

**EVALUATION OF ANTIMICROBIAL ACTIVITY OF
SOME PLANTS USED BY HERBALISTS IN
CENTRAL PROVINCE, KENYA**

LILLY WAMBUI GATHU

**A thesis submitted in partial fulfillment for the award of the
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Gathu, Lilly Wambui
*Evaluation of
antimicrobial*



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DECLARATION

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

LILLY WAMBUI GATHU

SIGNATURE  DATE 25/8/2006

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

PROFESSOR PAUL OKEMO
DEPT. OF BIOLOGICAL SCIENCES
KENYATTA UNIVERSITY

SIGNATURE  DATE 29.08.06.

PROFESSOR JULIUS MWANGI
FACULTY OF PHARMACY
UNIVERSITY OF NAIROBI.

SIGNATURE  DATE 28/8/06

DEDICATION

This work is dedicated to all the custodians of indigenous knowledge on plant uses and to all those who are working towards the discovery of curative remedies from plants.

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ABSTRACT

Herbal medicine is the use of plants and plant-based products to treat or prevent diseases. The traditional use of plants as a source of medicine still plays a major role in primary health care in Kenya today. The information on the use of these plants is often passed on from one generation to another through folk knowledge. Many of the plants still in use have neither been documented nor tested so as to confirm their efficacy. Six plants from different families, selected on the basis of their traditional medicinal uses in Central Province to treat diseases of infectious nature, were evaluated for antimicrobial activity. The plants screened were *Psidium guajava* L., *Persea americana* Mill, *Acacia mearnsii* De Wild, *Thunbergia alata* Sims, *Ocimum suave* Willd and *Periploca linearifolia* Dill and Rich. The aqueous, chloroform and methanol extracts of these plants were screened for their antifungal and antibacterial activities using the agar-well diffusion method. Four plants exhibited moderate activity against the tested microorganisms. Only one of the plants, *Acacia mearnsii* De Wild exhibited activity against the fungus. There was a significant difference in activity ($p < 0.05$) between the aqueous and the methanol extracts and between aqueous and chloroform extracts. There was however no significant difference in activity between the methanol and the chloroform extracts ($p > 0.05$). Extracts from *A. mearnsii* were the most active and exhibited moderate activity against ten out of the eleven microorganisms that were tested. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the active extracts were determined using the micro-broth dilution method. The MICs of the methanol extracts ranged from 0.1953 to 100 mg/ml while those of the aqueous extracts ranged from 0.09 to 50 mg/ml. The methanolic extract of *P. linearifolia* was fractionated using vacuum liquid chromatography and three active fractions obtained. Inhibition zones obtained on thin layer chromatogram (bioautoassay) revealed that the fractions obtained from this extract have more than one active component. These results are an important reference that confirms the use of the plants for treatment of bacterial and fungal diseases.

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CHAPTER 1

1.0 INTRODUCTION

Plants are precious and a valuable resource and have been used in many different ways. Man has over the years used plant materials as a source of food, shelter, medicine and transportation. Many animals also depend on plants for their food. World Health Organization (WHO) estimates indicate that eighty per cent of the population, (mostly in developing countries), still rely on plant-based medicines for primary health care (Fox, 1994). Medicinal plants and herbs contain substances known to modern and in some cases to the earlier civilizations for their healing properties. Herbal medicine is the use of plants and plant remedies in the treatment and prevention of disease (Barnes, 1998).

Of the estimated 250,000 to 500,000 plant species around the globe, only a small percentage has been investigated phytochemically and the fraction subjected to biological or pharmacological screening is even lower (Hostettmann et al., 1995). It is estimated that about 60,000 species of higher plants will probably have become extinct by the years 2050 (Akerale *et al.*, 1991). African plants, in particular medicinal plants, constitute a rich but still largely untapped pool of natural products (Hostettmann et al., 1996). Watt and Breyer (1962) have documented a large number of plants used in Eastern and Southern Africa for medicinal purposes.

Due to rapid population growth over the years, there is a lot of deforestation to give room for grazing, farming and settlement (Maunder *et al.*, 2002). Majority of the people in a developing country like Kenya live in rural areas and this has created a continuous need for grazing and farming land. Many plant species are therefore in danger of extinction due to deforestation or over-consumption. Some of the plants that are used as sources of medicines in Kenya are found in the wild while others are cultivated in various parts of the country where they are used for other purposes and have not been tested to confirm their efficacy. Harvesting of some of the parts like the roots and the bark is very destructive to the plants and unless their use is controlled and measures put to conserve the useful species there is danger of loss of particular species.

Traditional use of herbal medicines is a way of life in many cultures and it normally develops within an ethnic group before development and spread of modern medicine (Mukherjee, 2002). Due to the fact that different kinds of plants were found in different parts of the world, various people built up their own inventories of useful plants. A number of indigenous societies have taken up practices of the developed world and this foreign influence has resulted in the loss of some traditional systems like relying entirely on plants for their health care. Easy access to modern healthcare facilities in many parts of the country has resulted in many people visiting health centers as opposed to the traditional

health practitioners and hence a gradual loss of traditional health facilities in some areas.

Most herbal specialists have accumulated a lot of folk knowledge which they have acquired over generations. This information is normally passed on orally from one generation to the next and a lot of vital details can be lost or distorted due to lack of proper records. Furthermore not all plants used by herbalists are documented and the efficacy of many herbal preparations has not been investigated in order to confirm their activities.

Although there is a wide range of antibiotics for treatment of bacterial infections, the development of resistance to the existing drugs is increasingly becoming a pressing problem (Abimbola *et al.*, 1993). This is partly due to the widespread and often indiscriminate use of antibiotics together with poor hygiene and has led to the need for new active principles that can then be used as compounds for drug synthesis in future. Many important drugs in use today were derived from plants or plant compounds as starting molecules and this search for novel compounds is a continuous task. Plants are important to modern pharmaceutical firms which rely heavily on the same active principles, be they natural or synthetic, in order to manufacture new drugs.

1.1 Early use of drugs from plants

Records from ancient Egypt, Assyria, China and India show that the use of plants for medicinal purposes dates back to the earliest recorded history. A book known as Assyrian herbal of 7th Century BC gives a list of medicinal plants while the Chinese emperor Shen Nung described use of herbal medicines in 2800 BC. The earliest known text of Chinese herbal medicine was recently unearthed in Hunnan province and dated from the fourth Century BC and it lists over two hundred herbs with instructions for pharmaceutical preparations (Evans, 1996).

From as early as 1550 BC, the Egyptians used Elbers medical papyrus to arrange medicinal plants. The antibacterial properties of essential oils and resins were extensively used by the Egyptians in embalming of dead bodies. The Ayurvedic texts of the Indian healers of around 1000 BC list many hundreds of plant extracts and their use for medicinal and spiritual purposes. Some of these ancient systems of medicine such as the Chinese herbal medicine and the Indian Ayurveda and Unani systems of medicine were preserved in written form since time immemorial (Kleinman, 1976, Vogel, 1991). Among the earliest recorded uses of plant medicinals were those of the herb called “Ma hung”, a species of *Ephedra* used medicinally in China for over 500 years, and one called “Chang Shang “ and later identified as *Dichroa febrifuga* Lour.

Interest in medicinal plants has recently increased enormously after the discovery of several natural product drugs like vincristine and vinblastine from *Catharanthus roseus*. The untapped wealth of the plant Kingdom has become a target for the search for new drugs and lead compounds that can be used as templates for the design of more effective drugs. Investigation of traditional remedies of botanical origin on which a worldwide majority of the population still relies for its source of medicine has become prominent. Folk medicines naturally varied according to the plants available in a particular climatic area and can be studied today in those societies that the practice still exist. A lot of research work has been done by scientists as an attempt to justify, on a scientific basis, many aspects of the traditional medicinal practices in Africa and in the world.

The drugs that are used in modern medicine are expensive to most people, unavailable in some areas or is presented in a way that is inconsistent with many traditional beliefs (Smolan *et al.*, 1990) and therefore herbal medicine still has its place in the society.

1.2 Hypothesis

1. Plant extracts used in traditional medicine to treat microbial infections have no activity against the microorganisms that cause these diseases.
2. Different types of extracts from the same plant material do not differ in their efficacy against microorganisms.

3. Concentration of the extracts and exposure time has no affect on their performance.

1.3 Objectives

The overall objective of the study is to screen some plants for their microbial properties.

Specific objectives

1. Screen crude extracts for antimicrobial activity against both standard strains and hospital pathogenic isolates.
2. Determine the minimum inhibitory concentrations (MIC s) and the minimum bactericidal concentrations (MBC s) of the active extracts.
3. Determine the Kill kinetics of active plant extracts.
4. Separate and locate the active principles using thin layer chromatography and auto bioassay.

1.4 Justification

Many pathogenic microorganisms have acquired resistance to specific antibiotic treatments and this has created a need to look for new anti microbial agents. There are many plant species that have not been surveyed for chemical or biologically active constituents, and they could be harbouring new molecules with antimicrobial properties. One plant may be used to treat more than one disease while a single disease may be treated by several plant species. In such a case it would be difficult to ascertain which

plant is actually effective for a particular complaint unless we carry out tests to find out the sensitivity of particular pathogens to the extracts.

In the traditional systems of medicine, there is lack of proper diagnosis and the underlying disease may be quite difficult to interpret. This means that drug administration is based mainly on the patient's symptoms and it is therefore important to test the extracts against the pathogens to establish whether the activity exists or not. The results obtained give a scientific backing to the use of these plants. Preparations of the herbalists are mainly extracted with water and it would be important to compare the performance of the aqueous extract with the extracts obtained with other solvents.

Three of the plants in this study grow wild in bushes while the other three are cultivated for other uses other than for medicinal purposes. It is only after their medicinal value has been confirmed that the local communities can then be encouraged to adapt and cultivate the wild plants to avoid their extinction due to deforestation. Knowledge on the use of medicinal plants is most often passed on orally from one generation to the next and is normally the fruit of many years of plant use. One way of preserving this knowledge is to confirm and document the medicinal use and value of the plants. Modern medicines may be expensive to some people or unavailable in some areas while traditional medicine is easily accessible and affordable. Traditional use of plants in healthcare delivery is

important in many parts of this country and efforts should be made to improve and support it.

1.5. Selection criteria of plants screened in this study

Several herbalists in Central Kenya, which is a big geographical area, were interviewed on the plants they use to treat wounds and other diseases of infectious nature. Out of the plants they use, six plants were selected for the initial screening. These plants are from different families but they are used to treat diseases of the same nature and they are, *Psidium guajava* L., *Persea americana* Mill, *Acacia mearnsii* De Wild, *Thunbergia alata* Sims, *Ocimum suave* Willd and *Periploca linearifolia* Dill and Rich.

Psidium guajava L (Myrtaceae) is a branched short tree native of tropical America and is cultivated for its edible round fruits (Gachathi, 1989). It grows in Central Kenya where it is locally known as “Mubera” and at the coastal region. The flower and leaf have been found to contain tannins and sterol and the plant is used in Hawaii as a medicinal tea and as a remedy for deep cuts, sprains, diarrhoea and intestinal haemorrhages, while in India the leaf and bark have been used as remedy for diarrhoea (Watt and Breyer, 1962). The herbalists use the leaves to treat diarrhoea.

Persea americana Mill (Lauraceae) is a cultivated true native of Central America (Gachathi, 1989). It is about fifteen meters high with pear-shaped edible fruits.

The leaves contain 0.5 percent volatile oils and is used as a tea (Watt and Breyer, 1962). Methanolic extracts of the leaves test positive for alkaloids, triterpene glycosides and coumarins while the aqueous extracts contain alkaloids, saponins, flavonoids and tannins (Adeboye *et al.*, 1999). *P. americana* Mill is widely cultivated in Central Kenya where it is locally known as “Mukorobebe” and the herbalists use the leaf extracts to dress wounds.

Acacia mearnsii De Wild (Mimosaceae) is a cultivated Australian tree and has become naturalised in Kenya. This species is indigenous to Australia and is widely grown in a number of countries because of its bark. It was first introduced into Kenya by the Reverend Stuart Watt, an early missionary, who brought many seeds from Australia where he had lived before coming to Kenya (Stuart watt, undated, Trzebinski, 1985). It is planted for its bark and grows well in Central highlands (Gachathi, 1989) where it is locally known as “Muthanduku”. The herbalists use the bark to treat mouth rashes, wounds, as a cough remedy and at times they use the leaves to treat diarrhoea.

Thunbergia alata Sims (Acanthaceae) is a twinning herb growing amongst bushes in grass lands (Gachathi, 1989) and it is locally known as “Kanyanja.” The leaves are used traditionally in the treatment of mouth, tongue and teeth (Kokwaro, 1976) and the herbalists use this plant to treat allergies and dry cracking skin.

Ocimum suave Willd is a plant belonging to the family Lamiaceae. The genera *Ocimum* consists of about one hundred and fifty species. The leaves of this plant are used in African traditional medicine to treat fevers, colds, coughs, and for oral hygiene (Iwu, 1993) and for blocked nose, ear problems, abdominal pains, sore eyes, as a disinfectant and an insecticide (Kokwaro, 1976). The local herbalists know it as “Mukandu” and use the roots and stem to treat diarrhoea and tonsils.

