ANTIMICROBIAL ACTIVITY, PHYTOCHEMICAL PROFILE AND CYTOTOXICITY OF SELECTED MEDICINAL PLANTS FOUND IN KAPTUMO DIVISION IN NANDI COUNTY, KENYA

BY

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DECLARATION

DECLARATION BY CANDIDATE

This thesis is my own original work and has not been presented in any other University for any other awards. Works from other sources have been dully acknowledged.

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DEDICATION

I dedicate this work to my parents, brother and sisters for their support an encouragement.

ABSTRACT

In Kenya, medicinal plants have been used by the local people as traditional medicine to treat different human ailments from time immemorial. The efficacy and safety of most of these plants has not been determined hence the present study conducted at Kaptumo Division in Nandi County seeks to determine to ethnobotanical survey, antimicrobial activities, profile the phytochemicals and to determine cytotoxicity of the six selected medicinal plants namely: *Kigelia africana* (Lam.) and Benth, *Periploca linearifolia* Dill & A. Rich, *Ekebergia capensis* Sparrm, *Ehretia cymosa* Thonn, *Fagaropsis angolensis* (Engl.) Dale and *Dovyalis abyssinica* (A. Rich) Warb. that are commonly used to treat infectious diseases.The ethnobotanical survey was carried out using a structured questionnaire. Fresh plants were collected from the field and air dried under shade at 25^{o}C and later ground into powder and extracted using acetone and water. Phytochemicals from the extracts were profiled using thin layer chromatography method while *in vitro* cytotoxicity test was carried out following a modified rapid calorimetric assay. The extracts were tested against standard pathogenic microorganisms *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 32602 and clinical isolates of Methicillin resistant *Staphylococcus aureus*, *Shigella dysentriae, Microsporum gypseum* and *Trychophyton mentagrophytes* using disc diffusion and broth dilution methods. The solvents used for extraction were used as negative control while Fluconazole for fungi and Gentamycin for bacteria were used as positive controls. Minimum inhibitory concentrations (MIC), Minimum bactericidal concentrations (MBC) and cytotoxicity test for extracts with inhibition zone diameters of 10.0 mm and above (significance activity) were determined. All the plant extracts indicated presence of phenols, terpenoids and flavonoids. In disc diffusion assay water extracts of *E. capensis* were the most active and those of *F. angolensis* were the least against *S. aureus* with inhibition zone diameters of 14.7 mm and 6.0 mm, respectively. Acetone extracts of *E. capensis* and *K. africana* had a MIC of 3.125 mg/ml and 6.25 mg/ml, respectively. All the selected plant extracts were bactericidal except *E. cymosa* which was bacteriostatic. There was no activity at 100 mg/ml observed against fungi tested. Cell toxicity showed that most of the plant extracts tested were not cytotoxic having a CC_{50}(µg/ml) of ≥100 against Vero cell lines except the acetone extracts of *E. capensis* that were moderately toxic with a CC_{50}(µg/ml) of 12.5 , suggesting former extracts may be safe as antimicrobials. Significant variability within groups of plants, solvents and organisms at 95% confidence interval ($p_{=}0.05$) was determined, and the results were found to be significant. This study enhances the validity of the medicinal plants as resources that can be relied on to provide effective and affordable healthcare to the local communities. Further research is therefore recommended to isolate, purify and characterize these chemical constituents with a view to supplementing conventional drug development especially in developing countries. The antibacterial activity of the plants under investigation demonstrated support for the claimed antimicrobial uses of the plants in the traditional medicine and provides scientific proof for their medicinal.

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LIST OF ABBREVIATIONS/ACRONYMS

ANOVA Analysis of Variance

ATCC American Type Culture Collection

DMSO Dimethylsulfoxide

CC_{50} Cytotoxic Concentration killing 50% of the cells

CLSI: Clinical Laboratory Standard Institute

CMR Centre for Microbiology Research

CTMDR Centre for Traditional Medicine and Drug Research

FBS Fetal bovine serum

FDA Food and Drugs Act

HIV: Human Immunodeficiency Virus

KEMRI: Kenya Medical Research Institute

MEM: Minimum Essential Media

MBC: Minimum Bactericidal Concentration

MHA: Muller Hinton Agar

MIC: Minimum Inhibition Concentration

MR*SA*: Methicillin Resistant Staphylococcus Aureus

MTT Thiazolyl blue tetrazolium bromides

NCCLS: National Committee for Clinical Laboratory Standard

OR*SA*: Oxacillin Resistant Staphylococcus Aureus

OD: Optical Density

PBP Penicillin Binding Proteins

PBS Phosphate buffered saline

SDA: Sabouraund Dextrose Agar

SPSS Statistical package for Social scientists

WHO: World Health Organization

DEFINITION OF TERMS

Climbers: Herbaceous plants species twining or provided with tendrils to aid their Support

Family: A unit of classification of species including the genera of species.

Habitat: An area occupied by an organism.

Herbs: Non-woody stemmed plants that die back after flowering and seeding.

Shrubs: Woody stemmed plants with lateral branches coming from just below the ground and under 7.5 m height.

Species: Smallest unit of classification of an organism.

Tree: Woody plant 7.5 m or more in height branch.

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CHAPTER ONE

INTRODUCTION

1.1 Background information

The use of herbs and medicinal plants as primary health care is a universal phenomenon. Today, as much as 80% of the people in the world depend on traditional medicine as primary health care (Khaleel and Sudarshanam, 2011). Every culture on the earth, through written or oral tradition, has relied on the vast variety of natural chemical compounds found in plants for their therapeutic properties**,** (Anowi *et al.*, 2012) such as drug Artemisia isolated from Artemisia plant in 1972 by a Chinese scientist. The therapeutic potential of many plants has been utilized by Indian traditional medicine practitioners' like Siddha and Ayurveda. The Islamic system of medicine for instance the unani are backed by a large number of texts, thereby enjoying standardized theoretical as well as practical support (Ranjit *et al.*, 2001). The Siddha and Ayurveda systems may thus be regarded to constitute the classical systems of Indian medicine, Owing to their availability and affordability, there has been resurgence in the consumption and demand for medicinal plants all over the world, (Jayashree and Maneemegalai, 2008).

In Kenya traditional medicine is widely practiced as documented in ethnobotanical surveys (Miaron *et al.*, 2004; Kareru *et al*., 2007; Njoroge and Bussman, 2007). The high cost of imported conventional drugs and/or inaccessibility to western conventional health care system has led to over reliance on traditional medicine. On the other hand, even when conventional health care facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective (Munguti, 1997; Miaron *et al.*, 2004).

Some plants like *Heteromorpha trifoliata (*Wendl.) Eckl. & Zeyh. are used traditionally to treat stomach problems. This plant has been shown to have strong activity against several species of bacteria including *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella gallinarum* and S*taphylococcus albus* (Baker *et al*., 1995). Although, plants have been extensively used, assessment of their activity and scientific proof of their evidence and specific evaluation on toxicity is not readily available and could lead to serious complications (Rahman *et al*., 1996).

There is also an increase in resistance of pathogenic microorganisms to antibiotics, hence the need to develop new antimicrobial compounds. Infections associated with bacterial pathogens are among some of the indications treated using traditional remedies in Kenya (Njoroge and Bussman, 2007). Bacterial infections are prevalent due to various factors such as the HIV/AIDS pandemic, poor hygiene, overcrowding and resistance to conventional antimicrobials but natural products obtained from higher plants may provide a new source of antimicrobial agents with possibly novel mechanisms of action (Adenisa *et al.,* 2000). With the increase in number of people infected with human immunodeficiency virus (HIV) there has been intensive investigation into the plant derivatives which may be effective, especially for use in developing nations with little access to conventional medicines (De Clercq, 2005).

The increasing number of scholarly work on medicinal plants serve as clear evidence to the growing interest on ethnobotanical plants in Kenya and other parts of the world (Njoroge and Bussman, 2007). However, given the wide usage of these medicinal plants, only a small proportion of these plants have been studied so far. The study is

therefore aimed at determining the antimicrobial activities of water and acetone extracts of six selected medicinal plants used in Nandi county against pathogenic microorganisms.

1.2 Statement of the problem

Infectious diseases are major health problems that influence the general quality of life in Kenya and the other developing countries. In the past decade it has been witnessed that there is significant increase in the prevalence of microorganism's resistant to antibacterial and antifungal agents present in conventional drugs such as tetracycline, sulfonamides and chloramphenicol by enteric bacteria such as *Shigella dysentriae and* ketoconazole, flucanazole,terbinafine,amphotericin B ,nystatin. This resistance contributes to the increase in morbidity, mortality and health care costs. Secondly, the antibacterial and antifungal agents currently used in convectional drugs are costly and affordable to low income earners.Thirdly, the safety of the medicinal plants used in traditional herbal remedies needs to be investigated Therefore, medicinal plants present new therapeutic options for the treatment of infections caused by resistant microorganisms, though some of the plants that are utilized by the local people have not been tested scientifically, hence there is need for hence there is need for scientific validation of the plants used, alternative prevention and treatment options that are effective, safe, and economical.

Hence, the search for novel drugs that have minimal side effects and are affordable is necessary. Plants used as traditional medicines are considered as one of the possible alternative sources.

1.3 Justification of the study

Traditional medicine has remained as the most affordable and easily accessible source of treatment among the resource constrained communities. The efficacy and safety of most of the plants used among traditional communities in Kenya has not been established. Hence there is a lot of undiscovered potential in plants.

Plants and natural products remain a reservoir of potentially useful chemical compounds not only as drugs but also as unique templates that could serve as a starting point for synthetic drugs. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in the drug development programme in the pharmaceuticals industries, such as drug quinine isolated from *Cinchona* plant that is used all over the world to treat malaria. In addition, it is widely acknowledged that many current drugs were discovered on the basis of their indigenous use. Such indigenous knowledge also needs to be documented before it is lost forever with the passing on of the older generation. Hence this study seeks to determine the phytochemicals present, efficacy and safety of the selected medicinal plants from Kaptumo division. It is expected that this study will provide the baseline data on the antimicrobial activity, phytochemicals present and cytotoxicity of the selected plants used in the division.

1.4 Objectives

1.4.1 General objective

To determine an ethnobotanical survey, profile phytochemicals, antimicrobial properties and cytotoxicity of the selected medicinal plants used to treat infections related to microbial diseases from Kaptumo Division in Nandi County.

1.4.2 Specific objectives

1. To identify collected medicinal plants used in kaptumo Division

2. To carry out an ethnobotanical survey of the medicinal plants used in Kaptumo Division

3.To determine the phytochemical profiles of the selected medicinal plants.

4.To determine antimicrobial activities and compare the water and acetone extracts of selected medicinal plant extracts.

5.To determine the *in vitro* cytotoxicity of the active plant extracts of the selected medicinal plants.

1.5 Hypothesis

Null hypothesis

1.There are medicinal plants used found in kaptumo Division

2. There is ethnobotanical study of the medicinal plants used in Kaptumo Division

3. The selected medicinal plant extracts from kaptumo Division have no antimicrobial activity

4. The selected medicinal plant extracts from kaptumo Division have no active ingredients

5. The selected medicinal plant extracts from kaptumo Division have very high cytotoxicity

Alternative hypothesis

1.There no medicinal plants used in kaptumo Division

2. There are no ethnobotanical study of the medicinal plants used in Kaptumo Division

3.The selected medicinal plant extracts from Kaptumo Division have antimicrobial activities

4. The selected medicinal plant extracts from kaptumo Division have active ingredients

5. The selected medicinal plant extracts from kaptumo Division have no cytotoxicity

CHAPTER TWO

LITERATURE REVIEW

2.1 Documentation and the use of medicial plants

For a long period of time, plants have been a valuable source of natural products of therapeutic value to human health, especially in the last decade, with more intensive studies in natural therapies. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (Arunkumar and Muthuselvam, 2009).

The usage of herbal healthcare preparations is popular among the majority of people in the world especially in Asia, parts of Latin America and Africa. For example, it is estimated that 80% of the population in the aforementioned regions use such remedies as they are reported to have minimal side effects (Doughari, 2006). The same percentage of individuals from developed countries also makes use of traditional medicines. However, there is need to investigate such plants to understand their properties, safety and efficacy (Arunkumar and Muthuselvam, 2009).

