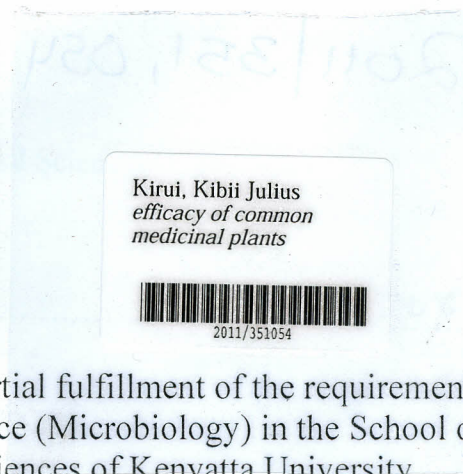


EFFICACY OF COMMON MEDICINAL PLANTS USED BY SAMBURU  
COMMUNITY TO TREAT WATERBORNE DIARRHEAL DISEASES //

By

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A thesis submitted in partial fulfillment of the requirements for award of the  
degree of Master of Science (Microbiology) in the School of Pure and Applied  
Sciences of Kenyatta University

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## DECLARATION

I, Kibii Kirui , declare that this thesis is my original work and has not been presented for the award of a degree in any other University or any other award.

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## DEDICATION

To my entire family and mostly my dear wife Rose, children, Rolus and Faith who, despite my long stay away from home during the course of this work, stood by me, persevered and provided me with much inspiration and energy.

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## ACRONYMS AND ABBREVIATIONS

UN	-	United Nations
WHO	-	World Health Organization
APHA	-	American Public Health Association
GITI	-	Gastrointestinal Tract Infections
ETEC	-	Enterotoxigenic Escherichia coli
HLT	-	Heat Labile
ST	-	Heat Stable
EIEC	-	Enteroinvasive Escherichia coli
EPEC	-	Enteropathogenic Escherichia coli
EAggEC	-	Enterotoxigenic Escherichia coli
EHEC	-	Enterohemorrhagic Escherichia coli
US	-	United States
UK	-	United Kingdom
IUPAC	-	International Union of Pure and Applied Chemistry
CFU	-	Colony Forming Unit
MIC	-	Minimum Inhibitory Concentration
MBC	-	Minimum Bactericidal Concentration
ANOVA	-	Analysis of Variance
MPN	-	Most Probable Number

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## ABSTRACT

Samburu District is characterized by limited availability of water resources. Competition for water between humans, livestock and wildlife is a common feature in the district. The pressure of water use in Samburu combined with a diversity of users results in contamination of water in different water sources. Past studies have revealed that water from various sources open to use by the different populations living in the area are usually contaminated with *Escherichia coli*, *Salmonella typhi* and other potentially pathogenic microorganisms. This exposes the Samburu community to waterborne diseases such as dysentery, typhoid fever and cholera. Most community members rely on traditional medicine that is cheap and readily available within the community to cure various ailments. This study aimed at determining the physico-chemical and microbial quality of water from different sources and assessing the efficacy of common medicinal plants used by the Samburu people to treat waterborne diarrheal diseases. Seven medicinal plants used by Samburu herbalists for the treatment of stomach illnesses were investigated for *in vitro* antibacterial activity and water disinfection. Water extracts of the dried powdered plant material were directly used to treat the water samples collected while the extracts for testing effectiveness against the fecal pathogens, *E. coli*, *S. dysenteriae* and *S. typhi* were freeze-dried using standard methods. Susceptibility testing was carried out using disc diffusion test (Kirby-Bauer) and minimum inhibitory concentration (MIC) carried out using broth microdilution method. The finding on microbial quality of water confirms the presence of indicator bacteria in water from most sources. The water sources investigated varied widely in the physico-chemical properties (pH, electrical conductivity, orthophosphate phosphorus, total phosphorus and total alkalinity). However, there were no significant differences in the physico-chemical properties of water among the study sites. Efficacy of water treatment with medicinal plants expressed as percentage reduction in bacteria colonies revealed that *A. nilotica* (L.) Del. extracts with a mean percentage reduction of 99.86 % was the most effective in reducing the number of bacterial colonies. *A. anthelmintica* (A. Rich.) extracts with a mean of 9.47 %, had the lowest reduction of bacterial colonies. The study also revealed a possible interaction between plant extracts and water source ( $P < 0.05$ ,  $df = 54$ ). *A. nilotica* registered lower MIC value of 1.56 mg ml<sup>-1</sup> against *S. typhi* and *S. dysenteriae* while *E. divinorum* Hiern, *A. tortilis* (Forssk.) Hayne and *A. etbaica* Schweinf had MIC greater than 25 mg ml<sup>-1</sup> against the three fecal bacteria used. Antimicrobial phytochemical compounds tannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids were present in all the seven plants. However, *A. anthelmintica*, *E. divinorum*, *A. tortilis*, *A. etbaica* were weakly effective *in vitro*, suggesting that water may not be extracting enough of such phytochemicals. This study confirms the efficacy of water extracts from *A. nilotica* and *A. seyal* Del. (Talh) against diarrheal diseases associated with drinking water. The sensitivity of the test bacteria to extracts from these plants makes them potential sources of antimicrobial drugs.

## CHAPTER ONE: INTRODUCTION

### 1.1 Background to the study

Globally, the microbial quality of water is declining rapidly (Ford and Colwell, 1996). Water related diseases are presently the most important human health concern worldwide in terms of morbidity, mortality and cost. Microbial contamination of water is responsible for many of the world's most persistent and widespread diseases (Gleick, 1993). Infectious diseases related to water include cholera, typhoid, paratyphoid, dysentery, brucellosis, leptospirosis, hepatitis and poliomyelitis, which are widespread despite all measures that have been taken to eradicate them (UN, 2005). The major source of water pathogens is fecal contamination from infected humans, pets, farm animals and wildlife (Geldreich, 1997). Animal and human wastes also act as a major source of organic matter in aquatic environments. This organic matter contains nutrients, which sustain the survival of pathogenic bacteria implicated in causing waterborne diseases. The number of bacteria present in freshwater is therefore closely associated with the amount of organic matter present.

Hospital treatment of waterborne diseases in poor communities is not affordable since most community members do not have a consistent source of income. Most members of such communities opt for herbal medicine. Many plant species contain potent biologically active compounds and at least 25% of the drugs currently used in modern medicine are derived from plants (Wanyoike, 2000). Many patients opt for traditional remedies because modern drugs are too expensive, not easily accessible or may no longer be effective because of resistance. Although the Kenya Government recognizes the value of traditional medicine as an alternative source of medication, most of the plants used in the treatment of diarrheal diseases have not been screened for their antimicrobial activity. It is therefore possible for people to use the plants, which may not be effective hence making the disease chronic. There is therefore an urgent need to evaluate the efficacy of plants used for medicinal purposes.

## 1.2 Problem statement and justification

Samburu District is semi-arid savannah grassland with an annual rainfall of between 250 mm-500 mm. Because of the low and erratic rainfall, this region experiences frequent shortage of water. During periods of low water availability, competition for water between humans, livestock and wildlife is common. The pressure for use combined with a diversity of users may result in contamination of water in different sources. Human and animal wastes that may get into water sources through surface run off may contain pathogenic bacteria such as *Salmonella typhi*, *Shigella dysenteriae* and *Escherichia coli*.

A survey to determine the bacteriological quality of water from common sources in Wamba Division, Samburu District was recently carried out by an Earthwatch Research Team (unpublished). According to these studies, water from a number of sources, which include rivers, dams and shallow dry riverbed wells are contaminated with fecal pathogenic bacteria. Hence, community members in this region are at risk of contracting waterborne diseases such as typhoid fever, dysentery and other gastrointestinal complications. However, these studies did not address measures that can be taken to both prevent and cure waterborne diseases, which the current study explored.

Identification of medicinal plants that can disinfect water can be of great value to the Samburu people for they will use them to prevent infections related to drinking water contaminated with fecal pathogens. The plants can be obtained easily since they are readily available and the mode of preparation and use do not require specialized skills. The potential of medicinal plants to disinfect water compared to commercial wastewater treatment plants reduces the dangers caused by residual compounds like trihalomethane, which occur in chlorinated water.

## 1.3 Research questions

The following research questions were formulated to address the problem stated.

- i. Which plant species are used to treat diarrheal diseases known to arise from drinking contaminated water in Wamba Division?
- ii. How effective are the identified plants in reducing bacterial loads in water of different physico-chemical properties?
- iii. Are the extracts from the plant species effective in reducing bacterial loads in water also effective against fecal pathogenic bacteria known to exist in contaminated water?
- iv. What groups of antimicrobial compounds are present in the medicinal plant species found to be effective against pathogenic bacteria?

## **1.4 Hypotheses**

The assumptions taken to guide this research were as follows

- i. Plants used for treatment of waterborne diseases are available within Wamba Division of the Samburu Eco-region.
- ii. Medicinal plants used by the Samburu people are effective in reducing bacterial load in water of different physico-chemical properties.
- iii. Medicinal plants used by the Samburu people to treat waterborne diseases are effective against fecal pathogens known to exist in contaminated water.
- iv. Medicinal plants species effective against fecal pathogens contain various known antimicrobial compounds.

## **1.5 Objectives of the study**

The main objective of the study was to determine the antimicrobial properties of medicinal plant species used by the Samburu people to treat waterborne diseases. To enable to achieve this objective, the study was guided by the following specific research objectives.

- i. Identify medicinal plant species and plant parts used to treat waterborne bacterial diseases.
- ii. Test the efficacy of identified medicinal plant species in reducing bacterial loads in water of different physico-chemical properties.
- iii. Test the efficacy of plant extracts shown to reduce bacterial load against bacterial pathogens known to exist in contaminated water.
- iv. Determine the groups of antimicrobial compounds present in plants showing efficacy against bacterial pathogens known to exist in contaminated water.

## CHAPTER TWO: LITERATURE REVIEW

### 2.1 Determination of bacterial water quality

Consumption or contact with water contaminated by fecal wastes washed into rivers, streams, pools or seeping into wells or boreholes cause serious ill health (Cheesbrough, 2000). Hence, routine testing of water for fecal contamination is necessary. Microbial quality of water has traditionally been assessed by monitoring the concentration of fecal indicator bacteria such as fecal coliforms and enterococci originating from warm-blooded animals (Chao *et al.*, 2003; Lamendella *et al.*, 2007).

In 1914, the United State of America Public Health Service adopted the use of coliform bacteria as “indicator microorganisms” to indicate the presence of fecal contamination in water. Ideally, if indicator microorganisms are detected in a substance, it indicates the presence of fecal contamination and therefore possible presence of pathogenic microorganisms in the water (APHA, 1998). The most common indicators are total coliform bacteria, fecal coliforms, and *Escherichia coli*. Total coliforms are defined as any bacteria capable of fermenting lactose (milk sugar) with the production of acid and gas in 48 hours at 35 °C under aerobic conditions. This group of bacteria may contain several genera and species of bacteria including *Enterobacter*, *Klebsiella*, *Aeromonas* and *Escherichia coli*.

Since coliforms were adopted as an indicator of fecal contamination in water in 1914, their use has since been questioned. This is because, although they are found naturally in the intestines of warm-blooded animals including humans, they may also be found naturally in other sources that are not associated with fecal contamination. Fecal coliform bacteria are a sub-set of the total coliform group that grow at 44.5 °C. The reason for testing for fecal coliforms is that they are more restricted in their source to the gastrointestinal tract of

warmblooded animals. Again, their presence in water could indicate fecal contamination and therefore presence of pathogens.

## 2.2 Waterborne diseases

Water related diseases continue to pose a major health challenge globally. The World Health Organization (WHO) estimates that 1.1 billion people do not have access to improved water supplies (UN Development Program, 1996). Drinking contaminated water contributes substantially to the estimated 2.2 million annual deaths from diarrhea (WHO, 1999). Despite the current efforts to increase the number of people with access to adequate water and sanitation, the proportion of people with access to adequate water and sanitation is not increasing due to population growth, insufficient continued investment, inefficient systems and lack of training to maintain systems in working order (Cheesbrough, 2000).

Infectious diseases related to water include bacterial, parasitic and viral diseases. However, this study focused on common bacterial diseases. Bacterial waterborne diseases which are prevalent include; cholera, typhoid fever, shigellosis, campylobacter enteritis and *E. coli* diarrhea.

### 2.2.1 Typhoid fever

#### 2.2.1.1 Description

Typhoid fever is caused by *Salmonella typhi* and *Salmonella paratyphi* A, B and C bacteria. These are typical members of *Enterobacteriaceae* family. They are gram negative bacilli, which are able to grow on a wide range of relatively simple media and distinguished from other members of the family by their biochemical characteristics and antigenic structure (Greenwood *et al.*, 2002). Their normal habitat is the animal intestine. Typhoid fever is a disease occurring more commonly among people after a travel to or residence in places where there is fecal contamination of food and water. Typhoid fever remains an important public

health problem particularly in developing countries with approximately 10 million cases, which results in 700,000 deaths annually (Talaro, 2007). Morbidity resulting from typhoid fever is more severe among patients with immunosuppressant and urinary tract abnormalities (Talaro, 2007). A small number of patients that recover from typhoid fever become chronic carriers (1-3%). They harbor the pathogen in gall bladder and continue to shed the organism indefinitely (Greenwood *et al.*, 2002).

#### **2.2.1.2 Pathogenesis and clinical manifestation**

Infection is by ingesting the salmonella organisms in contaminated food or water. Once ingested, the organism penetrates the ileum mucosa where they multiply and invade the bloodstream via the thoracic duct (Greenwood *et al.*, 2002). In the first 7-10 days, the organisms invade the liver, gall bladder, spleen, kidney and bone marrow where they further multiply before the bacilli pass into the blood causing fever. Invasion extends from the gallbladder to the intestine. Payer's patches and other gut lymphoid tissues become involved in an inflammatory reaction and infiltration with mononuclear cells, followed by necrosis, sloughing, and formation of characteristic typhoid ulcers. Symptoms are a high fever of 40 °C and a continued headache. Diarrhea appears only during the second or third week and the fever tends to decline (Greenwood *et al.*, 2002).

#### **2.2.1.3 Treatment**

Typhoid fever can be treated using either chloramphenicol or sulfatrimethoprim, these being the drugs of choice. Though some resistant strains occur, antimicrobial drugs are usually effective in treating the chronic carriers, but surgical removal of the gallbladder may be necessary in individuals with chronic gall bladder inflammation. Drug companies have produced two new vaccines; one is a live attenuated oral vaccine, and the other is the capsular polysaccharide (Talaro, 2007).

