

**E FFICACY OF SELECTED MEDICINAL PLANTS USED BY THE
OGIEK COMMUNITIES AGAINST MICROBIAL RELATED
INFECTIONS**

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**A Thesis Submitted in Fulfillment of the Requirements for the Award of the Degree of
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DECLARATION

This thesis is my own work and has not been presented for a degree award in any other institution.

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DEDICATION

This work is dedicated to my elder brother, Peter Sumba-Okayo Amuka, through whose self denials earlier in life, made it possible for me to obtain University Education.

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immuno Deficiency Syndrome
df	degree of freedom
Gram positive	Gram Positive Bacteria
Gram negative	Gram Negative Bacteria.
HIV	Human Immuno-deficiency Virus
HS	Hospital Strain
ITC	International Trade Centre.
MBC	Minimal Bacterial Concentration
MDR	Multi drug resistant
MFC	Minimal Fungicidal Concentration
MIC	Minimal inhibitory concentration
MRSA	Methicilin Resistant <i>Staphylococcus aureus</i>
ORSA	Oxycellin Resistant <i>Staphylococcus aureus</i>
STD dev	Standard deviation
Syn.	Synonyms
TB	Tuberculosis
TM	Traditional Medicine
Typhi	<i>typhiranium</i>
Var.	Variety
WHO	World Health Organization
µg	micrograms
µML	Microlitres

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ABSTRACT

Ethnobotany and traditional medicines have been used for bioprospection for modern pharmaceutical in all the world continents. In Kenya the ethnobotanical of several communities has revealed that plants are important source of medicinal products that require further research to establish their validity, efficacy and quality of health services. The Ogiek communities who currently live in harmony with nature and have minimal with modern cultural practices exhibit high potentials for discovery of new pharmaceutical products through ethnobotanical, phytochemical, and microbial strategic studies. Furthermore, such studies have been necessitated by newly emerging and reemerging diseases and the development of resistance of diseases to the drugs that are currently in use. The Ogiek community, who has lived in Mau forest for over six centuries, is fast being assimilated into other culturally stronger communities. However, their life is shrouded in secrecy and their rich cultural practices including ethnomedicine are being lost. Ethnobotanical field surveys revealed that over 80% of the Ogiek community has constructive knowledge of the forests and forest products. They have clear knowledge of medicine plants, their pharmaceutical procedures and pharmacological manifestations. It was evident that majority of the human health complaints were parasitic, bacterial, and fungal in nature. Symptomatic areas were the abdomen, chest, and the head. A total of 49 plant species in 33 families were collected. Bioassay of the extracts showed that some of the plant species possessed promising antimicrobial activities. In order to verify the efficacy of the drugs, selected pathogens were selected and *in vitro* studies carried out on individual crude extracts and essential oils. It emerged that 16 plant species showed reasonable biological activities on the selected human pathogens. Further studies to confirm the efficacies of the drugs were successfully done to establish the susceptibility (MIC, MBC and MFC) individually. Several metabolites known for their efficacy were identified. There is need to further document the Ogiek culture, *in situ* and *ex-situ* conservation of the Mau forest complex so as to conserve the biodiversity for the future generations. Further elucidation of the extracts may lead to discovery of new pharmaceutical compounds that could be used in the synthesis of new drugs for the currently challenging medical conditions.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

There is a general belief that traditional forms of treatments involving the use of plants and plant extracts are archaic and ineffective. The notion is an ill-conceived one. This may not necessarily be true. To remove this myth from the minds of scholars, there are records in various parts of the world to the effect that human has made judicious selections of plants that can be used for curative purposes. The development of such art has been gradual and over a long period of the civilization evolutionary process (Heinrich, 2000).

Some records to that effect are found in the Indus civilization dating back to 900 BC and the second millennium BC (Ali, 2008). There is even a lot of evidence contained in hymns found in the *Rigveda* as well as the *Atharvaveda* which contains the records of useful plants (Rajan *et al.*, 2005). In the Indian classical medicine it may be traced that plants form an important and an integral part of Ayurvedic *pharmacopoeia* (Patwarthan *et al.*, 2005). In other studies, a total of 341 different plant species are documented in the *Charaka Samhita* (900 BC), as useful in the management of human health (Ali, 2008). In the *Susruta Samhita*, there are a total of 395 plant species listed for the same purpose (Majumdar, 1971). It is evident that other treatise authors, from South East Asian region in this field, have over 70 species of plants with the list being expanded to 600 of plants that are used in Ayurvedic (Namjoshi, 1979). Such a culture depending on Mother Nature has been practised for over 2000 years (Namjoshi, 1979). Similarly, as the Indus civilization took root, the Chinese were also evolving the culture of phytomedicine the *Kanpo* which was systematised in the *Shang Han Tsu Ping Lun*, a 16-volume compendium. It is believed that the compendium which was compiled by Chang Chung Ching must have been done in the 2nd century (456-536 AD).

The compilation *Shiri-Nung Pen Tso Ching* of Tao Hung-Ching comprises about 365 crude drugs, all of which are of plant origin (Shibata, 1981). It is only in the last three decades that scientific evaluation of plant efficacy has generated interest (Farnworth and Bingel, 1977). With the advent of scientific methods of analysis, many of these reported medicinal plants came under scrutiny leading to the elucidation of their active principles. In the Amazonia, the early South and Central American culture dating back to 1000 BC, had systematic studies of the indigenous flora and documented knowledge of the advantage of the local inhabitants which confirms that a pharmacopoeia existed for the Indian population (Gotlieb, 1979).

From the ancient Egyptian culture in Africa, at about 1600 BC, there is enormous literature pertaining to the use of plants as food and for curative purposes (Diop, 1989). An Egyptian medical treatise (papyrus), drawn up in Thebes, during the aforementioned period, contains an inventory of 700 plants used in medicine (Pelt, 1979; Diop, 1989). There are also Egyptian motifs depicting appreciation and celebrations of bountiful harvests after a successful agricultural cropping year (Diop, 1989). In West Africa in more recent times, such as amongst most communities, for example, the Yoruba prior to the European colonisation, it was mandatory that a young boy before initiation into adulthood had to learn the names of all the useful plants in relation to future uses by the pupil in life (Rodney, 1971). The Greeks and the Romans, subsequent cultures that emerged after the Egyptian, contain all that it inherited from the latter. This is evidenced by the works of Hippocrates (370-287 BC) and Dioskorides (Taylor, 1965) that had extensive knowledge of medicinal plants (Kochhar, 1989). Dioskorides, a Roman soldier physician, made the first taxonomic compendium of useful medicinal plants in the Roman Empire (Sharma, 1982).

One reason for choosing plants is that they are readily available either for free or at minimal cost which majority of the rural poor communities in the developing and the developed world can afford (Samie *et al.*, 2005). Of the entire world flora, 250,000 species have been identified and used for curative purposes (Patwadharn, 2005). This number represents only 15% those species that have been effectively investigated and found useful (Okeke, 2005). Consequently, there is a staggering over 85% of higher plants which are yet to be investigated. Through ethnobotany, the useful plants can be deciphered from a large list of higher plants numbering a total of 850, 000 plant species. Most of these plants occur in the tropical and sub- tropical floral diversity (Ernst, 2005), and a large number of this floral diversity has not so far been prospected (Buchman, 1980). Reliance on Ethnobotany to carry out or do bio-prospecting has enabled humans to identify, recognize, and incorporate certain compounds into various pharmacopoeias of the world (Buchman, 1980). A few of such plants and their identifiable compounds are codeine, ephedrine, digoxin, atropine, quinidine, theophylline and caffeine (Swaminathan, 1989). Some of the aforementioned compounds are now used in modern allopathic medicine without any modification. However, some drugs which are of plant-based sources can now be synthesized in laboratories due to the low cost of production (Dev, 1989).

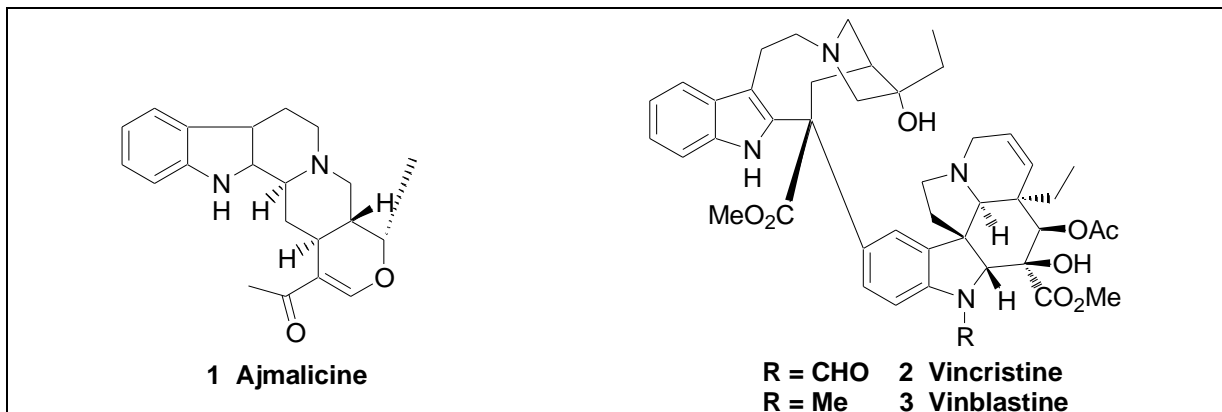
Based on the ethno-botanical information, there are some plants that have been found scientifically and economically important and are used in modern medicine (Kapur, 1982). Examples include *Prunus africana* used in the management of hyperplasia and *Artemisia annua*, an important source of Artimesinin currently used in malaria management. Some of the compounds included are not medicine *per se*, but serve as important raw materials that provide skeletons that are used in the manufacture of several pharmaceutical compounds. Although plants may not necessarily provide the drug wholly, their crude extracts may be

used as a skeleton for the synthesis of certain modern allopathic medicines. Such cases are found in diosgenin and sapogenin which are steroidal compounds used in the synthesis of steroidal and hormonal human drugs (Kapur, 1982).

Some plants which yield alkaloidal based drugs are widely distributed amongst higher plants, especially in the dicotyledons, that number in excess of 10,000 genera of which 9% contain such compounds (Asres *et al.*, 2005). The families; Amaryllidaceae, Apocynaceae, Liliaceae, Rubiaceae, Rutaceae and Solanaceae are known to possess alkaloids and 4000 alkaloids have been isolated from them (Suchs, 1983). These are plants which are considered as invasive in nature. Yet, they have been used in nature as source of medicine. Cough grass *Agropyron repens*, rhizome has a long history of medicinal use which was even known and recorded by Dioscorides (Dewick, 2002). Extracts from the rhizomes is used as demulcent and as diuretic for bladder and kidney complaints. Such uses were recognized even in the Greek times.

The Madagascar periwinkle, *Catharanthus roseus*, G. Dn, (Plate 1.1), an Apocynaceae which has potential as cancer remedy and its usefulness came into prominence in 1959-60. While studying the plant's ability to treat diabetes mellitus, some scientists accidentally found it effective in the management of human maladies like leukaemia and *caposis sarcoma* (Protzen, 1993). The hypoglycaemic principles could not however be substantiated. Some alkaloidal fractions contained in the extracts caused bone-marrow depression in the studies with rats. The scientists who were studying the extracts of the plant were able to isolate, from the alkaloidal fractions, Vinblastine which has anti-leukaemia activity (Dev, 1989). Eli Lilly scientists, an American pharmaceutical multinational company succeeded in isolating Vinblastine and other potent anticancer alkaloids. Since these alkaloids are present in the

plant at very low concentrations and in a mixture of 90 other alkaloids, their acquisition has only been possible with judicious systematic separation using appropriate pharmacological assays leading to elucidation of structures of ajmalicine (1), and vincristine (2) (Swaminathan and Kochhar, 1989).



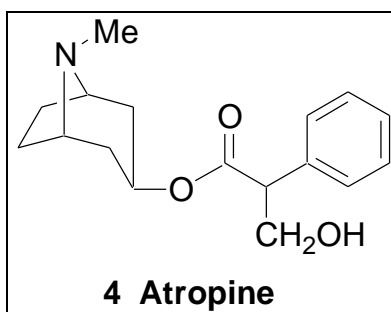
Vincristine (2) and Vinblastine (3) are amongst the therapeutically most useful antineoplastic agents. The sulphate of the latter is used to treat Hodgkin's disease while the sulphate of the former is used in paediatric and lymphatic leukaemia. They are administered intravenously. Often the drugs are used in cocktail with other therapeutic agents (ITC, 1982). The drugs are produced from two plant species which are erect shrubs with opposite and oblong leaves, grow up to 1m high, branching at the base with a spread up to 70 cm in diameter. The plant has two varieties based on the flower colours *C. roseus*, (Plate 1.1) which produces pink flowers and *C. alba* L. which has white flowers. The two varieties are grown as ornamental. Commercial cultivations are found in India, Israel and USA (ITC, 1982).



Plate 1.1: *Catharanthus alba*; left and *Catharanthus roseus*; right.

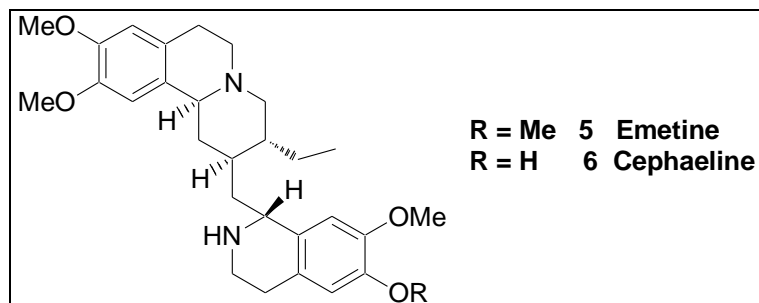
There are several plant species whose extracts have been incorporated into various pharmacopoeias of several countries. They include *Cephaelis spp*; *Cinchona spp*; *Papaver somniferum* (L); *Rhamnus purshiana* DC; *Digitalis spp* and *Dioscorea spp* (Schultes and Farnworth, 1980). *Celphaelis spp* Rubiaceae, which is a straggling evergreen shrub, produces rhizomes that have been used to cause vomiting and treat dysentery for centuries by the South American Indians and tribes (Taylor, 1965). By 17th century, the plant preparation was in use in Europe against amoebic dysentery. To date emetine hydrochloride, from *Celphaelis spp*, is still considered important in the treatment of amoebiasis through both subcutaneous and intramuscular injections and is effective against hepatic and bowel infections. Emetine-bismuth iodide is however, administered orally. The low doses of the drug preparations are used in cough and whooping cough (Schultes and Farnworth, 1980).

There are certain plants that have been used for curative purposes by various civilizations. Henbane (*Hyoscyamus niger* L. seeds were used by the Babylonians to relieve problems of toothache (Dev, 1989). Belladonna (*Atropa belladonna* L. has been used in Europe for centuries to relieve pain and was recorded in the London Pharmacopoeia in 1809. Belladonna roots and leaves are reliable source of atropine (4), which is important in the treatment of eye diseases (mydriasis).



Other plants yielding these important alkaloids are *Hyocymus muticus* (L), *Datura inoxia* Miller, *Datura metel* L., *Datura stromanium* L. and *Duboisia lechahardtii* F.v. Muell. Other uses of the drugs from this group of plants are used in the treatment of asthma, whooping cough and as an antidote to poisoning by cholinesterase inhibitors. Scopolamine and Hyoscine are used in the treatment of duodenal ulcer (Cordell, 1981).

The world requirement of the drug emetine (5) is met by synthetic sources. However, *Belladonna* is still commercially produced in Brazil and India with USA being the major importer. The demand for preparations based on the whole crude drug, such as ipecacuanha is expected to remain stable (ITC, 1982). Below are the structures of a closely related alkaloid, cephaeline (6).



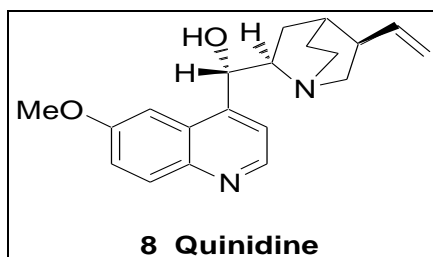
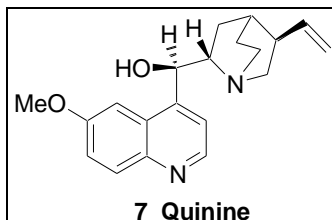
In malaria infested countries, quinine was a household name. These are alkaloids that occur naturally in *Cinchona* spp, Rubiaceae and are an indigenous of the slopes of Eastern Andes. The bark of the plant was used by the Peruvian Indians to cure fever (Dev, 1989). The drug reached European medicine and appeared in the British pharmacopoeia in 1677. Currently there are commercial plantations in Africa, Asia and South America (Schlutes and Farnworth, 1980).



Plate 1.2: *Artemisia annua*, commercially grown at Maseno University Farm

During the last half of the 20th century, patients suffering from malaria developed resistance to quinine and were replaced with chloroquine as a drug of choice but have also been withdrawn as first line of treatment Quinine (7) was also reintroduced in the 1990s as a

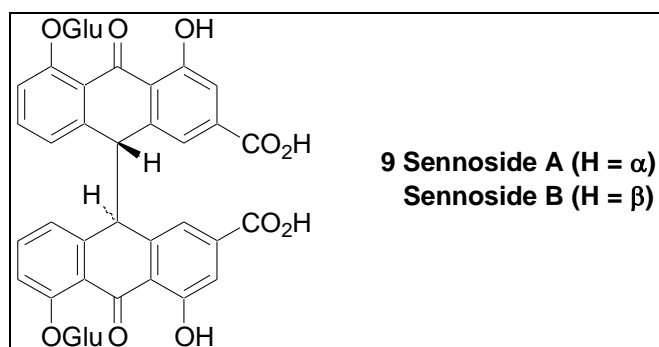
reliable source of treatment against malaria (Kirk-Othmer, 1978). Over 40 species of *Cinchona* are known but most important ones are *C. succirubra* Pavon ex klotzsch; *C. calisaya* Weddel; *C. officinalis* L; *C. ledgeriana* Moens ex Tremen and some hybrids do exist (Atal and Kapur, 1982). Quinidine (8) isolated from the *Cinchona* is a natural antiarithmetic drug.



A combination of quinine and (8) quinidine, an aminoquinolone, is recommended for malaria and relief for nocturnal leg cramps (Kirk-Othmer, 1978). Quinine sulphate is also used in food and drinks preparations while quinidine sulphate is used in cardiac arrhythmias (Kirk Othmer, 1978). Currently, there is a world increase in demand for *Cinchona* products (ITC, 1982). The above drugs are also fast being replaced by extracts of *Artemisia annua* L. (Plate 1.2) as the first line of treatment.

Anthroquinone glycosides compounds are found in several higher plant species and plant choice as source is variable (Dev, 1989). Some important plants currently used in various countries include *Aloe* spp., *Cassia* spp., *Rhamnus purshiana* DC (cascara) and *Rheum* spp (rhubarb) Basically, the major constituents of such drug plants are hydroxyan anthroquinone

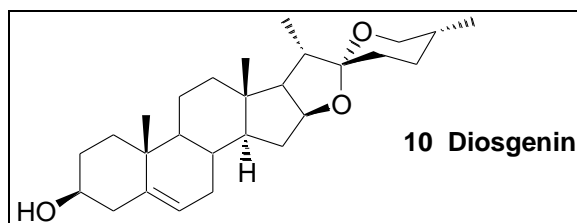
derivatives and their glycosides, which have huge markets as laxatives, reaching annual sale of \$ 300 million as imports into the USA (ITC, 1982). *Aloe barbadensis* Mill. Liliaceae, (Syn *A. vera* L. and *A. ferrox* Mill.) have become important; and all over the world farmers are turning to establish these plants (MoA, 2004). Mature plants, are squeezed, and the sap exported for use in pharmaceutical and cosmetic industries. Rhubarb that comprises rhizomes of *Rheum palmatum* L. is an ancient drug in China since 2700 BC (Codd, 1972). Major chemical constituents include emodin, aloe-emodin, rhein, chrysophanol and their glycosides. Cascara was at one time a major drug for constipation and was extracted from the bark of *Rhamnus purshiana* DC (Rhamnaceae). There is a reasonable world output of the raw material from which categories of cascariosides A B C and D are extracted (Codd, 1972).



The sennoside A and B (9) is extracted from *Senna aungustifolia* Delile (Fabaceae) and is used as a laxative in lidoginom of Alexandria (Codd, 1972). Currently, seeds of the plant are exported to Europe. The main constituents are glycosides sennoside A and B used as “tea”. Calcium sennosides are manufactured in Switzerland, the USA and India and used as carminative (ITC, 1982).

Steroids are some of the natural products extensively used in pharmaceuticals (Dev, 1989). Unfortunately, it was believed that animal source was the only available avenue for their acquisition. In 1936, Marker discovered a sapogenin from the roots *Dioscorera* spp

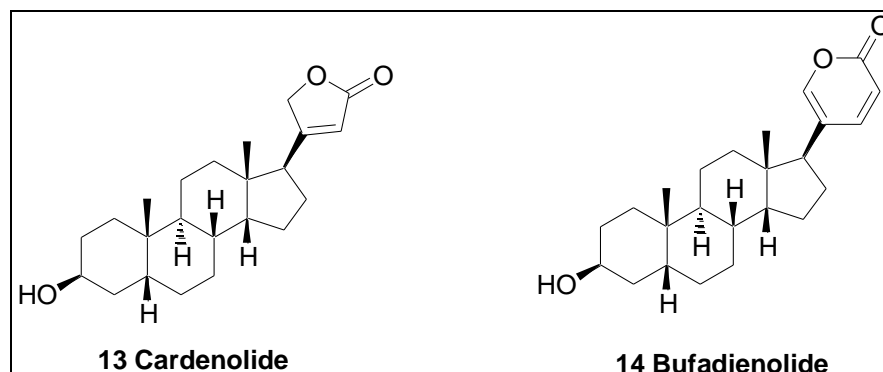
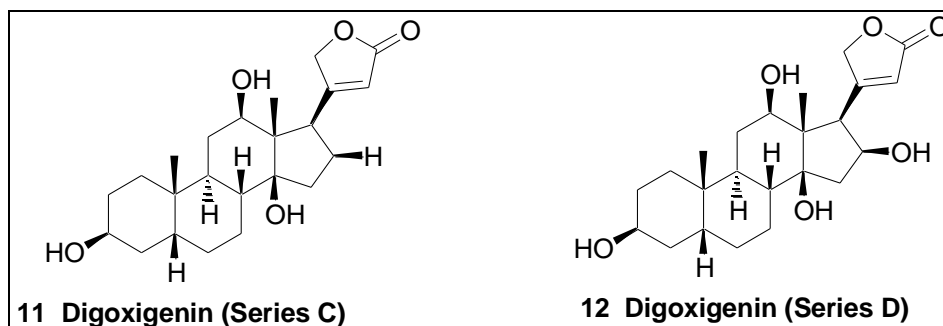
(Dioscoreaceae). In the same year it was converted to progesterone. Further, researchers found Mexican *Dioscorea* as an abundant source of diosgenin. Diosgenin (10) soon became a competitive source for steroid synthesis (Kirk-Othmer, 1978). However with the advancement of natural products chemistry it is now possible to synthesize the drug by side-chain degradation of cholesterol and sitosterol, this method has reduced the demand for such raw materials (Kirk-Othmer, 1978).



Yams still remain an important source of raw materials for steroid drugs synthesis and the major species used are *D. composita* Hemst; *D. zingiberinsis* C.H. Wright; *D. deltoidea* Wall; and *D. panthacia* Prain and Bark. More recently, plantations of *D. floribunda* Mart and Gal have been established in India (ITC, 1982).

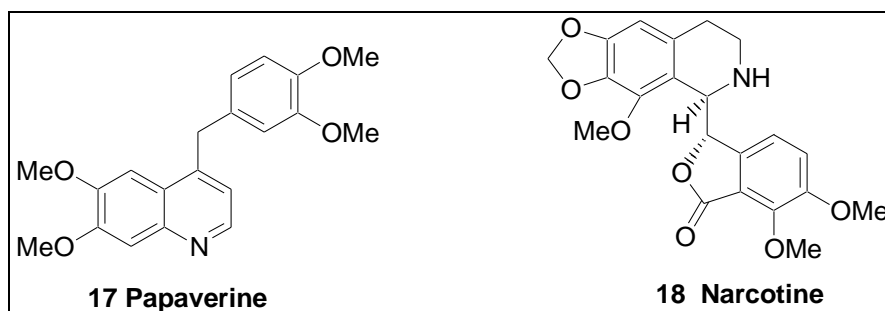
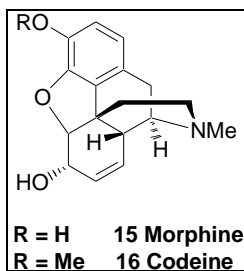
The other important natural product compounds are the steroids and are capable of acting directly on the heart. Such glycosides are referred to as cardiac glycosides as they have specific increase in the hearts excitability and contractibility (Rastogi and Mehrotra, 1995; Foye, 1981; Kirth-Othmer, 1978). These plant products are invaluable in the treatment of heart disorders. The examples are cardenolides (13) and bufadienolide (14), which are considered pivotal in the management of heart problems. The occurrences of these two categories of compounds are restricted to angiosperms and especially *Digitalis purpurea* (Scrophulariaceae), *Nerium stropharithus* and *Acokanthera* spp. (Apocynaceae), *Asclipias* (Asclepadiaceae) and *Erysimin* (Brassicaceae). Most of these species found in the tropical regions of South America and Africa have been used by the indigenous people as arrow poisons or drugs (Dev, 1989).

Digitalis spp., which is native of Europe, is worth mentioning. The plant has been used for therapeutic purposes since the medieval times for the purposes of poison preparations. However, it has also been used for dropsy (Taylor; 1965) and was introduced for heart treatment in the mid-20th century management. The most important species, which are used in production of current pharmaceutical drugs, are *D. purpurea* Ehrh. which produces cardenolides digoxin A and B Gitoxin, gitaloxin glucoverodoxin and odoroside are cardenolides; while *D. lanata* Ehrh. is rich in cardio active glycosides whose digoxigenin (11, 12) and diginatenin are important drugs of choice for heart ailments (Kirk-Othmer, 1978). Thirteen are also some of the glycosides that are of plant origin and are used in the management of heart problems.



There are cases where plants like Opium poppy, *Papaver somniferum* L. Papaveraceae, Family have been used for curative purposes across several cultures. The plant is native of Western Mediterranean region but has been grown in Egypt, Turkey, Greece, Asia Minor Balkans and Italy since ancient treatment times. Pliny recommended its products for the treatment of arthritis, headaches and for curing wounds (Taylor, 1965; Lewis and Elvin-Lewis, 1977). Its importance is contained in the history books with specific reference to British- Chinese opium wars (Taylor, 1965; Lewis, 1977). The milky extract from the fruit of the plant contains a complex mixture of compounds of triterpenoids and alkaloids and other compounds. The most important 40 species have yielded alkaloids. Morphine (15), whose structure was established in 1952, though isolated in 1803 by Derosne, remains the most important and the strongest narcotic (Foye, 1981). Narcotine (18) occurs as an admixture and a mild antitussive and is used in the preparation of cough lintus. Morphine (15) is biosynthesed from codeine (16) as an antitussive, which is widely used in medicine as an analgesic, a central nervous system stimulant (Dewick, 2002). There are also other important alkaloids which are also used for curative purposes. They include papaverine (17) which is a smooth muscle relaxant, cerebral vasodilator and also used in the treatment of asthma. Thebaine is a convulsant poison and is only used as a raw material in the manufacture of codeine or other semi- synthetic analgesics and narcotic antagonists like nalorphine and etorphine (Weiner, 1972).

There is a trend of emerging new diseases like AIDS, as a result of HIV infection and candidiasis. Re-emerging of diseases like tuberculosis and legionella, which were believed to be under control but are almost becoming epidemic and require urgent attention to save the human race from such scourges (Okeke *et al.*, 2005).



The main reason for resistance to antibiotics is due to the fast spread of resistant organisms due to lack of firm control of dispensation of antibiotics and lack of compliance to the treatment regimen (Vicente *et al.*, 2006). The other reason for the emergence of resistances to synthetic antibiotics is the emergence of spontaneous mutants *in vitro* that confer resistance to virtually any antibiotic (Woodford and Ellington, 2006). Solutions to such problems are embedded in the diverse plant resources and other innovations like biotechnology.

An increasing number of patients with HIV infection and or AIDS cannot use the currently approved anti-HIV drugs (Asres *et al.*, 2005). There is free provision of ARVs in all Hospitals and Health facilities Nationwide, nevertheless HIV cases are ever increasing and there are adverse side effects and the emergence of resistant strains of pathogens. This has been experienced more frequently in TB cases (Okeke *et al.*, 2005). The only avenue of treatment considered safe and sustainable for a reasonable period of time is the use of plant

products or their derivatives (Li *et al.*, 2004). Compounds extracted from many plants have been found to be effective in chronic and terminal cases like cancer (Laus, 2004). However, the goals of finding curative agents from plant sources can only be achieved through consistent screening of plants supported from the background by basic ethno-botanical studies of the indigenous people.

It is estimated that only 1% of the medicinal plants are known by scientists and accepted for commercial purposes (Aguilar, 2001). This would mean that the market for herbal medicine is still a goldmine and stands at US\$ 60 billion (Timmermanns, 2003). There would be a further impetus to this research if only the world could provide intellectual property protection to the natural product discovery particularly traditional herbal medicine and herbal medicinal products (Kartal, 2007). This overstatement means that very little has been done as pertains to medicinal plants and a lot remains outside there undiscovered and untapped. After all, a large proportion of drugs have been discovered with the aid of ethno-botanical knowledge of the traditional uses of plants (Kartal, 2007). Lots of useful compounds still remain to be prospected.

It is a fact that plants have been used extensively in folklore medicine in Africa, elsewhere and more so in East Africa (Chhabra *et al.*, 1993). However, knowledge on their bio-efficacy phytochemistry, silviculture and their pharmacological profiles has not been exhaustively done (Busia, 2005).

1.2 Statement of the problem

The Ogiek of the Rift Valley in Kenya are some of the oldest indigenous people of Sub-Saharan Africa and are a rich source of ethno-botanical information (Towett, 2002). They

have led a secretive life in the Tropical Montane Forests of Central Rift Valley as hunters and wild plant product gatherers. The tasks of documenting their vast and conservative ethnology are urgent because this indigenous knowledge is not properly documented. Furthermore, their natural habitats have been encroached by foreigners. Unfortunately the ecosystems of forests are fragile and are getting disseminated because of population pressure. Consequently, there is an accelerated reduction in the biodiversity in such areas. Ogiek are some of the oldest indigenous inhabitants in sub-Saharan Africa that feel threatened by these new developments.

1.3 Justification for the study

Plants have in the past been source of remedies for many diseases. There is need for new drugs to manage emerging and re-emerging diseases. Currently, microorganisms have a tendency of developing resistance to antibiotics that are in the market. It is believed plants can possibly offer solution to such challenges. Large population in the world (80%) use traditional forms of treatment that is mainly plant based. Areas where these plants are found are getting encroached by people thus the flora is fast being disseminated leading to loss of biodiversity. The indigenous people, the Ogiek, are also fast losing their traditional way of life and their ethno-medicine practices will be lost without any documentation.

1.4 Hypotheses

- (i) There is scanty documentation of the medicinal plants used by the Ogiek.
- (ii) The plants used by the Ogiek have no medicinal value and effect against any ailments as claimed by the Ogiek.
- (iii) There are no tangible phytochemicals and essential oils in these medicinal plants used by the Ogiek.

1.5 Objectives of the study

1.5.1 General objective

To document the ethno-medicine of the Ogiek with specific reference to plants in folklore medicine and verify their efficacy.

1.5.2 Specific objectives

- (i) To document some medicinal plants used in folklore medicine by Ogiek.
- (ii) To determine biological activities of crude extracts from the medicinal plants against selected human characteristic and pathogenic bacteria and fungi.
- (iii) To determine classes of phytochemicals found in these plants' using wet chemical reactions.
- (iv) Analyse the essential oils in some of the medicinal plants using gas chromatographic methods and mass spectrometry.

CHAPTER TWO

LITERATURE REVIEW

2.1 An of overview the medicinal plants past and present uses

A global review of phytomedicine in relation to ethnology reveals that the science of plants in the early days was based on the utilitarian approach (Wallis, 2005). This is evident because there are several records of highly prized plant species which have been mentioned several times in literature. An example is found in the Chinese civilization where there are records of one plant, *Artemisia annua* L. whose records are available in the tomb of Mawangdui Han dating back to 168 BC (Shibata, 1981). It is known to have treated over 52

kinds of diseases (Beriskyand and Gamble, 1993). The plant is further mentioned in Zhou Hou Bei Ji Fang (Handbook of Prescriptions for Emergency Treatments) which was written in 340 AD. The realisation of the potential of the plant in the mid-1960s led to its commercialisation. It is, therefore not new to South East Asians since the Chinese have used it for over 2,000 years against fever and other related ailments. In such a rich culture there are records of other plant species that were and are used for curative purposes (WHO, 1978; 1985; Dubey *et al.*, 2004).

Against such a rich cultural background embedded in the traditions, there has been gradual but dynamic penetration of the traditional Chinese medicine to other regions of the world like Japan via Korea (Ali, 2008). Chinese influenced Korean medicine was adapted by the Japanese during the reign of Emperor Ingyo (411-453 AD). Medical envoys continued to arrive from Korea throughout the next century and by the time of the Emperor Suiko (592-628 AD); Japanese envoys were sent directly to China to study medicine referred to as Kampo. Later in life, the Japanese evolved their traditional oriental medicine. Since culture is dynamic, the Chinese medicine was gradually integrated into Kampo and simplified (Beriskyand and Gamble, 1993). People still use the same practice in modern Japan to date.

Chinese and Kampo forms of treatments remain the most ancient forms with philosophical and experimental approaches (Patwardhan *et al.*, 2005). Such scientific development is rather gradual, systematic and more reliable compared to the allopathic approach. Because of little or no side effects, it is forecast that the Western world will have turned to alternative medicine by the year 2010 (WHO, 2001). Use of plant or plant related preparations are also common in India and their surrounding communities. The reason is that Ayuverdic medicine practice has conserved most of their traditions (Patwardhan *et al.*, 2005).

Beginning with Pakistan and India, which have only political boundaries, but culturally identical, previous and current efforts have been made to elucidate effective compounds from plants in this region for pharmacological uses with little success (Kamuhabwa *et al.*, 2000). For example, bio-assay guided fractionation of the ethanolic extracts of *Polyalthia longifolia* var *pendula* stem, showed promising antibacterial activity against thirteen Gram negative and nine Gram positive bacteria. However, pure compounds obtained from fractionations only showed some activities against fungi, *Mycobacterium stegmatis* and *M. fortuitum* (Faizi *et al.*, 2003). Elucidation of the structures of ethanolic extracts of the above plants revealed the presence of: (3S, 4R) trihydroxypentanoic acid as the active principle and a rich source of antibacterial agents. The compounds are safe, more potent, life saving may be used for a long period of time particularly in light of frequent emergence of multi-drug resistant micro-organisms (Normark and Normark, 2002).

Although most countries prefer allopathic medicine, such practices are only sustainable before chronic cases arise (Healy and Aslam, 2002). It is, therefore, a request by other intending users that Chinese and Indian art are made more accessible. It is imperative that the governments of both countries, India and China provide both moral and material support for the traditional industries to thrive globally (Dubey *et al.*, 2004).

In the Middle East, the scenario of herbal and art of medicine was knowledge learned from Greco-Roman period emanating from remote places (Majundar, 1971). The Arab inhabitants introduced to the West the Chinese technique of chemically preparing minerals (Patwarthan, 2005). These works are mainly recorded in the *-Materia Medica* of Jami of Ibn Baiar who died in 1248. His work had more than 2000 substances including many plant products (Ali, 2008). This entire body of knowledge was further introduced to Western Europe by Christian

doctors travelling with crusaders, and in the medieval ages, herbs became an important source of remedy and vast international commodity of commerce (Kochhar, 1989).

In the ancient Greek Empire, there are records of taxonomy of useful plants particularly those that were used by humans as food, for building, tools and medicines (Aslam, 2002). Such records are found in the works of Diocles, one of the first *Materia Medica*, the Rhizotomikon who was a Greek philosopher and a student of Aristotle (Sofawara, 2002). Unfortunately, his work is now lost, but he gave an insight into plant taxonomy. With the Roman Empire succeeding the Greek Empire, a Roman soldier-physician by the name Diokorediodes (1st AD) wrote the “*De Materia Medica*” which was the compendium containing only useful plants in the world of medicine (Sharma, 1982). The work had 950 curative substances of which 600 were plant products, the rest are of animal and mineral origin (Ackerknecht, 1973). Each document has botanical illustrations, medicinal qualities, method of preparation and warnings about undesirable effects. If any tradition exists that has withstood the test of time, it is in the traditional use of plants for curative purposes.

East India which is incidentally sandwiched between some major influential cultures, that is, China and the West also underwent a similar process in development of medicine in the case of China and Japan. Prior to emergence of Ayurvedic medicine, during the rise of philosophies of the Upanishads, Buddhism and other schools of thought that relied on nature were also developed (Sushruta, 1963). The practices in the preceding paragraphs employed Ethnobotany which is a multidisciplinary endeavour and encompasses several disciplines seeking to analyse how humans interact with plants in their environs. It means that it would combine anthropology, linguistics and botany as independent disciplines that are combined to form a solid background against which interpretations can be made (Martin, 1995).

There has been massive universal degradation of vegetation and this has led to decline on the flora diversity the world over (Schulte, 1980). Such unfortunate are being witnessed in the Mau Forest Complex where the Ogiek live in. Suggestions of urgent need for universal conservation where by organized method of management of land resources are used to maintain a balance between natural resources and evolutionary changes (Lincoln *et al.*, 1982). To achieve such goals, ethnobotany may be used to carry out many investigations in plant studies which are not possible without anthropological studies. Certain phytoscientists have taken advantage of ethnology and made outstanding scientific researches some of which have been used for therapeutic purposes (Martin, 1995).

In the Nilgiri Hills, the blue hills of South India, and has tropical, sub-tropical and temperate climate is inhabited by conservative tribal people, numbering about six tribes (Rajan *et al.*, 2002). Over 80 indigenous and endemic plants have been studied by several scientists, and in a review of their findings, it is recorded that different plant species are used by different minority groups in the area. The same plants are put to similar or different uses as detected from cross cultural relationship in other studies. Such uses of the same plants by different groups may be a predictable indicator for their therapeutic efficacy (Rajan *et al.*, 2002). The work further reaffirms that heterogeneity of the knowledge of medicinal plants by any indigenous community or communities explores the diverse knowledge about medicinal plants in their surroundings.

