BACTERIA, AFLATOXINS AND FLUORIDE LEVELS IN LOCALLY PROCESSED HERBAL MEDICINES FROM NAIROBI COUNTY, KENYA

BY

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DECLARATION

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

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DEDICATION

This work is dedicated to my lovely wife Sylvia Sitawa my parents Mr and Mrs Mukundi and the Lord Almighty.

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LIST OF ABBREVIATIONS

EMB	Eosin Methylene Blue
GACP	Guidelines on Good Agricultural and collection Practices
GHP	Hydrophilic polypropylene
GMPs	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Point
HPLC	High Pressure Liquid Chromatography
HRP	Horse radish peroxidase
IDMA	Indian Drug Manufacturers Association
IRCH	International regulatory cooperation for herbal medicines
MRVP	Methyl Red and Voges-Proskauer
NDA	New Drug Application
OD	Optical Density
PPB	Pharmacy and Poisons Board
TMB	Tetramethylbenzidine
USFDA	United States Food and Drugs Administration
WHO	World Health Organization

ABSTRACT

The increased use of herbal medicines has come with challenges of safety, quality and efficient utilization. This study assessed the safety of the herbal medicines with regards to microbial contamination, aflatoxins and fluoride. It also assessed the quality of the herbal medicines with regards to harvesting, processing and storage. A cross-sectional survey, targeting key stakeholders dealing in herbal medicines in Nairobi County was undertaken. A non probability sampling involving 50 traders was done and 3 samples were obtained from each one of them. From the Nairobi Central business district 30 herbalists were sampled while in Kawangware and Dagoretti markets 10 herbalists were sampled from each market. The microbial contamination by Coliforms was tested using the membrane filtration method, while the Aflatoxins contamination was determined by using ELISA and High Pressure Liquid Chromatography. Fluoride was measured by direct potentiometric method. A total of 90 solid samples and 60 liquid samples were analyzed. The solid samples were not contaminated with *Escherichia Coli*, but 26.7% of samples contained *Klebsiella pneumonia.* The liquid herbal preparations were contaminated with both *Klebsiella pneumonia* and *Escherichia coli* 13.3% and 6.67% respectively. Liquid samples were not contaminated with *aflatoxins*, while, 24.0% of the solid samples contained *aflatoxins* subtypes B_1, B_2, G_1 and G_2 at various concentrations. Although the levels of *aflatoxins* reported in this study are within the acceptable limits, and because *aflatoxins* especially *aflatoxin* B_1, are potent carcinogens there is an interest in the effects of long-term exposure to low levels of these important mycotoxins to humans. Safety of herbal medicines is therefore an important Public Health issue. If action is not taken, the levels of contamination are likely to increase adverse effect to public. Generally, the solid and liquid samples had fluoride concentrations within the range of 0.532 mg/L to 1.718 mg/L which was within the recommended WHO limits being 1.5

mg/L. This is expected given that the herbal medicine practitioners surveyed were from the same geographical area of Nairobi County and were most likely to share the same water source in preparation of the extracts. The liquid samples assessed for fluoride contamination were not hazardous to the consumer as fluoride levels were within acceptable levels. This study has shown that during preparations of various herbal medicines there is a need to use clean safe water and observe proper storage conditions in order to eliminate contamination of medicines.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Increasing use of herbal medicines in Kenya and the expansion of their market pose challenges in safety, quality and efficacy. More recently, urbanization has changed the face of traditional medicine. Previously, village healers would provide services using herbs obtained from nearby forests and fields. Urban markets, on the other hand, have many herb sellers, each giving advice and selling both raw plant material and preparations that they have produced themselves. Their products are mainly packaged in bottles or small plastic bags, perhaps wrapped in newspapers, but have no indication of the appropriate dosage. Quality control is a challenge under these circumstances. So is the conservation of the botanical resource. Thus traditional medicine in Kenya flourishes unrecognized and unregulated by the Government or other institutions. This has resulted in the proliferation of herbal practitioners dispensing various forms of herbal medicines that are touted as able to resolve just about any health problem. Little is known about the safety and efficacy of these proposed therapies, or their negative consequences. While unregulated use of traditional medicine can have negative effects, a claim that herbal medicines can cure every disease brings even good practice into disrepute. With increased use the questions of safety, efficacy and quality are some of the challenges that need to be addressed. More work is also needed to raise public awareness on appropriate use of traditional medicines.

1.2 Problem Statement

The history of using herbs is inextricably intertwined with that of modern medicine. Many synthetic drugs listed as conventional medication were originally derived from plants, for example the antimalarial drug quinine from Cinchona species (Janetzang, 1994). The World Health Organization

estimates that about 70 – 80% of the world population particularly in developing countries rely on non conventional medicines mainly of herbal origins for their primary health care.

This is because herbal medicines are accessible and cheap (Sofowora, 1993). Therefore, the quality and safety of herbal preparations are also of great concern. The WHO (2004) explained that quality is the basis of reproducible efficacy and safety of herbal drugs, and to ensure the standard of research on herbal medicines, the quality of the plant materials or preparations is of utmost importance. There is limited research and published work which has been conducted in this area especially in Nairobi County.

1.3 Study objectives

Main objective

The main objective of this study was to carry out an assessment of bacterial, aflatoxins and fluoride levels in locally processed herbal medicines from Nairobi County.

Specific Objectives

The specific objectives of the study were:

1. To determine the bacterial contamination, presence of aflatoxins and levels of fluoride in herbal medicines from Nairobi County.
2. To assess quality of the herbal medicines during harvesting, processing and storage from Nairobi County.

1.4 Justification

Along with increased popularity of herbal medicine there is an increased interest on safety and quality of the products. Though there has been research on microbial contamination of herbal products in the market not much has been done to assess Nairobi County, which is a great market for the products. Due to poor processing and storage practices which are done without standards it creates the need to

evaluate whether there is aflatoxins contamination of which can be lethal when consumed. Also due to the water and materials used to process the products it is necessary to assess whether Colliform bacteria are present as well as the fluoride levels.

1.5 Significance of the Study

The study aimed at assessing safety and quality standards of herbal medicines used in the Nairobi County. Being a public health concern, the information is vital to the general population in order to ascertain the safety of the products used as medication. The study also provided vital information for regulatory bodies, policy makers and other stake holders in the healthcare industry in order to identify the need to regulate remedies used in alternative medicine. Researchers will also benefit from information in this area which can be used as a foundation for further research.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

With the ever-increasing use of herbal medicines worldwide and the rapid expansion of the global market for these products, the safety and quality of medicinal plant materials and finished herbal medicinal products have become a major concern for health authorities, pharmaceutical industries and the public (Steven *et al*, 2003, Kosalek and Tomić 2009). National regulation and registration of herbal medicines varies from one country to another. Where herbal medicines are regulated, they are categorized either as prescription medicines or non-prescription medicines. Within a country, a group of herbal products categorized other than as medicines may coexist. Herbal products categorized other than as medicines and foods, are becoming increasingly popular and there is potential for adverse events due to lack of regulation, weak quality control systems and loose distribution channels which include mail order, street vending and internet sales.

2.2 Microbial and Aflatoxins contamination

Medical plants are associated with a broad variety of microbial contaminants, mainly bacteria and fungi. Also broad diversity of bacterial, fungal cells and viruses can be found either in or on the plant material (Ashiq, Hussain and Ahmad 2014, Ruparel, 2011). Among micro-organism, occurrence of pathogens particularly limits the use of these plants. Microbial contamination can transform the benign chemicals in the plant into harmful substances, or through the microbe's production of toxic compounds. For examples, the moulding of sweet clove (Melious officinalis) causes a chemical transformation of clove's constituents; the resultant compounds can cause haemorrhaging (Bogusz, al Tufail and Hassan 2002). The potentially toxic effects of bacterial and fungal endotoxins such as *Escherichia coli* and aflatoxins from *Aspergillus spp*. are well known (Chan 2003). Commonly found

pathogenic bacteria on botanicals include *E. coli*, *Salmonella typhi*, *Pseudomonas aeroginosa* and *Staphylococcus aureus*.

Mycotoxins are toxic metabolites produced by certain fungi that can infect and proliferate on various medicinal plants in the field and/or during storage. Mycotoxin may exhibit various toxicological manifestations; some are teratogenic, mutagenic and/or carcinogenic and are associated with toxins, various diseases. The different mycotoxins of relevance to human health are aflatoxins, ochratoxins, zearalenone, fumonisins, and trichothecenes (Efferth and Kaina 2011).

Studies have been conducted to determine the types of fungi and their toxins contaminating medicinal plants, processed and non-processed foods and other materials of plant origin. The fungal species commonly encountered are Fusarium, Aspergillus, Penicillium, Rhizopus, Absidia, Alternaria, Cladosporium and Trichoderma. On the contrary, considerable risk levels of aflatoxins in several botanical medicinal samples of different taxa have been detected. Risk assessment of the microbial load of medicinal plants has become an important subject in the establishment of Modern Hazard Analysis and Critical Control Point (HACCP) schemes. Various guidelines such as WHO, British Herbal Pharmacopoeia (BHP), Indian Herbal Pharmacopoeia, European Pharmacopoeia has issued special guidance for assessing microbial contaminations of both raw as well as processed botanicals. All these guidelines provide specific limits for the contaminants. These limits give due consideration to the level of treatment given to the processing material. WHO Quality Control Methods for Medicinal Plant Materials mentions that the presence of aflatoxins can be hazardous to health if absorbed even in very small amounts (Fong, 2002). The document provides the procedure for qualitative determination of aflatoxins B_1, B_2, G_1 and G_2 by TLC. It also gives the procedure for total viable count for bacteria and fungi, qualitative and quantitative determination of Enterobacteriaceae

and certain other Gram-negative bacteria, qualitative tests for determination of specific organisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella* species.

Microbial contamination of plants is influenced by environmental factors such as temperature, humidity, extent of rainfall during the pre-harvesting, harvesting, and post-harvesting periods, handling practices and storage conditions of crude and processed medicinal plants materials (Chotchoungchatchai, et al 2012**)**. This reflects the importance of indicator organism and framing of limits for microbial contamination based on the existing environmental conditions in the country. Further, the microbial risk inherent to botanical may vary with regard to the microbial quality of the final product. The application of hot water extraction (herbal infusion, herbal tea) usually compensates for microbiological contamination, since it can be expected that boiling water markedly reduces the viable counts by several log units and also inactivates possible pathogens. However, the drugs, which are subjected to cold-water extraction (herbal maceration), may host a considerable amount of microbes, and the extraction procedure carried out at ambient temperature usually enables microbial multiplication. In principle, most quality aspects of botanical drugs can be compared with those considered in food microbiology, since spices, herbs, tea, vegetables, cereals may exhibit similar microbiological tendencies. However, unlike foods, botanicals contain specific compounds of particular pharmaceutical and medical relevance with dose-dependent properties, and are not consumed for a nutritive or relishing function. Moreover, the consumers of medicinal plants are people who undergo some form of therapeutic treatment. USFDA strategies to minimize mycotoxins in the food supply include establishing guidelines (e.g., action levels, guidance levels), monitoring the food supply, through formal compliance programmes (domestic and import) and taking regulatory action against product that exceeds action levels, where action levels have been established (Zhan et al, 2012, Sahoo, Manchikanti and Dey 2010)

2.3 Fluoride occurrence in Water and Plants

Fluoride gets into the water cycle by leaching from soils and minerals into ground water and surface water. The fluoride concentration in water is affected by several factors such as availability and solubility of fluoride containing minerals, porosity of rocks or soil through which the water passes, pH, temperature and the presence of other elements which may complex with fluoride (Fleisher and Robinson, 1963) Fluoride water concentrations in Kenya are among the highest in the world with the occurrence mainly being found in some springs, boreholes, and lakes in Rift Valley, (WHO 1970, 1973, Gikunju et al., 1992).

