EFFECTS OF SELECTED MEDICINAL PLANT EXTRACTS AGAINST COMMON OPPORTUNISTIC BACTERIA ISOLATED FROM SEPTIC HERPETIC LESIONS AMONG HIV and AIDS PATIENTS

BY

MAMO UMURO ABUDO

REGISTRATION NUMBER - 156/7510/2002

A Thesis Submitted in Partial Fulfillment for the Award of the Degree of Master of Science (Infectious Diseases Diagnosis) in the school of Pure and Applied Sciences, Department of Biochemistry and Biotechnology Kenyatta University, Nairobi
DECLARATION

This Thesis is my original work and has not been presented for a degree or other award in any other university.

MAMO UMURO ABUDO

Signature.................................................. Date 11th May 2006

We confirm that the candidate under our supervision carried out the work reported in this thesis.

DR. FAITH W. MULI

Department of Biochemistry,
Kenyatta University.

Signature.................................................. Date 11th May 2006

PROF. MOHAMMED S. RAJAB

Department of Chemistry,
Kenyatta University.

Signature.................................................. Date 11th May 2006
DEDICATION

I dedicate this Thesis to my mother, Adho Umuro Abudo whose tenderness and love made me what I am today.
ACKNOWLEDGEMENT

This work has been made possible by contribution of many people. I am grateful to them all. Some of them however, deserve a special acknowledgement for the key role they played. This work could not have been possible without the following persons and institutions that I hereby sincerely acknowledge.

I recognize with appreciation the great support and tremendous encouragement from my supervisors, Dr. Faith W Muli and Prof. Mohammed S Rajab who ensured that all the work went on smoothly and were always available for consultations despite the tight schedules of their office.

I would like to thank the Ford Foundation who supported this research through the project on “Determination of the effects of Apocynaceae Extracts on viral opportunistic infections in patients living with HIV and AIDS”, Project Code Number 1010-1837. I feel much indebted to Dr Muluvi, the Chairman Department of Biochemistry for providing an enabling environment to undertake the project. I wish to express my sincere gratitude to Mr. Ndegwa chief technician department of health science, Kenyatta University for assisting in field collection and Identification of the medicinal plants used in this study. I wish to extend my special gratitude to all Nafsi members who stood by me when the going was tough. I wish to thank members of my family; Mother, brothers and sisters for their understanding and encouragement both which inspired me to complete the programme. Finally, I extend my sincere gratitude to my wife, Tume for her inspiring moral support.
Opportunistic bacterial infections have been identified as a major complication in immune compromised persons. Many of the antibiotics used in management of bacterial infections are experiencing increased resistance posing enormous public health concern. Herbal extracts have in the past been found to contain antibacterial compounds. There is therefore need to continue searching for new drugs to expand the choice of compounds needed to fight these conditions. In this study, crude extracts from fifteen Kenyan medicinal plants were investigated for antibacterial activity against 60 strains of common opportunistic bacteria (Staphylococcus, Pseudomonas, Klebsiella, Proteus and Escherichia coli) isolated from confirmed HIV and AIDS patients with septic herpetic lesions. The plants were collected within Kenya and were selected from among medicinal plants widely used in the treatment of various ailments using the information obtained from ethnomedical practice and literature. Polar and non-polar solvents were employed for extraction of plants. Disc diffusion technique was used to preliminarily determine invitro antibacterial activity for the extracts by evaluating the ability of the extracts to inhibit the growth of the five bacterial species. The minimum inhibitory concentration and minimum bactericidal concentration of the extracts with the most significant predominant activity were evaluated by plate dilution method. The opportunistic bacteria were isolated using standard culture techniques and identified by colonial morphology, gram stain and biochemical test for metabolic and enzymatic reactions. The activity exhibited by plant extracts was against both gram positive and gram-negative bacteria. Of the fifteen plants screened, four extracts i.e. from Ehretia cymosa, Combretum molle, Ekerbagia capensis and Plectranthus barbatus showed significant antibacterial effects against most bacterial isolates tested. The minimum inhibitory concentration reached by 50% (MIC\textsubscript{50}) and 90% (MIC\textsubscript{90}) were between 0.25-1mg/ml and 0.5-2mg/ml. The most effective plant extract was Plectranthus barbatus at mic\textsubscript{50} and mic\textsubscript{90} concentration of 0.5 –1mg for all categories of bacterial isolates. It is concluded that selected plant extracts had significant effects on both gram negative and gram-positive bacteria and that carefully guided extraction and characterization of these plant compounds may yield useful antibiotic compounds.
TABLE OF CONTENT

Declaration ........................................................................................................................................ ii
Dedication ......................................................................................................................................... iii
Acknowledgements ..................................................................................................................... iv
Abstract .......................................................................................................................................... v
Table of contents ........................................................................................................................... vi
List of Tables .................................................................................................................................... xi
List of Figures ................................................................................................................................... xii
List of abbreviations ....................................................................................................................... xiii

CHAPTER ONE .................................................................................................................................. 1
1.0 INTRODUCTION ........................................................................................................................... 1
1.1 Justification .................................................................................................................................. 3
1.2 Hypothesis ..................................................................................................................................... 4
1.3 Objectives ..................................................................................................................................... 4
1.3.1 Main objectives ..................................................................................................................... 4
1.3.2 Specific objectives ................................................................................................................ 4

CHAPTER TWO .................................................................................................................................. 5
2.0 LITERATURE REVIEW .................................................................................................................. 5
2.1 Opportunistic bacterial infection .................................................................................................. 5
2.1.1 Staphylococci ....................................................................................................................... 5
2.1.1.1 Clinical manifestation of Staphylococcus ..................................................................... 7
2.1.1.2 Pathogenesis ................................................................. 7
2.1.1.3 Laboratory diagnosis .................................................. 8
2.1.1.4 Resistance of Staphylococci to antimicrobial drugs .......... 10
2.1.1.5 Treatment ................................................................. 11
2.1.2 Pseudomonas aeruginosa .................................................. 12
2.1.2.1 Pathogenesis ............................................................. 13
2.1.2.2 Resistance of Pseudomonas to antimicrobial drugs .......... 13
2.1.2.3 Treatment ................................................................. 14
2.1.3 Escherichia coli ............................................................... 14
2.1.3.1 Pathogenesis ............................................................. 15
2.1.3.2 Treatment ................................................................. 16
2.1.4 Klebsiella species ............................................................. 16
2.1.4.1 Pathogenesis ............................................................. 17
2.1.4.2 Treatment ................................................................. 18
2.1.5 Proteus species ............................................................... 19
2.1.5.1 Pathogenesis ............................................................. 20
2.1.5.2 Treatment ................................................................. 21
2.2. Medicinal plants and traditional medicine .......................... 22
2.2.1. Traditional medicine ..................................................... 22
2.2.2. Plants as source of antibacterial agent ............................ 23
2.2.3. Plants included in the study .......................................... 25
2.2.3.1 Combretum molle ...................................................... 25
CHAPTER THREE .............................................................................. 28

3.0 MATERIALS AND METHODS ................................................. 28

3.1 Study bacterial isolates ................................................... 28

3.1.1 Collection of specimens for bacterial isolation ............... 28

3.1.2 Specimen processing .................................................. 28

3.1.3 Identification of the isolates ....................................... 29

3.1.3.1 Klebsiella screening media .................................... 29

3.1.3.2 Citrate utilization test ........................................... 29

3.1.3.3 Indole test .......................................................... 30

3.1.3.4 Urea test ........................................................... 30

3.1.3.5 Oxidase test ....................................................... 30

3.1.3.6 Catalase test ....................................................... 30

3.1.3.7 Coagulase test .................................................... 31

3.1.3.8 Confirmation of the bacterial isolates ....................... 31

3.1.4 Bacterial isolates preparation .................................... 31

3.1.5 Preservation of bacterial isolates ............................... 32

3.1.6 Antibiotic sensitivity testing ...................................... 32
3.2 Medicinal plants ................................................................. 33
3.2.1 Study plants ................................................................. 33
3.2.2 Collection of medicinal plants ........................................ 34
3.2.3 Processing of medicinal plants ....................................... 34
3.2.3.1 Leaves ................................................................. 34
3.2.2.2 Latex ................................................................. 35
3.2.4 Extractions of plants material .......................................... 35
3.2.4.1 Extraction with two organic solvents ............................ 35
3.2.4.2 Extraction with three organic solvents .......................... 36
3.2.5 Bioassays ................................................................. 36
3.2.5.1 Disc diffusion method .............................................. 36
3.2.5.2 Agar dilution method .............................................. 37
3.2.5.2.1 Minimum inhibitory concentration .......................... 37
3.2.5.2.1.1 Stock Preparation (Crude Extracts) ....................... 37
3.2.5.2.2 Minimum bactericidal concentration ....................... 39

CHAPTER FOUR ......................................................................... 41

4.0 RESULTS .............................................................................. 41
4.1 Isolation of bacterial species ............................................. 41
4.2 Antibiotic susceptibility .................................................... 46
4.3 Susceptibility to plants extracts ........................................ 48
4.4 Minimum inhibitory concentration .................................... 50
4.4.1 MIC of Methanol plants extracts .............................................. 50
4.4.2 MIC of Hexane plants extracts .............................................. 52
4.4.3 MIC of Dichloromethane plants extracts ............................... 54
4.5 Minimum bactericidal concentration ....................................... 56
4.6 Activity of medicinal plants preparation compared to

Conventional antibiotic .......................................................... 58

CHAPTER FIVE .................................................................................. 59

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATION 59

5.1 DISCUSSION ............................................................. 59

5.2 CONCLUSIONS ......................................................... 63

5.3 RECOMMENDATIONS .................................................. 64

REFERENCE ................................................................. 65

APPENDIX ................................................................. 74
LIST OF TABLES

Table 1  Medicinal plants of study.................................................................33
Table 2  Dilution series of MIC.................................................................38
Table 3  Dilution series of MBC.................................................................40
Table 4  Organism recovered from septic herpetic lesion.........................42
Table 5  Zone of inhibition (millimeters) of antibiotic ................................47
Table 6  Zone of inhibition (millimeters) for selected
  medicinal plant extracts against bacterial isolates .............................49
Table 7  MIC (mg/ml) of methanol plant extracts ...........................................51
Table 8  MIC (mg/ml) of hexane plant extracts ..............................................53
Table 9  MIC (mg/ml) of Dichloromethane plant extracts ...............................55
Table 10  MBC (mg/ml) of plant extracts against strains of
  bacterial isolates.......................................................................................57
LIST OF FIGURES

Figure 1  Gram- positive *Staphylococcus aureus* ...........................................43

Figure 2  Gram -negative *Klebsiella* isolates ..................................................44

