

**EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME
PLANTS USED BY TRADITIONAL HEALERS FOR TREATMENT
OF MICROBIAL INFECTIONS IN KAKAMEGA DISTRICT:
KENYA**

BY

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Declaration by the candidate

“I hereby declare that this thesis is a result of my own work except where acknowledgement is in text.

It has not been presented or submitted nor is it being currently submitted for a degree in any other university or any other award”.

Signed:

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Dedication

To my wife and children for their co-operation at the time I was away in college for studies.

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Abstract

Resistance to drugs especially antibiotics has become a major challenge facing the medical fraternity today. Most antibiotics that were once effective against pathogenic micro-organisms have now been rendered ineffective owing to resistance developed by these pathogens. This scenario has been complicated by the emergence of HIV/AIDS, which renders the victims immuno-compromised, and open to opportunistic infections. At the same time, most of the African population lives below the poverty line and cannot afford the expensive conventional medicines. These challenges call for renewed strategies on treatment, especially in the development of new antimicrobials. According to the World Health Organization (WHO), medicinal plants can provide the best alternative source to obtain a variety of drugs. Therefore there is need to investigate such plants in order to understand better their properties, safety and efficiency. Renewed interest in plant antimicrobials has also been prompted by the rapid rate of extinction of plant species owing to overharvesting and deforestation. In this study, some 18 plant species used as herbal medicine in Kakamega district were collected and authenticated at the East African Herbarium where voucher specimens are deposited. The plants were soxhlet extracted at the Kenya Medical Research Institute with petroleum ether, dichloromethane and methanol. Total extraction with water was also performed and all extracts screened at 1 g/ml against the organisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Candida albicans*, *Candida parapsilosis*, *Microsporum gypseum* and *Trichophyton mentagrophyte* by disc diffusion method. MIC tests by agar dilution method were performed on the active extracts at concentration range 0.5 - 0.004 g/ml. MBC and MFC tests were performed on the most active water extracts. Extracts of 6 plants with the highest activity were screened for phytochemicals using methods by Harborne. Of the 144 preliminary tests performed on all the water extracts, activity (inhibition zone >9 mm) was recorded in 44 cases as compared to 35 for methanol, 16 for dichloromethane and 4 for petroleum ether. *Entada abyssinica* water extract had the widest range of activity, with inhibitions against 7 of the 8 test organisms. However, the aqueous extract of *Warbugia ugandensis* was most inhibitory with MIC ($\leq 12.5 \times 10^3 \mu\text{g/ml}$) against 7 (85%) of the 8 tested organisms. *Entada abyssinica* water extract had the broadest spectrum of bactericidal activity, with MBC ($\leq 50 \times 10^3 \mu\text{g/ml}$) against 3 (75%) of the 4 tested bacteria. Only *W. ugandensis* water extract was fungicidal (MFC $\leq 50 \times 10^3 \mu\text{g/ml}$), with activities against *C. parapsilosis* and *M. gypseum*. These activities were not significantly different when compared with ketoconazole ($P = 0.217$). Phytochemical screening of *E. abyssinica*, *W. ugandensis*, *N. macrocalyx*, *Albizia coriaria*, *Rhamnus prinoides* and *Albizia amara* extracts revealed differences in the presence and abundance of alkaloids, phenolics, terpenoids, anthraquinones, flavonoids and saponins. Phenolics were most abundant and widespread, while anthraquinones were least abundant. Influence of these phytocompounds on the activities of the named plants varied. The ability by some of the plant extracts to inhibit microorganisms that have proved resistant to conventional medicine confirms the therapeutic potential that such plants hold, and the need to incorporate them into our healthcare systems, as well as conserving them.

Keywords; Phytochemical; Soxhlet; Bactericidal; *Entada abyssinica*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Like other living organisms, man has natural enemies such as microorganisms which pose one of the greatest problems. Among the harmful microorganisms to man are the pathogenic fungi, bacteria, and viruses which are causative agents for various diseases. Man has therefore developed conventional medicine through which these microbes and the diseases they cause are controlled. However it is evident that modern medicine does not provide the cure to some of the known human diseases. Even though a number of new antibiotics have been produced by pharmacological industries in the last three decades resistance to these drugs by microorganisms has increased (Nascimento *et al.*, 2000), owing to the mutations that these micro-organisms have undergone (Hadi and Bremmer, 2001). For example, drugs like quinine which were once used as antimalarials are no longer effective in the control of malaria (Robert and Meunier, 1998). These current negative health trends therefore call for renewed strategies on prevention and treatment of infectious diseases. The situation has been further complicated by the emergence of HIV/AIDS, which has resulted in the resurfacing of previously controlled diseases such as tuberculosis, typhoid, pneumonia and candidiasis, as opportunistic infections.

A disease such as tuberculosis is a chronic infectious disease caused by several species of mycobacteria (Sandra *et. al.*, 2000). But due to multi-drug resistant strains of mycobacteria and to a high prevalence of tuberculosis in patients who have acquired human immunodeficiency syndrome (AIDS), the number of patients infected with the

disease is increasing worldwide. Thus there is an urgent need for new effective antimycobacterial agents to replace those currently in use.

A wide range of plants used as traditional medicine have been found to cure various human diseases, which are associated with microbial infections (Kokwaro, 1993). This application of plants in disease management can be traced back to several generations. The Chinese for example, are known to have used moulds to treat ulcers 2000 years before the birth of Christ (Oldfield, 1984), while the antimalarial drug obtained from the plant Qinghao has records dating back to 168 BC when it was originally used to treat haemorrhoids (Hien and White, 1993).

Most African countries are very poor, with highly underdeveloped healthcare systems. A larger percentage of the African population is living below the poverty line and cannot afford expensive conventional medicines (Sofowora, 1982). Herbal medicine has therefore provided the best alternative method for disease treatment and management. Many people are now turning to herbal medicine as a source of medication (Nyazema, 1987; Pamplona-Roger, 1999). The World Health Organization (WHO) estimates that 80% of the inhabitants in the developing countries, most of which are in Africa rely on traditional medicine and medicinal plants for their primary health care (Sofowora, 1982). The belief among certain African communities that certain diseases are associated either with evil spirits (witchcraft) or curses and can only be managed traditionally, has greatly contributed to the continued use of these medicines.

In the last decade some very useful drugs with undisputed clinical efficacy have been isolated from plants (Dorsche *et al.*, 1991; Balandrin *et al.*, 1995; Mitscher *et al.*, 1975). In fact, 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 (Iwu *et al.*, 1999). It is estimated that about 26% of the active components of currently prescribed medicines in North America and United Kingdom were first identified in higher plants (Balandrin *et al.*, 1995). It is also estimated that today, plant materials are present in, or have provided the models for 50% of Western drugs (Robbers *et al.*, 1996).

The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Iwu *et al.*, 1999). Plant based antimicrobials therefore represent a vast untapped source of medicines. This source is rapidly shrinking due to over-population which has necessitated the clearing of forests for settlement. The demand for herbal medicine has also increased, leading to overharvesting of some medicinal plants. As a result, the rate of extinction of the plant species has been rapid in the past twenty years (Lewis and Elvin-Lewis, 1995). Thus the multitude of potentially useful phytochemical structures in plants is at a risk of being lost irretrievably (Borris, 1996). At the same time, knowledgeable indigenous people are dying with information on curative medicinal plants. It is imperative therefore that this wealth of knowledge is tapped before its holders are extinct.

1.2 Area of study

The area of study was Kakamega district in Western province of Kenya (Figures 1.1 and 1.2). Kakamega is located on latitude 0 degrees 20 minutes north and longitude 34 degrees 46 minutes east (Philip's, 2004), with an average altitude of 2,000 metres above sea level, and a population density of 406 persons per square kilometre (Phillips, 2004). This region boasts of a high rainfall pattern which has sustained some of the indigenous tropical rainforests of Kenya, such as Kakamega and Malava forests.

1.3 Statement of the problem

The current negative health trends call for a renewed interest in infectious diseases and renewed strategies on treatment and prevention. Particularly with the emergence of HIV/AIDS, a lot of opportunistic infections have resurfaced, which include tuberculosis, typhoid, pneumonia, and candidiasis. The Centre for Disease Control and Prevention (CDC) has proposed monitoring and development of new treatments which encompasses the development of new antimicrobials (Fauci, 1998). Plants would form one such source of antimicrobials. However, due to overpopulation, there has been clearance of forests in order to settle the human population. This has resulted in drastic reduction of very useful medicinal plants to the extent that some of these plants are now faced with extinction. This has further been enhanced by lack of data to support the usefulness of these plants for conservation purposes. This study aimed at screening some medicinal plants and their compounds for antimicrobial activities, in order to fill the gap in knowledge of their efficacy.

1.4 Significance of the study

It is evident that some disease causing micro-organisms that were previously controlled by conventional medicine have undergone mutations and developed resistance to these drugs. Therefore there is need for continuous search for new drugs in order to overcome this emerging resistance, which has been complicated by the emergence of HIV/AIDS. Since many people in Kakamega rely on herbal medicine, it was necessary therefore that some of the plants used as herbal medicine be investigated in order to validate their efficacy. Although Kakamega district has a conducive climate that supports a diversity of plants, some of the medicinal plants have been overharvested and are threatened with extinction. Therefore there is need to conserve these useful plants. This can only be done with sufficient information that supports their efficacy (usefulness). On the other hand, the usage of traditional medicine has met criticism because it has been associated with witchcraft and backwardness. Scientific information was necessary to change this school of thought, and encourage people to use herbal medicine which is cheaper and within reach of the majority population who are poor, in Kakamega, and the whole nation in general. Through this study also, information was gathered from the traditional healers about the medicinal values of some herbal medicine used in Kakamega. It was necessary that this information be tapped before its holders die. The 18 plants used in this study have been commonly and successfully applied as herbal medicine by certain communities of Kakamega district. This study was used to verify the efficacy of these plants against microorganisms that cause diseases which the same plants are known to cure. The results obtained will be helpful in supporting and promoting the usage of these plants in disease control, and their conservation by the people of Kenya. Knowledge of the compounds

contained in these plants is expected to be useful in the development of new antimicrobial drugs.

Figure 1:1 Map of Kenya, showing the location of Kakamega district

(Source: Philip's E.A.E.P. atlas, 2004)

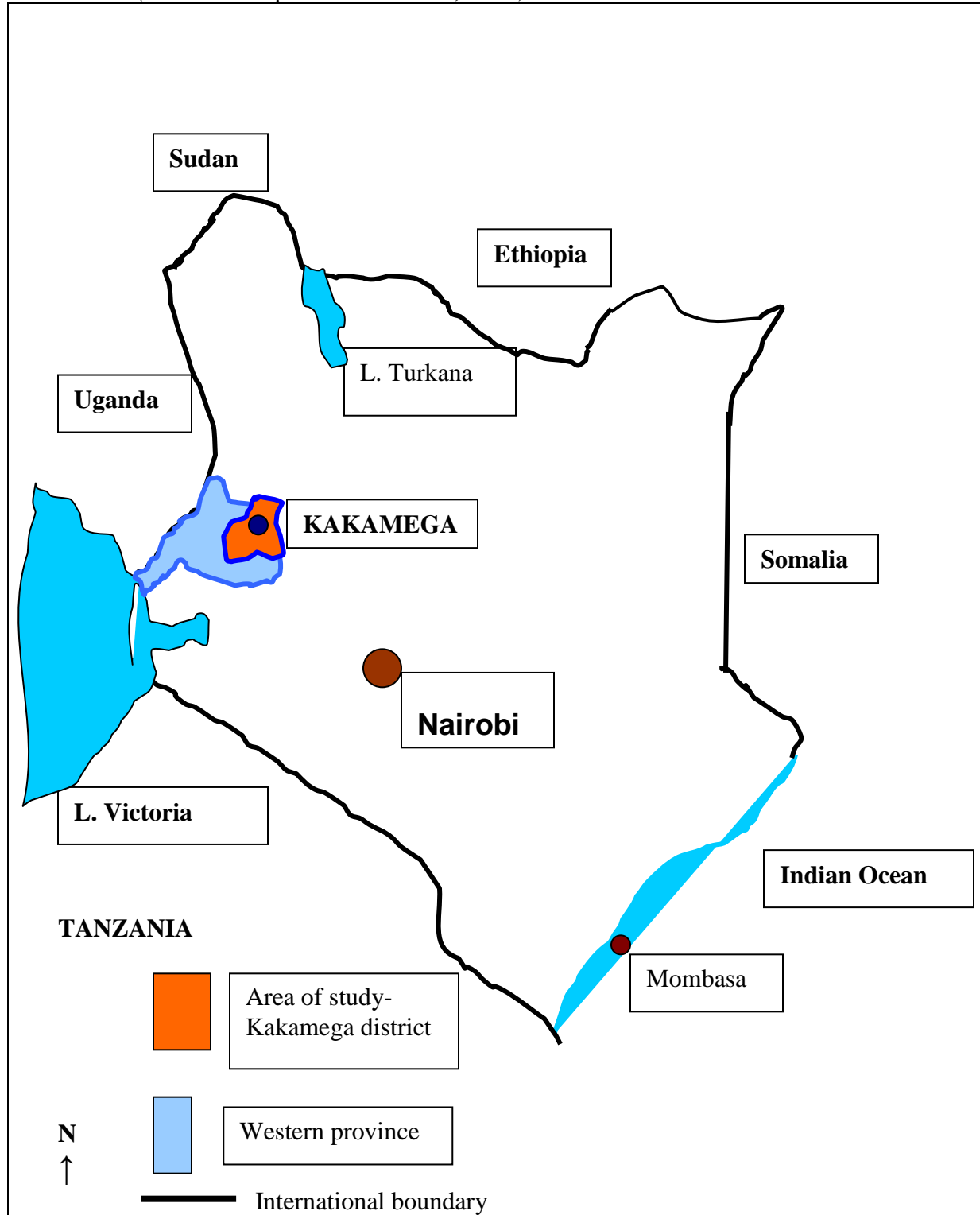
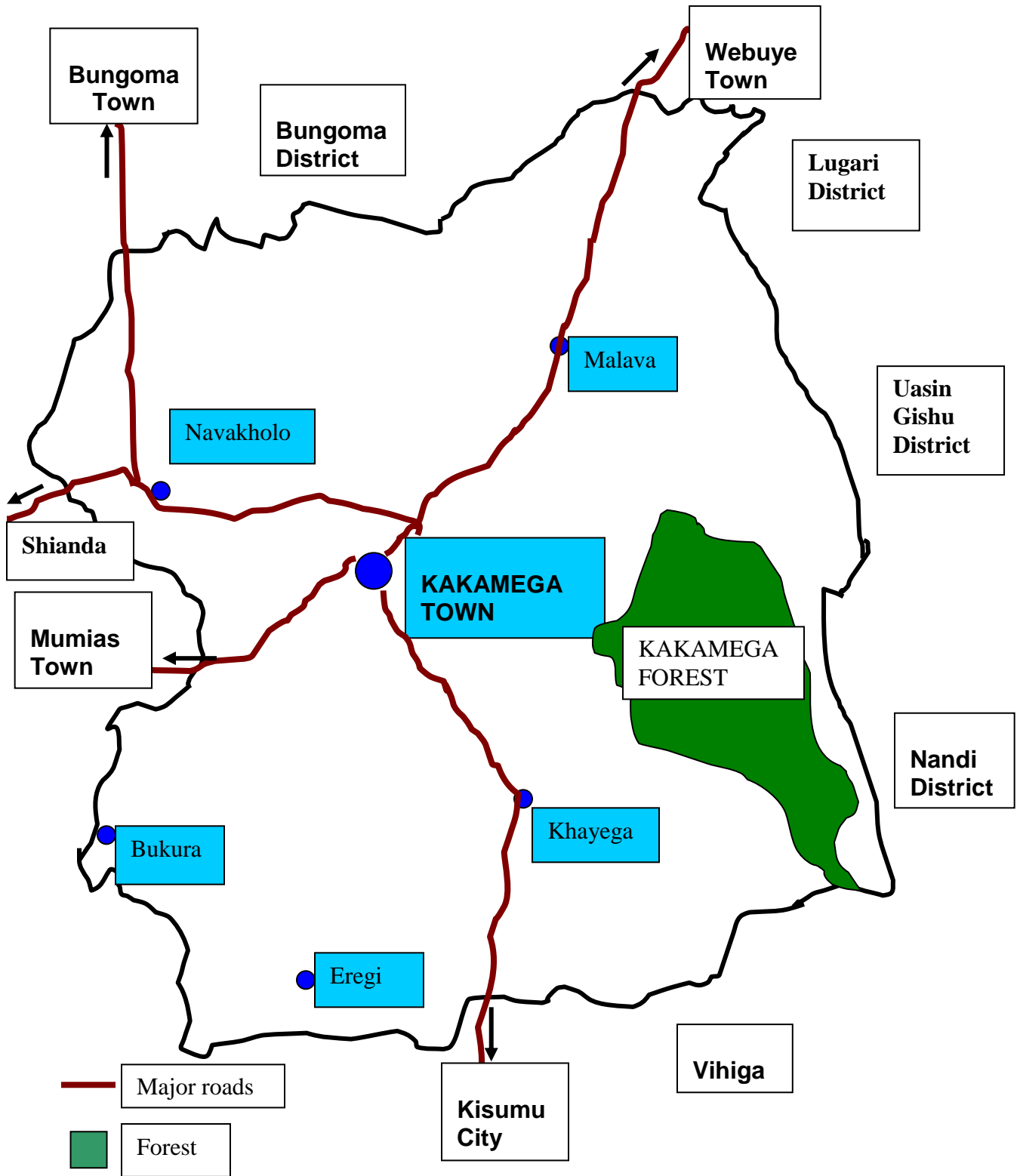


Figure 1:2 Map of Kakamega District

(Source: Philip's E.A.E.P. atlas, 2004)



1.5 Research questions

- (i) Do the selected medicinal plants have potential to inhibit microbial growth?
- (ii) What are the possible inhibitory effects of plant extracts on microbial growth?
- (iii) Do these medicinal plants possess any known groups of active phytochemicals?

1.6 Hypotheses

- (i) The growth of bacteria and fungi is not affected by extracts from the selected plants.
- (ii) The activity of extracts from the selected plants against bacteria and fungi is not different from that of conventional drugs against the same organisms.
- (iii) Crude extracts from the selected plants do not have detectable Minimum Inhibitory Concentrations against bacteria and fungi.
- (iv) The extraction of active principles does not depend on the extraction solvent used.
- (v) the cidal activities of plant extracts are not significantly different from those of conventional drugs.
- (vi) Crude plant extracts do not contain phytochemicals that influence their activity against bacteria and fungi.

1.7 Objectives

1.7.1 General objective

To screen some plant extracts and their active principles for antimicrobial activity against known pathogens.

1.7.2 Specific objectives

- a) To authenticate some medicinal plants used by traditional healers in Kakamega district

using identification keys.

- c) To test crude plant extracts against known microbial pathogens for antimicrobial activity.
- d) To determine the MIC, MBC and MFC values of the active plants.
- d) To determine the groups of compounds presumably present in the crude plant extracts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The history of medicinal plant use

Plants have been an integral part of life in many indigenous communities (Bussmann *et al.*, 2006), and moreso for curative purposes. Before the advent of conventional medicine, man depended on plants for their healing properties (Crevost and Petalot, 1929; Mayorga and Espinoza, 1970; Soleeki, 1975; Watt and Breyer-Brandwijk, 1962). Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections (Iwu *et al.*, 1999). The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years (Cowan, 1999). Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are surveying the earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases.

How man was prompted to start using plants as a source of medicine is still not clear, and therefore suggestions have been put forward to explain this, and his acquisition of knowledge on herbalism. One such suggestion implies that man accidentally discovered traditional medicine through random selection of plants for treatment of ailments (Akpata, 1979; Lambo, 1979). However, traditional medical practitioners argue that knowledge of traditional medicine was passed to them by their ancestors. The use of traditional medicine therefore has records which date back to several generations. The Chinese for example, are known to have used moulds to treat ulcers 2000 years before

Christ (Oldfield, 1984). The Greeks on the other hand are known to have had a rich tradition of folk medicine since 146 AD. (Vokou *et al.*, 1993).

Globally, every region has had at one time in its history, a form of traditional medicine (Chhabra *et al.*, 1984; Bussmann *et al.*, 2006). Therefore there is reference to Chinese, African or Arabic traditional medicine, and thus there are as many traditional medicines as there are communities.

In Northern and Southern American region, among the plants with a long medicinal history are *Bidens pilosa* L., *Aretium lappa* L., *Cassia alata* L. *Mangifera indica* L., *Eupatorium purpureum* L., *Solanum nigrescens* Mart. and Gal., *Annona salzmanii* DC. and *Ptelea trifoliata* L. In Jamaica, the plant *B. pilosa* L. has been used as an antiseptic and antibiotic on wounds, while *A. lappa* L. is used to treat chancre sores (Mechling, 1959; Lucas *et al.*, 1951; Mitscher *et al.*, 1975). *Acacia angustissima* (Mill.) is used in Mexico to treat mouth infections (Dominguez and Algon, 1985) while *Acacia salzmanii* DC. is used by Brazilians in the treatment of dysentery (Paul *et al.*, 1992). In North America, the plant *Rhus glabra* L. is used by the native Indians for the treatment of bacterial diseases, especially those associated with *Pseudomonas aeruginosa* and *Staphylococcus aureus* (McCutcheon *et al.*, 1992).

In the Asian region, *Eclipta alba* L., *B. pilosa* L. and *Matricaria chamomila* L. are among the plants reportedly used as traditional medicine (Burkill, 1966; Chiang-su, 1977; Chopra *et al.*, 1958). *B. pilosa* is used in China, Java and Malaysia for the treatment of eye diseases, wounds and ulcers while *E. alba* L. is used to treat skin disorders.

In Europe the French dandelion *Taraxacum officinale* Weber, and *Areticum lappa* L. are among the plants used to treat boils, abscesses and skin disorders (Hartwell, 1968). In Italy, efforts to evaluate the antibacterial effect of plant extracts as alternative for, or as active agents supporting antibiotics for treating bacterial infections, have shown a significant *in vitro* effect of ethanolic extracts of *Cuminum cyminum* L. and Propolis against *H. pylori* (Nostro *et. al.*, 2005).

2.2 Medicinal plants in Africa

Traditional plant use is of tremendous importance in most rural African communities (Bussmann *et al.*, 2006). Apart from providing building materials, fodder, weapons and other commodities, plants are especially important as traditional medicines (Sidigia *et al.*, 1990). It must be emphasized that many tribes in Africa have sophisticated plant knowledge (Barrow, 1996). Owing to the attempts by the Western world to outlaw the use of traditional medicine such as through enacting of the "Witchcraft Act" of 1925, in Kenya, the practice continued in secret and most knowledge was transferred entirely orally in many communities (Sidigia *et al.*, 1990). Western style healthcare supplied by the government has expanded in the last few decades, but is still often not readily available, and many regions remain completely underserved. Consequently, most communities still use herbal remedies as a readily and cheaply available alternative.

Not much historical information on medicinal plant use in the African region has been documented. However a few medicinal plants of Africa have been investigated for their chemical components and some of the isolated compounds have been shown to possess interesting biological activity (Iwu, 1993).

Among the plants reported for medicinal use in West Africa are *Dissotis triana* L. and *Lonchocarpus serceus* HBK which are used in the treatment of tuberculosis and wounds respectively (Watt and Breyer-Brandwijk, 1962). Other commonly used plants in West Africa include *Garcinia kola* T. Anders., *Tetrapleura tetraptera* Taub., *Acacia nilotica* L. ex. Del., *Cryptolepis sanguinolenta* (Lindl.) Schlechter and *Mitracarpus villosus* L. *G. kola* T. Anders. is used against bronchitis and throat infections (Iwu, 1993) while *Cryptolepis sanguinolenta* (Lindl.) Schlechter and *A. nilotica* L. are used to treat hepatitis and upper respiratory tract infections (Iwu, 1993; Watt and Breyer-Brandwijk, 1962). The plant *Acalypha wilkesiana* Muell-Arg. which grows in Southern Nigeria inhibits *S. aureus* and *Trichophyton rubrum* (Alade and Irobi, 1993), while *Dalbergia melanoxylon* Guill. and Perr., which is used in the treatment of wounds and gonorrhoea is active against *Salmonella typhimurium*, *Yersinia pestis* and *Klebsiella pneumoniae* (Gundidza and Gaza, 1993). In Ivory Coast, crude ethanol extracts of *Dioscorea minutiflora* tubers have been found to contain metabolites with specific effects against the pathogenic fungi *C. albicans* and *Cladosporium cucumerinum*. In the same country, the activity of *Erythrina vogelii* L. against the Gram positive *S. aureus*, *Enterococcus faecalis*, *C. albicans* and *C. cucumerinum* has been reported (Kamanzi *et al.*, 2002).

Among the commonly used medicinal plants in Eastern Africa are *Senna didymobotrya* L., and *Gnidia kraussiana* L. which have been used in Tanzania to treat anaemia and stomachache respectively (Watt and Breyer-Brandwijk, 1962)The roots of *G. kraussiana* L. are also used to treat conjunctivitis and malaria (Kokwaro, 1976). The Tongwe tribe in Tanzania use *Vernonia amygdalina* Del. against parasitic infections (Watt and Breyer-Brandwijk, 1962). In Sudan and Ethiopia, the plant *Acacia nilotica* Willd. ex. Del. has

been used to treat various conditions such as colds, bronchitis, pneumonia haemorrhage and diarrhoea (Abd El Nabi *et al.*, 1992) while in Zambia, *Mangifera indica* L. and *Pterocarpus angolensis* DC. are used in the treatment of diarrhoea and malaria respectively. In Lesotho, the plants *Alepidia longifolia* E. Mey. and *Helichrysum cymosum* (L.) Less. have been used to treat tuberculosis while *Senna petersiana* Bolle has been used to cure respiratory tract infections. In Southern Africa, the bark of *Warbugia salutaris* Engl., known to contain mannitol is used in the treatment of colds and coughs (van Wyk *et al.*, 1999) whereas *Bridelia cathartica* Bertol., with its methyl-salicylate content is commonly used in Mozambique to treat malaria. *Haemanthus coccineus* L., is a herb known among the Zulus as “Uzaneke”. A decoction of the plant is used as an emetic, while the bulbs are used as diuretics in dropsy, as squill substitutes, and for the treatment of asthma (Watt and Breyer-Brandwijk, 1962). The plant leaves are applied as antiseptic agents to sores, ulcers and anthrax pustules. In another survey conducted in Limpopo in which some twenty one plant species were investigated for their antibacterial activity, extracts obtained from *Punica granatum* L. and *Indigofera daleoides* L. were most active (Mathabe *et al.*, 2006). Water extracts of *P. granatum* were equally active as organic extracts against bacteria such as *Staphylococcus aureus*, *Shigella sonnei* and *Shigella flexneri*. The MIC values for the active extracts ranged between 0.039 and 0.6 mg/ml. The results obtained appeared to confirm the antibacterial potential of the plants investigated, and their usefulness in the treatment of diarrhoea.

