13*(2)**, 81–93.

Deitersen, J., El-Kashef, D. H., Proksch, P., & Stork, B. (2019). Anthraquinones and

autophagy - Three rings to rule them all? *Bioorganic & Medicinal Chemistry*, ***27*****(20)**.

Dirar, A. I., Alsaadi, D. H. M., Wada, M., Mohamed, M. A., Watanabe, T., & Devkota, H. P. (2019). Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *South African Journal of Botany*, *120*, 261–267.

Dixit, V. A. (2019). A simple model to solve a complex drug toxicity problem. *Toxicology Research*, ***8*****(2)**, 157–171.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis*, ***22*****(3)**, 296–302.

Drummond, R. A., Gaffen, S. L., Hise, A. G., & Brown, G. D. (2014). Innate Defense against Fungal Pathogens. *Cold Spring Harbor Perspectives in Medicine*, ***5*****(6),** 1–19.

Effah, C. Y., Sun, T., Liu, S., & Wu, Y. (2020). Klebsiella pneumoniae: An increasing threat to public health. *Annals of Clinical Microbiology and Antimicrobials*, ***19*****(1)**, 1–9.

El-Shiekh, Y. W. A., El-Din, N. H., Shaymaa, M. A. A., & El-Din, K. A. Z. (2012). Antifungal activity of some naturally occurring compounds against economically important phytopathogenic fungi. *Nature and Science*, ***10*****(6),** 114–123.

Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, ***64*****(08)**, 711–713.

Evans, W. . (2002). *Trease and Evans Pharmacognosy* (15th Edition). W.R Sauders.

Faria, R., Pereira, C., Alves, R., Mendonça, T., Farinha, F., & Vasconcelos, C. (2015). *Chapter 15 - Opportunistic Infections and Autoimmune Diseases* (Y. Shoenfeld, N.

Agmon-Levin, & N. R. B. T.-I. and A. (Second E. Rose (eds.); pp. 251–277). Academic Press.

Fish, D. N. (2002). Optimal antimicrobial therapy for sepsis. *American Journal of Health-System Pharmacy*, ***59*(Suppl_1)**, S13–S19.

Githinji, C. W., & Kokwaro, J. O. (1993). Ethnomedicinal study of major species in the family Labiatae from Kenya. *Journal of Ethnopharmacology*, ***39*(3),** 197–203.

Gomes, T. A. T., Elias, W. P., Scaletsky, I. C. A., Guth, B. E. C., Rodrigues, J. F., Piazza, R. M. F., Ferreira, C. S., & Martinez, M. B. (2016). *Medical Microbiology Diarrheagenic Escherichia coli*.

Gow, N. A. R., & Yadav, B. (2017). Microbe Profile: Candida albicans: a shape-changing, opportunistic pathogenic fungus of humans. *Microbiology*, ***163*(8)**.

Grandjean, P. (2016). Paracelsus Revisited: The Dose Concept in a Complex World. In *Basic and Clinical Pharmacology and Toxicology* (Vol. 119, Issue 2, pp. 126–132).

Guclu-Ustundag, Ö., & Mazza, G. (2007). Saponins: properties, applications and processing. *Critical Reviews in Food Science and Nutrition*, ***47*(3),** 231–258.

Gurib-Fakim, A., & Mahomoodally, M. F. (2013). African Flora as Potential Sources of Medicinal Plants: Towards the Chemotherapy of Major Parasitic and Other Infectious Diseases-A Review. *Jordan Journal of Biological Sciences*, ***6*(2),** 77–84.

Hamidi, M. R., Jovanova, B., & Kadifkova Panovska, T. (2014). Toxicological evaluation of the plant products using Brine Shrimp (Artemia salina L.) model. *Macedonian Pharmaceutical Bulletin*, ***60*(01)**, 9–18.

Harborne, J. B. (1984). Phytochemical Methods. In *Phytochemical Methods*. Springer Netherlands.

Harborne, J. B. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media.

Harborne, J. B., & Baxter, H. (1993). *PHYTOCHEMICAL DICTIONARY*. Taylor & Francis.

Heidary, M., Nasir, M. J., Dabiri, H., & Tarashi, S. (2018). Prevalence of Drug-resistant Klebsiella pneumoniae in Iran: A Review Article. *Iranian Journal of Public Health*, ***47*****(3)**, 317.

Heinrich, M. (2010). Ethnopharmacology and drug discovery. *Comprehensive Natural Products II. Elsevier, Oxford*, *351*.

Heinrich, M. (2014). Ethnopharmacology: Quo vadis? Challenges for the future. *Revista Brasileira de Farmacognosia*, ***24*****(2)**, 99–102.

Heinrich, M., Edwards, S., Moerman, D. E., & Leonti, M. (2009). Ethnopharmacological field studies: A critical assessment of their conceptual basis and methods. In *Journal of Ethnopharmacology* (Vol. 124, Issue 1, pp. 1–17). Elsevier.

Hiltunen, R., & Holm, Y. (1999). *Basil: the genus Ocimum*. CRC Press.

Hossan, M. S., Jindal, H., Maisha, S., Raju, C. S., Sekaran, S., Nissapatorn, V., Kaharudin, F., Yi, L. S., Khoo, T. J., Rahmatullah, M., & Wiart, C. (2018). Antibacterial effects of 18 medicinal plants used by the Khyang tribe in Bangladesh. *Pharmaceutical Biology*, *56*(1), 201–208.

Jacob, J. E. (2009). Natural products-based drug discovery: some bottlenecks and considerations. *Current Science*, ***96*****(6),** 753–754.

Javanmardi, J., Stushnoff, C., Locke, E., & Vivanco J.M. (2003). Antioxidant activity and total phenolic content of Iranian Ocimum accessions. *Food Chemistry*, ***83*****(4)**, 547–550.

Kabir, M. A., Hussain, M. A., & Ahmad, Z. (2012). Candida albicans: A Model Organism

for Studying Fungal Pathogens. *ISRN Microbiology*, *2012*, 1–15.

Kadosh, D. (2019). Regulatory Mechanisms Controlling Morphology and Pathogenesis inCandida albicans. *Current Opinion in Microbiology*, *52*, 27.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. T. (2004). Pathogenic Escherichia coli. *Nature Reviews Microbiology 2004 2:2*, ***2*****(2),** 123–140.

Karau, G. M., Njagi, E. N. M., Machocho, A. K., Wangai, L. N., & Nthinga, M. J. (2015). Chemical Composition and in vitro Antioxidant Activities of Ocimum americanum. In *Advances in Analytical Chemistry* (Vol. 5, Issue 2, pp. 42–49).

Kaufman, P., Cseke, L., Vogler, B., Setzer, W., & Kirakosyan, A. (2006). Traditional, Analytical, and Preparative Separations of Natural Products. In *Natural Products from Plants, Second Edition* (pp. 263–317).

Khatri, D., & Chhetri, S. B. B. (2020). Reducing Sugar, Total Phenolic Content, and Antioxidant Potential of Nepalese Plants. *BioMed Research International*, *2020*.

Kling, D. N., Marcial, G. E., Roberson, D. N., Lorca, G. L., & Gonzalez, C. F. (2016). The Synergistic Contribution of Lactobacillus and Dietary Phytophenols in Host Health. *Probiotics and Prebiotics in Human Nutrition and Health*.

Kokwaro, J. (2009). *Medicinal Plants of East Africa* (Third Edition). University Of Nairobi Press.

Kregiel, D., Berlowska, J., Witonska, I., Antolak, H., Proestos, C., Babic, M., Babic, L., & Zhang, B. (2017). Saponin-Based, Biological-Active Surfactants from Plants. *Application and Characterization of Surfactants*.

Krua, G. M., Njagi, E. M., Machocho, A., Wangai, L. N., & Nthinga, J. M. (2015). *Chemical Composition and in vitro Antioxidant Activities of Ocimum americanum*.

Kukula-Koch, W. A., & Widelski, J. (2017). *Chapter 9 - Alkaloids* (S. Badal & R. B. T.-P. Delgoda (eds.); pp. 163–198). Academic Press.

Kumar Pandey, A., Singh, P., & Nath Tripathi, N. (2014). Chemistry and bioactivities of essential oils of some Ocimum species: an overview Asian Pacific Journal of Tropical Biomedicine. *Document Heading Asian Pac J Trop Biomed*, ***4*(9),** 682–694.

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal*, *2013*.

Kuo, P.-C., Damu, A. G., Lee, K.-H., & Wu, T.-S. (2004). Cytotoxic and antimalarial constituents from the roots of Eurycoma longifolia. *Bioorganic & Medicinal Chemistry*, ***12*(3)**, 537–544.

Kurek, J. (2019). Introductory Chapter: Alkaloids - Their Importance in Nature and for Human Life. *Alkaloids - Their Importance in Nature and Human Life*.

Lahlou, M. (2013). The Success of Natural Products in Drug Discovery. *Pharmacology & Pharmacy*, ***04*(03)**, 17–31.

Limon, J. J., Skalski, J. H., & Underhill, D. M. (2017). Commensal Fungi in Health and Disease. *Cell Host and Microbe*, ***22*(2)**, 156–165.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., & Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. In *Molecules* (Vol. 21, Issue 10).

Madhiyazhagan, P., Murugan, K., Kumar, A. N., & Nataraj, T. (2014). Extraction of mosquitocidals from Ocimum canum leaves for the control of dengue and malarial vectors. *Asian Pacific Journal of Tropical Disease*, *4*, S549–S555.

Mailu, James K. (2021). *Ethnopharmacological and Toxicological Study of Medicinal Plants Used Against Respiratory Infections in Kisumu East Sub- County*. University of Nairobi.

Mailu, James Kiamba, Nguta, J. M., Mbaria, J. M., & Okumu, M. O. (2021). Qualitative and quantitative phytochemical composition, antimicrobial activity, and brine shrimp cytotoxicity of different solvent extracts of Acanthus polystachyus, Keetia gueinzii, and Rhynchosia elegans. *Future Journal of Pharmaceutical Sciences*, ***7*(1).**

Malik, E. M., & Müller, C. E. (2016). Anthraquinones As Pharmacological Tools and Drugs. *Medicinal Research Reviews*, ***36*(4)**, 705–748.

