PHYTOCHEMICAL SCREENING, ANTIMICROBIAL ACTIVITY AND ACUTE TOXICITY OF *ALOE TURKANENSIS*

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DECLARATION

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

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DEDICATION

To my parents Esther and Joseph for their committed parenthood, my son Achilles, my wife Damaris, my siblings Peter, Lilian and Shiro for their support and encouragement in academic endeavors.

"…For I know the plans I have for you, 'declares the Lord', plans to prosper you and not to harm you, plans to give you hope and a future"……..Jeremiah 29:11

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LIST OF ACRONYMS AND ABBREVIATIONS

BECA:	Biosciences Eastern and Central Africa
BSLT:	Brine Shrimp Lethality Test
CBS:	Central Bureau of Statistics
FAO:	Food and Agriculture Organization
FEC:	Feacal Egg Count
GOK/LWF:	Government of Kenya/Lutheran World Federation
GTZ:	Germany Technical Cooperation
HDP:	Herbal Drug Preparation
HIV/AIDS:	Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome
HPLC:	High Pressure Liquid Chromatography
ICBN:	Internal Conference on Botanical Identification
KIHBS:	Kenya Integrated Household Baseline Survey
KNBS:	Kenya National Bureau of Statistics
KWS:	Kenya Wildlife Service
MHB & MHA:	Mueller Hinton Broth & Muller Hinton Agar

MIC:	Minimum Inhibitory Concentration
NCAPD:	National Coordinating Agency for Population Development
PBS:	Physiological Buffer Saline
PHPT:	Public Health, Pharmacology and Toxicology
PWHC:	Price Water House Coopers
TH:	Traditional Healers
TMDDP:	Traditional Medicine and Drug Development Programme
TMPs:	Traditional Medical Practitioner
WHO:	World Health Organization

ABSTRACT

The use of plants to cure diseases and relieve physical sufferings started from the earliest times of mankind's history. Due to antimicrobial resistance becoming a global problem with far reaching implications for the survival of human race, efforts are continuously made to overcome this. Current efforts include research in finding new and safe antimicrobials from plants. Among these plants is *Aloe turkanensis* which is a widely used medicinal shrub in Kenya. The plant grows naturally in Turkana and West Pokot counties where it is used in ethnomedicine and ethinoveterinary medicine. It is also cultivated in Baringo, Isiolo, Laikipia counties and natural resource conservatory institutes.

Traditional Medical Practitioners (TMPs) in Turkana County uses herbal preparation of the plant to manage various ailments despite lack of proper scientific evidence on efficacy, safety and sustainability of the plant. In this study, *in-vitro* antimicrobial, phytochemical and toxicity profile of a naturally growing and cultivated *Aloe turkanensis* plant was determined. This was done in order to find out whether the plant has the presumed bioactivity and also to explore the possibility of conservation through cultivation in other ecological zones which would help in meeting the demand of the consumers incase the hypothesis was true.

An ethno botanical study on the use of *Aloe turkanensis* was done in Turkana County where qualitative data was collected through observation, photographing and questionnaires. A sample of the naturally growing whole

plant was harvested in Natira sublocation, in Turkana County in February 2012 after identification by Aloe-working group herbalists who voluntarily provided information on its medicinal uses. Botanical identification was done at Kenya Forest Service Research Centre in Karura where a voucher specimen was deposited. A cultivated species of *A. turkanensis* was harvested at Karura forest courtesy of the Kenya Forest Service Research Center. The naturally growing and cultivated plants ecotypes were treated independently where cold maceration using 70% methanol and distilled water was used for extraction. Bioassays to determine the effects of the extracts from the two plants ecotypes on brine shrimp and selected bacterial and fungal cultures were done. The extracts were tested for *in-vitro* activity against standard cultures of *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC25923), *Pseudomonas. aeroginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and a human infection clinical isolate of *Candida albicans*.

The data from laboratory experiments was analyzed using descriptive statistics and analysis of variance (ANOVA) and it was found that methanol extracts of naturally growing *Aloe turkanensis* plant inhibited the growth of *B. cereus* (100 mg/ml), *S. aureus* (100 mg/ml), and *P. aeroginosa* (200 mg/ml) with mean diameters of inhibition zones for *S. aureus* and *B. cereus* being 18.5±0.7 mm and 16.5±0.7 mm, respectively. Aqueous extract of naturally growing *A. turkanensis* inhibited the growth of *B. cereus and S. aureus* at a Minimum Inhibitory Concentration of 200 mg/ml and 50 mg/ml respectively with mean

diameter of inhibition zones for *S. aureus* and *B. cereus* being 19.75±1.0 mm and 11.5±0.0 mm respectively.

Methanol extracts of *cultivated A. turkanensis* plant inhibited the growth *B. cereus* (100 mg/ml), *S. aureus* (50 mg/ml), *E. coli* (400 mg/ml) and *P. aeroginosa* (200 mg/ml) with mean diameters of inhibition zones for *S. aureus* and *B. cereus* being 18.5±0.7 mm and 11.5±0.0 mm respectively.

On analysis of variance, it was noted that there was a significant difference in antibacterial activity between the naturally occurring plant and the cultivated one ($p<0.05$)

Phytochemical screening showed the presence of alkaloids, terpenoids, steroids, quinones, saponins and tannins in the plant extracts. The extract was found to be non-toxic at a concentration of 1000 µg/ml with a 100% survival of Brine Shrimp larvae.

The results of this study show that methanol and aqueous extracts of *A. turkanensis* growing naturally in the study areas had more phytochemicals compared to a cultivated plant. These phytochemicals inhibit the growth of bacteria making it an effective ethnomedicine when administered at appropriate doses. However, there is need for further studies to validate the *in-vivo* bioactivity of the plant and generate adequate toxicological data to support its ethno medicinal use, conservation, value chain of its products and its widespread use as herbal remedy.

CHAPTER ONE: INTRODUCTION

1.1 Background

Bacterial resistance to antibiotics is increasingly becoming a concern to public health. Most of the antibiotic agents in the market are failing to bring an end to many bacterial infections due to super resistant strains. Scientists have therefore continued to search for new antimicrobial agents, either by design and synthesis of new agents or through the search of natural sources. Herbal medications in particular have seen a revival of interest due to a perception that there is a lower incidence of adverse reactions to plant preparations compared to synthetic antimicrobial drugs. This together with the reduced costs of plant preparations makes the search for natural therapeutics from plants an attractive option (Cock, 2008).

Medicinal plants are a reservoir of principal therapeutic compounds of novel structure (Williams, 2006). Many drugs used in western medicine today are derived from plants and other natural resources (Ramalivhana *et al.*, 2010). Medicinal plants are traded for the extraction of their active ingredients (Lubia *et al.,* 2008). Plants have been studied to evaluate the effectiveness of traditional medicines used in the treatment and management of bacterial, fungal and viral infections (Hostettman and Merson, 1994; Mathabe *et al*., 2006; Buwa and Staden, 2006; Bessong and Obi, 2006; Rangasamy *et al*., 2007).

Another important characteristic of a medicinal plant is related to toxicity which in a number of species, the toxicity profile is not well documented. In a review of 406 articles on medicinal species in Plant Resource of Tropical Africa database (PROTA 11) it was noted that 33% of the documented plants are not known whether they are toxic or not; 32% are considered highly toxic (many plant species in *Apocynaceae, Menispermaceae* and *Loganiaceae* family) while, another 30% of the species are considered moderately toxic (Schmelzer *et al.*, 2010). This indicates that more than 60% of the species treated in PROTA 11 should be used with much precaution.

Therefore, in order to utilize plants as medicines, man has had to discriminate between poisonous and non-poisonous materials in his environment. Several toxicity studies on medicinal plants extract have been documented and include *in-vivo* and herbal-drug interactions (Gathumbi, 1995; Gathumbi *et al.,* 2000; Wojcikowski *et al.,* 2004). World Health Organization (WHO) has outlined a few guidelines on the responsibilities of all Africa nations for the realistic development of Tradition African Medicine, in order to sustain our health agenda and perpetuate our culture. This strategy provides for the institutionalization of traditional medicine in health care systems of the member states of the WHO African Region (WHO, 2003).

The gradual extinction of the forests and the inevitable disappearance of the aged Traditional Medicinal Practitioners (TMPs) pose an impending

deadline for researchers to learn, acquire and document our medical cultural endowment for the benefit of all Africans and indeed the entire humanity. For this reason, there is need to involve TMPs in national healthcare systems through training and evaluation of effective remedies, as they are a large and influential group in primary healthcare (Akerele, 1987; Anyinam, 1987; Good, 1987).

This study addresses a gap created by inadequate technical specifications and quality control standards for *Aloe turkanensis* plant an ethnomedicine used by Turkana community. The lack of such standards is also a major barrier to regional and international trade and an important reason why traditional medicine has not been integrated into African primary health care as it should be. For many promising species, the elaboration of adequate technical specifications and quality control standards is required. For instance, *Aloe turkanensis* exudates lacks quality control standard (Schmelzer *et al.,* 2010) despite the fact that plants in the family Aloaceae have been reported to treat many ailments among many communities (Wren, 2008; Kokwaro, 2009). *Aloe turkanensis* particularly has been widely used in management of wounds among other ailments (Bosch, 2006). This study documents work on the antibacterial, antifungal and toxicity profile of *Aloe turkanensis*.

Another important aspect addressed by this study is the issue of sustainable utilization of medicinal plants. Concern has been raised locally

and internationally about levels and impact of the exploitation to wild populations. Unsustainable harvesting of plants poses many threats ranging from overexploitation to ecological imbalance and possible loss of the species of interest. For instance, overexploitation of the commercial *Aloe* species in Kenya prompted a Presidential decree in 1986 banning harvesting of aloes from the wild for commercial purposes. In Kenya, Wildlife Conservation and Management Act, 1989 mandates KWS to formulate policies and regulations to govern conservation of all fauna and flora. One of the important conservation measure cited is propagation of plant biological materials (Lubia *et al.,* 2008). Scientists should therefore invest time in unveiling whether such propagated biological materials have a similar biological activity as the wild fauna considering the effect of ecological zones on quantity and quality of yielded secondary metabolites (Kerry *et al.*, 2009)

Prior to laboratory experiments of this study, an assumption (based on ethnobotanical survey) was made that aqueous and methanol crude extract of *Aloe turkanensis* have antibacterial and antifungal activities and that the extracts are safe for ethnomedicinal use at doses administered for therapeutic efficacy, and there is no difference in potency between a plant extract of *Aloe turkanensis* that grows naturally and a propagated one.

1.2 General objective

The general objective of the study was to evaluate the efficacy and safety of the aqueous and methanol extracts of *Aloe turkanensis* grown in Kenya.

1.2.1 Specific objectives

To determine the antibacterial and antifungal activity of aqueous and methanol extracts of naturally occurring and cultivated *Aloe turkanensis*

To find out the bioactivity of the plant extracts on Brine shrimp larva using Brine Shrimp Lethality Test (BSLT).

To carry out a qualitative phytochemical screening test of methanol and aqueous extracts of *Aloe turkanensis.*

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Since the discovery of the first antimicrobial agents in 1928, there has been widespread use of antimicrobials in treatment of infectious diseases in both man and animals. However, in recent years there is concern regarding the emergence of antimicrobial resistance in previous susceptible microbial populations. Overuse, inappropriate or inadequate therapy in medical clinics and hospitals has enhanced selection of resistant bacterial strains (Kapil, 2005). If an improper antimicrobial agent happens to be chosen for the treatment of infection with drug-resistant microorganisms, the therapy may not achieve any beneficial effects. In a situation where multidrug-resistant organisms have spread widely, there may be quite a limited choice of agents for antimicrobial therapy. At present, fewer brands of new antimicrobial agents are coming onto the market. Considering this situation together with the increasing awareness on drug safety, we are now facing a situation of severely limited options among antimicrobial use (Saga and Keizo, 2009)

Scientists have continued to search for new antimicrobial agents. Herbal medications in particular have seen a revival of interest (Cock, 2008). The majority of people in tropical Africa depend on traditional healthcare that is largely based on the use of medicinal plants. Plants have an almost limitless ability to synthesize phytochemicals (Williams, 2006). The

knowledge of plant-based household remedies is considerable; for common ailments people often rely on their own traditional knowledge and for more complicated health problems traditional healers are consulted. Only for the most serious problems do patients refer to hospitals and academically trained medical doctors. In many cases cultivation of medicinal plants in home gardens are useful, as they provide a solution for health problems and promotes sustainable utilization of plants. This is especially useful in urgent or recurrent cases (Schmelzer *et al.*, 2010). Tapping of this indispensable resource is the way forward to beat the challenges of 21st century and beyond. There is need to identify, collect, test and validate the medicinal plants used by traditional healers.

Herbal medication has seen a revival of interest in the recent past, issues of conservation of biological material through sustainable utilization with optimal propagation cannot be overlooked. At the same time scientists need to investigate the effects of these conservation endeavors on the biological activity of the plant. This includes checking whether such conservation measures as propagation in different ecological zones will compromise the quality and quantity of secondary materials derived from plants since these metabolites are considered to be responsible for their bioactivity (János, 2005)

2.2 Traditional medicine and herbal remedies

World Health Organization defines Traditional Medicine as the sum total of knowledge or practices; whether explicable or inexplicable, used in diagnosing, preventing or eliminating physical, mental or social diseases. This may rely on past experiences or observations passed from generation to generation, verbally or in writing. It comprises therapeutic practices that may have been in existence often for hundreds of years before development of modern scientific medicine and are still in use today without any documented evidence of adverse effects (WHO, 1978a). The explicable form of Traditional Medicine can be described as the simplified, scientific and the direct application of plant, animal or mineral materials for healing purposes and which can be investigated, rationalized and explained scientifically. The inexplicable form of traditional medicine include the spiritual, supernatural, magical, occultic, mystical or metaphysical forms that are not easily investigated, rationalized or explained scientifically.