Herbs and natural remedies are imported mainly from Europe and Asia; these include Chinese and Indian traditional medicines. Bulgaria which is in Eastern Europe is one of the largest producers of medicinal herbs in the world, exporting a total of 12,000 tons per year, most of it to the European Union and the United States. This is because Asian Medicinal Plants (AMP) is an integral part of the Bulgarian traditions and folk botanical knowledge and have had their place on the Bulgarian market from the past until now (Anonymous, 2011). The use of medicinal plants for pharmaceutical purposes has also gradually increased in Latin America especially in Brazil and

Uruguay (Gislene *et al*., 2008). Many studies have been conducted in Brazil and one of which documented that fifty eight plants were found to be active against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Muroi and Kibe, 2005).).

In Africa, it is estimated that seventy five percent (75%) of the population still relies on traditional healing practices and medicinal plants for their healthcare needs (Beentje, 1994). Africa has an ancient and rich tradition in the field of herbal medicine, which makes the use of medicinal plants in treating diseases an integral part of their culture.

In Kenya, according to Kokwaro, (2003) 10,000 plant species have been documented as medicinal especially among the pastoral communities in Kenya such as the Maasai, Samburu and the Kalenjins (Beentje,1994). Herbal knowledge is widespread in the Maasai community. Families are often able to care for their own health. For example, berries and other wild fruits are used to supplement the diet before they are eaten, mostly by women and children (Rainer *et al.,* 2006). Traditionaly the Samburu like the Maasai attribute most illnesses to the effect of pollutants that block or inhibit digestion. This can include "polluted" food, contagion through sick people as well as witchcraft. In most cases the treatment of illness involves herbal purgatives to cleanse the patient. There are however frequent indications of plant use for common problems like wounds, parasites, body aches and burns (Maundu *et al.*, 2001).

An ethnobotanical study carried out in Aldai division in South Nandi district on 25 medicinal plants used by the Kalenjins indicated their usefulness in treatment of various ailments caused by viruses, bacteria and fungi. The diseases mentioned to be common apart from malaria included; skin conditions, gastrointestinal worms,

rheumatism and HIV/Aids (Jeruto *et al,* 2011). Conventional treatment is inadequate in Nandi South District as there is only one government hospital and a few health centers. This explains why several plant species from the wild are still being used for treating a variety of medical and other related conditions (Jeruto *et al*., 2011).

The other plants that have been researched on and used by the kalenjin community include the *Amaranthus spp* which is distributed in Africa, Asia, Oceania and the Americas. The leaves and roots are used as decoction (internal) for management of malaria, diarrhoea and fungal infections among the Kalenjins while the Mbeere and Ameru use them to treat diabetes (Kareru *et al*.,2007). The flour made from *Amaranthus spp* is sold in supermarkets and is mainly used by diabetics. *Urtica massaica* (stinging nettle) is clinically used in treatment of symptoms associated with enlarged prostate (non-cancerous) and is also used for diabetes (Witsell *et al*., 2001).

Cassia didymobotrya is used for the treatment of wounds, burns and skin diseases, the active constituents are anthraquinones. *Ekebergia capensis* infusion is used for treatment of colds and malaria. *Kalanchoe spp.* has been used for pain and abortion and *Acacia spp.* is mainly used as a stabilizer in treatment of diarrhoea in HIV patients; this is ascribed due to the presence of the tannins. *Croton megalocarpus* is used for treating pneumonia and is reported to contain compounds that can be used to treat cancer. The main compound is crotonin (Albino *et al.,* 2003).

Conventional medicine is costly, often inaccessible and unaffordable. This alludes to ethnobotanical uses of many of these plants in which majority have not even undergone initial phytochemical investigations. The vast majority of plants used in traditional medicine, as well as those traded, are collected from the wild (Cunningham, 1997). Some plant species are also cultivated on farms, for example as

hedge rows, but this supply is still insufficient to meet growing demand. There is an increased concern not only from the conservation point of view, but also reduced availability of medicinal plants which is a challenge to people living in East and Southern Africa (Doughari, 2006).

Furthermore, the demand for medicinal plants by most of the people in developing countries has led to indiscriminate harvesting of these resources. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants. As a result, many plant species have become extinct or endangered (Marshall, 1998). It is therefore necessary that systematic cultivation of medicinal plants be introduced in order to protect endangered species and document the traditional use of these plants (Neha, 2011). Among the plants used as herbal remedies include several documented and non-documented.

2.2 Medicinal Plants commonly used in Kaptumo Division, Nandi County.

There are many plant species, which are used in traditional remedies that have been documented by Kokwaro (2003). Studies in Kaptumo Division indicate that the use of traditional medicine among the Nandi community is very common due to cultural beliefs coupled with scarcity of resources to access conventional medicine (Jeruto *et al*., 2007). Some of the most commonly used plants include; *Ekebergia capensis, Ehretia cymosa, Periplocca linearifolia, Kigelia africana, Dovyalis abyssinica* and *Fagaropsis angolensis.*

2.2.1 *Ekebergia capensis* Sparrman

It is a tree belongs to the Meliaceae family. Its range extends from the Eastern Cape of South Africa to Sudan and Ethiopia (Pooley, 2003). *Ekebergia capensis* (Plate 2.1) is commonly used traditionally for treatment of gonorrhea, tuberculosis and diarrhea (Jeruto *et al.,* 2007). It is commonly known as "Teldet" by the Nandi community. A study done in South Africa has showed activity against the resistant strain of *Mycobacterium tuberculosis* at 0.1 mg/ml by (Mitchison,2004).

Plate 2.1: The upper part of *Ekerbergia capensis*

Source: (Author, 2011)

2.2.2 *Ehretia cymosa* Thonn

It belongs to the family Boraginaceae. *Ehretia cymosa* (Plate 2.2) is widely distributed from 1200 to 1850 m above sea level, usually at forest margins in higher rainfall regions in Kenya. The local name is "Mororwet". The bark and leaf juice of *Ehretia cymosa* are used to treat wounds because they are considered to be astringent. The bark and roots are boiled and are used traditionally in the treatment of epilepsy and menstruation problems. The bark decoction is applied externally in treating skin diseases. The roots and leaf extract are known to be poisonous, but with apparent inconsistency they have a reputation as an aphrodisiac (Lemmens, 2009). The Maasai

people use the roots to treat brucellosis while in Ethiopia crushed roots soaked in water are used to treat stomach complaints and the root juice is applied to wounds (Mamo, 1997).

Plate 2.2: A habit of *Ehretia cymosa*
Source: (Author, 2011)

2.2.3 *Periploca linearifolia* Dill & A. Rich

Periplocca linearifolia (Plate 2.3) is a climber that belongs to the family Asclepiadaceae, (Brown *et al*., 1996). It grows best in rain forests and the Nandi people refer to it as "*Sinendet*". The harvested materials consist of stem bark, the roots and the whole plant. It is used traditionally by the Kalenjin people to treat stomach problems in human and animals having bloody diarrhea (Jeruto *et al*., 2011).

Plate 2. 3: *Periplocca linearifolia* shoots with leaves twining on the tree
Source: (Baerts & Lehmann,1989)

2.2.4 *Kigelia africana* (Lam.) Benth.

Kigelia africana belongs to the family Bignoniaceae, It is found mostly in riverine areas where distribution is restricted to the wetter areas (Cordell, 2000). It is a tree with a rounded crown and low-hanging branches, widely distributed from the coast to 1850 m above sea level regions of Kenya (Plate 2.4). The fruits are very unusual, like grey sausages up to a metre in length hanging from the long, distinctive stalks and weighing upto 10 kilograms. The vernacular name among the Nandi people is "*Ratinwet*". It's fruits and barks are boiled in water and taken orally as a laxative in treating stomach ailments (Burkill, 1995). The ripe fruit are used to ferment local beer but not the unripe fruits because they are poisonous. The ethanolic stem bark extract of *K. africana* has been found to possess antibacterial and antifungal activity against *S. aureus* and *C. albicans* (Omonkhelin *et al.*,2007).

Plate 2.4: Mature tree *Kigelia africana* (Lam.) Benth. with hanging fruits
Source: (Author, 2011)

2.2.5 *Dovyalis abyssinica* (A. Rich.) Warb.

Dovyalis abyssinica (Plate 2.5) belongs to the family Salicaceae (synonym Flacourtaceae) (Gill *et al.,* 2005). It is commonly distributed in highland forest areas from 1800 to 2500 m above sea level. Its vernacular name is "*Nukiat*" .The root barks are used traditionally by the Nandi people to treat gonorrhea, brucellosis and teeth problems in humans, in animals it is used to treat mastitis (Jeruto *et al.,* 2011).

Plate 2. 5: Mature *Dovyalis abyssinica* (A. Rich.)
Source: (Author, 2011)

2.2.6 *Fagaropsis angolensis* (Engl.) Dale.

Fagaropsis angolensis (Plate 2.6) belongs to the family Rutaceae, it grows scattered at the edges of highland forests. The vernacular name is "*Noiwet*", and the plant (Stem bark) is used traditionally by the Nandi people to treat respiratory infections in humans especially those suffering from pneumonia. A solution of bark and roots are boil and used by the Embu people for the treatment of back aches and joins (Kareru *et al.*,2007).

Plate 2.6: A young *Fagaropsis angolensis* (Engl.) Dale
Source: (Author, 2011)

2.3 Action of phytochemicals in medicinal plants

Many plants have been used because of their antimicrobial properties, which are due to the presence of compounds synthesized in the secondary metabolism of the plant. For example, medicinal plants are known to contain some natural products which perform definite physiological action on the human body and some of these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids and phenolic (Edeoga *et al.,* 2005). For example, the phenolic compounds which are germicidal and are used in formulating disinfectants. The antimicrobial activity noted in plants is attributed to the presence of the aforementioned compounds and most notably, the alkaloids, terpenoids and flavonoids (Nostro *et al.,* 2000). Alkaloids

which are one of the largest groups of phytochemicals in plants have been reported to have some effects on humans which has led to the development of powerful pain killers, anesthetic and stimulants for example, cocaine, caffeine, nicotine, and antimalarial drug quinine (Omulokoli and Chhabra, 1997)

Terpenoids are active antimicrobials, their mechanisms of action is assumed to involve membrane disruption by the lipophilic compounds (Barrett and Lawrence1999). Flavonoids and flavonoids-derived plant natural products have long been known to function as antimicrobial defense compounds in plants (Kazmi *et al*., 1994). Their role is to protect plants against microorganisms and insect attack (Cowen, 2008). The antimicrobial activities of the plants studied were attributed to the presences of these secondary metabolites.

Many herbalists use more than one plant for the treatment of the diseases with the notion that one plant may not work alone. An example is the use of bitter remedies to neutralize the poison (antidote), (Jeruto *et al*., 2011). It is possible that some of the compounds that are active *in vitro* could exhibit activity *in vivo* due to enzyme catalyzed transformation into potent derivatives and therefore are playing the role of pro-drugs (Omulokoli and Chhabra, 1997). This phenomenon has been demonstrated for *Azadirachta indica* extracts (Parida *et al*., 2002).

2.4 Phytochemical Compounds founds in plants

2.4.1 Terpenoids

These are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, (Mendoza *et al*., 1997). Preliminary studies have shown that several classes of terpenes have inhibitory antimicrobial activities due to their

ability to disrupt membrane by the lipophilic compounds (Mendoza *et al*,1997) . Terpenes are hydrocarbons resulting from the combination of several isoprene units (Figure 2.1) for example, hemiterpenoids, have one isoprene unit (5 carbons)

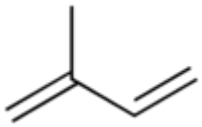

Figure 2. 1: Isoprene structure of terpenoids

The sesquiterpenes consist of three isoprene units and have the molecular formula C_{15} $H_{24,}$ those with the highest antibacterial activity are copaenol, cubenol, and torreyol. The presence of an OH group, which is an efficient uncoupler of the bacterial plasma membrane creates instability and breaks the membrane's phospholipid-sterol interactions and is often lethal (Hammond and Lambert,1981).