Malik, K., Ahmad, M., Bussmann, R. W., Tariq, A., Ullah, R., Alqahtani, A. S., Shahat, A. A., Rashid, N., Zafar, M., Sultana, S., & Shah, S. N. (2018). Ethnobotany of Anti-hypertensive Plants Used in Northern Pakistan. *Frontiers in Pharmacology*, *9*.

Martins, N., Ferreira, I. C. F. R., Barros, L., Silva, S., & Henriques, M. (2014). Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. *Mycopathologia*, ***177*(5–6),** 223–240.

Martorell, M., Castro, N., Victoriano, M., Capó, X., Tejada, S., Vitalini, S., Pezzani, R., & Sureda, A. (2021). An Update of Anthraquinone Derivatives Emodin, Diacerein, and Catenarin in Diabetes. *Evidence-Based Complementary and Alternative Medicine : ECAM*, *2021*.

Matara, D. N., Nguta, J. M., Musila, F. M., & Mapenay, I. (2021). Phytochemical Analysis and Investigation of the Antimicrobial and Cytotoxic Activities of Croton dichogamus Pax Crude Root Extracts. *Evidence-Based Complementary and Alternative Medicine*, *2021*, 1–9.

Matasyoh, J. C., Bendera, M. M., Ogendo, J. O., Omollo, E. O., & Deng, A. L. (2006). Volatile leaf oil constituents of Ocimum americanum L. occuring in Western Kenya.

Bulletin of the Chemical Society of Ethiopia, ***20*(1),** 177–180.

McCann, S. E., Freudenheim, J. L., Marshall, J. R., & Graham, S. (2003). Risk of human ovarian cancer is related to dietary intake of selected nutrients, phytochemicals and food groups. *Journal of Nutrition*, ***133*(6),** 1937–1942.

McDowell, R. H., Sands, E. M., & Friedman, H. (2021). Bacillus Cereus. *Food Microbiology: Fundamentals and Frontiers*, 541–554.

McLaughlin, J. L. (1991). Crown gall tumours on potato discs and brine shrimp lethality: two simple bioassays for higher plant screening and fractionation. *Methods in Plant Biochemistry*, *6*, 1–32.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. j, & McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, ***45*(05)**, 31–34.

Middleton Jr, E. (1988). Some biological properties of plant flavonoids. *Annals of Allergy*, ***61*(6 Pt 2)**, 53–57.

Middleton Jr, Elliott, & Kandaswami, C. (1992). Effects of flavonoids on immune and inflammatory cell functions. *Biochemical Pharmacology*, ***43*(6),** 1167–1179.

Moreau, R. A., Nyström, L., Whitaker, B. D., Winkler-Moser, J. K., Baer, D. J., Gebauer, S. K., & Hicks, K. B. (2018). Phytosterols and their derivatives: Structural diversity, distribution, metabolism, analysis, and health-promoting uses. *Progress in Lipid Research*, *70*, 35–61.

Morkunas, I., & Ratajczak, L. (2014). The role of sugar signaling in plant defense responses against fungal pathogens. *Acta Physiologiae Plantarum*, ***36*(7),** 1607–1619.

Morsy, N. F. S., & Hammad, K. S. M. (2021). Extraction of essential oil from methyl

cinnamate basil (Ocimum canum Sims) with high yield in a short time using enzyme pretreatment. *Journal of Food Science and Technology*, ***58*****(7),** 2599–2605.

Muia, B. M., Mbaria, J. M., Kanja, L. W., Gitahi, N., Okumu, P. O., & Okumu, M. O. (2020). Acute and sub-acute toxicity study of the root extracts of *Fagaropsis hildebrandtii* in mice and evaluation of their antimicrobial effects. *F1000Research 2020 8:1444*, *8*, 1444.

Mustafa, A., & El-kamali, H. (2019). Chemical Composition of Ocimum americanum In Sudan. *Research in Pharmacy and Health Sciences*, *05*, 172–178.

Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic Escherichia coli. *Clin. Microbiol. Rev.*, ***11*****(1),** 142–201.

Nguta, J M, Mbaria, J. M., Gakuya, D. W., Gathumbi, P. K., & Kiama, S. G. (2010). Antimalarial herbal remedies of Msambweni, Kenya. *Journal of Ethnopharmacology*, *128*, 424–432.

Nguta, Joseph Mwanzia, Mbaria, J. M., Gakuya, D. W., Gathumbi, P. K., Kabasa, J. D., & Kiama, S. G. (2012). Cytotoxicity of antimalarial plant extracts from Kenyan biodiversity to the brine shrimp, Artemia salina L.(Artemiidae). *Drugs and Therapy Studies*, ***2*****(1),** e12–e12.

Njue, L., Kanja, L. W., Ombui, J. N., Nduhiu, J. G., & Obiero, D. (2014). Efficacy of antimicrobial activity of garlic extracts on bacterial pathogens commonly found to contaminate meat. *East African Medical Journal*, ***91*****(12),** 442–448.

Nobile, C. J., & Johnson, A. D. (2015). Candida albicans Biofilms and Human Disease. *Annual Review of Microbiology*, ***69*****(1),** 71.

Okigbo, R. ., & Mmeka E.C. (2006). An Appraisal of Phytomedicine in Africa. *KMITL Science and Technology Journal*, ***6*****(2),** 83–94.

Okigbo, R. N., Anuagasi, C. L., & Amadi, J. E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. In *Journal of Medicinal Plants Research* (Vol. 3, Issue 2).

Ouchari, L., Boukeskasse, A., Bouizgarne, B., & Ouhdouch, Y. (2019). Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomic diversity. *Biology Open*, ***8*(2)**.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*, 1–15.

Pandey, A. K., Singh, P., & Tripathi, N. N. (2014). Chemistry and bioactivities of essential oils of some Ocimum species: an overview. *Asian Pacific Journal of Tropical Biomedicine*, ***4*(9),** 682–694.

Parhusip, Adolf J N, Angel, V., Anugrahati, N. A., Honga, J., & Sinaga, W. S. L. (2020). Ethyl Acetate Extract of Red Melinjo (Gnetum Gnemon L.) Peel as Antibacterial Compound. *5th International Conference on Food, Agriculture and Natural Resources (FANRes 2019)*, 189–198.

Parhusip, Adolf Jan Nexson, & Sitanggang, A. B. (2011). Antimicrobial activity of melinjo seed and peel extract (Gnetum gnemon) against selected pathogenic bacteria. *Microbiology Indonesia*, ***5*(3),** 2.

Parra, A. L., Yhebra, R. S., Sardiñas, I. G., & Buela, L. I. (2001). Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine*, ***8*(5),** 395–400.

Paton, A., Harley, R. M., & Harley, M. M. (1999). Ocimum-an overview of relationships and classification. *Ocimum Aromatic Plants-Industrial Profiles. Amsterdam: Harwood Academic*.

Pattnaik, S., & Chand, P. K. (1996). In vitro propagation of the medicinal herbs Ocimum americanum L. syn. O. canum Sims.(hoary basil) and Ocimum sanctum L.(holy basil). *Plant Cell Reports*, ***15*****(11),** 846–850.

Pengelly, A. (2021). *The Constituents of Medicinal Plants: An Introduction to the Chemistry and Therapeutics of Herbal Medicine*. Taylor & Francis Group.

Perveen, S. (2018). Introductory Chapter: Terpenes and Terpenoids. *Terpenes and Terpenoids*.

Podschun, R., & Ullmann, U. (1998). Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews*, ***11*****(4),** 589–603.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. In *Pathogens and Global Health* (Vol. 109, Issue 7, pp. 309–318). Taylor & Francis.

Ramadwa, T., Awouafack, M. D., Sonopo, M., & Eloff, J. (2019). Antibacterial and Antimycobacterial Activity of Crude Extracts, Fractions, and Isolated Compounds From Leaves of Sneezewood, Ptaeroxylon obliquum (Rutaceae). *Natural Product Communications*, *14*, 1–7.

Ramawat, K. ., Dass, S., & Mathur, M. (2009). The Chemical Diversity of Bioactive Molecules and Therapeutic Potential of Medicinal Plants. *Springer*.

Riganti, C., Campia, I., Kopecka, J., Gazzano, E., Doublier, S., Aldieri, E., Bosia, A., & Ghigo, D. (2011). Pleiotropic Effects of Cardioactive Glycosides. *Current Medicinal Chemistry*, ***18*****(6),** 872–885.

Runyoro, D. K., Matee, M. I. N., Ngassapa, O. D., Joseph, C. C., & Mbwambo, Z. H. (2006). Screening of Tanzanian medicinal plants for anti-Candida activity. *BMC Complementary and Alternative Medicine*, ***6*****(1),** 1–10.

Runyoro, D., Ngassapa, O., Vagionas, K., Aligiannis, N., Graikou, K., & Chinou, I. (2010). Chemical composition and antimicrobial activity of the essential oils of four Ocimum species growing in Tanzania. *Food Chemistry*, ***119*****(1),** 311–316.

Salehi-Sahlabadi, A., Varkaneh, H. K., Shahdadian, F., Ghaedi, E., Nouri, M., Singh, A., Farhadnejad, H., Găman, M. A., Hekmatdoost, A., & Mirmiran, P. (2020). Effects of Phytosterols supplementation on blood glucose, glycosylated hemoglobin (HbA1c) and insulin levels in humans: a systematic review and meta-analysis of randomized controlled trials. *Journal of Diabetes and Metabolic Disorders*, ***19*****(1),** 625.

S**alehi, B., Quispe, C., Sharifi-Rad, J., Cruz-Martins, N., Nigam, M., Mishra, A. P., Konovalov, D. A., Orobinskaya, V., Abu-Reidah, I. M., Zam, W., Sharopov, F., Venneri, T., Capasso, R., Kukula-Koch, W., Wawruszak, A., & Koch, W. (2021).** Phytosterols: From Preclinical Evidence to Potential Clinical Applications. *Frontiers in Pharmacology*, *0*, 1819.

Sarma, D. S. K., & Babu, A. V. S. (2011). Pharmacognostic and phytochemical studies of Ocimum americanum. *J. Chem. Pharm. Res*, ***3*****(3),** 337–347.