Figure 3  *Pseudomonas aeruginosa* colonies .........................................................45
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>AMO</td>
<td>Amoxycillin</td>
</tr>
<tr>
<td>AMP</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>API</td>
<td>Appareils et procedes d' Identification</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for disease control</td>
</tr>
<tr>
<td>CEP</td>
<td>Cephalosporin</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>CHL</td>
<td>Chloraphenicol</td>
</tr>
<tr>
<td>CIT</td>
<td>Citrate</td>
</tr>
<tr>
<td>COT</td>
<td>Cotrimoxazol</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DIZ</td>
<td>Diameter of inhibition zone</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>EHEC</td>
<td>Enterohemorrhagic <em>Escherichia coli</em></td>
</tr>
<tr>
<td>EIEC</td>
<td>Enteroinvasive <em>Escherichia coli</em></td>
</tr>
<tr>
<td>EPEC</td>
<td>Enteropathogenic <em>Escherichia coli</em></td>
</tr>
<tr>
<td>ESBL</td>
<td>Extended Spectrum beta lactamase</td>
</tr>
<tr>
<td>ET</td>
<td>Epidermolytic toxin</td>
</tr>
<tr>
<td>ETEC</td>
<td>Enterotoxigenic <em>Escherichia coli</em></td>
</tr>
<tr>
<td>GEN</td>
<td>Gentamicin</td>
</tr>
</tbody>
</table>
HIV: Human Immunodeficiency virus
H$_2$O$_2$: Hydrogen peroxide
HUS: Haemolytic uremic syndrome
IV: Intravenous
KAN: Kanamycin
KSM: *Klebsiella* screening media
MBC: Minimum Bactericidal Concentration
MDR: Multi Drug Resistant
MEOH: Methanol
M-H: Muller Hinton agar/broth
MIC: Minimum Inhibitory Concentration
MRSA: Methicillin resistant *Staphylococcus aureus*
NCCLS: National Committee for Clinical Laboratory Standards
TET: Tetracycline
TNF: Tumor Necrosis Factor
TSST: Toxic shock syndrome toxin
UTI: Urinary tract infection
WHO: World Health Organization
CHAPTER ONE

1. INTRODUCTION

Opportunistic infections are mild to severe infectious diseases caused by microorganisms that exist as part of the normal body flora but become pathogenic when the normal body defense mechanism is impaired (Baily and Scotts’, 1994). Opportunistic infections among patients with impaired immune systems are caused by wide range of microorganisms such as bacteria, viruses, fungi, or parasites (Gilks, 1998; Kaplan, 1996). Many of these organisms are pervasive in the environment but do not cause disease until host immunity declines. Occurrence of opportunistic infection often marks the progression from early to advanced disease (AIDS). In most cases infection follows some form of injury to the skin that allows the usually non-virulent microbes to invade. Then with secondary host defenses impaired, significant local or disseminated disease may result.

Infection with HIV leads progressively to profound immune dysfunction (Borkowsky et al., 1987). The primary targets of HIV in the immune system are CD4 positive helper –T lymphocytes. The invasions of these cells result into a progressive decrease in their numbers as well as qualitative defects in T cell function (Borkowsky et al., 1987). The major consequence of this immune dysfunction is an increased susceptibility to infection by a number of opportunistic organisms. Opportunistic bacteria have been identified as the major complication of HIV infection (Bernstein et al., 1995; Smith et al., 1999). If the bacteria that cause opportunistic infections acquire resistance to the commonly used antibiotics, they will be difficult to treat (Neu, 1992).
The most common of these bacteria are species of Mycobacterium, Listeria, Pseudomonas, Staphylococcus, Klebsiella, Providentia, Nocardia, Seratia, Corynebacterium, Streptococci, Heamophilus, Proteus and Escherichia coli among others (Bailey and Scott’s, 1994; Boudreau et al., 1988; Gilks, 1998). Bacterial infections in HIV positive individuals are essentially opportunistic, hence usually appearing only due to immunosuppression of the individual. These bacteria take advantage of both the cell-mediated and humoral immunodeficiency, which is particularly true in intravenous (IV) drug users, who often have subcutaneous bacterial infections (Gilks, 1998). Smith et al., (1999) found that approximately 71% of their study groups were infected with S.aureus skin infections. Smith et al. (1999) cite a study performed by Nicholas, Ballogh and Silverman in 1989, which found that 83% of AIDS patients were infected with a bacterial infection on autopsy, with S.aureus being the most common. S. aureus manifests itself as bullous impetigo and superficial folliculitis or deep cutaneous infections such as echzyma, plaque like folliculitis, botryomycosis or abscesses (Ronald, 1995).

The skin and mucosa are the body’s first defence mechanism against countless microbial threats (Stone & Lynch, 1990). However, this line of defence depends on a functional immune system. When the immune system is suppressed disorders of the skin may appear. Herpes virus is a common cause of dermatoses among HIV and AIDS patients. The infection with this virus causes primary infection that goes latent in the infected hosts and reactivates in the immune suppressed state. The lesion appears creating a potential portal of entry for opportunistic bacteria, which are frequent colonizers of normal skin (Pape et al., 1983). These secondary infections may not respond to the usual therapies at all or may require higher doses of medication for prolonged periods or
repeated surgical procedures (Morse et al., 1986). In today’s battle against infectious agents, conventional medicine may be effective however, due to the high cost of drugs and increasing bacterial resistance to the drugs, alternative medicine seems to be the latest weapon. As a result, growing number of health care consumers are turning to plants medicines with a believe that these product has fewer side effects (Stephen, 1999; Huffman and Wrangham, 1993).

1.1 JUSTIFICATION

Opportunistic bacterial infections have been identified as a major complication of HIV infection (Bernstein et al., 1985) and a main cause of considerable suffering and early death in HIV patients. Treatment of this emerging and re emerging strains of bacteria has become difficult due to their increase tolerance to present available antibiotics. New therapeutic agents with different modes of antibacterial actions are therefore desired. Many conventional antibiotics have been developed, however, due to their high cost they are out of reach to most people in developing world where the burden of infectious diseases is on the increase. There is therefore, need to identify and develop alternative drugs, which are effective, affordable and easily accessible to these people.
1.2 HYPOTHESIS

Medicinal plants are effective in the treatment of opportunistic bacterial infections in septic herpetic lesions among HIV/AIDS infected patients.

1.3 Objectives of the study

1.3.1 Broad Objective

The main objective of the study was to assess potential of herbal extracts from fifteen medicinal plants growing in Kenya for antibacterial activity.

1.3.2 Specific Objective

The specific objective of the study were

1. To isolate and identify bacteria in herpetic lesion of HIV infected patients.
2. To determine antibiotic susceptibility profiles of the bacterial isolates
3. To determine susceptibility of the isolated bacteria to selected medicinal plant extracts.
4. To compare the effectiveness of medicinal plant preparations to that of conventional antibiotics.
CHAPTER TWO

2. LITERATURE REVIEW

2.1 Opportunistic bacterial infection

Bacterial infections originating in the skin appear to be increased in incidence and severity in immune compromised patients (Cone et al., 1992). These infections are commonly caused by both Gram positive and Gram-negative opportunistic bacteria, which normally colonize normal skin. The skin and subcutaneous tissue occupy a central position in any consideration of infection in immune compromised host. The skin and mucosal surfaces of the body interface the environment and are the primary host barriers against infection. These primary barriers assume even greater importance in patients whose secondary host defences are impaired. Secondary bacterial infections among HIV and AIDS patients follow either some form of injury to the skin or due to underlying dermatoses which provide a potential portal of entry for life threatening opportunistic bacteria.

2.1.1 Staphylococci

The name *staphylococci* are derived from the Greek term for grape like cocci (Bhakdi and Tranum-Jensen, 1991). This name is appropriate because the cellular arrangement of these gram-positive cocci resembles a cluster of grapes. The bacteria normally grow on the skin and the mucus membrane of humans as well as that of other mammals and birds (Baily and Scotts, 1994). Their presence as endogenous flora allow
many species of *Staphylococcus* to cause opportunistic infections when the host defence mechanisms breaks down (Cheesbrough, 2000). Traditionally they were divided into two groups on the basis of their ability to clot blood plasma (the coagulase reaction). The coagulase-positive *staphylococci* constitute the most pathogenic species *Staphylococcus aureus*. The coagulase-negative *staphylococci* (CNS) are now known to comprise over 30 other species (Bhakdi and Tranum-Jensen, 1991).

The Coagulase negative staphylococci are common commensals of skin, although some species can cause infections. It is now obvious that the division of *staphylococci* into coagulase positive and negative is artificial and indeed, misleading in some cases. Coagulase is a marker for *Staphylococcus aureus* but there is no direct evidence that it is a virulence factor. Also, some natural isolates of *Staphylococcus aureus* are defective in coagulase. Nevertheless, the term is still in widespread use among clinical microbiologists (Bhakdi and Tranum-Jensen, 1991).

*Staphylococcus aureus* expresses a variety of extra cellular proteins and polysaccharides, some of which are correlated with virulence. Virulence results from the combined effect of many factors expressed during infection. Antibodies will neutralize staphylococcal toxins and enzymes, but vaccines are not available (Bhakdi and Tranum-Jensen, 1991). Both antibiotic treatment and surgical drainage are often necessary to cure abscesses, large boils and wound infections. *Staphylococci* are common causes of infections associated with indwelling medical devices. These are difficult to treat with antibiotics alone and often require removal of the device. Some strains that infect hospitalized patients are resistant to most of the antibiotics used to treat infections,
vancomycin being the only remaining drug to which resistance has not developed (Bhakdi and Tranum-Jensen, 1991).

2.1.1.1 Clinical Manifestations of *Staphylococcus aureus*

*Staphylococcus aureus* is notorious for causing boils, furuncles, styes, impetigo and other superficial skin infections in humans (Schlievert, 1993). It may also cause more serious infections, particularly in persons debilitated by chronic illness, traumatic injury, burns or immunosuppression. These infections include pneumonia, deep abscesses, osteomyelitis, endocarditis, phlebitis, mastitis and meningitis, and are often associated with hospitalized patients rather than healthy individuals in the community. *S. aureus* and *S. epidermidis* are common causes of infections associated with indwelling devices such as joint prostheses, cardiovascular devices and artificial heart valves (Schlievert, 1993).

2.1.1.2 Pathogenesis of *Staphylococcus aureus* Infections

*Staphylococcus aureus* expresses many cell surface-associated and extracellular proteins that are potential virulence factors. For the majority of diseases caused by this organism, pathogenesis is multifactorial. Thus it is difficult to determine precisely the role of any given factor (Tenovar et al., 1994). However, there are correlations between strains isolated from particular diseases and expression of particular factors, which suggests their importance in pathogenesis. In order to initiate infection the pathogen must gain access to the host and attach to host cells or tissues. *Staphylococcus aureus* cells express on their surface proteins that promote attachment to host proteins such as laminin
and fibronectin that form part of the extra cellular matrix (Vaudaux et al., 1994). In addition, most strains express a fibrinogen/fibrin binding protein (the clumping factor) that promotes attachment to blood clots and traumatized tissue. Most strains of \textit{S. aureus} express fibronectin and fibrinogen-binding proteins (Foster and McDevitt, 1994). The receptor, which promotes attachment to collagen, is particularly associated with strains that cause osteomyelitis and septic arthritis. Interaction with collagen may also be important in promoting bacterial attachment to damaged tissue where the underlying layers have been exposed (Schlievert, 1993). Evidence that these staphylococcal matrix-binding proteins are virulence factors, has come from studying defective mutants in invitro adherence assays and in experimental infections. Mutants defective in binding to fibronectin and to fibrinogen have reduced virulence in a rat model for endocarditis, suggesting that bacterial attachment to the sterile vegetations caused by damaging the endothelial surface of the heart valve is promoted by fibronectin and fibrinogen. Similarly, mutants lacking the collagen-binding protein have reduced virulence in a mouse model for septic arthritis. Furthermore, the soluble ligand-binding domain of the fibrinogen, fibronectin and collagen-binding proteins expressed by recombinant methods strongly blocks interactions of bacterial cells with the corresponding host protein (Foster and McDevitt, 1994).