As a sign of commitment to the promotion of the use of traditional medicine in Africa, the African Union has declared the period 2001-2010, the decade of African Traditional Medicine (AU, 2001), and the World Health Organization, African Region (WHO, 2001)

has provided protocols relating to promotion of traditional medicine research, to be adopted by African member states.

2.3 Medicinal plants in Kenya

In Kenya, several plants are used as herbal medicine (Kokwaro, 1993). Among these, *Aspillia mossambicensis* (Oliv.) Wild found in many parts of Kenya, is used as a remedy for gonorrhoea, cholera and typhoid (Sawer *et al.*, 1995). Leaf extracts of *Vernonia brachycalyx* O. Hoffm. Schreber have shown strong antimalarial activity (Oketch-Rabah *et al.*, 1999), while *Commiphora rostrata* Engl. which grows in the arid areas of Kenya has shown antimicrobial activity against fungi like *Aspergillus flaxus* and *A. niger* (McDowell *et al.*, 1988). *Warbugia ugandensis* Sprague is a plant known for its polygodial content. It is commonly referred to as “Apacha” among the communities of Western Kenya. Its stem bark is pounded, boiled in water and the decoction drunk as remedy for stomachache, coughs, fever and muscle pains (Kioy, 1989). The plants *Acacia nilotica* Willd., *Senna didymobotria* Fres. and *Rhus vulgaris* Meikle have been commonly used in the treatment of sexually transmitted diseases. *Cassia abbreviata* Oliv., *Grewia bicolor* Juss., and *Markhamia obtusifolia* (Bak.) Sprague are among the plants used to treat chest illnesses (Kokwaro, 1993).

Among the Kamba of the Eastern province of Kenya, roots of *Securinega virosa* (Wild.) Baill. are pounded and mixed with water, and the solution drunk to treat headache (Kokwaro, 1993). In Kakamega district, the Maragoli use pounded leaves of *Cassia falcinella* Oliv. as a remedy for rheumatism. *Mondia whitei* (Hook. f.) Skeels, popularly

known as “mgombera” is widely used in Western Kenya (Kokwaro, 1976). Its roots are used both as a remedy for gonorrhoea and as an aphrodisiac. Among the Luo in Kenya, it is estimated that some 330 species in 254 genera are used for the treatment of ailments (Johns *et al.*, 1990). Some of the plants commonly used by this community include *E. Abyssinica* A. Rich., which is used to treat stomach ailments, and *Warbugia ugandensis* Sprague which is used to treat malaria.

The *in vitro* antiplasmodial activity and chloroquine potentiation effects of fifty five organic and aqueous extracts of plants in malaria therapy in Central Kisii district of Kenya have been reported (Chhabra *et al.*, 2003). It was noted that synergistic effects were produced by the combination of some extracts of these plants with chloroquine against the multi-drug resistant *Falciparum* isolate VI/S.

2.4 Drug resistance by human pathogens

Even though a number of new antibiotics have been produced by pharmacological industries in the last three decades, resistance to these drugs by micro-organisms has increased tremendously (Nascimento *et al.*, 2000). Bacteria for instance, have the genetic ability to transmit and acquire resistance to drugs which are utilised as therapeutic agents (Cohen, 1992). This resistance is mediated by (i) the acquired genes, whose presence in a cell is usually synonymous with a resistance phenotype; (ii) mutations in resident genes that alter a variety of phenotypes, including alteration of target sites and enhanced efflux mechanisms; or (iii) changes in outer membrane proteins, which limit the access of drugs to the cell (Rasheed and Tenover, 2003). The resistance factor in these micro-organisms

is a cause for concern, considering the number of patients with suppressed immunity, and the emergence of new multi-resistant bacterial strains, worldwide.

Kariuki *et al.*, (1994) reported ofloxacin-resistance in *E. coli*, and norfloxacin-resistance in *Shigella* spp. In a study carried out on Sudanese patients, *Shigella dysenteriae* type 1 and enteropathogenic *E. coli* from 497 bacterial isolates from patients with diarrhoea and urinary tract infections showed high resistance levels against the common antimicrobials tetracycline, chloramphenicol, ampicillin, amoxyllin, nalidixic acid sulphonamide and neomycin. These pathogens were however sensitive to ciprofloxacin.

In Kenya, Okemo *et al.*, (2004) established that the *S. typhi* causing typhoid is a multi-drug resistant (MDR) strain. Similarly, *Shigella* serotypes isolated from patients with diarrhoea in Kwale district showed that 29% of *S. flexneri* were resistant to tetracycline (Shori, 2000).

Multi-drug resistant enterotoxigenic, enteroinvasive and enteropathogenic *E. coli* were isolated from 346 children with diarrhoeagenic *E. coli*, in Tanzania (Vila, 1999). These strains were resistant to ampicillin, clotrimoxazole and chloramphenicol.

These current trends call for renewed strategies in microbial therapy. This involves the development of new treatments, which encompasses development of new antimicrobial agents.

2.5 Bioactive compounds in medicinal plants

Generally, antibiotics are produced by soil micro-organisms or fungi. However, higher plants have also been a source of these antibiotics (Trease and Evans, 1972).

In the last few years concerns over the use of medicinal plants have increased, and plants with anti-microbial activity have been screened in a number of studies (Al-Shamma and Mitscher, 1979; Watt and Breyer-Brandwijk, 1962). As a result of these recent studies, some very useful drugs with undisputed clinical efficacy have been isolated from higher plants (Balandrin *et al.*, 1995; Mitscher *et al.*, 1987). For example the drug 'Forskolin' isolated from *Coleus forskohlii* Briq. has effectively been used for the treatment of glaucoma (Phillipson *et al.*, 1993). It has also been shown that some diseases which have been difficult to treat with conventional medicine, such as bronchial asthma can be managed by traditional medicine (Sheehan *et al.*, 1992; Dorsch *et al.*, 1991).

Even after searching for synthetic molecules against the human immuno deficiency virus (HIV), only reverse transcriptase inhibitors have been made. However investigation into plant extracts has produced a wide range of compounds that have resulted in non-viral proliferation (Kinghorn and Balandrin, 1993).

Plants produce biologically active secondary metabolites, often with highly complex chemical structures. Such compounds are believed to be mainly concerned with the survival of the plant (Waterman, 1992), by protecting it from disease causing micro-organisms. Compounds such as saponins, flavonoids, phenolics and glycosides have been extracted from plants like *Plumbago zeylanica* L. and *Embilica officinalis* L (Ahmad *et*

al., 2000). These compounds have been found to inhibit among other micro-organisms, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Other antimicrobial compounds isolated from plants include coumarins, steroids, crinitol, papaverine, anonaine and mannitol which have inhibitory effects on *B. subtilis*, *S.aureus*, *C. albicans*, *C. utilis*, measles, and HIV (Taniguchi and Kubo, 1993; Kubo *et al.*, 1992; Turano *et al.*, 1989).

Recent research on plants in Kenya has revealed various compounds of great medicinal value (Wanyonyi *et al.*, 2003, Muregi *et al.*, 2002). For example the antimalarial alkaloids, arborinine, tecleanthine, dictamnine and normelicopicine were isolated from the methanolic extract of *Techlea trichocarpa* Enge. leaves (Muriithi *et al.*, 2002). Other species of *Techlea* are used elsewhere in Africa, and include *T. nobilis*, whose bark is used in South Africa as a remedy for gonorrhoea, while in Ethiopia the leaves of this species are used as analgesics (Watt and Breyer-Brandwijk, 1962). *Solanum aculeastrum* Dunal is used to treat sexually transmitted diseases. The aqueous and methanolic extracts of its berries contain glucoalkaloids and glucosaponins which show moderate antimicrobial activity (Wanyonyi *et al.*, 2003). The chemistry and biological activity of *Vernonia* species such as *V. brachycalyx* O. Hoffm. and *V. amygdalina* Del. have been reported (Oketch-Rabah, 1999). For *V. brachycalyx*, 16,17-dihydrobrachycalyxolid was shown to be the major antiplasmodial principle. Crude extracts of *V. lasiopsus* O. Hoffm. have also exhibited good antiplasmodial activity comparable to those of *Cinchona* (0.5 µg/ml) (Muregi *et al.*, 2002). The activity of extracts of the plants *Entada abyssinica* (stem bark), *Terminalia spinosa* (young branches), *Harrisonia abyssinica* (roots), *Ximenia caffra* (roots), *Azadirachta indica* (leaves and stem bark) and *Spilanthes*

mauritiana (roots and flowers) found in Kenya, was evaluated against strains of *Helicobacter pylori* (Fabry *et. al.*, 1996 a). It was found that these plants contained compounds with antimicrobial activity against *H. pylori*. In addition, the stem bark of the plant *T. spinosa* which has long been used in traditional medicine in Kenya was found to have antibacterial action against *H. pylori* and antifungal activity against *Candida* spp.

Tephrosia is a genus of perennial woody shrubs, whose 30 species are found in Kenya (Beentje, 1994). The roots of *Tephrosia aequilata* Baker are used to treat venereal diseases, and the leaves to relieve stomachache (Kokwaro, 1993; Gillet *et al.*, 1971). Five flavonoids were isolated from the petrol extracts of the roots of *T. aequilata*, and among them pterocarpan was reported for the first time (Tarus *et al.*, 2002). *Embelia schimperi* Vatke is a plant used by the Maasai of Kenya as an antibacterial and antihelminthic (Kokwaro, 1976). These claimed biological activities have been supported by systematic studies done by Boegh *et al.*, (1996). Recent studies have shown that a methanolic extract of the fruit of *E. schimperi* has inhibitory effects on hepatitis C protease (Hussein *et al.*, 2000). Studies on the stem bark of *E. abyssinica*, found in Kenya, revealed activity of this plant against bacteria of the genera *Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Escherichia*, *Klebsiella*, *Salmonella* and *Mycobacterium* (Fabry *et. al.*, 1998). The minimum bactericidal concentrations by 50% (MBC 50%) and MBC 90% were all between 0.5 and > 8 mg/ml. Similarly, antifungal properties of *E. abyssinica* stem bark have been reported. Fungistatic and fungicidal activities were investigated against *Candida* spp. and *Aspergillus* spp, and the minimum inhibitory concentrations (MICs) ranged from 0.006 to > 8 mg ml⁻¹ while the minimum fungicidal concentrations (MFCs) ranged from 0.06 to > 8 mg ml⁻¹ (Fabry *et. al.*, 1996 b). Chloroform extracts of the stem

bark of *E. schimperi* have also revealed the presence of pentacyclic triterpenoids, of which some are antifungal (Machocho, 2002).

Alkaloids have been isolated from the bulbs of *Ammocharis tinneana* (Kotschy & Peyr.) Milne-Redh & Schweick (Machocho, 1999), of which ambelline is reported to have inhibitory activity against lymphocytic leukemia (Pettit *et al.*, 1984). Alkaloids have also been isolated from several species of *Albizia*. They have been isolated from the dichloromethane extract of *Albizia gummifera* L., while those isolated from *A. schimperana* L. have been found to be active against Gram negative and Gram positive bacteria (Rukunga and Waterman, 1996). Extracts prepared from seeds of *A. amara*, have demonstrated an interaction with DNA. On the basis of this interaction, six new spermine macrocyclic alkaloids, budmunchiamines D-I, were isolated (Pezzuto *et al.*, 1992). Previous studies also resulted in the isolation of macrocyclic pithecolobine alkaloids from *A. amara* L. (Woongchon *et al.*, 1991). As determined with *Salmonella typhimurium* strain TM677, the alkaloids were bactericidal, and also inhibited DNA polymerase, RNA polymerase, and HIV- 1 reverse transcriptase.

Antimicrobial activity has been reported among members of the genus *Croton*. For example the hexane-extracted resin of the roots of *C. sonderianus* L. showed antimicrobial activity in standardized bioassays. Fractionation of the resin yielded two acid diterpenes, (-)-hardwickic acid and the new 3,4-secotrachylobanoic acid as major bioactive materials (McChesney *et al.*, 1991).

Bees collect a resinous substance propolis from plant buds. This compound has been found in beehives, and is used in the treatment of wounds (Magro-Filho and de Carvalho, 1994), in the same way honey is used.

The use of plant compounds for pharmaceutical purposes has gradually increased. According to the WHO, medicinal plants would provide the best source to obtain a variety of drugs (Santos *et al.*, 1995). About 80% of individuals in developing countries use traditional medicine which has compounds derived from medicinal plants. Such compounds as tannins, terpenoids, alkaloids, and flavonoids, which are produced by plants as secondary metabolites have been found *in vitro* to have antimicrobial properties (Cowan, 1999). Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patients self-medicating with these preparations. Therefore there is need to investigate such plants to understand better, their properties, safety and efficacy (Ellof, 1998).

Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of extinction of plant species (Lewis and Elvin-Lewis, 1995). Thus the multitude of potentially useful phytochemical structures which could be synthesized chemically is at a risk of being lost irretrievably (Borris, 1996).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and preparation of plant material

3.1.1 Collection of plant material

The choice of plants for screening purposes may involve selecting plants with known medicinal history, or randomly, or by basing on their taxonomic characteristics (Taniguchi and Kubo, 1993). Alternatively, selection may be based on a common denominator such as family, genus or species (Watt and Breyer-Brandwijk, 1962).

The plants for this study were collected from Malava, Navakholo, Khayega, Eregi, Bukura, Kakamega forest and Kakamega municipality regions of Kakamega district, in the month of September, 2003. During this time, there were heavy rains that are characteristic of this region, which promoted more vegetative growth among most plants.

In this study, plants were selected on the basis of their folklore history. The selected plants and their active plant parts were those reported to have been used by the herbalists in the treatment of various diseases affecting people in Kakamega district. Using a questionnaire (Appendix i), some twenty healers who were randomly chosen from the selected areas of study were interviewed about the plants that they commonly used for curative purposes, the specific parts of the plants used, the conditions treated and the methods of preparation and administration of these plants. Plants that featured more frequently among the healers were selected for analysis screening.

3.1.2 Preparation of plant material

The collected material except the leaves were chopped into small pieces and dried under shade at 20-27 °C for a period ranging from 2 to 6 weeks, and later carried to the Kenya Medical Research Institute (KEMRI), Nairobi for the extraction process. Twigs bearing flowers and, or fruits of each plant under study were also collected pressed, dried and forwarded to the East African Herbarium for identification (authentication). Voucher specimens are deposited at the East African Herbarium.

3.2.0 Extraction of plant material

The extraction of plant materials was done as described by Ikan (1969), and Harborne (1984).

3.2.1 Extraction with water

50 g of powdered plant material was soaked in about 300 ml of sterile distilled water, boiled for 15 min and allowed to cool. The mixture was filtered and filtrate collected in a freeze-drying flask. The bottom of the flask was rotated in a trough of dry ice to freeze the extract. The flask was then transferred to the freeze-drying machine for 24 h, after which the dry powder of extract was obtained and stored in plastic bags at -20 °C, until the time for bioassays.

3.2.2 Sequential extraction with organic solvents

Sequential soxhlet extraction was performed on 50 g of each plant material using solvents of increasing polarity. The solvents used were petroleum ether, dichloromethane and methanol. Plant material was soaked in 300 ml petroleum ether and then soxhlet extracted for about 10 h, or until the extract was clear. The residue was soaked in 300 ml

dichloromethane and then soxhlet extracted for an additional 10 h. The final residue was soaked in 300 ml methanol and also soxhlet extracted. The extract obtained in each step was filtered, concentrated using a rotary evaporator at 50 °C, dried by vacuum evaporation and stored in plastic bags at -20 °C, until the time for bioassays.

3.3.0 Bioassay

In bioassays, disc diffusion methods were used qualitatively while agar dilution and micro/macrobrot h methods were used quantitatively.

3.3.1. Susceptibility testing

Susceptibility testing was performed using the disc diffusion method (Bauer *et al.*, 1966; Barry, 1976; Reiner, 1982; Rasoanaivo and Ratsimamanga- Urveg, 1993).

3.3.1.1 Preparation of water extracts solutions

One gram of the freeze dried water extract of each plant under study was weighed on a balance. Using a sterile pipette, 1ml of sterile distilled water (SDW) was added to each extract in a vial, and the mixture vortexed. This formed the stock solution for each plant extract (1 g/ml), and was stored at -20 °C until time for use.

3.3.1.2 Preparation of ketoconazole solution

100 µg of ketoconazole powder was weighed in a vial, and 1 ml of dimethylsulfoxide (DMSO) added and vortexed. This formed the stock solution for ketoconazole (100 µg/ml), and was stored at -20 °C until time for use.

3.3.1.3 Preparation of antimicrobial agent discs

Using a paper punch, paper discs of size 6 mm in diameter were cut off from a sheet of Whatman paper No. 3. The paper discs were placed in a clean, dry capped bottle and autoclaved at 121°C for 15 min. These were stored in a cool, dry cupboard until the time for use. 20 µl of the stock solution of each plant extract was pipetted onto a sterile paper disc of 6 mm diameter in a sterile petri dish, to give a concentration of approximately 20 mg/disc. This was replicated three times. The extract soaked discs were left overnight on a clean bench to dry.

3.3.1.4 Preparation of discs for organic solvents' extracts

Discs for methanolic, dichloromethane and petroleum ether extracts of each plant under test were prepared in a similar way to those of the water extracts as described above, except that the respective extraction solvents were used in place of the sterile distilled water.

3.3.1.5 Preparation of agar medium

3.3.1.5.1 Muller Hinton Agar (MHA)

The recommended medium for disc diffusion testing is Mueller-Hinton agar (MHA) (NCCLS, 2000 a). This is because:

- (i) it demonstrates good batch-to-batch reproducibility for susceptibility testing;
- (ii) it is low in sulfonamide, trimethoprim, and tetracycline inhibitors;
- (iii) it supports growth of most non fastidious bacterial pathogens and
- (iv) years of data and clinical experience regarding its performance have accrued. MHA (Oxoid, UK) was prepared from a commercially available dehydrated base, according to

the manufacturer's instructions. The prepared medium was autoclaved and immediately placed in a 50°C water bath. On cooling, the medium was poured into round plastic flat-bottomed petri dishes on a level surface to give a uniform depth of about 4 mm (25 ml of medium for 100 mm plates) and allowed to cool to room temperature.

3.3.1.5.2 Blood Agar

Since sheep blood should not be added to Mueller-Hinton medium for the testing of non fastidious organisms because the blood can significantly alter the zone diameters for several agents and bacterial species (Brenner and Sherris, 1972), tripticase soy agar (TSA) (Oxoid, UK) was used in the preparation of blood agar as follows:

20 g of TSA was weighed and dissolved in 475 ml of water in a conical flask. The flask was corked and the medium autoclaved for 15 mins at 121°C. The medium was transferred to a water bath where it was cooled to 40 °C. 25ml of defibrinated sheep blood was added to the medium and the flask gently agitated to form a homogeneous blood agar mixture containing 5% blood. This was poured into sterile petri-dishes to a depth of 4mm (25ml per 90mm plate), and allowed to set on a level surface. Freshly prepared plates were used on the same day or were wrapped in plastic paper and stored in a refrigerator at 4 °C, and used within 1 week of storage.

3.3.1.6 Inoculation for susceptibility testing

For reproducibility of disc diffusion susceptibility test results, the inoculum must be standardized (Bauer, *et al.*, 1966; NCCLS, 2000 a).

3.3.1.6.1 Bacterial inoculum for susceptibility testing

Inocula were prepared for the following bacteria, which were obtained from the KEMRI-JICA opportunistic infection laboratory in Nairobi: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 43320 and *Streptococcus pneumoniae* ATCC 49619. These organisms were representatives of Gram positive and Gram negative bacteria.

Four to five isolated colonies of each bacterium, of same morphological type were picked using an inoculating loop from overnight growth on agar medium (Oxoid, UK), and suspended in sterile saline (0.89%). This was agitated on a Vortex mixer and the turbidity of suspension matched with that of the 0.5 McFarland standard, by addition of saline or inoculum. This approach eliminated the time needed for growing the inoculum in broth (NCCLS, 2000 a).

3.3.1.6.2 Fungal inoculum for susceptibility testing

The *in-vitro* tests of anti-fungal agents are similar in design to those of antibacterial agents, that is serial dilutions and agar diffusion tests. Inocula were prepared for the following fungi, which were obtained from the KEMRI-JICA opportunistic infection laboratory in Nairobi: *Candida albicans* ATCC 90028, *Candida parapsilosis*, ATCC 22019, *Microsporium gypseum* and *Trichophyton mentagrophyte*. These were representatives of yeasts and filamentous fungi.

A few colonies were picked by a sterile wire loop from 24h culture plates of *C. albicans* and *C. parapsilosis*, and suspended in sterile saline (0.89%) in separate glass tubes. Each

tube was vortexed and the turbidity of the suspension adjusted to Macfarland 0.5, to give an inoculum concentration of 1×10^6 to 5×10^6 CFU/ml (Shadomy and Pfaller, 1985).

Using a wire loop, mycelia from 1-2 week cultures of *T. mentagrophyte* and *M. gypseum* were picked separately and suspended in sterile saline in glass tubes. The mycelia were macerated using a sterile loop to loosen the spores. The mixture was decanted into a glass tube to give a homogenous spore suspension, whose turbidity was matched with that of McFarland 0.5 standard.

Prepared suspensions for the non fastidious bacteria, and fungi were inoculated on MHA plates while that for *S. pneumoniae* was inoculated on blood agar plates. This was done within 15 min after inoculum suspension had been adjusted. The plates were inoculated by dipping a sterile swab into the inoculum. Excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid. The swab was streaked over the surface of the medium three times, rotating the plate through an angle of 60° after each application to ensure even distribution of the inoculum. A final sweep of the swab was made around the agar rim. The swab was finally passed round the edge of the agar surface. This was left to dry for 2-5 minutes at room temperature with the lid closed before drug/extract-impregnated discs were applied.

3.3.1.7 Application of extract/drug loaded discs on the seeded plates

Within 15 min of inoculation of the plates, the drug/extract-impregnated discs were distributed evenly on the agar surface, with at least 24 mm (centre to centre) between them (Jorgensen and Turnidge, 2003). For every extract tested, three discs impregnated with same extract were applied per plate for each organism. The discs were placed

individually with sterile forceps and then gently pressed down onto the agar surface to provide uniform contact. No more than 12 discs were placed on one 150 mm plate and no more than 6 discs were placed on a 100 mm plate to avoid overlapping zones. As positive controls, standard chloramphenicol discs (30 µg/disc) were similarly applied on plates seeded with *E. coli* ATCC 25922, *S. aureus* ATCC 43320 and *S. pneumoniae* ATCC 49619. For plates seeded with *P. aeruginosa* ATCC 27853, standard gentamicin discs (10 µg/disc) were applied. This is because *P. aeruginosa* is not sensitive to chloramphenicol. On fungal plates, discs impregnated with 20 µl of Ketoconazole (100 µg/ml) and dried, were used as positive controls. As negative controls, sterile discs loaded with 20 µl of SDW (sterile distilled water) and dried, were applied in triplicate for all the organisms.

3.3.1.8 Incubation of the seeded plates

Within 15 min of applying the discs, the plates were inverted and incubated. Plates for *E. coli* ATCC 25922, *S. aureus* ATCC 43320, *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 were incubated at 35 °C for 24 h in ambient air. *S. pneumoniae* ATCC 49619 was incubated at 35 °C for 24 h but in 5% CO₂, while *T. mentagrophyte* and *M. gypseum* were incubated at 25 °C for 5-7 days in ambient air (Peterson and Shanhaltzer, 1992). The diameters of the zones of inhibition, including the diameter of the disc were measured with a ruler to the nearest millimetre. For unsupplemented MHA plates, the ruler was held on the back of the inverted petri dish which was illuminated with reflected light against a black non-reflecting background (Barry *et al.*, 1979). Zones on blood agar were measured at the agar surface. The measurements taken were recorded. Extracts that inhibited micro-organism growth by zone diameters of more than 10mm were subjected

to quantitative tests whereby their minimum inhibitory concentrations (MICs) and minimum cidal concentrations (MBC and MFC) were investigated.

3.3.2.0 Determination of Minimum inhibitory concentrations (MICs) by agar dilution method

MICs and MBCs are taken as measurement parameters to quantify the effects of antimicrobial agents. MIC is the lowest concentration of an antimicrobial agent that inhibits growth as determined visually after a standard incubation period of 18-24h at 35°C (Peterson and Shanholtzer, 1992). The MBC is the lowest concentration of the agent that shows no growth after a subculture of all the dilutions that showed no growth in the MIC test.

The agar dilution method was used to determine the MICs of the extracts whose zone of inhibition was 10 mm diameter and above, after the susceptibility testing.

3.3.2.1 Agar dilution

In the agar dilution method, the liquid containing the antimicrobial agent is further diluted by use of agar as solid media for growth. Agar dilution was preferred to the broth dilution method because of its convenience for testing a number of strains simultaneously, its ability to detect microbial heterogeneity or contamination, and its slightly better reproducibility than the broth dilution method (Ericsson and Sherris, 1971).

3.3.2.2 Preparation of drug dilutions

Dilutions of drugs are prepared from stock solutions at 10 times the concentrations required in the final test. Two-fold (Log_2) dilutions are normally used for determining MICs (Washington and Sutter, 1980).

3.3.2.2.1 Preparation of extracts' stock solutions

A stock solution of the required extract was prepared using the following formula:

$$\text{Weight of extract} = \frac{\text{Volume required (mls)} \times \text{desired concentration of extract solution}}{\text{Potency of extract}}$$

The desired extract concentration for the stock solution was 500mg/ml. Since extract potency was unknown, a value that is lower than that for a known antibiotic (tetracycline-900 $\mu\text{g}/\text{mg}$) was used, based on the assumption that the crude extract is impure, and therefore has a lower concentration of the antimicrobial principles. Thus a value of 1 $\mu\text{g}/\text{mg}$ was assumed as the potency. The desired extract was dissolved in the respective extraction solvent in a glass tube, to give a final concentration of 500 mg/ml.

3.3.2.2.2 Dilution of extracts

The stock solution was serially diluted with the extraction solvent, in sterile test tubes labelled and arranged from the highest to lowest concentration of extract desired. Using a sterile pipette, 2 ml of solvent was added to each of the 9 tubes, except the first tube. 2 ml of the extract (0.5 g/ml) was added to the first and second tubes, and the contents of the second tube agitated on a Vortex mixer. 2 ml of the solution in the second tube was transferred to the third tube, and the process continued through the next-to-the 8th tube from which 2 ml was removed and discarded. Thus the resulting solutions had the

following concentrations: 0.500g/ml, 0.250g/ml, 0.125g/ml, 0.063g/ml, 0.032g/ml, 0.016g/ml, 0.008g/ml and 0.004g/ml. No extract was added to the 9th tube which served as a growth control. A similar approach was used to prepare solutions of chloramphenicol (2560 - 2.5 µg/ml), gentamicin (320-0.31 µg/ml), and ketoconazole (1280 - 0.63 µg/ml). Chloramphenicol and gentamicin stock solutions (2560/320 µg/ml) were diluted in SDW while ketoconazole (1280 µg/ml) was diluted in DMSO.