The high prevalence of fluoride in soil, water and rocks results in the occurrence of fluoride in many plant tissues. However, it is known that fluoride content of most plants, with the exception of root, is not readily affected by the amount of fluoride in soil in which they grow. Exception to this rule are the *Camelia spp*, tea plant, of which they have been found to contain upto 2000 ppm and 150 ppm respectively (Allcroft et al., 1965). Plants generally have limited ability to accumulate fluoride from soils, although acidic soils can augment uptake (Underwood, 1977).

2.3.1 Fluoride Toxicity

Acute poisoning associated with massive ingestion of ascaricides (sodium fluoride), rodenticides (sodium fluorosilicate), or oral dental products will produce clinical disease within 2 hr. The fatal dosage of sodium fluoride is 5–10 mg/kg. Toxic manifestations may be evident after consumption of 1 mg/kg. Serum calcium and magnesium concentrations decline rapidly after the onset of the clinical syndrome. Severe gastroenteritis, salivation, restlessness, sweating, anorexia, muscle weakness, stiffness, dyspnea, ventricular tachycardia, and convulsions followed by depression and death are typically seen. Chronic fluorosis is characterized by weakness with skeletal and dental abnormalities (Obi et al, 2006). Reduced appetite accompanied by poor weight gain reflects dental lesions and

impaired mastication. Mottled, chalky, pitted and stained enamel, and uneven and excessive wear on the teeth are frequently seen. Skeletal abnormalities associated with increased bone resorption and remodeling produces severe lameness and stiffness. In later stages of the syndrome, severely affected people may be forced to move on their knees because of spurring and bridging of joints. Periosteal hyperostosis is seen on ribs. Metabolically active and growing bones of young people are more severely affected. Anemia and hypothyroidism manifested by reduced T_3 and T_4 levels plus reduced serum calcium concentrations are often present.

Severe gastrointestinal inflammation and degenerative changes in other organs such as the liver, kidney, and lungs reflect the cytotoxic effects of acute fluorosis. After chronic exposure during pregnancy, offspring are more severely affected. Bilateral and symmetrical skeletal abnormalities are present. The bones are chalky white with disrupted osteogenesis, accelerated bone remodeling, and resorption in association with production of abnormal bone osteoid results in exostoses, sclerosis, and osteoporosis. The mandible, ribs, metacarpals, and metatarsals are most often affected. Exostoses are most evident in the long bones. In addition to the mottled, chalky, stained teeth exhibiting uneven wear, eruption of permanent incisor teeth may be delayed.

2.4 Regulatory aspects of Herbal Medicines.

World Health Organisation (WHO) has tried to establish internationally recognizable regulatory guidelines to define basic criteria for the evaluation of quality, safety and efficacy of botanical medicines (WHO 2002). WHO assists national regulatory authorities, scientific organization and manufactures to undertake an assessment of the documentation/submissions /dossiers in respect of such products. Guidelines for assessing the quality of such products are formulated. Guidelines for assessing the quality of botanical materials mainly emphasize the need to ensure the quality of medicinal plant products by using the modern techniques and applying suitable standards (Routledge,

2008). A series of tests for assessing the quality of medicinal plant material have been described. For physical evaluation, parameters like ash value, extraction matter, volatile matter etc. have been recommended. Pharmacological evaluation has been recommended for certain norms like bitterness value and haemolytic activity. Detection of pesticidal residue, arsenic and heavy metal content, microbial load and radioactive contaminants has been recommended for safety of the botanical materials (WHO, 2002).

In 1997, WHO developed draft guidelines for methodology on research and evaluation of traditional medicine (TM). It mainly focuses on current major debates on safety and efficacy of traditional medicine. It also tries to provide answer for some of the challenging questions concerning evidence base of the evaluation of botanical medicine, and also recommend new approaches for carrying out clinical research Specific objectives of these guidelines are to harmonize the use of certain accepted and important terms in Traditional Medicine (WHO, 1997).

Under the overall context of quality of botanical medicines, WHO developed the Guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plant. GACP provides general technical guidance on obtaining medicinal plant materials of good quality for the sustainable production of herbal products classified as medicines. The main objectives of these guidelines are to guide the formulation of national and/or regional GACP guidelines and GACP monographs for medicinal plants and related standard operating procedures and to encourage and support the sustainable cultivation of medicinal plants of good quality. The following are some of the areas under which safety evaluation is reviewed for the purpose of providing quality herbal medicines.

Over the past decade, several adverse effects of botanical medicines due to chemical composition of botanicals or extraneous matters present in/on the plant material have been reported. This has raised many questions regarding safety of the botanicals. Botanical medicines may be associated with

contaminants like micro-organisms, excessive or banned pesticides, heavy metals, chemical toxic either by mycotoxins, and radioactive substances etc.

2.4.1 Safety Regulatory aspects and approval of herbal drugs in various countries

The legal process of regulation and legislation of herbal medicines varies from country to country. The reason for this involves mainly cultural aspects and also the fact that herbal medicines are rarely studied scientifically. Thus, few herbal preparations have been tested for safety and efficacy. The WHO has published guidelines in order to define basic criteria for evaluating the quality, safety, and efficacy of herbal medicines aimed at assisting national regulatory authorities, scientific organizations and manufacturers in this particular area. Furthermore, the WHO (2002) has prepared pharmacopoeia monographs on herbal medicines and the basis of guidelines for the assessment of herbal drugs. Several regulatory models for herbal medicines currently exist, including prescription drugs, over-the-counter drugs, traditional medicines and dietary supplements. Thus, the need to establish global and/or regional regulatory mechanisms for regulating herbal drugs seems obvious. A summary of the regulatory processes related to herbal drugs in some selected countries is presented below.

2.4.2 Herbal drug regulations in India

India is a significant contributor to this field of herbal medicines. Recognizing the global demand, Government of India has realized Good Manufacturing Practices (GMPs) for the pharmacies manufacturing Ayurvedic, Siddha and Unani medicines to improve the quality and standard of drugs. The new rules came into force from June 2000 as an amendment to the Drugs and Cosmetics Act, 1940. These rules give details regarding essential infrastructure, personnel and quality control requirements for herbal drug manufacturing. Implementation of GMP requirements is mandatory to the industry. Qualifying units can get the GMP certificate immediately. Exemption has been given to the registered practitioners and teaching institutions that prepare medicines for their patients.

Department of Indian Systems of Medicine and Homeopathy (ISM&H) is trying to frame safety and efficacy regulations for licensing new patent and proprietary botanical medicines. Indian Pharmacopoeia covers few Ayurvedic medicines. Monographs have been given for some Ayurvedic drugs like clove, guggul, opium, menthe, senna, the ayurvedic pharmacopoeia of India gives monographs for 258 different Ayurvedic drugs. The standards mentioned are quite inadequate to build quality of the botanical materials. Indian Drug Manufacturers Association (IDMA) has published Indian Herbal Pharmacopoeia (2002) with 52 monographs of widely used medicinal plants found in India. The latest available scientific data has been incorporated in theses monographs.

Provisions relating to the manufacture and control of Ayurvedic, Siddha and Unani drugs have been prescribed in the Drugs and Cosmetics Act.

2.4.3 Safety and quality regulation in United States of America

The USA government has established the Office of Alternative Medicine at the National Institutes of Health (NIH) with the following aims: 1) to explore the potential role of dietary supplements in the improvement of health; 2) to promote the scientific study of supplements for maintaining health and preventing chronic diseases; 3) to compile a database of scientific research related to supplements; 4) to coordinate NIH funding for dietary supplements related to the treatment of chronic disease (USFDA, 2000)

In the USA, herbal remedies are referred to as homeopathic remedies. All such remedies , because these are offered for treatment of disease, are regarded as drugs. This means that if a herbal remedy is included in United States Pharmacopoeia, the official Homeopathic Pharmacopoeia or the National formulary, it will be recognized officially as a drug.

The only way that a drug application for its intended use in USA is by approval of new drug application by the Food and Drug Administration. Up to now, no homeopathic drug has been

approved for administration under a New Drug Application. This however does not necessarily mean that it is illegal to market these herbal preparations. These could be marketed without the approval of the Food and Drug Administration in certain circumstances. All marketed drugs have to be listed with the Food and Drug Administration.

The position in the United States of America is that there are many homeopathic preparations in the market which have not gone through the process of approval by the Food and Drug Agency. Some of these may be mentioned in the pharmacopoeias concerned or in the national formularies. If so, these homeopathic remedies are officially recognized but not officially approved for marketing. They are however marketed but not illegally. They are marketed as homeopathic remedies recognized (if it is in the Formulary or pharmacopoeia) but not approved for marketing. It is not illegal for use. The United States of America Government did not, up to now, stringently regulate the use of marketing of homeopathic remedies because, in the past, these have really been marketed only by a very few manufacturers on a very limited scale. These firms have been serving the need mainly of homeopathic consumer. The labels were intended for use by the homeopathic physician who would make a diagnosis and then either dispense homeopathic medicine himself or give the patient a homeopathic prescription. The patient could have that prescription made out at a homeopathic pharmacy.

In the United States of America, there is a legal requirement that all drug have to be approved for their intended uses through the approval of a new Drug Application (NDA) by FDA. Herbal medicines are drugs because these are used for the treatment of disease conditions. Yet, homeopathic drugs, which include medicinal plant products, are legally allowed to be marketed, and, at this time, are also being promoted for use, with this being frowned up.

2.4.4 Safety and quality regulations in Africa

A 2005 WHO global survey found that 60% of WHO's Member States in Africa had no national policy, laws or regulations for traditional medicine, although more than half of these countries proposed developing them (WHO 2005 National policy on Traditional Medicine) Interestingly, approaches to licensing, dispensing, manufacturing and trading of traditional remedies differ greatly even among those countries with national policies and legal and regulatory frameworks. The lack of regulation in many countries means there are just as many fake remedies and false practitioners as there are genuine treatments-a situation, which can have fatal consequences.

The survey also found that around 110 countries regulate herbal medicines in response to a dramatically increased use globally and demand for more vigorous requirements to ensure quality, safety and efficacy. A number of countries also review and strengthen existing regulations for herbal medicines in a continued effort to improve their use and efficacy. A global network of regulatory agencies responsible for regulation of herbal medicines, the "International regulatory cooperation for herbal medicines (IRCH)" was established in 2006 under the coordination of WHO and currently has 19 members.

Generally, the use of herbal medicines in the Region is based on oral tradition within a family or a community. As a result, most herbal medicines claimed to provide "effective cures" for various diseases lack scientific evidence for safety, efficacy or quality-essential requirements for evaluating traditional medicines. Yet, they are openly sold in markets, stores, homes and even in pharmacies as over-the-counter medicines and dietary supplements, with little, if any, advice offered on their use. Consumers may often be unaware of how and when herbal medicines may be safely taken, or of their potential side effects. Despite this, most countries in the Region have not established safety-monitoring mechanisms for imported and locally produced traditional medicines, as demonstrated by

a survey conducted by WHO in 2002, which showed that only 8 out of the 34 countries covered had regulations on traditional medicines (WHO 2005 National policy on Traditional Medicine). This would seem to reflect the inadequacy of facilities for researchers in the Region for assessing the quality, safety and efficacy of traditional medicines whose composition is unknown.