Schulz, V., Hänsel, R., & Tyler, V. E. (2001). *Rational Phytotherapy: A Physician's Guide to Herbal Medicine.* Springer Berlin Heidelberg.

Seo, J., Lee, S., Elam, M. L., Johnson, S. A., Kang, J., Arjmandi, B. H., & Bahram Arjmandi, C. H. (2014). Study to find the best extraction solvent for use with guava leaves (Psidium guajava L.) for high antioxidant efficacy. *Food Science & Nutrition*, ***2*****(2),** 174–180.

Shadia, E., El-Aziz, A., Omer, E. A., & Sabra, A. S. (2007). Chemical Composition of *Ocimum americanum* Essential Oil and Its Biological Effects Against, Agrotis ipsilon, (Lepidoptera: Noctuidae). *Research Journal of Agriculture and Biological Sciences*.

Shaw, D., Leon, C., Kolev, S., & Murray, V. (1997). Traditional remedies and food supplements. A 5-year toxicological study (1991-1995). *Drug Safety*, ***17*****(5),** 342–356.

Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G., & Jiang, Y. (2004). Saponins from edible legumes: chemistry, processing, and health benefits. *Journal of Medicinal Food*, ***7*****(1),** 67–78.

Shiri, N.-V., Kondratyeva, K., & Carattoli, A. (2017). Klebsiella pneumoniae: A major worldwide source and shuttle for antibiotic resistance. In *FEMS Microbiology Reviews* (Vol. 41, Issue 3, pp. 252–275). Oxford Academic.

Shukla, V., Asthana, S., Gupta, P., Dwivedi, P. D., Tripathi, A., & Das, M. (2017). *Chapter One - Toxicity of Naturally Occurring Anthraquinones* (J. C. Fishbein & J. M. B. T.-A. in M. T. Heilman (eds.); Vol. 11, pp. 1–50). Elsevier.

Si, W., Zhang, Y., Li, X., Du, Y., & Xu, Q. (2021). Understanding the Functional Activity of Polyphenols Using Omics-Based Approaches. *Nutrients*, ***13*****(11),** 3953.

Simon, J. E., Morales, M. R., Phippen, W. B., Vieira, R. F., & Hao, Z. (1999). Basil: A Source of Aroma Compounds and a Popular Culinary and Ornamental Herb*. In *hort.purdue.edu*.

Sims, M., Tomkins, S., Judge, K., Taylor, G., Jarvis, M. J., & Gilmore, A. (2010). Trends in and predictors of second-hand smoke exposure indexed by cotinine in children in England from 1996 to 2006. *Addiction*, ***105*****(3),** 543–553.

Sutili, F J, Murari, A. L., Silva, L. L., Gressler, L. T., Heinzmann, B. M., de Vargas, A. C., Schmidt, D., & Baldisserotto, B. (2016). The use of Ocimum americanum essential oil against the pathogens Aeromonas hydrophila and Gyrodactylus sp. in silver catfish (Rhamdia quelen). *Letters in Applied Microbiology*, ***63*****(2),** 82–88.

Sutili, Fernando J, Velasquez, A., Pinheiro, C. G., Heinzmann, B. M., Gatlin III, D. M.,

& Baldisserotto, B. (2016). Evaluation of Ocimum americanum essential oil as an additive in red drum (Sciaenops ocellatus) diets. *Fish & Shellfish Immunology*, *56*, 155–161.

Suvajdžić, L., Djendić, A., Sakač, V., Čanak, G., & Dankuc, D. (2016). Hippocrates: The father of modern medicine. *Scindeks-Clanci.Ceon.Rs*, ***73*(12),** 1181–1186.

Tajo, A., & Thoppil, J. (1998). *Cytotoxic properties of Ocimum americanum L. 17 B*, 49–53.

Taylor, T. A., & Unakal, C. G. (2021). Staphylococcus Aureus. *Food Microbiology: Fundamentals and Frontiers*, 555–584.

Thaweboon, S., & Thaweboon, B. (2009). *In vitro antimicrobial activity of Ocimum americanum L. essential oil against oral microorganisms.*

Tong, S. Y. C., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G. (2015). Staphylococcus aureus infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, ***28*(3),** 603–661.

Trease, G. E., & Evans, M. C. (1989). Text book of Pharmacognosy 13th Edition. In *Bailiere Tindall, London.*

T**ruong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., & Nguyen, H. C. (2019).** Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of severinia buxifolia. *Journal of Food Quality*, *2019.*

Tyler, V. E. (2000). Herbal medicine: from the past to the future. *Public Health Nutrition*, ***3*(4a),** 447–452.

UNESCO. (1996). *Culture and Health Orientation Texts World Decade for cultural Development 1988-1997, Document CLTDECPRO.*

UNESCO. (1998). *Promotion of Ethnobotany and the Sustainable Use of Plant resources in Africa. Terminal Report: FIT/504-RAF-48.*

V**algas, C., Machado De Souza, S., Elza, ;, Smânia, F. A., & Smânia, A. (2007).** Screening methods to determine antibacterial activity of natural products. *SciELO Brasil*, *38*, 369–380.

V**an Gorkom, B. A. P., De Vries, E. G. E., Karrenbeld, A., & Kleibeuker, J. H. (1999).** Review article: Anthranoid laxatives and their potential carcinogenic effects. *Alimentary Pharmacology and Therapeutics*, ***13*(4),** 443–452.

Vidhya, E., Vijayakumar, S., Rajalakshmi, S., Kalaiselvi, S., & Pandiyan, P. (2020). Antimicrobial activity and phytochemical screening of Ocimum americanum L extracts against pathogenic microorganisms. *Acta Ecologica Sinica*, ***40*(3),** 214–220.

Vieira, P. R. N., de Morais, S. M., Bezerra, F. H. Q., Ferreira, P. A. T., Oliveira, Í. R., & Silva, M. G. V. (2014). Chemical composition and antifungal activity of essential oils from Ocimum species. *Industrial Crops and Products*, *55*, 267–271.

Vieira, R. F., Grayer, R. J., & Paton, A. J. (2003). Chemical profiling of Ocimum americanum using external flavonoids. *Phytochemistry*, ***63*(5),** 555–567.

Waghulde, S., Kale, M. K., & Patil, V. (2019). Brine Shrimp Lethality Assay of the Aqueous and Ethanolic Extracts of the Selected Species of Medicinal Plants. *Proceedings*, ***41*(1)**, 47.

Walsh, C. (2003). Where will new antibiotics come from? *Nature Reviews Microbiology*, ***1*(1)**, 65–70.

Wang, J., Xu, C., Wong, Y. K., Li, Y., Liao, F., Jiang, T., & Tu, Y. (2019). Artemisinin, the Magic Drug Discovered from Traditional Chinese Medicine. *Engineering*, ***5*(1),** 32–39.

WHO. (2013). *WHO Traditional Medicine Strategy: 2014-2023*.

WHO. (2018, February 23). *WHO | Regional Office for Africa - WHO | Regional Office for Africa*. World Health Organization.

WHO. (2021, November 17). *Antimicrobial resistance*. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

Xu, L., Sun, X., & Ma, X. (2017). Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant Klebsiella pneumoniae. *Annals of Clinical Microbiology and Antimicrobials*, ***16*(1),** 1–12.

Yoon, S. H., Jeong, H., Kwon, S. K., & Kim, J. F. (2009). Genomics, biological features, and biotechnological applications of Escherichia coli B: Is B for better? *Systems Biology and Biotechnology of Escherichia Coli*, 1–17.

Zengin, G., Ferrante, C., Gnapi, D. E., Sinan, K. I., Orlando, G., Recinella, L., Diuzheva, A., Jekő, J., Cziáky, Z., & Chiavaroli, A. (2019). Comprehensive approaches on the chemical constituents and pharmacological properties of flowers and leaves of American basil (*Ocimum americanum* L). *Food Research International*, *125*, 108610.

Zhang, Q.-W., Lin, L.-G., & Ye, W.-C. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*, ***13*(1),** 20.

APPENDICES

Appendix 1: Ethical approval of the faculty of biosafety, animal use and ethics committee

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

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

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

REF: FVM BAUEC/2021/305

Ali Hashim Mohamed
Dept. of PHP & Toxicology
University of Nairobi
02/07/2021

Dear Dr. Ali,

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

Evaluation of Anti-microbial activity, cytotoxicity and phytochemical composition of *Ocimum americana.*

Ali Hashim Mohamed J56/34824/ 2019

We refer to your MSc. proposal submitted to our committee for review and your application letter dated 17th June 2021. We have reviewed your application for ethical clearance for the study. The antimicrobial, cytotoxicity protocols and phytochemical analysis procedure meets the minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

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

Yours sincerely,

Kaluwa

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

Appendix 2: Letter of plant identification

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

07/01/2021

REF: NMK/BOT/CTX/5/1/3

Hashim Ali

University of Nairobi

Tel. 0722 - 783666

Nairobi

Dear Sir,

PLANT IDENTIFICATION

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

03. Ocimum americanum L. **(Family: Lamiaceae)**

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

Yours Sincerely

Mathias M. Mbale

For: Head, Botany Department.

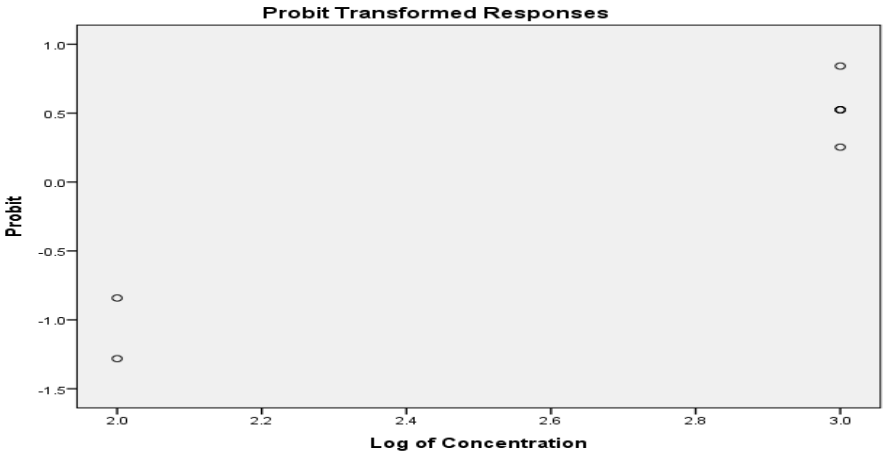
NATIONAL MUSEUMS OF KENYA
09 MAR 2021
HERBARIUM NAIROBI

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

Appendix 3: Probit analysis of acetone crude extract of *Ocimum americanum* L.