2.1.1.3 Laboratory diagnosis

\textit{Staphylococci} are Gram-positive cocci about 0.5 - 1.0 \textmu m in diameter. They grow in clusters, pairs and occasionally in short chains. The clusters arise because \textit{Staphylococci} divide in two planes. The configuration of the cocci helps to distinguish
Micrococci and Staphylococci from Streptococci, which usually grow in chains. Observations must be made on cultures grown in broth, because Streptococci grown on solid medium may appear as clumps. Several fields should be examined before deciding whether clumps or chains are present (Duvic, 1987; Bhakdi and Tranum-Jensen, 1991).

The presence of staphylococci in a lesion might first be suspected after examination of a direct Gram stain. However, small numbers of bacteria in blood preclude microscopic examination and require culturing first (Rupp and Archer, 1994). The organism is isolated by streaking material from the clinical specimen onto solid media such as blood agar, tryptic soy agar or heart infusion agar (Bhakdi and Tranum-Jensen, 1991). Specimens likely to be contaminated with other microorganisms can be plated on mannitol salt agar containing 7.5% sodium chloride, which allows the halo-tolerant staphylococci to grow. Ideally a Gram stain of the colony should be performed and tests made for catalase and coagulase production, allowing the coagulase-positive S. aureus to be identified quickly. Another very useful test for S. aureus is the production of thermos table deoxyribonuclease. S. aureus can be confirmed by testing colonies for agglutination with latex particles coated with immunoglobulin G and fibrinogen which bind protein A and the clumping factor, respectively, on the bacterial cell surface. These are available from commercial suppliers (such as Staphaurex). The most recent latex test (Pastasurex) incorporates monoclonal antibodies to serotype 5 and 8 capsular polysaccharide in order to reduce the number of false negatives (Tenovar et al., 1994).

The association of S. epidermidis (and to a lesser extent of other coagulase-negative staphylococci) with nosocomial infections associated with indwelling devices means that isolation of these bacteria from blood is likely to be important and not due to
chance contamination, particularly if successive blood cultures are positive. Nowadays, identification of *S. epidermidis* and other species of *Staphylococcus* is performed using commercial biotype identification kits, such as API Staph Ident, API Staph-Trac, Vitek GPI Card and Microscan Pos Combo. These comprise preformed strips containing test substrates.

2.1.1.4 **Resistance of *Staphylococci* to antimicrobial drugs**

Hospital strains of *S. aureus* are often resistant to many different antibiotics. Indeed strains resistant to all clinically useful drugs, apart from the glycopeptides vancomycin and teicoplanin, have been described (Lyon and Skurray, 1987). Plasmid-associated vancomycin resistance has been detected in some *Enterococci* and the resistance determinant has been transferred from enterococci to *S. aureus* in the laboratory and may occur naturally. *S. epidermidis* nosocomial isolates are also often resistant to several antibiotics including methicillin. In addition, *S. aureus* expresses resistance to antiseptics and disinfectants, such as quaternary ammonium compounds, which may aid its survival in the hospital environment (Easmon and Adlan, 1983).

Since the beginning of the antibiotic era *S. aureus* has responded to the introduction of new drugs by rapidly acquiring resistance by a variety of genetic mechanisms including acquisition of extrachromosomal plasmids or additional genetic information in the chromosome via transposons or other types of DNA insertion and by mutations in chromosomal genes (Prevost *et al.*, 1995).

Many plasmid-encoded determinants have recently become inserted into the chromosome at a site associated with the methicillin resistance determinant. There may
be an advantage to the organism having resistance determinants in the chromosome because they will be more stable. There are essentially four mechanisms of resistance to antibiotics in bacteria which include enzymatic inactivation of the drug, alterations to the drug target to prevent binding, accelerated drug efflux to prevent toxic concentrations accumulating in the cell, and a by-pass mechanism whereby an alternative drug-resistant version of the target is expressed (Tenovar et al., 1994; Foster, 1991).

2.1.1.5 Treatment

Minor skin infections, such as folliculitis and tiny patches of impetigo are usually treated with an ointment such as nonprescription triple-antibiotic mixture (bacitracin, neomycin, polymyxin B) or prescription mupirocin. For most other skin infections, oral antibiotics (such as cloxacillin, dicloxacillin, and cephalexin) are adequate. More severe infections, especially blood infections, require intravenous antibiotic therapy, often for up to 6 weeks. The choice of antibiotic depends on the site of infection, the severity of the illness, and the susceptibility of the particular staphylococcal strain. Some strains are resistant to many antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to nearly all antibiotics and is becoming increasingly common in big city and university hospitals. Among the few antibiotics that are still effective against MRSA are vancomycin and trimethoprim-sulfamethoxazole. Antibiotics alone do not cure abscesses; they must also be drained. Abscesses deeper in the body may require surgery (Skinner and Ahmad, 1994).
2.1.2 Pseudomonas aeruginosa

Pseudomonas aeruginosa is the epitome of an opportunistic pathogen of humans. The bacterium almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner (Robin and Janda, 1984). Pseudomonas aeruginosa is a Gram-negative rod measuring 0.5 to 0.8 μm by 1.5 to 3.0 μm. Almost all strains are motile by means of a single polar flagellum. The bacterium is ubiquitous in soil and water, and on surfaces in contact with soil or water. Its metabolism is respiratory and never fermentative, but it will grow in the absence of oxygen if nitrates are available as respiratory electron acceptor. In its natural habitat Pseudomonas aeruginosa is not particularly distinctive as a pseudomonad, but it does have a combination of physiological traits that are noteworthy and may relate to its pathogenesis. Pseudomonas aeruginosa has very simple nutritional requirements (Robin and Janda, 1984). It is often observed, "growing in distilled water" which is evidence of its minimal nutritional needs. In the laboratory, the simplest medium for growth of Pseudomonas aeruginosa consists of acetate for carbon and ammonium sulfate for nitrogen. Its optimum temperature for growth is 37 degrees Celcius, and it is able to grow at temperatures as high as 42 degrees Celcius. It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics (Cheesbrough, 2000).

Pseudomonas aeruginosa has a predilection for growth in moist environments, which is probably a reflection of its natural existence in soil and water. These natural properties of the bacterium undoubtedly contribute to its ecological success as an
opportunistic pathogen. They also help explain the ubiquitous nature of the organism and its prominence as a nosocomial pathogen (Robin and Janda, 1984).

2.1.2.1 Pathogenesis

For an opportunistic pathogen such as *Pseudomonas aeruginosa*, the disease process begins with some alteration or circumvention of normal host defenses (Cheesbrough, 2000). The pathogenesis of *Pseudomonas* infections is multifactorial, as suggested by the number and wide array of virulence determinants possessed by the bacterium. Multiple and diverse determinants of virulence are expected in the wide range of diseases caused, which include septicemia, urinary tract infections, pneumonia, chronic lung infections, endocarditis, dermatitis, and osteochondritis. Most *Pseudomonas* infections are both invasive and toxinogenic. The ultimate *Pseudomonas* infection may be seen as composed of three distinct stages: bacterial attachment and colonization; local invasion; disseminated systemic disease. However, the disease process may stop at any stage. Particular bacterial determinants of virulence mediate each of these stages and are ultimately responsible for the characteristic syndromes that accompany the disease (Gilardi, 1991).

2.1.2.2 Resistance of *Pseudomonas* to antimicrobial drugs

*Pseudomonas aeruginosa* is notorious for its resistance to antibiotics and is, therefore, a particularly dangerous and dreaded pathogen. The bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane lipopolysaccharides. Also, its tendency to colonize surfaces in a biofilm form
makes the cells impervious to therapeutic concentrations of antibiotics. Since its natural habitat is the soil, living in association with the bacilli, actinomycetes and molds, it has developed resistance to a variety of their naturally occurring antibiotics. Moreover, *Pseudomonas* maintains antibiotic resistance plasmids, both R-factors and RTFs, and it is able to transfer these genes by means of the bacterial processes of transduction and conjugation. Only a few antibiotics are effective against *Pseudomonas*, including fluoroquinolones, gentamicin and imipenem, and even these antibiotics are not effective against all strains. The futility of treating *Pseudomonas* infections with antibiotics is most dramatically illustrated in cystic fibrosis patients, virtually all of whom eventually become infected with a strain that is so resistant that it cannot be treated (Gilardi, 1991).

2.1.2.3 Treatment

*Pseudomonas aeruginosa* is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to Gentamicin, tobramycin, colistin, and amikacin, resistant forms have developed. The combination of gentamicin and carbenicillin is frequently used to treat severe *Pseudomonas* infections. Several types of vaccines are being tested, but none is currently available for general use (Gilardi, 1991).

2.1.3 *Escherichia Coli*

The genus *Escherichia* is named after Theodor Escherich who isolated the type species of the genus. They are Gram-negative bacilli occurring singly or in pairs. *Escherichia coli* are facultative anaerobic with both a fermentative and respiratory type of metabolism. They are either non motile or motile by peritrichous flagella (Schlievert,
1993). *E. coli* is a major facultative inhabitant of the large intestine. It is one of the most frequent causes of some of the many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infections (UTI), and traveler's diarrhea.

2.1.3.1 Pathogenesis

*Escherichia coli* account for 28.5% of the vast majority cases of neonatal meningitis. Pregnant women are at a higher risk of colonization with the K1 capsular antigen strain of *E. coli*. This strain is also commonly observed in neonatal sepsis. The mortality rate is 8%, and most survivors have subsequent neurologic or developmental abnormalities. Respiratory tract infections due to *E. coli* are uncommon and are almost always associated with *E. coli* urinary tract infections (UTI). No virulence factors have been implicated. However, *E. coli* pneumonia can also be community-acquired in patients who are immunosuppressed (Gilardi, 1991).