According to WHO (1978b) Traditional Healer (TH) or Traditional Medical Practitioner (TMP) is described as a person who is recognized by the community in which she/he lives as competent to provide health care by use of vegetables, animal and mineral substances and certain other methods. They may be serving as herbalists, bonesetters, traditional psychiatrists, traditional pediatricians and general practitioners.

The various types of herbal preparations used include concoctions, decoctions, infusions, dried powders, ointments, tinctures and macerates. Plants via their metabolic pathways contain various secondary metabolites which makes them the main source of medicinal treatment. Some of the drugs derived from plants include; Warfarin a dicoumarol from sweet clover, anticancer like paclitaxel from yew tree, vinca alkaloids (vincristine and vinblastine) from periwinkle, antimalarial agents like quinine from cinchona tree and artemisinin derivatives from *Artemisia annua* and several antibiotics from fungal metabolites (TDR 2007). However, while many conventional drugs or their precursors were/are derived from plants, there is fundamental difference between administering a pure chemical and the same chemical in plant matrix. Synergy is an important aspect in medicinal plant use, where pharmacological action of the chemical mixture is greater than the arithmetic of the actions of individual components. Example is in the use of insecticidal pyrethrins. Piperonyl butoxide has little insecticidal activity but interferes with insects' ability to break down the pyrethrins, thus increasing their toxicity (Kakko *et al*., 2000). Eder and Mehnert (1998) described the basic advantages from chemical complexity leading to enhanced solubility or bioavailability.

2.3 Status of traditional medicine in Kenya

Traditional medicine is a vital yet often neglected part of health care in Kenya. The conventional system provides for only 30 per cent of the population, implying that more than two-thirds of Kenyans depend on traditional medicine for their primary health care needs (NCAPD, 2008). This wide usage of traditional medicine has been documented by ethnobotanical surveys (Miaron *et al*. 2004; Kareru *et al*., 2007; Njoroge and Bussman 2007; Kokwaro, 2009); Nguta *et al.,* 2010). The high cost of imported conventional drugs and/or inaccessibility to western health care facilities has led to overreliance on traditional medicine (Munguti 1997). Although about 80% of Kenyans live within 5 kilometers of a health facility, medical services are not always available. Many facilities lack drugs, basic services and amenities and the cost of medicine is high. In addition, there are shortages of health professionals and the ratio of doctors to the population remains low at 15 per 100,000[2]. In addition, many Kenyans believe in the potency of herbal medicine, even when they can access modern medicine. In many cases they would choose to combine both herbal and modern medicine, especially if they are afflicted with chronic ailments such as HIV/AIDS, hypertension, infertility, cancer and diabetes (NCAPD, 2008; Nagata *et al.*, 2011).

According to Traditional Medicine and Drug Development Programme (TMDDP) newsletter, TMDDP is one of the six research programmes of

the Kenya Medical Research Institute (KEMRI) aligned to the institute's Strategic Master Plan and the Vision 2030 (TMDDP, 2013).

Growing use of herbal medicines and expansion of their market pose challenges in safety, quality and efficacy of traditional remedies since historically this practice has been on small scale and without government involvement. However with expansion of the sector, the government is the process of public consultation, awareness creation and discussions in different part of the countries to address the needs of the sector. This process has resulted in the development of a draft policy on Traditional Medicine and Medicinal Plants aimed at achieving conservation of medicinal plants, equitable sharing of benefits while ensuring the safety and efficacy of the products (NCAPD, 2008).

2.4 Drug discovery and medicinal plants

The process of deriving drugs from plant sources is not new. The ancient Egyptians have described several useful preparations such as opium and castor oil. They used "rotten bread" for treating infections which "resembles" our use of antibiotics produced by moulds and fungi. The Roman physician, Discorides, studied the medical uses of hundreds of plants and wrote the first systematic *materia medica* during the first century. He also described the medicinal properties of wines (Parfitt, 1978)

Later, drug discoveries from plants led to isolation of digoxin (*Digitalis pupurea*), quinine (*Cinchona officinalis)*, codeine and morphine (*Papaver somniferum*), ephedrine (*Ephedra sinica)* during the 18th and 19th century (Samuelsson, 2004). The first *British Pharmacopoeia* of 1863 contained descriptions of 187 crude extracts including *Digitalis, Datura, Belladona* and *Hyoscyamus* and the families of plants which digitalis-like glycosides have been reviewed (Melero *et al.,* 2000).

The approaches taken to identify potential medicinal plants are varied and have been reviewed by Fabricant and Farnsworth (2001). These processes include; random phytochemical screening, follow-up of biological activity reports and investigation geared to compare the reports with the claimed ethnomedical uses of plants.

It must be emphasized that clinical studies with human subjects represent the only assessment of effectiveness and safety that can translate into medical practice and National Policy. There should be more patient observation and follow up, this is of importance for further ethnopharmacological investigation and involvement but not limited to molecule elucidation and coming up with viable synthetics.

Graz *et al.,* (2007) noted that clinical trials involving ethnopharmaceuticals need not be expensive. They noted that the best way is to create interdisciplinary research group involving traditional

practitioners, research institute(s), physicians, pharmacologists, epidemiologists/statisticians and patients. Concarto *et al.,* (2000) suggested strict principles for follow-up studies. These include: first, choose inclusion and exclusion criteria similar to those in experimental trials; secondly, adjust for differences in base-line susceptibility to the outcome; thirdly, use statistical methods similar to randomized controlled trials, including "intention to treat" analysis.

2.4.1 Classification of natural products

In his book 'THE ORIGIN AND THE NATURE OF NATURAL PRODUCTS, Cock (2008) uses four schemes to classify natural products:

2.4.1.1 Classification based on the molecular skeletal structure

These are open-chain aliphatic, alicyclic and cycloaparaffinic, aromatic, benzenoid and heterocyclic compounds.

2.4.1.2 Classification based on physiological activity

The interest in natural products is frequently initiated by attempts to isolate and clarify a physiologically active principle of plant or animal origin. Actually, many medicines currently in use are natural products, e.g. alkaloids, such as morphine and penicillin G.

2.4.1.3 Classification based on chemotaxonomy

The field of chemotaxonomy attempts to review plant constituents according to plant taxa. Namely, constituents are regarded as markers for evolution as well as the classification of plants.

2.4.1.4 Classification based on biogenesis

It has been established that the primary synthetic process in nature is photosynthesis by which green plants utilize the energy of the sun for the production of organic compounds from carbon dioxide. The initial products of photosynthesis are carbohydrates. Further metabolic alterations lead to the formation of a pool of organic compounds of low molecular weight and simple structures such as carboxylic and amino acids, which are vital for the living organisms. They form the synthetic starting materials for specific, genetically controlled, enzymatically catalyzed reactions that lead to the complex compounds that characterize the secondary metabolism of plants and mammals. The reaction pathway leading to a particular natural product is called the biosynthetic pathway and the corresponding event is known as biogenesis.

Of the four major classes of primary metabolites/biochemicals (carbohydrates, proteins, nucleic acids and lipids), experiments have shown that the first three classes could have arisen through prebiotic chemistry. Although the biosynthesis of many natural products can be traced back to acetate (e.g., fatty acids, terpenes and polyketide

biosynthesis) or amino acids (e.g., alkaloid biosynthesis), there are many whose biosynthetic origins are either obscure or result from a complex combination of pathways. Figure 2.1 is an illustration that explains the biogenesis/biosynthesis of secondary metabolites.

The most important building blocks employed in the biosynthesis of secondary metabolites are derived from the intermediates acetyl coenzyme A (acetyl-CoA), shikimic acid, and mevalonic acid.

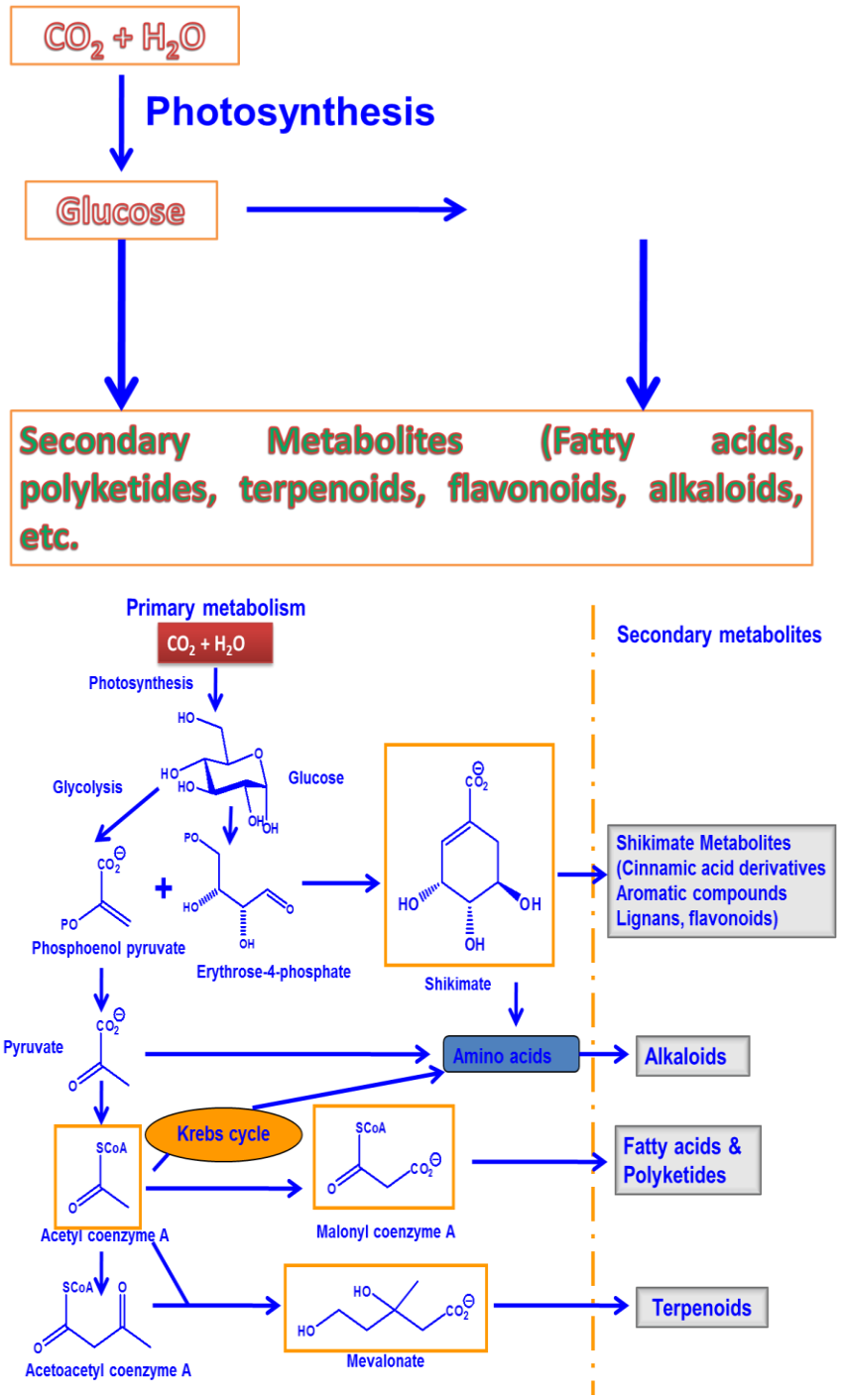

Figure 2.1 An illustration on biosynthesis of secondary plant metabolites

2.4.2 Adulteration and evaluation of crude drugs

Medicinal plants collected from the wild population may be contaminated by other species or plant parts through misidentification, accidental contamination or intentional adulteration, all of which may have unsafe consequences. The belief that herbal remedies are safe is deep rooted among the population and is not based on any conclusive scientific basis. It is important to note that botanicals are complex mixtures of chemicals substances. Ernst (2002) described presence of heavy metals and synthetic drugs in traditional Indian and Chinese medicines respectively. Most of these are not disclosed by manufacturers and they present potential hazard to users. The drugs mostly encountered were ephedrine, chlorpheniramine, methyltesterone and phenacetin. For example, PC-SPES is a patented herbal preparation marketed to "enhance prostate health," but commonly used to treat prostate cancer. Reports of its effectiveness have appeared in major medical journals (Sovak *et al*; 2002, Marks *et al*., 2002). However, after chemical analysis of PC-SPES the presence of diethylstilbestrol, indomethacin, warfarin or a combination of these drugs was revealed; and this led to the product being withdrawn from the market (Sovak *et al*; 2002). Marcus and Grollman (2002) noted that the medical professionals have been slow to respond to the public health and educational problems associated with botanical supplements.

Consistency in composition and biological activity are essential for safe and effective use of therapeutic agents. The use of chromatographic techniques and marker compounds to standardize herbal preparations promotes batch-batch consistency but does not ensure consistent pharmacologic activity or stability. Moreover, analyses of purportedly standardized herbal preparations reveal that botanical products often do not contain the amount of the compound stated on the label (Goldman, 2001).

2.5 Scientific evidence of activity of plant remedies

2.5.1 Antimicrobial activity

In Kenya, a number of plants used in traditional medicine have been *found* to have antimicrobial activity. The methanol extracts of *Tetracera boiviniana* (roots and aerial parts) showed antimicrobial activity against *in-vitro* cultures of *B. cereus, E. coli, P. aeruginosa, S. typhimurium, Proteus vulgaris* and *C. pyogenes* (Mbaria *et al*., 2005).