Periploca linearifolia Dill and Rich is a milky vigorous climber common along the river-banks. Its roots are used in soups, to treat chest pains and fever (Gachathi, 1989). Phytochemical screening of this plant has revealed the presence of one sterol and four triterpenes (Rukunga *et al.*, 1986). The herbalists use the stem together with the leaves to treat diarrhoea and blood disorders and is locally known as “Muimba-iguru”.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Chemical constituents of Medicinal plants

Plants have been a rich source of medicines because they produce a host of bioactive molecules. The active principles may be carbohydrates, glycosides, tannins, alkaloids or lipids and differ from plant to plant due to the plants genetic coding. Alkaloids and steroids are the two major classes of plant-derived compounds used in medicine today (Simpson and Ogorzaly, 1986). Compounds in both these classes can occur in forms with one or more sugar molecules attached. Such forms, called glycosides, are often the medicinally active form of the compound.

Tannins are also among the most abundant active principles in plants particularly in trees and shrubs where they are mainly found in the bark (Kokwaro, 1976). Tannins have the ability to form complexes with other molecules, have antiseptic effects and are often used to treat diarrhoea (Ben-Eric *et al.*, 1997). Flavonoids are another group of compounds found to have several medicinal properties like anti-inflammatory, anti-oxidant, anti-allergic, anti-bacterial and anti-viral effects (Ben-Eric *et al.*, 1997). Saponins are compounds that make a soaplike foam when shaken with water. The medicinally important saponins have an additional ring added to the steroid backbone that makes them similar to human sex hormones.

Threats of loss of plant species and indigenous knowledge has forced mankind to examine plants for novel antimicrobial compounds. Ethnobotanical studies are conducted in order to document the uses of plants in a particular area or community. Surveys on the use of plants as a source of medicines are common in many parts of the world and most of the times a lot of useful information is obtained.

2.2 Review of studies done worldwide

In Papua New Guinea, *Micrechites navoguineensis* Schum is used in traditional medicine to treat dysentery, peptic ulcers, sores and asthma. Fractions of partitioned leaves and stem bark extracts were tested against micro-organisms and showed antibacterial activity against Gram positive bacteria such as *Bacillus cereus*, *Streptococcus faecalis* and *Staphylococcus aureus* (Khan and Omoloso, 1999). *Drancantomelon dao* which is used in the same country to treat sore throat, dermatitis and as a general drug source (Perry, 1980) has also been tested and the extracts of the stem, leaves and root bark found to exhibit good antibacterial activity (Khan and Omoloso, 2002).

Persea cordata Vell. Mez grows in Brazil and is used in rural communities as a remedy for many infectious diseases. The ethyl acetate fraction of the stem bark has been shown to exhibit antibacterial activity against both Gram positive and Gram negative bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae* as well as *Streptococcus pyogenes* (Schlemper *et al.*, 2001). *Borreria veticillata* (L) roots are taken as a tea here and the methanolic extract was tested

against six multi drug resistant strains of *Pseudomonas aeruginosa* isolates and showed high activity against six different strains with inhibition zones of between 10 and 18 mm (Neto *et al.*, 2002). The root of *Cephaelis ipecacuanha* A. Richard was used as an emetic by natives of Brazil and Peru mainly for dysentery and the emetic principle (emetine) was isolated in 1817 (Foye, 2002).

India has a long tradition of medicinal plant uses and there are approximately 1,250 documented Indian medicinal plants (Chatterjee and Pakrashi, 1991) that are used in formulating therapeutic preparations. The traditional plant preparations have significant historical background and most have now been evaluated to establish the active principles. The bark of *Litsea glutinosa* (Lour.) is widely used in India for the treatment of diarrhoea and dysentery. Methanol extracts tested against a wide range of both Gram negative and Gram positive bacteria, show antibacterial activity comparable to that of chloramphenicol and gives positive tests for alkaloids, steroids, triterpenoids, saponins and tannins (Subhash *et al.*, 2000).

Two Bangladeshi medicinal plants, *Toona ciliata* M. J. Roem and *Amoora rohituka* Weight and Arn. have been screened for antimicrobial activity. The petroleum ether, dichloromethane and methanol extracts of the stem barks exhibited significant *in vitro* antibacterial activity against *Bacillus* species, *Staphylococcus aureus*, *Klebsiella* species, *Salmonella typhi* and *Pseudomonas*

aeruginosa and also mild antifungal activity against *Macrophomia phaseolina* (Chowdhury *et al.*, 2003).

In Poland where Herba solidaginis from *Solidaga virgaurea* (L) has been used to treat urinary tract infections, nephrolithiasis and prostrate gland, the ethanolic and methanolic extracts of the micro propagated plant showed moderate bactericidal activity with MICs ranging from 1.8 to 125 mg/ ml against *Bacillus pumillis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and the fungi *Aspergillus niger* (Thiem and Goslinska, 2002).

The genus *Hypericum* is known as a valuable resource of anti-Staphylococcal leads as indicated by a study where chloroform and methanol extracts of thirty four species and varieties of the genus *Hypericum*, showed significant activity against a clinical isolate of methicillin resistant *S. aureus* (Gibbons *et al.*, 2002). Studies were carried on *Agrimonia eupatoria* L , which is locally known as an ethnomedicinal herb (Giggs, 1997), where the n-hexane extract inhibited *Bacillus cereus* and *Bacillus subtilis* while fractions that were obtained from the methanol extract showed significant bacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *B. subtilis*, and *B. cereus* (Copland, 2003).

2.3. Review of studies done in Africa

Early Africans could have gained some specific knowledge by watching the effects produced by various plants when eaten by domestic animals (Sofowora,

1996). Information on the use of medicinal plants in Africa is obtained from herbalists, herb sellers and indigenous people for many years (Wondergem *et al.*, 1989, Baba *et al.*, 1992). A number of plants that are currently in use traditionally in treating microbial and other infections have been screened to confirm their activities and to study the nature of the active components.

Leaves of *Bixa orellana* L (Bixaceae) are used traditionally in Ghana to treat gonorrhoea, for oral hygiene and as a gargle for sore throats while the seeds are used in the treatment of buccal tumors (Pamplona, 1998, Burkill, 1985 and Caceres *et al.*, 1995). The ethanolic extract of the leaves and seeds show a broad spectrum of antimicrobial activity (Fleischer *et al.*, 2003). The leaves have been found to contain alkaloids (Adegoke, 1968) while the seeds contain carotenoids (Windholz, 1976). This plant is cultivated for the colouring matter of its seeds and is used as an edible colourant.

In Sudan, root extracts of *Ximenia americana* L. are used as a local antiseptic after child birth while the leaves are used to treat measles (Omer and Elnima, 2003). Saponins and phenolics have been isolated from the roots (D'Agostino *et al.*, 1994 and Mwangi *et al.*, 1994). Extracts of the bark, leaves and stem exhibit high activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* (Mwangi *et al.*, 1994, Omer and Elnima 2003).

Uvavia chamae P. Beauv. is a tree found in Nigeria and is used in Ibo traditional medicine to treat fever and skin diseases (Oguntimein *et al.*, 1989). The

methanolic extract of the stem bark and its fractions show antimicrobial activity against both Gram- negative and Gram-positive bacteria as well as fungal pathogens (Ebi *et al.*, 1999). The Hausa tribe of Nigeria use the roots and twigs of *Vernonia amygdalina* Del. to treat stomach ache and gastro intestinal troubles. The methanolic extract of the leaves has been shown to exhibit activity against Gram positive and Gram negative bacteria (Akinpelu *et al.*, 1999). The root of *Vernonia amygdalina* Del. is used in many African homes for oral hygiene (Sofowora, 1993 and Lewis, 1980). Previously isolated compounds from this plant include flavonoids, saponins and alkaloids (Sayed *et al.*, 1982). *Alchornea cordifolia* (Schum) stem bark is also used in Nigerian traditional medicine to treat fever, and rheumatic pains. It has shown anti-microbial activity against Gram negative organisms like *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. Gram positive bacteria inhibited by the same include *Staphylococcus aureus* and *Bacillus subtilis* (Ajali, 2000).

Leaves of *Helichrysum pedunculatum* Hilliard and Burrt are used in South Africa to dress wounds in traditional male circumcision and as anti-inflammatory and antibacterial agents (Watt and Breyer, 1962, Meyer and Dilika, 1996). Antibacterial activity guided fractionation of the dichloromethane extract of the leaves has resulted in the isolation of linoleic and oleic acids. Both acids inhibit the growth of several Gram positive bacteria such as *Bacillus cereus*, *Bacillus pumilus* and *Staphylococcus aureus* (Dilika *et al.*, 2000).

Euphoria hirta L is used in treating diarrhoea and dysentery in African traditional medicine and has been shown to be effective *in vitro* and *in vivo* against entamoeba which causes amoebic dysentery (Keita, 1994). *Cryptolepis sanguinolenta* is used for treating urinary tract infections in Africa and has shown strong antimicrobial activity. Cryptolepine was identified as the active alkaloid and another alkaloid named cryptospirolepine has since been characterized from its root by Tackie *et al.*, (1993).

Polygala nyikensis is used by the highlanders of Malawi and bordering countries to treat various skin problems of fungal origin (Sofowora, 1996). The root of this plant was shown to exert its antifungal activity due to the presence of xanthenes (Marston *et al.*, 1993).

Two commonly used plants in Cameroon, *Zingiber officinale* Roscoe (ginger) and *Garcinia kola* (bitter kola) have been found to have antibacterial activity on four respiratory tract pathogens (Akoachere *et al.*, 2002). *Bridelia ferruginea* leaves, bark and fruits also used in Cameroon to treat dysentery and thrush in children (Dalziel, 1937) have been screened for antibacterial activity and found to be active against both Gram positive and Gram negative bacteria (Talla *et al.*, 2002). In Ethiopia, *Dodonala viscosa*, *Rumex nervosus* Vahl and *Rumex abyssinicus* Jacq are used to treat various diseases of microbial nature and when screened for anti-microbial activity, the methanolic extracts exhibited activity against *S.*

aureus, *S. pyogenes* and *C. diphtheria* but none was active against the Gram-negative bacteria as well as the fungal pathogens tested (Getie *et al.*, 2003).

Ageratum conyzoides L is commonly used in African traditional medicine for dressing wounds and ulcers, for scabies and as an eyewash (Sofowora, 1996) and these common uses are as a result of its possession of antimicrobial properties which have since been demonstrated (Durodola, 1977, Wiedenfeld and Roder, 1991).

2.4 Review of studies done in Kenya

A number of plants that are used in folk medicine in Kenya have also been screened for antimicrobial activity. *Hymenodictyon parvifolium* (Oliv.) is used traditionally as a remedy for skin diseases, venereal diseases and dysentery and the methanolic extract exhibits both antifungal and antibacterial activities against a wide range of micro-organisms like *Bacillus subtilis*, *Staphylococcus aureus* and *Trichophyton spp.* while the stem bark extracts have been found to contain flavonoids, terpenoids and glycosides (Kariba, 2002).

Newtonia hilebranditii (Vatke) Torre from Meru is used traditionally to treat wounds and other skin conditions and the methanolic extract of the stem bark is found to be active against fungal and bacterial strains such as *Escherichia coli*, *Staphylococcus aureus* and *Trichophyton interdigitale* (Kariba and Houghton, 2001). The neem tree, *Azadirachta indica* A. (Juss) is used to treat a variety of ailments like malaria and gastro-intestinal problems in Kenya. The aqueous leaf

extract is active against strains of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella ozaena* (Kofi-Tsepko *et al.*, 1989). All parts of this plant continue to receive extensive phytochemical and pharmacological investigations and the seed oil that is widely used in Asian medicines has now been confirmed as an anti-inflammatory and antibacterial agent and contains many bitter limonoids to which most of the activity can be ascribed (Mohammed Aslam, 1996).

The decoction of the root bark of *Solanum aculeastrum* Dunal. is used for the treatment of sexually transmitted diseases while the juice from the berries is used for treating jigger infestations. The aqueous and methanolic extracts of the berries show antimicrobial activity against *Staphylococcus aureus* and *Bacillus cereus*. (Wanyonyi *et al.*, 2003).

Oils from two Kenyan species of *Lippia*, *L. javanica* (Burm. F.) Spreng and *L. grandifolia* have been shown to exhibit strong antimicrobial activity (Mwangi *et al.*, 1994). Fruits of *Maesa lanceolata* var. *goulungensis* Weir (Myrsinaceae) are widely used in East Africa to treat ailments like sore throat, tapeworms, hepatitis and cholera (Kubo *et al.*, 1987). In a study to evaluate the microbiological activity of the plant, the methanol extract was found to have strong anti fungal activity (Okemo *et al.*, 2003).