As a cause of enteric infections, 6 different mechanisms of action of 6 different varieties of *E. coli* have been reported. Enterotoxigenic *E. coli* (ETEC) is a cause of traveler's diarrhea. Enteropathogenic *E. coli* (EPEC) is a cause of childhood diarrhea (Mandell *et al.*, 2000). Enteroinvasive *E. coli* (EIEC) causes Shigella-like dysentery. Enterohemorrhagic *E. coli* (EHEC) causes hemorrhagic colitis or hemolytic uremic syndrome (HUS). Enteroaggregative *E. coli* (EAaggEC) is primarily associated with persistent diarrhea in children in developing countries, and enteroadherent *E. coli* (EAEC) is a cause of childhood diarrhea and also traveler's diarrhea in Mexico and North Africa. ETEC, EPEC, EAaggEC, and EAEC colonize the small bowel, and EIEC and EHEC preferentially colonize the large bowel prior to causing diarrhea. *E. coli* is a
leading cause of nosocomial infections originating from gastro-intestinal and genito-urinary tracts (Clark et al., 1984).

2.1.3.2 Treatment

Escherichia coli organisms are resistant to multiple antibiotics (Mandell et al., 2000). This is thought to be a Plasmid-mediated property. The choice of a specific antimicrobial agent depends on local susceptibility patterns. Once bacteremia is confirmed, treatment may be modified. Escherichia coli are sensitive to sulphonamides, cephalosporins, aminoglycoside, ampicillin and tetracycline (Clark et al., 1984).

2.1.4 Klebsiella species

The genus Klebsiella belongs to the tribe Klebsiellae, a member of the family Enterobacteriaceae. The organisms are named after Edwin Klebs, a 19\textsuperscript{th} century German microbiologist. Klebsiellae are non-motile, rod-shaped, Gram-negative bacteria with a prominent polysaccharide capsule. This capsule accounts for their large appearance on samples stained with Gram stain (Cheesbrough, 2000). Serotypes are based on the structural variability of the capsular polysaccharides (K antigens) and lipopolysaccharides (O antigens). There are 77 K antigens and 8 O antigens. The virulence of all serotypes appears to be similar (Cheesbrough, 2000). The genus was originally divided into 3 main species based on biochemical reactions. Today, 7 species with demonstrated similarities in DNA homology are known. These are Klebsiella pneumoniae, Klebsiella ozaenae, Klebsiella rhinoscleromatis, Klebsiella oxytoca, Klebsiella planticola, Klebsiella terrigena, and Klebsiella ornithinolytica. K. pneumoniae
is the most medically important species of the group. *K. oxytoaca* and *K. rhinoscleromatis* have also been demonstrated in human clinical specimens. In recent years, *Klebsiellae* have become important pathogens in nosocomial infections. Important manifestations of Klebsiellae infection in the hospital setting include UTI, pneumonia, bacteremia, wound infection, cholecystitis, and catheter-associated bacteriuria. The presence of invasive devices in hospitalized patients greatly increases the likelihood of infection. Patients with these infections have similar presentations to those with infections caused by other organisms. Other nosocomial infections in which Klebsiellae may also be implicated include cholangitis, meningitis, endocarditis, and bacterial endophthalmitis (Cheesbrough, 2000). The latter occurs especially in patients with liver abscesses and diabetes. These infectious presentations are relatively uncommon.

### 2.1.4.1 Pathogenesis

Host defense against bacterial invasion depends on phagocytosis by polymorphonuclear granulocytes and the bactericidal effect of serum, which is mediated, in large part, by complement proteins. Both classic-pathway and alternate-pathway complement activation have been described, but the latter, which does not require the presence of immunoglobulins directed against bacterial antigens, appears to be the more active pathway in *K. pneumoniae* infections (Ronald, 1990).

The bacteria overcome innate host immunity through several means. They possess a polysaccharide capsule that is the main determinant of their pathogenicity. The capsule is composed of complex acidic polysaccharides. Its massive layer protects the bacterium from phagocytosis by polymorphonuclear granulocytes. In addition, the capsule prevents
bacterial death caused by bactericidal serum factors. This is accomplished mainly by inhibiting the activation or uptake of complement components, especially C3b. The bacteria also produce multiple adhesins. These may be fimbrial or non fimbrial, each with distinct receptor specificity. These help the microorganism to adhere to host cells, which is critical to the infectious process. Lipopolysaccharides (LPS) are another bacterial pathogenicity factor. They are able to activate complement, which causes selective deposition of C3b onto LPS molecules at sites distant from the bacterial cell membrane. This inhibits the formation of the membrane attack complex (C5b-C9), which prevents membrane damage and bacterial cell death (Cheesbrough, 2000).

Availability of iron increases host susceptibility to *K. pneumoniae* infection. Bacteria are able to compete effectively for iron bound to host proteins because of the secretion of high-affinity, low molecular weight iron chelators known as siderophores. This is necessary because most host iron is bound to intracellular and extracellular proteins. In order to deprive bacteria of iron, the host also secrete iron-binding proteins.

### 2.1.4.2 Treatment

*Klebsiella* organisms are resistant to multiple antibiotics (Mandell *et al*., 2000). This is thought to be a plasmid-mediated property. Length of hospital stay and performance of invasive procedures are risk factors for acquisition of these strains. Treatment depends on the organ system involved. In general, initial therapy of patients with possible bacteremia is empirical. The choice of a specific antimicrobial agent depends on local susceptibility patterns. Once bacteremia is confirmed, treatment may be modified.
Agents with high intrinsic activity against *Klebsiella* should be selected for severely ill patients. Examples of such agents include third-generation cephalosporins (eg, cefotaxime, ceftriaxone), aminoglycosides (eg, gentamicin, amikacin), and quinolones. These agents may be used as monotherapy or combination therapy. Some experts recommend using a combination of an aminoglycoside and a third-generation cephalosporin as treatment for non–ESBL-producing isolates. Others disagree and recommend monotherapy. Aztreonam may be used in patients who are allergic to beta-lactam antibiotics. Quinolones are also effective treatment options for susceptible isolates in patients with major beta-lactam allergy. Other antibiotics used to treat susceptible isolates include ampicillin ticarcillin/clavulanate, ceftazidime, cefepime, levofloxacin, norfloxacin, gatifloxacin, moxifloxacin, meropenem, and ertapenem (Roberts, 1999).

### 2.1.5 *Proteus* species

*Proteus* species are part of the Enterobacteriaceae family of gram-negative bacilli (Braunwald *et al.*, 2001). *Proteus* organisms are implicated as serious causes of infections in humans, along with *Escherichia*, *Klebsiella*, *Enterobacter* and *Serratia* species. *Proteus* species are most commonly found in the human intestinal tract as part of normal human intestinal flora, along with *Escherichia coli* and *Klebsiella species*, of which *E. coli* is the predominant resident (Mandell *et al.*, 2000). *Proteus* is also found in multiple environmental habitats, including long-term care facilities and hospitals. In hospital settings, it is not unusual for Gram-negative bacilli to colonize both the skin and oral mucosa. Infection primarily occurs from these reservoirs. *Proteus mirabilis* causes
90% of *Proteus* infections (Braunwald *et al.*, 2001) and can be considered a community-acquired infection.

*Proteus vulgaris* and *Proteus penneri* are isolated from individuals in long-term care facilities and hospitals and from patients with underlying diseases. Patients with recurrent infections, those with structural abnormalities of the urinary tract, those who have had urethral instrumentation, and those whose infection were acquired in the hospital have an increased frequency of infection caused by *Proteus* and other organisms (eg, *Klebsiella, Enterobacter, Pseudomonas, Enterococci, Staphylococci*) (Roberts, 1999).

### 2.1.5.1 Pathogenesis

Proteus species possess an extracytoplasmic outer membrane, a feature shared with other Gram-negative bacteria (Mandell *et al.*, 2000). In addition, the outer membrane contains a lipid bilayer, lipoproteins, polysaccharides, and lipopolysaccharides. Infection depends on the interaction between the infecting organism and the host defense mechanisms. Various components of the membrane interplay with the host to determine virulence. Inoculum size is important and has a positive correlation with the risk of infection. Certain virulence factors have been identified in bacteria. The first step in the infectious process is adherence of the microbe to host tissue. Fimbriae facilitate adherence and thus enhance the capacity of the organism to produce disease (Roberts, 1999). Proteus has fimbriae (ie, pili), which are tiny projections on the surface of the bacterium. Specific chemicals located on the tips of pili enable organisms to attach to selected host tissue sites (eg, urinary tract endothelium). The presence of these
fimbriae has been demonstrated to be important for the attachment of *P. mirabilis* to host tissue. The attachment of *Proteus* species to uroepithelial cells initiates several events in the mucosal endothelial cells, including secretion of interleukin 6 and interleukin 8.

*Proteus* organisms also induce apoptosis and epithelial cell desquamation. Bacterial production of urease has also been shown to increase the risk of pyelonephritis in experimental animals. Urease production, together with the presence of bacterial motility and fimbriae, may favor the production of upper urinary tract infections (UTIs) by organisms such as *Proteus*. Enterobacteriaceae (of which *Proteus* is a member) and *Pseudomonas* species are the microorganisms most commonly responsible for Gram-negative bacteremia (Mandell *et al.*, 2000). When these organisms invade the bloodstream, endotoxin, a component of Gram-negative bacterial cell walls apparently triggers a cascade of host inflammatory responses and leads to major detrimental effects. Because *Proteus* and *Pseudomonas* organisms are Gram-negative bacilli, they can cause gram-negative endotoxin-induced sepsis, resulting in systemic inflammatory response syndrome (SIRS). SIRS has a mortality rate of 20-50% (Roberts, 1999).

### 2.1.5.2 Treatment

*Proteus mirabilis* remains susceptible to nearly all antimicrobials except tetracycline (Engel and Schaeffer, 1998). Resistance does not appear to be a significant clinical factor, but 10-20% of strains can acquire resistance to ampicillin and first-generation cephalosporins. *P. mirabilis* is likely to be sensitive to ampicillin; broad-spectrum penicillins (eg, ticarcillin, piperacillin); first-, second-, and third-generation cephalosporins; imipenem; and aztreonam ((Engel and Schaeffer, 1998).). *Proteus*
_vulgaris_ and _P. penneri_ are resistant to ampicillin and first-generation cephalosporins. Activation of an inducible chromosomal beta-lactamase (not found in _P. mirabilis_) occurs in up to 30% of these strains. Fourth-generation cephalosporins, aminoglycosides, and quinolones have excellent activity (90-100%) (Mandell *et al.*, 2000).