The surveys carried out, in most of the cases, took note of endangered species in which proper preservation is an urgent requirement. Some few selected plants used in Ethnomedicine by the people in Nilgiri Hills were noted including their phytochemical and pharmacological principles. They were *Adhatoda vasica* (Nees) C.B.Cl. and *Alstonia*

schloraris (R. Br) (Asclepiadaceae). The above plants contained; echiternie and ditamine inositol which are active as antitumors, anticancer and thrombopoietic. Other plant species with active principles like *Artemisia nilagrica* CB Clarke Pamp (Asteraceae) has alkaloids that have antimalarial activities. *Embelia ribes* Burm. f. (Myrsinaceae) has embelin that functions as antifungal/antibacterial and *Mormodica charantia* L. (Cucurbitaceae) contains Momoridine that works as antidiabetic and antifertility are also part of the flora. Such knowledge may be useful in furthering pharmaceutical studies and thus synthesis of medically important compounds. The same approach may be applicable in pharmaceutical studies in the case of the Ogiek.

Anthropology, ethno-biology and aspects related to the presence of so many medicinal plant species as well as traditional significance of these plants have been used to draw comprehensive medico-ethno biological studies (Rajan *et al.*, 2002). Such a study formed the basis for establishing the potential of the flora in any locality that might be generating interest. The approach through the studies of traditional plant usage when based on the indigenous population and their medicinal value revealed that Euphorbiaceae family, amongst many families led to the evolution of certain substances which have proved to be anticancer, antitumor, cardiac stimulants, and central nervous system drugs. It is important to deviate and make a treatise consideration of individual plants used in folklore medicine in Asia and elsewhere. Currently, lots of interests have been generated as pertains to phytomedicine. Basing the facts on ethnobotany, selective and advanced studies are presently carried out to augment the use of certain plants in allopathic medicine (Barnes, 2003).

The genuses *Uncaria* which are pantropical plants have found widespread use in traditional medicine (Laus, 2004). Based on ethnological information, a few species from the family have been selected and elucidated to understand their chemistry by one worker and are

available in public domain. Some species of the genus *Uncaria* have been subjected to chemical and their ethnomedicinal values verified. They include *U. ianosa* var *glabrata* (Bl) (Ribsd) that was studied in west Sumatra where it is used in traditional medicine as a remedy for food poisoning, *U. rynchophylla* Miq. the plant extracts are used in Phytomedicine in China against epilepsy *U. guianensis* (Aub) dried and powdered leaves are used in the treatment of dysentery and the healing of wounds. Water extracts of the latter are used for the osteoarthritis treatment. In Peru, *U. hirsute* Havil. water extracts with its hooks in combination with other drugs are used as “thang-kau- tin” which on testing exhibited liver protection activity and it also exhibited activity as an antihypertension drug. *U. macrophylla* (Wall) is commonly found in the Chinese pharmacopoeia and was found to be useful in the treatment of hypnosis (Laus, 2004). *U. sinensis* (Oliv) Havil is used in Japan as a herbal sedative and analgesic and is important in the treatment of vascular dementia. In Peru, *U. tomentosa* Wild DC. is commonly used in traditional medicine against blood disorders and the treatment of arthritis. In East Africa, *U. africana* G. Don. is used by the Ogiek community against stomach disorders and as tonic (Kokwaro, 1993).

Ayurveda traditional India medicine and traditional Chinese medicine remain the most ancient form of medicine yet the practical living traditions in medicine. This is because they both possess great philosophical and experimental basis. Their attributes are based solely on patience and cultural values of the two traditions (Ali, 2008). This has made them more popular and preferred. Elsewhere, the majority of patients are turning to Phytomedicine due to several reasons as stated earlier; increased side effects; lack of curative treatment for several chronic diseases. High costs of new drugs have been realized because of microbial resistance to new drugs and emerging diseases (Ernst, 2005). It has, therefore, been postulated that by 2010 at least two thirds for example, of the United States population will

be using one or more of the alternative therapeutic treatments. There is a fast growing market for botanicals at a rate between 5 to 15% annually (WHO, 2001). Most of the knowledge and usage of the raw materials are largely dependent on the traditional knowledge (Ali, 2008).

In the majority of cultural medical practices, plants remain the bulk source of medicaments employed in folklore medicine. Many hundreds of plants are used in Asian Chinese, Indian and Japanese medical practices (Ali, 2008). Comparatively, Unani practised by the Muslims is derived from many herbs and its final preparations include pills, syrups, confections and alcoholic extracts. Minerals, metals and metalloids such as gold, mercury silver, tin or arsenic were also employed, although some of these metals are very toxic (Aslam, 2002). The Unani medicine originated from ancient Greece and has been influenced by Persian, Egyptian and African medicine. The other fundamental difference between Ayuverdic and *Unani* is that the Unani had hospitals where patients could be treated with any of the drugs but Ayuverdic was carried out within family households. Now the opium and mercury used in Ayuverdic are not recorded the ancient in traditional Ayuverdic texts but sneaked into the system through the interaction with its Muslim counterpart (Aslam, 2002). That implies that *Unani* employs more metallic use as compared to Ayuverdic.

Hundreds of plants were used in Asian medicine by the Hakims or the healers of Unani medicine (Ali, 2008). For example in one ailment, at least 88 species belonging to about 44 families are used in the control of high blood pressure. Modern research has upheld the pharmacological aspects such as hypoglycaemic effect of many of these traditional drugs. The remaining task is to establish their efficacy and safety to humans. Some of these plant species used in the past and present by the Hakims to treat diabetes are from the families: Curcubitaceae. *Mormodica charantia*, *Cucumis sativus*, *Curcubita pepo* (seeds) and *Citrullus*

vulgaris. They are all herbalist armamentarium for the control of diabetes, although; their efficacies have not been established. Another ancient plant which the Hakims have used for centuries against run down conditions and depression is *Hypericum perforatum* A. Rich (Lusaciaceae). To date, the plants remain the most prescribed placebo herbal drug preparation for depression in the USA (Dewick, 2002).

The Unani and Ayurvedic medicines include *Melia* and *Azadiracta indica* (Meliaceae) and *Abrus precatorius* (Fabaceae) which have been used from antiquity to date. There are many more which were in use, but the fact that they have not been mentioned does not mean that they are less important. The mentioned ones only underscore the important role of ethnobotany in the current medical systems all over the world (Williamson, 2002). In trying to conserve the use of Phytomedicine and further support folklore medicine, the World Health Organization redefined traditional medical practice (TM) recently, as comprising therapeutic practices that have been in existence, often for hundreds of years, before the spread of modern scientific medicine and are still in use today (WHO, 2001). The practices are variable and contain considerable mysticism and secrecy. The previous definition which took into account sociological environment, whether living or dead and the metaphysical forces of the universe are still valid (WHO, 1978). The validity arises from the sociological aspect which validates its sustainability.

In the whole world, China traditional knowledge of medicine has been used in selecting and evaluating the efficacy of plant extracts against current and recently emerging viral infections. In the Mediterranean and the Near East areas, the family Clausiaceae the genus *Hyperacid* has generated lots of interests due to its variety of compounds with the ability for different biological activities and use in folklore medicine. They demonstrated antimicrobial, antifungal and antiviral activities (Kamuhabwa *et al.*, 2000).

2.2 Current studies of plants as medicine

Bangladesh is a country with rich flora. Some of this flora has been screened for their anti-bacterial cytotoxicity. Ethyl acetate and chloroform extracts of dried leaves of *Sida rhombifolia* demonstrated activity against selected Gram-positive and Gram-negative bacteria (Aslam *et al.*, 2002). It was also suggested that the lethality of brine shrimps could demonstrate the presence of some antitumour compounds. Its active principles could lead to development of cheaper and dependable antibiotics instead of the synthetic ones. However, this would require studies on their efficacies and safety including clinical trials. In the same regional location that is in Japan, further quest for compounds which could assist in alternative natural antibiotics, has been carried out. It emerged that several compounds found in *Erythrina poeppigiana* possessed antimicrobial agents with anti-candidial and anti-MRSA (Methicillin resistant *Staphylococcus aureus*) (Sato *et al.*, 2003). It is rare to find such drug combinations in allopathic systems, though in demand, particularly in cases of immuno-compromised and immuno-suppressed conditions.

Nigella sativa (Ranunculaceae), commonly referred to as black cumin and, is used traditionally in the Indian sub-continent, Arabian countries and Europe for culinary and medicine as a natural remedy for a number of ailments (Ali *et al.*, 2003). Diseases it cures are even described in the *Melanthion* of Hippocrates and Dioscorides and, as the Gith of Pliny (Worthen *et al.*, 1998). Many of the claimed folklore medicinal values of the plant have been tested and scientifically verified (Ali *et al.*, 2003). The pharmacological tests in animal models have revealed that the oil has strong antinociceptive depressing properties on the central nervous system, anticarcinogenic and mutagenic, antihepatic and nephrotoxic, antidiabetic mellitus effects. It is also effective in reducing ulcer index, antihypertensive,

immuno-potentiating properties in human T cells. The oil has strong activities against human pathogenic Gram-positive Gram-negative bacteria, and fungi. More bioactivity was also detected in cestoda and nematode. At the levels that are comparable to those of piperazine in *Schistosoma mansoni*. Lastly, it was reported that the administration of the plant extracts intraperitoneally or otherwise in rats consecutively for a period of 5 days did not significantly affect the activities of several enzymes and metabolites indicative of hepatic and renal function. In other words, the plant has low toxicity in mammals. In this review, special credit and recognition is given to over 40 authors whose works have been enumerated without being quoted as they have been quoted by Ali and Blunden (2003) in their extensive review.

Uses similar to those of *Nigella sativa* was extended to cover black pepper, black cummin and revealed similar results showing that tested essential oils and acetone extracts exhibited broad spectrum antimicrobial and antioxidants. A conventional method involving ultra sound assisted and solvent extraction method showed that the extracts of Ginger (*Z. officinale*) fingerroot (*B. pandurata*) and tumericin (*C. longal*) have high anti-microbial activities against *L. monocytogenes* and *S. typhimurium* (Thongson *et al.*, 2004; Singh *et al.*, 2005).

With ever emerging resistance of bacteria to various antibiotics, it is empirical that new methods of combating the problem are invented. *Helicobacter pylori* which causes gastrointestinal infections in adults and children is often treated with antibiotics. However, the antibiotic therapy requires a cocktail of more than three drugs (Nariman *et al.*, 2004). Studies of various organic solvents of six Iranian plants, *Juglans regia*, *Xanthium brasiliicum*, *Ligustrum vulgare*, *Thymus kotschyanus* and *Trachyspermum copticum* indicated that all the plants contain bioactive principles which have inhibitory growth principles against bacteria. This gave hope to use them as substitute antibiotics. There have been studies of Gram-

negative bacteria, *H. pylori*, and its problem to human and there are possibilities to link it to gastric cancer and adenocarcinoma. Studies on one single plant, *Plumbago zeylanica* L. on its non-polar extracts exhibited reasonable bacterial activity at pH ranges of 1-7 (Nostro *et al.*, 2006; Wang and Huang, 2005).

Anti-microbial and cytotoxic studies of three Neoligans were isolated from *Magnolia officinalis* Rehd et Wils. (Magnoliaceae) (Syu *et al.*, 2004). Further studies *in vitro* on enterobacteriaceae produced promising results. This was necessitated by the observation that a number of strains of Enterococci ssp pose resistance to Vancomycin while *Staphylococcus aureus* has more often demonstrated resistance to Methicillin. It was confirmed that certain neoligans extracted from the plant exhibited reasonable anti-microbial activities up to the minimal bacteriacidal concentration (MBC) levels (Mascini and Bonten, 2005).

Pseudomonas ssp. is an opportunistic pathogenic organism that may exhibits multi- drug resistance characteristics (Papadopoulos *et al.*, 2006). Recent studies in Australia cite 30 isolates of *Pseudomonas aeruginosa*, 11 isolates of *P. fluorescens* and 15 isolates of *P. putida* using oil and components of *Melaleuca alternifolia* L. tests indicated that *Pseudomonas* ssp are susceptible to the extract (Papadopoulos *et al.*, 2006). It was concluded that many oil products from the test plant could be employed in wound management or hand washing and contain 5-10% (w/v) oil. Given that MBC of the oil for *P. aeruginosa* was 4%, it is possible that the use of such topical agents in both treatment of wounds and other skin washing situations could be of benefit in preventing or reducing infection and transmission. However, clinical trials are currently on the same extracts (Cowan, 1999; Edris, 2007).

Studies of the activities of ten traditional plants extracts against 35 clinical isolates of Methicillin resistant *Staphylococcus aureus* also indicated that ethanolic extracts of the plants, 9 of the study species exhibited activity against all isolates tested (Voravuthikunchai and Kitpipit, 2005). Some of the native traditional plants whose extracts were used were *Garcinia mangostana*, *Punica granatum* and *Quercus infectoria*. They were found to be effective. It was concluded that these plants which showed significant activity against MRSA isolates could possess metabolic toxins or broad spectrum antimicrobial compounds. Further analysis of the extracts could yield inexpensive alternative drugs to conventional treatment of the bacteria. Traditional herbal medicine has been used for a long time in the treatment of infectious diseases in many countries and in China *in vitro* studies of plant extracts have shown activities against HIV (Li *et al.*, 2004). Some of the families screened include Asteraceae, Rutaceae, Rosaceae, Acanthaceae Lamiaceae, and Araliaceae among others. It was noticed that they give reasonable and promising results. Preliminary results of the evaluation of *Garcinia mangostana* L. (Clusiaceae) have significant anti-microbial activity against MRSA isolates. The plant is known to possess compounds such as tannins metabolites whose antimicrobial activity is well-known (Voravuthikunchai and Kitpipit, 2005).

Further studies to evaluate the efficacy of Thai basil oils and their micro emulsions *in vitro* activities against *Propionibacterium acnes* revealed promising results (Viyoch *et al.*, 2006). The essential oils were obtained from: *Ocimum basilicum* L., *O. sanctum* L. and *O. americanum* L. using disc diffusion and tube dilution methods to obtain areas of inhibition and minimal bacteriacidal concentration (MIC), respectively. *Ocimum basilum* and *O. sanctum* also showed 2 and 3% v/v MIC. However, *O. americanum* did not exhibit growth inhibition even at the highest concentration although it had initially inhibited the growth of

the test organism. It was further demonstrated that *P. acne*, which is a common teenage problem, could be managed effectively by incorporating the oils from *O. basilicum* and *O. sacrum* in common facial creams (Viyoch *et al.*, 2006). Several bacterial strains and plant species were used *in vitro* studies in the laboratory, in which various parts of plants used in folklore medicine were selected. The plants included: *Lantana camara*, *Hymenea courbaril*, *Myroxylon balsamum*, *Aristolochia cymbifera*, *Chondodendron platyphyllum*, *Drimys winteri* and *Xyanthoxylum tingoassuoiba*. It was established that several of these plant extracts had efficacies against both the Gram-positive and Gram-negative bacteria such as, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. They could in future prove as alternative agents for therapy of infections caused by the micro-organisms. The use of solvents such as ethanol, ethyl acetate and hexane, and further phytochemical screening revealed the presence of various classes of compounds like, flavonoids, tannins, alkaloids and glycosocoles all of which have been known to possess anti-microbial activities (Dimmock *et al.*, 1999).

Many hundreds of plants worldwide are used in the management of bacterial infections (Martin and Ernst, 2003). Conventional drugs usually provide effective therapeutic results however, this benefit is limited by development of resistance; thus a need for drug discovery. Plant products have been found to be safer than synthetic products (Ali, 2008). Therefore, many patients often prefer to use plant sourced medicines. Thus there is need to have trained human power on the use of herbal/plant in the management of diseases. The evidence summarized in the above chapters suggests possible benefits from some plant preparations with antimicrobial activity. *Micromeria cristata* (Lamiaceae) and subsp. *Phrygia* was studied and the results were that; essential oils and components of extracts of the plant leaves used

as herbal tea, has got profound activity against *Proteus vulgaris*, *Salmonella typhimurium* and *Candida albicans* (Tabanco *et al.*, 2001).

2.3 Viral infections still elusive

The search for affordable anti- HIV and AIDS drug is not necessarily an academic curiosity but a necessity. Natural products are important sources of new drugs but may also lead to tailored synthesis (Martin and Ernst, 2003). Flavonoids and other related polyphenols possess good anti HIV properties since they inhibit reverse transcriptase induce interferons and inactivate viral protease (Harsteen, 2002). Plants which are capable of providing anti HIV flavonoid components are: *Scutellaria baicalensis*, *Thevatia peruviana*, *Desmos* ssp, *Maytenus senegalensis*, *Juglans mandshurica* and *Camellia sinensis*. A few of these plants yield flavonoids which act as enzyme inhibitors and immuno toxins in their own ways (Bocklandt *et al.*, 2003). There are other plants which yield classes of compounds like coumarins which are a distinct subgroup of non-nucleotide reverse transcriptase inhibitors which have received special attention as antiretroviral (Li *et al.*, 2004). Coumarins have been isolated from plants such as *Fraxinus tschunganica*, and *Ferula sumbul*. Terpenoids and triterpenoids from these two plants have been found to exhibit antiretroviral activity with different mechanisms of action such as replication in HIV-1 (Cichewics and Kouzi, 2004). *Clausena exacanta* showed anti HIV activity with very low cytotoxicity (Cichewics and Kouzi, 2004).

Globalisation has resulted in a massive increase in trade and human mobility. This has facilitated the rapid spread of human infectious agents such as *Staphylococcus aureus* other pathogens, including those that are drug resistant (Meyer, 2005). A particularly serious threat to human health is posed by methicillin resistant *staphylococcal* strains which have acquired

molecular mechanisms to evade the action of β -lactam antibiotics (BLAS). Studies by Elgayar *et al.* (2006) revealed that, classical studies of the species *H. triquetrifolium* Turra. which has been used in ethnomedicine in parts of Turkey as sedative and antiseptic were effective in the treatment of burns in which *S.aureus* is common in secondary infections (Baytop, 1984). However, an all out and exhaustive study was not possible since the plant is not used independently. It was, therefore, necessary to make a collective study of the whole genus and species available. The species included: *H. triquetrifolium*, *H. scabrum* and *H. scabroides*. The studies involved their essential oils which had been extracted using steam distillation (Kizil *et al.*, 2004). The oils extracted proved to be of variable broad spectrum activities against both Gram positive and negative bacteria as well as *Candida albicans* (Kizil *et al.*, 2004). Their efficacies validated their traditional use against diseases caused by these organisms.

In North America, early explorers traded knowledge with the indigenous American Indians (Schultes and Farnworth, 1980). The indigenous Indians taught them the type of plant herbs used to sharpen their senses for hunting, to build endurance and to bait their traps. Some of these plant species were so potent that they became articles of trade in the old and the new worlds (Duke, 1989). Ginseng (*Panax quinquefolius* L.) growing in the Iroquois territory in the new world was dug up plentifully by Jesuits and sold to the Chinese. The money generated from this commercial concern was used to build schools. Todate ginseng remains a sizeable crude phytomedicinal product exported from the USA. The red Indians of the American continent have been using it as an oral stimulant since the prehistoric civilisations todote (Schulte, 1980).

In Central America, the search for plant active compounds against infectious agents has been noted (Navorro *et al.*, 2003). Certain Guatemalan plants such as *Guazuma ulmifolia* ILam.

Acalypha guatamalensis Pax. Haffm, *Smilax spinosa* Kill, *Ocimum micranthum* Wild and *Piper auritum* were screened to ascertain their antibacterial, antiprotozoal and antioxidant activities. The aforementioned study was targeted at ascertaining their activities against infectious agents to correlate the oxidant potential of their different extracts in order to validate their popular use in traditional medicine by the local population. It was established that all classes of active compounds were present amongst them flavonoids, anthroquinones, condensed tannins and sugars. Such compounds have been synthetically incorporated into allopathic therapy. Furthermore, the trials revealed that compounds found in them confirm the traditional ways in which they are used in the area.

In the entire America, herbal drugs have been used in vogue by Indians. Some crude extracts have even been incorporated into Brazilian pharmaceuticals for the treatment of infectious diseases (Rodrigues, 2006). A list of such plants is also available (Machado *et al.*, 2005). Some of these plants, as indicated in the previous chapters, have been known to be able to combat nosocomial bacterial, Gram negative and Gram-positive pathogens.

Plants are forever emigrants either through nature or by humans to satisfy human needs and desires. With such human behaviour, plants have been translocated into different continents of the world where they naturalized to assume the status of being indigenous (Rodrigues, 2006). *Thuja occidentalis* L. commonly referred to as white cedar, is indigenous to Eastern North America and is grown in Europe as an ornamental tree. To native Indians in Canada, during 16th century expedition, it was found as a source of remedy for scurvy. It was later established to be adjuvant to antibiotics in severe bacterial infections such as bronchitis, angina pharyngitis, otitis media and sinusitis (Naser *et al.*, 2005). An up-to-date review of the various uses of the plant which are evidence based for the plant has established such uses.

The essential oil extracted from *T. occidentalis* that is α -thujone and β -thujone, were the major constituents. The other useful metabolites included; flavonoids tannins and coumarins. The immunopharmacological potential of *Thuja* spp has been investigated in various *in vitro* and *in vivo* test models (Naser *et al.*, 2005).

Further studies found in the review of studies which were done *in vivo* indicated that, in mice, the leukocyte count was dose dependent and the percentage of segmented granulocytes decreased from 36 to 9% and stab granulocytes from 15 to 3%. The number of lymphocytes in the control mice also decreased from 89 to 48%. There was also stimulation of cytokine production in the mice treated with the extracts. There was also increase in response in the number of splenic plaque- forming cells and titters of specific antibodies in sera from mice treated with the extracts. After long-term treatment of mice with ethanolic mixture of *Thujae occidentalis* Hera. *Baptisiae tinctoriae*, *Echinacea purpureae* and *Echinacea pallidae*, it emerged that there was increase in the plaque forming cells in the mice. The immune stimulation went on without necessarily affecting the weight of the spleen. Separate studies on the influenza virus type, an infection in mice indicate that there was increase in the survival rate after the mice were treated to the aforementioned mixture from ethanolic extracts (Naser *et al.*, 2005).

2.4 Plants as a major source of medicines

The search for plants with biological and therapeutic activities has been a continuous process the world over (Dimayuga and Garcia, 1990). In Mexico, several field surveys have been carried out to isolate and elucidate active compounds in plants. Laboratory tests of the undermentioned plants' ethanol extracts against Gram-positive bacteria have revealed that: *Bursera microphylla* (Burseraceae), *Wislizenia refracta* (Capparidaceae), *Baccharis glutinosa* (Asteraceae), *Pithecellobium dulce* (Mimosaceae), *Fraximum uhlei* (Olacaceae), *Ludwigia octovalvis* (Onagraceae), *Solanum nigrum* L (Solanaceae) *Asclepias subulata* (Asclepadiaceae) had high antibacterial activities. The selections were based on the ethnobotanical survey, which was carried out on the local populations over a period of time. Most of the plant preparations were known to treat notorious and chronic diseases that were caused by non-bacterial pathogens.

2.5 Fungal infections and their challenges

Perhaps the most stubborn disease causing micro-organisms are the fungi. All the way from oomycetes to *fungi imperfecti*, they are known to inflict suffering both to plants and animals (Turchetti *et al.*, 2005). The cost implications and losses caused by them are enormous. At the moment, the remedies remain elusive. However, it has been proved that higher plants have the potential of providing solutions to these problems in that they have active principles, which are antifungal (Bandara *et al.*, 1988). The bark of *Butea monosperma* (Ceacelpiniaceae) extracted with petroleum and ethyl acetate yielded biologically active compounds against *Cladosporium cladosporioides*. Due to the upsurge in the number of immuno-suppressed and immuno-compromised infections the world over, it has become imperative to develop new antifungal drugs, which can be of benefit to humans (Bandara *et al.*, 1988). Although most of the plant extracts from native plants from North America had

antifungal activities on various parasitic fungi; they were only slightly active against the more susceptible dermatophytes like *Microsporum cookerii*, *Microsporum gypserum* and *Trichophyton mentagrophyte*.

There are better prospects of obtaining dermofungicides from the tropics, which have a larger biodiversity as compared to that of the northern temperate regions. This holds true because most of the species like *Rhus glabra* (Anacardiaceae), *Opuntia fragilis* (Cactaceae) and *Achillea millefolium* ssp *pubens* have a wider and diversified genus representation in the tropics (McCutcheon, 1994).

From South America in Surinam, it was also revealed that certain family members of the Apocynaceae and Loganiaceae yielded indole alkaloids, which were active against Gram negative bacteria (Verpoorte, 1983). Some of the tests on organism individually included *E. coli*, which is a mild pathogen, *B. subtilis* that causes food poisoning, *S. aureus*, and *C. albicans* which could be disease causing organisms and are difficult to deal with particularly when they have turned infectious. The local populations used selected plants from the aforementioned families of plants in traditional medicine and had their activities verified by the scientists through *in vitro* and animal studies.

Studies in other regions as in the case of Indians living in the Amazonia by other scholars like Spruce and Martins concluded that the communities had limited vegetal pharmacopoeia (Dewick, 2000). Later workers disapproved this fact and actually proved that numerous collections of the plants were of high medicinal value (Schulte, 1983). Further researches in this field are limited in that for one to carry out such studies, it is tedious and often dangerous. Moreover, the risk is more of a universal experience than individual one.

It is true that plants manufacture secondary, tertiary and quaternary metabolites which are used as source of protection against adverse ambient conditions. Such conditions are hot and arid, cold and arid, cold and humid. These conditions may occur once throughout the year or alternately in a year in the tropical areas. Whereas the adverse dry and arid environments, humid tropics, and temperate conditions have been well-investigated; the altitudes modify tropics to give afro-montane climates which have not been documented.

Since the invasion of North America by settlers, Phytomedicine has evolved and the knowledge was passed from physician to physician and through generations (Rodrigues, 2006). During this period, there was partnership between home folk medicine and family doctors (Majrio, 1975). It is clear that all the common ills were treated by physicians using preparations from plants. This fact is supported by the earlier pharmacopoeias (Sikirik, 2000; Buchman, 1980). However, with the technological advances in the 20th century, simple plant and water remedies were gradually discarded. Today, several Americans have lost touch with herbal preparation. This fact notwithstanding the re-emergence of Native Indian American culture has increased interest in Native Indian herbal medicines. Some reasons for these resurgences of the popularity of traditional medicines are because pharmaceutical drugs are seen increasingly as oversubscribed, expensive and dangerous, yet herbal remedies are seen as less expensive and less toxic. Second, people increasingly are willing to self doctor their medical needs by investigating and using herbs and herbal preparations particularly those with chronic illness such as arthritis, diabetes, cancer and AIDS. Successful management of such ailments is elusive. People having such maladies are turning to herbs as adjuncts for treatments (Gurau, 2006). This has also led to enhancement of regulations status of herbal

medicine the world over thus further re-enforcing the role such medicine plays in enhancing provision of health.

2.6 Role of plants in traditional medical practices

The World Health Organisation (WHO) estimates that 80% of the 4 billion people in the world depend on plants or plant related products for primary health care (Martin and Ernst, 2003). The sophistication of herbal remedies around the world varies with the technological advancement of the countries that make them and use them (Busia, 2005). These remedies range from medicinal teas and crude tablets used in traditional medicine to concentrated, standardised extracts produced in modern pharmaceutical facilities and used in modern medical systems under a physician's supervision (Homer *et al.*, 2000).

Diseases caused by infectious microbes are some of the outstanding maladies of human race and livestock (Pauli, 2006). From time immemorial, the use and search for antimicrobials has and will always occupy the centre stage for research activities. There is need for research and innovation to combat new opportunistic infections as a result of HIV pandemic. *Candida* species are some of the commonest micro-organisms, which are responsible for nosocomial blood stream infection with mortality rate of almost 50%. Risk groups are dilapidated persons or patients with compromised immune systems and include infants, pregnant women, diabetics, cancer, and AIDS patients treated previously with antibiotics (CDC, 2004). Most antifungal drugs which have been licensed have therapeutic limitations in that there is fungal drug resistances, drug related toxicity, hazardous drug interactions or insufficient bio-availability. This explains why it is tricky to overcome or manage fungal problems that are encountered in life (Pauli, 2006). Since the beginning of scientific researches in medicinal plants, these plants have been a subject of investigations. Such studies have led to structure

elucidation, which has caused an increase in publications dealing with pharmacological examinations of individual compounds of plant origin (Pauli, 2006).

2.7 Pharmaceutical product development from plants

To obtain information about the usefulness of such natural resources for development of anticandidal/antifungal drugs, the identities of isolated compounds and their described properties have been compiled and are assisting in finding solutions against such complications that previously have been mentioned. With regard to pharmaceutical drugs discovery, it is evident that over the past two decades, interest in drugs derived from higher plants, especially the therapeutic ones, has increased markedly (Iauk *et al.*, 2003). Currently, the major pharmaceutical companies have demonstrated renewed interest in investigating higher plants as sources of new lead structures and source of new drugs. The evaluation of such new compounds against nosocomial pathogens that have proved resistant to conventional antibiotics is a common phenomenon (Machado *et al.*, 2005). With this in mind about seven plants from Brazil were selected for screening against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Enterococcus coli*, *Staphylococcus aureus*, *A. calcoaceticus* and *P. microbilis*. These organisms are known to cause both surgical and post-surgical ailments as well as medical complications in human (Pauli, 2006). Quite a good number of them were extracted with various solvents and found to have antimicrobial activities against the above listed microbes (Machado *et al.*, 2005).

2.8 Role of ethnobotany in drug development

Traditional systems all over the world are in the provision of primary health using the art they have inherited from ancient times (Takashi *et al.*, 2004). The use of medicinal plants in the management of bacterial and fungal maladies is not a new phenomenon or art but a

carryover from such antique periods as in the cases of traditional use of essential oils from medicinal plants. The Indian physician, Sushruta reported in 600 BC the medicinal use of essential oils. Oils from plants were used for the treatment of infections of the mouth, jaw and teeth (Sushruta, 1963). Going through other literature, it is possible to compile a list of plant families from which essential oils that possess antimicrobial activity are found. They include families such as Asteraceae, Apiaceae, Lamiaceae, Fabaceae, Rutaceae, Zingiberaceae, Myrtaceae, Lauraceae, Cuperaceae and many others. Approximately 20% of the extracts are consumed as fragrances in perfumery and cosmetics, 5% in pharmacy and 15% are used for the isolation of components respectively (Protzen, 1993). Such compounds have been extracted from plants such as *Larrea divirata* Cav (Quiroga, 2004). It is reported that the plant has *in vitro* fungal toxic activities which is safe to the non-targeted cells and gives results which are comparable to Ketoconazole. The elucidation of compounds from antifungal activities is desired particularly from tropical plants (Buzzini and Pieroni, 2004). A further reason for such desires is that the side effects of such extracts are minimal and therefore considered safe.

Ethnobotany is and remains an integral part of human life and through folklore, compounds extracted from plants and specific plant parts are worthy of further investigations for their use as potent sources of antimicrobial and antihelminthic agents. Herbal and plant medicines have become a popular form of healthcare. Even though several differences exist between herbal and conventional pharmacological treatments, herbal medicine can be tested for efficacy using conventional trial methodologies (Ernst, 2005). Moreover, many of today's synthetic drugs originated from the plant kingdom, and only 200 years ago our pharmacopoeia was dominated by herbal medicines. The non-complexity of herbal products in their physical structures, methods of preparations and investigations of their efficacy makes them desirable,

particularly vis-à-vis their popularity. For some but by no means all herbal medicines, efficacy data are now emerging (De Smet, 2002). Most of the herbal drugs reviewed are efficacious for certain conditions. Generally speaking, research into phyto-medicines is much less active than research into conventional drugs (Machado *et al.*, 2005).

In most countries, Phytomedicine is considered or given as dietary supplements and thus lacks legal status to prove their efficacy and safety. Based on the data available to date, it is impossible to draw general conclusions about the therapeutic value of Phytomedicine, more so herbal ones for that matter. However, many of the plants presented in the reviews show promising activity in various antimicrobial agents and the efforts to regularise their therapeutic exploitation should not be relaxed (Machado *et al.*, 2005).

Ethnomedicine has been used in selecting and justifying continued use of certain traditional plants (Akinyemi *et al.*, 2005). In combating methicillin resistant *Staphylococcus aureus* certain plant crude ethanol extracts were used. It was evident in the scientific records that water and ethanol extracts of certain traditional plants like *Acalypha wilkesiana*, *Ocimum gratissimum*, *Ageratum conyzoides*, *Briddellia ferruginea*, *Terminalia avicennoides* and *Phyllanthus discoideus* were ineffective (Atindenou *et al.*, 2002).

Antimicrobially active alkaloids have also been isolated from alcohol extract of certain wild plants like *Tabernaemontana chippi* L. which are found along the tropical rainforest, in Ivory Coast, Liberia and Ghana (Atindenou *et al.*, 2002). The extractions yielded several alkaloids, which are active against both Gram positive and Gram-negative bacteria (Van Beet *et al.*, 1985). Ethno medical survey also showed that they were used against various ailments, the majority of which are bacterial in nature extracts but were ineffective *in vitro* in the study in

that their efficacies were too low to be recommended for traditional use (Akinyemi *et al.*, 2005).

Elsewhere and in Africa, traditional medicine is well recognized and is of great value. Based on fact and faith, many potent drugs have been purified from traditional medicinal plants by persistent research (Bessong *et al.*, 2006). They include: anticancer, antimalaria, antibacterial and antidiabetic compounds (Samie *et al.*, 2005). In South Africa, there has been a trend to treat gastrointestinal complaints with traditional herbal preparations. Based on this traditional information, about fourteen traditionally used medicinal plants by the Venda rural communities were selected for clinical bacterial screening. Two plants, *Warburgia salutaris* and *Maerua edulis* showed antimicrobial activities that were higher than synthetic and conventional antibiotics. Further screening of other plants; *Piper capense*, *Lippia javanica*, *Berchemia discolor*, *Cassine tranvaalensis* and *Pseudlastylis marouneifolia* not only possess antimicrobial efficacy but also fungicidal effects on *Candida albicans*, *C. brusei* and *Cryptococcus neoformas* (Samie *et al.*, 2010). It is now evident that indigenous knowledge can be employed in the development of today's pharmaceutical industry. Some of the success in pharmaceutical industries is attributed to ethno-biological information obtained from local people (Ernst, 2003).

Plants have remained a major source of medicine for human. Only a few families for purposes of review have been selected in this chapter. There are certain families which are relatively small as compared to the others though being included in studies. Araliaceae are tropical plants which are trees, shrubs or some are climbers that have been taken into account during the reviews. Some genera in this family are traditional drug yielders. The best known drug which has been used for centuries from the family is *Panax schinseng* (Williamson, 2002). Oleaceae is important in that *Olea hochstetteri*, which is found in the bible as olive

plant yields high herbal drugs quality used both in pharmaceutical industries and culinary (Swaminathan, 1989). Asclepiadiaceae too is important and has been used in folk medicine although currently they do not yield any medicine (Williamson, 2002). The Convolvuliaceae, Boraginaceae, Verbenaceae, Lamiaceae, Solanaceae, Acanthaceae, Scrophulariaceae and Pedaliaceae families majority of which yield a variety of drugs like: hypotensive, expectorant and carminative. The plant species have been used in cooking since the advent of human time to date. They are also important constituents of perfumery because of their essential oils. Some selected genera are *Lavandula*, *Ajuga*, *Origanum*, *Mentha*, *Basils* and *Salvia*.

Solanaceae family is best known for its alkaloids and has been used in medicine, both in the new and the old worlds. It also has those important vegetables like *Lycopersicum* spp, *Solanum tuberosum*, *Capsicum annum* and *Solanum melongena* to mention but a few. Some of the aforementioned species have been used in folklore and allopathic medicine. The genus *Datura* comprising; *D. stromanium*, *D. metel*, *D. inoxia* and *Duboisia* spp., *Solanum dulcamara*, *Atropa belladonna* and, *N. tobaccum* which are used as tobacco, currently provide source of modern drugs (Dev, 1989). Most of the plants found in Solanaceae family are important in that they yield important alkaloids which are used as a base for the manufacture of various drugs in today's pharmaceutical industries. Adopting them in Western medicine depended largely on ethnological observation by European explorers and missionaries who visited the new Worlds and Asia (Machado *et al.*, 2005).

Considering the gradual and steady developments in Ethnomedicine, herbs may be a cheaper option for medical problems in the provision of therapy in that their efficacy and acceptance are growing and that they have very few side effects (Aslam, 2002). Herbs grow everywhere and are inexpensive to collect and prepare. In view of the current crisis in the provision of

healthcare in most African countries, there is every reason to promote knowledge of understanding which type of herb is used for treating which ailment in the various communities. Some health planners have given ways through which the aforementioned justification can be used to achieve the provision of primary healthcare (Busia, 2005).

In many areas where these plants are found, they are subjected to destructive harvesting by greedy traders. Second, majority of the rural and urban poor have strong attachment to herbal medicine and third, there are traditional healers who rely on herbs where they combine spiritual beliefs with therapeutic efficacy. Birth attendants and bone setters also use a lot of herbal medicine. It is a strong belief that all these categories of traditional healers acquire their skill through oral inheritance (Kokwaro, 1993). The facts reinforce the warning given by (Busia, 2005; Carsenti *et al.*, 2001) that the African authorities can only afford to ignore herbal medicine at their own peril. Moreover, African healthcare has never been and probably will never be, adequately and equitably availed. This is because there is uncontrollable rapid population growth, poor economic performance and political instability to mention but a few (WHO, 1985). In West Africa, *Garcinia cola* has been used as chewing stick and to treat stomach problems. When its ethanolic extract was screened against *E. feacalis* and *S. aureus*, the result showed high level of efficacy in the *in vitro* studies (Atindehou *et al.*, 2002).

2.9 Missing data in phytomedicine

The shortcomings with regard to medicinal plant studies and its use in the sub-Saharan Africa and the rest of the new world is that most of the knowledge were derived through oral traditions, subsequently; there were no written records prior to the advent of colonial epoch (Rodney, 1971). But this fact does not infantilise the art and the use of plants for treatments

amongst the African folk. Those above facts on Africa were later fortified in the quest for solutions to several maladies like malaria, asthma and later HIV or AIDS.

Practitioners of traditional medicine (TM) in African include herbalists, plants and plant products sellers, traditional birth attendants, bonesetters, diviners, faith healers, traditional surgeons, spiritualists and others. There is a period and method through which such skills are mastered. The training for these practitioners is still by apprenticeship for a minimum of 7 years. During this period, the apprentice is expected to master all the ethno-practices which encompass all the botanicals and zoological which are used in the practices and their methods of use. There are specializations and the practitioners also refer patients to one another appropriately (Sofawara, 2002).

The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs in the African continent (Busia, 2005). Herbal medicine has been widely used and formed an integral part of primary healthcare all over the world (Akiyemi *et al.*, 2005). Traditional healers in Kenyan communities use herbal and phytotherapy to treat different kinds of ailments. The reputed efficacies of these medicinal plants have been experienced and passed on from one generation to the other. Apparently, of scientific proof of efficiencies of the medicinal plants as claimed by traditional healers in the Ogiek communities is lacking. Further, medicinal plants constitute an effective source of both traditional and modern medicine. It is estimated that about 80% of rural population depends on plants (WHO, 1978).