3.3.2.3 Media preparation

MHA is the recommended medium for the testing of most commonly encountered aerobic and facultatively anaerobic bacteria (NCCLS, 2000 b). The dehydrated agar base was prepared in a conical flask as described by the manufacturer. The agar was molten to a homogeneous liquid by heating the flask. Before sterilization, 18 ml of the agar was distributed into capped glass tubes. Tubes, one for each drug/extract concentration to be tested were sterilized by autoclaving at 121°C for 15 min and were allowed to equilibrate to 50 °C in a preheated water bath. 2 ml of each of the diluted extract, ranging in concentration from 0.500 g/ml to 0.004 g/ml (see 3.3.2.2.2) was added to 18 ml of the sterilized MHA. This gave an extract concentration range of 50 - 0.04 mg/ml. Similarly, control drugs were diluted in agar to give concentration range of 256 - 0.25 µg/ml for chloramphenicol, 32 - 0.032 µg/ml for gentamicin, and 128 - 0.063 µg/ml for ketoconazole. The tubes were capped and gently agitated to mix their contents. The tube contents were poured into sterile petri dishes set on a level surface, and allowed to solidify. MHA plates containing drug/extract-free agar were also prepared as positive controls. For the testing of *S. pneumonia*, blood agar was prepared in tubes by supplementing TSA with 5% defibrinated sheep blood (see 3.3.1.5.2). Thereafter 2 ml of

the extract or control drug was added to the tubes as described above. Plates that were not required for use immediately were sealed in plastic bags and stored at 4 °C, to be used within 5 days of preparation.

3.3.2.4 Inoculum preparation for MIC testing

The recommended final inoculum for agar dilution is 10^4 CFU per spot (0.001 ml) for bacteria (NCCLS, 2000 b) and 10^2 CFU for fungi (Shadomy and Pfaller, 1985).

Bacterial and fungal inocula were prepared as described in section 3.3.1.6.1 and 3.3.1.6.2 respectively.

3.3.2.5 Inoculation for MIC testing

Inoculation of the antimicrobial agent plates was accomplished within 30 min of inoculum preparation. For convenience, use of a replicator is preferred because of a consistent volume, for up to 36 different isolates are simultaneously achieved (Steers *et al.*, 1959). By using this device, 1 to 2 μ l of the suspension (10^7 CFU/ml of bacteria and 10^6 CFU/ml of fungi) was delivered to the agar surface, resulting in 10^4 and 10^3 CFU per spot respectively (Jorgensen and Turnidge, 2003; Shadomy and Pfaller, 1985). An aliquot of the adjusted inoculum for each organism was pipetted into the appropriate well of an inoculum seed plate. The inocula (1-2 μ l) were picked by the inoculating rods of the replicator and gently transferred onto the agar surface to avoid splashing (Washington and Sutter, 1980). Except for *S. pneumoniae* inoculum which was transferred onto blood agar plates, all other inocula were transferred onto MHA agar plates. Plates containing the lowest concentration of the antimicrobics were seeded first to avoid transfer of significant amounts of antimicrobial back to the wells. To check the viability of each

organism, control plates without drug/extract were inoculated last (Jorgensen and Turnidge, 2003). The plates were marked so that the locations of the different organisms being tested on each plate were known.

3.3.2.6 Incubation of the plates and determination of the MIC

The inoculated plates were incubated as described in section 3.3.1.8. Endpoints for each drug/extract were determined by placing the plates on a dark background and examining the plates for the lowest concentration that inhibited visible growth. This was recorded as the MIC.

3.3.3.0 Minimum bactericidal concentration (MBC)

MBC is the lowest concentration of an antibacterial agent that causes at least a 3 log₁₀ reduction in the number of surviving cells (compared with the initial, preincubation concentration) after 18 to 24 h of incubation (Peterson and Shanholtzer, 1992)

Due to the marked variations in the methods used to perform tests that assess the bactericidal activity of antimicrobial agents, two methods for performing the MBC have been proposed. These are the NCCLS (1987) and the Stratton and Cooksey (Stratton and Cooksey, 1991) methods. In this study the NCCLS method was used to determine the MBCs for the most active water extracts which recorded MICs. Water extracts were selected because they had exhibited higher antimicrobial activity. These were extracts of *R. prinoides* (root bark), *A. amara* (stem bark), *W. ugandensis* (stem bark), *A. coriaria* (stem bark), *C. macrostachyus* (stem bark) and *E. abyssinica* (stem bark).

In the MIC determination, the agar dilution was preferred to the broth dilution method because of its convenience for testing a number of strains simultaneously, its ability to detect microbial heterogeneity or contamination, and its slightly better reproducibility than the broth dilution method (Ericsson and Sherris, 1971). However agar dilution method was not suitable for MBC determination and hence the broth macrodilution method was applied in this study.

3.3.3.1 Dilution of antimicrobial agents

Samples (1ml) of MHB containing serial dilutions of an antimicrobial agent (water extract or control drug) were used. The tested plants were *R. prinoides*, *A. amara*, *A. coriaria*, *W. ugandensis*, *C. macrostachyus* and *E. abyssinica*. Desired extract concentration range was 50 - 0.05 mg/ml, while the control drug concentration range was 256 - 0.25 µg/ml for chloramphenicol and 32 - 0.031 µg/ml for gentamicin. Water solutions of concentration 0.500 g/ml, of each of the plant extracts to be tested were prepared as already described above and filter-sterilized. A set of twelve sterile test tubes were labelled and arranged in a rack. To the first tube was added 400 µl of the extract, of concentration 0.500 g/ml followed by 1600 µl of MHB. This resulted in 2 ml of extract of concentration 100 mg/ml, twice the final desired concentration of 50 mg/ml (Sherris and Washington, 1980; Jorgensen and Turnidge, 2003). 1 ml of MHB was delivered to the rest of the test tubes. Using a sterile pipette, 1 ml of tube one contents was transferred to tube two and vortexed. This dilution process was continued up to tube 11, resulting in a concentration range of 100 - 1.0 mg/ml. The last tube received no extract, to serve as a growth control. Similar procedure was used to prepare dilutions of chloramphenicol and gentamicin of range 512 - 0.5 µg/ml and 64 - 0.0632 µg/ml respectively.

3.3.3.2 MBC determination

10 µl samples were picked with a sterile pipette from each tube with a concentration at and above the MIC of the antimicrobial being tested (NCCLS, 2000 b). To avoid the carryover antimicrobial agent interference, the sample was placed onto the agar plate in a single streak down the centre. The broth was allowed time (10 mins) to be absorbed into the agar until the plate surface appeared dry, and the inoculum was then spread over the plate with a sterile wire loop (Shanholtzer *et al.*, 1984). This method has been successfully used for both Gram-positive cocci (Peterson *et al.*, 1987; Shanholtzer *et al.*, 1984) and Gram-negative bacilli. MHA plates were incubated in air at 35 °C for 24 h while blood agar plates were incubated in 5% CO₂ at 35 °C for 24 h. After incubation, any visible colonies were counted on each subculture plate, as well as the initial inoculum plates. The MBC was recorded as the lowest concentration of the antimicrobial in which there was a reduction of the initial inoculum by 99.9% (Peterson and Shanholtzer, 1992; Jorgensen and Turnidge, 2003)

3.3.4.0 Minimum Fungicidal Concentration (MFC)

Although a consensus recommendation of a standardized susceptibility test method is not available from the NCCLS Subcommittee on Antifungal Susceptibility Testing (Shadomy and Pfaller, 1985), a macrobroth dilution method outlined by Shadomy and Pfaller was used in this study.

3.3.4.1 Preparation of the antifungals (extracts and ketoconazole)

The dilution procedure for antifungals should give a dilution series that is ten times the final desired concentration (Shadomy and Pfaller, 1985). For this study, the desired range

was 50 - 0.05 mg/ml for the plant extracts (*R. prinoides*, *A. amara*, *A. coriaria*, *W. ugandensis*, *C. macrostachyus* and *E. abyssinica*) and 128 - 0.125 µg/ml for Ketoconazole. Using the method described above dilutions were performed in MHB to give the concentration range of 500 - 0.5 mg/ml for plant extracts, and 1280 - 0.635 µg/ml for ketoconazole.

3.3.4.2 Determination of the MFC

The MICs were determined as described above in the case of bacteria. The MFC was determined by subculturing 10 µl from each negative tube and from positive growth control tubes onto plates of antimicrobial-free MHA. 10 µl of the initial inoculum for each organism was also streaked onto antimicrobial-free MHA. All plates for *Candida* and the moulds were incubated as described above. The MFC was determined as the lowest concentration of the antifungal from which subcultures were negative (Shadomy and Pfaller, 1985).

3.4.0 Phytochemical Screening of the extracts

3.4.1 Thin Layer Chromatography (TLC)

TLC was carried out on water, methanol, dichloromethane and petroleum ether extracts of *E. abyssinica*, *W. ugandensis*, *N. macrocalyx*, *A. amara*, *A. coriaria* and *R. prinoides* by the methods described by Harborne (1984).

3.4.1.1 Preparation of plates

Plates were prepared with slurry consisting of approximately 1 part silica gel (Makall, China) and 1 part water. After the slurry was mixed and was homogeneous, plates were prepared. The glass plates were carefully cleaned with acetone-soaked cotton wool to

remove grease. The spreader gate was adjusted so that the thickness of the gel was approximately 250 microns. After spreading, the plates were placed on a smooth surface, and allowed to set. They were then activated for 30 to 35 mins in an oven set at 110 °C, to 120 °C, after which they were removed from the oven, placed in a desiccator box and allowed to cool to room temperature.

3.4.1.2 Extract application and development of the plates

3 to 6 µl of diluted sample of the extract was spotted one inch from the bottom and an inch from the left hand margin of a plate. Further to the right and on the same line of origin, the same quantity of extract from stage one was applied, and then further to the right the extract from stage two. When the spots were dry, the plates were developed in selected mobile solvent phases (Table). Sufficient solvent was poured into chromatography jar to a level of ½ inch from the bottom. The spotted plates were mounted vertically in the jars which were paperlined to saturate the atmosphere inside with the solvent phase. The mounting was such that the extract spots were just above the solvent level. The jars were covered with greased lids and allowed to develop. As the solvent rose by capillarity, ascending chromatographic separation was obtained resulting in discrete spots. When the solvent front reached a point one inch below the top of each plate, the plate was removed from the jar and allowed to dry. The separated spots were located by fluorescence under ultraviolet light to determine the presence of flavonoids (Harborne, 1984). Terpenoids were located by spraying plates with vanillin sulphuric acid and heating in a pre-heated oven at 110 °C. To locate alkaloids, plates were sprayed with Dragendorff, while phenolics were located by spraying plates with 1% ferric chloride and 1% potassium ferricyanide FeCl_3 $\text{K}_3\text{Fe}(\text{CN})_6$. Plates sprayed with

methanolic potassium hydroxide were used to locate anthraquinones while flavonoids were located by placing plates in a chromatography jar in which a few drops of liquid ammonia had been added, and covered with a lid. To test for the presence of saponins, a solution of the extract in a little water was shaken vigorously in a test tube and any foaming indicated.

3.5.0 Data analysis

Statistical package SPSS was used for statistical analysis. t-test was used to compare the activities of the different plant extracts, as well as with the standard control drugs against the test organisms.

CHAPTER FOUR

4.0 RESULTS

4.1.1 Authentication of the selected plant material

The authentication of the 18 selected plants was performed by Mr. Tom Kirika, a taxonomist at the East African Herbarium, in Nairobi, Kenya, where voucher specimens of the same plants were deposited.

Table 4.1 Selected and authenticated plants

PLANT SPECIES	FAMILY	FREQUENCY OF PLANT USE AMONG HERBALISTS (out of 20)	MEDICINAL USE (References made courtesy of Kokwaro, 1993)
<i>Rauvolfia caffra</i> Sond.	Apocynaceae	10	Decoction of stem bark drunk as medicine for pneumonia, rheumatism and general body swellings. Leaves are used as beddings for circumcised boys.
<i>Spathodea companulata</i> Beauw. P.	Bignoniaceae	12	Bark of stem chewed and sprayed over swollen cheeks. Bark also boiled in water and used to treat body rashes in newborns

<i>Maytenus heterophylla</i> (Eckl. & Zeyh.) Robson	Calatraceae	8	Stem bark boiled and drunk to cure hernia and syphilis
<i>Warbugia ugandensis</i> Sprague	Carnellaceae	17	Juice from stem bark used to treat stomachache, coughs, fever, diarrhoea, muscle and general body pains
<i>Aspillia mossambicensis</i> (Oliv.) Wild.	Compositae	10	Decoction of roots used to cure cystitis and gonorrhoea
<i>Vernonia glabra</i> (Steetz) Vatke	Compositae	11	Twigs pounded, mixed with water and decoction drunk to cure stubborn coughs
<i>Cyperus articulatus</i> L.	Cyperaceae	8	Roots chewed as treatment for headaches.
<i>Croton macrostachyus</i> Del.	Euphorbiaceae	13	Decoction of leaves used for coughs, and to hasten blood clotting. Bark of stem is used to treat skin rashes while roots are used to treat malaria and venereal

			diseases
<i>Hyptis pectinata</i> Poit.	Labiatae	12	Twigs pounded and mixed with water and drunk to remedy stomachache and congested lungs. Sap applied to skin of children with rashes.
<i>Cassia occidentalis</i> L.	Leguminosae	14	Decoction of roots used to treat severe stomachache and swollen testicles. Leaves are boiled in water and vapour inhaled to treat fever. Also used for snakebites and kidney troubles.
<i>Neobutonia macrocalyx</i> L.	Leguminosae	13	Stem bark pounded, mixed with water and drunk to cure chest and stomach disorders
<i>Trichilea dregeana</i> Sond.	Meliaceae	11	Decoction of stem bark used to remedy pneumonia
<i>Cissampelos pareira</i>	Menispermaceae	9	Pounded twigs mixed

L.			with water and used to treat sore throat, colds, coughs, wounds and snakebites
<i>Albizia amara</i> L.	Mimosaceae	12	Decoction of stem bark used to treat venereal diseases and skin complications
<i>Albizia coriaria</i> Oliv.	Mimosaceae	16	Decoction of stem bark used to remedy meorrhagia, haemorrhage and threatened abortion. Roots are used to treat venereal diseases and sore eyes
<i>Entada abyssinica</i> A. Rich.	Mimosaceae	16	Decoction of stem bark and roots used to treat rheumatic pains. Used as an ingredient in rain making medicine and other ceremonies
<i>Antiaris toxicaria</i> L	Moraceae	9	Stem bark used to treat stomach upsets
<i>Rhamnus prinoides</i> L'Herit	Rhamnaceae	10	Decoction of roots used to treat gonorrhoea.

4.2.1 Susceptibility testing

Results for preliminary screening of the extracts of 18 plants are as shown in tables 4.2 and 4.3. Comparisons by t-test were made between the extract activity and that of the control drugs, and also between that of the different extracts (water, methanol, dichloromethane and petroleum ether) of the same plant.

4.2.1.1 Preliminary screening of extracts of *Rhamnus prinoides* L' Herit

Water extracts of *R. prinoides* were active against the bacteria *S. aureus*, *S. pneumoniae* and *P. aeruginosa* (Table 4.2). Most activity was recorded against *S. aureus* (16mm), while *E. coli* was resistant (6mm) against this extract. This activity varied significantly among the test bacteria ($t = 5.633$, $df = 3$, $P = 0.011$). However, the difference in the activity of the water extract, 10 $\mu\text{g}/\text{disc}$ gentamicin ($t = -1.372$, $df = 3$, $P = 0.264$) and 30 $\mu\text{g}/\text{disc}$ chloramphenicol ($t = -1.745$, $df = 3$, $P = 0.179$), against these bacteria was not significant. On the other hand *R. prinoides* water extracts were active against the fungi *M. gypseum*, *C. albicans* and *C. parapsilosis* (Table 4.3), and the activity varied significantly among these fungi ($t = 5.222$, $df = 3$, $P = 0.014$). Similarly there was no significant difference between the activity of water extracts against fungi and ketoconazole ($t = -5.172$, $df = 3$, $P = 0.014$).

Methanol extracts of *R. prinoides* were active against the two Gram positive bacteria, *S. aureus* and *S. pneumoniae* (Table 4.2), and the activity varied significantly among the bacteria ($t = 4.264$, $df = 3$, $P = 0.024$). The Gram negative bacteria *E. coli* and *P. aeruginosa* were not sensitive to this extract, and there was no significant difference between the activity of methanol extract against bacteria, when compared with 10 $\mu\text{g}/\text{disc}$

TABLE 4.2 – 2 PAGES

TABLE 4.3 – 2PAGES

gentamicin ($t = -1.559$, $df = 3$, $P = 0.217$) and 30 $\mu\text{g}/\text{disc}$ chloramphenical ($t = -2.937$, $df = 3$, $P = 0.061$). Methanol extract was active against only one fungus, *T. metagrophyte*, and there was significant difference between the activity of this extract and ketoconazole, against the fungi ($t = -3.338$, $df = 3$, $P = 0.044$).

Dichloromethane extract of *R. prinoides* was active against only *S. aureus* (Table 4.2). This was a slight activity of 10 mm diameter. When compared with the standard drugs, there was no significant difference in the activity of dichloromethane extract, and that of gentamicin ($t = -2.946$, $df = 3$, $P = 0.06$) and chloramphenical ($t = -2.946$, $df = 3$, $P = 0.06$). Dichloromethane extract was not active against fungi, while ketoconazole was significantly more active than dichloromethane extract against fungi ($t = -3.976$, $df = 3$, $P = 0.028$).

Like for dichloromethane, petroleum ether extract of *R. prinoides* was active against only *S. aureus* (Table 4.2). This was also a slight activity of 10 mm diameter. No activity was shown by the extract against *E. coli*, *P. aeruginosa* and *S. pneumoniae*. But there was however no significant difference in the activity of petroleum ether extract, gentamicin ($t = -2.946$, $df = 3$, $P = 0.06$) and chloramphenical ($t = -2.946$, $df = 3$, $P = 0.06$), against bacteria. All the test fungi were insensitive to petroleum ether extract, and ketoconazole was more active than this extract against the fungi ($t = -3.976$, $df = 3$, $P = 0.028$).

A comparison of the activities of the different extracts of *R. prinoides* revealed that there was no significant difference in the activity of the water extract compared to methanol ($t = 1.252$, $df = 3$, $P = 0.299$), dichloromethane ($t = 2.922$, $df = 3$, $P = 0.061$) and petroleum

ether ($t = 2.922$, $df = 3$, $P = 0.061$) extracts against bacteria (Table 4.2). Similarly there was no significant difference in the activity of methanol extract compared to dichloromethane ($t = 1.414$, $df = 3$, $P = 0.252$) and petroleum ether ($t = 1.414$, $df = 3$, $P = 0.252$) extracts against bacteria. The activity of dichloromethane extract compared to that of petroleum ether against bacteria was also not significantly different ($t = 3.14$, $df = 3$, $P = 0.288$).

Against the fungi, there was no significant difference in the activity of water extract of *R. prinoides* compared to that of methanol ($t = 1.715$, $df = 3$, $P = 0.185$), dichloromethane ($t = 2.556$, $df = 3$, $P = 0.084$) and petroleum ether ($t = 2.556$, $df = 3$, $P = 0.084$) (Table 4.3). Similarly there was no significant difference in the activity of methanol extract compared to dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts, against fungi. The activity of dichloromethane extract compared to petroleum ether extract against bacteria was not significantly different ($t = 1.24$, $df = 3$, $P = 0.097$).

4.2.1.2 Preliminary screening of extracts of *Albizia amara* L

Water extracts of *Albizia amara* stem bark were active against *S. aureus* and *P. aeruginosa*, and these activities were not significantly different among bacteria ($t = 2.996$, $df = 3$, $P = 0.058$) (Table 4.2). There was also no significant difference in the activity of water extract compared to that of the control drugs gentamicin ($t = 1.552$, $df = 3$, $P = 0.218$) and chloramphenicol ($t = -1.19$, $df = 3$, $P = 0.320$) against bacteria. The water extract of *A. amara* had inhibition of 16 and 19 mm diameter against *C. albicans* and *C. parapsilosis* respectively, and the activity varied significantly among the fungi (t

= 3.846, df = 3, P = 0.04). There was a significant difference between the activity of water extract of *A. amara* against fungi and ketoconazole (t = 4.361, df = 3, P = 0.022).

Methanol extract of *Albizia amara* was only active against the bacterium *S. aureus* with inhibition of 19 mm diameter (Table 4.2). This activity was however, not significantly different when compared with gentamicin (t = -2.391, df = 3, P = 0.065) and chloramphenicol (t = -2.609, df = 3, P = 0.080), against the bacteria. Similarly methanol extract showed antifungal activities against *T. metagrophyte* and *C. albicans*, and these activities varied significantly among the fungi (t = 4.020, df = 7, P = 0.028). This activity was however not significantly different from that of ketoconazole (t = -2.668, df = 3, P = 0.076).

All the test bacteria were not sensitive to dichloromethane extracts of *Albizia amara* except *S. aureus* (Table 4.2), and there was no significant difference in the activity of this extract compared to gentamicin (t = -2.974, df = 3, P = 0.059) and chloramphenicol (t = -2.929, df = 3, P = 0.061) against the bacteria. Dichloromethane extract was not active against all test fungi, while ketoconazole was more active than dichloromethane extract against fungi (t = -3.976, df = 3, P = 0.028).

Petroleum ether was active against *S. aureus* only (Table 4.2) and there was no significant difference in the activity of this extract compared to gentamicin (t = -2.946, df = 3, P = 0.059) and chloramphenicol (t = -2.942, df = 3, P = 0.06), against bacteria. This extract was not active against fungi, and ketoconazole was more active than the extract against fungi (t = -3.976, df = 3, P = 0.028).

A comparison of the different extracts of *A. amara* revealed that there was no significant difference in the activity of water extract compared to methanol, ($t = 1.53$, $df = 3$, $P = 0.223$), dichloromethane ($t = 1.658$, $df = 3$, $P = 0.196$) and petroleum ether ($t = 1.692$, $df = 3$, $P = 0.189$) extracts, against bacteria (Table 4.2). There was also no significant difference in the activity of the methanol extract compared to dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against bacteria. The activity of dichloromethane extracts compared to petroleum ether against bacteria was not significantly different either ($t = 1$, $df = 3$, $P = 0.391$).

The difference in the activity of water extracts against the fungi was not significant compared to methanol ($t = 0.607$, $df = 3$, $P = 0.587$), dichloromethane ($t = 1.703$, $df = 3$, $P = 0.187$) and petroleum ether ($t = 1.703$, $df = 3$, $P = 0.187$) (Table 4.3). Similarly there was no significant difference in the activity of methanol extract compared to dichloromethane ($t = 1.481$, $df = 3$, $P = 0.346$) and petroleum ether ($t = 1.481$, $df = 3$, $P = 0.346$) extracts against fungi.

4.2.1.3 Preliminary screening of extracts of *Neobutonia macrocalyx* L

The water extract of *N. macrocalyx* showed activity against *S. aureus*, while none was shown against the other test bacteria (Table 4.2). The activity against bacteria was however not significant compared to that of gentamicin ($t = -1.805$, $df = 3$, $P = 0.169$) and chloramphenicol ($t = -2.791$, $df = 3$, $P = 0.068$). Water extract was not active against any fungi, and ketoconazole was more active than this extract against fungi ($t = -3.976$, $df = 3$, $P = 0.028$).

Methanol extract was active against the two Gram positive bacteria *S. aureus* and *S. pneumoniae* and inactive against the Gram negative *E. coli* and *P. aeruginosa* (Table 4.2), but the activity was not significantly varied among these bacteria ($t = 2.835$, $df = 3$, $P = 0.066$). There was no significant difference between the activity of methanol extract compared with gentamicin ($t = 1.130$, $df = 3$, $P = 0.341$) and chloramphenical ($t = 2.255$, $df = 3$, $P = 0.109$). Apart from the water extracts of *C. pareira*, and *C. macrostachyus*, methanol extract of *N. macrocalyx* was the only other extract with activity against all the test fungi, *T. metagrophyte*, *M. gypseum*, *C. albicans* and *C. parapsilosis*. These activities varied significantly among the fungi ($t = 9.00$, $df = 7$, $P = 0.0001$). However, there was no significant difference between the activity of the methanol extract and ketoconazole ($t = 2.435$, $df = 3$, $P = 0.093$).

Dichloromethane extract was active against the Gram positive *S. aureus* and *S. pneumoniae*, and inactive against the Gram negative *E. coli* and *P. aeruginosa* (Table 4.2). But there was no significant difference in activity of this extract among bacteria ($t = 3.082$, $df = 3$, $P = 0.054$). There was similarly no significant difference in the activity of dichloromethane extract compared with gentamicin ($t = 1.895$, $df = 3$, $P = 0.154$) and chloramphenical ($t = 2.640$, $df = 3$, $P = 0.078$). Dichloromethane extracts was active against *T. metagrophyte*, and *C. parapsilosis*, and the activity varied significantly among fungi ($t = 7.66$, $df = 3$, $P = 0.005$). Ketoconazole was more active than the dichloromethane extract against fungi ($t = 3.976$, $df = 3$, $P = 0.028$).

Petroleum ether did not show activity against any bacteria (Table 4.2). This extract was also not active against fungi and ketoconazole was more active than this extract against fungi ($t = -3.976$, $df = 3$, $P = 0.028$).

The activity of water extracts against bacteria did not vary significantly in comparison to that of methanol ($t = -1.06$, $df = 3$, $P = 0.391$), dichloromethane ($t = 0.550$, $df = 3$, $P = 0.621$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts (Table 4.2). Similarly there was no significant difference in the activity of methanol extracts compared to dichloromethane ($t = 1.732$, $df = 3$, $P = 0.182$) and petroleum ether ($t = 1.501$, $df = 3$, $P = 0.230$) extracts against the test bacteria. This was the same case for dichloromethane and petroleum ether extracts against bacteria ($t = 1.278$, $df = 3$, $P = 0.291$).

Against the fungi, the activity of water extract of *N. macrocalyx* did not differ significantly with that of methanol ($t = -8.485$, $df = 3$, $P = 0.187$) and petroleum ether ($t = -1.321$, $df = 3$, $P = 0.841$) However, methanol extract was more active than dichloromethane ($t = 2.967$, $df = 3$, $P = 0.003$), while there was no significant difference in the activity of methanol and petroleum ether extracts against fungi ($t = 8.485$, $df = 3$, $P = 0.278$). The activity of dichloromethane extract compared to petroleum ether extract against fungi was not significantly different ($t = 1.321$, $df = 3$, $P = 0.278$).

4.2.1.4 Preliminary screening of extracts of *Cyperus articulatus* L

Some little activity was shown by the water extract of *C. articulatus* against the Gram positive bacteria, *S. aureus* (10 mm) and *S. pneumoniae* (10 mm), while no activity was shown against the Gram negative bacteria (Table 4.2).

The activities were significantly different among the test bacteria ($t = 7.159$, $df = 3$, $P = 0.005$). However the activities of the water extract compared with gentamicin ($t = -2.387$, $df = 3$, $P = 0.097$) and chloramphenical ($t = -2.986$, $df = 3$, $P = 0.058$), against the test bacteria did not vary significantly. Water extract did not show any antifungal properties, and therefore ketoconazole was significantly more active against the fungi than this extract ($t = -3.976$, $df = 3$, $P = 0.028$).