### 2.2 Medicinal plants and traditional medicine

#### 2.2.1 Traditional medicine

Herbal medicines, which formed the basis of health care throughout the world since the earliest days of mankind, are still widely used. Indeed, medicinal plants have been a common source of medicaments, either in form of crude preparations or as pure active principles (Philipson and Anderson, 1994). The use of herbal remedies in the treatment of the diseases is universal and traceable to the ancient times when man acquired the skills of herbal healing through deliberate selection of plants or by accidental discovery (Wiselogle, 1940; Spencer *et al.*, 1947). Traditional medicine involves the use of herbal medicine, animal parts and minerals. However, herbal medicines are the most widely used of the three. Herbal medicines contain as active ingredients, aerial or underground parts of plants or other plants materials, or combination thereof, whether in the crude state or as plant preparations (World health organization, 1996). The basic principles of current herbal medicines are a mixture of ancient traditions applied to modern conditions without, in many cases, the benefit of modern science and technology (Tyler, 1994).
In recent years the importance of the utilization of herbal medicine in primary health care system has been emphasized in Africa as well as other countries (Sheeham et al., 1992). In Kenya, herbs are traditionally consumed in various ways, most commonly in form of infusion or decoction prepared from the dried plants material. Despite the use of herbal medicine over many centuries, only a relatively small number of plant species has been studied for possible medical application. Safety and efficacy data are available for an even smaller number of plants, their extracts and active ingredients and preparation containing them (World health organization, 1998).

2.2.2 Plants as source of antibacterial agent

Historically, plants have provided a source of inspiration for novel drug compound, as plant derived medicines have made large contributions to human health and well-being. It is estimated that today, plant materials are present in, or have provided the models for 50% western drugs (Robbers et al., 1996). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices or for other purposes that suggested potentially useful biological activity (Trease and Evans, 1972). Natural products from medicinal plants have shown to contain antibacterial compounds (Kaul, 1976; Li et al., 1997; and Koo et al., 2000). Much of the exploration and utilization of natural products as antimicrobial arise from microbial source. It was the discovery of penicillin that led to later discoveries of antibiotics such as streptomycin, aureomycin and chloromycin (Trease and Evans, 1972).

Though soil microorganisms or fungi produce most of the clinically used antibiotics, high plants have also been a source of antibiotics (Trease and Evans, 1972;
Examples of these are the bacteriostatic properties of lichens, the antibiotic action of allinine in Allium sativum (garlic) (Trease and Evans, 1972; Boakye-Yiadom, 1977). An investigation carried out by Li et al. (1997) in search for antibacterial agents revealed that out of the 18 medicinal plants examined, 5 methanolic extracts arising from five individual plants showed activity against bacterial growth. Okemo and Mwatha, (2002) evaluated methanolic extracts of six Kenyan medicinal plants for antibacterial activity and found that the extracts from Entanda abyssinica, X. caffra, A. indica and H. abyssinica showed the most potent antibacterial activity. McCutcheon et al. (1992) tested 100 methanolic extracts of the plants used by British Colombian Native people, against 11 bacterial isolates. They found 85% of the plants with antibacterial. Pedersen et al. (1999) examined antibacterial activity of 27 medicinal plant extracts of Rubiaceae and found 11 active.

Based on these previous observations, fifteen medicinal plants locally used as traditional medicines in Kenya were investigated for their potential antibacterial activity. Their organic solvent extracts were first evaluated for antibacterial activity in vitro by disk diffusion method (Bauer et al., 1966). Water was not used as a solvent due to lack of lyopholization equipment. Those extracts that showed high inhibitory activity were selected and examined further to determined the minimum inhibitory concentration and minimum bactericidal concentration.

### 2.2.3 Plants included in the study

Extracts from fifteen medicinal plants were screened for antibacterial activity. The plants samples were randomly selected from various localities in Kenya. Each plant
picked had some history of medicinal usage. The plants were: *Ajuga remota, Artemisia anua, Plumeria alba, Ehertia cymosa, Rhus natalensis, Cassia didymorbatyra, Ekerbagia capensis, Clemaentis species, Lantana camara, Combretum molle, Caesalpinia volkensis, Trichilia emetica, Ziziphus mucranata, Vernonia brachycaalyx and Plectranthus babartus.*

The selections of the plants were based on the following combined approach:

1. Ethno-medical use (Kubo and Taniguchi, 1993; Chhabra *et al.*, 1981)
2. Common denominator such as family (Chhabra *et al.*, 1990b).

Out of the 15 medicinal plants five of them (*Ehretia cymosa, Combretum molle, Ekebergia capensis, Plectranthus barbatus* and *Plumeria alba*) showed antibacterial Activity by disc diffusion technique and they were chosen for background information details as described below.

2.2.3.1 *Combretum molle*

*Combretum molle* belong to the family *Combretaceae*. It is a common tree of the dry wooded grassland. It tolerates most soils and it’s about 10 metres tall. It has a grey dark bark and large leaves, covered with soft brownish hairs. Fruits are bright brownish yellow with 4 wings (Gachathi, 1989). Boiled root decoction is taken for hookworm, stomach pains, snakebite, leprosy, fever, dysentery, general body swellings and as an abortifacient. Both the leaves and roots are used for snakebite treatment. Leaves are chewed or pounded, soaked in water and the juice taken for chest complaints, as an anthelminthic, or used as an inhalant in hot steam bath. Leaves are also placed on a
wound on which have been applied pounded roots of *Senecio lyratipartitus* and finally banana leaves are used as a bandage (Kokwaro, 1976)

### 2.2.3.2 *Ehretia cymosa*

*Ehretia cymosa* belong to the family *Boraginaceae*. It is a short tree about 8m tall with a round crown and white flowers in large clusters. Leaves are dark green with many smooth fruits that are yellowish when ripe. It is common in forest edges, and readily coppicing (Gachathi, 1989). Wood is used for tool handles. The roots and leaves are used as an aphrodisiac, but are also toxic. Leaf juice is styptic and used for healing wounds. Roots are also used in treatment of diarrhea (Kokwaro, 1978).

### 2.2.3.3 *Pletranthus barbatus*

*Pletranthus barbatus* belong to the family *Labiatae*. It is a common shrub usually planted around homesteads and along boundaries (Gachathi, 1989). It is a softly hairy, strongly aromatic shrub about 4m tall with fleshy angled stem and thick soft opposite leaves. The flowers are bright purple – blue. This is a useful hedge plant.

The leaves are crushed and the juice taken as a remedy for stomachache and as a purgative. The Luo community uses this plant for bathing babies suffering from measles. The leaves are normally pounded and soaked in warm water (Kokwaro, 1978).

Roots are used for chest troubles and cleaning circulatory system. Roots are also used in treatment of arthritis/rheumatism and high blood pressure.
2.2.3.4  *Ekebergia capensis*

*Ekebergia capensis* belong to the family Meliaceae. It’s a large tree attaining 30m high with short ash-like bole and rounded crown. Leaves that are compound (5-12-leaflet) are confined to the end of branches. It is a widely distributed tree but uncommon in the forest (Gachathi, 1989).

Stem is used for making traditional stools and beehives. Roots are used for the treatment of diarrhea.

2.2.3.5  *Plumeria Alba*

The plant belongs to the family Apocynaceae. It is a tall sparingly branched conical tree with large fragmented yellow flowers (Kokwaro, 1978). Traditionally it is used to treat wound infections. The plant is thought to possess antimicrobial activity.
CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study bacterial isolates.

Bacterial isolates used were recovered from confirmed HIV/AIDS patients with septic herpetic lesions and characterized as described below.

3.1.1 Collection of specimens for bacterial isolation

Specimens were collected from Herpetic lesions of patients confirmed to be infected with HIV and have developed AIDS. The skin surface was cleaned using normal saline and the specimens collected by aseptically swabbing the ulcer with sterile cotton wool (Forbes et al., 1990). The specimens were labeled and transported to the laboratory for processing.

3.1.2 Specimen processing

 Cultures for the isolation of bacteria were performed on all samples collected. On the receipts of the samples the patients and samples number were verified and details entered into laboratory register. The samples were then inoculated into blood agar and MacConkey agar (Krieg and Holf, 1984; Cheesbrough, 2000). This was done by use of cotton tipped swab and spread into about one-third of the plate. Using a flame sterilized wire-loop, the inocula were then streaked into four quadrants of the plate with flaming after each quadrant had been streaked, to be able to obtain discrete colonies after over
night incubation. All the procedures were carried out aseptically. All the inoculated plates were then incubated both aerobically and an aerobically at 37°C for 24 hours. Then the plates were removed from the incubator and examined for the growth.

3.1.3 Identification of the isolates

The following biochemical tests Citrate, Indole, Urea, Oxidase, Catalase, Coagulase and culture on Klebsiella screening media (KSM) were performed to identify the bacterial isolates. These are tests done to identify bacteria that may cause wound infection though they may cause infections on other areas of the body.

3.1.3.1 Klebsiella screening media

Lactose fermenters from MacConkey agar were picked and inoculated into KSM tube by stabbing in a single down and up motion in the center of the agar going three-fourth of the way down the tube and keeping the wire as vertical as possible. The test tubes were then incubated at 37°C for 24 hours and the results interpreted as per biochemical reaction chart for enterobacteriaceae (Krieg and Holm, 1984).

3.1.3.2 Citrate Utilization Test

Lactose fermenters from MacConkey agar were picked and inoculated into simmon’s citrate tube by stabbing in a single down and up motion in the center of the agar going three-fourth of the way down the tube and keeping the wire as vertical as possible. The test tubes were then incubated at 37°C for 24 hours. The development of bright colour indicate positive test (Cheesbrough, 2000).
3.1.3.3 Indole test

Kovac’s reagent (0.5mls) was added to the test organism grown overnight in broth culture at 37°C in a Bijou bottle containing 3mls of tryptone water. Development of a red colour indicated positive reactions. *E.coli* gives positive reaction (Cheesbrough, 2000).

3.1.3.4 Urease test

Sterile Christensen’s modified urea broth was inoculated heavily with test organism in a bijou bottle. Development of a pink colour after incubation at 37°C for 6 hours indicates positive reaction. *Proteus* gives positive reaction (Cheesbrough, 2000; Krieg and Holt, 1984).

3.1.3.5 Oxidase test

Oxidase test was performed to identify *Pseudomonas* by adding 2 drops of freshly prepared Oxidase reagent to a piece of filter paper in a clean petri dish. Using sterile wooden stick a colony of test organism was smeared onto the filter paper. Development of blue purple colour within 2-3 seconds indicated positive reaction. *Pseudomonas* gives positive reaction (Cheesbrough, 2000).

3.1.3.6 Catalase test

Catalase test was performed on gram-positive cocci by pouring 2mls of 3% H₂O₂ solution in to a test tube. Using a sterile wooden stick colonies of test organisms were immersed in to H₂O₂ solution Active bubbling indicates positive reaction. *Staphylococcus* gives positive reaction.
3.1.3.7 Coagulase test

Coagulase was performed by adding 0.2mls of plasma to 0.8mls of test broth culture in sterile test tube. These were mixed well and incubated at 37°C. Clotting of tube contents or formation of fibrin clot indicate positive reaction. *Staphylococcus aureus* give a positive reaction.

3.1.3.8 Confirmation of the bacterial isolates

This was done by use of the API 20E system following the manufacturer's instructions. The API 20E commercial test system is an identification system for enterobacteriaceae and other gram-negative rods. It uses 23 miniaturized biochemical test and database. The strips consist of 20 micro tubes containing dehydrated substrate. These micro tubes are inoculated with a bacterial suspension, which reconstitutes the media. During incubation, bacterial metabolism produces colour changes that are either spontaneous or revealed by the addition of specific reagents. This consists of inoculation of the chamber by a suspension medium and incubating at 37°C for 24hours.