Scientific screening of various plant extracts against microbes; bacteria, viruses and fungi is one sure way through which the continued use of such plant preparations could be justified.

In the same vein a lot of plants have been used in culinary and this has also led to testing of their extracts for medicinal uses for example, the antimicrobial screening of *Decalepis hamiltonic* (Singh *et al.*, 2005) against food-related pathogenic micro-organisms (Thangadurai *et al.*, 2004). It has been demonstrated that plants indeed possess metabolites which are biologically active against pathogenic micro-organisms (Thangadurai *et al.*, 2004). This explains why such plants have the capabilities of prolonging shelf lives of foodstuff. It further demonstrated the usefulness of plant extracts in food preservation management and against contamination.

In sub-tropical Africa, bacterial and fungal infections represent an ever-increasing problem, more so in patients and people who are immunocompromised (Ebi and Kamalu, 2001). It is imperative to establish the efficacy of the plants since majority of the local population are rural and over 70% of them depend on plants as therapeutic sources (Pousset, 1994). However, most of the previous studies have been descriptive and exploratory. Biological, pharmacological and phytochemical data are lacking (Atindehou *et al.*, 2002). Attempts have been made to highlight the potential and verify the continued use of medicinal plants from West Africa with tests ranging from antibacterial to antifungal properties (Ebi and Kamalu, 2001).

Other infectious diseases like diarrhea have threatened the lives of millions of people around the world. It is estimated that 20% of infants in developing countries die before their fifth birthday due to diarrhea (Asbolt, 2004). The problem is more compounded by antimicrobial resistances to antibiotics. The alternative antibiotics are too exorbitant and beyond the affordability of the rural people (McGaw *et al.*, 2000). Traditional setups had means of treatment. For example in South Africa, majority of the people have well organized

traditional curative practices. In an attempt to document such practices of antibacterial screening of medicinal plants from South Africa; some workers have investigated certain aromatic plants by extracting their essential oils and using them against some enteropathogens. They achieved comparable results, >40% of the tested plants that showed justified and continued use in traditional medicine (Samie *et al.*, 2005).

There are also cases of comparing indigenous plant species with commercial species as in the case of *Tulbaghia alliacea* with *Allium cepa* in the management of candidiasis in South Africa (Thumbran *et al.*, 2006). It emerged that the *T. alliancea* extracts had comparable results to *A. sativum*, which is known to be a traditional commercial preparation and source of treatment in case of *Candida* spp. However, the commercial preparation is too expensive for the rural poor. This problem is further complicated by the fact that most people who are affected by modern maladies in the developing world have limited access to conventional drugs due to high costs that are prohibitive (Stagmann, 2001). This is the commonest scenario particularly in the light of near epidermis AIDS deaths which reach 2500 daily and 10-20% having fatal results (Mackenzie *et al.*, 2005).

The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs on the African continent (Okeke, 2005). Herbal medicine has been widely used and formed an integral part of primary healthcare all over the world (Akiyemi *et al.*, 2005). The screening therefore, justifies continued traditional use of several plant species against opportunistic fungal bacterial infections due to immuno suppressed conditions.

Some screenings have been carried out elsewhere on indigenous plants in other continents and have however naturalized in other places where their potentials have been identified to

possess other uses such as cosmetics, antiseptic agents' germicides and carminatives (Willis, 1995). In Egypt, it emerged that the species; *M. armillaris*, *M. alternifolia*, *M. leucadendron*, and *M. stypheloides* each yielded essential oils from the leaves that possessed bioactivity against Gram positive bacteria, *Aspergillus* fungi, viral conjutivitis disease and represent a detoxifant in first line of defence against peroxidation of polyunsaturated fatty acids and phospholipids (Farag *et al.*, 2004).

Shigella dysenteriae, type A, is a common problem in the world and its scientific importance was recognized in Japan in 1893. However, to date there is no known vaccine for it (Kamgang *et al.*, 2005). Certain African Savannah medicinal flora for example, *Mallotus oppositifolium* extracts are currently used in the management of diarrhea and dysentery. *In vitro* hexane extracts have been demonstrated to have antimicrobial activity against *Shigella* spp. (Ogundipe *et al.*, 2000, McGaw *et al.*, 2000; Kamgang *et al.*, 2006).

Across Africa, typhoid fever, caused by *Salmonella typhi* remains a major problem in rural areas where there is poor sanitation (Ogundipe *et al.*, 2000). To manage and overcome the malady *Cleistropholis patens* Benth (Anonaceae) is traditionally used in Nsukka, Nigeria to manage the disease (Ebi and Kamalu, 2001; Bischoff *et al.*, 2004).

There are certain botanicals which possess multiple uses in life (Abaineh and Sintayehu, 2001). In Ethiopian livestock with mastitis, the herb *Persicaria senegalense*, is used as a remedy as well as topical antiseptic by women after delivery. In addition, for veterinary trials, it showed that the inflammation subsided within five to seven days of substituting animal cabbage fodder with herbs.

2.10 Role of aromatic plants in medicine in African traditional medicine

For centuries, the antimicrobial properties of essential oils from medicinal plants have been recognized, but scientifically studied only recently. It has been confirmed that some of them have antibacterial activities against food borne bacteria thus extending shelf lives of processed food (Cosentino *et al.*, 1999). Several species of the genus *Thymus* covering *T. pectinatus*, *T. capitatus* and *T. herba-barona* all of which are wild were collected and steam distilled then the volatile oil extracts subjected to some Gram-negative and Gram-positive bacteria to ascertain the efficacy of the extracts; it emerged that the extracts were active against the bacteria and could be effectively employed in the preservation of food (Vardar-Untu, 2003).

African traditional treatments use holistic approach (Busia, 2005). The point demonstrates the uniqueness of the African medicine. Such types of treatments are variable and indicative of the specializations. The medicines include vegetable organs such as leaves, barks, roots, seeds, flowers, resins, latex or whole part of plant and/or together with parts of animals and/or some minerals like alum salts. African medicines may contain just one active ingredient but, flavourings, preservatives or colouring agents are also incorporated into the mixture (Sofora, 2002). Such colourings may act but are not necessarily synergists. The African TM also has ingredients comprising several preparations which have ingredients for all ailments that need to be managed to restore the patient's balance.

These make the African (TM) fundamentally different from allopathic ones whereby several prescriptions may be made for reported case illness (Sofora, 2002). In Africa (TM) is administered through liquid, solid, semi-solid or gas formulation, although intravenous or other forms of injections are absent. Other specialised forms of treatment used in Africa (TM) include obstetrics and gynecology, dry heat therapy, hydrotherapy, treatment of burns,

fasting and dieting spinal manipulation, psychotherapy, spiritual healing, occultism and massage. Africans have their form of surgeries which include male circumcision, female genital mutilation, tribal marks, cutting of the umbilical cord, tooth abstraction, piercing of the ear lobes uvulectomy, whitlow operation, trephination and abdominal surgery. However, there is no x-ray or anaesthesia used in Africa. After the surgeries, the patients are treated with herbal preparations to heal the wounds (De Smet, 1998).

Such practices are mainly dependent on plant uses that have evolved over centuries and therefore, still remain part and parcel of rural cultures. The *A. conyzoides* collected are heated over fire and the resultant sap squeezed into palm oil expressed from the mesocarp of *Elais guiniensis*. The concoction is used to rub the whole body. *A. conyzoides* has been used in vogue by Africans in dressing wounds and treatment of ulcers (Okeke *et al.*, 2005). It is used as a styptic in East Africa (Kokwaro, 1993). The common uses is due to its antimicrobial activities which have been demonstrated scientifically but the occult power it is claimed to possess when collected at night cannot easily be rationalized on scientific basis, especially when there is no precise diagnosis of the disease. Some other examples are: in many African homes chewing sticks are used to clean the teeth. The chewed ends are used to clean the teeth thoroughly. The sticks impart varying sensations, a tingling, peppery taste and numbing is provided by *Zanthoxylum zanthoxloides* Waterman, a strong bitter taste and frothing by *Masularia acuminata* (G. Don) and initial bitterness becoming sweeter later by *Vernonia amygdalina* Del. Buffered extracts of most of these sticks showed varying antimicrobial activities (Sofawara, 1993). The other practices in African (TM) such as; the collecting of medicinal plants is only done at a certain season, using cold extraction as opposed to boiling, using young leaves instead of old ones, using fallen dead leaves instead of young ones and using fresh ones, have been rationalized as being due to seasonal, diurnal

or age variations in accumulation of active constituent of plants or the thermolability of active ingredients of certain plants (Okeke *et al.*, 2005).

In Malawi, *Polygala nyikensis* which is used to treat skin conditions, proved both *in vitro* and *in vivo* to contain xanthenes which have high antifungal activities (Marston *et al.*, 1993). Further researches by Sofowara (1993) to ascertain the efficacy of African medicinal plants showed that the use had very positive sides. For example, the use of *Rauwolfia vomitoria*, roots to treat mentally ill patients; *Plumbago zeylenica*, roots for treatment of various fungal skin diseases, *Ocimum gratissimum* leaves which has essential oils to treat diarrhea are all justifiable (Sofora, 1993). Such plants like *Combretum mucroriatum* and *Mitragyna stipulosa* have proved effective as antihelminths but active ingredients have not been elucidated (Sofora, 1993). There are a lot more plants which are currently being used in folklore medicine but their characterization are yet to be done.

2.11 Challenges from antibiotic resistance by pathogens

Two pathways may be used by the organisms in acquiring the resistance. One is by modification of their own genes and two by acquisition of resistant genes from other bacteria. The resistance genes that encode systems to either expel or inactivate antibiotics occur naturally because many antibiotic producing organisms need them to avoid self-destruction (Homer *et al.*, 2000). Thus, we can say that antibiotic usage boosts the frequent development of resistant organisms (Homer *et al.*, 2000). Mutation occurs even during the single treatment and therefore, their target can be modified to confer resistance in a very short time after the introduction of new drug as was in the case of penicillin and more recently in linezolid, an oxazolidinone that interacts with the peptidyl - +RNA binding P site at the 50s submit (Homer *et al.*, 2000). The emergence of resistant micro-organisms has even

preceded the clinical use of some antibiotics (Bush, 2004). It can be said with certainty that the development of new classes of antibiotics and their introduction into medical use has been met by further development in antibiotic resistance such that multi-drug resistant bacterial pathogens are now common (Woodford and Ellington, 2006).

Bacterial resistance to conventional antibiotic treatment is most critical in hospital environment than anywhere else (Cavanagh *et al.*, 2001). The spread of antibiotic resistance in clinical and community setting is therefore, a pragmatic phenomenon (Gonzales *et al.*, 2005). In Industrialized, countries bacterial multi-resistance to drugs is responsible for over 50% of infections. The problem of resistance is related to the degree of exposure to antibiotics and is exacerbated by inappropriate use, both in developed and developing countries. In a nutshell, antibiotic resistance poses one of the greatest challenges facing public health officials since it increases healthcare costs (Ernst, 2005). A good example is the reemergence of tuberculosis especially *M. tuberculosis* that is multi-drug resistant and whose treatment is a hundred times more costly than the normal therapy (Murray, 2006). The cost implications in the provision of healthcare may, therefore, be a major component in antibiotic therapy compliance. Those victims who cannot afford combination therapy or other costly prescribed drugs may altogether assume that they have recovered and default the treatment.

Methicillin resistance involves a complex network of molecules and primarily depends on sufficient expression of penicillin binding protein with low sensitivity towards BLAS (Cavanagh *et al.*, 2001). It further requires that other factors include the fine tuned regulation of autolytic activity of cell wall components, as well as optimal rate of peptidoglycan precursor formation and highly specific peptidoglycan precursor structure.

Regarding the evolution of various resistances, studies show that the resistance of bacteria to antibiotics has been a progressive one (Gonzales *et al.*, 2005). However, β -lactamase production evolved rapidly in *S. aureus* and > 50% of hospitals acquired *S. aureus* isolates were penicillin G. resistant by 1948. This proportion has reached between 80-90% to date (Livermore, 2000). The resistance is due to the introduction of various broad spectrum antibiotics including Methicillin which was introduced in 1960 (Woodford and Ellington, 2006). It is unfortunate that Methicillin resistant strains of *S. aureus* have spread with speed to unknown proportions in many countries thus rendering it ineffective. Most countries have since then ceased to use the drug as an ultimate drug of choice. There have been genetic studies of the vancomycin resistant strains of *S. aureus* which reveal that *Van A* gene is the one that has mutated to lead to this resistance (Weigel *et al.*, 2003). In *P. aeruginosa*, about 116 rectal swabs over a period of time revealed that: 78% the test organisms were resistant to penicillin. Of these, five strains were resistant to penicillin G and to other antibiotics in the following order: erythromycin, clindamycin (one strain), erythromycin A and tetracycline (one), erythromycin A (one), tetracycline (one) and fusidic acid (one) (Lindberg *et al.*, 2004). It finally emerged that antibiotics did not seem to impede the survival fitness of *S. aureus* in intestinal commensal microflora. Strains that have acquired resistance in one host may, therefore, spread to other hosts unhindered by their resistance phenotype (Lindberg *et al.*, 2004).

There is indication that *S. aureus* exists in variant colonies. Such colonies referred to as small variant have been implicated in persistent and spread of chronic infections (Kolar *et al.*, 2006). Data may lead to the understanding of the methicillin resistance of the *S. aureus* and the ways through which such complications could help in assisting the design of new

therapeutic strategies. This loaded statement does not resolve the mystery of the current antibiotic resistances (Aguila *et al.*, 2001).

Epidemiology in human communities may present challenges in that certain diseases could remain pandemic, re-emerging and endemic. Respiratory tract infections are commonly caused by bacteria (Marrie, 2004). *P. aeruginosa*, *K. pneumoniae*, *S. pneumoniae*, *S. aureus*, *H. influenzae* and *Legionella* ssp. *S. pneumoniae* is one of the commonest causative organism in common respiratory tract infections (RTI) (Marrie, 2004). Currently, antibiotic resistant *S. pneumoniae* has emerged and this threatens successful treatment of the disease which is common in urban communities (Furuno *et al.*, 2006). To consolidate future antibiotic therapy, 649,552 patients: on treatment with selected macrolides, β -lactams like amoxicillin, amoxicillin/clavulanate, azithromycin, cephalexin and levofloxacin produced interesting results (Andersson *et al.*, 2005). Out of the 649,552 available patients of RTI and 7,252 susceptibility tests performed on *S. pneumoniae* isolates, there were no statistically significant trends in resistance for resolution proportion following treatment by either β -lactams or macrolides among any of the RTI'S. Further, to this, there was no positive significant association between *S. pneumoniae* susceptibility and RTI treatment results apart from significant positive association between erythromycin. Also, there was non-susceptibility in ear isolates and macrolides treatment resolution for supportive a cuter otitis media. It was concluded that on the population level *in vitro* *S. pneumoniae* nonsusceptibility to macrolide or β -lactam antibiotics were not associated with treatment failure conditions of probable *S. pneumoniae* etiology (Marrie, 2004).

With the foregoing revelations in mind, it has been noticed that there are prospects and challenges in developing new agents for tough Gram-negatives like *P. aeruginosa* and *K.*

pneumoniae which are all known pathogens (Meyer, 2005). There is emphasis on the mechanisms through which the resistance is developed against fluoroquinolones and aminoglycoside through the development of extended spectrum β -lactamase which is capable of inactivating both amino glycoside and fluoroquinolone (Meyer, 2005). The other reason for the development of resistance *P. aeruginosa* is due to reduction in the cell permeability and efflux of the drug, which occurs as cell mobilization (Nikaido, 2003). The tendency of mutation to occur and the Mutator cells persisting is a very common phenomenon which leads to resistance to β -lactam and amino glycosides. There occurs a mismatch which is common in *P. aeruginosa* (Nikaido, 2003) multi-drug efflux transport may also cause high resistance in pathogenic bacteria to amino glycoside in the case of *Acetivobacter* ssp (Magnet *et al.*, 2001).

In prostates and mid-ear infections there is a tendency of multi-drug resistance development which is attributed to microfilm colony development (Mylotte and Keagle, 2005). After such development, there is a tendency of reduction of the cell permeability with the interior of the colony recruiting more cells to behave in that manner by the bacteria using quorum sensing mechanism (Meyer, 2005).

The prospects for the discovery of new antibacterial agents given the seriousness of hospital acquired infections which are frequently multi-drug resistant; is to carry out empirical therapy (Marrie, 2004). This may be achieved by delaying the treatment until culture and susceptibility data for a particular infection are available. These resistances of the organisms to new antibiotics paint rather bleak pictures for new antibiotics (Cornaglia *et al.*, 2004). Scientists work round the clock to ensure that they keep pace with pathogens to counter newly emerging strains (Vicente *et al.*, 2006). Antibiotic use exerts influence on the

resistance to several pathogens which were previously controlled by vancomycin as the drug of choice. This implies for example, that Enterococci have certain strains which are vancomycin resistant (VRE). In such situations, the first drugs of choice are ampicillin and aminoglycosides in nosocomial environments. However, in the event that resistance to aminoglycoside and ampicillin is experienced, certain glycopeptides are employed (Kolar *et al.*, 2006). Further, it was revealed that in order to curb VRE it is empirical not only to control infection but to regulate and have careful administration of antibiotics like cephalosporin. The reason for this desire of restrictions is because such complicated antibiotics lead to the emergence of VRE's and a tendency to colonization pressure from within the communities.

Food is another conduit through which transmission of antibiotic resistance occurs (Wang *et al.*, 2006). This is why resistant pathogens are continually emerging rapidly. Surfacing of these resistant pathogens, poses a real threat to public health. Unfortunately, most of such foodstuffs such as milk products shrimps and groceries are bought from traders and eaten raw (Wang *et al.*, 2006). Such foodstuff could provide a time bomb and challenge to fight against antibiotic resistance by microbes in humans. Although it would be a long and tedious effort to clean up AR gene pool from the environment, interrupting the transmission of AR bacteria into human by focusing efforts on the food chains could be an effective strategy to combat the AR challenge in humans. This effort, however, remains a pipe dream.

2.12 Erratic use of antibiotics

Research in Croatia, one of the teaching hospitals, showed that tight control on antibiotics is necessary (Palcevski *et al.*, 2004). Mainly antibacterials were evaluated in two years and it emerged that the antimicrobial utilization was similar in both years. However, the pattern of

utilization had changed in contrast to the outpatient setting where the pattern of antimicrobial prescribing remained the same. There was more liberal prescription over the counter sales as compared to the previous years when certain drugs were restricted (Palcevski *et al.*, 2004). Furthermore, it established the fact that when the use of antibiotics is controlled, there is likelihood that the bacteria do not develop resistance to the used antibiotics.

Common typhoid fever is generally caused by *Salmonella enterica serovar. Typhirunium* (common name *S. typhi*.) are Gram-negative whose treatments are difficult (Barnes, 2003). The antibiotics used in the management of the diseases include chloramphenicol as the first drug of choice. Other drugs which may be employed are ampicillin, penicillin G, Norfloxacin, Streptomycin and Rifampicin. However, there are cases of resistances to antibiotics which were later traced to genetic changes within the cell structure (Macvanin and Hughes, 2005). This was noticed while certain strains were subjected to fusidic acid which is a steroid like antibiotic and which establishes EF-G. GDP on the ribosome after translocation has occurred thus blocking protein synthesis. Against this backdrop of genetic modifications, phenotypes of the organism with variable susceptibility to the mentioned antibiotics have emerged.

Nosocomial systemic bacterial infections have revealed that there is correlation between nosocomial infections and mortality. Such mortalities have been further complicated by the fact that pathogens so studied have strains that are resistant to treatments by more conventional or the β lactam antibiotics (Guillemot *et al.*, 2005). The research was carried on strains of both Gram-positive and Gram-negative bacteria which included *Staphylococcus*, *E. coli*, *Klebsiella* spp, *Enterobacter* spp., *Serratia* spp and other Gram- negative Bacilli. It was

evident that Methicillin resistant strains of Staphylococci and penicillin G resistant Streptococci, ampicillin resistant enterococci and cefotaxime resistant enterobacteriaceae *Pseudomonas* spp., were resistant to the drugs that were currently in use in hospital. The above data provided hospital decision makers with pertinent information on the clinical consequences of nosocomial infections caused by antibiotic resistant bacteria. It is important to mention that there are possibilities of bacterial attacks as epidemics universally. In the 21st century, affluent societies lived under the impression that they were free from the attack of pathogenic bacteria and if they by accident suffer, there would be an antibiotic to cure the malady (Machado, 2005). This is false and it is prognosed that if the conditions of discovering new drugs remain (status quo) the fatalities from such cases may be alarming. The impression that the availability of antibiotics within short notices to combat diseases is a fallacy and such a notion may consequently result into a catastrophic event (Vicente *et al.*, 2006). This is so because antibiotics have kept human and livestock from many plagues that were the scourges of humanity until the second half of the 20th century. However, ironically antibiotics are weak marketable goods in that patients stop buying them once their health returns, after short courses of treatment before the completion of the stipulated period of treatment (Mylotte and Keagle, 2005).

To date a vast majority of infections caused by *Staphylococcus aureus* are penicillin resistant (Palumbi, 2001). Hitherto new ones only provide short respite until new resistance emerges which are staphylococcal based. Bovine mastitis induced by *S. aureus* is an example of infection difficult to eradicate by conventional antimicrobial therapies (Brouillette *et al.*, 2004). *In vivo* studies in mice indicated that the pathogens persisted in mice despite being treated with antibiotics. Such studies were carried using several mutants of the bacteria which included isogenic hem mutants that had small colony variant (scv) phenotype. The latter

mutant expressed a marked inability to colonise tissues. Although the hem mutant and *S. aureus* were susceptible *in vitro* to cephalosporins, it was 100 times more persistent than the parental strains in the mammary glands when the antibiotics were administered at the rate of 1 or 2mg/ kg (Brouillette *et al.*, 2004). The studies concluded that despite the fact that hem. B mutant has reduced ability to colonise mammary glands, scv phenotype may account for the persistence of *S. aureus* under antibiotic *in vitro*.

Although it may seem obvious, it is essential to point out that the antibiotics, that were easy to discover have already been found, the search for new ones involves a substantial amount of high quality and laborious research (Ernst, 2005). Currently, there are various antibiotics which can combat most bacterial diseases but several alarms have been raised to develop new antimicrobials which could be used to combat the newly emerging resistant ones (Seguin *et al.*, 2006).

The need has been prompted by several reasons, among them the spread of multi-drug resistant organisms, the spread of emerging and re-emerging pathogens and the consequential high social and economic impacts of infectious diseases. Although vaccinations are a classical way of controlling infections they can only be used for preventive measures and not curative once the diseases is established (Webster *et al.*, 2004; Howard, 2004). Vaccines such as the pneumococcal one may also help in the reduction of antibiotic resistant isolates. However, there have been controversies since studies have failed to establish the role of vaccination in the reduction of resistance but have suggested that reduction of antibiotic pressure may be needed to reduce the resistance frequency (Frazao *et al.*, 2005). Research may also be slowed down because of the attitude of the clinicians who are satisfied by the fact that what they have is good enough for the time being.

The belief that the need for new antibiotics is not pressing may then appear as justified. However, for cases involving elderly or immunocompromised patients, for whom the prognosis is so dangerously poor, the development of new treatment should be a matter of priority. Patients with diverse medical conditions including those immunocompromised are in one way or the other likely to create another segment with the risk of succumbing to infections in any given human populations. In developed countries, nosocomial infections occur in 5-7% of patients hospitalized for other reasons, thus there is increase in hospital stay for at least an average time of four extra days, increasing the cost of hospitalization by U\$500 (Howard, 2004; Sade *et al.*, 2003). It is more complicated when the patients are in the intensive care unit in that both the risk and the cost are more than doubled; their additional stay can extend up to nineteen days with a concomitant higher mortality rate, often associated with antibacterial therapeutic failure (Kollerf, 2003).

2.13 Mortalities from antimicrobials

The (WHO, 1996), hinted that bacterial infectious diseases have impact with far-reaching effects in terms of morbidity and mortality. In developing countries alone, according to the (WHO, 1996), statistics on bacterial and bacterial related infections are responsible for 60% of the annual mortality. Amongst the dilapidated and elderly, despite existing antibiotic therapies and vaccine, bacteria remain the leading source of morbidity and mortality worldwide (Mackenzie *et al.*, 2005). Pathogens also contribute to a third of the deaths and ailments both in Europe and worldwide. Although vaccination contributes substantively towards curbing pneumonia, it does not provide full protection because not all individuals respond equally well to the immunization and because the immunity provided by the

available capsular polysaccharide based vaccines do not cover all the possible serotype variants of the pathogen (Bernatoniene and Finn, 2005).

It would be imperative to look for therapeutic techniques that seek to minimize accelerated development of resistance by micro-organisms to antibiotics; there has been marginal impact of patient education on antibiotics prescribed to children with pharyngitis and adults with acute bronchitis in private practices (Ron, 2003; Gonzales *et al.*, 2005). Profiling the physicians' prescription, records revealed that there was increase in antibiotic prescription for children with pharyngitis in distant control but decrease in local control practices and intervention cases had the highest decrease from 34 to 30%. In adults control through education reduced substantively from 55 to 44% in local control, from 50 to 44% in distant control, and from 60 to 36% at the intervention practices.

2.14 Patient education on drug abuse

Patient education can help to reduce antibiotic use for adults through physician directed efforts and restrictions. The foregoing statement has more to do with ethical perspective as opposed to the open happy physicians who prescribe antibiotics at the mention of ailment without carrying out diagnostic survey and investigations (Barnes, 2003). With the unending problems with antibiotics, the studies for alternatives are necessary to evolve potent antibiotics with unending problems with antibiotics, the studies for alternatives are necessary to evolve potent antibiotics with activities against both Gram-positive and Gram-negative bacteria. Antibiotic activities of the extracts from the study of plants were comparable to the standard antibiotics with a zone of inhibition seen to be quite potent (Chhabra and Usio, 1991). Local communities in Western Kenya claim that there are certain plant extracts, which are known to have antibiotic activities and such plants include: *Chamaecrista abrus* against

measles, *Emilia cocnea* against syphilis and *Philiostigma thoningii* against gonorrhoea (Kokwaro, 1993). Some plants like *Kedrostis foedisima* and *Melia Azadiracta* are used for the treatment of general viral rashes and swellings like measles and mumps. *Aloe* ssp. is used for treatment of herpes zoestra.

2.15 Remedy from plants against viral infections

Currently, tackling “modern” arising infections is crucial (Li *et al.*, 2004). An increasing number of HIV infections and AIDS cannot use the currently approved anti HIV drugs, including the reverse transcriptase and protease inhibitors due to the adverse effects and the emergence of drug resistance (Thumbran *et al.*, 2006). Many of the antivirals presently in use have narrow spectrum activity and restricted therapeutic usefulness (Asres *et al.*, 2005). It is known that antiretrovirals are expensive and beyond the reach of the victims. For the last two decades, scientists have been forced to address the epidemic, which has drastically, retarded socio-economic development and in turn resulted in a wildfire rate of its spread. There are other plant species, which yield similar compounds with the same activities (Akiyemi *et al.*, 2005). Such species are from the Euphorbiaceae family which include plants like *Euphorbia poissonii*; some plants with anticancer principles like *Pedilanthus* ssp. and showed activities against HIV–1 Virus (Petit *et al.*, 2002). Oleanolic acid was also identified as an anti HIV principle from several plants, which include *Rosa woodsii*, leaves, *Hyptis capitata* (whole plant) *Syzigium clariflorum* leaves and *Terenstromia gymnathera*. There are also other classes of compounds from plants, which have useful effects against the HIV virus (Petit *et al.*, 2002) which include alkaloids, proteins, polyphenols and polysaccharides. There are even other plant extracts, which are unclassified but are useful in the control of the virus. The plant extracts, however, have been studied *in vivo* and *in vitro* animal experimental models but lack clinical trials in human beings due to drug safety uncertainties.

Next to malaria, AIDS is the leading infectious cause of death in the world. Untreated disease caused by HIV has a fatality rate of 100%. Nevertheless, it should be stressed that a number of natural products mainly derived from plants have proved effective in suppressing HIV replication and progress (Petit *et al.*, 2000). In regions where herbal medicine could be considered to be at par with the current dominant allopathic medicine, most of the therapeutic plants are from the traditional data bank (Li *et al.*, 2004). Several plants were investigated to establish their scientific potential values in the management of HIV. It emerged that quite a good number had antiretroviral activities and potential drug development that are comparable to the drugs currently in the market (Li *et al.*, 2004). This revelation enhances the use and encourages further studies on the use of plants in tackling modern problems and conservation of indigenous knowledge in Africa. However, only a meagre portion of East African plants has been currently covered in the ongoing work *in vitro* as in the case of (Muregi *et al.*, 2004) on trying to find out new antiplasmodia compounds from the indigenous flora from the East African region.

2.16 Emerging and reemerging diseases: a burning issue

Problems of emerging and reemerging resistant micro-organisms and their strains are common phenomena (Ron, 2006). There are certain bacteria which were not previously a cause for concern but are currently receiving special attention due to their turn around and are virulence. An example is *E. coli*, under virulent conditions causes extra intestinal infections a wide diverse spectrum of diseases including: urinary tract infections (UTI), new born meningitis, abdominal sepsis and septicemia (Ron, 2006). All these infectious diseases are ever increasing problems of human health, especially in patients who are immunocompromised. Owing to these diseases, chemotherapy and old stream infections are

difficult to treat due to high incidence of drug resistance being transmitted by plasmids (Meyer, 2005). Antibiotic resistance is only perceived as a problem when it occurs in clinical cases but the use of antibiotics has been closely followed by the emergence of antibiotic resistant microbial populations that in some cases are prevalent (Gonzales *et al.*, 2004). In contrast to other drugs, it has emerged that; antibiotics can start to lose their efficacy at the beginning of their clinical use through the development of antibiotic resistance by bacterial pathogens (Garau, 2006).

Challenges in antimicrobial resistance are more real due to emerging and reemerging diseases. It is, therefore, empirical that further studies on antibiotic discovery is vital considering the threat posed by the emergence of drug resistance (Morens *et al.*, 2004). The best example is the frequent upsurge of *Legionella* an organism which becomes a threat due to change of lifestyle of using air conditioning systems more frequently. Such a condition enhances quick multiplication of the organism and its delivery to human respiratory system. Based on metagenomics in microbiology it is possible to identify the gene pool which is either cultivable or not cultivable which may be used in future identification of unexpected potential pathogens following the identification of all the virulence related genes—“resistone” present in the environment (D’Costa *et al.*, 2006).

The ecological impact of human activity is well-illustrated by the changes seen in antibiotic resistance through the years (Marrie, 2001). Bacteria quickly develop resistance to antibiotics usually within a few years of their commercial manufacture and marketing. For example in 1943 when penicillin was first prescribed, virtually all Gram-positive infections were susceptible to its effects on cell wall synthesis (Marrie, 2001). However, the bacteria now developed resistances to the drug and by 1946 resistant strains were already well-recognized.

Mycobacteria require special attention, but lightly, because it causes infections of a killer disease, tuberculosis (TB). Currently, the disease is the leading cause of deaths worldwide from a single human pathogen. The disease claims more adult lives and children than diseases like AIDS, malaria, diarrhoea, leprosy and all the other tropical diseases combined (Zumla and Grange, 1998). It is estimated that a third of the world's population is currently infected with *M. tuberculosis*, where 10% of those infected will develop clinical diseases, more so those that are immunosuppressed or immunocompromised as a result of HIV (Zumla and Grange, 1998). Although there are several broad spectrum antibiotics, there has been an upsurge in the mortality rate due to the emergence of multi drug resistant strains of *M. tuberculosis* (WHO, 1985). By the year 2000 the mortality due to the disease had reached over 3.5 million (Newton *et al.*, 2000; WHO, 2001). The upsurge in the incidences of TB mortality is further attributed to poor physical infrastructure in the rural areas where majority of the developing world live, lack of diagnosis and drug supply. There is also poor supervision and medical care and as a result of drug resistance development (WHO, 2001). This means that there is need for broad effective spectrum to curb this malady.

Tuberculosis was considered as a conquered malady that was disappearing, but has made a comeback in the recent past (WHO, 2001). This new scenario is only due to the associated of *Mycobacterium* with AIDS but due to evolution of strains of the pathogens that are resistant to several drugs which are currently in use (WHO, 2001). It seems clear that besides biomedical research, additional social measurements are urgently required to deal with the problem of infectious diseases in a global scenario.

CHAPTER THREE

ETHNOBOTANICAL SURVEY

3.1 Study area

The Community is located in the Rift Valley Province, Kenya (Figure 3.1). They live in Kericho around Tinnet; Nessuit and Mariashoni locations in Nakuru District and Kaptuiget Forest in Koibatek District within the bearings of: latitudes 0.5 and 1°, and longitudes of 34°10' and 34°50'E (Survey of Kenya, 1974). The area could possibly cover almost the greater Molo division of Nakuru District; if consolidated. These areas have equatorial rain forest, which have been modified by the high altitude and are more or less Afromontane in nature.

The topography of the area is rugged, rather undulating, being the western flank of the famous Mau ranges of the Great Rift Valley. The soil types are basically volcanic Andosols. Such soils are ash type with very low inherent fertility. However, due to the physiography and altitude coupled with vegetative littering, the organic matter content has considerably changed for the better. The vegetation is varied because of the sudden changes in altitude and it ranges from tropical savannah over acacias to a few shrubs on the lower portion of the Rift Valley floor. These changes modify temperate forests as one ascends the Mau escarpment to the alpine type. By the time one reaches the Mau summit, there is such a steep ascent from 11 km from the floor to the summit the tremendous and change in the flora. The altitude changes from about 1500m to over 3000m above sea level. The temperature has very low diannual range. However, temperatures at night may drop to near freezing (5°C). The rainfall is bimodal with two peaks during April and May with the short ones occurring in December. Largely, the precipitation spreads throughout the year up to over 1800mm annually. The

relative humidity in these areas ranges between 75 and 85%. Virtually, all the wild tropical African game animals are found in this environment with the exception of reptiles hardly inhabiting the higher altitude areas (GoK, Soil Survey 1974).

3.2 Materials and methods

A purposive sampling technique and structured questionnaire was administered while ensuring single administration of the questionnaire to each respondent. The respondents were recruited with assistance of the Ogiek Welfare Council (Towett, 2002), a non-governmental organization interested in the conservation of Ogiek culture.

A total of twenty (20), traditional healers of the ages of between 50 and 70 years were selected as recognized by the community as custodians of indigenous knowledge. Transects to represent the study area starting with the plain to trigonometric point was taken to represent the flora of the study area. This was meant to ensure that there was no duplication or falsification of the information. Records on the plants, time of collection, parts of plant used, method of preparation, precautions on use, formulation, dosage and other mixtures were noted (Appendix 1).

Photographs, availability and environmental status of each plant were taken during the excursions. Voucher specimens of each plant were made and deposited at the National Museum of Kenya for authentication assigned voucher numbers and kept there for future reference. Each plant part was collected and dried under shade to obtain moisture content of 13% before being ground to pass through 0.5mm sieve and hermetically stored in refrigerators for future processing to be used in biological and chemical studies. Each plant was accurately described and discussed based on several references which gave their

geographical location. The descriptions of the plants were based on the approaches used by International Code of Botanical Nomenclature (ICBN) unless otherwise, the plants have been indicated with an arrow for purposes of clarity.

Simple tabulation and descriptive statistics of the plants in common and scientific names, percentages of use and their therapeutic uses was prepared. Use of each plant species was calculated by:

$$\text{Respondents} \times 100 \div \text{total number of respondents interviewed.}$$

3.3 Results and discussion

The Ogiek communities constitute a kaleidoscope of the cultural heritage of the Kalenjin speaking people. The origin of the Ogiek ethnic group is believed to have been the Nile valley but they moved southwards with the onset of the Sahelisation of North Africa and the spread of the Sahara desert. Their southward migrations must have been in search of pasture and game animals (National Archives, 1913). Cultural development, being a dynamic process, the Ogiek community was assimilated into other ethnic groups who are their immediate neighbours. A few of them, however still partially retain their cultural identities. It is noted in history that the Kikuyus leased or acquired the land for livestock grazing from the community. The greater Kiambu and the better parts of Muranga districts were originally occupied by the Ogiek until the late 19th century and the first two decades of the 20th century (National Archives, 1913).

To their neighbours, the Kalenjin and Maasai, the Ogiek people have remained veterinarians. Besides being veterinarians to their neighbours they also initiated them into adulthood through circumcision (Towett, 2002). However, towards the mid-20th century most of the

Ogiek communities had been assimilated into the communities that neighbour them. They have empirical and vast knowledge on the forest and forest product uses.