## 2.2.2 Bacillary dysentery

### 2.2.2.1 Description

Members of the genus *Shigella* cause this disease. This genus is subdivided into four species namely; *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonni*. They are typical members of the *Enterobacteriaceae* family, which are gram-negative bacilli, non-motile and non-capsulated (Greenwood *et al.*, 2002). These bacteria are resident on the intestinal tract of humans, apes and monkeys. *Shigella dysenteriae* serotype 1 is the most virulent hence have been responsible for many cases of the hemolytic uremic syndrome accompanying outbreaks of dysentery in several countries. It is estimated that there are 164.7 million annual episodes of shigellosis throughout the world (Cheesbrough, 2000). In tropical areas of the developing world, shigellosis is endemic. It has been estimated that some 5 million cases require hospital treatment and about 600,000 die every year. Young children are particularly vulnerable (Greenwood *et al.*, 2002). *S. dysenteriae* is prevalent in tropical areas hence the high mortality rate in these areas (Tortora *et al.*, 2004).

### 2.2.2.2 Pathogenesis and clinical manifestation

The infective dose required to cause disease is as low as 10 viable *S. dysenteriae* bacteria cells. The bacteria are not much affected by stomach acidity and this gives them a chance to proliferate to immense numbers in the small intestine (Greenwood *et al.*, 2002). In the small intestine, the bacteria attach themselves to epithelial cells. The membranous cellular walls ruffle because of this attachment that enables the *Shigella* to be engulfed. There the bacteria multiply releasing shiga toxins, which destroy tissues and causes dysentery. The onset of symptoms is usually sudden and frequent. Abdominal colic followed by onset of watery diarrhea with fever and malaise are initial symptoms which progress to abdominal cramps and the frequent passage of small volumes of stool, predominantly consisting of bloody mucus. *Shigella* bacteria rarely invade the bloodstream (Tortora *et al.*, 2004).

### 2.2.2.3 Treatment

Symptomatic treatment is done by use of oral rehydration salt solution. Antibiotic treatment is by use of ampicillin, co-trimoxazole, tetracycline or ciprofloxacin, which are the antibiotics of choice (Tortora *et al.*, 2004).

## 2.2.3 *Escherichia coli* Gastroenteritis

### 2.2.3.1 Description

*E. coli* colonizes the gastrointestinal tract (GIT) of most warm-blooded animals within hours or few days after birth. This enteric bacterium belongs to a large bacterial family, *Enterobacteriaceae*. *E. coli* is facultative anaerobic gram-negative rod that lives in the intestinal tracts of animals in health and in disease. The ability of *E. coli* to ferment glucose producing acid and gas distinguished it from other fecal coliforms. It is oxidase negative and when motile produces peritrichous flagella (Greenwood *et al.*, 2002). Physiologically, *E. coli* is versatile and well adapted to its characteristic habitats. It can grow in media with glucose as the sole organic constituent. *E. coli* is a constituent inhabitant of human intestinal tract and it is the predominant facultative organism in the human GIT. The regular presence of *E. coli* in the human intestine and feces has led to tracking the bacterium in nature as an indicator of fecal pollution and water contamination. As such, it is taken to mean that, wherever *E. coli* is found, there may be fecal contamination by intestinal parasites of humans.

### 2.2.3.2 Pathogenesis and clinical manifestation

*E. coli* is normally carried in the gut as a harmless commensal, but some strains may cause gastrointestinal tract infections (GITIs), ranging in severity from mild self-limiting diarrhea to hemorrhagic colitis. Such strains fall into five groups each associated with specific serotypes and with different pathogenic mechanisms (Greenwood *et al.*, 2002).

**a. Enterotoxigenic *E. coli* (ETEC)**

Enterotoxigenic *E. coli* (ETEC) are an important cause of diarrhea in infants and travelers in regions of poor sanitation. The disease varies from minor discomfort to a severe cholera like syndrome. ETEC adhering structures are called fimbriae. Fimbriae are species specific and help the bacterium to adhere to the intestinal mucosa of the small intestine. The disease requires colonization and elaboration of either heat labile (LT) or heat stable (ST) toxins. Both traits are plasmid encoded (Greenwood *et al.*, 2004). Symptoms of ETEC infections include diarrhea without fever.

**b. Enteroinvasive *E. coli* (EIEC)**

EIEC penetrate and multiply within epithelial cells of the colon causing widespread cell destruction. The clinical syndrome is identical to dysentery caused by *Shigella* and includes diarrhea with fever. Unlike ETEC, Enteroinvasive *E. coli* lack fimbrial adhesins but possess a specific adhesin that, as in *Shigella*, is thought to be an outer membrane protein (Greenwood *et al.*, 2002).

**c. Enteropathogenic *E. coli* (EPEC)**

EPEC induce a watery diarrhea similar to ETEC, but do not produce ST or LT toxins. They produce a non-fimbrial adhesin, which is an outer membrane protein that mediates the final stages in adherence. Adherence of EPEC strains to the intestinal mucosa is a very complicated process and produces dramatic effects in the ultrastructure of the cells resulting in the rearrangement of actin within the vicinity of adherent bacteria. EPEC strains are said to be moderately invasive compared with *Shigella*, but unlike ETEC or enteroaggregative *E. coli* (EaggEC), they cause an inflammatory response. The diarrhea and other symptoms of EPEC infections are caused by bacterial invasion of host cells and interference with normal cellular signal transduction, rather than by production of toxins.

#### d. **Enteroaggregative *E. coli* (EAggEC)**

The distinguishing feature of EAggEC strains is their ability to attach to tissue culture cells in an aggregative manner. These strains are associated with persistent diarrhea in young children. They resemble ETEC strains in that the bacteria adhere to the intestinal mucosa and cause non- bloody diarrhea without invading or causing inflammation.

#### e. **Enterohemorrhagic *E. coli* (EHEC)**

EHEC are represented by a single strain (serotype 0157:H7), which causes a diarrhea syndrome distinct from EIEC (and *Shigella*) in that there is copious bloody discharge without fever. It can progress to hemolytic uremic syndrome with renal failure. EHEC has been reported mainly in Europe, North America, refugee camps in Mozambique, Swaziland and Malawi (Cheesbrough, 2000). The bacteria do not invade mucosal cells as readily as *Shigella*, but it produces a toxin that is virtually identical to shiga toxin. The toxin plays a role in the intense inflammatory response produced by EHEC strains (Greenwood *et al.*, 2002).

### 2.2.3.3 **Treatment and control**

*E. coli* gastroenteritis is usually self-limiting and patients rarely require more than fluid and electrolyte replacement. Ciprofloxacin and other fluoroquinolones are effective drugs of choice (Greenwood *et al.*, 2002). Basic control measure is the purification of water.

## 2.2.4 ***Campylobacter* gastroenteritis**

### 2.2.4.1 **Description**

There are more than an estimated 2 million cases of *campylobacter* gastroenteritis in the United States (US) annually, usually caused by *Campylobacter jejuni* (Tortaro *et al.*, 2004). In most industrialized countries, *Campylobacter jejuni* is the most frequently identified cause of acute infective diarrhea and as a result causes much morbidity and economic loss.

*Campylobacter* was first isolated in 1906 from aborting sheep in the United Kingdom (UK) (Greenwood *et al.*, 2002). The discovery that *C. jejuni* causes acute enteritis in man was not made until the late 1970s. *C. jejuni* is a small, spirally curved gram-negative rod with a single flagellum at one or both poles, which endows the bacteria with exceptionally rapid motility. This bacterium is usually sensitive to high levels of molecular oxygen and super oxides such as hydrogen peroxides. Hence, micro aerophilic conditions must be provided for their cultivation. They grow best at 42-43 °C.

#### **2.2.4.2 Pathogenesis and clinical manifestation**

Infection follows by ingestion of either contaminated water or food. The jejunum and ileum are the first sites to become colonized but the infection extends distally to affect the terminal ileum and usually the colon and rectum. The main sources of infection are animals that constantly shed off bacteria into the surface waters, lakes, rivers and streams (Tortora *et al.*, 2004). In water, the bacteria can survive for many weeks at low temperatures. Following ingestion, incubation period is 3 days. Illness may start with abdominal pain and diarrhea. Nausea is common but vomiting is seldom pronounced. Severe watery diarrhea with blood may lead to prostration.

#### **2.2.4.3 Treatment and control**

*Campylobacter* gastroenteritis is usually self-limiting and patients rarely require more than fluid and electrolyte replacement. Ciprofloxacin and other fluoroquinolones are effective drugs of choice (Greenwood *et al.*, 2002). Basic control measure is the purification of water.

### **2.2.5 Cholera**

#### **2.2.5.1 Description**

This is a disease caused by bacteria of the family *Vibrionaceae* (Greenwood *et al.*, 2002). The genus *Vibrio* is the most extensively characterized and medically important group within

this family. The genus includes more than 30 species that are commonly found in aquatic environment. Some cause disease in man as well as in marine vertebrates and invertebrates. The most important pathogens of man are *Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus*. Various other species are occasionally implicated as opportunistic pathogens (Greenwood *et al.*, 2004). Until recently, it was thought that man was the only natural host of *V. cholerae* and that all infections resulted from direct or indirect contact with human waste. It is now recognized that *V. cholerae* like other *Vibrio*, is commonly found as a natural resident of aquatic environments in areas free of cholera (Tortora *et al.*, 2004) hence its presence is not necessarily associated with fecal contamination.

*Vibrio cholerae* the causative agent of cholera; is a slightly curved, gram-negative rod with a single polar flagellum. The serogroup 0:1, which caused a cholera pandemic in 1880s in Europe and North America (Tortora *et al.*, 2004), is known as the classical strain. A later pandemic was caused by strain of serogroup 0:1 named *El Tor* or eltor (for the El Tor quarantine camp for pilgrims to Mecca, where it was first isolated). Until 1990s, it was thought that only *V. cholerae* 0:1 caused cholera, but a widespread epidemic in India and Bangladesh by a new serogroup, 0:139 changed this view (Tortora *et al.*, 2004).

#### **2.2.5.2 Pathogenesis and clinical manifestations**

*V. cholerae* 0:1 penetrates the mucus layer of small intestine and adheres to the enterocyte surface with the help of proteolytic enzymes. Adherent bacteria produce a potent enterotoxin, which causes the transfer of adenosine diphosphoribose to form adenylyl cyclase that is responsible for the generation of intracellular cyclic adenosine monophosphate. This in turn causes the inhibition of uptake of Na<sup>+</sup> and Cl<sup>-</sup> ion by cells lining the villi, together with hypersecretion of Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> ion. This blocks the uptake of water, which normally accompanies Na<sup>+</sup> and Cl<sup>-</sup> ion absorption. There is passive net flow of water across mucosal

cells, leading to serious loss of water and electrolytes, taking on a typical appearance of “rice water stools” from masses of intestinal mucus, epithelial cells and bacteria.

Sudden loss of these fluids and electrolytes causes shock, collapse and often death. Because of the loss of fluid, the blood becomes so viscous that vital organs are unable to function properly. Violent vomiting sometimes occurs. The microbes are not invasive, and a fever is usually not present. Recovery from the disease results in an effective immunity based on the antigenic activity of both the cells and the enterotoxin. However, because of the antigenic differences among bacterial strains, the same person can have cholera more than once.

### **2.2.5.3 Treatment and control**

Oral rehydration therapy is administered as a replacement of fluid and electrolytes, but severe cases may require intravenous rehydration. Antibiotics such as tetracycline, chloramphenicol and co-trimoxazole are used. Control measures include provision of safe drinking water supplies and the proper disposal of human feces.

## **2.3 Common water treatment methods**

Water can play a major role in the transmission of enteric infections and virtually all of the agents that cause water related diarrhea may be present in contaminated water. Water treatment is a central challenge in the 21<sup>st</sup> century (Acra *et al.*, 1990). An array of disinfection methodologies and technologies are available for the treatment of water. These include; chlorination, ozonisation, ultraviolet disinfection, sterile filtration, thermal disinfection and slow sand filtration.

### **2.3.1 Chlorination**

Chlorination is the least expensive methods for home treatment of drinking water that have been proved to reduce waterborne diarrheal disease (Quick *et al.*, 2002). In this method,

chlorine gas, sodium or calcium hypochlorite is added to water. The efficiency of the chlorination is dependent on the pH, concentration of the organic matter, temperature of the water and contact time. When sodium hypochlorite solution is added to highly turbid water the chlorine rapidly binds to the organic matter and so is unavailable to kill pathogens (Crump *et al.*, 2004). A lower concentration of chlorine agent or decreased temperature requires a longer contact time for disinfection. However, a minimum reaction period of 20 minutes is required for effective water disinfection. Disinfection of water contaminated by organic waste with chlorine requires high dosages. Although the high dosage can render the water microbiologically safe, such high levels of chlorine adversely, affect the taste of the water and may decrease the willingness of people to treat the water (Reller *et al.*, 2003; WHO, 2003; Acra *et al.*, 1990). Large doses of chlorine agent can also lead to high concentration of potential mutagens and carcinogens like trihalomethanes (Karol, 1995; Monarca *et al.*, 1998). Trihalomethanes have been cited to be associated with increased cancer risks in the urinary and gastrointestinal tract (Koivusalo *et al.*, 1997). Despite the risks mentioned, chlorination of drinking water has played a leading role in reducing mortality rates associated with waterborne pathogens.

### 2.3.2 Ozonization

Ozone is a triatomic oxygen molecule consisting of an oxygen molecule with an extra oxygen atom. It is the strongest disinfectant and oxidant suitable for water treatment. When exposed to sunlight ozone decomposes to release an oxygen atom. The released oxygen atom breaks down the bacteria cells by means of oxidation (Acra *et al.*, 1990). Its major advantage is that ozonisation does not produce undesirable by-products as ozone itself decomposes to oxygen. Its disadvantage is a low half-life and a weak solubility in water.

### 2.3.3 Solar disinfection

Solar treatment of water has been cited as the least expensive method for home treatment of drinking water (Conroy *et al.*, 1999). In this technique, water is exposed to short wave ultraviolet light, which is an effective germicide that does not affect the water quality. Treating highly turbid water using this method prevents adequate penetration of ultraviolet light that may protect organisms from thermal activity of solar disinfection (Crump *et al.*, 2004).

### 2.3.4 Sterile filtration

This process uses ultra filtration membranes with pore sizes of less than 0.5  $\mu\text{m}$ , which can only allow water molecules to pass through but not the bacterial cells. Simple ultra filters are also used in small potable water filters like those for domestic use. Two major limitations of this approach are that there is always a danger of bacteria passing through the filters and its high costs (Acra *et al.*, 1990). These limitations have confined sterile filtration to mainly pharmaceutical application.

### 2.3.5 Slow sand filtration

Slow sand filtration with filter speeds of approximately  $0.1 \text{ m h}^{-1}$ , achieves a significant reduction of the microbial counts (Acra *et al.*, 1990). Filters work by physically removing infectious agents from the water. Filters have the advantage of providing immediate access to drinking water without adding an unpleasant taste. Major disadvantage is that micro cracks or eroded channels within the filter may allow passage of unfiltered water. This technology is used primarily for wastewater treatment and potable water treatment. However, due to the large filter surface required and the time needed to maintain the filter system, slow sand filters are becoming increasingly rare in portable water treatment.