3.1.4 Bacterial isolates preparation

Before the assay each organism was streaked on to blood agar plates and incubated at 37°C for 24hrs. A McFarland standardized suspension of the test organisms was made in normal saline from which a final concentration of $1.5 \times 10^6$ colony forming units was prepared.
3.1.5 Preservation of the Bacterial isolates

The isolated bacteria were preserved in nutrient agar slope at 4°C (Difco laboratory) until when required for biological assay.

3.1.6 Antibiotic sensitivity testing

Antibiotic sensitivity testing was performed on all the identified bacterial isolates using single discs of the antibiotic panel that included Ampicillin, Tetracycline, Co-trimoxazole, Gentamycin, Chloramphenicol, Kanamycin, and Cephalosporin. The above antibiotic panel was chosen on the basis of their use in the treatment of such conditions commonly caused by the bacterial species isolated.

Testing method employed was the Kirby-Bauer agar disc diffusion technique (Bauer et al., 1966). Briefly, isolated organisms were emulsified in sterile water to conform to 0.5 McFarland turbidity. This was then diluted further to give a density of approximately $10^6$ CFU/ml resulting into confluent growth. Muller-Hinton agar plate was then inoculated with 0.1ml of the diluted organism and the isolates spread with the aid of a sterile swab on the plate and the antibiotic discs applied. The plates were incubated at 37°C for 24 hours. The sensitivity and resistance of isolates were interpreted according to the National Committee for Clinical Laboratory standards (NCCLS, 1987). The activities were rated into three classes as, low (Diameter of inhibition zone (DIZ) < 12mm), medium (DIZ 12<15) and high (DIZ > 15 or higher) (Monsouri, 2001; Forbes et al., 1990).
3.2 Medicinal plants

3.2.1 Study plants

The following 15 medicinal plants shown in Table 1 were investigated for antibacterial effect.

Table 1: Medicinal plants of the study

<table>
<thead>
<tr>
<th>NAME</th>
<th>FAMILY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajuga remota.</td>
<td>Labiatae</td>
</tr>
<tr>
<td>Artemisia anua</td>
<td>Compositae</td>
</tr>
<tr>
<td>Plumeria alba</td>
<td>Apocynaceae</td>
</tr>
<tr>
<td>Ehertia cymosa</td>
<td>Boraginaceae</td>
</tr>
<tr>
<td>Rhus natalensis</td>
<td>Anacardiaceae</td>
</tr>
<tr>
<td>Cassia didymorbatyra</td>
<td>Leguminosae</td>
</tr>
<tr>
<td>Ekerbagia capensis</td>
<td>Meliaceae</td>
</tr>
<tr>
<td>Clemantis species</td>
<td>Ranunculaceae</td>
</tr>
<tr>
<td>Lantana camara</td>
<td>Verbenaceae</td>
</tr>
<tr>
<td>Combretum molle</td>
<td>Combretaceae</td>
</tr>
<tr>
<td>Caesalpinia volkensis</td>
<td>Caesalpiniaeeae</td>
</tr>
<tr>
<td>Trichilia emetica</td>
<td>Meliaceae</td>
</tr>
<tr>
<td>Ziziphus mucranata</td>
<td>Rhamnaceae</td>
</tr>
<tr>
<td>Vernonia brachycalyx</td>
<td>Compositae</td>
</tr>
<tr>
<td>Plectranthus barbatus</td>
<td>Labiatae</td>
</tr>
</tbody>
</table>
3.2.2 Collection of medicinal plants

The medicinal plants used in this study were collected from their natural habitats in different geographical zones of Kenya. *Plectranthus babartus, Rhus natalensis, Cassia didymorbatyra, Ekerbagia capensis* and *Clemantis* species were collected from Njoro division, Nakuru district, in Rift valley province, Kenya. *Ehertia cymosa, Caesalpinia volkensis, Trichilia emetica, Ziziphus mucranata* and *Vernonia brachycalyx* were collected from Egoji division, Meru district in Eastern province Kenya. *Lantana camara, Combratum molle, Ajuga remota, Artemisia anua* and *Plumeria alba* were collected from Nairobi province, Kenya. The plants were identified on the ground before collection by a plant taxonomist who was part of the collection team. The plants parts were collected with consideration of the bio-conservation aspects.

3.2.3 Processing of medicinal plants

3.2.3.1 Leaves

Leaves of the plants were used except *Plumeria alba* for which latex was used. The leaves were collected while still green and dried at room temperature for 1 week. When completely dried each plant's leaves were separately ground using an electric mill. The powdered plant materials were kept separately in closed plastic containers at room temperature.
3.2.3.2 Latex

Latex of *Plumeria alba* was collected in plastic bags after making a cut at the tree branch and letting the liquid flow freely in the bags. One hundred grams of the latex was weighed and mixed with 40g of silica gel (mesh size 70-230). The mixture formed a solid paste, which was dried and ground to form homogenous fine mixture.

3.2.4 Extraction of plant materials

3.2.4.1 Extraction with a mixture of two organic solvents

A mixture of two organic solvents, one polar and the other non-polar, i.e. hexane, and methanol, respectively was used in this process to obtain broad range of compounds that could be tested for preliminary bioassay. The plant materials were individually extracted using 20g of each powdered material, weighed and put into extraction flasks. Fifty milliliters of each organic solvent was sequentially added and gently hand shaken to mix. Each plant extraction process took 10 hours or until the extract was clear. Each extract was concentrated on a rotary evaporator at 50° C and the resulting concentrated sample transferred to clean sterile sample bottles. This was further dried under vacuum over anhydrous copper sulphate to give a dry solid or paste of the extract for the preliminary bioassay.
3.2.4.2 Extraction with three organic solvents separately

Three organic solvents used in this process were hexane, dichloromethane, and methanol (Cichewicz et al., 1996). The plant materials were extracted individually using a soxhlet system for each respective solvent. Fifty grams of each powdered material was weighed and put into extraction flasks. Two hundred and fifty milliliters of each organic solvent were sequentially added to respective plant powders and gently hand shaken to mix. Each solvent extraction process proceeded as described above.

3.2.5 Bioassays of extracts

3.2.5.1 Disc diffusion method

Preliminary screening of the medicinal plant extracts against common opportunistic bacteria was carried out using disc diffusion method. By this method, 10mg of each sample was dissolved in 1ml of dimethylformamide and kept as a stock solution. Two milligrams equivalent of each stock solution was applied on a sterile 6mm disc (Becton diskson). The discs were allowed to dry over night in the oven set at room temperature. Organic solvents used in the extraction were included in the assay as controls. The technique used was the same as for the antibiotic sensitivity test (Kirby-Baur, 1966) above. The activities were rated into three classes as, low (DIZ < 12mm), medium (DIZ 12<14) and high (DIZ > 14 or higher). The extracts, which showed highest antibacterial activity, were selected for quantitative test.
3.2.5.2 Agar dilution method

3.2.5.2.1 Minimum inhibitory concentration

The following medicinal plants, which showed high activity during preliminary screening, were further bio-assayed to determine their minimum inhibitory concentration (MIC). These plants were *Ehretia cymosa*, *Combretum mole*, *Ekerbagia capensis*, *Plectranthus barbatus* and *Plumeria alba*.

3.2.5.2.1.1 Stock preparation (crude extracts)

Four hundred eighty milligrams (480mgs) of each extract were dissolved in 1ml N, N-dimethyl formamide (DMF) and made to a final volume of 3ml using nutrient broth (160mg/ml). The extracts were then serially diluted in six test tubes to a range of 80mg/ml-2.5mg/ml. Six sterile test tubes were arranged and labeled as follows, 80mg/ml -2.5mg/ml, a positive and a negative control tubes were then added (Ayof et al., 1994; Hess et al., 1995). Three milliliters of nutrient broth prepared according to manufacturer’s instructions was added to all test tubes. Three milliliters of the stock solution (160mg/ml) was added to the 1st test tube labeled 80mg, mixed well and serially diluted by removing 3ml to the next test tube up to the last test tube, 3mls were discarded from the last test tube (Hess et al., 1995; Cheesbrough, 2000). Racks carrying the dilution test tubes were gently hand shaken to mix the contents. Negative and positive test tubes contained only the nutrient broth. Muller Hinton agar (oxoid) was prepared according to manufacturer’s instructions and 18mls was distributed into well-labeled universal bottles. This was sterilized by autoclaving at 121°C for 15 minutes. The sterile molten agar was
kept at 50°C in water bath until poured into plates. For each extract, 2mls of the respective dilutions were added to corresponding well-labeled plates and 18mls of molten Muller Hinton agar added to each plate. These were mixed well and allowed to set yielding concentration range of 8 - 0.25mg/ml. A bacterial suspension containing 1.5 x 10^6 colony forming units/ml (cfus/ml) of the test organisms was added to each plate except the negative control. All the plates were incubated at 37°C for 24hrs. The lowest concentration of each extract showing no growth was recorded as the minimum inhibitory concentration (MIC)

Table 2.0: Dilution series of minimum inhibitory concentration

<table>
<thead>
<tr>
<th>Reagent used</th>
<th>Dilutions</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Nutrient broth 3ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stock solution (160mg/ml)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Dilution transfer (3ml)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Final conc. Mg/ml</td>
<td>80</td>
<td>40</td>
</tr>
</tbody>
</table>

Key: * Discard 3ml
+ Reagents added
- No reagents added
3.2.5.2.2 Minimum bactericidal concentration

The medicinal plants that showed significant minimum inhibitory concentration (MIC$_{50}$ and MIC$_{90}$ range of 0.5 – 2mg/ml) were chosen for minimum bactericidal concentration assay. These plants were *Ehretia cymosa, Combratum mole, Ekerbagia capensis* and *Plectranthus barbatus*. Forty-eight milligrams (48mgs) of each extract were dissolved in 1ml N, N-dimethyl formamide (DMF) and made to a final volume of 3mls using nutrient broth (16mgs/ml). Six sterile test tubes were arranged and labeled as follows, 8mg/ml -0.25mg/ml a positive and a negative control tubes were then added (Ayof *et al.*, 1994; Hess *et al.*, 1995). Three milliliters of nutrient broth prepared according to manufacturer’s instructions was added to all test tubes. Three milliliters of the stock solution (16mg/ml) was added to the 1st test tube labeled 8mg. This was mixed well and serially diluted by removing 3mls to the next test tube up to the last test tube, 3mls were discarded from the last test tube (Hess *et al.*, 1995; Cheesbrough, 2000). Racks carrying the dilution test tubes were gently handshake to mix the contents. Negative and positive test tubes contained only the nutrient broth. A bacterial suspension containing $1.5 \times 10^6$ colony forming units/ml (cfus/ml) of the test organisms was added to each test tube except the negative control. Calibrated loopful (0.01ml) of the content of the test tubes, which showed turbidity was streaked over a quadrant of a 90-mm Muller Hinton agar plate (NCCLS, 1987). Colonies on subculture plates were counted after incubation for 24hrs at 37°C. The Minimum bactericidal concentration was defined as the lowest concentration of the crude extract which resulted in at least a 99.9% kill of the inocula and was such that no higher concentration yielded less than 99.9% kill.
### Table 3.0: Dilution series of minimum bactericidal concentration