Unlike water extract, methanol extract of *C. articulatus* was not active against any of the test bacteria (Table 4.2), nor against any of the test fungi.

Dichloromethane extract was only active against the Gram positive bacteria, *S. aureus* and *S. pneumoniae* (Table 4.2), and the activity significantly varied among these bacteria ($t = 9.667$, $df = 3$, $P = 0.002$). The difference in activity of this extract compared with gentamicin ($t = -2.961$, $df = 3$, $P = 0.094$) and chloramphenical ($t = 1.667$, $df = 3$, $P = 0.06$) was however not significant, while no activity was shown against the Gram negative *E. coli* and *P. aeruginosa*. Among the fungi, only *T. mentagrophyte* was sensitive to dichloromethane extract, and the activity significantly varied among these fungi ($t = 5.222$, $df = 3$, $P = 0.014$). Ketoconazole was significantly more active than dichloromethane extract against fungi ($t = -4.048$, $df = 3$, $P = 0.027$).

The petroleum ether extract of *C. articulatus* was also active against the Gram positive bacteria, *S. aureus* and *S. pneumoniae* (Table 4.2), and the inhibitions varied significantly among the test bacteria ($t = 9.667$, $df = 3$, $P = 0.002$). There was however no significant difference in the inhibition activity of petroleum ether extract compared with gentamicin

($t = -2.574$, $df = 3$, $P = 0.082$) and chloramphenical ($t = -2.970$, $df = 3$, $P = 0.059$), against bacteria.

This extract also inhibited *T. mentagrophyte*, *C. ablicans* and *C. parapsilosis*, and the activities were significantly different among fungi ($t = 3.538$, $df = 3$, $P = 0.038$). However, ketoconazole was more active than this extract against fungi ($t = -4.014$, $df = 3$, $P = 0.028$).

A comparison in the activities of the four extracts of *C. articulatus* shows no significant variation in the activities of water extract of this plant compared to methanol ($t = 1.698$, $df = 3$, $P = 0.188$), dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1.732$, $df = 3$, $P = 0.182$) extracts against bacteria (Table 4.2). Similarly there was no significant difference in the activity of methanol extracts compared to dichloromethane ($t = -1.667$, $df = 3$, $P = 0.194$) and petroleum ether ($t = -1.667$, $df = 3$, $P = 0.194$) extracts against bacteria. The activity of dichloromethane extract compared to petroleum ether against bacteria was not significantly different ($t = 0.0001$, $df = 3$, $P = 1$).

There was no significant difference in the activity of water extract of *C. articulatus* compared to dichloromethane ($t = -1.321$, $df = 3$, $P = 0.278$) and petroleum ether ($t = -1.608$, $df = 3$, $P = 0.206$), against the test fungi. There was also no significant difference in the activity of methanol extract compared to dichloromethane ($t = -1.321$, $df = 3$, $P = 0.278$) and petroleum ether ($t = -1.608$, $df = 3$, $P = 0.206$) extracts against the test fungi. The activity of dichloromethane extracts compared to petroleum ether against fungi was not significantly different ($t = -1.667$, $df = 3$, $P = 0.194$).

4.2.1.5 Preliminary screening of extracts of *Warbugia ugandensis* Sprague

Water extract of *W. ugandensis* was very active against the Gram positive bacteria, *S. aureus* (24 mm diameter), and also against *S. pneumoniae* (16 mm) (Table 4.2). No activity was shown against the Gram negative *E. coli* and *P. aeruginosa*. These activities were not significantly different among the test bacteria ($t = 2.982$, $df = 3$, $P = 0.058$). Similarly, the activity of the water extract against the test bacteria was not significantly different from that shown by gentamicin ($t = -1.040$, $df = 3$, $P = 0.375$) and chloramphenicol ($t = -2.345$, $df = 3$, $P = 0.101$). Water extract was also very active against *C. parapsilosis* (20 mm), but slightly active against *M. gypseum* (10 mm) and *C. albicans* (13 mm) (Table 4.3). The activity varied significantly among these fungi ($t = 5.8$, $df = 3$, $P = 0.010$). There was no significant difference between the activity of the water extract and ketoconazole against the fungi ($t = -3.976$, $df = 3$, $P = 0.035$).

Methanol extract of *W. ugandensis* was active against two bacteria namely *S. aureus* and *S. pneumoniae* (Table 4.2), and the activity varied significantly among the bacteria ($t = 5.657$, $df = 3$, $P = 0.011$). There was no significant difference between the activity of the methanol extract with that of gentamicin ($t = -2.003$, $df = 3$, $P = 0.139$) and chloramphenicol ($t = -2.925$, $df = 3$, $P = 0.061$) against bacteria. No activity was recorded against the Gram negative bacteria. Methanol extract was also not active against any of the fungi, and ketoconazole was more active than this extract against fungi ($t = -3.976$, $df = 3$, $P = 0.028$).

Dichloromethane extract of *W. ugandensis* recorded 25 % inhibition, with activity against only *S. aureus* and *S. pneumoniae* among all the test organisms (Table 4.2). This activity

was significantly different among the test bacteria ($t = 4.7$, $df = 3$, $P = 0.018$), but not significantly different from that shown by gentamicin ($t = -2.105$, $df = 3$, $P = 0.126$) and chloramphenicol ($t = -2.971$, $df = 3$, $P = 0.059$).

Similarly, petroleum ether extract of *W. ugandensis* was active against only the Gram positive *S. aureus* and *S. pneumoniae* (Table 4.2), and these activities varied significantly among the test bacteria ($t = 12.124$, $df = 3$, $P = 0.001$). On comparing with the standard drugs, there was no significant difference in the activity of petroleum ether extract with that of gentamicin ($t = -2.587$, $df = 3$, $P = 0.081$) and chloramphenicol ($t = -2.959$, $df = 3$, $P = 0.06$) against bacteria.

Water extract of *W. ugandensis* did not show a significant variation in the activity against bacteria when compared with methanol ($t = 1.321$, $df = 3$, $P = 0.278$), dichloromethane ($t = 1.633$, $df = 3$, $P = 0.201$) and petroleum ether ($t = 1.576$, $df = 3$, $P = 0.215$), (Table 4.2). There was also no significant difference in the activity of methanol extract compared to dichloromethane ($t = -5.777$, $df = 3$, $P = 0.604$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against bacteria. The activity of dichloromethane extract compared to that of petroleum ether extract against bacteria was not significantly different ($t = 1.414$, $df = 3$, $P = 0.252$).

There was no significant difference in the activity of water extract of *W. ugandensis* compared to methanol ($t = 1$, $df = 3$, $P = 0.391$), dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against fungi.

4.2.1.6 Preliminary screening of extracts of *Maytenus heterophylla* (Eckl. & Zeyh.)

Robson

Water extract of *M. heterophylla* stem bark showed very low activity of 12.5 %, with activity against only *S. pneumoniae* (Table 4.2). This activity against bacteria was however not significantly different when compared to that of gentamicin ($t = -1.805$, $df = 3$, $P = 0.169$) and chloramphenical ($t = -2.791$, $df = 3$, $P = 0.68$).

Methanol extract of *M. heterophylla* did not exhibit any activity against the bacteria nor fungi (Tables 4.2 and 4.3).

Among the 8 test organisms, dichloromethane extract of *M. heterophylla* was active against only *S. aureus* (Table 4.2) with a low inhibition of 11 mm diameter. The activity of this extract against bacteria varied significantly ($t = 5.8$, $df = 3$, $P = 0.01$), but did not vary when compared with that of the standard drug gentamicin ($t = -2.919$, $df = 3$, $P = 0.061$) and chloramphenical ($t = -2.932$, $df = 3$, $P = 0.063$).

Petroleum ether extract of *M. heterophylla* also showed very little activity against only *S. aureus* (Table 4.2), and its activity varied significantly among the test bacteria ($t = 13$, $df = 3$, $P = 0.001$), but did not vary in comparison to gentamicin ($t = -2.973$, $df = 3$, $P = 0.059$) and chloramphenical ($t = -2.938$, $df = 3$, $P = 0.061$).

There was no significant difference in the activity of water extract compared to methanol ($t = 1$, $df = 3$, $P = 0.391$), dichloromethane ($t = 0.279$, $df = 3$, $P = 0.789$) and petroleum ether ($t = 0.679$, $df = 3$, $P = 0.547$) extracts against bacteria (Table 4.2). Similarly a

comparison of the activity of methanol extract with that of dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) against bacteria, showed no significant difference. The activity of dichloromethane extract compared to petroleum ether extract against bacteria was also not significantly different ($t = 1$, $df = 3$, $P = 0.391$).

The activity of water extract compared to methanol, dichloromethane and petroleum ether, methanol extract compared to dichloromethane and petroleum ether and the activity of dichloromethane extract compared to petroleum ether against fungi were all not significant.

4.2.1.7 Preliminary screening of extracts of *Cissampelos pareira* L.

Only water and petroleum extracts of *C. pareira* exhibited some activity, while methanol and dichloromethane extracts were inactive against the test organisms. Water extract was active against the Gram positive bacteria, *S. aureus* and *S. pneumoniae* (Table 4.2) and this activity varied significantly among the test bacteria ($t = 9.667$, $df = 3$, $P = 0.002$).

There was however no significant difference in the activity of water extract compared with gentamicin ($t = -2.421$, $df = 3$, $P = 0.094$) and chloramphenicol ($t = -2.961$, $df = 3$, $P = 0.061$), against bacteria. Water extract of *C. pareira* exhibited antifungal properties against all the tested fungi, *T. mentagrophyte* (9 mm), *M. gypseum* (18 mm), *C. albicans* (13 mm) and *C. parapsilosis* (20 mm). The activities varied significantly among these fungi ($t = 9.879$, $df = 3$, $P = 0.002$). However the activity of the water extract and ketoconazole against these fungi did not vary significantly ($t = -2.686$, $df = 3$, $P = 0.075$).

Petroleum ether extract of *C. pareira* was active against only *S. aureus*, and this activity varied significantly among the bacteria ($t = 13$, $df = 3$, $P = 0.001$). There was however no significant difference in the activity of this extract compared with gentamicin ($t = -2.973$, $df = 3$, $P = 0.059$) and chloramphenicol ($t = -2.938$, $df = 3$, $P = 0.061$) against bacteria. This extract was not active against fungi (Table 4.3), and ketoconazole was more active than the extract against the fungi ($t = -3.976$, $df = 3$, $P = 0.028$).

There was no significant difference in the activity of water extract of *C. pareira* compared to that of methanol ($t = 1.667$, $df = 3$, $P = 0.194$), dichloromethane ($t = 1.667$, $df = 3$, $P = 0.194$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts, against bacteria (Table 4.2). There was similarly no significant difference in the activity of methanol extract compared to petroleum ether against bacteria ($t = 1$, $df = 3$, $P = 0.391$), while that of dichloromethane extract compared to petroleum ether against bacteria was not significantly different either ($t = 1$, $df = 3$, $P = 0.391$).

However, there was a significant difference in the activity of water extract compared to methanol ($t = 5.861$, $df = 3$, $P = 0.01$), dichloromethane ($t = 5.861$, $df = 3$, $P = 0.01$) and petroleum ether ($t = 5.861$, $df = 3$, $P = 0.01$) extracts against fungi (Table 4.3).

4.2.1.8 Preliminary screening of extracts of *Aspilia mossambicensis* (Oliv.) Wild.

There was no activity shown by the water extract of the root bark of *A. mossambicensis* against any of the bacteria or fungi (Tables 4.2 and 4.3).

Methanol extract was active against only *S. aureus* (15 mm) among the bacteria (Table 4.2), and the activity varied significantly ($t = 3.667$, $df = 3$, $P = 0.035$). This activity was lower than that of the control drug chloramphenicol (23 mm). However the difference in the activity of methanol extract against bacteria compared with gentamicin ($t = -2.717$, $df = 3$, $P = 0.073$) and chloramphenicol ($t = -2.828$, $df = 3$, $P = 0.066$), was not significant. Methanol extract had slight activity against only *C. parapsilosis* (13 mm) among the fungi. This was much lower than the activity of ketoconazole against *C. parapsilosis* (40 mm) and so ketoconazole was comparatively more active than this extract against the fungi ($t = -3.976$, $df = 3$, $P = 0.028$).

Dichloromethane extract had no activity against any of the test bacteria or fungi (Table 4.3).

Similar to the methanol extract, petroleum ether extract was slightly active against only *S. aureus*, and its activities varied significantly among the bacteria ($t = 25$, $df = 3$, $P = 0.001$) (Table 4.2). These activities were however not significantly different from those of gentamicin ($t = -2.974$, $df = 3$, $P = 0.059$) and chloramphenicol ($t = -2.929$, $df = 3$, $P = 0.061$) against bacteria. This extract was not active against fungi.

There was no significant difference in the activity of the water extract compared to that of methanol ($t = -1$, $df = 3$, $P = 0.391$) and petroleum ether extracts ($t = 1$, $df = 3$, $P = 0.391$), against bacteria (Table 4.2). There was also no significant difference in the activity of the methanol extract compared to the petroleum ether extract against bacteria ($t = 1$, $df = 3$, P

= 0.391), as well as that of dichloromethane extract compared to petroleum ether ($t = 1$, $df = 3$, $P = 0.391$).

4.2.1.9 Preliminary screening of extracts of *Vernonia glabra* (Steetz) Vatke

Water extract of *V. glabra* showed activity against one organism *S. pneumoniae*, with a slight inhibition of 8 mm disc diameter, which varied significantly among bacteria ($t = 13$, $df = 3$, $P = 0.001$) (Table 4.2). This was much lower than that of chloramphenicol (32 mm) against *S. pneumoniae*. The activity of water extract, compared with that of gentamicin ($t = -2.596$, $df = 3$, $P = 0.081$) and chloramphenicol ($t = -2.922$, $df = 3$, $P = 0.061$), against bacteria was not significantly different.

Methanol extract of *V. glabra* was active against the two Gram positive bacteria, *S. aureus* and *S. pneumoniae*, but showed no effect on the Gram negative bacteria (Table 4.2), and the activity varied significantly among these bacteria ($t = 4.483$, $df = 3$, $P = 0.021$). However, there was no significant difference between the activity of this extract compared with gentamicin ($t = -1.658$, $df = 3$, $P = 0.196$) and chloramphenicol ($t = -2.967$, $df = 3$, $P = 0.059$). *T. metagrophyte* and *C. parapsilosis* were the only fungi sensitive to the methanol extract, and the activity of this extract varied significantly among the fungi ($t = 12.124$, $df = 7$, $P = 0.001$). Similarly there was significant difference between the activity of methanol extracts and ketoconazole ($t = -3.662$, $df = 3$, $P = 0.035$).

The two Gram positive bacteria *S. aureus* and *S. pneumoniae* were also affected by the dichloromethane extract of *V. glabra* (Table 4.2). The activity by this extract against the

bacteria varied significantly ($t = 7.833$, $df = 3$, $P = 0.004$). This activity however was not significantly different when compared to that of gentamicin ($t = -2.555$, $df = 3$, $P = 0.084$) and chloramphenical ($t = -2.976$, $df = 3$, $P = 0.059$). Dichloromethane extract was not active against any of the fungi.

Petroleum ether extract of *V. glabra* was slightly active against only *S. pneumoniae* while no activity was recorded by this extract against the other test bacteria. There was a significant variation in the activity of this extract among the test bacteria ($t = 7$, $df = 3$, $P = 0.006$) (Table 4.2). There was no significant difference in the activity of the petroleum ether extract compared with that of gentamicin ($t = -2.287$, $df = 3$, $P = 0.106$) and chloramphenical ($t = -2.905$, $df = 3$, $P = 0.062$), against bacteria. Petroleum ether extract was not active against the fungi.

There was no significant difference in the activity of water extract of *V. glabra* compared to methanol ($t = 1.722$, $df = 3$, $P = 0.184$), dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against bacteria (Table 4.2). There was similarly no significant difference in the activity of methanol extract compared to dichloromethane ($t = 1.567$, $df = 3$, $P = 0.215$) and petroleum ether ($t = 1.616$, $df = 3$, $P = 0.205$) extracts, against bacteria. The same phenomenon was observed when activity of dichloromethane extract was compared to that of petroleum ether against bacteria ($t = 0.397$, $df = 3$, $P = 0.718$).

The difference in the activity of water extract of *V. glabra* compared to methanol extract, against fungi was not significant ($t = 1.732$, $df = 3$, $P = 0.182$) (Table 4.3). There was also

no significant difference in the activity of methanol extract compared to dichloromethane ($t = 1.732$, $df = 3$, $P = 0.182$) and petroleum ether ($t = 1.732$, $df = 3$, $P = 0.182$) extracts, against fungi.

4.2.1.10 Preliminary screening of extracts of *Rauvolfia caffra* Sond.

The water extract of *R. caffra* showed activity against *S. aureus* (Table 4.2), which was not significantly different from that of gentamicin ($t = -2.973$, $df = 3$, $P = 0.059$) and chloramphenical ($t = -2.938$, $df = 3$, $P = 0.061$). The same extract had no activity against the test fungi.

The activity of methanol extract of *R. caffra* against bacteria was recorded against the Gram positive *S. aureus* and *S. pneumoniae*, and the gram negative *E. coli* (Table 4.2). This activity varied significantly among the bacteria ($t = 6.789$, $df = 3$, $P = 0.007$), but did not vary significantly when compared with that of gentamicin ($t = -1.894$, $df = 3$, $P = 0.155$) and chloramphenical ($t = -2.967$, $df = 3$, $P = 0.059$) against bacteria. Methanol extract was slightly active against the filamentous *T. metagrophyte* with inhibition diameter of 13 mm. The activity of this extract varied significantly among the fungi ($t = 5$, $df = 7$, $P = 0.015$). The activity of methanol extract of *R. caffra* did not however vary when compared with that of ketoconazole against fungi ($t = -2.836$, $df = 3$, $P = 0.066$). Dichloromethane extract of *R. caffra* had no activity against any bacteria or fungi (Tables 4.2 and 4.3).

Petroleum ether extract of *R. caffra* was slightly active against *S. aureus* and *S. pneumoniae* with inhibitions of 9 mm in each case (Table 4.2). This antibacterial activity

varied significantly among the bacteria ($t = 8.66$, $df = 3$, $P = 0.003$). However there was no significant difference in the activity of the petroleum ether extract compared to that of gentamicin ($t = -2.407$, $df = 3$, $P = 0.095$) and chloramphenical ($t = -2.976$, $df = 3$, $P = 0.059$), against bacteria. This extract was not active against fungi.

The activity of water extract against bacteria was not significantly different in comparison to methanol ($t = -2.6$, $df = 3$, $P = 0.08$), dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether extracts ($t = -1.444$, $df = 3$, $P = 0.0252$) (Table 4.2). Similarly, there was no significant difference in the activity of methanol extract against bacteria compared to dichloromethane ($t = -2.6$, $df = 3$, $P = 0.08$) and petroleum ether extracts ($t = 3$, $df = 3$, $P = 0.058$). The activity of dichloromethane extract compared to petroleum ether extract against bacteria was likewise not significantly different ($t = -1.732$, $df = 3$, $P = 0.182$).

The activity of water extract of *R. caffra* compared to that of methanol extract against fungi was not significantly varied ($t = 1$, $df = 3$, $P = 0.391$) (Table 4.3). Similarly there was no significant difference in the activity of methanol extract against fungi compared to dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether extracts ($t = 1$, $df = 3$, $P = 0.391$).

4.2.1.11 Preliminary screening of extracts of *Trichilea dregeana* Sond.

Water extract of *T. dregeana* had inhibitions of 11 mm, 14 mm and 13 mm respectively, against *S. aureus*, *S. pneumoniae* and *P. aeruginosa*, while *E. coli* was not sensitive to this extract (Table 4.2).

The activity of water extract of *T. dregeana* varied significantly among the bacteria ($t = 6.181$, $df = 3$, $P = 0.009$), while the difference in these activities compared to gentamicin ($t = -1.516$, $df = 3$, $P = 0.227$) and chloramphenicol ($t = -1.832$, $df = 3$, $P = 0.168$), were not significant. Water extract of *T. dregeana* was also active against *C. parapsilosis*, but the activity was not significantly different among fungi ($t = 2.6$, $df = 3$, $P = 0.08$). However, ketoconazole with a large inhibition diameter of 30mm against *C. parapsilosis* was more active than the water extract against fungi ($t = -4.422$, $df = 3$, $P = 0.021$).

Among the test bacteria, sensitivity to the methanol extract of *T. dregeana* was shown by only *S. aureus* (17 mm) (Table 4.2), but this activity was not significantly different among the bacteria ($t = 3.182$, $df = 3$, $P = 0.05$). The difference between the activity of the methanol extract compared with that of gentamicin ($t = -2.566$, $df = 3$, $P = 0.083$) and chloramphenicol ($t = -2.732$, $df = 3$, $P = 0.072$) was also not significant. Methanol extract was active against *T. mentagrophyte* and *C. parapsilosis*, and the activity varied significantly among fungi ($t = 4.701$, $df = 3$, $P = 0.018$). This activity was however not significantly different from that of ketoconazole against fungi ($t = -2.810$, $df = 3$, $P = 0.067$).

Dichloromethane extract of *T. dregeana* had low activity against *S. pneumoniae* (Table 4.2), and there was a significant difference in activity among the test bacteria ($t = 5$, $df = 3$, $P = 0.015$). This activity of dichloromethane extract when compared with that of gentamicin ($t = -2.027$, $df = 3$, $P = 0.136$) and chloramphenicol ($t = -2.810$, $df = 3$, $P = 0.064$), against bacteria was not significantly different. Dichloromethane extract of *T. dregeana* was active against the yeast fungi *C. albicans* and *C. parapsilosis*, and the

activity varied significantly among the fungi ($t = 8.66$, $df = 3$, $P = 0.003$). Similarly there was a significant difference between the activity of ketoconazole and that of dichloromethane extract against fungi ($t = -4.273$, $df = 3$, $P = 0.025$).

Petroleum ether extract of *T. dregeana* had no activity against any bacteria or fungi (Tables 4.2 and 4.3).

The difference in the activity of water extract compared to methanol ($t = 0.687$, $df = 3$, $P = 0.541$), dichloromethane ($t = 2.251$, $df = 3$, $P = 0.110$) and petroleum ether ($t = 2.810$, $df = 3$, $P = 0.067$) extracts against bacteria was not significant (Table 4.2). Similarly a comparison in the activity of methanol extract to that of dichloromethane ($t = 0.353$, $df = 3$, $P = 0.748$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against bacteria showed that there was no significant difference. The activity of dichloromethane extract compared to petroleum ether extract against bacteria was also not significantly different ($t = 1$, $df = 3$, $P = 0.391$).

There was no significant difference in the activity of water extract compared to methanol ($t = 0.082$, $df = 3$, $P = 0.940$), dichloromethane ($t = 0.676$, $df = 3$, $P = 0.547$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against fungi (Table 4.3). The difference in the activity of methanol extract compared to dichloromethane ($t = 0.910$, $df = 3$, $P = 0.430$) and petroleum ether ($t = 1.732$, $df = 3$, $P = 0.182$) extracts against fungi was also not significant. Similarly, the activity of dichloromethane extract compared to petroleum ether against fungi was not significantly different ($t = 1.732$, $df = 3$, $P = 0.182$).

4.2.1.12 Preliminary screening of extracts of *Albizia coriaria* Oliv.

Water extract of *A. coriaria* recorded activities against the bacteria, *S. aureus*, *S. pneumoniae* and *P. aeruginosa* (Table 4.2). These activities varied significantly among the bacteria ($t = 5.387$, $df = 3$, $P = 0.013$). But there was no significant difference between the activity of this extract, gentamicin ($t = -1.169$, $df = 3$, $P = 0.3327$) and chloramphenicol ($t = -1.476$, $df = 3$, $P = 0.236$), against bacteria. Among the fungi, water extract of *A. coriaria* showed activity against *M. gypseum* and *C. parapsilosis*, and the activity varied significantly among the fungi ($t = 3.357$, $df = 3$, $P = 0.044$). Ketoconazole recorded high activities against the fungi, which was significantly different from that of the water extract ($t = -2.360$, $df = 3$, $P = 0.099$).

Methanol extract of *A. coriaria* showed high activity against *S. aureus*, with inhibition of 18 mm (Table 4.2). The activity of this extract compared with that of gentamicin ($t = -2.481$, $df = 3$, $P = 0.089$) and chloramphenicol ($t = -2.673$, $df = 3$, $P = 0.075$), against bacteria was not significantly different. Similarly the extract was active against *T. metagrophyte* and *C. albicans*, but the activity was not significantly different among the fungi ($t = 3.178$, $df = 7$, $P = 0.05$). There was no significant difference between the activity of this extract and ketoconazole against fungi ($t = -2.36$, $df = 3$, $P = 0.099$).

Dichloromethane extract of *A. coriaria* was slightly active against *S. pneumoniae* (Table 4.2), and the activity was significantly different among the bacteria ($t = 7$, $df = 3$, $P = 0.006$). There was however no significant difference between the activity of this extract and that of gentamicin ($t = -2.905$, $df = 3$, $P = 0.106$) and chloramphenicol ($t = 1$, $df = 3$, $P = 0.062$), against bacteria. Dichloromethane extract was not active against any fungus.

Very slight activity was exhibited by the petroleum ether extract of *A. coriaria* against *S. aureus* (Table 4.2), with significant differences in activity among the test bacteria ($t = 7$, $df = 3$, $P = 0.006$). There was no significant difference in the activity of petroleum ether extract, gentamicin ($t = -2.946$, $df = 3$, $P = 0.063$) and chloramphenical ($t = -2.941$, $df = 3$, $P = 0.060$), against bacteria. This extract was not active against any of the fungi.

A comparison of the activities of the different extracts of *A. coriaria* showed that there was no significant difference in the activity of the water extract compared to methanol ($t = 1.545$, $df = 3$, $P = 0.22$), dichloromethane ($t = 2.574$, $df = 3$, $P = 0.82$) and petroleum ether ($t = 2.926$, $df = 3$, $P = 0.061$) extracts against bacteria (Table 4.2). Similarly there was no significant difference in the activity of methanol extract compared to that of dichloromethane ($t = 0.577$, $df = 3$, $P = 0.604$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against bacteria, and also that of dichloromethane extract compared to petroleum ether extract against bacteria ($t = 0.0001$, $df = 3$, $P = 1$).

The activity of the water extract did not vary significantly when compared to that of methanol ($t = 0.122$, $df = 3$, $P = 0.911$), dichloromethane ($t = 1.567$, $df = 3$, $P = 0.215$) and petroleum ether extracts ($t = 1.567$, $df = 3$, $P = 0.215$) against the fungi (Table 4.3). There was also no significant difference in the activity of methanol extract compared to dichloromethane ($t = 1.362$, $df = 3$, $P = 0.266$) and petroleum ether extracts ($t = 1.362$, $df = 3$, $P = 0.266$), against fungi.