## 2.4 Medicinal plants

### 2.4.1 Brief history

Finding healing powers in plants is an ancient idea. Humankind has long used poultices and infusions of many indigenous plants dating back to prehistory (Cowan, 1999). Poultices are herbal remedies applied directly on rashes and wounds and are also used as topical pain relieving remedies, while infusions are herbal remedies which are boiled and taken together with other drinks. There is evidence that Neanderthals living 60,000 years ago in the present day Iraq used plants such as hollyhock to treat various ailments (Stockwell, 1988). This plant is still widely used for medical purposes around the world (Cowan, 1999). Early records on the use of natural products to cure various ailments have been found on Babylonian writings, among Egyptian documents and in ancient cultural records of Asia, especially India and China. Hippocrates (in the 100 B.C) mentioned 300 to 400 medicinal plants (Scultes, 1978).

In the 100 A.D, Dioscorides wrote a medicinal plant catalogue, *De materia medica*, which became the prototype for modern pharmacopoeias (Cowan, 1999). The bible offers descriptions of approximately 30 healing plants. Indeed, plants such as frankincense and myrrh probably enjoyed their status of great worth due to their medicinal properties. These plants are reported to have antiseptic properties, such that they were even employed as mouthwashes (Cowan, 1999). American groups have used 1,625 species of plants as food, 2,564 species have found use as drugs (Klink, 1997).

### 2.4.2 Medicinal plant use

Worldwide the number of individuals using and searching for drugs and dietary supplements derived from plants has increased in recent years in both developed and developing countries (Okello and Ssegawa, 2007). In an attempt to rescue some of the secrets of the wild before they disappear completely, many countries are sending botanists into the forests and savannas

to tap the knowledge of communities whose way of life is threatened by the destruction of their habitats (Arms, 1999). Plants are rich in secondary metabolites such as tannins, terpenoids, alkaloids and flavanoids that have been shown to exhibit antimicrobial activity *in vitro* and *in vivo*. This antimicrobial activity is the reason for increased research on traditional medicine, which aim at characterizing the antimicrobial activity of these plants (Martinez *et al.*, 1996).

African traditional medicine is mainly based on medicinal plants and most traditional communities still rely mostly on herbal medicines. In many remote parts of Africa where hospitals and clinics are scarce, herbal medicine plays an important role in healthcare systems (Magassouba *et al.*, 2007). For example, the Samburu community uses medicinal plants for the treatment of various diseases. A widespread usage of medicinal plants by the Samburus can be attributed to their rich indigenous knowledge of herbal medicine, poverty and inadequate healthcare facilities. In such communities, traditional herbalists operate closer to the people, taking advantage of the biodiversity of plant species in such areas to establish the cure for various diseases and ailments (Magassouba *et al.*, 2007). Although the use of herbal medicine is well established in many cultures and traditions of Africa, not much information has been documented in scientific literature (Magassouba *et al.*, 2007). Most of the time, information on medicinal plants is orally inherited, and is therefore in danger of being lost since most of the herbalists are elderly and may not pass the information wholly to their offspring.

It is estimated that there are about 2.5 million species of higher plants throughout the world and most of them have not been examined in details for their pharmacological activities (Jeevan *et al.*, 2004; Voravunthikunchai *et al.*, 2004). Since a large number of medicinal plants have not been investigated for their antibacterial activities, the use of their crude

extracts can result in health complications. Need for scientific evaluation of medicinal plant extracts is also important in assisting in the development of effective drugs. It is against this background that the present study was undertaken to assess the efficacy of selected plants as antimicrobial agents.

### **2.4.3 Phytochemical constituents**

The medicinal value of plants relates to their possession of chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952).

#### **2.4.3.1 Alkaloids**

Alkaloids are arguably the most potent therapeutic compounds and have been manufactured as various allopathic drugs including the pain killer morphine and the antimalarial quinine. Derived from amino acids, alkaloids represent a varied and complex class of nitrogenous crystalline or oily compounds. Presence of alkaloids appears to be most prevalent in the *Fabaceae*, *Papaveraceae*, *Ranunculaceae*, *Rubiaceae*, *Solanaceae* and *Berberidaceae* families (Cowan, 1999). Quinoline alkaloids is a group of alkaloids named from quinoline in the Cinchona plant, and refers to the quinoline alkaloids developed in the nucleus from tryptophan. The therapeutic value of this class of alkaloids differs according to the sub-categories, which include protoberberines, which are antibacterial and antiprotozoan. Other alkaloids include morphine, diterpenoids and solamargine. Diterpenoid have been found to have antimicrobial properties (Omulokoli *et al.*, 1997).

#### **2.4.3.2 Tannins**

Tannins are polyphenolic compounds, which are astringent (pore closing) and antiseptic. Tannin is a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution. Hamamelitannin is a source of pharmacologically used

tannin and is often found in men's aftershave lotions. Many human physiological activities such as stimulation of phagocytic cells, host mediated tumor activity and a wide range of anti-infective actions have been assigned to tannins (Haslam, 1996). Mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, and cell envelope and transport proteins. They are also known to complex with polysaccharide.

#### 2.4.3.3 Flavonoids

The term flavonoid refers to a class of plant secondary metabolites. According to the International Union of Pure and Applied Chemistry (IUPAC) nomenclature, they can be classified into flavonoids, isoflavonoids and neoflavonoids. They are widely distributed in plants fulfilling many functions including producing yellow, red or blue pigmentation in flowers and are involved in protection of plants from attack by microbes and insects (Dixon *et al.*, 1983). Flavonoids have been referred to as "nature's biological response modifiers" because of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-inflammatory, anti-allergic, antimicrobial and anti-cancer activities. These compounds have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms (Cowan, 1999). Their activity is probably due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell walls (Cowan, 1999). Flavonoids, which occur in Oolong green tea, have been demonstrated *in vitro* to exert antimicrobial activity (Borris, 1996). Flavonoids have also been shown to inhibit *in vitro* *Vibrio cholerae* 01 (Borris, 1996), *Shigella* (Vijaya *et al.*, 1995) and other bacteria and microorganisms (Sakanaka *et al.*, 1992).

#### 2.4.3.4 Terpenoids

The fragrance of plants is carried in the essential oil fraction. These oils are secondary metabolites that contain compounds based on an isoprene structure, called terpenes. Terpenes

occur as monoterpenes, diterpenes, triterpenes, tetraterpenes, hemiterpenes and sesquiterpenes. When the compounds contain additional elements, usually oxygen, they are termed terpenoids. Examples of common terpenoids are methanol and camphor, which are monoterpenes, while farnesol and artemisin are sesquiterpenes. Artemisin and its derivatives are currently used as antimalarials (Vishwakarma, 1990). Terpenoids are active against bacteria (Amaralal *et al.*, 1998), viruses (Xu *et al.*, 1996) and protozoa (Ghoshal, 1996). Terpenes are effective against both Gram-positive and Gram-negative bacteria, which include *Staphylococcus aureus*, *V. cholerae* and *P. aeruginosa* (Batista *et al.*, 1994).

#### 2.4.3.5 Lectins and polypeptides

Peptides, which are inhibitory to microorganisms were first reported in 1942 (Balls *et al.*, 1942). Since then, further studies have demonstrated the antibacterial and antifungal properties of lectins and polypeptides (De Bolle *et al.*, 1996). Their mechanism of action is attributed to the formation of ion channels in the microbial membrane (Zhang and Lewis, 1997). Thionins are peptides commonly found in barley and wheat and consist of 47 amino acid residues. They are toxic to yeasts, gram-negative and gram-positive bacteria. Fabatin, an amino acid residue from fava beans inhibits *E. coli*, *P. aeruginosa* and *Enterococcus hirae* (Zhang and Lewis, 1997).

#### 2.4.3.6 Quinones

Quinones have an aromatic ring with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds are responsible for the browning reaction in cut or injured fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin (Schmidt, 1988). Quinones are known to complex irreversibly with nucleophilic amino acids in proteins (Stern *et al.*, 1996), often leading to inactivation of the protein and loss of function. For this reason, probable targets in the

microbial cell are surface exposed adhesins, cell wall polypeptides, and membrane bound enzymes. Quinones may also render substrates unavailable to microorganisms (Cowan, 1999).

...the following... Report 11...  
...to the north...  
...with...  
...the...

...people

...District are...  
...a "Mas" language and...  
...The Samburu are mostly...  
...of Northern Kenya (Mazmani, 2006)...

...the Samburu, who...  
...in the dry...

...covered with vegetation...  
...frequent cloud...  
...from cows, camel, sheep and goats, plus some...  
...parts of the Samburu diet. The Samburu...

...group rules enforced by...  
...and wise use of...

...Climate  
...annual rainfall...  
...and...  
...The district...

## CHAPTER THREE: MATERIALS AND METHODS

### 3.1 Site of study

#### 3.1.1 Location

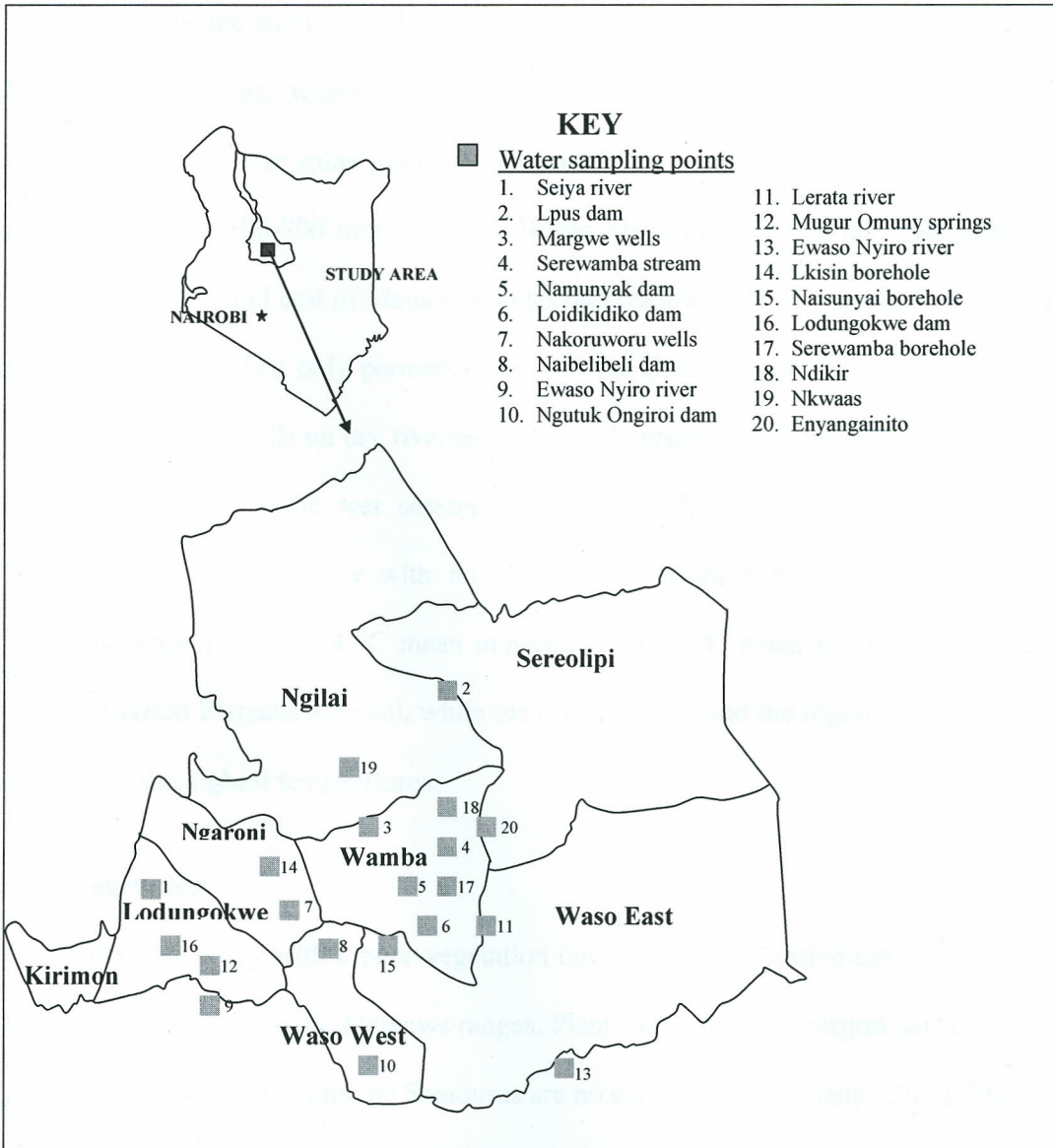
Samburu district borders the following districts: Turkana to the North West, Baringo to the southwest, Marsabit to the north east, Isiolo to the east and Laikipia to the south (**Figure 1**). The district lies between latitudes 0° 40'S and 2° 31'S and longitudes 36° 2' and 38° 10'N (Kasusya, 1998).

#### 3.1.2 The Samburu people

The inhabitants of Samburu District are mainly the Samburu people. Like their cousins the Maasai, they speak a "Maa" language and belong to the Chari-Nile branch of the Nilo-Saharan language family. The Samburu are mostly nomadic pastoralists, inhabiting the plains and highlands of Northern Kenya (Bussmann, 2006). Traditional way of nomadic life is still much more prevalent amongst the Samburu, who migrate from their lowland wet season grazing grounds up to the humid mountain areas in the dry season (Spencer, 1965). Most mountain areas like Mathews ranges are covered with vegetation, which could be the result of humidity received from mist condensation and frequent cloud formation in the peak areas. Milk and blood from cows, camel, sheep and goats, plus soups derived from wild collected herbs are the main parts of the Samburu diet. The Samburu people, who are pastoralists living in group ranches, have strong group rules enforced by appointed elders which have traditionally been essential in the conservation and wise use of communal tree and forest resources.

#### 3.1.3 Climate

The area is arid to semi-arid with annual rainfall of between 250 – 500 mm, which is erratic varying significantly in time and space (Kasusya, 1998) and falling in two seasons; the short rain and long rain seasons. The driest months are January and February in the whole District.



**Figure 1: Map of the study area, showing the location of the sampling sites and the location of the study area in Samburu District.**

The patterns for the short rains differ between the high altitude areas such as Lerroki and the lowlands of Wamba, where it occurs from July to August and October to November respectively. The long rains start at the end of March and end in May. Lerroki Plateau receives between 500-700 mm while the Ndoto Mountains receive between 750-1250 mm. The central plains and east of Mathew Ranges receive lower rainfall amounts of between 250-500 mm annually. The only permanent river is the Ewaso Nyiro. Occasional water sources include ephemeral wells on dry riverbed, man-made reservoirs and natural ponds, which only contain water during the wet season. For most of the year, the area experiences high temperatures during the day with no cloud cover. Temperatures vary with altitude and generally range between 24 °C mean minimum and 33 °C mean maximum. The highlands belts of Lerroki Plateaus are cool, while the central plains and the region east of the Mathews range have the highest temperatures.