<table>
<thead>
<tr>
<th>Reagent used</th>
<th>Test tubes</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrient broth 3ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stock solution (16mg/ml)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Dilution transfer (3ml)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Final conc. Mg/ml</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Key:  
* Discard 3ml  
+ Reagents added  
- No reagents added
CHAPTER FOUR

4. RESULTS

4.1 Isolation of bacterial species

Gram-positive bacteria isolated from the herpetic lesion of the HIV/AIDS patients included *Staphylococcus aureus* and coagulase negative *Staphylococcus* while gram negative isolates was *Escherichia coli, Klebsiella species, Pseudomonas species* and *Proteus species*. Figure 1 below represents gram-positive coccis while figure 2 represents gram-negative bacilli.
Table 4.0: Organisms recovered from hepatic HIV and AIDS patients

<table>
<thead>
<tr>
<th>Sample</th>
<th>Organism recovered</th>
<th>Staphylococcus</th>
<th>Pseudomonas</th>
<th>Klebsiella</th>
<th>E.coli</th>
<th>Proteus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Legend: + Bacteria was isolated from sample
- Bacteria was not isolated from sample
Figure 1: A Gram stain showing one of Gram-positive *Staphylococcus aureus* isolated from HIV and AIDS patients with septic herpetic lesions.
Figure 2: Gram stain showing Gram- negative *Klebsiella* isolated from one of the HIV and AIDS patients with septic herpetic lesions.
Figure 3: Culture plate showing mucoid pyocyanin producing strains of *Pseudomonas aeruginosa* isolated from one of HIV and AIDS patients with septic herpetic lesions
4.2 Antibiotic susceptibility testing

Antibiotics inhibited the growth of bacterial isolates over a diameter of 6 to 18mm as shown in table 5. Ampicillin produced the lowest zone of inhibition to microorganisms isolated. These results also show that chloraphenicol was the most inhibitory antibiotic (p= 0) with inhibitory zones ranging from 10mm to 18 mm. These results also show that all the bacterial isolates were resistant to Ampicillin, Cotrimoxazole, Tetracycline and Amoxycillin. In terms of sensitivity to antibiotic, Escherichia coli was the most sensitive bacterial isolates (p=0) while Pseudomonas was the least sensitive (p< 0.05).
Table 5.0: Zone of inhibition (millimeters) of respective antibiotics against bacterial isolates.

<table>
<thead>
<tr>
<th>BACTERIAL ISOLATES</th>
<th>ANTIBIOTIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMP</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>7</td>
</tr>
<tr>
<td>aureus ATCC</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>6</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>6</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>6</td>
</tr>
<tr>
<td>E.coli</td>
<td>6</td>
</tr>
<tr>
<td>Proteus</td>
<td>6</td>
</tr>
</tbody>
</table>

Legend:  
AMP – Ampicillin  
COT – Cotrimoxazole  
TET – Tetracycline  
CHL – Chloraphenicol  
GEN – Gentamicin.  
KAN – Kanamycin  
AMO- Amoxycillin  
CEP – Cephalosporin

The antibiotics with low activity were Ampicillin, Cotrimoxazol, Tetracycline and Amoxycillin. Those with medium inhibition zone were Gentamycin, Kanamycin and Cephalosporin while Chloraphenicol was the only one with high inhibition zone.
4.3 **Susceptibility of bacterial isolates to plants extract**

Table 4 and 5 below show the effect of plant extracts against bacterial isolates. *Clemantis* species, *Ziziphus mucranata*, *Trichilia emetica*, *Artemisia anua* and *Ajuga remotata* showed no antibacterial activity. *Cassia didymorbatyra*, *Lantana camara*, *Plumeria alba* and *Vernonia brachycalyx* were fairly active against *E. coli*, *Klebsiella* species and *Proteus* species. *Rhus natalensis* and *Caesalpinia volkensis* were active against *Staphylococcus* species only. *Ehretia cymosa*, *Combretum mole*, *Ekerbagia capensis* and *Plectranthus barbatus* had antibacterial activity against several bacterial isolates. *Plectranthus babartus* had the highest inhibitory effect while *Clemantis* species, *Ziziphus mucranata*, *Trichilia emetica* and *Artemisia anua* were the least (p<0). The most sensitive organism was *Escherichia coli* (p=0) while the least was Pseudomonas (p<0.05).
Table 6.0: Zone of inhibition (millimeters) of selected medicinal plant extracts against bacterial isolates.

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Medicinal plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M₁</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>14</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>15</td>
</tr>
<tr>
<td><em>Klebsiella species</em></td>
<td>18</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>19</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>15</td>
</tr>
</tbody>
</table>

**Keys:**
- M₁ - *Ehertia cymosa.*
- M₂ - *Rhus natalensis.*
- M₃ - *Cassia didymorbatyra*
- M₄ - *Ekerbagia capensis.*
- M₅ - *Clemantis species.*
- M₆ - *Caesalpinia volkensis.*
- M₇ - *Ziziphus mucranata.*
- M₈ - *Plectranthus babartus.*
- M₉ - *Trichilia emetica.*
- M₁₀ - *Lantana camara.*
- M₁₁ - *Vernonia brachycalyx.*
- M₁₂ - *Combratum mole.*
- M₁₃ - *Artemisia anua.*
- M₁₄ - *Ajuga remota.*
- M₁₅ - *Plumeria alba*
4.4 Minimum inhibitory concentration of organic solvents plant extracts

Tables 7 to 9 show results of activity for plant extracts made using three organic solvents tested against the various strains of bacterial isolates. Methanol extracts showed a significant activity (\( p=0 \)) with MIC range of 0.5-2mg/ml across the extracts for all categories of bacteria compared to DCM (\( p=0 \)) and Hexane, 1 – 4mg/ml and 4 –8mg/ml respectively (\( P<0.05 \)).

The activities of methanol extracts are as shown in the table 7. *Plectranthus barbatus* was active over the range 0.5 – 1mg/ml, *Combratum mole*, *Ehretia cymosa* and *Ekerbagia capensis*, ranging from 0.5 –4mg/ml and *Plumeria alba* at 4 –8mg/ml. Statistical analysis of the results shows that *Plumeria alba* had no significant inhibition on the growth of all the bacteria (\( p <0.503 \)).

The result shows that the Hexane extracts had minimal inhibition to the bacterial isolates tested (Table 8). The MIC ranged from 4 –8mg/ml across the extracts for all categories of bacteria. According to these results the Hexane extracts was significantly inactive as compared to DCM and methanol extracts (\( P=0 \)).

The result indicates that the DCM extracts of *Ehretia cymosa* and *Plectranthus barbatus* had a fairly significant activity on selected bacteria (Table 9). *Ehretia cymosa* inhibited the growth of *Escherichia coli* with MIC 50% and 90% range of 1-2 mg/ml while *Plectranthus barbatus* inhibited the same bacteria with MIC 50% and 90% range of 0.5 – 2 mg/ml. The other DCM extract had no significant inhibition on the growth of the bacteria. There was no significant difference within bacterial species under DCM extracts (\( p=0.625 \)).
Table 7.0: MIC of methanol plant extracts (mg/ml)

<table>
<thead>
<tr>
<th>Medicinal plant species</th>
<th>Bacterial species</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus</td>
<td>E. coli</td>
<td>Klebsiella</td>
<td>Pseudomonus</td>
<td>Proteus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIC 50%</td>
<td>MIC 90%</td>
<td>MIC 50%</td>
<td>MIC 90%</td>
<td>MIC 50%</td>
<td>MIC 90%</td>
<td>MIC 50%</td>
<td>MIC 90%</td>
</tr>
<tr>
<td>Ehretia cymosa</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Combretum mole</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ekerbegia capensis</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Plectranthus barbatus</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Plumeria alba</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Legend: 50licer 50licer and 90% MIC value that inhibit growth of 50 and 90% of the tested strains
Table 8.0: MIC (mg/ml) of Hexane plant extracts against strains of bacterial isolates.

<table>
<thead>
<tr>
<th>Medicinal plant species</th>
<th>Staphylococcus</th>
<th>E. coli</th>
<th>Klebsiella</th>
<th>Pseudomonus</th>
<th>Proteus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC 50%</td>
<td>MIC 90%</td>
<td>MIC 50%</td>
<td>MIC 90%</td>
<td>MIC 50%</td>
</tr>
<tr>
<td>Ehretia cymosa</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Combretum mole</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Ekerbegia capensis</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Plectranthus barbatus</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Plumeria alba</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Legend: 50\(^1\) and 90\(^1\) MIC value that inhibit growth of 50 and 90% of the tested strains.
Table 9.0: MIC of Dichloromethane (DCM) plant extracts against strains of bacterial isolates.

<table>
<thead>
<tr>
<th>Medicinal plant species</th>
<th>Bacterial species</th>
<th>Staphylococcus</th>
<th>E. coli</th>
<th>Klebsiella</th>
<th>Pseudomonus</th>
<th>Proteus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC 50%</td>
<td>MIC 90%</td>
<td>MIC 50%</td>
<td>MIC 90%</td>
<td>MIC 50%</td>
<td>MIC 90%</td>
</tr>
<tr>
<td><strong>Ehretia cymosa</strong></td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Combretum mole</strong></td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><strong>Ekerbegia capensis</strong></td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Plectranthus barbatus</strong></td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Plumeria alba</strong></td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Legend: 50\(^{1}\) - 50\(^{1}\) and 90% MIC value that inhibit growth of 50 and 90% of the tested strains
4.5 Minimum bactericidal concentration

Table 10 below shows results of Minimum bactericidal concentration (MBC) of four plant extracts against the various strains of bacterial isolates. Methanol extract that had a highest inhibitory effect (P< 0.05) compared to hexane and dichloromethane was used to determine the bactericidal concentration. *Plectranthus barbatus, Combratum mole* and *Ehretia cymosa* had a significant bactericidal effect on all categories of bacterial isolates tested. Where they all show MBC value that kill 50 and 90% of the tested strains ranging from 1 – 4mg/ml and 2- 4mg/ml respectively.
Table 10. MBC (mg/ml) of plant extracts using methanol

<table>
<thead>
<tr>
<th>Medicinal plant species</th>
<th>Bacterial species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
</tr>
<tr>
<td></td>
<td>50%</td>
</tr>
<tr>
<td>Ehretia cymosa</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Combretum mole</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Ekerbegia capensis</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Plectranthus barbatus</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: 50\(^1\) - 50\(^1\) and 90% MBC value that kills 50 and 90% of the tested strains
4.6 Activity of medicinal plant preparations compared to conventional antibiotics

Four out of the fifteen plants extracts tested by disc diffusion technique had inhibitory activity on most bacterial isolates with highest inhibition diameter of 20mm (Tables 6). Seven of the eight antibiotics tested had no effect on majority of bacterial isolates examined (Table 5). Pseudomonas strains were resistant to all of the eight antibiotic tested (DIZ ranging from 6-10mm) while Proteus strains were resistant to seven of the eight antibiotic tested. Extracts from three of the plant tested (Plectranthus barbatus, Combratum mole and Ehretia cymosa) had significant inhibitory and bactericidal effect against both isolates (Tables 7 and 10).
CHAPTER FIVE

5 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

The organisms isolated in the present study showed a slight variation compared with those of other earlier study (Noble, 1969). Organisms of the genera *Streptococci* and *Citrobacter* were not encountered in the study, in contrast to other reports. Coagulase negative *Staphylococci* formed the largest group, whereas in other studies the most commonly isolated organism was coagulase negative *Staphylococcus*. This might be due to changing trends in the frequency pattern of the organisms isolated from septic herpetic lesion. It is clear from the present evidence that coagulase negative *Staphylococcus* has changed from the status of a non-pathogen to that of an opportunistic pathogen. Although once regarded as an innocuous member of the normal skin flora, coagulase negative *Staphylococcus* is now recognized as an important opportunistic pathogen. It is routinely found on the skin, with prevalence of 85% (Noble, 1969). Noble believed that coagulase negative *Staphylococcus* was found excessively on damaged skin surface in normal persons. Skin surface with herpetic lesion is a damaged skin surface, this may be the reason for the high frequency of coagulase negative *Staphylococcus* in this study.