Chloroform and ethanol extracts of *Myrica salicifolia, Erythrina abyssinica, Solanum aculeastrum* and *Croton megalocarpus* showed antimicrobial activity against *in-vitro* cultures of *E. coli*, *Klebsiella pneumonia* and *S. typhi*. Water extracts except those of *Solanum aculeastrum* and *Croton megalocarpus* showed wider sensitivity to the bacterial strains (Kariuki *et al*., 2005).

Dichloromethane extract of *Warbugia ugandensis* had *in-vitro* antimycobacterial activity against *Mycobacterium aurum, M. fortuitum, M. phlei* and *M. Smegmati* (Wube *et al*., 2005). Forty crude extracts of twenty Cameroonian medicinal plants screened for antibacterial activity showed bacteriostatic effect on gram-negative pathogenic bacteria. *Euphorbia hirta* had the lowest inhibitory concentration against *S. aureus* and *Ps. aeruginosa* (Ngemenya *et al*., 2006). Antiviral effects of some plants and herbs are documented. The use of *Aloe secundiflora* in free range chicken in Tanzania was observed to reduce mortality and severity of clinical signs of Newcastle disease as compared to non-treated ones (Waihenya *et al*., 2002, 2005).

In Burkina Faso, extracts of three plants were screened for *in-vitro* activity against *Plasmodium* spp. The methanol extracts of *Swartzia madagascariensis* showed the highest antimalarial activity. The experiment showed that the extracts of *S. madagascariensis*, *Combretum glutinosum* and *Tinospora bakis* possess some measure of antimalarial activity. Methanol and alkaloid extracts showed higher activity than the aqueous extracts which are used in traditional medicine (Ouattara *et al*., 2006).

2.5.2 Pesticidal activity

Larvicidal activities of extracts from *Aloe turkanensis* against the common malaria vector, *Anopheles gambiae* has been determined (Matasyoh *et al.*, 2008). Other plants reported to have similar activity includes *Meliaceae* spp. Crude methanol extracts of *Turraea wakefieldii* and *Turraea floribunda* were more potent than azadiractin against larvae of *A gambiae* (Ndung'u *et al*., 2004). Larvicidal effects of neem extracts have been reported elsewhere (Vatandoost and Vaziri, 2004).

In India, the alcoholic and hexane extracts of seventeen (17) plants were found to be toxic to the egg, larval and pupal stages of the external gregarious larval parasitoid *Bracon brevicornis Wesm* (Srivastava *et al*., 1997). In Saudi Arabia, acaricidal effects of some plant extracts are documented against *Hyalomma dromedarii* (Al-Rajhy *et al.*, 2003).

2.5.3 Socio-economic uses

Kenya and the rest of Africa are rich sources of medicinal plants. Perhaps, the best- known species is *Phytolacca dodecandra*. Extracts of the plant, commonly known as endod, are used as an effective molluscicide to control schistosomosis (Lemma *et al*., 1991). *Prunus africana* bark is exploited and exported mostly to U.S.A, Belgium and France, for the treatment of benign prostatic hyperplasia. This has led to an annual

international trade worth approximately US$ 220 million in the final pharmaceutical product (Cunningham *et al*., 1997).

Other notable examples are *Catharanthus roseus*, which yields anti-tumour agents such as vinblastine and vincristine; and *Ricinus communis*, which yields the laxative (castor oil). In Botswana, Lesotho, Namibia and South Africa, *Harpagophytum procumbens* is produced as a crude drug for export. Similarly, *Hibiscus sabdariffa* is exported from Sudan and Egypt. Other exports are *Pausinystalia yohimbe* from Cameroon, Nigeria and Rwanda, which yields yohimbine, and *Rauwolfia vomitoria*, from Madagascar, Mozambique and Zaire and is exploited to yield reserpine and ajmaline. The markets for branded non-prescription herbal medicines have grown from $ 1.5 billion in 1994 to $4.0 billion in 2000 in U.S.A alone with the same trend being followed in European countries as well (De Smet *et al*., 2000). According to WHO (2003), the trade in herbal products was estimated to be worth US$ 60 billion and growing.

Plants have been an indispensable source of both preventive and curative medicinal preparations for human beings (Dery *et al*., 1999). Medicinal plants in Africa and other developing countries frequently provide economically disadvantaged groups such as small holders and landless people with their only form of cash income. Medicinal plants are also important sources of therapeutic agents in the industrial production of pharmaceuticals (Lambert *et al*., 1997).

2.6 Collection and preparation of crude drugs

Identification of medicinal plants (involvement of traditional practitioners and their informed consent is crucial) is the step that should not be over-looked. Collection of medicinal plants should be done sustainably taking into account environmental protection. The rising demand of herbal products is estimated by WHO (2003) to be US$ 14 billion/year and this is putting a lot of strain on the environment hence the need for sustainable harvesting.

Labeling and pressing of collected samples is done by placing the sample plant between two pressing boards and papers for transport and subsequent confirmatory identification in a Herbarium. The drying of plant samples can be done under shade, direct sunlight or in an oven. The effect of each method of drying on chemical constituent should be taken into account (WHO, 2002). Garbling involves separation and cleaning of dried plant samples by picking of dirt, debris, foreign organic matters and other unwanted plant parts. Gloves and air mask should be worn in case one is dealing with poisonous plants. During pulverization, milling can be done using pestle and mortar or electric grinder and the powder collected in an air-tight container.

2.6.1 Extraction methods and isolation

Water is universal solvent used to extract active ingredients. Dried plants are ingested as teas, or rarely as tinctures or inhaled via steam from boiling suspensions. Dried plant/herbal parts can be added to petroleum jelly and used topically. Poultices can be made from concentrated teas or tinctures. In alcoholic extractions, plant parts are dried, ground to fine powder and soaked in methanol or ethanol for extended period. Techniques for further chemical analyses include chromatography, radioimmunoassay, fast atom bombardment mass spectrometry and tandem mass spectroscopy. Other methods include high performance liquid chromatography, capillary zone electrophoresis, nuclear magnetic resonance spectroscopy and x-ray crystallography (Bhattaram *et al*., 2002).

2.6.2 Active phytochemicals in medicinal plants

These are primarily secondary metabolites and include alkaloids, phenolic and polyphenols, steroids, quinones, tannins, saponins, terpenoids, glycosides and cardenolids.

2.6.3 Phenols

Flavonoids occur both in free-state and as glycosides and are the largest group of naturally occurring phenols. They have been used extensively as chemotaxonomic markers. Phenolic compounds are important plant secondary products that are non-nutrients, but are useful for the plant's

defense against foreign bodies. They also contribute to the flavor, color and astringency of plants. These phenolic compounds also have antioxidant properties that enable them to quench free radicals in the body. It is probable that herbal remedies contain active flavonoids. Their capability to interact with protein phosphorylation and the antioxidant, iron chelating, and free radical scavenging activity may account for the wide pharmacological profile of flavonoids. These include vasoprotective, anticarcinogenic, antineoplastic, antiviral, anti-inflammatory, antiallergic, antiproliferative activity on cancer cells, antimicrobial activity and hepatoprotective activity (Jain *et al*., 2006; Li *et al*., 2007).

2.6.4 Quinones

These are aromatic rings with two ketone substitutions. They are abundant and highly active. They are responsible for the brown coloration of cut vegetables and are an intermediate in melanin synthesis in human skin (Schmidt, 1988). Anthroquinone is known to be bacteriostatic (Kazmi *et al*., 1994). Hypericin from St. John's wort has antidepressant activity.

2.6.5 Terpenoids

This refers to compounds with basic skeletons derived from mevalonic acid or closely related precursor. The fragrance of plants is carried in the so-called quinta essential, or essential oil fraction. The oils are based on isoprene structure. When the compounds contain additional elements,

usually oxygen, they are termed terpenoids. Based on their chemical structures, the following groups are identified:

a) Normal monoterpenes: these include all aliphatic and cyclic steam distillable monoterpenes. Non-steam distillable monoterpene glycosides have been found naturally. Based on their ring closure they are further divided into acyclic, monocyclic and bicyclic.

b) Iridoids (cyclopentanoid monoterpenes): these are characterized by a cyclopentanopyran ring nucleus. Most occur as -D glucosides, but those of the nepetalactone type are without the sugar molecule and are volatile, occurring in essential oils. They are bitter in nature. They have medicinal properties (antimicrobial, hypotensive, antileukemic, laxative effect etc.).

c) Tropolones: have seven-membered ring with double bond system conjugated with keto group and a hydroxyl group. They are restricted and identified in certain fungi and conifers. They structurally resemble phenols and are strongly fungicidal. The most common terpenoid is artemisinin. Terpenoids have been documented to having antimicrobial activity (Ghoshal *et al*., 1996).

2.6.6 Alkaloids

These are heterocyclic nitrogen compounds. They are basic in nature and contain one or more nitrogen atoms. They have pronounced pharmacological actions in animals and man. Morphine was first useful

alkaloid to be isolated in 1805. They are distributed in various parts of plants in different quantities including the leaves, bark, fruits, seeds, aerial parts, roots and rhizomes. They include diterpenoid alkaloid, glycoalkaloid and berberine. Alkaloids have antimicrobial activity and smooth muscle contractility (Rattmann *et al.,* 2005).

2.6.7 Tannins

These are complex substances containing mixture of polyphenols and are widely distributed in plant kingdom. They are protective and most tannins have molecular weight of 1000-5000. They are subdivided into hydrolysable and condensed (proanthocyanidins) tannins. Hydrolyzable tannins (HT) are polymers esterified to a core molecule, commonly glucose or a polyphenol such as catechin. Hydrolyzable tannins are potentially toxic to ruminants. Proanthocyanidins (condensed tannins) are relatively stable in the digestive tract of the animal, and rarely have toxic effects. They have antiparasitic properties (Githiori, 2004).

2.6.8 Glycosides

These are compounds that contain aglycone and glycone molecules. Widely present in plants where -D glucose sugar moiety is present. On hydrolysis glycosides produce aglycone (genin) and glycone (sugar) molecules. They are classified on basis of linkages between glycone and aglycone moiety, and on basis of chemical nature of aglycone molecule.

The most important in this group in medical circles are steroidal (cardiac) glycosides they contain cyclopentophenanthrene nucleus. About 50 cardenolides exist and are primarily found in: Apocynaceae, Asclepiadiaceae, Brassicaceae, Celastraceae, Euphorbiaceae, Liliaceae, Moraceae, Ranunculaceae, Scrophulariaceae, Sterculiacea and Tiliaceae families. They affect the dynamics and rhythm of dysfunctional heart muscle. Important sources of the digitalis glycosides (digoxin, digitoxin) include: *Digitalis lanata* (white foxglove) and (red foxglove). Digitalis drugs are agents used for people in congestive heart failure. Cardenolides and bufenolides (occur in free-state or glycoside form) are triterpenoids which are highly poisonous and which come from 23- and 24-carbon steroids, with an isoprenoid substituent (Okunade *et al*., 2004).

2.6.9 Essential oils/volatile oils

Essential oils are a group of natural organic compounds that are predominantly composed of terpenes (hydrocarbons) and terpenoids (oxygen containing hydrocarbons). Essential oils also contain simple phenols, sulphur containing mustard oils, methyl anthranilate and coumarins. These are mixtures of hydrocarbons terpenes, sesquisterenes and polyterpenes and their oxygenated derivatives obtained from various plant parts. They evaporate at room temperature and thus are called etheral oils. They are generally insoluble in water and soluble in organic solvents. Volatile oils mainly contain terpene. Monoterpenes are major constituents

of volatile oils. They may be acyclic, monocyclic or bicyclic, either as hydrocarbons or their oxygenated derivatives (terpenoids). The terpenoid are responsible for odor and taste. These have been reported to have antibacterial, antifungal, antinoceptive, spasmolytic, antiplasmodial and insecticidal activity (Cimanga *et al*., 2002, Abena *et al*., 2007).

2.7 Environmentally induced phytochemical variation

Various studies have been conducted to demonstrate environmental induced variations in chemical composition of plants (Yernes *et al*., 2008). Plants are greatly influenced by ecological factors. Much of the phenotypic variations encountered are the result of plastic response of the individual to factors of the environment (Okarech *et al*., 2012). However, species with wide distribution give clear evidence of hereditary adaptation to varying environmental conditions as diverse environmental conditions engender diverse patterns of species variation. A phytochemical screening done by Okarech (2012) on *Aloe vera* grown in different environments showed some slight differences in chemical composition with some plants giving negative results for flavonoids. A qualitative study of ethanolic extracts of *Annona squamosa* collected from different eco-zones done using High Perfomance Liquid Chromatography showed almost the same kind of compounds based on their retention factor as observed in both long and short wavelength. However the leaf ethanolic extract were screened for mosquito larvicidal activity against larvae of *Culex quinquefasciatus*

Say and *Annona gambiae* s.s. The leaf ethanolic extract of *A. squamosa* from T6 eco-zone was shown to be the most rich chemotype agent for mosquito control (Daniel *et a.,l* 2011).

2.8 Phytochemical screening

The subject of phytochemistry has developed in recent years as a distinct discipline. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deal with chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function. Ideally, fresh plants tissues should be used for phytochemical analysis (Harborne, 1973). However, alternative methods have been developed where the plant material is air dried under a shade, crushed into powder and subjected to solvents extraction. The acquired extract is then subjected to chemical test to identify the constituents using standard procedures (Sofowora, 1993).

Medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The variation in biological activities of these plants is mainly attributed to the differences in chemical composition. Qualitative phytochemistry screening has shown that plants have different phytochemicals; Water extract of *Carica papaya* leaves has shown positive results for alkaloids, terpenoids and saponins while a similar extract of *Cymbopogan citratus*

has shown negative results for alkaloids but positive for saponins and terpenoids (Njoku and Obi 2009). The anti-inflammatory and analgesic activity of *Cyclea pelteta* plant can be attributed to its high levels of steroid (Savithramma *et al* 2011)

2.9 *In-vitro* bioassay techniques

2.9.1 Brine shrimp lethality test

Plants used in folkore are assumed to be safe based on their long usage, but have been shown to be potentially carcinogenic, toxic and mutagenic (Elgorashi *et al*., 2003). The brine shrimp lethality test (BST) was originally proposed by Meyer *et al.* (1982) and later modified by McLaughlin *et al.,* (1991). It is based on the ability to kill laboratory cultured *Artemia salina* leach. This assay is considered a useful tool in preliminary assessment of bioactivity. It has been used for detection of fungal toxins, heavy metals, cyanobacteria toxins, pesticides, cytotoxicity testing of dental materials (Pelka *et al*., 2000) and plant toxicity testing (McLaughlin *et al*., 1991).

The brine shrimp assay is a very useful tool since it is simple, rapid and an inexpensive bench top bioassay. It also allows the use of small quantities of extracts. The *Artemia* spp eggs are readily available at low cost price in pet shops as food for tropical fish and remain viable for years in dry state. The eggs hatch between 24-48 hours, providing a large number of nauplii

upon being placed in brine solution. The method was used for screening extracts of plants used in herbal medicine in Kenya (Mwangi *et al*., 1999; Gakuya, 2001), Tanzania (Moshi *et al*., 2004), India (Alluri *et al*., 2005) and Jordan (Alkofahi *et al*., 1997).

2.9.2 Antimicrobial testing techniques

Antimicrobial agents have been used for over 40 years (Zhanel *et al*., 1991). Dosing regimes for agents were designed from research done on penicillin G (Eagle *et al*., 1950). These dosing regiments are supposed to maintain antimicrobial serum concentrations above the minimum inhibitory concentration (Kunin, 1981). The activity of plant extract is defined and measured in terms of its ability to inhibit the growth of a microbial population. According to Hostettmann, (1991) detection of antibacterial activity needs fulfillment of three conditions. First, the plant extract must be brought into contact with microbial cell wall. Secondly, optimal conditions for microbial growth must be present. Thirdly, selection of an appropriate means of judging the amount of growth.

In-vitro methods are divided into groups such as diffusion, dilutions, impedance and optical density methods (Koutsoumanis *et al*., 1999; Tassou *et al*., 2000). Among these, the dilution method provides more quantitative results (Manou *et al*., 1998). Results obtained with other methods may not be comparable (Tassou *et al*., 2000; Skandamis *et al*., 2001). Bioautography combines thin layer chromatography with bioassay

in-situ allowing for localization of active compounds within a sample. Bioautography is a useful method for the bioassay-guided fractionation of compounds with antimicrobial activity. However, some compounds show poor migration through the agar overlay and may not be detected (Gibbons and Gray, 1998).

2.9.3 Broth dilution methods

Broth of the antimicrobial agent(s) to be tested is usually prepared in serial two fold dilutions and placed in tubes of a broth medium that will support growth of test microorganisms. Antimicrobial agents are prepared in concentrated solutions and diluted to the appropriate concentrations in broth. Minimum inhibitory concentration is determined after overnight incubation; the tubes are examined for turbidity which indicates growth of microorganism. The microorganisms will grow in control tubes (negative) and any other tubes that do not contain enough antimicrobial agents to inhibit growth. The lowest concentration of the agent that inhibits growth of microorganisms as detected by lack of visual turbidity on marching the positive control is designated as minimum inhibitory concentration. Minimum inhibitory concentration (MIC) is used as 'gold' standards for determining the susceptibility of organisms and performance of other methods of susceptibility testing (William, 2000)

In broth techniques different methods exist for determining the MIC (Burt, 2004). Knowledge of MIC is important in order to apply the minimum

essential concentration capable of preventing microbial growth (Lambert and Pearson, 2000).

2.9.4 Impedimetric analysis

This is an automated technique which is a combination of absorbance measurements with the common dilution method (Lambert and Pearson, 2000; Lambert *et al*., 2001). Better results were obtained when optic density measurement was replaced with conductance measurements overcoming the inherent problems in optical density technique (Chorianopoulos *et al*., 2006).

2.9.5 Turbidometry

This technique detects only the upper part of microbial growth curves, and requires calibration in order to correlate the results with viable counts obtained on agar media (Daalgard and Koutsoumanis, 2001; Skandamis *et al*., 2001).

2.10 Formulation and value addition of herbal remedies

Herbal remedies can be put to commercial use. Scientists are demanding that traditional knowledge should be validated to verify efficacy of treatment (Wanzala *et al*., 2006). Preparation and dosages of the same remedy may often vary greatly. The required plant materials may not be available through the year and the pharmacological active ingredients may

vary according to season, site, harvest time, maturity and other factors (Martin *et al*., 2001).

Indigenous knowledge particularly on medicinal plants is speeding up as firms and research groups that seek to patent ingredients or preparations. Outsiders are bypassing the communities that developed them and commercializing the herbal remedies. Bioprospectors often apply for permits without indicating their commercial intent. Improving packaging of herbal products is one of the ways of value addition. To improve on value of herbal products, pharmacokinetics, bioavailability and interactions of some herbal remedies have been reviewed (Bhattaram *et al*., 2002). Bhattaram *et al*. (2002) noted that the determination of the pharmacokinetics and use of pharmacodynamics modeling can aid in more rational use of herbal products. Concerted research should be geared towards gathering data on toxicological and bioavailability of the products in-vivo.

2.10.1 Quality criteria and standardization

Phytopharmaceuticals are composed of many constituents and are thus capable of variation. The variability depends on ecological zone, season, and part of plant/shrub used, harvest, drying and growth conditions. The other conditions are polarity of the solvent, mode of extraction and instability of constituents which may influence quality and composition of plant extract.

Products are pharmaceutically equivalent if they contain the same amount of active substance(s) in same dosage forms that meet the same or comparative standards according to the Note for Guidance on the investigation of bioavailability and bioequivalence. This should apply for herbal drug preparation (HDP). Reproducible efficacy and safety of phytopharmaceuticals is based on reproducible quality. Thus in order for them to be considered rational drugs, they need to be standardized and quality approved. In addition to pharmacological, toxicological, and clinical studies of the herbal drugs, their composition needs clear documentation in order to obtain reproducible results (Bauer, 1998).

The WHO (2002) strategy for traditional medicinal plants had four main objectives of: farming policy, enhancing safety, efficacy and quality, ensuring access and promoting rational use. The WHO has recognized this problem and has published guidelines to ensure the reliability and repeatability of research on herbal medicines (WHO, 2000). A survey conducted by WHO, 2005 found that the main constraint in regulation of herbal products and medicine was lack of research data. Of the 129 countries polled, 109 reported this as a constraint among other. According to Di Stasi, (2005) standardization of phytomedicines could be best realized by considering three essential aspects: (i) selection of the plant species for use and studies of new phytomedicines is based on traditional knowledge(ethnopharmacological approach) and consequent benefit

sharing; (ii) pharmacological, toxicological and phytochemical evaluation of the selected medicinal plants is appropriately done to ascertain their efficacy, safety and quality control; and (iii) strategic utilization of the biological material for the sustainable use of multiple forest resources and conservation of ecosystems.

2.11 Literature on Aloe turkanensis

2.11.1 Species description

Aloe species are a large group of succulent monocotyledons plants, currently placed in the family *Asphodelaceae* (ICBN). *Aloe turkanensis* is a succulent sprawling shrub with stems of up to 70 cm long. It grows in loose clumps up to 2 m diameter. On average the plant produces 14-18 leaves that are borne in a compact rosette, are erect to spreading. The stipules and petiole are absent. The blade is lanceolate and measures up to 70 cm x 9 cm. It has a long-acuminate apex, margin with sharp deltoid teeth, 2mm long, whitish, brown-tipped, 12-18 mm apart. The blade is pinkish green with elongated whitish spots on both surfaces. The inflorescence is many-branched, up to 30 cm long and bright pink in colour (Mukiama, 2005; Wabuyele, 2006).

Figure 2.2: A photograph of *Aloe turkanensis* growing in its natural habitat in Natira sub-location, Turkana County

Figure 2.3: A photograph of cultivated *Aloe turkanensis* in Karura Forest, Kiambu County

2.11.2 Geographic distribution and ecology

*Aloe turkanensis (*endemic and propagated species) is indigenous to Kenya and is mainly found in Baringo, Isiolo, Laikipia, Turkana and West Pokot counties. The plants grow on stony, sandy soil or lava, usually in the shade of shrubs in arid areas at 600–1250 m altitude. It naturally occurs in north-western Kenya and in the Karamoja District of Uganda (Bosch, 2006; Lubia *et al*, 2008)

2.11.3 Uses

The diverse traditional uses of plants in the genera *Aloe* in Kenya have been documented by Wren (2008) in her project of Laikipia Aloe bio-enterprise. In his ethno-botanical survey, Bosch (2006) reported a diverse use of the plant especially for human medicine where sap is used as a laxative and as an antihelmintic while the gel is used in management of stomach ulcers, colon dysfunction, as wound salve, in treatment of malaria and for skin softening in beauty treatment. Both the sap and gel are used for veterinary treatment of skin conditions mainly in cattle and goats. The roots of *Aloe* plant are used as a traditional ingredient in brewing of alcohol and as rodents poison. *Aloe* plant is used as live fence and plays an important role in control of soil erosion by encouraging growth of grass in the rangelands (Bosch, 2006).

The traditional use of *Aloe turkanensis* is documented by PROTA database. *Aloe turkanensis* is well known and revered for its curative properties utilized by a diversity of cultures for a diverse number of ailments, the best known being in the treatment of eyes, wounds and burns. The juice from boiled roots is added to a drink to induce vomiting, which is said to relieve persistent headaches. The roots are used to flavour beer (Schmelzer *et al*, 2008).

Scientific studies support the claims from ethno-botanical survey where antileishmanial, antimalarial, anticancer, larvicidal and antibacterial activities of *Aloe* species of plant have been documented (Waihenya *et al*., 2005; Matasyoh *et al.,* 2008; Wagate *et al.,* 2009; Thiruppathi *et al.,* 2010 and Irshad *et al* 2011).

2.11.4 Bioactivity and bioactive principles

The main components of leaf exudate of *Aloe turkanensis* are aloin A, aloin B and aloesone. Out of 70 *Aloe* species screened, *Aloe turkanensis* had the highest aloin content, both in the exudate and in the leaf (31% and 6.6% of dry weight, respectively). Aloin is a mixture of the stereoisomers aloin A (barbaloin) and aloin B (isobarbaloin), and is responsible for the laxative properties. The compound is present in what is commonly referred to as the aloe latex that exudes from cells adjacent to the vascular bundles, found under the rind of the leaf. When dried, it has been used as a bittering agent for alcoholic beverages (Reynolds, 1996).

CHAPTER THREE:MATERIALS AND METHODS

3.1 Study areas

The naturally growing *A. turkanensis* plant sample was obtained from Natira community aloe garden at the outskirts of Kakuma town, Turkana County while the cultivated plant was obtained in Karura Forest in Kiambu County. Turkana County is located in the northwestern part of Kenya and lies between latitude 3^0 37´ North and longitude 36^0 0´ East. Turkana County covers a surface area of 68,680km^2, ranked as the 2nd largest county in Kenya. It has a population density of 13 persons per km^2 and total population of 855,399 persons. The urban population is distributed in four major towns Lodwar (48,316), Kakuma (36,875), Lokichogio (17,695) and Lokichar (31,610).

County poverty data have been computed based on the Kenya Integrated Household Baseline Survey (KIHBS) and have shown that 94.3% of Turkana people are below the baseline therefore ranking the county as the poorest in Kenya. The KIHBS aims at providing a key database to update various indicators and provide a baseline for measuring and monitoring progress through future surveys in the National Statistical System. To achieve this objective, Central Bureau of Statistics (CBS) has set new standards of best practice by which KIHBS was designed as an integrated

modular set of questionnaire instruments, which together provide a baseline database that is unprecedented in its level of detail, coverage and quality. In addition to collecting an integrated set of socio- economic indicators, the KIHBS was designed to enable updating and strengthening of three vital aspects of the national statistical database: the Consumer Price Index, measures of living standards, poverty and inequality; and the System of National Accounts. Out of 47 counties, Turkana rank number 46 in health service delivery. Approximately 6.9% of newborns are delivered in health centre (hospital, clinic or maternity home) by qualified medical assistant (doctor, midwife or nurse) (KNBS 2009).

Kiambu County on the other hand lies between latitude 10 10´ south and longitude 360 40´ east. The county covers a surface area of 2,543 km^2 with an estimated population of 1,623,282 persons. The population density is 638 people per km^2. The region is well endowed with natural resources as arable land, forests, water falls, which form a good base for tourist attractions. The main economic activities include farming, food processing, manufacturing (Leather), Mining (Carbacid), Textile (Cotton), among others. Kiambu rank among the wealthiest counties in Kenya.