Table 3.1: Medicinal plants used by the Ogiek

Family	Scientific Name	Common Name (Ogiek)	Plant Part (s) Used	% usage (Healers)	Remedies
Acanthaceae	<i>Hypoestes verticillaris</i>	Narubat	Roots	100	Tuberculosis/pneumonia
Araliaceae	<i>Schefflera abyssinica</i>	Chelembut	Stem bark wax	100	Pneumonia
Asclepiadaceae	<i>Periploca linearlifolia</i>	Senendet	Root/whole plant	100	Roots tonic/circumcision
Asclepiadaceae	<i>Gomphorcapus semilunatus</i>	Toiliotilio	Roots	10	Toothache/syphilis
Asparagaceae	<i>Asparagus racemosus</i>	Kaptalelit	Roots	10	Stomachache
Asteraceae	<i>Vernonia lasiopus</i>	Segumeriet	Roots	20	Liver problems
Asteraceae	<i>Vernonia brachycalyx</i>	Segumeriet	Leaves	30	Colds, stomachache
Asteraceae	<i>Helicherycum schimperi</i>	Manarariat	Leaves	10	Stomachache
Asteraceae	<i>Helichrycum schimperi</i>	Chesaleit	Flowers	10	Colds/coughs
Asteraceae	<i>Solinecio manii</i>	Masageriet	Leaves	100	Otitis media
Asteraceae	<i>Solinecio nadensis</i>	Molosuet	Leaves	100	Conjunctivitis
Balsaminaceae	<i>Impatiens tinctoria</i>	Pumbuetiet	Stem	20	Placenta removal
Clausiaceae	<i>Garcinia buchananni</i>	Nderiot	Fruit/ stem bark	20	Bowel disorders
Curcubitaceae	<i>Zehneria scabbra</i>	Sumeito	Stem	100	Malaria
Curcubitaceae	<i>Lagenaria spherica</i>	Mtondoruet	Fruits	50	Placenta removal
Curcubitaceae	<i>Mormodica rostrata</i>	Chepkologolio	Whole stem	100	Tuberculosis/malaria
Dracaenaceae	<i>Dracaena afromantana</i>	Lebekuet	Leaves	100	Stomachache/dysentery
Euphorbiaceae	<i>Clutia robusta</i>	Kibarnyat	Root bark	100	Tonic/aphrodisiac/cookery
Euphorbiaceae	<i>Macaranga kilimandschrica</i>	Logomaita	Roots	100	Bilharzia/coughs/stomache
Flacourtaiceae	<i>Dovyalis abyssinica</i>	Nkuyiat	Fruits/root bark	100	Tonic/food/culinary
Fabaceae	<i>Senna didymobotrya</i>	Senetuet	Leaves	100	Malaria
Lamiaceae	<i>Satureia biflora</i>	Chepsagitiet	Roots/stem/leaves	90	Coughs/tonic/pneumonia
Melanthaceae	<i>Bersama abyssinica</i>	Sagaweita	Stem bark	100	East coast fever
Meliaceae	<i>Ekabergia capensis</i>	Teldet	Stem bark	100	Dysentery
Myrsinaceae	<i>Embelia schimperi</i>	Sachuonet	Stem bark	100	Pneumonia/colds/tuberculosis
Myrsinaceae	<i>Rapanea melanophloes</i>	Kwarabariet	Seeds	100	Stomachache/anthelminthes
Oleaceae	<i>Olea africana</i>	Masaieta	Stem bark	90	Malaria/East coast fever/ body ache

Oleaceae	<i>Olea hochstetteri</i>	Murunguyet	Stem bark	90	UTI/malaria/pneumonia
Olinaceae	<i>Olinia rochetiana</i>	Kabideleliet	Leaves	20	Malaria/colds/coughs/pneumonia
Piperaceae	<i>Piper umbellatum</i>	Ketagan	Roots	70	Pneumonia
Pittosporaceae	<i>Pittosporum lanatum</i>	Mtabonit	Root bark	100	Vomiting/malaria
Protaceae	<i>Faurea saligna</i>	Mosomboriet	Stem bark	70	Liver problems/UTI/tonic
Ranunculaceae	<i>Clematis hirsute</i>	Pissiinda	Stem bark	100	Gonorrhoea/syphilis/backache
Rhamnaceae	<i>Rhamnus prinoides</i>	Kosisito	Ripe fruits	100	Tonic/pneumonia
Rhamnaceae	<i>Scutia myrtina</i>	Sumbeyet	Stem bark	100	Tonic/pneumonia
Rosaceae	<i>Rubus keniensis</i>	Tagaimaiet	Root bark	10	Stomachache/fruits edible
Rosaceae	<i>Prunes africana</i>	Tenduet	Stem bark/leaves	100	Malaria/prostatitis
Rubiaceae	<i>Gallium simensis</i>	Tipsoliet	Whole stem/leaves	20	Malaria /rashes
Rutaceae	<i>Zanthosylum chalybeum</i>	Kikomit	Stem bark	30	Tonic/UTI
Rutaceae	<i>Toddalia asiatica</i>	Chepindoruet	Stem bark	60	Tonic / chest complains
Solanaceae	<i>Solanum eculeastrum</i>	Sigowet	Ripe fruit	100	Milk preparation/vomiting
Solanaceae	<i>Solanum mauense</i>	Kimoyat	ripe fruits	70	Tuberculosis
Solanaceae	<i>Solanum nakuruense</i>	Tiliet	Ripe fruits	70	Otitis media
Streculiaceae	<i>Dombeya goetziii</i>	Silibuet	Stem bark	100	Chest problems coughs
Urticaceae	<i>Urtica massaica</i>	Ila ila	Leaves	100	Circumcision ceremonies
Verbenaceae	<i>Lippia javanica</i>	Labotuet	Leaves	80	Fresh wounds
Vittaraceae	<i>Pteris ssp</i>		Roots	40	Stomachache
Xyleriaceae	<i>Engleromyces goetziii</i>	Puindaii	Whole plant	100	Fever/malaria/vomiting

As shown from Table 3.1, on knowledge and use of plants by the respondents it could be seen that they were medical conditions within the Ogiek community and a majority would resort to using plants or plant parts as remedy. From Table 3.2, it was evident from the 32 plant families collected that majority are used in treating malaria (21.88%), pneumonia (21.88%), stomachache (21.88%) and tonic diseases (21.88%). Other remedies used fewer plant families.

Table 3.2: Diseases/other uses showing % number of families and scores

Diseases treated/ other uses	Scores	Percentage out of 32 families
Treating fresh wounds	1	3.12
Malaria	7	21.88
TB	3	9.38
Pneumonia	7	21.88
Tonic	7	21.88
Circumcision ceremonies	2	6.25
Toothache	1	3.12
Syphilis	1	3.12
Liver Problem	2	6.25
Otitis	1	3.12
Placenta removal	1	3.12
Arthritis	1	3.12
Dysentery	2	6.25
Bowel disorder/Stomachache	7	21.88
Coughs	4	12.5
East coast fever	2	6.25
Gonorrhoea	1	3.12
Backache	1	3.12
Leprosy	1	3.12

The medical conditions are, possibly, due to the fact that the Ogiek are forest inhabitants, who rely on forest products that are generally used while fresh. The foodstuffs are thus liable to contamination if they are not washed or well preserved. In addition, the environmental conditions, being windy with night temperatures falling to minus 1°C, lead to frequent chest infections, forcing the Ogiek to look for quick and locally available

remedies. It would be encouraging to use such local remedies since the majority of the Ogiek are yet to be accessed by the allopathic medical facilities (Busia, 2005).

The high incidence of malaria and hepatitis in the community means that useful drugs such as tonics for the liver are well-known and widely are used. Such plants have been cited repeatedly (Table 3.2) as species that are used for such purposes. The high incidences of gastrointestinal infections are further supported by the use of root (Table 3.3). The Chi-Square indicated that there was no significant difference use of any part of the plants used. This could also assist in explaining the reasons for the scarcity and red listing of plant species whose barks and roots are used as medicine. The plants' parts that were used were; mainly roots stem, bark, leaves, tubers, fruits, and seeds. However, a whole plant could be in some families.

Table 3.3: Percentage use of plant parts

Parts of the plant	Mean percentage in the plant family	S.E.
Roots	57.27	12.07
Stem bark	89.38	4.95
Tubers	20.0	-
Fruits	80.0	12.24
Leaves	52.73	12.7
Seeds	100	-
Whole Plant	55.0	45.0
Chi-value	61.357	
P- value	0.810	

From the study it was found that most frequently used part of the plants was the stem bark (Tables 3.5 and 3.6). Tubers and seeds were not always used except in the family of Balsaminaceae and Myrsinaceae, respectively. Level of usage of the plant parts may reinforce the fact that a careless use of certain species would endanger their existence in the Mau flora and its environs.

Table 3.4: Frequency and percentage usage of plant parts

Parts of the plant	Frequency	Percentage
Roots	12	37.5
Stem bark	16	50
Tubers	1	3.12
Fruits	5	15.63
Leaves	13	40.63
Seeds	1	3.12
Whole Plant	2	6.25

In the plant family Myrsinaceae, 100% usage of the seed was recorded. The percentage level at which the plant parts were used varied, although not significantly $\chi^2 = 61.357$, $p = 0.810$. The plant families identified during the floral survey ranged from Araliaceae to Xylariaceae. The plants so far documented were found to be used for more than one ailment or health disorder. Interpretations of the symptoms as described by the traditional healers were made based on the descriptions and interpretations perceived at the interview stage.

It was evident that from some 49 different plant species, 100% of the traditional healers were aware about and use plant products for treatment of stomachache, malaria, pneumonia and chest-related complaints (Table 3.4). These findings were consistent with the fact that the community depends almost entirely on forest products. This is also partly majority of the still rely on traditional forms of treatment in rural areas (Busia, 2005). The use of plants for treatments was about 85%, which tallies with the WHO and other surveys conducted elsewhere (Busia, 2005; WHO, 1980). There are also specific plants that are highly prize for other uses although they could still be used for other ailments.

The methods of preparations of these herbs revealed that a majority of the drugs are formulated in water with an exception of a few which are formulated in honey or cooked with meat, with the soup taken as a source of medicinal remedy. A few of the plant raw

materials are soaked in water for some time since the bio-active ingredients are polar in nature and are formulated in water. The aliquot is then taken as a curative portion. In female genital mutilation and male circumcision rituals, for instance, *Urtica massaica* and *Periplocca linearlifolia* are applied. The plants may also be used for other purposes. For example, when boiled, the *P. linearlifolia* roots may be taken as a tonic drink while the *U. massaica* leaves may be cooked as vegetable or the seeds may be eaten as grain during famine period (Towett, 2002).

It was also found that majority of the respondents could categorize plant parts into three main groups (i) those that are used to resolve veterinary problems (ii) human health problems and diseases, and (iii) those that are used in both. It also emerged that there are various categories of traditional healers and forest product users in the community (Tables 3.1). There are forest product gatherers and hunters. This category involves nearly everyone within the Ogiek community. Then the herbalists who treat people and livestock using animal extracts alone. As we noted earlier, the Ogiek were found to have undergone a significant cultural assimilation. However, a significant proportion of members of this community still adhere to their culture. For instance, some of the respondents admitted possessing the ability to communicate with friendly spiritual powers or their ancestors through the use of plant preparations.

Table 3.5: Plant families and the number of remedies for which they are used

Plant family	No. of remedies	Plant parts used
Acanthaceae	2	Roots
Araliaceae	1	Stem bark
Asclepiadaceae	4	Root/Whole plant
Asparagaceae	1	Roots
Asteraceae	1	Roots/Leaves
Balsaminaceae	1	Tubers
Carusselaceae	1	Leaves
Curcubitaceae	2	Stem/Fruits/Whole plant
Dracaenaceae	2	Leaves
Euphorbiaceae	2	Root
Fabaceae	2	Whole plant/Leaves
Flacourtaiceae	1	Fruits/Root
Lusaciaceae	1	Leaves/Stem bark
Laminiaceae	3	Root/Stem/Leaves
Melanthaceae	1	Stem bark
Meliaceae	1	Stem bark
Myrsinaceae	3	Stem bark
Oleaceae	4	Stem bark
Piperaceae	1	Roots
Pittosporaceae	1	Roots
Protaceae	2	Stem bark
Ranunculaceae	2	Stem bark
Rhamnaceae	2	Fruits/Root/ Stem bark
Rosaceae	2	Roots/Stem bark/Leaves
Rubiaceae	1	Whole stem /Leaves
Rutaceae	1	Stem bark
Solanaceae	2	Roots/Fruits
Streculiaceae	1	Stem bark
Urticaceae	2	Leaves
Verbenaceae	1	Leaves
Vittaraceae	1	Roots
Xylaraiceae	1	Whole plant

3.4 *Hypoestes verticillaris* L. Acanthaceae (Narubat) (Voucher No. AO/O37/NMK/12/12/2008)

3.4.1 Plant description

A medium sized herb about 1m high. Slender stem, leaves opposite and somehow lanceolate, entire margin, dark green. Flowers white and sometimes yellow bilobed. The plant grows as lianas at an altitude of 0-2820m. Found in secondary forests and where the biomass is of high percentage. The species being an under growth is still abundant in the region.



Plate 3.1: *Hypoestes verticillaris* lianas, Mariashoni Dec, 2008

3.4.2 Usage and dosage of the medicine

Three hundred grams of its roots are mixed with equal of roots of *Toddalia asiatica*, 300 gm of each and boiled in 1½ litres of water and 250 ml of the extract drunk to cure TB, various chest complaints and dry coughs.

3.5 *Schefflera abyssinica* (A. Rich) Harms. Araliaceae (Chelumbut) (Voucher No. AO/037/NMK/12/12/2008)

3.5.1 Plant description

Tree or epiphyte 4.5-20 (30) m bark corky deeply fissured, grey black or black or brown, leaves with 12-38 cm long; leaflets 5-7, ovate or elliptic, base rounded or subcordate, apex acuminate, margin creminate 9-20 by 4.5–12 cm, glabrous; petioles 3-12 cm long. Inflorescence up to 25 spikes together each spike up to 40 cm long small stalked umbellules of yellow green or orange – yellow flowers each about 3mm long. Fruits red urceolate or subglobose, up to 5mm across, sulcate, glabrous or puberulous. Found in wet upland forest or riparian forest. Population drastically reduced in the last 10 years.



Plate 3.2: *Schefflera abyssinica*, Mariashoni (Dec, 2007)

3.5.2 Usage and dosage of the medicine

Wax is formed from the exudates in mature trees directly scrapped to form one teaspoonful of powder. The powder is then mixed with three tablespoonfuls of honey and then eaten once daily for about four days to treat asthmatic and respiratory disorders like bronchitis and coughs. About 600gm of the bark also dried or directly boiled thoroughly in about 2 litres of water, may be bottled and 200 ml taken twice daily to restore vitality (aphrodisiac) in men.

3.6 *Periploca linearifolia* Dill and Rich. Asclepiadaceae (Senendet) (Voucher No. AO/003/NMK/20//08/2006)

3.6.1 Plant description

Genus *Periploca*. A hairless woody climber with linear leaves and terminal complex cymes; sepals purple, rounded; petals creamy white-hairy above, to 5 mm long; corona lobes forming a lantern above stamens; pods diverging at 180°. Linear to 10 cm long common in upland forest edges but seldom flowering between July-September, altitude 1900-2900 m. The plant species forms berry.



Plate 3.3: *Periploca linearifolia* foliage Kaptenga Forest (August, 2006)



**Plate 3.4: left: *Periploca linearifolia* flowering, Kaptenga Forest (August, 2006)
Right: flower close-up Kaptenga Forest (August, 2006)**

3.6.2 Usage and dosage of the medicine

There are four main uses for Genus *Periploca*: (a) It is precious amongst the Kalenjin, Maasai, and Ogiek particularly during the circumcision, it is used in initiation amongst the Ogiek by making a big ring which is taken to the river by the person to be circumcised. Medicinal uses are:(b) About 200 gm pieces of the roots are washed and

cooked in 1 litre of water. The water extract is decanted, and about 250 ml is drunk to cure colds. (c) About 250 gm roots are cooked in mutton to add flavour to the soup. The soup prepared is also known for vitality to men. (d) The leaves are dried pounded and the resulting powder is applied to wounds inflicted by herpes zoster and also mixed with 30 ml of honey and eaten once daily to cure oral thrush.

3.7 *Gomphorcapus semilunatus* A. Rich. Apocynaceae (Toililotio) (Voucher No. AO/035/NMK/01/06/2007)

3.7.1 Plant description

A large erect perennial with crowded lanceolate leaves and purple pink flowers, corona mostly erect in the central hollow, rather square-edged outside fruits more hairy or less semi-circular in outline, equally rounded at each end, thickly covered with minutely rough bristles. Found in open lands.



Plate 3.5: *Gomphorcapus semilunatus* A. Rich. Asclepadiaceae Mariashoni, (June, 2007)

3.7.2 Usage and dosage of the medicine

The leaves chewed to relieve tooth ache and the milky sap from the plant is applied twice daily to cover the whole part of the affected areas of the gum in the mouth twice till the lesions disappear. About ½ kg of the roots boiled in about 2 litres of water, and 200 ml of the decoction is drunk to cure syphilis, gonorrhoea and gastenteroritis

**3.8 *Asparagus racemosus* Wild. syn. *A. buchannani*. Asparagaceae (Kaptalelit)
(Voucher No. OA/033/NMK/10/08/2007)**

3.8.1 Plant description

A woody climber or scrambling shrub with yellowish brown to grey stems with zigzag branches branchlets often in threes, grooved; spines 6-20 mm long flattened towards the base, stout chestnut brown with axillary pale brown spur; cladodes clustered, needlelike, smooth or grooved, slightly curved, to 3 cm long, flowers white in racemes replacing branchlets on stalks jointed just below the middle; ovary stalked, berry red, 1 to 2 seed. Occurs in forest margins and drier bushland, 1160-2800 m. Has always preferred open fields and support from other vegetation.



**Plate 3.6: *Asparagus racemosus*,
Kaptenga Forest, (Dec, 2007)**

3.8.2 Usage and dosage of the medicine

1 Kg of the roots cooked as soup which is believed to act as immuno – booster. 1 kg of the roots are mixed with same amount of roots of *Dovyalis abyssinica* and *Solanum eculeastrum* cooked in 1.5 l of water and 250 ml of resulting infusion drunk twice daily, to cure syphilis and malaria till recovery.

3.9. *Helichrycum schimperi* O Haffm. Asteraceae (Manarariat) (Voucher No. AO/032/NMK/12/12/2007)

3.9.1 Plant description

A trailing soft shrub, white-hairy exception the upper side of stalked, broad-elliptic leaves; inflorescences rather diffuse of tightly packed yellowish, pinkish or whiteheads; phyllaries 4.5 mm long. Common on roadsides and wastelands.

3.9.2 Usage and dosage of the medicine

Fresh roots, about 30 g, are washed, chewed and the resultant liquid swallowed to cure abdominal problems like stomach-ache and other related ailments twice daily till recovery.

3.10 *Solanecio manii* (Hoof K.), C. Jeffrey. Asteraceae (Molsuet) (Voucher No. AO/038/NMK/14/12/2008)

3.10.1 Plant description

A hairless tree to 3 m with large rosettes of elliptic lanceolate leaves; involucre 4-6 mm long, of 5-8 phyllaries. Found in places where fire was used in clearing bushes.



Plate 3.7: *Solanecio manii* Plant, Mariashoni (Dec, 2007)

3.10.2 Usage and dosage of the medicine

About three fresh leaves are squeezed into sorous eyes of people twice daily till recovery.

3.11 *Solinecio nandensis* (S. Moore) C. Jeffery (*Senecio nandensis* S. Moore) Asteraceae (Masagariet) (Voucher No. AO/038/NMK/14/12/2008)

3.11.1 Plant description

A sparsely cobwebby, semi-succulent trailing climber with ovate, coarsely toothed and shallowly lobed leaves; heads in terminal corymbs; phyllaries 8-10, inner 8-10 mm long; achenes hairy. Foetid-smelling climber at the edge of dry upland forest.



Plate 3.8: *Solinecio nandensis* flowering plant and **Plate 3.9:** *Solinecio nandensis* shrub (Dec, 2008)

3.11.2 Usage and dosage of the medicine

The leaves approximately ½ kg are pounded and boiled in 2 litres of water cooled and decanted. The 250 ml of the aliquot is taken once daily for 3 days against malaria and stomach-ache.

**3.12 *Helichrycum schimperi* Sensus, Hedbergenson (L) Moench–Asteraceae (Chesalit)
(Voucher No. 032/NMK/12/12/2007)**

3.12.1 Plant description

An erect glundar hairy annual branching above into lanceolate to oblong, clasping at the base, 5 cm long; flowers numerous corymbs of golden yellow heads. Locally common in grassy clearings in dry upland forest areas of the Afromontane between 2400-3000 m.

3.12.2 Usage and dosage of the medicine

of fruits and about 200 gm of the roots washed and chewed once daily against colds and A handful chest infections.



Plate 3.9: *Helichrycum foeditum*, Mariashoni (Dec, 2007)

3.13. *Vernonia lasiopus*, O. Haffm. Asteraceae (Segumeriet) (Voucher No. AO/NMK/10/08/2007)

3.13.1 Plant description

This is a small shrub which forms storage in bulbous root system. Leaves ovate or elliptic, base cuneate or decurrent, apex acute, and margin roughly dentate, 4-18 by 1.6-9 cm, tomentose beneath. Florets pale mauve or white appendages 1-3mm long; heads 5-10 cm across. It is common in disturbed vegetation (bushed) grassland and riverine woodland or forest.



Plate 3.10: Flowering *Vernonia lasiopus*, Kaptuiget Forest (Dec, 2007

3.13.2 Usage and dosage of the medicine

Freshly harvested root are pounded when fresh soaked in water for about one hour and about 200 ml of the liquid is drunk once to expel worms, and also used to treat dysentery and respiratory ailments.

3.14 *Vernonia brachycalyx* O. Haffm. Asteraceae (Gugutu) (Voucher No. AO/03/NMK/12/12/2007)

3.14.1 Plant description

(Scandent) shrub 1-4(6). Leaves ovate or elliptic, base decurrent, apex acute margin entire or dentate, 3.5-16 by 1.5-7 cm, slightly sandpapery above, pubescent beneath. Florets mauve or white, phyllaries 2-3 mm long; heads 3-5 mm across in dense infloresces; pappus violet at flowering stage, later brown. It is common in dry forest (edges) and semideciduous clump bushland; also riverine and in roadsides. This species was common in wastelands of the floor of the Rift Valley to Mau Summit.



Plate 3.11: *Vernonia brachytylax* in a flowering stage, Mau Summit Forest (Dec, 2007)

3.14.2 Usage and dosage of the medicine

About 100-150 g fresh leaves are boiled in 1 litre of water. The infusion is decanted, cooled and 250 ml taken once daily for five days to cure stomachache and cough.

3.15 *Impatiens tinctoria* A. Rich. Balsaminaceae (Pumbuetiet) (Voucher No. AO/O29/NMK/12/12/2007)

3.15.1 Plant description

An erect nearly hairless herb 1-3 m tall from a tuberous rootstock; leaves alternate; elliptic; racemes with 3-7 flowers almost trumpet shaped, white with red or pink spots, with very large lower petiole lobes 20-40 mm long, spur swollen and often 2 lobed at tip. Common near streams, banks and waterfalls in selected wetter highland forest areas.



Plate 3.12: *Impatiens tinctoria* Mariashoni (Dec, 2007)

3.15.2 Usage and dosage of the medicine

Six pieces of the roots each measuring 3-4 inches are washed, boiled in 300 ml of water, decanted, cooled and given to an expectant woman to enhance safe baby delivery during labour.

3.16 *Basella alba* L. Basellaceae (Nderemiet) (Voucher No. AO/039/NMK/12/12/2008)

3.16.1 Plant description

A hairless twining shrub with glossy heart-shaped stalked leaves; spikes axillary, short 20 mm long, elongating to 17 cm in fruits, flowers white, tubular to 5 mm long. Common in riverine forest margins and hedges 1500 m – 3000 m altitudes.



Plate 3.13: *Basella alba* Plant, Mariashoni Forest, (Dec, 2008)

3.16.2 Usage and dosage of the medicine

About 2 kg of the leaves and stems are pounded and mixed with the same amount of powdered bark of *Bersama abyssinica* and 200 ml of *Solanum eculeastrum* seeds. The mixture soaked in 3 litres of water, strained and the decoction given to a cow whose placenta has refused to be expelled from the uterus. Leaves are cooked and eaten as vegetable.

3.17 *Kalanchoe densiflora* Rolfe. Crassularaceae (Misigiliet) (Voucher No. AO041/NMK/12/12/2008)

3.17.1 Plant description

An erect hairless herb, perennating from innovations on the rootstock, leaves crate to circular, round, toothed red, stalked; flowers yellow to red in dense terminal corymbs of about 10 cm diameter, sepals thin, hardly fleshy; petal tube 12 mm long. Occurrence at an altitude of 1500-3000 m. Found only in a few wastelands.



**Plate 3.14: *Kalanchoe densiflora*, Left: by the Roadside, Mariashoni
Right: flowering head (Dec, 2008)**

3.17.2 Usage and dosage of the medicine

The leaves heated over fire, when tender, used to massage the whole body in cases of rheumatism, muscular bone dislocations and general muscular aches.

3.18 *Zehneria scabra* L.F. Sond. Curcubitaceae (Sumeito) (Voucher No. AO/051/NMK/10/12/2009)

3.18.1 Plant description

A perennial climber often covered with short hairs and shallowly lobed to ovate, rounded rough leaves; stems becoming thick, white and jointed; male flowers often white stalked in cluster, 7mm on separate plants from the females. Fruits red, spherical, in clusters about 10mm long, on very short stalks. It is the commonest climber in the forest edges. Altitude 750-1500 m. Common in wastelands.



Plate 3.15: *Zehneria scabra*, plant, Mariashoni Forest station, (Dec, 2009)

3.18.2 Usage and dosage of the medicine

The bark of the stem, about 1 kg dried; finely ground boiled and allowed to cool for about fifteen minutes. The resultant water extract strained out, and drunk twice for one day to cure malaria. Fresh exudes from the plants are applied to fresh wounds to enhance the healing process.

3.19 *Mormodica rostrata* A. Zimm. Cucurbitaceae (Chepkolokiolio) (Voucher No. AO/041/NMK/12/12/2008)

3.19.1 Plant description

A climber with slender stem originated from a fleshy stock on the soil surface with simple tendrils and leaves often with ovate wavy toothed leaflets in 3 groups of 3s. Flower sexes on separate plants, orange with brownish base; males about 20mm diameter up to 14 without an enclosing bract, each with 3 stamens; fruits up to 70 mm long, egg shaped beaked, almost smooth, bright orange-red. Common in drier areas. Altitude 750-1500 m.



Plate 3.16: *Mormodica rostrata*: Left; on dead tree trunk, May and right, a fruit; Nessuit, (Dec, 2008)

3.19.2 Usage and dosage of the medicine

The underground tuber which acts as plants food reserve during the dry or dormant seasons, chopped into small pieces and dried completely. The dried material, 200 gm, ground into very fine powder. Three teaspoonfuls (15 ml) of powder mixed with same amount of honey and swallowed at once in a single dose to treat malaria and acts as brain stimulant. As a matter of caution, it has nauseating effect and must be handled with care.

3.20 *Lagenaria sphaerica* (Sond) Naud. Cucurbitaceae (Mtondoruet) (Voucher No. AO/028/NMK/20/12/2007)

3.20.1 Plant description

A climbing herb, with divided tendrils; leaves kidney shaped, with two tiny glands at the base of lamina; flower sexes on different plants, 2-10 in a raceme, white corolla; fruit spherical, blotchy green 10 cm in diameter. Riverine vegetation. Altitude upto 2000 m, occasionally found in lowlands.



Plate 3.17: *Lagenaria sphaerica*, a vine, Mariashoin (Dec, 2007)



Plate 3.18: *Lagenaria sphaerica*, fruit, Mariashoin (Dec, 2007)

3.20.2 Usage and dosage of the medicine

About 1kg of fresh flowers and young fruits which are freshly forming are pounded and soaked in about 5 litres of water. The decoction used to bathe human body daily inflicted with scabies, candidiasis, ringworms or leprosy till the areas heal.

3.26 *Garcinia buchananni* Bak. syn. *G. huillensis* Clausiaceae (Nderiot) (Voucher No.AO/006/NMK/12/12/2006)

3.26.1 Plant description

Tree 6-15 m leaves elliptic, base cuneate, apex acuminate, 6-12 by 2-5 cm, glabrous. Flowers white, yellow or orange, axillary or terminal, solitary (female) or in short cymes (male), petals 8-9 mm long. Fruit orange to red, round, 2-2.5 cm. Occasionally found in moist forest or dense wooded land.



Plate 3.19: *Garcinia buchananni* Left Branches and Right Close up of Branches, Nessuit, Dec, 2006



Plate 3.20: *Garcinia buchananni*, Bottom Right Stem, Bottom Left, Close up of Branches Nessuit, (Dec, 2006)

3.26.2 Usage and dosage of the medicine

Fruit readily edible, the 500 gm bark is cleaned and soaked in 1½ litre of water. Decanted and the resultant decoction 20 ml given to a child with stomach problems twice daily till recovery.

3.21 *Dracaena afromantana* Mildbr. Dracaenaceae (Lebekuet) (Voucher No. AO/004/NMK/04/03/2006)

3.21.1 Plant description

Tree with very few branches and somewhat palm like habit 2.5–7 m high; twice reported as scandent. Leaves narrowly lanceolate, base gradually narrowing but suddenly amplexicaul, apex acute, 12–35 by 12–22 cm glabrous. Flowers white or cream in panicles 20-60 cm long; flowers 13-15 mm long fruits oranges, round 15-18 mm. Found in areas of highlands 1900-2400 m. It has become extremely rare to find and only available in riverines at high altitudes.



Plate 3.21: *Dracaena afromantana*: Trigonometric point, Kaptuiget Forest, (March, 2006)

3.21.2 Usage and dosage of the medicine

Approximately 250 g of fresh young growing leaves crushed and soaked in about 500 ml of cold water for about two hours, decanted and then 125 ml given twice daily till

recovery to children suffering from stomach problems and swollen spleen. The stem is also used as toothbrush.

3.22 *Macaranga kilimandscharica* Pax. Euphorbiaceae. (Legomaita) (Voucher No. AO/02/NMK/08/12/2007)

3 22.1 Plant descriptions

Tree 6-24 m, bark grey. Leaves triangular – ovate, base cuneate, rounded, truncate or rarely subcordate, occasionally peltate, apex acuminate, 5-15 by 3-10 cm. 3-7 nerved from the base, rusty –tomentellous but glabrescent, densely glandular punctate beneath. Inflorescences 2-10 cm long, with yellow green flowers, fruits dull green, subglobse or two lobed 4-6 by 5-11 cm densely glandular. 1660-2400 m altitude, moist upland forest in the study area, mostly forest edges.



Plate 3.22: *Macaranga kilimandscharica*: Left, flowering branch; right trunk Kaptenga Forest, (Dec, 2007)

3.22.2 Usage and dosage of the medicine

About 500g of the roots washed pounded boiled in 1 litre of water, the extract is decanted and 250 ml is drunk once to cure bilharzia. The 500 g of the roots may be cooked in a litre of water and 250 ml decoction drunk once daily for coughs. A decoction of the leaves amounting to 0.5 g is cooked and 250 ml drunk daily to cure stomach problems.

3.23 *Clutia abyssinica* Jaub. and Spach. Syn. (*C. pedicellaris* (Pax), Hut; *C. richiardiana* (Mull Arg, *C. Mollis* Pax) Euphorbiaceae. (Kirbanyat) (Voucher No. AO/026/NMK/08/2007)

3.23.1 Plant description

Shrub 1-3 m, rarely to 6 m. Leaves turning orange (when old and dying) ovate or elliptic, base cuneate, apex obtuse or acute. 2-10 by 1-5 cm, glabrous to densely pubescent. Flowers yellow – green or greenish cream, in few – flowered fascicles, sepals 2–2.5 mm long. Fruit green, turning red, 4.5–5.5 mm across 1600–3100 m altitude. Drier forest type, forest remnants, also in secondary bushland. Wooded grassland and in bushland on rocky hills. Also in waste uplands.



Plate: 3.23 *Clutia abyssinica*, right; a thicket and left, stem branch Mariashoni, (Dec, 2007)

3.23.2 Usage and dosage of the medicine

The roots of the plant dug and washed. Approximately 250 gm is chopped and cooked with 1kg. of mutton; the soup is then taken to enhance vitality.

3.24 *Senna didymobotrya* (Fresen.) H.S. Irwin and Barneby (Syn. *Cassia didymobotrya* Fabaceae (Senetuet) (Voucher No. AO/005/NMK/12/12/2006)

3.24.1 Plant description

Shrub or in optimal condition may be a tree 1.75 m. Leaves with 8-18 pairs of leaflets, bipinnately; leaflets elliptic apex rounded or obtuse with a mucro 1–3mm long, 2-6.5 by 0.6–2.5 cm more or less pubescent. Flowers yellow, petals 18-2.7 by 1–1.6 cm; fruits flattened, oblong 8–12 by 1.5–2.5 cm Common in occurrence at an altitude of 1500-2000 m.



Plate 3 23: (A): *Senna didymobotrya*, Left: a shrub, (B): Right; flowering branches, (Nessuit, 2006)

3.24.2 Usage and dosage of the medicine

About 500 g of the leaves boiled in 1½ litres of water to form two glasses (500 ml) of the resultant decoction drunk twice daily for three days to cure malaria and fever.

3.25 *Dovyalis abyssinica* A. Rich Warb. Flacourtaiceae (Nukchat) (Voucher No. AO/.042 /3/NMK/09/03/2008)

3.25.1 Plant description

Shrub or tree to 13 m bark, pale grey. Branchlets with axillary spines. Leaves shiny above, ovate or elliptic base cuneate rarely rounded, apex rounded or obtuse, margin entire to subserrate, shortly pubescent on veins but glabrescent; petiole and veins reddish.

Flowers yellow – green or greenish white, 1-3 flowered fascicles in axils of fallen leaves. Fruits yellow to red. Floral status is that there are a few plants remaining and stand isolated since its prickly nature offers it an automatic protection from predators.



Plate 3.24: *Dovyalis abyssinica*, Mariosioni ((March, 2008)) a whole tree



Plate 3.24: *Dovyalis abyssinica*, Mariosioni fruiting branch (March, 2008)

3.25.2 Usage and dosage of the medicine

The roots 500 g crushed and cooked with meat to boost body immunity. About 500 g of the roots are also crushed and mixed with 500 g of *Clutia robusta* roots then boiled, the resultant concoction decanted. 250 ml drunk daily to cure stomachache and chest ailments till recovery.

3.27 *Hoslundia opposita* Vahl. Laminiaceae (Cherunguet) (Voucher No.AO/007/NMK/12/2006)

3.27.1 Plant description

Shrub 0.5-3 m. Leaves ovate or elliptic, base cuneate, apex acute, and margin serrate, 1-10 by 0.5-4 cm, pubescent. Flowers white or yellowish, in terminal racemes. 1-6 cm long; corolla 3-6 mm long. Fruit with yellow to white red fleshy calyx, to 9 mm long. (Secondary) bushland bushed or wooded grassland.



Plate 3.25: *Hoslundia opposita*, Kaptenga Forest (Dec, 2006)

3.27.2 Usage and dosage of the medicine

About 10 freshly collected leaves are boiled in 1litre of water and the resultant infusion taken hot tonic.

3.28 *Satureia biflora* D. Don. Benth. Lamiaceae. (Labiatae) (Chepsagitiet) (Voucher No. AO/08/2/12/2006)

3.28.1 Plant description

An erect woody herb to 50 cm, hairless to hairy with elliptic to circular entire leaves 12 (-20) x 8 mm; inflorescence bracts leaf like flowers 2–20 in dense auxiliary clusters, pink, 8 mm long; sepal teeth all equal in lowland individuals but flower up to 2 x as long upper ones in alpine forms. Common in wastelands and roadsides.



Plate 3.26: *Satureia biflora* (See arrow) Plate left, regenerating plant and right full grown plant Mariashoni (Dec, 2006).

3.28.2 Usage and dosage of the medicine

The preparations from the plant are used in the following manner: About 200 gm of the roots crushed and boiled in one litre of water, decanted and 200 ml of decoction drunk daily for 4 days against stomachache, gastroenteritis and pneumonia.

3.29 *Bersama abyssinica* Fres. spp., *abyssinica* Verdc. Family Melianthaceae (Sagawaita) (Voucher No. AO/025/NMK/15/04/2007)

3.29.1 Plant description

Shrub or tree 1.5-24; bark smooth or rough, splitting lengthwise. Rachis of pinnate leaves round to broadly winged, in this specie, winged, the rachis wingless or only slightly winged capsule < 2 cm long, globose, usually red-velvety; leaves normally glabrous. Stamens 5; disc annul pentagonal 4-lobed; capsule often grooved. The species is endangered since only on tree remains in the whole of Mau Forest complex (Towett, 2002).

3.29.2 Usage and dosage of the medicine

About 1kg of freshly collected bark cooked along with 5 ripe seeds of *S. eculeastrum* in 4 litres of water. The resultant infusion, decanted and 1litre given daily for 3 days to animals suffering from black water, east coast fever and Rift Valley fever.



Plate 3.27: *Bersama abyssinica* Left, canopy Plant, Right, Fruits, Nessuit (April, 2007)

3.30 *Ekebergia capensis* C. DC. Meliaceae. (Ororouet) (Voucher No. AO/024 /NMK/17/12/2007)

3.30.1 Plant description

A beautiful and elegant tree grows between 20-30 m high. The bark brown and rough with age. Branchlets dotted with whitish breathing pores. Leaves; compound, mostly crowded at the end of branches, young leaflets hairy, later thin, and shiny up to 5 pairs of lateral leaflets, leaflets blades unequal-sided. Flowers in loose sprays up to 8 cm, each flower small and white and heavily scented. Fruit; rounded, thin skinned berries on long stalks, yellow red when ripe. Altitude 0-1500 m. A pioneer in forest edges, shady used as a meeting place. It has become rare due to over exploitation for timber which is highly prized. Endangered and in all the forests surveyed but only two adult trees were found.

3.30.2 Usage and dosage of the medicine

The bark is used as medicine. About 1 kg of the bark pounded, boiled in about 2 litres of water, cooled and decanted 250 ml of the decoction taken with juice daily against dysentery and diarrhea due to HIV related opportunistic infections till recovery (Towett, 2002).



Plate 3.29: *Ekebergia capensis*: left: Tree trunk, centre: Branches and right: Fruits, Tinnet (Dec, 2007)

3.31 *Embelia schimperi* Vatke. Myrsinaceae (Sochuonet) (Voucher No. AO/043/NMK/12/12/2008)

3.31.1 Plant description

A Climber or shrub, leaves obvate to lanceolate base cuneate (decurrent); apex acute to shortly acuminate 5-8 (11) by 3-4 (6.5 cm) shiny dark green above paler beneath, slightly fleshy with new leaves sometimes red, margin revolute. Raceme to 5 cm on leafless part of the stem; petals 4-5 usually speckled and sometimes finged pink. Fruits red to purple speckled dry. Altitude 1500-2600 m. Rare and found, only in a few remnant forests.



Plate 3.28: *Embelia schimperi*: Twig; Mariashoni (Dec, 2008)

3.31.2 Usage and dosage of the medicine

Stem bark measuring about 1 Kg when fresh boiled in 1.5 litres of water to one litre concentrate, bottled; 10 mls of the concentrate drunk four times daily indefinitely to cure rheumatic fever, tuberculosis and other chest complaints like pneumonia.

3.32 *Rapanea melanophloeos* (L) Mez Syn. *R. pulchra* Gilg and Schellenb *R. rhododendroides* (L) Mez–Myrsinaceae (Kwarabariet) (Voucher No. AO/044/NMK/04/07/2008)

3.32.1 Plant description

Tree 4–20 m very green bark grey/brown, thick, blood red and granular inside. Young branches purplish, glabrous occasionally hairy, rough with prominent leaf scaly. Leaves sub sessile, clustered at branch ends, elliptic to obvate base cuneate (decurent), apex obtuse to acute, 5 5-11 (17) by 1.7-5 cm glossy above, glabrous often with resin dote steaks. Midrib and petiole usually red when young. Flower clusters usually below on old wood from scaly knobs. Flowers to 5 cm across on stout stalks to 7 mm, white to yellow/green with comparatively large anthers white, Fruits purple, globose, 5 mm. Secondary vegetation widespread in upland forest edges of moorlands.



Plate3. 29: *Rapanea melanophloeos*: Growing branch, Kaptuiget Forest (July 2008)

3.32.2 Usage and dosage of the medicine

3-5 berries chewed and swallowed or the seeds may be pounded together with millet or finger – millet at the rate of 20 seeds to 0.5 kg of the cereal grains, cooked and drunk as medicated gruel to cure removal of intestinal worms. Alternatively 20 g ground berries is mixed with milk and drunk to act as a purgative and antihelminths.