2.4.2 Phenols

These are a class of chemical compounds consisting of a hydroxyl group (—OH) bonded directly to an aromatic hydrocarbon group (Figure 2.2).

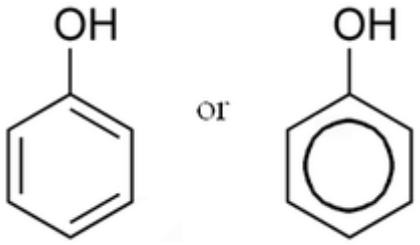

Figure 2. 2: Phenol structure

They are plant metabolites widely spread throughout in the plant kingdom. In some cases the natural phenols are present in vegetative foliage to discourage herbivorous, such as in the case of Western poison oak (Michael, 2008). Phenolic compounds are found in fruits and vegetables and are essential for the growth and reproduction of plants. They are produced as a response for defending injured plants against

pathogens. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant has made them to be highly utilized in the recent years while some phenols such as Xylenol are used in antiseptic and as a germicidal in formulating disinfectants. Others possess estrogenic or endocrine disrupting activity (Coll, 1993).

Phenolics have been reported to have the inhibitory effect on microorganisms by probably due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes (Scalbert *et al.,* 1991). Studies showed that phenolic group joined to a single hydroxyl group confers lipophilicities and acidity, important factors in antifungal activity, to the molecule. Antimicrobial activity studies showed that the phenolic diterpenes with more hydroxyl groups have greater activity over bacteria and fungi (Becerra, 2002).

Phenols are classified depending on the number of carbon atoms ,or aromatic rings. Athraquinones are considered to be phenols or phenolic compounds that have 14 carbon atoms while flavonoids have 15 carbon atoms (Harbone,1998).

2.4.3 Anthraquinones

They are also called anthracenedione and are aromatic organic compounds, with the formula $C_{14}H_8O_2$. Anthraquinones have a laxative effect on the body. Anthraquinones naturally occur in certain plants (e.g. *Senna*, rhubarb, and Cascara buckthorn), fungi, lichens, and in insects where they serve as a basic skeleton for their pigments. Natural anthraquinone derivatives tend to have laxative effects (Harborne, 1998). The presence of anthraquinone glycosides in the extract of *Ficus sycomorus* have been shown to contribute to antifungal activity (Hassan, 2005).

2.4.4 Flavonoids

They are polyphenolic compounds possessing 15 carbon atoms (Figure 2.3); such as quercetin found in citrus fruit, buckwheat and onions (Paul *et al.*, 2002).

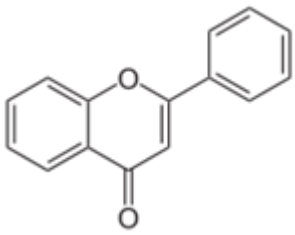

Figure 2. 3: Molecular structure of the flavone backbone(2-phenyl-1,4-benzopyrone)

They belong to a class of secondary metabolites produced by plants and are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants (Spencer, 2008). Plants including all fruits, vegetables, herbs and spices contain flavonoids. Flavonoids isolated from different plant species including mango leaves have been reported to possess antibacterial activities and mechanisms of action of different flavonoids have been proposed, including inhibition of nucleic-acid synthesis, inhibition of cytoplasmic membrane function and inhibition of energy metabolism (Tsuchiya *et al.*, 1996)

2.4.5 Alkaloids

These are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms due to the presence of double bond between carbon atom and nitrogen atom in pyridine ring (left) (Figure 2. 4). This group also includes some related compounds with neutral and even weakly acidic properties (Manske, 1995).

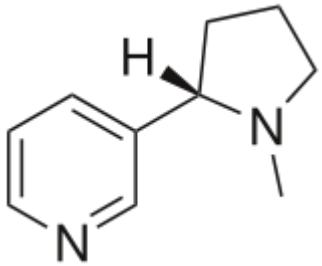

Figure 2. 4: Alkaloid structure containing nitrogen molecule with both pyrrolidine rings (left) and pyridine ring (right)

Plant alkaloids are natural, they often produce menacing effects of the remedies. However, many herbalists are of the opinion that the instant discomfort caused by the medications containing plant alkaloids are an important and necessary part of the stimulating action required for healing from any disorder (Cheeke,1985). Alkaloids display a good antimicrobial activity against several microorganisms (Dassonneville *et al*., 2008).For instance, it has been reported that antimicrobial activities that have been assesed showed that the response of *Escherichia coli* to alkaloids was faster than that of *Shigella dysentriae*.

2.4.6 Saponins

They are mainly produced by plants, but also by lower marine animals and some bacteria (Riguera, 1997;Yoshiki *et al*.,1998). Saponins are naturally occurring surface-active glycosides or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition, and in terms of structure, by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative (Hostettmann *et al*., 1995).

Several biological effects have been ascribed to saponins including significantly affectig growth, feed intake and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and mollusks. Also recent

research has established saponins as the active components in many herbal medicines (Liu and Henkel, 2002)

2.5 Medicinal plants as antimicrobials

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, parasites or viruses (Davey and Warrell, 2002).The property of the medicinal plants to combat the microbes is due to the presence of the phytochemical compounds which disrupt microbial processes or structures that differ from those of the host. Damage to pathogen may occur through inhibition of cell wall synthesis, protein and nucleic acid synthesis, disruption of membrane structure and function and blockage of metabolic pathways through inhibition of key enzymes (Atlas, 1995).

Though the antimicrobials are effective against most pathogens, some pathogens such as bacteria may develop their own defense against the drug. The bacteria may produce enzymes that can destroy a drug hence becoming resistant to the antibiotic. The cell wall may also become resistant to being broken by the action of the antibiotic. This usually happens when an antibiotic is used most incorrectly during the treatment eventually makig the treatment more inefficient. For example, some strains of *Staphylococcus aureus* that cause pneumonia may become resistant to all antibiotics (Mark *et al.,* 2003).

Antibacterial resistance especially among gram negative bacteria is an important issue that has created a number of problems in treatment of infectious diseases and necessitates the search for alternative drugs of natural anti-bacterials (Jensen. *et al.*, 1996). This increasing failure of chemotherapeutics and antimicrobial resistance in pathogens affecting humans, and animals, combined with the increasing awareness of the consumers on chemical substances used as food preservatives, necessitates

research for new antimicrobial that lacks side-effects on humans. This has led to the screening of several medicinal plants for their potential antimicrobial activities (Njenga *et al*., 2005). Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens (Cragg *et al.,* 1997). Recently, the antimicrobial effects of various plant extracts against certain pathogens have been reported (Onakoya, 2006)

In the last few years, a number of studies have been conducted in different countries to prove such efficacy and safety in these used dugs(Akinyemi *et al.,* 2007), especially in Latin America. In Argentina, researchers tested 122 known plant species used for therapeutic treatments (Nascimento *et al.,* 2000). It was documented that among the compounds extracted from these plants, twelve inhibited the growth of *Staphylococcus aureus*, ten inhibited *Escherichia coli,* and four inhibited *Aspergillus niger* and also it was reported that the most potent compound was the one extracted from *Tabebuia impetiginosa* (Martinez *et al.,* 1996).

In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. For example plants such as Moringa plant (*Moringa oleifera*) with Plant-derived biopharmaceuticals are cheap to produce, store, and easy to use and it has been the object of much research.This is due to its multiple uses and well-known bactericidal potential apart from a range of industrial and medicinal applications,it is used to purify water for human consumption (Ghebremichael *et al.,* 2005).

2.6 Herbal remedies preparation and synergism

The World Health Organization (WHO) has long recognized the role plyed by traditional medicinal plants and proposed an integrated health care system which involves all members of patient health care team and the goal is to ensure optimal care and avoid errors that result from overuse or misuse of prescribed medication. The proposition was made after analysis of the potential risks due to toxicity and microbial/parasites contamination of such plants during preparation methods and dosage administration (WHO, 2002).

The herbal remedies are prepared using different methods such as decoctions, infusion, roasting, concoctions, paste and powder (ash). For example, preparation of compounds from dry parts of one plant or several plant parts as ashes by using grinding stones, burning, chewing, roasting, pounding, and boiling or soaking in hot or cold water and milk added as a solvent and orally administered are the most frequently used methods for preparing medicinal plants (Jeruto *et al.*, 2011).

Use of concoctions suggests that the drugs may only be active in combination due to synergistic effects of several compounds in plants (Gessler *et al.*, 1994). This synergistic effect is important in the elimination of the resistant pathogens especially those that resist the drugs that are used most frequently during treatment. Synergistic effects resulting from the combination of antibiotics with extracts were documented in the literature (Muroi and Kibe, 2005). The study involved the association of anacardic acid and totarol with methicillin to inhibit strains of *S. aureus* resistant to methicillin (MRSA). The association of thyme (20 μg/mL) with ampicillin was also able to cause such an effect. The use of plant extracts and phytochemicals, both with

known antimicrobial properties, can be of great significance for treatment of diseases (Gislene *et al.*,2008).

2.7 Common test microorganisms used in antimicrobial bioassays analyses

The most commonly used microorganism are bacteria and fungi .The bacteria include, Gram positive; *Staphylococcus aureus* and Methicillin resistant *Staphylococcus aureus* while Gram negative are; *Escherichia coli*, *Shigella dysentriae* and *Pseudomonas aeruginosa* (Sebhat *et al*., 2007). The most commonly used fungi include dermatophytes namely; *Trichophyton mentagrophytes* and *Microsporum gypseum* while yeasts are *Candida albicans* and *Cryptococcus neoformans* (Miller *et al*.,2006).

2.7.1 *Escherichia coli*

This is a Gram-negative, rod-shaped, facultative bacteria ,most are normal flora, but some for instance *E. coli* O157:H7 cause severe anemia or kidney failure, which can lead to death.The symtoms include, hemorrhagic diarrhea, and occasionally to kidney failure, especially in young children and elderly persons (Karch, 2005).

2.7.2 *Shigella dysentriae*

It is a Gram-negative, rod (bacillus) shaped, non-motile, non-spore-forming, facultative anaerobic bacteria that are non-capsulated causes Shigellosis, an infectious disease associated with hemorrhagic diarrhea and is caused by various species of *Shigella* (Swapan, 2005).

2.7.3 *Pseudomonas aeruginosa*

Is a gram-negative rod and an opportunistic pathogen of immunocompromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infection (Govan and Deretic,1996).

2.7.4 *Staphylococcus aureus*

It is a facultative anaerobic Gram-positive coccus bacterium. It is frequently found as part of the normal skin flora on the skin and nasal passages (Voet *et al*., 2012). *Staphylococcus aureus* can cause a range of illnesses, from minor skin infections, such as pimples, to life-threatening diseases such as pneumonia (Muroi and Kibe, 2005).

2.7.5 Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA is any strain of *Staphylococcus aureus* that has developed, through the process of natural selection, resistance to beta-lactam antibiotics. It is also called multidrug resistant *staphylococcus aureus* and oxacillin resistant *Staphylococcus aureus* ORSA (Holten *et al.*, 2000).

2.7.6 *Trichophyton mentagrophytes*

This is a fungus that causes a skin infection consisting of an eruption of a number of concentric rings of overlapping scales. It also causes athlete's foot, ringworm, jock itch, and similar infections of the nail, beard, skin and scalp (Voet *et al*, 2012).

2.7.7 *Microsporum gypseum*

This affects the keratinous tissue causing diseases such as *tinea capitis* and *tinea corporis* (Kwon-Chung and Bennett, l992).

2.7.8. *Candida albicans*

This is a dimorphic fungus that exists as a commensal of warm-blooded animals including humans. It colonizes mucosal surfaces of the oral and vaginal cavities and the digestive tract causing candidiasis (Gupta and Kohli, 2003).