		Confidence Limits					
	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
Probit	.010	102.075	49.035	159.873	2.009	1.691	2.204
	.020	115.972	58.787	179.068	2.064	1.769	2.253
	.030	125.755	65.845	192.752	2.100	1.819	2.285
	.040	133.655	71.638	203.931	2.126	1.855	2.309
	.050	140.445	76.671	213.644	2.148	1.885	2.330
	.060	146.496	81.193	222.388	2.166	1.910	2.347
	.070	152.015	85.341	230.442	2.182	1.931	2.363
	.080	157.133	89.206	237.979	2.196	1.950	2.377
	.090	161.938	92.845	245.119	2.209	1.968	2.389
	.100	166.490	96.303	251.942	2.221	1.984	2.401
	.150	186.738	111.743	283.029	2.271	2.048	2.452
	.200	204.574	125.349	311.472	2.311	2.098	2.493
	.250	221.227	137.992	338.981	2.345	2.140	2.530
	.300	237.334	150.123	366.492	2.375	2.176	2.564
	.350	253.304	162.031	394.667	2.404	2.210	2.596
	.400	269.451	173.928	424.067	2.430	2.240	2.627
	.450	286.053	186.000	455.256	2.456	2.270	2.658
	.500	303.390	198.424	488.862	2.482	2.298	2.689
	.550	321.777	211.396	525.651	2.508	2.325	2.721
	.600	341.602	225.147	566.620	2.534	2.352	2.753
	.650	363.378	239.976	613.152	2.560	2.380	2.788
	.700	387.830	256.299	667.274	2.589	2.409	2.824
	.750	416.067	274.740	732.175	2.619	2.439	2.865
	.800	449.936	296.323	813.315	2.653	2.472	2.910
	.850	492.910	322.947	921.258	2.693	2.509	2.964
	.900	552.859	358.835	1080.761	2.743	2.555	3.034
	.910	568.399	367.926	1123.745	2.755	2.566	3.051
	.920	585.778	377.995	1172.589	2.768	2.577	3.069
	.930	605.500	389.307	1228.999	2.782	2.590	3.090
	.940	628.313	402.243	1295.528	2.798	2.604	3.112
	.950	655.382	417.400	1376.216	2.816	2.621	3.139
	.960	688.678	435.773	1478.016	2.838	2.639	3.170
	.970	731.939	459.234	1614.383	2.864	2.662	3.208
	.980	793.682	491.989	1816.806	2.900	2.692	3.259
	.990	901.741	547.515	2192.231	2.955	2.738	3.341

a. Logarithm base = 10.



Appendix 4: Probit analysis of chloroform fraction of acetonic extract

		Confidence Limits					
	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
Probit	.010	102.075	49.035	159.873	2.009	1.691	2.204
	.020	115.972	58.787	179.068	2.064	1.769	2.253
	.030	125.755	65.845	192.752	2.100	1.819	2.285
	.040	133.655	71.638	203.931	2.126	1.855	2.309
	.050	140.445	76.671	213.644	2.148	1.885	2.330
	.060	146.496	81.193	222.388	2.166	1.910	2.347
	.070	152.015	85.341	230.442	2.182	1.931	2.363
	.080	157.133	89.206	237.979	2.196	1.950	2.377
	.090	161.938	92.845	245.119	2.209	1.968	2.389
	.100	166.490	96.303	251.942	2.221	1.984	2.401
	.150	186.738	111.743	283.029	2.271	2.048	2.452
	.200	204.574	125.349	311.472	2.311	2.098	2.493
	.250	221.227	137.992	338.981	2.345	2.140	2.530
	.300	237.334	150.123	366.492	2.375	2.176	2.564
	.350	253.304	162.031	394.667	2.404	2.210	2.596
	.400	269.451	173.928	424.067	2.430	2.240	2.627
	.450	286.053	186.000	455.256	2.456	2.270	2.658
	.500	303.390	198.424	488.862	2.482	2.298	2.689
	.550	321.777	211.396	525.651	2.508	2.325	2.721
	.600	341.602	225.147	566.620	2.534	2.352	2.753
	.650	363.378	239.976	613.152	2.560	2.380	2.788
	.700	387.830	256.299	667.274	2.589	2.409	2.824
	.750	416.067	274.740	732.175	2.619	2.439	2.865
	.800	449.936	296.323	813.315	2.653	2.472	2.910
	.850	492.910	322.947	921.258	2.693	2.509	2.964
	.900	552.859	358.835	1080.761	2.743	2.555	3.034
	.910	568.399	367.926	1123.745	2.755	2.566	3.051
	.920	585.778	377.995	1172.589	2.768	2.577	3.069
	.930	605.500	389.307	1228.999	2.782	2.590	3.090
	.940	628.313	402.243	1295.528	2.798	2.604	3.112
	.950	655.382	417.400	1376.216	2.816	2.621	3.139
	.960	688.678	435.773	1478.016	2.838	2.639	3.170
	.970	731.939	459.234	1614.383	2.864	2.662	3.208
	.980	793.682	491.989	1816.806	2.900	2.692	3.259
	.990	901.741	547.515	2192.231	2.955	2.738	3.341

a. Logarithm base = 10.

Appendix 5: Probit analysis of ethyl acetate fraction of acetonic extract

	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	102.075	49.035	159.873	2.009	1.691	2.204
	.020	115.972	58.787	179.068	2.064	1.769	2.253
	.030	125.755	65.845	192.752	2.100	1.819	2.285
	.040	133.655	71.638	203.931	2.126	1.855	2.309
	.050	140.445	76.671	213.644	2.148	1.885	2.330
	.060	146.496	81.193	222.388	2.166	1.910	2.347
	.070	152.015	85.341	230.442	2.182	1.931	2.363
	.080	157.133	89.206	237.979	2.196	1.950	2.377
	.090	161.938	92.845	245.119	2.209	1.968	2.389
	.100	166.490	96.303	251.942	2.221	1.984	2.401
	.150	186.738	111.743	283.029	2.271	2.048	2.452
	.200	204.574	125.349	311.472	2.311	2.098	2.493
	.250	221.227	137.992	338.981	2.345	2.140	2.530
	.300	237.334	150.123	366.492	2.375	2.176	2.564
	.350	253.304	162.031	394.667	2.404	2.210	2.596
	.400	269.451	173.928	424.067	2.430	2.240	2.627
	.450	286.053	186.000	455.256	2.456	2.270	2.658
Probit	.500	303.390	198.424	488.862	2.482	2.298	2.689
	.550	321.777	211.396	525.651	2.508	2.325	2.721
	.600	341.602	225.147	566.620	2.534	2.352	2.753
	.650	363.378	239.976	613.152	2.560	2.380	2.788
	.700	387.830	256.299	667.274	2.589	2.409	2.824
	.750	416.067	274.740	732.175	2.619	2.439	2.865
	.800	449.936	296.323	813.315	2.653	2.472	2.910
	.850	492.910	322.947	921.258	2.693	2.509	2.964
	.900	552.859	358.835	1080.761	2.743	2.555	3.034
	.910	568.399	367.926	1123.745	2.755	2.566	3.051
	.920	585.778	377.995	1172.589	2.768	2.577	3.069
	.930	605.500	389.307	1228.999	2.782	2.590	3.090
	.940	628.313	402.243	1295.528	2.798	2.604	3.112
	.950	655.382	417.400	1376.216	2.816	2.621	3.139
	.960	688.678	435.773	1478.016	2.838	2.639	3.170
	.970	731.939	459.234	1614.383	2.864	2.662	3.208
	.980	793.682	491.989	1816.806	2.900	2.692	3.259
	.990	901.741	547.515	2192.231	2.955	2.738	3.341

Confidence Limits

a. Logarithm base = 10.

Appendix 6: Probit analysis of aqueous crude extract of *Ocimum americanum. L*

		Confidence Limits					
	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	42.836	13.083	82.376	1.632	1.117	1.916
	.020	57.889	20.103	104.548	1.763	1.303	2.019
	.030	70.078	26.368	121.767	1.846	1.421	2.086
	.040	80.911	32.313	136.675	1.908	1.509	2.136
	.050	90.946	38.100	150.229	1.959	1.581	2.177
	.060	100.462	43.813	162.901	2.002	1.642	2.212
	.070	109.622	49.502	174.965	2.040	1.695	2.243
	.080	118.530	55.198	186.595	2.074	1.742	2.271
	.090	127.258	60.924	197.913	2.105	1.785	2.296
	.100	135.860	66.697	209.006	2.133	1.824	2.320
	.150	178.113	96.624	263.029	2.251	1.985	2.420
	.200	220.883	128.923	317.730	2.344	2.110	2.502
	.250	265.675	164.117	375.906	2.424	2.215	2.575
	.300	313.590	202.588	439.873	2.496	2.307	2.643
	.350	365.671	244.684	512.071	2.563	2.389	2.709
	.400	423.068	290.781	595.391	2.626	2.464	2.775
	.450	487.160	341.356	693.474	2.688	2.533	2.841
Probit	.500	559.709	397.085	811.091	2.748	2.599	2.909
	.550	643.062	458.970	954.739	2.808	2.662	2.980
	.600	740.483	528.517	1133.650	2.870	2.723	3.054
	.650	856.711	608.023	1361.603	2.933	2.784	3.134
	.700	998.995	701.052	1660.406	3.000	2.846	3.220
	.750	1179.164	813.399	2067.137	3.072	2.910	3.315
	.800	1418.279	955.202	2651.070	3.152	2.980	3.423
	.850	1758.855	1146.385	3560.283	3.245	3.059	3.551
	.900	2305.869	1434.430	5187.573	3.363	3.157	3.715
	.910	2461.726	1513.181	5685.266	3.391	3.180	3.755
	.920	2643.005	1603.234	6281.790	3.422	3.205	3.798
	.930	2857.777	1707.977	7012.136	3.456	3.232	3.846
	.940	3118.337	1832.501	7931.095	3.494	3.263	3.899
	.950	3444.628	1984.930	9130.313	3.537	3.298	3.960
	.960	3871.858	2179.354	10777.517	3.588	3.338	4.033
	.970	4470.369	2443.313	13222.511	3.650	3.388	4.121
	.980	5411.593	2842.082	17365.888	3.733	3.454	4.240
	.990	7313.321	3601.494	26725.240	3.864	3.556	4.427

a. Logarithm base = 10.