#### **3.1.4 Vegetation**

Soil is dry and sandy with a poor vegetation cover. Thick vegetation cover is only found in high altitude places like the Mathews ranges. Plants have been an integral part of life in many indigenous communities, and the Samburus are no exception (Bussmann, 2006). The Samburu people do not only use plants as building materials, fodder and weapons but they also use them as traditional medicines to treat diseases of varied causes. Government healthcare service is not readily available in most parts of Samburu district thereby leaving many regions underserved with medical care. Subsequently most communities still use herbal remedies as readily and cheaply available alternative.

### **3.2 Water sampling**

Water samples were collected from twenty common ground and surface water sources previously investigated by an Earthwatch Research Team (Unpublished Report). The sites

included Lodungokwe pan, Serewamba river, Mugur omuny dry riverbed well, Enyangainito river, Ewaso nyiro river, Ndikir, Nagoruworu dry riverbed wells, Naibelibeli dam, Nkwaas and Namunyak dam among others. Water was collected in sterile 125 ml and 500 ml plastic bottles fitted with screw caps for microbiological and physico-chemical analyses respectively. All water samples were transported to the laboratory in an icebox and analyzed within six hours.

### **3.3 Field measurements**

Electrical conductivity and pH were measured on site using a Universal Multiline P4 WTW (Wilhelm, Germany) meter. Where possible, each probe was in turn lowered directly into a suitable portion of the water body and readings taken after the meter stabilized (APHA, 1998). Where this was not possible, samples were carefully collected with a water scooper and readings taken immediately after lowering each probe into the water sample.

### **3.4 Chemical analyses**

#### **3.4.1 Total alkalinity**

Total alkalinity was determined by the titration of 100 ml water samples with 0.02 N standard hydrochloric acid using mixed methyl red bromocresol green indicator to determine the titration end point. For samples with pH greater than 8.3, phenolphthalein indicator was used to first determine the phenolphthalein alkalinity (APHA, 1998). Total alkalinity was calculated based on the volume of sample, normality and the amount of titrant used.

#### **3.4.2 Phosphorus**

Orthophosphate phosphorus was determined by ascorbic acid reduction procedure (APHA, 1998). Water samples were first filtered with prewashed glass fiber filters. A combined reagent of acidified ammonium molybdate and antimonyl potassium tartrate was added to the filtered sample to form complex, which was reduced to an intensely blue molybdenum blue

complex by ascorbic acid. Orthophosphate phosphorus concentration was next determined by the colorimetric procedure using a portable spectrophotometer (Nanocolor 300D). Standards of known orthophosphate phosphorus concentration were subjected to same treatment as the filtered samples and used to determine actual orthophosphate phosphorus concentration. To determine the total phosphorus concentration, all forms of phosphorus in water samples were first oxidized to orthophosphate by autoclaving a 25 ml of the sample at 121 °C for 40 minutes in presence of potassium persulphate oxidizing agent. The amount of total phosphorus and orthophosphate present was thereafter determined by the colorimetric procedure using a portable spectrophotometer.

### **3.5 Bacteriological quality of water samples**

#### **3.5.1 Total coliforms**

Analysis of water for the presence of total coliforms was carried out using multiple tube fermentation technique (APHA, 1998). This was done in two steps, namely; presumptive and confirmatory steps. In presumptive test, three series of five test tubes containing 10 ml, 1 ml and 0.1 ml portions of water samples were inoculated in tubes containing lauryl tryptose broth and incubated at 37 °C for 24 hours where any growth and gas production were indicative for possible presence of coliforms. Confirmatory test was done by transferring a loopful of all positive tubes into brilliant green lactose bile broth and incubated at 37 °C for 24 hours. Growth and gas production indicated positive test. Most probable number (MPN) was calculated from the number of positive tubes.

#### **3.5.2 Fecal coliforms**

Analysis of water for the presence of fecal coliforms was carried out using multiple tube fermentation technique (APHA, 1998). Using a sterile loop, a growth was transferred from each presumptive tube showing gas, growth or acidity to lauryl tryptose broth. The inoculated

tubes were incubated in water bath at 44.5 °C for 24 hours. Gas productions with growth in the tubes within 24 hours were considered positive fecal coliform reaction. Failure to produce gas with little or no growth constituted a negative reaction (APHA, 1998). MPN was calculated from the number of positive tubes.

### **3.6 Plant collection and identification**

The plant materials were collected in July, August and November 2007. The healers were convinced that their cooperation would be of great benefit to them and to the country. The identification was conducted by interviewing traditional healers using the local language. Each interview followed a semi-structured questionnaire (**Appendix 5**), designed to obtain the following information; plants used for the treatment of stomach ailments, vernacular plant names, plant parts used, mode of preparation and administration. Local scouts were used to translate the interview and to collect identified plants from the field. Plant collection was done with utmost care to minimize damage to the plants. The collected plants were identified by taxonomist at the field and voucher specimens were deposited at Kenyatta University Herbarium.

### **3.7 Preparation of plant extracts**

#### **3.7.1 Extracts for water treatment**

The fresh plant materials were washed with plenty of tap water followed by distilled water. The washed plant parts were cut into small pieces, air dried under shade and then crushed to powder using a crushing machine (Model 8 Lab., Mill Christy and Norris, England). The plants were extracted using maceration extraction procedure employed by the Samburu community to obtain the crude plant extract. Briefly, an amount of 15 g of the powdered plant material was each soaked in 50 ml of distilled water for 30 minutes. After 30 minutes, the mixture was sieved using a tea strainer into a clean and sterile container as ground material

was squeezed for maximum liquid extraction. The sieved extract was further filtered using Whatman filter paper. The extract filtrate was stored in a refrigerator at 4 °C.



**Figure 2: Medicinal plant extract filtrate ready for water treatment**

### 3.7.2 Extract used against pathogenic bacteria

Plants whose extracts were effective in reducing bacterial load in water were chosen for further sensitivity testing. A two hundred gram portion of each powdered plant was soaked separately in 500 ml of distilled water. Samples of the soaked plant powder were left on a mechanical shaker at 150 rpm for 24 hours at room temperature. The mixture of each powdered plant obtained from the extraction procedures was decanted into a dry clean conical flask, then filtered through Whatman filter paper and the filtrate freeze-dried. The resultant powder was stored at 4 °C in airtight containers in readiness for use in bioassay.

## 3.8 Water treatment

Sample water was sterilized by autoclaving at 121 °C for 15 minutes. Some 15 ml of the sterile water sample was spiked with 1 ml of fecal coliform cultures containing approximately  $1.0 \times 10^8$  colony forming unit (cfu) then treated with 5 ml of freshly prepared plant extract. The colony forming units (cfu) were determined within two minutes after treatment. This was

achieved by plating 0.1 ml of serially diluted treated water sample in duplicate on nutrient agar and incubated at 35 °C for 48 hours after which all the bacterial colonies were counted. The same procedure was repeated after 30 minutes and one hour in order to determine the optimum time for maximum antibacterial action. The efficacy of the plant extract was expressed as a percentage using the following equation:

$$FC-IC/IC \times 100 = \% \text{ EFFICACY}$$

Where: FC – final number of cfu, IC – initial number of cfu.

### **3.9 Testing of plant products against pathogenic bacteria**

#### **3.9.1 Test cultures**

The bacterial strains used in this study were, *Escherichia coli* – STD 25922, *Salmonella typhi* – ATCC 2202 and *Shigella dysenteriae* that were obtained from the Department of Plant and Microbial Sciences, Kenyatta University. All the bacterial strains were grown and maintained on nutrient agar slants. The inoculum's size of each test bacterial strain was 0.5 McFarland standards ( $1.5 \times 10^7$  cfu mL<sup>-1</sup>).

#### **3.9.2 Preparation of extract solution**

The extract were dissolved in sterile distilled water to a final concentration of 10 mg ml<sup>-1</sup> for disc diffusion assay and a range of 50 mg ml<sup>-1</sup>- 0.78 mg ml<sup>-1</sup> concentration for microwell diffusion assay. The choice of dose concentration was based on the finding that crude plant extract was capable of inhibiting the growth of bacteria at 10 mg ml<sup>-1</sup> (Basri and Fan, 2005). All extracts were sterilized by passing through a 0.45 µm membrane filter.

### **3.9.3 Antibacterial sensitivity testing**

#### **3.9.3.1 Inhibition zones**

The disk diffusion method was used to evaluate the antibacterial activity of the plant extracts (Bauer and Kirby, 1966). Mueller Hinton agar was prepared in plates as the media for the test microorganism. Sterile filter paper discs (Whatman filter paper) were impregnated with 100  $\mu\text{l}$  of plant extract with a concentration of 500  $\text{mg ml}^{-1}$  to give a final concentration of 10  $\text{mg disc}^{-1}$  and left to dry under the laminar flow cabinet overnight. Bacterial inocula were prepared as follows: four to five colonies of the test cultures were emulsified in sterile distilled water and the turbidity adjusted to  $1.5 \times 10^8 \text{ cfu ml}^{-1}$  (corresponding to 0.5 McFarland standards).

A sterile cotton swab was dipped into the standardized bacterial suspension and spread evenly onto the surface of the Mueller Hinton agar plates before the extract discs were positioned on the inoculated agar surface. Each extract was assayed in triplicate. Streptomycin was used as standard to confirm that the antibiotic inhibited all the microorganisms tested. All plates were incubated for 24 hours at 37 °C. The antibacterial activity was interpreted from the size of the diameter of the zone of inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the discs. Data were analyzed using a one way ANOVA, and Tukey's test was used to separate means where there was a significant difference.

#### **3.9.3.2 Minimum inhibitory concentration (MIC)**

The extracts that produced inhibition zones were used to determine the MICs using broth dilution assay. The bacterial inoculum was prepared by emulsifying four to five colonies of the isolates in sterile distilled water and the turbidity adjusted to correspond to 0.5 McFarland standards. The extracts were prepared at the highest concentration of 100  $\text{mg ml}^{-1}$ . Two-fold serial dilution of plant extract were prepared in sterile polystyrene 96 well plates to obtain

concentrations ranging from 50.00 to 0.78 mg ml<sup>-1</sup> then 50 µl of the test organisms was added to each diluents in micro plate. Bacterial growth controls were made of broth and inoculums only, while a mixture without inoculums was used as a control for extract color (to avoid the interference of the plant extract color in the results). The plates were covered with sterile plate sealer and then incubated at 37 °C for 24 hours. Microbial growth in each medium was determined by observing and comparing the test wells with the control. The lowest concentration (highest dilution) of the plant extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC.

### 3.9.3.3 Minimum bactericidal concentration (MBC)

The MBC was determined by streaking the test dilution that showed no growth onto fresh Mueller Hinton agar medium and incubated for 18-24 hours. The lowest concentration (highest dilution) that yielded no bacteria colony on a growth medium was taken as MBC.

## 3.10 Phytochemical screening methods

The freshly prepared extracts were tested qualitatively for the presence of tannins, alkaloids, saponins, flavonoids, cardiac glycosides and terpenoids phytochemical compounds. They were identified by their color changes using standard procedures as described by Edeoga *et al.* (2005).

### 3.10.1 Test for tannins

0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride were added to 5 ml of the filtrate and observed for brownish green or black coloration, which indicates the presence of tannins.

### **3.10.2 Test for saponins**

2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was then mixed with 5 ml of distilled water and shaken vigorously for a stable and persistent froth. The frothing was mixed with 3 drops of olive oil, shaken vigorously and then observed for formation of emulsion, which indicates the presence of saponins.

### **3.10.3 Test for flavonoids**

2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. An amount of 5 ml of dilute ammonia solution was added to 10 ml portion of the aqueous filtrate of each plant extract followed by addition of concentrated sulphuric acid. A yellow coloration observed in each extract indicated the presence of flavonoids.

### **3.10.4 Test for terpenoids (Salkowski test)**

2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. An amount of 5 ml of each extract was mixed with 1 ml of chloroform and 3 ml of concentrated sulphuric acid was carefully added to form a bottom layer. Formation of a reddish brown coloration at the interface indicated the presence of terpenoids.

### **3.10.5 Test for cardiac glycosides (Keller-Killani test)**

2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. Some 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride ( $\text{FeCl}_3$ ) solution. 1 ml of concentrated sulphuric acid was next carefully added to form a bottom layer. A brown ring at the interface indicated the presence of a deoxysugar, which is characteristic of the cardiac glycosides group.

### 3.10.6 Test for alkaloids

0.2 g of grounded plant material was introduced into 10 ml of distilled water, thoroughly mixed and then filtered. Some drops of Wagner's reagent was added to 2 ml of the filtered plant extract and shaken to mix. Creamish/ brownish-red/ orange precipitate observed indicated the presence of alkaloids.

### 3.11 Statistical analysis

After the data was collected, descriptive and inferential statistics were used to summarize and analyze the data respectively. The data collected was summarized in appropriate tables and their presentation done graphically. Data analysis to establish if there were significant differences among the various treatments employed the Analysis of Variance (ANOVA) test. Tukey's post-hoc test was used for separation of statistically significant means. Pearson's correlation coefficient was used to establish if there was a relationship between the efficacies of plant extract with the physico-chemical properties of water samples.

## CHAPTER FOUR: RESULTS

### 4.1 Preliminary microbiological water quality results

A preliminary microbial analysis of water from various common sources in the study area confirmed the presence of fecal coliforms in most water samples (**Table 1**). These findings demonstrated that water from many of these sources are not safe for human use and therefore intervention measures are required to reduce the chances of waterborne pathogenic bacteria affecting the human population of the study area.

**Table 1: Results of the preliminary microbial analyses of water from various common sources in Wamba Division Samburu District**

Water source	MPN index of total coliforms	MPN index of fecal coliforms
Lkisin borehole	2	<2
Namunyak dam	≥1600	461
Lpus dam	1600	254
Seiya river	≥1600	129
Lerata river	≥1600	184
Naisunyai borehole	8	<2
Margwe wells	475	64
Ewaso nyiro river	≥1600	865
Ngutuk ongiroi dam	≥1600	480
Serewamba river	≥1600	149
Serewamba borehole	20	2
Mugur omuny dam	≥1600	140
Loidikidiko dam	260	19
Naibelibeli dam	≥1600	125
Enyangainito well	865	485
Nkwaas well	≥1600	265
Lodungokwe dam	485	150
Ndikir dam	1600	90
Nagoruworu wells	885	50

### 4.2 Medicinal plants used by Samburu people to treat diarrhea

Seven plants were identified using the questionnaire. The plants identified are *Acacia nilotica*, *Acacia seyal*, *Acacia tortilis*, *Acacia etbaica*, *Albizia anthelmintica*, *Euclea divinorum* and *Plumbago zeylanica* (**Table 2**). The community uses these plants to manage diarrhea related to consumption of contaminated water. Majority of the plants are used as maceration and the

essential route of administration is oral. Most of the people interviewed commented on the effectiveness of the plants in managing diarrhea and general stomach complaints. *Plumbago zeylanica* was described as a wonder herb in treating stomach ailments in children while *Albizia anthelmintica* was described as being used by both humans and livestock as a de-wormer. The interviewees also volunteered information on modes of collection and preparation. The ethnobotanical data of seven medicinal plants species including botanical names, local names, plant parts and mode of preparation are summarized in **Table 2**.