2.7.9 *Cryptococcus neoformans*

This is encapsulated yeast that is a facultative intracellular pathogen. The fungus is very common in the environment mainly in soil, dirt and bird droppings. It enters into the body through inhalation into the lungs through to the blood, spinal column and the brain where it cause severe diseases condition (*Eisenman*, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Kaptumo division is situated in Rift region, Kenya; its geographical coordinates are 0º 40’ 0” North, 35º 4’ 0” East (0.07º N, 35.07 ºE). There are four administrative locations in the division; it borders South Nandi forest to the north–west that covers an area of approximately 1800 ha between altitudes levels 1700 and 2000 m (Kigomo, 1991). Kaptumo division is in Nandi county which is situated in the western part of Kenya and borders Uasin-Gishu county to the north-east, Kericho county to the south-east, Kisumu county of Nyanza region to the south east, Kakamega county of western region of kenya to the north–east (Anon, 2001)(Figure 3.1)

3.2 Plant materials

3.2.1 Collection and identification of medicinal plants

The information on the medicinal plants was gathered from the traditional practitioners, herbalists using structured questionnaires (Appendix 3) in order to obtain information on the medicinal plants that are traditionally used for management of infectious diseases in Kaptumo Division, after conducting the survey of the area. The information gathered included; the common/local name of plants, the source, the part(s) used, methods of preparation, mode of administration, the disease it cures, dosage, frequency of use, their effectiveness and duration of treatments. Selection of the plants was based on available ethnobotanical information from traditional health practitioners consulted during the pilot study as well as available literature and plant collection was guided by the number of plants available within a locality.

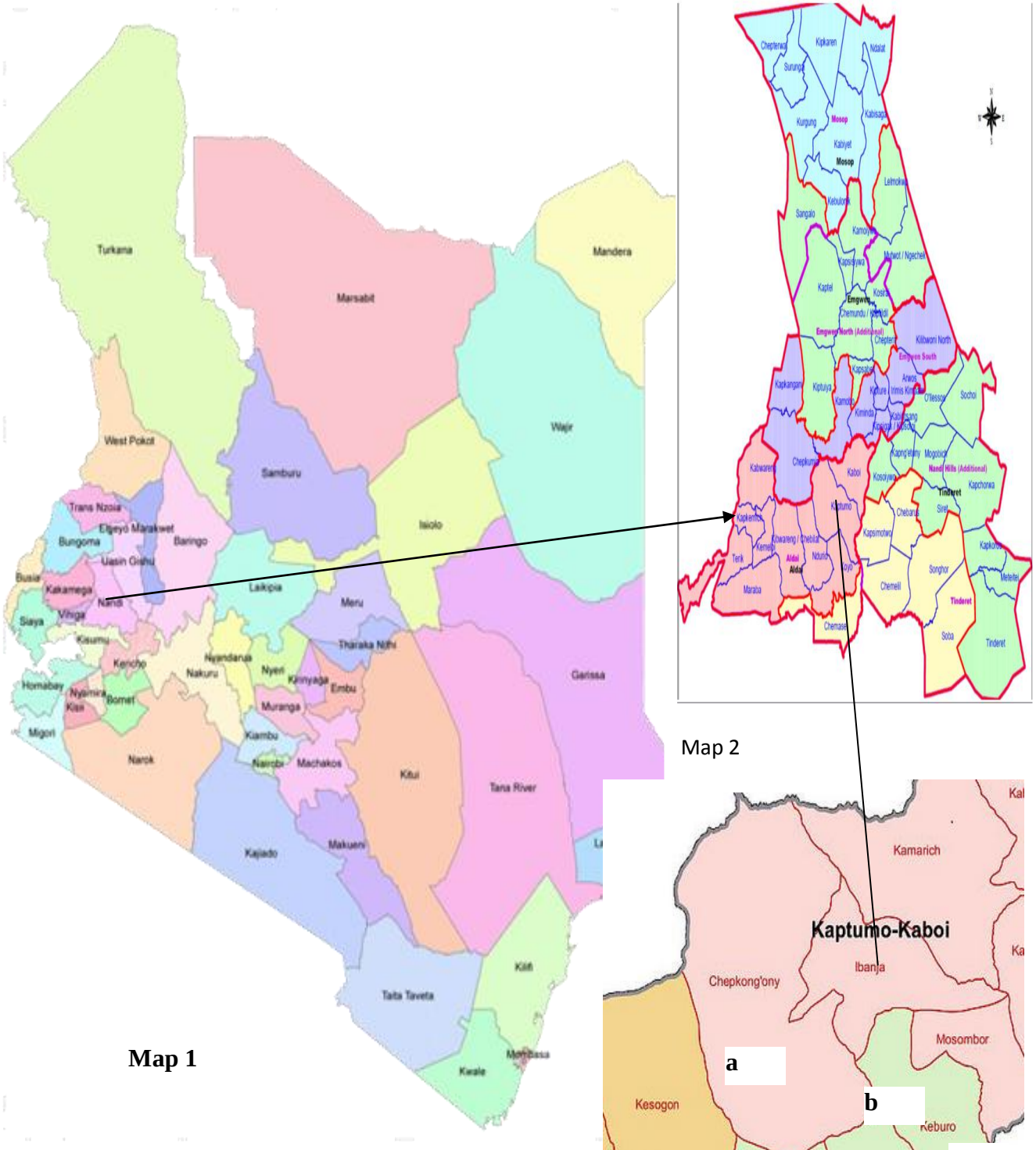

Figure 3. 1: map of the study area

1.map of kenya

2.Map of Nandi county

3: Map of Aldai constituency showing study areas, a-chepkongony,b-Ibanja,c-Mosombor and d-keburo

Source: Google Maps, 2013

Where a species was abundant, a minimum of five plant material was collected, but where materials were scarce, a minimum of two plants per species were collected.

The plant parts collected for identification consisted of flowers, roots, stems and leav

County and identified at the Department of Biological sciences herbarium University of Eldoret where the Voucher specimens were deposited, Table 4.1. The criteria that was used to decide the medicinal plants collected in the study included the disease it cures, their effectiveness and how common is it used in the region. Those plants that were used to treat other aspects than infectious disease were left out. Six medicinal plants used to cure bacterial and fungal diseases were selected. The plant parts collected for extraction included leaves, barks, stem and leaves obtained from their natural habitats. The scientific name and herbarium numbers of the six medicinal plants specimen collected include; *Dovyalis abyssinica* (Kim/Kap/12/11/ 001), *Fagaropsis angolensis* (Kim/Kap/12/11/ 002), *Kigelia africana* (Kim/Kap/12/11/003), *Periploca linearifolia* (Kim/Kap/ 12/11/004), *Ekebergia capensis (*Kim/Kap/12/11/005) and *Ehretia cymosa (*Kim/Kap/12/11/006) (Table 4.1).

3.2.2 Preparation of plant material

After collection, each of the plant samples was separated into different parts: barks for *F. angolensis, K. africana, E. capensis* and *E. cymosa* while for *E. cymosa* and *D. abyssinica* leaves were used. The stems and leaves were used for *Periploca linearifolia.* The plant materials were transported to Kenya Medical Research Institute (KEMRI) Phytochemistry laboratory and washed thoroughly with running tap water. They were then chopped into small pieces and air-dried for two weeks at room temperature by spreading evenly in the open drying area. The dry samples were ground separately into fine powder using a grinder (Willy mill). The powdered

samples were bagged in black plastic bags and labeled appropriately using their voucher numbers and stored in air-tight containers.

3.2.3. Solvent extraction of plant materials

A sample of the powdered bark and leaves weighing 50 grams were exhaustively extracted with acetone and water. Acetone was used to obtain the organic extracts based on the study by Eloff (1998) who determined that acetone extracts the most inhibitors, is less toxic and very volatile compared with other organic solvents making it a preferred extractant. The extraction was carried out in 250 ml conical flask with 200 ml of the respective solvent added. The extracts were left to stand for 24 hours at room temperature (acetone) and for the water extracts were heated in water bath for 1 hour at 65^{o}C. The extracts were filtered through Whatman No. 1 filter paper and the solvents removed using a rotary evaporator and air dried for a day. The former was put in sterile airtight vials weighed and kept in desiccator at 4^{o} C in readiness for use (Ana *et al*., 2005).The water extracts were evaporated to dryness for three days using freeze drying machine. The lyophilized dry powder was then put in a stoppered sample vial, weighed and kept in desiccator to avoid absorbing moisture.

3.3 Phytochemical profiling

Phytochemical screening of the crude water and acetone extract of the plants was carried out on TLC(Thin Layer Chromatography) using standard phytochemical methods as described by (Harborne, 1998) using chromatography visualizing agents. Spotting was done on the TLC plate using a micro-tube and the TLC plate was put in a solvent system of acetone: petroleum ether at the ratio of 1:9 in a solvent tank, and allowed to develop till it reached solvent front. It was removed and allowed to dry in

an open air and then observed under U.V light at a wavelength of 365 nm and thereafter sprayed with the appropriate reagents for further development. (Harborne, 1973).

3.3.1Test for alkaloids

Dragendroff spraying reagent was use for testing of alkaloids. It was made by reacting two portions of 1g of bismuth substrate dissolved in a solution of 10 mls acetic acid and 40mls of water and 2g of potassium iodide (KI) dissolved in 20 mls of water (Harborne, 1973). Detection of alkaloids and other nitrogen compounds was by the formation of brown spots on a yellow background (Harborne, 1973)

3.3.2 Test for phenolics

Ferric Ferricyanide reagent for phenolic test was prepared by mixing 0.1g of ferric chloride and 0.1g of potassium ferric cyanide ($K_3F_3CN_3$) freshly prepared by dissolving in 10ml of distilled water. Equal portions of ferric chloride and potassium Ferric cyanide were mixed, sprayed on the TLC plates and heated at 110^0C in an oven. Change of colour to blue (instant) indicated the presence of phenolics (Harborne, 1998).

3.3.3 Test for terpenoids

The vanillin reagent for terpenoids test was prepared by mixing ten percent (10%) vanillin dissolved in ethanoic acid in ratio of 2:1 and sprayed onto the plates followed by spraying with concentrated sulphuric acid and put in the oven for 15 minutes. Presence of terpenoids was indicated by the separation into different colours; brown, dark green and purple colour (Harborne, 1998).

3.3.4 Test for flavonoids

The test for flavonoids was carried out by exposing ammonia fumes on TLC plate. The presence of flavonoids was indicated by coloured spots e.g. yellow, pink and brown spots (Harborne, 1973).

3.3.5 Test for anthraquinones

A test for anthraquinones was carried out by spraying the TLC plates with a solution of mixture of 10 ml ethanol (C_2H_5OH) and10 g potassium hydroxide (KOH). Change of the original yellow brown colour to purple showed a positive test for anthraquinones (Harborne, 1973).

3.3.6 Test for saponins

Tests for saponins was carried out by shaking 0.5 grams of each of the plant extract in a test tube and left for 5 minutes, a persistent foam showed a positive test for presence of saponins (Harborne, 1973;1998).

3.4 Antimicrobial assay

3.4.1. Microorganisms used

The microbial organisms and clinical isolates used in the study were obtained from the Centre for Microbiology Research (CMR) for bio-assay. The gram positive bacteria were *Staphylococcus aureus* (ATCC 25923) and clinical isolate of Methicillin resistant *Staphylococcus aureus* (MR*SA*) while the Gram negative were *Escherichia coli (*ATCC 25922), *Pseudomona*s *aeruginosa (*ATCC 27853) and clinical isolate of *Shigella dysentriae.* Fungi consisted of yeast comprising *Candida albicans (*ATCC 90028) and *Cryptococcus neoformans* (ATCC 32602), and clinical

isolates of *Microsporum gypseum* and *Trychophyton mentagrophytes* which are dermatophytes.

3.5. Preparation of microorganisms

This involved sub-culturing of bacterial strains on Muller Hinton agar number CM0337 (Oxoid Ltd, Basingstock, Hamshire, England) followed by incubation at 37 ºC for 18 hours to obtain freshly growing strains. Yeast and moulds were sub-cultured onto Sabouraund Dextrose Agar (SDA) number CMOO4 (Oxoid Ltd, Basingstock, and Hamshire, England). Each media was prepared according to the manufacturer's instructions. Yeasts were incubated at 35º C for 24 hours while moulds were incubated for 72 hours at 30º C to obtain freshly growing culture (Rajakarunas and Towere, 2002).

