ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF THREE SELECTED MEDICINAL PLANTS USED TO TREAT BACTERIAL AND FUNGAL INFECTIONS IN KENYA

BY

CATHERINE K. KITONDE

SECOND CLASS HONOURS (LOWER DIVISION), B.Sc MICROBIOLOGY AND BIOTECHNOLOGY, UNIVERSITY OF NAIROBI

A THESIS SUBMITTED TO THE SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF NAIROBI, IN THE FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS IN SCIENCE IN BOTANY (MICROBIOLOGY).

SCHOOL OF BIOLOGICAL SCIENCES

UNIVERSITY OF NAIROBI.

JUNE, 2012

DECLARATION

This Research Thesis is my original work and has not been presented for the award of any degree at this or any other University or Institute.

Catherine K. Kitonde

Signature ----------

Date 4/6/2012

This Thesis has been submitted with our approval as the University supervisors

Dr. Saifuddin F. Dossaji

School of Biological Sciences

University of Nairobi

Signature ----------

Date 4/06/2012

Dr. Catherine W. Lukhoba

School of Biological Sciences

University of Nairobi

Signature ----------

Date 4/6/2012

Dr. Miriam Jumba

School of Biological Sciences

University of Nairobi

Signature ----------

Date 4/6/2012

DEDICATION

Dedicated to my family members who encouraged and gave words of advice while studying. My nephew Alexander Kyalo, who was always there even when things got tough. My second family, Kajiado Children's Home staff and children, the directors: Mike D. Keen, Mike Chester, Larry and Linda Rupp, Linda Bannister, Pede Self, and Joel Baugher, who valued the search for Knowledge above most things and gave moral support and encouragement to my research work.

ACKNOWLEDGEMENT

This thesis marks the culmination of a process that has been made possible through the contribution of various institutions and individuals whom I am grateful to. First I thank the University of Nairobi for the scholarship award and financial support accorded to this research work and enabling learning environment. Second I thank V*icRES* under the Inter-University Council of East Africa (IUCEA) for partial financial support for this project.

I extend my deep gratitude to my first supervisor, Dr. Saifuddin Dossaji for moral support, encouragement and advice especially on both phytochemistry and microbiology work. It was always a relief sharing taxing issues for you always had a way out and in very encouraging attitude. My appreciation goes to my second supervisor, Dr. Catherine Lukhoba for accompanying me to the field for collection of plant materials, advice on ethnomedicine, and bioassays. You have been an inspiration and provided creative insights during my studies. You are really honoured for your motherly words of encouragement, inspiration to finish the study, counselling, and of course critic when the right direction for this research work was much needed. My thanks also go to my third supervisor, Dr.Miriam Jumba for encouragement and advice on handling microbiology experiments.

I recognise School of Pharmacy, University of Nairobi for providing bacterial strains (*Staphylococcus aureus* ATCC 259213, and *Escherichia coli* NC 35218) for research. Also my thanks go to Ted White, Seattle Biomedical Research Institute U.S.A, for providing *Candida albicans* SC5314 for research through Judith Odhiambo. My acknowledgment and honour go to the late Professor George M. Siboe University of Nairobi, for using his research microbial collection (*Aspergillus niger* ATCC 16404).

I acknowledge School of Biological Sciences for providing laboratory equipment for microbiology experiments and Professor Abiy Yenesew for advice on chemical analysis and allowing me to use his research laboratory for TLC analysis. My acknowledge goes to Dr. Peter Wachira for providing some chemicals for my research work.

I extend my appreciation to School of Biological Sciences administration and teaching staff for facilitation and guidance throughout this study. I thank technical staff: Mrs. Margret Mwai, Mr. P. Wachira, Mr. M.K. Ndii, and Mr. B. Komu for support and guidance in operating laboratory equipment. My thanks go to Mr. Patrick Mutiso and Mr. Willy Chebii, University of Nairobi Herbarium for accompanying me in field for collection, identification and deposition of plant material in University of Nairobi Herbarium. Your company during field work was fantastic.

Part of this work was done at the National Museums of Kenya. I therefore thank Dr. Adan, Head of phytochemistry Department for allowing me to carry out the initial organic extractions in their laboratory and the technical support received from Solomon Cheboi, Peter Ngolo, Daisy Nyawira, and Rodgers Wayaya is acknowledged. My gratitude also goes to Prof. Francis J. Mulaa, department of biochemistry, University of Nairobi, for allowing me to carry out aqueous extractions using their freeze drier equipment. I also thank the herbalist from Kisumu County; Kakola village, for providing ethnomedicinal information on *Kigelia africana* medicinal plant.

I recognise Dr. Nelson Owour, department of Mathematics, University of Nairobi and Mr. Willis Ochillo, School of Biological Sciences, University of Nairobi, for advice in data analysis.

To my class mates and friends especially Judith Odhiambo, you have been instrumental in the success of my research work. Your warm and friendly atmosphere kept me going smoothly. I have appreciated your company and strong words of advice when I was conducting my research.

My heartfelt appreciation to my family members for the love, understanding and encouragement during this period of hard work. Your support has been steadfast. My nephew Alexander Kyalo, you have always extended love and kindness with regular visits and encouragement during my research work. More so, my thanks goes to Kajiado Children's Home in Kenya, my second family and beyond for your parental love, moral support and regular communication that has carried me through this period.

While I may not be in a position to thank each and every one, I extend a vote of appreciation to all persons who in one way or the other made my studies a success.

Lastly, I give honour to God for being my provider and strength during this research and the far He has brought me.

TABLE OF CONTENTS **PAGE**

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

ATCC:	American Type Culture Collection
NC:	National Collection
SC:	Suspension Culture
CM:	Centimetre
^{O}C:	Degree Celsius
DCM-MeOH	Dichloromethane and Methanol
F.A.O:	Food and Agriculture Organization
GM:	Gram
HIV/AIDs:	Human Immunodeficiency Virus/Acquired Immune Deficiency
IUCEA:	InterUniversity Council of East Africa
MIZ:	Mean Inhibition Zone
µL:	Microlitre
MIC:	Minimum Inhibitory Concentration
CLSI:	Clinical and Laboratory Standard Institute
NCCLS:	National Committee for Clinical Laboratory Standards
%:	Percentage
SAD:	Saboroud's Dextrose Agar
TLC:	Thin Layer Chromatography
H_2O:	Water
W.H.O:	World Health Organization
n. d:	No date

ABSTRACT

Infectious diseases are prevalent and life threatening in Kenya. The majority of the sick are seeking herbal remedies in search of effective, safe, and affordable treatments. This study investigated the antimicrobial activity and presence of alkaloids, terpenoids, sapogenins, flavonoids and quinones in different parts of *Vernonia glabra, Senna didymobotrya,* and *Kigelia africana.* Traditionally, these medicinal plants are used to treat microbial infections in Kenya. The plants were selected based on the available traditional medical knowledge and literature and collected in January 2010 in Machakos and Kisumu counties. Different parts were dried at room temperature under shade, ground into powder and extracted in dichloromethane: methanol (1:1) and water. The crude extracts were tested against *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative) bacteria, *Candida albicans* (yeast fungus), and *Aspergillus niger* (filamentous fungus) for antimicrobial activity and Minimum Inhibitory Concentrations (MICs) determined using disc diffusion technique under sterile conditions. Discs impregnated with standard antibiotics (Streptomycin for bacteria and Nystatin for fungi) were used as positive controls while the extraction solvents were used as negative controls. Antimicrobial activity was determined by measuring the diameter of the clear inhibition zones around the paper discs using a transparent ruler (cm) after 24 to 48 hours for bacteria and yeast fungus, and up to 72 hours for filamentous fungus. Thin Layer Chromatography (TLC) was used to determine the chemical compounds present in selected active crude extracts. Results showed that, organic extracts of *V. glabra* leaf (Mean inhibition zone of 1.85 cm) and flower (MIZ of 1.78 cm) recorded the highest activity against *S. aureus* than the standard antibiotic (Streptomycin MIZ of 1.30 cm). Organic extract of *V. glabra* flower showed significant activity only against *S. aureus*, with the lowest MIC of 1.5625 mg/100 µl compared to streptomycin at high MIC of 6.25 mg/100 µl. Qualitative spray reagents on TLC plates, showed the *V. glabra* and *S. didymobotrya* flavonoids highly present; terpenoids, sapogenins and quinones sufficiently present and *V. glabra* flower alkaloids greatly present. The results of this study suggest that the three plants have significant antimicrobial properties and justify their use in traditional herbal medicine for the management of microbial based diseases. The presence of chemical compounds in most extracts of *V. glabra* indicates its potential to produce novel compounds. Bioassay-guided fractionations are recommended to identify the compounds responsible for antimicrobial activity. Cytotoxicity assays are highly recommended for *V. glabra* in order to verify, validate and document its safety in medicine.

Key words: Microbial infections, *Vernonia glabra, Senna didymobotrya, Kigelia africa*

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Infectious diseases are the leading cause of death in children and young adults accounting for one in every two deaths in developing countries (WHO, 1999, 2000, as cited in Smolinski and Hamburg, 2003). The issue of population health and socioeconomic development is particularly acute in Sub-Saharan Africa with a high burden of bacterial, fungal, and viral infections, such as: tuberculosis, skin rushes, mouth rush, diarrhoea, gonorrhoea, syphilis, and ringworms among others. (Bloom and Canning, 2008). It has been estimated that every hour, 1,500 people die from an infectious disease. Over half of them are children under 5 years of age.

Microbial infections are prevalent in Kenya and have contributed to unsustainable socio-economic development due to high mortality rate of the infected patients, emergence of antibiotic resistance in pathogens which threatens to overwhelm modern healthcare systems, side-effects of these antibiotics to the hosts, unaffordable medicine to the poor, and eventually the suffering people result to use of alternative medicine/or herbal remedies in such of effective and safe cure. Natural products of higher plants may provide a new source of antimicrobial agents with possibly novel mechanisms of action (Adenisa *et al.*, 2000).

Throughout history, microbial infections have been a major threat to human and animal health and a prominent cause of morbidity and mortality which lead to reduced work productivity and long-term poverty (WHO/FAO/OIE, 2003). In our increasingly interconnected world, new diseases are emerging at unprecedented rates, often with the ability to cross borders rapidly and spread (World Health Organization, 2012).

Increased antibiotic resistance has become a global concern, coupled with the problem of microbial persistence, thus highlighting the need to develop novel microbial drugs that are not only active against drug resistant microbes, but more importantly, kill persistent micro-organisms and shorten the length of treatment. Apart from toxicity, lengthy therapy also creates poor patient compliance (Mariita *et al.*, 2010).

The high cost of important conventional drugs and / or inaccessibility to modern health care facilities has led to overreliance on traditional medicine since it is affordable and available to rural people. On the other hand, even when conventional health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective (Munguti, 1997). Given today's universal vulnerability to these threats, better security calls for global solidarity in search for safe, effective, and affordable drugs (WHO, 2007).

It is estimated that about 75% of the population in Kenya seeks health care among traditional healers (Sandiga *et al.*, 1995). In certain instances people utilize both traditional and modern medicine simultaneously. Traditional medicine is widely practiced in Kenya, where this has been documented by ethnomedical surveys (Miaron *et al.*, 2004; Kareru *et al.*, 2007). Infections associated with bacterial and fungal pathogens are among some of the indications treated using traditional remedies in Kenya (Njoroge and Bussmann, 2007).

Therefore, the aim of this study was to determine antimicrobial activity (efficacy) in different parts of *Vernonia glabra*, *Senna didymobotrya*, and *Kigelia african* claimed to treat microbial infections in Kenya, against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*, and thereafter, find out the chemical profiles of the active crude extracts, which would be a prospective open for future search to identify the active compounds.

1.2 LITERATURE REVIEW

1.2.1 Traditional Medicine

Traditional medicine is the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in maintenance of health as well as in prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2000). It involves use of herbal medicines, animal parts, and minerals; but herbal medicines are the most widely used of the three, therefore, it is to be accessed in this study.

For centuries, people have used plants for healing. Plant products as part of food or botanical portions and powder have been used with varying success to cure and prevent diseases throughout history (Maryam, *et al.*, 2010).

During the past decade, use of traditional medicine has expanded globally and has gained popularity (WHO, 1999). Practices of traditional medicine vary greatly from country to country, and from region to region, as they are influenced by factors such as culture, history, personal attitude and philosophy. In many cases, their theory and application are quite different from those of conventional medicine (WHO, 2000). Traditionally, instructions are generally given in the field, where the inheriting person is shown the plant, told the vernacular name, and how to prepare the drug from the plant or plant part for specific diseases (Kokwaro, 1976).

The historical use of traditional medicine that has been passed on from generation to generation, has demonstrated some degree of safety and efficacy through trial and error. However, scientific research is needed to provide additional evidence of its safety and efficacy (WHO 1998).

In developing countries, broad use of traditional medicinal plants is often attributed to its accessibility and affordability as a source of primary healthcare especially for the world's poorest patients. The world health Organization (WHO) estimates that around 80% of the population in Africa use traditional medicine and about 85% of the population in Africa involve use of plant extracts (Farnsworth, 1988).

About 20% of patients, who seek conventional medical care, first consult traditional healers and therefore is a significant source of healthcare for significant number of Africans (Dejong, 1991). The overall number of drug plants used is large, and includes both indigenous and introduced plants (Kokwaro 1993; Fowler 2006).

To appreciate the extent of this dependence, it is estimated that in Sub-Saharan Africa there is one traditional healer for every 50,000 people, whereas there is only one medical doctor for every 40,000 people (Falodun, 2010).

Moreover, distribution of such personnel may be uneven, with most being found in cities or other urban areas, and therefore difficult for rural populations to access. Herbal medicines for treating microbial infections are considerably cheaper and many times are paid for in kind and/ or according to the "wealth" of the client. However, increased use of traditional medicinal plants has not been accompanied by scientific evidence in the efficacy and safety to support traditional medicine claims (WHO 2000-2005). Traditional medicine is also highly popular in many developing countries because it is firmly embedded within wider belief systems and yet little effort has been devoted to their documentation, validation, utilization and conservation.

With respect to diseases caused by microorganisms, the increasing resistance in many common pathogens to currently used therapeutic agents, such as antibiotics and antiviral agents, has led to renewed interest in the discovery of novel anti-effective compounds

(Palombo, 2009). Hence the aim of this study is to provide scientific justification for the efficacy of the medicinal plants and search for plant based drugs with less/or no adverse effects, effective and economical.

Although the traditional herbalists may not know the actual bioactive compounds in the medicinal plants they use, it is the presence of bioactive compounds that is the secret behind the effectiveness of the traditional herbal medicine against a wide range of human ailments (Balick, 1990; Farnsworth *et al.*, 1985).

Hence the focus of this study is to access the efficacy and screen for phytochemical compounds present in the three medicinal plants claimed to treat microbial diseases in Kenya and scientifically verify the traditional ethnomedicine of these plants.

1.2.2 Herbal Medicine

Herbal medicine also called botanical medicine or phytomedicine refers to the use of any plant part, i.e. seeds, berries, roots, leaves, bark, or flowers for medical purposes. Long practiced outside of conventional medicine, herbalism is becoming more mainstream as scientific research has shown their value in the management of diseases (Altschuler *et al.*, 2007).

Herbal medicines are efficient in the treatment of fungal related infections in Kenya, (Olembo *et al.*, 1995; Kokwaro, 1993) and they constitute a great economic and strategic value for the people in Kenya. However, the development of these potentially useful drug plants has been impeded by poor documentation together with little or no scientific validation of the herbal drugs, their safety and efficacy. As reported by Ng'etich, (2005) the use of ethnomedicinal leads to identify medicinal plants is a very slow process because herbalists maintain secrecy of their knowledge regarding it as a personal property. In order to prove the full potential of

these medicinal plants, it is therefore necessary to scientifically verify for their efficacy and produce a reliable and valid scientific documentation.

However, only a small portion of plant species that provide medicinal herbs have been scientifically evaluated for their possible medical application. Safety and efficacy data are available for even few plants, their extracts and active ingredients, and the preparations containing them. Furthermore, in most countries the herbal medicines market is poorly regulated and herbal products are often neither registered nor controlled. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and developing countries. Both the general consumer and healthcare professionals need up to date authoritative information on the safety and efficacy of medicinal plants (WHO, 1999).

Traditional herbal medicines are widely used in much of the world. However, their usefulness is limited by lack of clinical trials that would definitively demonstrate efficacy and safety. Without clinical trials, regulatory approval cannot be obtained, and the products cannot be marketed as drugs (Mendel and Hollis, 2010).

1.2.3 Microbial Diseases Caused By Bacteria and Fungi in Kenya

Microbial infections pose a health problem throughout the world, and plants are possible source of antimicrobial agents (Burapadaja and Buchoo 1995; Adenisa *et al.*, 2000). In developing countries where microbial diseases are endemic, depend strongly on medicinal plants as a source for inexpensive treatment of these diseases. However, scientific data to validate the antimicrobial properties of these herbal remedies is scarce (Maryam *et al.*, 2010). Kenya is endowed with a wide variety of indigenous medicinal plants, many of which have not been studied (Mwangi *et al.*, 2009). These are used by traditional practitioners for treatment of different microbial diseases.

Common bacterial infections that cause death include those associated with HIV/AIDS such as Tuberculosis caused by *Mycobacterium tuberculosis*, syphilis caused by *Treponema pallidum*, gonorrhoea caused by *Neisseria gonorrhoea*, skin and wound infections caused by *Staphylococcus aureus*, diarrhoea caused by strains of *Escherichia coli*, and typhoid fever caused by *Salmonella typhus* (Topley and Wilson, 1998).

Fungal infections are common opportunistic infections that cause quick death in about 10 % of HIV/AIDS patients (Saag, 1997). For example, candidiasis/Oral lesions/ oral thrush caused by *Candida albicans* have been recognized as prominent features of AIDs infection since the beginning of the epidemic, and it is estimated that about 5.8 % of people with AIDS have oral lesions (Greenspan and Greenspan, 1997). *Aspergillus* species (*Aspergillus niger*) are a major cause of Aspergillosis and respiratory allergies associated with HIV/AIDS due to exposure to their spores (Kozkiewicz, 1989).

The current antibacterial and antifungal agents used to treat microbial infections are expensive, and there is increasing occurrence of resistance to the available antimicrobial therapies (Frontling, 1984; Colombo *et al.*, 1994). Several have been found to have cytotoxic effects. For example, Ketoconazole useful in the treatment of cetaceous and systemic fungal diseases has been known to cause liver damage (Frontling *et al*; 1984), thus reduces compliance by the patients.

1.2.4 Antimicrobial Activity of Medicinal Plants

An antimicrobial agent is a chemical compound that in low concentrations can kill or inhibit the growth of a micro-organism without causing the host (such as a human or an animal) significant damage (WHO/FAO/OIE, 2003).

Antimicrobial agents can be naturally produced (like penicillin) by a mould, bacterium, or plants, or synthetically made (like the fluoroquinones). Antimicrobials have had a more

positive impact on human health than any other medical discovery. In the early history of antimicrobial agents, it was perceived that infectious diseases had been conquered and were no longer a major threat to human and animal health. However, it was soon observed that bacteria could develop resistance to antimicrobial agents. Due to the spread and persistence of antimicrobial resistance, antimicrobials are losing their effectiveness (WHO/FAO/OIE, 2003).

Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Nair *et al.*, 2004).

The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered leads for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Sexana and Sharma, 1999).

The beneficial medicinal effects of plant materials typically result from the combination of secondary metabolites/ or compounds present in plants such as alkaloids, steroids, tannins, flavonoids, terpenoids, quinones, saponins, fatty acids, and gums which are capable of producing definite physiological action on a body (Bishnu *et al.*, 2009).

Recent research has shown that *Artemisia annua* (Asteraceae family), better known in the west as sweet wormwood, a traditional Chinese herbal medicine contains artemisinin substance (classified as a sesquiterpene lactone) which has proved to be a dramatically effective anti-malarial against multi-drug resistant plasmodium spp. (WHO, 2006). The medicinal plant, *Opium poppy* (*Papaver somniferum*) is been reported to contain morphine

which is the most abundant alkaloid used to relieve severe or agonizing pain and suffering (Sharon, 2010).

Ethanol extracts of *Cassia occidentalis* (Leguminoceae family), used in traditional Nigeria folk medicine for the treatment of dysentery, gastrointestinal disorders and typhoid fever are reported as antibacterial against *Salmonella typhi* and revealed the presence of anthraquinones, terpenoids, saponins, tannins and cardiac glycoside which might explain the ethnomedicinal use (Sadiq *et al.*, 2012). Acetone extract of *Senna obtusifolia* L. leaf (Cassia synonymy) is reported to show antibacterial and antifungal activities against *Staphylococcus aureus*, *Neisseria gonorrhoea*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* with phytoconstituents including: saponins, alkaloids, tannins, flavonoids and no presence of anthraquinones (Doughari *et al.*, 2008).

Vernonia amygdalina (Asteraceae family) leaves have been reported to have medicinal properties in the herbal remedies for leprosy, cough, gastro internal disorders and toothache. In addition, the leaves have been screened for sesquiterpene lactones which exhibited a significant bactericidal activity against *S. aureus* and antifungal activity against *Aspergillus niger*, *A. flavus*, and *Penicillium notatum* (Erasto *et al.*, 2006). Also leaf and bark extracts of *V. tenoreana* are reported to show a broad spectrum to varied number of bacteria, while the bark extracts showed antifungal activity against *C. albicans*, *A. niger* and *A. flavus*. Phytochemical analysis revealed the presence of saponins, tannins, anthraquinones in the bark extract, while tannins, anthraquinones, and cardenolides were present in the leaf extract (Ogundare *et al.*, 2006).

The medicinal plant, *Jacaranda cuspidifolia* Mart. (Bignoniaceae family) is traditionally used in Brazil to treat syphilis and gonorrhoea. Methanol and chloroform extracts of *J. cuspidifolia* bark exhibited antibacterial activity against *Staphylococcus aureus*, *Neisseria gonorrhoea*,

Streptococcus pyogenes, and *Staphylococcus epidermidis*. These extracts showed presence of saponins, flavonoids, tannins, quinones, alkaloids, triterpenes, and steroids (Arruda *et al.*, 2011).

The success of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microbial pathogens (Khan *et al.*, 2003), which have evolved numerous defence mechanisms against antimicrobial agents, hence resistant to old and newly available drugs (Poovendran *et al.*, 2011). Antimicrobial activity of medicinal plants is evaluated first by performing an *in vitro* experiment and then *in vivo* studies follow. Therefore in order to confirm the efficacy of medicinal plants, there is a need to evaluate their antimicrobial activity against the diseases they are claimed to treat.

Previous reports on biological activities and phytochemical compounds of the three medicinal plants being investigated in this study are shown below.

1.2.4.1 Previously Reported Biological Activities and Phytochemical Compounds Present in *Senna didymobotrya* (Fresen.) Irwin & Barneby.

The species *Senna didymobotrya* (previously described as *Cassia didymobotrya*) used traditionally to treat skin conditions and stomach problems in Bomet district was reported to exhibit antibacterial and antifungal activities. Methanol and Dichloromethane stem bark extracts of *S. didymobotra* exhibited antibacterial activity against *S. aureus*, *E. coli*, and *Klebsiella pneumonia*. Hexane stem bark extract was antifungal on *Microsporium gypseum*, while Dichloromethane stem bark extract was antifungal on *Trychophyton mentagrophyte* and *M. gypseum*. The classes of compounds reported present in stem bark extracts were quinones, Phenolics, flavonoids, terpenoids and alkaloids (Korrir *et al.*, 2012). In Egypt dried

entire plant showed presence of quinones (El-Sayyad *et al.*, 1988), while the leaf extract in Rwanda also showed presence of quinones (Classen, 1977).

Ethyl acetate leaf extract of *Cassia didymobotra* exhibited pronounced antibacterial activity on *Staphylococcus aureus*, *Salmonella paratyphi*, *Bacillus cereus*, *Enterobacter faecalis* and *Escherichia coli* (Reddy *et al.*, 2010). In Rwanda dried root showed antibacterial activity against *Bacillus subtilis* (Boily and Puyvelde, 1986). In Kenya, leaf and root extracts of *S. didymobotrya* are reported to be antigiardiasis against *giardia lamblia*- a protozoan (Johns *et al.*, 1995). Aqueous extracts of leaves, stem and root barks at concentrations of 1, 0.1 and 0.01% w/v showed larvicidal activities on Anopheles mosquito larvae (Ojewole *et al.*, 2000).

1.2.4.2 Previously Reported Biological Activities and Phytochemical Compounds Present in *Kigelia africana* (Lam) Benth.

In the light of the traditional uses of this plant, antimicrobial activities of the aqueous, ethanol, and ethyl acetate extracts of stem bark and fruit were reported to show significant antibacterial activity against *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* using microtitre plate assay (Sangita *et al.*, 2009). The crude ethanol extract of stem bark is reported to exhibit antibacterial and antifungal activities against *S. aureus* and *C. albicans* respectively, while butanol crude extract of stem bark exhibited *in vitro* antiamoebic activity against *Entamoeba histolytica* (using micro dilution method) (Sangita *et al.*, 2009). The dried bark is reported to be antimalarial against *Plasmodium falciparum* (Okech *et al.*, 1999).

Dichloromethane extracts from the root and stem bark of *K. africana* contains naphthaquinones (Jackson *et al.*, 2000) which showed antitrypanosomal activity *in vitro* against *Trypanosoma brucei brucei* (Moideen *et al.*, 1999). Flavonoids and iridoids have been isolated from the fruit and leaves (Guoda *et al*, 2006) and steroids, coumarins, and

iridoids from the root bark (Akunyili and Houghton, 1993; Khan and Mlungwana, 1999). The dried fruit pulp is reported to have alkaloids (Cabalion *et al.*, 1980).

1.2.4.3 Previously Reported Biological Activities and Phytochemical Compounds Present in *Vernonia glabra* ((Steetz) Oliv. & Hiern.

V. glabra (Asteraceae family) dried bark water extract is reported to show antifungal activity against *C. albicans* using test tube dilution method (Gundidza, 1986). Leaf and root methanol extracts were reported to be antigiardiasis against *Giardia lambria* at concentration of 1000ppm (Johns *et al.*, 1995). The dried aerial parts are reported to have sesquiterpene lactones, namely; loliolide, vernodalin, and vernolepin (Jakupovic *et al.*, 1985).

1.2.5 Ethnomedicine and Its Role in Research

Ethnomedicine refers to the study of traditional medical practice which is concerned with the cultural interpretation of health diseases and illness and also addresses the healthcare seeking process and healing practices. The practice of ethnomedicine is a complex multi-disciplinary system constituting the use of plants, spirituality and the natural environment and has been the source of healing for people for millennia (Williams, 2006).