3.33 *Olea hochstetteri* L. (syn. *O. hochstetteri* (Baker) Fiiis and P.S. Green, *O. welwitshii* Knobl.) Oleaceae (Yemdit Murunguyet) (Voucher No. OA/045/NMK/13/09/2008)

3.33.1 Plant description

A tall tree, up to 40 m with steeply ascending branches and a dense crown. Bark smooth grey white. Leaves stiff and occur in opposite pairs to 10 cm long, apex sharply tipped, margin wavy, midrib pale and clear below underside not white, with scales, stalk to 3 cm long. Flowers small and white mostly in heads at the tip of the branchlets. Fruits are oval about 2 cm long. Mechanized lumbering has led to its wanton logging and as a result the reserves in valleys are fast dwindling.



Plate 3.30: *Olea hochstetteri* L, syn. *O. hochstetteri*: Left: Thicket and flowering branch, Right: fruits Mariashoni (Sep, 2008)

3.33.2 Usage and dosage of the medicine

The barks of *Olea hochstetteri* and *Olea africana* (Yemdit) are mixed in the proportions of about one kilogram of each fresh bark, boiled in 5 litres of water allowed to cool. 1 litre of the cooled water extract is given to animal, livestock to cure East Coast and Rift Valley fevers. About 125 ml same extract is given once to humans to cure malaria, general body ache and pneumonia.

3.34: *Olea africana* L (Mill.) P. Green [*O. africana* Mill., *O. chrysophylla* Lamm., *O. klimandschandrlica* Knobl.] Oleaceae (Masaieta) (Voucher No. OA/023/NMK/12/12/2007)

3.34.1 Plant description

Evergreen shrub or tree 3-24 m.; bole often gnarled; bark grey or dark brown longitudinally fissured. Leaves glossy dark green above dull or metallic golden or silvery beneath, leathery (narrowly) elliptic, base cuneate, apex acute or obtuse and often apiculate 2-9 by 0.5-3 cm, glabrous above, densely scaly beneath. Flowers many, in terminal and lateral paniculate cymes, usually shorter than suspending leaf; flowers white or creamy yellow, scented about 2.5-3 mm long. Fruit purple or black, ellipsoid, 0.5-1 cm

long. Found in dry upland evergreen forest (edges, remnants) often associated with *Juniperus*; may be co-dominant; also in woodland on larva flows.



Plate 3.31: *Olea africana*; Left; Close up of ripening fruits, Right; a plant, Mariashoni (Dec, 2007)

3.34.2 Usage and dosage of the medicine

Approximately 150 g of fresh bark peeled, coarsely smashed and boiled in 2.5 litres of water, cooled and decanted; 250 ml of the aliquot drunk twice daily for three days to cure malaria and fever.

3.35 *Olinia rochetiana* A. Juss Oliniaceae (Syn. *O. usambarensis* Gilg). (Kabideleliet) (Voucher No. OA/046/NMK/13/09/2008)

3.35.1 Plant description

Shrub or tree (2-24) m; bark grey, smooth or rough, sometimes flaking; young branches square; young, sometimes petioles red. Leaves elliptic base cuneate apex (cuminated and obtuse), 2-10 by 1-4 cm glabrous or minutely puberulous. Flowers cream fading to pink and red, scented, in round pyramidal cymes to 7.5 cm across; petals 2-5 mm long. Fruit pink or red round 5.10 mm. Cedar forest; also in other types of drier upland forests; also in forest remnants as fire induced thickets. Altitude 1700-3050 m.



Plate 3.32: *Olinia rochetiana* Syn. *O. usambarensis*: Fruiting branch, Kaptuiget Forest (Sep, 2008)

3.35.2 Usage and dosage of the medicine

A handful of young growing leaves at the growth terminals are chewed from the growing tips daily to cure cold coughs and other chest related complaints like pneumonia.

3.36 *Piper umbellatum* L. Piperaceae (Ketagan) (Voucher No. AO/022/NMK/04/07/2007)

3.36.1 Plant description

Soft wooded herb 1-2 m, sometimes subscaudent leaves broadly ovate, base deeply cordate, apex acute, 10-23 cm, glabrous. Flowers white, minute in umbel of spikes 2-8 cm long. Fruit not seen. Rainforest or moist riverine forest at altitudes 1150-2100 m, rarely common.



Plate 3.33: *Piper umbellatum*: Young regenerating plant, Kaptuiget Forest Stream (July, 2007)

3.36.1 Usage and dosage of the medicine

A few (10) pieces 6 cm of the roots chewed once daily to cure bronchitis and general body ailments till recovery.

3.37 *Pittosporum lanatum* Hutch. and Bruce var. *lanatum* (Syn. *P. abyssinicum* Del. Pittosporaceae (Mtabonit) (Voucher No. AO/08/NMK/14/08/2006)

3.37.1 Plant description

Tree 6-18 m, bark ash grey, scaly. Leaves elliptic to slightly obvate, base cuneate, apex obtuse, acute or bluntly acuminate, 5-10 by 1.5–3.5 cm, yellowish–tomentose beneath. Flowers yellow, in short panicles; petals 6-10 mm long. Fruit round 6-8 mm, tomentellous, with style on top, splitting into two. Found in evergreen drier evergreen forest, often in isolated forest clumps. Altitude 2100-2850 m.



Plate 3.34: *Pittosporum lanatum*: Left: a twig. Right: branch. Mariashoni (August, 2006)

3.37.2 Usage and dosage of the medicine

About 1.5 kg of the stem bark boiled in 1½ litre of water then decanted and 250 ml taken once to induce vomiting in malaria and nausea cases.

3.38 *Faurea saligna* Harr. Proteaceae (Mosomboriet) (Voucher No. AO/021/NMK/04/12/2007)

3.38.1 Plant description

Tree about 20 m high, bark grey black deeply fissured. Leaves (narrowly) ovate to elliptic, base cuncate, apex acute, (5) 8-15 by 15-35 (45) cm glabrous or nearly so. Flowers cream with pink calyx in dense spikes, 6–14 cm long, and corolla 15-18 mm long. Fruit small, round very hairy found in Afromontane forests. Endangered rare to find and only a few plants are found in Kaptuiget forest.



Plate 3.35: *Faurea saligna* Branch, Kaptuiget Forest (Dec., 2007)

3.38.2 Usage and dosage of the medicine

Scrapped fresh stem bark about 1 Kg, boiled in 2 litres of water cooled and 250 ml of resultant decoction taken daily as: tonic to restore vitality in males, relive liver conditions, and pneumonia

3.39 *Clematis brachiata* Thumb Syn *C. hirsuta* Guil. and Perr. Ranunculaceae (Piisinda) (Voucher No. AO/020/NMK/04/07/2008)

3.39.1 Plant description

A woody climber, leaves with 5-7 almost circular to ovate, often lobed dull pale green leaflets. Flowers cream or white, about 25 mm diameter, in a narrow panicle, very common in the area's forest edges found in the altitudes of 720-3150 m.



Plate 3.36: *Clematis brachiata* (Syn *C. hirsuta*): Flowering branches, Mariashoni (July, 2007)

3.39.2 Usage and dosage of the medicine

It is used against several ailments. Firstly, about 400 g of the roots boiled in 1 litre of water and the decoction used to cure backache ailments by drinking 250 ml thrice daily indefinitely till recovery, Secondly 800 g of the roots of the plant, mixed with about 3-4 ripe seeds of *S. aculaestrum* and boiled in about two litres of water and one 25 0ml of the resultant extract drunk three times daily till recovery against syphilis and gonorrhoea.

3.40 *Scutia myrtina* (Burm. F) Kurz. Rhamnaceae (Sumbeyet) (Voucher No. AO/047/NMK/04/07/2008)

3.40.1 Plant description

Shrub, often scrambling or tree 1.5-9 m rarely a lianas (to 20 m), bark smooth, grey, corky and fissured when older. Thorns axillary recurved often per node, 2-12 mm. Leaves shiny above, apex rounded and epiculate, emarginated or rarely slightly acuminate, margin entire or somewhat crenulate to dentate in upper two thirds, 2-6 by 1.5-4 cm, glabrous. Flowers greenish white to 2 mm. Fruit to green, red purplish when ripe ovoid, 7-9 mm across. Common in moist and dry parts of the Mau Forest. Survives against predators because of its prickly nature.



Plate 3.37: *Scutia myrtina* left, a thicket and Plate3. 38: right: a flowering twig, Mariashoni, July, 2008

3.40.2 Usage and dosage of the medicine

Fresh bark scrapped and approximately 200 g boiled in 1 litre of water to make tonic drink. About 300g of roots are chopped and combined with other ten plant species in equal proportions to form a cocktail which is decanted and stored. 250 ml taken once daily for 28 days to cure female sterility.

3.41 *Rhamnus prinoides* L'Herit. Rhamnaceae (Kosisito) (Voucher No. AO/048/NMK/04/07/2008)

3.41.1 Plant description

A tree or shrub which grows to medium size (12) m rarely scrambling. Bark grey to chocolate. Leaves shiny above, ovate or elliptic, base rounded or cuneate, apex acuminate, margins glandular. Flowers (yellowish) green in fascicles of 2-3(8), about 2 mm long. Fruit red, turning (purple-) black, 3-lobed, subglobose, 5-6 mm across. Found in forest (edges), less often in secondary bushland or bamboo/heath zone. The populations of the plant species found are only secondary growth.



Plate 3.39: *Rhamnus prinoides*, left mature fruits on branch stem and right, a thicket Mariashoni, (Dec, 2008)

3.41.2 Usage and dosage of the medicine

The roots dug and four pieces amounting 500 gm are boiled in 1½ litres of water then decanted. About 250ml of the decoction mixed with an extract of *Dovyalis abyssinica* roots also prepared separately through the same process. The mixtures of both the decoctions drunk once to cure amoebiasis and bacillary dysentery. Soup of meat may also be used as a vehicle by mixing the decoction with *S. eculeastrum* roots.

3.42 *Prunus africana* (Hook.f.) Kalkm. (Syn. *Pygeum africanum* Hook.f.) Rosaceae (Tendwet) (Voucher No. AO/019/NMK/07/07/2008)

3.42.1 Plant description

Evergreen tree 6-25 (rarely a shrub in lava forest); bark grey-black, corrugated. Leaves often with red petiole and mid rib, ovate, elliptic or obvate base cuneate or rounded, apex obtuse to shortly acuminate, margin crenate-serrate, 4-16 by 1.5-7 cm, glabrous. Flowers white or cream in axillary racemes 3-8 cm long; corolla lobes to 2 mm long. Fruit red to purple-black, ellipsoid, up to 8 by 12 mm. It is found in moist evergreen forest, riverine; often in remnants or on margins.



Plate 3.40: *Prunus africana* Syn. (*Pygeum africanum* :) Plants, Mariashoni, (June, 2007)

3.42.2 Usage and dosage of the medicine

Mature bark, about 500 g cooked in 2 litres of water then the infusion, 250 ml, taken once daily till recovery against prostate cancer.

3.43 *Rubus keniensis* Standl. Rosaceae (Tagaimaiet) (Voucher No. AO/049/NMK/04/07/2008)

3.43.1 Plant description

Scrambling shrub 1-2.5 m; stems reddish, hairy with hooked prickles to 3 mm. leaves with 3 leaflets; leaves ovate or elliptic with serrate margins, the terminal one 8-11 by 4-8 cm, densely greenish hairy beneath flowers white or pink, in loose panicles to 26 cm long; petals 11-15 mm long. Fruit red, ovoid nearly always galled, to 4 cm long. Montane forest margins; also riverine or in secondary forest. Rare to find.



Plate 3.41: *Rubus keniensis*, Mau Summit, July, 2008

3 43.2 Usage and dosage of the medicine

The roots are dug and 200 g boiled in 1.5 litres of water, cooled, decanted and 250 ml taken once against stomach ache.

3.44 *Gallium simense*. Fres. Rubiaceae (Tipsoliet) (Voucher No. AO/018/NMK/04/12/2007)

3 44.1 Plant description

“Sticky” climber with large linear leaves under 3 cm long and small axillary inflorescence, Petals green yellow, smaller. Fruits about less than 3.5 mm. Not very common in forest edges in the Afromontane zones.



Plate 3.42: *Gallium simense* above, on a dead tree trunk and below, in lianas, Mariashoni, see the arrow Dec, 2007

3.44.2 Usage and dosage of the medicine

About 500 g of the leaves of the plant pounded and then soaked in 1 litre of water. After about 30 minutes of soaking the mixture used to bathe cancerous areas of the body and the infusion also used to cure general body rashes.

3.45 *Toddalia asiatica* (L) Lam. Rutaceae (Chepindoruet) (Voucher No. AO/017/NMK/04/07/2007)

3.45.1 Plant description

Semi-climber, spiny, stem green when young turning yellowish-brown, leaves green, palmately compound; leaves found in clusters. Flowers greenish yellow orange fruits when ripe in axillary and terminal panicles; petals 2-3 mm long. Commonly distributed in forest margin or secondary regrowth and grassland thickets, 450 m coast and 1200-3000 m has profile growth at high altitudes.



Plate3.43: *Toddalia asiatica*: left, Thicket, right, fruiting branch; Kaptuiget Forest (July, 2007)

3.45.2 Usage and dosage of the medicine

The roots chewed to cure various body ailments ranging from malaria, stomachache and sore throat. A handful of the roots boiled in 1 litre of water and the decoction taken as tonic against harsh environmental conditions.

3.46 *Zanthoxylum chalybeum* Engelm. Rutaceae (Kikomit) (Voucher No. AO/010/NMK/01/2/2006)

3.46.1 Plant description

A medium tree in savanna up to 10 m high stems and branches, armed with woody spines. Due to careless harvesting the plant is endangered only a few shrubs remain.



Plate 3.44: *Zanthoxylum chalybeum*: Left; regenerating plant, Right; Fruiting branch, Tinet (Dec, 2006)

3.46.2 Usage and dosage of the medicine

It has several medicinal uses which include: (a) the branches are used as tooth brushes and mouth freshener (b) in combination of *Carissa edulis* Forsk. Vahl. *Withania somnifera* (L) Dunal. a handful of the roots of each mixed boiled extract cooled and glassful drunk to cure chest pains and stomach problems and (c) the same mixture put in a pot and about 5 litres of water added tightly sealed boiled, and used in steam bath to treat diseases such as anthrax and measles.

3.47 *Solanum mauense* Bitter. Solanaceae (Labotuet) (Voucher No. AO/011/NMK/12/12/2006)

3.47.1 Plant description

An erect hairy or rarely almost hairless woody herb or shrub, with ovate leaves mostly angular leaves, unlobed; grows up to 2 m tall. Found in wastelands in the lower parts of the Afromontane forests.



Plate 3.45: *Solanum mauense*, Kaptuiget Forest, Dec, 2006

3.47.2 Usage and dosage of the medicine

Produces small orange seeds, when ripe, which dried and ground into fine powder. About a 100 g put into boiling water 250 ml then decanted. 10 ml. decoction liquid drunk by adults while 5 ml is drunk by children, once to expel worms and to cure chest ailments and act as a purgative.

3.48 *Solanum aculaestrum* Dunal. Solanaceae. (Sigowet) (Voucher No. AO/01/NMK/06/07/2006)

3.48.1 Plant description

A large shrub approaching a small tree with soft white hairy covering and coarse nearly unhooked prickles on stems and undersides of leaves; leaves broad-elliptic, with pointed lobes, becoming hairless and glossy above; flowers white to mauve, few, in lateral

umbel-like cymes about 22 mm diameter; petals almost free, oblong; fruit. It is found in upland forest edges and clearings, especially in the Mau Summit at altitudes ranging between 1760-2635 m. Currently restricted to roadsides where it provides fence to cultivated land.



Plate 3.46: *Solanum aculeastrum*, Nessuit, June, 2006

3.48.2 Usage and dosage of the medicine

The plant preparation is used for various ailments amongst the community. The ripe fruits are used in various ill health conditions; both in domestic animals and human. (a) in cattle, a few ripe fruits, about ten are squeezed into 3 litres of hot water, cooled decanted and 1 litre of the decoction is given to sick animal 3 times daily for five days to cure Rift Valley and East Coast fevers.(b) branches of the stem are used as tooth –brush and are known to cure tooth ache. (c) ripe fruits are squeezed into a tablespoonful (10 ml) and the resultant juice taken once to cure nusea fever by inducing vomiting. (d) ripe fruits are cut and the juice squeezed once daily after bathing on areas affected by what is believed to be osteomyelitis till recovery.

3.49 *Solanum nakurense* Bitter. Solanaceae (Tiliet) (Voucher No. AO/01/NMK/10/12/2006)

3.49.1 Plant description

An erect unarmed herb or shrub from trailing woody rootstock, stems with many raised lenticels; leaves broad-elliptic, entire, with pointed apex and wedge shaped base, often 5cm long, often pale-hairy below; flowers white to pale blue, 12-15 mm diameter, in subterminal umbels; fruits red or orange, 6-8 mm diameter.

3.49.2 Usage and dosage of the medicine

Ripe fruits juice numbering about 2, are squeezed into the ears of human daily to cure otitis media till recovery.



Plate 3.47: *Solanum nakurense*, a climber stem on a dead wood, Nessuit, Dec, 2005

3.50 *Dombeya goetzenii* Gerrad. Streculiaceae. (Silibuet) (Voucher No. AO/013/NMK/01/12/2006)

3.50.1 Plant description

Deciduous tree up to more than 10 m high, the crown rounded. Bark grey, smooth. Leaves heart shaped like in all *Dombeya* spp; the leaf base overlapping to 30 cm long, vein network very clear below hairy, flowers pale pink or white, with red centres full of nectar in showy clusters on branched hairy stalk to 30 cm. Fruit small capsule, densely

hairy about 10 small seeds; found in altitudes of 1850 – 2700 m. Endangered and found only in secondary growths or where tree felling has been restricted.



Plate 3.48: *Dombeya goetzenii*: left and right flowering plant Nessuit, Right, flowering, Marioshoni below, thicket dominated by the species, Dec, 2006

3.50.2 Usage and dosage of the medicine

The plant is highly prized in the Ogiek community since it provides source of nectar to the foraging bees. Honey is considered a staple food in this community. The 200 gm bark is mixed with the leaves of *Senna didymobotrya* (Senetuet), 300 g crushed and boiled in 1 litre of water. The resultant extract cooled and one 250 ml drunk three times daily for three days against chest problems.

3.51 *Urtica massaica* Mildbr. Urticaceae (Ila Ila) (Voucher No. AO/02/NMK/01/12/2006)

3.51.1 Plant description

An erect herb from rhizome, with ovate, closely doubly toothed leaves 6(-8) cm wide; inflorescence of axillary groups of spike-like, unbranched racemes; male and female flowers on separate plants. A painful stinger, often growing on abandoned tracks in Montane forest areas, and often associated with buffaloes. However, there were no buffaloes in the vicinity.



Plate 3.49: *Urtica massaica*, Mariashoni, Dec, 2005

3.51.2 Usage and dosage of the medicine

Leaves are cooked and eaten as vegetables. The seeds are used as grain cereals in times of famine. Plants are cut and placed on sting line to form two rows; the boys who are to be circumcised run through it several till the whole body is numb and ready for the ritual.

3.52 *Pteris pteridioides* (Hook) F. Ballard Vittaraceae (Voucher No. AO/014/NMK/01/06/2006)

3.52.1 Plant description

Fronds 1-2 m long with the middle one of the three divisions somewhat longer than the lateral ones; sori short (0.25-0.5x length of lobe), medial on the pinnule lobes. Dense shade in moist, intermediate to Montane forests, uncommon east of the Rift Valley.



Plate 3.50: *Pteris pteridioides*: left and right, Fronds, Nessuit, June 2006

5.52.2 Usage and dosage of the medicine

Fresh roots of young plants are dug, washed and about 30 g chewed, the sap from it swallowed once to cure stomachache.

3.53 *Lippia javanica* (Burm.f.) Spreng. Verbenaceae. (Mwokyot) (Voucher No. AO/015/NMK/06/12/2007)

3.53.1 Plant description

Shrub 0.5-3 m leaves opposite (rarely in threes), aromatic ovate or elliptic, base cuneate, apex acute margin crenate, 2-8 by 0.6-3 cm, sandpapery above pubescent beneath. Flowers white or cream with yellow throat, in short-peduncled (rarely long-stalked) crowded spikes 0.5-1cm long; corolla tube about 2 mm long. Locally abundant in secondary bushland or grassland; found abundantly in Mau wastelands.



Plate 3.51: *Lippia javanica* Plants, Mariosioni, Dec, 2007

3.53.2 Usage and dosage of the medicine

About 50 grams of fresh leaves a bunch carefully wrapped around fresh wound to enhance healing.

3.54 *Engleromyces goetzii* P. Hennings. Xylaraiceae (Puindaii) (Voucher No. AO/050/NMK/07/12/2009)

3.54.1 Plant description

Thrives in substrate. Stomata distributed above the ground and beneath perithecia; stomata layers unipartite, stomatal surface level; erumpent or superficial. Stomata interior homogeneous; granules surrounding perithecia missing, potassium hydroxide missing, ascomatal ostioles present, ascomatal configuration not valsoid, ascomatal orientating mostly vertical; ascus ring present. Rare to find due to fast receding Bam vegetation.



Plate 3.52: mycelia of *Engleromyces goetzii*. Courtesy of D. Ndegwa, 2009

3.54.2 Usage and dosage of the medicine

200gm of the fungi is boiled in about one litre of water over a long period of time. The extract is cooled decanted and about 250 ml drunk as a purgative and against malaria. If taken in low doses of about 20 ml once it will malaria, and cure bacillary dysentery and amoebiasis by acting as a purgative.

3.55 Discussion

It was possible to document the identities of 49 plant species. A systematic topographical record, coupled with the geographical location and time of collection was also included. For instance, the order Campanulales has two major families, Campanulaceae and Asteraceae. The latter has the largest genera from which plants have been found that are

useful in the provision of medication as is the case with *Artemisia annua*. Asteraceae contains a wide variety of chemical constituents, one of which is inositol (Rajan *et al.*, 2002). The Ogiek community uses *Vernonia lasiopus* in the treatment of malaria and hepatitis, both of which remain a worldwide problem (Paulo *et al.*, 1994). In the same family there have been observations which suggest that chimpanzees use *Vernonia amygdalina* as a remedy against dysentery related cases (Huffmann *et al.*, 2000).

Being forest dwellers, the Ogiek must have observed the animals using the plants for remedies similar to humans, thereby applying this knowledge for similar human ailments. Based on ethnology, it has been adduced that certain plants from the family Asteraceae in East Africa, like *Aspilia mossambicensis* (Ofulla, 1996), are effective against malaria. *Cladosporium ssp.* (Sofora, 1993; Okeke *et al.*, 2005) from the same family, Asteraceae, is also effective against malaria parasites. From the genus *Vernonia* it was established to possess antibacterial characteristics, especially *V. amygdalina* Del. whose stem branches are used by chewing in West Africa (Sofora, 1993).

The Ogiek community also uses *Gomphorcapus semilunatus* (Asclepadiaceae) against sexually transmitted infections. Other plants from the same family such as *Calotropis procera* (Ait.Ait.f.) are known to have bacteria lytic substances (Aslam, 2002). Possibly, this is why *G. semilunatus* from the same family is claimed by the community to treat gonorrhoea and syphilis. Other communities elsewhere in the world likewise reported to use plant species that are members of this family, but have since shifted from Asclepadiaceae to Apocynaceae (Okeke *et al.*, 2005). Some of these plant species such as *Adhadota vasica* Lindau and *Alstonia scholaris* respectively yield natural organic compounds which have since been used in allopathic medicine. They have been traditionally used in Ayurvedic, to treat thrombopoietic tumors and cancer (Rajan *et al.*,

2002). Some root extracts from *Rauwolfia vomitoria* still remain the treatment of mentally ill patients in USA (Marston *et al.*, 1993). Till recently, *Cartharanthus roseus* was the sole source of vincristine and vinblastine, which remain the drugs of choice for leukemia, lymphomas in children and caposis sarcoma in adults (Dev, 1989).

Balsaminaceae Family has one plant, *Impatiens tinctoria*, whose roots are chewed or soaked in water, (Table 3.1), by mothers in labour to enhance baby safety and faster delivery in the Ogiek community. There is no mention of this plant or its relatives as source of medicine anywhere in literature except as bedding plants in ornamental horticulture (Fish, 2004). Certain plants may be used both as pot herb vegetables and as medicines (Kokwaro *et al.*, 1998). One such plant having multiple uses in the Ogiek community is *Basella alba* (Basellaceae). Its medicinal value amongst the people is more pronounced (3.3 and 3.5), as compared to its value as a vegetable. When mixed with other plant species, the concoction is used in veterinary gynecology to enhance expulsion of the uterus after calf delivery in cows.

There are certain plants that are used in the Ogiek community whose medicinal value only became explicit towards the end of the interviews. These include Carusselaceae *Cotyledon barbeyi* Scheinf whose leaves are heated before they are rubbed on the body as remedy for body pains by the Maasai (Beentje, 1994). From the same plant family, the Ogiek community uses the genus *Kalanchoe densiflora* in the same manner. In allopathic medicine, an extract of bufadienolides obtained from Carusselaceae (*Kalanchoe pinnata*) L. and the toad venom are also used as expectorant in human treatment (Houghton, 2002). The fruits from the order Curcubutales also have several uses in some communities within East Africa. For example, the fruit may be used in making containers and utensils (Kokwaro, 1998). *Curcubita moschata* is used by the Ogiek as an important

vegetable. Both its fruits and leaves are cooked and eaten. The Ogiek also cut *Zehneria scabbra*'s stem so the sap is squeezed onto fresh wounds to enhance healing. There are other members of the family whose fruits are taken elsewhere as table dessert, as in the case of cucumber and watermelon (Pursglove, 2004). Among the Ogiek, *L. spherica* is externally used for skin conditions ranging from scabies, leprosy, to *candidiasis*. Elsewhere, most of the extracts from the family have been used as antitumours (Houghton, 2002). There are also instances where the Cucurbitaceae have been reported as being highly toxic to humans and to higher animals (Kokwaro, 1993). The management of diabetes, which some people claim to treat, in Ayurvedic treatment was achieved through the use of *Mormodica charantia* (Rajan *et al.*, 2002). There are some members of this genus like *Mormodica reticulate* that are capable of accumulating selenium which is highly and fatally toxic although other members of the genus like *M. citrifolia*, *M. elleptica* and *M. lucida* have been used in folklore medicine as antifungals. There is also the mention of *Kedrostis feotidissima* L as having antiviral properties. The species is used in the treatment of small pox (Kokwaro, 1993). Information regarding other medicinal use of the plant is however scanty as there is no laboratory documentation with regard to its efficacy as an antiviral.

Dracaenaceae family is a relatively small family but has resins, which produce medicinally useful steroids (Mills, 2002). The Ogiek community use *Dracaena afromontana* in the treatment of enlarged spleen in children. Stems of the same plant are also used as toothbrush. The family Euphorbiaceae has plants which possess several general ethnopharmacological values in the Ogiek community. For instance, *Clutia robusta* roots are used as tonic when cooked along with meat by the Ogiek. Most members of this family produce latex, which is known to be carcinogenic. Unfortunately, *in vitro* studies carried out to establish their efficacy against selected bacteria; it was

revealed that the responses were too low to justify their continued use (Akinyemi *et al.*, 2005). Although it may be argued that *in vitro* studies do not necessarily translate into the same thing as *in vivo* studies. There are also records of the family members being used in traditional medicine in South East Asia or India for various physiological disorders (Rajan *et al.*, 2002). Most of these species were also verified to possess antidiabetic activities.

Fabaceae is an important source of grain legumes for the supply of proteins to human beings. Besides being medicinal and source of grain legumes, the plant is also capable of fixing molecular nitrogen and making it available for plant uptake as an essential nutrient, thus increasing soil fertility. The medicinal value of the family members dates back past the medieval ages. They were listed as medicinal as early as the Egyptian civilization. *Senna acustifolia*, Del. and *Cassia angustifolia* Vahl have in their leaves and seeds medicinal components (Houghton, 2002).

From antiquity to date, there are certain plants that have been used in Ayurvedic and Unani medical practices but are poisonous when ingested. Such plants include *Abrus precatorious*, Papilionaceae whose seeds may lead to blindness when ingested (Kokwaro, 1993). In Ayurvedic and Unani medicine, the leaves are used in the treatment of syphilis, coughs and gonorrhoea (Dewick, 2002). Uses of other species that range from antifungal, antibacterial and laxative and more recently their antitussive activities have also been demonstrated (Newton *et al.*, 2000). The Ogiek use several species that have been reported as having activities against several ailments and conditions, some of which are physiological. Other conditions that are treated by preparations from other species of the family range from anti helmentic to antiprotozoal activities (Kokwaro, 1993). Antifungal activities have also been reported. For example, the Ogiek use the leaves from *Senna*

didymobotrya against malaria, stomach pains, and as bathe against fungal infections on the skin.

Flacourtaiceae family is known to yield such metabolites which have bacteriocidal effects as in the case of the seed extracts of *Hydnocarpus ssp* that have been known to have bacteria effects on *Micrococcus ssp* of leprosy (Kochhar, 1989). Similarly, the Ogiek community derives several benefits from *Dovyalis abyssinica* whose fruits are picked when ripe and eaten, while the roots are cooked along with mutton to yield a tonic soup. It is believed that decoction of the roots treats gonorrhoea too and also serves as a tonic against harsh environmental conditions.

The Ogiek also use an infusion made from the bark of *Garcinia buchananni* to treat stomach ailments in children. From elsewhere, the medicinal values of some of these plants have also been documented. In West Africa for instance, *Garcinia cola* yields nuts, the branches are used as chewing stick, while when boiled, the bark is used to treat stomach ailments (Atindehou *et al.*, 2002). In Thailand and South East Asia, *G. mangostana* produces the bark used to treat various infections since its ethanolic extract is active against MRSA (Voravuthikunchai and Kitpitpit, 2005). In East Africa, the *G. livingstonei* infusion from the roots is mixed with milk and drunk by women for pains in the abdomen during pregnancy or shortly after birth (Kokwaro, 1993).

Lamiaceae family has several therapeutic uses to the Ogiek. They may be used as tonic drinks, as antiseptic, as tea leaves, as anti cough, and as remedy against stomach ache and emetics. In other cases, they have been used as culinary, as source of volatile oils, and as anti-insect growth hormones (Dev, 1989; Kokwaro, 1993). *Ajuga remota* is highly medicinal for various ailments, malaria being one of them. *Mentha peparita* and

Basilcum spp. are used in confectionery and in cookery. Among the Ogiek, the plant uses range from antibiotic to culinary. For example, *Satureia biflora* roots are used in the treatment of upper respiratory tract ailments. Extracts from the same genera are currently being incorporated into Vaseline. They have also been used in hot fomentation to alleviate cold infections. *Hoslundia opposita* leaves are used in tea making by the Kipsigis of Kenya (Kokwaro, 1993) the Ogiek also use its leaves for a similar purpose. Being hunters, they use certain species in cases of accidental injuries. The leaves of *Lippia javanica* are used to wrap fresh wounds to enhance healing.

Although nearly extinct as a result of exploitation for its prized timber, the bark of *Ekabergia capensis* Sparm is effective antiseptic/antibiotic to communities in South Africa and the surrounding regions (Samie *et al.*, 2005). Among the Ogiek, the decoction from the bark is used against dysentery. Recently, it has also been found to manage HIV related dysentery. The community also uses the extracts of *E. capensis* in cases of pneumonia and against other stomach related problems.

Meliantaceae family is less mentioned in the literature except for having extracts with cardiovascular activities (Williamson, 2002). The decoctions from the leaves of *Bersama abyssinica* are used as emetic in animals. The decoction from the bark is given to animals with East Coast fever and those bearing symptoms similar to Brucellosis. The same decoction maybe used against general body pains, chest pains and stomach problems in humans by the community.

In the literature, certain plants have been found to bear high medicinal value. For instance, *Embelia ribes* and *Embelia tsejram cottam* from the Myrsinaceae family, have been proved effective against tapeworms, chest infections, skin ailments, cholera,

diarrhea, weak pulse, pleurisy, infertility and pneumonia (Mills, 2002; Rajan *et al.*, 2002). Among the Ogiek, it was found that *Embelia schimperi* (a species from the same genera) is used in the treatment of several conditions such as gastroenteritis, rheumatics, fever, tuberculosis and other chest related problems. The community uses seeds of *Rapanea melanophloeos* L. Mez. that are pounded with finger millet, cooked or ground, then drunk as purgative or as antihelmintic.

The family Oleaceae is also ranked high as a source of salad oil particularly by several communities in the Mediterranean regions. *Olea hochestetteri* is widely used in salad and parenteral preparations due to its low acid level. It is also used in preparations like plaster soap that may require low peroxide level and no water. Within the Ogiek community, it is used for the treatment of animals suffering from east coast fever, and against malaria and fever in human beings. *O. africana* and *O. hochestetteri* are the two main species used as medicine in the community for the treatment of malaria and fever (Tables 3.4, 3.5 and articles. 3.34 and 3.35), the bark from the plant is quite exploited which partly explains why the species are endangered.

There is a dearth of current literature on Oliniaceae. Nonetheless, the Ogiek use the bark as well as freshly forming leaves for chewing as toothbrush/mouth freshener and against pneumonia. The Maasai also use the decoction from the bark against coughs (Beentje, 1994). Williamson's (2002) taxonomic approach to the study of medicinal plants and animals enlists a host of species from Piperaceae that are used as medicine but which are now obsolescent. In South Pacific Island, aqueous extract of the roots of *Piper methysticum* is consumed as a ritual stimulant. However, large doses of this may cause intoxication (Williamson, 2002). In Phytomedicine, the root of the plant is used as a diuretic, as stimulant and as tonic. During the survey, it emerged that the Ogiek wash the

roots of *Piper umbellatum* (L) and chews a handful daily against bronchitis and general body ailments.

From the family Pittosporaceae is found *Pittosporum lanatum* whose bark is boiled and 250 ml of the decoction taken once to induce vomiting in cases of nauseating conditions particularly when one suspects to be having malaria. The Kipsigis use the infusion of the bark as a purgative, emetic and antimalarial (Beentje, 1994); a use almost similar with the Ogiek to induce vomiting when someone is nauseated or has fever.

With about 62 genera and about 1050 species, the Protaceae family is well represented in Australia, New Zealand and South Africa. However, the family is sparingly mentioned in pharmacognosy. In East Africa though, there are certain uses worth mentioning; the roots of *Faurea saligna* are used against indigestion (Kokwaro, 1993), the bark decoction is used as a tonic, while the timber is used for furniture. The stembark of *Faurea saligna* is used by the community as tonic and against ailments such as respiratory tract infections. Currently there is an over exploitation of the species.

Members of the Ranunculaceae family have also been used in herbal medicine in the past. *Cimicifuga racemosa* is now used in menopausal and other female disorders as well as against various rheumatic conditions (Mills, 2002) while *C. simplex* is used in Chinese medicine (Mills, 2002). In East Africa, the Ogiek use the root decoction of *Clematis hirsuta* Fresen against malaria and diarrhea (Beentje, 1994). The Ogiek use freshly collected stembark mixed with seed of *S. eculeastrum* to prepare a decoction, which is taken at the rate of 125 ml twice daily to cure syphilis and/ or gonorrhoea

Members of the Rosaceae family have been used either as fruits for table dessert or ornamentals and as medicine. Oil from rose (*Rosa damascena*, *Prunus Africana*) is used in perfumery and in the preparation of rose waters whose decoction of the stem bark is used against prostatitis (Beentje, 1994). There are several other species that provide table fruits. In the Ogiek communities, a decoction of the stem bark of *Prunus africana* is used by the community against malaria. In combination with *Gallium sinensis* and *Senna didymobotrya*, the decoction is used in the treatment of urinogenital problems. The aforementioned preparation is taken for an unspecified period of time till the ailment ceases. Besides being bee forage at flowering, and fruits, which are gathered and eaten by the community, *Rubus keniensis* roots are collected and boiled; the resultant decoction is taken against stomachache and food poisoning.

In the Rhamnaceae family, the genera of *Rhamnus*, *Scutia* and *Zizyphus* are widely distributed in East Africa (Beentje, 1994). Some of the species are of medicinal importance as they produce important metabolites and have also been used for treatment in various parts of the world. In the new world, *Rhamnus purshiana* (Rhamnaceae) yields active cascariosides (Dev, 1989). Reports from China confirm that *Zizyphus jujuba* yields edible fruits and are used as mild sedative in Chinese medicine (Williamson, 2002). Within the Ogiek community, *Rhamnus prinoides* is used in the treatment of amoebic dysentery. The roots are chopped, boiled, after which the decoction is taken at the rate of one glass (250 ml) daily till recovery. When the roots are cooked with meat, the soups are known to cure rheumatism. The soup may also be taken as tonic. The stem bark of *Scutia myrtina* is scraped, boiled, decanted, and then the portion is taken as tonic by the community. Most of the respondents indicated that they use it as a substitute for tea leaves.

Rubiaceae are mainly tropical trees and shrubs. A few members are herbs growing in temperate areas like *Gallium spp.* The former is known for its medicinal value while the latter (*Coffea spp.*) yields a beverage. The medicament from *Cinchona spp.* yields hallucinogens, painkillers, as well as the famous quinine. *Rubia* and *Gallium* are used as dyes. The genus *Uncaria* is used for various complaints in the far eastern Asia in Sumatra. *U. ionosa* is used in food poisoning; *U. rynchophylla* against epilepsy, while when dried and powdered the leaves from *U. guianensis* are used against dysentery and in the healing of wounds (Laus, 2004). There are also several species in this genera found in the Chinese pharmacopeia for centuries, suggesting that a good number of them are currently being used in Chinese folklore medicine. In the Ogiek community, *Gallium simense* Fres. is used to treat prostate gland hypertrophy and benign prostate hyperplasia. As is evident from 3.4.40, the whole plant is pounded fresh. The resultant pasties are then applied on cancerous wounds so they are covered till recovery. The aforementioned uses in the Far Eastern and amongst the Ogiek would suggest that *G. simense* could be a good subject for physiological dysfunction studies like cancer.

The family Rutaceae with about 150 genera and 900 species is mainly made up of shrubs and trees. Members of this family are distributed in both tropical and temperate countries; but more so in South Africa and in Australia. Oil glands are present in the leaves and other parts. There are various types of fruits especially in the orange sub-family, *Hesperidium spp.* has mainly been used in traditional medicine. Each of the genuses is represented by more than one species except *Toddalia*. A majority of these have been used in traditional medicine. Their uses range in several ailments from immunomodulatory as in the case of *Aegle mermolos* anti-rheumatic *Zanthoxylum-clava-herculis* (Mitcher *et al.*, 1975; Williamson, 2002) and *Murraya pariculata* as anti implantation (Partwadhan *et al.*, 2002). *Toddalia asiatica*, a prickly climber or shrub, is

used for several ailments ranging from malaria, coughs, stomach-ache to skin rashes as an important remedy from the decoction from root preparations in several communities in East Africa. In the Ogiek community, it is used as a tonic. In India *Toddalia* is used as textile dye for its yellowish shade (Rajan *et al.*, 2002). The Ogiek community also uses the stem bark *Zanthoxylum chalebeum* and leaves as milk sweetener.