3.6. Preparation of McFarland Standard

Precisely 0.5 McFarland equivalent turbidity standards was prepared by adding 0.6ml of 1% barium chloride solution to 99.4 ml of 1% sulphuric acid solution and mixed thoroughly. Two milliliters of the turbid solution was transferred to the tube of the same type that was used to prepare the test and control innocula and kept in the room temperature (25ºC). Exactly 0.5 McFarland gives an equivalent approximate density of bacteria $1x10^6$ colony forming unit (CFU)/ml (Ana *et al.*, 2005).

3.7. Antimicrobial assays

3.7.1 Disc diffusion assay

The antimicrobial activity of the extracts was determined based on the inhibition zones using disc diffusion method described by (Bauer *et al.*, 1996). The test microorganisms were sub-cultured for 18 hours at 37^o C for bacteria (Bauer *et al.*, 1996) while moulds and yeast cultures were incubated at 30^o C for 72 hours and 35^0 C

for 24 hours, respectively, in their prescribed nutrient agar to obtain working cultures. Nutrient media for growth of the test microorganisms were prepared as per the manufacturer's instructions, sterilized and left to cool at around 50^{o}C. Each of the cultured microorganisms from an overnight growth of the test organism, 4-6 colonies were emulsified and the suspension was adjusted to match the 0.5 McFarland's standard so as to produce inoculated agar with $1x10^{6}$ colony forming units/ml. The suspension was inoculated into the respective growth medium using a sterile cotton wool swab.

Briefly, 100 mg of each extract was dissolved in 1ml to come up with a concentration of 100 mg /ml. Ten microliters of each prepared plant extracts was measured and impregnated onto 6 mm sterile filter paper disk with a diameter of 6 mm and air dried. The disk was then placed aseptically onto the inoculated plates and incubated for 18 hours at 37^{o} C for bacteria (Bauer *et al.*, 1996) while moulds and yeast cultures were incubated at 30^{o} C for 72 hours and 35^{0} C for 24 hours respectively.

3.7.2 Determination of minimum inhibitory concentration (MIC)

Broth micro dilution method was used to determine minimum inhibitory concentration (MIC) for the active crude extracts against the test microorganisms in disc diffusion test. The method is recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002) now Clinical Laboratory Standard Institute (CLSI). MIC interpretation for the standard drugs Gentamycin was adapted from NCCL's interpretive standards for dilution and disc diffusion susceptibility testing tables (Murray *et al.*, 1999).The reference was the 0.5 McFarland turbidometry

to achievement of inoculums approximately $1x10^6$ colony forming units (cfu) (Burt and Reinders, 2003).

A serial dilution was carried out to give final concentrations between 100-0.05 of crude extracts that were dissolved in respective solvents. During serial dilution, a 96 well-micro-titer plate was fed with 100 µl of sterile water except the first well. Plants extracts were transferred into micro-titer plate, using micro-titre pipette, starting with 200 µl of plant extracts in the first well that was left empty. Half of the solution in the first well was transferred to the second well containing 100 µl of water and mixed thoroughly. Half of the solution in the second well was transferred to the third and mixed thoroughly and this was continued up to the 10^{th} well as outlined by Eloff (1998; 1999) to make serial dilutions ranging from 10^{-1}, 10^{-2}, 10^{-3} upto 10^{-10} . The final volume in each well was 100 µl. The wells were then inoculated with 20 µl of microbial suspension to come up with 120 µl mixture. Wells that were not inoculated served as the controls. The bacteria were then incubated at 37^o C for 24 hours in ambient air.

The Minimum Inhibitory Concentration (MIC) value was determined as the lowest concentration of the crude extract in broth medium that inhibited the visible growth of the test microorganism as compared to the control (Motamedi *et al*., 2009). The MIC was recorded as the lowest extract concentration demonstrating no visible growth as compared to the control broth turbidity (Michael *et al.,* 2003). All the experiments were carried out in triplicates and the average results were recorded.

3.7.3 Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by collecting a loop full of broth from those wells which did not show any growth in MIC assay, two wells above and two below the lowest

MIC value and inoculated on sterile Muller-Hinton agar by streaking. The plates were incubated at 37 º C for 18-24 hours. The highest dilution that yielded no colony fraction on a solid medium was considered as MBC (Motamedi, 2009).

3.8 Cytotoxicity Assay

The cytotoxic concentration causing 50% cell lysis and death (CC_{50}) was determined for the active extracts by following a modified rapid calorimetric assay, a method described by Mosmann (1983). The extracts of the active plants were tested for *in vitro* cytotoxicity, using actively dividing sub-confluent Vero E6 cells. Briefly, preparation of plant extracts involved 100µg of each extract was dissolved in 1ml of Dimethyl sulfoxide (DMSO) because it is a good stabilizer for both organic and water extracts to come up with a concentration of 100 µg /ml. The extracts were then diluted in minimum essential medium (MEM) to reduce the toxicity of DMSO.

The cells were thawed to revive them from the stock cultures by placing on warm water bath. Once thawed they were poured into a 75ml culture flasks containing minimum essential medium (MEM) supplemented with 10% Fetal bovine serum (FBS) and incubated at 37^0C and 5% CO_2. Upon attainment of confluence, cells were detached by trypsinization and pooled in a 50 ml centrifuge tube to aid in cell density count to attain 2×10^5 . This was determined using tryphan blue exclusion test by placing the cells centrifuged in four wells and tryphan blue added then transferred to a haemocy-tometer (each) to aid in counting viable cells the unstained(viable) cells while blue-stained(dead) was seen under 40x objective lens of an inverted microscope to obtain a field containing cells in 4x4 columns. The cell average density was obtained to be 32.5 by adding all the values obtained from the 4×4 wells and

averaged, the cell density was obtained from the formula C_1 = A× dilution factor×10^4 as below. Where A = 32.5

Dilution factor (constant) = $2x10^4$

$C_1=32.5\times2\times10^4$

The volume of cells (V_1) obtained from the centrifuge and diluted with minimum essential media (MEM) was calculated by multiplying a constant (C_2) $2x10^5$ by volume of the MEM used and that of cells used in the setting (V_2) (96 wells ×100) 9600 µl, and divided by (C_1) $65x10^4$ this gave 2.9 as the Volume of cells to be diluted with MEM to make 9.6 ml, the total volume that was put in the 96 well plate.

$V_1=C_2\times V_2 \div C_1$

Where C_2= Concentration of cells in the wells (plate)

V_2= volume of the MEM and Cells used.

C_1=cell density

$V_1=2\times10^5\times9.6ml\div65\times10^4$

=2.9ml`

The volume of MEM to be added to the cells was obtained by subtracting the number of cells obtained from the centrifuge (V_1) from the total volume of cells used in the setting and that of MEM added (V_2) that is V_2- V_1, 9.6-2.9=6.7 .The calculated volumes were then mixed and transferred to the 96 well plate using micro-titre pipette.

One hundred (100) µl of the cell suspension at 2 x 10^5 cells per ml were seeded into each well of a 96- well plate and incubated at 37^0 C in 5% CO_2 for 24 hours to attach. The test sample extracts diluted with MEM at a ratio of 1:99 to a starting concentration of 100 µg/ml and a volume of (10+990) 1000 µl were seeded in duplicate in columns in a 96 well plate while the third column was left blank as the control; Columns 1, 2, 4, 5, 7, 8 and 10, 11 "had drug extracts and cells whereas columns 3, 6, 9 and 12 that served as Controls only had cells but no drug extracts.

Row H had the highest drug concentration and a volume of 150 µl a serial dilution of 50 µl was carried out upwards from row H to row B, (row A had no drug extracts). Chloroquine drug with an Initial concentration of 10 µg/ml in 1% DMSO was used as the control standard for the experiment. The plates were then incubated for 48 hours at 37^o C in a 5 % CO_2 incubator. At the end of the incubation time, 10 µl of Thiazolyl blue tetrazolium bromides

(MTT) dye was added into each well and cells were incubated for another 4 hours, (with 0.8 mg/ml of MTT), dissolved in Phosphate buffered saline (PBS). After 4 hours of incubation with MTT, the cells were observed for dye intake, after which all media was removed from the plates and 100 µl of DMSO added into each well. The plates were then read on a scanning multiwall spectrophotometer (Mullikan Ex labs systems) at 562 nm and 620 nm as reference. Data was analyzed as follows:

$$\% \text{ Cell viability } (CC_{50}) = \frac{[OD_{sample562} - OD_{620}]}{[OD_{control562} - OD_{620}]} \times 100$$

Where OD = optical density

CC_{50} = Concentration or the dose of the extract that kills 50% of the cells.

3.9. Data Analysis

Data obtained was entered into Excel Spreadsheets for cleaning and encoding. After this, it was exported to SPSS 16.0 programme for analysis. ANOVA(one way) test was used to establish the significance variability between and within groups (plants, solvents and microorganisms). Bioactivity was used as an independent variable to establish significance at 0.05 levels of confidence.For non-parametric data Chi-square was used to determine significance. The data was presented in form of tables.

CHAPTER FOUR

RESULTS

4.1 Plants collected for antimicrobial studies

Six medicinal plants belonging to six different families namely, *Dovyalis abyssinica, Fagaropsis angolensis, Kigelia africana, Periploca linearifolia* and *Ekebergia capensis* and *Ehretia cymosa*,parts collected,Herbarium number,family ,vernacular name, preparation and administration, targeted diseases, duration and frequency of use are indicated in Table 4.1and 4.2

Table 4.1: Plants collected for antimicrobial studies from Kaptumo Division

Botanical name	Part collected	Herbarium No.	Family Name	Vernacular Name
Dovyalis abyssinica	Leaves	Kim/Kap/12/11/001	Flacourtiaceae	Nukiat (Nandi)
Faragopsis angolensis	Bark	Kim/Kap/12/11/002	Rutacea	Noiwet (Nandi)
Kigelia africana	Bark	Kim/Kap/12/11/003	Bignoniaceae	Ratinwet (Nandi)
Periplocca linearifolia	Leaves & stem	Kim/Kap/12/11/004	Asclepiadaceae	Sinendet (Nandi)
Ekebergia capensis	Bark	Kim/Kap/12/11/005	Meliaceae	Teldet (Nandi)
Ehretia cymosa	Leaves, bark	Kim/Kap/12/11/006	Boraginacea	Mororwet (Nandi)

Table 4.2: Ethnobotanical information of the selected medicinal plants for antimicrobial studies

Botanical name	Preparation/ Administration	Disease targetted.	Duration of treatment	Frequency
Dovyalis abyssinica (A.Rich.)	Decoction (oral)	Gonnorhea & Brucellosis	3months	Frequently
Fagaropsis angolensis (Engl.) Dale	Concotion (oral)	Pneumonia	6 months	Frequently
Kigelia africana (Lam.) Benth.	Concotion (oral)	Dysentry	1 month	Occasionaly
Periploca linearifolia Dill and A. Rich	Concotion (oral)	Dysentry	2 months	Rarely
Ekebergia capensis Sparrman	Concotion (oral)	Tuberculosis	2 months	Rarely
Ehretia cymosa Thonn	Decoction (pouitice)	Wound infections	1 month	Occasionally

4.2 Yields of Extracted Plant Materials

Maximum and minimum yields obtained from the water extracts of *K. africana* (*bark)* produced the highest yield (9.19%) while the bark of *F. angolensis* (4.42%) was the lowest. The acetone extract of *E. capensis* produced the highest yield (2.50%) and bark of *E. cymosa* was (0.55%) as the lowest. Water extracts produced significantly higher yields than acetone. (χ^2 =4.23; df = 1; p =0. 04). The yields are shown in table 4.2 below. The differences in percentage extract yields between plants did varied significantly when water was used as the extract (χ^2 = 1.215; df = 6; p = 0.944) and also when acetone was used as the extract (χ^2 = 1.644; df = 6; p = 0.896).