Appendix 7: Probit analysis of chloroform fraction of aqueous crude extract

		Confidence Limits					
	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
Probit	.010	102.075	49.035	159.873	2.009	1.691	2.204
	.020	115.972	58.787	179.068	2.064	1.769	2.253
	.030	125.755	65.845	192.752	2.100	1.819	2.285
	.040	133.655	71.638	203.931	2.126	1.855	2.309
	.050	140.445	76.671	213.644	2.148	1.885	2.330
	.060	146.496	81.193	222.388	2.166	1.910	2.347
	.070	152.015	85.341	230.442	2.182	1.931	2.363
	.080	157.133	89.206	237.979	2.196	1.950	2.377
	.090	161.938	92.845	245.119	2.209	1.968	2.389
	.100	166.490	96.303	251.942	2.221	1.984	2.401
	.150	186.738	111.743	283.029	2.271	2.048	2.452
	.200	204.574	125.349	311.472	2.311	2.098	2.493
	.250	221.227	137.992	338.981	2.345	2.140	2.530
	.300	237.334	150.123	366.492	2.375	2.176	2.564
	.350	253.304	162.031	394.667	2.404	2.210	2.596
	.400	269.451	173.928	424.067	2.430	2.240	2.627
	.450	286.053	186.000	455.256	2.456	2.270	2.658
	.500	303.390	198.424	488.862	2.482	2.298	2.689
	.550	321.777	211.396	525.651	2.508	2.325	2.721
	.600	341.602	225.147	566.620	2.534	2.352	2.753
	.650	363.378	239.976	613.152	2.560	2.380	2.788
	.700	387.830	256.299	667.274	2.589	2.409	2.824
	.750	416.067	274.740	732.175	2.619	2.439	2.865
	.800	449.936	296.323	813.315	2.653	2.472	2.910
	.850	492.910	322.947	921.258	2.693	2.509	2.964
	.900	552.859	358.835	1080.761	2.743	2.555	3.034
	.910	568.399	367.926	1123.745	2.755	2.566	3.051
	.920	585.778	377.995	1172.589	2.768	2.577	3.069
	.930	605.500	389.307	1228.999	2.782	2.590	3.090
	.940	628.313	402.243	1295.528	2.798	2.604	3.112
	.950	655.382	417.400	1376.216	2.816	2.621	3.139
	.960	688.678	435.773	1478.016	2.838	2.639	3.170
	.970	731.939	459.234	1614.383	2.864	2.662	3.208
	.980	793.682	491.989	1816.806	2.900	2.692	3.259
	.990	901.741	547.515	2192.231	2.955	2.738	3.341

a. Logarithm base = 10.

Appendix 8: Probit analysis ethyl acetate fraction of aqueous crude extract

	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	102.075	49.035	159.873	2.009	1.691	2.204
	.020	115.972	58.787	179.068	2.064	1.769	2.253
	.030	125.755	65.845	192.752	2.100	1.819	2.285
	.040	133.655	71.638	203.931	2.126	1.855	2.309
	.050	140.445	76.671	213.644	2.148	1.885	2.330
	.060	146.496	81.193	222.388	2.166	1.910	2.347
	.070	152.015	85.341	230.442	2.182	1.931	2.363
	.080	157.133	89.206	237.979	2.196	1.950	2.377
	.090	161.938	92.845	245.119	2.209	1.968	2.389
	.100	166.490	96.303	251.942	2.221	1.984	2.401
	.150	186.738	111.743	283.029	2.271	2.048	2.452
	.200	204.574	125.349	311.472	2.311	2.098	2.493
	.250	221.227	137.992	338.981	2.345	2.140	2.530
	.300	237.334	150.123	366.492	2.375	2.176	2.564
	.350	253.304	162.031	394.667	2.404	2.210	2.596
	.400	269.451	173.928	424.067	2.430	2.240	2.627
	.450	286.053	186.000	455.256	2.456	2.270	2.658
Probit	**.500**	**303.390**	**198.424**	**488.862**	2.482	2.298	2.689
	.550	321.777	211.396	525.651	2.508	2.325	2.721
	.600	341.602	225.147	566.620	2.534	2.352	2.753
	.650	363.378	239.976	613.152	2.560	2.380	2.788
	.700	387.830	256.299	667.274	2.589	2.409	2.824
	.750	416.067	274.740	732.175	2.619	2.439	2.865
	.800	449.936	296.323	813.315	2.653	2.472	2.910
	.850	492.910	322.947	921.258	2.693	2.509	2.964
	.900	552.859	358.835	1080.761	2.743	2.555	3.034
	.910	568.399	367.926	1123.745	2.755	2.566	3.051
	.920	585.778	377.995	1172.589	2.768	2.577	3.069
	.930	605.500	389.307	1228.999	2.782	2.590	3.090
	.940	628.313	402.243	1295.528	2.798	2.604	3.112
	.950	655.382	417.400	1376.216	2.816	2.621	3.139
	.960	688.678	435.773	1478.016	2.838	2.639	3.170
	.970	731.939	459.234	1614.383	2.864	2.662	3.208
	.980	793.682	491.989	1816.806	2.900	2.692	3.259
	.990	901.741	547.515	2192.231	2.955	2.738	3.341

a. Logarithm base = 10.

Appendix 9: Probit analysis of hydroethanolic crude extract of *Ocimum americanum L.*

	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	111.424	43.859	185.322	2.047	1.642	2.268
	.020	127.323	53.335	206.381	2.105	1.727	2.315
	.030	138.567	60.343	221.114	2.142	1.781	2.345
	.040	147.675	66.189	232.976	2.169	1.821	2.367
	.050	155.524	71.341	243.159	2.192	1.853	2.386
	.060	162.532	76.025	252.228	2.211	1.881	2.402
	.070	168.936	80.370	260.502	2.228	1.905	2.416
	.080	174.884	84.460	268.178	2.243	1.927	2.428
	.090	180.475	88.348	275.389	2.256	1.946	2.440
	.100	185.780	92.076	282.229	2.269	1.964	2.451
	.150	209.453	109.126	312.782	2.321	2.038	2.495
	.200	230.401	124.704	339.944	2.362	2.096	2.531
	.250	250.034	139.648	365.593	2.398	2.145	2.563
	.300	269.088	154.409	390.725	2.430	2.189	2.592
	.350	288.037	169.292	416.007	2.459	2.229	2.619
	.400	307.251	184.541	441.981	2.487	2.266	2.645
	.450	327.059	200.383	469.162	2.515	2.302	2.671
Probit	**.500**	**347.800**	**217.057**	**498.104**	2.541	2.337	2.697
	.550	369.856	234.837	529.466	2.568	2.371	2.724
	.600	393.700	254.062	564.096	2.595	2.405	2.751
	.650	419.962	275.183	603.157	2.623	2.440	2.780
	.700	449.536	298.836	648.352	2.653	2.475	2.812
	.750	483.792	325.984	702.359	2.685	2.513	2.847
	.800	525.018	358.211	769.770	2.720	2.554	2.886
	.850	577.527	398.453	859.491	2.762	2.600	2.934
	.900	651.118	453.233	992.473	2.814	2.656	2.997
	.910	670.256	467.169	1028.417	2.826	2.669	3.012
	.920	691.684	482.624	1069.317	2.840	2.684	3.029
	.930	716.037	500.003	1116.630	2.855	2.699	3.048
	.940	744.251	519.895	1172.532	2.872	2.716	3.069
	.950	777.789	543.215	1240.475	2.891	2.735	3.094
	.960	819.127	571.496	1326.406	2.913	2.757	3.123
	.970	872.969	607.612	1441.854	2.941	2.784	3.159
	.980	950.063	658.025	1613.853	2.978	2.818	3.208
	.990	1085.624	743.437	1934.465	3.036	2.871	3.287

Confidence Limits

a. Logarithm base = 10.

Appendix 10: Chloroform fraction of Hydroethanolic extract of *ocimum americanum L.*

		Confidence Limits					
	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	.023	.	.	-1.636	.	.
	.020	.034	.	.	-1.471	.	.
	.030	.043	.	.	-1.367	.	.
	.040	.052	.	.	-1.288	.	.
	.050	.060	.	.	-1.224	.	.
	.060	.068	.	.	-1.169	.	.
	.070	.076	.	.	-1.122	.	.
	.080	.083	.	.	-1.079	.	.
	.090	.091	.	.	-1.040	.	.
	.100	.099	.	.	-1.004	.	.
	.150	.139	.	.	-.856	.	.
	.200	.183	.	.	-.738	.	.
	.250	.231	.	.	-.637	.	.
	.300	.284	.	.	-.546	.	.
	.350	.345	.	.	-.462	.	.
	.400	.415	.	.	-.382	.	.
	.450	.496	.	.	-.305	.	.
Probit	**.500**	**.591**	**.**	**.**	-.229	.	.
	.550	.704	.	.	-.153	.	.
	.600	.841	.	.	-.075	.	.
	.650	1.010	.	.	.005	.	.
	.700	1.227	.	.	.089	.	.
	.750	1.512	.	.	.180	.	.
	.800	1.908	.	.	.281	.	.
	.850	2.503	.	.	.399	.	.
	.900	3.523	.	.	.547	.	.
	.910	3.826	.	.	.583	.	.
	.920	4.184	.	.	.622	.	.
	.930	4.618	.	.	.664	.	.
	.940	5.155	.	.	.712	.	.
	.950	5.844	.	.	.767	.	.
	.960	6.773	.	.	.831	.	.
	.970	8.119	.	.	.909	.	.
	.980	10.331	.	.	1.014	.	.
	.990	15.105	.	.	1.179	.	.

a. Logarithm base = 10.