The family Solanaceae, *S. eculeastrum*, is used by the community to a large extent in combination with other drugs to manage animal ill health. Its use in combination with *B. abyssinica* summed up to 100%. *S. eculeastrum* roots are also used in milk preparation for preservation. There were also two other species from the Family that are extensively used by the community. The uses were predominantly against bacterial infections. These were: *S. nakuruense* and *S. mauense*. There is very limited mention of the genus as medicines elsewhere in literature.

Cocoa and cola drinks, which are famous worldwide, are products of *Theobroma cacao*, *Cola vera* and *C. acuminata*. There are several genera in this family that are reportedly used in medicine in Tanzania (Chhabra *et al.*, 1993). It was reported that the genus *Dombeya* alone has six species that are known for their medicinal value in Northern Tanzania (Kokwaro, 1993). *Dombeya burgesseae* Gerr that is one of the species is used against malaria. A decoction prepared from the leaves of *Dombeya cincnaa* K. Schum together with the root decoction is used against vomiting, chest pains and dysmenorrhoea. Root decoctions from *Dombeya shupangae* K. Shum, *Dombeya rotundoka* Hochst Sun and *Dombeya reticulata* are rubbed on the whole body to rid it of evil effects from witchcraft. The stem bark is used against rheumatism, diarrhea, colic and abdominal pains (Kokwaro, 1993). There are other species which are used in Northern Tanzania like *Melhania volutmia* Forsk and several species of the genus *Sterculia* and *Waltheria*

(Chhabra *et al.*, 1993). The Ogiek use the bark of *Dombeya goetzenii* Gill in combination with the leaves of *Senna didymobotrya* at the rate of 1 kg of each boiled in about 3 litres of water, decanted, after which 200 ml is taken once daily for one week to cure pneumonia, coughs and colds. The stem bark of the plant is also used in making ropes and dyes for fabrics.

The Urticaceae family-stinging nettle is able to alleviate prostatitis (Mitcher *et al.*, 1987; William, 2002). The plant *Urtica massaica* is used in circumcision by several communities living with and around the Ogiek. There are incidences of the plant being used as a vegetable and the seed eaten as grain during famines. The community in a combination of *Prunus africana* uses the plant to treat urine blockage.

Although some members of the family Vittaraceae are used in traditional medicine, they are rarely mentioned in. Most of the information available on usage is not found in scientific research materials. However, in the Ogiek community, *Pteris catoptera* roots are used in the treatment of gonorrhoea and some stomach ailments. The Chinese use it as an immune modulator (Li *et al.*, 2004).

Xylaraiceae is the most unique family since it is a saprophyte that thrives in substrate. The Ogiek use the bracket fungi against malaria and inducement for vomiting. In China, the plant is used in traditional medicine and is indicated as a plant which contains noble natural organic compounds (Hyde *et al.*, 2002). Amongst the Mbeere of Kenya, the plant is used against malaria (Kareru *et al.*, 2007).

CHAPTER FOUR

ANTIMICROBIAL ACTIVITY OF CRUDE METHANOL EXTRACTS AND ESSENTIAL OILS

4.2 Materials and methods used in the evaluation of the crude extracts

In the preparation of plant materials, there were three separate reconstitutions: Methanol extracts, essential oils, and sequential extractions. Specified portions of the individual plants were collected, and dried at room temperature under shade till all the moisture was finished, milled to pass through a sieve of 0.50 mm diameter. The powder, of each sample, was hermetically sealed in polythene bags, and stored till the time of use if not extracted immediately.

4.2.1 Methanol extraction

The plant powders from the 49 plants were individually extracted with methanol while fresh leaves were steam distilled. Some powdered material (50 g) were weighed into conical flasks (250 ml), covered with aluminium foil and then filled with about 200 ml of methanol. This was allowed to stand for 24 hrs.

The solution was then decanted and filtered through whatman filter paper No 1. The filtrate was transferred into aluminium-foiled beakers and allowed to evaporate at room temperature to dryness. Various forms of residues were obtained as crude extracts. The resultant materials were put into individual vials labelled and stored in the refrigerator at 4 C for future use.

4.2.2 Steam distillation

This method was meant to extract essential oils from plants that were identified to be containing the substances. Fresh leaves of *L javanica* Burm. F. and *S. biflora* D. Don,

Benth, Speng. A 2 kg of each of the plants were separately steam distilled through hydrodistillation using Cleveland apparatus until no more of the oil was recoverable from the samples. Two yellowish substances obtained from each were put into separate vials, tightly corked, sealed with parafilm and frozen at 4⁰C in a dark referigerator for future use. The same procedure of extraction used in *L. javanica* and *S. biflora* was further applied in the extractions of fruits and leaves of *T. asiatica*

4.3 Sequential extraction

Sequential extractions of various selected plant species part which had shown antibacterial and antifungal activities. Some powdered material (50 g) were weighed into conical flasks (250 ml), covered with aluminium foil and then filled with about 250ml of petroleum ether. This was allowed to stand for 24 hrs. The solution was then decanted and filtered through whatman filter paper No 1. The aliquot was evaporated us rotor evaporator to dryness. The filtrate was further extracted with ethyl acetate in the same way and the process repeated on the residue with methanol. In further extractions plants that were further extractions each of the extracts was put into individual vials tightly corked, labeled and stored in darkness in 4⁰C for future use. The accuracy was checked against known concentrations of Gentamycin for bacteria and fluconazole for fungi.

4.3.1 Reconstitution of the test samples

Each of 0.1g individually dried material was dissolved in about four drops of dimethyl sulphoxide (DMSO), so as to make it absorbable by the test organisms, and topped up to 1ml. of water. Discs made from Whatman filter paper no. 1 and measuring 6mm in diameter, previously sterilized, and were directly soaked in the extract the solution, removed, desiccated in Silica gel and later placed onto the Plate. With the help a micro-syringe, required amounts of Essential oils were also individually loaded onto the

sterilized paper discs at the rate of 0.1ml. in the case of methanol extracts (Elgayyar *et al.*, 2000).

4.3.2 Screening for antimicrobial activity

Antimicrobial efficacies were tested using the filter paper disc diffusion method (Elgayyar *et al.*, 2000). A solution of each was prepared by dissolving 200 mg in 1 ml of methanol and 10 μ l of the solution were dispensed onto 6 mm sterile filter paper discs and dried (2 mg/disc). The Muller-Hinton and Potato Dextrose Agar (PDA) were used in the culture of bacteria and fungi, respectively.

Each Plate media Plate was inoculated seeded with 0.1 ml of bacterial and yeast culture directly from the 24 hr broth culture diluted to match 0.5 and 1.0 McFarland's standard, respectively (10^8 Colony Forming Units (CFU)/ ml) and fungi diluted to match 1.0 McFarland standard (10^8 spores/ml). The discs loaded with the extracts were then placed onto the seeded media and incubated at 37°C for 24hrs, and at 25°C for 5 days for fungi. After the incubation period the zones of inhibition were measured and recorded in mm as described by (Elgayyar *et al.*, 2000). Negative control Plates had discs with sterile methanol. The plants which were found to posses bioactivities were further subjected to screening to ascertain their zones of inhibition at various concentrations. antimicrobial sensitivity and resistance were confirmed by use of standard discs containing ampicillin (10 μ g), chloramphenicol (30 μ g), erythromycin (15 μ g), gentamycin (10 μ g), ciprofloxacin (10 μ g), tetracycline (30 μ g), amikacin (30 μ g) and an additional oxacilin (1 μ g) for *S. aureus* (oxoid, London). *Candida albicans*, *Candida brusei*, *Cryptococcus neoformas*, *Trichophyton mentagrophytes* and *Microsporium gypseum* were incubated at 25°C for a period of five days to ascertain the activities of the extracts their standards for fungi were

discs containing fluconazole. Broths without any treatments and inoculations were taken as positive controls (Barry, 1980).

4.4 Results and discussion

4.4.1 Methanol extracts

Of the 49 plant species only 16 showed activities against both bacteria and fungi. Using a ruler the zones of inhibition were measured across each petri dish. The activities were indicated as: no inhibition (6mm), slight (8 mm) inhibition, moderate (>9 but 15 mm) and high (>16mm).

From the pathogenic bacteria tested, *Salmonella* spp. exhibited resistance to the methanol extracts of *B. abyssinica*. So were the strains of the sub-cultures of the pathogens listed herein: *Salmonella* (*S. mim*), *S. typhi*, *S. Group D* (Clinical isolates) *S. typhi* types and *R. colidale* amp, Similar results were obtained from methanol extracts from *Bersama* stem bark extracts with the strains of: *E. coli* (0125/B15), *E. coli* (086), *E.coli* (0125 K70), *E. coli* (0127/8), *E. coli* (D126/B16), and *E. coli* (087/B7). However, several extracts from various species of plants were found to be effective against microbial pathogens (Table 4.1).

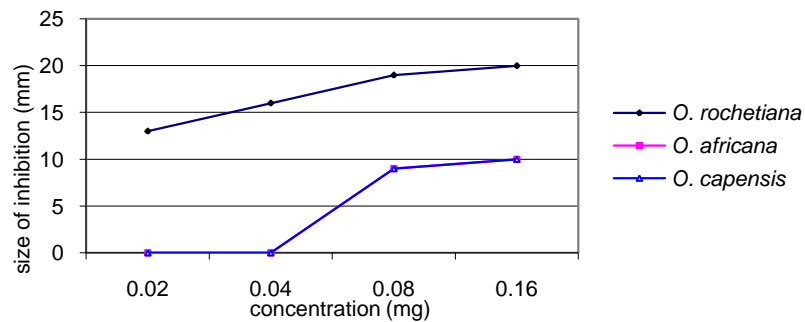
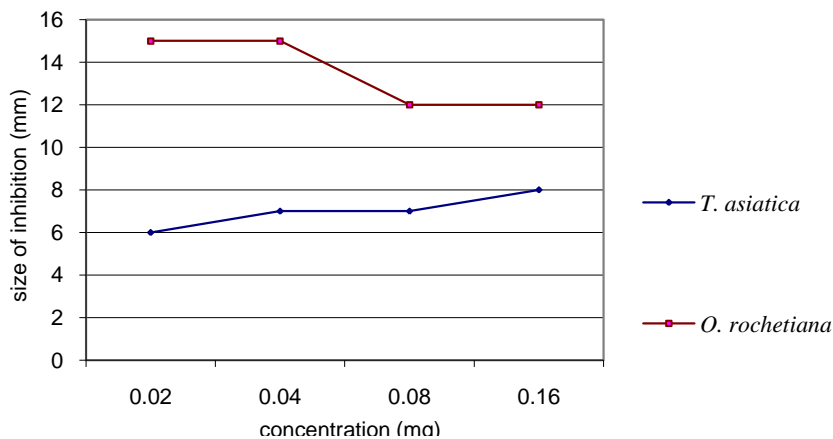
F. saligna and *B. abyssinica* showed antimicrobial activities across the entire test organisms. There were also varied inhibitions from one organism to the including the fungi (Table 4.1). The plants of methanol extracts that processed antimicrobial properties were further subjected to the same organisms but at different serial concentrations showing progressive inhibitions. The Figures 4.1 to 4.4 show the increase in activities against the increase in concentrations. There was resistance on fungi against the majority of the plant extracts with only a few showing activities.

Table 4.1: Screening for Activity

Name of plants	<i>S.a.</i>	<i>P.a.</i>	<i>E.f.</i>	<i>K.p.</i>	<i>E.c.</i>	<i>S.t.</i>	<i>C.a.</i>	<i>C.b.</i>	<i>C.n.</i>	<i>T.m.</i>	<i>M.g.</i>
<i>R. prinoides</i>	++	-	-	+	-	-	-	-	-	-	-
<i>O. Africana</i>	++	-	++	+	-	-	-	-	-	-	-
<i>O. hochstetteri</i>	+++	-	-	+	-	-	-	-	-	-	-
<i>F. saligna</i>	+++	++	-	++	-	-	+++	+++	+++	++	+++
<i>E. capensis</i>	+++	+++	++	++	-	-	-	-	-	-	-
<i>B. abyssinica</i>	+++	-	+	+++	-	-	++	+++	++	+++	++
<i>S. myrtina</i>	+++	++	-	+++	-	-	-	-	-	-	-
<i>G.buchananni</i>	++	-	-	-	-	-	-	-	-	-	-
<i>C.robusta</i>	+++	++	+	+++	-	-	-	-	-	-	-
<i>T. asiatica</i>	+++	-	-	-	-	-	+++	+++	+++	++	++
<i>I. tinctoria</i>	++	-	-	-	-	-	++	++	+++	+	++
<i>L. javanica</i>	++	-	+	-	-	-	+	+	+	-	-
<i>S. biflora</i>	+++	+	+	-	+++	-	+++	+++	-	++	+
<i>E. schimperii</i>	+++	+++	+++	++	++	+	-	-	-	-	-
<i>O. rochetiana</i>	+++	+++	+++	+++	-	+++	-	-	-	-	-
<i>R. keniensis</i>	++	+++	+	++	-	+	-	-	-	-	-

Legend: *Staphylococcus aureus* (*S.a.*) *Pseudomonas aeruginosa*(*P.a.*) *Klebsiella pneumoniae* (*K.p.*) *Escherichia coli* (*E.c.*) *Salmonella typhi* (*S.t.*) *Candida albicans* (*C.a.*) *Candida brusei* *C.b.* *Cryptococcus neoformas*(*C.n.*) *Trichophyton mentagrophyte* (*T.m.*) *Microsporium gypseum*(*M.g.*)

High activity (16 mm); moderate activity (>9-15 mm); slight activity (8 mm) and no activity (6 mm)

**Figure 4.1: Various inhibitions in mm of methanol extracts against *E. coli*****Figure 4.2: Stem bark methanol extracts of *T. asiatica* and *O. rochetiana***

Inhibition of *E. coli* strains with *O. rochetiana*, though concentrations, showed the same trend in increase and finally stabilizing at 20 mm for *O. rochetiana* and 10mm for *Olea* spp. (Figure 4.3).

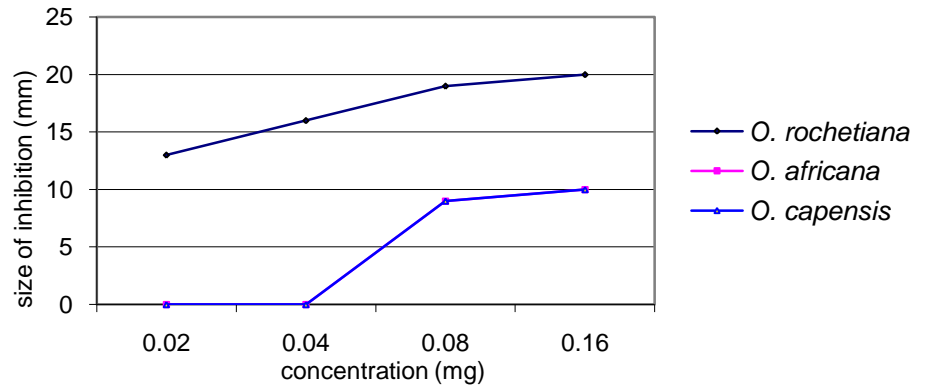


Figure 4.3: Stem bark methanol extracts of *O. rochetiana*, *O. africana* and *O. hochstetteri*

Inhibition of *Staphylococcus* spp. showed varied but minimally to changes in concentrations (Figure 4.3). *Klebsiella* spp. did not show susceptibility despite the increase in concentrations of the methanol extracts except in *O. africana* stem bark methanol extracts which had a sharp increase between (Figure 4.4).

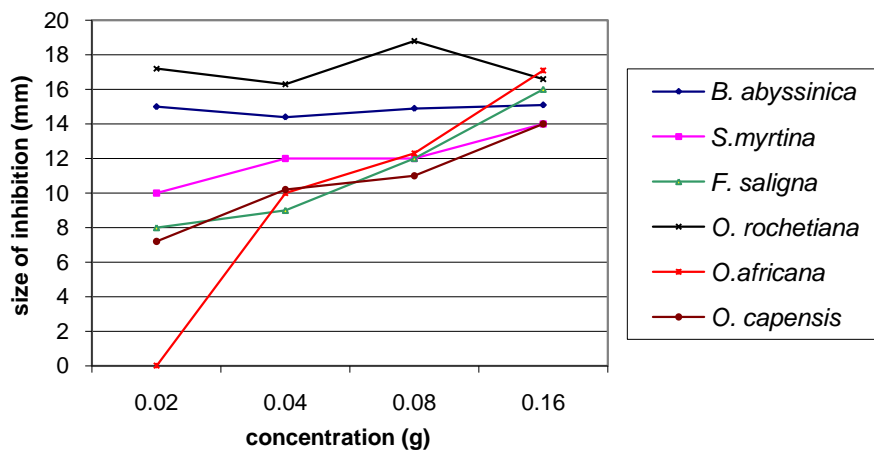


Figure 4.4: Stem bark Methanol extracts of various Drugs against *Klebsiella* spp

Tables 4.2 to 4.37 contain individual extracts efficacies with varied concentrations against different organisms including sub cultures. Universally known resistant sub

cultures to current broad spectrum antibiotics were also tested and the extracts showed good antimicrobial activities.

The following strains of *Pseudomonas* used and all had similar reactions. *P. aeruginosa*, *P. aeruginosa* ATCC27853 and *P. aeruginosa* (clinical isolates) (Table 4.2).

Table 4.1: Zones of inhibition (mm) by stem bark methanol extract of *B. abyssinica* against *P. aeruginosa*

conc. mg/ml		control
20	10	6
40	10	6
80	10	6
160	10	6
	6	

There was moderate inhibition of various strains of *S. aureus* by the extract (Table 4.3)

Table 4.3: Zones of inhibition (mm) by methanol extracts of *B. abyssinica* against strains of *S. aureus*

Pathogens	Conc. mg/ml			
	20	40	80	160
<i>S. aureus</i> (Pigmented with staphylokinase)	15	15	15	15
<i>S. aureus</i> (ATCC 20591)	15	14	15	15
<i>S. aureus</i> (ORSA) and MRSA	15	14	15	15
Control	6	6	6	6

The strains of *P. aeruginosa* showed similar response to the drug (Table 4.4)

Table 4.2: Zones of inhibition (mm) by methanol extract of stem bark of *S. myrtina* against *P. aeruginosa*

Pathogens	conc. in mg/ml			
	20	40	80	160
<i>P. aeruginosa</i> (ATCCC27853)	8	8	11	14
<i>P. aeruginosa</i> (Clinical isolates)	8	8	10	12
Control	6	6	6	6

There were similar in this showed the same activities to the extracts of *S. myrtina* (Table 4.5)

Table 4.3: Zones of inhibition (mm) by methanol extracts of *S. myrtina* stem bark against *Klebsiella* species

Pathogens	conc. in mg/ml			
	20	40	80	160
<i>K. pneumoniae</i> (Clinical isolate)	11	11	14	15
<i>K. pneumoniae</i> (Belgium Strain)	11	12	16	16
<i>K. pneumoniae</i> (MDRS)	11	11	14	15
<i>K. pneumoniae</i> (WHO)	11	11	14	15
Control	6	6	6	6

There was moderate susceptibility of *P. aeruginosa* and its strains to the methanol extracts of *S. myrtina* (Table 4.6)

Table 4.4: Zones of inhibition (mm) by *S. myrtina* methanol extract against *S. aureus* and its strains

Pathogens	Conc. in mm/ml			
	20	40	80	160
<i>S. aureus</i> (Clinical isolate)	8	12	12	14
<i>S. aureus</i> (ATCC 20591)	8.0	12	12	14
<i>S. aureus</i> (haemolytic strain)	12	12	12	14
<i>S. aureus</i> (Pigmented strain)	12	12	12	14
Control	6	6	6	6

Table 4.5: Zones of inhibition (mm) by methanol extracts of *R. pirinoides* stem bark against *Klebsiella* species

Pathogens	conc. in mg/ml			
	20	40	80	160
<i>K. pneumoniae</i> (Clinical isolate)	11	11	14	15
<i>K. pneumoniae</i> (Belgium Strain)	11	12	16	16
<i>K. pneumoniae</i> (MDRS)	11	11	14	15
<i>K. pneumoniae</i> (WHO)	11	11	14	15
Control	6	6	6	6

There was high susceptibility of *P. aeruginosa* and its strains to the *F. saligna* (Table 4.8)

Table 4.6: Zones of inhibition (mm) by of *R. pirinoides* methanol extract against *S. aureus* and its strains

Pathogens	Conc. in mm/ml			
	20	40	80	160
<i>S. aureus</i> (Clinical isolate)	8	12	12	14
<i>S. aureus</i> (ATCC 20591)	8.0	12	12	14
<i>S. aureus</i> (haemolytic strain)	12	12	12	14
<i>S. aureus</i> (Pigmented strain)	12	12	12	14
Control	6	6	6	6

There was minimal to moderate susceptibility of *K. pneumoniae* and its strains to the *F. saligna* (Table 4.9).

Table 4.7: Zones of inhibition (mm) by methanol stem bark extracts of *F. saligna* Species and strains of *Klebsiella*

Pathogens	Conc. in mg /ml			
	0.02	0.04	0.08	0.16
<i>K. pneumoniae</i> (WHO std)	8	10	10	12
<i>K. pneumoniae</i> (MDRS)	8	10	10	12
<i>K. pneumoniae</i> (Clinical isolates)	8	10	10	12
Control	6	6	6	6

There was minimal to moderate susceptibility of *K. pneumoniae* and its strains to the *F. saligna* (Table 4.10).

Table 4.8: Zones of inhibition (mm) by Methanol stem bark extracts of *F. saligna* against Strains of *S. aureus*

Conc. in mg/ml	Zones of inhibition (mm)
20	8
40	9
80	12
160	16
Control	6

There was minimal to moderate susceptibility of *K. pneumoniae* and its strains to the *F. saligna* (Table 4.11).

Table 4.9: Zones of inhibition (mm) by *T. asiatica* root bark methanol extracts against Strains of *S. aureus*

Pathogens	Conc. in mg/ml			
	20	40	80	160
<i>S. aureus</i> (Clinical isolate)	-	7	12	16
<i>S. aureus</i> (β Haemolytic)	-	7	14	16
<i>S. aureus</i> (Pigmented)	-	7	12	16
<i>S. aureus</i> (Pigmented with staphylokinase)	-	7	12	16
<i>S. aureus</i> (ATCC 20591)	-	7	13	17
<i>S. aureus</i> (ORSA)	-	7	14	16
<i>S. aureus</i> (MRSA)	-	7	12	16
Control	6	6	6	6

There was no significant inhibition of the extract on *E.coli* (Table 4.12).

Table 4.10: Zones of inhibition (mm) by methanol extracts of root bark of *T. asiatica* against *E. coli* and its strains

Pathogens	Conc in mg/ml			
	20	40	80	160
<i>E. Coli</i> (086)	6	7	7	8
<i>E. Col</i> (std)	6	7	7	8
<i>E. Coli</i> (std 25922)	6	7	7	8
<i>E. Coli</i> (35218)	6	7	7	8
<i>E. Coli</i> (0125/B15)	6	7	7	8
<i>E. Coli</i> (087/B7)	6	7	7	8
<i>E. Coli</i> (0126/B16)	6	7	7	8
<i>E. Coli</i> (125/K70)	6	7	7	8
<i>E. Coli</i> (0127/8)	6	7	7	8
Control	6	6	6	6

There was a high antimicrobial activity of the methanol extract against *S. aureus* (Table 4.12).

Table 4.11: Zones of inhibition (mm) by methanol extracts *O. rochetiana* against *E. faecalis* spp:

Pathogens	Conc in mg/ml			
	20	40	80	160
<i>E. faecalis</i> (clinical isolates)	13	16	19	20

<i>E. faecalis</i> ATCC 29212	13	16	19	20
Control	6	6	6	6

There was a high antimicrobial activity of the methanol extract against *Pseudomonas* spp (Table 4.13)

Table 4.12: Zones of inhibition (mm) by *O. rochetiana* activities against *Pseudomonas* spp:

Pathogens	Conc in mg/ml			
	20	40	80	160
<i>P. aeruginosa</i> (Clinical isolates)	10	10	12	12
<i>P. aeruginosa</i> ATCC 27853	10	10	12	12
Control	6	6	6	6

There was little activity of the methanol extract against *Salmonella* spp (Table 4.145)

Table 4.13: Zones of inhibition (mm) by *O. rochetiana* against *Salmonella* spp.

Pathogens	Conc in mg/ml			
	20	40	80	160
<i>S. paratyphi</i>	6	10	10	10
<i>S. typhi</i> (type R. colidale amp)	6	10	10	12
<i>S. enteridis</i>	6	11	11	11
<i>Salmonella</i> (clinical isolate)	6	10	10	10
<i>S. typhi</i> N2202	6	10	10	10
Control	6	6	6	6

There was no variation in activities of the extracts of *R. keniensis* against various strains pathogens (Tables 4.16 to 4.20).

Table 4.14: Zones of inhibition (mm) by *R. keniensis* root bark methanol extracts

Pathogens	Conc in mg/ml			
	20	40	80	160
<i>P. aeruginosa</i> (Clinical isolates)	10	16	20	22
<i>P. aeruginosa</i> ATCC 27853	10	15	20	22
Control	6	6	6	6

The strains used included: *E. faecalis* (hospital strain) and *E. faecalis* (ATCC 29212)

(Table 4.17)

Table 4.15: Zones of inhibition (mm) by *R keniensis* root bark against *E. faecalis* and its strains

Conc. in mg/ml	Zones of inhibition (mm)
20	6
40	6
80	7
160	10
Control	6

Other strains used were: (clinical strain) *K. pneumoniae* (Belgium strain) *K. pneumoniae*

(WHO Std) and MDRS): There was similar activity in all the strains (Table 4.18).

Table 4.16: Zones of inhibition (mm) by *R. keniensis* root bark against *K. pneumoniae oxytoca*

Conc. in mg/ml	Zones of inhibition (mm)
20	7
40	10
80	12
160	14
Control	6

S. aureus (*B. haemolytic*), *S. aureus* (oxford strains) *S. aureus* (Danida strain) *S. aureus*

(pigmented) *S. aureus* (pigmented with staphylokinase) *S. aureus* (ATCC 20591) and *S.*

aureus (clinical isolates): There was similar activity in all of them (Table 4.19).

Table 4.17: Zones of inhibition (in mm) by *R. keniensis* root bark methanol extracts against *S. aureus*

Conc. in mg/ml	Zones of inhibition (mm)
20	7
40	12
80	13
160	15
Control	6

There was similar activity in all the strains and (ORSA). The positive control had no growth (Table 4.20).

Table 4.18: Zones of inhibition (mm) by *R. keniensis* on *S. aureus* oxycillin resistant (ORSA)

Conc. in mg/ml	Zones of inhibition (mm)
20	14
50	14
100	16
200	20
Control	6

There was similar activity in all the strains and (ORSA). The positive control had no growth (Table 4.21).

Table 4.19: Zones of inhibition (mm) by *R. keniensis* on *S. aureus* oxycillin resistant (ORSA)

Conc. in mg/ml	Zones of inhibition (mm)
20	14
50	14
100	16
200	20
Control	6

All the strains of *P. aeruginosa* showed similar response to the drug (Table 4.22).

Table 4.20: Zones of inhibition (in mm) by methanol extracts of root bark of *C. abyssinica* against *Pseudomonas* spp.

Pathogens	Conc in mg/ml			
	20	40	80	160
<i>P. aeruginosa</i> (Clinical isolates)	7	9	10	11
<i>P. aeruginosa</i> ATCC 27853	7	9	10	11
Control	6	6	6	6

Table 4.21: Zones of inhibition (in mm) by methanol extracts of root bark of *C. abyssinica* against *Staphylococcus* spp:

Pathogens	Conc. in mg/ml			
	20	40	80	160
<i>S. aureus</i> (Clinical isolate)	7	7	11	16
<i>S. aureus</i> (β Haemolytic)	7	7	11	15
<i>S. aureus</i> (Pigmented)	7	7	11	16
<i>S. aureus</i> (Pigmented with staphylokinase)	7	7	11	16
<i>S. aureus</i> (ATCC 20591)	7	7	11	16
<i>S. aureus</i> (ORSA)	7	7	11	16
<i>S. aureus</i> (MRSA)	7	7	11	16
Control	6	7	6	6

No reaction at in the corresponding concentration column *E. faecalis* (Table 4.24)

Table 4.22: Zones of inhibition (in mm) by methanol extracts of root bark of *C. abyssinica* against *E. faecalis*

Pathogens	Conc in mg/ml			
	0.02	0.04	0.08	0.16
<i>E. faecalis</i> (clinical isolates)	6	7	10	13
<i>E. faecalis</i> ATCC 29212	6	7	10	12
Control	6	6	6	6

There little inhibitory activities from the extract (Table 4.25)

Table 4.23: Zones of inhibition (in mm) by methanol extracts of root bark of *C. abyssinica* against *Salmonella* spp

Pathogens	Conc in mg/ml			
	20	40	80	160
<i>S. paratyphi</i>	6	7	7	10
<i>S. typhi</i> (type R. colidale amp)	6	7	9	11
<i>S. enteridis</i>	6	7	7	11
<i>Salmonella</i> (Clinical isolate)	6	7	8	11
<i>S. typhi</i> N2202	6	7	7	11
Control	6	6	6	6

The positive control had no growth (Table 4.26).

Table 4.24: Zones of inhibition (in mm) by methanol extracts of root bark of *C. abyssinica* against *Klebsiella* spp.:

Pathogens	Conc. mg/ml			
	20	40	80	160
<i>K. oxytoca</i>	6	11	12	15
<i>K. pneumoniae</i> (clinical isolate)	6	12	12	16
<i>K. pneumoniae</i> (Belgium stain)	6	11	13	15
<i>K. pneumoniae</i> (WHO std)	6	12	12	16
<i>K. pneumoniae</i> (MDRS)	6	11	12	14
Control	6	6	6	6

Only three strains of the pathogen were used (Table 4.27)

Table 4.25: Zones of inhibition (in mm) by methanol extracts of stem of *E. schimperi* against *P. aeruginosa*

Pathogens	Conc in mg/ml			
	20	40	80	160
<i>P. aeruginosa</i> (Clinical isolates)	7	9	12	16
<i>P. aeruginosa</i> ATCC 27853	7	12	12	16
Control	6	6	6	6

There was increase in activity to high inhibition of the organism in relation to increase in concentration of the drug (4.28).

Table 4.26: Zones of inhibition (in mm) by methanol extracts of stem of *E. schimperi* against *S. aureus*

Pathogens	Conc. in mg/ml			
	20	40	80	160
<i>S. aureus</i> (Clinical isolate)	11	12	14	18
<i>S. aureus</i> (β Haemolytic)	11	12	14	18
<i>S. aureus</i> (Pigmented)	11	12	14	18
<i>S. aureus</i> (Pigmented with staphylokinase)	11	12	14	18
<i>S. aureus</i> (ATCC 20591)	11	12	14	18
<i>S. aureus</i> (ORSA)	7	8	11	12
<i>S. aureus</i> (MRSA)	7	8	11	12
Control	6	7	6	6

Clinical isolates of *E. faecalis* and ATCC 29212 were used (Table 4.29).

Table 4.27: Zones of inhibition (mm) by methanol extracts of stem of *E. schimperi* against *E. faecalis*

Conc. in mg/ml	Zone of inhibition (mm)
5	11
10	11
20	12
40	15
Control	6

There were no changes in the zones of inhibitions at any other concentrations (4.30).

Table 4.28: Zones of inhibition (mm) by methanol extracts of stem of *E. schimperi* against Strains of *K. pneumoniae*

Conc. in mg/ml	Zone of inhibition (mm)
12.5	9
25	10
50	12
100	16
Control	6

Three strains of the pathogen were used in the test (Table 4.31).

Table 4.29: Zones of inhibition (mm) by methanol stem bark extract of *E. capensis* against *Klebsiella* ssp.

Pathogens	Conc. in mg/ml				
	25	50	100	200	400
<i>K. pneumoniae</i> (Clinical isolates)	11	14	14	14	16
<i>K. pneumoniae</i> (WHO std)	11	14	14	14	16
<i>K. pneumoniae</i> (MDRS)	11	14	14	14	16
Control	6	6	6	6	6

In the test the following strains were used: *S. aureus* (β - haemolytic), *S. aureus* (Clinical isolates), *S. aureus* (pigmented) *S. aureus* pigmented with staphylokinase), *S. aureus* (local strains *S. aureus* (ATCC 20591), *S. aureus* (ORSA), and *S. aureus* (MRSA): Similar results were obtained with *E. faecalis* and their strains (Tables 4.31-4.36).

Table 4.30: Zones of inhibition (mm) by *G. Buchananni* (methanol) leaf extract against *S. aureus* and *E. faecalis*

Organism	Conc. (100mg/ml)
<i>S. aureus</i> (ORSA)	10
<i>S. aureus</i>	10
<i>C. albicans</i>	10

At the similar concentrations similar zones of inhibition were realized from three different organisms listed in Table 4.33. The positive control had no growth.

Table 4.31: Zones of inhibition (mm) by *I. tinctoria* 100 mg/ml root methanol extract

Organism	Conc. (100mg/ml)
<i>S. aureus</i>	25
<i>S. aureus</i> (ORSA)	10
<i>B. subtilis</i>	10

Only *E. faecalis* (ATCC 29212) and *E. faecalis* (clinical isolates) were used in the study (Table 4.34).

Table 4.32: Zones of inhibition (mm) by drug conc.100 mg/ml *I. tinctoria* root methanol extract against *E. faecalis*

Conc. in mg/ml	Conc. (100mg/ml)
25	12
50	14
100	18
200	20
Control	6

Essential oils *S. biflora* and *L. javannica* had similar activities (Table 4.35)

Table 4.33: Zones of inhibition (mm) by *S. biflora* and *L. javannica* essential oils activity (100 mg/ml)

Organism	Zones of inhibition (mm)	Control (mm)
<i>S. aureus</i> (ORSA)	19	6
<i>S. aureus</i>	10	6
<i>K. pneumoniae</i>	10	6
<i>K. pneumoniae</i> (MDRS)	11	6
<i>E. coli</i>	17	6

Table 4.34: Zones of inhibition (mm) by: *L. javannica* essential oils against *S. aureus* (100 mg/ml)

Organism	Zones of inhibition (mm)
<i>S. aureus</i> (ORSA)	13
<i>S. aureus</i>	13
Control	6

Activities of the organisms were achieved in the last two plants extracts. The positive control had no growth (Table 4.37).

Table 4.35: Zones of inhibition (mm) by *S. biflora* (conc.100 mg/ml) against organisms

Pathogens	Zones of inhibition (mm)	Control (mm)
<i>S. aureus</i> (ORSA)	19	6
<i>S. aureus</i>	10	6
<i>K. pneumoniae</i>	10	6
<i>K. pneumoniae</i> (MDRS)	11	6
<i>E. coli</i>	17	6

4.3.5 Discussion

Several medicinal plant species yielded methanol extracts which were screened against six bacterial pathogens and three fungal species. There were a total of 49 plant species, out of which 16 species 32.6% of the plant methanol extracts inhibited *S. typhi*, 12.1% of all the methanol extracts inhibited growth of *S. aureus*, 6.8% and *P. aeruginosa*. Extracts from *S. biflora*, *F. saligna* and *B. abyssinica* were effective against most of the bacteria and *C. albicans*, *C. neoformans* and *T. mentagrophyte*. *S. biflora* were found to be slightly inhibiting, *S. aureus*, and *P. aeruginosa* and high inhibition of *E. coli*. *F. saligna* highly inhibited *S. aureus*, moderately *P. aeruginosa* and *K. pneumoniae*. The extract from the plant were also inhibitory on the test fungi while *B. abyssinica* was highly inhibiting the *K. pneumoniae*, *S. aureus*, *E. faecalis* and all the test fungi.

This could be attributed to the fact that the drugs are able to penetrate the cell wall of the organisms. The inability may be, due to the fact that fungi being plants, though from the lower classes may have similar compounds as the extracts. *S. biflora* was found to be effective on *K. pneumoniae*, *S. aureus*, *P. aeruginosa* and *E. coli*. *F. saligna* was effective on *E. coli*, *S. typhi* and *E. faecalis* while *B. abyssinica* was effective on *K. pneumoniae*, *S. aureus*, *E. coli* and *C. albicans* (Table 37) with StDev= 0,456 which was significantly different. There were variable activities of individual methanol extracts from one species to the other.

Individually *B. abyssinica* stem bark had extracts showed significant effect of inhibition against *K. pneumoniae* at various levels of conc. $P=0.045$ suggesting that these results confirms the ethnobotanical surveys whereby the plant is used for various ailments in human like fever and rheumatism and livestock east coast fever. Although the extract was active against *S. aureus* there was not much difference in response to increase in

concentrations in the case of *Klebsiella spp.* The $P=0.040$ in the case *S. aureus*, *B. abyssinica* is not mentioned in other literature as a medicinal plant of significance. However, in East Africa several communities use it to treat colds, aphrodisiac, purgative emetic and anti diarrhea (Kokwaro, 1993). The records on its efficacy and bioactivities are limited in literature. The information on efficacy on growth inhibition at 50% at concentration 0.08gm/ml, against *K. pneumoniae* (Fig. 4.5) and this could be the reason for of its use as a remedy for colds and chest related complaints by the community in the study. The experience of inhibition was the same in case of *S. aureus* and its strains.

Rhamnaceae family has *S. myrtina* and *R. prinoides*, both of which are used as medicines. The latter by incorporating the root bark in cookery, and the former stem bark as tea tonic and treatment of chest ailments. There was a significant effects, 12 mm and 15 mm, inhibitions, of *S. myrtina* extracts at the concentration used in the studies, at 100% $P=0.043$ in inhibiting growth of *P. aeruginosa* at 160 mg/ml (Table 4.6).

This was the highest zones of inhibition and did not was not varying from strain to strain. Concentrations of 20 mg/ml to 80mg/ml had $P=0.079$ in the inhibition until a threshold of 160 mg/ml was reached. *K. pneumoniae* and its strains (including multidrug resistant one) had the maximum inhibitory zone of 22 cm at 80 mg/ml. Although *S. aureus* was sensitive to the treatment at relatively low concentration, reaching the peak at 40mg/ml, any further increase in the drug concentration did not matter. So the responses of the other strains of the organism remained constant when pooled (StDev=1.069). There is a clear indication that the extract has efficacy on Gram negative bacteria with, seemingly, higher sensitivity as compared to Gram positive *Klebsiella spp.* increased proportionally in zones of inhibition with the increase in plant extract concentration. The effect was the same with *S. aureus*. However, it was interesting to note that there was no activity against *C.*

albicans although it is considered to be a weak organism. There is a possibility of the organism having been infiltrated indirectly with foreign genetic material that enables it to exhibit resistance to the drug (Livermore, 2000).