CHAPTER 3

3.0 ANTIMICROBIAL ACTIVITY

3.1 Introduction

The concept of antibiosis was described by Louis Pasteur in 1877 when he wrote that amongst the lower organisms, life hinders life (Sneider, 1986). The antibiotic revolution can be accurately dated to the early 1940s when Howard Florey and his colleagues in Oxford seized upon Alexander Flemings penicillin and turned it into a major therapeutic compound. Antimicrobial agents are among the most commonly prescribed of all drugs (Greenwood, 1997). Antimicrobial activity of plants can be detected by observing the growth response of various microorganisms to the plant extracts placed in contact with them and the currently available methods for anti microbial screening fall in three groups, diffusion, dilution, and bioautographic methods.

In the diffusion technique, a reservoir containing the plant extract is brought into contact with an inoculated medium and after incubation, the diameter of the zone around the reservoir is measured. If the extract is active against the particular strain of bacteria, a clear zone of inhibition is seen around the well. The diameter of the zone is directly proportional to the degree of sensitivity of the bacterial strain and the concentration of the extract. In the dilution method, the samples being tested are mixed with a suitable medium that has been previously

inoculated with the test organism. After incubation, the organism is detected by direct visual observation.

Some antimicrobial agents are able to inhibit a wide range of bacteria belonging to both Gram positive and Gram negative cultures and so are called broad spectrum. Others inhibit only a few bacterial types and are termed narrow spectrum. Gram positive bacteria are generally more sensitive to antimicrobials than the Gram negatives due to the difference in the composition of bacterial cell walls of the two groups.

3.2. Materials and methods

3.2.1. Preparation of the Plant materials

The plants were collected from different habitats where they grow in Central Kenya, and authenticated by Mr. Patrick Mutiso of the Department of Botany University of Nairobi. Voucher specimens LW MM 01- 06 are deposited in the herbarium of the department of Pharmacology and Pharmacognosy, University of Nairobi. The plant materials were then air-dried under shade at room temperature and ground into powder using an engine grinder 2 mm diameter (Muharata Food Company, Kenya). The powders were put in plastic bags and stored at 4 °C until the time of extraction.

3.3. Extraction of the plant materials

3.3.1. Aqueous extracts

About 100 g. of powdered plant material was boiled at about 98 ° C in distilled water for 15 min. with frequent shaking to macerate. This solution was centrifuged at 2000 revolutions per minute and then filtered using Whatmans filter paper number one and the water reduced in a rotar evaporator (VV 2000 Heidolph, Germany), to give a crude aqueous extract.

3.3.2. Organic solvents extraction

One hundred grams of powder was first extracted with chloroform at room temperature for 48 h. with frequent shaking. The material was filtered using Whatmans filter paper number one, dried and was then extracted for a further 48 h. with methanol. The two filtrates were then reduced by evaporation in a rotar evaporator (VV 2000 Heidolph, Germany), to give dried extracts for the bioassay.

3.4 The microorganisms that were used as test cultures

The following clinical isolates, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Salmonella typhi*, *Shigella dysenteriae* and *Bacillus pumilis* were obtained from the microbiology departments of Kenyatta National Hospital and the University of Nairobi and were used together with the standard cultures *Escherichia coli* (ATCC 35 218), *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853) for quality control. All cultures were maintained on tryptone soya agar

(TSA) and subcultured periodically to keep them in log phase until the time of use.

3.5 Bioassays of the plant extracts

Antimicrobial activity of the plant extracts was detected by use of the agar well diffusion method, Minimum Inhibitory Concentration (MIC) method, and the Minimum Bactericidal Concentration (MBC) method.

3.5.1 Agar well diffusion assay

The agar-well diffusion method as described by Rios *et al.*, (1988) was used. 10^7 Cfu/ml of the test organism was seeded into sterile, molten Tryptone soya agar (TSA) at 48° C. It was mixed and 20 ml poured into each of the sterile petridishes. After the agar solidified, six holes of uniform diameter were made using a sterile borer. Fifty micro litres of each of the test solutions at four concentrations 80, 40, 20 and 10 mg/ml as well as chloramphenicol (0.2 mg/ml) were placed in separate wells under aseptic conditions. Gentamicin (0.01 mg/ml) was used as a control in one of the tests.

The plates were maintained at room temperatures for 2 h. to allow the solutions to diffuse into the media before the organisms start to grow, and were then incubated at 37° C for 24 h. The diameters of the inhibition zones were measured using an antibiotic zone reader. Every concentration was done in triplicates and the size of the zone of inhibited growth was regarded as indicative of the degree of antibacterial /anti fungal activity of the test extract.

3.5.2 Determination of the Minimum Inhibitory Concentrations (MICs) of the crude extracts

Micro-dilution test as described by Gavan and Barry (1980) was used to determine the minimum concentrations of the extracts required to inhibit or kill the different bacteria grown in Tryptone Soya Broth (TSB). Stock solutions of both aqueous and methanol extracts of *Periploca linearifolia* Dill and Rich, *Acacia mearnsii* De Wild bark and *Psidium guajava* L. leaves and the aqueous extract of *Thunbergia alata* were prepared as follows; 200 mg of each of the extract was dissolved in 0.5 ml of sterile distilled water and then diluted with 0.5 ml of sterile Tryptone soya broth to give stock solutions of 200 mg/ml. Serial dilutions of the extracts were done in micro-titre well plates containing 8 rows to give a range of 100 to 0.0488 mg/ml with each well holding 50 µl of extract dilution. The last well received no extract and acted as a control.

An inoculum was prepared so as to contain 10^5 to 10^6 CFU/ml as follows; colonies of an actively growing culture were suspended directly into a small volume of saline and diluted until it matched the turbidity visually comparable to that of a Mc Farland 0.5 standard and the inoculum suspension was used immediately (Barry *et al.*, 1973). An inoculum of 50 µl was added into each of the wells to make a final volume of 100 µl and the plates were incubated at 37°C for 18h. The MIC was taken as the lowest concentration of extract at which the micro-organism tested did not show growth.

3.5.3 Determination of the Minimum bactericidal concentrations (MBCs) of the crude extracts

From each well that did not show visible growth in the MIC test, 0.01 ml. was transferred into sterile extract-free nutrient broth. After 18h incubation, a loopful was subcultured onto Tryptone soya agar plate and incubated at 37 °C for 18 h. The Minimum Bactericidal Concentration (MBC) was recorded as the lowest concentration of extract that produced complete killing of the inoculum.

3.6 Results

3.6.1 Antimicrobial activities of the plant extracts

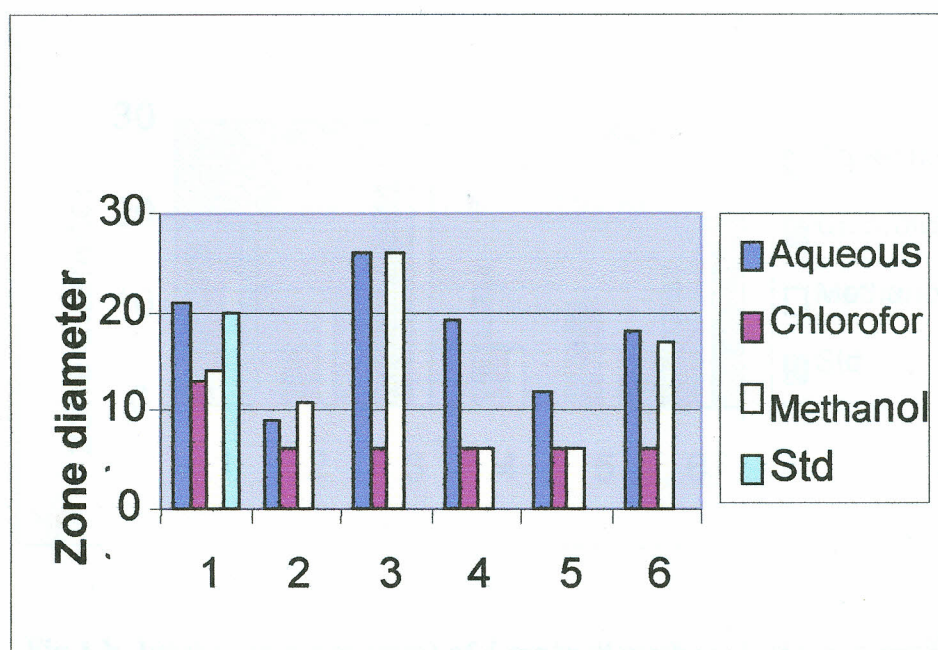


Fig 1a. Inhibition zones (mm) of 4 mg/well methanol, chloroform and aqueous extracts of the six plants against *S. aureus* isolate.

(1) *P. guajava* (2) *P. americana* (3) *A. mearnsii* (4) *T. alata* (5) *O. suave* (6) *P. linearifolia*. Std =chloramphenicol 10 µg /well.

One concentration (4 mg /well) was selected and the mean inhibition zone diameter of the extracts plotted for each organism. *Staphylococcus aureus* isolate responded to eleven out of eighteen extracts and was one of the most susceptible microorganisms (Fig 1a). It was inhibited by the aqueous extracts of all the six plants with zone diameters ranging from 12 to 26 mm. The methanol extracts were also fairly active with zone diameter of the *Acacia mearnsii* extract at 26 mm. Only one chloroform extract, that of *Psidium guajava*, inhibited the growth of this isolate with a mean zone diameter of 13 mm.

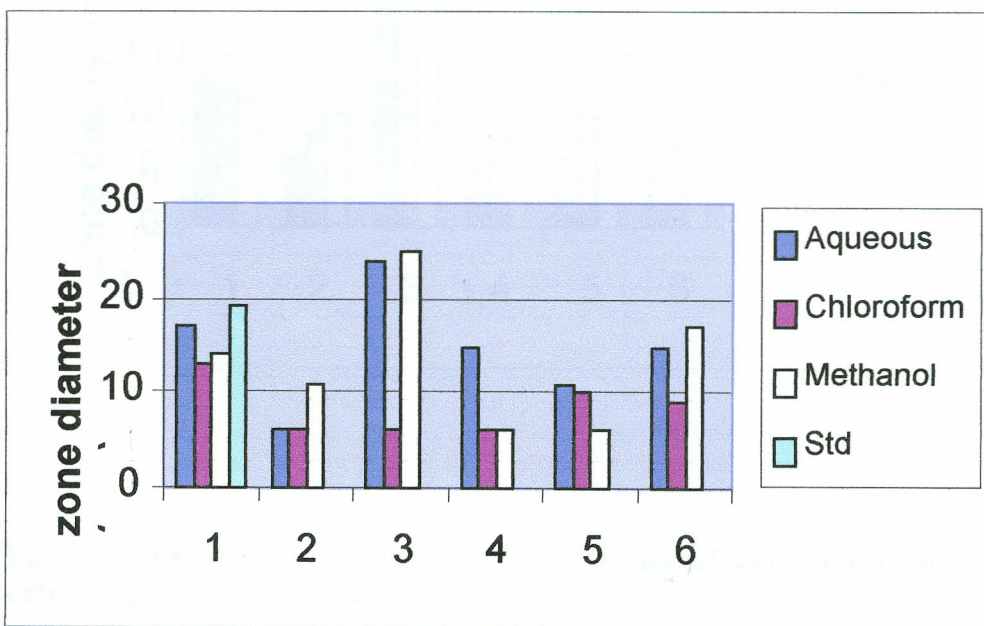


Fig 1 b. Inhibition zones (mm) of 4 mg/well methanol, chloroform and aqueous extracts against *S. aureus* ATCC 29213.

(1) *P. guajava* (2) *P. americana* (3) *A. mearnsii* (4) *T. alata* (5) *O. suave* (6) *P. linearifolia* std = chloramphenicol 10 µg /well.

All the aqueous extracts apart from that of *Persea americana* were effective against *Staphylococcus aureus* ATCC 29213 with zone diameters ranging from 11 to 24 mm (Fig. 1b) Three chloroform extracts, *Psidium guajava*, *Ocimum suave* and *Periploca linearifolia* inhibited the growth with zone diameters ranging from 9 to 13 mm.

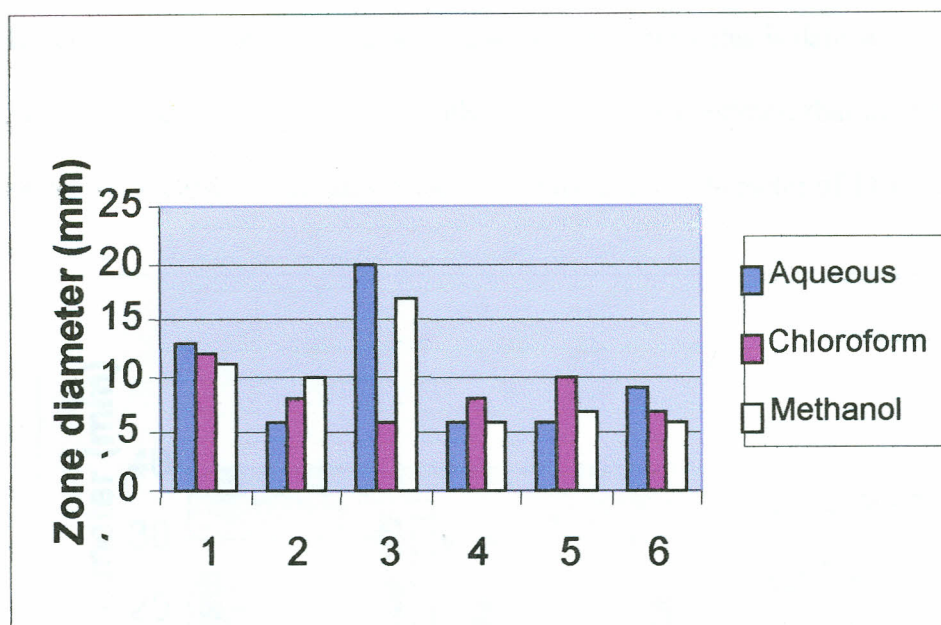


Fig. 1c. Inhibition zones (mm) of 4mg/well methanol, chloroform and aqueous extract against *Bacillus pumilis* isolate.