The use of ethnomedical information has contributed to healthcare worldwide, even though efforts to use it have been sporadic. The plants that are used are often kept secret by the practitioner, so little information about them is recorded; thus there is less dependence on scientific evidence as in systems of traditional medicine that can be subject to scrutiny (Fabricant and Farnsworth, 2001).

The value of ethnomedicine in scientific research is recognised when scientists go to certain geographical areas to assess the use of ethnomedicinal plants by traditional healers and collect plants to evaluate them for validity in the laboratories. To discover plants that contain potential drugs, ethnomedical approach, could be the best way to select plants on the basis of ethnomedical claims related to human illnesses. Much local knowledge regarding medicinal

plants is being lost. Having never been written down, the indigenous knowledge of the elderly is slipping away day by day and a number of plant species or varieties of species are on their way to extinction. Therefore, medicinal plant indigenous knowledge can serve as a powerful search engine which will greatly facilitate a starting point in scientific research and generate scientific knowledge useful for the preservation of cultural and biological diversity and thus there is need to document it (GFHR, 2004).

1.2.5.1 Correlation between Plants used in Traditional Medicine and Drugs Obtained From Them

Traditional medicine usually uses multiple plants which contain a complex mixture of compounds and act multi-systematically, meaning they tend to have broad and non-specific actions on a number of physiological systems simultaneously. These reactions are usually in the same therapeutic action and are complementary or synergistic with mild adverse effects. A drug usually consists of a single chemical which is single target-based and elicit specific metabolic reactions in the body. Their associated side effects are usually traded as a risk against the benefit of the primary effect (Eagleton, 2006).

Pharmaceutical drugs involve chemical analysis techniques which allow scientists to isolate and extract beneficial plant compounds and go through extensive testing before being available to the public. In contrast, traditional use of medicinal plants involves utilizing an entire portion of the plant like the root or leaf rather than extracting a single compound (Audain, 2012). There is lack of standardization with respect to raw material, methods of production and in quality control of the finished product. The problems with traditional medicine is that, it does not keep pace with scientific and technological advancement, and its methods, techniques, medicines and training are often kept secret (Taylor *et al.*, 2001).

Crude plant extracts cannot be patented or approved as drugs. The drug researcher's goal is to come up with a single chemical with good biological activity, one that can be changed in

some way (without losing activity), so that it can be patented as a novel chemical and then be synthetically manufactured into a new patented drug. Sometimes the isolated chemical might not be quite as effective as the crude extract in which it was found. Researchers can even improve on the activity of the plant chemical by modifying it in some way, which also makes it patentable (Taylor, 2005).

One of the major approaches in developing new drugs from plants is to examine the uses claimed for a traditional preparation. Although investigators involved in the development of drugs from natural products usually argue that there is a close relationship between a traditional preparation and a drug obtained from the same plant, data supporting such claims have not been presented (Farnsworth *et al.*, 1985).

1.2.6. Phytochemical Profiling

Phytochemistry is the study of secondary metabolite compounds produced by plants (Croteau *et al.*, 2000). Many of these are produced by the plant to provide protection against insect attacks and plant diseases. They also exhibit a number of protective functions for human consumers (Veilleux and Steven, 2006 as cited in Falodun, 2010). The techniques commonly used in the field of phytochemistry for crude extracts are; extraction using solvents of varying polarity (Houghton and Raman 1998) and then the classes of compounds are identified using Thin Layer Chromatography were by the extracts are spotted on Aluminium TLC plates and the chromatogram developed in a glass tank (Harborne, 1998). Visible light is used to locate the coloured compounds, while Utra-violet lamp is used for identification of non visible fluorescent classes of compounds at long wavelength of 366nm and also at short wavelength of 254 for compound which absorb the light (Markham, 1982). Appropriate qualitative spray reagents are also used for clear identification of the classes of compounds on TLC plates, which take different colours and show band separations (Harborne, 1998). Therefore,

phytochemical analysis techniques are needed in this study for quick identification of chemicals present in these medicinal plants and documentation.

1.2.7 Description, Ecology, and Ethnomedicinal Information on *S. didymobotrya*, *K. africana*, and *V. glabra*

In this study, *S. didymobotrya*, *K. africana*, and *V. glabra* were selected based on available literature and Ethnomedicinal information as given below per plant.

1.2.7.1 *S. didymobotrya* (Fresen.) Irwin & Barneby (Leguminosae)

It is an indigenous plant with common name as candle bush and local name; Owino according to Luo community. A bushy, poisonous shrub, occasionally growing to a tree of up to 6 m height; commonly found in riverines and lake-shore areas, and along forest edges, in dump and well-watered sites. Occurs at altitude of 700 m to 2100 m.

Leaves, stems and roots serve as purgative, as a remedy for gonorrhoea, and backache in women. The burnt ash is applied by smearing areas infected with ringworms. The leaves, pods, and roots are poisonous; leaves are used as a fish poison. The bark contains tannins. Roots are used as an antidote (neutralizes poison or counteracts its effects) to general poisoning (Dharani, 2002; Kokwaro, 1993).

Figure 1. *S. didymobotrya* plant with its yellow inflorescences- collected from Kibuye in Kisumu County. Picture by Catherine Kitonde

1.2.7.2 *K. africana* (Lam.) Benth (Bignoniaceae)

The genus *Kigelia* comprises of only one species *Kigelia africana*, which occurs throughout tropical Africa. Although some synonymys are still acceptable by a few horticulturists as distinct species, such as *Kigelia pinnata* (Jacq.) DC., *K. aethiopica* Decne, *K. abyssinica* A. Rick, *Crescentia pinnata* Jacq., and *Tecoma africana* (Lam.) G. Don, botanical studies agree that the genus contains only one species (Joffe, 2003).

It is an indigenous plant known as Sausage tree due to its long sausage shaped fruits. Its local name is Yago with reference to Luo community. A semi-deciduous tree with a rounded crown grows to a height of 9 m in open woodland, but 18 m beside rivers. It is widespread throughout East Africa; found in wet savannah and along rivers in dry areas, from coast to the highlands, at altitudes from sea level to 1850 m.

A decoction from the bark serves as a remedy for headaches and dysentery. A leaf decoction is taken for malaria. Dried fruit is powdered and used as a dressing for ulcers, sores, and syphilis, and also applied locally as rheumatism. Beer made from the fruit appears to be an excellent cure for childhood measles. The fruit is also known to induce abortion. A decoction of fruit and bark may also be taken for juvenile stomach ailments (Dharani, 2002; Maundu and Tengnas 2005; Kokwaro, 1993).

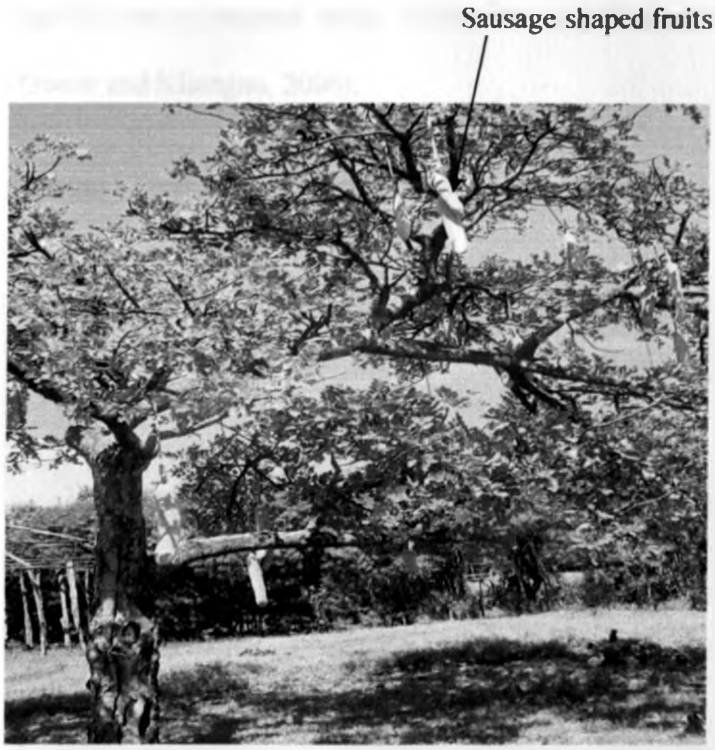

Figure 2. *K. africana* tree showing long sausage shaped fruits – collected from Kakola village in Kisumu County. Picture by Catherine Kitonde

1.2.7.3 *V. glabra* (Steetz) Oliv. & Hiern. (Asteraceae).

Its common name is Wild heliotrope. Herbaceous perennial that flowers throughout the summer producing masses of flowers that look like little mauve paintbrushes, at tips are many upright stems. From a woody rootstock, tall, a robust stems, shoot stiff and upright,

branching sparingly to about 1.2 m high. Flowers are grouped in dense clusters at the tip of the stems. Tight buds are light green to straw coloured, opening into light and dark purple flowers, which turn into fluffy white seedheads. Buds, flowers and seedheads on the same plant at the same time. Occurs naturally in Kenya, usually as a pioneer in disturbed ground (Van der Walt, 2003).

A decoction of leaf plus root is used to treat gastrointestinal problems (Johns *et al.*, 1995). The leaf ash or crushed leaves rubbed into scarification around the snake bite as antidote (Owour and Kisangau, 2006).

Terminal inflorescence of flower buds and dark purple flowers.

Figure 3. *V. glabra* herb collected from Kathiani in Machakos County. Picture by Catharine Kitonde

1.2.8. Justification of the Study

Microbial diseases in humans in Kenya, have contributed to unsustainable socio-economic development due to high mortality rate of the patients, resistance of microbial pathogens to antibiotics, side-effects of antibiotics, limited accessibility and unaffordable medicine to the poor.

Sufficient information on the efficacy and safety of medicinal plants is limited by inadequate knowledge of bioassay tests and identification of phytochemicals present among different medicinal plants.

It is therefore necessary to incorporate antimicrobial and phytochemical studies to determine the effectiveness and identify compounds present in *V. glabra*, *S. didymobotrya*, and *K. africana* for quick scientific verification and documentation of these three medicinal plants.

1.2.9 Objectives of the Study

1.2.9.1 Broad Objective

This study was designed to evaluate antimicrobial activity and phytochemical profiles of *V. glabra*, *S. didymobotrya*, and *K. africana* used traditionally for the treatment of microbial infections in Kenya.

1.2.9.2 Specific Objectives

1. To screen for antimicrobial activity of the crude extracts of the three selected plants against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*.

2. To determine the minimum inhibitory concentration of the active crude extracts.

3. To screen for the presence of five classes of chemical compounds: alkaloids, sapogenins, terpenoids, flavonoids and quinones.

1.2.9.3 Hypothesis of the Study

It is hypothesized that plants used in traditional medicine for treatment of bacterial and fungal infections in Kenya, posses antimicrobial properties that may be scientifically verified and documented.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Study site

Below is a map of Kenya showing floral regions (Top right) and study sites (Top left) - Kisumu county and (bottom left) - Machakos county.

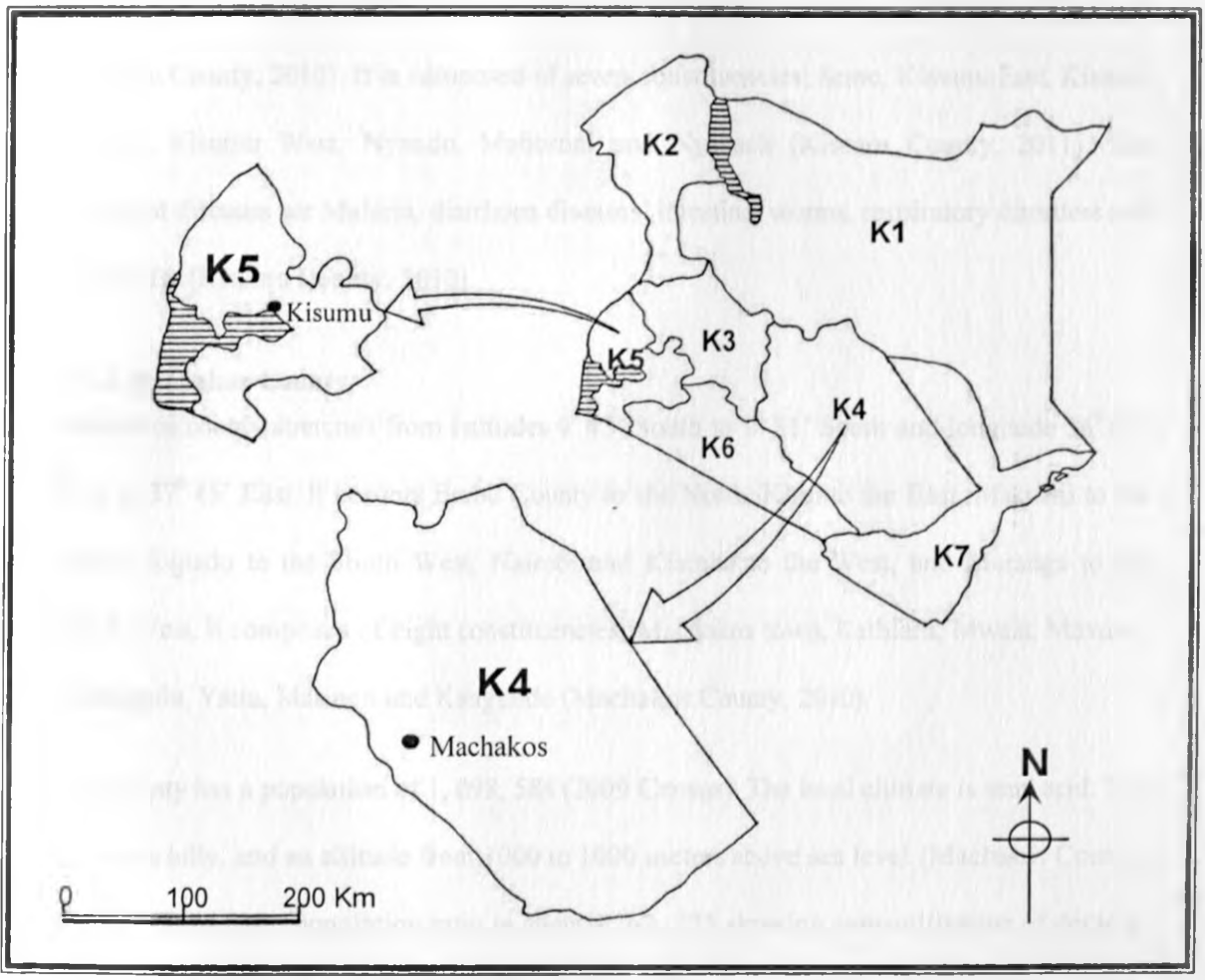

Figure 4. Floral Regions of Kenya according to Flora of Tropical East Africa (FTEA) (Beentje, 1994) (Top Right); and the Study Collection Sites (Top Left and Bottom left).

2.1.2 Kisumu County

Kisumu County is located at 0.1^0 South latitude, 34.75^0 East longitude and about 1132 m altitude above the sea level (Kisumu maps, 2012). It borders Vihiga to the North, Nandi County to the North East, Kericho County to the East, Nyamira to the South, Homa bay to the South West and Siaya to the West with an area of 2, 085.9 km^2 (Kisumu County, 2012). It has a population of 968, 909 (2009 Census) with a population density of 465 people per km^2 (Kisumu County, 2010). It is composed of seven constituencies; Seme, Kisumu East, Kisumu Central, Kisumu West, Nyando, Muhoroni and Nyakach (Kisumu County, 2011). The prevalent diseases are Malaria, diarrhoea diseases, intestinal worms, respiratory disorders and HIV/AIDs (Kisumu County, 2010).

2.1.3 Machakos County

Machakos county stretches from latitudes 0^0 45' South to 1^0 31' South and longitude 36^0 45' East to 37^0 45' East. It borders Embu County to the North, Kitui to the East, Makueni to the South, Kajiado to the South West, Nairobi and Kiambu to the West, and Muranga to the North West. It comprises of eight constituencies: Machakos town, Kathiani, Mwala, Mavoko, Matungulu, Yatta, Masinga and Kangundo (Machakos County, 2010).

The county has a population of 1, 098, 584 (2009 Census). The local climate is semi arid. The terrain is hilly, and an altitude from 1000 to 1600 metres above sea level (Machakos County, 2010). The doctor/ population ratio is about 1: 62, 325 showing over-utilization of doctors. The average distance to a health facility is 5 km. The most prevalent diseases are malaria, skin diseases and HIV/AIDs, while the childhood diseases include anaemia, marasmus, eye infection, pneumonia, malaria, and kwashiorkor (Machakos County, 2010).

2.2 Collection of Plant Materials

The three plants were selected on the basis of information from literature and previous ethnobotanical study. These plants with information on treatment of microbial infections were collected in January, 2010 in Kisumu and Machakos counties which fall within the floral regions K4 and K5 (Figure 4).

Based on the existing ethnomedical and literature information on biological activity and phytochemical analysis of the three plants; leaves, stems, roots, flowers and seeds were selected to be evaluated each plant part separately and/ or entire plant, because the secondary metabolites are specifically found in certain taxa of plants and at varying presence among different parts of plant tissues (Gottlieb, 1990).

Voucher specimens; *V. glabra* (CK 2010/01), *S. didymobotrya* (CK 2010/02), and *K. africana* (CK 2010/03) were collected and carefully arranged on drying paper, tagged, pressed in a plant press and identified using keys (Agnew and Agnew, 1994; Beentje, 1994) and by comparison with authentic herbarium materials. They were finally mounted according to standard herbarium procedures and deposited in the University of Nairobi Herbarium.

2.2.1 Ethnomedicinal data collection on *K. africana*

Ethnomedicinal information on *K. africana* was collected through oral interviews from one herbalist at Kakola village, Nyando constituency in Kisumu County. Information provided included the human ailments treated, local name, plant species, plant parts used, method of preparation, and administration. The fruit, leaves, bark and roots were collected.

2.2.2 Preparation of crude plant extracts

The plant material collected from the field was air-dried in the shade at room temperature for two weeks. The dried plant material was ground into powder, divided into two portions and

extracted using sterile distilled water, and organic solvents; dichloromethane and methanol mixed in the ratio 1:1 (DCM-MEOH=1:1), according to standard extraction methods (Harborne, 1998). Twenty grams of powdered plant material was mixed thoroughly with the appropriate amount of solvent, left to stand for 24 hours and filtered twice. The filtrates were combined and filtered using a Buchner funnel. Dry crude extracts were obtained by concentrating filtrates from organic solvents using a rotary evaporator at 40°C. The water crude extract filtrates were freezed to ice in 24 hours using a deep freezer, and dried into powder using a freeze drier. The dry extracts were stored in vials at low temperatures (4°C) awaiting antimicrobial test. Figure 5 below shows steps in preparation of crude extracts.

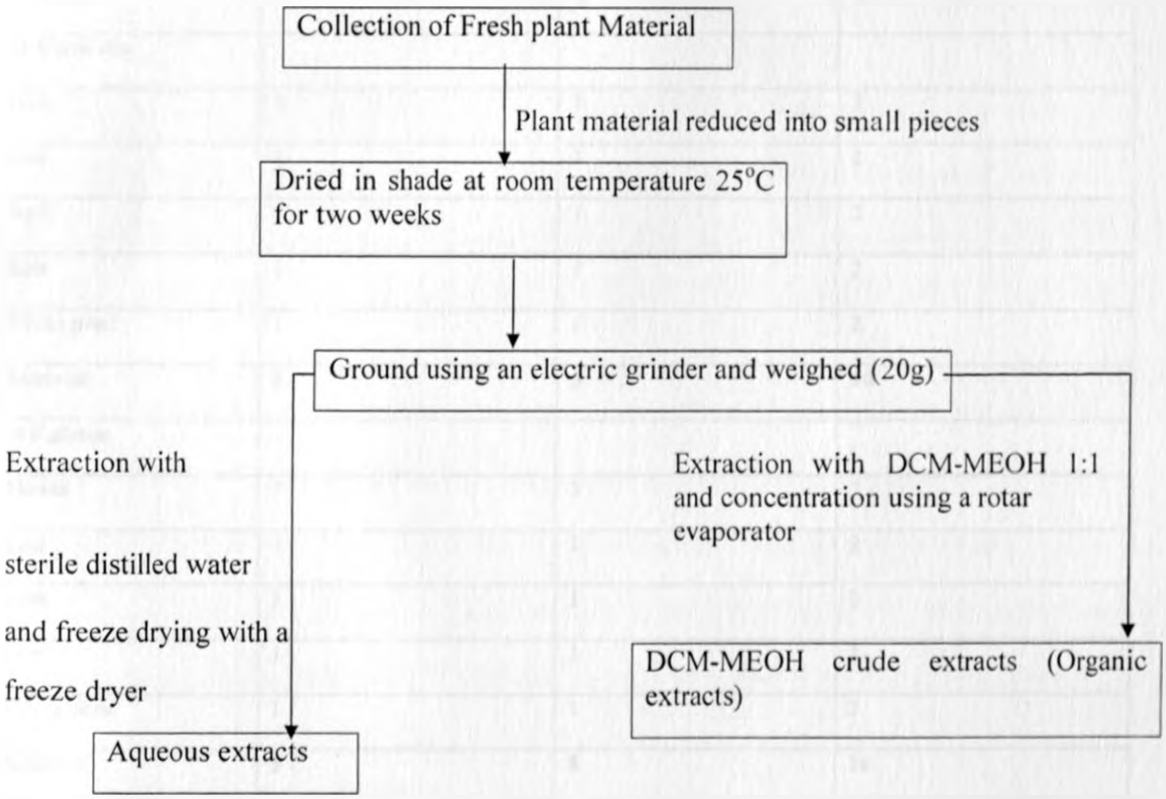

Figure 5: A flow diagram for crude plant extracts preparation

A total of 34 crude extracts were prepared from the mixture of the three plants, each plant part separately, and/or entire plant (Table 1).

Table 1. Number of Crude Extracts Prepared from *S. didymobotrya*, *K. africana*, *V. glabra* and Mixture of the three plants

Plant	Organic extracts	Aqueous extracts	Total number of extracts
a) *S.didymobotrya*			
Seed	1	1	2
Seedpod	1	1	2
Leaf	1	1	2
Stem	1	1	2
Root	1	1	2
Whole plant	1	1	2
Subtotal	**6**	**6**	**12**
b) *K.africana*			
Fruit	1	1	2
Leaf	1	1	2
Bark	1	1	2
Root	1	1	2
Whole plant	1	1	2
Subtotal	**5**	**5**	**10**
c) *V.glabra*			
Flower	1	1	2
Leaf	1	1	2
Stem	1	1	2
Root	1	1	2
Whole plant	1	1	2
Subtotal	**5**	**5**	**10**
d)Mixture of *S.didymobotrya*, *K.africana*, and *V.glabra*	1	1	**2**
Total	**17**	**17**	**34**

2.3 Sources of Micro-organisms

Pure cultures of bacteria; *Staphylococcus aureus* ATCC 259213, *Escherichia coli* NC 35218 (from School of Pharmacy, Microbiology Laboratory, University of Nairobi) were maintained on nutrient broth slants at 4° C. Two fungi; *Candida albicans* SC 5314 (Provided by Ted White, from Seattle Biomedical Research Institution U.S.A), and *Aspergillus niger* ATCC 16404 (a collection of the late Professor George M. Siboe, School of Biological Sciences, University of Nairobi) were maintained on Sabourauds' Dextrose broth slants at 4° C.

2.3.1 Preparation of Standard Inoculums

After confirmation tests of the four micro-organisms was conducted, direct colony and/ or mycelia and spores were transferred into 0.9% normal saline, using a sterile wire loop for each test organism. The suspension was adjusted to a 0.5 McFarland standard turbidity solution using a spectrophotometer at wavelength of 625 nm to give a concentration of 1 $x10^8$ cells or spores/ml (Nostro *et al.*, 2000).

2.4 Stock Solutions and Serial Dilutions Preparation for Organic and Aqueous Extracts

2ml stock solution at concentration 2 g/2 ml (1g/ml or 1mg/µl) was prepared for each plant part, entire plant, and mixture of the three plants extracted (Aqueous and DCM-MEOH 1:1 extracts), and nine serial dilutions prepared from each stock solution. Nine sterile glass vials were arranged in a vial rank and 1ml of diluent solvent added into vials one to nine. Then, 1 ml of stock solution was transferred into the 1st vial to total to 2ml, then 1ml was transferred from 1st vial to 2nd vial that totalled to 2ml. This was done up to the ninth vial. This way the concentration of each dilution reduced by half to form ten concentrations including stock solution, i.e., 1g/ml, 0.5g/ml, 0.25g/ml, 0.125g/ml, 0.625g/ml, 0.3125g/ml, 0.15625g/ml, 0.078125g/ml, 0.0390625g/ml and 0.01953125g/ml. The same concentrations were prepared

for standard antibiotics for easy comparison of the antimicrobial activity. From each concentration prepared, 100µl were pipetted on to individually placed sterile paper discs on flat-bottomed glass plates and allowed to dry waiting for antimicrobial assays (Ochei and Kolhatkar, 2000). The potency for each paper disc per extract for the concentrations prepared was; 100mg/100µl, 50mg/100µl, 25mg/100µl, 12.5mg/100µl, 6.25mg/100µl, 3.125mg/100µl, 1.5625mg/100µl, 0.78125mg/100µl, 0.390625mg/100µl and 0.1953125mg/100µl.

2.5 Antimicrobial Assays and Minimum Inhibitory Concentrations (MICs) for Organic and Aqueous Crude Extracts

The standard disc diffusion assay was used to evaluate the antibacterial and antifungal activities according to standard antimicrobial techniques (Clinical and Laboratory Standards Institute formerly NCCLS, 2012). The paper discs were prepared from Whatman filter paper No.1 using a paper punch with a diameter of 6mm. These paper discs impregnated with various concentrations of crude plant extracts were transferred onto agar plates inoculated with the test organisms using a sterile forceps, labelled, sealed with parafilm and incubated at 37°C for *S. aureus*, *E. coli*, and *C. albicans* for 24 to 48 hours. *A.niger* was incubated at 25° C for 72 hours after which the inhibition zone diameter was measured in centimetres (cm) and recorded against the corresponding concentrations (Das *et al.*, 2010; Elgayyar *et al.*, 2001).

These were done in duplicates under sterile conditions. The antimicrobial activity of crude plant extracts were compared with the activity of two Standard commercial antibiotics at the same concentrations; Nystatin for fungi, and Streptomycin for bacteria.

2.5.1 Determination of Minimum Inhibitory Concentrations (MICs)

The disc diffusion technique was used to determine MICs of each active organic and aqueous extracts of *V. glabra, S. didymobotrya, and K. africana* against the four test organisms after

the failure of broth dilution and agar dilution methods. These two methods made determination of MICs impossible because of the opacity that was produced by the crude extracts in the media. The active extracts screened for MICs in this study ranged from a concentration of 100mg/100µl to 0.02mg/100µl. The MICs were compared with those of standard antibiotics against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*.