**Table 2: Ethnobotanical information on medicinal plants used by the Samburu community living in Northern Kenya**

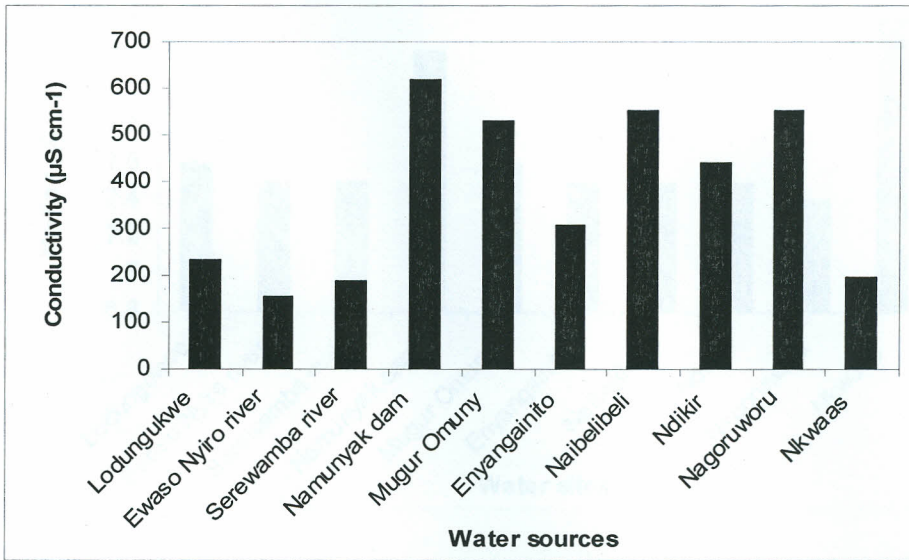
Plant species	Family	Samburu name	Parts used	Mode of preparation	Medicinal use
<i>Albizia anthelmintica</i> (A. Rich.) Brongn.	Mimosaceae	Lomurtana	Roots	Maceration	De-worming both humans and livestock
<i>Plumbago zeylanica</i> (L)	Plumbaginaceae	Lkiriantus	Roots	Maceration	Treatment of diarrhea
<i>Acacia nilotica</i> (L.) Del.	Fabaceae	Lkiloriti	Bark	Maceration	Treatment of diarrhea and stomachache
<i>Acacia etbaica</i> Schweinf	Fabaceae	Lchakwai	Bark	Maceration	Treatment of diarrhea
<i>Acacia seyal</i> Del. (Talh)	Fabaceae	Lerai	Bark	Maceration	Treatment of diarrhea
<i>Acacia tortilis</i> (Forssk.) Hayne	Fabaceae	Ntepes	Bark	Maceration	Treatment of indigestion mainly of meat and general intestinal complication
<i>Euclea divinorum</i> Hiern	Ebenaceae	Lchingei	Roots	Decoction	Purgative and indigestion

### 4.3 Physico-chemical properties of water

Water from ten sources from Samburu district, Wamba division was sampled and the physico-chemical properties (electrical conductivity, pH, total alkalinity, orthophosphate phosphorus and total phosphorus) determined. Selection of these water sources was based on the finding of a previous study that indicated that they harbor high number of indicator bacteria (Unpublished report), and as confirmed during the preliminary microbial analyses.

#### 4.3.1 Electrical conductivity (EC $\mu\text{S cm}^{-1}$ )

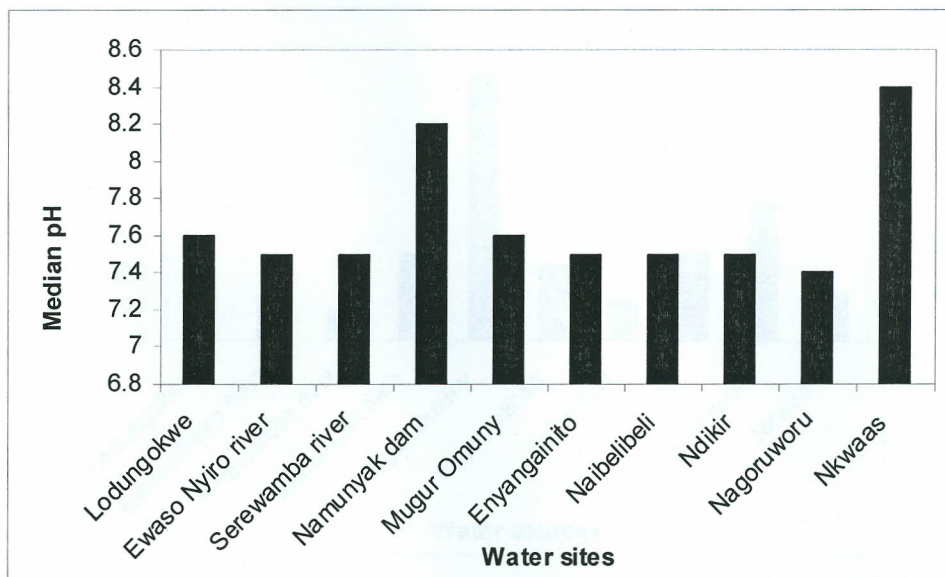
Electrical conductivity ranged from 118  $\mu\text{S cm}^{-1}$  at Ewaso Nyiro river to 1060  $\mu\text{S cm}^{-1}$  at Nagoruworu dry riverbed wells. The mean electrical conductivity values for each site varied from 155  $\mu\text{S cm}^{-1}$  at Ewaso Nyiro to 619.33  $\mu\text{S cm}^{-1}$  at Namunyak dam (Fig. 3). Using the one-way ANOVA, it was observed that the electrical conductivity values were not significantly different among the sites investigated ( $P = 0.1146$ ,  $df = 27$ ).



**Figure 3:** Mean electrical conductivity of water from various sources in Samburu between July and August 2007

### 4.3.2 pH

The pH values ranged from 7.4 in Nagoruworu to 9.9 at Namunyak dam. However, median pH values varied between 7.4 at Nagoruworu to 8.4 in Nkwaas (Fig. 4).



**Figure 4:** Median pH values of water from various sources in Samburu between July and August 2007

### 4.3.3 Total alkalinity (TA mg L<sup>-1</sup>)

Total alkalinity (mg L<sup>-1</sup> CaCO<sub>3</sub>) ranged from a mean of 47.5 at Serewamba River to a mean of 411.7 at Mugur omuny (Fig. 5). Subjecting the total alkalinity values of the various sites to a one way ANOVA test revealed that the total alkalinity values of the water from various sources was not significantly different (P = 0.44, df = 13).

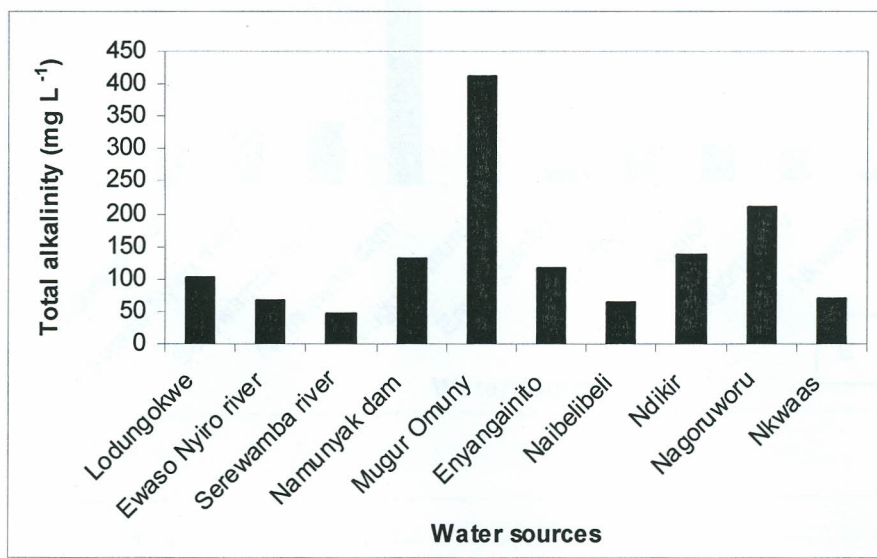
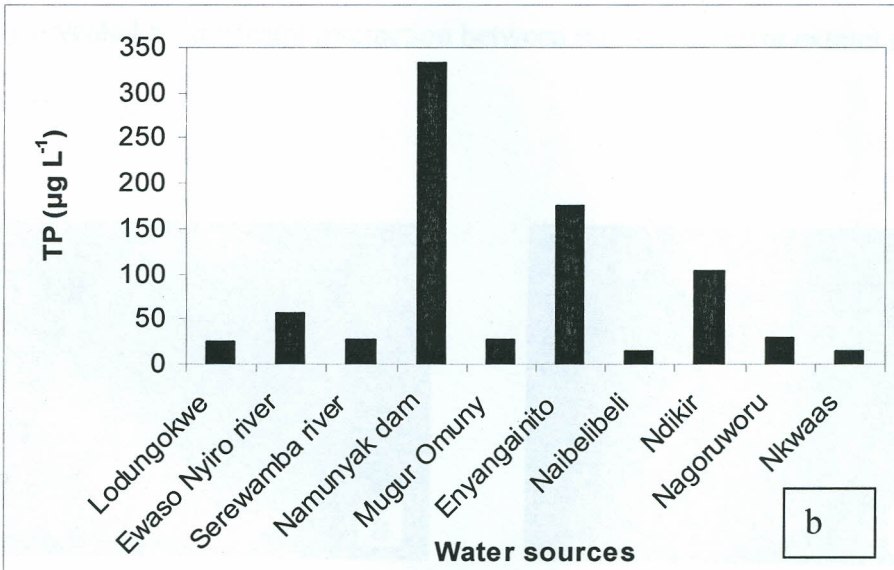
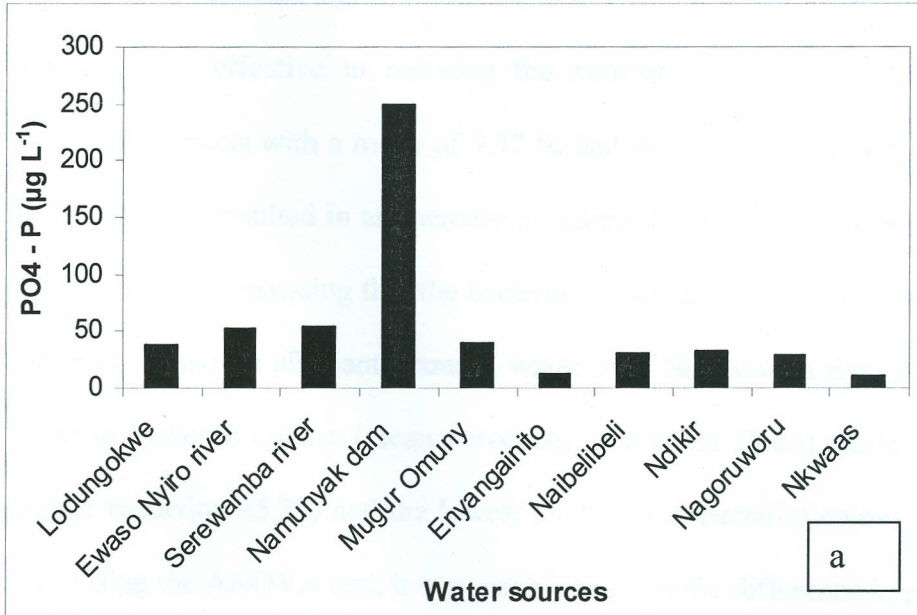


Figure 5: Total alkalinity of sampled water from different sources in Wamba Division, Samburu District

### 4.3.4 Orthophosphate phosphorus (PO<sub>4</sub>-P) and total phosphorus (TP)

Orthophosphate phosphorus ranged from 12.62 µg L<sup>-1</sup> at Enyangainito to 357.76 µg L<sup>-1</sup> at Namunyak dam. Mean orthophosphate phosphorus values in the selected sampling sites varied between 10.94 µg L<sup>-1</sup> in Nkwaas to 249.73 µg L<sup>-1</sup> in Namunyak dam. Total phosphorus registered the lowest value of 14.73 µg L<sup>-1</sup> in Naibelibeli dam while Namunyak dam gave the highest value of 387.05 µg L<sup>-1</sup>. Mean total phosphorus ranged from 14.74 µg L<sup>-1</sup> in Naibelibeli dam to 332.22 µg L<sup>-1</sup> in Namunyak dam (Fig. 6). Using a one-way ANOVA test, it was established that there was no significant difference in PO<sub>4</sub>-P concentration of the selected sites

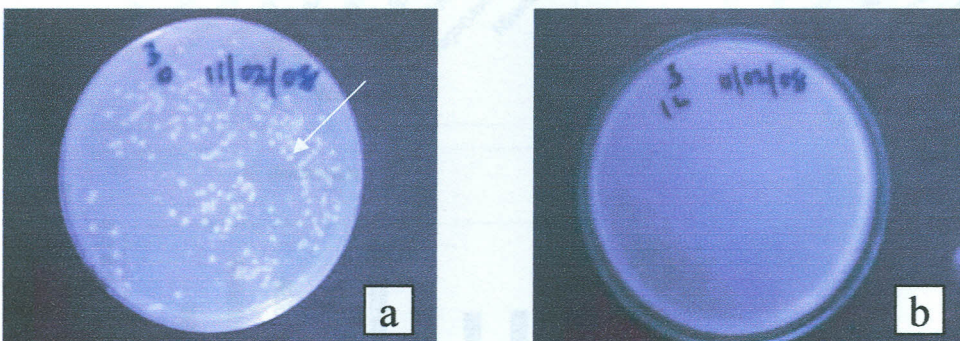
( $p=0.723$ ,  $df=9$ ). Similarly, total phosphorus did not significantly differ among the study sites ( $df = 9$ ,  $P= 0.769$ ).