F. saligna gave results which indicated a high activity on *P. aeruginosa* and its strains, in that it was the only single plant species whose extract gave an ever-increasing zone of inhibition proportional to the increase in concentration without deviation. The inhibition was the same for both *S. aureus* and *K. pneumoniae*. Apart from the concentrations in other species of the pathogens, the same concentrations there were no inhibitions in all the strains and species of *E. coli*, *E. faecalis* and *S. typhi*.

Toddalia asiatica root bark extracts of the plant had significant activity, $P < 0.05$ with a pooled $StDev = 0.725$ and all the tested strains of *S. aureus* and *E. coli* with growth being inhibited significantly. Its inhibition increased proportionally with the increase in the drug concentration without any or much variation. Sensitivity of the organism to the plant extract justifies the fact that the community uses the plant against respiratory tract ailments such as cough, cold, stomachache and other chest related conditions. The plant was also mentioned to be used as tonic, possibly as an immune booster whereby, the roots are boiled decanted and the decoction taken by the Ogiek. However, members of the Rutaceae are known to possess strong antimicrobial agents which have been tested (Quiroga, 2004). The genera *Pseudomonas*, *Enterococcus* and *Salmonella* were all resistant to various levels of treatments of the root bark extracts.

Olinia rochetiana was indicated to be used in the treatment of colds and chest related conditions, it was found to be active against several organisms, both Gram positive and Gram negative. The results showed that the higher the concentration of the extract, the

higher the inhibition with high efficacy being reached in the case of *Pseudomonas* spp. without any variations. However, *Salmonella* spp. manifested varied species and strains responses to the extract at $P = <0.45$ without any variation in the changes of the concentrations. In the entire tests against other pathogenic organisms, the methanol extracts was active against all the other six; namely *E. faecalis*, *S. typhi*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae* and *E. coli* at varied concentrations. These results are important for the selection of the plant for future anti-bacterial studies using plants extracts (Fig. 4.4).

The family Oleaceae was represented by *O. africana* and *O. hochestetteri*. Both plant species gave results with significant effect at $P < 0.045$, with changes of extracts concentrations on inhibitions growth of *S. aureus* and its other strains. There was more activity, individually, in that the area the test organisms inhibited increased proportionally with the increase in the drug concentrations. *O. hochesetteri* stem bark extracts showed high inhibitory activity against species of *E. faecalis* and its other strains at higher concentrations. This was evident since the significant effect was reliable at 95% confidence level ($p < 0.045$). Besides *O. hochstetteri* which yields olive oil that is used in cooking and pharmaceutical preparations, members of the same genus are used in ethno medicine as in the case of East African communities. There are not many mentions of the other uses of the family members in traditional medicines (Williamson, 2002).

Root bark methanol extracts from the family Rosaceae, *R. keniensis* were active with varied efficacies against several microorganisms. There was significant effect $P < 0.05$ might help give actual efficacies with change of the extracts concentrations on inhibition of the growths of all the species and strains of *Pseudomonas*. Higher concentrations of the extract was more efficient as compared to the lower ones with pooled $StDev = 0.258$. The

results were the same as in the cases of *E. faecalis*, *K. pneumoniae* and *S. aureus*. However, in the entire last cases the readings were the same. Studies to ascertain the efficacy or the safety of this genus have not been done either locally or elsewhere to justify their continued use in folklore medicine.

Four of the test organisms including their strains were highly inhibited by methanol extracts of *C. abyssinica* at increasing concentrations. *S. aureus* had the highest area of inhibition in relation to increase in concentration with a mean of 15.7 mm at a concentration of 160 mg/ml. *K. pneumoniae* and its strains also showed similar trend of inhibition. *Pseudomonas* spp and their strains also showed the same trend of inhibition with increased concentrations of the drug. Similar activities as in the case of *Pseudomonas* and *Klebsiella* were exhibited in *E. faecalis* regardless of the strains or species. The plant was, however, not regarded as medicinal but as inclusion as tonic during cookery particularly when cooking mutton could explain its continued use by the community. Root bark extracts from *C. abyssinica* plant, the test result further showed that there was a significant effect $P=0.05$. Change of the extracts concentration showed increase on inhibitions of *K. pneumoniae*. Higher concentrations of the extract inhibited more of the bacterial growth. However, there was no significant difference in concentrations 0.04 and 0.08 gm it was noted that there was no significant difference in inhibition on the various strains of *K. pneumoniae* even though, *K. oxytoca* was more inhibited than the other strains.

Embelia schimperi was widely mentioned for its medicinal value, as an antibiotic and antihelminths (Rajan *et al.*, 2002). Uses of the stem bark as medicine were verifiable since the methanol extract was capable of inhibiting *S. aureus*, *P. aeruginosa* and *E. faecalis*, and all their different strains. During the laboratory studies, some of the

organisms, in all the cases, had decreases in area of inhibition of the pathogens in relation to increase in the extracts concentration with a significant effect at $P=0.044$. The inhibition value remained the same despite varying concentrations of the drug from one organism to the other. Similarly, there are other members of the same genus, *E. ribes* and *E. tsjenam-cattam* which have been used as taenicides, treatment of chest infections, skin ailments, cholera, pneumonia and diarrhea in Ayurvedic medicine with lots of success (Rajan *et al.*, 2002; Patwardhan *et al.*, 2005).

Ekabergia capensis was most effective progressively and significantly against all types of the genus *Klebsiella* and species of *S. aureus* at a peak concentration of 40 mg/ml ($P<0.05$). Various extracts from the plant have been used as antibiotics in folklore medicine by Venda communities of South Africa with reasonable success (Samie *et al.*, 2005). Most of the uses are against diarrhea, stomachache and pneumonia. Such uses tally with the cases amongst the Ogiek.

Garcinia buchananni, Lusaciaceae family, a medium sized tree, whose wild fruits are common and a delicacy in this area, the portion from the roots is used against stomach pain in pediatrics. Its root bark methanol extract showed high inhibition on *S. aureus*, and its resistant strains. This further demonstrates the accuracy with which the community have understood and judiciously selected products of Mother Nature to meet their daily livelihoods. Another study on the same genus elsewhere was on *G. mangostana* has been reported to possess strong antimicrobial activities against several strains of the species *S. aureus* (Voravuthikunchai and Kitpitpit, 2005).

Impatiens tinctoria, a Balsaminaceae, was mentioned by the community to be useful in enhancing delivery process in human. The root extract from the plant when screened

against the test organisms had high inhibitory efficacy on *S. aureus* and its strains. Furthermore, the extracts showed high inhibition on all the test fungi except marginally *M. mentagrophyte*. The medicinal uses of the species is not cited in literature elsewhere in the world to authenticate this report and for that matter further investigations are recommended on this plant.

Lippia javanica (Table 4.35) Verbenaceae and *S. biflora* Laminiaceae are used in fresh wounds treatment, pneumonia, and colds, and in tea as tonic respectively. Oil extracted from *S. biflora* was effective against *S. aureus*, *K. pneumoniae* and *E. coli* including all their drug resistant strains. The essential oils from *L. javanica* possessed activities against all the strains of *S. aureus*, at the levels shown in the Tables 4.35 and 4.36 *L. javanica* is amongst other medicinal essential oils that have been studied elsewhere and proved useful medically. Essential oils from higher plants have been used in folklore medicines from time immemorial, and the uses are universal in all the continents, civilisations (Samie *et al.*, 2005). There are other plants that have shown activities include the genus *Ocimum* from which oils capable of controlling *Propionibacterium acnes* is found (Viyoch *et al.*, 2006). *Thymus kotschyanus* yielded oils that were active against *Helicobacter pylori* which are found in human duodenal ulcer sites (Nariman *et al.*, 2004). *Pseudomonas aeruginosa* which is a common opportunistic pathogen may as well be controlled by oils extracted from *Melaleuca alternifolia* (L) (Chelsea *et al.*, 2006). However, *S. biflora* which has varied activities when screened in the laboratory *in vitro* has not been studied exhaustively. The plant oil extracts gave promising and astounding results against both Gram negative and Gram positive bacteria (Table 4.37).

Laboratory results indicated strong activities of essential oil extracts from *S biflora* against *S. aureus*, *E. coli* and *K. pneumoniae* which is a clear justification of its use by the

communities of freshly prepared decoction against respiratory ailments and abdominal complaints.(Tables 4.35and 4.37) Coincidentally, a decoction from the plant is also taken as tonic. However, the use of zones of inhibition was not conclusive enough to determine the efficacy of a drug.

CHAPTER FIVE

ANTIMICROBIAL SUSCEPTIBILITY TESTING

5.1 Materials and methods

5.1.1 Disc diffusion and serial dilution

Antimicrobial susceptibility testing was meant to establish minimum inhibitory concentrations (and minimum bactericidal/fungicidal concentrations MIC/MBC/MFC). 10 mg of individual test plant extracts which had previously shown bioactivity during the bioassay tests were weighed out into sterile test tubes and then reconstituted with four droplets of dimethyl sulphoxide just to dissolve it and then topped with sterile distilled water to the required volume of 10 ml. Further, serial dilutions were made to give a total multiple of four concentrations 10, 20, 30 and 40 µg/ml. Each dilution was impregnated onto paper discs each measuring 6mm in diameter. The discs were desiccated before being placed onto pathogen-seeded Mueller–Hinton agar. Similarly, various concentrations of the essential oils were also prepared to give 10, 20, 30 and 40µl/ml. The discs were then dipped into the oil and then placed onto the dishes using the same procedure as in the case of the reconstituted methanol extracts. Each of the agar materials was autoclaved for 15 minutes at 121°C and 1.5 kg f/cm³ before being poured onto sterile Plates; each Plate holding 20 ml of it. Each Plate was then seeded with the organism separately using nutrient broth no. 2 as diluting agent of the organisms previously prepared by scooping the organism with a sterile wire loop then dipping into the broth.

The same process was repeated with reference strains from the National Public Health Laboratory services (NPHLS) in Nairobi. The discs, having a diameter of 6.0mm, previously treated with the drugs were carefully placed on the clearly marked portions of the Plates and then incubated for 24 hours at relative humidity of 98% and temperature of 37 °C. The records of zones of inhibitions diameters in (mm) were measured using a

ruler. Where there was no growth of each species of micro-organism or strains were taken individually based on the concentrations. Gentamycin was used as control and for comparative purpose and represented other standard antibiotics. The active extracts from the antimicrobial screening were tested for minimum inhibitory concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBCs/MFCs). The MICs were determined using doubling serial dilution method in a peptone water solution for bacteria and PDA broth for fungi for the active extracts to give a final extract concentration of between 1.95 and 8000 µg/ml. Each tube was then inoculated with 0.1 ml of standardized bacterial suspension (1×10^8 CFU/ml) and fungal suspension (1×10^8 spores/ml). The cultures were incubated at 37 °C for 24 h for bacteria, 48 h for yeast and at 25 °C for 5 days for moulds. The first tube showing no growth was taken as the MIC, MBC and MFC were determined by sub-culturing 0.1 ml of all the tubes showing no growth on Nutrient Agar (NA) for fungi and PDA Plates for yeast and moulds. After 24 h incubation at 37°C for bacteria, the first Plate showing no growth was considered as the MBC, while after 48 h at 37°C for *Candida* and 5 days at 25°C for fungi, respectively, the first Plate showing no growth was taken as the MFC (Barry, 1980; Michael *et al.*, 2003).

5.1.2 Disc diffusion and MIC ratings of the extracts

The negative controls of the disc diffusion testing was done by use of methanol that showed no inhibition, while positive control was done by use of known antibiotic profile standard discs from (Oxoid). The average zones of inhibition were measured. A clearing zone above 9 mm for Gram-positive and Gram-negative bacteria and 10 mm for fungi or greater was used as the criterion for designating significant antibacterial and antifungal activity (Faizi *et al.*, 2003). The *in vitro* MIC results were classified as per Pessini *et al.*, (2003). The extracts that displayed MIC < 100 µg/ml, the antimicrobial activity was

considered high activity; from 100-500 µg/ml, moderate activity; 500-1000 µg/ml, slight activity; and 1000-4000 µg/ml, low (Barry, *et al.*, 1986)

+++ High activity ++ moderate activity + slight activity - no activity

5.2 Results and discussion

The results obtained were reflective of the information gathered in the field in that certain extracts exhibited both bacteriostatic and bacteriocidal activities, for examples in the case of *B. abyssinica* inhibited the growth of *S. aureus* at 50µg/ml are called (MBC) as in Table 5.1. Three plants: *G. buchananni*, *T. asiatica* and *O. rochetiana* only showed bacteriostatic activities and but no bacteriocidal abilities in all the test organisms (5.1). The details of the individual results of the plant extracts were as in Table 5.1. Sequential exactions of stem barks of *B. abyssinica* and *F. saligna* showed varied fungicidal activities at various concentrations (tables 5.2, 5.3, 5.4 and 5.5). The laboratory value given in the graph below would otherwise demonstrate the organism as being bacteriostatic to contain the organism as the body builds its immune system (Gorretty *et al.*, 2004; Aguilla *et al.*, 2001).

From Tables 5.1 to 5.5 it was clear that there was activity across all the pathogens with MeOH root extracts but with highest potency from root bark petroleum ether extracts. This signified that most of the active principles are non polar soluble and are deposited in the roots. However, ethyl acetate, a weaker solvent had the least activity from the root bark extract. Table 5.5 contains three sequential extracts from three parts of the plant and it is evident that the petroleum ether from the root bark is the most active. This was the same in the stem bark methanol and petroleum extracts. There were however no activities from the all the extracts from the leaves.

MIC $\mu\text{l/ml}$ or $\mu\text{g/ml}$ ((conc. at activity stopped) (Table 5.1).
MBC $\mu\text{l/ml}$ or $\mu\text{g/ml}$ (conc. At activity stopped)

Table 5.1: MIC/MBC of Various plants extracts in $\mu\text{g/ml}$ or $\mu\text{l/ml}$

Plant	Test Organism	MIC	MBC
<i>B. abysinica</i>	<i>Pseudomonas</i> spp.	500	1000
	<i>Klebsiella</i> spp. including MDRS	1000	2000
	<i>S. aureus</i> including ORSA	50	50
<i>S. myrtina</i>	<i>Pseudomonas</i> , spp., <i>Klebsiella</i> spp.	500	1000
	<i>S. aureus</i> including (ORSA)	500	1000
<i>R. prinoides</i>	<i>K. pneumoniae</i> .	250	400
	<i>Pseudomonas</i> spp.	400	1000
<i>F. saligna</i>	<i>Pseudomonas</i> spp.	25	1000
	<i>S. aureus</i> including (ORSA)	<25	2000
	<i>K. pneumoniae</i>	<25	<25
<i>S. biflora</i>	<i>Pseudomonas</i> spp	1000	2000
<i>I. tinctoria</i>	<i>S.aureus</i> including (ORSA)	31	60
	<i>E. faecalis</i> and its strains	16	35
<i>G. buehananni</i>	<i>S. aureus</i> and (ORSA)	40	No reaction
	<i>E. faecalis</i>	50	No reaction
<i>T. asiatica</i>	<i>E. coli</i> its strains	20	No reaction
	<i>S. aureus</i> and (ORSA)	400	No reaction
<i>O. rochetiana</i>	<i>Pseudomonas</i> spp.	25	No reaction
	<i>E. faecalis</i> and its strains	1000	No reaction
	<i>S.aureus</i> and (ORSA)	12.5	No reaction
	<i>K. pneumonia</i>	50	No reaction
<i>O. africana</i>	<i>S.aureus</i> and (ORSA)	1000	2500
<i>O. hochestetteri</i>	<i>S. aureus</i> and (ORSA)	250	500
	<i>E. faecalis</i> and its strains	400	400
<i>R. keniensis</i>	<i>S. aureus</i> and (ORSA)	500	
	<i>E. faecalis</i> and its strains	1250	1250
	<i>K. pneumoniae</i>	1000	1250
<i>C. abyssinica</i>	<i>Pseudomonas</i> spp.	25	-
	<i>S. aureus</i> and (ORSA)	3.1	No reaction
	<i>E. faecalis</i> and its strains	6.2	25
	<i>K. pneumoniae</i>	<25	50
<i>E. schimperi</i>	<i>Pseudomonas</i> spp.	50	100
	<i>S.aureus</i> and (ORSA)	1250	1250
	<i>E. faecalis</i> and its strains	3000	No reaction
	<i>K. pneumoniae</i>	1250	1250

Steam distillate of *T. asiatica* fruits and leaves (MFC in $\mu\text{g/ml}$) of (*C.a*) *C. albicans* (clinical Isolates), (*C.b*) *C. brusei*, (*Cr.n*) *C. neoformas*, (*T.m*) *T mentagrophytes* (clinical isolates), (*M.g*) *M. gypseum* (clinical isolates) (Table 5.2).

Table 5.2: MFC of fungi to various stem distillate of *T. asiatica* ($\mu\text{l/ml}$)

Plant part used	Fungi/Conc. ($\mu\text{l/ml}$)				
	<i>C.a</i>	<i>C.b</i>	<i>Cr.n</i>	<i>T.m</i>	<i>M.g</i>
Fruits	-	-	4.1	1.2	1.0
Leaves	-	-	-	45.0	-

Sequential extracts of *F. saligna* root bark and stem bark (MFC in $\mu\text{g/ml}$) of (*C.a*) *C. albicans* (clinical isolates), (*C.b*) *C. brusei*, (*Cr.n*) *C. neoformas*, (*T.m*) *T mentagrophytes* (clinical isolates) and (*M.g*) *M. gypseum* (clinical isolates) showed activities (Table 5.3).

Table 5.3: MFC of fungi of sequential extracts of root bark and stem bark of *F. saligna* ($\mu\text{g/ml}$)

Plant Part	Extract	Fungi/Conc. ($\mu\text{g/ml}$)				
		<i>C.a</i>	<i>C.K</i>	<i>Cr.n</i>	<i>T.m</i>	<i>M.g</i>
Stem Bark	MeOH	25	50	-	-	110
	Petroleum ether	10	20	-	100	70
	Ethyl acetate	50.0	80.0	-	-	250
Root Bark	MeOH	12.5	30	150	100	200
	Petroleum ether	10.0	12.5	60	25	150
	Ethyl acetate	-	-	-	-	300

MFC in $\mu\text{g/ml}$ sequential extracts of *I. tinctoria* (MFC) results in (μml) (*C.a*) *C. albicans* (clinical Isolate), (*C.b*) *C. brusei*, (*Cr.n*) *C. neoformas*, (*T.m*) *T. mentagrophytes* (clinical isolates) and (*M.g*) *M. gypseum* (clinical isolates); showed activities: (Table 5.4).

Table 5.4: Antifungal activity (MFC) of root extracts of *I. tinctoria* ($\mu\text{g/ml}$)

Extract	Fungi/ Conc. ($\mu\text{g/ml}$)				
	<i>Ca</i>	<i>C.K</i>	<i>Cr.n</i>	<i>T.m</i>	<i>M.g</i>
Methanol	10.0	10.0	-	-	25
Petroleum ether	1.5	105	4.5	-	7.5
Ethyl acetate	-	-	-	-	-

The organisms: (C.a) *C. albicans* (clinical isolate), (Cb) *C. brusei*, (Crn.) *C. neoformas*, (T.m) *T. mentagrophytes* (clinical isolates), and (M.g) *M. gypseum* (clinical isolates) (Table 5.5)

Table 5.5: Antifungal activity (MFC) of root extracts of *B. abyssinnica* (µg/ml)

Plant Part	Extract	Fungi/ Conc. (µg/ml)				
		C.a	C.k	Cr.n	T.m	M.g
Stem bark	Methanol	5.5	5.25	4.0	11.5	17.0
	Petroleum ether	2.75	4.5	3.0	15.0	5.75
	Ethyl acetate	-	-	-	-	20.0
Leaves	Methanol	-	-	-	-	-
	Petroleum ether	-	-	-	-	-
	Ethyl acetate	-	-	-	-	-
Root bark	Methanol	4.0	6.0	3.75	12.5	5.5
	Petroleum ether	1.25	3.0	2.5	10.0	4.5
	Ethyl acetate	-	-	-	-	-

5.4 Discussion

Extracts, predominantly, methanol, from 16 plants were tested for their bactericidal activities and confirmed some results obtained earlier on African plants (Atindenou *et al.*, 2002). Although certain plants which were mentioned several times by the Ogiek traditional healers and showed antimicrobial activities at the initial screening stage later exhibited no activities when their extracts were subjected to micro dilution studies in the bactericidal stages. Such plants were excluded from the MBC and MFC studies.

Antifungal activity (MBC) of *B. abyssinnica* root stem bark and leaves sequential extracts (MFC) results in (µg/ml) showed activities from all the extracts except ethyl acetate ones from all the three different plant parts. Leaves distillate of the lant showed higher dosage as compared to others against *T. mentagrophyte*. The lowest activity was obtained from *T. mentagrophyte*. Extracts from *Bersama abyssinnica* showed activities against both Gram negatives like *Pseudomonas aeruginosa*. The case was the same when tested

against all the other strains including hospital drug resistant ones at an MIC of 500 µg/ml and at 100 µg/ml. *K. pneumoniae* including MDR strains had MIC of 100 µmg/ml and MBC of 200 µg/ml. The *S. aureus* including ORSA had MIC and MBC at 50 µg/ml, indicating that the extract was active against the organism. These studies concur with uses to which the community put the plant products at the local levels. However, the plants of the Melianthaceae family are mentioned in cases involving cardiovascular pharmacopoeia of biological origin (Houghton, 2002). Both the root and the stem methanol and petroleum ether extract possessed activities against all the test organisms with having the degree P value ≤ 0.5 at a concentration of 2000 µg/ml. This is a good indication of the plant being a high potential for drug development. The African species have not been fully explored to ascertain their ability to provide scientific base for pharmaceutical development.

Methanol extracts of *Olinia rochetiana* leaves, (Tables 4.11 to 4.17) were active and killed every organism the extract was subjected to. The lowest MIC recorded was that of *S. aureus* including the ORSA at 12.5 µg/ml for MIC. This activity is attributed to the presence of prunaslin, hydrogen cyanide and a functional, benzenoid (Bukenyia *et al.*, 2000). Several species from the family have been reported to be containing various substances of which *O. cymosa* has prunaslin and is used for stomach disorders, *O. macrophylla* leaves are used for control of malaria in Kenya and contains caffeic acid 4-0-β-D-glucoside. *O. ventosa* from South Africa has leaves containing benzenoid (Simon and Lamla, 1991). However, it was interesting that none of the extract studied provided bacteriacidal level even to *S. aureus*. It is possible that the organisms could regain some vigour and overcome the poisoning effect of the drug with time over a period of 24h.

There are two plant species from the genus *Olea*, in the Oleaceae family, used as medicine in the traditional treatments by the Ogiek communities. Such plants include: *O. africana* used against malaria and fever *O. hochestetteri*. The former plant's methanol extract when subjected to the six species of the Gram +ve and Gram -ve bacteria showed activity against ORSA and *S. aureus* at an MIC of 1000 µg/ml. The extracts also showed active against all the test organisms except; *Pseudomonas* and its strains, *E. coli* and *S. typhi* and its strains. Potent anti- microbial activities were exhibited by the extracts on *S. aureus* and *E. faecalis* with *S. aureus* had an MIC of 250 µg/ml, MBC at 500 µg/ml and *E. faecalis* 400 µg/ml. Olive oil from *O.hochestetteri* is the most mentioned in literature (Gunstone *et al.*, 1994). There seems to be very little or no evidence of scientific studies on these plants on the African continent.

The family Rosaceae is of importance in that *Rubus keniensis* roots are used by the Ogiek as a source of remedy against stomach ache and dysentery. This was verifiable by the fact that the methanol extracts was effective, with an MBC, against *E. faecalis* at 1250 µg/ml. unfortunately literature on the antibacterial activities of the genus is lacking. There were significant $P \geq 1$ antibacterial activities of the extracts, except for *Salmonella typhi*, *E. coli* and their stains that were resistant. *Pseudomonas* and its strains showed MIC at 1000 µg/ml and MBC of 2000 µg/ml as the highest, and an MBC of 500 µg/ml, in *S. aureus* and its strains. From this genus, there is *R. rigidus* which is used as remedy against coughs and colds in children, *R. steudneri* indigestion and *Rubus sp.* against abdominal complaints (Kokwaro, 1993).

Lippia javanica in the family of Verbenaceae are used to treat fresh wounds. The same species has been used as a mosquito repellent by populations in East Africa up to South Africa for a long time (Lukwa, 1996). Previous studies in Kenya have shown that oils

from *L. javanica* have very strong and lasting repellent activity against Bruchids (Tarus, 2006). Other studies showed that extracts of *L. javanica* were not active against *E. coli* and other bacteria (McGraw *et al.*, 2000). However, during this study methanol extract and essential oils of this plant inhibited *S. aureus* at MIC of 140 µl/ml and 11µl/ml. traditionally, this plant is used by the community as mosquito repellent and fresh leaves rapped onto fresh wounds to assist in healing. Its action might be directed at the fresh wounds and thus combats the organism directly by preventing it from penetrating the host.

It was recorded during the ethnobotanical survey that the bark of *Faurea saligna* is used by the community as aphrodisiac and tonic. Its methanol extract was active against three organisms tested, that is, *Pseudomonas*, *Klebsiella* and *Staphylococcus* with an MBC at less than 25 µg/ml. This indicates a high of potency often (Mallorqui-Fernandez *et al.*, 2004). Although this plant has medicinal value very little or no work has been done on its pharmacognosy. Its activity against *P. aeruginosa* makes it a good potential for research since this organism is common in nosocomial infections (Guillemot *et al.*, 2005). Because of the nature of certain metabolites to respond to solvents differently, it became necessary to use conventional extractive solvents. This in the end yielded levels of activities, as shown in the tables, MIC/MBC and MFC.

Furthermore, sequential extractions of various portions of the plant had significant activities against selected human pathogenic fungi. When various extracts were set against the organisms with fluconazole as control, the ANOVA for all the extracts from both parts of the plant showed $df=2$ with leading results in the petroleum ether followed by methanol. Although ethyl acetate was not active against most of the test organisms, it was significantly active against *Microsporum gypseum* with a test of $df=2$ when

compared to other extracts. Outstanding activities across the board were also reached when dealing with the extracts from the root bark. The phenomena are that most of the metabolites are transported from the sites of synthesis and ultimately stored in the roots in majority of the higher plants. This is perhaps why the activity is higher in root extracts.

Embelia schimperi methanol extract was active with the lowest MIC and MBC of 50 µg/ml recorded in *Pseudomonas* and the highest in *E. faecalis* (3000 µg/ml) as well as their strains. Although the least MBC and MIC were recorded in *Pseudomonas*, previous studies indicated that chloroform extracts had no antibacterial activity on *P. purida* and *E. coli* (Machocho *et al.*, 2003). *Embelia ribes* and *Embelia jeram-cattam* are in the same genera and are used in Ayuverdic treatments of chest infection, expulsion of tapeworms' cholera, diarrhea, weak pulse, pneumonia, pleurisy and skin ailments (Mills, 2002). Majority of these curatives coincidentally agrees with the uses that the Ogiek put the root bark preparations. There are other pathogens which are commonly known to cause these ailments that showed positive results when subjected to the methanol extracts with MIC/MBC and MFC being established in some organisms. They were: *E. faecalis* with MIC at 3000 µg/ml, though MBC was not achieved in certain species, in *S. aureus* plus ORSA both MIC/ MBC were 1250 µg/ml., in *K. pneumoniae* including other strains and MDR, the MIC and MBC was 1250 µg/ml, and lastly, the MFC for *C. albicans* was 1500 µg/ml. The value obtained justifies the use of the plant in the traditional medicine in East Africa more so by the Ogiek. However, the plant extracts did not inhibit *S. typhi* and its strains and *E. coli*.

Clutia robusta is a plant whose roots are used by the Ogiek community in cookery of meat to yield quality soup. Its methanol extract under *in vitro* conditions demonstrated relatively a wide spectrum of antimicrobial activities. Although *Pseudomonas* had an

MIC at 25 µg/ml., MBC was not achievable. This implies that the plant acts as bacteriostatic as in the cases of antibiotics like erythromycin (Cameron *et al.*, 2004). The plant extract was bacteriostatic to *S. aureus* at 3.1 µg/ml, while its MIC was 2 µg/ml and MBC 50 µg/ml for *E. faecalis*. Several species of the family Euphorbiaceae have been used in Ayurvedic and folklore treatments in South East Asia and South America for over 3000 years (Rajan *et al.*, 2002). In the Ogiek community, the plants are used in cooking to provide protection against harsh cold weather and as an aphrodisiac. Other species of the family, not the same genus, like *Euphorbia hirta* have been used as antibiotics against diarrhea, and *in vitro* and *in vivo* activity against *Entamoeba* which causes amoebic dysentery. There are reports of the species being toxic (Pousset, 1994).

Garcinia buchananni syn. *G. hulliensis* leaf extract showed activity against *S. aureus* and its strains with an MIC at 40 µg/ml and *E. faecalis* MIC at 50 µg/ml, although MBC was not obtained. This compares very closely with other species and justifies its use as a mild antibiotic in the treatment of stomach ailments mainly in children by the community as indicated during the field survey. Other species of the same genus like *G. kola* and *G. mangostana* have been commercialised as articles of trade for their medicinal values in Africa and Asia (Sofowara, 2002; Voravuthikunchai and Kitpitpit, 2006).

Toddalia asiatica root bark methanol extract showed MIC against *S. aureus*, and *E. coli* and their strains at 400 and 20 µg/ml, respectively, indicating that the community is justified to use it as tonic besides being a remedy for coughs and stomach ailments as revealed by the ethnobotanical survey. There are also several antibiotics used in allopathic medicines that are no better than certain plants that studies reveal that have high potentials (Woodford and Ellington, 2006). Studies show that resistances to synthetic antibiotics are as a result of mutations which occur within the nucleic acids as

one of the major factors underlying evolution, providing the working material for natural selection.

Furthermore, allopathic synthetic medicine involves one or two active ingredients as opposed to plant extracts. In crude form, plant extracts for example; polar ones contain a cocktail of compounds which could run into hundreds. Some of these compounds may not be necessarily be active, but play roles as promoters, synergists, formulators, and carriers (Woodford and Ellington, 2006). The reactions leading to their activity may be more complex. This is why it is possible to use Phytomedicine and will continue to be used in folklore without worrying of failure chances due to development of resistance by the targeted organisms.

In this study *T. asiatica* leaves and fruits distillates were active an MBC of 3-5 µl/ml. against some fungi. In another study the chemicals showed potentials for control of protozoan such as *Trypanosoma cruzi* which cause Chagaa disease. This is because of the presence monoterpenes, Limonene and myrcene, that have antimicrobial activity (MIC) at 42.1 and 46.5 µl/ml (William *et al.*, 2007). The Ogiek similarly have been known to use the root bark and the steamed leaves of the same plant against malaria stomachache and chest-related conditions. *T. asiatica* are known for their antibacterial and antifungal activities (Meccia *et al.*, 2007).

Further activities of various sequential extracts of root and stem barks were observed against: *C.albicans*, *C. neoformas*, *T. mentagrophytes* and *M. gypseum* as indicated in the above tables. It has also been reported that euginol, commonly occurring in the three plants, has been used in the formation of musco adhesive tablets for the treatment of periodontal diseases (Jadher *et al.*, 2004). This validates the Ogiek traditional use of the

plant stems as tooth brushes and the incorporation of its root bark when preparing soup. It is believed that by its incorporation to the soup, it enhances the people's disease resistance. Furthermore, β -caryophyllene found in *S. biflora*, *L. javanica* and *T. asiatica* have been known to have cytoprotection of the non-steroidal anti-inflammatory properties (Tambe *et al.*, 1996). The two plants, *T. asiatica* and *S. biflora* are used as tonic drinks by the community. β -caryophyllene, found in all the three plants, have been known to contain cytoprotection of the non-steroidal anti-inflammatory properties (Tambe *et al.*, 1996). Two of the plants are used as tonic drinks by the community.

Essential oils obtained from *Lippia javanica* and *Satureia biflora* have been used for several purposes in many parts of Africa as a mosquito repellent and studies have proved that essential oils from *L. javanica* have very strong and lasting repellent activity against starved *Anopheles arabiensis* (Lukwa, 1994). There is also strong indication of its essential oils extracts acting as repellent in pest control of field and stored grain legumes (Tarus *et al.*, 2005). Previous studies on the essential oils had shown that the extracts were not active against certain Gram -ve bacteria like *E. coli*. In this study, however, it emerged that methanol extracts of this plant were active against all strains of *S. aureus* including MRSA and ORSA with MIC/MBC at 1.3 μ l/ml. Some variations in the activities of the oil are believed to be due to the impact of geographical and climatic differences (Lukwa, 1994). Traditionally, this plant is used to treat fresh wounds and stomach ailments and fever by the Ogiek. Its action might then be directly on the micro-organisms or indirectly affecting the immune system of the individuals (Viljoen *et al.*, 2005). *Satureia biflora* oils had varied activities in that it was effective against both Gram negative and Gram positive bacteria. Its oil possessed activities against *S. aureus*, *K. pneumoniae* and *E. coli* at 12 μ l/ml and its strains. Such activities may be attributed to different chemical composition of the oil as compared to *L. javanica*. The activities

confirm the use of the leaves and roots by the Ogiek community to combat upper respiratory tract ailments like colds, pneumonia, coughs and stomachache.

The mode of antimicrobial action of the oil may be due to the inhibition of respiration and disrupting the permeability of the cell wall structures. The other speculation on the enhanced efficacy of the oil is due to differential permeabilities as a result of molecular actions which have been prompted by adhesive activities of the oil molecules (Lukwa, 1994). Ultimately, even some Gram negative bacteria which possess complex cell wall structures that more often resist foreign molecules penetration will succumb to the oil.

CHAPTER SIX

PHYTOCHEMICAL SCREENING

6.1 Materials and methods used in phytochemical screening and gas chromatography (GC)/mass spectrophotometer (MS)

Different methods were employed to carry out phytochemical screening of the 16 plant species extracts that showed antimicrobial activities. It is known plants that are found to have antimicrobial activities have such metabolites like saponins, flavonoids, and anthroquinones. The compounds that were tested included: flavonoids, anthroquinones, anthrocyanins, tannins, saponins, coumarins, reducing sugars, alkaloids, polyoses, fatty acids and essential oils

The methods used are those contained in natural products (Ikan, 1991) to profile the classes of compounds. The chemicals used were of analytical grade (BDF Britain). Using wet bench method the presence of the following classes of compounds were determined:

6.2.1 Anthrocyanins

To about 4-5 ml of the plant methanol extracts in a test tube 5ml of conc. hydrochloric was added to maintain a pH of 3-4 and later treated with sodium hydroxide to change to pH 8-9 then added the acid to give a pH of 2.22. At every stage of pH change the colour of the mixture was observed. If the colour changed from blue to green then anthocyanin was present in the extract.

6.2.2 Flavonoids

The test of flavonoids was carried out on samples that were extracted with ethanol (Shinoda's reaction). After completely evaporating the solvent, about 1gm of the conc.

hydrochloric acid was added to the residue. The solution was then warmed up and the colour change observed. Positive results were that the colour changed to red on warming.

6.2.3 Anthroquinones

5 g of crude extract, previously extracted with methanol was further extracted with ethyl acetate, filtered and the filtrate evaporated to dryness. About 1g of the resulting extract was dissolved in 5 ml of benzene and 2 ml of 25% ammonium hydroxide added to it. The colour change was observed. If a red colour was obtained then an anthroquinone was present in the extract.

6.2.4 Tannins

2g of the methanol extract was treated with 1% gelatin solution and 10g of sodium chloride aqueous solution (5-10ml) and the colour change observed to confirm the presence or the absence of the product. About four drops of dilute ferric chloride was added to the test tube and the change of colour was observed. If the mixture turned green black, then tannins were present.

6.2.5 Coumarins

Using methanol extract, the test material was subjected to evaporation till all the solvent was removed. The residue (5 g), was then dissolved in distilled water (5 ml), and observed through UV lamp at 254 nm. The intensity of the light was increased after the addition of a few drops of 10% ammonium hydroxide and the fluorescence observed. If the UV fluorescence intensified and colour yellow observed, then coumarins were present.

2.6.6 Saponins

Water extracts amounting to (4 ml) was dissolved in distilled water and libermanns–Buchard's acetic anhydride + conc. H_2SO_4 at the ratio of 4:1 v/v added to it, then observed. Appearance of a greenish colour indicated the presence of saponins.

6.2.7 Starch

5 ml of methanol extract was treated to form a liquid with Lugol's solution and the colour changes observed. Instantaneous dark blue colour confirmed the presence of starch.

6.2.8 Reducing sugars

Reducing sugar was tested in methanol extracts by adding a few drops of Fehling's solution A to 5 ml of the extract, shaken to dissolve, followed by a few drops of Fehling's solution. B. The colour changes were then observed. If a brick red precipitate appeared, then the extract had reducing sugars.

6.2.9 Polyoses

To about 2 g of the water extracts all the water was evaporated and a few drops of conc. sulphuric acid added, followed by a few drops of alcoholic thymol. The colour changes were then observed. Appearance of red colour indicated presence of polyoses.

6.2.10 Alkaloids

After evaporating all the alcohol, 2 g of the residue was put in a test tube and macerated with 5 ml concentrated hydrochloric acid then apportioned in 1.5ml filtered and then basified with 10% ammonium hydroxide. Formation of a reddish ring indicated the presence of alkaloids.

6.2.11 Fatty acids

Both extracts of water or methanol of each sample were evaporated on a piece of “transparency paper”, and the transparency of the paper observed. If transparency was observed, then fatty acids were present.

6.3 Essential oils: Gas chromatography (GC)/mass spectrophotometer (MS)

GC and MS co-injection were performed on capillary gas chromatograph Hewlett Packard (HP) 5890 A series II, equipped with a split- less capillary injector system, cross-linked Hewlett Packard ultra 1-methyl silicone (50m length, 0.22 m internal diameter, 0.33 μ m carbowax film thickness) capillary column and a flame ionization detector coupled to Hewlett Packard 3396 series II integrator. Hydrogen gas was used as source of fuel, while nitrogen gas flowing at a speed of 0.8ml/min was used as carrier gas. GC-MS analysis was carried out on a Hewlett Packard 5790A series II Gas Chromatograph coupled to a VG Analytical Organic Mass Spectrophotometer (Micromass, United Kingdom) formally known as VG Biotech. The mass spectrophotometer (MS) was operated in the electron ionization (EI) mode at 70 electron volts (eV) and an emission current of 200 micro amperes (μ A). The temperature of the source was held at 180 °C and a multiplier voltage of 300 volts. The pressure of the ion source and MS detector were held at 9.4×10^{-6} and 1.4×10^{-5} milli-bar, respectively. The MS had a scan cycle of 1.5 seconds (scan duration of 1s and inter-scan delay of 0.5 s). The mass and scan ranges were set at mass charge ration (m/z) of 1-1400 and 38-650 respectively. The instrument was calibrated using heptacosafuorotributyl amine, $[\text{CF}_3-(\text{CF}_2)_3]_3\text{N}$. The column used for GC-MS was the same as the one described for GC above except that the film thickness was 0.5 μ m. The temperature programme was similar to the ones earlier described for GC. Helium was used as a carrier gas. In both GC and GC-MS, High Performance Liquid Chromatography (HPLC) grade dichloromethane (DCM) was used as a dilution solvent.