(1) *P. guajava* (2) *P. americana* (3) *A. mearnsii* (4) *T. alata* (5) *O. suave* (6) *P. linearifolia*

Acacia mearnsii aqueous and methanol extracts inhibited the growth of *Bacillus pumilis* isolate and gave zone diameters of 20 and 17 mm respectively (Fig 1c). The

chloroform extracts of *Ocimum suave* and *Psidium guajava* were moderately active and gave zone diameters of 10 and 13 mm respectively.

Large zones of inhibition of 33 mm were obtained with *Acacia mearnsii* aqueous and methanol extracts against *Pseudomonas aeruginosa* isolate (Fig 1d). The aqueous extracts of all the other plants were also active against this isolate with zone diameters ranging between 15 and 25 mm. Only one chloroform extract, that of *Psidium guajava* was active against this organism and gave a mean zone diameter of 11 mm.

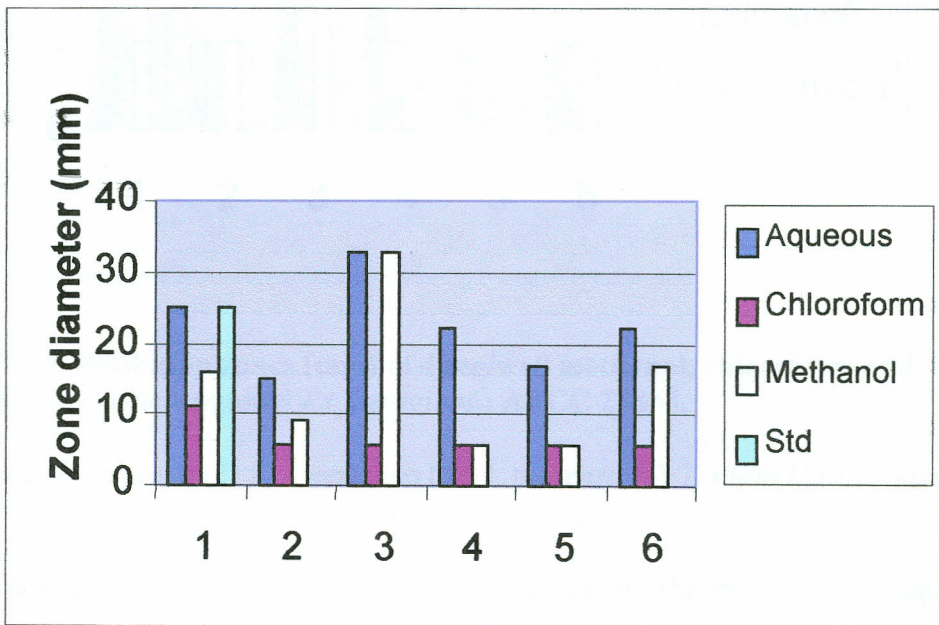


Fig. 1d Inhibition zones (mm) of 4 mg/ well methanol, chloroform and aqueous extracts against *Ps. aeruginosa* isolate.

(1) *P. guajava* (2) *P. americana* (3) *A. mearnsii* (4) *T. alata* (5) *O. suave* (6) *P. linearifolia* std = chloramphenicol 10 µg /well.

Pseudomonas aeruginosa ATCC 27853 responded to extracts from three plants, *Psidium guajava*, *Acacia mearnsii* and *Thunbergia alata* with zone diameters of between 9 and 15 mm (Fig 1e).

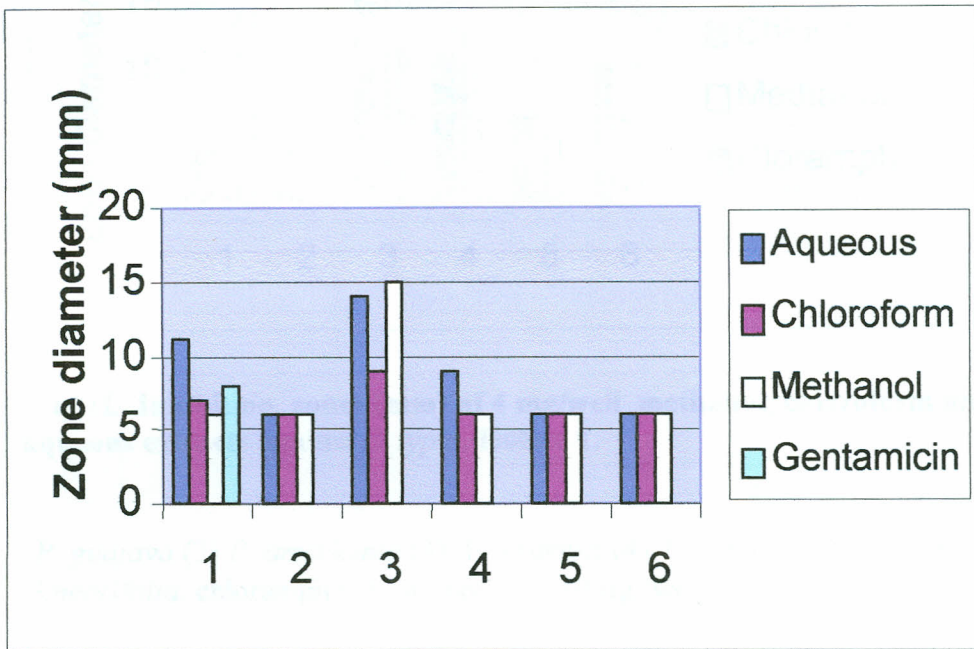


Fig. 1 e. Inhibition zones (mm) of 4 mg/well methanol, chloroform and aqueous extracts against *Ps. aeruginosa* ATCC 27853.

- (1) *P. guajava* (2) *P. americana* (3) *A. mearnsii* (4) *T. alata* (5) *O. suave* (6) *P. linearifolia*, Gentamicin = 0.5 μg / well.

Salmonella typhi isolate (I) was inhibited equally by the methanol and aqueous extracts of *Acacia mearnsii* with zone diameters of 16 mm (Fig 1f). *Thunbergia alata* aqueous and chloroform extracts inhibited its growth but with smaller zones of 11 and 10 mm respectively. The only other extract active against this isolate was *Periploca linearifolia* aqueous extract with a zone diameter of 10 mm.

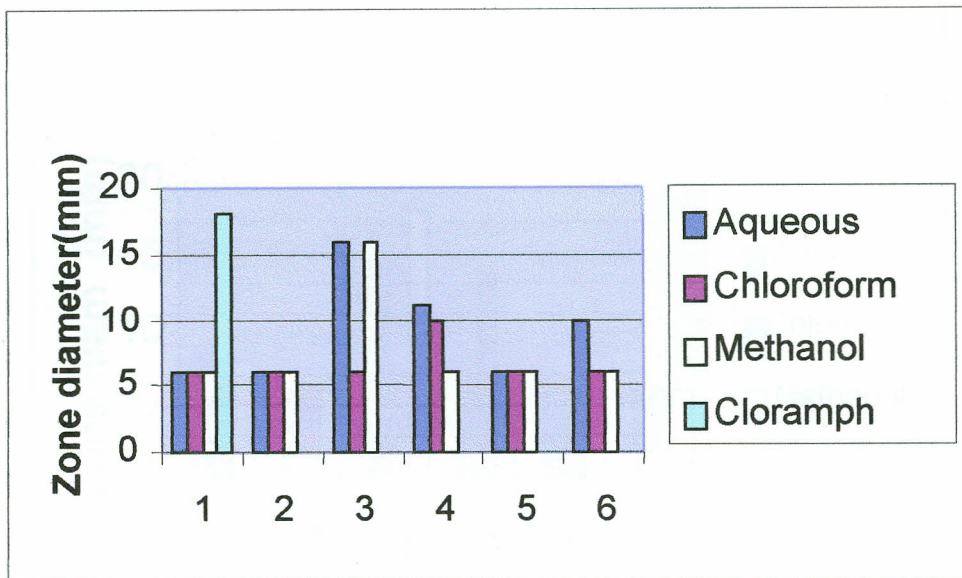


Fig 1f. Inhibition zones (mm) of 4 mg/well methanol, chloroform and aqueous extracts against *S. typhi*. Isolate 1.

P. guajava (2) *P. americana* (3) *A. mearnsii* (4) *T. alata* (5) *O. suave* (6) *P. linearifolia*. chloramph =chloramphenicol 10 µg /well.

An isolate of *Shigella dysenteriae* was tested against the extracts and only three plants had minimal activity against this isolate. All the three extracts of *Psidium guajava* exhibited moderate activity with mean zone diameters of 8 to 9 mm while *Acacia meansii* methanol and aqueous extracts gave big zones of inhibition, 18 and 15 mm respectively (Fig 1g). The only other plant that was active against this isolate was *Thunbergia alata* whose chloroform extract gave zones of 14 mm. in diameter.

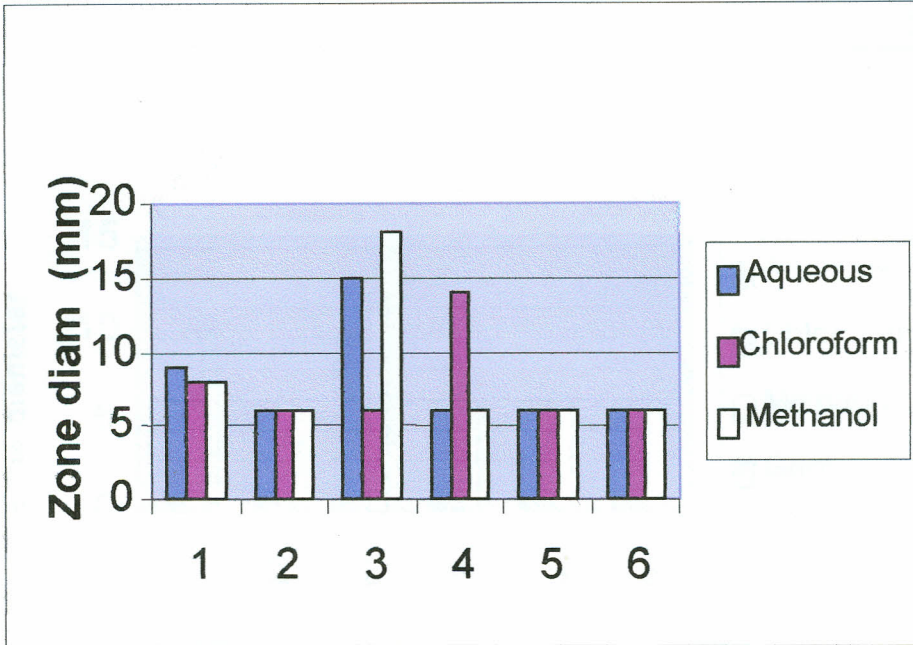


Fig. 1g. Inhibition zones (mm) of 4 mg/ well methanol, chloroform and aqueous extracts against *Shigella dysenteriae* isolate.

(1) *P. guajava* (2) *P. americana* (3) *A. mearnsii* (4) *T. alata* (5) *O. suave* (6) *P. linearifolia*.

The performance of most of the extracts against *E coli* ATCC 35218 was quite low as evidenced by the small inhibition zones that were obtained (Fig. 1h).

None of the extracts from four plants, *P. guajava*, *P. americana*, *O. suave* and *P. linearifolia* inhibited its growth. *Acacia mearnsii* aqueous and methanol extracts gave zones of 10 and 12 mm respectively while *T. alata* chloroform extract gave an inhibition zone of 8 mm.