Appendix 11: Probit analysis of ethyl acetate fraction of hydroethanolic extract

	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	15.623	.076	34.644	1.194	-1.120	1.540
	.020	17.669	.123	37.255	1.247	-.909	1.571
	.030	19.103	.168	39.018	1.281	-.776	1.591
	.040	20.259	.211	40.404	1.307	-.675	1.606
	.050	21.250	.255	41.571	1.327	-.594	1.619
	.060	22.132	.299	42.593	1.345	-.524	1.629
	.070	22.935	.344	43.512	1.361	-.463	1.639
	.080	23.679	.390	44.353	1.374	-.409	1.647
	.090	24.377	.437	45.134	1.387	-.359	1.655
	.100	25.037	.486	45.867	1.399	-.314	1.661
	.150	27.966	.750	49.045	1.447	-.125	1.691
	.200	30.536	1.058	51.754	1.485	.024	1.714
	.250	32.929	1.421	54.221	1.518	.153	1.734
	.300	35.237	1.852	56.560	1.547	.268	1.753
	.350	37.520	2.365	58.843	1.574	.374	1.770
	.400	39.823	2.982	61.124	1.600	.475	1.786
	.450	42.185	3.730	63.447	1.625	.572	1.802
Probit	**.500**	**44.647**	**4.646**	**65.860**	1.650	.667	1.819
	.550	47.252	5.783	68.415	1.674	.762	1.835
	.600	50.056	7.216	71.179	1.699	.858	1.852
	.650	53.128	9.061	74.246	1.725	.957	1.871
	.700	56.570	11.497	77.759	1.753	1.061	1.891
	.750	60.535	14.824	81.962	1.782	1.171	1.914
	.800	65.278	19.583	87.320	1.815	1.292	1.941
	.850	71.278	26.832	94.910	1.853	1.429	1.977
	.900	79.617	38.898	108.064	1.901	1.590	2.034
	.910	81.773	42.279	112.214	1.913	1.626	2.050
	.920	84.182	46.115	117.338	1.925	1.664	2.069
	.930	86.912	50.478	123.871	1.939	1.703	2.093
	.940	90.066	55.446	132.534	1.955	1.744	2.122
	.950	93.804	61.102	144.588	1.972	1.786	2.160
	.960	98.394	67.539	162.405	1.993	1.830	2.211
	.970	104.345	74.926	191.007	2.018	1.875	2.281
	.980	112.817	83.731	243.425	2.052	1.923	2.386
	.990	127.588	95.788	371.518	2.106	1.981	2.570

Confidence Limits

a. Logarithm base = 10.

Appendix 12: Probit analysis of positive control drug - Vincristine

Confidence Limits

	Probability	95% Confidence Limits for Concentration			95% Confidence Limits for log(Concentration)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	3.294	.	.	.518	.	.
	.020	3.826	.	.	.583	.	.
	.030	4.208	.	.	.624	.	.
	.040	4.520	.	.	.655	.	.
	.050	4.790	.	.	.680	.	.
	.060	5.033	.	.	.702	.	.
	.070	5.257	.	.	.721	.	.
	.080	5.465	.	.	.738	.	.
	.090	5.662	.	.	.753	.	.
	.100	5.849	.	.	.767	.	.
	.150	6.692	.	.	.826	.	.
	.200	7.448	.	.	.872	.	.
	.250	8.165	.	.	.912	.	.
	.300	8.867	.	.	.948	.	.
	.350	9.571	.	.	.981	.	.
	.400	10.291	.	.	1.012	.	.
	.450	11.039	.	.	1.043	.	.
Probit	**.500**	**11.828**	**.**	**.**	1.073	.	.
	.550	12.673	.	.	1.103	.	.
	.600	13.595	.	.	1.133	.	.
	.650	14.617	.	.	1.165	.	.
	.700	15.778	.	.	1.198	.	.
	.750	17.134	.	.	1.234	.	.
	.800	18.782	.	.	1.274	.	.
	.850	20.905	.	.	1.320	.	.
	.900	23.919	.	.	1.379	.	.
	.910	24.710	.	.	1.393	.	.
	.920	25.599	.	.	1.408	.	.
	.930	26.613	.	.	1.425	.	.
	.940	27.793	.	.	1.444	.	.
	.950	29.204	.	.	1.465	.	.
	.960	30.952	.	.	1.491	.	.
	.970	33.246	.	.	1.522	.	.
	.980	36.561	.	.	1.563	.	.
	.990	42.469	.	.	1.628	.	.

a. Logarithm base = 10.

Appendix 13: Analysis of variance comparing growth inhibition of acetone extract

ANOVA (Comparison of growth inhibitions among various concentrations of acetone extract under each microbe to detect significant differences in terms of growth inhibitions)						
		Sum of Squares	df	Mean Square	F	Sig.
Inhibition of *E.coli* by acetone crude extracts	Between Groups	.000	6	.000	NA	.
	Within Groups	.000	14	.000		
	Total	.000	20			
Inhibition of *S. aureus* by acetone crude extracts	Between Groups	66.500	6	11.083	14.778	**.000**
	Within Groups	10.500	14	.750		
	Total	77.000	20			
Inhibition of *K. pneumoniae* by acetone crude extracts	Between Groups	.000	6	.000	NA	.
	Within Groups	.000	14	.000		
	Total	.000	20			
Inhibition of *B. cereus* by acetone crude extracts	Between Groups	55.119	6	9.187	17.538	**.000**
	Within Groups	7.333	14	.524		
	Total	62.452	20			
Inhibition of *C. albicans* by acetone crude extracts	Between Groups	728.786	6	121.464	364.393	**.000**
	Within Groups	4.667	14	.333		
	Total	733.452	20			
*. The mean difference is significant at the 0.05 level.						

Analysis of variance comparing growth inhibition of chloroform fraction of acetonic extract

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Inhibition of *S. aureus* by chloroform crude extracts A	Between Groups	278.286	6	46.381	139.143	.000
	Within Groups	4.667	14	.333		
	Total	282.952	20			
Inhibition of *B. cereus* by chloroform crude extracts A	Between Groups	73.952	6	12.325	26.547	.000
	Within Groups	6.500	14	.464		
	Total	80.452	20			
Inhibition of *C. albicans* by chloroform crude extracts A	Between Groups	796.452	6	132.742	371.678	.000
	Within Groups	5.000	14	.357		
	Total	801.452	20			

Appendix 14: Analysis of variance comparing growth inhibition of hydroethanolic crude extract and fractions

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Inhibition of S .aureus by hydroethanolic crude extracts	Between Groups	505.333	6	84.222	7.032	**.001**
	Within Groups	167.667	14	11.976		
	Total	673.000	20			
Inhibition of B. cereus by hydroethanolic crude extracts	Between Groups	110.905	6	18.484	40.860	**.000**
	Within Groups	6.333	14	.452		
	Total	117.238	20			

Analysis of variance comparing growth inhibition of chloroform fraction of hydroethanolic crude extract

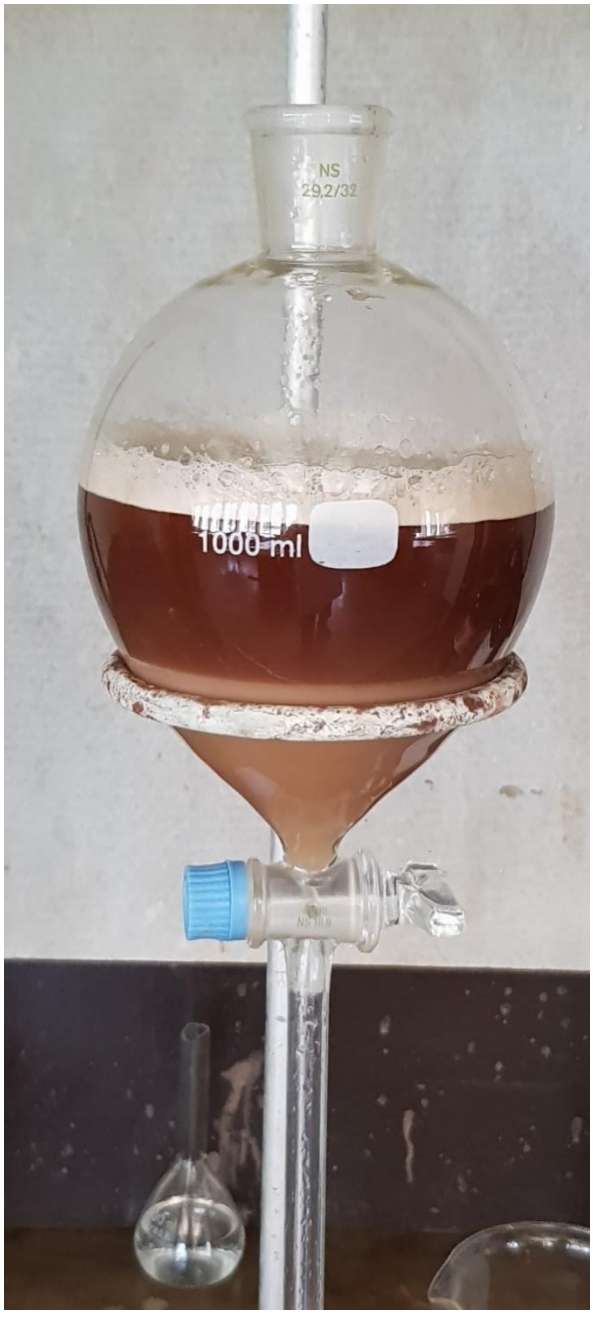
ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Inhibition of S.aureus by chloroform crude extracts B	Between Groups	686.143	6	114.357	28.006	.000
	Within Groups	57.167	14	4.083		
	Total	743.310	20			
Inhibition of B.cereus by chloroform crude extracts B	Between Groups	800.000	6	133.333	12.108	.000
	Within Groups	154.167	14	11.012		
	Total	954.167	20			

Analysis of variance comparing growth inhibition of ethyl acetate of hydroethanolic crude extract