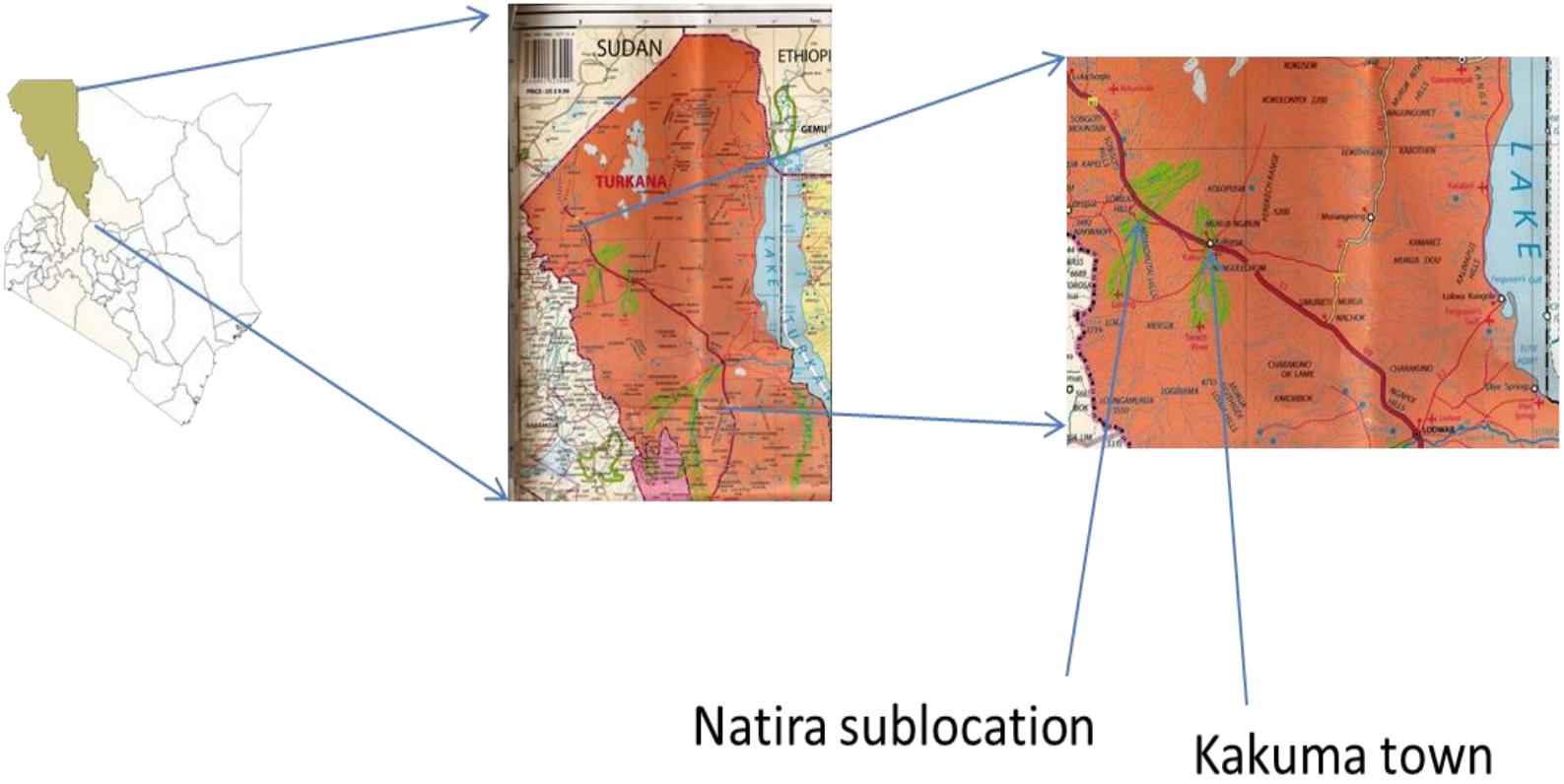

Figure 3.1: A map of Kenya showing the location of Turkana County where *Aloe turkanensis* growing naturally was obtained.

3.2 Identification and cultivation of *Aloe turkanensis*

A pilot study was done prior to plant collection to familiarize with the study area, investigate the local uses of the herb, its availability and conservation statues, to create an interactive rapport between the indigenous people and the investigating team on the matter of indigenous knowledge and assess the possibilities/opportunities of biosprospecting and value chain on herbs used for management of ailments

The study covered Turkana Central and Turkana West districts. Areas visited included Lodwar, Kolakol, Kakuma towns and the outskirts. Lodwar and Kolakol town lies in Turkana Central while Kakuma and Natira are in Turkana West districts.

Plant collection was done in Natira sublocation, Kolobeiyei location, Oropoi division currently located in Turkana West district. In this location, *Aloe turkanensis* plants grew on stony terrain, 42 km from Kakuma town along Lodwar-Lokichogio road. The plants are under the custody of the local people most of whom are women. There is a chairman, a secretary (who are both elected by the local people) and one government representative who is the assistant chief. In this region, there is abundant species of *Aloe turkanensis* which occurs naturally on the hilly rocks extending all the way to Lokichogio.

The plant collection was aided by 5 employees from Government of Kenya and Lutheran World Federation (GOK-LWF) humanitarian

organization who provided the means of transport and security in the field, and 10 people from the local community who voluntarily provided information on medicinal plants. A number of questions about their past experience with the plant as a herb were asked prior to the actual collection exercise and provided answers recorded as shown in table 4.1.

Data was collected through observation, photographing, interviews and sampling. The samples were collected and deposited at the herbarium in Kenya Forestry Research Centre in Karura. A thorough literature search was done on the plants from electronic databases, putting into account the uses given by the herbalists.

Figure 3.2: A photo taken in Turkana County during field collection of *Aloe turkanensis* for laboratory testing

3.3 Preparation of plant samples for extraction

Two ecotypes of *Aloe turkanensis* specimen were obtained; a whole plant from naturally growing species in Turkana County and the other one from cultivated species in Kiambu County. The materials were thoroughly cleaned with tap water then rinsed with distilled water, chopped into small pieces using a knife then dried at room temperature. The dried and chopped plant sample was placed in Cunningham® grinder that had both high and low speed. The sample was ground using one level of high speed and two levels of low speed each lasting 15 seconds. This was repeated until all the dry sample was turned into powder. The grinding process was done in a fume chamber for protection from the fumes emitted. The powder obtained was packed in 500 grams portions and was placed in clean airtight polythene paper (Gakuya, 2001).

3.4 Extraction procedure

The two plant ecotypes were extracted using the same method of cold maceration using 70% v/v methanol in water and distilled water as described by Gakuya, (2001). Two hundred grams of the plant powder were extracted separately. The powdered sample was placed in two conical flasks; methanol was added into one flask while distilled water was added into the other until the powders were submerged. The flasks were corked with appropriate stoppers and shaken thoroughly. The process

took 4 days at room temperature during which proper agitation was regularly done to allow proper percolation and extraction. On the fifth day, the extracts were filtered using Whatman No. 1 filter papers into other conical flasks. Each of the extract was then evaporated to dryness under pressure in a rotary evaporator.

The resultant viscous substances were weighed separately, put in sterile Falcon tubes, tightly covered using aluminum foil and stored in a refrigerator at +4°C pending freeze drying, bioassay and antimicrobial activity testing. Freeze-drying was done using Christ Beta lyophilizer courtesy of BECA-International Livestock Research Institute-Kenya. The samples in the Falcon tubes were placed on a cooled, temperature controlled shelf within the freeze dryer. After complete freezing, the pressure was lowered. Moisture from the methanol extract was lost by sublimation. The extracts were left in freeze dryer overnight. The following day the dried samples were removed from the freeze drier and the brim of the Falcon tubes sealed using paraffin film. During bioassay, samples were treated the same; where 0.1 grams of plant extracts were transferred into a centrifuge bottle and ten milliliters of distilled water added to form a concentration of 10 mg/ml. Sonication was done to dissolve the powder. Marine salt solution (33 g of marine salt in one (1) liter distilled water) was added to dissolve and stirred using electrical mixer (Voltex Reamix 2789®) at 2800 rpm to make final stock

concentration of 10,000 µg/ml. Serial dilutions were prepared from this stock solution (Gakuya, 2001).

3.5 Tests for antimicrobial activity

3.5.1 Test for *in vitro* antimicrobial activity

3.5.1.1 Reference strains of micro-organisms

The reference strains used for the screening were *Escherichia coli* (ATCC 25922), *Pseudomonas aerugenosa* (ATCC 27853), *Staphylococcus aureus* (ATCC25923), *Bacillus cereus* (ATCC 11778) and a clinical isolate of *Candida albicans*. Bacteria were obtained from stock cultures from the Department of Public Health, Pharmacology and Toxicology, University of Nairobi. The bacterial stock cultures were maintained on cooked meat media stored at 4°C. The five microorganisms sub-cultured in blood agar base were used to assess the antimicrobial activity of the methanol and aqueous plant extract.

3.5.1.2 Standard antimicrobial drugs

Antibiotics Benzyl penicillin and Gentamycin (Vet genta®, sourced from Dawa chemicals limited) powder were used as reference standards for gram positive and gram-negative bacteria, respectively, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2000) while antifungal Amphotericin B (sourced from Sarabhai chemicals in India) was used as reference standard for antifungal activity.

3.5.1.3 Determination of antibacterial activity

The preliminary studies were done using Agar well diffusion method. A loopful of stock cultures of standard organisms that were stored in cooked meat media were sub inoculated on Blood Agar (BA) and incubated for 24 hours at 37°C. The sub- cultured bacteria were used as stock cultures and were kept refrigerated at +4°C. Using a sterile loop, a single colony was picked and streaked on pre-prepared Mueller Hinton Agar (MHA) and incubated for 18 hours at 37°C. Standard well were made on the agar plate (1cm in diameter). The 50 ul of the plant extracts at different concentrations were added into the wells and incubated for 24 hours at 37^0C. The diameters of the zones of inhibition were measured and displayed in the figure 3.4, 3.5 and 3.6.

Broth dilution technique as described by Suffredini (2006) was used to test for inhibitory activity of plant extracts. Pre-sterilized Muller Hinton Broth was dispensed into sterilized 10 ml test tubes using sterile 10 ml pipette. The test tubes were clearly labeled and put in test tube rack. For test with gram positive bacteria 200 mg of extract was dissolved in 2 ml sterile MHB. For the inhibitory tests on gram negative bacteria 500 mg of plant extract was dissolved in 2 ml sterile PBS. Serial two fold dilution of plant extracts was made. Using sterile 1 ml pipette 0.1 ml of bacterial suspension was dispensed into each of the test tubes. They were incubated at 37°C for 24 hours. All experiments were performed in triplicates.

Another tube containing the bacterial inoculum without plant extract was used as negative control. After overnight incubation, visual turbidity was noted and 0.1 ml from non-turbid tubes was sub-cultured to MHA plates. The inocula were spread on agar using sterile glass rods. The plates were incubated for 24 hours at 37°C. For the positive controls, two-fold dilution of gentamycin powder at concentration of 40 mg/ml and Benzyl penicillin 10 mg/ml were made as above for gram negative and gram positive bacteria respectively.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited any visible bacterial growth on the culture plates (Prescott *et al*., 1999; Shahidi, 2004; Aibinu *et al*., 2007). This was determined from readings on the culture plates after incubation.

3.5.1.4 Determination of antifungal activity

The preliminary studies were done using Agar Well Diffusion Method. A loopful of stock cultures of standard organisms that were stored in cooked meat media were sub inoculated on BA (Oxoid) and incubated for 48 hours at room temperature. The sub- cultured fungal were used as stock cultures and were kept refrigerated at +4°C. Using a sterile loop, a single colony was picked and streaked on pre-prepared MHA (Oxoid) and incubated for 48 hours at room temperature. Standard wells were made on the agar plate (1cm in diameter). The 50 ul of the plant extracts at different concentrations were added into the wells and incubated for 48 hours at

room temperature. There was growth of *Candida albicans* even in the highest concentration of plant extract which was an indication that the extract had no antifungal activity at the preselected plant treatment.

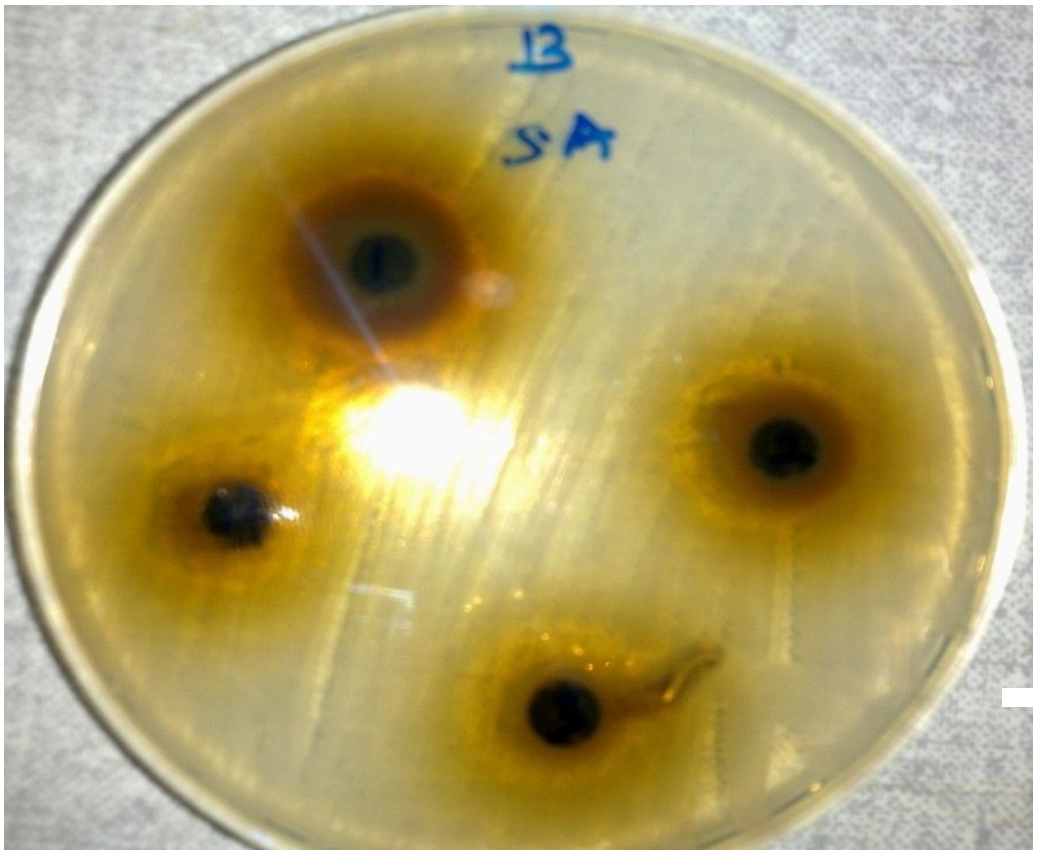

Figure 3.3: A culture plate showing diameter of zones of inhibition of microbial growth for aqueous extract of naturally growing *Aloe turkanensis* against *Staphylococcus aureus*