Five organic extracts of *V. glabra* tested for MICs were Flower, Leaf, Stem, Root, and Whole plant, and one aqueous extract of whole plant. Three organic extracts each for *S. didymobotrya* (Seed, Stem, and Root) and *K. africana* (Fruit, Root, and Whole plant) were screened for MICs. Of all the mixture extracts of the three plants, only organic extract was screened for MIC as the only active extract.

2.6 Phytochemical Screening of Crude Extracts Using Thin Layer Chromatography

Only DCM-MEOH crude extracts were evaluated for chemical profiling. Extracts that exhibited MICs of 12.5-1.5625 mg/100 µl against any of the test organisms were selected for chemical analysis.

They were screened for the presence of five classes of compounds: alkaloids, sapogenins, terpenoids, quinones, and flavonoids using Thin Layer Chromatography (TLC) techniques. The developed TLC plates were viewed under Ultra-Violet light and then sprayed with appropriate reagents for the detection of the chemical groups according to Harborne (1998). The analyses were carried out as shown in Table 2.

Table 2. Solvent Systems and Spray Reagents used in TLC Analyses

Phytochemicals	Solvent system	Ratio	Spray reagent	Colour of the positive spot
Alkaloids	Dichloromethane/Methanol	92 : 8	Dragendorff; Solution A consists of 0.85 basic bismuth nitrate dissolved in a mixture of 10ml acetic acid and 40ml water; solution B consists of 8g potassium iodide in 20ml of water. Equal volumes of solutions A and B are mixed to make Dragendorff reagent.1ml is mixed with 2ml acetic acid and 10ml water before use.	Orange spots.
Sapogenins	Dichloromethane/Methanol	11.5:1	Antimony trichloride in concentrated Hydrochloric acid and heating the sprayed TLC plate at 110°C for 10 minutes.	Pink to purple spots.
Terpenoids	Chloroform/Ether	5:1	Concentrated Sulphuric acid sprayed on TLC plates and heated at 100-110°C for 5 minutes.	Green, brown, blue, yellow, or red spots
Quinones	Ethyl acetate/Methanol	6.25:1	Exposure to ammonia fumes	Yellow, pink, green spots
Flavonoids	Dichloromethane/Methanol	4:1	Add 8ml ethanol cooling to 0.5g of vanillin in 2ml of concentrated sulphuric acid and heating at 110°C for 5 minutes.	Red, Purple, yellow, and blue. Spots fluorescent in long-UV light at 366nm.

(Adapted from Harborne, 1998).

2.7 Data Analysis

Data analysis was done by use of multivariate Analysis of Variance (ANOVA) to determine significant factors in production of inhibition zones; Tukey's Honest Significant Difference Test was used for separation or comparison of mean inhibition zones (Brown, 2012).

CHAPTER THREE

3.0 RESULTS

3.1 Ethnomedicinal Data on *K. africana*

The traditional practitioner used sustained boiling of the hard parts of the plant or soaked powdered plant material in water or mixture with various herbs, or burned into ash with some additives to treat different ailments such as measles/skin diseases, diarrhoea, mouth rush, malaria, typhoid, and ulcers (Table 3).

Table 3. Ethnomedicinal data on *K. africana* (Lam.) Benth. Collected from Kakola village, Nyando constituency in Kisumu County

Part used	Preparation method and administration	Ailment treated
Fruit	Cut into small pieces, boiled and drunk. 1/4 glass for babies, 3/4 glass-adults, 2x a day	Skin rushes, Measles, Malaria, Typhoid, and Stomach problems
Leaves	Burned to ash, ghee added and licked	Ulcers
Bark	Dried bark, add group of medicinal herbs, boiled in water and drunk	Diarrhoea, Mouth rush in children
Seeds	Pound, boiled and drunk	Stomach problems
Roots	Boiled and drunk or put in basin and bath	Diarrhoea and skin lesions

K.africana (Bignoniaceae) collected from Kakola village. Local name Yago (Luo), voucher specimen CK 2010/03.

3.2 Antimicrobial Activity of Organic and Aqueous Crude Extracts of *V.glabra, S. didymobotrya, K. africana* and three plants Mixed at 100mg/100µl

Out of 34 crude extracts screened, 44% extracts (15 extracts) were active against at least one of four micro-organisms tested. Organic extracts of *V. glabra* represented 14.7% of the active

extracts (5 extracts) and its aqueous extracts represented 2.9% of active extracts (1extract), with most of its extracts inhibiting *S. aureus*, *E. coli*, *A. niger*, or *C. albicans*. 3 organic extracts of *S. didymobotrya* represented 8.8% of active extracts, and its aqueous extracts showed 5.9% activity of the active extracts (2 extracts) against *S.aureus*. Organic extracts of *K. africana* represented 8.8% of the active extracts (3 extracts) only against *S. aureus*, while its aqueous extracts were inactive. Organic extract of the mixture of these three plants represented 2.9% of the active extracts (1 extract) only against *S.aureus*. Its aqueous extract was inactive. A summary of the activity of the active crude extracts are shown in figure 6.

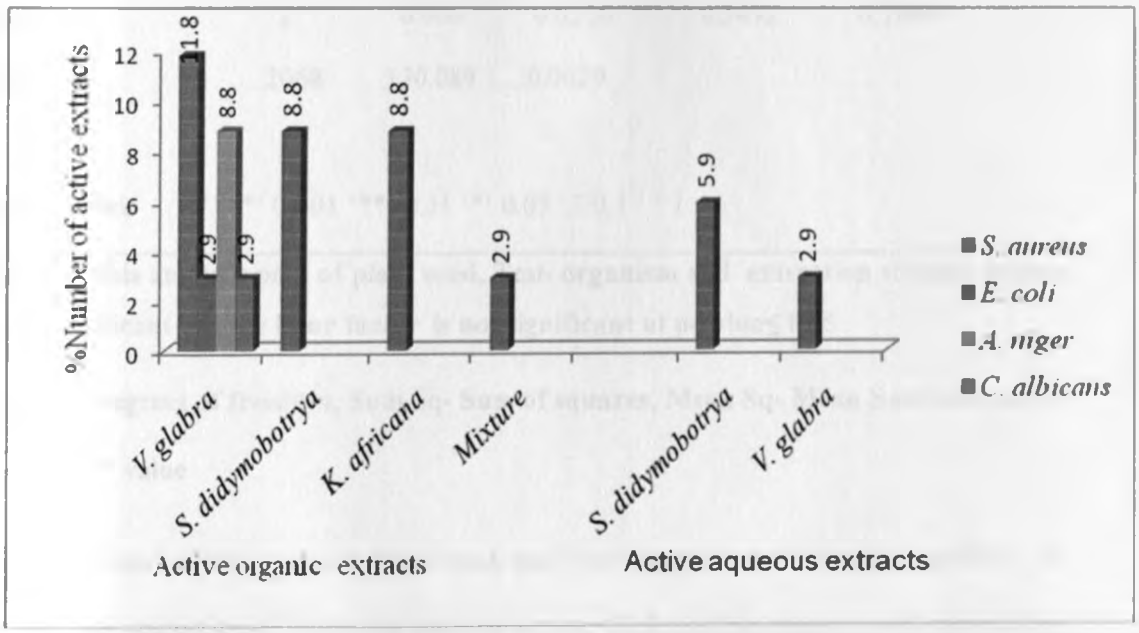

Figure 6. Percentage number of active organic and aqueuos crude extracts of *V. glabra*, *S. didymobotrya*, *K. africana*, and three plants mixed.

Key: VG- *V. glabra*, SD- *S. didymobotrya*, KA- *K. africana*

3.3 Antimicrobial Activity of Organic and Aqueous Extracts of *V. glabra* at 100mg/100µl Concentration

The antimicrobial activity of *V. glabra* crude extracts varied with the plant parts used, extraction solvent (organic and aqueous) and test-organisms as the significant factors that

affected the production of inhibition zones. Time factor (incubation period) was insignificant for the production of inhibition zones at p≤ 0.05, (Table 4).

Table 4. Significance of plant part used, test-Organism, and extraction solvent in the production of inhibition zones by *V. glabra* extracts

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Extraction Solvent	1	5.610	5.6103	89.1855	< 2.2e-16 ***
Plant part	4	1.985	0.4963	7.8902	2.612e-06 ***
Organism	3	11.268	3.7560	59.7081	< 2.2e-16 ***
Time	3	0.066	0.0220	0.3492	0.7898
Residuals	2068	130.089	0.0629		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Based on this analysis part of plant used, Test- organism and extraction solvent factors were significant but the hour factor is not significant at p-value≤ 0.05

Key: Df- Degrees of freedom, Sum Sq- Sum of squares, Mean Sq- Mean Sum of squares, Pr (□F) - P value

The extraction solvent, part of plant used, and test-organism were highly significant in determining production of inhibition zones at p-value≤ 0.0 less than the accepted statistical p-value≤ 0.05 in this study. The time factor was insignificant in determining formation of inhibition zones at p-value= 0.7898, greater than the accepted p-value≤ 0.05 and therefore it was dismissed as a factor.

3.3.1 Comparison Of Antimicrobial Activity Of Organic And Aqueous Extracts Of *V. Glabra* Parts To Standard Antibiotics (Streptomycin And Nystatin) At 100mg/100µl

The organic and aqueous extracts of *V. glabra* parts used were flower, leaf, stem, root, and the whole plant for antimicrobial activity. The mean inhibition zones comparison among the

V. glabra extracts to standard antibiotics (streptomycin and nystatin) were significantly different or insignificantly different from each other at p-value≤ 0.05, (Table 5).

Table 5. Means inhibition zones (cm) comparison among organic and aqueous extracts of *V. glabra* parts used compared to Streptomycin and Nystatin

	Organic crude extracts				Aqueous crude extracts			
Crude extract	***S. aureus***	***E. coli***	***A. niger***	***C. albicans***	***S. aureus***	***E. coli***	***A. niger***	***C. albicans***
Flower	1.78d	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Leaf	1.85d	0.35b	1.43b	0.00a	0.00a	0.00a	0.00a	0.00a
Stem	0.00a	0.00a	0.18a	0.00a	0.00a	0.00a	0.00a	0.00a
Root	1.05bc	0.00a	0.00a	1.13c	0.00a	0.00a	0.00a	0.00a
Whole Plant	1.50b	0.00a	0.26a	0.00a	1.23c	0.00a	0.00a	0.00a
Control	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Streptomycin	1.30c	2.36c	NT	NT	1.30b	2.36b	NT	NT
Nystatin	NT	NT	0.83b	1.05b	NT	NT	0.83b	1.05b
Control (DCM-MEOH or H_2O)	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a

Means, within a column, followed by the same letter are not significantly different from each other at p ≤ 0.05 (Tukey's Honest Significant Difference Test).

Key: DCM-MEOH –Dichloromethane–Methanol, H_2O- sterile distilled water), NT-Not tested.

Figure 7 below shows that the organic extract of *V. glabra* flower recorded higher inhibition zone (1.78 cm) than the standard antibiotic (streptomycin MIZ of 1.30 cm). The same extract inhibited *S. aureus* at the lowest minimum inhibitory concentration (MIC of 1.5625 mg/100 µl) than streptomycin at M.I.C of 6.25 mg/100 µl.

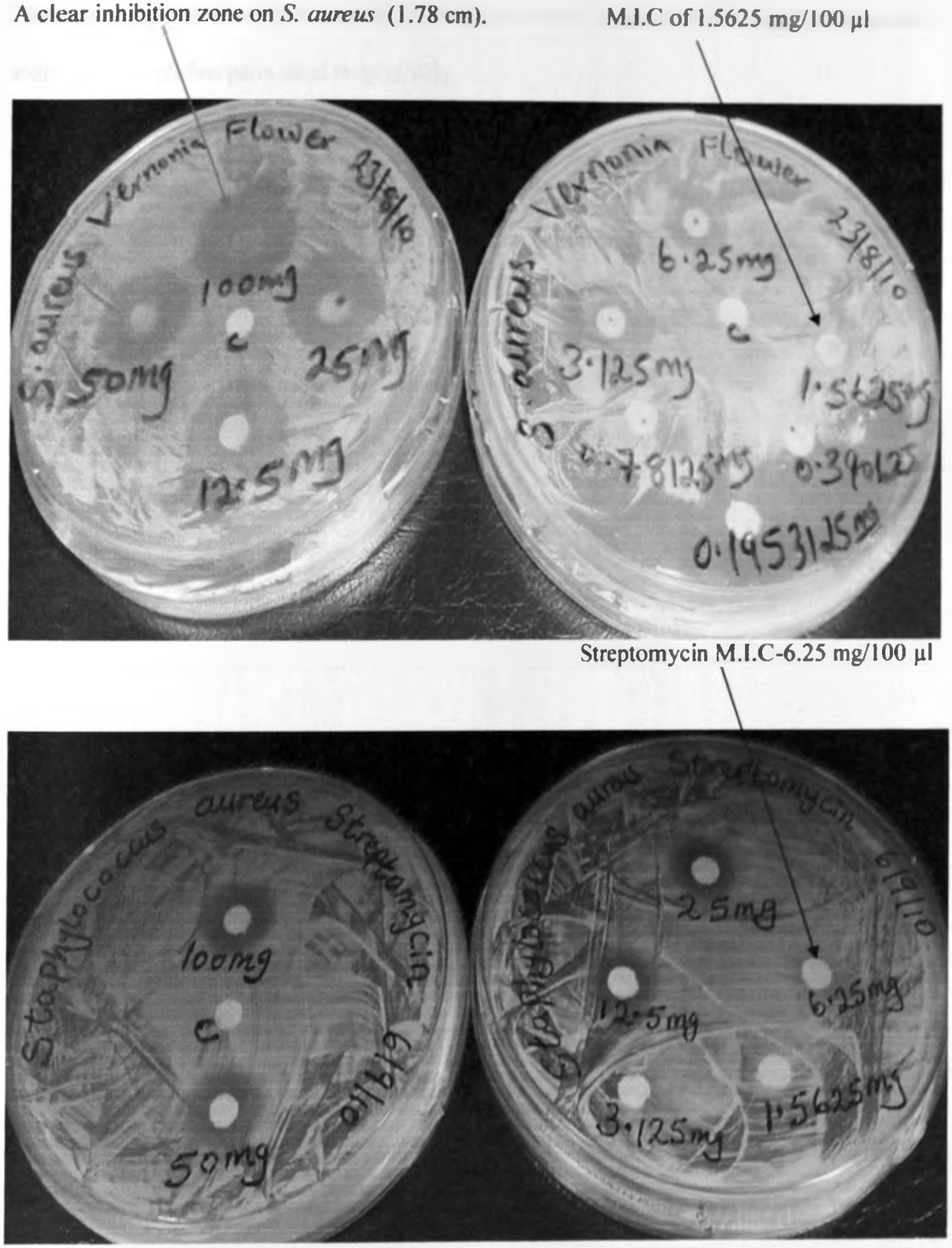

Figure 7. Inhibition zones exhibited by organic crude extract of *V. glabra* flower and streptomycin on *S. aureus* plates. Note that the flower had a lower MIC of 1.5625 mg/100 µl compared to the latter at 6.25 mg/100 µl

Figures 8 and 9 below show the mean inhibition zones produced by organic and aqueous extracts of *V. glabra* parts used respectively.

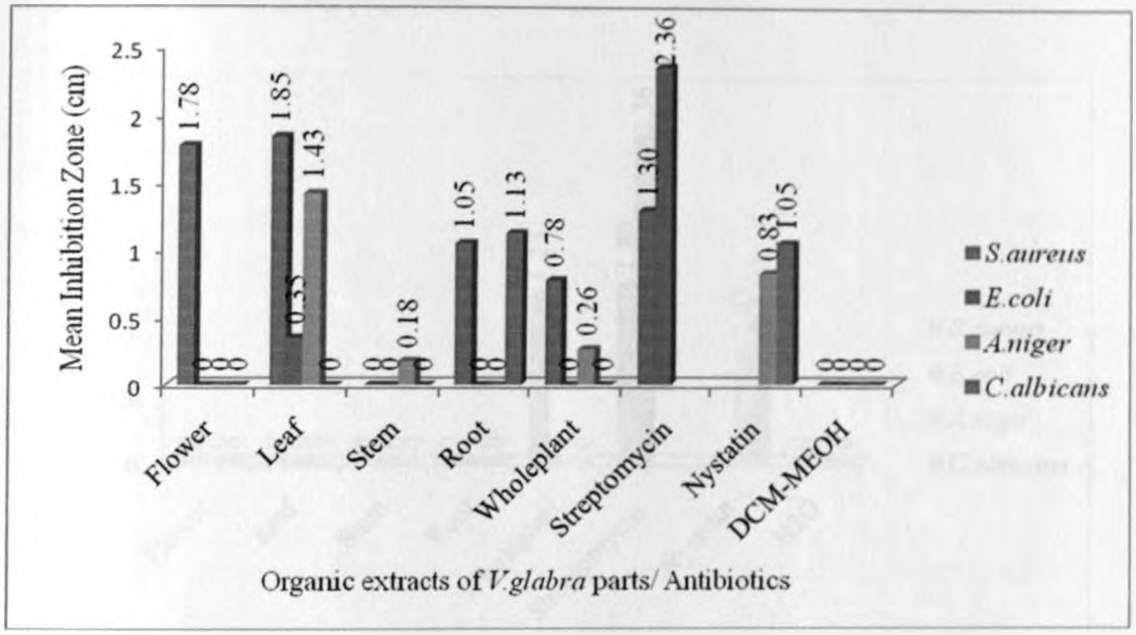

Figure 8. Antimicrobial activity of organic extracts of *V. glabra* parts compared to streptomycin and nystatin against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*

All the organic extracts of *V.glabra* tested were active at least against one of the four micro-organisms. The leaf and flower extracts showed the greatest activity against *S. aureus* (mean inhibition zones of 1.85, and 1.78 respectively). These were both higher than streptomycin activity (MIZ of 1.30). However, the leaf extract showed less activity against *E. coli* (MIZ of 0.35) compared to streptomycin (MIZ of 2.36) and moderate activity against *A. niger* (MIZ of 1.43) compared to nystatin (MIZ of 0.83). The stem extract showed activity only against *A. niger* (MIZ of 0.18); lower than that of nystatin (MIZ of 0.83). Root extract was active against *S.aureus* (MIZ of 1.05) and *C.albicans* (MIZ of 1.13), while the whole plant extract was active against *S. aureus* (MIZ of 1.50) higher than streptomycin (MIZ of 1.30), and *A.niger* (MIZ of 0.26) lower than nystatin (MIZ of 0.83).

Among the aqueous extracts of *V.glabra* parts screened, only that of the whole plant was active against *S.aureus* (MIZ of 1.23) with no significant difference in activity from Streptomycin (MIZ of 1.30) (Figure 9).

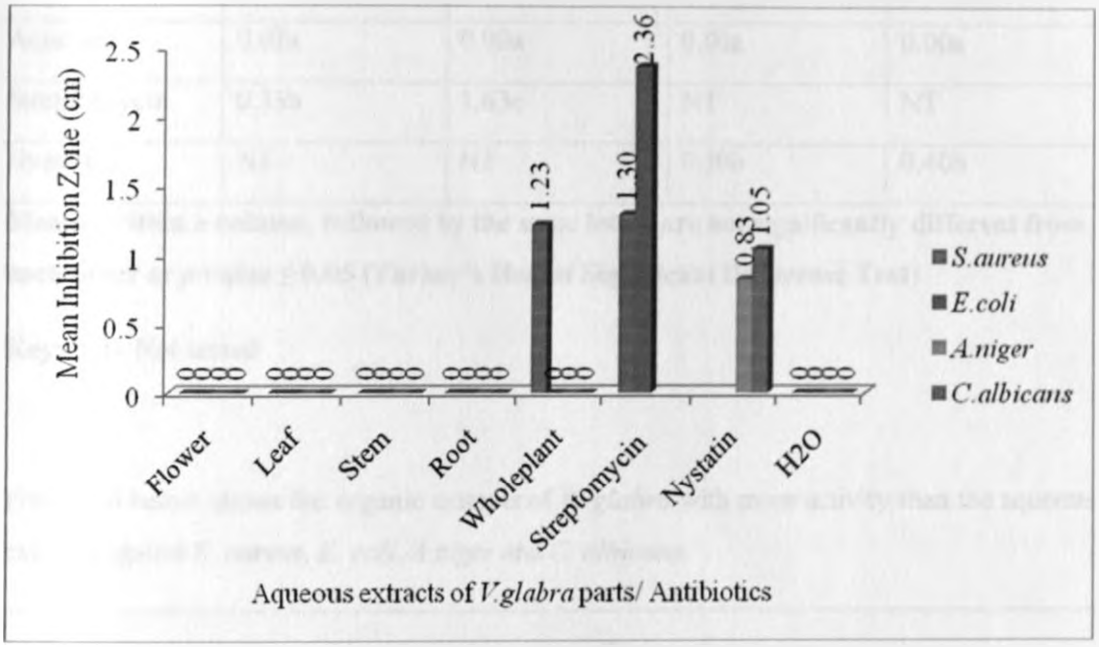

Figure 9. Aqueous extracts of *V. glabra* parts antimicrobial activity compared to streptomycin and nystatin against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*

3.3.2 Extraction solvent: Organic versus aqueous crude extracts of *V. glabra* antimicrobial activity compared to streptomycin and nystatin

The organic extracts of *V.glabra* were more active than the aqueous extracts and significantly different in activity from the aqueous extracts at 100mg/100µl concentration (Table 6).

Table 6. Means comparison among organic, aqueous extracts of *V. glabra*, Streptomycin, and Nystatin against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*

Extract	*S. aureus*	*E. coli*	*A. niger*	*C. albicans*
Organic	0.35b	0.01a	0.05a	0.03a
Aqueous	0.02a	0.00a	0.00a	0.00a
Streptomycin	0.33b	1.63c	NT	NT
Nystatin	NT	NT	0.30b	0.40b

Means, within a column, followed by the same letter are not significantly different from each other at p-value ≤ 0.05 (Turkey's Honest Significant Difference Test)

Key: NT- Not tested

Figure 10 below shows the organic extracts of *V. glabra* with more activity than the aqueous extracts against *S. aureus*, *E. coli*, *A.niger* and *C. albicans*.

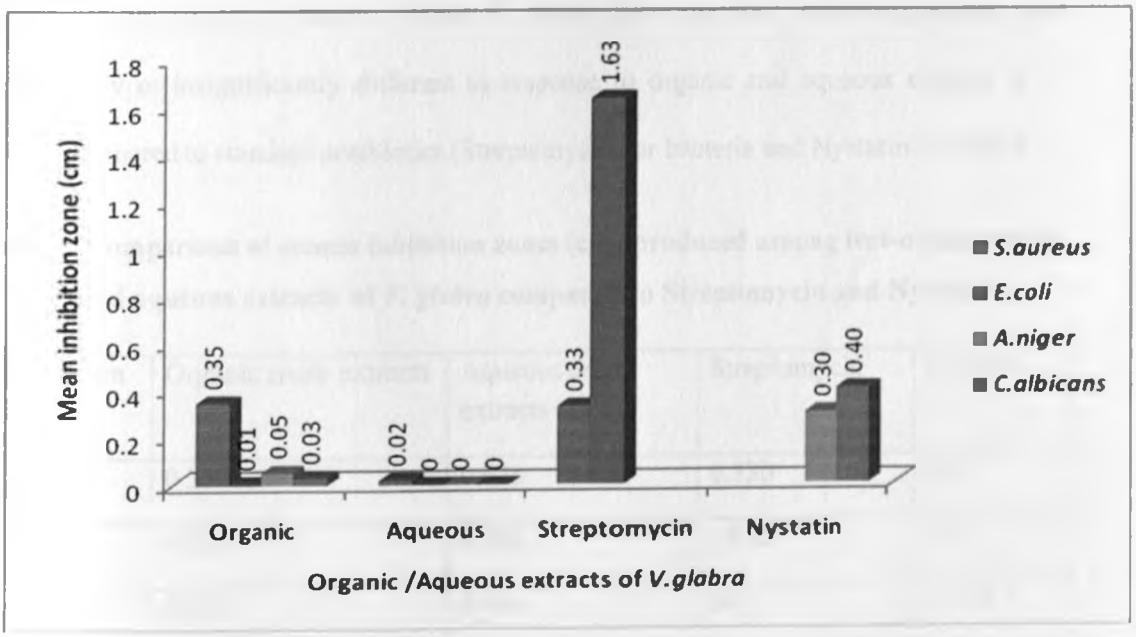

Figure 10. Organic versus aqueous extracts of *V. glabra* antimicrobial activity compared to streptomycin and nystatin

The organic crude extracts (mean inhibition zone 0.35) appear more effective than the aqueous extracts (MIZ of 0.02) against *S. aureus*, with significant difference in activity from each other, while there is no significant difference in activity of streptomycin (MIZ of 0.33) from the organic extracts against *S. aureus*. There was no significant difference in activity of organic (MIZ of 0.01) and aqueous extracts (MIZ of 0.00) against *E. coli*, where as streptomycin (MIZ of 1.63) was significantly effective against *E. coli* . The organic extracts were less effective against *A. niger* (MIZ of 0.05) and *C. albicans* (MIZ of 0.03) with no significant difference from each other, compared to nystatin with higher activity against *A.niger* (MIZ of 0.30) and *C. albicans* (MIZ of 0.40) with no significant difference in activity in both organisms (Table 6).

3.3.3 Effects of *V. glabra* Extracts on *S. aureus*, *E. coli*, *A. niger* and *C. albicans*.

The different effects on the four test-organisms by organic and aqueous extracts of *V.glabra* are shown on figure 10 above. Table 7, shows how the four micro-organisms were significantly or insignificantly different in response to organic and aqueous extracts of *V. glabra* compared to standard antibiotics (Streptomycin for bacteria and Nystatin for fungi).

Table 7. Comparison of means inhibition zones (cm) produced among test-organisms by organic and aqueous extracts of *V. glabra* compared to Streptomycin and Nystatin

Test-organism	Organic crude extracts	Aqueous crude extracts	Streptomycin	Nystatin
S. aureus	0.35b	0.02a	0.33b	NT
E. coli	0.01a	0.00a	1.63c	NT
A. niger	0.05a	0.00a	NT	0.12b
C. albicans	0.03a	0.00a	NT	0.15b

Means within the columns followed by same letter are not significantly different from each other at p-value≤ 0.05 (Tukey's Honest Significant Difference Test)

S. aureus was more sensitive to organic extracts of *V. glabra* (mean inhibition zone 0.35), with no significant difference in susceptibility to streptomycin (MIZ of 0.33), but less susceptible to aqueous extracts. *E. coli* was less susceptible to organic extracts (mean inhibition zone 0.01), while it was not sensitive to any aqueous extract, but highly sensitive to Streptomycin (MIZ of 2.36). *A. niger* and *C. albicans* were less affected by organic extracts with mean inhibition zones of 0.05, and 0.03 respectively, compared to higher activity of Nystatin (MIZ of 0.30 for *A. niger* and 0.40 for *C. albicans*) to both organisms with no significant difference in susceptibility from each other. *A. niger* and *C albicans* were not susceptible to any aqueous extracts.