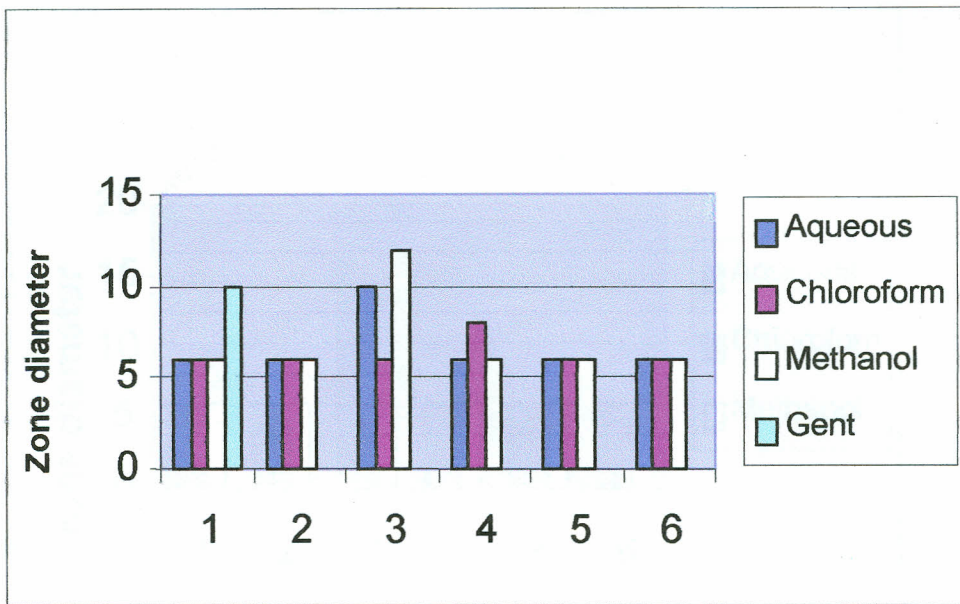


Fig. 1h. Inhibition zones (mm) of 4 mg/ well methanol, chloroform and aqueous extracts against *E. coli* ATCC 35218.

(1) *P. guajava* (2) *P. americana* (3) *A. mearnsii* (4) *T. alata* (5) *O. suave* (6) *P. linearifolia*. Gent = Gentamicin 0.5 μ g / well.

Candida albicans was the least susceptible microorganism and responded to only one of the extracts, *Acacia mearnsii* aqueous extract (Fig 1i).

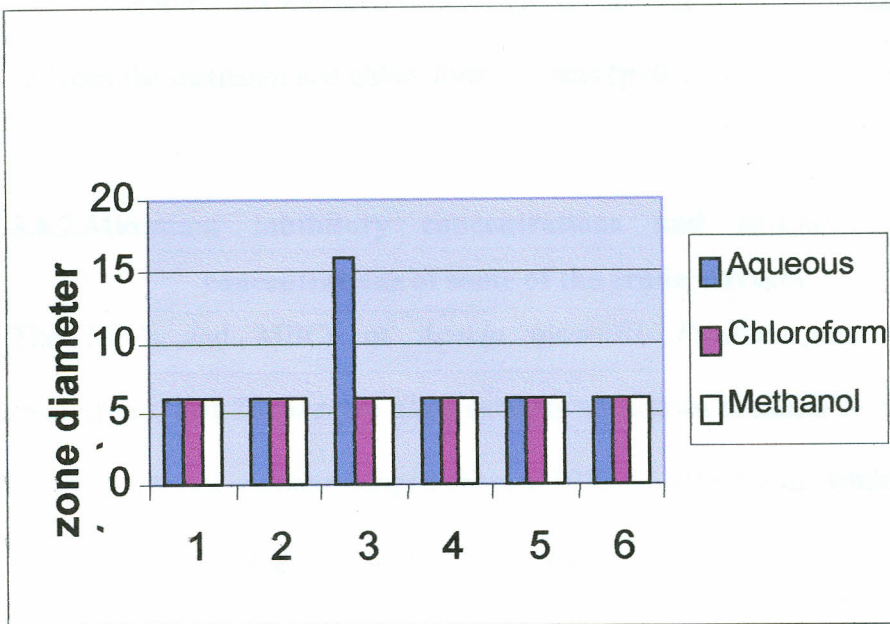


Fig. II Inhibition zones (mm) of 4 mg/ml methanol, chloroform and aqueous extracts against *Candida albicans* isolate.

(1) *P. guajava* (2) *P. americana* (3) *A. mearnsii* (4) *T. alata* (5) *O. suave* (6) *P. linearifolia*

Staphylococcus aureus ATCC 29213 together with the clinical isolates *S. aureus* and *Pseudomonas aeruginosa* were the most susceptible microorganisms having responded to eleven out of the eighteen extracts tested (Fig 1a, 1b, 1d). Analysis of variance was done on the performance of the three extracts from each plant on the tested microorganisms. It was found that for each of the plants there were significant differences in the activity of the aqueous, chloroform and the methanol extracts. Post anova tests to separate the means revealed significant differences between the aqueous and methanol extracts ($p=0.017$) and between the aqueous

and the chloroform extracts ($p=0.049$) but no significant difference in activity between the methanol and chloroform extracts ($p=0.139$).

3.6.2. Minimum inhibitory concentrations and Minimum bactericidal concentrations of some of the crude extracts

The MICs and MBCs of *Acacia mearnsii*, *Psidium guajava*, *Periploca linearifolia*, and *Thunbergia alata* extracts are shown in tables 1- 4 below. MICs of the methanol extracts ranged from 0.1953 to 100 mg/ml while those of the aqueous extracts ranged from 0.09 to 50 mg/ml.

Micro-organisms	Methanol extract		Aqueous extract	
	MIC (mg/ml)	MBC	MIC	MBC
1. <i>Escherichia coli</i> ATCC 35218	3.125	6.25	50	nd
2. <i>Staphylococcus aureus</i> ATCC 29123	0.1953	nd	0.09	3.125
3. <i>Pseudomonas aeruginosa</i> ATCC 27853	1.5625	3.125	1.5625	3.125
4. <i>Salmonella typhi</i> isolate	1.5625	1.5625	1.5625	3.125
5. <i>Shigella dysenteriae</i> isolate	0.78125	1.5625	0.78125	1.56
6. <i>Pseudomonas aeruginosa</i> isolate	1.5625	3.125	nd	nd
7. <i>S. aureus</i> isolate	0.1953	0.1953	0.1953	0.1953
8. <i>Bacillus pumiliis</i> isolate	nd	nd	0.7812	1.5625

Table 1. Concentrations of *Acacia mearnsii* bark extracts that kill /inhibit the tested bacterial strains

MIC = Minimum inhibitory concentration, MBC = minimum bactericidal concentration, nd = not determined.

The methanol extract of *Acacia mearnsii* was more active against *E. coli* ATCC at 3.125 mg/ml as compared to the aqueous extract at 50 mg/ml (Table1). The aqueous extract on the other hand gave a lower value against *S. aureus* ATCC

29213 as compared to the value of the methanol extract (0.09 and 0.1953 mg/ml respectively). Both the aqueous and the methanol extracts gave similar values with the rest of the bacteria. The *Acacia mearnsii* extracts were generally very active and the values obtained were as low as 0.09 mg/ml for *S. aureus* ATCC 29123.

	Micro-organisms	Methanol extract		Aqueous extract	
		MIC (mg/ml)	MBC	MIC	MBC
1.	<i>E. coli</i> ATCC 35218	50	nd	nd	-
2.	<i>S. aureus</i> ATCC 29123	0.3903	0.7812	0.7812	0.7812
3.	<i>Ps. aeruginosa</i> ATCC 27853	100	nd	12.5	25
4.	<i>Salmonella typhi</i> isolate	12.5	25	12.5	nd
5.	<i>Shigella dysenteriae</i> isolate	6.25	nd	3.125	nd
6.	<i>Pseudomonas aeruginosa</i> isolate	25	nd	nd	nd
7.	<i>S. aureus</i> isolate	1.562	nd	1.5625	nd
8.	<i>Bacillus pumiliis</i> isolate	nd	nd	3.125	nd

Table 2. Concentrations of *Psidium guajava* leaf extracts that kill / inhibit the tested bacterial strains

MIC = Minimum inhibitory concentration, MBC = minimum bactericidal concentration, nd = not determined.

The values obtained with the extracts of *Psidium guajava* were low especially against the Gram-positives *S. aureus* isolate and *Bacillus pumillis* (1.56 and 3.125 mg/ml respectively) (Tab. 2). Among the Gram negatives that gave low values were *Shigella dysenteriae* and *Salmonella typhi* (3.125 and 12.5 mg/ml respectively). The methanolic extract of *Psidium guajava* had greater activity

against *S. aureus* (MIC= 0.3903 mg/ml) as compared to the aqueous extract (MIC =0.7812). Low values were obtained for the Gram negatives *Pseudomonas aeruginosa* and *Shigella dysenteriae* with the aqueous extract (MIC= 12.5 and 3.125 mg/ml respectively) as compared to the methanol extract (100 and 6.25 mg/ml respectively). Similar MIC values were obtained with the two extracts for *Salmonella typhi* (12.5).

Micro-organisms	Chloramph mg/ml	Methanol extract		Aqueous extract	
		MIC (mg/ml)	MBC	MIC	MBC (mg/ml)
1. <i>E. coli</i> ATCC 35218	0.125	100	nd	50	nd
2. <i>S. aureus</i> ATCC 29123	0.0078	25	25	12.5	nd
3. <i>Ps. aeruginosa</i> ATCC 27853	0.015625	25	Nd	50	nd
4. <i>Salmonella typhi</i>	0.007812	25	50	25	50
5. <i>Shigella dysenteriae</i>	0.0625	6.25	12.5	1.565	nd
6. <i>Pseudomonas aeruginosa</i>	nd	50	50	nd	nd
7. <i>S. aureus</i>	nd	6.25	nd	3.125	3.125
8. <i>B. pumiliis</i>	nd	nd	nd	25	50

Table 3. Concentrations of *Periploca linearifolia* leaves/ stem extracts that kill / inhibit the tested bacterial strains

MIC = Minimum inhibitory concentration, MBC = minimum bactericidal concentration, nd = not determined. Chloramph = chloramphenical.

The MIC values obtained with *Periploca linearifolia* extracts are shown in table 3. The aqueous extract gave low values for *Shigella dysenteriae* and the *S. aureus* isolate (1.56 and 3.125 mg/ml respectively). The aqueous extract gave lower values than the methanol extract for *E. coli*, *S aureus* standard and isolate and *S. dysenteriae*.

	Micro-organism	MIC mg/ml	MBC mg
1	<i>S. aureus</i> ATTC 29213	1.56	25
2	<i>Ps. aeruginosa</i> ATCC 27853	100	nd
3	<i>S. aureus</i> isolate	6.25	25
4	<i>Bacillus pumillis</i>	100	nd
5	<i>Shigella</i> (2)	3.125	>100
6	<i>Salmonella</i> (1)	0.78125	>100

Table 4. Concentrations of the aqueous extract of *Thunibergia alata* that kill / inhibit the tested strains.

MIC = Minimum inhibitory concentration, MBC = minimum bactericidal concentration, nd = not determined.

The aqueous extract of *Thunbergia alata* gave low values in the minimum inhibitory concentration tests against *Staphylococcus aureus* standard, *Shigella dysenteriae* and *Salmonella typhii* isolate 1 but the values obtained for the minimum bactericidal concentrations for the same organisms were quite high (Tab 4).

The activities of the two extracts from each plant were tested for any significant differences. There were no significant differences in MICs of the *Psidium guajava* aqueous and methanol extracts (p=0.0590 and p=0.088 respectively) and of the two *Acacia mearnsii* extracts (p=0.065 and p=0.134) respectively. There was however a significant difference in MICs of *Periploca linearifolia* aqueous and methanol extracts (p=0.023 and p=0.03)

3.7 Discussion

Psidium guajava L is a member of the family Myrtaceae, a family of about 100 genera with many of them providing important volatile oils and spices. In the diffusion assay the aqueous and the methanol extracts exhibited activity against both Gram positive (*S. aureus* and *Bacillus pumillis*) and Gram negative (*Pseudomonas aeruginosa* and *Shigella dysenteriae*) bacteria but were not effective against the fungi, *Candida albicans*. The MICs of these two extracts ranged from 0.3903 to 100mg/ml. The chloroform extract was also fairly active against *S. aureus*, *Bacillus pumilis* and *Pseudomonas aeruginosa*. From these results, the mechanism of activity appears not to be cell-wall mediated since activity was against both the Gram positives and the Gram negatives.

The results of this study agree with those of a study done in Sudan where the water extract of the leaves is used to treat many ailments like bronchitis, asthma and dysentery while the bark is used to treat diarrhoea and stomachache and investigations done on the aqueous and methanol extracts found them to be effective against both Gram positive (*S. aureus* and *B. subtilis*) and Gram negative (*E. coli* and *Pseudomonas aeruginosa*) with MICs ranging from 1.69 to 18.06 mg/ml (Abdelrahim *et al.*, 2002). The leaves have been found to contain an essential oil rich in cineol, tannins and triterpenes and three oils have already been isolated (Olajide *et al.*, 1999). Various other compounds that have been confirmed to be present include tannins from the bark (Tanaka *et al.*, 1992),

terpenoids from the leaves (Sagrero *et al.*, 1994), essential oils also from the leaves (Turker *et al.*, 1995, Ekundayo *et al.*, 1991) and mono terpenoids and esters from the ripe fruit (Chyau *et al.*, 1992). Essential oils of plants belonging to this family are known for their biological activities and this is due to the presence of 1,8- cineole (Lis-Balchin *et al.*, 1998).