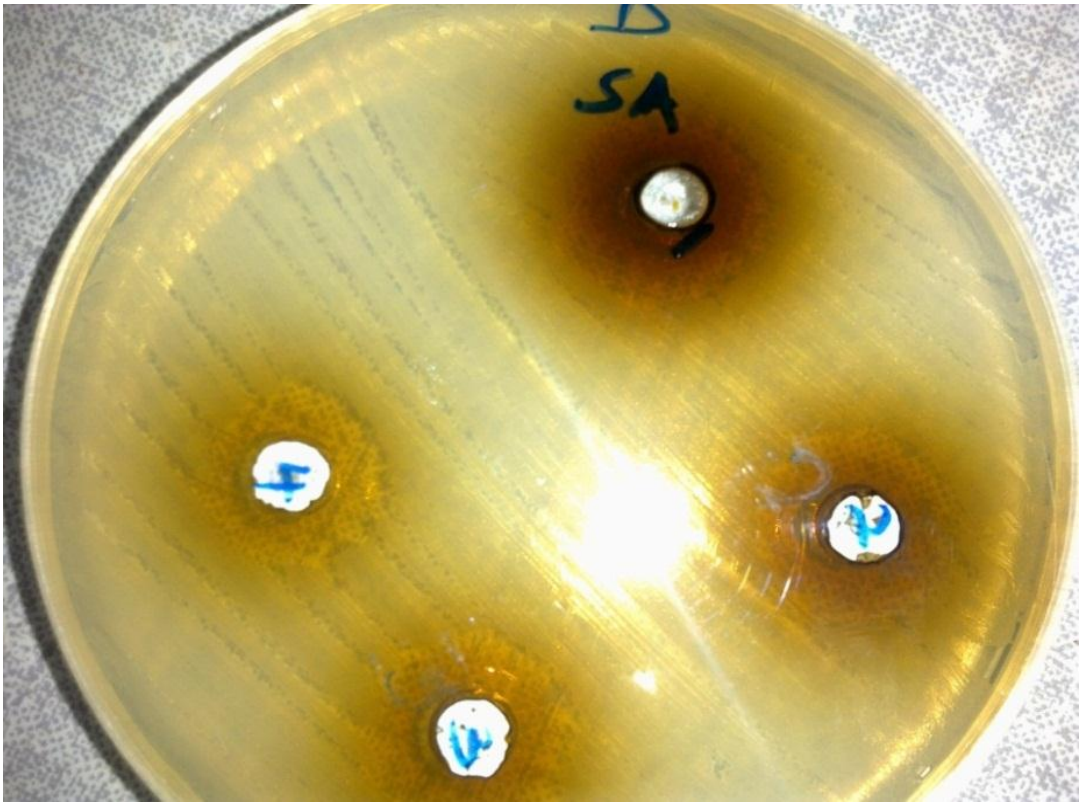

Figure 3.4: A culture plate showing diameter of zones of inhibition of microbial growth for methanol extract of naturally growing *Aloe turkanensis* against *Staphylococcus aureus*.

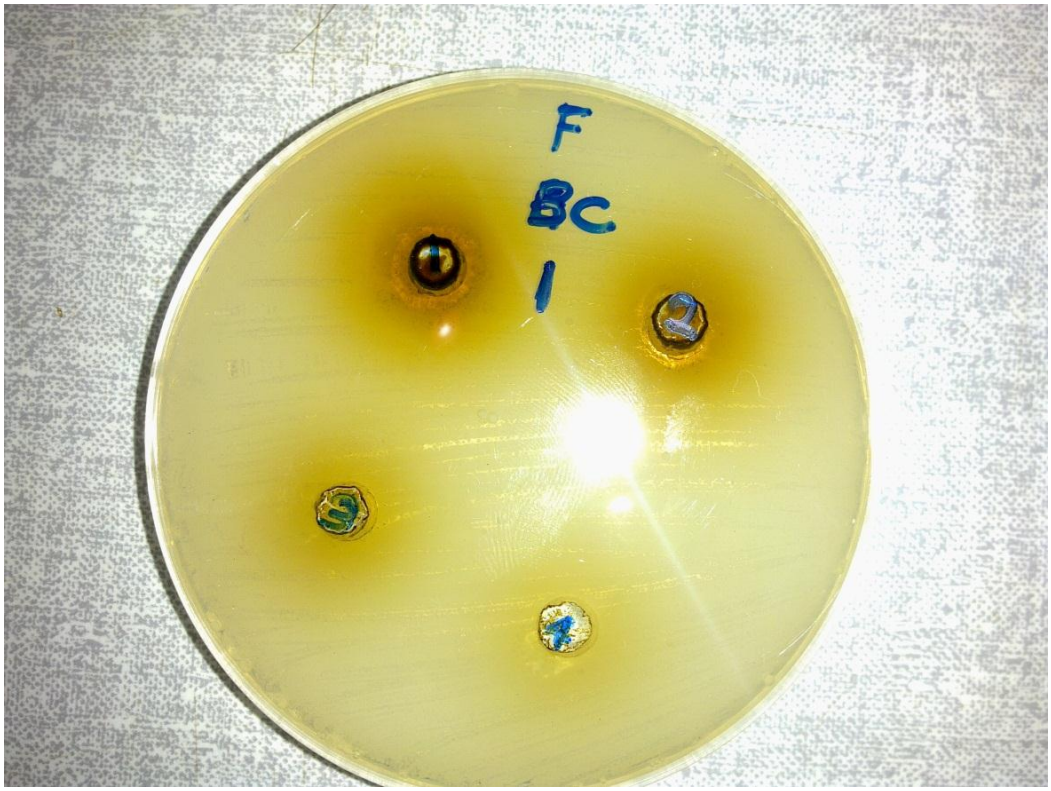

Figure 3.5: A culture plate showing diameter of zones of inhibition of microbial growth for methanol extract of cultivated *Aloe turkanensis* against *Bacillus cereus.*

3.6 Test for bioactivity using Brine Shrimp Lethality Test

3.6.1 Source of Brine Shrimp eggs

Standard Brine shrimp eggs were sourced from JBL Novo Termaad GMBH and CO in Germany.

3.6.2 Hatching the Brine Shrimp eggs

Thirty-three (33) grams marine salt was weighed on an electric weighing machine and transferred into 1liter conical flask. Distilled water was added gradually concurrently stirring to dissolve marine salt. When all marine salt had dissolved distilled water was added to 1 liter mark to constitute the marine salt solution.

Brine shrimp eggs were hatched in shallow rectangular plastic double chambered box with a dividing wall which had 1-2 mm holes. The box was filled with marine salt solution (33g of marine salt in 1 liter of distilled water). Using a spatula about 50 mg of brine shrimp eggs was sprinkled and about 5 mg of dry yeast which served as food for the nauplii was sprinkled in the dark compartment. The other compartment was illuminated through a hole in the lid of the box and kept under a light source using a 40 watts electric bulb. After 48 hours, the phototropic nauplii were collected by use of a Pasteur pipette from the lighted compartment and subjected to brine shrimp lethality test (Gakuya, (2001).

3.6.3 Cytotoxicity bioassay

Three dilutions were prepared by transferring 500 µl, 50 µl and 5 µl of plant extract each to a set of five graduated tubes. Ten shrimps were transferred into each of the vial using Pasteur pipette and marine salt solution was added to the 5 ml mark to make dilutions of 1000 µg/ml, 100 µg/ml and 10 µg/ml. Five graduated vials were set for each dilution and a further five for the control. The tubes were left at room temperature and the number of live larvae counted after 24 hours. The percentage mortality was determined for each dilution and controls. Where control deaths occurred within 24 hours, the data was corrected using the formulae: % death= {(test-control)/ control} ×100 (Gakuya, 2001). The results were interpreted using probit method of Finney computer program acquired from Department of Pharmacology and Pharmacognosy, Faculty of Pharmacy, University of Nairobi. The program uses the number of dose level, number of brine shrimp for every concentration, percentage mortality for every concentration and the dose level. The lethal concentration 50 (LC_{50}) and 95% confidence intervals were determined using the computer program (McLaughlin *et al*., 1991).

3.7 Phytochemical screening

Preliminary qualitative phytochemical screening was carried out on the aqueous and methanol extracts to identify the constituents using

standard procedures (Harborne, 1973; Trease and Evans,1989; Sofowora, 1993). The following compounds were tested:

3.7.1 Test for tannins

To test for tannins, 0.5 g of each plant extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of blue-black, green or blue green precipitate was considered positive for tannins.

3.7.2 Borntrager's test for anthraquinones

To test for presence of anthraquinones 0.2 g of each plant extract was shaken with 10 ml of benzene and then filtered. Five milliliters of 10% ammonia solution was then added to the filtrate and thereafter shaken. Appearance of a pink, red or violet color in the ammoniacal (lower) phase was considered positive.

3.7.3 Liebermann-Burchard Test for steroids and terpenoids

To test for terpenoids, two 0.2 g of each plant extract 2 ml of acetic acid was added, the solution was cooled well in ice followed by the addition of concentrated sulfuric acid ($H_2SO_{4)}$ carefully. Color development from violet to blue or bluish-green was considered positive

3.7.4 Test for saponins

To test for presence of saponins, 1 gram of each portion was boiled with 5 ml of distilled water and then filtered. To the filtrate, about 3 ml of distilled water was added and shaken vigorously for about 5 minutes. A persistence frothing was considered positive

3.7.5 Test for flavonoids

Shinoda's test was used to test the presence of flavonoids where 0.5 g of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by few drops of concentrated hydrochloric acid (HCL). A pinks, orange, or red to purple colouration was considered positive.

3.7.6 Test for alkaloids

To test for presence of alkaloids, 1 gram of the each portion of the plant extract was stirred with 5 ml of 1% aqueous HCL on water bath and then filtered. Of the filtrate, 1 ml was taken individually into 2 test-tubes. To 1 ml, Mayer's reagent was added and appearance of buff-coloured precipitate was considered positive

3.8 Data handling and analysis

Two softwares were used to analyse the data; Microsoft excel and Gen stat. The Microsoft excel 2010 software was used in calculating the mean,

standard deviation and drawing charts while Gen Stat program was used to for analysis of variance between the two plant ecotypes.

CHAPTER FOUR: RESULTS, DISCUSSION AND CONCLUSIONS

4.1 Results

4.1.1 Medicinal use of *Aloe turkanensis*

During the pilot study, the local people in Turkana County claimed that they use various part of the plant to manage malaria, wounds, stomach ache, pain, fatigue and as a laxative, etc. Ten people were interviewed on the use of the plant and the results on percentage usage are displayed in Table 4.1.

Table 4.1: Use of Aloe turkanensis, methods of preparation, routes of administration and disease ethnodiagnosis among the TMP in Turkana County

AILMENT	Percentage number of TMP who have used the plant	Part used	Method of disease diagnosis	Reconstituting the medicine	Route of administration
Malaria	100	Whole	Clinical signs	Boiled	Oral
Wounds	90	Leaf	Clinical signs	Fresh juice	Topical
Pain	80	Whole	Clinical signs	Boiling when dry	Oral
Stomach ache	80	Leaf	Clinical signs	Boiling when dry	Oral
Tiredness	90	Leaf	Clinical signs	Boiling when dry	Oral
Detoxify	60	Whole	Clinical signs	Boiling when dry	Oral
Laxative	100	Leaf	Clinical signs	Boiling when fresh	Oral
Emetic	30	Leaf	Clinical signs	Boiling when fresh	Oral
Cosmetic	100	Leaf	Clinical signs	Fresh sap & gel	Topical
Ringworms	70	Leaf	Clinical signs	Fresh sap	Topical
Health Drink	100	Leaf	Clinical signs	Gel	Oral
Retained After birth in cows	20	Leaf	Clinical signs	Sap	Intrauterine
Poultry disease	20	Leaf	Clinical signs	Freshly harvested sap	Drinking water

4.1.2 Antimicrobial activity of *Aloe turkanensis*

Results on the *in-vitro* antimicrobial activity of the plant extracts is displayed in Table 4.2 and 4.3 as well as Figure 4.1 and 4.2.