2.4.5 Safety and Regulation in Kenya

In the Republic of Kenya, a national policy, laws and regulations on Traditional medicine are being developed. No national programme has been issued, and no national office or expert committees have been established. A national research institute (Kenya Medical Research Institute) that conducts research on traditional medicine was established in 1984. Herbal medicines are not regulated in Kenya. Neither a national pharmacopoeia nor national monographs exist or are being developed. No other pharmacopoeias or monographs are used in their place.

No information was provided on manufacturing requirements, but special regulatory requirements for safety assessment of traditional use without demonstrated harmful effects and reference to documented scientific research on similar products apply to herbal medicines. These have been established by the Kenya Medical Research Institute, but no control mechanism exists to ensure their implementation.

There is no registration system for herbal medicines and they are not included on the essential drug list. A post marketing surveillance system is in development. Herbal medicines in Kenya are sold without restriction.

There have been a few studies carried out to investigate microbial contamination and heavy metal poisoning, though most have not been published yet. Some include: Identification and characterisation of microbial contaminants of herbal medicines in Kenya (Meshack et.al, 2011).

The pharmacy and poisons board (PPB, 2010) Kenya, has come up with guidelines to ensure that only good quality, safe and efficacious herbal and complementary products are available in Kenya; and to contribute towards their accessibility, cost effectiveness and appropriate use with the current state of knowledge. These guidelines have been drawn to address the many issues on the quality of herbal and complementary medicines that have been used for a long period of time in Kenya. These issues include;

1. Misconception amongst herbalists that documentation requested for by PPB is intended to steal their indigenous knowledge and thus, there has been hesitation to submit applications.
2. Lack of documented evidence on quality, safety and efficacy of Herbal and complementary products.
3. Unethical practices that include; Adulteration of herbal and complementary products with conventional medicines. Advertising of Herbal and complementary products in print media, electronic and bill boards. Peddling of products with no therapeutic benefits. Unsubstantiated medicinal claims by herbal practitioners. Dealing with herbal products whose toxicological profile is not known.
4. Poor standards of preparation/manufacture and sale of herbal and complementary products.

The quality control according to the Pharmacy Poisons Board guidelines (PPB, 2010) starts from the raw materials being acquired followed by the processing, to the finished product and storage. Highlights to the key safety and quality regulations as per recognized pharmacopoeias stating the minimum range of specifications include:

1) Microbiological contamination and tests for other toxins. 2) Uniformity of weight (for tablets, single-dose powders, suppositories, herbal tea in sachets and capsules, etc.), disintegration time (for tablets, capsules, suppositories and pills), hardness and friability (for example, uncoated tablets),

viscosity (for internal and external fluids), consistency (semisolid preparations), and dissolution (tablets or capsules), if applicable. 3) Physical appearance such as colour, odour, form, shape, size and texture 4) Loss on drying or water content 5) Identity tests, qualitative determination of relevant substances of the plants (e.g. fingerprint chromatograms).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

A cross-sectional survey design which targeted key stakeholders dealing with herbal medicines in Nairobi County was used.

3.2 Study Sites

The area of sampling was Nairobi County because it has a large market for the products. As most of the herbal practitioners and traders are not registered with the Registrar of Companies, the study targeted herbalist based on location and mainly their key areas of operation. According to the County Government records, there are about 57 herbal practitioners and traders licensed to conduct this trade within Nairobi County.

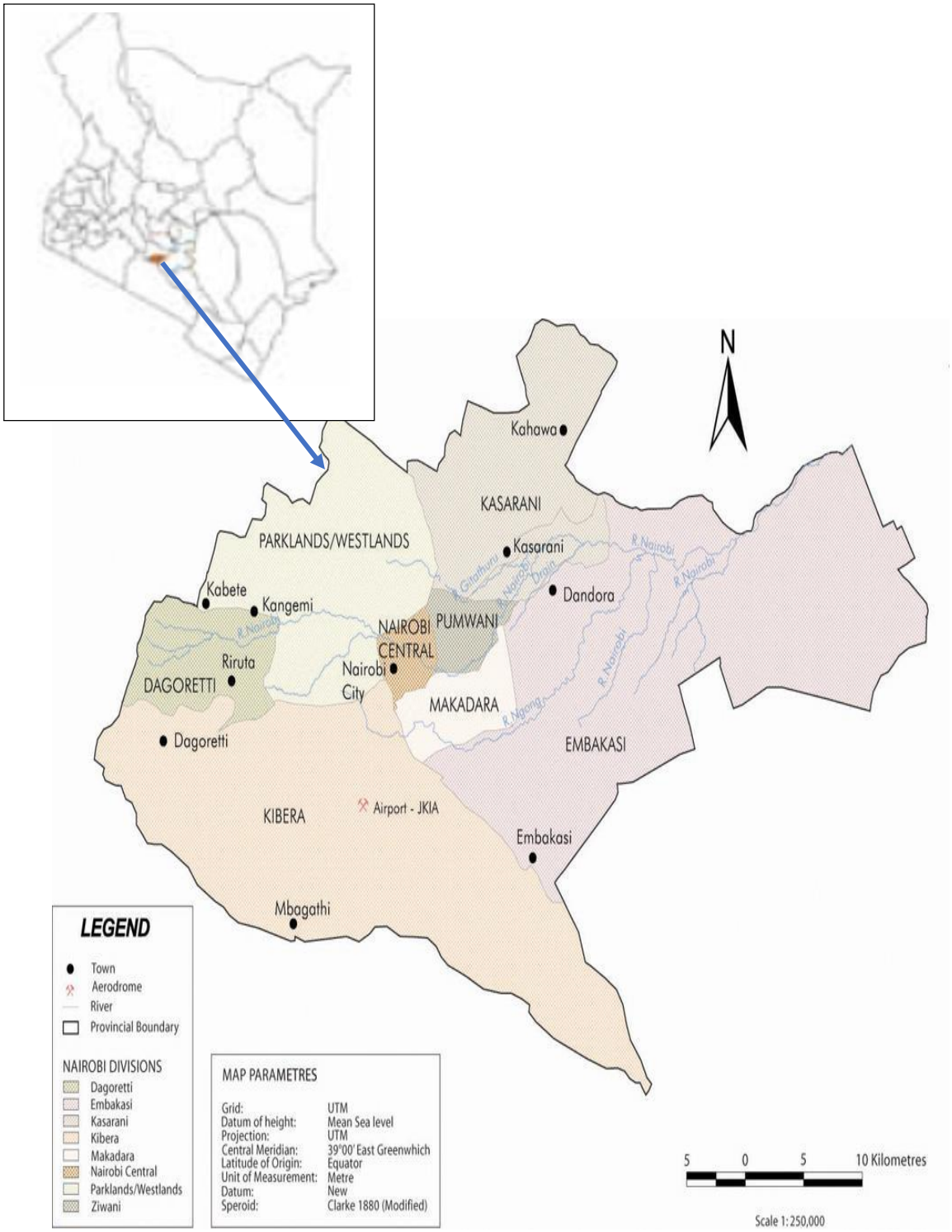

Figure 1: Map of Nairobi County

(Source: Google maps, 2015)

The target sites were classified as; Central business district and satellite towns around the city which included Kawangware and Dagoretti markets. The Central business district was picked on the basis of being a high volume transit area with a lot of commercial activities. Kawangware and Dagoretti had a high population of poor people who could only afford the herbal medicines rather than visiting the hospitals dispensing conventional medicines.

3.2.1 Sample size Calculation

There are about 57 herbal practitioners and traders licensed by the Nairobi County Government from which the study sample was drawn. The researcher adopted a sampling formula (Yamane, 1967) where the sample size was determined as follows:

$$n = \frac{N}{1 + N\,(e)^2}$$

Where n = the sample size

N= the population

e = error term

1 is a constant

$$\text{Therefore } n = \frac{57}{1 + 57\,(0.05)^2}$$

$$= \frac{57}{1 + 57 \text{ X } 0.0025}$$

$$= \frac{57}{1 + 0.1425}$$

$$= \frac{57}{1.1425}$$

$$= 49.8906$$

n = 50 herbal practitioners

This study used cluster sampling strategies. It involved selecting respondents from certain areas only, or certain time-periods only. Cluster sampling is an example of 'two-stage sampling' or 'multistage sampling': in the first stage a sample of areas is chosen; in the second stage a sample of respondents within those areas is selected before random sampling (Mugenda, 2008 and Rukwaru, 2007)The clusters were from the Nairobi central business district, Kawangware and Dagoretti markets. Cluster sampling was used due to the difficulty of obtaining a sampling frame of the herbal practitioners in the entire Nairobi (WHO, 2014). Therefore, the fifty traders dealing with herbal medicines were selected and 3 samples obtained from each one of them. From the Central business district 30 herbalists were sampled while in Kawangware and Dagoretti markets 10 herbalists of each were sampled.

3.3 Data collection procedures

Assessing quality in regards to harvesting, processing and storage was carried out with the help of questionnaires. Questionnaires were administered to the 50 herbalists while collecting samples because the information was obtained without delay and with precision.

3.4 Experimental Procedures

3.4.1 Determination of colliform microbes by membrane filtration method

The Colliforms contamination was determined by membrane filtration method (Dufour, 1981). The membrane filter (MF) technique is highly reproducible, can be used to test relatively large volumes of sample, and yields numerical results more rapidly than the multiple-tube procedure. A sterile absorbent pad was placed in a sterile petri dish, taking care not to touch the pad or the inside of the petri dish. Forceps were dipped in alcohol to sterilize them. Contents were carefully poured evenly over absorbent pad. The membrane filter assembly was set up and the sterilized forceps used to replace a membrane filter, grid side up, into the assembly. The sample was inverted for 30 seconds

and vacuum applied to filter the sample. The vacuum was released and the funnel wall rinsed with 20 to 30 mL of sterile buffer dilution water. With a slight rolling motion, the filter was centered grid side up on the absorbent pad. The pad was checked for air trapped under the filter ensuring that the filter touches the entire pad after which the petri-dish lid was replaced. The petri-dish was inverted and incubated at 35 ± 0.5 °C for 24 hours. After incubating, a 10 to 15X microscope was used to count the red colonies that have a greenish-gold metallic sheen.

As related to the membrane filter technique, the coliforms group may be defined as comprising all aerobic and many facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that develop a red colony with a metallic sheen within 24h at 35^{o}C on an Endo-type medium containing lactose. Some members of the total coliform group produce a dark red or nucleated colony without a metallic sheen. When the groups were verified they were classified as typical coliform colonies. When purified cultures of coliform bacteria were tested they produced a negative cytochrome oxidase (CO) and positive Beta-galactosidase (ONPG) reaction. Generally, all red, pink, blue, white, or colourless colonies lacking sheen were considered non-coliforms by this technique.

3.4.1.1 Materials and Equipment

Equipment: All of the bottles, pipettes, and graduated cylinders used were made of sterilizable glass or plastic. The specific glassware needed for this procedure included: sample plastic bottles, dilution bottles, pipettes and graduated cylinders (Jaytec England), containers for culture medium which included; Erlenmeyer flasks (Class A) with metal caps, metal foil covers, or screw caps. Disposable culture dishes (Greine bio-one ltd) and 1-L filtering flask with a side tube.

In addition, the following equipments were used:

Filtration units (Sigma-Aldrich® vacuum filtration assembly) included a seamless funnel fastened to a base by a locking device or by magnetic force. Funnels with deep scratches on the inner surface or with chipped surfaces were discarded.

Vacuum line, electric vacuum pump (Charles Austen ltd), Membrane filters (0.45 µm,47 mm nitrocellulose, Tekno ltd). The filters had a pore diameter which was to retain all coliform bacteria. The filters were non-toxic to bacteria and did not influence the pH. The membranes were grid-marked in a manner which neither inhibits nor stimulates bacterial growth along the grid-marks.