Antibiotics inhibited the growth of bacterial isolates over the range 6mm to 18mm as shown in table 5. Ampicillin produced the lowest zone of inhibition to microorganisms isolated while *chloraphenicol* was the most inhibitory antibiotic (p= 0) with inhibitory zones ranging from 10mm to 18mm. This agrees with other previous
study (Skinner and Ahmad, 1994). The result also shows that all the bacterial isolates were resistant to Ampicillin, Cotrimoxazole, Tetracycline and Amoxycillin. In terms of sensitivity to antibiotic, Escherichia coli were the most sensitive bacterial isolates (p=0) while Pseudomonas were the least sensitive (p< 0.05). This result agrees with other previous studies (Sawer et al., 1995; Mitscher et al., 1972).

The finding reveals that for most of the bacterial isolates common antibiotics like chloramphenicol, gentamicin and cephalosporin are fairly active (p< 0.05). Species of Pseudomonas and Proteus that are known to be resistant pathogens (Cheesbrough, 2000) did not respond to seven of the eight antibiotics tested (p <0.001).

Four out of the fifteen plant extracts tested by disc diffusion technique had inhibitory activity on most bacterial isolates with inhibition diameter ranging from 10mm to 20mm (Table 6). The plant extracts showed antibacterial effects on the growth of the bacterial isolates. This is in agreement with other investigators (Anand and shanmuga, 1998; Okemo, 1996; Sawer et al., 1995; Mitscher et al., 1972). The active extracts were further extracted using three different organic solvents and they effects determined using agar dilution technique (tables 7 and 9). Methanol extracts showed a significant activity with mic range of 0.5-2mg/ml across the extracts for all categories of bacteria compared to DCM and Hexane, 1 – 4mg/ml and 4 –8mg/ml respectively (P<0.05). The most effective plant extract was Plectranthus barbatus at mic50 and mic90 concentration of 0.5 –1mg for all categories of bacterial isolates. Combratum mole, Ehretia cymosa and Ekerbagia capensis follows respectively with MIC ranging from 0.5 –4mg/ml.and finally Plumeria Alba 4 –8mg/ml.
Plectranthus barbatus and Combratum mole inhibited the growth of most bacteria tested. The most inhibited were the E. coli strains. These were inhibited by the extract of Plectranthus barbatus at mic50 and mic90 concentration as low as 0.5mg/ml. Staphylococcus strains were significantly inhibited by Plectranthus barbatus and Combratum mole at mic50 and mic90 concentration of 0.5 and 1mg/ml. Similar observation had been made. McCutcheon et al. (1992) tested 100 methanolic extracts of the plants used by British Colombian Native people, against 11 bacterial isolates. McCutcheon observed that the growth of Staphylococcus was significantly inhibited.

Pseudomonas strains, which were the most resistant isolates, was significantly inhibited by Ehretia cymosa at mic50 and mic90 of 1mg/ml and 2mg/ml respectively. Plants have activity against microorganisms (Sawer et al., 1995) but the nature of the activity is not known. However the selection of bacteria isolates along susceptibility lines of convection drugs and subsequently testing of these strains against various extracts of selected medicinal plants could aid in the understanding of the problem. According to the results the most active extract were from Plectranthus barbatus and Combratum mole, range of 0.5 – 1mg/ml. The activity of these plant extracts has no specific pattern, they seems to be similar in most categories of the selected bacterial isolates. However Proteus strains and Pseudomonas strains showed some resistance. These were followed by activity of Ehretia cymosa and Ekerbagia capensis with range of 1– 2mg/ml and 1 – 4mg/ml respectively. The least active was extract of Plumeria alba in the range of 4 - 8mg/ml. Plumeria alba is commonly used by various traditional medicine men as a drug of choice in treatment of herpetic lesions. The extract is externally applied onto the septic lesion once every day. This results into drying of the lesion and finally healing. However,
according to these results *Plumeria alba* had no significant inhibition on the growth of the bacteria with MIC 50% and 90% range from 4mg to 8mg/ml. The bacteria were therefore, relatively resistant to these plant extracts, and it's healing action may be through others on the body.

Result indicates that *Plectranthus barbatus* and *Combratum mole* had low MIC 50 and MIC 90 and are likely to have compounds that act effectively on wide range of bacteria. However the *Combratum mole* extracts was more active against gram-positive cocci. This may be related to their mode of action and permeability of the bacterial cell wall. The cell walls of gram-positive bacteria are more permeable than the cell wall of gram negative (Franklin and Snow, 1985).

*Plectranthus barbatus* extracts were effective against both Gram-positive and Gram negative (Table 6 and 7) bacteria. Since the cell walls of the Gram-positive and Gram-negative bacteria are different in composition, it is not clear what mechanisms of activity *Plectranthus barbatus* extracts used to affect structural components of the different bacteria. *Ekerbagia capensis* only inhibited the growth of *Esherichia coli* and *Proteus*; the mic 50 and mic 90 range from 1-1 and 1-2 respectively. However, the plant had mbc50 range of 2- 4mg/ml and mbc90 of 4 –8mg/ml indicating that it is marginally active. Except for *Plumeria alba* and *Ekebergia capensis* there is a significant bactericidal effect by the other three extracts, *Plectranthus barbatus*, *Combratum mole* and *Ehretia* cymosa on all categories of bacterial isolates tested Where they all show a low mbc50 range of 1 – 4mg/ml and mbc90 of 2- 4mg/ml. It appears that accessibility of the extracts into the cell leads to the death of the cell. However, the mechanism of killing is not clear.
These results and similar ones in the past (Okemo and Mwatha, 2002; McCutcheon et al., 1992; Sawer et al., 1995) indicate that there may be plenty of unexploited natural resources of compound in higher plants that can be used to control bacteria. Therefore, bioassay guided research could reveal new and more potent compounds in these plants.

5.2 Conclusion

The plants extracts showed antibacterial effects on the growth of the bacterial isolates. This is in agreement with other investigators (Anand and Shanmugan, 1998; Okemo, 1996; Sawer et al., 1995; Mitscher et al., 1972). This means that it is possible to obtain inexpensive renewable sources of compound from plant that can be readily transformed into drugs. Seven of the eight antibiotics tested had no effect on Pseudomonas and Proteus (Table 5) comparatively three of the fifteen plants extracts tested had both inhibitory and bactericidal effect against both isolates (Table 7 and 10). this is an important significant finding, indicating a possibility of difference in the mechanism of the extracts to that of the antibiotic.

The extracts from Ehretia cymosa, Combratum mole and Plectranthus barbatus clearly demonstrated their ability in inhibiting the growth of the bacterial isolates in vitro, Plectranthus barbatus extract being the most potent of the three. These confirm the observation that some difficult diseases conventional medicines cannot treat may be managed by traditional medicines.

Latex of Plumeria alba was the commonly used traditionally in treatment of septic herpetic conditions in comparison to other plants. However, the extract had minimal
antibacterial effect indicating that bacteria are relatively resistant to the extracts from this plant.

5.3 **Recommendations**

1. There is need to determine the mechanism of antibacterial action for the three medicinal plants extracts of *Ehretia cymosa, Combratum mole* and *Plectranthus barbatus* which showed the best activity.

2. It's evident that plants have compound active against pathogenic organisms therefore working relation should be strengthened between traditional healers and scientific institutions to rapidly identify and evaluate these plants.

3. Effort should be made to isolate and identify bioactive compounds, which are present in the four extracts. Such an effort could lead to the identification of a new range of compounds for management of bacterial infections.

4. Minimum inhibitory concentration and Minimum bactericidal concentration are recommended for future extracts or compound evaluation.

5. There is need to evaluate *in vivo* antibacterial effect and toxicity of the three active extracts.

6. Finally efforts should be made to determine effects of the active extracts on viral pathogen.
REFERENCE


*Microbial Reviews, 55*:733.


Microbiology, Washington, D.C.


*Journal of Ethnopharmacology,* 47: 97-100.


Kokwaro, J.O. 1976. Medicinal plants of East Africa Literature Bureau, Kampala, Nairobi, Dare salaam.


1972b. Antimicrobial agents from high plants IV. Zanthoxylum elephantiasis. 

tetracycline resistance in Neisseria. Journal of Antimicrobial Agent and 
Chemotherapy, 30: 664 –667.

bactericidal activity of antimicrobial agents. Documents M26-P. National 
Committee Clinical Laboratory Standard, 7: 35 – 76


Nobel, W. C. 1969. Skin carriage of micrococcaceae. Journal of Clinical Pathology, 
22:249-53

Okemo, P. O. 1996. Antimicrobial efficacy of selected medicinal plants used by Kenya 

extracts against pathogenic bacteria and HIV/AIDS related Mycobacterium 

conditions in Haiti N.Euge. Journal of Medicine, 309: 495.

properties of seven medicinal plants of Rubiaceae from Mauritius pharmaceutical. 
Biomedicine, 37: 202-207.


### Antibiotic sensitivity panel

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>25 microgram</td>
</tr>
<tr>
<td>Cotrimoxazol</td>
<td>25 microgram</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>25 microgram</td>
</tr>
<tr>
<td>Chloraphenicol</td>
<td>30 microgram</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10 microgram</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 microgram</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>25 microgram</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>30 microgram</td>
</tr>
</tbody>
</table>

This antibiotic panel has been chosen on the basis of their use in treatment of gram positive and negative pathogen.