4.1.2.1 Bioactivity on Agar well diffusion test

Bioactivity on Agar well diffusion test is listed in Table 4.2

Table 4.2: Diameters of zone of inhibition of microbial growth for aqueous and methanol extract of *Aloe turkanensis* recorded on Agar well diffusion test against various species of micro-organisms

Plant extract/Test Organism	Diameter of zone of inhibition in mm			
	Naturally growing plant		Cultivated plant	
	Methanol extract	Water extract	Methanol extract	Water extract
S. aureus	18.5±0.7	19.75±1.0	16.5±0.7	6
B. cereus	16.5-0.7	11.5	11.5	6
E.coli	6	6	6	6
P.aeroginosa	6	6	6	6

Diameter of disk = 6.0 mm; 6 mm = no inhibition; 7 - 10 mm = weak activity; 11 - 13 mm = moderate activity and > 14 mm = strong activity

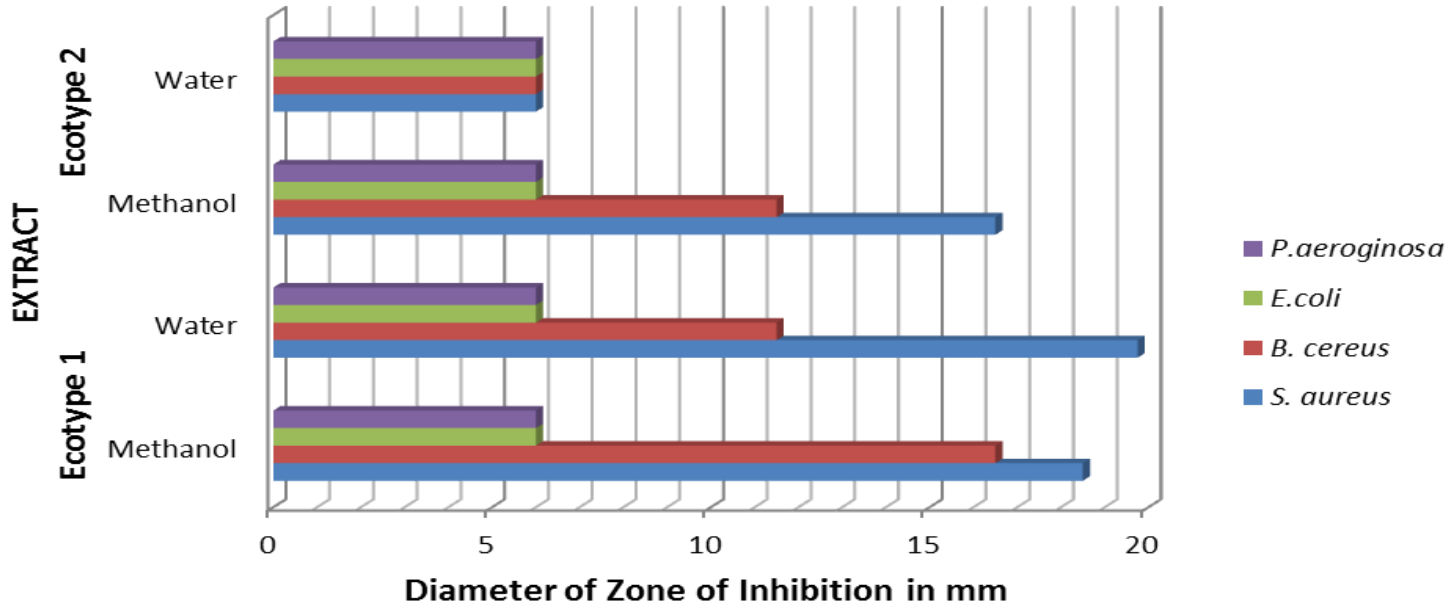

Figure 4.1: A chart showing diameter of zones of inhibition of microbial growth for aqueous and methanol extracts of *Aloe turkanensis* against various species of micro-organisms

KEY:

Ecotype 1: *Aloe turkanensis* growing naturally in Turkana County, Kenya

Ecotype 2: *Aloe turkanensis* cultivated in Kiambu County, Kenya

4.1.2.2 Bioactivity on broth dilution test

Bioactivity on broth dilution test is listed in Table 4.3

Table 4.3: Minimum inhibitory concentrations (MIC) mg/ml for methanol and aqueous extracts different ecotypes of *Aloe turkanensis*

Test material	Plant material	Extract	*P. aeroginosa*	*E. coli*	*S. aureus*	*B. cereus*	*C. albinans*
A. turkanensis	Naturally growing *Aloe turkanensis*	Aqueous	-	-	50	200	-
		Methanol	200	-	100	100	-
	Cultivated *Aloe turkanensis*	Aqueous	-	-	-	-	-
		Methanol	400	200	50	100	-
Benzyl penicillin		-	-	-	0.625	0.625	
Gentamycin		-	0.0049	0.0049	-	-	-
Amphotericin B		-	-	-	-	-	0.0125

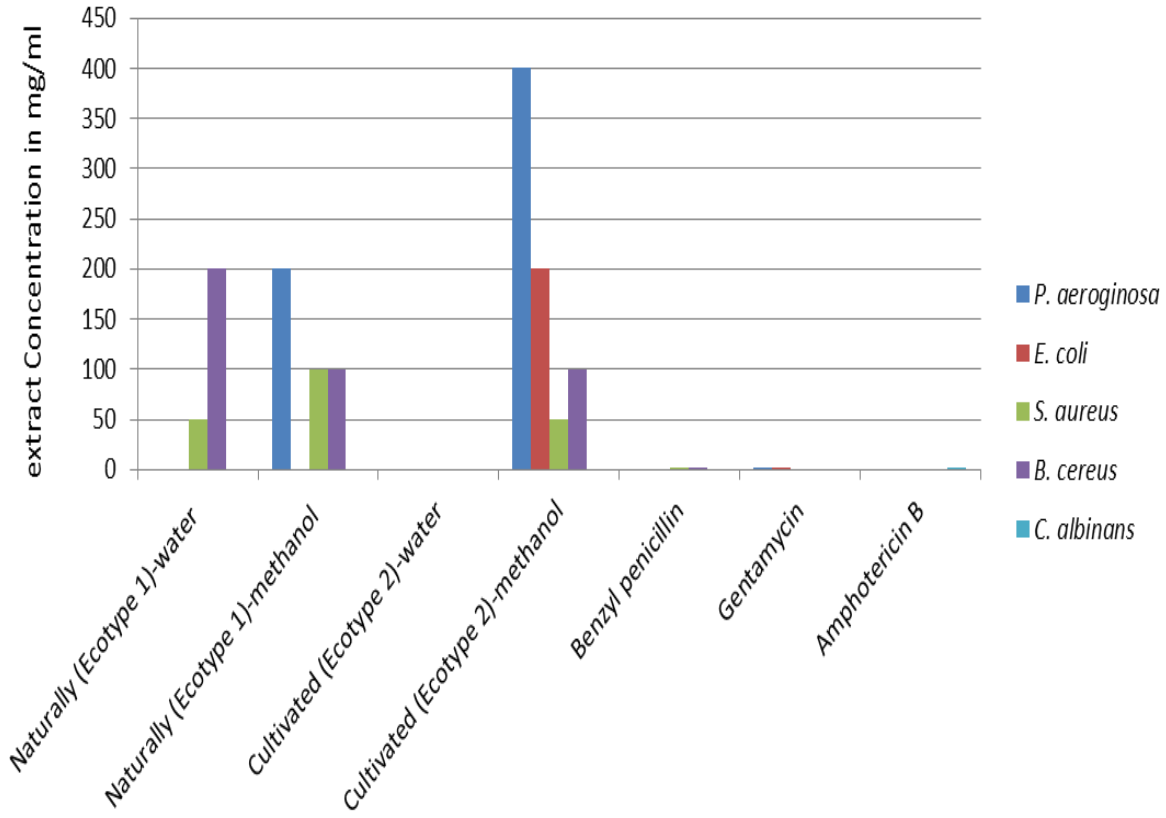

Figure 4.2: Comparative minimum inhibitory concentrations of cultivated and naturally growing *Aloe turkanensis* against various species of micro-organims

On both broth dilution and agar well diffusion method, the methanol and water extracts were found to have statistically significant antibacterial activities except for water extract of the cultivated plant. However no antifungal activity against the clinical isolate of *Candida albicans* was found for both the water and methanol extracts even on the highest concentration of 500 mg/ml. On broth dilution method, aqueous extract of naturally growing *A. turkanensis* inhibited *S. aureus* bacteria at the concentration of 50 mg/ml which had a similar activity as methanol extracts from cultivated *Aloe turkanensis*. This activity was not different from the result gotten on a different test (agar well diffusion) where the water extract zone of inhibition for the growth of *S. aureus* was recorded as 19.75±1mm while for methanol it was 18.5±0.7mm. For the methanol extract of naturally growing *Aloe turkanensis*, there was a slight difference in activity compared to water extract after inhibiting the growth of the same bacteria at a concentration 100 mg/ml. However, the activity of methanol extract of cultivated *A. turkanensis* against *S. aureus* on agar well diffusion was seen to decrease with zone of inhibition recorded as 16.5±0.7mm.

On broth dilution method, water and methanol extracts activity against *B. cereus* was lower compared to *S. aureus* where the naturally growing *Aloe turkanensis* extracts inhibited the growth of *B. cereus* at the concentration of 200 mg/ml and 100 mg/ml, respectively. The activity of water extract of

naturally growing *A. turkanensis* was similar to that of methanol extracts of cultivated *Aloe turkanensis*. On agar well diffusion, methanol extracts of naturally growing *Aloe turkanensis* zone of inhibition for *B. cereus* was 16.5±0.7 mm while water extract zone of inhibition was recorded as 11.5 mm.

Methanol extracts of naturally growing *Aloe turkanensis* and cultivated *Aloe turkanensis* inhibited the growth of *P. aeroginosa* at a concentration of 200 mg/ml and 400 mg/ml respectively. However no activity was reported on agar well diffusion. The lowest activity of the plant was reported from methanol extract of cultivated *A. turkanensis* plant where it inhibited the growth of *E. coli* at a concentration of 400 mg/ml.

More analysis carried out using Analysis of variance to determine the significance of the differences in biological activity of cultivated and naturally growing plant extracts as summarized in table 4.2 shows that there was a significant difference ($P<0.05$) in bacterial growth inhibition.

4.1.3 Effects of *Aloe turkanensis* on Brine shrimp larvae

At a dosage of 1000 µg/ml, methanol and aqueous extract of the naturally growing *A. turkanensis* and the cultivated one exhibited a zero percent mortality when tested against *Artemia salina.*

Table 4.4: Extract yield following extraction of the two ecotypes of *Aloe turkanensis*

Plant specimen	Amount of Powder (gms)	Solvent (1:10)	Yield after extraction (gms)
Naturally occurring AT	200	Methanol	14.96
	200	Aqueous (Water)	13.32
Cultivated AT	200	Methanol	12
	200	Aqueous (Water)	15

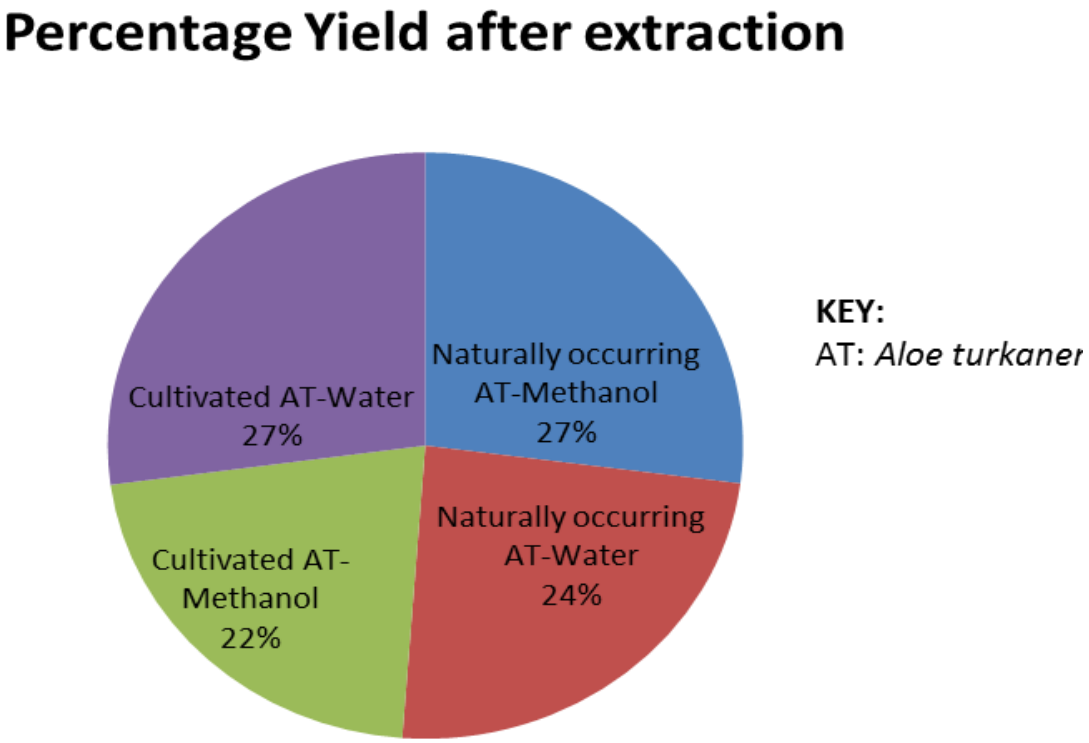

Figure 4.3: Relative percentage plant yield following extraction of cultivated and naturally growing *Aloe turkanensis* using different types of solvents

4.1.4 Phytochemicals present in *Aloe turkanensis*

On qualitative phytochemical screening, methanol and aqueous extracts of naturally growing *Aloe turkanensis* exhibited high concentration of tannins but gave negative results for flavonoids. Aqueous extracts from naturally growing *Aloe turkanensis* were shown to have high concentration of alkaloids with moderate concentration of anthraquinones, terpenoids, steroids and saponins. Methanol extract of naturally growing *Aloe turkanensis* had a lower concentration of anthraquinones, steroids and terpenoids with moderate concentration of saponins and alkaloids. For the cultivated species (cultivated *Aloe turkanensis*), aqueous extract had a moderate concentration of anthraquinones, lower concentrations of terpenoids and steroids with a negative results for saponins and alkaloids while methanol extract had a moderate concentrations of terpenoids and steroids with a negative results for anthraquinones, saponins and alkaloids. The high bioactivity of naturally growing *Aloe turkanensis* as compared to cultivated *Aloe turkanensis* plant species can be attributed to the difference in the level of phytochemicals/secondary metabolites with naturally growing *Aloe turkanensis* having higher concentration of alkaloids and anthraquinones as opposed to cultivated *Aloe turkanensis* species. These metabolites have been documented to be responsible for the antibacterial activity (Kazmi *et al.*, 1994; Ghoshal *et al.*, 1996; Rattmann et al., 2005).