4.2.1.13 Preliminary screening of extracts of *Hyptis pectinata* Poit.

Water extract of *H. pectinata* was active against *S. pneumoniae* with an inhibition of 16 mm (Table 4.2).

The activity of the water extract of *H. pectinata* varied significantly among the bacteria ($t = 3.4$, $df = 3$, $P = 0.042$). Although the control drugs had higher activities than the water extract, there was no significant difference between the activity of the extract against bacteria, compared with gentamicin ($t = -1.113$, $df = 3$, $P = 0.205$) and chloramphenical ($t = -2.693$, $df = 3$, $P = 0.074$). Water extract of *H. pectinata* was active against *M. gypseum* (Table 4.3), but there was no significant difference between the activity of this extract and ketoconazole against the fungi ($t = -3.041$, $df = 3$, $P = 0.056$).

Methanol extract of *H. pectinata* showed activity against the Gram positive *S. aureus* and *S. pneumoniae* (Table 4.2). This activity was very low (9 mm and 10 mm respectively) and was significantly different among the bacteria ($t = 7.519$, $df = 3$, $P = 0.005$). However, there was no significant difference between the activity of methanol extract compared with gentamicin ($t = -2.251$, $df = 3$, $P = 0.11$) and chloramphenical ($t = -2.976$, $df = 3$, $P = 0.059$) against bacteria. Methanol extract was not active against any fungus.

Low activity was shown by the dichloromethane extract of *H. pectinata* against *S. aureus* and *S. pneumoniae* (Table 4.2), and this activity was significantly different among these bacteria ($t = 6.532$, $df = 3$, $P = 0.007$). The difference in activity between the dichloromethane extract and that of gentamicin ($t = -2.362$, $df = 3$, $P = 0.099$) and chloramphenical ($t = -2.989$, $df = 3$, $P = 0.058$), against bacteria was not significant. This extract was not active against fungi.

Petroleum ether extract was not active against any bacteria or fungi (Tables 4.2 and 4.3).

There was no significant difference in the activity of water extract of *H. pectinata* compared to methanol ($t = 0.397$, $df = 3$, $P = 0.718$), dichloromethane ($t = 0.203$, $df = 3$, $P = 0.852$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against bacteria (Table 4.2). There was also no significant difference in the activity of methanol extract compared to dichloromethane ($t = 0.397$, $df = 3$, $P = 0.718$) and petroleum ether ($t = 1.698$, $df = 3$, $P = 0.188$) extracts against bacteria. Similarly, the activity of dichloromethane extract compared to petroleum ether extract against bacteria was not significantly different ($t = 1.633$, $df = 3$, $P = 0.201$).

There was no significant difference in the activity of water extract of *H. pectinata* compared to methanol ($t = 1$, $df = 3$, $P = 0.391$), dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against fungi.

4.2.1.14 Preliminary screening of extracts of *Croton macrostachyus* Del.

The activity of water extract of *C. macrostachyus* against bacteria was very low and was recorded against *S. pneumoniae* only (11 mm) (Table 4.2). There was no significant difference in the activity of water extract compared with gentamicin ($t = -2.152$, $df = 3$, $P = 0.121$) and chloramphenical ($t = -2.887$, $df = 3$, $P = 0.063$), against bacteria. Considerably high antifungal activities were shown by the water extract of *C. macrostachyus*. These activities were recorded against all the test fungi, *C. parapsilosis*, *C. albicans*, *T. mentagrophyte* and *M. gypseum* (Table 4.3). The differences in activities of the water extract of *C. macrostachyus* however did not vary significantly among these fungi ($t = 7.651$, $df = 3$, $P = 0.005$). Neither were these activities significantly different

when compared with those of ketoconazole ($t = -0.804$, $df = 3$, $P = 0.48$) against the same fungi.

Methanol extract of *C. macrostachyus* had varied levels of inhibition towards the test organisms. Against the bacteria, activity was exhibited on the Gram positive organisms *S. aureus* and *S. pneumoniae* (Table 4.2), and this activity differed significantly ($t = 7.833$, $df = 3$, $P = 0.004$). However, there was no significant difference between the activity of this extract against bacteria compared to gentamicin ($t = -2.269$, $df = 3$, $P = 0.108$) and chloramphenicol ($t = -2.956$, $df = 3$, $P = 0.060$). The methanol extract recorded high activities against the fungi *C. albicans* (20 mm), and *M. gypseum* (22 mm), and lesser activity on *C. parapsilosis* (14 mm), while *T. mentagrophyte* was not sensitive to this extract. These antifungal activities were significantly different among the fungi ($t = 4.313$, $df = 3$, $P = 0.023$), but were not significant in comparison with those recorded by ketoconazole against the same fungi ($t = -2.446$, $df = 3$, $P = 0.092$).

Dichloromethane extract of *C. macrostachyus* had poor antimicrobial activity. This extract showed very low activity, and only against *S. pneumoniae* (10 mm) (Table 4.2). This activity of dichloromethane extract against bacteria was not significantly different from that of gentamicin ($t = -2.287$, $df = 3$, $P = 0.106$) and chloramphenicol ($t = -2.905$, $df = 3$, $P = 0.062$) against the same bacteria. Dichloromethane extract was not active against the test fungi but ketoconazole was more active than this extract against fungi ($t = -3.976$, $df = 3$, $P = 0.028$).

Petroleum ether extract of *C. macrostachyus* was active against the Gram positive *S. aureus* and *S. pneumoniae*, with very low inhibitions of 8 mm and 9 mm respectively (Table 4.2), and these varied significantly among the bacteria ($t = 13$, $df = 3$, $P = 0.001$). However, there was no significant difference in the activity of this extract compared with that of gentamicin ($t = -2.973$, $df = 3$, $P = 0.059$) and chloramphenical ($t = -2.938$, $df = 3$, $P = 0.061$), against these bacteria. Petroleum ether extract was not active against the test fungi while ketoconazole was more active than this extract against fungi ($t = -3.976$, $df = 3$, $P = 0.028$).

The antibacterial activity of the water extract of *C. macrostachyus* had no significant difference in the activity compared to methanol ($t = -0.391$, $df = 3$, $P = 0.718$), dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 0.502$, $df = 3$, $P = 0.651$) extracts, against bacteria (Table 4.2). Similarly there was no significant difference in the activity of methanol extract of this plant compared to dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against bacteria. The activity of dichloromethane extract compared to that of petroleum ether against bacteria was not significantly different ($t = 0.397$, $df = 3$, $P = 0.718$).

There was no significant difference in the activity of water extract compared to methanol ($t = 1.134$, $df = 3$, $P = 0.339$), dichloromethane ($t = 5.356$, $df = 3$, $P = 0.013$) and petroleum ether ($t = 5.356$, $df = 3$, $P = 0.013$) extracts against fungi (Table 4.3). There was also no significant difference in the activity of methanol extract compared to dichloromethane ($t = 2.643$, $df = 3$, $P = 0.078$) and petroleum ether ($t = 2.643$, $df = 3$, $P = 0.078$) extracts against fungi.

4.2.1.15 Preliminary screening of extracts of *Cassia occidentalis* L.

Very low activities were recorded for the four extracts of *C. occidentalis*. The water extract of this plant was not active against any bacteria or fungi (Tables 4.2 and 4.3).

Methanol extract of *C. occidentalis* was slightly active. Very low activity of 7 mm inhibition diameter was noted against both *S. aureus* and *E. coli* (Table 4.2). The activity of this extract against the bacteria was significantly varied ($t = 22.517$, $df = 3$, $P = 0.0001$). However, there was no significant difference between the activity of this extract against bacteria when compared with the antibiotics gentamicin ($t = -2.984$, $df = 3$, $P = 0.058$) and chloramphenical ($t = -2.897$, $df = 3$, $P = 0.063$). Methanol extract was also not active against any fungi.

The dichloromethane extract of the root of *C. occidentalis* showed activity against only *S. aureus* with very low inhibition of 7 mm (Table 4.2), and there was a significant difference in activity among the bacteria ($t = 25$, $df = 3$, $P = 0.0001$). The activity of dichloromethane extract against bacteria compared with that of gentamicin ($t = -2.974$, $df = 3$, $P = 0.059$) and chloramphenical ($t = -2.929$, $df = 3$, $P = 0.061$), was however not significantly different. Dichloromethane extract was not active against the fungi.

Petroleum ether extract was active against *S. aureus* with very low inhibition of 7 mm (Table 4.2). There was however no significant difference in the activity of petroleum ether extract compared with that of gentamicin ($t = -2.421$, $df = 3$, $P = 0.094$) and chloramphenical ($t = -2.961$, $df = 3$, $P = 0.061$), against bacteria. Petroleum ether extract was not active against the test fungi.

Although water extracts of *C. occidentalis* showed no activity on any of the organisms, there was no significant difference when compared with the activity of methanol ($t = -1.732$, $df = 3$, $P = 0.182$), dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = -1.667$, $df = 3$, $P = 0.194$) extracts against bacteria (Table 4.2). Similarly there was no significant difference in the activity of methanol extract compared to dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = -0.878$, $df = 3$, $P = 0.444$) extracts against bacteria. The activity of dichloromethane extract compared to petroleum ether against bacteria was not significantly different ($t = -1.414$, $df = 3$, $P = 0.252$).

4.2.1.16 Preliminary screening of extracts of *Antiaris toxicaria* L.

Very low activity was shown by all the extracts of *A. toxicaria* against the test organisms. The water extract was not active against any bacteria (Table 4.2), but active only against *M. gypseum* among the fungi (Table 4.3). This activity was significantly different in comparison to that of ketoconazole ($t = -3.976$, $df = 3$, $P = 0.028$).

Methanol extract was not active against any bacteria or fungi (Tables 4.2 and 4.3).

Dichloromethane extract had low inhibition action against the gram positive bacteria. Zone diameters against *S. aureus* and *S. pneumoniae* were 10 mm and 9 mm respectively (Table 4.2), and the difference in these activities was significant ($t = 7.519$, $df = 3$, $P = 0.005$), but not so when compared with the activity of gentamicin ($t = -2.387$, $df = 3$, $P = 0.097$) and chloramphenical ($t = -2.967$, $df = 3$, $P = 0.058$). Dichloromethane extract of *A. toxicaria* was not active against the test fungi.

Petroleum ether extract of *A. toxicaria* was not active against any bacteria or fungi (Tables 4.2 and 4.3).

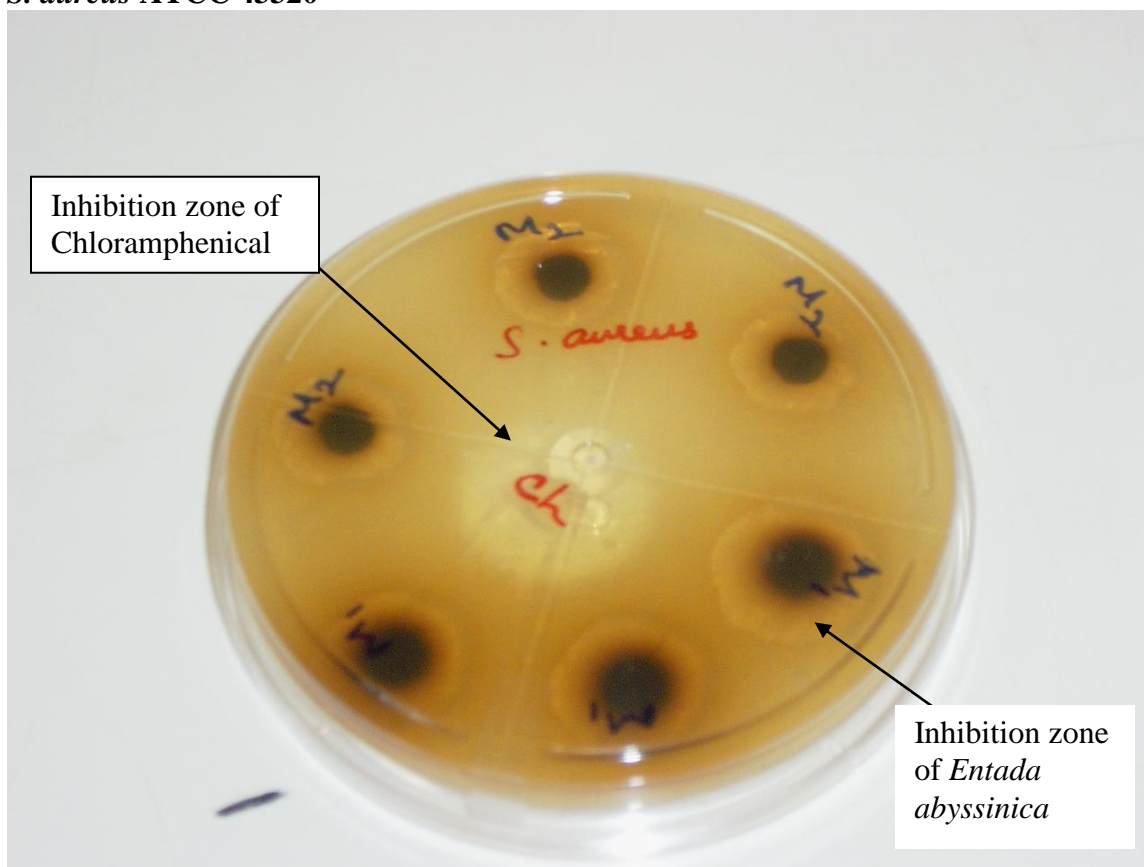
The difference in the activity of water extract compared to methanol, against bacteria was not significant ($t = -1.698$, $df = 3$, $P = 0.188$) (Table 4.2). There was also no significant difference in the activity of methanol extract compared to dichloromethane extract ($t = 1.698$, $df = 3$, $P = 0.188$), and the activity of dichloromethane extract compared to petroleum ether extract against bacteria ($t = 1.698$, $df = 3$, $P = 0.188$).

Although activity was recorded only by the water extract against *M. gypseum* among the fungi, this activity was not significantly different when compared to that of methanol ($t = 1$, $df = 3$, $P = 0.391$), dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts. Similarly, there was no significant difference in the activity of methanol extract compared to dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = -0.878$, $df = 3$, $P = 0.444$) extracts against fungi, and also between the activity of dichloromethane extract compared to petroleum ether extract against these fungi ($t = -1.414$, $df = 3$, $P = 0.252$).

4.2.1.17 Preliminary screening of extracts of *Entada abyssinica* A. Rich.

Generally, the activity of *E. abyssinica* reduced with decrease in polarity of the extraction solvent. Quite high activity was recorded for the water extract of *E. abyssinica*. Activity was observed on 7 of the 8 (87.5%) test organisms (Tables 4.2 and 4.3). This extract exhibited activities against all the test bacteria, *S. aureus* (Plate 4.1), *E. coli*, *S. pneumoniae* and *P. aeruginosa*.

Plate 4.1: Inhibition action of *Entada abyssinica* A. Rich. water extract (M) against *S. aureus* ATCC 43320



M1 = *E. abyssinica* A. Rich. extract disc (1 g/ml); M2 = 0.5 g/ml; Ch = Chloramphenical disc (30 µg/ml)

The inhibition was highest against *S. aureus*, *S. pneumoniae* and *P. aeruginosa* (22 mm, 17 mm and 20 mm respectively), and these activities varied significantly among the bacteria ($t = 7.298$, $df = 3$, $P = 0.005$). There was however no significant difference between these activities compared with those of the antibiotics gentamicin ($t = 0.516$, $df = 3$, $P = 0.508$) and chloramphenical ($t = 0.749$, $df = 3$, $P = 0.391$), against the same bacteria. The water extract of *E. abyssinica* was active against *M. gypseum*, *C. albicans* and *C. parapsilosis*, and the activity varied significantly among these fungi ($t = 4.34$, $df = 7$, $P = 0.023$). However, *T. mentagrophyte* did not respond to this extract. There was a

significant difference between the activity of this extract and ketoconazole against the fungi ($t = -7.66$, $df = 3$, $P = 0.005$).

The activity of methanol extract of *E. abyssinica* was reduced as compared to the water extract, with activity of 19 mm inhibition diameter only against *S. aureus* (Table 4.2). No activity was recorded against *E. coli*, *S. pneumoniae* and *P. aeruginosa*. There was no significant difference between the activity of methanol extract and that of gentamicin ($t = -2.391$, $df = 3$, $P = 0.097$) and chloramphenicol ($t = -2.609$, $df = 3$, $P = 0.08$) against bacteria. The extract recorded some activity against the fungi. *T. mentagrophyte* (Table 4.3), *C. parapsilosis* and *C. albicans* were sensitive to the methanol extract, and this activity was significantly different among fungi ($t = 6.754$, $df = 7$, $P = 0.007$). However, there was no significant difference between the activity of this extract and ketoconazole against the fungi ($t = -2.975$, $df = 3$, $P = 0.059$)

All the test bacteria, except *S. aureus* did not respond to the dichloromethane extract of *E. abyssinica*, representing 12.5 % inhibition by this extract (Table 4.2). This was very low activity and was significantly different among the bacteria ($t = 5.8$, $df = 3$, $P = 0.01$). There was no significant difference between the activity of dichloromethane extract with that of gentamicin ($t = -2.919$, $df = 3$, $P = 0.061$) and chloramphenicol ($t = -2.932$, $df = 3$, $P = 0.062$), against bacteria, while no activity was shown by this extract against the fungi.

Petroleum ether extracts of *E. abyssinica* were not active against any bacteria or fungi (Tables 4.2 and 4.3 respectively).

With the activity of *E. abyssinica* reducing with the polarity of extraction solvent, a comparison of the activities of the four extracts of *E. abyssinica* revealed a significant difference in the activity of the water extract compared to that of methanol ($t = 3.22$, $df = 3$, $P = 0.049$), dichloromethane ($t = 5.341$, $df = 3$, $P = 0.012$) and petroleum ether ($t = 4.796$, $df = 3$, $P = 0.017$) against bacteria (Table 4.2). However, there was no significant difference in the activity of the methanol extract compared to dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) extracts against the bacteria. Similarly the activity of dichloromethane extract compared to petroleum ether extract against bacteria was not significantly different ($t = 1$, $df = 3$, $P = 0.391$).

Against the fungi, there was no significant difference in the activity of water extract of *E. abyssinica* compared to methanol ($t = 1.661$, $df = 3$, $P = 0.195$), dichloromethane ($t = 2.873$, $df = 3$, $P = 0.064$) and petroleum ether ($t = 2.873$, $df = 3$, $P = 0.064$) (Table 4.2). A similar scenario was observed when the activity of methanol extract against fungi was compared to that of dichloromethane ($t = 2.895$, $df = 3$, $P = 0.063$) and petroleum ether ($t = 2.895$, $df = 3$, $P = 0.063$).

4.2.1.18 Preliminary screening of extracts of *Spathodea companulata* P. Beauw.

Very little activity was observed in the methanol and dichloromethane extracts of *S. companulata*. Water extract of this plant was not active against any of the test bacteria or fungi (Tables 4.2 and 4.3).

Methanol extract of *S. companulata* was active against only *S. aureus* with very low inhibition of 10 mm (Table 4.2). The difference in the activity of this extract compared

with gentamicin ($t = -2.946$, $df = 3$, $P = 0.060$) and chloramphenical ($t = -2.941$, $df = 3$, $P = 0.061$), against bacteria was however not significant. Methanol extract was not active against any of the fungi.

Dichloromethane extract was active against the Gram positive *S. aureus* and *S. pneumoniae* (Table 4.2), and the activity was significantly varied among the test bacteria ($t = 3.667$, $df = 3$, $P = 0.035$). However, there was no significant difference between the activity of this extract with that of gentamicin ($t = -2.717$, $df = 3$, $P = 0.073$) and chloramphenical ($t = -2.828$, $df = 3$, $P = 0.066$), against bacteria, while this extract showed no antifungal activity.

Petroleum ether extract was not active against any bacteria or fungi (Tables 4.2 and 4.3).

The difference in the activity of water extract compared to methanol ($t = 1$, $df = 3$, $P = 0.391$), dichloromethane ($t = 1$, $df = 3$, $P = 0.391$), and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) against bacteria was significant (Table 4.3), while there was no significant difference in the activity of methanol extract compared to dichloromethane ($t = 1$, $df = 3$, $P = 0.391$) and petroleum ether ($t = 1$, $df = 3$, $P = 0.391$) against bacteria. Similarly, the activity of dichloromethane extract compared to petroleum ether against bacteria was not significantly different ($t = 1$, $df = 3$, $P = 0.391$).

4.2.2 Minimum Inhibitory Concentration by Agar dilution

4.2.2.1 MIC test results for plant water extracts

MIC tests were performed on nine water extracts and the results were as shown in Table 4.4.

Water extract of *W. ugandensis* had varied MICs against all the test bacteria. The MIC values were 25×10^3 $\mu\text{g/ml}$ for *E. Coli* and *S. pneumoniae*, 0.78×10^3 $\mu\text{g/ml}$, for *S. aureus*, and 12.5×10^3 $\mu\text{g/ml}$ for *P. aeruginosa* (Table 4.4). These MIC values were not significantly different among these bacteria ($t = 2.721$, $df = 3$, $P = 0.073$). There was no significant difference in the MIC values of this extract compared to those registered by the antibiotics gentamicin ($t = 2.721$, $df = 3$, $P = 0.073$) and chloramphenical ($t = 2.721$, $df = 3$, $P = 0.073$).

Against the fungi, MIC values were 12.5×10^3 $\mu\text{g/ml}$ for each of *M. gypseum*, *C. albicans* and *C. parapsilosis* (Table 4.4). However, these values were not significantly different among the fungi ($t = 3$, $df = 3$, $P = 0.058$), and there was also no significant difference in the MIC values between this extract and the antifungal drug, ketoconazole ($t = 3$, $df = 3$, $P = 0.058$).

T. dregeana water extract recorded MICs at 12.5×10^3 $\mu\text{g/ml}$ for *S. aureus* and 25×10^3 $\mu\text{g/ml}$ for *S. pneumoniae* (Table 4.4), and this activity against bacteria was not significantly different ($t = 1.567$, $df = 3$, $P = 0.125$). Although the control antibiotics had much lower MICs, the difference in the MIC values of this extract against bacteria, compared to gentamicin ($t = 1.562$, $df = 3$, $P = 0.216$) and chloramphenical ($t = 1.526$, df

TABLE 4.4 – 1 PAGE

= 3, $P = 0.224$), was not significant. *T. dregeana* water extract had no detectable MICs against fungi.

E. abyssinica water extract had detectable MICs against all the test organisms except *T. mentagrophyte*. This extract inhibited all the test bacteria (Table 4.4), and MICs were detected at 25×10^3 $\mu\text{g/ml}$ for *E. coli*, 12.5×10^3 $\mu\text{g/ml}$ for *S. aureus*, 6.25×10^3 $\mu\text{g/ml}$ for *S. pneumoniae* and 25×10^3 $\mu\text{g/ml}$ for *P. aeruginosa*. These values were significantly different among the bacteria ($t = 3.667$, $df = 3$, $P = 0.035$), and were also significantly different when compared to gentamicin ($t = 3.669$, $df = 3$, $P = 0.037$) and chloramphenicol ($t = 3.611$, $df = 3$, $P = 0.036$). The MIC values for fungi were detected at 50×10^3 $\mu\text{g/ml}$ for *M. gypseum*, 12.5×10^3 $\mu\text{g/ml}$ for *C. albicans*, and 25×10^3 $\mu\text{g/ml}$ for *C. parapsilosis*, and were significantly different ($t = 2.049$, $df = 3$, $P = 0.133$). Similarly there was a significant difference in the MICs of this extract and ketoconazole against the test fungi ($t = 2.049$, $df = 3$, $P = 0.133$).

Water extract of the stem bark of *C. macrostachyus* had detectable but high MIC at 50×10^3 $\mu\text{g/ml}$ for only *P. aeruginosa*, among the bacteria (Table 4.4). This activity was not significantly varied when compared to that of the antibiotic gentamicin ($t = 1$, $df = 3$, $P = 0.391$) and chloramphenicol ($t = 0.990$, $df = 3$, $P = 0.391$). This extract also recorded activity against all the test fungi, with varying MICs. These were 50×10^3 $\mu\text{g/ml}$ for *M. gypseum*, 12.5×10^3 $\mu\text{g/ml}$ for *T. mentagrophyte*, and 25×10^3 $\mu\text{g/ml}$ for each of *C. albicans* and *C. parapsilosis*. The MIC values recorded for the fungi were significantly different ($t = 7$, $df = 3$, $P = 0.006$). Similarly there was significant difference in these MIC values compared to ketoconazole ($t = 7.001$, $df = 3$, $P = 0.0060$).

Water extract of *A. amara* had various detectable MICs for different test organisms (Table 4.4). The extract registered MIC for *S. aureus* at $12.5 \times 10^3 \mu\text{g/ml}$, *S. pneumoniae* at $6.25 \times 10^3 \mu\text{g/ml}$ and *P. aeruginosa* at $12.5 \times 10^3 \mu\text{g/ml}$. These values were not significantly different among bacteria ($t = 2.611$, $df = 3$, $P = 0.08$), and also when compared to gentamicin ($t = 2.612$, $df = 3$, $P = 0.08$) and chloramphenicol ($t = 2.536$, $df = 3$, $P = 0.085$). The MIC values for *A. amara* water extract were $25 \times 10^3 \mu\text{g/ml}$ for each of *C. albicans* and *C. parapsilosis*, and these MICs were not significantly different ($t = 1.732$, $df = 3$, $P = 0.182$). The MIC values of the extracts were also not significantly different from those of ketoconazole ($t = 1.732$, $df = 3$, $P = 0.182$) against the fungi.

Except for *E. coli*, *A. coriaria* water extract recorded MICs against all the other test bacteria (Table 4.4). These were $12.5 \times 10^3 \mu\text{g/ml}$ for *S. aureus*, $25 \times 10^3 \mu\text{g/ml}$ for *S. pneumoniae* and $25 \times 10^3 \mu\text{g/ml}$ for *P. aeruginosa*. The MICs for this extract, against bacteria were not significantly different ($t = 2.611$, $df = 3$, $P = 0.080$). There was also no significant variation in the MIC values of the water extract compared to gentamicin ($t = 2.610$, $df = 3$, $P = 0.082$) and chloramphenicol ($t = 2.602$, $df = 3$, $P = 0.081$). Among the fungi, MICs were detected only against the yeast group of fungi. There were high values of $50 \times 10^3 \mu\text{g/ml}$ for each of *M. gypseum* and *C. parapsilosis*, and the MICs were not significantly different among these fungi ($t = 1.732$, $df = 3$, $P = 0.182$). Similarly, there was no significant difference between the MIC values of this extract and ketoconazole ($t = 1.732$, $df = 3$, $P = 0.182$). There were no detectable MICs for the filamentous fungi.