Membrane filters (0.45 µm; Merck Millipore ltd England) these were disks of filter paper not toxic to bacteria and did not influence the pH.

Forceps were smooth and flat without corrugations on the inner sides of the tips.

Incubators provided a temperature of 35+O.5°C and a humidity of 60%.

Microscope and light source (OLYMPUS, Japan). The microscope had a magnification of 10 to 15 diameters.

Reagents

The reagents used included:

Culture media; Eosin Methylene blue (HiMedia lab, India, lot 0000146845), MacConkey Agar, Sterile distilled water, Methyl Red (MR) and Voges-Proskauer (VP) broth (HiMedia lab, India, lot 0000147140), Tryptone water, Erlich's Indole reagent ((HiMedia lab, India, lot 0000143127)

3.4.1.2 Confirmation of Colliforms

A 100 ml of the sample was filtered through a 47-mm, 0.45-µm pore size cellulose ester membrane filter that retains the bacteria present in the sample. The filter was placed on a 5-mL plate of Eosin Methylene Blue (EMB) agar and the plate was incubated at 37°C for up to 24 hours. The bacteria formed deeply coloured nucleated colonies with metallic surface lustre.

The colonies were sub cultured into MacConkey Agar media which is a differential plating medium used in the detection and isolation of all types of dysentery, typhoid and paratyphoid organisms. When grown on MacConkey medium,colonies of coliform bacteria were brick-red in color and were surrounded by a zone of precipitated bile. These reactions were due to the acid produced by the fermentation of lactose.

Procedure

The equipments were sterilized in the autoclave at 120^{o} C. A total of 10 gm of sample was weighed and mixed thoroughly in 100 ml of distilled water. The solution was filtered through a 0.45 μm sterilized filter membrane that was placed on a vacuum filtration unit. Once run the filter membrane was placed with the help of sterilized forceps on a petri dish containing Eosin Methylene Blue (EMB) media and incubated at 37ºC for 18 hrs. After incubation petridish with the filter membrane was observed for colony growth to confirm presence of Colliform.

3.4.1.3 Biochemical Tests for lactose-fermenting Enterobacteriaceae

In detecting *E. coli* exclusively as the lactose fermenting "coliform" that invariably indicates fecal contamination four biochemical tests were used to distinguish *E. coli* from other lactose-fermenting *Enterobacteriaceae*: The biochemical tests used included:

1.Indole test detects indole production from tryptophane. *E. coli* is positive (+); many other coliforms are negative.

2.Methyl Red test was used to detect acid production in the medium; intended to distinguish between type of fermentation reaction (mixed acid vs. butylenes glycol). *E. coli* is positive and some other coliforms are negative.

3.Voges-Proskauer test was used to detects acetoin, an intermediate in the butylene glycol pathway. Acetoin is oxidized to diacetyl under alkaline conditions in the presence of air, and when reacted with creatine, it forms a pink color. *E. coli* is negative and some other coliforms are positive.

4.Citrate utilization as sole carbon source. *E. coli* is negative and *klebsiella* is positive.

Procedure

For determination of *E.coli* (lactose fermentes) four colonies were transferred into a petri dish containing MacConkey agar and incubated at 37ºC for 18 hrs. The colony morphology was determined to confirm presence of lactose Fermentes bacteria. The colonies were subcultered for purification, and then one colony from the subculture was suspended in distilled water in a centrifuge tube which was then vortexed. A loopful of the solution was then transferred to test tubes containing 3 ml tryptone water, 5 ml MRVP media and 5 ml Simmion citrate solution respectively. This was then incubated at 37ºC for 18 hrs. In the tryptone water containing tube was added Erlich's Indole reagent, while in the MRVP medium was added methyl red and potassium hydroxide with creatine respectively and left at room temperature for 2 hrs and the results were recorded.

3.4.2 Determination of Aflatoxins

This was conducted under total aflatoxin ELISA Assay which is a solid phase direct competitive enzyme

An aflatoxin specific antibody optimized to cross react with all four subtypes of aflatoxin was coated to a polystyrene microwell. Toxins were extracted from a ground sample with 70% methanol. The extracted sample and horse radish peroxidase (HRP)-conjugated aflatoxin (B_1) were mixed and added to the antibody-coated microwell. Aflatoxin from the extracted sample and HRP-conjugated aflatoxin (B_1) competed to bind with the antibody coated to the microwell. Microwell contents were decanted and non-specific reactants were removed by washing. An enzyme substrate (TMB) was added and

blue colour developed. The intensity of the colour was directly proportional to the amount of bound conjugate and inversely proportional to the concentration of aflatoxin in the sample or standard. Therefore, as the concentration of aflatoxin in the sample or standard increased, the intensity of the blue color decreased. An acidic stop solution was added which changed the chromagen color from blue to yellow. The microwells were measured optically by a microplate reader with an absorbance filter of 450 nm (OD_{450}). The optical densities of the samples were compared to the OD's of the kit standards and an interpretative result was determined.

The positive samples were further passed through High pressure liquid chromatography in order to determine the specific subtypes of aflatoxin contamination

3.4.2.1 Materials and equipments used

A Helica® Total Aflatoxin Assay kit, a grinder sufficient to render samples to particle size of fine instant coffee, collection container of minimum 125 ml capacity, balance, 20 g measuring capability, graduated cylinder 100 ml, methanol, 70 ml reagent grade per sample, distilled or deionized water: 30 ml per sample, filter paper, whatman ® number 1 or equivalent, filter funnel, pipettor with tips: 100 µl and 200 µl, timer, wash bottle, absorbent paper towels, microplate reader (Multiskan,Thermo Electron Corp) with 450 nm filter were used in the study.

A HPLC column used had the following specifications:

Shimadzu; Column: Novapak®, oven, CT 10ASVP, C18 4um, 3.9×150 mm, Mobile phase: Water/MeoH/ACN; 50/40/10 (V/V/V); Auto sampler SIL 20AHTand an integrator operated by shimadzu LC software, Temperature: Ambient; Fluorescence Detector: RF 20A; Injection volume: 10 µL (0.044 mg/mL.

3.4.2.2 Extraction and derivatization of Aflatoxins (B_1, B_2, G_1 and G_2) from the samples

A 20 g of homogenized sample was weighed and 80 ml acetonitrile-water solution (84:16) or 70% MeoH was added to the samples. The samples were mixed for 2 hrs with a magnetic stirrer. Extraction jar was rinsed with the extraction solution and filtered through a Buhner-funnel with a sucker. The sample was evaporated to dryness with nitrogen stream. A total of 200 µl trifluoroacetic acid (TFA) was added and the cap of the Vortex blender closed and then mixed for 1 minute and incubated for 30 min at 25^{o}C. Sample was diluted to 500 µl with acetonitrile-water solution (30:70) and mixed for 1 minute and then filtered with 0.2 um membrane filter (GHP). The samples were analyzed with High Pressure Liquid Chromatography and fluorescence detector on the same day of analysis in order to avoid deterioration.

3.4.2.3 Identification and quantification

Aflatoxins were analysed as Trifluoroacetic acid derivatives and eluted in the following order: G_1, B_1, G_2, and B_2. They were identified according to their retention times and quantified using external standard curve.

Aflatoxin Content Per Sample was calculated using the following formulae:

$$\text{µg/kg (ng/g)} = \frac{C \times Vtot \times Vf}{VIA \times m}$$

C = concentration from HPLC run (ng/mg)

Vtot = total volume of extract (5 ml)

VIA = volume of extract for IA clean up ml (15ml)

Vf= final volume for measurement ml (3 ml)

m = sample weight (5gms)

Total Aflatoxin was compared with **ELISA** results

3.4.3 Determination of Fluoride Levels

3.4.3.1 Introduction

Fluoride was determined by direct potentiometric method. This was done using fluoride electrode. The electrode has been used to determine fluoride in drinking water, industrial waste, seawater, air, food and beverages, (Jacobsen and Weinstein, 1977). It is relatively easy to use the fluoride electrode in water samples, but analysis of food, animal feeds and tissue samples require special preparation (Mwaniki and Gikunju, 1995).

3.4.3.2 Preparation of Standard Calibration curve

Fluoride standard solution (0.1, 1.0 and 10.0 ppm) were prepared by diluting the 100 ppm standard solution with deionised water. Two parallel tubes were filled with 3.0 ml standard fluoride solution and 0.3 ml buffer (TISAB 3) added to each tube before analysis. A calibration curve was prepared from these standards. The average relative millivolt value for each standard was plotted against the fluoride concentration on a 4 cycle semi-logarithmic paper.

In this method a series of fluoride standards are prepared in a background matrix of TISAB (Total Ionic Strength Adjustment Buffer). The unknown is prepared using TISAB in the hope that the matrix will be similar to the standards.

Since $E = K + S \log C$

3.4.3.4 Reagents, Equipments and Instruments

The following reagents were used for analysis: perchloric acid (Riedel-de Haen AG, Hannover, Germany), nitric acid (Riedel-de Haen AG, Hannover, Germany), acid mixture equal parts of perchloric acid and nitric acid, base mixture: sodium hydroxide and trisodium citrate in the ratio of 3:10, blank II solution was a mixture of sodium hydroxide, trisodium citrate and acid mixture in the

ratio of 15:50:10, respectively, 100 ppm sodium fluoride was used as stock solution (Orion, Massachuset, USA).

The instruments used were: Fluoride combination electrode (96-09 Orion Research Incorporated, Cambridge, Massachuset, USA), Digital pH meter (3020 Orion), Electrode filling solution (Orion, 90-00-01). pH electrode storage solution (91-00-01). Magnetic stirrer and Teflon coated bar, 5mm x 11mm polyethylene tubes (15ml), Twenty milliliter plastic cups (NDD/TL, Norsk Dental Depot, Oslo Norway), Pipette tips (1000 µl, 9604, Treff, Degershein, Switzerland). Ten milliliter plastic disposable straight pipettes, 500 µl digital transfer pipette (Transferpette, Germany), glass beakers, measuring beakers, measuring flasks and Metle AE 163, mettle instrument AG, CH-8606 Greifensee, Switzerland. Others used were electronic weighing balance (Metler AE 163 Switzerland).

3.4.3.5 Fluoride analysis in liquid samples

Preparation of Standard solution with TISAB 2

One ml of standard fluoride was added to one ml of TISAB 2. Each sample was then prepared and analysed in two parallels. Firstly without dilution then after dilution with deionised water, 1:10, 1:25,1:50,1:75,1:100,1:150 and 1:200.As for standards one ml of TISAB 2 was added to one ml of samples.

Preparation of Standard solution with TISAB 3

Three ml of standard fluoride solution was added to 0.1 ml of TISAB 3 solution. Each sample was prepared and analysed in two parallels. First without dilution then after dilution with deionised water, 1:10, 1:25,1:50,1:75,1:100 and 1:150. As for standards 0.3 ml of TISAB 3 was added to three ml volume of samples.

3.4.3.6 Fluoride analysis in solid samples

Samples were analysed using a method described by Birkeland (1970) and modified by Gikunju et al, (1992). After drying the sample at 105ºC for 24hrs, it was then ground and homogenized. 50mg of each sample was dissolved in polypropylene tube containing a mixture of 0.2ml, 11.6 M perchloric acid and 0.2 ml, 14.3 M nitric acid at 60ºC for 60 min. The mixture was then buffered to ph 5.2-5.5 with a base mixture of 7.8 M sodium hydroxide and 1.0 M trisodium citrate. The whole dissolution process took place in a closed double tube arrangement (Birkeland, 1970, Gikunju et al, 1992) in order to minimize loss of fluoride from the sample. The digested sample was then analysed as liquid samples against the background of a standard.