Table 4.5: Qualitative phytochemical screening of methanol and water extracts of different ecotypes of *Aloe turkanensis*

Samples	Tannins	Anthraquinones	Terpenoids/ steroids	Saponins	Flavonoids	Alkaloids
Aq-Eco 1	+++	++	++	++	-	+++
Met-Eco 1	+++	+	+	++	-	++
Aq-Eco 2	+++	++	+	-	-	-
Met-Eco 2	+++	-	++	-	-	-

KEY:

Aq-Eco-1	Aqueous extract of naturally growing *Aloe turkanensis*
Met-Eco 1	Methanol extract of naturally growing *Aloe turkanensis*
Aq-Eco-2	Aqueous extract of cultivated *Aloe turkanensis* plant
Met-Eco 2	Methanol extract of cultivated *Aloe turkanensis* plant
+++	Higher Concentration
++	Moderately higher concentration
+	Lower Concentration
-	Negative results

4.2 Discussion

Turkana community has a rich cultural knowledge of medicinal plants including *Aloe turkanensis* which is a potential plant for drug development and bioprospecting. Majority of the Turkana people uses *Aloe turkanensis* for management of many disease conditions, including skin conditions and treatment of malaria. This could probably be due to cultural trends and the fact that there are fewer health facilities in the region as compared to the urban centers. However, only a few individual use the plant for management of livestock disease. The latter could be attributed to the fact there are a number of livestock projects spearheaded and partially funded by government and non-governmental organizations such as LWF and FAO which willingly offer veterinary services to livestock at no cost with an objective of improving the livelihoods of the community. This study shows a variation of phytochemicals in *A. turkanensis* grown in different geographical environments. This variation in biological activity, yields and phytochemical analysis may be caused by differences in kinds and/or concentration of the active compounds present in the plants species. The phytochemicals content is an attribute of differences in soil, age, seasons, climate and type of vegetation among the ecological zones (Daniel *et al.*, 2011). These factors contribute in determination of the phytochemicals that a plant is able to concentrate during metabolism. Furthermore, phytochemical screening indicates presence of some secondary

metabolites in some plants while others are either lacking or have in small quantity (Table 4.5). Phytochemical production in plants varies with the geographical location (Shannon *et al*., 2002). Plant developmental stage influences secondary metabolism; defense compounds are generally more concentrated and diverse when plants are young and more "apparent" to herbivores, but they are known to decrease with age as structural defenses are developed (Feeny, 1976).

The results on antibacterial tests indicate that some plants used in traditional medicine have antibacterial activity and lack antifungal activity. The growth of bacterial organism on some crude methanol extracts can be due to natural resistant of the organism or lack of antimicrobial principle. The plant under study was identified by traditional healers as important in the management of infectious diseases. The extracts from naturally occurring *Aloe turkanensis* and methanol extract of the cultivated plant showed a narrow spectrum of antibacterial activity by inhibiting the growth of gram positive bacteria. Their use by TMPs in management/treatment of opportunistic bacteria is therefore justifiable.

The indiscriminate use of antimicrobials has led to development of antimicrobial resistance to drugs. Several researchers have reported the cost effective use of medicinal plants remedies. Stermitz et al (2000) has reported synergy of herbal remedies with convectional antimicrobials. The current study supports the continued intensive study of traditional

remedies, conservation and value addition of *A. turkanensis* in ethnomedicinal use. Smith *et al*., (1999) noted that the emergence of bacterial resistance threatens to return us to the era before the development of antibiotics due to increase in antimicrobial resistance in health care associated pathogens. The rapid development of resistance including the emergence of multi-drug resistant tuberculosis (MDR-TB) shows that the potency of prevalent antibiotics is decreasing steadily. This situation calls for urgent need for new and safe antimicrobials for replacement of invalidated antimicrobials or use antibiotic in a rotation programs (Quale and Atwood, 1996).

However while exploiting the natural products, scientists need to embrace the concept of sustainability as well as ecological impact on the value of medicinal plants. Many types of action can be taken in favour of conservation and sustainable use of medicinal plants. Some of these are undertaken directly at the places where the plants are found, while others are less direct, such as some of those relating to commercial systems, *ex situ* conservation and bioprospecting. However, the results on antimicrobial activity and the phytochemistry of the two ecotypes under study indicate that there is a difference in the bioactivity expressed when the plants are cultivated in different environment. For this reason, most work by conservationists on medicinal plants should be with those people who own, manage or make use of these species, or else own or manage the

land on which they grow. The conservationists need to identify the conditions at field sites that are most favourable for releasing the potential offered by medicinal plants to achieve maximum value of the plant in terms of bioactivity, conservation and sustainable development. It is in working with such stakeholders that the special meanings of medicinal plants to people can best be 'exploited' (Alan, 2004).

Sustainability requires the consideration of the economic, environmental and social aspects of products and product systems. Therefore, responsible decision-making in public policy, industry and related fields should consider those issues for present and future relevance (Mary, 2009).

The use of bioassay of bioactivity using brine shrimp lethality test has been done for some medicinal plants in Kenya (Mwangi *et al*., 1999, Gakuya, 2001). Mwangi *et al*., 1999 indicated that lack of lethality to brine shrimp does not mean absence of biological activity. The current studies showed that most of extracts had biological activity yet showed no lethality. Therefore the safety of *Aloe turkanensis* when used as a herbal remedy by Turkana community is justifiable.

4.3 Conclusion

1. The aqueous and methanol extract of naturally growing and cultivated *Aloe turkanensis* showed appreciable antibacterial properties but lacked antifungal activity against *Candida albicans*.

2. The aqueous extract of naturally growing *Aloe turkanensis* showed higher antibacterial activity against *S. aureus* compared to methanol extract.
3. Methanol extracts of the cultivated *Aloe turkanensis* showed higher antibacterial activity against *S. aureus* compared to aqueous extract.
4. Aqueous extract of the cultivated *Aloe turkanensis* showed no antibacterial activity.
5. Both aqueous and methanol extracts of naturally growing and cultivated *Aloe turkanensis* showed moderate to high concentrations of phytochemicals
6. Both aqueous and methanol extracts of naturally growing and cultivated plant were found non-toxic at a concentration of 1000µg/ml with a 100% survival of Brine Shrimp larva

4.4 Recommendations

1 Based on the ethnobotanical study and laboratory screening of *Aloe turkanensis*, further phytochemical, antibacterial and toxicological studies need to be done on *Aloe turkanensis* in order to quantify the amount of phytochemicals present, find out the *in-vivo* toxicity profile, identify the active principles and consider taking the phytotherapeutic products to clinical trials.

2 Antimalarial activity of *Aloe turkanensis* need to be studied to validate the claims made by Turkana herbalist of its efficacy.

3 Scientists involved in natural products screening need to embrace the ecological impact on the value of medicinal plants. This will enlighten the conservationists in identifying the conditions at field sites that are most favourable for releasing the potential offered by medicinal plants to achieve maximum value of the plant in terms of bioactivity.

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APPENDICES

Appendix 1: Data acquisition questionnaire

EVALUATION OF THE ETHNOMEDICAL USES OF *ALOE TURKANENSIS*

Serial number of the questionnaire……

Name of the interviewer……..

Date……

A. PART ONE: CONSENT

RESEARCHER'S DECLARATION:

1. The following research will be undertaken respecting the indigenous knowledge and intellectual proprietary of the herbal practitioners.
2. We will at no time initiate or conduct practices that are deemed to obtain information from the respondents by intimidation, coercion or false pretense.
3. We will be under no obligation to edit or tamper with the information provided by the respondents.
4. The information collected will be used for the described research purpose and not any other undisclosed intentions.

Investigating team:

Dr. Z. M. Rukenya (BVM) Signature…….

Date……

Prof. J.M. Mbaria J (PhD) Signature……

Date……

Prof. S.G. Kiama (PhD) Signature…….

Date……

Dr. P. M. Mbaabu (PhD) Signature…….

Date……

RESPONDENTS CONSENT AGREEMENT

I……hereby agree to participate in this study with my full consent and conscience and declare that to the best of my knowledge the information that I have provided is true, accurate and complete.

Signature/thumb

print…….Date…….

B. PART TWO

RESPONDENT BIODATA

Name……Age

(yrs)……Gender……

Location of practice…….

Number of years/months in practice……..

How did you acquire the skills?

Level of education

(None…..Primary….Secondary……College…..Other……)

Contact(s) address?.....

INFORMATION ON TRADITIONAL HERBAL PRACTICE

1. What do you call this plant?
    a. Local name……
    b. Common name……
    c. Scientific name……
2. Do you use it? (……) if yes, for what purpose?
3. What conditions do you treat using this plant?
4. For the above conditions (s) (……), what are the signs/symptoms?……
5. Where do you harvest the plant?.....
6. When do you harvest it? ……
7. Which part and how do you harvest the plant?.....
8. How do you prepare the medicine?.....……
9. How do you administer the medicine to the patient for the above condition?……
10. How much of the extracts to you administer?.....
11. For how long do you prescribe that the patient take the medicine?.....
12. How are you able to determine how much to give to a patient and for how long (how do you gauge the level of infection?)
13. How long does the patient take before feeling well…after finishing the dose? How do you know/any follow-up?

14. What are some of the side effects during the period of treatment?
15. What are the contraindications…Which patients would you recommend not taking this medicine because of age, health statues, genetics, any other?……
16. If the patient takes too much medicine, what happens?……
17. What can you give to a patient who has taken too much of the medicine?
18. How do you store/preserve the medicine?
19. How long do you keep the medicine before it goes bad?

Appendix 2: Procedure used in preparation of blood agar medium

Suspend 40g of blood agar base in 1 liter of distilled water and boil to dissolve. Sterilize by autoclaving at 121°C for 15 minutes. Cool the base to 45-55°C. Add 7% sterile blood to the sterile media. The blood is warmed to 37°C before adding. Dispense the mixture into sterile Petri dishes.

Appendix 3: Procedure used in preparation of Mueller Hinton Agar (Oxoid®) medium

Typical formula (g/l):

Beef dehydrated infusion 300

Casein hydrolysate 17.5

Starch 1.5

Agar 17.7

Suspend 38g in 1L of distilled water. Bring to boil to dissolve medium completely. Sterilize by autoclaving at 121°C for 15 minutes.

Appendix 4: Procedure used in preparation of Mueller Hinton Broth medium (Oxoid®)

Typical formula (g/l):

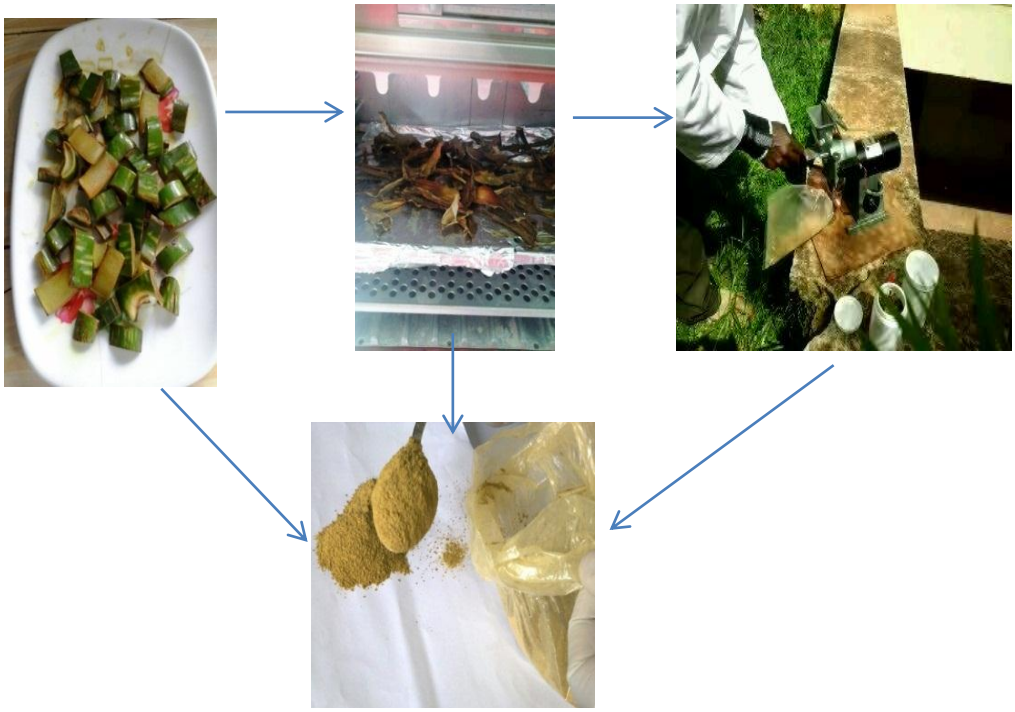
Beef dehydrated infusion 300

Casein hydrolysate 17.5

Starch 1.5

Dissolve 21g in 1L of distilled water. Bring to boil to dissolve medium completely. Sterilize by autoclaving at 121°C for 15 minutes.

Appendix 5: An illustration showing how the plant was prepared prior to extraction



Appendix 6: An illustration showing the procedure used for plant extraction in the laboratory