Water extracts from the root bark of *R. prinoides* had MICs against various test organisms, and these values were relatively high (Table 4.4). The MIC values for the

water extract of this plant were $50 \times 10^3 \mu\text{g/ml}$ for *E. coli*, $12.5 \times 10^3 \mu\text{g/ml}$ for *S. aureus* and $25 \times 10^3 \mu\text{g/ml}$ for *P. aeruginosa*, and were not significantly varied among the bacteria ($t = 2.049$, $df = 3$, $P = 0.133$). Similarly, no significant differences were noted in the MIC values of this extract compared to those obtained by the activity of gentamicin ($t = 2.049$, $df = 3$, $P = 0.133$) and chloramphenicol ($t = 2.034$, $df = 3$, $P = 0.135$). MIC was obtained for the water extract of *R. prinoides* against the yeast fungi as $50 \times 10^3 \mu\text{g/ml}$ for each of *C. albicans* and *C. parapsilosis*. These MICs were not significantly different among the fungi ($t = 2.611$, $df = 3$, $P = 0.081$), and also when compared with ketoconazole ($t = 1.732$, $df = 3$, $P = 0.182$).

Low activity was exhibited by the water extract of *H. pectinata* (Table 4.4). This extract had no detectable MIC against the tested bacteria, and MIC value of $25 \times 10^3 \mu\text{g/ml}$ for *M. gypseum* only. This activity against fungi was not significantly different from that of ketoconazole ($t = 1$, $df = 3$, $P = 0.391$).

The *C. pareira* water extract had no detectable MIC against bacteria (Table 4.4). Among the fungi, MICs were recorded at $6.25 \times 10^3 \mu\text{g/ml}$ for each of *M. gypseum*, *C. albicans* and *C. parapsilosis*, and these were not significantly different among these fungi ($t = 3$, $df = 3$, $P = 0.058$). There was no significant difference between the MIC values of this extract and ketoconazole ($t = 2.997$, $df = 3$, $P = 0.056$).

4.2.2.2 MIC test results for plant methanol extracts

MIC tests were performed on eleven methanol extracts and the results were as shown in table 4.4.

Methanol extract of *W. ugandensis* had MIC of $12.5 \times 10^3 \mu\text{g/ml}$ for only *S. pneumoniae* (Table 4.4), while the other bacteria had no detectable MICs. There was no significant difference between the extract activity and that of gentamicin ($t = 0.996$, $df = 3$, $P = 0.393$) and chloramphenicol ($t = 0.986$, $df = 3$, $P = 0.397$), against bacteria. Methanol extract of *W. ugandensis* was not active against fungi.

The methanol extract of *T. dregeana* recorded MICs against few organisms. The extract had a lower MIC of $3.13 \times 10^3 \mu\text{g/ml}$ against *S. aureus* and a higher MIC of $50 \times 10^3 \mu\text{g/ml}$ for *C. parapsilosis* (Table 4.4). There was no significant variation in the activity of this extract against bacteria compared to gentamicin ($t = 1.564$, $df = 3$, $P = 0.216$) and chloramphenicol ($t = 1.564$, $df = 3$, $P = 0.216$). Similarly there was no significant difference in the activity of this extract against fungi compared to ketoconazole ($t = 1$, $df = 3$, $P = 0.391$).

E. abyssinica methanol extract had detectable MIC against 4 of the 8 test organisms. A lower MIC of $1.56 \times 10^3 \mu\text{g/ml}$ was recorded for *S. aureus* while MIC against *P. aeruginosa* was $12.5 \times 10^3 \mu\text{g/ml}$ (Table 4.4). The MIC values were not significantly different among bacteria ($t = 1.165$, $df = 3$, $P = 0.328$) and also when compared to gentamicin ($t = 1.164$, $df = 3$, $P = 0.329$) and chloramphenicol ($t = 1.120$, $df = 3$, $P = 0.344$). MICs against fungi were $25 \times 10^3 \mu\text{g/ml}$ for *C. albicans* and $25 \times 10^3 \mu\text{g/ml}$ for *C. parapsilosis* (Table 4.4). These MICs were significantly different among the fungi ($t = 1.732$, $df = 3$, $P = 0.182$), and also significantly different when compared with ketoconazole ($t = 1.732$, $df = 3$, $P = 0.182$).

The *C. macrostachyus* methanol extract had no detectable MIC for the bacteria tested (Table 4.4), but showed activity against all the test fungi. The MICs for the fungi were detected at $3.13 \times 10^3 \mu\text{g/ml}$ for *M. gypseum*, $6.25 \times 10^3 \mu\text{g/ml}$ for *T. mentagrophyte*, $50 \times 10^3 \mu\text{g/ml}$ for *C. albicans* and $25 \times 10^3 \mu\text{g/ml}$ for *C. parapsilosis*. These MIC values were however not significantly varied among the fungi ($t = 1.957$, $df = 3$, $P = 0.145$), and also when compared with ketoconazole ($t = 1.957$, $df = 3$, $P = 0.145$).

The MIC of *A. amara* methanol extract was detected at $3.13 \times 10^3 \mu\text{g/ml}$ for *S. aureus* (Table 4.4). There were no detectable MICs for the other test bacteria. This activity of the extract was not significantly different compared to that of gentamicin ($t = 0.983$, $df = 3$, $P = 0.398$) and chloramphenical ($t = 0.863$, $df = 3$, $P = 0.451$). The methanol extract of *A. amara* was not active against fungi.

MICs were recorded for the methanol extract of *A. coriaria* against one Gram positive and one Gram negative bacteria (Table 4.4). Against *S. aureus*, the detected MIC was $3.13 \times 10^3 \mu\text{g/ml}$ while MIC against *P. aeruginosa* was $25 \times 10^3 \mu\text{g/ml}$. These MIC values were not significantly different when compared among the test bacteria ($t = 1.165$, $df = 3$, $P = 0.328$). Similarly there was no significant difference in the MIC values of the methanol extract of *A. coriaria* compared to gentamicin ($t = 1.165$, $df = 3$, $P = 0.328$) and chloramphenical ($t = 1.143$, $df = 3$, $P = 0.336$). MICs were detected against the yeast fungi as $25 \times 10^3 \mu\text{g/ml}$ for *C. albicans* and $12.5 \times 10^3 \mu\text{g/ml}$ for *C. parapsilosis*. However, these MIC values were not significantly different among these fungi ($t = 1.567$, $df = 3$, $P = 0.215$), and also when compared with ketoconazole ($t = 1.566$, $df = 3$, $P = 0.215$).

R. prinoides methanol extract recorded activity against all the test bacteria and fungi, except *T. mentagrophyte* (Table 4.4). Where detectable, the MICs ranged between 3.13×10^3 $\mu\text{g/ml}$ and 50×10^3 $\mu\text{g/ml}$. The MICs against bacteria were 25×10^3 $\mu\text{g/ml}$ for *E. coli*, 3.13×10^3 $\mu\text{g/ml}$ for *S. aureus*, 25×10^3 $\mu\text{g/ml}$ for *S. pneumoniae* and 12.5×10^3 $\mu\text{g/ml}$ for *P. aeruginosa*, and were not significantly different among the bacteria ($t = 3.086$, $df = 3$, $P = 0.054$). Similarly, these MICs were also not significantly different when compared to gentamicin ($t = 3.086$, $df = 3$, $P = 0.054$) and chloramphenicol ($t = 3$, $df = 3$, $P = 0.278$). Methanol extract of *R. prinoides* recorded a high MIC of 50×10^3 $\mu\text{g/ml}$ against *M. gypseum*, 12.5×10^3 $\mu\text{g/ml}$ for *C. albicans* and a lower MIC of 6.25×10^3 $\mu\text{g/ml}$ for *C. parapsilosis*. However, the MICs though widely varied, were not significantly different among these fungi ($t = 1.53$, $df = 3$, $P = 0.223$), and were also not significantly different in comparison to those recorded by ketoconazole ($t = 1.530$, $df = 3$, $P = 0.224$).

Except for *M. gypseum*, MICs for *N. macrocalyx* methanol extract were detected on all the other test organisms, and ranged between 3.13×10^3 $\mu\text{g/ml}$ and 50×10^3 $\mu\text{g/ml}$ (Table 4.4). The MIC values for this extract were 50×10^3 $\mu\text{g/ml}$ for *E. coli*, 3.13×10^3 $\mu\text{g/ml}$ for *S. aureus*, 12.5×10^3 $\mu\text{g/ml}$ for *S. pneumoniae* and 25×10^3 $\mu\text{g/ml}$ for *P. aeruginosa*. These MICs were not significantly different among the bacteria ($t = 2.231$, $df = 3$, $P = 0.112$), and were also not significantly different in comparison to those of gentamicin ($t = 2.231$, $df = 3$, $P = 0.112$) and chloramphenicol ($t = 2.218$, $df = 3$, $P = 0.113$). The MIC value against *T. mentagrophyte* was 50×10^3 $\mu\text{g/ml}$, *C. albicans*, 12.5×10^3 $\mu\text{g/ml}$ and *C. parapsilosis*, 3.13×10^3 $\mu\text{g/ml}$. These MIC values were similarly, not significantly different ($t = 1.426$, $df = 3$, $P = 0.249$). They were also not significantly

different from the MICs recorded by ketoconazole against the fungi ($t = 1.426$, $df = 3$, $P = 0.249$).

Low MIC values were recorded for the methanol extract of *A. mossambicensis*, but against few organisms (Table 4.4). The MICs ranged between $0.39 \times 10^3 \mu\text{g/ml}$ and $6.25 \times 10^3 \mu\text{g/ml}$. The MIC values of this extract were $0.78 \times 10^3 \mu\text{g/ml}$ for *S. aureus* and $0.39 \times 10^3 \mu\text{g/ml}$ for *P. aeruginosa*. These activities were not significantly different among bacteria ($t = 1.567$, $df = 3$, $P = 0.215$) and also in comparison the activities of gentamicin ($t = 1.516$, $df = 3$, $P = 0.227$) and chloramphenical ($t = 0.894$, $df = 3$, $P = 0.437$). The MIC value was $6.25 \times 10^3 \mu\text{g/ml}$ for *C. parapsilosis*, and this was not significantly different from the activity of ketoconazole ($t = 0.998$, $df = 3$, $P = 0.392$) against the fungi.

The MIC values for the methanol extract of this plant were $25 \times 10^3 \mu\text{g/ml}$ for *S. pneumoniae* and $3.13 \times 10^3 \mu\text{g/ml}$ for *P. aeruginosa* (Table 4.4), and these activities were not significantly varied among bacteria ($t = 1.165$, $df = 3$, $P = 0.328$). There was no significant difference in the MIC value of this extract compared to gentamicin ($t = 1.163$, $df = 3$, $P = 0.329$) and chloramphenical ($t = 1.163$, $df = 3$, $P = 0.329$). This extract had no detectable MIC against the test fungi except for *C. parapsilosis* where a high MIC of $25 \times 10^3 \mu\text{g/ml}$ was recorded. Similarly the difference in the MIC values between this extract and ketoconazole was not significantly varied ($t = 0.633$, $df = 3$, $P = 0.572$).

The MIC value of *R. caffra* methanol extract was $12.5 \times 10^3 \mu\text{g/ml}$ for each of *E. coli*, *S. aureus* and *S. pneumoniae*, while it was undetectable for *P. aeruginosa* (Table 4.4). This

activity was not significantly different among the bacteria ($t = 3$, $df = 3$, $P = 0.058$). Similarly, this activity was not significantly different compared to that of gentamicin ($t = 3$, $df = 3$, $P = 0.058$) and chloramphenical ($t = 2.999$, $df = 3$, $P = 0.059$). There was no detectable MIC against tested fungi.

4.2.2.3 MIC test results for plant dichloromethane extracts

MIC tests were performed on nine dichloromethane extracts and the results were as shown in Table 4.4.

The MIC value of *W. ugandensis* dichloromethane extract was detected at 1.56×10^3 $\mu\text{g/ml}$ for *S. aureus* (Table 4.4). There was no significant difference in the MIC value of this extract compared to gentamicin and chloramphenical ($t = 0.966$, $df = 3$, $P = 0.405$) and ($t = 0.727$, $df = 3$, $P = 0.520$), respectively. A high MIC value of 50×10^3 $\mu\text{g/ml}$ was observed for dichloromethane extract of *W. ugandensis* against *C. parapsilosis*. Similarly, there was no significant difference in the MIC values of this extract and ketoconazole against fungi ($t = 1$, $df = 3$, $P = 0.391$).

The MIC value for dichloromethane extract of *T. dregeana* was detectable at 50×10^3 $\mu\text{g/ml}$ for *S. pneumoniae* (Table 4.4). The variation in the activity of this extract compared to gentamicin ($t = 1.564$, $df = 3$, $P = 0.216$) and chloramphenical ($t = 0.983$, $df = 3$, $P = 0.398$), against bacteria was however not significant. This extract was not active against fungi (Table 4.4).

E. abyssinica dichloromethane extract had no detectable MICs against bacteria and fungi (Table 4.4).

The dichloromethane extract of *C. macrostachyus* had MIC of 25×10^3 $\mu\text{g/ml}$ for *S. pneumoniae* only (Table 4.4). This activity was not significantly different when compared to that of gentamicin ($t = 0.993$, $df = 3$, $P = 0.394$) and chloramphenical ($t = 0.998$, $df = 3$, $P = 0.392$) respectively. The dichloromethane extract had no detectable MIC for the fungi tested.

A low MIC of 3.13×10^3 $\mu\text{g/ml}$ was noted for the dichloromethane extract of *H. pectinata* against *S. aureus* (Table 4.4). The activity against bacteria was not significantly different in comparison with that of gentamicin ($t = 0.983$, $df = 3$, $P = 0.398$) and chloramphenical ($t = 0.863$, $df = 3$, $P = 0.451$). Against *C. albicans*, the MIC value was also 3.13×10^3 $\mu\text{g/ml}$, and this activity was similarly not significantly different from that of ketoconazole ($t = 1$, $df = 3$, $P = 0.391$).

Dichloromethane extract of *N. macrocalyx* showed lesser activity and recorded MIC of 50×10^3 $\mu\text{g/ml}$ for *S. aureus* only (Table 4.4). This activity was not significantly varied when compared to that of gentamicin ($t = 0.999$, $df = 3$, $P = 0.391$) and chloramphenical ($t = 0.991$, $df = 3$, $P = 0.395$). There was no detectable MIC against the tested fungi.

Large MIC values were recorded for dichloromethane extract of *M. heterophylla* (Table 4.4). These were 50×10^3 $\mu\text{g/ml}$ for each of *E. coli*, *S. aureus*, and *P. aeruginosa*, and were not significantly different among bacteria ($t = 3$, $df = 3$, $P = 0.058$). Similarly there

was no significant difference in the MIC value of this extract compared to gentamicin ($t = 3$, $df = 3$, $P = 0.058$) and chloramphenical ($t = 2.98$, $df = 3$, $P = 0.059$). *M. heterophylla* dichloromethane extract reported activity against all the test fungi, also with high MIC values of $50 \times 10^3 \mu\text{g/ml}$ for each of *M. gypseum*, *T. mentagrophyte*, *C. albicans* and *C. parapsilosis*. These activities were not significantly different among the fungi ($t = 1.426$, $df = 3$, $P = 0.249$). However, the difference between the MICs of this extract and ketoconazole was significant ($t = 326.6$, $df = 3$, $P = 0.0001$).

Little activity was shown by the dichloromethane extract of *S. companulata* with an MIC of $50 \times 10^3 \mu\text{g/ml}$ for *S. aureus* only (Table 4.4). The difference in activity against the bacteria was not significant when compared to that of gentamicin ($t = 0.999$, $df = 3$, $P = 0.391$) and chloramphenical ($t = 0.991$, $df = 3$, $P = 0.395$), while there was no detectable MIC against the tested fungi.

A wide MIC range was shown by the dichloromethane extract of *C. articulatus* against some test organisms (Table 4.4). A high MIC of $50 \times 10^3 \mu\text{g/ml}$ was noted against *E. coli* and lower MIC of $6.25 \times 10^3 \mu\text{g/ml}$ for *S. aureus*. These MICs were not significantly different among the bacteria ($t = 1.165$, $df = 3$, $P = 0.328$), and were also not significantly different from those of gentamicin ($t = 1.164$, $df = 3$, $P = 0.329$) and chloramphenical ($t = 1.156$, $df = 3$, $P = 0.331$). The activity was recorded against the fungi *C. albicans* and *C. parapsilosis* with MICs of $6.25 \times 10^3 \mu\text{g/ml}$ and $3.13 \times 10^3 \mu\text{g/ml}$ respectively. Similarly, these activities were not significantly different among the fungi ($t = 1.426$, $df = 3$, $P = 0.249$), and in comparison with ketoconazole ($t = 1.565$, $df = 3$, $P = 0.216$).

4.2.3.0 Results for the MBC and MFC tests on 6 plants, by broth macro dilution method

The water extract of *W. ugandensis* exhibited bactericidal action against bacteria at 50×10^3 $\mu\text{g/ml}$ for *S. aureus* and 12.5×10^3 $\mu\text{g/ml}$ for *S. pneumoniae* (Table 4.5). These MBC values were not significantly different among the bacteria ($t = 1.321$, $df = 3$, $P = 0.278$), and were also not significantly different when compared to gentamicin ($t = -0.082$, $df = 3$, $P = 0.940$).

The water extract of *W. ugandensis* was also fungicidal against *M. gypseum* at 25×10^3 $\mu\text{g/ml}$ and *C. parapsilosis* at 50×10^3 $\mu\text{g/ml}$ (Table 4.5). However, these activities were not significantly different among the fungi ($t = 1$, $df = 3$, $P = 0.391$), and there was no significant difference between the MFC values of *W. ugandensis* water extract and ketoconazole ($t = -1.411$, $df = 3$, $P = 0.113$).

Water extract of *A. amara* was bactericidal at 25×10^3 $\mu\text{g/ml}$ for *P. aeruginosa* (Table 4.5), and there was a significant difference in the activity of this extract when compared to gentamicin ($t = -3.476$, $df = 3$, $P = 0.040$). However, this extract had no detectable MFC against the test fungi.

A. coriaria water extract was bactericidal against both Gram positive and negative bacteria (Table 4.5). The MBC values were 12.5×10^3 $\mu\text{g/ml}$ for *S. aureus*, 12.5×10^3 $\mu\text{g/ml}$ for *S. pneumoniae* and 50×10^3 $\mu\text{g/ml}$ for *P. aeruginosa*. These activities were not significantly different among the bacteria ($t = 1.732$, $df = 3$, $P = 0.182$). Similarly there

TABLE 4.5

was no significant difference in the MBC values of *A. coriaria* water extract compared to gentamicin ($t = 0.237$, $df = 3$, $P = 0.828$).

The MFC value of *A. coriaria* water extract was $25 \times 10^3 \mu\text{g/ml}$ for *M. gypseum*, and was not significantly different when compared with that of ketoconazole ($t = 1.411$, $df = 3$, $P = 0.253$).

The *R. prinoides* water extract had no detectable MBC against the test bacteria (Table 4.5). Similarly, this extract had no detectable MFC against the test fungi.

E. abyssinica water extract recorded bactericidal activity at $6.25 \times 10^3 \mu\text{g/ml}$ for *S. aureus*, $50 \times 10^3 \mu\text{g/ml}$ for *S. pneumoniae* and $6.25 \times 10^3 \mu\text{g/ml}$ for *P. aeruginosa* (Table 4.5). These MBC values were not significantly different among bacteria ($t = 1.353$, $df = 3$, $P = 0.269$), and were also not significantly different compared to gentamicin ($t = -1.050$, $df = 3$, $P = 0.923$).

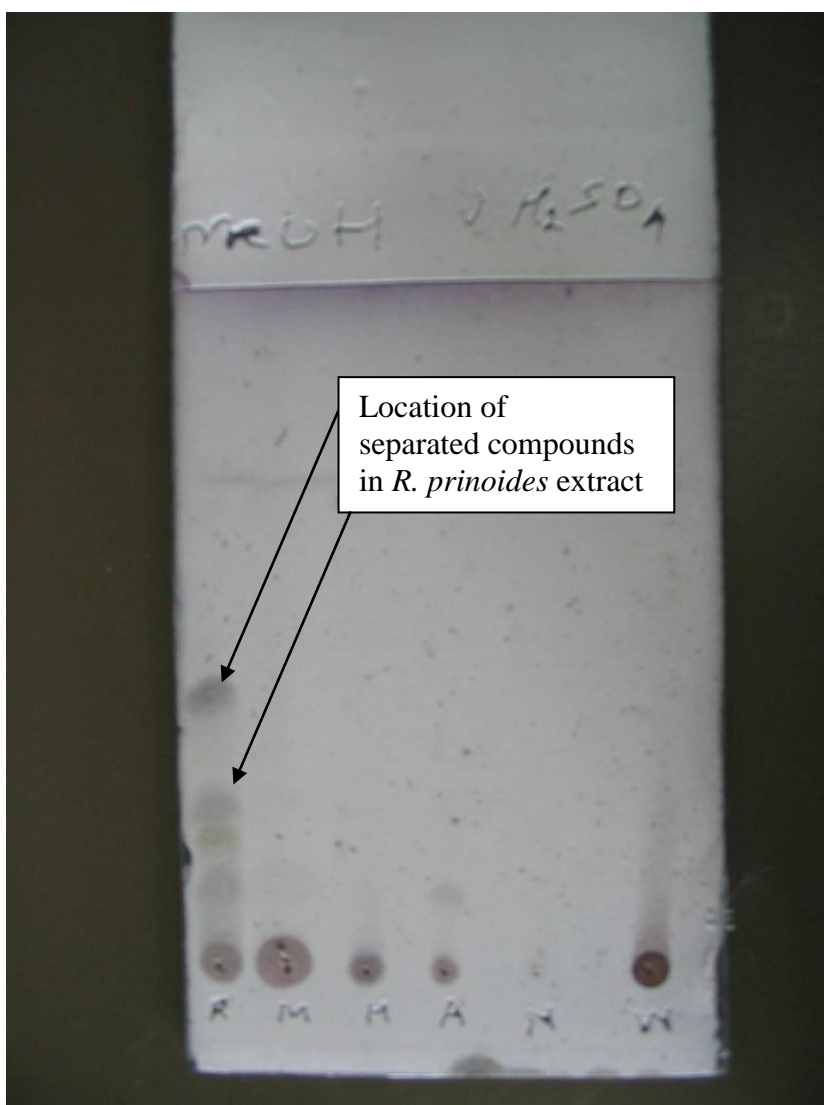
E. abyssinica water extract had fungicidal action on *M. gypseum* at $3.13 \times 10^3 \mu\text{g/ml}$. There was no significant difference in the MFC value of this extract compared to ketoconazole against fungi ($t = -2.101$, $df = 3$, $P = 0.126$).

The *C. macrostachyus* water extract had no detectable MBC against bacteria (Table 4.5). The same extract had no detectable MFC against the tested fungi.

4.2.4.0 Results of the phytochemical screening of the plant extracts

Results for the TLC test to determine the presence or absence of alkaloids, phenolics, terpenoids, anthraquinones, flavonoids and saponins in the plant extracts of *R. prinoides*, *A. amara*, *N. macrocalyx*, *W. ugandensis*, *A. coriaria*, and *E. abyssinica*, are as shown in table 4.6.

Plate 4.2: Separation of terpenoids in methanol extracts of 6 plants by Thin Layer Chromatography



R = *Rhamnus prinoides*; M = *Entada abyssinica*; H = *Albizia coriaria*; A = *Albizia amara*; N = *Neobutonia macrocalyx*; W = *Warbugia ugandensis*.

TABLE 4.6

CHAPTER FIVE

DISCUSSION

Results of the disc diffusion assays demonstrated that more water extracts had inhibitory effects against the test organisms than methanol, dichloromethane and petroleum ether extracts. Of the 144 preliminary tests performed on all the water extracts, activity (inhibition zone > 9 mm) was recorded in 44 cases as compared to 35 for methanol, 16 for dichloromethane and 4 for petroleum ether (Table 4.2).

The activity of different solvent extracts of *E. abyssinica* stem bark against bacteria and fungi varied. The range of activity reduced with the decrease in polarity of the extraction solvent (Table 4.2). *E. abyssinica* water extract had the widest range of activity, with inhibitions against 7 (87.5%) of the 8 test organisms. The activity of the water extract against bacteria was significantly different compared to that of methanol ($t = 3.22$, $df = 3$, $P = 0.049$), dichloromethane ($t = 5.341$, $df = 3$, $P = 0.012$) and petroleum ether ($t = 4.796$, $df = 3$, $P = 0.017$). But there was no significant difference in activity of the water extract against the fungi compared to methanol ($t = 1.661$, $df = 3$, $P = 0.195$), dichloromethane ($t = 2.873$, $df = 3$, $P = 0.064$) and petroleum ether ($t = 2.873$, $df = 3$, $P = 0.064$) (Table 4.3). Thus a variety of the active principles in this plant were aqueous (polar), and were extracted by water. *E. abyssinica* water extract exhibited significant differences in activity against the tested bacteria ($t = 7.298$, $df = 3$, $P = 0.005$) and fungi ($t = 4.34$, $df = 7$, $P = 0.023$). Although *E. abyssinica* methanol extract was active against fewer organisms than the water extract, its activity against *S. aureus* and *P. aeruginosa* was higher than that exhibited by the water extract (Table 4.2). The MICs of methanol extract against *S. aureus* and *P. aeruginosa* were $1.56 \times 10^3 \mu\text{g/ml}$ and $12.5 \times 10^3 \mu\text{g/ml}$

respectively, while MICs for water extract against the same bacteria were 12.5×10^3 $\mu\text{g/ml}$ and 25.0×10^3 $\mu\text{g/ml}$ respectively. Methanol would therefore be the best solvent for extraction of antistaphylococcal and antipseudomonal agents from *E. abyssinica* stem bark. The activity against the Gram positive and negative bacteria, yeasts and *Microsporium gypseum* confirms the antibacterial and antifungal properties *E. abyssinica* reported in earlier studies (Fabry *et. al.*, 1996 a,b). *E. abyssinica* has been used to treat severe coughs and chest pains associated with *S. pneumoniae*, and, diarrhoea and boils associated with *E. coli* and *Candida spp.*, and *S. aureus* respectively (Fabry *et. al.*, 1996 a,b). *E. abyssinica* water extract had no activity against *T. mentagrophyte* (Table 4.3), but had bactericidal activity against *S. aureus*, *S. pneumoniae* and *P. aeruginosa* (Table 4.5) with MBCs of 6.25×10^3 $\mu\text{g/ml}$, 50×10^3 $\mu\text{g/ml}$ and 6.25×10^3 $\mu\text{g/ml}$ respectively. However, bacteriostatic activity was recorded against *E. coli* $>50 \times 10^3$ $\mu\text{g/ml}$. The extract had fungicidal activity against *M. gypseum* 3.13×10^3 $\mu\text{g/ml}$ and fungistatic action against *C. albicans* and *C. parapsilosis* with an MFC value of $>50 \times 10^3$ $\mu\text{g/ml}$ in each case. The difference in activity against the filamentous and yeast forms of fungi could be attributed to differences in cell wall composition. Filamentous fungi contain chitin and glycan in their cell wall while yeast forms contain glucans and mannan proteins. Depending on composition, the cell wall can act as a barrier, preventing drugs from reaching the site of action. The presence of chitin in the cell wall has been reported to be essential for effectiveness of some antifungal drugs. Polyoxin is a powerful competitor of chitin synthase and it is not effective on fungi without chitin. An earlier evaluation of the stem bark of *E. abyssinica* reported antibacterial activity against *Helicobacter pylori*, and bacteria of the genera *Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Escherichia*, *Klebsiella*, *Salmonella* and *Mycobacterium*, with MIC₅₀ and MIC₉₀ ranges of 0.13-8

mg/ml, and 0.5 to > 8 mg/ml, respectively (Fabry *et al.*, 1998). Similarly, antifungal properties of *E. abyssinica* stem bark have been evaluated, with fungistatic and fungicidal activities being recorded against *Candida* spp. and *Aspergillus* spp (Fabry *et al.*, 1996 b).