Table 4. 3 Percentage yields for sequential extractions

Plant	Solvent	Part extracted in grams	Initial weights in grams	Extract weight in grams	% yield
Kigelia africana	Acetone	Bark	50.0	0.74	1.47
	Water	Bark	50.0	4.59	9.19
Periplocca Linearifolia	Acetone	Stem & leaves	50.0	1.07	2.14
	Water	Stem & leaves	50.0	3.56	7.12
Ehretia cymosa	Acetone	Bark	50.0	0.27	0.55
	Water	Bark	50.0	4.53	9.05
Ehretia cymosa	Acetone	Leaves	50.0	0.51	1.02
	Water	Leaves	50.0	2.77	5.54
Ekebergia capensis	Acetone	Bark	50.0	1.25	2.50
	Water	Bark	50.0	3.99	7.99
Dovyalis abyssinica	Acetone	Leaves	50.0	0.90	1.78
	Water	Leaves	50.0	4.275	8.550
Fagaropsis angolensis	Acetone	Bark	50.0	0.391	0.782
	Water	Bark	50.0	2.211	4.422

4.3 Thin layer Chromatography

All the screened water and acetone extracts were found to contain terpenoids indicated by the separation into different colours; brown, dark green and purple colours. Phenols and flavonoids were also found in all plants, this was indicated by Change of colour to blue (instant) and coloured spots e.g. yellow, pink and brown spots respectively. Acetone extracts of *K. africana* (bark) showed high amount these phytochemical while those of *E. cymosa* (bark) contained traces of terpenoids, phenols and flavonoids. The water extracts indicated moderate to high amount of terpenoids, phenols and flavonoids except the leaves of *D. abyssinica* and *E. cymosa* which indicated traces of terpenoids and flavonoids, respectively. The other secondary metabolites were varied from one plant to the other. For example, anthraquinones were not detected in acetone extracts of *F. angolensis* (bark*), D. abyssinica* (leaves*),* and leaves of *E. cymosa* the other plant extract showed a positive test for anthraquinones indicated by change of the original yellow brown colour to purple. Alkaloids were not detected on both *P. linearifolia (*stem and leaves) and *E. cymosa* (leave) acetone extracts and present in the other plants showed by the formation brown spots on yellow background. Saponins were absent on *P. linearifolia, D. abyssinica* and *F. angolensis* acetone and water extracts and present in the other plants extracts screened. The barks were found to contain more phytochemicals than the leaves, example are the leaves of *E. cymosa* which did not indicate the presence of alkaloids and anthraquinones while the bark indicated the presence of all the phytochemicals screened. *D. abyssinica* (leaves) did not show the presence of anthraquinones and saponins. The results are as shown in table 4.3.

Table 4. 4 Phytochemical Profile of the Plant extracts

Plant species	**Solvent**	**Plants compounds**					
		Terpenoids	**Alkaloids**	**Flavonoids**	**Phenolics**	**Anthraquinones**	**Saponins**
Ekebergia capensis	A	+	++	++	++	+	++
(bark)	W	+++	++	+++	+++	++	+
Eheretia cymosa	A	+	+	+	++	+	++
*(*bark*)*	W	++	++	+	+++	+	+
Eheretia cymosa	A	+++	_	+	++	_	+
*(*leave*)*	W	++	+++	+	++	+	+
Kigelia africana	A	+++	+++	+++	+++	++	+
*(*bark)	W	++	+++	+++	+++	+	+
Periploccalinearifoia	A	++	_	+++	+++	++	_
*(*Stem&leaves*)*	W	++	++	+++	++	+	_
Dovyallis abyssinica	A	++	+	+	+++	_	_
*(*leaves*)*	W	+	++	++	+++	+	_
Fagaropsis	A	+	+++	++	+++	_	_
angolensis	W	+++	+++	++	+++	+	_
*(*bark*)*							

*Key: – = Not detected; + = Trace; ++ = moderate, +++ = High1. W-Water 2 A- Acetone

4.4 Antimicrobial assays

4.4.1 Disc diffusion

The activities of the test plants were expressed in inhibition zone diameters, (Plate 4.1) that were averaged for the triplicate tests. The highest activity was noted in the water extracts of *Ekebergia capensis* against *S. aureus* with a zone of inhibition of 14.7 mm and lowest in *Fagaropsis angolensis* with 6.0 mm. Generally water extracts were more active with an inhibition zone of 14.7 to 7.0 mm compared to acetone extracts; the acetone extracts of *Periplocca linearifolia* leaves were active against *S. aureus,* 12.3 mm as shown in table 4.4. There were differences in the levels of antibacterial activities of plant extracts from different plants. These differences were statistically significant (Anova: F = 287.34; df = 6; p< 0.001).Appedix 2,anova Table 6.2. The selected plant extracts showed no activity against the selected fungal isolates tested at 100 mg/ml,hence they were not used for any other analysis.

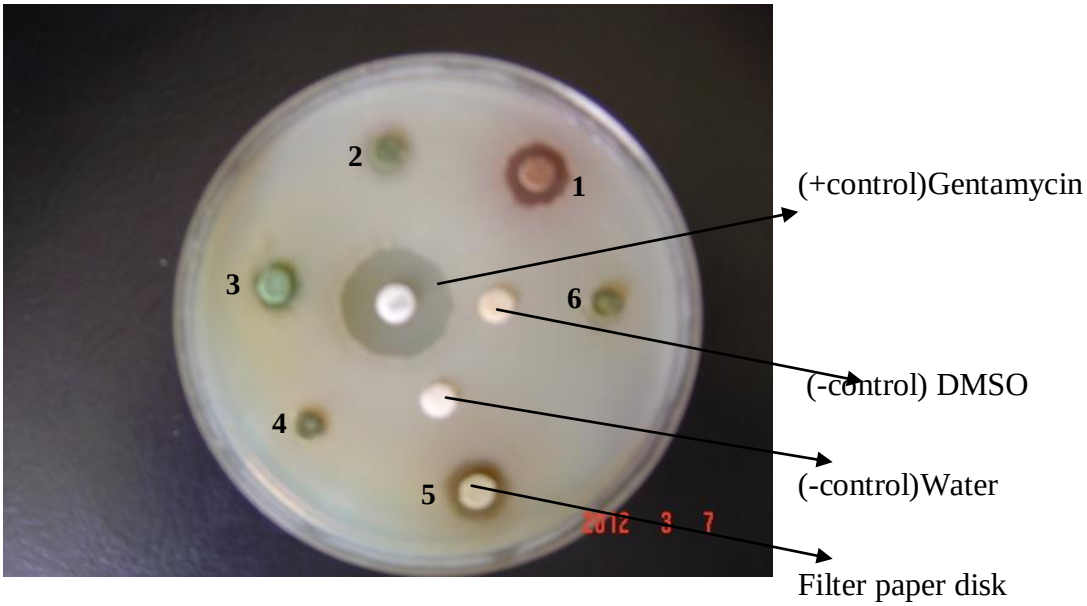

Figure 4. 1: MullerHinton agar plate, showing zones of inhibition of acetone extracts against Pseudomonas aeruginosa.

from different plants as shown on Table 4.4.The numbers indicated on the plate was used to assign the impregnated actone disks that belong to a particular plant:1- *E. capensis*, 2-*P. linearifolia*, 3-*E. cymosa* , 4- *D. abyssinica*, 5- *K. africana*, 6-*F. angolensis.*

Table 4. 5 Inhibition zones in millimeters of six selected medicinal plants against five selected bacterial test microorganisms .

Plants	**Test samples extracts/drug**	**Conc. mg/ml**	**Average inhibition Zone diameters in millimeter for each *Test organisms***				
			S. aureus	MR*S. Aureus*	*P. aeruginosa*	*E. coli*	*S. Dysentriae*
Ekebergia capensis(Bark)	Acetone	100.0	12.3	11.0	9.3	7.0	6.0
	Water	100.0	14.7	11.6	10.3	7.0	6.0
+ Control	Gentamycin	*30.0	21.0	19.3	21.7	26.3	22.3
- Control	DMSO	100.0	6.0	6.0	6.0	6.0	6.0
-control	Water	100.0	6.0	6.0	6.0	6.0	6.0
Ehretia cymosa (Bark)	Acetone	100.0	7.0	7.3	6.0	6.0	6.0
	Water	100.0	6.0	6.0	12.6	6.0	6.0
+ Control	Gentamycin	*30.0	21.0	19.3	21.7	26.3	22.3
Ehretia cymosa (Leave)	Acetone	100.0	6.0	7.0	6.0	6.0	6.0
	Water	100.0	6.0	6.0	11.0	6.0	6.0
+ Control	Gentamycin	*30.0	21.0	19.3	21.7	26.3	22.3
Kigelia africana (Bark)	Acetone	100.0	11.3	10.0	7.3	7.0	6.0
	Water	100.0	7.7	6.0	6.0	6.0	6.0
+ Control	Gentamycin	*30.0	19.0	9.0	21.0	19.0	17.0
Fagaropsis angolensis(Bark)	Acetone	100.0	6.0	6.0	6.0	6.0	6.0
	Water	100.0	6.0	6.0	6.0	6.0	6.0
+ Control	Gentamycin	*30.0	21.0	19.3	21.7	26.3	22.3
Dovyalis abyssinica (Leaves)	Acetone	100.0	6.0	6.0	6.0	6.0	6.0
	Water	100.0	6.0	7.0	6.0	6.0	6.0
+ Control	Gentamycin	*30.0	21.0	19.3	21.7	26.3	22.3
Periplocca linearifolia (Leaves &stem)	Acetone	100.0	6.0	6.0	6.0	6.0	6.0
	Water	100.0	10.3	7.0	7.0	6.0	6.0
+ Control	Gentamycin	*30.0	21.0	19.3	21.7	26.3	22.3

***The concentration of the control standards drugs Gentamycin are in ug/ml.**
***The values are an average of three replicates for each.**

4.4.2 Minimum Inhibitory Concentration (MIC)and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) of the plant extracts which had inhibition diameters of 10.0 mm and above in the disc diffusion assay (significance activity) was determined and the results were recorded. *Ekebergia capensis* acetone extracts recorded the lowest concentration of 3.125 mg/ml against *S. aureus* whereas water extracts of *Periplocca linearifolia* and *Ehretia cymosa* (bark) recorded the highest concentration of 50.0 mg/ml against *S. aureus* and *P. aeruginosa,* respectively. Table 4.6 below.

The active plant extracts that had a zone of inhibition of 10.0 mm and above in the disc diffusion assay were considered active and MBC was carried out to determine whether they were cidal or static. The four plants investigated for MBC showed that *Ekebergia capensis* , *Kigelia africana* and *Periplocca linearifolia* were bactericidal at different concentrations against different strains of bacteria while *Ehretia cymosa* (bark) was bacteriostatic against *P. aeruginosa* at 50mg/ml . The results are as shown in table 4.6 below.

Table 4. 5 showing the Minimum Inhibitory Concentration (MIC) in mg/ml and Minimum Bactericidal Concentration (MBC) in mg/ml, results of active plant extracts

Plants	**Plant part**	**Type of extract**	**Test organism**	**MIC Mg/ml**	**MBC mg/ml**
Kigelia africana	Bark	Acetone	*S. aureus*	6.25	6.25
Kigelia africana	Bark	Acetone	*MRS. aureus*	100.0	100.0
Ekebergia capensis	Bark	Acetone	*S. aureus*	3.13	3.13
Ekebergia capensis	Bark	Acetone	MR*S. aureus*	12.25	12.25
Ekebergia capensis	Bark	Water	*P. aeruginosa*	6.25	6.25
Periplocca linearifolia	Stem& leaves	Water	*S. aureus*	50.0	50.0
Ehretia cymosa	Bark	Water	*P. aeruginosa*	50.0	50.0
Standard (Gentamycin)		Water	All tested bacteria	0.5	50.0

Key

*****The concentration of the control standards drugs Gentamycin is in ug/ml.

MR. - Methicillin resistant

4.5. Cytotoxicity test

The extracts of the active plants were tested for *in vitro* cytotoxicity, showed that aqueous extract of *E. capensis* was moderately cytotoxic with CC_{50} of 12.5 µg/ml while the rest of the extracts were not cytotoxic at 100µg/ml ;the highest concertration that was tested.This is shown in table 4.8 below which summarises cytotoxicity against vero cell lines.