3.4.4 Data analysis and Presentation

The completed questionnaires were edited and coded in order to facilitate Statistical analysis. The aim was to eliminate unusable data, interpret ambiguous answers and contradictory data from related questions. A coding scheme was developed for the responses to each question. The coding scheme facilitated the development of an appropriate data structure which was entered into the computer. Data entry, storage and analysis were done using Statistical Package for Social Sciences (SPSS).

Orodho and Kombo, (2004) points out that qualitative data is not always computed with arithmetic relationship. The responses were categorized into various classes or as categorical variables. Since the purpose of this study was to describe the situation as it is, a simple descriptive analysis was done. The categorized data was arranged in order to determine how the independent and dependent variables were related. Since the study was descriptive in nature, descriptive statistics were used in the analysis. The data was presented in form of frequency tables and graphs yielded by the SPSS and Microsoft Excel.

3.4.5 Ethical Consideration

All participants consented after explaining the purpose of the study, potential risks and benefits. Interviewees were informed that they are entitled to decline or stop the interview at any given moment of the survey without any negative consequences to them. Each participant was informed that the information supplied will be kept and used confidentially.

CHAPTER FOUR

RESULTS

4.1 Microbial Contamination with Total Colliform

A total of 90 solid samples and 60 liquid samples were analyzed for total colliforms. Solid samples did not register any contamination with *Escherichia coli*, but 26.7% were contaminated by *Klebsiella pneumonia.* In contrast the liquid samples registered contamination with both *Klebsiella* and *E.coli* 13.3% and 6.67% respectively (Table 1), (Figure 2).

Table 1: Results of microbial contamination using various biochemical tests

SOLID SAMPLES

S.NO	MORPHOLOGY	I	MR	VP	C	REMARKS
1	Mucoid pink colonies	Negative	Positive	Negative	Negative	
2	Mucoid pink colonies	Negative	Positive	Negative	Negative	
3	Mucoid pink colonies	Negative	Positive	Negative	Negative	
4	Mucoid pink colonies	Negative	Positive	Negative	Negative	
5	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
6	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Negative	
7	Mucoid pink colonies	Negative	Positive	Negative	Negative	
8	Mucoid pink colonies	Negative	Positive	Negative	Negative	
9	Mucoid pink colonies	Negative	Positive	Negative	Negative	
10	Mucoid pink colonies	Negative	Positive	Negative	Negative	
11	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
12	Mucoid pink colonies	Negative	Positive	Negative	Negative	
13	Mucoid pink colonies	Negative	Positive	Negative	Negative	
14	Mucoid pink colonies	Negative	Positive	Negative	Negative	
15	Mucoid pink colonies	Negative	Positive	Negative	Negative	
16	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
17	Mucoid pink colonies	Negative	Positive	Negative	Negative	
18	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
19	Mucoid pink colonies	Negative	Positive	Negative	Negative	
20	Mucoid pink colonies	Negative	Positive	Negative	Negative	
21	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
22	Mucoid pink colonies	Negative	Positive	Negative	Negative	
23	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
24	Mucoid pink colonies	Negative	Positive	Negative	Negative	

S.NO	MORPHOLOGY	I	MR	VP	C	REMARKS
25	Mucoid pink colonies	Negative	Positive	Negative	Negative	
26	Mucoid pink colonies	Negative	Positive	Negative	Negative	
27	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
28	Mucoid pink colonies	Negative	Positive	Negative	Negative	
29	Mucoid pink colonies	Negative	Positive	Negative	Negative	
30	Mucoid pink colonies	Negative	Positive	Negative	Negative	
31	Mucoid pink colonies	Negative	Positive	Negative	Negative	
32	Mucoid pink colonies	Negative	Positive	Negative	Negative	
33	Mucoid pink colonies	Negative	Positive	Negative	Negative	
34	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Negative	
35	Lactose fermentes flat serratted colonies	Negative	Positive	Negative	Negative	
36	Mucoid pink colonies	Negative	Positive	Negative	Negative	
37	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Negative	
38	Mucoid pink colonies	Negative	Positive	Negative	Negative	
39	Mucoid pink colonies	Negative	Positive	Negative	Negative	
40	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Positive	Klebsiella
41	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Negative	
42	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Negative	
43	Mucoid pink colonies	Negative	Positive	Negative	Negative	
44	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
45	Mucoid pink colonies	Negative	Positive	Negative	Negative	
46	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
47	Mucoid pink colonies	Negative	Positive	Negative	Negative	
48	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
49	Mucoid pink colonies	Negative	Positive	Negative	Negative	
50	Mucoid pink colonies	Negative	Positive	Negative	Negative	
51	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
52	Mucoid pink colonies	Negative	Positive	Negative	Negative	
53	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
54	Mucoid pink colonies	Negative	Positive	Negative	Negative	
55	Mucoid pink colonies	Negative	Positive	Negative	Negative	
56	Mucoid pink colonies	Negative	Positive	Negative	Negative	
57	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
58	Mucoid pink colonies	Negative	Positive	Negative	Negative	
59	Mucoid pink colonies	Negative	Positive	Negative	Negative	

S.NO	MORPHOLOGY	I	MR	VP	C	REMARKS
60	Mucoid pink colonies	Negative	Positive	Negative	Negative	
61	Mucoid pink colonies	Negative	Positive	Negative	Negative	
62	Mucoid pink colonies	Negative	Positive	Negative	Negative	
63	Mucoid pink colonies	Negative	Positive	Negative	Negative	
64	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Negative	
65	Lactose fermentes flat serratted colonies	Negative	Positive	Negative	Negative	
66	Mucoid pink colonies	Negative	Positive	Negative	Negative	
67	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Negative	
68	Mucoid pink colonies	Negative	Positive	Negative	Negative	
69	Mucoid pink colonies	Negative	Positive	Negative	Negative	
70	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Positive	Klebsiella
71	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Negative	
72	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Negative	
73	Mucoid pink colonies	Negative	Positive	Negative	Negative	
74	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
75	Mucoid pink colonies	Negative	Positive	Negative	Negative	
76	Mucoid pink colonies	Negative	Positive	Negative	Negative	
77	Mucoid pink colonies	Negative	Positive	Negative	Negative	
78	Mucoid pink colonies	Negative	Positive	Negative	Negative	
79	Mucoid pink colonies	Negative	Positive	Negative	Negative	
80	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
81	Lactose fermentes raised smooth colonies	Negative	Positive	Negative	Negative	
82	Mucoid pink colonies	Negative	Positive	Negative	Negative	
83	Mucoid pink colonies	Negative	Positive	Negative	Negative	
84	Mucoid pink colonies	Negative	Positive	Negative	Negative	
85	Mucoid pink colonies	Negative	Positive	Negative	Negative	
86	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
87	Mucoid pink colonies	Negative	Positive	Negative	Negative	
88	Mucoid pink colonies	Negative	Positive	Negative	Negative	
89	Mucoid pink colonies	Negative	Positive	Negative	Negative	
90	Mucoid pink colonies	Negative	Positive	Negative	Negative	

LIQUID SAMPLES

S.NO	MORPHOLOGY	I	MR	VP	C	REMARKS
1	Mucoid pink colonies	Negative	Positive	Negative	Negative	
2	Mucoid pink colonies	Negative	Positive	Negative	Negative	
3	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
4	Mucoid pink colonies	Negative	Positive	Negative	Negative	
5	Mucoid pink colonies	Negative	Positive	Negative	Negative	
6	Mucoid pink colonies	Negative	Positive	Negative	Negative	
7	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
8	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli
9	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli
10	Lactose fermentes flat smooth colonies	Negative	Positive	Negative	Negative	
11	Mucoid pink colonies	Negative	Positive	Negative	Negative	
12	Mucoid pink colonies	Negative	Positive	Negative	Negative	
13	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
14	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli
15	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli
16	Lactose fermentes flat smooth colonies	Negative	Positive	Negative	Negative	
17	Mucoid pink colonies	Negative	Positive	Negative	Negative	
18	Mucoid pink colonies	Negative	Positive	Negative	Negative	
19	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
20	Mucoid pink colonies	Negative	Positive	Negative	Negative	
21	Mucoid pink colonies	Negative	Positive	Negative	Negative	
22	Mucoid pink colonies	Negative	Positive	Negative	Negative	
23	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
24	Mucoid pink colonies	Negative	Positive	Negative	Negative	
25	Mucoid pink colonies	Negative	Positive	Negative	Negative	
26	Mucoid pink colonies	Negative	Positive	Negative	Negative	
27	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
28	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli
29	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli
30	Lactose fermentes flat smooth colonies	Negative	Positive	Negative	Negative	
31	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli

S.NO	MORPHOLOGY	I	MR	VP	C	REMARKS
32	Lactose fermentes flat smooth colonies	Negative	Positive	Negative	Negative	
33	Mucoid pink colonies	Negative	Positive	Negative	Negative	
34	Mucoid pink colonies	Negative	Positive	Negative	Negative	
35	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
36	Mucoid pink colonies	Negative	Positive	Negative	Negative	
37	Mucoid pink colonies	Negative	Positive	Negative	Negative	
38	Mucoid pink colonies	Negative	Positive	Negative	Negative	
39	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
40	Mucoid pink colonies	Negative	Positive	Negative	Negative	
41	Mucoid pink colonies	Negative	Positive	Negative	Negative	
42	Mucoid pink colonies	Negative	Positive	Negative	Negative	
43	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
44	Mucoid pink colonies	Negative	Positive	Negative	Negative	
45	Mucoid pink colonies	Negative	Positive	Negative	Negative	
46	Mucoid pink colonies	Negative	Positive	Negative	Negative	
47	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
48	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli
49	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli
50	Lactose fermentes flat smooth colonies	Negative	Positive	Negative	Negative	
51	Mucoid pink colonies	Negative	Positive	Negative	Negative	
52	Mucoid pink colonies	Negative	Positive	Negative	Negative	
53	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
54	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli
55	Lactose fermentes flat smooth colonies	Positive	Positive	Negative	Negative	E.coli
56	Lactose fermentes flat smooth colonies	Negative	Positive	Negative	Negative	
57	Mucoid pink colonies	Negative	Positive	Negative	Negative	
58	Mucoid pink colonies	Negative	Positive	Negative	Negative	
59	Mucoid pink colonies	Negative	Positive	Negative	Positive	Klebsiella
60	Mucoid pink colonies	Negative	Positive	Negative	Negative	