The antimicrobial activity of *E. abyssinica* is attributed to the phytochemical compounds present in this plant. Phytochemical screening revealed that water, methanol, dichloromethane and petroleum ether extracts contained phenolics and terpenoids (Table 4.6), which have been associated with antimicrobial activities (Clark, 1981; Mather and Gonzalez, 1982). Phenolics were present in all the extracts of *E. abyssinica*, and their inhibitory properties have been reported against bacteria (Brantner *et al.*, 1996; Thomson, 1978), fungi (Duke, 1985) and viruses. Polyphenolic compounds like gallic acid and epigallocatechin have inhibited bacteria such as *Streptococcus mutans* (Sakanaka *et al.*, 1989). Similarly terpenoids, which were abundant in the dichloromethane extract of *E. abyssinica* (Table 4.6), have been reported to be active against bacteria and fungi (Amaral *et al.*, 1998; Ayafor *et al.*, 1994; Cichewicz and Thorpe, 1996). Despite all the solvent extracts containing same groups of compounds (Table 4.6), differences in their antimicrobial activities were noted (Tables 4.2 and 4.3). These differences either suggest that the solvents extracted different concentrations of the active principles in each group of compounds, or the active principles extracted in each group were different altogether. For example, different types of terpenoids exist in plants, and include isoprene, mono-, di- and triterpenoids (Harborne, 1984). Varying concentrations of these classes of terpenoids would contribute to differences in the efficacy of the extract. The difference in activity of the extracts could also have been due to the presence or absence of other phytochemical compounds which were not tested for in this study. These could probably

be reducing sugars, steroids, or tannins. For instance, tannins and reducing sugars which were not investigated in this study are among compounds with claimed antimicrobial activity (Fleischer *et al.*, 2002). Their presence in plants like *Acanthospermum hispidum*, explains the activity of these plants against a wide range of pathogenic bacteria. Odebiyi (1985) reported the antimicrobial properties of the extracts of *Jatropha podagrica* stem bark which contains tannins. In another study, bioassay-guided fractionation of the dichloromethane root bark extract of *E. abyssinica* (Leguminosae), a plant used by traditional healers in Uganda for the treatment of sleeping sickness, led to the isolation of a diastereoisomer of the clerodane type diterpene kolavenol (Freiburghausa *et al.*, 1998). This was the first report on this compound. It showed a trypanocidal activity with an IC₅₀ value of 2.5 µg/ml against *Trypanosoma brucei rhodesiense*, the causing agent of the acute form of human African trypanosomiasis.

Alkaloids, anthraquinones, flavonoids and saponins were not contained in any of the four extracts of *E. abyssinica* (Table 4.6). Therefore these compounds did not contribute to the activity exhibited by this plant.

Although there was no significant difference between MBC and MFC values of *E. abyssinica* water extract, and those of gentamicin ($t = -1.050$, $df = 3$, $P = 0.269$) and ketoconazole ($t = -2.101$, $df = 3$, $P = 0.126$), the effectiveness of *E. abyssinica* should be exploited for the management of pathogenic bacterial and fungal conditions. The antimicrobial activities demonstrated by crude extracts of this plant justify some of the ethnopharmacological claims about the plant (Fabry *et. al.*, 1996 a; Hugo and Russel, 1983; Sleigh and Timbury, 1981).

W. ugandensis water extract was potent against six of the eight test organisms, compared to the methanol, dichloromethane and petroleum extracts, each of which had inhibition against two of the organisms (Tables 4.2 and 4.3). *W. ugandensis* water extract showed both antibacterial and antifungal properties. Inhibition was recorded against the growth of Gram positive bacteria, yeast forms of fungi and *M. gypseum*. These results help to support the antimicrobial properties that *W. ugandensis* is claimed to possess (Kokwaro, 1976). *W. ugandensis* extracts have been used for the treatment of skin infections. Skin diseases are caused by bacteria and viruses, but most commonly by dermatophyte infections and yeasts. Fractions from *W. ugandensis* have proved effective on *Candida utilis* and *Saccharomyces cerevisiae*, and also on *Penicillium crustosum*, *Aspergillus niger*, and mucor with MIC of 50 µg/ml for *Hansenula anomala* (Taniguchi *et al.*, 1988).

From the disc diffusion assays, no effect was recorded against the tested Gram negative bacteria and *T. mentagrophyte* by the water extract of *W. ugandensis* (Table 4.2). However all extracts of this plant showed activity against *S. aureus*, indicating that the plant contains potent antistaphylococcal agents. The difference in activity against Gram positive and negative bacteria could be attributed to the difference in cell wall compositions of these groups of bacteria. The presence of lipoproteins and lipopolysaccharides in the Gram negative bacteria prevents large hydrophilic molecules from reaching an otherwise susceptible cellular target (Greenwood, 1995).

From the agar dilution assay however, except for *T. mentagrophyte*, the water extract of *W. ugandensis* inhibited all the other organisms including the Gram negative bacteria. MIC values were 25×10^3 µg/ml for *E. coli* and 12.5×10^3 µg/ml for *P. aeruginosa*

(Table 4.4). *W. ugandensis* water extract had bactericidal effects on the Gram positive bacteria, and fungicidal effects on *M. gypseum* and *C. parapsilosis* (Table 4.5). The activity against *E. coli* and *P. aeruginosa* was bacteriostatic, while it had fungistatic effect against *C. albicans*. Fungistatic activity of *W. ugandensis* on *C. albicans* was earlier reported by Kubo *et al.*, (1992). Fungicidal activity on *M. gypseum* and *C. parapsilosis* could have been caused by the ability of *W. ugandensis* to destroy the cells of these organisms. Compounds such as the sesquiterpene dialdehyde and polygodial from *W. ugandensis* have been known to destroy the plasma membrane which leads to cell destruction (Kioy, 1989; Taniguchi *et al.*, 1988). *In vitro* activity of polygodial was reportedly stronger than amphotericin B, with MICs of 0.78 and 1.56 µg/ml respectively, against *Saccharomyces cerevisiae* IFO 0203 (Taniguchi *et al.*, 1988).

The high antimicrobial activity of *W. ugandensis* may be attributed to the anthraquinones which were present only in the water extract (Table 4.6). The distribution of anthraquinones in higher plants has been mainly studied because certain anthraquinones have cathartic action and are used in the Pharmacopiea of many countries as purgatives (Harborne, 1984). Similarly, flavonoids may have contributed to the activity of *W. ugandensis* water extract against fungi (Table 4.6). Flavonoids are synthesized by plants in response to microbial infections (Dixon *et al.*, 1983). It is therefore not surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide range of microorganisms. It is believed that their activity is conferred by their ability to complex with bacterial cell walls (Cowan, 1999). Lipophilic flavonoids may also disrupt microbial membranes (Tsuchiya *et al.*, 1996). Due to its high antimicrobial potential, the

effectiveness of *W. ugandensis* in the management of microbial infections needs to be exploited.

Although all test extracts of *R. prinoides* exhibited activity in the preliminary screening tests, the water extract had a wider range of antimicrobial activity (Tables 4.2 and 4.3). This was another indication that a wider range of the active principles in this plant were more polar and therefore extracted in aqueous form. However, further bioactivity tests by agar dilution showed that the methanolic extract of *R. prinoides* was more effective than the water extract (Table 4.4). Methanol extract had lower MIC values than the water extract. Phytochemical tests showed that water extract of *R. prinoides* contained alkaloids, phenolics, terpenoids, anthraquinones, flavonoids and saponins (Table 4.6), which could be regarded as the active principles that confer antimicrobial properties to this plant. Possession of alkaloids by some plants for instance has been shown to give distinct fungicidal activities (Bringman *et al.*, 1992), most likely to give the plants specific advantage against fungal attacks in the humid rain forest where such plants exist.

The activity of *R. prinoides* water extract against Gram positive bacteria (*S. aureus* and *S. pneumoniae*), Gram negative bacteria (*P. aeruginosa*), yeasts (*C. albicans* and *C. parapsilosis*) and moulds (*M. gypseum*), confirm the anti-bacterial and antifungal potentials of this plant. *R. prinoides* has been used in the treatment of skin fungal infections, diarrhoea and throat infections (Kokwaro, 1993).

Bioactivity tests revealed that at concentrations below $50 \times 10^3 \mu\text{g/ml}$, *R. prinoides* water extract has bacteriostatic and fungistatic effects on bacteria and fungi respectively (Table 4.5). At these concentrations the extract cannot be effective on patients suffering from

infective endocarditis, whereby bacteria are protected against phagocytic activity within vegetations present on the deformed or prosthetic heart valve or adjacent endocardium (Greenwood, 1995). In such circumstances it is necessary to use a bactericidal drug or combination of drugs which penetrate the vegetations to eradicate the infection. Similarly the extract would not be useful to patients with neutropenia from cytotoxic chemotherapy or other causes of bone marrow aplasia, who are extremely vulnerable to infection.

The abundance of phenolics and terpenoids in water, methanol, dichloromethane and petroleum ether extracts of *R. prinoides* (Table 4.6) explain the inhibitory effects of *S. aureus*. It is also interesting to note that *R. prinoides* contained all the phytochemical compounds tested for, although alkaloids, anthraquinones, flavonoids and saponins were detected in slight amounts (Table 4.6). Diterpenoid alkaloids, commonly isolated from some plants have commonly been found to have antimicrobial properties (Omulokoli *et al.*, 1997), while their mechanism of action is attributed to their ability to intercalate with DNA (Hopp *et al.*, 1976; Phillipson and O'Neill, 1987).

The presence of flavonoids in the water extract of *R. prinoides* may have contributed to the activity of this extract against bacteria (Table 4.6). This agrees with earlier studies on the activity of flavonoids on bacteria whereby, some flavonoids such as dimethylenedioxypterocarpan, praecansone A and praecansone B, extracted from plants like *Tephrosia equilata* have been reported to have antimicrobial properties against *E. coli* and *P. aeruginosa* (inhibition zone = 10 mm), and *Bacillus subtilis* and *Micrococcus lutea* (≤ 8 mm) (Tarus *et al.*, 2002). The effectiveness of *R. prinoides* should be exploited

to determine the active principles, which may serve as novel compounds for development of new drugs.

Disc diffusion assays showed that *C. macrostachyus* water and methanol extracts had inhibitory effects against equal number of the tested organisms, which was higher than that of the extracts of dichloromethane and petroleum ether (Table 4.2 and 4.3). However water extract had broader spectrum of inhibition (Table 4.4) than methanol when MIC tests were performed. Water extract of *C. macrostachyus* inhibited only *S. pneumoniae* among the bacteria and all the fungi tested. The difference in activity against the tested bacteria was however not significant ($t = 1$, $df = 3$, $P = 0.391$), as well as the difference in activity when compared with gentamicin ($t = 1$, $df = 3$, $P = 0.391$) and chloramphenicol ($t = 0.990$, $df = 3$, $P = 0.391$). There was a significant difference in activity of the aqueous extract against the tested fungi ($t = 7$, $df = 3$, $P = 0.006$). This activity also varied significantly with that of ketoconazole ($t = 7.001$, $df = 3$, $P = 0.006$).

Thus *C. macrostachyus* water extract has higher antifungal and lower antibacterial activity (Table 4.2 and Table 4.3). Poor antibacterial activity of *C. macrostachyus* was also reported in earlier studies (Matu and Staden, 2003). However, antibacterial activity was previously detected in the bark of *C. macrostachyus* collected from East Africa (Taniguchi and Kubo, 1993). This study did not confirm these previous results. This variation could be explained by the fact that therapeutic potency of biologically active components in plants can be affected by the locality of the plant species, the age of the plant, time of collection and storage conditions among other factors (Matu and Staden,

2003). In comparison, ketoconazole had no varied MFC values with the water extract of *C. macrostachyus* against the fungi ($t = -2.222$, $df = 3$, $P = 0.113$).

Although water extract had a broad spectrum of activity, methanol extract of *C. macrostachyus* had greatest inhibition against *T. mentagrophyte* and *M. gypseum* with MIC values of $3.13 \times 10^3 \mu\text{g/ml}$, and $6.25 \times 10^3 \mu\text{g/ml}$ respectively (Table 4.4). Methanol is therefore the appropriate solvent for the extraction of antifungal agents against filamentous fungi, from *C. macrostachyus*. MBC/MFC tests revealed that *C. macrostachyus* water extract at concentrations of $\leq 50 \times 10^3 \mu\text{g/ml}$ has no detectable MIC against *P. aeruginosa* and *T. mentagrophyte* while it is fungistatic on *M. gypseum*, *C. albicans* and *C. parapsilosis* (Table 4.5). Similarly, ketoconazole had no varied MFC values than this extract ($t = -2.222$, $df = 3$, $P = 0.113$), against the test fungi.

Antimicrobial activity has been reported among members of the genus *Croton*, and diterpenes, isolated as the major bioactive materials (McChesney *et al.*, 1991). The broad spectrum of potency against yeasts and filamentous fungi suggests the presence of antifungal agents in this plant, which need to be exploited. If confirmed, the extracts of *C. macrostachyus* would be useful, through incorporation in creams for topical applications against dermatophytes. Traditionally, *C. macrostachyus* is used in the treatment of various human diseases (Kokwaro, 1976; Gachathi, 1989; Beentje, 1994; Maundu *et al.*, 1999). Known in Kakamega as “Musutsu”, the roots of this plant are used as anti-helminthics and for treatment of malaria and venereal diseases. The bark is used to treat skin rashes while leaves are used to treat coughs and improve blood clotting. Venereal diseases which the plant is known to treat such as gonorrhoea and syphilis, as well as

some coughs are caused by bacterial infections. On the other hand, skin rashes may be caused by fungal infections of the skin. This may confirm the antibacterial and antifungal properties of *C. macrostachyus*.

Water extract of *A. coriaria* stem bark inhibited more organisms than extracts of methanol, dichloromethane and petroleum ether (Tables 4.2 and 4.3). The extract had inhibitory effects on Gram positive bacteria (*S. aureus* and *S. pneumoniae*), Gram negative bacteria (*P. aeruginosa*), yeasts (*C. parapsilosis*) and moulds (*M. gypseum*) (Table 4.3). However, *E. coli*, which is known to be multi-drug resistant (Nascimento *et al.*, 2000), *C. albicans* and *T. mentagrophyte* were resistant to this plant extract.

A. coriaria water extract had bactericidal effects on *S. aureus*, *S. pneumoniae* and *P. aeruginosa* with MBC values of 12.5×10^3 $\mu\text{g/ml}$ for *S. aureus* and *S. pneumoniae*, and 50×10^3 $\mu\text{g/ml}$ for *P. aeruginosa*, and fungicidal effect on *M. gypseum* (MFC 25×10^3 $\mu\text{g/ml}$) (Table 4.5). The extract had a fungistatic effect on *C. parapsilosis*. Although these inhibitory effects were lower than those of the control drugs gentamicin, chloramphenicol and ketoconazole (Table 4.5), the differences in the activities of the extract and the controls were not significant.

Phytochemical tests revealed the presence of phenolics, saponins, and abundance of alkaloids and terpenoids in the stem bark of *A. coriaria* (Table 4.6). The presence of phenolics like thymol in plants such as *Thymus vulgaris* has been used to explain the antiviral, antibacterial and antifungal properties of these plants (Cowan, 1999). These compounds are known to exhibit their antimicrobial properties either by substrate

deprivation (Peres *et al.*, 1997), membrane disruption (Toda *et al.*, 1992), enzyme inhibition (Haslam, 1996) or by binding to adhesins (Perrett *et al.*, 1995) which could explain the inhibitory effects of the water extract. Therefore *A. coriaria*, known among the Luhya as “Musenzeli” is a potential source for novel compounds, which should be harnessed for the development of new antimicrobial drugs.

In vitro antibacterial and antifungal tests showed that the stem bark of *A. amara* possesses some potent antimicrobial properties. The water extract of this plant had activity against a wider range of pathogenic micro-organisms than extracts of methanol dichloromethane, and petroleum ether (Tables 4.2 and 4.3).

Quantitative bioactivity tests showed that *A. amara* water extract had higher activity against bacteria with MICs of 6.25×10^3 $\mu\text{g/ml}$ against *S. aureus*, and 12.5×10^3 $\mu\text{g/ml}$ against *E. coli* and *P. aeruginosa* (Table 4.4). However the extract was bacteriostatic against *E. coli* and *S. aureus* with MBCs of $>50 \times 10^3$ $\mu\text{g/ml}$ in both cases (Table 4.5). It is interesting to note that the lowest concentration at which this extract inhibited *P. aeruginosa* (MIC of 25×10^3 $\mu\text{g/ml}$) also killed the pathogen (MBC of 25×10^3 $\mu\text{g/ml}$). This activity (MBC) was significantly different from that of gentamicin ($t = -3.476$, $df = 3$, $P = 0.040$). It is therefore necessary that more studies be performed on this plant to establish the antipseudomonal principles present, which could be used in place of the presently used aminoglycosides, which besides being expensive are also toxic to the human body (Greenwood, 1995). No activity was shown against *S. pneumoniae*. This may be explained by the evolving antimicrobial resistance that *S. pneumoniae* has been reported to have (Kathryn *et al.*, 2003). Penicillin resistance in pneumococci has been reported in areas all over the world (Doern *et al.*, 1999), and its incidence appears to be

increasing. The reported frequencies of penicillin-resistant pneumococcal strains are variable, depending on the geographical location (Sahm *et al.*, 2000).

Lower activity of *A. amara* was shown against the fungi, with MICs of 25.0×10^3 $\mu\text{g/ml}$ against each of the yeasts *C. albicans* and *C. parapsilosis* (Table 4.4). The extract was fungistatic against these two fungi, with MFC values $>50 \times 10^3$ $\mu\text{g/ml}$ for both (Table 4.5). No activity was shown against the moulds.

Phytochemical tests revealed that *A. amara* water extract contains moderate amounts of alkaloids, saponins and terpenoids, and small amounts of phenolics (Table 4.6). However the antimicrobial properties of this extract could not be attributed to anthraquinones and flavonoids which were both absent in this extract.

The antimicrobial activities of these compounds need to be investigated so as to explain the antimicrobial properties of *A. amara*. Since there is no reported adverse or toxic effects of this plant used in different African communities for various ailments (Adeniyi and Odufowora, 2003), more phytochemical studies need to be performed so as to isolate, characterize and identify the specific active compounds in this plant responsible for the antimicrobial activity.

N. macrocalyx methanol extract had higher activity than water, dichloromethane and petroleum ether extracts of the same plant (Tables 4.2 and 4.3). Among the methanolic extracts of all the plants tested, *N. macrocalyx* had the highest activity, with 75% of the test organisms being susceptible. From the quantitative bioassay tests, *N. macrocalyx* methanol extract inhibited all organisms tested except *M. gypseum*. Therefore it has both

antibacterial and antifungal properties. It exhibited highest activity against *C. parapsilosis* and *S. aureus*, giving an MIC value of 3.13×10^3 µg/ml in each case (Table 4.4).

The absence of all the tested compounds in the methanol extract of *N. macrocalyx* suggests that the potent bioactivity is not conferred by these compounds. Therefore further phytochemical tests need to be carried out to determine the antimicrobial agents present in this plant. It is possible that the activity could have been conferred by such groups of compounds as sugars, steroids and tannins which were not tested for in this study, and which have been reported to have antimicrobial activity against a wide range of pathogenic micro-organisms (Fleischer *et al.*, 2002). The presence of these compounds has been reported in some plants such as *Acanthospermum hispidum*, which had antimicrobial activity against a wide range of pathogenic bacteria. If determined, the antimicrobial agents in this plant would be used as prototypes for development of new antifungal and antibacterial drugs. Also, MBC and MFC tests should be carried out to determine the efficacy of the extract. This would be useful for the purpose of administration of the drug especially to HIV immuno compromised patients who have to be regularly on drugs, in case such drugs are bacteriostatic or fungistatic. This is because the phagocytic cells in such patients are incapable of destroying the pathogens after the bacteriostatic or fungistatic action of the the drug, which relies on the host defenses (Greenwood 1995).

Trichilea dregeana water extract recorded some antibacterial activity (Table 4.2). When MIC tests were performed, it inhibited only the Gram positive and not Gram negative bacteria (Table 4.4). Similar results were earlier recorded, where a pectinolide isolated

from *H. pectinata* inhibited the Gram positive bacteria, *S. aureus* and *B. subtilis* (Pereda-Miranda *et al.*,1993).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1.1 CONCLUSION

Of the 18 plants tested, extracts of *E. abyssinica*, *W. ugandensis*, *A. coriaria*, *A. amara*, *R. prinoides* and *N. macrocalyx* exhibited greatest antimicrobial activity (Tables 4.2 and 4.3).

The water extracts have higher spectra of antimicrobial activity than extracts of methanol, dichloromethane and petroleum ether. Aqueous extraction by boiling therefore is adequate in preparation of herbal medicine, and should be encouraged.

Petroleum ether extracts had the least spectra of activity. This means the active principles in these plants are more polar, or are more concentrated in the water extracts than the other extracts.

S. aureus was the most susceptible organism tested, having been inhibited by all except three of the test plants, while *E. coli* was the most resistant organism, having been inhibited by only *E. abyssinica* water extract (Table 4.2).

There is the presence of phytochemicals with reputed antimicrobial properties, in these plants.

Some extracts have bactericidal and fungicidal effects, rendering them the most appropriate therapeutic agents for immuno-compromised people, who cannot be helped by bacteriostatic and fungistatic antimicrobials.

The fact that some of the organisms susceptible to the extracts have shown multi-drug resistance to conventional drugs, confirms the potential held by herbal medicine as the best alternative for therapy of the emerging diseases.

The use of combination-therapy in the application of such plants for medicinal purposes, especially in cases of multiple infections such as opportunistic diseases in HIV-infected victims, should be encouraged. This is so because this study recognized the differential activity of the plants against different groups of microorganisms. For instance *C. macrostachyus* was active against the fungi but less active against bacteria, while the reverse was true for *A. amara*.

6.1.2 RECOMMENDATIONS

- There is need for conducting more studies to identify and characterize the medicinal principles in the tested plants, which may serve as novel compounds for development of new and more effective antimicrobial drugs. This would prove very useful especially in this era when drug resistance is a major issue.
- There is need for enacting conservational measures that will ensure the continued existence of these plants so as to tap their potential in disease management.
- There is need for toxicity tests to be performed on these extracts before clinical trials are initiated.

- Since this study involved the evaluation of activity of specific plant organs, it would be interesting to investigate other organs of the same plants to establish their activity as well.
- It would also be valuable to perform synergistic studies to evaluate the performance of these plants when combined with conventional medicine.

REFERENCES

- Abd el Nabi, O.M., E.C. Reisinger, F.F. Reinthaler, F. Still, U. Eibel, and G.J. Krejs. 1992. Antimicrobial activity of *Acacia nilotica* (L.) Wild. ex Del. Var. *nilotica* (Mimosaceae). *Journal of Ethnopharmacology*, 37: 77-99
- Adeniyi, B.A., and R.O. Odufowora. 2003. *In vitro* antimicrobial properties of *Aspilia Africana* (Compositae). *Journal of Biomedical Research*, 3: 3, 2000, pp. 167 -170
- African Union. 2001. Decade for African Traditional Medicine (2001-2010) (AU/AHG Dec. 164 XXXVII, 2001) Lusaka: 171-174.
- Akpata, L. 1979. The practice of herbalism in Nigeria. In: *African Medicinal Plants* (ed. Sofowora, E.A.) University of Ife Press, Ife, Nigeria: 13-20.
- Alade, P.I., and O.N. Irobi. 1993. Antimicrobial activities of crude extracts of *Acalypha wilkesiana*. *Journal of Ethnopharmacology*, 39: 1-9.
- Al-Shamma, A., and L.A. Mitscher. 1979. Comprehensive survey of indigenous Iraqi plants for potential economic value. 1. Screening results of 237 species for alkaloids and antimicrobial agents. *Journal of natural products*, 42: 633-642.
- Amaral, J.A., A.Ekins, S.R. Richards, and R. Knowles. 1998. Effect of selected monoterpenes on methane oxidation, denitrification and aerobic metabolism by bacteria in pure culture. *Applied Environmental Microbiology*. 64:520-525.
- Ayafor, J.F., M.H.K., Tchuendem, and B. Nyasse. 1994. Novel bioactive diterpenoids from *Afromum aulacocarpos*. *Journal of Natural Products*, 57:917-923.
- Balandrin, M.F., J.A. Klocke, E.S. Wurtele, and W.H. Bollinger. 1995. Natural plant chemicals: sources of industrial and medicinal materials. *Science* 228: 1154-60.
- Barrow, E.G.C. 1996. *The dry lands of Africa: Local participation in tree management*. Initiative Publishers, Nairobi.
- Barry, A. L., M. B. Coyle, C. Thornsberry, E. H. Gerlach, and R. W. Hawkinson. 1979. Methods of measuring zones of inhibition with the Bauer-Kirby disc susceptibility test. *Journal of Clinical Microbiology*, 10:885-889.
- Barry, A.L. 1976. *Principles and practices of microbiology*, Lea and Fabager, Philadelphia
- Bauer, A. W., W. M. M. Kirby, J. C. Sherris and M. Truck. 1966. Antibiotic susceptibility testing by standardized single disc method. *American Journal of Clinical Pathology*, 45: 493-496.
- Beentje, H. 1994. *Kenya trees, shrubs and lianas*. National Museums of Kenya,

Nairobi.