3.4 Antimicrobial Activity of Organic and Aqueous Crude Extracts of *S. didymobotrya* compared to Streptomycin and Nystatin at 100mg/100µl

Analysis of variance showed that the antimicrobial activity (production of inhibition zones) of *S. didymobotrya* extracts was influenced by plant parts used, test-organisms, and extraction solvent (Table 8).

Table 8. Significance of plant parts, test-organism, and extraction solvent in production of inhibition zones by *S. didymobotrya* extracts

	Df	Sum Sq	Mean Sq	F-value	Pr(>F)
Extraction Solvent.	1	0.090	0.09009	4.9056	0.02687 *
Plant part	5	2.024	0.40482	22.0441	< 2e-16 ***
Organism	3	3.357	1.11908	60.9388	< 2e-16 ***
Time	3	0.020	0.00678	0.3691	0.77533
Residuals	2327	42.733	0.01836		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

*** Based on this analysis, plant parts, test- Organism, and extraction solvent factors are significant but time (hour) factor is not significant in production of inhibition zones at p-value≤ 0.05**

Key: Df- Degrees of freedom, Sum Sq- Sum of squares, Mean Sq- Mean of Squares, Pr (□F)- P value

Based on data analysis, only plant parts used and test organisms were highly significant at p-value≤ 0.0, while the extraction solvent (DCM-MEOH and Water) was significant at p-value≤ 0.05 in determining the production of inhibition zones (response). Time factor was insignificant in that, its p-value≥ 0.77533 is greater than the accepted statistical p-value≤ 0.05 and thus it's rejected.

3.4.1 Comparison of Antimicrobial Activity of Organic and Aqueous Extracts of *S. didymobotrya* parts with that of Standard Antibiotics (Streptomycin and Nystatin) at 100mg/100µl

The organic extracts of *S. didymobotrya* were significantly different in activity from those of aqueous extracts and standard antibiotics (Table 9).

Table 9. Means inhibition zones comparison among organic, aqueous extracts of *S. didymobotrya* parts, streptomycin and nystatin against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*

	Organic crude extracts				Aqueous crude extract			
Crude extracts	***S. aureus***	***E. coli***	***A. niger***	***C. albicans***	***S. aureus***	***E. coli***	***A. niger***	***C. albicans***
Seed	0.18b	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Seedpod	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Leaf	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Stem	1.10c	0.00a	0.00a	0.00a	1.10b	0.00a	0.00a	0.00a
Root	1.58e	0.00a	0.00a	0.00a	0.76d	0.00a	0.00a	0.00a
Whole plant	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Control	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
St	1.30d	2.36b	NT	NT	1.30c	2.36b	NT	NT
Ny	NT	NT	0.83b	1.05b	NT	NT	0.83b	1.05b

Means, within a column, followed by the same letter are not significantly different from each other at $p \leq 0.05$ (Tukey's Honest Significant Difference Test)

Key: St-Streptomycin; Ny-Nytatin, NT-Not tested

Control (DCM-MEOH 1:1 and/ or H_2O- Sterile distilled water)

Among the organic extracts of *S. didymobotrya* parts used, only the Stem, root, and seed extracts were active only against *S. aureus* (Figure 11).

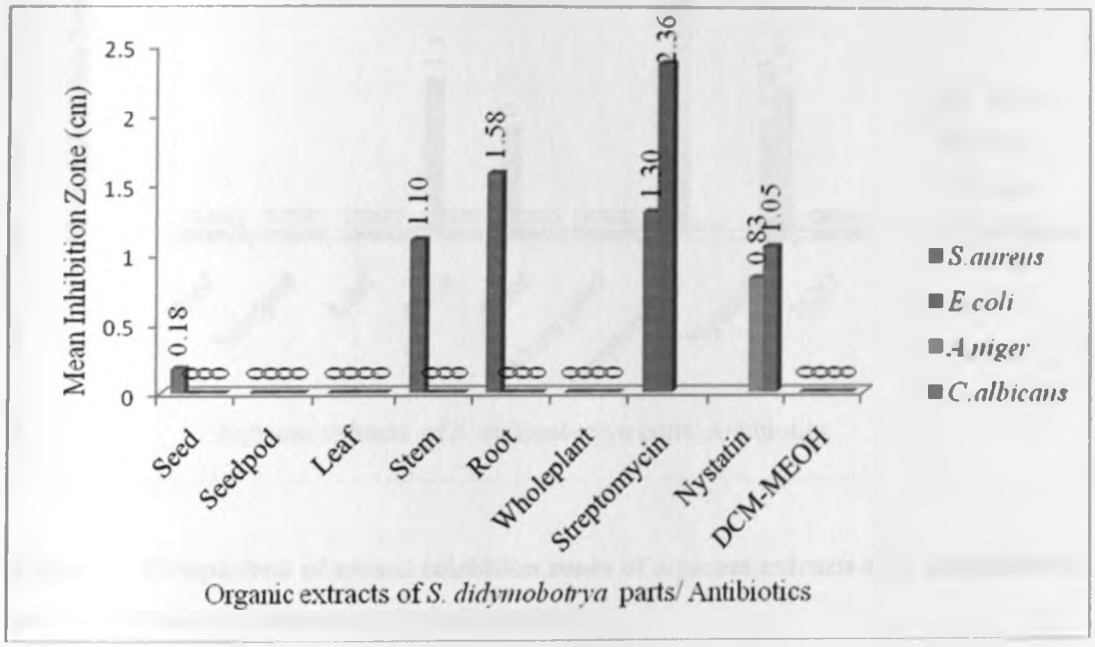

Figure 11. Mean inhibition zones of organic extracts of *S. didymobotrya* parts compared with streptomycin and nystatin

The organic crude extract of root was more active (MIZ of 1.58) against *S. aureus* with significant difference in activity from streptomycin (MIZ 1.30). Stem extract (MIZ of 1.10) was significantly different in activity from the root extract against *S. aureus*, but insignificantly different in activity from Streptomycin (MIZ of 1.30). The seed extract was less effective against *S. aureus* (MIZ of 0.18) with significant difference in activity from Streptomycin (MIZ of 1.30), root, and stem extracts. The seedpod, leaf, and whole plant extracts were not active against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*.

Only the stem and root aqueous extracts of *S. didymobotrya* were effective only against *S. aureus* (Figure 12).

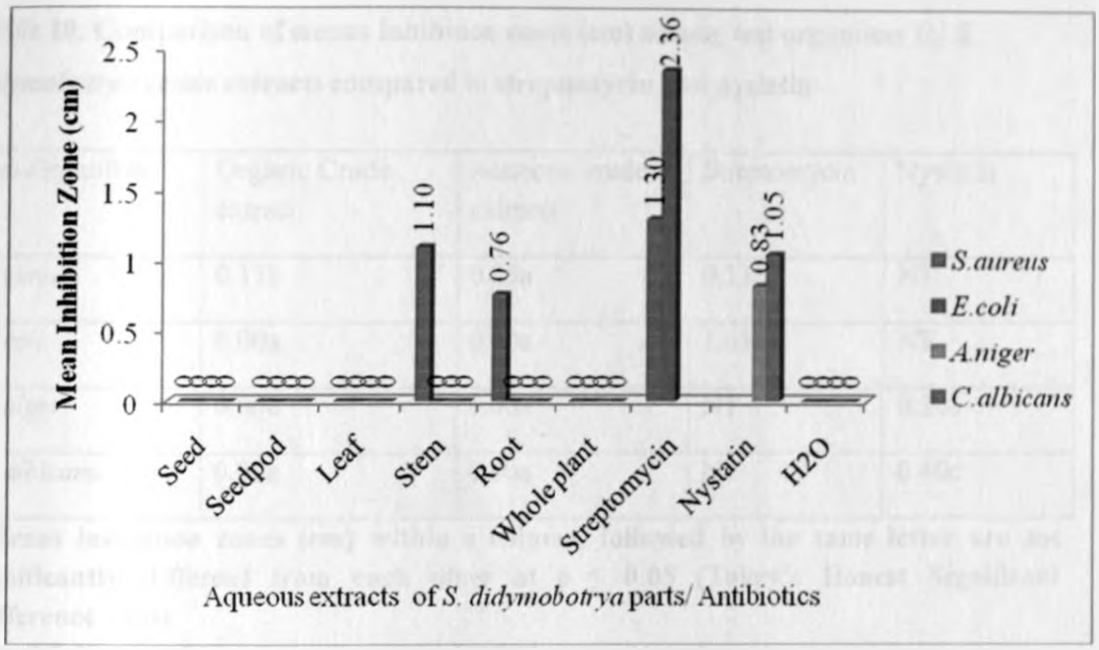

Figure12. Comparison of means inhibition zones of aqueous extracts of *S. didymobotrya* parts with those of streptomycin and nystatin

The stem extract was slightly more active (MIZ of 1.10) than the root extract (MIZ of 0.76) against *S. aureus*, with significant difference from each other. Streptomycin (MIZ of 1.30) was more active against *S. aureus* as compared to stem and root extracts.

3.4.3 Effects of *S. didymobotrya* Extracts on *S. aureus*, *E. coli*, *A. niger* and *C. albicans*

Table 10 below shows the significant difference in susceptibility of the four micro-organisms by *S. didymobotrya* extracts.

Table 10. Comparison of means inhibition zones (cm) among test organisms by *S. didymobotrya* crude extracts compared to streptomycin and nystatin

Test-Organism	Organic Crude extract	Aqueous crude extracts	Streptomycin	Nystatin
S. aureus	0.11b	0.05a	0.33c	NT
E. coli	0.00a	0.00a	1.63d	NT
A .niger	0.00a	0.00a	NT	0.30c
C. albicans	0.00a	0.00a	NT	0.40c

***Means inhibition zones (cm) within a column, followed by the same letter are not significantly different from each other at $p \leq 0.05$ (Tukey's Honest Significant Difference Test)**

Key: NT-Not tested

S. aureus was the only susceptible organism to organic extracts (MIZ of 0.11) and aqueous extracts (MIZ of 0.05) of *S. didymobotrya* with significant difference in susceptibility from *E. coli*, *A. niger* and *C. albicans* which were not affected by any of the extracts (Figure 13).

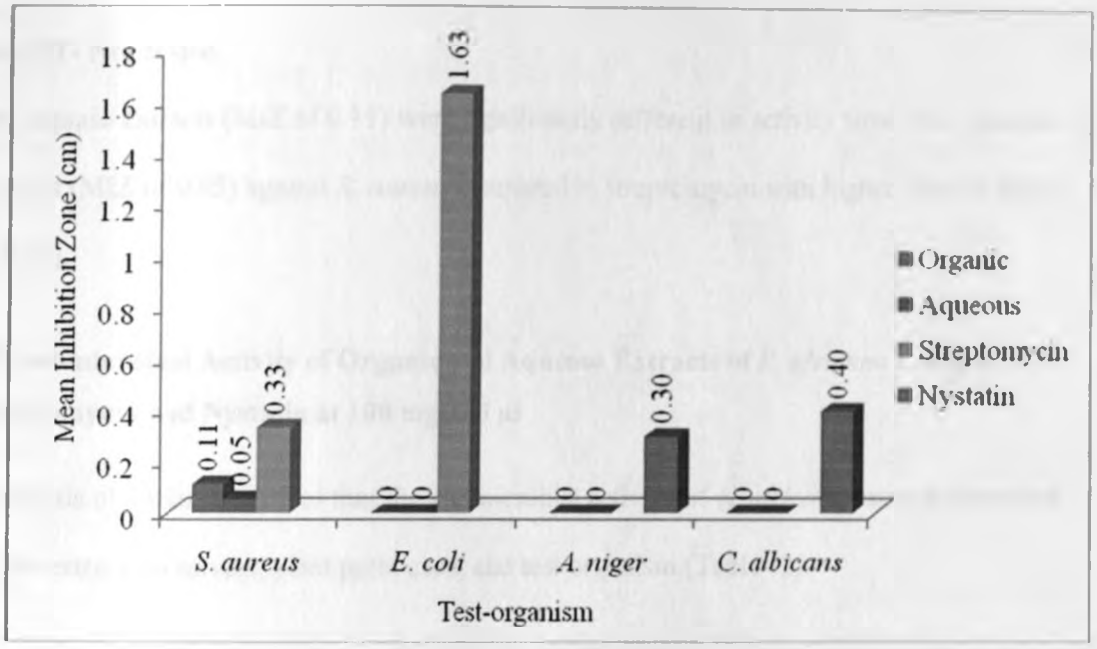

Figure 13. Mean inhibition zones (cm) comparison for different test pathogens by organic and aqueous extracts of *S. didymobotrya* and antibiotics

3.4.4 Extraction Solvent: Organic versus aqueous extracts of *S. didymobotrya*, antimicrobial activity compared to streptomycin and nystatin

Data analysis showed that extraction solvent was significant at p-value≤ 0.01, where by the organic and aqueous extracts were significantly different from each other in activity (Table 11).

Table 11. Means inhibition zones comparison among organic, aqueous extracts of *S. didymobotrya* against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*

Extracts	*S.aureus*	*E.coli*	*A.niger*	*C.albicans*
Organic	0.11b	0.00a	0.00a	0.00a
Aquous	0.00a	0.00a	0.00a	0.00a
Streptomycin	0.33c	1.63d	NT	NT
Nystatin	NT		0.30b	0.40b

***Means inhibition zones (cm) within a column, followed by the same letter are not significantly different from each other at p≤ 0.05 (Tukey's Honest Significant Difference Test)**

Key:NT- Not tested.

The organic extracts (MIZ of 0.11) were significantly different in activity from the aqueous extracts (MIZ of 0.05) against *S. aureus* compared to streptomycin with higher activity (MIZ of 0.33).

3.5 Antimicrobial Activity of Organic and Aqueous Extracts of *K. africana* Compared to Streptomycin and Nystatin at 100 mg/100 µl

Analysis of variance showed that the antimicrobial activity of *K. africana* extracts depended on the extraction solvent, plant parts used, and test-organism (Table 12).

Table 12. Significance of extraction solvent, plant parts used, and test-organism in production of inhibition zones by *K. africana* extracts

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Extraction Solvent	1	0.0812	0.081250	15.7005	7.672e-05 ***
Plant.part	5	0.1010	0.020198	3.9031	0.001593 **
Organism	3	0.2438	0.081250	15.7005	4.261e-10 ***
Time	3	0.0110	0.003683	0.7116	0.545003
Residuals	2067	10.6967	0.005175		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Based on this analysis, extraction solvent, plant part, and test-organism factors are significant, but time was not significant in determining production of inhibition zones

Key: DF- Degrees of freedom, Sum Sq- Sum of squares, Mean Sq- Mean Sum of squares, Pr (□F) - P- value

3.5.1 Comparison of organic and aqueous extracts of *K. africana* parts used in production of inhibition zones compared to streptomycin and nystatin at 100mg/100µl

Table 13 below shows significant differences in activity among the organic and aqueous extracts of *K. africana* parts used compared to standard antibiotics

Table 13. Means inhibition zones (cm) comparison among organic and aqueous extracts of *K. africana* parts to streptomycin and nystatin against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*

	Organic crude extracts				Aqueous crude extracts			
	S. aureus	*E. coli*	*A. niger*	*C. albicans*	*S. aureus*	*E. coli*	*A. niger*	*C. albicans*
Fruit	0.66c	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Leaf	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Bark	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Root	0.20b	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Whole plant	0.96d	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Streptomycin	1.30e	2.38f	NT	NT	1.30b	2.38b	NT	NT
Nystatin	NT	NT	0.83d	1.05e	NT	NT	0.83b	1.05b
Control	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a

***Means inhibition zones (cm), within a column, followed by the same letter was not significantly different from each other at p-value≤ 0.05 (Tukey's Honest Significant Difference Test).**

Key: NT- Not tested.

Only three organic extracts of *K. africana* (fruit, root, whole plant) were active only against *S. aureus*, but none of the aqueous extracts were active (Figure 14).

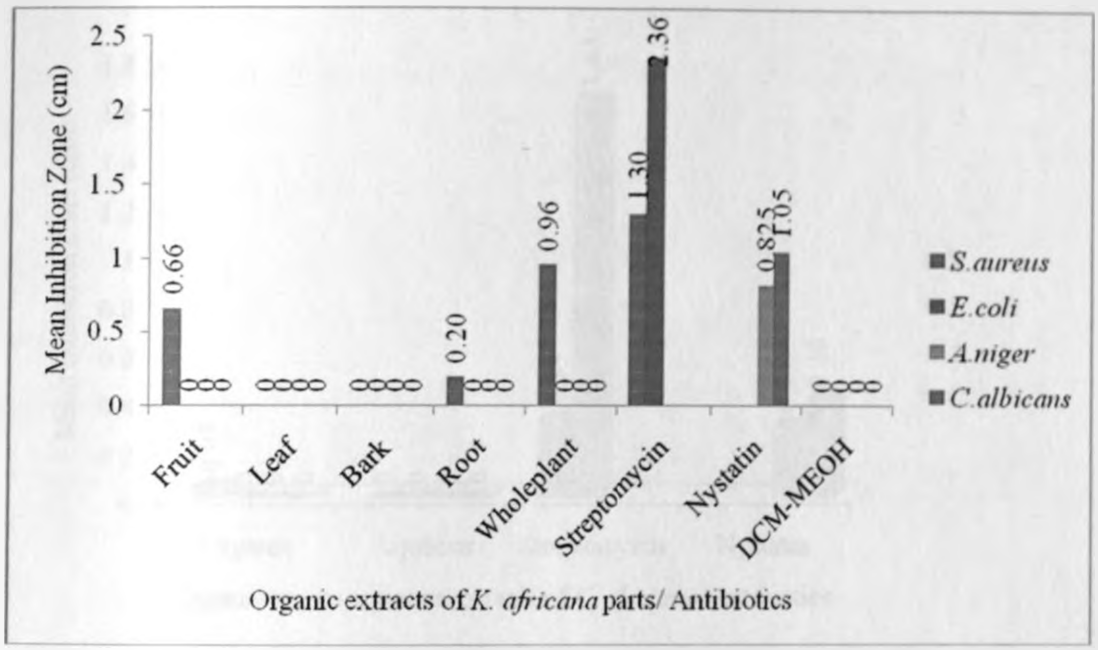

Figure 14. Organic extracts of *K. africana* parts used that determined production of inhibition zones compared to streptomycin and Nystatin against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*

The whole plant extract was high in activity (MIZ of 0.96) against *S. aureus* than the fruit extract (MIZ of 0.66), while streptomycin (MIZ of 1.30) was significantly different in activity from both whole plant and fruit extracts. The root extract (MIZ of 0.20) showed least activity against *S. aureus* compared to Streptomycin (MIZ of 1.30). All the aqueous extracts of *K. africana* were not effective against the four micro-organisms.

3.5.2 Extraction solvent: Organic versus aqueous extracts of *K. africana* as a determinant for inhibition zones production compared to streptomycin and nystatin

Only the organic extracts of *K. africana* were active against *S. aureus* (Figure 15).

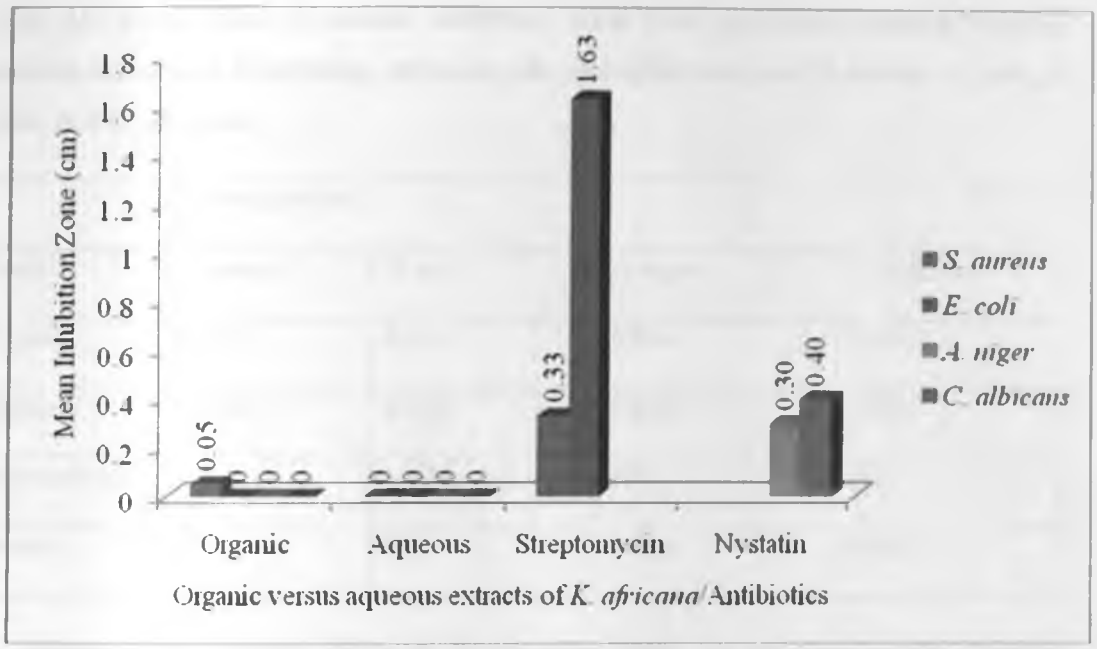

Figure15. Organic versus aqueous extracts of *K. africana* as a determinant for inhibition zones production compared to Streptomycin against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*

Only organic extracts (MIZ of 0.05) of *K. africana* were active only against *S. aureus* with significant difference in activity from the inactive aqueous extracts. Streptomycin (MIZ of 0.33) was significantly different in activity from the organic extracts against *S. aureus* (Table 14).

Table 14. Comparison of means inhibition zones (cm) production among organic, aqueous extracts of *K. africana*, streptomycin, and nystatin against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*

	Test-organisms			
Extracts	*S.aureus*	*E.coli*	*A.niger*	*C.albicans*
Organic	0.05b	0.00a	0.00a	0.00a
Aqueous	0.00a	0.00a	0.00a	0.00a
Streptomycin	0.33c	1.63d	NT	NT
Nystatin	NT	NT	0.30c	0.40c

***Means, within column followed by the same letter are not significantly different from each other at p-value≤ 0.05 (Tukey's Honest Significant Test)**

Key: NT-Not Tested

3.5.3 Effects of *K. africana* Extracts on *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*

S. aureus was the only affected test-organism by organic extracts of *K. africana* with significant difference in susceptibility from *E. coli*, *A. niger*, and *C. albicans* which were unsusceptible to any extract. *S. aureus* was moderately susceptible to streptomycin (MIZ of 0.33) at p- value ≤ 0.05 (Table 15).

Table 15. Means inhibition zones (cm) comparison among different test organisms by organic, aqueous extracts of *K. africana*, streptomycin and nyststin

Test-organism	Organic extracts	Aqueous extracts	Streptomycin	Nystatin
S. aureus	0.05b	0.00a	0.30c	NT
E. coli	0.00a	0.00a	1.63d	NT
A. niger	0.00a	0.00a	NT	0.30c
C. albicans	0.00a	0.00a	NT	0.40c

***Means within a column, followed by the same letter are not significantly different from each other at p-value ≤0.05**

Key: NT-Not Test

3.6 Comparison of Antimicrobial Activity of Organic and Aqueous Extracts of *V. glabra* whole plant, *S. didymobotrya* whole plant, *K. africana* whole plant, and the Mixture to Streptomycin and Nystatin at 100 mg/100 µl

The antimicrobial activity of the three single plants and their mixture was influenced by the extraction solvent, plant used, and test-organism factors based on the analysis of variance at p-value ≤ 0.05 (Table 16).

Table 16: Significance of extraction solvent, plant used, and test-organism in production of inhibition zones

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Extraction solvent	1	0.8113	0.81126	12.6133	0.0004321 ***
Plant used	3	1.2997	0.43324	6.7359	0.0001953 ***
Test-organism	3	5.3092	1.76975	27.5158	4.267e-16 ***
Time	3	0.2946	0.09820	1.5268	0.2071193
Residuals	373	23.9904	0.06432		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Based on this analysis, extraction solvent, plant (Treatment) used, and test-organism factors are significant at p-value ≤0.05, but the time factor is not significant

Key: Df- Degrees of freedom, Sum Sq- Sum of squares, Mean Sq- Mean of Squares, Pr (□F) – P value

3.6.1 Effects of Organic and Aqueous Extracts of Three Single Plants and their Mixture on different Test Organisms

Among the four micro-organisms used, *S. aureus* and *A. niger* were the only affected organisms. *S. aureus* was significantly affected by *V. glabra* whole plant organic and aqueuos extracts (MIZ of 1.50 and 1.23 respectively), organic extract of *K. africana whole* plant (MIZ of 0.96), and organic extract of mixture (MIZ of 1.26) compared to streptomycin (MIZ of 1.30). *A. niger* was only affected by organic extract of *V. glabra* whole plant (MIZ of 0.07), but was insignificantly different in susceptibility from *E. coli* (MIZ 0.0) and *C. albicans* (MIZ of 0.0), while they were significantly different in susceptibility from and *S. aureus* at p-value ≤ 0.05 (Table 17, Figure 16 and 17 below).

3.6.2 Extraction solvent: Organic versus aqueous extracts of three single plants and their Mixture as a determinant of inhibition zone production compared to Streptomycin and Nystatin

The organic extract of *V. glabra* (MIZ of 1.50) had a significant difference in activity from the aqueous extract (MIZ of 1.23) against *S. aureus*, but not significantly different in activity from streptomycin (MIZ of 1.30). Also, the organic extract of *V. glabra* whole was less active against *A.niger* (MIZ of 0.26) and significantly differenct in activity from that of nystatin (MIZ of 0.83). The means inhibition zones comparison among the organic and aqueous extracts of the single plants , mixture and antibiotics are shown in Table 17, and Figures 16 and 17 below.

Table 17. Means inhibition zones (cm) comparison among organic and aqueous extracts of *V. glabra* whole plant, *S. didymobotrya* whole plant, *K. africana* whole plant and their mixture compared to streptomycin and nystatin

	Organic crude plant extract				Aqueous crude plant extract			
Extracts	***S. aureus***	***E. coli***	***A. niger***	***C. albicans***	***S. aureus***	***E. coli***	***A. niger***	***C. albicans***
Mixture	1.26bc	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
V. glabra whole plant	1.50d	0.00a	0.26b	0.00a	1.23c	0.00a	0.00a	0.00a
S. didymobotrya whole plant	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
K. africana whole plant	0.96b	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
Streptomycin	1.30c	2.36d	NT	NT	1.30c	2.36d	NT	NT
Nystatin	NT	NT	0.83b	1.05c	NT	NT	0.83b	1.05c
Control	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a

***Means inhibition zones (cm), within a column, followed by the same letter are not significantly different from each other at $p \leq 0.05$ (Tukey's Honest Significant Difference Test)**

Key: Mixture- Mixture of *V. glabra* whole plant, *S. didymobotrya* whole plant, and *K. africana* whole plant, C- Control (Dichloromathane : Methanol (1:1) , or Sterile distilled water)

The three plants (*V. glabra*, *S. didymobotrya*, and *K. africana*) were tested individually and compared to their mixture and standard antibiotics. Their significant differences in activity at p-value ≤ 0.05 are shown in Table 18.