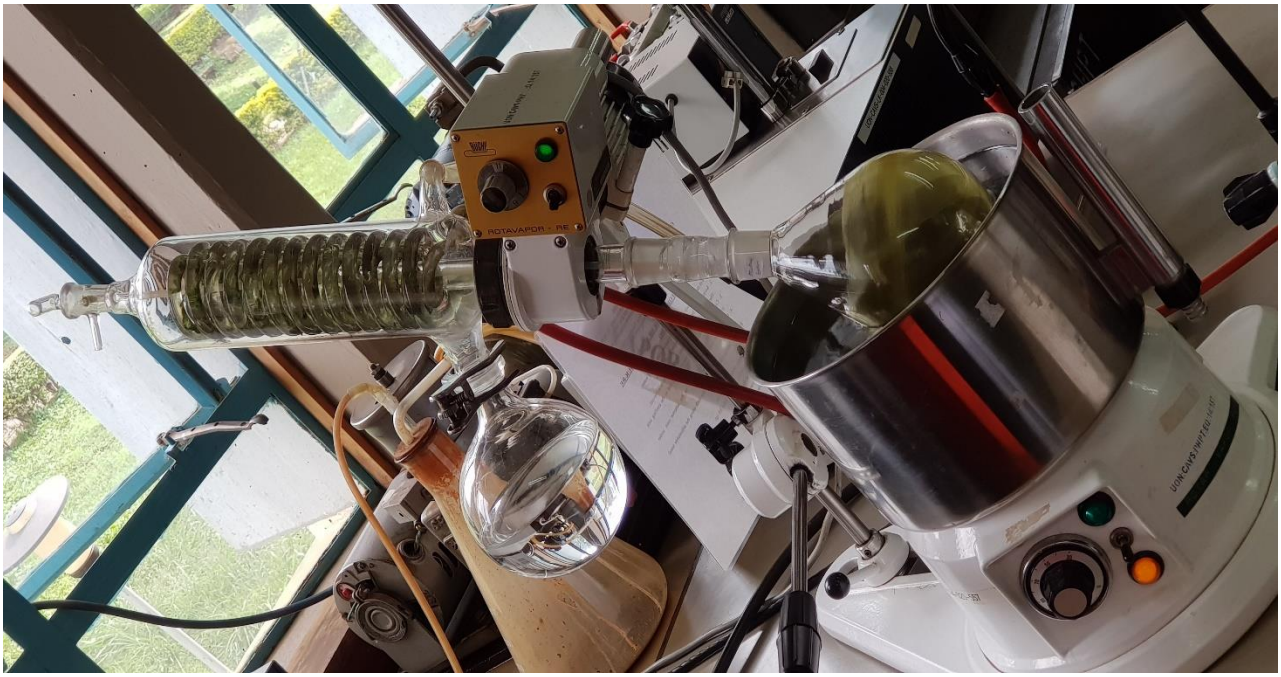
ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Inhibition of S.aureus by Ethlyacetate crude extracts B	Between Groups	872.405	6	145.401	33.462	.000
	Within Groups	60.833	14	4.345		
	Total	933.238	20			
Inhibition of B.cereus by Ethlyacetate crude extracts B	Between Groups	533.238	6	88.873	191.419	.000
	Within Groups	6.500	14	.464		
	Total	539.738	20			

Appendix 15: Photos for plant specimen collection Pate Island Lamu County

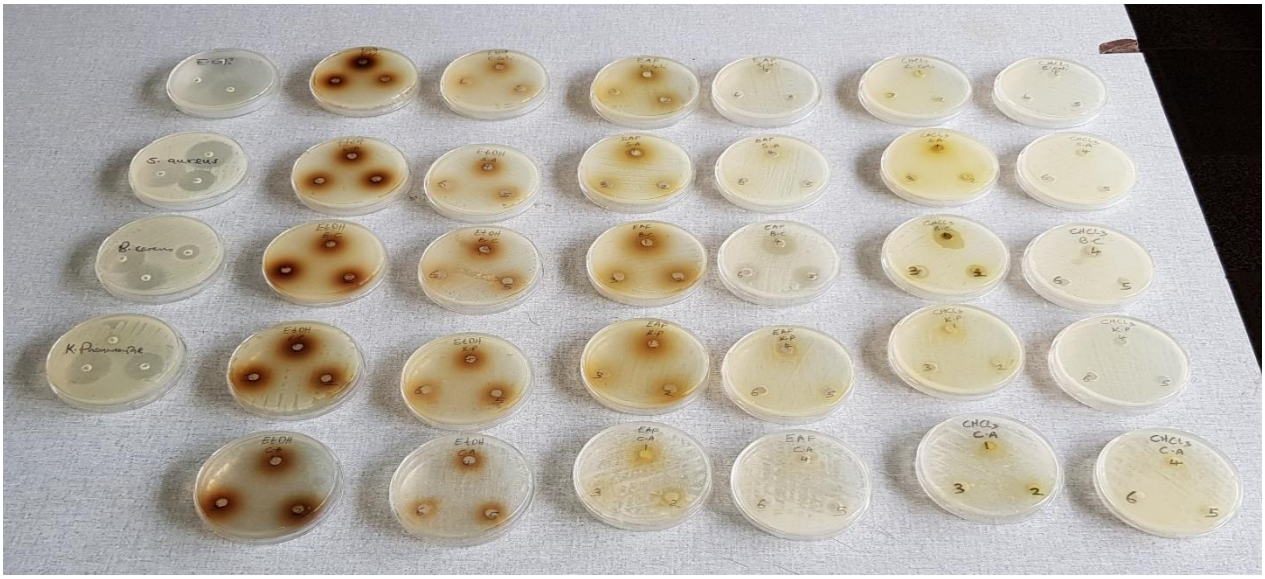
Appendix 16: Photos for crude extract extraction process


NS
29,2/32
1000 ml

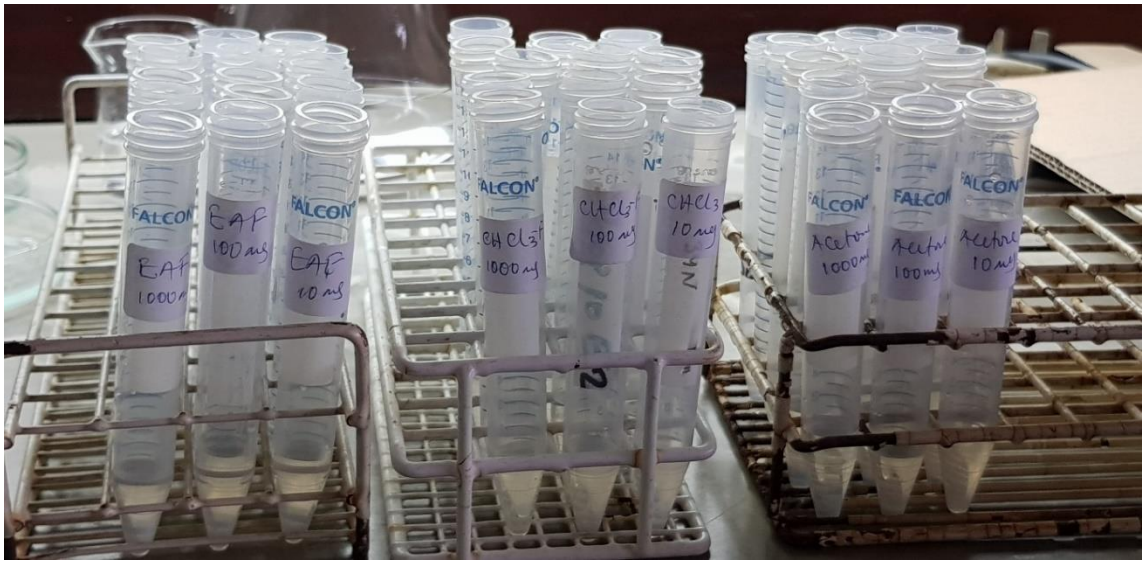

PYREX
600ml
500
400
300
200
100



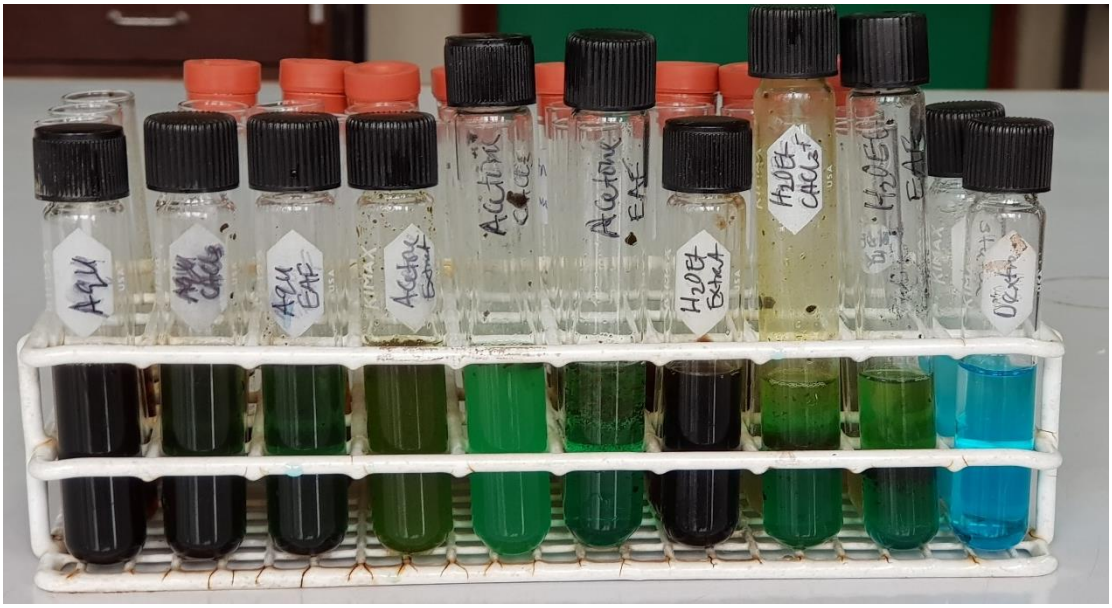
Appendix 17: Photos for Agar well diffusion