Results

From the analysis it was evident that several classes of compounds are present in the extracts in all the plants that were tested it was evident that they contained classes of compounds that are known to have medicinal values. This may explain why the plants are preferred as source of remedies by the Ogiek. Chromatographic studies also showed several compounds that qualify the plants to be of medicinal value both traditionally and in other forms of treatments (Tables 6.1 and 6.2).

Table 6.1: Essential oils from *T. asiatica*, *S. biflora* and *L. javanica* (GC)

<i>T. asiatica</i>	<i>S. biflora</i>	<i>L. javanica</i>
beta-phellandrene, 1,6-Octadien-3-ol,3,7-dimethyl-trans-Sabinenehydrate, 2-Cyclohexene-1-ol,methyl-4-(1-methyl-1-Octene,6-methyl-2-Cyclohexen-1-ol, Bicyclo[3.1.1]hept-2-ene-2-methanol, (S)-(+)-6Methyl-1-octenol,Caryophyllene oxide	<i>Mycene</i> , <i>Linalool</i> , <i>β</i> - <i>Caryophyllene</i> , Sabinene, Trans- Sabinene, Humulene, <i>Dihydro Tagotene</i> , 3,9-Epoxy P-Menthyl, Cyclohexyl-1-Pentyne	<i>Limonene</i> , <i>β</i> <i>Caryophyllene</i> , <i>Mycerene</i> , <i>Camphor</i>

Table: 6.2 Qualitative, wet bench tests, classes of compounds

Family	Species	Part Used	flavonoids	anthrocyanins	Anthroquinones	tannins	coumarins	saponins	steroids	starch	reducing sugars	polyoses	alkaloids	Fatty acids
Balsaminaceae	<i>I. tinctoria</i>	Root	+++	-	+		+++	-	-	-	+++	++	-	-
Euphorbiaceae	<i>C. robusta</i>	Root Bark	+	-	+++	++	-	-	-	-	++	+	-	-
Lamiaceae	<i>S. biflora</i>	Whole Plant	-	-	+++	-	+++	-	++	-	+	+++		-
Lusaciaceae	<i>G. buchananii</i>	Leaves/Stem Bark	+	++	+	+++	+	++						-
Meliaceae	<i>E. capensis</i>	Stem	+	-	-	-	++	+++	-	-	-	-	-	-
Meliaceae	<i>B. abyssinica</i>	Stem Bark	+++		+++	+++	+++	+++	+	-	-	++	-	-
Myrsinaceae	<i>E. schimperi</i>	Stem Bark	++	-	-	-	++	+	+	+	+	+++	+++	-
Oleaceae	<i>O. hochstetteri</i>	Stem Bark	+++	+	++	+++	++	+	++	-	+++	-	-	-
Oleaceae	<i>O. africana</i>	Stem Bark	+	++	-	++	+++	-	+++	-	-	+	-	-
Oliniaceae	<i>O. rochetiana</i>	Leaves	+++	+	++	+	+++	-	++	+	++	+	-	-
Proteaceae	<i>F. saligna</i>	Stem Bark	+ -	-	-	-	+	+	++	++	-	+++	+	-
Rhamnaceae	<i>R. prinoides</i>	Stem Bark	++	+++	++	+++	++	+++	++	+	++	++	+	-
Rhamnaceae	<i>S. myrtina</i>	Stem Bark	-	-	+									-
Rosaceae	<i>R. keniensis</i>		+++	-	+++	+++	-	+++	+	-	++	-	-	-
Rutaceae	<i>T. asiatica</i>	Root Bark	+++	-	-	+++	-	+++	+	-	+	++	-	-
Verbenaceae	<i>L. javanica</i>	Leaves	+++	-	-	+++	-	+++	+	-	+	++	-	-

Key: The qualitative (wet bench tests) were either positive (+) or negative (-) signifying presence or absence of the metabolites. The results are given in a summary Table 6.1 (wet bench analyses of some plants found to be antimicrobially active or are of new phytochemical interests).

+++ =High presence; ++ = Moderate presence; + = minimal presence; - = Absent

6.4 Discussion

There are various families which are of biological interest in the study. Curcubitaceae usually poses curcubutacin triterpenoids which are responsible for their bitter principles and various chemical characteristics (Table 6.2). Balsaminaceae, perhaps the smallest family studied generated interest since quite a majority of the compounds tested were present. Second, it is not cited anywhere in phytochemicals studies or any other studies of biomedical importance- rather, it is reported here for the first time.

Euphorbiaceae family is important in that its members are used as minor drugs (Dev, 1989). Anthroquinones and coumarins found to be abundant in the *C. robusta* which is a justification for its use as tonic and in culinary, as a carminative as well as stimulant. In Asian medicine *Chrozophora prostrata* is used in the treatment of leprosy, as a purgative, depurative and against coughs. *Phyllanthus emblica* is used for several ailments and is the most reliable natural source of vitamin C and may safely be used in curative cases (Singh, 1982). *Clusia robusta* although used as tonic in cookery, has some advantage because it can safely be used against some common Gram-positive and negative micro-organisms which are associated with everyday food contamination. Classes of compounds which were found in *C. Robusta* include majorly: anthroquinones, tannins, some flavonoids and polyphenols. Such compounds may be accountable for the efficacy of the plant extracts.

Flacourtaiceae is seldomly mentioned in literature but commonly used in the treatment of leprosy, thus seed oils from this family, particular species of *Hydnocarpus* which contain cyclic unsaturated compounds that are bactericidal towards *Micrococcus* spp which causes leprosy (Houghton, 2002). Other constituents of the family include cyanogenetic glycosides,

tannins and phenolic acids. These classes of compounds were not found in extracts of, *D. abyssinica* used in cooking meat and to treat stomachache and chest ailments, except anthroquinones. On profiling it was found that alkaloids, tannins and acids existed in the roots. This suggests the presence of biologically active metabolites that are responsible for the antimicrobial activities at the initial screening stage, although there was no activity during the MIC/MB/MFS studies.

The genus *Garcinia* from the family Clusiaceae (Gutifarea) has been used for curative purposes in South East Asia and elsewhere in Africa to treat various ailments that are bacteria related (Voravuthikunchai & Kitpitpit, 2005). From the studies, qualitatively, there were abundant occurrences of flavonoids, anthroquinones, coumarins, and polyses. Tannins were moderately present *G.buchananni*. Perhaps these are the classes of compounds that are responsible for its efficacy.

The family Lamiaceae has been used in Asian medicine and involves several species in the family. Besides steroidal compounds which are found in the family, essential oils are of universal occurrence and have been used in medication in Asia and elsewhere successfully (Rajan *et al.*, 2002). The presence of these oils, steroidal compounds confirm the ability of *S. biflora* to combat pneumonia and stomachache. Furthermore, the plant is also used in cooking and warm drinks by the local community of the Ogiek. It is also often boiled and the decoction taken as a hot cup due to its strong aroma from the Artimesinin, terpenes and other aromatics. It is believed that the plant's strong aroma is able to alleviate common cold. The essential oils are found in the leaves which are freshly harvested. Some of these include *O. basilcum* (L), *O. sanctum*, *M. piperita* all of which yield eugenol and caryophyllene

(Mehrotra and Rastogi, 1995). The extract had *S. biflora*, flavonoids and steroidal. Other classes of compounds found in the plant include reducing sugars, coumarins, saponins and anthocyanins. These groups of compounds are perhaps responsible for the plant's ability to combat bacteria. The same inferences conform to the other medicinal uses of *Dracocephalum moldavica* whose volatile oils are used in culinary, astringent and tonic. There are many plants like *Ocimum basilicum*, *Rosmarinus officinalis* and *Mentha piperita* whose extracts are used in perfumery and in the treatments of various antibacterial ailments (Willis, 1995) and are known to contain monoterpenes (Wagner, 1991).

Methanol stem bark extracts of *B. abyssinica*, Melianthaceae, exhibit activities against known fungi as seen in Table 6.1. Phytochemical profiling revealed the presence of flavonoids, tannins, and saponins. However, there was also an indication of the presence of steroidal, polyoses and reducing sugars. These may explain its uses as antihelminths and antibiotic both in livestock and humans (Kokwaro, 1993). Previous studies quoted in a text indicate the presence of bufadienolides which are cardioactive principles (Derrick, 2002) and thus explains why the plant is considered toxic in the traditional medicine in East Africa (Kokwaro, 1993).

E. capensis which is effective against bacteria was found to contain various flavonoids, tannins and saponins. Alkaloids, polyoses and steroidal were also present. Two members of the family, *Azadiracta indica* and *Melia azadiracta* have notable insecticidal properties as well as medicinal uses due to the presence of certain alkaloids and modified Terpenoids as indicated in literature (Bohnenstengel *et al.*, 1999). *E. capensis* medicinal qualities make it a highly priced medicinal plant amongst the Ogiek communities.

The family Myrsinaceae is not common in that it has few genera known in medicinal use elsewhere. *Embelia ribes* Burm. F. contains embelin, embolic acid and anthroquinones as reported in India (Rajan *et al.*, 2002). The therapeutic value for the plant in traditional India and Chinese medicine is that it is used against infertility, helminthes, chest infections, cholera, diarrhea, pneumonia, pleurisy and weak pulse (Asolkar *et al.*, 1992). For comparative purposes of members of the same genus, the Ogiek communities use *E. schimperi*, which was found to be containing flavonoids, anthrocyanins, anthroquinones, tannins, coumarins, saponins, steroidal and polyoses in reasonable amounts. Oleaceae family has two species which are used in the Ogiek communities. *O. africana* which is used against malaria fever and other related ailments. *O. hochstetteri* both contain anthroquinones, coumarins and polyoses in large quantities. Steroidals and reducing sugars were found in moderate quantities. Other than *O. hochstetteri* which yields vegetable and pharmaceutical oils, the plants are seldom mentioned elsewhere in (Wills, 2002).

The ability of *O. rochetiana* to act *in vivo* against most of the micro-organisms is attributed to the presence of prunaslin which is cytotoxic. The tests carried out on the methanol leaf extracts of the plant revealed the presence of flavonoids.

Proteaceae, one of the smallest families studied was represented by *F. saligna*. It emerged that the methanol extracts had *in vitro* activities against Gram +ve and -ve bacteria including those that have varied responses. Phytochemical screening revealed the presence of several coumarins, saponins, tannins, anthroquinones and alkaloids. These results corroborate the other studies on several genera which generally contain tannins, cyanogenetic compounds, arbutin, sugar alcohol, polygalitol and alkaloids. The presence of various classes of

compounds is possibly responsible for its ability to possess varied antibiotic activities (William, 2002). This fact confirms why the stem bark preparation is used as tonic and aphrodisiac by the Ogiek communities. *R. prinoides* and *S. myrtina* from the Rhamnaceae family both have flavonoids anthroquinones, coumarins and polyoses in their stem bark methanol extracts. However, starch and reducing sugars were also present. It has been reported that the family contains some important species which produce drugs like cascara purgative from *R. purshiana*.

The screening of *R. keniensis* of the family Rosaceae revealed the presence of flavonoids, anthroquinones and saponins but only a very small presence of steroidal and reducing sugars. The extracts showed good activities against both Gram –ve and +ve bacteria. Coumarins and alkaloids are reported to be rare (Williamson, 2002). The family is known for its several genera which are of medicinal importance. However, the genus *Rubus* is hardly found in any literature.

Toddalia asiatica, of Rutaceae family, whose methanol root bark extracts yielded flavonoids, coumarins, polyoses and alkaloids in good amounts saponins, starch, steroids and reducing sugars were present in small quantities. Studies of the plant extracts indicate that it can be used in abortion in India due to the presence of berberine, toddaline and toddanaline as selected chemical constituents (Choppra *et al.*, 1980; Rostogi and Mehrotra, 1993). The families are also known to contain triterpenoids as limonoids which are important in pharmaceutical chemistry due to their use in traditional medicine (Rajan *et al.*, 2002). The essential oils, like limnoides, were contained both in the leaves and in the fruits but with higher concentration in the fruits than in the leaves (Table: 6.1).

Lippia javanica from the Verbenaceae family had two categories. One was the methanol leaf extracts and two the steam distilled fresh leaf essential oils. The methanol leaf extracts contained all the tested classes of compounds (Table 6.2) except fatty acids and alkaloids that were missing. However, the extracts were not of any efficacy as pertains to its activity against gram +ve and -ve except on the test strains of *S. aureus*. Traditionally, amongst the Ogiek the infusion of the plant is used to treat fresh wounds, colds and stomach complaints. The variable antimicrobial activity might be due to chemotypes (Vijoen *et al.*, 2005). The oils found in freshly harvested and steam distilled leaves yielded limonene, camphor, β -caryophyllene, and myrcene which give the same results as the leaf when tested against the test organisms. It has been observed that geographical location and time of plant collection has a lot of influence on the type and quantities of the oil collected through steam distillation of the plant leaves (Samie *et al.*, 2005).

CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

This study set out with the objectives to: document the ethnomedicine of the Ogiek people, document the plant medicines used by the Ogiek. The community has empirical and vast knowledge in the use of the forests and forest products. Observations revealed that there are wanton and excessive environmental degradations which have led to drastic reduction in the biodiversity in the study area. Hence ascertain plant species are rare to find with the Mau forest complex and elsewhere. Such species are thus endangered

Bioassay of the extracts from 16 of the 49 plant species studied showed that some of the species possess promising antimicrobial activities that justify their continued use by the community. *R. keniensis* and *I. tinctoria* showed activities although they have never been studied anywhere. Three other plants that are; *B. abyssinica*, *F. saligna* and *T. asiatica* all have antimicrobial on both fungi and bacteria. This could be the reason for their continued use by the community and the reason for their scarcity.

Some of the plants used possess oils that have medicinal value. They were: *T. asiatica*, *S. biflora*, and *L. javanica*. Oils extracted from the leaves and fruits of *T. asiatica* showed high activities against both human pathogenic fungi bacteria. *S. biflora* whole plant distillate showed high activities against pathogens. This supports and justifies their continued use as sources of medicines by the community.

7.2 Recommendations

The study has demonstrated that further studies can be carried concerning the medicinal plants used by Ogiek Community and the following recommendations came up;

- (i) Documentation using other methods, other than ethnomedical approach should be employed to close the data gaps.
- (ii) Both *in situ* and *ex situ* conservation efforts are *introduced* in the area to counter the fast increasing population that is encroaching the fragile habitats of these biologicals.
- (iii) An exclusive reserve is created since it will allow natural regeneration of the ecosystem to take place thus the restoration of degraded areas. Failure to do so will lead to drastic reduction in the floral diversity which would otherwise be used in future studies.
- (iv) A comprehensive natural resource inventory, type on the plant species used by the people and the methods employed in their exploitation be made so that a holistic approach be employed for sustenance of the diversity.
- (v) The plants which did not show any bio-activities against the test pathogens be further studied to ascertain their efficacies and the plants that showed bioactivities be studied *in vivo* using small laboratory animals to validate their efficacies.
- (vi) An interdisciplinary approach is employed in studying the community's ethnomedicine involving the pharmaceutical industry for purposes of new drug discovery and development.

- (vii) There should be a proper epidemiological record keeping so as establish the efficacy of the drugs used in traditional systems on endemic diseases and the newly emerging and reemerging ones.
- (viii) The toxicity of plant medicines to human and livestock be carried out by laboratory cytological evaluations using small animal models.
- (ix) More organisms should be covered in future studies so as to leave no doubt on the validation of the efficacy of such plants.
- (x) For purposes of scientific progress, phytochemists should be carry out comprehensive elucidation of the active principles. Thereafter, pharmacists may work on the several combinations and cocktails which could be effective and used in the manufacture of new drugs to combat the maladies currently afflicting humankind
- (xi) Further assay these sequential extracts against some common human pathogenic fungi and profile the classes found in the biologically active plants.
- (xii) Several plants that were found to contain essential oils of reasonable efficacies against known human and animal pathogens, further researches be carried on their possible incorporation into toiletries, and other oral medicines for pursoses of pharmaceutical uses.

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Appendix I

ETHNOBOTANICAL DATA COLLECTION SHEET

Collection number | _____

Date

Genus species variety

Local name

Ethnic community

Locality Sub location Location

Division District

Altitude Longitude

Equipment used GPS () Map ()

Specify

Soil type

General description of the study area

Habit tree () shrub () herb ()

Climber () grass () vine ()

Others specify

Height Diameter

Other notes on plant's appearance (leaves, flowers, ~~fruit colour, seeds etc~~)

Fruit type Drupe () Nut () Achene () Caryopsis ()

Pod () Berry () others specify

Local name

Translation _____

Habit/vegetation _____

Associated species _____

Uses _____

If medicinal, state diseases/symptoms used for _____

Any other use _____

Part of the plant used Bark () Root () Leaves ()

Others specify

Harvesting method _____

Method of preparation _____

Any other information

Informant's name _____

Gender _____

Age

Thank you very much

APPENDIX II
MAP OF THE STUDY AREA

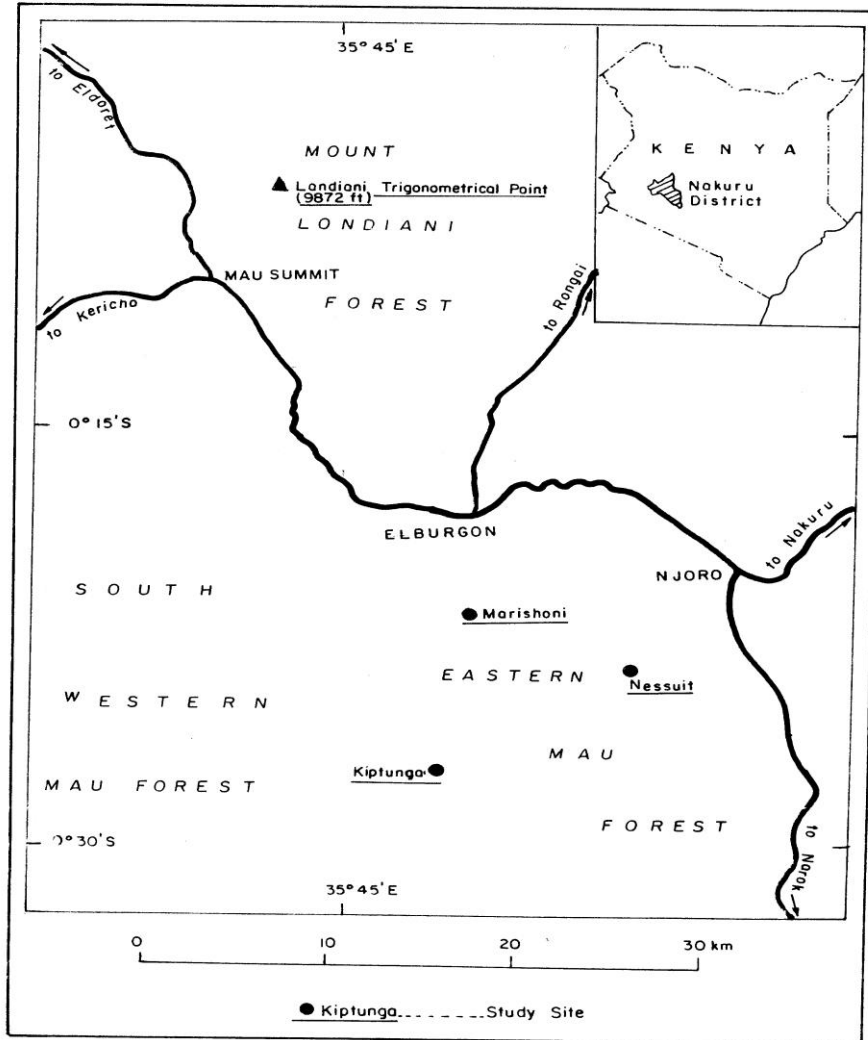


Figure 1: The map of the study areas in the Mau Complex
KEY: □ Road map, ▲ Trigonometric point

APPENDIX III

**EFFICACY OF CERTAIN MEDICINAL PLANTS FROM THE OGIEK
COMMUNITIES AGAINST MICROBIAL RELATED CONDITIONS. (Preliminary
studies)**

Observation made after 24 hours of incubation of the Plates with six organisms obtained from Aga Khan Hospital and NPHLS at 37 degrees

Drug/ extract	Micro organisms					
	<i>K. pneumoniae</i>	<i>S.aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>E.faecalis</i>
<i>E. schimperi</i>				+	+	+
<i>S. biflora</i>	+				+	
<i>S. mauense</i>					+	+
Kosisito	+				+	
<i>S.didymobotyra</i>					+	
<i>E.capensis</i>			+			
<i>O. africana</i>			+			
<i>Z. scabbra</i>		+				
<i>Z. chalybeum</i>			+			
Kabageriet		+				
Korapariet		+				
<i>F. saligna</i>		+				

(+) - indicate effective

41.7% of the plant extracts were inhibitory to *salmonella typhi* while 33.3% of the effective plant extracts were able to inhibit growth of *Staphylococcus aureus*. The plant extract observed to be effective on most bacteria was from Sachuonet plant. This was effective on *E. coli*, *S.typhi* and *E. faecalis* bacteria.

Laboratory results from the plant extracts tested against various bacteria species showed that,

Plant extract	Bacteria inhibited				
	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>
<i>S. myrtina</i>	12.3	15.2	14		
<i>Faurea saligna</i>	16	12	16.3	8	
<i>O. rochetiana</i>	12	23	16.4	12	10.6

<i>Olea africana</i>				17	
<i>Olea europaea</i>	22			14	
<i>Rubus keniensis</i>	22	14		15	
<i>Clutia abyssinica</i>	11	15		16	10.4
<i>Embelia schimperi</i>	16			9.8	

The average Plant extracts bacterial inhibition

S. aureus had its growth inhibited by plant extracts but mainly from *Olea africana*. The highest inhibition was from extract of *O. rochetiana* on the *K. pneumoniae* bacteria.

Stem bark of (*Bersama abyssinica*)

Methanol extraction results

There was a significant effect of plant extract concentration on zones of inhibition by *Klebsiella* ssp. $P < 0.05$.

One-way ANOVA: zones of inhibition versus drug concentration

Analysis of Variance for zones of

Source	DF	SS	MS	F	P
Drug con	3	293,792	97,931	175,40	0,000
Error	20	11,167	0,558		
Total	23	304,958			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	
0.02	6	10,000	0,632	(-*)
0.04	6	15,500	0,837	(-*)
0.08	6	15,833	0,753	(-*)
0.16	6	19,833	0,753	(-*)

Pooled StDev = 0,747 10,5 14,0 17,5 21,0

The higher concentration (0.16 gm) had a significantly higher inhibition effect. There was, however, no significant difference in concentration 0.04 and 0.08 gm.

It did not matter whichever species the bacteria *Klebsiella* was, as the study realized no significant difference on the effect of the plant extract inhibition.

One-way ANOVA: zones of inhibition versus *Klebsiella ssp*

Analysis of Variance for zones of

Source	DF	SS	MS	F	P
Klebsiella	5	8,2	1,6	0,10	0,991
Error	18	296,8	16,5		
Total	23	305,0			

There was no significant effect, $p > 0.05$, of plant extract concentration on zones of inhibition neither of *Staphylococcus aureus* nor on the various strains of the organism.

One-way ANOVA: zones of inhibition of *Staphylococcus* versus drug concentration

Analysis of Variance for zones of

Source	DF	SS	MS	F	P
drug con	3	2,000	0,667	2,95	0,053
Error	24	5,429	0,226		
Total	27	7,429			

One-way ANOVA: zones of inhibition of *S.a* versus *staph. ssp*

Analysis of Variance for zones of

Source	DF	SS	MS	F	P
staph. s	6	0,929	0,155	0,50	0,801
Error	21	6,500	0,310		
Total	27	7,429			

Stem bark of *Scutia myrtina* family Rhamnaceae

The bark of *Scutia myrtina* was tested against *Pseudomonas* bacteria.

The result showed;

There was a significant effect, $p < 0.05$, of the plant extract concentration on the inhibition of *Pseudomonas aeruginosa* bacterial growth. Concentration with 0.16gm extract had a significantly higher inhibition effect. There was no significant difference in inhibition caused at concentration 0.02gm with 0.04gm.

The effect was not varying with the strains of the bacteria.

One-way ANOVA: *P. aeruginosa* inhibition versus concentration

Analysis of Variance for *P. aeruginosa*

Source	DF	SS	MS	F	P
--------	----	----	----	---	---

Concentr	3	77,458	25,819	123, 93	0,000
Error	20	4,167	0,208		
Total	23	81,625			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	
0,02	6	8,000	0,000	(-*--)
0.04	6	8,000	0,000	(-*--)
0.08	6	10,167	0,408	(--*-)
0.16	6	12,333	0,816	(-*--)

Pooled StDev = 0,456 9,0 10,5 12,0

Scurtia myrtina extract tested against *Klebsiella* species

The plant extract from *S. myrtina* concentration had significant effect, $p < 0.05$, on *Klebsiella* bacteria. Concentration of 0.08gm and 0.16gm of the extract significantly inhibited more bacterial growth than the 0.04gm concentration. However, there were no significant differences in inhibition at 0.08 and 0.16gm concentrations. Inhibition was the same in all the *Klebsiella* strains.

One-way ANOVA: *Klebsiella* zones of inhibition versus concentration of *S.mrytina*

Analysis of Variance for s.m.inhi

Source	DF	SS	MS	F	P
Concentr.	3	73,200	24,400	81, 33	0,000
Error	16	4,800	0,300		
Total	19	78,000			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	
0.02	5	11,000	0,000	(--*---)
0.04	5	11,200	0,447	(---*--)
0.08	5	14,600	0,894	(--*---)
0.16	5	15,200	0,447	(--*---)

Pooled StDev = 0,548 10, 5 12,0 13,5 15,0

Scurtia myrtina extract tested against *Staphylococcus aureus*

Plant extract concentration significantly, $p < 0.05$, inhibited *S.aureus* bacterial growth. It was noted that there was no significant difference in inhibition at 0.04gm with 0.08gm extract concentrations. The differences did not vary with the *Staphylococcus aureus* bacterial strains.

One-way ANOVA: s.m.inhibition versus concentration of *S. myrtina*

Analysis of Variance for s.m.inhi

Source	DF	SS	MS	F	P
Concent.	3	64,00	21,33	18,67	0,000
Error	28	32,00	1,14		
Total	31	96,00			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----
0.02	8	10,000	2,138	(---*---)
0.04	8	12,000	0,000	(---*---)
0.08	8	12,000	0,000	(---*---)
0.16	8	14,000	0,000	(---*---)
				-----+-----+-----+-----

Pooled StDev = 1,069 10, 5 12, 0 13, 5

Faurea saligna extract tested against *Pseudomonas*

The inhibition of *Pseudomonas* bacteria increased with an increase in plant extract concentration. Higher concentration of the extract inhibited more on the growth.

One-way ANOVA: P.a inhibition versus concentration of *F.saligna*

Analysis of Variance for p.inhibi

Source	DF	SS	MS	F	P
Concentr	3	100,0000	33,3333	*	*
Error	16	0,0000	0,0000		
Total	19	100,0000			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev		
0.02	5	10,0000	0,0000	*	
0.04	5	12,0000	0,0000		*
0.08	5	14,0000	0,0000		*
0.16	5	16,0000	0,0000		*

-----+-----+-----+-----

Pooled StDev = 0,0000 12,0 14,0 16,

Toddalia asiatica extract tested against *S.aureus*

Plant extract concentration had a significant effect, $p < 0.05$, on inhibition of all the tested strains of *S.aureus*

One-way ANOVA: inhib. S.a inhibition versus concentration of *T.asiatica*

Analysis of Variance for inhibition. S

Source	DF	SS	MS	F	P
Concentr	2	378,296	189,148	334,89	0,000
Error	24	13,556	0,565		
Total	26	391,852			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev		
0.04	9	7,222	0,667	(-*-)	
0.08	9	12,667	0,866		(-*-)
0.16	9	16,333	0,707		(*-)

-----+-----+-----+-----

Pooled StDev = 0,752 9,0 12,0 15,0

Olinia rochetiana leaf extract tested against *E.faecalis*

Using leaf extract from this plant, the test result showed that the higher concentration of the extract had the most inhibition effect on the growth of *E.faecalis* bacteria

One-way ANOVA: *E.faecalis* inhibition versus conc. of *O.rochetiana*

Analysis of Variance for O. roche

Source	DF	SS	MS	F	P
--------	----	----	----	---	---

Conc. of	3	150,0000	50,0000	*	*
Error	16	0,0000	0,0000		
Total	19	150,0000			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----+--
0.02	5	13,0000	0,0000	*
0.04	5	16,0000	0,0000	*
0.08	5	19,0000	0,0000	*
0.16	5	20,0000	0,0000	*

-----+-----+-----+-----+--

Pooled StDev = 0,0000 14,0 16,0 18,0 20,0

Olinia rochetiana leaf extract tested against *Pseudomonas ssp.*

Using leaf extract from *O. rochetiana* plant, the test result showed that the higher concentration of the extract had the most inhibition effect on the growth of *Pseudomonas spp.* bacteria

One-way ANOVA: inhibition of P.a versus conc. of o.r

Analysis of Variance for inhibiti

Source	DF	SS	MS	F	P
Conc. of	3	20,00000	6,66667	*	*
Error	16	0,00000	0,00000		
Total	19	20,00000			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----+--
0.02	5	10,0000	0,0000	*
0.04	5	10,0000	0,0000	*
0.08	5	12,0000	0,0000	*
0.16	5	12,0000	0,0000	*

-----+-----+-----+-----+--

Pooled StDev = 0,0000 10,20 10,80 11,40 12,00

Using leaf extract from *O. rochetiana* plant, the test result showed that there was no significant effect of change of the extract concentration on inhibition effect on the growth of *Salmonella ssp.* Bacteria. However, the effect of the plant extract significantly, $p < 0.05$, varied with the *Salmonella* species

One-way ANOVA: *Salmonella typhi*. Inhibition versus *Salmonella* species

Analysis of Variance for Sal. INH

Source	DF	SS	MS	F	P
S.specie	9	9,367	1,041	5,20	0,001
Error	20	4,000	0,200		
Total	29	13,367			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----+-
S. paratyphi	3	10,000	0,000	(-----*-----)
S. typhi	3	11,333	0,577	(-----*-----)
S. typhi	3	10,000	0,000	(-----*-----)
S. typhi	3	11,333	0,577	(-----*-----)
S. type R	3	10,667	1,155	(-----*-----)
S.t(mim)	3	10,000	0,000	(-----*-----)
S. enteridis	3	11,000	0,000	(-----*-----)
S.t type A	3	10,000	0,000	(-----*-----)
S.group d.k	3	10,000	0,000	(-----*-----)
1S.t. N2202	3	10,000	0,000	(-----*-----)

-----+-----+-----+-----+-

Pooled StDev = 0,447 9, 80 10, 50 11, 20 11, 90

Using leaf extract from *O. rochetiana* plant, the test result showed that there was a significant effect $p < 0.05$, of change of the extract concentration on inhibition of the growth of *Klebsiella ssp.* Bacteria. Higher concentration of the plant extract inhibited more of the bacterial growth.

One-way ANOVA: *Klebsiella* spp. inhibit versus plant concentration

Analysis of Variance for Kleb. In

Source	DF	SS	MS	F	P
Plant co	3	268, 5000	89, 5000	*	*

Error	20	0,0000	0,0000
Total	23	268,5000	

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----+--		
0.02	6	14,0000	0,0000 *			
0.04	6	19,0000	0,0000 *			
0.08	6	21,0000	0,0000 *			
0.16	6	23,0000	0,0000 *			
-----+-----+-----+-----+--						
Pooled StDev =		0,0000	15,0	17,5	20,0	22,5

Using leaf extract from *O. rochetiana* plant, the test result showed that there was a significant Effect $p < 0.05$, of change of the extract concentration on inhibition of the growth of *Staphylococcus aureus* strains. However, a significantly high inhibition was experienced from concentration of 0.08 gm. There was no significant variation in inhibition within the *S aureus* species

One-way ANOVA: *Salmonella* spp. inhibition versus extract concentration

Analysis of Variance for Sa inhib

Source	DF	SS	MS	F	P
Extract	3	45,275	15,092	23,93	0,000
Error	36	22,700	0,631		
Total	39	67,975			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----	
0.02	10	17,300	0,823 (---*---)		
0.04	10	16,300	0,483 (---*---)		
0.08	10	19,000	1,054 (---*----)		
0.16	10	16,500	0,707 (---*----)		
-----+-----+-----+-----					
Pooled StDev =		0,794	16,8	18,0	19,2

Using leaf extract from *O. rochetiana* plant, the test result showed that there was a significant effect $p < 0.05$, of change of the extract concentration on inhibition of the growth of *E. coli*. However, a significantly high inhibition was experienced from lower concentrations (0.02 and 0.04gm) of the extract. There was no significant variation in inhibition within the *E. coli* strains.

One-way ANOVA: *E.coli* inhibition versus *O.rochetiana* concentration

Analysis of Variance for E.coli i

Source	DF	SS	MS	F	P
<i>O.rochetiana</i>	3	81,00000	27,00000	*	*
Error	32	0,00000	0,00000		
Total	35	81,00000			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----	
0.02	9	15,0000	0,0000	*	
0.04	9	15,0000	0,0000	*	
0.08	9	12,0000	0,0000	*	
0.16	9	12,0000	0,0000	*	
-----+-----+-----+-----					
Pooled StDev =		0,0000	13,0	14,0	15,0

Using stem bark extract from *O. africana* plant, the test result showed that there was a significant effect $p < 0.05$, of change of the extract concentration on inhibition of the growth of *S. aureus*. Higher inhibition was experienced in higher concentrations of extract.

One-way ANOVA: *Sa* inhibition versus *O.africana* concentration.

Analysis of Variance for *Sa* inhibition

Source	DF	SS	MS	F	P
O.Afric.	2	262,467	131,233	1181,10	0,000
Error	27	3,000	0,111		
Total	29	265,467			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	+-----+-----+-----+-----
0.04	10	10,000	0,000 (*)	

Total 23 509, 8333

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	
0.02	6	10,000	0,000	(*
0.04	6	15,667	0,516	(*
0.08	6	20,000	0,000	*)
0.16	6	22,000	0,000	(*

Pooled StDev = 0,258 10,5 14,0 17,5 21,

Root bark extract from *Clutia abyssinica* plant, the test result showed that there was a significant effect $p < 0.05$, of change of the extract concentration on inhibition of the growth of *Pseudomonas* ssp. Higher concentrations of the extract inhibited significantly high bacterial growth more than lower concentrations.

One-way ANOVA: *P* spp. inhibition versus *C* .*abyssinica* conc.

Analysis of Variance for P.spp. i

Source	DF	SS	MS	F	P
c.abysyi	3	43,75000	14,58333	*	*
Error	16	0,00000	0,00000		
Total	19	43,75000			

Individual 95% CIs For Mean

Based on Pooled StDev

Level	N	Mean	StDev	
0.02	5	7,0000	0,0000	*
0.04	5	9,0000	0,0000	*
0.08	5	10,0000	0,0000	*
0.16	5	11,0000	0,0000	*

Pooled StDev = 0,0000 7,2 8,4 9,6 10,8

Root bark extract from *Clutia abyssinica* plant, the test result showed that there was a significant effect $p < 0.05$, of change of the extract concentration on inhibition of the growth of *Klebsiella pneumoniae*. Higher concentrations of the extract inhibited more of the bacterial growth. However

there was no significant difference in concentrations 0.04 and 0.08gm. it was noted that there was no significant difference in inhibition on the various strains of *K. pneumoniae* even though, *K. oxytoca* was more inhibited than the other strains.

One-way ANOVA: *K. pneumoniae* inhib. Versus *C.abbyssinica* Concentration

Analysis of Variance for *K. pneumoniae*

Source	DF	SS	MS	F	P
C.a Conc	3	59,429	19,810	47,54	0,000
Error	24	10,000	0,417		
Total	27	69,429			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----+---		
0.02	7	10,429	0,535	(---*--)		
0.04	7	11,571	0,787	(--*--)		
0.08	7	12,143	0,378	(--*--)		
0.16	7	14,429	0,787	(--*---)		
-----+-----+-----+-----+---						
Pooled StDev =		0,645	10, 5	12, 0	13, 5	15, 0

Stem bark extract from *Embelia schimperi* plant, the result showed that there was a significant effect $p < 0.05$, of change of the extract concentration on inhibition of the growth of *Staphylococcus* ssp. Higher concentration (0.1gm) inhibited the bacterial growth more than the subsequent lower concentrations.

One-way ANOVA: *S. aureus* inhibit. Versus *E.schimperi* concentration

Analysis of Variance for *S. a* inhibition

Source	DF	SS	MS	F	P
<i>E. schimperi</i>	3	170, 96	56, 99	12, 43	0,000
Error	24	110, 00	4, 58		
Total	27	280, 96			

Individual 95% CIs for Mean

Based on Pooled StDev

Level	N	Mean	StDev	-----+-----+-----+-----+---
0.1	7	16,286	2,928	(----*-----)

0.05 7 13,143 1,464 (-----*-----)

0.025 7 10,857 1,952 (----*-----)

0.0125 7 9,857 1,952 (-----*-----)

---+-----+-----+-----+---

Pooled StDev = 2,141 9,0 12,0 15,0 18,

Plant	Microorganism inhibited	Bacterial inhibition by the extract (mm) at four different extract concentrations			
		0.02	0.04	0.08	0.16
<i>R. keniensis</i>	<i>Pseudomonas ssp.</i>	10	15.7	20	22
<i>C. abyssinica</i>	<i>Pseudomonas ssp</i>	7.7	9	10	11
<i>R. keniensis</i>	<i>E. faecalis</i>	-	-	7	10
<i>R. keniensis</i>	<i>Klebsiella ssp</i>	7	10	12	14
<i>C. abyssinica</i>	<i>Klebsiella ssp</i>	10.4	11.6	12.1	14.6
<i>R. keniensis</i>	<i>Staphylococcus ssp</i>	7	12	13	15
<i>C. abyssinica</i>	<i>Staphylococcus ssp</i>	7	7	11	15.7
<i>C. abyssinica</i>	<i>Enterococcus faecalis</i>	-	7	10	12.3
<i>C.abyssinica</i>	<i>Salmonella sp</i>	-	7	7.7	10.4

MIC accuracy was checked against standard antibiotics gentamycin for bacteria and fluconazole for fungi. Sterility of media and growth of the organism was controlled by use of broth only in negative control tubes and broth plus microorganism in question in positive control tubes. All the controls were subjected to the same conditions as the tests.