The activity of organic extract of *V. glabra* whole plant (MIZ of 1.50) against *S. aureus* was significantly higher than that of the mixture (MIZ of 1.26) as well as streptomycin (MIZ of 1.30) only against *S. aureus*. Conntrastingly, *K. africana* whole plant organic extract (MIZ of 0.96) was significantly lower in activity, compared to mixture, *V. glabra* whole plant extract, and streptomycin. *V.glabra* whole plant actvity (MIZ of 0.26) against *A. niger* was lower than that of nystatin (MIZ of 0.83), while *S. didymobotrya* whole plant extracts were not active (Figure 16).

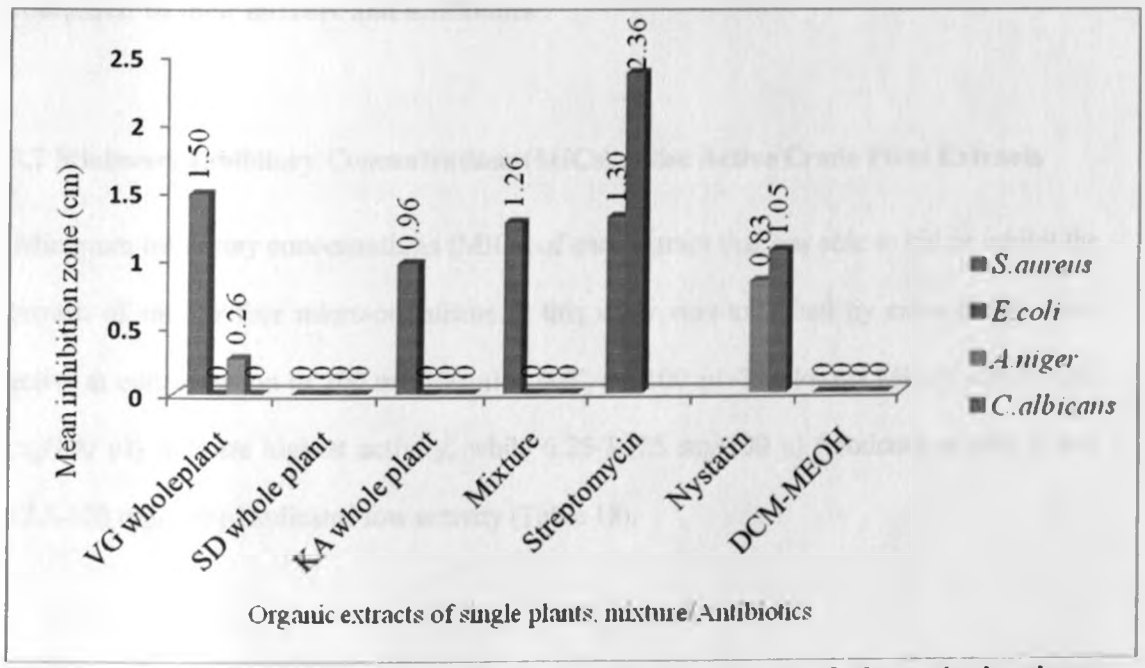

Figure 16. Mean inhibition zones (cm) of organic extracts of three single plants compared to their mixture and antibiotics

Only *V. glabra* whole plant aqueous extract was active only against *S. aureus* (Figure 17).

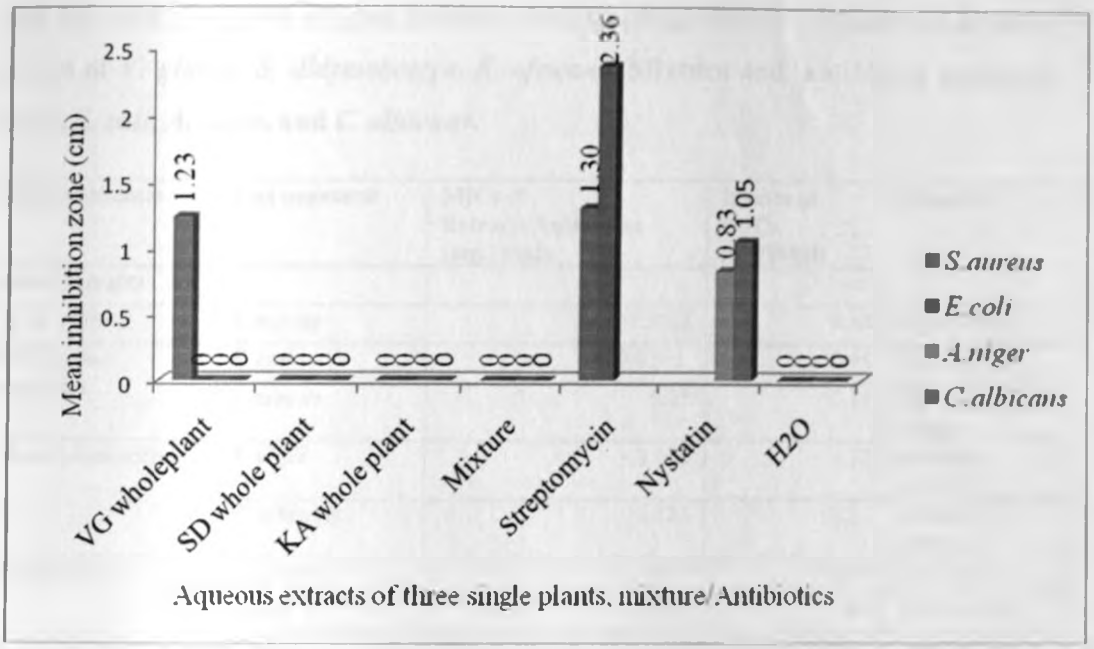

Figure 17. Mean inhibition zones (cm) of aqueous extracts of three single plants compared to their mixture and antibiotics

3.7 Minimum Inhibitory Concentrations (MICs) of the Active Crude Plant Extracts

Minimum Inhibitory concentrations (MICs) of each extract that was able to kill or inhibit the growth of one or four micro-organisms in this study was exhibited by extracts that were active at concentration of 100 mg/100 µl to 0.02 mg/100 µl. The lowest MICs (1.5625-0.02 mg/100 µl) indicate highest activity, while 6.25-3.125 mg/100 µl (moderate activity), and 12.5-100 mg/100 µl indicated low activity (Table 18).

Table 18. MICs (mg/100 µl) and inverse of MICs (mg/100µl) of organic and aqueous extracts of *V. glabra*, *S. didymobotrya*, *K. africana*, Mixture and antibiotics against *S. aureus*, *E. coli*, *A. niger*, and *C. albicans*.

Extract/Antibiotic	Test organism	MICs of Extracts/Antibiotics (mg/100µl)	Inverse of MICs (mg/100µl)	Remarks
Organic extracts				
V. glabra flower	*S. aureus*	1.5625	0.64	High activity
Streptomycin (Antibiotic)	*E. coli*	0.02+1	0.98	High activity
	S. aureus	6.25	0.16	Moderate activity
Nystatin (Antibiotic)	*A. niger*	3.125	0.32	Moderate activity
	C. albicans	3.125	0.32	Moderate activity
V. glabra leaf	*S. aureus*	12.5	0.08	Low activity
	E. coli	100	0.01	Low activity
	A. niger	25	0.04	Low activity
V. glabra stem	*A. niger*	100	0.01	Low activity
V. glabra root	*S. aureus*	25	0.04	Low activity
	C. albicans	50	0.02	Low activity
V.glabra whole plant	*S. aureus*	25	0.04	Low activity
S. didymobotrya seed	*S. aureus*	100	0.01	Low activity
S. didymobotrya stem	*S. aureus*	6.25	0.16	Moderate activity
S. didymobotrya root	*S. aureus*	6.25	0.16	Moderate activity
K. africana fruit	*S. aureus*	50	0.02	Low activity
K. africana root	*S. aureus*	100	0.01	Low activity
K. africana whole plant	*S. aureus*	100	0.01	Low activity
Mixture	*S. aureus*	25	0.04	Low activity
Aqueous extract				
V. glabra whole plant	*S. aureus*	100	0.01	Low activity
	A. niger	100	0.01	Low activity
S. didymobotrya stem	*S. aureus*	25	0.04	Low activity
S. didymobotrya root	*S. aureus*	50	0.02	Low activity

Note*: 0.02+1- 1 was added to 0.02 in order to get a value that will minimize the big range among the inverse numbers, because values without whole numbers tend to have high inverse numbers.

The inverse of MICs is displayed in Figure 18. Organic extract of *V. glabra* flower showed the highest activity with an inverse MIC of 0.64 mg/100 µl (MIC of 1.5625 mg/100 µl)

against *S. aureus*, which was high in activity than the standard antibiotic (Streptomycin) with inverse MIC 0.16 mg/100 µl. The organic extracts of *V. glabra* root and whole plant against *S. aureus*, and leaf against *A. niger and S. aureus* had low activity (Figure 18).

The organic extracts of *S. didymobotrya* stem and root against *S. aureus* recorded moderate activity (inverse MICs of 0.16 mg/100 µl each) as well as streptomycin, while its aqueous extracts of stem and root displayed low activity (inverse MIC of 0.04 and 0.02 mg/100 µl respectively).

Organic extracts of *K. africana* fruit, root and whole plant showed low activity only against *S. aureus* (inverse MICs of 0.02, 0.01 and 0.01 mg/100 µl respectively). Nystatin was moderately active against *A. niger* and *C. albicans* (inverse MICs of 0.16 mg/100 µl each) (Figure 18).

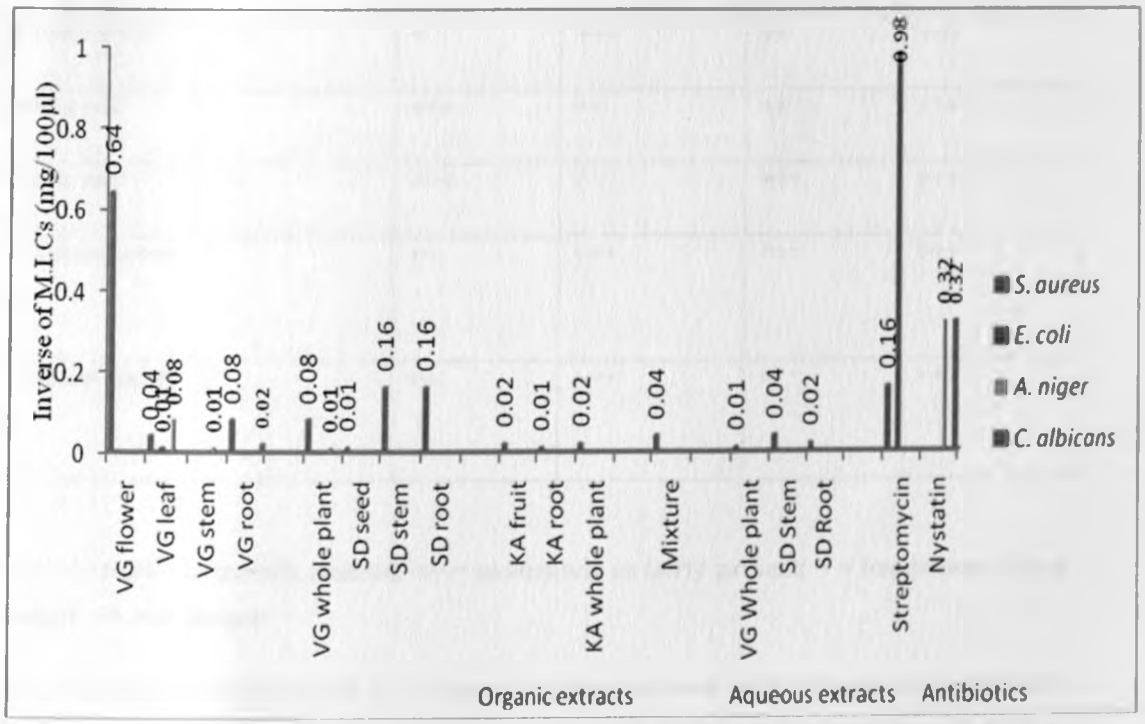

Figure 18. MICs for active organic and aqueous extracts of *V. glabra*, *S. didymobotrya*, and *K. africana* and mixture compared to streptomycin and nystatin

Key: VG- *V. glabra*, SD- *S. didymobotrya*, KA- *K. africana*.

Note: Lower inverse of MICs indicate low activity while high inverse of MICs represent high activity.

3.8 Chemical Profile of five classes of Compounds identified in Different Plant crude Extracts

The crude extracts of *V. glabra* (flower, leaf, and root) and *S. didymobotrya* (stem and root) were screened for alkaloids, sapogenins, terpenoids, quinones, and flavonoids. The presence of classes of compounds was displayed by simple scoring (Table 19).

Table 19. Five classes of compounds screened present in *V. glabra* extracts (flower, leaf, and root) and *S. didymobotrya* extracts (stem and root)

	Five classes of compounds screened present				
Extracts	**Alkaloids**	**Sapogenins**	**Terpenoids**	**Quinones**	**Flavonoids**
V. glabra flower	+++	+	+++	++	+++
V. glabra leaf	–	+++	++	++	+++
V. glabra root	++	+++	+	+++	+++
S. didymobotrya stem	+	++	+++	+++	+++
S. didymobotrya root	–	++	+++	+	+++

Key: +++= highly or greatly present; ++ = moderately or fairly present; + = less present (trace amounts); - = Not present

***Note: *K. africana* extracts were not screened for the presence of five classes of compounds, because only crude extracts that showed lowest minimum inhibition concentrations (1.5625 to 12.5 mg/100 µl) in antimicrobial activity were used for TLC analysis.**

Following screening of the five classes of compounds in all the five crude extracts, flavonoids were highly present in all extracts. Terpenoids, sapogenins and quinones sufficiently present, while alkaloids were least present because they were found only in a few extracts; *V. glabra* flower, root and *S. didymobotrya* stem.

Alkaloids were greatly present in *V. glabra* flower extract, while the root extract had moderate number of alkaloids. *S. didymobotrya* stem extract had less alkaloids. Both *V. glabra* leaf and *S. didymobotrya* root extracts had no alkaloid.

V. glabra root and leaf extracts showed high presence of sapogenins, followed by *S. didymobotrya* stem and root extracts, each showing moderate presence of sapogenins, while *V. glabra* flower had less sapogenins.

Terpenoids were highly present in *V. glabra* root, flower extracts, and *S. didymobotrya* root and stem extracts. *V. glabra* leaf extract had moderate presence of terpenoids.

S. didymobotrya root, stem and *V. glabra* root extracts showed high presence of quinones, while *V. glabra* flower and leaf extracts showed moderate number of quinones.

Flavonoids were highly present in all the five extracts; *V. glabra* flower, leaf, root, *S. didymobotrya* root and stem.

CHAPTER FOUR

4.0 DISCUSSION

Ethnomedicinal data of *K. africana* from traditional herbalist in this study revealed that, the herbal drugs were prepared through boiling, soaking in water, burning into ash and some additive (ghee) added and administered orally or dermal to treat different human ailments; such as skin lesions, diarrhoea, stomach problems, malaria, and ulcers (Table 3).

This observation is in agreement with studies by Sangita *et al.*, (2009), who reported on traditional preparation methods of *K. africana*, as powder or infusion for application or drunk to treat a wide range of skin ailments, such as fungal infections, boils and internal ailments like dysentery.

In addition, considering different ethnomedicinal uses of *K. africana* parts to manage various ailments, some of the traditional information used as a guide in evaluating the antimicrobial activity in this study turned positive; such as the antistaphylococcal activity of the organic extracts of fruit and root traditionally used to treat skin rushes/lesions (Table 4). *S. aureus* is known for causing skin rushes, boils and sores (Greenwood *et al.*, 2002 p. 170). Therefore, *K. africana* fruit and root extracts could be used to manage skin rushes by traditional healers.

This result is in agreement with studies of Houghton (2002) who worked on *K. africana* fruit and root methanol extracts following their ethnomedicinal information and found out that they were active against *S. aureus*. Therefore, there is a correlation between ethnomedicinal uses of a plant and bioactivity in a biological screening system.

The results obtained in this study showed that 44% (15 extracts) of 34 crude extracts (17 organic and 17 aqueous extracts) screened demonstrated antimicrobial activity. *V. glabra* plant (Asteraceae) was reported to have the highest number of active extracts (6 extracts)

against at least one or four micro-organisms tested (*S. aureus*, *E. coli*, *A.niger*, and *C. albicans*). *S. didymobotrya* (Leguminosae) was second in presence of active extracts (5 extracts) only against *S. aureus*. *K. africana* (Bignoniaceae) was third with little number of active extracts (3 extracts) only against *S. aureus*. The mixture of the three plants had only one active extract against *S. aureus*. According to literature done on these plants, no previous work has reported the comparison of the antimicrobial activity of these three plant families.

However, not all information on ethnomedical uses is reliable, as noted by Marston and Hostettman (1987); Silkkerveer and Silkkerveer (1995). This statement supports the findings in this study whereby, 19 crude extracts (56%) (against) *S. aureus*, *E. coli*, *A. niger*, and *C. albicans)* screened to give positive results failed. Some of these ineffective plant extracts are probably used to treat diseases caused by non bacterial and non fungal pathogens. Among other possible reasons for this are the methods used in preparation and administration of the traditional medicine compared to scientific methodology applied in this study. For example, the leaf extracts from *K. africana* had no bacteriotoxic or fungitoxic effects on any of the four micro-organisms, although traditionally leaves are burned, mixed with ghee and licked for treatment of stomach ulcers (Table 4) caused by a bacterium known as *Helicobacter pylori*. In this case, the addition of ghee could have potentiated bacteritoxicity or fungitoxicity of the extract. This is in agreement with the studies of Otieno, *et al.*, (2007) who reported that, a mineral *Kadosero* supplemented to plant extracts increased antimicrobial activity and thus used in traditional healing systems in Siaya district- Kenya. Also, burning might have affected its antimicrobial activity too.

The extent of the antimicrobial activity in this study was related to the extract of the plant part used. The organic extract of *V. glabra* leaf elicited the highest mean inhibition zone of 1.85, followed by organic extract of flower (MIZ of 1.78) against *S. aureus*. Still all demonstrated greater activity than streptomycin (MIZ of 1.30). These two organic extracts

with higher MIZs than the standard antibiotic, have never been reported in any antimicrobial research work. This could be the first report and they can be used for management of Staphylococal infections such as skin rushes. These findings are in agreement with the studies of Abubakar *et al.*, 2011, who also reported on other species in the same genus. They found organic extracts of aerial parts of *Vernonia ambigua*, *Vernonia blumeoides*, and *Vernonia oocephala* exhibited large inhibition zones on *S. aureus*, but less inhibition compared to streptomycin. These data provided some justification in their use by traditional medicinal practitioners for treatment of boils, sores, and open wounds.

Intermediate active extracts were reported in organic extracts parts of *V. glabra* leaf against *A. niger* (MIZ of 1.43) compared to nystatin (MIZ of 0.83) and organic extract of root (MIZs of 1.13 against *C. albicans* and 1.05 against *S. aureus*). Aqueous extract of *V. glabra* whole plant was also moderately active against *S. aureus* (MIZ of 1.23) compared to streptomycin (MIZ of 1.30) and organic extracts of *S. didymobotrya* root and stem (MIZs of 1.58 and 1.10 respectively) against *S. aureus*. These results are significant in early stages of drug research as reported by Ochei and Kolhatkar, (2000 p. 811) that intermidiate susceptibility results indicate that the therapy with the drug can be successful only if it is administered in larger doses or when the drug is concentrated at a certain site such as urinary tract or dermal.

Some of extracts of plant parts were less active against test micro-organisms, including organic extract of *V. glabra* leaf against *E. coli* (MIZ of 0.35), stem against *A. niger* (MIZ of 0.18), organic extract of *S. didymobotrya* seed agaist *S. aureus* (MIZ of 0.18) ,aqueous extract of *S. didymobotrya* root (MIZ of 0.76) against *S. aureus*, and organic extracts of *K. africana* root, fruit, and whole plant (MIZs of 0.20, 0.66, and 0.96 respectively) aginst *S. aureus*. Some extracts of plant parts were not active against any of the four micro-organism tested in this study. These were *S. didymobotrya* leaf, seedpod, and *K. africana* leaf and bark. The less

activity and inactivity of these extracts could have been caused by the seasonal variations in collection of plant material and the part of plant species at different stages of the plant growth and development that affected the chemical composition of the plants and thus its antimicrobial activity. Therefore, the antimicrobial agents were either present in very little quantities or totally absent. This argument is in line with that of Birdi *et al.*, (n.d) who argues that seasonal variations and part of plant species harvested at different stages of growth and development, can affect chemical composition of the plants, and thus its biological activity. In most cases, maximum accumulation of chemical constituents occur at the flowering season and declines at the beginning of the fruiting stage and this can lead to either presence of chemicals in high or very low quantities or totally absent in matured plants.

Results of this study did show some extracts of plant parts were more active than those of the entire plant extracts. Organic extract of *V. glabra* leaf (MIZ of 1.85) and flower (MIZ of 1.78) had higher activities compared to that of whole plant (MIZ of 1.50), while organic extract of *S. didymobotrya* entire plant was inactive compared to its single parts; stem and root extracts (MIZs of 1.10 and 1.58 respectively).

This observation indicates that, the interactions of the active chemical substances present in crude extracts of plant parts used, formed strong synergistic effects to be able to inhibite the growth of test organisms, compared to those of the entire plants which had intermediate effect, less effect or no effect against the four micro-organisms tested. This observation is in line with the studies of Ibrahim *et al.*, (2009) who did research on *V. amygdalina* and *Occimum gratissimum* and found out that parts of these plants were more active when used singly than when the entire plant parts are combined.

Moreover, when the antimicrobial activity is compared among the three entire plant species and their mixture, the organic extract of *V. glabra* (MIZ of 1.50) showed high activity than

the mixture (MIZ of 1.26), yet traditional practitioners usually deliver these in combination. The organic extract of *K. africana* entire plant (MIZ of 0.96) and *S. didymobotrya* inactive extract (MIZ of 0.0) were not significantly different in activity from the mixture extract. This result may have indicate that chemicals present in *V. glabra* extract had synergistic effects or potentiated the high effecacy of this extract, while the chemical compounds present in the extracts of the mixture, *K. africana*, and *S. didymobotrya* exhibited antagonistic effect that could have been caused by the interaction of its active parts extracts, and therefore negating or lessening the activity of each other. This study may be an eye open for traditional medicine, in that, the idea of crude extracts combination may not always work better for management of some infectious diseases.

These findings correlate with the results of Maryam, *et al.*, 2010 who did research on different plant parts and mixtures (combinations) of plant extracts and found that, plant extracts or phytocompounds exhibiting strong antimicrobial activity may interact with each other and the interactions may be synergistic or antagonistic. Therefore, the synergistic effects of these plant extracts in this study would be of significant importance in further search of novel compounds, with desirable synergistic effects, to kill persistent micro-organisms that are resistant to known antibiotics and delay emergency of microbial resistance.

Based on a literature review of *V. glabra*, no previous work had reported the antimicrobial activity of organic extracts of *V. glabra* leaf (against *S. aureus*, *A. niger*, and *E. coli*), flower extract against *S. aureus*, stem extract against *A. niger*. This could be the first report from the results of this study and exhibiting a broad-spectrum activity against four test organisms, it could be a start point for further drug research on a wide diversity of micro-organisms.

The patterns of antimicrobial activity varied with the plant extract and the solvent used for extraction. The organic crude extracts showed more inhibition than the aqueous extracts on the average for all micro-organisms tested. This is seen in more active organic extracts of *V. glabra* (MIZ of 0.35) compared to its aqueous extracts (MIZ of 0.02) against *S. aureus*, organic extracts of *S. didymobotrya* (MIZ of 0.11) compared to its aqueous extracts (MIZ of 0.05), and organic extracts of *K. africana* (MIZ of 0.05) compared to its inactive aqueous extracts (MIZ of 0.0) against *S. aureus*. These were compared with streptomycin (MIZ of 0.33) against *S. aureus*.

Since traditional herbal remedy preparation use water as the extractant, it is a paradox that the aqueous extracts were inactive in this study. It is possible that the aqueous crude extracts may contain antimicrobial constituents insufficient for efficacy in our study and which may explain why large amounts of the decoctions must be drunk by the patients. This observation is supported by Jigna and Chanda, (2007) who also found out that, aqueous extracts showed little or no antimicrobial activity in contrast to those made using organic extracts.

Another reason for the ineffectiveness of aqueous extracts could be that the active compounds were not soluble in water, or the aqueous extracts in this study were not prepared according to traditional methods, which in many cases involves boiling and soaking with water for several hours (Clarkson *et al.*, 2004). Success in traditional medicines may be due to administration of the extracts in large quntities and over a long period of time (Yineger *et al.*, 2008).

Sensitivity of the test organisms to the plant extracts varied depending on the micro-organism. Most of the extracts were antibacterial especially against *S. aureus*, which was the most susceptible bacterium to the extracts compared to less affected *E. coli*. Differential sensitivity of bacteria to plant extracts, may be explained by the cell wall composition of

Gram-positive and Gram-negative bacteria. The cell wall of gram-negativeve bacterium (*E. coli*), contains an outer membrane and lipid bilayer embedded with proteins and porins (carrier proteins). These proteins allow passege of certain small molecules or ions either into or out of the cell periplasm. The active compounds may not be able to pass into the cells, making them inactive. The size of the porin channel particularly determines the size of the molecule that can pass through it and thus, the outer membrane serves as a barrier to the passage of many molecules and excludes many toxic compounds, and hence less sensitive to many extracts. However, the Gram-positive bacterium (*S. aureus*) has a relatively thick membrane consisting of layers of peptidoglycan, but regardless of its thickness, peptidoglycan is fully permeable to many substances including sugars, ions, and amino acids, and thus sensitive to most extracts (Nester *et al*., 2004).