Table 4.6 showing cytotoxicity (CC_{50}) of the active plants extracts to VERO E6 cells

Plant	**Plant part**	**Type of extract**	**CC_{50}(µg/ml)**
Ekebergia capensis	Bark	Acetone	12.5
Ekebergia capensis	Bark	Water	≥100
Periplocca linearifolia	Leaves & stem	Water	≥100
Eheretia cymosa	Bark	Water	≥100
Kigelia africana	Bark	Acetone	≥100
$_{+}$Control	Chloroquine		24.78

Key.

- CC50 - 2< µg/ml- Cytotoxic

-CC_{50} between 2-89 µg/ml- Moderately toxic

- CC_{50}>90 µg/ml- Not toxic

CHAPTER FIVE

DISCUSSION

A total of six plants were studied which are known for their healing properties and are used for the treatment of various human diseases ranging from stomachache, headache, diarrhoea, chest infection, skin diseases and wound infections among others. The criteria that was used to decide the medicinal plants collected in the study included the disease they cure, parts used, method of preparation, their effectiveness, their habitat and how common they are used in the region.(Table 4.2).Majority of the studied plants were cultivated, and the herbalists collect only a few from the wild, for example is *Fagaropsis angolensis*. The most commonly used method was oral application of decoctions especially for internal diseases like tuberculosis and gastrointestinal ones, for example dysentery, (Jeruto *et al*., 2007) (Table 4.2). It was found that the most commonly used part of the plant was found to be the bark which due to high concentration of secondary metabolites hence high activitry, this agrees with the findings of (Grace,2002) (Table 4.1).

The leaves were noted to have a high percentage yields of secondary metabolites as compared to the stem bark in the two solvents used (Kigondu, 2007) also got similar results (Table 4.3). This is because the leaves manufacture secondary metabolites and contain little fibres as compared with the barks (Kigondu, 2007). However, they had low activity compared with the barks, hence the locals should use the bark for treatment(Table 4.6). For example, the leaf extracts from *Dovyalis abyssinica* and *Ehretia cymosa* had inhibition zone diameter of (7.0 mm) against MR*S. aureus*. This is attributed to the absence of medicinal plant phytochemicals that are known to contribute to the activity against the selected pathogens and also the leaves

manufacture secondary metabolites and are transported away and stored at barks at high concentration for being excretory and storage organs (Bibitha *et al*., 2002).

A total of 14 water and acetone extracts from different parts of the six plant species were investigated. The comparison of average percentage yields of all the solvents used showed that water extract had the highest yields in all the plants parts extracted in comparison to the organic solvent (acetone) (Table 4.3). This is due to the fact that water is very polar than organic solvent hence it is able to extract more compounds from a plant material, (Muthaura *et al*., 2007).

Furthermore, the antimicrobial activity of acetone was lower than that of water, these variations observed on potency of the extractants used to extract phytochemicals are in conformity with the reports by (Vinoth-Raja, 2009) which revealed that water extract of the plants were more effective than acetone extracts particularly on *Pseudomonas sp*. For example, *Ehretia cymosa* (bark) water showed activity zone inhibition of 11.0mm against *P. aeruginosa* only while the acetone extracts showed lower activity with a zone inhibition of 7.0 mm against *S. aureus* and *MR S. aureus*(Table 4.5). The most plausible reason for this is that water is very polar and it extracted some compounds that were not extracted by acetone (Nostro *et al*., 2000). However, water and acetone extracts from *Fagaropsis angolensis* did not show any activity against bacteria and fungi tested at 100 mg/ml. This does not mean that the plant extracts are not medicinal because they phytochemicals present may be active in combination with other plant extracts due to synergistic effect of several compounds that are active singly (Gessler *et al.*,1995).This is the reason why traditional

practioners mix several plants when preparing concotions. It is also possible that some of the compounds found in *Fagaropsis angolensis* could exhibit activity *in vivo* due to enzyme catalyzed transformation into potent derivatives and therefore are playing the role of prodrugs, (Omulokoli and Chhabra, 1997).

All plants screened contained terpenoids, phenolics and flavonoids and were also active against *Staphylococcus aureus* with inhibition zone ranging from 14.7 mm for *E. capensis* (bark) to 7.0 mm for *E. cymosa* (bark) except *F. angolensis* (bark) and *D. abyssinica* (leaves) with an inhibition zone of 6.0 mm (Table 4.5). The medicinal value of these plants lies in their secondary metabolites, some of the most important of these bioactive constituents of the medicinal plants are alkaloids, anthraquinones, flavonoids, and phenolic compounds (Edeoga *et al*., 2005). The properties of the phytochemical ingredients could explain the results of the antibacterial activities observed in the present study. This is corobarated by previous studies by (Oyewole *et al*., 2012) for *Telfairia Occidentalis* (fluted pumpkins) leaf extract which were active against selected intestinal pathogens.

Ekebergia capensis and *Ehretia cymosa* were found to contain saponins yet they did not show any activity on the fungi and yeast which were not in agreement with the finding of (Delmas *et al*, 2000). This may be due to the possibility of the existence of the saponins in different structures. For example, those known to exhibit toxicity against fungi as observed by (Miyakoshi *et al*., 2000).) have a branched-chain trisaccharide moiety without any oxygen-containing groups at C2 and C12 exhibited the anti-yeast activity, while saponins with 2β-hydroxyl or 12 keto groups showed very weak or no activity (Miyakoshi *et al.*, 2000). Saponins with a disaccharide

moiety exhibit relatively low activity and the aglycones saponins have no activity. This is supported by the cytotocity test on the active plant extracts which showed the aqueous extract of *Ekebergia capensis* being moderately cytotoxic with CC_{50} of 12.5µg/ml.(Table 4.8)

Many naturally-occurring compounds found in plants have been shown to possess antimicrobial activities and could thus serve as a source for both traditional and conventional medicine (Kim *et al.*, 1995; Akinyemi *et al.,* 2007). Antibacterial activity of phytochemicals flavonoids (Tsuchiya *et al*., 1996), saponins (Soetan *et al.,* 2006) isolated from plant materials have been studied. The antibacterial activity of plants that were found to be active suggest that the extract contain effective active phytochemicals responsible for the elimination of microorganisms. For instance, *E. capensis* (bark) that contain both flavonoids and saponins and had the highest zone of inhibition of 14.7 mm against *Staphylococcus aureus*. *Kigelia africana* (bark) and *Periplocca linearifolia* (stem and leaves) plants extracts also showed high activity probably due to the presence of flavonoids in high amounts. Similar findings were also made by Lutterodlt *et al*., (1994) that showed flavonoids are active against bacteria. Flavonoids are known for their anti-allergic effect as well as a wide variety of activity against Gram-positive and Gram-negative bacteria, fungi and viruses (Afolayan and Meyer, 1997). This may be the reason why *E. capensis* and *E. cymosa* plant extracts were active against *Pseudomonas aeruginosa* and *Staphylococcus aureus* both being Gram-negative and Gram-positive with an inhibition zone of 14.7mm and 11.0mm. The plants containing more of these metabolites demonstrated

stronger antimicrobial properties. This observation is in agreement with findings by Geyid *et al*, (2005).

The water extract from *E. capensis* showed antibacterial activity against *S .aureus, P. aeruginosa* and MR *S. aureus* which are associated with skin diseases and wound infections, this agrees with the information given by the traditional practitioners. This is also supported by the findings of Tabuti (2007) *didymobotrya* DCM extracts which was active against MR S. *aureus* and *P. aeruginosa*, and were considered as potential for control of wound infections

The water extracts from *Periploca linearifolia* showed high antibacterial activity against *S .aureus* and low activity against *P. aeruginosa*, this is because *P. aeruginosa* is a gram negative bacteria which is rich in lipopolysaccharides in the outer membrane which exclude certain drugs and antibiotics from penetrating the cell, partially accounting for why Gram-negative bacteria are generally more resistant to antibiotics than are Gram positive bacteria (Kaplan, 2012) (Table 4.5).

The variations observed in the potency of the plant parts used to inhibit bacterial growth are in conformity with the reports of Duke (1992) and Yusha' *et al.* (2008) who observed that antibacterial activity may vary from one plant part to another. For example, the acetone extracts of *Ehretia cymosa* (leaf) was 7.0 mm. against MRS .*aureus* while those of *E. capensis (*bark*)* extracts was 12.3 mm. *K. africana (*bark) acetone extract were active against the four bacteria tested with an inhibition zone of 11.0 mm, 7.0 mm, 7.0 mm and 10.0 mm against *S .aureus, P. aeruginosa, E. coli* and

MR S. aureus respectively while water extracts were active against *S .aureus* only. This also supports Bibitha *et al.* (2002) who reported variations in the antibacterial activities of different plant extracts.

Among the six plants screened, the largest inhibitory zones were observed with the extracts of *E. capensis* (14.7 mm) and the bark of *E. cymosa* against *S .aureus,* with that of *E. cymosa* (bark) having 12.7 mm against *P. aeruginosa.* Compared with the drug of choice Gentamycin which had an inhibition zone of 17.0-24.0 mm, the plants were comparable since they were crude extracts. All the plant extracts were more effective against two Gram positive bacteria *S. aureus* and MR *S. aureus* than against the selected Gram negative bacteria due to the resistantance to antibiotics by gram negative bacteria as explain by Kaplan, (2012).

Moreover, the crude extracts were not active against the fungal pathogens selected with an inhibition zone of 6.0 mms at 100 mg/ml. Fungi are eukaryotes thus the cell is difficult to be penetratd by the plant extracts due to the presence of cell wall made of cellulose. Many plant products are of low molecular weight in vitro. These compounds may be preformed inhibitors that are present constitutively in healthy plants or synthesize by plants in response to pathogen attack (phytoalexins). Successful pathogens must be able to overcome these antifungal defenses. Bacterial infections are prevalent due to various factors such as the HIV/AIDS pandemic, poor hygiene, overcrowding and resistance to conventional antimicrobials but natural products obtained from higher plants may provide a new source of antimicrobial agents with possibly novel mechanisms of action, (Adenisa *et al*., 2000). Infections

associated with bacterial pathogens are among some of the indications treated using traditional remedies in Kenya (Njoroge and Bussman 2007). This is the reason why the medicinal plants under investigation found effective on bacteria than the fungi.

The MIC values ranged from 3.125 mg/ml as the most potent to 100 mg/ml as the least potent. The most potent plant extracts with MIC 3.125 against *S. aureus* proved to be *E. capensis* and 6.25 mg/ml for *K. africana* against the same microorganism. The high MICs and MBCs of the plant extracts investigated of up to 100.0 mg/ml for *Kigelia africana* (bark) acetone extracts against MR *S. aureus* is attributed to high resistance rates of the test isolates isolated from humans. This is concurs with Bibitha *et al.*, (2002).Though, MR *S. aureus* has been known to be resistant pathogen to conventional drugs such as methicillin and oxacillin antibiotics, *E. capensis* water extracts and *K. africana* acetone extracts proved to be effective against MR *S. aureus* with inhibition zones of 11.6mm and 10.0mm respectively (Table 4.5). Therefore, the two plants can be used for the treatment of the infections associated with this resistant pathogen. The emergence of antibiotic resistance is an evolutionary process that is based on selection for organisms that have enhanced ability to survive doses of antibiotics that would previously be lethal (Cowen, 2008).

The other plant extracts were not as active as those from *E. capensis* and *K. africana*. The concentrations and proportions of the active compounds in plant extracts components depend on the plant variety, origin, time of harvest, solvent used, conditions of processing and storage (Deans and Ritchie, 1987).

The MBC assay, revealed the concentrations at which the active plant extracts were bacteriostatic or bactericidal on the test organisms. The cidal and static activities of

the active plant extracts was in the trend of its antibacterial assay. The active plant extracts that had an inhibition zone of 10.0 mm and above were considered active and MBC was carried out to determine whether they are cidal or static.