**Figure 6:** Amounts of orthophosphate phosphorus (a) and total phosphorus (b) in water from various sources in Wamba division, Samburu District

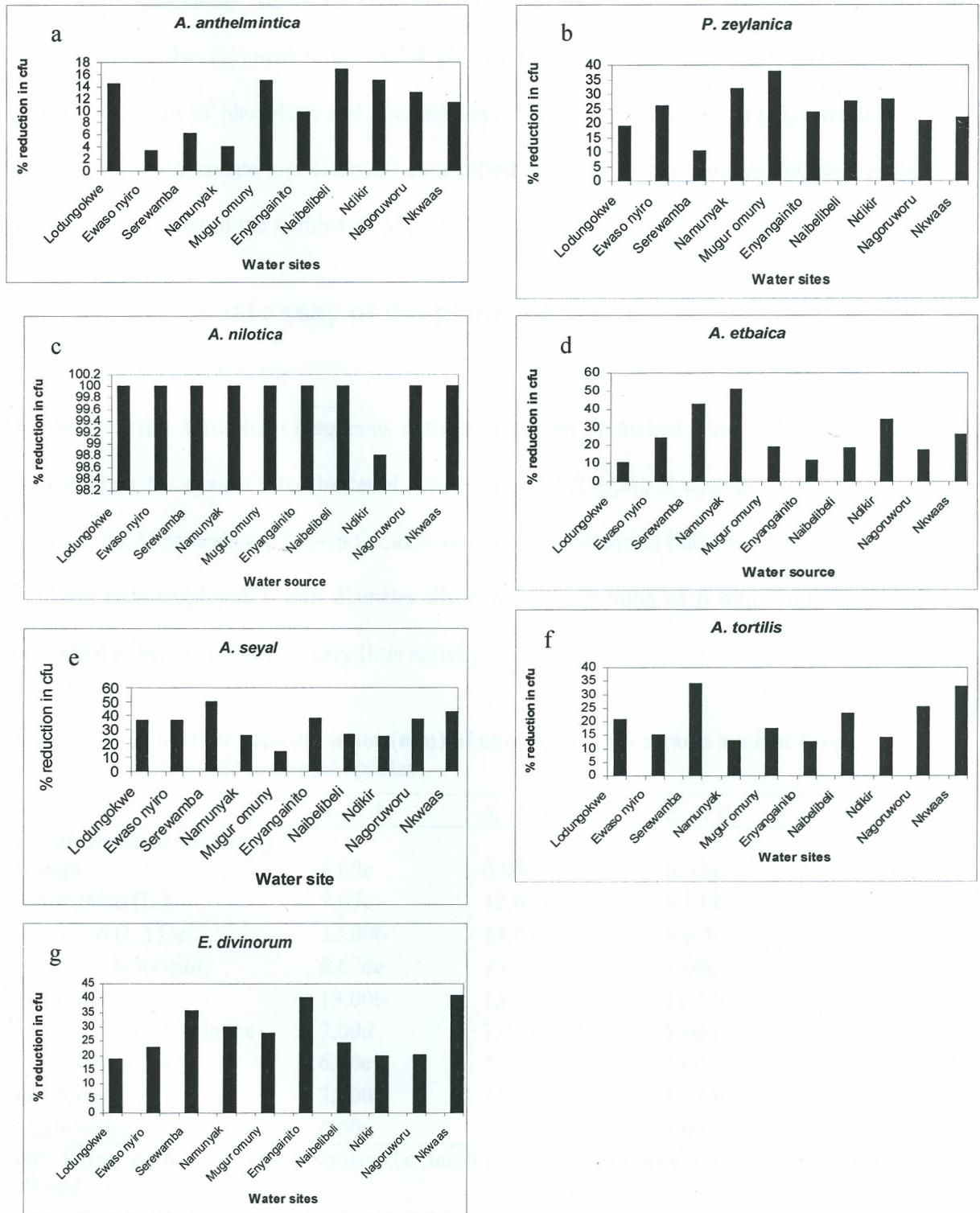
#### 4.4 Efficacy of medicinal plants in water treatment

Efficacy of water treatment with medicinal plants expressed as percentage reduction in bacteria colonies revealed that *A. nilotica* extracts with a mean percentage reduction of 99.86 % was the most effective in reducing the number of bacterial colonies (Figure 7). *A. anthelmintica* extracts with a mean of 9.47 % had the lowest reduction of bacterial colonies. Control treatments resulted in an increase in colony forming units (mean percentage increase of 2.37) clearly demonstrating that the bacteria continued to grow in the absence of medicinal plant extract. Based on all plant extracts, water from Serewamba river registered the highest reduction in bacterial colony (mean percentage reduction 33.84) while Lodungokwe (mean percentage reduction 25.22) had the lowest reduction in bacterial colony with the same plant extracts. Using the ANOVA test, it was established that the difference in percentage reduction of bacterial colonies by the different plant extracts was significant ( $P < 0.05$ ,  $df = 6$ ). The study revealed a significant interaction between the type of plant extract and water source ( $P < 0.05$ ,  $df = 54$ ).



**Figure 7:** Colony forming units (cfu) in water treated with *Acacia nilotica* plated at the beginning of treatment (a) and after one-hour (b).

**Legend:** White dots in Figure 7a indicate bacteria colonies



**Figure 8:** Mean percentage reduction in bacterial colonies per plant per water source

Based on Pearson correlation test, it was found that there was no significant correlation between the percentage reduction in the number of bacteria in the water samples after treatment with the commonly measured physico-chemical properties such as pH, total alkalinity, amount of phosphate and conductivity. However, an increase in pH, reduction in total alkalinity, phosphate and electrical conductivity tended to result into an increase in the percentage reduction of the number of bacteria.

## 4.5 Antimicrobial assay of the plants effective in water treatment

### 4.5.1 Disc diffusion assay

The antibacterial activities of aqueous extracts of seven medicinal plants (Table 3) were evaluated *in vitro* against three bacterial species namely; *S. typhi*, *S. dysenteriae* and *E. coli*. These strains of bacteria are known to cause waterborne diseases in humans. Bacterial growth inhibition tests employed 6 mm diameter discs; hence, a reading of 6 mm implied that an extract had either no activity or very little activity.

**Table 3: Inhibition zone diameter (mm) of aqueous plant extracts against three pathogenic bacterial species**

	<i>S. typhi</i>	<i>S. dysenteriae</i>	<i>E. coli</i>
<i>A. anthelmintica</i> (A. Rich.) Brongn.	6.00e	6.00e	6.00e
<i>P. zeylanica</i> (L.)	8.67c	12.67d	8.33d
<i>A. nilotica</i> (L.) Del.	13.00b	14.00b	9.67c
<i>A. etbaica</i> Schweinf.	6.67de	7.00e	7.00e
<i>A. seyal</i> Del.	13.00b	13.33c	11.33b
<i>A. tortilis</i> (Forssk) Hayne	7.00d	7.00e	8.00d
<i>E. divinorum</i> Hiern	6.00e	7.33e	6.00e
Streptomycin	22.00a	22.00a	19.33a
Sterile water	6.00e	6.00e	6.00e

Note: letters a, b, c, d and e shows the inhibition diameters which are not significantly different

All the plants investigated except *Albizia anthelmintica* showed some antimicrobial activity against the test bacteria. Highest zone of inhibition was caused by *Acacia seyal* (mean zone of inhibition 12.56 mm) while the lowest zones were noted in *Euclea divinorum* (mean zone of inhibition 6.44 mm). The antimicrobial activity profile of all species of plants (except *Albizia anthelmintica*) against the tested strains indicated that *S. dysenteriae* was the most susceptible bacterium of all the bacterial test strains (**Table 3**).

#### a) Plant extracts inhibition on *S. typhi*

Plant extracts inhibition on *S. typhi* ranged from 6.67 mm with *A. etbaica* to 13.00 mm with *A. seyal* and *A. nilotica* (**Table 3, Fig. 9**). However, *A. anthelmintica* and *E. divinorum* did not show any activity at all. Using the one-way ANOVA test, the plants were found to differ significantly in their activity against *S. typhi* ( $F = 1172.376$ ,  $df = 8$ ,  $P < 0.05$ ). Mean separation using Tukey's mean separation procedure revealed that *Acacia nilotica* and *Acacia seyal* (mean inhibition 13 mm) had a significantly higher inhibition on *S. typhi* than the other plant extracts tested. Streptomycin antibiotic used as control (mean 22 mm) showed that the test bacteria were sensitive to antibiotics.

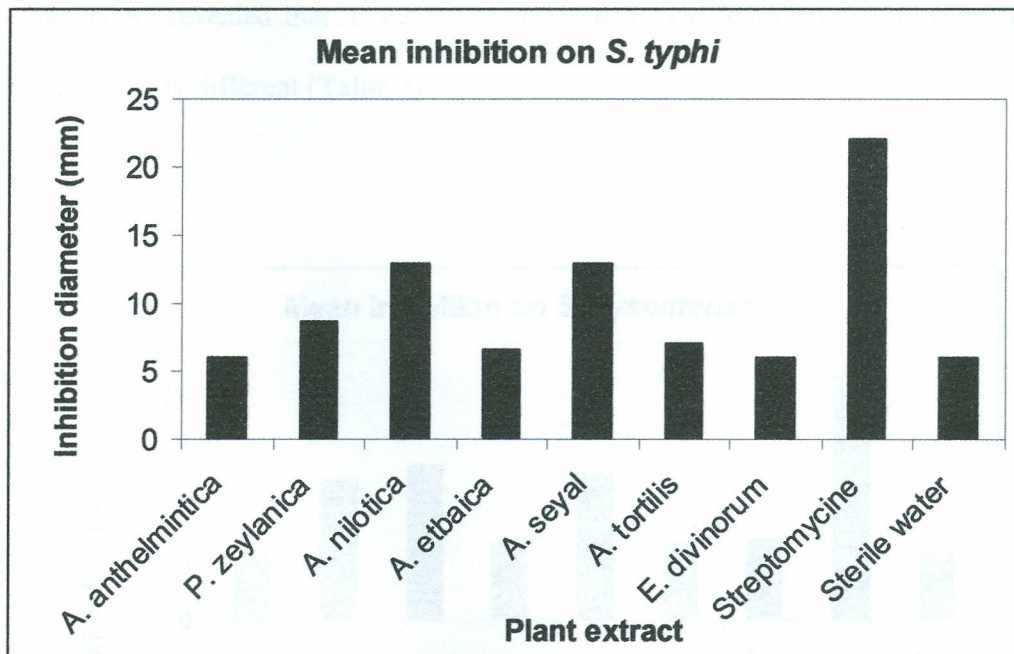


Figure 9: Mean inhibition zone diameter by the individual plant extracts on *S. typhi*

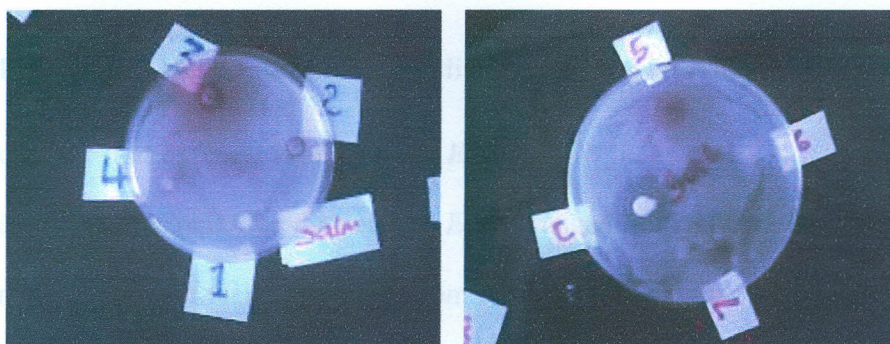


Figure 10: Inhibition of *S. typhi* by plant extracts

KEY: 1. *A. anthelmintica*, 2. *P. zeylanica*, 3. *A. nilotica*, 4. *A. etbaica*, 5. *A. seyal*, 6. *A. tortilis*, 7. *E. divinorum*, C. Streptomycin

#### b) Plant extracts inhibition on *S. dysenteriae*

Plant extracts inhibition on *S. dysenteriae* ranged from 7.00 mm with *A. tortilis* and *Acacia etbaica* to 14.00 in *A. nilotica*. An inhibition diameter of 6.00 mm as was the case with sterile water indicated no activity. The sizes of zones of inhibition were greater for *S. dysenteriae* than for *E. coli* and *S. typhi*, suggesting that *S. dysenteriae* was more susceptible to the plant extract. ANOVA test showed a significant difference in the effect by plant extracts on *S. dysenteriae* ( $F = 783.583$ ,  $df = 8$ ,  $P < 0.05$ ). Mean separation using Tukey's mean separation

procedure revealed that *A. tortilis*, *A. etbaica*, *A. anthelmintica* and *E. divinorum* were not significantly different (Table 3).

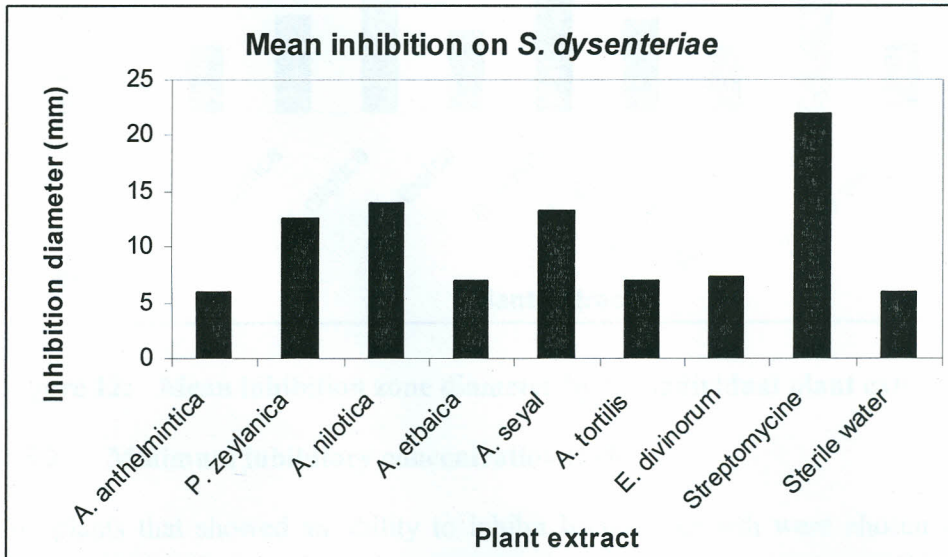
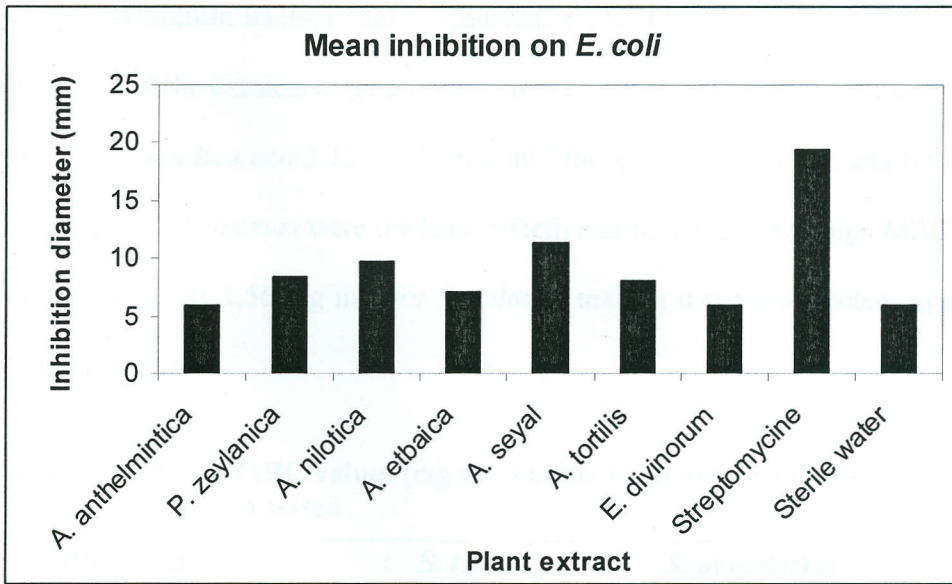


Figure 11: Mean inhibition zone diameter by the individual plant extracts on *S. dysenteriae*

### c) Plant extracts inhibition on *E. coli*

Plant extracts inhibition diameter on *E. coli* ranged from 6.00 mm in *A. anthelmintica* to 11.33 mm in *A. seyal*. Hence, extracts from *Acacia seyal* exhibited a greater inhibition of *E. coli* than the other extracts. Mean streptomycin inhibition diameter on *E. coli* (19.33 mm) was comparatively lower than the inhibition diameters of *S. typhi* and *S. dysenteriae* (22 mm each). The results also showed that *E. divinorum* and *A. anthelmintica* had the same inhibition zones with the sterile water. The extract of the different plants used differed significantly in their activity against *E. coli* ( $F = 209.821$ ,  $df = 8$ ,  $P < 0.05$ ). Mean separation using Tukey's mean separation procedure revealed that *A. nilotica* and *A. seyal* had a higher inhibition on *E. coli* than the other plant extracts (Table 3).