Key: I- Indole Test MR- Methyl Red Test VP- Voges-Proskauer C- Citrate

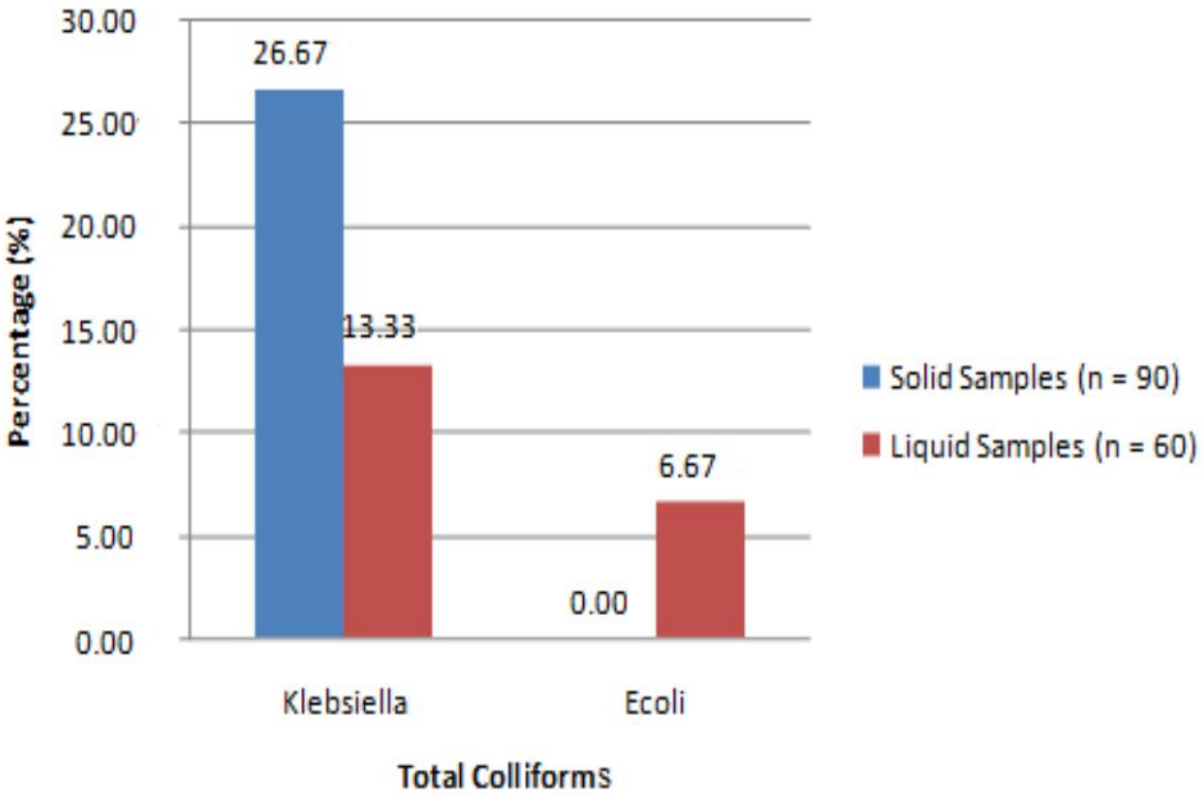

Figure 2: Contamination by Total Colliforms

4.2 Contamination with *Aflatoxin* B_1, B_2, G_1 and G_2

Liquid samples of the herbal extracts did not register contamination with *aflatoxins*. On the other hand, 24.0% of the solid samples were contaminated by *aflatoxins*. The contamination of the samples with the different sub-types of *aflatoxin* (B_1, B_2, G_1 and G_2) was not normally distributed (skewness and Kurtosis). This implied that some samples were more contaminated than others which depended on a variety of factors ranging from source of the extract, period before preparation, preparation method and storage conditions. These factors were further explored in this study.

Of the solid samples the following were found to have aflatoxin of various subtypes which are shown in the table (Table 2).

Table2: Concentration of Aflatoxins in various samples

	AFLATOXINS CONC IN ng/ml				
SAMPLES WITH AFLATOXIN	B_1	B_2	G_1	G_2	TOTAL
S2	0.0252	0	0.1928	0	0.218
S3	0.2084	0	0.4507	0	0.6591
S4	0.0574	0		0	0.0574
S5	0.0574	0	1.0852	0	1.1426
S6	0.0574	0		0	0.0574
S7	0.0574	0	0	0	0.0574
S8	0.0574	0	0	0	0.0574
S11	0.0574	0	0	0	0.0574
S13	0.0574	0	0.154	0	0.2114
S19	0.0382	0	0	0	0.0382
S22	0	0	1.4339	0	1.4339
S23	0.0348	0.2299	0	0.1472	0.4119
S28	0.0623	0	0	0	0.0623
S30	0.0363	0.0893	0.0531	0	0.1787
S31	0.0332	0	0.0574	0	0.0906
S32	0.0548	0	0.0574	0	0.1122
S33	0.2071	0	0.0574	0	0.2645
S34	0	0	0.0574	0	0.0574
S35	0.0382	0	0.0574	0	0.0956
S36	1.4339	0	0.0574	0	1.4913
S37	0.4119	0	0.0574	0	0.4693

4.3 Contamination of Extract with Fluoride

Fluoride was determined by direct potentiometric method. In this method a series of fluoride standards were prepared in a background matrix of TISAB (Total Ionic Strength Adjustment Buffer). The unknown was prepared using TISAB in the hope that the matrix would be similar to the standards.

The measured potential (E) can be represented by:

$E = K + S \log C$, where; K is a constant, S is the slope of the calibration curve that is equal to $\beta(0.05916)$ and C is the analyte ion (F^-) concentration.

A plot of E vs. log C yielded a working curve that was used to measure the unknown. The first thing we learnt from this plot is that our electrode was working properly, and had the expected linear response of E vs. Log C with slope measured at -59.4 mV, very near to the theoretical -59.2 mV at 25^{o} C. A Linear Least Squares analysis of this plot allowed us to measure the concentration of our unknown directly, and to compute the error of this determination. The results were tabulated based on relative milliequivalents (Table 3). In Figure 3, the unknown was found to be 2.28 $^{+}$. 0.05 mg/L.

Table 3: Fluoride electrode readings and calculated concentrations

FLUORIDE CONCENTRATION IN SOLID SAMPLES

STD CONC IN PPM	A(Rel mV)	pH	B(Rel mV)	pH	Mean	Conc mg/l
0.1	80.8		82.9		81.85	1.55515
1	74.6		71.6		73.1	1.3889
10	17.9		17.2		17.55	0.33345
100	-41.8		-44.4		-43.1	-0.8189
SAMPLES						
S1	92	5.01	91.5	5.1	91.75	1.74325
S2	53.3	5.44	51.1	5.44	52.2	0.9918
S3	48.7	5.7	54.2	5.72	51.45	0.97755
S4	58.3	5.75	51.6	5.75	54.95	1.04405
S5	56.3	5.65	54.5	5.65	55.4	1.0526
S6	53.9	5.3	54.1	5.3	54	1.026
S7	53.9	5.35	51.7	5.38	52.8	1.0032
S8	54.1	5.55	43.9	5.57	49	0.931
S9	53.4	5.59	53.4	5.6	53.4	1.0146
S10	51.5	5.74	52.1	5.7	51.8	0.9842
S11	55	5.8	55.9	5.8	55.45	1.05355
S12	52.9	5.35	53.5	5.36	53.2	1.0108
S13	52.2	5.37	53.8	5.37	53	1.007
S14	54.5	5.45	53.8	5.43	54.15	1.02885
S15	53.8	5.4	53.7	5.4	53.75	1.02125
S16	53	5.37	53.5	5.32	53.25	1.01175
S17	54.7	5.81	52.9	5.83	53.8	1.0222
S18	54.1	5.38	49.2	5.35	51.65	0.98135

SAMPLE	A(Rel mV)	pH	B(Rel mV)	pH	Mean	Conc mg/l
S19	50.4	5.65	50.1	5.65	50.25	0.95475
S20	51.9	5.39	48.3	5.38	50.1	0.9519
S21	51.2	5.38	52	5.35	51.6	0.9804
S22	51.4	5.75	50.2	5.73	50.8	0.9652
S23	49.2	5.25	50.1	5.25	49.65	0.94335
S24	49.1	5.45	48.8	5.42	48.95	0.93005
S25	52.5	5.4	49.3	5.4	50.9	0.9671
S26	45.4	5.46	46.5	4.42	45.95	0.87305
S27	47.3	5.5	48.6	5.5	47.95	0.91105
S28	50.4	5.65	46.5	5.63	48.45	0.92055
S29	47.4	5.43	48	5.39	47.7	0.9063
S30	53.6	5.83	57.7	5.75	55.65	1.05735
S31	33	5.35	32.2	5.65	32.6	0.6194
S32	4.9	5.65	2.6	5.3	3.75	0.07125
S33	76.6	5.38	88.3	5.35	82.45	1.56655
S34	52.9	5.35	53.5	5.36	53.2	1.0108
S35	52.2	5.37	53.8	5.37	53	1.007
S36	60.9	5.73	62.1	5.35	61.5	1.1685
S37	51.8	5.25	49.7	5.37	50.75	0.96425
S38	59.2	5.42	61.8	5.45	60.5	1.1495
S39	62.2	5.4	61.6	5.4	61.9	1.1761
S40	62.3	4.42	62.2	5.37	62.25	1.18275
S41	86.2	5.5	82	5.81	84.1	1.5979
S42	91.5	5.1	53.6	5.83	67.6	1.2844
S43	51.1	5.44	33	5.35	42.05	0.79895
S44	54.2	5.72	4.9	5.65	29.55	0.56145
S45	51.6	5.75	76.6	5.38	64.1	1.2179
S46	54.5	5.65	52.9	5.35	53.7	1.0203
S47	54.1	5.3	52.2	5.37	53.15	1.00985
S48	51.7	5.38	60.9	5.73	56.3	1.0697
S49	43.9	5.57	51.8	5.25	47.85	0.90915
S50	53.4	5.6	59.2	5.42	56.3	1.0697
S51	52.1	5.7	62.2	5.4	57.15	1.08585
S52	55.9	5.8	62.3	4.42	59.1	1.1229
S53	53.5	5.36	86.2	5.5	69.85	1.32715
S54	53.8	5.37	52.2	5.37	53	1.007
S55	53.8	5.43	54.5	5.45	54.15	1.02885
S56	53.7	5.4	53.8	5.4	53.75	1.02125
S57	53.5	5.32	53	5.37	53.25	1.01175
S58	52.9	5.83	54.7	5.81	53.8	1.0222

SAMPLE	A(Rel mV)	pH	B(Rel mV)	pH	Mean	Conc mg/l
S59	49.2	5.35	54.1	5.38	51.65	0.98135
S60	50.1	5.65	50.4	5.65	50.25	0.95475
S61	48.3	5.38	51.9	5.39	50.1	0.9519
S62	52	5.35	51.2	5.38	51.6	0.9804
S63	50.2	5.73	51.4	5.75	50.8	0.9652
S64	50.1	5.25	49.2	5.25	49.65	0.94335
S65	48.8	5.42	49.1	5.45	48.95	0.93005
S66	49.3	5.4	52.5	5.4	50.9	0.9671
S67	46.5	4.42	45.4	5.46	45.95	0.87305
S68	48.6	5.5	47.3	5.5	47.95	0.91105
S69	46.5	5.63	50.4	5.65	48.45	0.92055
S70	48	5.39	47.4	5.43	47.7	0.9063
S71	57.7	5.75	53.6	5.83	55.65	1.05735
S72	32.2	5.65	33	5.35	32.6	0.6194
S73	2.6	5.3	4.9	5.65	3.75	0.07125
S74	88.3	5.35	76.6	5.38	82.45	1.56655
S75	53.5	5.36	52.9	5.35	53.2	1.0108
S76	53.8	5.37	92	5.01	72.9	1.3851
S77	62.1	5.35	53.3	5.44	57.7	1.0963
S78	49.7	5.37	48.7	5.7	49.2	0.9348
S79	61.8	5.45	58.3	5.75	60.05	1.14095
S80	61.6	5.4	56.3	5.65	58.95	1.12005
S81	62.2	5.37	53.9	5.3	58.05	1.10295
S82	82	5.81	53.9	5.35	67.95	1.29105
S83	51.1	5.44	54.1	5.55	52.6	0.9994
S84	54.2	5.72	53.4	5.59	53.8	1.0222
S85	51.6	5.75	51.5	5.74	51.55	0.97945
S86	54.5	5.65	55	5.8	54.75	1.04025
S87	54.1	5.3	52.9	5.35	53.5	1.0165
S88	51.7	5.38	57.7	5.75	54.7	1.0393
S89	43.9	5.57	32.2	5.65	38.05	0.72295
S90	53.4	5.6	2.6	5.3	28	0.532