Most of the plant extracts were less antifungal or inantifungal. *A. niger* was moderately susceptible compared to less affected *C. albicans*. The less active and inactive plant extracts against yeast fungus (*C. albicans*) compared to filamentous fungus (*A. niger*) may be due to differences in cell wall composition. Yeast fungus cell wall contains polyssacharides and proteins, compared to chitin and glycan in the cell walls of filamentous fungi (Paiva *et al*., 2010). The proteins expression of *C. albicans* function as a selective transport system to expell wastes and compounds that are deleterious to the cell. This functions as an efflux which is medically important in that, it allows micro-organisms to oust antimicrobial medications that are made to destroy them, and therefore render them inactive (Nester *et al*., 2004). This could be one of the reasons why the plant extracts were ineffective against *C.albicans*. The report of this study is in agreement with the findings of Masakazu *et al*., (2010) who reported that expression of drug efflux pumps were responsible for the inactivity of drugs to *Candida* spp.

Minimum Inhibitory Concentration (MIC) is important to confirm resistance of micro-organisms to an antimicrobial agent and also monitor the activity of new antimicrobial agent (Das *et al.*, 2010). MIC of the plant extracts ranged from 100mg to 1.5625mg/100µl. The organic extract of *V. glabra* flower showed the lowest MIC of 1.5625mg/100µl against *S. aureus*, even lower than streptomycin (MIC of 6.25mg/100µl). The activity of *V.glabra* flower at very low MIC of 1.5625mg/100µl has not been reported in any research work and these findings from this study are being reported for the first time. *V. glabra* flower activity at low concentration even lower than the standard antibiotic (streptomycin), is significant in that, it has been identified as a new antstaphylococcal extract in this study. This extract may be used for discovery of novel compounds at low concentrations with new mechanisms to combat bacterial strains unsensitive to drugs, shorten the length of treatment and increase patient compliance, and avoid overdose which may lead to toxicity or side effects to patients. This observation is collaborated by the studies of Mariita *et al.*, (2010); Aiyegoro and Okoh, (2009), who reported that bioactive extracts of medicinal plants at low concentrations are not only active against unsusceptible bacteria, but may also kill persistent bacteria such *S. aureus*, shorten the length of treatment and reduce overdose and toxicity.

This study reports that flavonoids were highly present in all extracts that were screened; *V. glabra* flower, leaf, root extracts and *S. didymobotrya* stem and root extracts. The presence of flovonoids in *V. glabra* has not been reported in any other research work and this could be the first time to report in this study. The *Vernonia* genus is reported to produce flavonoids, for example, *V. amygdalina* has been reported for the production of flavonoids (Farombi and Owoeye 2011). *S. didymobotrya* stem has been reported to produce flavonoids (Korrir *et al.*, 2012), but the root extract has never been reported to have flavonoids. Flavonoids have been associated with inhibition of cytoplasmic membrane functions as well as inhibition of of DNA gyrase enzyme and carrier protein activities (Paiva *et al.*, 2010). This property of

flavonoids may explain why most of the crude extracts were active against atleast one or four micro-organisms tested in this study.

Terpenoids were the second class of compounds present in this study. They were adequately present in *V. glabra* root, flower extracts, and *S. didymobotrya* root and stem extracts. *V. glabra* leaf extract had moderate presence of terpenoids. Literature has reported presence of terpenoids (sesquiterpene lactones and triterpenes) in *V. glabra* aerial parts (Jokupovic *et al.*, 1985; Bohlmann *et al.*, 1983), but no work has reported presence of terpenoids in the root extract. The presence of terpenoids in *S. didymobotrya* stem has been reported (Korrir *et al.*, 2012), but for the root extract has not been reported in any other research work. Other species in genus *Senna* such as *Senna obtusifolia*, has been reported to produce terpenoids (Sudi *et al.*, 2011). The significance of terpenoids in medical application is reported to be useful in human healthcare. For instance, those with antimicrobial mechanism of action, influence membrane structures which increase membrane fluidity and permeability, changing the topology of membrane proteins and inducing disturbances in the respiration chain of a microbial pathogen (Paduch *et al.*, 2007). Therefore, these plant extracts with terpenoids in this study can be used to manage infectious diseases caused by microbial pathogens.

Sapogenins were also reported sufficiently present in this study; especially the *V. glabra* root and leaf extracts, while they were moderately present in *S. didymobotrya* stem and root extracts and in trace amounts in *V. glabra* flower extract. No previous work has reported the presence of sapogenins in *V. glabra* and *S. didymobotrya* extracts screened in this study. Kareru *et al.*, (2008) reported presence of saponins in *S. didymobotrya*, which are in the same class of compounds as sapogenins, but differ from each other in that saponins are linked to glycosyl group, while sapogenins are aglycosidic (Harborne, 1998). Sapogenins have been associated with hemolysis property of microbial cells (Segal *et al.*, 2006) and this property

could enhance the antimicrobial activity of plant extracts with sapogenins in this study for management of infectious diseases.

Quinones were sufficiently present in *S. didymobotrya* stem and *V. glabra* root extracts. *V. glabra* flower and leaf extracts had moderate amount of quinones, while *S. didymobotrya* root extract had trace amounts. The presence of quinones in *V. glabra* extracts have not been reported in any work and this is the first time to report in this study. Genus *Vernonia* has not been reported to produce any quinones in the previous research works, but *Calendula officinalis* in Asteraceae family has been reported to produce quinones (Muley et al., 2009). El-sayyad *et al.*, (1988); Classen, (1977) in their research findings reported presence of quinoids in *S. didymobotrya* entire plant and leaf. Quinones have been reported to have propable targets in the microbial cell such as, surface-exposure adhesins, cell wall polypeptides, membrane-bound enzymes and render substrates unavailable to the micro-organism (Review of literature, n.d). These properties associated with quinones could be significant in medicine for extracts with quinones in this study for use in management of microbial pathogens.

Alkaloids were present in greatly present in *V. glabra* flower extract, fairly present in root extract, and present in trace amounts in *S. didymobotrya* stem. *V. glabra* leaf and *S. didymobotrya* root extracts had no alkaloids. The presence of alkaloids in *V.glabra* flower and root extracts have not been reportedin any another work and are been reported in the first time . Other *Vernonia* species, for example, *V. ambigua*, *V. blumeoides*, and *V. oocephala* were reported to produce alkaloids (Abubakar, *et al.*, 2011). Nyaberi *et al.*, (2007) in their research reported that *S. didymobotrya* stem alkaloids were less present. Alkaloids are associated with the common biological property of toxicity against cells of organisms (Igbinosa *et al.*, 2009), and this could explain why the organic extract of *V. glabra* flower was highly active only against *S. aureus* at low concentration.

CHAPTER FIVE

5.0 Conclusions and Recommendations

5.1 Conclusion

The screening for antimicrobial activity of the thirty-four crude plant extracts from three species in different families collected using available ethnomedical information, has verified that Kenyan medicinal plants have potential as new sources of antibacterial and antifungal agents. Organic crude extracts of *V. glabra* (flower, leaf, stem and root), *K. africana* (fruit, root and whole plant) and *S. didymobotrya* (stem and root) were active against one or four microbial pathogens tested. This shows the potency of these medicinal plants to treat bacterial and fungal diseases. Therefore, this study justifies the use of these plants by traditional healers/ or herbalists and thus may be the starting point of research as phytomedicines for the society's healthcare.

The high antistaphylococcal activity of organic extract of *V. glabra* flower at lower MIC than the standard antibiotic (streptomycin), shows the high potency of this extract and validates its use in traditional medicine. This observation justifies that, medicinal plants in Kenya have potential antimicrobial agents that can be used to curb microbial diseases at lower concentrations, shorten lengthy treatment, and avoid overdose and toxicity.

Findings of this study showed that flavonoids were present in great amounts in all the extracts screened, while terpenoids, sapogenins and quinones were present in sufficient amounts and were the four major classes of phytochemical compounds present in almost every extract screened. Alkaloids were highly present only in *V. glabra* flower extract and moderately present in the root extract. The highly presence of these classes of compounds in different crude extracts , suggests the richness of Kenyan medicinal plants with diverse phytochemicals. These chemicals can be used for research in development of new compound with antimicrobial activities for management of infectious diseases.

5.1.2 Recommendations

As only *in vitro* method was used in assessing the antimicrobial activity of the plant crude extracts, further investigations using bioassay guided fractionations are recommended to isolate and identify the pure compounds responsible for antibacterial and antifungal activities of *V. glabra* which was more active of the three plants and less researched.

Toxicology research which is missing in this study, is recommended for *V. glabra* which showed more active extracts, in order to verify and document the safety of this medicinal plant to the society.

The antimicrobial activity of mixture of *V. glabra*, *S. didymobotrya* and *K. africana* medicinal plants was compared with those of their singles against the four test organisms. The high activity of *V. glabra* whole plant than the mixture against *S. aureus* is not well understood. Therefore, further investigation on synergistic and/ or antagonistic effects of these plants and their mechanisms of action is recommended.

While most of the major phytochemical classes of compounds were identified in most extracts, further investigation is recommended to identify the compounds responsible for antimicrobial activity and especially in *V. glabra* flower extract which was more active than the standard antibiotic (streptomycin) and may be can be used as phytochemical indicator.

6.0 REFERENCES

Abubakar, B.A., Aliyu, M.M., Mikhail, S.A., Hamisu, I., Adebayo, O.O., 2011. Phytochemical Screening and Antibacterial Activities of *Vernonia ambigua. Vernoniablumeoides*, and *Vernonia oocephala. Acta Poloniae Pharmceutica-Drug Research*, **68** (1): 67-73.

Adenisa, S.K., Idowu, O., Ogundaini, A.O., Oladimeji, H., Olugbade, T.A., ... Pais, M., 2000. Antimicrobial Constituents of Leaves of *Acalypha wilkesiana* and *Acalypha hispida. Phytotheraphy Research,* **14**: 371-374

Agnew, A.D.Q. and Agnew, S., 1994. *Upland Kenya Wild Flowers*, 2nd Ed. East Africa Natural History Society.

Aiyegoro, O.A. and Okoh, A.I., 2009. Use of bioactive plant products in combination with standard antibiotics: Implications in antimicrobial chemotherapy. *Journal of Medicinal Plants Research*, **3** (13): 1147-1152.

Akunyili, D., Houghton, P., 1993. Monoterpenoids and napthaquinones from *Kigelia pinnata* Phytochemistry. **32**: 1015- 1018.

Altschuler, J.A., Casella, S.J., Mackenzie, T.A., Curtis, K.M., 2007. Herbal Medicine. The effect of Cinnamon on AIC among adolescents with type 1 diabetes. *Diabetes Care.* **30** (4): 813-816.

Arruda, A. L. A., Vieira, B. J. C., Sousa, G. D., Oliveira, F. R., Castilho, O.R., 2011. Jacaranda cuspidifolia Mart. (Bignoniaceae) as an Antibacterial Agent. *Journal of Medicinal Food*, 14(12): 1604-1608.

Audain, K., 2012. History, Scope and Importance of Medicinal Plants. Available: www.ehow.com/about_6743739_history_scope-importance-medicinal-plants.html. [Accessed 16th April, 2012].

Balick. M.J., 1990. Ethnobotany and Identification of therapeutic agents from the rain forest. In Bioactive Compounds from Plants. CIBA foundation symposium 154. New York. John Willy and Sons: 23-39.

Beentje, H.J., 1994. *Kenya trees, shrubs and Lianas*. National Museums of Kenya. Nairobi.

Bishnu, J., Sunil, L., Anuja, S., 2009. Antibacterial Property of Different Medicinal Plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana*. *Kathmandu University Journal of Science, Engineering and Technology* **5**(1): 143-150.

Birdi, T.J., Brijesh, S., Daswan, P., (n.d). Approaches towards the preclinical testing and standardization of medicinal plants. *Foundation for Medical Research India*. Online: www.aifo.it/english/resources/online/books [accessed 25th April 2012]

Bloom, D.E. and Canning, D., 2008. Population Health and Economic growth. Commission on Growth and Development. Working Paper No. 24. Available: http://growthcommission.org [accessed 23rd September 2011].

Bohlmann, F., Ates, N., Jakupovic, J., 1983. Naturally Occurring Terpene Derivatives. 464. Hirsutinolides from South African *Vernonia* Species. *Phytochemistry*, **22** (5): 1159-1162

Boily, Y. and Puyvelde, V.L., 1986. Screening for Medicinal Plants of Rwanda (Central Africa) for Antimicrobial activity. *Journal of Ethno pharmacology* **16** (1): 1-13.

Brown, S., 2012. Comparing More Than Two Means: One-Way ANOVA. Available: www.tc3.edu/instruct/sbrown/stat [accessed 23rd May 2012]

Burapadaja, S. and Buchoo, A., 1995. Antimicrobial activity of tannins from Terminalia citrina. *Journal of Medicinal Plant and Natural Product Research*. **61**:365-366.

Cabalio, P., Fournet, A., Mangeney, P., Bouguet. A., 1980. Preliminary Chemistry Research of the Plants of the Peoples Republic of Congo. *Fitoterapia* **51**: 89-111.

Clarkson, C., Maharaj, V.J., Crouch, N.R., Olwen, M.G., Pillay, O.M., Matsabisa, M.G., Bhagwandin, N., Smith, P.J., Folb. P., 2004. *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *Journal of Ethnopharmacology*, **92**: 177-191

Classen, A., 1977. Anthraquinones of *Cassia didymobotyra*. *Journal of Plant Medicine and Phytotherapy*. **11:** 91.

Clinical and Laboratory Standards Institute, 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-11th Edition. **32**(1): 3-236. Available: www.clsi.org/source/orders/free/m02-a11.pdf [accessed 28th April 2012]

Colombo, L.A., Mcgough, D.A., Rinaldi, M.G., 1994. Discrepancies between MIC and MLC values of Amphoteric B against isolates of *Aspergillus* species. *Mycopathologia* **128**: 129-133.

Croteau, R., Kutchan, M. T., Lewis, G. N., 2000. *Biochemistry and Molecular Biology of Plants: Natural Products (Secondary Metabolites). American Society of Plant Physiologists*, pp. 1250-1317.

Das, K., Tiwari, R.K.S., Shrivastava, D.K., 2010. Techniques for Evaluation of Medicinal Plant Products as Antimicrobial Agents: Current Methods and Future Trends. *Journal of Medicinal Plants Research*, **4**(2): 104-111.

Dharani, N., 2002. *Trees and Shrubs of East Africa.* New Holland Publishing Cape Town (South Africa) Ltd): Struik Publishers. pp. 266

Dejong, J., 1991. Traditional Medicine in Sub-Saharan Africa. Its importance and potential Policy Options. The World Bank.

Doughari, J.H., El-mahmood, A.M., Tyoyina, I., 2008. Antimicrobial Activity of Leaf Extracts of *Senna obtusifolia* (L). *African Journal of Pharmacy and Pharmacology*, **2**(1): 7-13.

Eagleton, J., 2006. Safety and Toxicity Evaluation of Chinese Herbal Medicine. Available online: www.shen-nong.com. (accessed 24th April, 2012).

Elgayyar, M., Draughon, F.A., Golden, D.A., Mount, J.R., 2001. Antimicrobial Activity of Essential Oils from Plants against Selected Pathogenic and Saprophytic Micro-organisms. *Journal of Food Protection*, **64**: 1019-1024

El-Sayyad, S.M., Abdel-Baky, A.M., Backheet, E.Y., Glombitzx, K.W., 1988. Phytochemical Study of *Cassia didymobotrya* Fres. Cultivated in Egypt. Part 1, Study of Quinones and Uracil. *Bulletin of Pharmacetical Science Egypty*, **11** (2): 165-175.

Erasto, P., Grierson, D.S., Afolayan, A.J., 2006. Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina*. *Journal of Ethnopharmacology*, **106**(1):117-120. Online: www.ncbi.nlm.gov/pubmed [accessed 12th April, 2012].

Fabricant, S.D. and Farnsworth, N.R., 2001. The value of plants used in Traditional Medicine for Drug Discovery. *Reviews in Environmental health, Environmental Health Perspective*, **109** (1): 69-75.

Falodun, A., 2010. Herbal Medicine in Africa- Distribution, Standardization and Prospects. *Research Journal of Phytochemistry*, **4** (3): 154-161.

Farnsworth, N.R., Akere, O., Bingle, A.S., 1985. Medicinal Plants in therapy. Bulleting of the World Health Organization. **63** (6): 965-981.

Farnsworth, N.R., 1988. Screening Plants for New Medicines. Chapter 9 in *Biodiversity*, ed. E.O.Wilson.Washington, D.C.: National Academic Press. Available: www.ciesin.org/docs/002-256c/002-256c.html [accessed 21st April 2012-05-26].

Farombi, E.O, and Owoeye, O., 2011. Antioxidative and Chemopreventive Properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. *International Journal of Environmental Research. Public Health*, **8**: 2533-2555

GFHR, 2004. Global Forum for Health Research: Health Research for Policy Action and Practice. Version 2.

Fowler D.G., 2006. Traditional Fever Remedies: A List of Zambian Plants.

Frontling, R.A., 1984. Imidazoles as medically important antifungal agents. An Overview. Drugs Today **20**: 325-349.

Gottlieb, O.R., 1990. Phytochemicals: differentiation and function. *Phytochemistry*, **29**: 1715-1724.

Greenspan, J.S. and Greenspan, D., 1997. *Oral complications in HIV infections*. In. *The Medical Management in AIDs*. Eds. Merles, A., Sande, M., Paul, A., Volberding, M.D.W.D. London. Saunders Company, pp: 168-180.

Greenwood, D., Slack, B.C.R., Peutherer, F., J. eds. 2002. *Medical Microbiology. A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control.*

16th Ed. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto. Elsevier.

Guoda, Y. G., Abdel-Baky, A. M., Darwish, F.M., Mohamed, K. M., Kasai, R., Yamasaky, K., 2006. Phenylpropanoid and Phenylethanoid derivatives from *Kigelia pinnata* D.C Fruits. *Natural Products Research*, **20**(10): 935-940.

Gundidza, M., 1986. Screening of Extracts from Zimbabwean Higher Plants II: Antifungal Properties. *Fitoterapia*, **57** (2): 111-113.

Harborne, J.B., 1998. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman and Hall.

Houghton, P.J., 2002. The Sausage tree (*Kigelia pinnata*): Ethnobotany and recent scientific work. *South African Journal of Botany*, **68**(1) pp. 14-20.

Houghton, P. J., Raman, A., 1998. *Laboratory Handbook for the Fractionation of Natural Extracts*. London: Chapman and Hall.

Ibrahim, T.A., Lola, A., Adeluyi, F.A., Ojei, J., 2009. Assessment of the Antibacterial Activity of *Vernonia amygdalina* and *Occimum gratissimum* Leaves on Selected Food Borne Pathogens. *Elctronic Journal of Environmental, Agricultural and Food Chemistry*, **8**(11): 1212-1218.

Igbinosa, O.O., Igbinosa, E. O., Aiyegoro, O. A., 2009. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *African Journal of Pharmacy and Pharmacology*, **3**(2): 058-062.

Jackson, N. J., Houghton, P.J., Retsas, S., Photion, A., 2000. *In vitro* Cytotoxicity of Norviburtinal and Isopinnatal from *Kigelia pinnata* against Cancer cell lines. *Journal of Medicinal Plant and Natural Product Research*, **66**: 758-61.

Jakupovic, J., Baruah, R.N., Chau Thi, T.V., Bohlmann, F., Msonthi, J.D., Schmeda-Hirschmann, S., 1985. New Vernolepin derivatives from *Vernonia glabra* and Glaucolides from *Vernonia scorpoiides*. *Journal of Medicinal Plant and Natural Product Research*, **51** (5): 378-380.

Jigna, P. and Chanda, S.V., 2007. *In vitro* Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. *Turkey Journal of Biology*, **3**: 53-58.

Joffe, P., 2003. Plantzafrica: *Kigelia africana*. *Pretoria National Botanical Garden*. Available: http://plantZafrica.com [accessed 13th April, 2012].

Johns, T., Faubert, G.M., Kokwaro, J.O., Mahunnah, R.L.A., Kimanani, 1995. Antigiardial Activity of Gastrointestinal Remedies of the Luo of East Africa. *Journal of Ethnopharmacology*, **46** (1): 17-23.

Kareru, G., Kenji, M., Gachanja, N., Keriko, M., Mungai, G., 2007. Traditional Medicines among the Embu and Mbeere People of Kenya. *African Journal of Traditional, Complementary and Alternative Medicines*, **4**: 75-86.

Kareru, P.G., Keriko, J.M., Gachanja, A.N., Kenji, G.M., 2008. Direct detection of Triterpenoid Saponins in Medicinal Plants. *African Journal of Traditional Complementary and Alternative Medicines*, **5**(1): 56-60. Available: www.ncbi.nlm.nih.gov/pmc/articles/PMC/2816600 [accessed 22nd September 2011].

Kenya Census, 2009. *Counting Our People for Implementation of Vision 2030*. Available: www.knbs.or.ke/CensusResults/KNBSBrochure.pdf [accassed 30th April 2012].

Kisumu County, 2010. Population of Kisumu County. Online: www.softkenya.com/kisumu [accessed 27th April 2012].

Kisumu County, 2010. Health in Kisumu County. Online: www.softkenya.com/kisumu [accessed 27th April 2012].

Kisumu County, 2011. Kisumu County: Developing a Development Case, Our Case. Online: www.kisumu-county-forum.1048884.n5.nabble.com [accessed 30th April 2012].

Kisumu Maps, 2012. Kisumu Maps-roadmap, Satellite View, Street View, Terrain Map, Photos. Available: www.maps.live-translator.net [accessed 30th April 2012].

Khan, M.R., Kihara, M., Omoloso, A., 2003. Antimicrobial Activity of the Alkaloidal Constituents of the root bark of *Eupamatia laurina*. *Pharmaceutical Biology*, **41**: 277-280.

Khan, M.R. and Mlungwana, S.M., 1999. Gamma-Sitosterol, a cytotoxic sterol from *Markhamia zanzibarica* and *Kigelia africana*. *Fitoterapia*, **70**(1): 96-97.

Kokwaro J.O., 1993. Medicinal Plants of East Africa 2nd Ed. Literature Bureau, Nairobi.

Kokwaro J.O., 1976. Medicinal Plants of East Africa. East Africa Literature Bureau, Nairobi.

Korrir, K.R., Mutai, C., Kiiyukia, C., Bii, C., 2012. Antimicrobial Activity and Safety of Two Medicinal Plants Traditionally Used in Bomet District of Kenya. *Research Journal of Medicinal Plants*, **6**(5): 370-382.

Kozkiewicz, Z., 1989. *Aspergillus* Species on stored products. Mycological papers No.161. C.A.B. International Mycological Institute.

Machakos County, 2010. Population in Machakos County. Available: www.softkenya.com/machakos-county [accessed 27th April 2012].

Machakos County, 2010. Health in Machakos County. Available: www.softkenya.com/machakos-county [accessed 27th April 2012].

Mariita, R.M., Ogol, C.K.P., Oguge, N.O., Okemo, P.O., 2010. Antitubercular and Phytochemical Investigation of Methanol Extracts of Medicinal Plants used by the Samburu Community in Kenya. *Tropical Journal of Pharmaceutical Research*, **9** (4): 379-385.

Markham, K.R., 1982. *Techniques of Flavonoid Identification.* London: Academic press Inc.

Maryam, Z., Aqil, F., Khan, M.S.A., Ahmad, I., 2010. Ethnomedicinal plants derived antibacterial and their prospects. Ethnomedicine: *A source of Complementary Therapeutics,* 149-179.

Martson, A., Hostettman, K.1987. Antifungal, Molluscicidal and Cytotoxic compounds from plants used in traditional medicine. In: Biologically Active Natural Products. Proceedings of the phytochemical society of Europe.Eds, Hostettman, K. and Lea, P.J. Oxford, pp. 65-83.

Masakazu, N., Firth, N.A., Cannon, D.R., 2010. Antifungal drug resistance of oral fungi. Odontology, 98(1): 15-25. Review Article. www.springerlink3.metapress.com [accessed 13th September, 2011].

Maundu, P. and Tengnas, B., 2005. *Useful Trees and Shrubs for Kenya: World Agro-forestry Centre-East and Central Africa Regional Programme.* Technical Handbook, **35**. pp: 273.

Mendel, J. and Hollis, A., 2010. Incentives for Global Health: The Health Impact Fund and Traditional Medicines. Discussion Paper No. 8: 1-19.

Miaron, O.J., Kassim, O., Ekaya, N., 2004. Indigenous Knowledge: The basis of Maasai Ethnoveterinary Diagnostic Skills. *Journal of Human Ecology*, **16**: 43-48

Moideen, S. V. K., Houghton, P. J., Rock, P., Croft, S.L., Aboagye-Nyame, F., 1999. Activity of extracts and napthaquinones from *Kigelia pinnata* against *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense*. *Journal of Medicinal Plant and Natural Product Research*, **65**(6): 536-40.

Muley, B.P., Khadabadi, S.S., Banarase, N.B., 2009. Phytochemical Constituents and Pharmacological Activities of *Calendula officinalis* Linn (Asteraceae): A Review. *Tropical Journal of Pharmaceutical Research*. **8**(5): 455-465.

Munguti, K., 1997. Indigenous Knowledge in the Management of Malaria and Visceral Leishmaniasis among the Turgen of Kenya. *Indigenous Knowledge Development Monitor*, **5**: 10-12.

Mwangi, J.W., Thoithi, G.N., Kibwage, I.O., 2009. Essential Oil Bearing Plants from Kenya: Chemistry, Biological activity and Applications. Available from: www.pubs.acs.org/doi/abs/10.1021/bk-2009-1021.ch027 [accessed 29th May 2012].

Nair, R., Kalariya, T., Chanda, S., 2004. Antibacterial Activity of Some Selected Indian Medicinal Flora. *Turkey Journal of Biology*, **29** (2): 41-47.

Nester, E.W., Anderson, D.G., Roberts, C.E., Pearsall, N.N., Nester, M.T., 2004. *Microbiology*: *A Human Perspective*. 4th ed. New York. McGrow-Hill.

Ng'etich, K.A., 2005. Indigenous Knowledge, Alternative medicine and Intellectual Property Rights concerns Kenya. Paper presented in the 11th General Assembly Maputo, Mozambique, 6-10.

Njoroge, G.N., and Bussman, R.W., 2007. Ethnotherapeutic Management of Skin Diseases among the Kikuyus of Central Kenya. *Journal of Ethnopharmacology*, **111**:303-307.

Nostro, A., Germano, M., Marino, D.V., Cannatelli, M., 2000. Extraction Methods and Bioautography for Evaluation of Medicinal Plant Antimicrobial Activity. *Letters in Applied Microbiology*, **30**: 379-384.

Nyaberi, M.O., Onyango, C.A., Mathooko, F.M., Maina, J.M., Makobe, M., Mwaura, F., 2007. Bioactive Fractions in the Stem Charcoal of *Senna didymobotrya* Fresen Irwin and Barneby used by Pastoral Communities in West Pokot to Preserve Milk. Available: www.kari.org/biennialconference [accessed 22nd May 2012].