Oils from the leaves of two plants belonging to this same family, *Syzygium cumini* (L) and *S. travancoricum* Gamble are used in Indian traditional medicine and they have been shown to exhibit good anti-bacterial activity against *Bacillus subtilis*, *S. aureus*, *E. coli* and *Pseudomonas* species (Shafi *et al.*, 2002). The ethanolic extracts of two other *Syzygium* species, *S. andamanicum* (King), *S. samarangense* (Balakr) have shown activity against both Gram positive and Gram negative bacteria (Chattopadhyay and Sinha, 1998). These plants were also found to contain compounds like flavonoids, tannins and terpenoids all of which are known to be of medicinal value. Other members of this family have been shown to have essential oils, tannins, terpenoids, triterpenoids and flavonoids and activity may be due to the presence of these secondary metabolites. Tannins are known to have the ability to form complexes with other molecules, have anti-septic effects and are often used to treat diarrhoea (Ben-Eric *et al.*, 1997) while flavonoids have several medicinal properties like anti-inflammatory, anti-oxidant, anti- allergic anti bacterial and antiviral effects (Fransworth, 1966) and this may

explain why *Psidium guajava* L is used to treat different illnesses in different parts of the world.

Persea americana Mill leaf extracts were among the least active with moderate activity against only four strains, *Pseudomonas aeruginosa*, *S aureus* ATCC 29123 and the clinical isolate of *S. aureus* as well as *Bacillus pumilis*. The chloroform extract was slightly active against one organism, *Bacillus pumilis*. This plant belongs to the family Lauraceae that has thirty two genera with alkaloids and volatile oils occurring in many species. Infusion of the leaves has been found to contain flavonoids, alkaloids reducing sugars, tannins and saponins (Adeyemi *et al.*, 2002).

Persea cordata Vell Mez is another plant from this family whose stem bark is used in Brazil rural communities as a remedy to cure infectious processes (Da Silva, 1997). This plant has been screened for antimicrobial activity and the ethyl acetate fraction of the hydro-alcoholic extract exhibited activity suggesting that the non- polar constituents are likely to be the active principles responsible for the biological action of the plant (Schlemper *et al.*, 2001). These findings agree with the results of the current study in that although the methanol and the aqueous extracts of the other plants exhibited moderate activity against most of the tested microorganisms, those of *Persea americana* Mill had very minimal activity against all the tested micro organisms.

The aqueous and methanol extracts of *Acacia mearnsii* were the most active extracts and gave big zones of inhibition ranging from nine to thirty three millimeters in diameter in the agar well diffusion assay. These two extracts had a broad spectrum activity and were active against Gram positive bacteria, *Staphylococcus aureus* and *Bacillus pumilis* and the Gram negatives, *Pseudomonas aeruginosa* and *Salmonella typhi*. This was also evident in the low MICs obtained which ranged from 0.09 to 50 mg/ml. The aqueous extract from this plant was the only extract that was active against the fungal pathogen *Candida albicans*. The chloroform extract was only slightly active against *Pseudomonas aeruginosa*. The herbalists use this plant to treat mouth rashes that are likely to be oral thrush, a condition that is caused by *Candida albicans*.

These results agree with those of Bwambok and Ndaluti (2001) where they found that the stem bark extract of the same plant inhibits the growth of the fungi *Fusarium oxysporum*. This plant is known to be rich in tannins, plant-derived poly phenols with anti microbial effects. Condensed tannins obtained from the wattle tree are toxic to *E. coli* in aerobic medium (Smith *et al.*, 2003). Other members of this family screened for antimicrobial activity showed activity against various microorganisms. In a study conducted on Sudanese plants that are used in folk medicine, *Acacia nilotica* (L) fruits were found to exhibit antimicrobial activity and tested positive for tannins, sterols and alkaloids (Omer *et al.*, 1998). *A. nilotica* fruits are used in Sudan to treat many ailments of microbial nature like

cold, fever, pneumonia and meningitis. *Neptunia, oleracea* Lour. , a plant belonging to the same family and used as a food in Sudan has also been found to have antibacterial activity (El Egami *et al.*, 2001).

Thunbergia alata belongs to the family Acanthaceae. This family consists of 250 genera and is abundant in the tropics. In addition to alkaloids, the family contains tannins, diterpenoids, cyanogenetic compounds and saponins. The aqueous extract exhibited moderate activity against both Gram positive (*Staphylococcus aureus*), and also the Gram negatives (*Pseudomonas aeruginosa* and *Salmonella typhi*) but was inactive against *Candida albicans*. The methanol extract exhibited no activity at all while the chloroform extract was fairly active against *E.coli*, *Shigella dysenteria* and *Salmonella typhi* all of which are Gram negative. The MICs of the aqueous extract against *S. aureus*, *Shigella dysenteria* and *Salmonella typhi* were quite low.

Several other plants from this family have been screened against various microorganisms and found to have antimicrobial activity. *Astheracantha longifolia* (L) is used in Sudanese folkloric medicine and has been shown to exhibit antibacterial activity (El Egami *et al.*, 1998). Other locally used plants in Sudan with demonstrated antibacterial activity include *Barleria ruellioides* T. Anders, *Blepharis maderaspatensis* (L), *Lepidagathis collina* (Milne-Redn) and *Pupalia lappacea* (L) (El Fatih *et al.*, 1997).

The extracts from *Ocimum suave* Willd (Lamiaceae) exhibited slight activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus pumillis*. Lamiaceae is a family of about 200 genera and in addition to the volatile oils, the family also contain diterpenoids and triterpenoids, saponins, poly phenols and tannins. The essential oil from *Salvia tomentosa* Miller also in the family Lamiaceae has been shown to exhibit antibacterial activity against various microorganisms (Haznedaroglu et al., 2001). Similarly, *Salvia triloba* L., used in Pakistan traditional medicine for a variety of abdominal problems (Hasan et al., 1986) has been shown to contain triterpenoids and sterols (Ulubelen et al., 1968). *Hyptis suaveolens* L is used in Nigerian traditional medicine and the essential oil from the leaves was found to be active against both Gram positive and Gram negative bacteria (Olayinka et al., 1999).

Periploca linearifolia Dill and Rich is a member of the family Asclepiadaceae. Other constituents present in members of this family include alkaloids, glycosides, saponins, tannins and cyclitols most of which are known to have medicinal properties. In this study the methanol and the aqueous extracts exhibited a broad spectrum activity against both the Gram positive bacteria (*S. aureus* and *B. pumilis*) as well as Gram negative bacteria (*Ps.aeruginosa* and *S. typhi*). The chloroform extract did not have activity against any of the tested organism. Other members of this family that have been found to have antimicrobial activity include *Caralluma retrospiciens* Ehrenb that is used to treat

animal wounds in Sudan (El Egami et al., 1998). In India, *Decalepis hamiltonii* Wright et. Arn roots have been investigated and found to contain steroids, saponins, tannins, and fatty esters (Chanda, 1976, Parish and Davidson, 1993) and to exhibit antimicrobial activity (Ravishankar, 1999).

The extracting agent or solvent used in the preparation of drugs from plant materials must be suitable for dissolving the important drug constituents and it therefore determines the kind as well as the quantity of the active ingredients. Many plant constituents like flavonoids, polyglycosides, carbohydrates, quaternary alkaloids, saponins and tannins are soluble in water. The methanol and the aqueous extracts of the plants that were studied were in most cases more active than the chloroform extracts. This means that the active components in these plants are polar and are easily extracted by water and therefore supports their use in traditional medicine where extraction is mostly by water.

CHAPTER 4

4.0 TIME KILL KINETICS

4.1. Introduction.

After the initial screening by the diffusion assay and the determination of minimum inhibitory and bactericidal concentrations, the active aqueous extracts were further studied to evaluate how concentration and exposure times influence their activities. The bactericidal activity of these extracts was evaluated by time-kill kinetic studies on the bacteria that had responded to the extracts. The time kill gives a good overview of how fast an anti-microbial can kill certain bacteria and prevent their re-growth and these parameters are important for the assessment of the efficacy of bactericidal substances. The time-killing curve method has been used in many studies since bacterial death is evaluated with more information by this method than by end-point methods (Guerillot et al., 1993). The kinetics of anti-microbial activity are generally used to evaluate and compare new drugs and to study differences and changes in the anti-microbial sensitivities of clinically important bacterial isolates (Amsterdam, 1991). Bacterial kill kinetics are used to describe the interactions between drug and pathogens since antimicrobials are either time or concentration dependent or both.

4.2. Materials and methods

Two bacteria, *S. aureus* ATCC 29213 and *Ps. aeruginosa* ATCC 27853 strains were selected to study the bactericidal activity of *Acacia mearnsii*, *Psidium*

guajava and *Periploca linearifolia* aqueous extracts. Portions of five colonies of bacteria growing in tryptone soya agar were suspended in 10 ml of 0.85 % saline, mixed and made to a turbidity, 0.5 Mc Farland standard. 100 μ l of this suspension was added to 10 mls of tryptone soya broth containing the extracts to give an initial bacterial density of approximately 10⁶ CFU/ml. The final concentration of each extract was selected on the basis of the particular minimum inhibitory concentration. The assays were done in duplicates in universal bottles. A bottle with the media only and without the extract was used as a control. All the inoculated bottles were incubated in plastic packs in a water bath at 35°C.

Sampling

Two samples of 0.05 mls (50 μ l) were removed from each tube at 0, 2, 4, 6, and 24 h. after inoculation. One of the samples was plated directly on a petridish containing tryptone soya agar (TSA) while serial dilutions of 10⁻¹ to 10⁻⁶ were prepared for each of the other samples in sterile distilled water and 0.05 μ l of each dilution plated on TSA. All the plates were incubated for 24 h. at 35°C. The number of resultant colonies were counted where counts were between 30 and 300 and the number of colony forming units per millilitre (CFU/ml) was calculated for each strain. The killing curves were obtained by plotting the logarithm of number of CFU per millilitre against time for each of the concentrations.

4.3. Results.

Mathematical modeling was carried out with log-transformed data. The growth of *Pseudomonas aeruginosa* was slow in the first two hours as shown by the bottle with no extract (Fig 2a). The rate however increased exponentially in the next four hours and then slowed down.

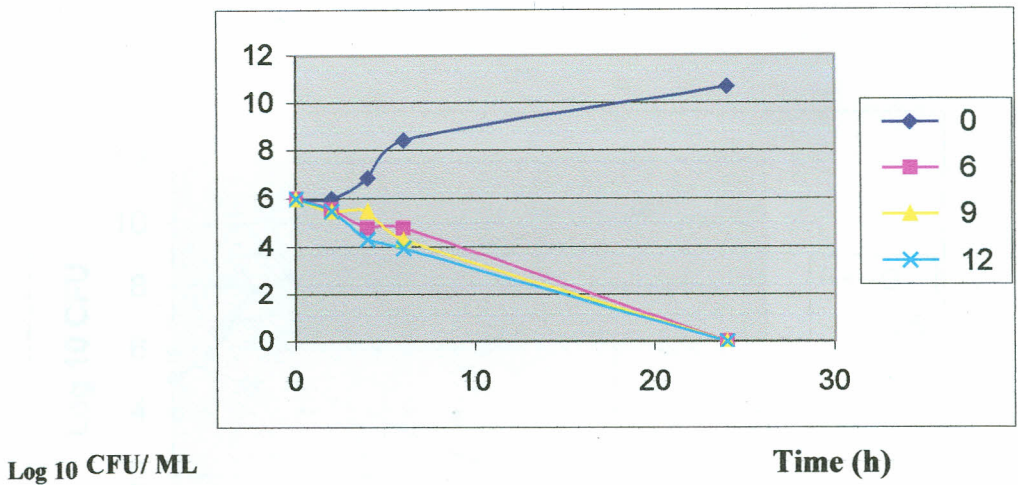


Fig. 2a Time kill curve of *Ps. aeruginosa* by varying concentrations of aqueous extract of *Acacia mearnsii* (mg/ml)

There was a slow decrease in the number of cells in the first four hours of incubation at a concentration of 6 mg/ml of *Acacia mearnsii* extract. The rate slowly declined but achieved complete killing within 24 hours. At 9 mg/ml the number of cells were reduced in the first two hours but almost remained the same in the next two hours. The rate of decline increased after four hours and

complete killing was obtained within 24 h. Further increase of concentration to 12 mg/ml gives a steady rate of cell reduction that was maintained in the first six hours unlike in the lower concentrations and killing was complete within 24 hours of incubation.

The growth of *Staphylococcus* is shown in Fig 2b. There was a sharp increase in the number of cells in the first four hours before slowing down in the next two hours in the control bottle.