**Figure 12:** Mean inhibition zone diameter by the individual plant extracts on *E. coli*

#### 4.5.2 Minimum inhibitory concentration (MIC)

Six plants that showed an ability to inhibit bacterial growth were chosen for an MIC test against the three bacterial species. MIC ranged from 4.16 – 12.50 mg ml<sup>-1</sup> for *P. zeylanica*; 1.56 – 3.12 mg ml<sup>-1</sup> for *A. nilotica* and 2.08 – 3.12 mg ml<sup>-1</sup> for *A. seyal*. Plant extracts of *A. etbaica*, *A. tortilis* and *E. divinorum* inhibited bacteria at concentrations greater than 25 mg ml<sup>-1</sup>. The lowest MIC concentration of 1.56 mg ml<sup>-1</sup> was recorded for *A. nilotica* (Table 4). *A. nilotica* extract was therefore the most potent among the seven plant extracts tested.

**Table 4:** Summary of the MIC values of the plant extracts on each bacterium tested

Plant extract	<i>S. typhi</i>	<i>S. dysenteriae</i>	<i>E. coli</i>
<i>Plumbago zeylanica</i> (A. Rich.) Brongn.	6.25	3.12	6.25
<i>Acacia nilotica</i> (L.) Del.	1.56	1.56	3.12
<i>Acacia etbaica</i> Schweinf	>25	>25	>25
<i>Acacia seyal</i> Del.	3.12	2.08	3.12
<i>Acacia tortilis</i> (Forssk) Hayne	>25	>25	>25
<i>Euclea divinorum</i> Hiern	>25	>25	>25

### 4.5.3 Minimum bactericidal concentration (MBC)

The MBC of the extracts ranged from 4.16 – 12.50 mg ml<sup>-1</sup> for *P. zeylanica*; 1.56 – 3.12 mg ml<sup>-1</sup> for *A. nilotica* and 3.12 – 6.25 mg ml<sup>-1</sup> for *A. seyal*. Plant extracts from *A. etbaica*, *A. tortilis* and *E. divinorum* were the least effective as they registered high MBC (Table 5). The lowest MBC was 1.56 mg ml<sup>-1</sup> for *A. nilotica* making it the most potent plant on the tested bacterial species.

**Table 5: Mean MBC values (mg ml<sup>-1</sup>) of the plant extracts on the three species of bacteria tested**

Plant extract	<i>S. typhi</i>	<i>S. dysenteriae</i>	<i>E. coli</i>
<i>Plumbago zeylanica</i> (L.)	10.25	4.16	12.50
<i>Acacia nilotica</i> (L.) Del.	1.56	1.56	3.12
<i>Acacia etbaica</i> (Schweinf)	>25	>25	>25
<i>Acacia seyal</i> Del.	3.12	3.12	6.25
<i>Acacia tortilis</i> (Forssk.) Hayne	>25	>25	>25
<i>Euclea divinorum</i> Hiern	>25	>25	>25

### 4.6 Phytochemical compounds in the studied plants

The present study carried out on the plant samples revealed the presence of medicinally active constituents. The phytochemicals detected in the plants used in the study included tannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids (Table 6). Terpenoids were the most common compound found in all plant samples except in samples from *Plumbago zeylanica*. Alkaloids were present in only *Acacia seyal* and *Acacia nilotica* (Table 6). The study revealed that cardiac glycoside was only present in *A. tortilis*, a member of *Fabaceae* family and notably absent in the other members of the same family. This finding therefore suggests that cardiac glycosides are not present in all species in the *Fabaceae* family.

**Table 6: Qualitative analysis of the phytochemicals of the medicinal plants studied**

Plant code	Tannin	Saponin	Flavonoid	Terpenoid	Cardiac glycoside	Alkaloid
<i>A. anthelmintica</i> (A. Rich.) Brongn.	-	+	-	+	+	-
<i>P. zeylanica</i> (L.)	+	-	+	-	+	-
<i>A. nilotica</i> (L.) Del.	+	+	+	+	-	+
<i>A. etbaica</i> Schweinf.	+	+	+	+	-	-
<i>A. seyal</i> Del	+	+	-	+	-	+
<i>A. tortilis</i> (Forssk.) Hayne	-	+	-	+	+	-
<i>E. divinorum</i> Hiern	-	-	+	+	+	-

Key: (-) absence, (+) presence

## CHAPTER FIVE: DISCUSSION

### 5.1 Microbiological quality of water

According to Reddy *et al.* (1995), the number of microorganisms in the drinking water visited by birds should have no more than 100 CFU ml<sup>-1</sup> for total bacteria and 50 CFU ml<sup>-1</sup> for coliforms. The findings of this study (**Table 1**) showed that the coliform counts in water from 17 sources were above the maximum acceptable level. Fecal coliforms were also detected in water from 18 sources, indicating the occurrence of fecal pollution. This could be due to free access by birds, wild animals and domestic animals to the surface water sources, which is a characteristic feature of all water points in Samburu except boreholes. Poor disposal of human excreta is also common since this nomadic community do not use pit latrines thereby raising the chances of runoff washing it into these water sources.

Although surface water sources are more prone to fecal contamination, the underground water is also exposed to this type of pollution (Amaral, 2004). Absence of fecal coliforms in all boreholes except Serewamba suggests that boreholes are safer than open waters for domestic use. Presence of fecal coliforms in Serewamba borehole may have been due to the drainage from the contaminated surface waters or passage of contaminated water through underground tunnels. It is, however, important to note that the presence of coliforms, fecal coliforms, or even *Escherichia coli* in water does not mean that pathogenic microorganisms are present. It only gives an indication that they could be present.

### 5.2 Ethnobotany

Among the seven plants used for the treatment of diarrheal diseases, the plant part mostly used by the community is the bark. Excessive debarking may however lead to the death of the affected tree. The community should therefore be encouraged to adopt sustainable harvesting methods and encouraged to domesticate some of these plants, a practice which is common in

other parts of the world (Kala, 2000). Four of the identified plants belong to the genus *Acacia*. The genus *Acacia* comprises over a thousand species spread all over the world (Ibrahim and Aref, 2000). Most of them grow in the arid and semi arid regions with average temperatures of 40-45 °C in summer and less than 5 °C in winter. Acacias are equipped with most of the features required to withstand severe climatic conditions, they are therefore considered as the most successful plant species in regions exposed to severe climatic conditions (Ibrahim and Aref, 2000). In Samburu, the acacias are among the dominant tree species and are exploited as fuel, forage and medicine. The Samburu community has been exploiting the acacias for centuries in treatment of diseases of varied etiology (Bussmann, 2006). *Acacia seyal*, *Acacia nilotica*, *Acacia tortilis* and *Acacia etbaica* are the most valued and are therefore the commonly used *Acacia* species as herbal medicine. The barks of these *Acacia* species are macerated in water and drunk mainly to cure stomach illnesses. Besides that, they also form a good habitat for honeybees that produces good quality acacia honey. *Acacia* species are also used in other countries as medicine; Ethiopians use the bark of *Acacia etbaica* to treat gonorrhoea (Hedberg and Edwards, 1989) while the Sudanese use *Acacia nilotica* to treat wounds and diarrhoea (Aref *et al.*, 2000).

*Albizia anthelmintica* belongs to the family *Mimosaceae*. The plant is widely used by the Samburu pastoralists to de-worm themselves and their livestock. For human use, small amounts of the plant extracts are blended with milk and administered to patients with gastrointestinal complaints. *Euclea divinorum* is found only on mountain regions of Samburu district and its decoction is used in the treatment of stomach upsets by inducing diarrhoea. The antimicrobial studies of *Euclea divinorum* have demonstrated the ability of this plant to inhibit proteolytic activities of strains of *Bacteroides gingivalis*, *Bacterioides intermedius* and *Treponema denticola* (Homer *et al.*, 1990). *Plumbago zeylanica*, which belongs to *Plumbaginaceae* family, is distributed in both thickets and grasslands of Samburu district. Its

roots are macerated and the extract taken as medicine in the treatment of diarrhea. In traditional India, *P. zeylanica* has been used in the formulation of compounds used as alternative medicine (Kirtikar and Basu, 1993). In southwestern Nigeria, *P. zeylanica* is used in folk medicine to treat parasitic diseases, scabies and ulcers (Magassouba *et al.*, 2007).

### 5.3 Water disinfection

The methods for treatment of drinking water that have proved to reduce waterborne diarrheal disease include the addition of sodium hypochlorite solution (Quick *et al.*, 2002) and solar disinfection (Conroy *et al.*, 1999) combined with safe storage of water in a narrow mouth containers (Mintz *et al.*, 1995). Despite the efficacy of these methods, they are out of reach by majority of the people particularly those in the remote regions like Samburu. The results obtained in this study indicated that *A. nilotica* could be an alternative to sodium hypochlorite as this registered a high percentage reduction of bacterial load in water (**Figure 8c**). *A. nilotica* extract was also consistently effective in all the different waters treated suggesting that its efficacy is not significantly affected by the physico-chemical properties of the water. Unlike *A. nilotica*, sodium hypochlorite may be affected by physico-chemical properties of water like turbidity, alkalinity and concentration of organic matter (Crump *et al.*, 2004).

High doses of sodium hypochlorite have been shown to render water microbiologically safe. However, such levels adversely affect the taste of water and therefore decrease the willingness of people to treat the water (Reller *et al.*, 2003). Likewise, treatment of water with crude plant extracts is also known to add color, taste and odor problems (Ndagigengesere and Narasiah, 1998). These problems can further be amplified by the storage of treated water for longer periods. Water treated with crude water extract should therefore be stored for periods not longer than 24 hours (Jahn, 1988) to prevent it from developing bad taste that may result from microbial decomposition of its organic compounds (Ndagigengesere and Narasiah, 1998).

Extracts from plants such as *Acacia nilotica* and *Acacia seyal* do not have a discouraging taste like the bitter taste of *A. anthelmintica*. Hence, potential users may not shy away from using them.

#### 5.4 Antimicrobial assay

The qualitative antibacterial assays of the seven plants were done using zones of inhibition and turbidity. Most of the tested plant extracts showed inhibitory activity against the three waterborne pathogens (*S. typhi*, *S. dysenteriae* and *E. coli*) with the exception of *Euclea divinorum* and *A. anthelmintica*, which had inhibition zone diameter similar to that of the control (6 mm). It can therefore be postulated that extracts of *Euclea divinorum* and *A. anthelmintica* may have contained antibacterial compounds at such low concentrations that could not be effective (Stainer *et al.*, 1986), or that the active constituents were not soluble in water. The substances responsible for antimicrobial activity have different polarities ranging from non-polar to polar. This therefore means that the plants that did not indicate activity in this work may show activity if a non-polar solvent was used instead of water.

The differences in the antimicrobial effects of plant species used can be attributed to the difference in the phytochemical properties of the species. The results of phytochemical screening of the seven plants indicated the presence of saponins, terpenoids, cardiac glycosides, flavonoids and alkaloids. It has been documented that tannins, saponins and alkaloids plant metabolites exerts antimicrobial activity (Akinyemi *et al.*, 2003). Phenolic compounds such as flavonoids have also been reported to possess antimicrobial activity (Edeoga *et al.*, 2005). The activity of flavonoids is attributed to their ability to complex with extra cellular soluble proteins and with bacterial cell walls in addition to disruption of microbial membranes (Cowan, 1999). The antidiarrheal activity of flavonoids has been

ascribed to their ability to inhibit intestinal motility by inhibiting the intestinal secretory response and hydroelectric secretion (Di carlo *et al.*, 1993., Sanchez *et al.*, 1997).

Tannins have also been known to evoke an antidiarrheal effect by precipitating proteins of the enterocytes, reducing peristaltic movement and intestinal secretion (Yu *et al.*, 2000). Tannins are known to bind cell walls of bacteria preventing growth and protease activity (Jones *et al.*, 1994). Only the plant extracts that registered lower MIC and MBC values against the bacterial strains in this study contained alkaloids (**Table 6**). It is therefore most likely that the presence of alkaloid enhanced the antimicrobial activity observed in *A. seyal* and *A. nilotica*. *A. nilotica* found to be most effective in water treatment was also most active plant with MBC of 2.08 mg ml<sup>-1</sup>. This plant also had a lot of tannins and alkaloids. These results are in agreement with the previous studies on *A. nilotica*, which show that its pods can be used for treating wounds and diarrhea (Aref *et al.*, 2000). The Zulus of South Africa take a decoction of the bark as a cough relieve, while extracts from roots are used in the treatment of tuberculosis, impotence, diarrhea, toothache, dysentery and gonorrhoea (Behr, 2005). The current study therefore confirms the medicinal value of *A. nilotica*. The MIC and MBC results of *A. nilotica* showed that the extracts had substantial inhibitory and bactericidal effect against the test cultures (**Tables 4 & 5**).

Although *A. nilotica* with MIC of 2.08 mg ml<sup>-1</sup> was closer to that of *A. seyal* (3.08 mg ml<sup>-1</sup>), the efficacy of *A. seyal* (33.64 %) in water treatment was low, while the efficacy of *Acacia nilotica* (99.86 %) was high (**Figure 8**). This observation suggests a possible interaction between antimicrobial compounds with intrinsic water properties from the different sources that may have enhanced the efficacy of *A. nilotica* and inhibited the efficacy of *A. seyal*. A possible synergistic interaction was also observed in the extract of *E. divinorum*. While *E. divinorum* greatly reduced bacterial colonies (28.35%) in water with a percentage reduction

value that was close to that of *A. seyal* (33.64 %), it rated poorly in efficacy testing against the bacterial strains with MIC greater than 25 mg ml<sup>-1</sup> compared with *A. seyal*, which had an MIC of 3.08 mg ml<sup>-1</sup>.