Ochei, J., Kolhatkar, A., 2000. *Medical Laboratory Science, Theory and Practice*. New Delhi. Tata McGraw-Hill.

Ogundare, O.A., Adetuyi, F.C., Akinyosoye, A.F., 2006. Antimicrobial Activities of *Vernonia tenoreana*. *African Journal of Biotechnology*, **5** (18): 1663-1668.

Ojewole, J.A.O., Rahim, S., Shode, F.O., 2000. Mosquito Larvicidal Properties of Aqueous Extracts of *Senna didymobotrya*. *Nigerian Journal of Natural Products and Medicine*, **4**: 46-47.

Oketch, R. H.A., Dossaji, S.F., Mberu, E.K., 1999. Antimalarial Activity of some Kenyan Medicinal Plants *Pharmaceutical Bio* **37** (5): 329-334.

Olembo, N.K., Fedha, S.S., Ngira, E.S., 1995. Medicinal and Agricultural plants of Ikolomani Division, Kakamega District. Signal press L.t.d Nairobi.

Otieno, N.J., Matengo, M.K.H, Lyaruu, H.V., 2007. The Effect of a Local Mineral *Kadosero* Towards the Antimicrobial Activity of Medicinal Plant's Extract: Case of Lake Victoria Basin, Tarime Tanzania. *African Journal of Traditional, Complementary and Alternative Medicines*, **4** (1): 1-6.

Owour, B.O. and Kisangau, D.P., 2006. Kenyan Medicinal plants used as antivenin: a comparison of plant usage. *Journal of Ethnobiology and Ethnomedicine*, **2**:7.

Paduch, R., Kandefer-Szerszein, M., Trytek, M., Fiedurek, J., 2007. Terpenes: Substances useful in human healthcare. Review. *Archives Immunology and Therapy Experintal*, **55**: 317-327.

Paiva, P.M.G., Gomes, F.H., Napoleao, T.H., Sa, R.A., Correia, M.T.S., Coelho, L.C.B.B., 2010. Antimicrobial activity of Secondary metabolites and lectins from plants. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Available: www.formatex.infor/microbiology2/396-406.pdf [accessed 20th May 2012].

Palombo, E.A., 2009. Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral diseases. Environment and Biotechnology Centre, Faculty of Life and Social Sciences, Swinburne University of Technology, Oxford Press.

Parek, J., Chanda, S., 2007. Antibacterial and Phytochemical Studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, **10**: 175-181.

Poovendran, P., Vidhya, N., Murugan, S., 2011. Antimicrobial Activity of *Coccinia grandis* against Biofilm and ESBL Producing Uropathogen *Escherichia coli. Global Journal of Pharmacology*, **5** (1): 23-26.

Reddy, J.L., Beena, J., Anjana, C.J., Ruveena, N.T., 2010. Evaluation of Antibacterial Activity of *Trichosanthes cucumerina* L. and *Cassia didymobotrya* Fres. Leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2: 4.

Review of Literature (n.d). Available: www.shodhgonga.inflibnet.acin [accessed 22nd May 2012)].

Saag, M.S., 1997. Cryptococcosis and other fungal infections (Histoplasmosis, Coccidioidomycosis) in the Management of AIDs. Eds. Merle, A., Sande, M.D., Paul, A., Volberding, M.D.W.D Sunders Company, London, pp. 327-342.

Sadiq, S. I., Shuaibu, M., Bello, B. A., Tureta, G. S., Isah, A., Izuagie, T., ... Kamaru, B.M., 2012. Phytochemistry and Antimicrobial Activities of Cassia occidentalis Used for Herbal Remedies. *Journal of Chemical Engineering*, **1**(1): 38-41.

Sandiga, I., Chacha, C.N., Kanunah, M.P., 1995. Traditional *Medicine in Africa*: *The Existence and Use of Traditional Medicine in Kenya* [e-book]. Nairobi: East African Educational Publishers Ltd. Available: http://books.google.com/books [accessed 19th July, 2011].

Sangita, S., Kaur, H., Verma, B., Singh, K.S., 2009. *Kigelia Africana* (Lam.) Benth. - An Overview. *Natatural Products Radiance*, 8(2): 190-197.

Segal, R., Shud, F., Milo-Goldzweig, I., 2006. Hemolytic properties of synthetic glycosides. Available: http://onlinelibrary.wiley.com [accessed 22nd May 2012].

Sexana, V.K. and Sharma, R.N., 1999. Antimicrobial activity of essential oils of *Tadalia asiatica*. *Fitoterapia*, **70** (1): 64-66.

Sharon, F., 2010. The Medicinal Value of *Opium poppy*. Healing Properties and Uses of the Opiate, Poppy. Available: http://sharonfalsetto.suite101.com [accessed 11th April, 2012].

Skikkerveer, L.J. and Skikkerveer M.K.L. 1995. Indigenous Indonesian Medicine for Self-reliance .In. The cultural dimensions of development. Indigenous knowledge systems.13-14

Smolinski, M.S. and Hamburg, M.A., 2003. *Microbial Threats to Health*: *Emergence, Detection, and Response*. Committee on Emerging Microbial Threats to Health in the 21st Century [e-book]. The National Academic Press. Washington D.C. Available: http://site.ebrary.com [accessed 21st September, 2011].

Sudi, I.Y., Ksgbiya, D.M., Muliuh, E.K., Clement, E., 2011. Nutritional and Phytochemical Screening *Senna obtusifolia* indigenous to Mubi, Nigeria. *Advances in Applied Science Research*, **2**(3): 432-437.

Taylor, L., 2005. *The Healing Power of Rainforest Herbs*: Differences and Similarities of Drugs and Medicinal plants. Available online: www.rain-tree.com/chapter2.htm [accessed 24th April, 2012].

Taylor, J.L.S., Rabe, T., McGaw, L.J., Jager, A.K., Van Staden, J., 2001. Towards the Scientific Validation of Traditional Medicinal Plants. *Plant Growth Regulation* **34**: 23-37.

Topley and Wilson's, 1998. Microbiology and Microbial Infections .Eds. Hausler, W.J., Sussman, J.M. 9th Ed. 603-604.

Van der Walt, L., 2003. *Vernonia glabra*. Family: Asteraceae (Daisy family), Common names: Wild heliotrope. Kirsten Bosch National Botanical Garden. Available: www.plantzafrica.com [accessed 23rd May 2012].

Williams, L.A.D., 2006. The Natural Products Unit of Scientific Research Council. *West Indian Medical Journal*, **55** (4): 125.

World Health Organization, 2012. World Health Report. International Spread of Disease Threatens Public Health Security. Available: www.who.int/mediacentre [accessed 24th May 2012].

World Health Organization, 2007. World Health Report. A safer future: Global public health security in the 21st century. Available: www.who.int/whr/2007 [accessed 23rd May 2012].

World Health Organization, 2006. WHO Monograph on good agricultural and collection practices (GACP) for *Atermisia annua* L. Available: http://Who.int/medicines/publications/ArtemisiaMonograph.pdf [accessed 11thApril, 2012].

World Health Organization- W.H.O Traditional Medicine Strategy 2002-2005.

World Health Organization, Food and Agriculture Organization, and World Organization for Animal Health, 2003. WHO/FAO/OIE Expert Workshop on Non-human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment, Geneva, Switzerland, 1-5.

World Health Organization, 2000. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO Geneva, pp. 1-74. Available: http://whql.bdoc.who.int/ht/2000/WHO_EDM_TRM_2000.1.

World Health Organization, 1999. WHO Monographs on Selected Medicinal Plants, Volume1.

World Health Organization, 1998. WHO-Traditional Medicine Regulatory Situation of Herbal Medicines. A World Review. WHO Geneva, Switzerland, 1-5.

Yineger, H., Kelbessa, E., Bekele, T., Lulekal, E., 2008. Plants used in traditional management of human ailments at Bale Mountains National Park, Southeastern Ethiopia. *Journal of medicinal plant research*, 2(6): 132-153.

7.0 APPENDIX

7.1 CERTIFICATE OF AWARD

CERTIFICATE OF PARTICIPATION

This SECOND PRIZE / Masters Category certificate is awarded to:

Catherine Kadogo Kitonde

This certificate is in recognition of diligent and dedicated participation by the above named individual in the first TEEAL Graduate Research Paper Competition 2011

The TEEAL Project, located within Cornell University, Mann Library, Ithaca, NY, USA, recognizes and congratulates you on your Second Prize achievement and overall participation in this competition.

Mary Ochs, Director
Albert R. Mann Library, Cornell University
26 October 2011

teeal

7.2 THE SCIENTIFIC RESEARCH PAPER WORN

Antimicrobial Activity and Phytochemical Study of *Vernonia glabra* (Steetz) Oliv. & Hiern. in Kenya.

Abstract

Infectious diseases are prevalent and life threatening in Kenya. Majority of the sick are seeking herbal remedies in search of effective, safe, and affordable cure. This project aims to investigate the antimicrobial activity and presence of active phytochemical compounds in different parts of *Vernonia glabra*; a plant used by herbalists in various regions of Kenya, for the treatment of gastrointestinal problems. The plant sample was collected in January 2010 in Machakos, and different parts dried at room temperature under shade, ground into powder and extracted in Dichloromethane: Methanol in the ratio 1:1, and water. These crude extracts were tested against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* for antimicrobial activity using disc diffusion technique. Minimum inhibitory concentrations (MICs) for active crude extracts were done using disc diffusion technique after the failure of agar and broth dilution methods. It was observed that the organic crude extracts of flower, leaf, stem, root, and/or entire plant, showed activity against one or four micro-organisms, and at concentrations lower than the aqueous crude extracts. Organic crude extract of the leaf showed the highest activity against *Staphylococcus aureus* (mean inhibition zone 1.85), recording higher activity than the commercially used standard antibiotic (Streptomycin mean inhibition zone of 1.30). The organic crude extract of flower showed significant activity only against *S.aureus*, with the lowest MIC of 1.5625mg/100µl, compared to streptomycin with M.I.C of 6.25mg/100µl. Thin Layer Chromatography-Bioautography Agar-Overlay showed that, flower alkaloids (50% active), root sapogenins (43.8% active), and root terpenoids (38.5% active) were identified as the potential antibacterial compounds against *S.aureus*. These results suggest that, *V.glabra* contains phytochemicals of medicinal properties and justify the use of *V.glabra* in traditional herbal medicine for the treatment of microbial based diseases. However, research on toxicity which is missing in this study is recommended for *V. glabra* in order to verify, validate and document the safety of this medicinal plant to the society.

Keywords: *Vernonia glabra*, Antimicrobial activity, and Phytochemicals.

1.0 Introduction

Infectious diseases caused by microbes are a major health hazards all over the world, Jigna *et al.*, (2007); Sasikumar *et al.*, (2007). Throughout history, infectious diseases have been a major threat to human and animal health and a prominent cause of morbidity and mortality, WHO/FAO/OIE, (2003). The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main source of drugs. For centuries, people have used plants for healing, Maryam *et al.*, (2010). About 80% of the worlds people rely on traditional plant based medicine, UNEP, (2010). Increased antibiotic resistance has become a global concern, coupled with the problem of microbial persistence, thus highlighting the need to develop novel microbial drugs that are not only active against drug resistant microbes, but more importantly, kill persistent micro-organisms and shorten the length of treatment. Apart from toxicity, lengthy therapy also creates poor patient compliance, Mariita *et al.*, (2010). It is estimated that about 75% population in Kenya seeks health care among traditional healers, Sandiga *et al.*, (1995). Traditional medicine is widely practiced in Kenya, where this has been documented by ethnomedical surveys, Miaron *et al.*, (2004); Kareru *et al.*, (2007). The high cost of important conventional drugs and/ or inaccessibility to western health care facilities has led to overreliance on traditional medicine since it is affordable and available to people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective, Munguti, (1997). Infections associated with bacterial and fungal pathogens are among some of the indications treated using traditional remedies in Kenya, Njoroge and Bussmann, (2007). Therefore, the aim of this project is to investigate the efficacy and phytochemical compounds present and active in different parts of *V. glabra*; an herb used by traditional practitioners in Kenya. A

decoction of leaf plus root taken orally is claimed to treat gastrointestinal problems in Kenya Johns *et al*., (1995). The leaf ash or crushed leaves rubbed into scarification around the snake bite is used as antidote, Owour and Kisangau, (2006).

2.0 Material and Methods

2.1 Collection of Plant Material

The plant was selected based on ethnomedicinal information from literature and collected from Kathiiani village in Machakos county, Kenya in January 2010 which falls within the floral region K4 (Fig.1). The specimen was authenticated by a plant taxonomist in University of Nairobi and a voucher specimen (CK 2010/01) deposited at University of Nairobi Herbarium.

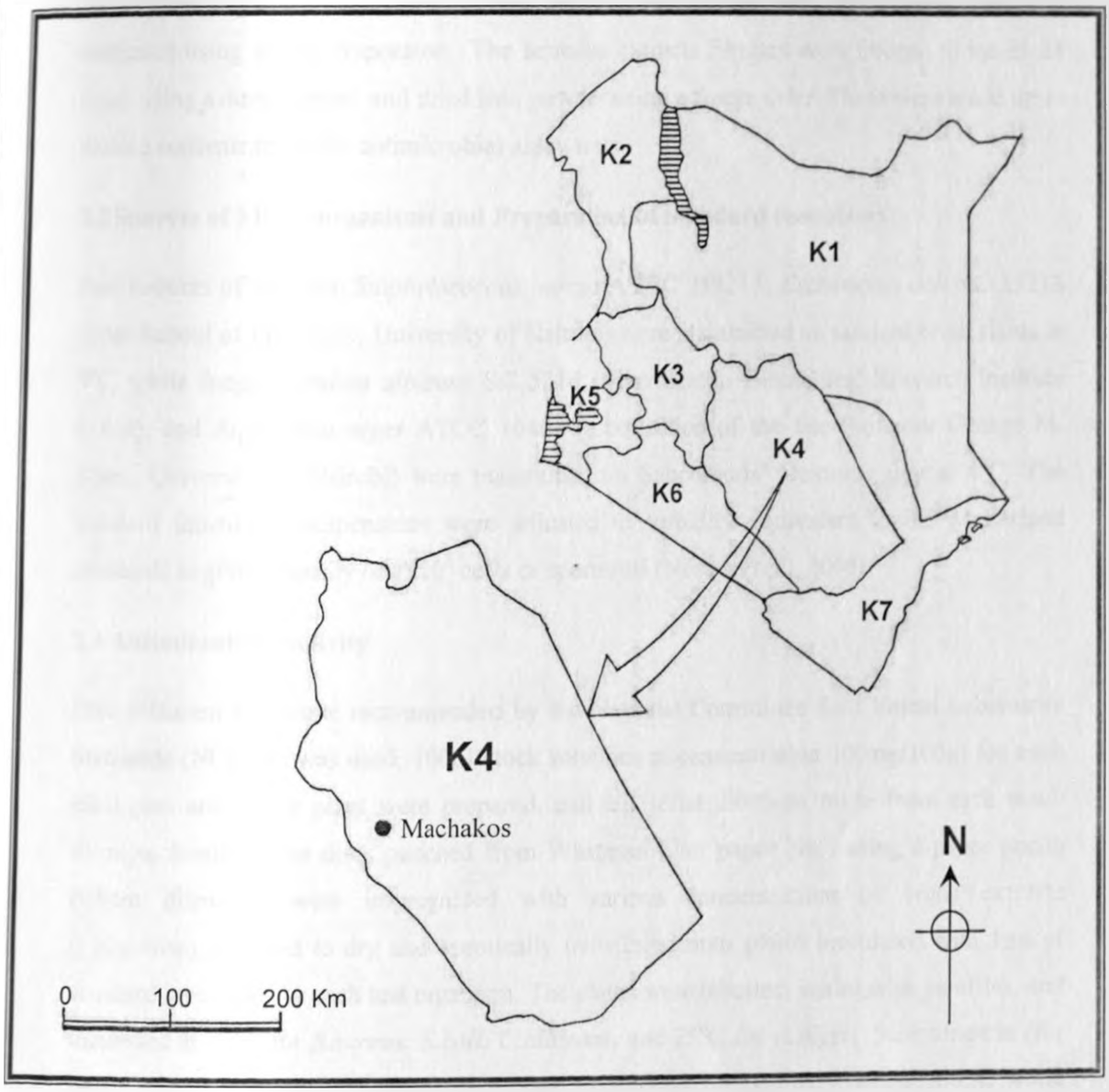

FIG. 1. A MAP OF KENYA SHOWING FLORAL REGIONS OF KENYA ACCORDING TO FLORA OF TROPICAL EAST AFRICA (FTEA) BEENTJE, (1994).

2.2 Crude plant Extracts preparation

The flowers, leaves, stem, and roots were air-dried under the shade at room temperature, ground into powder and extracted using Dichloromethane/Methanol in the ratio 1:1, and water, according to standard extraction methods (Harborne, 1998). 20g of powdered plant material was mixed thoroughly with appropriate amount of solvent, left to stand for 24 hours

and decanted (this was done twice). The filtrates were combined and filtered using a Buchner funnel. Dry organic crude extracts were obtained after evaporating Dichloromethane and Methanol using a rota evaporator. The aqueous extracts filtrates were freezed to ice in 24 hours using a deep freezer, and dried into powder using a freeze drier. These were made up to desired concentrations for antimicrobial assay tests.

2.3 Sources of Micro-organisms and Preparation of Standard inoculums

Pure cultures of bacteria; *Staphylococcus aureus* ATCC 259213, *Escherichia coli* NC 35218 (from School of Pharmacy, University of Nairobi) were maintained on nutrient broth slants at 4°C, while fungi; *Candida albicans* SC 5314 (from Seattle Biomedical Research Institute U.S.A), and *Aspergillus niger* ATCC 16404 (a collection of the late Professor George M. Siboe, University of Nairobi) were maintained on Sabourauds' Dextrose agar at 4°C. The standard inoculums suspensions were adjusted to turbidity equivalent to 0.5 McFarland standards to give a density of $1x10^8$ cells or spores/ml (Nostro *et al.*, 2000).

2.4 Antimicrobial Activity

Disc diffusion technique recommended by the National Committee for Clinical Laboratory Standards (NCCLS) was used. 100µl stock solutions at concentration 100mg/100µl for each plant part and entire plant were prepared, and ten serial dilutions made from each stock solution. Sterile paper discs punched from Whatman filter paper No.1 using a paper punch (0.6cm diameter) were impregnated with various concentrations of crude extracts (100µl/disc), allowed to dry and aseptically transferred onto plates inoculated with 1ml of standard inoculum for each test organism. The plates were labelled, sealed with parafilm, and incubated at 37°C for *S.aureus*, *E.coli*, *C.albicans*, and 25°C for *A.niger*. Streptomycin (for bacteria) and Nystatin (for fungi) were used as standards at similar concentrations, while discs with extraction solvents only were used as controls. These were done in duplicates under sterile conditions. The results were recorded after 24, 48, 72 and 96 hours. The antimicrobial activity was determined by measuring clear inhibition zones diameters formed around the discs using a transparent ruler (in cm). Minimum inhibitory concentrations (MICs) were determined by recording the lowest concentration of the active extracts that inhibited growth of the micro-organisms, Ochei and Kolhatkar, (2000).

2.5 Chemical Analysis of Selected Crude plant Extracts

Extracts that exhibited MICs of ≤25mg/100µl) against any of the test-organisms used were selected for chemical analysis. They were screened for the presence or absence of five classes of compounds namely: alkaloids, Sapogenins, terpenoids, quinones, and flavonoids using Thin Layer Chromatography (TLC) technique. The developed TLC plates were viewed under Ultra-Violet light and then sprayed with appropriate reagents for the detection of the chemical groups according to Harborne (1998).

2.6 Bioautography Agar Over-Lay

The active compounds were identified using bioautography agar over-lay method on commercially prepared Aluminium silica gel TLC plates against only the most affected test-organism. The separated spots were observed under UV lamp and the freshly prepared standard inoculum of *Staphylococcus aureus* in nutrient agar was uniformly overlaid on the TLC plates with the crude extracts and incubated at 37°C for 24 hours. then sprayed with aqueous solution of 2.5mg/ml thiazolyl blue (3-(4, 5 dimethylthiazolyl-2-2, 5-diphenyl tetrazolium bromide (MTT), Nostro *et al.*, (2000); Runyoro *et al.*, (2006) and inhibition zones noted.

Data Analysis

In order to analyse data, multiple way ANOVA was used to determine significant factors in formation of inhibition zones, Tukey's Honest Significant Difference Test (THSDT) was used for means comparison within the significant factors. Ms Word 2007 was used to draw tables; Ms Excel 2007 was used to determine means inhibition zones, percentage number of present and active compounds, and plotting of bar graphs.

3.0 Results

3.1 The antimicrobial activity.

The antimicrobial activity (formation of inhibition zones) of *Vernonia glabra* crude extracts depended on: (a) parts of plant used, (b) extraction solvent, and (c) test-organism factors (Table 1).

Table 1. Significance of Parts of plant used, Extraction solvent, and Test-organism in formation of Inhibition zones

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Extraction Solvent	1	5.610	5.6103	89.1855	< 2.2e-16 ***
Parts of plant used	4	1.985	0.4963	7.8902	2.612e-06 ***
Test-organism	3	11.268	3.7560	59.7081	< 2.2e-16 ***
Time	3	0.066	0.0220	0.3492	0.7898
Residuals	2068	130.089	0.0629		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

***Based on multiple-way ANOVA , part of plant used, test- organism, and extraction solvent factors were significant, but the time factor was not significant at statistical p-value≤0.05**

Three factors namely: extraction solvent, parts of plant used, and test-organism were highly significant in the formation of inhibition zones at p-value=0.00*** (0% error); less than the accepted statistical p-value≤0.05 (5% error) in this study (see Table 1). The time factor (incubation period) was insignificant in that it has p-value=0.7898, greater than the accepted p-value≤0.05 (5% error) and therefore it was rejected.

(a)Parts of plant used: All the organic crude extracts of *V.glabra* parts used were active at least on one or four test-organisms used (see Fig. 2). It was observed that organic extracts of *V.glabra* leaf had the highest activity (mean inhibition zone of 1.85) against *S.aureus*, followed by organic extract of flower at a mean inhibition zone (MIZ) of 1.78 against only *S.aureus*. The two extracts showed no significant difference in activity from each other, but were significantly different in activity from streptomycin (MIZ of 1.30).

Also organic extract of leaf recorded high activity (MIZ of 1.43) against *A.niger*, compared to nystatin (MIZ of 0.83 against *A.niger*), while the organic extract of whole plant (all parts mixed) showed significant activity (MIZ of 1.50) against *S.aureus* compared to streptomycin's (MIZ of 1.30) low activity

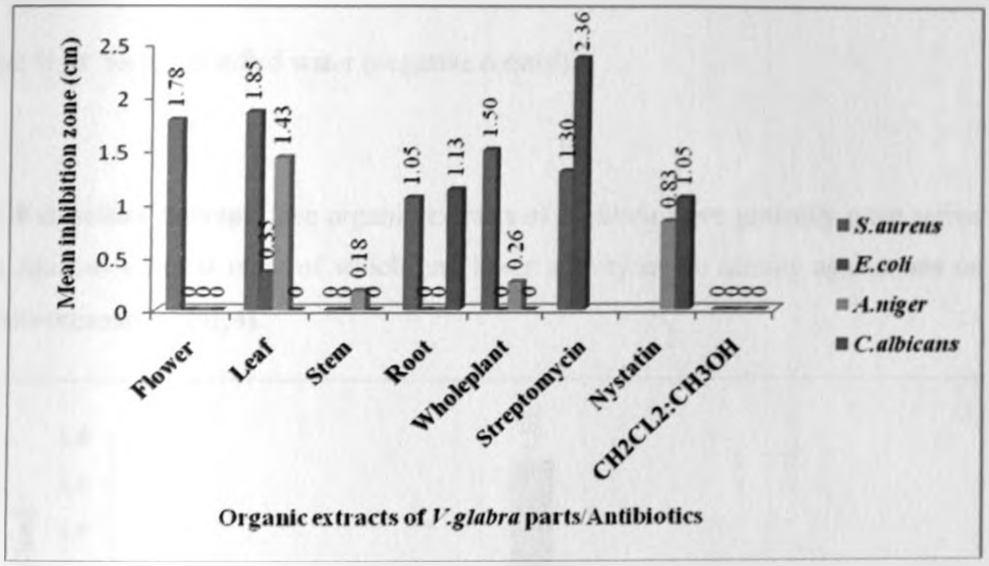

Fig.2 Antimicrobial activity of organic crude extracts of *V.glabra* parts compared to streptomycin and nystatin at 100mg/100µl

Note: DCM-MeOH- Dichloromethane and Methanol in the ratio 1:1(negative Control).

Aqueous extract of the whole plant of *V.glabra* was the only active aqueous extract against *S.aureus* (see Fig.3).

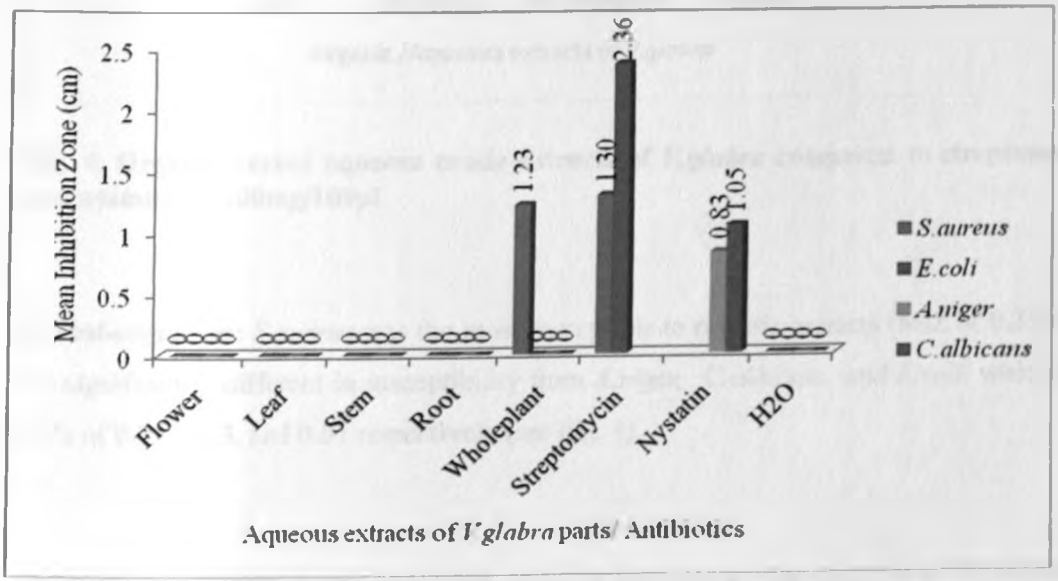

Fig.3 Antimicrobial activity of aqueous crude extracts of *V.glabra* parts compared to streptomycin and nystatin at 100mg/100µl

The controls (Dichloromethane: Methanol 1:1, and sterile distilled water) were not active against any test-organism

Note: H_2O: Sterile distilled water (negative control).