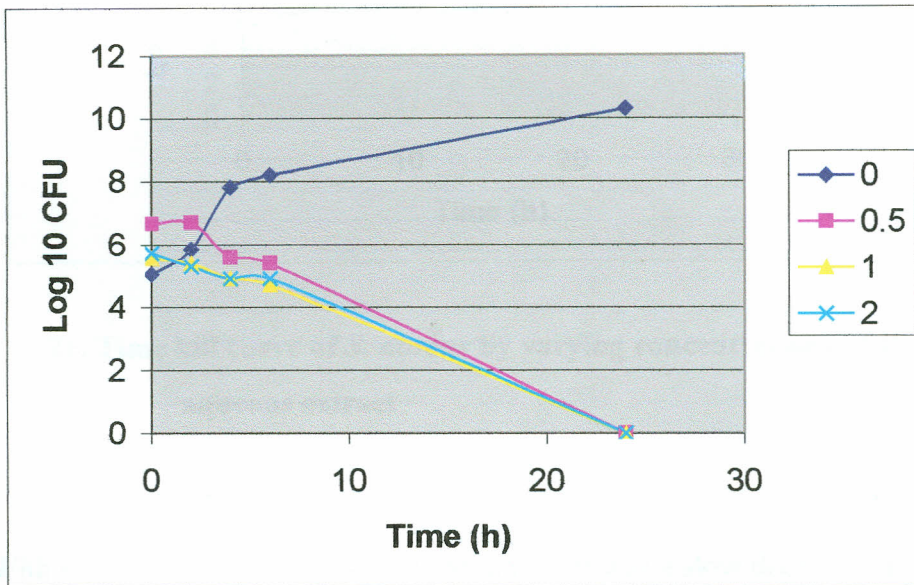


Fig. 2b. Time kill curve of *S. aureus* by varying concentrations of aqueous extract of *Acacia mearnsii*

With 0.5 mg/ml of the *Acacia mearnsii* extract, the number of cells remained unchanged in the first two hours but declined steadily in the next four hours and complete killing was attained within 24 h. The curves obtained with the next two

concentrations of 1 and 2 mg/ml were similar and in both of them, the number of cells decreased steadily from time zero and complete killing was achieved by 24 h.

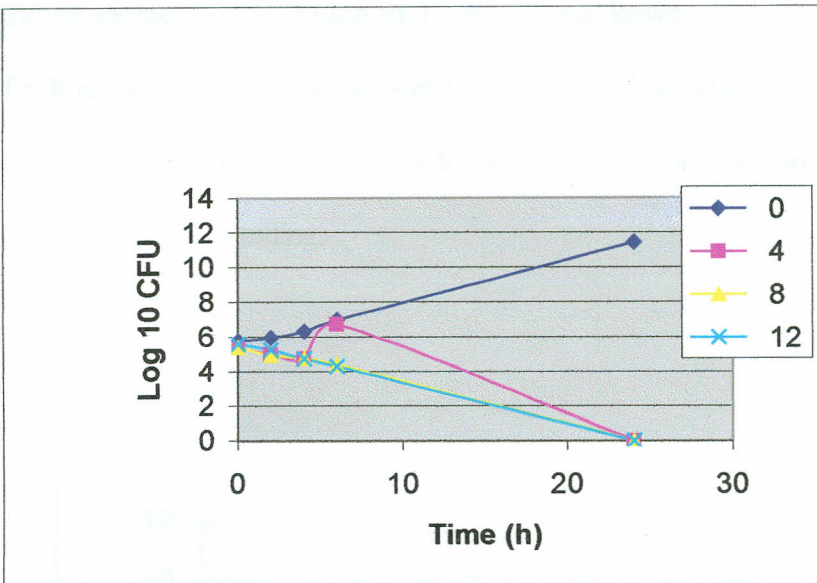


Fig. 2c. Time kill curve of *S. aureus* by varying concentrations of *P. guajava* aqueous extract

With 4mg/ml of *Psidium guajava* extract there was a slow decrease in the number of *S. aureus* cells in the first four hours, followed by a slight increase in the next two hours but complete killing was achieved by 24 h. (Fig 2c). The other two concentrations 8 and 12 mg/ml caused a steady decline in the number of viable cells and achieved complete killing within 24 h.

At 6 mg/ml, *Periploca linearifolia* extract slowly decreased the cell population of *S. aureus*, the rate being slightly higher in the first two hours before slowing down but this concentration did not achieve bactericidal effects within 24 h (Fig. 2d). An extract concentration of 12 mg/ml was however bactericidal and the rate of cell reduction was slow but steady in the first four hours and then increased to achieve complete killing within 24 h. Doubling the concentration to 24 mg/ml did not alter the rate of killing.

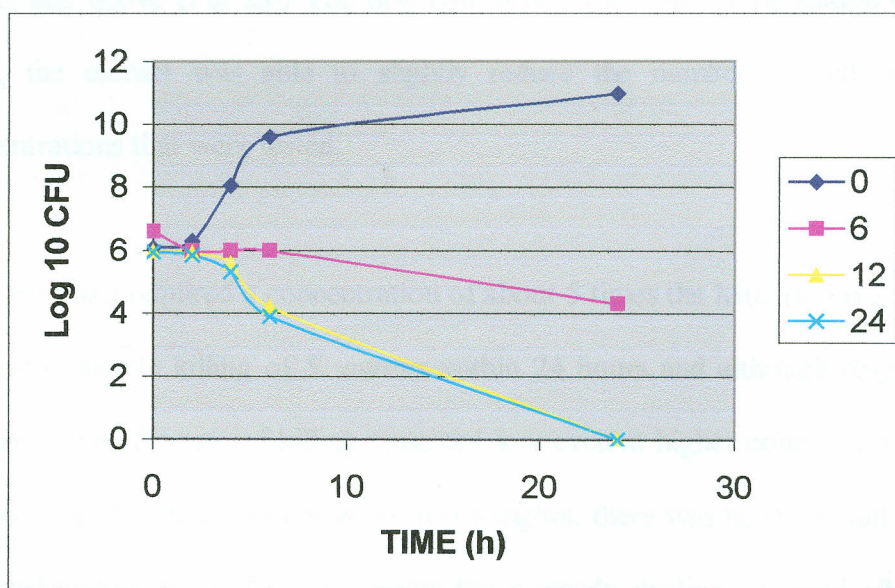


Fig. 2d. Time kill curve of *S. aureus* by varying concentrations of *P. linearifolia* aqueous extract.

4.4. Discussion.

The time kill curves were used to assess the effectiveness of the aqueous extracts of three plants, *Acacia meansii*, *Psidium guajava*, and *Periploca linearifolia* by following microbial killing and growth as a function of the effects of both time and concentration.

The *Acacia mearnsii* aqueous extract had given large zones of inhibition of between 12 and 19 mm against *Pseudomonas aeruginosa*, a Gram negative bacteria, in the agar diffusion assay and also had low values in the MICs (1.562 mg/ml.). Bactericidal effects were however achieved by higher concentrations of 6 mg/ml and above (Fig 2a). The first two hours when the multiplication of cells is slow, the extract was able to slightly reduce the number of cells at all the concentrations that were tested.

A. mearnsii required a concentration of about 5 times the MIC (0.5 mg/ml) to achieve complete killing of *S. aureus* within 24 hours and although re-growth did not occur, the rate of killing remained low even at higher concentrations of extract (Fig 2b). At a concentration of 0.5 mg/ml. there was no reduction in the bacterial counts in the first two hours but a steady decline occurred after six hours of extract exposure. Below this concentration the extract failed to eliminate the bacteria at 24 h but with higher concentrations, there was a decline in the number of colonies from two hours. The low bactericidal values evident in this test also correspond to the large zones of inhibition that were obtained

with *S. aureus* in the agar diffusion assay (25 to 10 mm) and the low MIC values that were obtained (0.09 mg/ml) and this points to the fact that this extract is highly effective against the *S. aureus* ATCC 29213. *Acacia mearnsii* is known to be rich in tannins and these may be attributed to the activity against both the Gram positive and the Gram negative bacteria as well as the fungi *Candida albicans*. Concentration effects of the *A. mearnsii* extracts had an influence mainly on the length of the lag phase and on the number of living cells observed.

Psidium guajava extract achieved bactericidal activity against *S. aureus* from concentrations of 4 mg/ml and above which is about five times the value obtained in the MIC test (Fig 2c). At low concentrations this extract affects the length of the lag phase but as the concentration increases the performance remains unchanged. Low concentrations of this extract may be just bacteriostatic but bactericidal effects can be achieved by increasing the concentration.

At 6 mg/ml which is $\frac{1}{2}$ the MIC value of *Periploca linearifolia* extract obtained against *S. aureus*, the extract slowly reduced the cell population of *S. aureus* although it did not achieve complete killing (Fig. 2d). Concentrations higher than this were bactericidal and therefore concentration of this extract has a direct influence on its performance.

CHAPTER 5

5.0 CHROMATOGRAPHIC FRACTIONATION OF *PERIPLOCA LINEARIFOLIA* METHANOL EXTRACT

5.1. Introduction

Periploca linearifolia is one of the plants that was found to have antibacterial activity and from literature, search it has not been fully investigated and was therefore selected for further screening of the antibacterial components. The methanolic extract was partitioned further using column chromatography. Chromatography is an analytical method that is widely used for the separation, identification and qualitative determination of the chemical components in complex mixtures (Douglas et al., 1997). All chromatography relies on the differential distribution of compounds between two phases, a mobile and a stationary phase (Roughton and Ralman, 1998). Components of a mixture are carried through the stationary phase by the flow of a mobile phase. Separations are based on differences in migration rates among the sample components.

Vacuum liquid chromatography (VLC) is a form of column chromatography that is especially useful for the rapid crude fractionation of extracts and was used to fractionate the methanol extract of *P. linearifolia*. This column is usually contained in a Buchner funnel and the eluant is collected in a Buchner flask. Elution is carried out by adding aliquots of mobile phase of increasing polarity to produce a stepwise gradient elution. The fractions are monitored using thin layer chromatography. The

ratio between the distance travelled on the paper by a component of the test solution and the distance traveled by the solvent is termed the Rf value.

5.2 Materials and methods

The methanol extract from *P. linearifolia* was passed through silica gel chromatographic column and eluted with solvent mixtures of increasing polarity starting with 100 % petroleum ether and ending with 100 % methanol.

5.2.1.Partitioning of the methanol extract using vaccum liquid chromatography

Two grams of the plant extract was dissolved in methanol and then mixed with a small amount of TLC grade silica gel to form a paste. This was completely dried by reducing the solvent in a rotary evaporator to give a free-flowing powder. The Buchner-Sintered- glass funnel was assembled and attached to a vacuum line and was three-quarter filled with about 50 g of TLC grade silica gel. The column was packed by sucking through about 100 milliliters of petroleum ether but not to dryness. The free-flowing powder was evenly packed on top of the silica bed and covered with some cotton wool. Elution was carried out by sucking through reduced pressure, aliquots of mobile phase of decreasing polarity as follows; 100 % petroleum ether, petroleum ether: dichloromethane 50:50, 100 % dichloromethane, dichloromethane: ethyl acetate 50:50, 100% ethyl acetate, ethyl acetate: methanol 50:50 and finally with 100 % methanol.

A total of seven fractions were collected in round bottomed quick fitting flasks and each one of them was concentrated in a rotary evaporator. All the fractions were monitored by spotting small volumes onto thin-layer chromatography (TLC) plates that were then developed in a chamber containing 100% chloroform. The fractions that appeared to have same compounds were pooled together and were screened for antibacterial activity using the agar well diffusion assay at 4 mg/well. Stock solutions of 80 mg/ml of each of the fractions were prepared by dissolving 80 mg in a few drops of dimethylsulphoxide (DMSO) and then making up to one milliliter with distilled water.

5.2.2. Detection of antimicrobial substances on Thin Layer Chromatograms (Bioautoassay)

The active components of each of the fractions obtained from the methanol extract were determined using bioautography, a method that localizes bacterial activity on a chromatogram as described by Rahalison *et al.*, (1991). 20x20 mm pre-coated silica gel 60F 254 normal phase plates were used. Stock solutions of each of the fractions as well as the original extract were prepared by dissolving 100 mg of each in 10 ml of the eluting solvent to give final concentrations of 10 mg/ml. Spots of 50, 100, and 200 µg of each of the solutions were made in series on one end of a TLC plate. The plate was then developed in 100% chloroform in an air-tight developing tank. The solvent front was marked and the positions of the separated compounds were visualized by use of iodine vapours as well as spraying with 1% vanillin in sulphuric acid reagent.

Similar plates were spotted and developed but were not stained and were inoculated as follows for the overlay assay: About 100 millilitres of tryptone soya agar (TSA) was poured into an assay dish and allowed to set in order to give a firm base. An inoculum of the test microorganism was prepared by adjusting an actively growing culture of the test bacteria to match the turbidity of a Mc Farland 0.5 standard. 100 µl of this inoculum was added to 100 millilitres of molten, sterile TSA that was maintained at 50 °C to give a final titre of 10⁵ CFU/ml. The developed plate was placed on the tryptone soya agar base and the medium containing the test organism poured over slowly and allowed to set. The assay tray was covered and incubated at 37 °C for twenty hours. The fractions were tested against *S. aureus* ATCC 29123, *E. coli* ATCC 35218 and *P. aeruginosa* ATCC 27853. Antibacterial zones appeared as clear spots against a background of bacterial colonies and the location and the size of the inhibition zones were compared with the previously developed plate.