Quite often, plants showing anti parasitic properties exert antimicrobial effects. For example, in west Africa, stem barks and roots of *Ximenia americana* are applied on wounds. In Zimbabwe, its fruits as well as leaves are consumed as an antihelmintic (Sofowora, 1986) while in Ivory Coast, its fruits, roots and stem bark have shown activity against worms and diarrhea (Diehl *et al.*, 2004). Contrary to this observation, *A. anthelmintica*, which has widely been exploited for treating livestock against internal parasites (Githiori *et al.*, 2003), did not display antibacterial properties despite the presence of saponins, terpenoids and cardiac glycoside. The same plant showed poor disinfection properties of water (9.47%). The poor performance of this plant could have resulted from either the drying process or the extraction process. The drying process may have caused structural changes to its chemical constituents (Parekh and Chanda, 2006). The water extraction method that was used may have yielded low amounts of antimicrobial constituents that would not be effective (Stainer, 1986). This could also have been the case with *A. etbaica*, *A. tortilis* and *E. divinorum*, which despite registering low reduction in colony forming units (18.66%, 18.41% and 28.35% respectively), recorded very high MIC and MBC.

According to Bever (1986), the *in vitro* inactivity of the plants on pathogens may not necessarily translate to an *in vivo* inactivity. This is because the extracts may be playing immuno modulatory roles in the body of an organism. Based on this suggestion, plants that were weakly effective in this study could be highly active *in vivo*. This therefore necessitates the *in vivo* study of these plants.

## CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusions

The following conclusions can be drawn from the findings of this study:

- The Samburu community uses a number of indigenous plants to cure various ailments. The plants include *Acacia nilotica*, *Acacia seyal*, *Acacia tortilis*, *Acacia etbaica*, *Euclea divinorum* and *Plumbago zeylanica*.
- A number of the plants traditionally used for treating various ailments by the Samburus are effective against bacteria that cause diarrhea such as *S. typhi*, *S. dysenteriae* and *E. coli*. Extracts from these plants, therefore, have the potential of treating the infections caused by waterborne pathogens.
- Most of the studied plants contain tannins, flavonoids, alkaloids, saponins and terpenoids, the phytochemicals which have been known to possess antidiarrheal activity.
- Water extracts of *A. nilotica* demonstrated higher efficacy in water disinfection. Hence the plant has the potential of being used as an alternative water disinfectant that is cheap and readily available.

### 6.2 Recommendations

Based on this study the following recommendations are made:

- *in vivo* studies on these medicinal plants are necessary and should find out the toxicity of the active constituents and their side effects.
- Further studies are required to standardize the method of water disinfection using medicinal plants.
- More research needs to be carried out on *A. nilotica* on aspects such as its effect on water properties and its toxicity.



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## APPENDICES

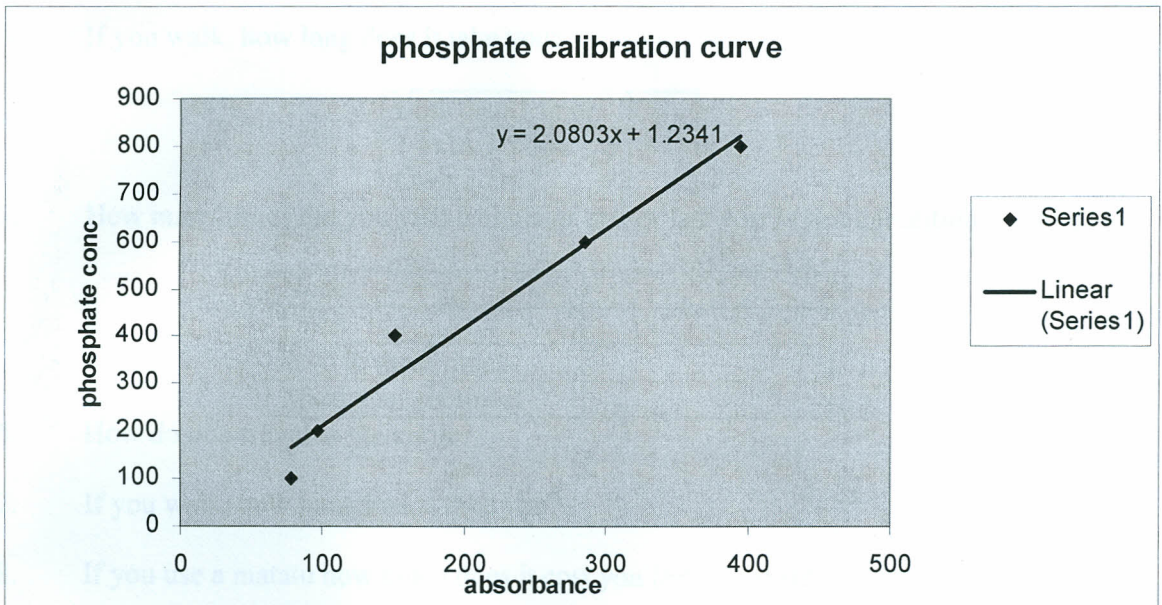
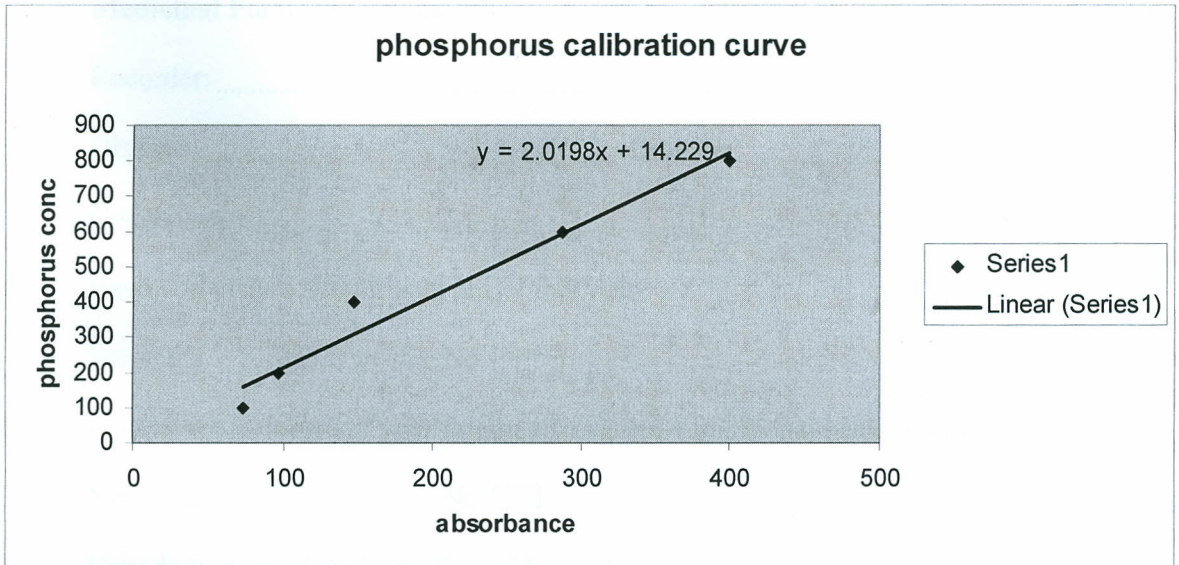
### Appendix 1: Bacterial counts before and after water treatment using medicinal plant extracts (300 g<sup>-1</sup>)

Water source	Plant 1		plant 2		Plant 3		Plant 4		Plant 5		Plant 6		Plant 7		Control	
	30 Min	60 min	30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min	30 min	60 min
Lodungokwe	150	140	147	120	94	0	126	114	170	106	150	144	141	115	159	157
	169	154	169	126	104	0	130	113	162	104	162	148	138	113	159	165
	161	147	158	123	99	0	128	114	166	105	158	146	141	114	158	161
Ewaso Nyiro River	216	201	180	144	168	0	270	192	190	117	248	203	227	175	250	256
	224	200	194	134	164	0	260	209	183	120	240	210	216	166	257	260
	210	201	187	139	166	0	265	201	187	119	244	207	222	171	254	258
Serewamba	240	221	230	225	183	0	304	260	313	150	264	180	263	139	289	283
	245	236	210	166	187	1	294	242	263	138	276	175	265	136	271	300
	243	228	222	185	172	1	299	251	288	144	270	178	244	138	280	292
Namunyak dam	232	221	252	170	267	0	207	184	242	177	296	263	241	135	295	298
	210	200	234	186	196	0	264	210	240	180	298	244	247	136	301	305
	221	216	243	178	232	0	236	191	241	179	292	254	244	136	298	301
Muguru omuny	240	200	203	123	225	0	250	220	250	200	271	200	200	128	280	294
	254	220	207	130	230	1	251	216	282	225	270	230	210	167	250	270
	247	210	204	127	228	1	286	231	296	244	221	185	205	148	255	277
Enyangainito	200	180	160	120	197	0	273	234	245	142	304	258	226	186	237	240
	214	184	167	130	164	0	241	220	195	131	300	261	248	178	250	253
	207	187	164	125	181	0	257	227	220	137	302	253	237	182	240	247
Naibelibeli	239	224	288	200	287	0	265	220	302	223	289	257	282	227	298	304
	226	206	292	213	282	0	266	217	302	220	250	220	288	218	290	294
	215	199	294	214	266	0	275	225	297	217	271	227	297	225	300	304
Ndikir	270	229	255	183	272	0	300	197	292	220	294	209	277	222	273	268
	252	221	240	180	280	2	288	189	293	250	295	253	266	215	282	287
	266	242	278	205	255	2	295	217	275	204	297	258	274	231	281	286
Nakoruworu	288	250	266	211	236	1	298	246	255	160	272	203	288	229	311	321
	287	252	272	209	237	0	288	244	265	177	262	201	288	229	300	307
	297	264	273	218	230	1	285	237	257	165	290	218	292	236	305	308
Nkwaas	273	237	266	207	240	0	255	189	281	161	289	194	270	159	304	317
	250	222	280	223	236	0	257	195	266	159	280	197	272	165	300	310
	261	234	282	221	238	0	270	217	267	150	270	187	278	163	298	310

## Appendix 2: Percentage reduction in bacterial colonies after water treatment with medicinal plant extract

Water source	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7	Untreated
1. Lodungukwe	6.67	18.36	100	9.52	37.64	4	18.4	1.25
	8.87	25.44	100	13.07	35.8	8.64	18.1	-3.7
	8.69	22.15	100	10.93	36.74	7.59	19.14	-1.8
2. Ewaso Nyiro	6.94	20	100	28.88	38.42	18.14	22.9	-2.4
	10.71	30.92	100	19.61	34.42	12.5	23.14	-1.16
	4.28	25.67	100	24.15	36.36	15.16	22.97	-1.57
3. Serewamba	7.91	21.73	100	14.47	52.07	31.81	47.14	2.07
	3.67	20.95	99.46	17.68	47.52	36.59	48.67	-1.07
	6.17	16.66	99.46	16.05	50	34.07	43.44	-4.28
4. Namunyak	4.74	32.53	100	11.11	26.85	11.14	43.98	-1.01
	4.76	20.51	100	20.45	25	18.12	44.93	-1.32
	2.26	26.74	100	19.06	25.72	13.01	44.26	-1
5. Mugurugemunye	16.67	39.4	100	12	20	26.19	36	-5
	13.38	37.19	99.56	13.94	20.21	14.81	20.47	-8
	14.97	37.74	99.56	19.23	17.56	16.28	25.22	-8.62
6. Nayankoinito	10	25	100	14.28	42.04	15.13	17.69	-1.27
	14.01	22.15	100	8.71	32.82	13	28.22	-1.2
	9.66	23.78	100	11.67	37.72	16.22	23.2	-2.91
7. Naibelibeli	6.27	30.55	100	16.98	26.15	11.67	19.5	-2.01
	8.84	27.21	100	18.42	27.15	12	24.3	-1.37
	7.44	27.21	100	18.18	26.93	16.23	24.24	-1.3
8. Ndikir	15	28.2	100	34.3	24.6	13.9	19.7	-1.6
	12.3	25	99.28	34.3	28.91	14.2	19.2	-1.77
	9.02	26.25	99.21	26.44	25.81	13.13	15.69	-1.77
9. Nakoruworu	12.9	20.68	99.56	17.4	37.2	25.36	20.4	-3.3
	12.1	23.16	100	15.27	33.2	23.28	20.4	-2.3
	11.14	20.14	99.56	16.84	35.79	24.82	19.17	-0.98
10. Nkwaas	13.18	22.18	100	25.8	42.7	32.87	41.11	-4.27
	11.2	20.35	100	24.12	40.22	29.64	39.33	-3.33
	10.34	21.63	100	19.62	43.82	30.74	41.36	-4.02

### Appendix 3: Total phosphorus and orthophosphate phosphorus calibration curve



### Appendix 4: Correlation of water properties and percentage reduction in bacteria colonies in treated water

	Conductivity	pH	Alkalinity	Orthophosphate Phosphorus	Total phosphorus
Correlation coefficient	-0.382	+0.44	0.239	-0.0054	-0.125
Degrees of freedom	8	8	8	8	8

## Appendix 5: Questionnaire

### Medicinal Plants Questionnaire

Recorder: ..... Date: .....

District: ..... Division: .....

Conservancy: .....

1. Have you visited a traditional healer this year?

Yes  No

2. Have other members of your family also visited a traditional healer this year?

Yes  No

3. How do you travel to the traditional healer?

4. If you walk, how long does it take you?

Less than 1 hour	
1 hr to 1.5 hrs	
Over 2 hrs	

5. How many times did you visit a clinic or doctor last year? (Not a traditional healer?)

None	
Once	
Two to three times	
More than six times	

6. How do you travel to the clinic?

7. If you walk, how long does it take you?

8. If you use a matatu how much does it cost you (return fare)?

9. If you could get treated at a clinic is it more expensive or cheaper than a traditional healer?

Cheaper	
Same	
More expensive	

10. If a clinic were closer to your house, would you use traditional medicines more or less often?

Less	
More	
It depends on what treatment I need	

11. Do you prefer traditional medicine for the treatment of stomach illness?

Yes  No

12. Why do you prefer traditional medicine to hospital medicine?

TM is cheap	
TM readily available	
Hospitals far away	

13. Which plants are used for treating people with stomachache?

1. 4.  
2. 5  
3. 6

14. Which parts of the plant in (5) above are commonly used?

15. How are the plants in (5) above prepared to administer to patients?

16. In what year were you born?

17. What religion do you belong to?

Protestant	
Catholic	
Pentecostal	
SDA	
Muslim	
Traditional	
Others	

19. What is the highest level of formal education you obtained?

Primary	
High School	
College	

20. What is your occupation?

Pastoralist	
Trader	
Other	

21. Do you have any other source of income? (Specify)