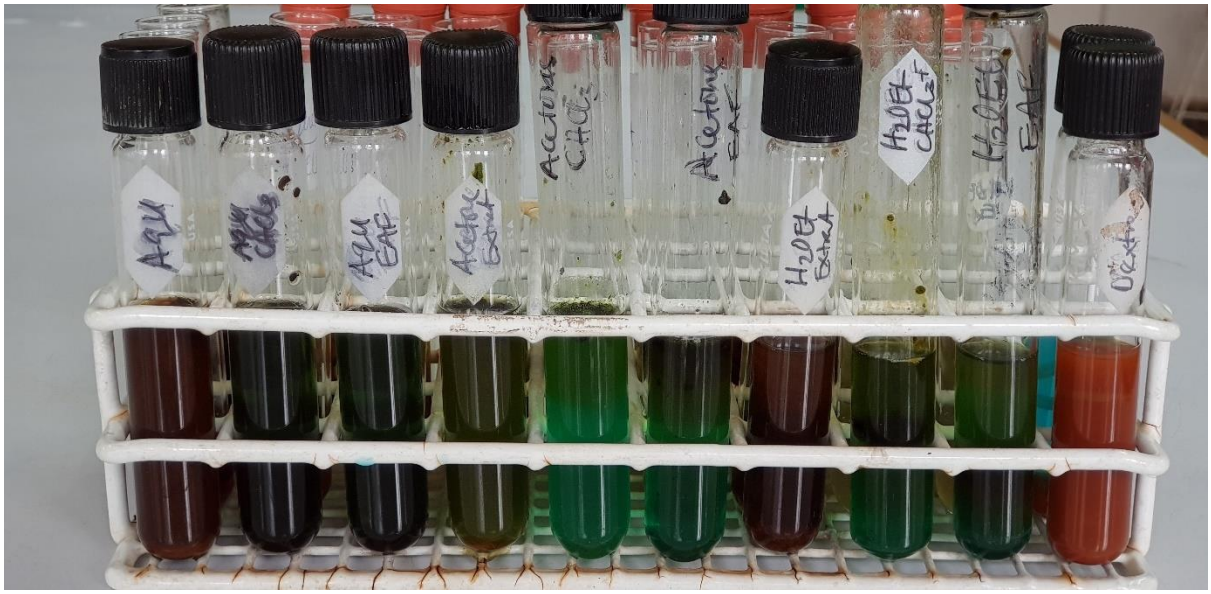


Appendix 18: Photo for Cytotoxicity



Appendix 19: Photo for phytochemicals





Appendix 20: Published papers related with the research

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 6484578, 11 pages
https://doi.org/10.1155/2022/6484578

Research Article

Evaluation of Antimicrobial Activity, Cytotoxicity, and Phytochemical Composition of *Ocimum americanum* L. (Lamiaceae)

Hashim Ali,[1] **Joseph Nguta**,[1] **Fredrick Musila**,[2] **Isaac Ole-Mapenay**,[1] **Dorine Matara**,[1] **and James Mailu**[1]

[1]*Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, Nairobi, Kenya*
[2]*Department of Applied and Technical Biology, School of Biological and Life Sciences, Technical University of Kenya, Nairobi, Kenya*

Correspondence should be addressed to Hashim Ali; hashimali@students.uonbi.ac.ke

Received 4 November 2021; Accepted 13 February 2022; Published 17 March 2022

Academic Editor: Olufunmiso Olusola Olajuyigbe

Background. Herbal plants are a natural source of novel biomolecules used widely in ethnomedicine. The present study was intended to examine the antimicrobial properties, cytotoxicity, and phytoconstituents of *Ocimum americanum* L., an herb traditionally used by the people of Swahili (Kenya) against microbial infections. *Methods.* The aerial parts of *Ocimum americanum* L. were sourced, dried, milled, and extracted using three solvents: aqueous, acetonic, and 70% hydroethanolic. Additionally, fractions of chloroform and ethyl acetate were obtained from all crude extracts of the plant. The antimicrobial property was evaluated using agar well diffusion and microdilution techniques against human opportunistic pathogens including *S. aureus*, *E. coli*, and *C. albicans*. The brine shrimp cytotoxicity test was used to analyze the lethality of the extracts and fractions. Phytochemical screening was used to qualitatively assay the presence of phytoconstituents. *Results.* The phytochemical assay confirmed the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, reducing sugars, anthraquinones, and glycosides. The lethality test demonstrated that all the extracts and fractions were toxic against *Artemia salina* nauplii with LC_{50} values ranging from 0.59 to 559.71 μg/ml. Chloroformic fraction of the hydroethanolic extract had the highest lethality with an LC_{50} value of 0.59 μg/ml. Two of the extracts and their fractions displayed antimicrobial activity against the Gram-positive bacteria (*B. cereus* and *S. aureus*) and fungus (*C. albicans*), while the same extracts had no activity against the Gram-negative bacteria (*E. coli* and *K. pneumoniae*). The highest antimicrobial activity was seen in the ethyl acetate fraction of the hydroethanolic extract at 250 mg/ml against *Bacillus cereus* which had an inhibition zone of 26.00 ± 0.00 and MIC value of 62.5 mg/ml. *Conclusion.* In the current study, we report that *Ocimum americanum* L. demonstrated moderate antimicrobial activity, contains numerous phytocompounds, and is highly cytotoxic; thus, further research is needed for bioprospecting a novel compound.

The Journal of Phytopharmacology 2021; 10(1): 56-60
Online at: www.phytopharmajournal.com

Review Article

ISSN 2320-480X
JPHYTO 2021; 10(1): 56-60
January- February
Received: 31-12-2020
Accepted: 01-02-2021

doi: 10.31254/phyto.2021.10111

Hashim Mohamed Ali
Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Nairobi, Kenya

Joseph Mwanzia Nguta
Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Nairobi, Kenya

Isaac Ole Mapenay
Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Nairobi, Kenya

Fredrick Mutie Musila
Department of Applied and Technical Biology, Technical University of Kenya, Nairobi, Kenya

Vincent Mokoro Omambia
Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Nairobi, Kenya

Dorine Nyak Matara
Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Nairobi, Kenya

Correspondence:
Dr. Hashim Mohamed Ali
Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Nairobi, Kenya
Email: hashimali@students.uonbi.ac.ke

Ethnopharmacological uses, biological activities, chemistry and toxicological aspects of *Ocimum americanum* var. *americanum* (Lamiaceae)

Hashim Mohamed Ali*, Joseph Mwanzia Nguta, Isaac Ole Mapenay, Fredrick Mutie Musila, Vincent Mokoro Omambia, Dorine Nyak Matara

ABSTRACT

The rationale for the use of *Ocimum americanum* var. *americanum* in herbal medicine is largely based on the longstanding experience of traditional medicine practitioners. The genus *Ocimum* is extensively used in Eastern Africa in folkloric practice against a wide range of illnesses. The present paper intends to bring a comprehensive overview of O. *americanum* var. *americanum* in regard to its biological activities, ethnopharmacological uses, phytochemical and toxicological effects. The literature search was conducted using Google, Google Scholar, Chemical abstracts, Sciverse; JSTOR, Medline, PubMed, Science Direct, Scopus and Springer Link. *O. americanum* var. *americanum* extracts have been shown to have antimicrobial, antioxidant, antiproliferative, insecticidal, and repellent activities. Literature on the activity of *O. americanum* var. *americanum* extracts against metabolic syndromes such as diabetes, hyperlipidaemias and hypertension is scanty. Toxicological data is also limited; however, the accessible information indicates non-toxicity of O. *americanum* var. *americanum* extracts. Substantial variations in phytochemical constituents of this particular species are observed, which may be attributed to edaphic differences as well as ecoclimatic regions.

Keywords: *Ocimum americanum* var. *americanum*, Ethnopharmacology, Phytochemistry, Toxicology, Traditional uses.

INTRODUCTION

Whilst it is often acknowledged that folkloric medicine works, there still exists gaps in the scientific study of the natural products from such traditions. Such is the challenge that faces ethnopharmacology even though it is increasingly being acknowledged that many types of diseases, including such common ones as vector-borne diseases; diarrhea or tuberculosis are still commonly treated and/or managed with herbal medicines [1]. African Traditional Medicine (ATM) have passed down through generations via oral tradition with very little documentation or none at all [2]. Traditional practice and phytomedicines of proven efficacy and safety, contribute to the primary healthcare goal and guarantees access to all people. The World Health Organization (WHO) estimates for millions of people, traditional herbal medicine is the main source of healthcare, and most often the only source of healthcare service. This is due to easy of accessibility, acceptability and affordability by millions of people. The cost factor of most phytomedicines makes them all the more agreeable at a time of spiralling healthcare expenses and nearly widespread austerity [3].

Africa is naturally endowed with abundance of flora, estimated to thousands of species. Botanists approximate that about 10% of Africa's flora is of medicinal significance and some of the herbal plants have been scientifically evaluated and folkloric role ascertained [1]. The herbal plant *Ocimum americanum* var. *americanum* (Lamiaceae) is indigenous to tropical Africa and India. The genus *Ocimum* contains about 50 to 150 species of shrubs and aromatic herbs [4]. *Ocimum americanum* var. *americanum* is a small branched erect, aromatic annual perennial shrub which grows up to 1m high. Stems are quadrangular or somewhat rounded, woody near the base, hairy and appressed. Leaves are narrowly elliptic, up to 2.5cm long, mostly hairless [5, 6]. The medicinal plant has a very wide geographical distribution in East Africa, making it the most popular ethnobotanical plant in the region [7]. In Kenya, it is widely distributed in the forest margins, secondary bushland and grassland, riverine sites and in dry areas, mainly in the hills [8].

Ocimum americanum var. *americanum* (Syn. *O. canum* Sims) is a high variable, polymorphic species with numerous forms, many of which have previously been treated as different species and subspecies. Three chemo-types are common; camphoraceous, floral-lemony and spicy. The tropical shrub from the mint family Labiate (Lamiaceae) is commonly known as American basil, hoary basil or mosquito plant [9,10]. American basil is an extensively scattered species in the tropical and subtropical regions of the globe, and