FLUORIDE CONCENTRATION IN LIQUIDS SAMPLES

STD CONC IN PPM	A(Rel mV)	pH	B(Rel mV)	pH	Mean	Conc mg/l
0.05	83.4		81.1		82.25	1.56275
0.1	78.6		77.2		77.9	1.4801
1	44.2		42.8		43.5	0.8265
10	-13.9		-15.5		-14.7	-0.2793
100	-74.7		-74.5		-74.6	-1.4174
SAMPLES						
L1	53.6	5.83	57.7	5.75	55.65	1.05735
L2	33	5.35	32.2	5.65	32.6	0.6194
L3	4.9	5.65	2.6	5.3	3.75	0.07125
L4	76.6	5.38	88.3	5.35	82.45	1.56655
L5	52.9	5.35	53.5	5.36	53.2	1.0108
L6	52.2	5.37	53.8	5.37	53	1.007
L7	60.9	5.73	62.1	5.35	61.5	1.1685
L8	51.8	5.25	49.7	5.37	50.75	0.96425
L9	59.2	5.42	61.8	5.45	60.5	1.1495
L10	62.2	5.4	61.6	5.4	61.9	1.1761
L11	62.3	4.42	62.2	5.37	62.25	1.18275
L12	86.2	5.5	82	5.81	84.1	1.5979
L13	52.2	5.37	53.8	5.37	53	1.007
L14	54.5	5.45	53.8	5.43	54.15	1.02885
L15	53.8	5.4	53.7	5.4	53.75	1.02125
L16	53	5.37	53.5	5.32	53.25	1.01175
L17	54.7	5.81	52.9	5.83	53.8	1.0222
L18	54.1	5.38	49.2	5.35	51.65	0.98135
L19	50.4	5.65	50.1	5.65	50.25	0.95475
L20	51.9	5.39	48.3	5.38	50.1	0.9519
L21	51.2	5.38	52	5.35	51.6	0.9804
L22	51.4	5.75	50.2	5.73	50.8	0.9652
L23	49.2	5.25	50.1	5.25	49.65	0.94335
L24	49.1	5.45	48.8	5.42	48.95	0.93005
L25	52.5	5.4	49.3	5.4	50.9	0.9671
L26	45.4	5.46	46.5	4.42	45.95	0.87305
L27	47.3	5.5	48.6	5.5	47.95	0.91105
L28	50.4	5.65	46.5	5.63	48.45	0.92055
L29	47.4	5.43	48	5.39	47.7	0.9063
L30	53.6	5.83	57.7	5.75	55.65	1.05735
L31	33	5.35	32.2	5.65	32.6	0.6194
L32	4.9	5.65	2.6	5.3	3.75	0.07125

SAMPLE	A(Rel mV)	pH	B(Rel mV)	pH	Mean	Conc mg/l
L33	76.6	5.38	88.3	5.35	82.45	1.56655
L34	52.9	5.35	53.5	5.36	53.2	1.0108
L35	92	5.01	91.5	5.1	91.75	1.74325
L36	53.3	5.44	51.1	5.44	52.2	0.9918
L37	48.7	5.7	54.2	5.72	51.45	0.97755
L38	58.3	5.75	51.6	5.75	54.95	1.04405
L39	56.3	5.65	54.5	5.65	55.4	1.0526
L40	53.9	5.3	54.1	5.3	54	1.026
L41	53.9	5.35	51.7	5.38	52.8	1.0032
L42	54.1	5.55	43.9	5.57	49	0.931
L43	53.4	5.59	53.4	5.6	53.4	1.0146
L44	51.5	5.74	52.1	5.7	51.8	0.9842
L45	55	5.8	55.9	5.8	55.45	1.05355
L46	52.9	5.35	53.5	5.36	53.2	1.0108
L47	92	5.01	53.5	5.36	72.75	1.38225
L48	53.3	5.44	53.8	5.37	53.55	1.01745
L49	48.7	5.7	62.1	5.35	55.4	1.0526
L50	58.3	5.75	49.7	5.37	54	1.026
L51	56.3	5.65	61.8	5.45	59.05	1.12195
L52	53.9	5.3	61.6	5.4	57.75	1.09725
L53	53.9	5.35	62.2	5.37	58.05	1.10295
L54	54.1	5.55	82	5.81	68.05	1.29295
L55	53.4	5.59	53.6	5.83	53.5	1.0165
L56	51.5	5.74	33	5.35	42.25	0.80275
L57	55	5.8	4.9	5.65	29.95	0.56905
L58	52.9	5.35	76.6	5.38	64.75	1.23025
L59	52.2	5.37	52.9	5.35	52.55	0.99845
L60	49.2	5.35	52.2	5.37	50.7	0.9633
WATER D	18.7		18		18.35	0.34865
WATER S	13.2		9		11.1	0.2109

Table 4: Mean mV Values for Liquid Herbal and Solid Herbal Extracts

Type of Sample	First Run		Second Run		
	Relative mV (A)	PH	B	PH	Mean
Solid	96.2	5.01	98.6	5.10	97.4
Liquid	95.1	5.44	97.7	5.44	96.4

Table 5: Determination of Fluoride Concentration for the Solid and Liquid Samples from the Standard Fluoride Curve

Concentration (mg/L) from Standard Fluoride Curve	Log C (mg/L)	mV	
	x	y	
200.000	2.301	-35.6	
100.000	2.000	-17.8	
50.000	1.699	0.4	
25.000	1.369	16.8	
12.500	1.097	34.9	
6.250	0.796	52.8	
3.125	0.495	70.7	
1.563	0.194	89.3	
0.781	-0.107	107.1	
0.391	-0.408	125.5	
0.195	-0.709	142.9	
known Standard	1.390	79.4	
Unknown (Solid Samples) [1.629]	0.212	97.4 (mean value)	
Unknown (Liquid Samples) [1.618]	0.209	96.4 (Mean Value)	
Linear Least Squares (LLS)Analysis	**LLS**	**Value**	**LLS Error Analysis**
	slope	-54.4	
	intercept	100.6	
	Log C (calc)	0.3576	+ - 0.010
	C (mg/L)	2.28	+ -0.054
	Relative Error %		-2.36

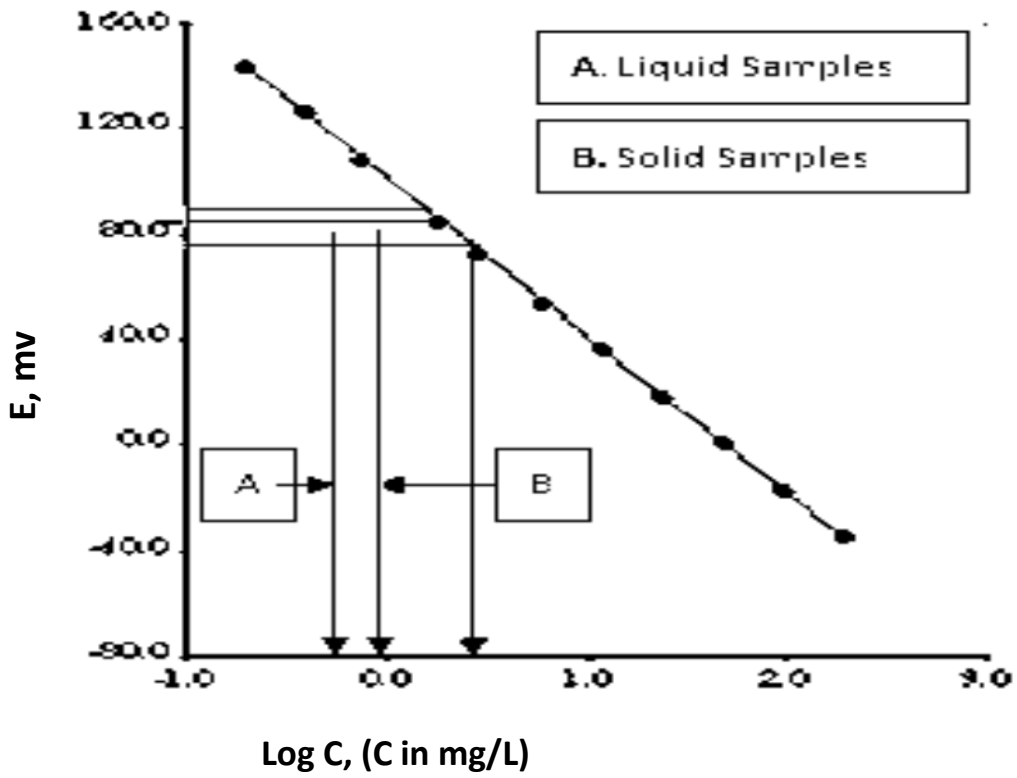

Figure 3: A plot of potential (E) against logarithm of concentration (Log C)

Generally the solid and liquid samples had fluoride concentration within acceptable limits 1.629 mg/L and 1.618 mg/L respectively. This was expected given that the herbal medicine practitioners were surveyed from the same geographical are of Nairobi County and were most likely to share the same water source in preparing the extracts. The permitted value of fluoride in drinking water is 1.5 mg/L. Generally for the samples which were assessed fluoride contamination is not an issue of public concern.

4.4 Results relative to the demographics

The study showed that majority of those practicing herbalism were males (80%) compared to females (20%). Majority of the herbalists were of the age group of 30 to 40 years. Most herbalists (44%) had at least 10 – 20 years of practice while 38% of these had less than 10 years practice on herbalism. Majority of practitioners were married 62%, 31% were single and 7% were widowed. A total 20% of

the surveyed herbal practitioners had received formal education while the rest (80%) indicated that they had never been in school (Table 6). A total 30% of those who were educated reported that they were trained herbal professionals and 33% had post-secondary education.

Table 6: Demographic Characteristics of the Respondents

	Disaggregates	**n**	**Percentage**
Demographic Characteristics			
Gender (n=50)	Males	40	80.00
	Females	10	20.00
Age (n=50)	Below 30 Years	14	28.00
	30 to 40 Years	23	46.00
	40 to 50 Years	10	20.00
	Above 50 Years	3	6.00
Number of Years Practicing as a Herbalist (n=50)	Below 5 Years	12	24.00
	5 to 10 Years	7	14.00
	10 to 20 Years	22	44.00
	Above 20 Years	9	8.00
Trained Professional (n=50)	Yes	10	20.00
	No	40	80.00
Highest Level of Training (n=10)	Diploma	3	30.00
	Degree	1	10.00
	Not Applicable	6	60.00
Other Income (n=50)	Yes	10	20.00
	No	40	80.00
Geographic Location of Clients (n=50)	Urban	33	66.00
	Rural	17	37.00

The herbal extracts are derived from a variety of sources, the predominant one being plant extracts such as; leaves, stems, barks and roots.

Formal education is not significantly associated with herbal extract contamination ($p > 0.05$). However, professional training (especially diploma and graduate) seems to have the effect of marginal reduction of the contamination levels for *Klebsiella* and *E. coli*. Professional training on herbal/phytomedicine is likely to have an impact in reduction of *Kelebsiella* and *Aflatoxins* contamination.