5.3. Results

<i>Organisms</i>	CF2	CF3	CF4	<i>Chloramphenicol</i> 10µg/well
<i>S. aureus</i> ATCC 29213	12	17	14	19
<i>S. aureus</i> (isolate)	14	17	14	19
<i>E. coli</i> . ATCC 35218	6	6	6	6
<i>Ps. aeruginosa</i> ATCC 27853	6	6	6	6
<i>Ps. aeruginosa</i> (isolate)	6	17	14	20

Tab 5. Antimicrobial activity of the column fractions of *P. linearifolia* at 4 mg/well against bacterial strains.

A total of seven fractions (100 ml each) were initially collected and after pooling together the fractions that appeared similar on TLC plates, three fractions were obtained, CF2, CF3 and CF4. In the agar diffusion assay, CF2, the ethyl acetate:dichloromethane 50:50 soluble fraction was active against both *S. aureus* ATCC 29213 and the *S. aureus* hospital isolate with zone diameters of 12 mm and 14 mm respectively (Tab.5). This fraction was however not active against the *Pseudomonas aeruginosa* ATCC 27853 and *Ps. aeruginosa* hospital isolate. The ethyl acetate soluble fraction CF3, exhibited activity against *S. aureus* with an equal mean zone diameter of seventeen millimeters for both the *S. aureus* standard and the *S. aureus* hospital isolate. This fraction was active against the *Pseudomonas aeruginosa* hospital isolate but not the *Pseudomonas aeruginosa* standard.

CF3 had larger zones of inhibition against *S. aureus* compared to the other two fractions. CF4, just like CF3, was active against *S. aureus* standard and *S. aureus* hospital isolate and *Pseudomonas aeruginosa* hospital isolate. On silica gel thin layer chromatograms, the methanolic extract of *Periploca linearifolia* was separated into three bioautographic spots with different Rfs which were shown to be active (Fig 5a). *S. aureus* ATCC 29213 was inhibited by the reference sample, and by two fractions CF3 and CF4 at the base where the extracts were spotted and at two other spots (Rfs = 0.56 and 0.83). CF2 had one active spot at Rf = 0.46. The fractions exhibited no activity against *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 35218 at the tested concentrations.

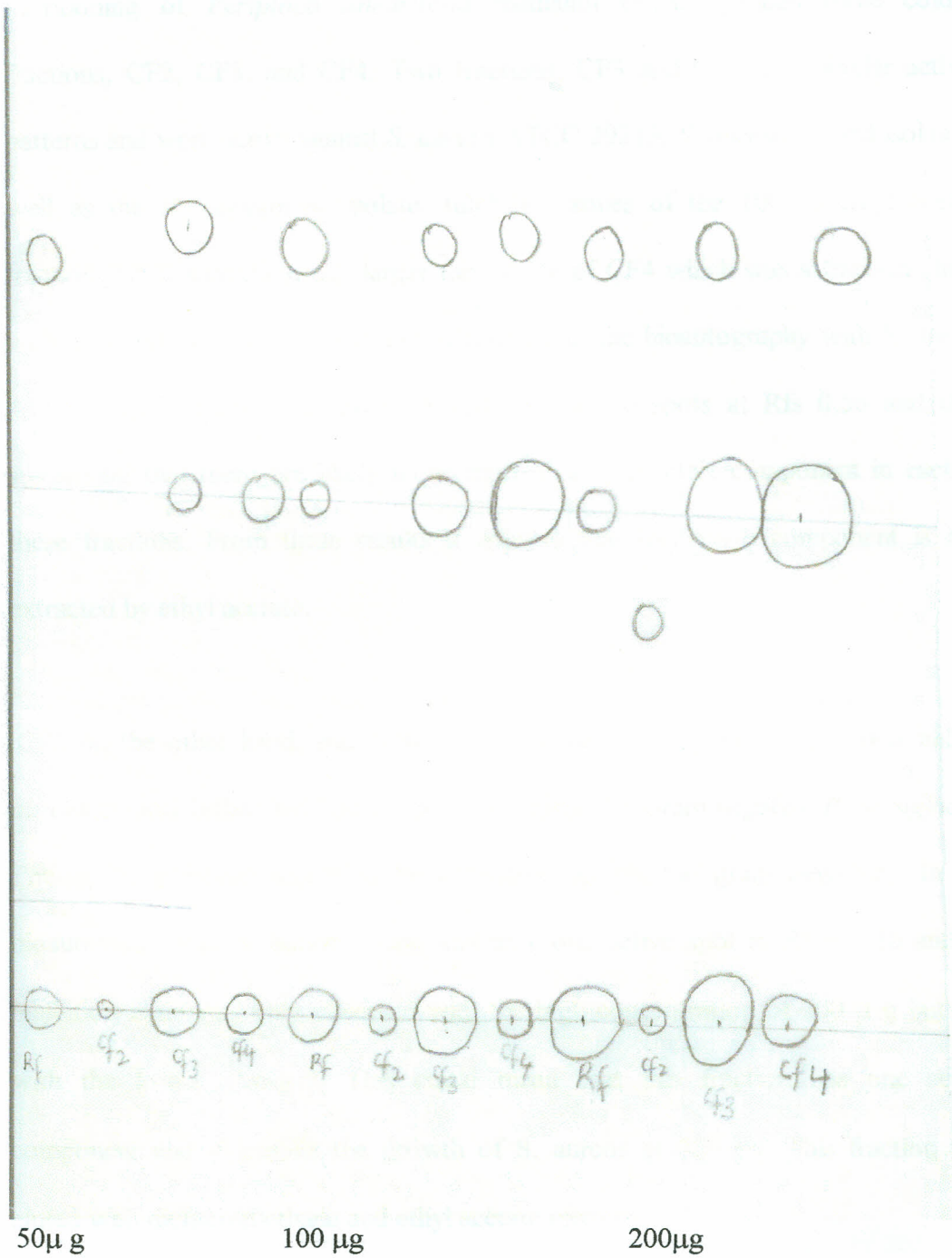


Fig 5a. Inhibition zones of the active components of the fractions of *P. linearifolia* against *S. aureus* ATCC 29213 on thin layer chromatogram

5.4 Discussion

Partitioning of *Periploca linearifolia* methanol extract yielded three column fractions, CF2, CF3, and CF4. Two fractions, CF3 and CF4, had similar activity patterns and were active against *S. aureus* ATCC 29213, *S. aureus* clinical isolate as well as the *P. aeruginosa* isolate. Inhibition zones of the 100 % ethyl acetate fraction (CF3) were however larger than those of CF4 which was a fraction eluted with a mixture of ethyl acetate and methanol. In the bioautography with *S. aureus* ATCC 29213, both CF3 and CF4 had two active spots at Rfs 0.56 and 0.83 suggesting that there are likely to be more than one active component in each of these fractions. From these results it appears that the active component is best extracted by ethyl acetate.

CF2 on the other hand, had activity against the Gram- positive *S. aureus* and *S. aureus* clinical isolate but had no activity against the Gram negative *P. aeruginosa*. The active principle seems to be neffective against the gram negatives. In the bioautoassay with *S. aureus* there was only one active spot at Rf = 0.46 and an inhibition zone was only observed with the high concentration of 200 μ g but not with the lower amounts. This could mean that this fraction has one active component and it inhibits the growth of *S. aureus* at 200 μ g. This fraction was eluted with dichloromethane and ethyl acetate mixture.

GENERAL DISCUSSION

Plants do not move and due to the fact that they grow in diverse and changing physical environments, there is a possibility of attacks by animals and other pathogens and this has necessitated the development of numerous chemical mechanisms for their own protection and defence. The Secondary metabolites from plants are known to be compounds with no apparent function in the primary metabolism of the plant and these substances tend to be of restricted taxonomic distribution. Alkaloids are thought to play a defensive role in plants against herbivores and pathogens (Castells and Penuelas, 1997), glucosinolates have a role in protecting against insect attack (Oleszek, 1995), while tannins act to preserve the wood in living trees from microbial decomposition and insects (Laks, 1991). Plants have also developed defence against other plants and many compounds are implicated in this including phenolics and terpenoids (Langenheim, 1994). In herbal practice the plants are either used alone or in some cases are combined to give a multi herbal recipe.

The indications for the administration of traditional medicines are based on the patient's symptoms and not on the underlying cause of the disease which is usually unknown (Mukherjee, 2002). A good example of this is *Digitalis purpurea* leaves which were formerly used in Europe for the treatment of dropsy, that is, for a set of symptoms and not for a particular disease or for the treatment

of the cardiac disorders which cause oedema. Like wise, *Cinchona* bark, the source of quinine, was used for the treatment of fever but not against Plasmodium, the malarial parasite. The Madagascan periwinkle *Catharanthus roseus* G. Don was used mainly as anti diabetic in traditional herbal remedies and research carried out to isolate the anti diabetic constituents led to the discovery of the two anticancer agents vincalucoblastine and leucocristine (Svoboda, 1975).

This work has shown that plants like *Psidium guajava*, *Acacia mearnsii*, *Thunbergia alata* and *Periploca linearifolia* have useful antimicrobial compounds. Each of the six plants screened in this study had at least some activity on the micro-organisms tested confirming their usefulness in the management of the various ailments. *Psidium guajava* leaves are reportedly used to treat diarrhoea and the results obtained confirm moderate activity of the aqueous extract against the Gram negatives *Salmonella typhi*, *Shigella dysenteriae* as well as *Pseudomonas aeruginosa*.

Persea americana leaves which the herbalists use to dress wounds exhibited very minimal antibacterial activities and slightly inhibited the growth of *S. aureus* and *P. aeruginosa* and this can be associated with the wound healing activities.

Extracts of *Acacia mearnsii* were very active against both the Gram-negatives and Gram-positives and the fungi, *Candida albicans* and this can confirm the

effectiveness of this plant in the treatment of mouthrashes, coughs and in the dressing of wounds as indicated by the herbal practitioners.

Thunbergia alata is used by the herbalists to treat allergic and cracking skin and it has been previously reported as a remedy for mouth, teeth and tongue. This study shows that the aqueous extract had activity against *S. aureus* and *P. aeruginosa* strains while the chloroform extract exhibited moderate activity against *E. coli*, *Salmonella typhi* isolate and *Shigella dysenteriae* isolate which are all known to cause diarrhoea.

In the chromatographic separation of the compounds, it emerged that some compounds did not move with the solvent system (chloroform) and so another solvent system may be needed to separate these components. The herbalists in this region of Central Kenya have indicated the conditions that they treat with the different plants. From literature survey, it has emerged that the use of some of these plants is similar to the uses recorded in other countries, possibly indicating authenticity of their usefulness in treating microbial infections.

CONCLUSIONS

Psidium guajava leaves gave high yields when extracted with water and with methanol. These two extracts were equally active against both the Gram positive and the Gram-negative bacteria. The chloroform extracts gave low yields and had minimal activity against these bacteria.

Acacia mearnsii bark is used to treat mouth rashes, wounds and coughs and the aqueous and methanol extracts were very effective against both Gram-positive and Gram-negative bacteria. The aqueous extract has antifungal activity. The chloroform extract did not have any activity.

Thunbergia alata plant is used by the healers to treat allergies and skin problems and the chloroform and aqueous extracts were found to be active against Gram-negative bacteria. The methanol extract had no activity.

Ocimum suave roots and stem are used to treat diarrhoea and tonsils. The aqueous extraction gave the highest yields and was the only active extract against a small range of bacteria, both Gram positive and Gram negative.

Periploca linearifolia stem and leaves are used to treat diarrhoea and blood disorders. The chloroform extracts did not have activity against the tested

organisms. The aqueous extract was more effective than the methanol extracts.

The aqueous extracts of majority of the plants were more active than the other extracts and the activity of the extracts depends on concentration as well as the exposure time.

Herbal remedies may consist of a single plant species though in some cases it may be made of two or more different plant remedies. Since the herbalists use single plant materials, these plants can therefore be considered to have the desired effects. Several extracts are promising due to their broad spectra of activity. Thus this study ascertains the value of these plants

From these results it can be concluded that the plant materials have the potential to meet the medical requirement of the community and the knowledge used by these herbalists is effective in the management of infections.

RECOMMENDATIONS

1. Indigenous Knowledge of this community on the use of these medicinal plants should be recognized, protected and promoted since they serve as curative measures for the various infections. Research and development of traditional medicines should be undertaken from the community level.
2. The community should be encouraged to conserve and cultivate the important medicinal plants. Plants like *Periploca linearifolia*, *Ocimum suave* and *Thunbergia alata* grow in the wild and since they have shown to be of medicinal value, their cultivation should be encouraged to avoid extinction once the indigenous forests where they grow are cleared.
3. A fairly large population of Kenyans use herbal drugs as an alternative to modern medicines and these preparations need to be evaluated for safety, efficacy and quality.
4. More work on *Periploca linearifolia* is recommended in order to isolate, purify and characterize the active component(s).

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APPENDIX

1. Mc Farland 0.5 Standard.

This is half the density of a Mc Farland number one Standard and is prepared by adding:

0.5 ml. of 0.048 M BaCl_2 (1.1755 w/v $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) to

99.5 ml of 0.36 N H_2SO_4 (1% V/ V).