- Boegh, H.O., J. Andreassen, and J. Lemmich. 1996. Antihelmintic usage of extract of *Embelia schimperi* from Tanzania. *Journal of Ethnopharmacology*, 50: 35-42.
- Borris, R. P. 1996. Natural products research: perspectives from a major pharmaceutical company. *Journal of Ethnopharmacology*, 51:29-38
- Brantner, A., Z. Males, S. Pepeljnjak, and A. Antolic. 1996. Antimicrobial activity of *Paliurus spina-christi* Mill. *Journal of Ethnopharmacology*, 44:35-40.
- Brenner, V. C., and J. C. Sherris. 1972. Influence of different media and bloods on the results of diffusion antibiotic susceptibility tests. *Antimicrobial Agents Chemotherapy*, 1:116-122.
- Bringman, G., S. Gramatokis, and P. Proksch. 1992. Feeding deterrence and growth retarding activity of the thylisoquinoline alkaloid dioncophylline A against *Spodoptera littoralis*. *Phytochemistry*, 32: 3821 – 3825.
- Burkill, I.H. 1966. A Directory of the economic products of the Malay Peninsula. Ministry of Agriculture and Co-operatives, Kuala Lumpur, Malaysia, 2444 pp.
- Bussmann, R.W., G.G. Gilbreath, J. Solio, M. Lutura, R. Lutuluo, K. Kunguru, N. Wood, and S.G. Mathenge. 2006. Plant use of the Maasai of Sekenani Valley, Maasai Mara Kenya. *Journal of Ethnobiology and Ethnomedicine*, 2: 22.
- Chhabra, S.C., F.C. Uiso, and E.N. Mshiu. 1984. Phytochemical screening of Tanzanian Medicinal Plants. *Journal of Ethnopharmacology*, 11: 157-179.
- Chhabra, S.C., F.W. Muregi, E.N.M. Njagi, C.C. Langat-Thoruwa, W.M. Njue, A.S.S. Orago, S.A. Omar, and I.O. Ndiege. 2003. *In vitro* antiplasmodial activity of some plants used in Kisii, Kenya against malaria and their chloroquine potentiation effects. *Journal of Ethnopharmacology*, 84: 235-239.
- Chiang-su New Medical Institute. 1977. Chinese Materia Medica, Shangai Scientific Technology Publishing Co., China, 906 pp.
- Chopra, R.N., I.C. Chopra, K.L. Handa, and D. Kapur. 1958. Chopras indigenous drugs of India. 2ND Edn., U.N. Dhur & Sons Private Ltd. Calcutta. 816 pp.
- Cichewicz, R.H., and P.A., Thorpe. 1996. The antimicrobial properties of Chile peppers (*Capsicum* species) and their uses in Mayan medicine. *Journal of Ethnopharmacology*, 52:61-70.
- Clark, W.S. 1981. Antimicrobial activities of phenolic constituents of *Magnolia grandiflora* L. *Journal of pharmaceutical sciences*, 70. 951-952.

- Cohen, M.L. 1992. Epidemiology of drug resistance: implications for a post-antimicrobial era. *Science*, 257, 1050-1055.
- Cowan, M.M. 1999. Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*. 12 (4) :564-582.
- Crevost, C., and A. Petalot. 1929. Catalogue des produits de l' indochine : Plantes medicinales. *Bulletin Economique de l' indochine* 37: 516-528.
- Dixon, R.A., P.M. Dey, and C.J. Lamb. 1983. Phytoalexins: enzymology and molecular biology. *Advanced Enzymology*, 55:1-69.
- Doern, G.V., A.B. Brueggemann, H. Huynh, and P. Rhomberg. 1999. Antimicrobial resistance with *Streptococcus pneumoniae* in the United States, 1998. *Emerging Infectious Diseases*, 5:757-765.
- Dominguez, X.A., and J.B. Alcorn. 1985. Screening of Medicinal plants used by Huastec Mayans of North Eastern Mexico. *Journal of Ethnopharmacology*, 13: 139-156.
- Dorsche, W., and H. Wagner. 1991. New antiasthmatic drugs from traditional medicine. *International Archives of Applied Immunology*, 94: 262 – 265.
- Duke, J.A. 1985. Handbook of medicinal herbs. CRC Press, Inc., Boca Raton, Fla.
- Ellof, J.N. 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology* 60, 1-6.
- Ericsson, H.M., and J.C. Sherris. 1971. Antibiotic sensitivity testing. Report of an International Collaborative Study. *Acta Pathol. Microbiol. Scand. Sect. B Suppl.* 217.
- Fabry, W., P. Okemo, and R. Ansorg. 1996 a. Activity of East African medicinal plants against *Helicobacter pylori*. *Chemotherapy*, 42(5): 315 – 317.
- Fabry, W., P. Okemo, and R. Ansorg. 1996 b. Fungistatic and fungicidal activity of East African medicinal plants. *Mycoses*, 39(1-2):67-70.
- Fabry, W., P. Okemo, and R. Ansorg. 1998. Antibacterial activity of East African medicinal plants, *Journal of Ethnopharmacology*, 60(1):79-84.
- Fabry, W., P. Okemo, W.E., Mwatha, S.C., Chhabra, and R. Ansorg. 1996. Susceptibility of *Helicobacter pylori* and *Candida spp.* to the East African plant *Terminalia spinosa*. *Arzneimittelforschung*, 46(5): 539 – 540.
- Fauci, A. 1998. New and reemerging diseases: The importance of biomedical research. *Emerging Infectious Diseases*. (www.cdc.gov/ncidod/EID/vol4no3/fauci). 4:3.

- Fleischer, T.C., E.P.K. Ameada, and I.K. Sawyer. 2002. Antimicrobial activity of the leaves and flowering tops of *Acanthospermum hispidum*. *Fitoterapia* 74. 130-132.
- Freiburghausa, F., A. Stecka, H. Pfandera, and R. Brunb. 1998. Bioassay-guided isolation of a diastereoisomer of kolavenol from *Entada abyssinica* active on *Trypanosoma brucei* rhodesiense. *Journal of Ethnopharmacology*. 61:179-183.
- Gachathi, F.N., 1989. Kikuyu Botanical Dictionary of Plant Names and Uses. AMREF, Nairobi.
- Greenwood, D. 1995. *Antimicrobial Chemotherapy*. Oxford University Press. 1: 13.
- Gundidza, M., and N. Gaza. 1993. Antimicrobial activity of *Dalbergia melanoxylon* extracts. *Journal of Ethnopharmacology*, 40: 127-130.
- Hadi, S., and J. B. Bremmer. 2001. Initial Studies on Alkaloids from Lombok Medicinal Plants. *Molecules*, 6:117 – 129.
- Harborne, J.B. 1984. *Phytochemical methods. A guide to modern techniques of plant analysis*, 2nd edition, London: Chapman and Hall, 2: 89.
- Hartwell, J.L. 1968. Plants used against cancer. A survey. *Lloydia*, 31: 71-170.
- Haslam, E. 1996. Natural polyphenolics (vegetable tannins) as drugs: possible modes of action. *Journal of Natural Products*, 59:205-215.
- Hien, T.T., and N.J. White. 1993. Quinghaosa. *Lancet* 341: 603-08.
- Hopp, K.H., L.V., Cunningham, M.C. Bromel, L.J. Schermeister, and S.J. Wahba Khalil. 1976. In vitro antitrypanosomal activity of certain alkaloids against *Trypanosoma lewisi*. *Lloydia*, 39: 375-377.
- Hugo W.B., and A.D Russel. 1983. *Pharmaceutical microbiology*, 3rd Edition. Blackwell Scientific publication, 47.
- Hussein, G., H. Miyashiro, N. Nakamura, M. Hattori, N. Kakiuchi and K. Shimotohno. 2000. Inhibitory effects of Sudanese medicinal plant extracts of hepatitis C virus (HCV) protease. *Phytother. Res.* 14:510-516.
- Ikan, R. 1969: *Natural products: A laboratory guide*, Jerusalem: Israel: Israel University Press. *International Archives of Applied Immunology*, 94: 262-265.
- Iwu, M. 1993. *Handbook of African medicinal plants*. CRC Press, Boca Raton.
- Iwu, M.W., A.R. Duncan, and C.O. Okunji. 1999. New antimicrobials of plant origin. p. 457–462. In: J. Janick (ed.), *Perspectives on new crops and new uses*. ASHS Press, Alexandria, VA.

- Johns, T., J.O. Kokwaro and E.K. Kimanani. 1990. Herbal remedies of the Luos of Siaya District, Kenya; Establishing criteria for concensus. *Economic Botany*, 44(3): 369-381).
- Jorgensen, J. H., and J. D. Turnidge. 2003. Susceptibility test methods: Dilution and Disc diffusion methods. *Manual of Clinical Microbiology*. 8th ed. 1108 - 1127.
- Kamanzi, K.A., M. Kone, C. Terreaux, D. Traore, K. Hostettmann, and M. Dosso. 2002. Evaluation of the antimicrobial potential of medicinal plants from the Ivory Coast. *Phytotherapy research*. 16 (5):497 – 502.
- Kariuki, S., C. Gilks, R. Brindle, B. Batchelor, J. Kimari, and P. Waiyaki. 1994. Antimicrobial susceptibility and presence of extachromosomal DNA in *Salmonella* and *Shigella* isolates from patients with AIDS. *East Africa Medical Journal*, 71(5): 292-296.
- Kathryn, L. Ruoff, R.A. Whiley, and D. Beighton. 2003. Streptococcus. *Manual of Clinical Microbiology*, 8:405.
- Kinghorn, A.D., and M.F. Balandrin. 1993. Human medicinal agents from plants. *American Chemical Society Symposium Ser.* 539: 1-348.
- Kioy, D.W. 1989. A study of the Chemistry and pharmacology of phytochemicals from three spp. Of Canellaceae. Ph.D. thesis, University of Stratheclyde, Scotland: 241.
- Kokwaro, J.O. 1976. Medicinal plants of East Africa. East African Literature Bureau, Kampala, Nairobi, Dar es salaam.
- Kokwaro, J.O. 1993. Medicinal plants of East Africa. East African Literature Bureau, Kampala, Nairobi, Dar es salaam.
- Kubo, I., M. Himejima, K. Tsujimoto, and H. Muroi. 1992. Antibacterial activity of crinitol and its potentiation. *Journal of Natural Products*, 55 (6): 780-785.
- Lambo, J.O. 1979. The healing powers of herbs with special reference to obstetrics and gynaecology. In: *African Medicinal plants* (ed. Sofowora, E.A.) University of Ife Press, Ife, Nigeria: 23-31.
- Lewis, W. H., and M. P. Elvin-Lewis. 1995. Medicinal plants as sources of new therapeutics. *Ann. Mo. Bot. Gard.* 82:16-24.
- Lucas, E.H., Lickfeldt, A., Gottshall, R.Y. and Jennings J.C. (1951). The occurrence of anti bacterial in seeds with special reference to *Mycobacterium tuberculosis*. *Bulletin of Torrey Botanical Club* 78; 310.
- Machocho, A.K., S.C. Chhabra, F. Viladomat, C. Codina, and J. Bastida. 1999. Alkaloids

- from *Ammocharis tinneana*. *Phytochemistry*, 51: 1185-1191.
- Machocho, A.K., P.C. Kiprono, S. Grinberg, and S. Bittner. 2002. Pentacyclic triterpenoids from *Embelia schimperi*. *Phytochemistry* 62: 573-577.
- Magro-Filho, O. and A. de Carvalho. 1994. Topical effect of propolis in the repair of sulcoplasties by the modified Kayanjian technique: Cytological and Clinical evaluation: *Journal of Nihom University School of Dentistry*, 36: 102-111.
- Mathabe M.C., R.V. Nikolova, N. Lall, and N.Z. Nyazema. 2006. Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. *Journal of Ethnopharmacology*, 105(1-2): 286-93.
- Mather, S.B., and L. Gonzalez. 1982. Identification of terpenoids from leaves of *Piptocarpha peretoxa* and their biological activities. *Journal of Natural Products* 45. 495-496.
- Matu, E.N., and J.V. Staden. 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *Journal of Ethnopharmacology* 87:35-41.
- Maundu, P.M., G.W. Ngugi, and C.H.S. Kabuye. 1999. *Traditional Food Plants of Kenya*. National Museums of Kenya, Nairobi.
- Mayorga, R.P., and H. Espinoza. 1970. Coccidiomycosis in Mexico and Central America. *Mycopathologia et mycologia applicata* 41, 3-11.
- McChesney, J.D., A.M. Clark, and E.R. Silveira. 1991. Antimicrobial Diterpenes Of *Croton sonderianus*, 1. Hardwickic and 3,4-secotrachylobanoic acids. *Journal of Natural Products*. 54 (6): 1625 – 1633.
- Mcdowell, P.G., W. Lwande, S.G. Deans, and P.G. Waterman. 1988. Volatile resin exudate from stem bark of *Commiphora rostrata*: Potential role in plant defence. *Phytochemistry* 27(8): 2519-2521.
- McCutcheon, A.R., S.M. Ellis, R.E.W. Hancock and G.H.N. Towers. 1992. Antibiotic screening of medicinal plants of the British Colombian native people. *Journal of Ethnopharmacology*, 37(3): 213-223.
- Mechling, W.H. 1959. The Malecite Indians, with notes on the Micmacs. *Anthropologia* 8 (1959) 239-263.
- Mitscher, L.A., M.S. Bathala, G.W. Clarke, and J.L. Beal. 1975. Antimicrobial agents from higher plants. The quaternary alkaloids of *Ptelea trifoliata*. *Lloydia* 38(2):109-116.

- Mitscher, L.A., S. Drake, S.R. Collapudi, and S.K. Okute. 1987. A modern look at Folklore use of anti-infective agents. *Journal of Natural Products* 50, 1025 (1987).
- Muregi, F.W., S.C. Chhabra, E.N.M. Njagi, C.C. Lang'at-Thoruwa, W.M. Njue, A.S.S. Orago, S.A. Omar and I.O. Ndiege. 2002. *In-vitro* Antiplasmodial Activity of Some Plants used in Kisii, Kenya against Malaria and their Chloroquine potentiation effects. *Journal of Ethnopharmacology*, 84: 235-239.
- Muriithi, M.W., W.R. Abraham, J. Addae-Kyereme, I. Scowen, S.L. Croft, P.M. Gitu, H. Kendrick, E.N.M. Njagi and C.W. Wright. 2002. Isolation and in-vitro Antiplasmodial Activities of Alkaloids from *Teclea trichocarpa*: *In vivo* Antimalarial Activity and X-ray Crystal Structure of Normelicopicine. *Journal of Natural products*, 65: 956-959.
- Nascimento, G.G.F., J. Locatelli, P.C. Freitas, and G.L. Silva. 2000. Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic-Resistant Bacteria. *Brazilian Journal of Microbiology*, 31:247-256.
- NCCLS, 1987. Methods for determining bactericidal activity of antimicrobial agents. Document M26-P. National Committee for Clinical Laboratory Standards, 7(2):35-76.
- NCCLS, 2000 a. Performance standards for antimicrobial disc susceptibility testing. Approved standard M2-A7. NCCLS, Wayne, Pa.
- NCCLS, 2000 b. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. NCCLS, Wayne, Pa.
- Nostro, A., L. Cellini, S. Di Bartolomeo, E. Di Campli, R. Grande, M. A. Cannatelli, L. Marzio, and V. Alonzo. 2005. Antibacterial effect of plant extracts against *Helicobacter pylori*. *Phytotherapy Research*, 19(3): 198 – 202.
- Nyazema, N. Z. 1987. Medicinal plants of wide use in Zimbabwe. In: A.J.M. Leeuwenberg, Medicinal and poisonous plants of the tropics- Proceedings of symposium 5-35 of the 14th International Botanical Congress, P.38, Pudoc Wageningen, Berlin.
- Odebiyi, O.O. 1985. Antimicrobial and antifungal properties of the extracts of *Jatropha podogrica* stem. *Fitoterapia*, 56, 297 – 299.
- Oldfield, M. 1984. The value of conserving genetic resources. Washington, D.C. U.S. Dept. of the Interior.
- Okemo, P.O., S.M. Kariuki and P.W. Ndung'u. 2004. Multi-drug resistance in *E.coli*, *Salmonella* and *Shigella* causing diarrhoea in children below 5 years in Kenyan hospitals. The Second Regional Microbiology Conference. *Journal of Tropical Microbiology and Biotechnology*, Nairobi, Kenya. 2: 28-35.

- Oketch-Rabah, H.A., S.F. Dossaji, and E.K. Mberu. 1999. Antimalarial activity of some Kenyan medicinal plants. *Pharmaceutical Biologia*, 37: 329-334.
- Omulokoli, E., B. Khan, and S.C. Chhabra. 1997. Antiplasmodial activity of four Kenyan medicinal plants. *Journal of Ethnopharmacology*, 56:133-137.
- Pamplona-Roger, G.D., (1999). *Encyclopedia of Medicinal Plants*. Editorial Safeliz, Spain.
- Paul, S., M.O.M. Tanira, and N.R. Bonna. 1992. The role of medicinal plants in the healthcare system of United Arab Emirates: Past, present and future. In: *First World Congress on Medicinal Aromatic Plants for Human Welfare Maastricht, the Netherlands (Abstracts)*. P. 16.
- Pereda-Miranda, R., M.J. Hernandez Villavicencio, M. Novelo, P. Ibarra, H. Chai, and J.M. Pezzuto. 1993. Structure and stereochemistry of pectinolides A-C, novel antimicrobial and cytotoxic 5,6-dihydro- α -pyrones from *Hyptis pectinata*. *Journal of Natural Products*, 56(4):583-93.
- Peres, M.T.L.P., F.D. Monache, A.B. Cruz, M.G. Pizzolatti, and R.A. Yunes. 1997. Chemical composition and antimicrobial activity of *Croton urucurana* Baillon (Euphorbiaceae). *Journal of ethnopharmacology*, 56:223-226.
- Perrett, S., P.J. Whitfield, L. Sanderson, and A. Bartlett. 1995. The plant molluscicide *Millettia thonningii* (Leguminosae) as a topical antischistosomal agent. *Journal of ethnopharmacology*, 47:49-54
- Peterson, L.R., and C.J. Shanholtzer. 1992. Tests for Bactericidal Effects of Antimicrobial Agents: technical performance and clinical relevance. *Clinical Microbiology Reviews*, 5(4): 420-432.
- Pettit, G. R., V. Gaddamidi, A. Goswami and G. M. Cragg. 1984. *Journal of Natural Products*, 47, 796.
- Pezzuto, J. M., M. Woongchon, L. Long-ze, G. A. Cordell, A. Neszmely, and H. Wagner. 1992. Budmunchiamines D-I from *Albizia amara*. *Phytochemistry*. 31 (5): 1795 – 1800.
- Philip's Atlas. 2004. East African Publishers, Nairobi, Kampala, Dar es Salaam. Pp. 33.
- Phillipson, J.D., and M.J. O'Neill. 1987. New leads to the treatment of protozoal infections based on natural product molecules. *Acta Pharm. Nord*. 1:131-144.
- Phillipson, J.D., C.W. Wright, G.C. Kirby, and D.C. Warhurst. 1993. Tropical plants as sources of antiprotozoal agents. In Downum K.R., Romeo, J.T., Stafford, H.A., eds., *New York: Plenum*, 1-40.
- Rasheed, J.K., and F.C. Tenover. 2003. Detection and characterization of antimicrobial

- resistance genes in bacteria. *Manual of Clinical Microbiology*, 75: 1196.
- Rasoanaivo, P., S. Ratsimamanga-Urveg. 1993. Biological evaluation of plants with reference to the Malagasy flora. Monograph of the IFS-NAPRECA Workshop on Bioassays, Antananarivo, Malagasy, pp. 72-79.
- Reiner, R. 1982. Antibiotics- An introduction, F. Hoffman La Roche & Co., Basle, Switzerland, P. 70.
- Robbers, J., M. Speedie, and V. Tyler. 1996. Pharmacognosy and pharmacobiotechnology. Williams and Wilkins, Baltimore. p. 1-14.
- Robert, A., and B. Meunier. 1998. Is Alkylation the Main Mechanism of Action of the Antimalarial Drug Artemisinin? *Chemistry Society Review*, 27: 273 – 279.
- Rukungu G.M., and P.G., Waterman. 1996. New macrocyclic spermine (Budmunchiamine) alkaloids from *Albizia gummifera*: With some observations on the structure- activity relationships of the Budmunchiamines. *Journal of Natural Products*, 59 (9): 850 – 853.
- Sahm, D.F., M.E. Jones, M.L. Hickey, D.R. Diak, S.V. Mani, and C. Thornsberry. 2000. Resistance in Surveillance of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* isolated in Asia and Europe, 1997-98. *Journal of Antimicrobial chemotherapy*, 45:457-460.
- Sakanaka, S., M. Kim, M. Taniguchi, and T. Yamamoto. 1989. Antibacterial substances in Japanese green tea extracts against *Streptococcus mutans*, a carcinogenic bacterium. *Agricultural Biological Chemistry* 53 (9): 2307-2311.
- Sandra M.N., L. Clara, and C.W. Wright. 2000. A review of antimycobacterial natural products. *Journal of Antimicrobial chemotherapy*, 14 (5) : 303 – 322.
- Santos, P.R.V., A.C.X. Oliveira, T.C.B. Tomassini. 1995. Controle microbiológico de produtos fitoterápicos. *Rev. Farm. Bioquím.* 31, 35-38.
- Sawer, I., M. Berry, M. Brown, and J. Ford. 1995. The effect of Cryptolepine on the morphology and survival of *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae*. *Journal of Applied Bacteriology*, 79: 314-321.
- Shadomy, S., and M. A., Pfaller. 1985. Laboratory studies with antifungal agents: Susceptibility Tests and Quantitation in Body Fluids. *Antimicrobial Agents and Chemotherapy*, 117: 1173-1183.
- Shanholtzer, C.J., L.R. Peterson, M.L. Mohn, J.A. Moody and D.N. Gerding. 1984. MBCs for *Staphylococcus aureus* as Determined by Macrodilution Techniques. *Antimicrobial Agents and Chemotherapy*, 26(2): 214-219.

- Sheehan, M.P., M.H.A. Rustin, and D.J. Atherton. 1992. Efficiency of traditional Chinese herbal therapy in adult atopic results of a double-blind placebo-controlled study. *Lancet*: 340: 13-17.
- Sherris, J. C., and J. A., Washington. 1980. Laboratory tests in chemotherapy. *Manual of clinical microbiology*. American society of microbiology, 44: 456-457.
- Shori, R. 2000. Impact of socioeconomic indicators on the health of children in Kenya households, with emphasis on poverty, malnutrition, child mortality, morbidity and HIV/AIDS. Malaguerra C, Fust W, Edition. International Association for Official Statistics Conference. Moutrex, Switzerland. pp 185-192.
- Sidigia, I., C. Nyaigotti-Chacha, and M.P. Kanunah. 1990. Traditional medicine in Africa. East African Educational Publishers, Nairobi.
- Sleigh J.D., and M.C. Timbury. 1981. Notes on medical bacteriology. Churchill Livingstone Edinburgh, London, Melbourne and New York, 130. © 2000 - Ibadan Biomedical Communications Group.
- Sofowora, F.A. 1982. Medicinal plants and traditional medicine in Africa. John Wiley and Sons, N.Y., U.S.A.
- Soleeki, (1975). Shanida iv, a Neanderthal flower burial in Northern Iraq. *Science* 190: 880-881.
- Steers, E., E.L. Foltz, B.S. Graves, and J. Riden. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiotic Chemotherapy*, (Basel) 9:307-311.
- Stratton, C.W., and R.C. Cooksey, 1991. Susceptibility tests: special tests, p. 1153-1165. In A. Balows, W.J. Hausler, Jr., K.L. Hermann, H.D. Isenberg and H.J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
- Taniguchi, M., and I. Kubo. 1993. Ethnobotanical drug discovery based on medicinemen's trials in the African savannah: screening of East African plants for antimicrobial activity II. *Journal of Natural Products*, 56(9): 1539-1546.
- Taniguchi, M., Y. Yano, K. Motoba, T. Tanaka, O. Susumu, H. Haraguchi, K. Hashimoto, and I. Kubo. 1988. Polygodial-induced Sensitivity to Rifampicin and Actinomycin D of *Saccharomyces cerevisiae*. *Agricultural Biological Chemistry*, 52 (7), 1881-1883.
- Tarus, K.T., A.K. Machocho, C.C. Lang'at-Thoruwa and S.C. Chhabra. 2002. Flavonoids from *Tephrosia aequilata*. *Phytochemistry*, 60: 375-379.

- Thomson, W.A.R. (ed.). 1978. Medicines from the Earth. McGraw-Hill Book Co., Maidenhead, United Kingdom.
- Toda, M., S. Okubo, H. Ikigai, T. Suzuki, Y. Suzuki, Y. Yara, and T. Shimamura. 1992. The protective ability of tea catechins against experimental infection by *Vibrio cholerae* O1. Microbiol. Immunol. 36:999-1001.
- Trease, G. and W. Evans. 1972. Pharmacognosy, Univ. Press, Aberdeen, Great Britain, 161-163.
- Tsuchiya, H., M. Sato, T. Miyazaki, S. Fujiwara, S. Tanigaki, M. Ohyama, T. Tanaka, and M. Linuma. 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. Journal of ethnopharmacology, 50:27-34.
- Turano, A., G. Scura, A. Caruso, C. Bonfanti, R. Luzzati, D. Basetti, and N. Manca. 1989. Inhibitory effect of papaverine on HIV replication *in vitro*. AIDS Res. Hum. Retrovir. 5: 183-191.
- van Wyk, B-E., B. Oudtshoorn, and N. Gericke. 1999. Medicinal plants of South Africa. Pretoria: Briza Publications, Pretoria.
- Vila, J., M. Vargas, H. Urassa, H. Mshinda, D. Schellemerge, and J. Gascon. 1999. Antimicrobial resistance of diarrhoeagenic *E. coli* isolated from children under age 5 years from Ifakara, Tanzania. Antimicrobial Agents and Chemotherapy, 43(12): 3002-3004.
- Vokou, D., K. Katradi, and S. Kokkini. 1993. Ethnobotanical survey of Zagori (Epirus Greece), a renowned centre of folk medicine in the past. Journal of Ethnopharmacology, 39(3): 187-96.
- Wanyonyi, A.W., S.C. Chhabra, G. Mkoji, W. Njue, and P.K. Tarus. 2003. Molluscidal and antimicrobial activity of *Solanum aculeastrum*. Journal of Ethnopharmacology.
- Washington, J.A., and V.L. Sutter. 1980. Dilution susceptibility test: agar and macro-broth dilution procedures. Manual of clinical microbiology. American Society of Microbiology. 4th ed. Pp 453.
- Waterman, P.G. 1992. Roles for secondary metabolites in plants. In: Chadwick, D.J., Whelan, J. eds. Secondary metabolites: their function and evolution. Ciba Foundation Symposium 171. Chichester: Wiley: 255-75.
- Watt, J.M., and M.G. Breyer-Brandwijk. 1962. Medicinal and poisonous plants of Southern and Eastern Africa, 2nd edn. E & S. Livingstone Ltd., Edinburgh, London.
- Woongchon M., G. T., Tan, G. A., Cordell, and J. M., Pezzuto. 1991. Biological activity

of novel macrocyclic alkaloids (Budmunchiamines) from *Albizia amara* detected on the basis of interaction with DNA. *Journal of Natural Products*, 54 (6): 1531-1542.

WHO (2001). Promoting the role of Traditional Medicine in Health Systems, A Strategy for the African Region (AFR/RC 509), Regional Office for Africa.

APPENDIX i

QUESTIONNAIRE

Information on medicinal plants

(One form to be completed for each plant)

1. Name of interviewer.....
2. Institution.....
3. Date.....
4. Name and address of interviewee.....
.....
5. Plant specimen number.....
6. Vernacular name.....
7. Disease condition treated by the drug.....
8. Plant organ used.....
9. Time of collection.....
10. Where plant is commonly located.....
11. Method of preparation of the plant.....
.....
.....
.....
12. Dosage and method of administration
13. Any other plants or ingredients that may be combined with the plant.....
.....
.....