The four plants investigated for MBC showed that *E. campensis* water extracts were bactericidal against *S. aureus* and *P. aeruginosa,* while *P. linearifolia* and *Kigelia africana* were bactericidal against S. *aureus.* This is attributed to the presence of terpenoids containing OH group which is an efficient uncoupler of the bacterial plasma membrane creating instability and breaks the plasma membrane hence killing the bacterial cells, (Hammond and Lambert, 1981). sssOther antibacterial compounds may target bacterial cell wall (penicillins, cephallosporins), cell membrane (polymixins,) and bacterial enzymes (quinolones and sulfonamides which are bactericidal in nature). Those which target protein synthesis such as the aminoglycosides, macrolides and tetracyclines are usually bacteriostatic (Finberg *et al*., 2004). This agrees with the results of *E. cymosa* (bark) which was bacteriostatic against *P. aeruginosa* as there was growth in the whole plate swabbed. This may be also be due to the presence of anthraquinones present that are known to be bacteriostatic against *P. aeruginosa* as described by Kazmi (1994), who found an anthraquinone from *Cassia italica*, a Pakistani tree, which was bacteriostatic for *Bacillus anthracis*, *Corynebacterium pseudodiphthericum*, and *Pseudomonas aeruginosa* and bactericidal for *Pseudomonas pseudomalliae*, (Kazmi *et al*., 1994).Although terpenoids are known to be bactericidal *E.cymosa* was bacteristatic yet it contained large quantities of this probably due to the terpenoids of different structures from those known to contribute bactericidal activity.

Toxicity studies are very important during the screening of medicinal plants in order to determine their safety. The plant extracts that had a zone inhibition of 10.0 mm and above on any microorganisms were considered potent and their cell toxicity were determined. The CC_{50} for the plants extracts was tested on concentration 100 µg /ml to determine their safety. Cell toxicity of the plant extracts were categorized into three: cytotoxic at CC_{50}<2 µg/ml, moderately toxic at CC_{50} between 2-89 µg/ml and not toxic at CC_{50}>90 µg/ml (Rukunga and Simons, 2006).The extracts of *E. cymosa* (water),*P. linearifolia*(water), *K. africana(*Acetone) and water extracts of *E. capensis* were considered to be safe because it recorded a CC_{50} which was greater than 90 µg/ml while the acetone extracts of *E.capensis* was moderately cytotoxic with a CC_{50} of 12.5 µg/ml. *In vitro* cytotoxicity does not mean that an extract cannot be used in humans (Kokwaro 1993) as there is potential for isolation of save non-toxic compounds. For instance, *galega officinalis* is a plant that has proved too toxic for widespread agricultural use, with the potential to induce tracheal frothing, hypertension, paralysis and even death and yet Metroformin the current gold standard for management of Type II diabetes was isolated from it. Experimental and clinical evaluation of Galegine, a substance produced by the herb *G. officinalis* provided the pharmacological and chemical basis for the subsequent discovery of Metformin (Bailey *et al.,* 2007).

These result agrees with the study done by Kigondu, (2007) on cell toxicity of medicinal plants. Chloroquine which was a positive control had a CC_{50} of 24.78. Water extracts were not cytotoxic while the acetone extracts of *E. capensis* were moderately toxic. This results are in agreement with other work done by Muthaura *et*

al., (2007) on antimalarial properties of *Boscia angustifolia* water extracts, which he found to have no cytotoxicity. Cepleanu *et al.*, (1994) also found out that the water stem/bark extracts of *B. angustifolia* had neither cytotoxicity nor brine shrimp lethality. These results seem to confirm the validity of their traditional uses, since traditionally herbs are boiled in water (Gessler *et al.*, 1995). The other plant extracts tested had no cytotoxicity (CC_{50}>90) against Vero cell lines, suggesting that they may be safe as antimicrobials.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the study six medicinal plants were collected and identified namely: *Kigelia africana* (Lam.) and Benth, *Periplocca linearifolia* Dill and A. Rich, *Ekebergia capensis* Sparrm, *Ehretia cymosa* Thonn, *Fagaropsis angolensis* (Engl.) Dale and *Dovyalis abyssinica* (A.Rich.) that are commonly used to treat infectious diseases. Only five out of the six plants were scientifically proved to be active against the test microorganisms and therefore this validated their use by the people of Kaptumo.

All the plant extracts indicated presence of phenols, terpenoids and flavonoids that may be responsible for the antimicrobial activities noted.

The antimicrobial activities were reported in tandem with the reported uses by herbalists hence supports and provides a scientific basis for use of these plants in herbal remedies The active plant extracts were active against gram positive and gram negative bacteria thus it is considered to be having broad spectrum of activity..

Cell toxicity showed that most of the plant extracts tested were not cytotoxic against Vero cell lines except the acetone extracts of *E. capensis* that were moderately toxic with a CC_{50} (µg/ml) of 12.5 , suggesting that the former extracts may be safe as antimicrobials.

Cytotocixity test, Minimum Inhibition Concentration and Minimum BactericidalConcentration values in mg/ml for active plants against different Bacteria

selected provides the guidance as to doses that can be effectively used for drug administration.

6.2 Recommendations

Those plants that were containing more of the secondary metabolites demonstrated stronger antimicrobial properties.This asserts the need for further investigations using fractionated extracts and purified chemical components to tested against microorganisms.

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APPENDICES

Appendix I: Microtitres plates

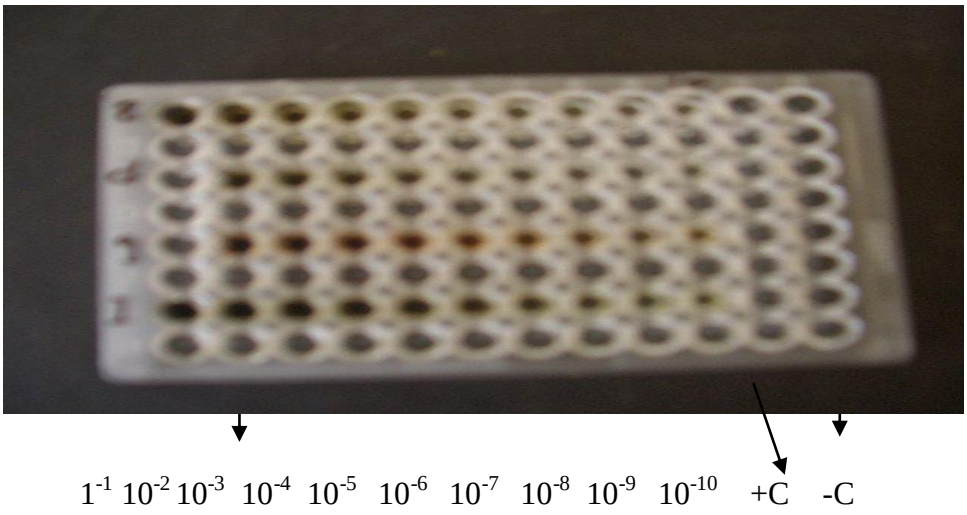

Plate 6.1, Microtitre plates showing MIC's

The plate shows extracts at different concentration beginning from 1-1– 10-10 dilution factor

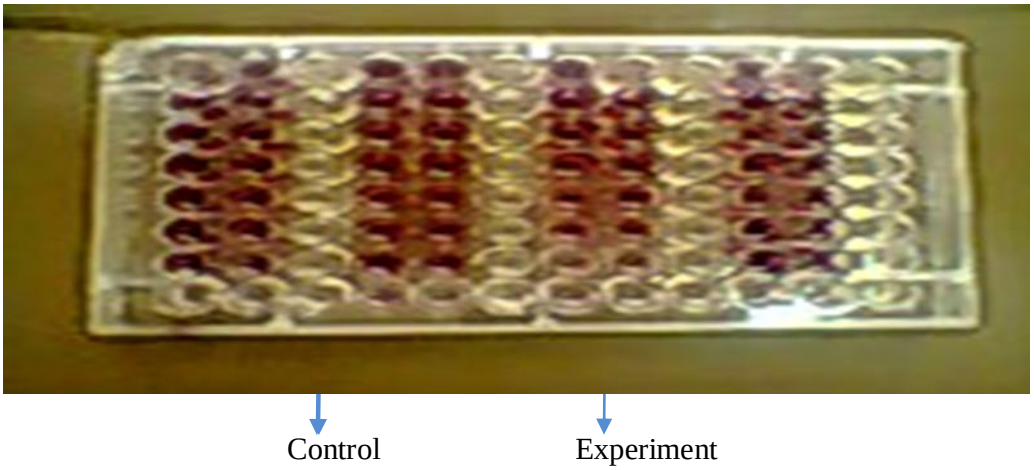

Plate 6.2: Microtitre plates showing cytotoxicity test

Appendix II: ANOVA TABLES

Table 6.1: Showing the mean zone of inhibition

Zone of inhibition

PLANT	Mean	N	Std. Deviation	Sum	Std. Error of Mean
Kigelia africana	7.30	30	1.95	219	0.36
Periplocca linearifolia	6.63	30	1.38	199	0.25
Ehretia cymosa bark	6.60	30	2.34	198	0.43
Ehretia cymosa leave	6.43	30	1.33	193	0.24
Ekerbergia capensis	8.77	30	3.20	263	0.58
Dovyallis abyssinica	6.10	30	.31	183	0.27
Faragopsis angolensis	6.00	30	.00	180	0.00
Positive control	23.13	30	2.34	694	0.43
Negative control	6.00	30	.00	180	0.00
Total	8.55	270	5.52	2309	0.34

Table 6.2: Showing F-test for mean zone of inhibition based on plants extract

		Sum of Squares	Df	Mean Square	F	Sig.
Zone of inhibition * PLANT P	Between (Combined) Groups	7357.407	8	919.676	287.341	0.001
	Within Groups	835.367	261	3.201		
	Total	8192.774	269			

Table 6.3:Showing mean zone of inhibition of the extracts

Treatment	Mean	N	Std. Deviation	Sum	Std. Error of Mean
Water	8.61	135	5.58	1162	0.48
Acetone	8.50	135	5.48	1147	0.47
Total	8.55	270	5.52	2309	0.34

Table 6.4:Showing F-test for the zone of inhibition on the treatment.

			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Zone of inhibition Treatment	Between Groups	(Combined)	0.833	1	0.833	0.027	0.89
	Within Groups		8191.941	268	30.567		
	Total		8192.774	269			

Appendix III: Questionnaire

NO……

SECTION A: Demographic data of the herbalists

1. Location……

2. Sex Male ☐ Female ☐

3. Age bracket (20-30) ☐ (31-40) ☐ (41-50) ☐

(51-60) ☐ (61-70) ☐ (71-over) ☐

4. Level of education……..

SECTION B: Use of medicinal plants

Instructions; Answer the questions by marking the appropriate answer ( )

1. Local name of the plant.....

2. Scientific name of the plant……..

3. What is the habit of the plant?

Tree ☐ Shrub ☐ Herb ☐ Climber ☐

Other specify……..

4. Where do you obtain the plant?

Forest ☐ Farms ☐ Swamp ☐ River banks ☐

Other specify……..

5. At what stage do you harvest the plant?

Seedling ☐ Middle stage ☐ Mature ☐

Other specify……

6. Which part of the plant is used?

Leaves Stem ☐ Bark ☐ Root ☐ Flower ☐ ☐

Other specify……

7. What quantities of the plant do you harvest?

1-5 kg ☐ 6-10kg ☐ 10 kg and over ☐

Other specify……

8. Is the plant available?

Very common ☐ Common ☐ Not-common ☐

Other specify……

9. How do you prepare the parts harvested?

……

10. Which disease(s) does the plant treat?

……

……

11. What are the other uses of the plant ……

……

12. How effective is the plant

Very effective ☐ Effective ☐ Fairly effective ☐

Other specify……

13. How often do you harvest the plant?

Frequently ☐ Occasionally ☐ Rarely ☐

Other specify……

SECTION C: Information on patients

1. Group of people treated Children ☐ outh ☐ Old

2. Gender of the patients Male ☐ Female ☐

3. Level of Education of the patients Primary ☐ Secondary ☐ Tertiary ☐

4. What quantities of the plant do you use to treat ……