(b) Extraction Solvent: The organic extracts of *V.glabra* were generally more active than the aqueous extracts most of which had lower activity or no activity against one or four micro-organisms (Fig.4).

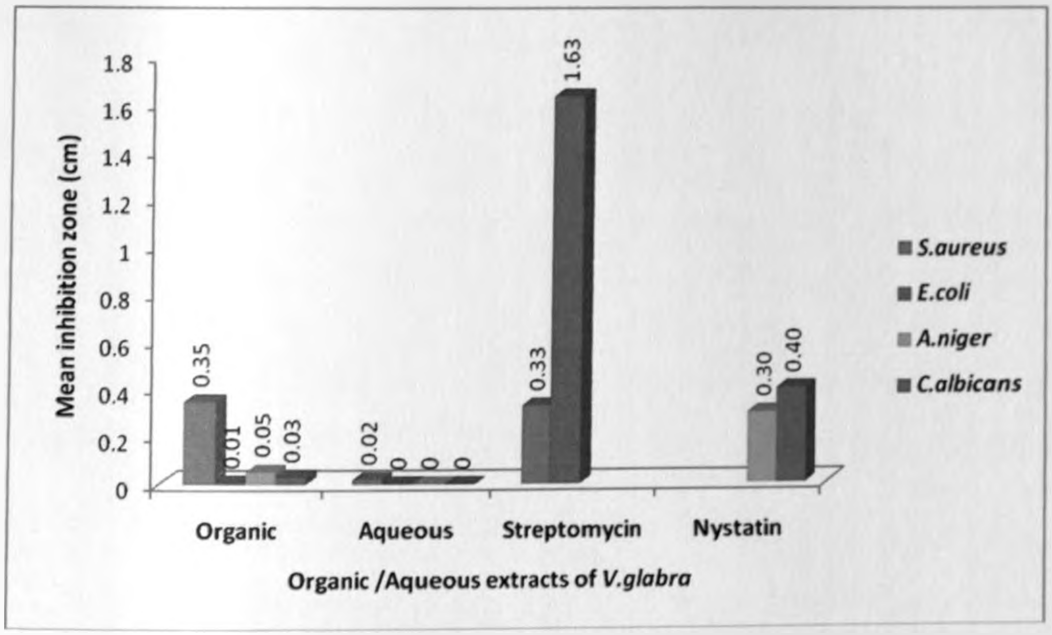

Fig. 4. Organic versus aqueous crude extracts of *V.glabra* compared to streptomycin and nystatin at 100mg/100µl

(c).Test-organism: *S.aureus* was the most susceptible to organic extracts (MIZ of 0.35) and was significantly different in susceptibility from *A.niger, C.albicans, and E.coli* which had MIZs of 0.05, 0.03, and 0.01 respectively (see Fig. 4).

3.2 Minimum inhibitory concentration: M.I.C of each extract that was able to kill or inhibit the growth of one or four micro-organisms in this study was exhibited by extracts that were active at concentration of 100mg/100µl to 0.02mg/100µl. The lowest MICs (1.5625-

0.02mg/100µl) indicate highest activity, while 6.25-3.125mg/100µl (moderate activity), and 12.5-100mg/100µl indicate low activity (Table 2).

Table 2. MICs (mg/100µl) and Inverse of MICs (mg/100µl) of organic and aqueous extracts of *V. glabra*/Antibiotics against *S. aureus*, *E.coli*, *A. niger*, and *C. albicans*.

Extract/Antibiotic	Test-organism	MICs of Extracts/Antibiotics (mg/100µl)	Inverse of MICs (mg/100µl)	Remarks
Organic extracts				
Flower	*S.aureus*	1.5625	0.64	High activity
Streptomycin (Antibiotic)	*E. coli*	0.02+1	0.98	High activity
	S. aureus	6.25	0.16	Moderate activity
Nystatin (Antibiotic)	*A. niger*	3.125	0.32	Moderate activity
	C. albicans	3.125	0.32	Moderate activity
Leaf	*S. aureus*	12.5	0.08	Low active
	E. coli	100	0.01	Low active
	A. niger	25	0.04	Low active
Stem	*A. niger*	100	0.01	Low active
Root	*S. aureus*	25	0.04	Low active
	C. albicans	50	0.02	Low active
Whole plant	*S. aureus*	25	0.04	Low active
Aqueous extract				
Whole plant	*S. aureus*	100	0.01	Low active

Note*: 0.02+1- 1 was added to 0.02 in order to get a value that will minimize the big range among the inverse numbers, because values without whole numbers tend to have high inverse numbers.

The inverse of MICs was displayed on fig. 5 below. Organic extract of flower showed highest activity recording the lowest MIC of 1.5625mg/100µl against *S.aureus*, which was lower than the standard antibiotic (streptomycin) with MIC of 6.25mg/100µl. The organic extracts of root, whole plant against *S.aureus*, and leaf against *A.niger and S.aureus* had low activity (Fig. 5). The organic extract of root against *C.albicans*, organic extract of whole plant against *A.niger*, organic extract of stem against *A.niger*, and aqueous extract of whole plant against *S.aureus*, and organic extract of leaf against *E.coli* were less effective at higher concentrations (Fig. 5).

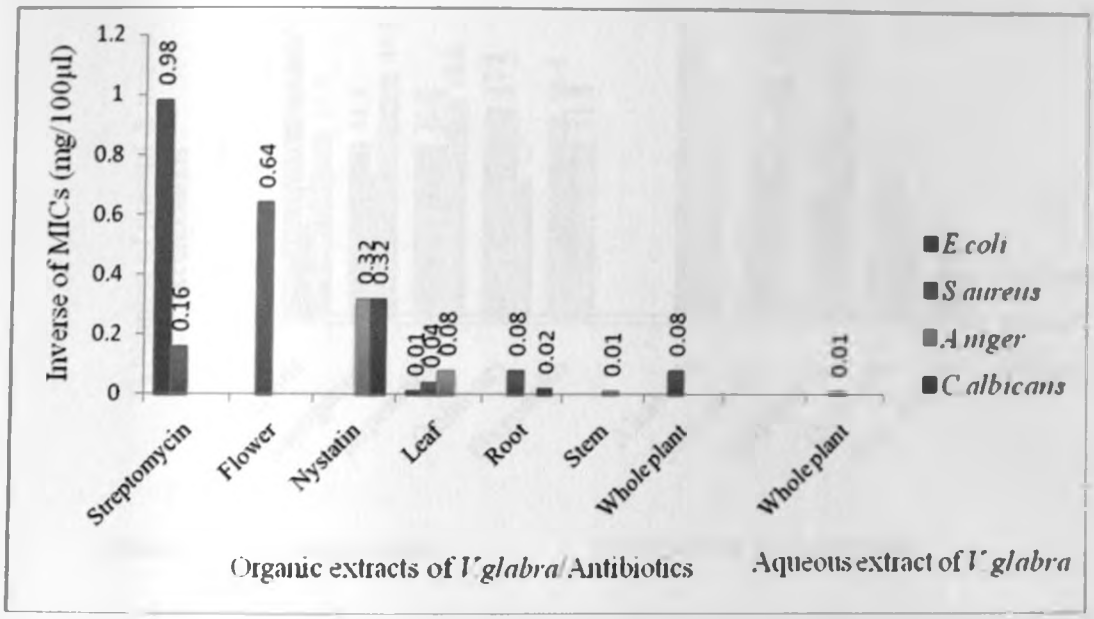

Fig. 5. MICs for organic and aqueous extracts of *V. glabra* compared to streptomycin and nystatin (antibiotics).

Note: Extracts/antibiotics with high inverse of MICs indicate higher activity (MIC 0.64-0.98mg/100µl) at low concentrations, extracts/antibiotics with inverse of MICs of 0.16-0.32mg/100µl indicate moderate activity, while the extracts with low inverse of MICs display low activity at higher concentrations (see Table 2).

3.3 Presence and Activity of Chemical Compounds.

Out of all the alkaloids screened present in different parts, flower alkaloids were 50% present and were all active against *S.aureus*. Root sapogenins made up 43.8% of all sapogenins present and were all active, while flower sapogenins followed with 31.3% presence and all were active against *S.aureus*. Root terpenoids showed 38.8% presence out of all terpenoids and almost all of them (38.5%) were active against *S.aureus*. Quinones of flower and leaf accounted for 37.5% presence, but only leaf quinones were moderately active at 25% activity, where as flower qiunones were less active at 12.5%. Flower flavonoids were present at 36.4%, while both leaf and root flavonoids showed 31.8% presence out of all flavonoids screened present in different parts, but all showed less activity of 9.1% against *S.aureus* (see Fig.6).

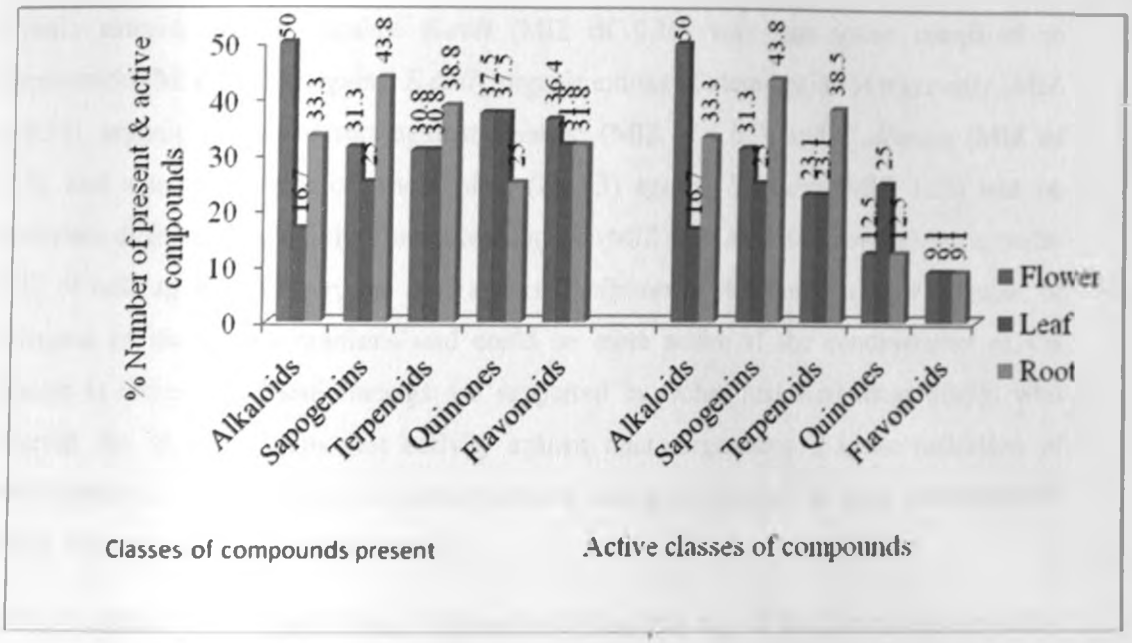

Fig. 6. Percentage number of five classes of compounds present and active in *V.glabra* crude extracts (flower, leaf, and root).

Note*: Most of the alkaloids and sapogenins present in the different parts, and root terpenoids were active against *S. aureus*. Leaf quinones were moderately active, while all flavonoids in all different parts were less active. (fig.6).

Discussion

The larger MIZs of leaf (1.85 against *S.aureus* and 1.43 against *A.niger*) and flower (1.78 against *S.aureus*) extracts compared to standard antibiotic streptomycin (1.30 against *S.aureus*) and nystatin (0.83 against *A.niger*), suggest that these extracts could be used for management of staphylococcal diseases caused by *Staphylococcus aureus* such as boils, sores, wounds as well as Aspergillosis diseases caused by *Aspergillus niger*, and also validate its use by traditional medicine practitioners in Kenya. These findings are in agreement with the studies of Chika *et al*., 2007 who reported large inhibition zones of organic extract of *Euphorbia hirta* leaf against *S.aureus* and justified its use by traditional medicinal practitioners for treatment of boils, sores and open wounds. Literature study done on *V.glabra*, elicited no previous research work on the antimicrobial activity of crude extracts of leaf and flower. As such, this could be the first report on such activity and could very well be a start point for further drug research of this species on a wide diversity of microbial pathogens.

Organic extracts of leaf against *E.coli* (MIZ of 0.35) was less active compared to streptomycin (MIZ of 2.36 against *E.coli*), organic extract of stem against *A.niger* only (MIZ of 0.18), organic extract of root against *S.aureus* (MIZ of 1.05), and *C.albicans* (MIZ of 1.13), and aqueous extract of whole plant (Fig. 3) against *S.aureus* (MIZ 1.23) had no significant difference in activity from streptomycin (MIZ 1.30 against *S.aureus*) and nystatin (MIZ of 0.83 against *A.niger*, and 1.05 against *C.albicans*). This could suggest presence of resistance of these test-organisms and could be more active if the concentration of the extracts is increased. These findings are supported by Ochei and Kolhatkar, (2006) who reported that if drugs show less activity against micro-organisms it is an indication of development of resistance by the test-organisms and that increase in drug concentration would increase the antimicrobial activity.

However, some of the crude extracts were not active against any of the four micro-organisms used. For instance, organic extract of flower (Fig.2) was not active against *E.coli*, *A.niger*, and *C.albicans*, while organic extract of stem was not active against *S.aureus*, *E.coli*, and *C.albicans*. These ineffective extracts are probably used to treat diseases caused by non bacterial and non fungal pathogens. These findings correlate with the previous studies of Johns *et al.*, 1995 who reported that decoction of *V.glabra* leaf and root was active against *Giardia lambria* which is a protozoa.

Moreover, this study shows that there are synergistic, intermediate, and antagonistic effects among the plant parts used and their mixture whereby, the organic extract of whole plant mixed (MIZ of 1.50 against *S.aureus*) showed intermediate activity ranging in between its highly active and less active single parts, yet traditional medicine prefer mixing (combining). This study may be an eye open for traditional medicine and could indicate that, the interactions of active chemical substances present in single crude extracts of plant parts used have strong synergistic effects to be able to inhibit the growth of test-organisms, compared to their mixture with intermediate effect which could be due to antagonising effects of less active or inactive extracts lessening the effect of highly active single crude extracts of *V.glabra*. These findings are in line with the findings of Maryam *et al.*, (2010) who did research on different plant parts and mixtures (combinations) of plant extracts and found that, plant extracts or phytocompounds exhibiting strong antimicrobial activity may interact with each other and the interactions may be synergistic or antagonistic. Therefore, the synergistic effects of *V.glabra* leaf and flower extracts in this study would be of significant importance in further search of novel drugs, with desirable synergistic effects, to kill persistent micro-

organisms that are resistant to known antibiotics and probably delay emergency of microbial resistance.

The organic extracts were active against *S.aureu*, *A.niger*, *C.albicans*, and *E.coli* with Mean inhibition zones of 0.35, 0.05, 0.03 and 0.01 respectively. The aqueous extracts were less active against *S.aureus* only (MIZ of 0.02) and inactive against *E.coli*, *A.niger*, and *C.albicans*. Noting that the traditional herbal remedy preparation from this species is by use of water, it is a paradox that the aqueous extracts were inactive in this study. This may be due to the absence or insufficient and effective concentration of the antimicrobial constituents in the aqueous extracts of *V.glabra*. These findings are in agreement with the studies of Koua *et al.*, (2011) who reported that inactivity of aqueous and organic extracts of *Striga hermonthica* may be due to absence or insufficient and effective concentration of the antimicrobial agents of *S.hermonthica* extracts. It is well known that patients using the traditional herbs take in large amounts of the concoctions and hence may eventually consume sufficient amounts of the curative drugs to elicit healing.

Differential sensitivity of bacteria to *V.glabra* extracts may be explained by the cell wall composition of the gram-positive and gram-negative bacteria. The cell wall of gram-ve bacteria (*E.coli*), contains the outer membrane and lipid bilayer embedded with proteins and porins that determine and allow different molecules or ions either into or out of the cell and thus the outer membrane serves as a barrier to the passage of many molecules and hence less sensitive to many extracts and render *E.coli* resistant to plant extracts. The gram+ve bacterium (*S.aureus*) has a relatively thick layer of peptidoglycan sheets of interconnected glycan chains made up of polymer which is fully permeable to many substances, and thus sensitive to most extracts, Nester *et al.*, (2004).

Less effects of the crude extracts against *C.albicans* (yeast fungus) compared to *A.niger* (filamentous fungus) may also be due to differences in cell wall composition of the organisms. Yeast fungus cell wall contains polysaccharides and proteins, compared to chitin and glycan found in the cell walls of filamentous fungi, Paiva *et al.*, (2010). The protein expression on *C.albicans*, function as selective transport system used to expel wastes and compounds that are otherwise deleterious to the cell; a function called efflux which is medically important in that, it allows micro-organisms to oust antimicrobial medications that are made to destroy them and therefore render them resistance, Nester *et al.*, (2004). This explanation could be one of the reasons why *C.albicans* was resistant to most crude extracts.

This observation reported in this study is in agreement with the findings of Masakazu *et al.*, (2010), who did research on antifungal drug resistance of *Candida* spp and reported that expression of drug efflux pumps were responsible for the resistance.

The higher activity of organic extract of flower compared to the standard antibiotic (streptomycin) is significant. Such comparisons may be used as yardstick to identify novel drugs with new mechanisms of action to combat bacterial strains resistant to known antibiotics, shorten the length of treatment, increase patient compliance, and avoid overdose which may lead to toxicity or side effects to patients. The findings of this study are in line with the studies of Mariita *et al.*, (2010); Aiyegoro and Okoth, (2009), who reported that bioactive extracts of medicinal plants at low concentrations are not only active against drug resistant bacteria, but kill persistent bacteria, shorten the length of treatment, reduce overdose and toxicity or side-effects.

The higher antimicrobial activity of *Vernonia glabra* flowers could be attributed to the presence of large amounts of flower alkaloids 50% (Fig. 6). Alkaloids are pharmacogenically active basic principles of flowering plants, Das *et al.*, (2010). The observed activity of these compounds in *V.glabra* is in line with that of Sawer *et al.*, (2005 (as cited in Karou *et al.*, 2006) who demonstrated that the Indoloquinoline alkaloid causes cell lysis and morphological changes of *S.aureus*. The presence of alkaloids in *V.*glabra extracts has not been reported in any previous research work and the findings in this study could be the first one to report.

Root sapogenins (43.8% present and active) have been associated with hemolysis property and it's probably why significant anti-staphylococcal activity has been reported in this study. This finding is in agreement with the studies of Pinarosa, *et al.*, (2006) who did research on sapogenins of *Medicago arabica* root and reported a high anti-staphylococcal activity against *S.aureus*. According to literature on *V.glabra* sapogenins, no previous work has reported the presence of sapogenins in *V.glabra*. This could be the first time to report.

The antibacterial activity of root terpenoids (38.5% active) may be due to presence of terpenes combined and associated with bacterial membrane disruption. This finding is in line with the studies of Chiruvella *et al.*, (2007) who did research on terpenoids isolated from callus cultures of *soymida febrifuga* and reported that antimicrobial activity of terpenoids is probably due to membrane disruption by the terpenes.

On the other hand, leaf quinones (37.5% present but 25% active) had moderate activity, while flavonoids (31.8% - 36.4% present but 9.1% active) had low activity against *S.aureus*.

Conclusions and Recommendations

The screening of ten crude extracts (5 organic and 5 aqueous) of *V.glabra* collected using available ethnomedical information, has justified the medicinal potency in *V.glabra* used by traditional practitioners in Kenya to treat gastrointestinal problems and other different diseases and that ethnomedicinal information is important as a start point for research in drug development.

The high activity of *V.glabra* flower reported at lowest MIC of 1.5625mg/100µl than the standard antibiotic (streptomycin MIC of 6.25mg/100µl), is significant in treatment of resistant microbial pathogens. The low concentrations needed for treatment means that cases of overdose, toxicity, and side-effects may be reduced when using *V.glabra* flowers. However, research on toxicology which is missing in this study is recommended for *V.glabra* in order to verify and document the safety of this medicinal plant to the society.

Acknowledgement

This work was fully funded by the University of Nairobi, and partially V*iCRES* under the Inter-University Council of East African Universities (IUCEA). We gratefully thank them.

References

Aiyegoro, O.A., and Okoth, A.I. (2009). Use of bioactive plant products in combination with standard antibiotics: Implications in antimicrobial chemotherapy. *Journal of Medicinal Plants Research*, 3(13), pp.1147-1152.

Beentje, H.J., 1994. *Kenya trees, shrubs and lianas*. National Museums of Kenya. Nairobi

Chiruvella, K.K., Mohammed, A., Dempuri, G., Gopal, R.G., and Raghavan, S.C. (2007). Phytochemical and Antimicrobial Studies of Methyl Angolensate and Luteolin of-O-glucoside Isolated from Callus Cultures of *Soymida febrifuga*. *International Journal of Biomedical Science*, 3(4) pp. 269-278.

Chika, C.O., Jude, N.O., Ifeanyi, C.O., & Anyanwu, N.B. (2007). Antibacterial Activities and Toxicological Potentials of Crude Ethanolic Extracts of *Euphorbia hirta*. *Journal of American Science*, 3(3), pp.11-16.

Das, K., Tiwari, R.K.S., & Shrivastava, D.K. (2010). Techniques for Evaluation of Medicinal Plant Products as Antimicrobial Agent: Current Methods and Future Trends. *Journal of MedicinalPlantsResearch*,4(2),pp.104-111.Availableonlineat http://www.academicjournals.org/JMPR

Harborne, J.B. (1998). *Phytochemical Methods*: *A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman & Hall.

Jigna, P., and Chanda, S.V. (2007). *In vitro* Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. *Turk J Biol*, **3**: 53-58.

Johns, T., Faubert, G.M., Kokwaro, J.O., Mahunnah, R.L.A., & Kimanani, E.K. (1995). Anti-giardial Activity of Gastrointestinal Remedies of the Luo of East Africa. *Ethnopharmacol*, **46** (1), pp.17-23.

Kareru, G., Kenji, M., Gachanja, N., Keriko, M., & Mungai, G. (2007). Traditional Medicines among the Embu and Mbeere People of Kenya. *Afr J Trad CAM*, **4**: 75-86.

Karou, D., Savadogo, A., Canini, A., Yameogo, S., Montesano, C., Simpore, J., ... Traore, A. (2006). Antibacterial activity of alkaloids from *Sida acuta*. *African Journal of Biotechnology*, **5**(2), pp.195-200. Available online at http://www.academicjournals.org/AJB

Koua, F.H.M., Babiker, H.A., Halfawi, A., Ibrahim, R.O., Abbas, F.M., Elgaali, E.I., ... Khlafallah, M.M. (2011). Phytochemical and biological study of *Striga hermonthica* (Del.) Benth Callus and intact plant. Research In Pharmaceutical Biotechnology, **3**(7), pp. 85-92. Available online at http://www.academicjournals.org/rpb

Mariita, R.M., Ogol, C.K.P., Oguge, N.O., & Okemo, P.O. (2010). Antitubercular and Phytochemical Investigation of Methanol Extracts of Medicinal Plants used by the Samburu Community in Kenya. *Tropical Journal of Pharmaceutical Research*, **9** (4):379-385.

Maryam, Z., Aqil, F., Khan, M.S.A., & Ahmad, I. (2010). Ethnomedicinal plants derived antibacterial and their prospects. Ethnomedicine: A source of Complementary Therapeutics, pp.150-177.

Masakazu, N., Firth, N.A., Cannon, D.R. (2010). Antifungal drug resistance of oral fungi.Odontology, 98(1):15-25, DOi:10.1007/s/0266-009-0118-3. *Review Article*. Available online https:/springerlink3.metapress.com [accessed 13th September, 2011].

Miaron, O.J., Kassim, O., & Ekaya, N. (2004). Indigenous Knowledge: The basis of Maasai Ethnoveterinary Diagnostic Skills. *J Hum Ecol*, **16**: 43-48.

Munguti, K. (1997). Indigenous Knowledge in the Management of Malaria and Visceral Leishmaniasis among the Turgen of Kenya. *Indig-Knowl Dev Monitor*, **5**: 10-12

Nester, E.W., Anderson, D.G., Roberts, C.E., Pearsall, N.N., & Nester, M.T. (2004). *Microbiology*: *A Human Perspective*. 4th ed. New York. McGraw-Hill.

Nostro, A., Germano, M., Marino, D.V., & Cannatelli, M. (2000). Extraction Methods and Bioautography for Evaluation of Medicinal Plant Antimicrobial Activity. Letters in Applied Microbiology, **30**: 379-384. Doi: 10.1046/j. 1472-765x.2000.00731.x

Ochei, J., & Kolhatkar, A. (2000). *Medical Laboratory Science. Theory and Practice*. India: Tata McGraw Hill, p.803.

Owour, B.O. and Kisangau, D.P. (2006). Kenyan Medicinal plants used as antivenin: a comparison of plant usage. Journal of Ethnobiology and Ethnomedicine, 2:7.

Paiva, P.M.G., Gomes, F.H., Napoleao, T.H., Sa, R.A., Correia, M.T.S., & Coelho, L.C.B.B. (2010). Antimicrobial activity of Secondary metabolites and Lectins from Plants Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, pp. 396-406.

Pinarosa, A., Rosella, B., Tava, A., Vitali, C., Rosato, A., Zbigniew, B., ... Marian. J. (2006). Antimicrobial Activity of Sapons from Medicago sp: Structure-Activity Relationship. *Phytother. Res*. **20**:454-457.

Runyoro, D.K.B., Matee, M.I., Ngasapa. O.D., Cosam, C.J., & Mwambo, Z.H. (2006). Screening of Tanzanian Medicinal Plants for Anti-Candida Activity. *BMC Complementary and Alternative Medicine*, **6**:11.

Sandiga, I., Chacha, C.N., & Kanunah, M.P. (1995). *Traditional Medicine in Africa*: *The existence and Use of Traditional Medicine in Kenya* [e-book]. Nairobi: East African

Educational Publishers Ltd. Available from http://books.google.com/books [cited 19 July 2011].

Sasikumar, J.M., Thayumanavan, T., Subashkumar, R., Janardhanan, K., & Lakshmanaperumalsamy, P. (2007). Antibacterial activity of some ethnomedicinal plants from the Nilgiris, Tamil Nadu, India. *Natural Product Radiance*, **6** (1), pp. 34-39.

United Nations Environment Program- UNEP. (2010). Global Strategy for Plant Conservation. Biodiversity is life 2010 International Year of Biodiversity. Website: www.cbd.int/gspc

World Health Organization, Food Agricultural Organization of the United Nations and World Organization for Animal Health- W.H.O/FAO/OIE. (2003). Expert Workshop on Non-human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment, pp. 1-5.