Table 8: Correlation between Professional Training and Herbal Extract Contamination

			Klebsiella		*E. Coli*		*Aflatoxins*	
			No	Yes	No	Yes	No	Yes
Formal Training	No	Count	81	24	101	4	42	21
		Percentage within Professional Training	77.1	22.9	96.2	3.8	40.0	20.0
	Yes	Count	37	8	45	0	12	15
		Percentage within Professional Training	82.2	17.8	100.0	0.0	26.7	33.3
Professional Training (Diploma, Degree)	No	Count	76	24	96	4	39	21
		Percentage within Highest Training	76.0	24.0	96.0	4.0	39.0	21.0
	Yes	Count	42	8	50	0	15	15
		Percentage within Highest Training	84.0	16.0	100.0	0.0	30.0	30.0

The herbal practitioners who used tap water had 23.7% of their herbal extracts contaminated with *Klebsiella*, 3.0% with *E. coli* and 22.2% with *aflatoxins*. For the practitioners, who reported using river water, 40% of the extracts were contaminated by *Klebsiella*, almost twice the contamination levels observed with tap water. This implied that using safe water had a huge potential to reduce contamination by aflatoxins. Except for aflatoxins contamination, herbal extracts which were prepared by boiling and were less likely to be contaminated by coliforms. Herbal extracts prepared by distillation registered no contamination. Grinding was a common initial preparation method for solid and liquid herbal raw materials. It is also a likely source of *Klebsiella* contamination as these bacteria are present in soil (Table 8).

Table 9: Factors contributing to Contamination of the Herbal Extract

			Klebsiella		*E. Coli*		*Aflatoxin*	
			No	Yes	No	Yes	No	Yes
Source of Water	River	Count	15	0	15	0	3	6
		Percentage within Source of Water	100.0	0.0	100.0	0.0	20.0	40.0
	Tap Water	Count	103	32	131	4	51	30
		Percentage within Source of Water	76.3%	23.7	97.0	3.0	37.8	22.2
Method of Herbal Extract Preparation								
Boiling	No	Count	76	24	96	4	42	18
		Percentage within Boiling	76.0	24.0	96.0	4.0	42.0	18.0
	Yes	Count	42	8	50	0	12	18
		Percentage within Boiling	84.0	16.0	100.0	0.0	24.0	36.0
Distillation	No	Count	113	32	141	4	51	36
		Percentage within Distillation	77.9	22.1	97.2	2.8	35.2	24.8
	Yes	Count	5	0	5	0	3	0
		Percentage within Distillation	100.0	0.0	100.0	0.0	60.0	0.0
Grinding	No	Count	42	8	50	0	12	18
		Percentage within Grinding	84.0	16.0	100.0	0.0	24.0	36.0
	Yes	Count	76	24	96	4	42	18
		Percentage within Grinding	76.0	24.0	96.0	4.0	42.0	18.0
Storage and Safety								
Storage time before preparation	Less than 3 days	Count	70	15	81	4	24	27
		Percentage within Storage time before Preparation	82.4	17.6	95.3	4.7	28.2	31.8
	More than 3 Days	Count	48	17	65	0	30	9
		Percentage within Storage time before Preparation	73.8	26.2	100.0	0.0	46.2	13.8
Storage Conditions	Refrigerator	Count	2	3	5	0		
		Percentage within Storage Conditions	40.0	60.0	100.0	0.0		
	Room Temperature	Count	111	29	136	4		
		Percentage within Storage Conditions	79.3	20.7	97.1	2.9		

Storage time although its association with contamination is not statistically significant, it has a bearing on the safety of the final herbal preparations.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The study shows that herbal products found in Nairobi County generally had some levels of contamination. Bacteria contamination with *klebsiella* and *Escherichia coli* were found in a few samples. This is an indication that there is a need for surveillance and regulation in the sector.

5.1.1 Contamination of Herbal Extracts by Colliforms

Safety is a fundamental principle in the provision of herbal medicines and herbal products for health care, and a critical component of quality control. This study has shown that out of a batch of herbal preparation there is a proportion that is contaminated by Coliforms and aflatoxins. *Klebsiella spp*. are natural inhabitants of many water environments, (Podschun. et.al, 2001) and they may multiply to high numbers in water which is rich in nutrients. The herbal preparation can provide these nutrients which can support large numbers of bacteria loads. The findings also suggest that some contamination from tap water that is considered safe for drinking. The contamination of such water can be at the point of use or at the source. *Escherichia coli* occurs in high numbers in human and animal feces, sewage and water subject to recent faecal pollution. An excellent case study undertaken by (Elizabeth Wambui Kimani-Murage and Augustine Ngindu 2007) provides an explanation for the severe contamination of drinking water in Kenya. The study shows that the severe bacteria contamination of water was largely due to the close distance between pit latrines and wells.Water temperatures and nutrient conditions present in drinking-water distribution systems are highly unlikely to support the growth of these organisms.

5.1.2 Contamination of Herbal Extracts by Aflatoxins

Aflatoxins were found in 24% of samples and is an indication of significant contamination of the products. Due to the nature of handling the raw materials and finished products contamination by Mycotoxins that are fungal is bound to happen as Fungi are a large group of diverse eukaryotic organisms which include yeasts and moulds. Moulds (filamentous fungi) are widely distributed in nature. Due to their versatile nutritional requirements, they are common contaminants and under favourable conditions of humidity and temperature, propagate on different commodities and beverages and produce mycotoxins (Brera et al., 1998).

Aflatoxins are naturally occurring mycotoxins which are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, species of fungi. *Aflatoxins* are toxic and are among the most carcinogenic substances known. After entering the body, *aflatoxins* are metabolized in the liver in to a reactive epoxide intermediate or hydroxylated to the less harmful metabolites (Moss. and Neal, 1985)

The European Pharmacopeia has set limits for aflatoxin B_1 and total aflatoxins at 2 and 4 μg/kg respectively, for some medicinal herbs (Pharmacopeia, 2007). Although in one study in South Africa, no aflatoxin contamination was found in some medicinal plants (Sewram, Shephard, van der Merwe, & Jacobs, 2006), while others reported levels ranging from 2.90–32.18 μg/kg (Yang, Chen, & Zhang, 2005). Roy et al. (1988) reported both high incidence (>93%) and high levels ranging from 90–1200 μg/kg in some common drug plants. Piper nigrum reported a concentration of 1200 μg/kg which was the highest contamination level in the study. The second highest reported value was in the seeds of Mucuna prurita at a level of 1160 μg/kg. The third highest value was 1130 μg/kg, which was found in the roots of Plumbago zeylanica (Roy, Sinha, & Chourasia, 1988). Aflatoxins were only found in 1 out of 5 Aerra lanata medicinal plant samples from Sri Lanka at 500 μg/kg (Abeywic krama & Bean, 1991). In another survey in India, 60% samples of medicinal plant seeds were contaminated with B_1,

ranging from 20 to 1180 µg/kg (Trucksess & Scott, 2008). In Thailand, five out of 28 herbal medicinal products were found to be contaminated with aflatoxins at 1.7–14.3 µg/kg using an immunoaffinity column (IAC) and high performance liquid chromatography (HPLC) method (Tassaneeyakul et al. 2004). None of the samples contained aflatoxins at levels above 20 ng/g(Tassaneeyakul, Razzazi-Fazeli, Porasuphatana, & Bohm, 2004). In Malaysia and Indonesia, 16 of the 23 commercial traditional herbal medicines, jamu and makjun, analyzed using IAC/HPLC method contained a low level of total aflatoxins 0.36 µg/kg.

5.1.3. Presence of Fluoride

Fluoride is extensively found throughout the environment and its levels in the herbal products were below the recommended concentrations (WHO, 2004). Both the solid and liquid samples had an average fluoride concentrations of about 1.629 mg/L and 1.618 mg/L respectively. This is expected given that the herbal medicine practitioners were surveyed from Nairobi County and most likely they shared the same water source (tap water) in preparing the extracts. The permitted value of fluoride in drinking water is 1.5mg/L (WHO, 2004) thus for the samples assessed fluoride contamination was not an issue of great concern.

It was noted that solid plant samples also contained some levels of fluoride. This is in agreement with studies done previously. Appreciable amounts of fluoride (Leone et al., 1956) are generally found in plants and the actual amounts depends on plant species, part of the plant and age of the plant, mature plants generally containing higher amounts than young plants. Sanni (1982) reported that the commonly eaten Nigerian vegetables have fluoride ion contents of between 20 and 91 mg kg- 1. The most commonly eaten vegetable in Kenyan urban areas is 'kale' (Brassica spp.) and has been shown to have fluoride concentration ranging from 7 to 55 mg kg - 1. Cow peas (Vigna sinensis) are the second most widely used vegetables in Kenya and the fluoride concentration ranged from 12 to 115 mg kg-1. Cow peas (Vigna sinensis) form part of the most regular meals in rural areas of Kenya. The

ingestion of fluoride from cow peas is therefore considerable. Pumpkin leaves (Curcarbita maxima) are widely eaten vegetable in the Central parts of Kenya and, to lesser extent, in other parts of Kenya. The pumpkin leave samples taken from Murang'a and Meru Counties show that fluoride content of the leaf blades varies from 21 to 50 mg kg-.

5.2 Conclusion

The risk of contamination of herbal preparation is real and prevalent in the Kenyan Market. Although the levels of *aflatoxins* reported in this study are within the acceptable range, and because *aflatoxin* especially *aflatoxin* B_1, are potent carcinogens there is an interest in the effects of long-term exposure of the low levels of these important mycotoxins on humans. Safety of herbal medicines is therefore an important public health concern. If action is not taken, the levels of contamination are likely to increase adverse effects to the public. The following actions are suggested in order to mitigate contamination of herbal medicines.

1. Legal and Institutional frameworks

Aflatoxins are considered unavoidable contaminants of food and feed, even where good manufacturing practices have been followed. This is also true for herbal preparation. While it may not be possible to eliminate contamination by *aflatoxins*, it is important to put in place legislations and specific guidelines on acceptable levels of *aflatoxins* in herbal preparations which are sold to the people. The action level for human food is 20 ppb of total *aflatoxins*, with the exception of milk which has an action level of 0.5 ppb for *aflatoxin* M_1. These levels can be starting points in setting the acceptable levels of *aflatoxins* in herbal medicines. To function properly, the Kenyan Drugs and Pharmaceutical Board's National Safety Monitoring Programme for herbal medicines should operate alongside an effective national drug regulatory system with a will and a potential to react to concerns emanating from reports of adverse effects of herbal medicines and to take proper regulatory

measures. These are proxy indicators of use of potentially contaminated herbal products. It is also necessary through parliament to institute laws that will govern the sector under the general public health system. This would go a long way in standardizing the herbal medicines in strict hygiene and superior quality like the conventional medicines.

2. Quality Assurance and Control

The herbal preparations should also be subject to the regulations of the Kenya Bureau of Standards certification of quality because this will allow removal of non-compliant lots from the market. These measures are vital for ensuring safety and efficacy of herbal medicines. If regulatory and quality control measures are weak, the practitioners have space to continue to sell potent substances and/or contamination with potential hazardous substances. As with other medicines for human use, herbal medicines should be covered by a drug regulatory framework in order to ensure that they conform to required standards of safety, quality and efficacy.

3. Training of Herbal Practitioners

Most herbal practitioners have little training on drugs and understanding aspects of safety of the products. Increasing awareness on safety issues can lead to a change in the way the practitioners prepare the products. Information on safety and the adverse effects of the products can influence sales to the consumers. In addition, any available legislation and guidelines can be highlighted so that the practitioners can understand the implications of non-compliance.

4. Educating the Community

Among the consumers, there is a widespread misconception that “natural” always means “safe”, and a common belief that remedies from natural origin are harmless and carry no risk. This has led to an upsurge of “herbal” products which have permeated commerce in recent years. However, some

medicinal plants are inherently toxic. Furthermore, as with a number of medicines, herbal medicines are expected to have side effects, which are harmful to consumers. Some adverse events reported in association with herbal products are attributable to problems of quality like the ones highlighted by the findings of this research study.

5.3 Recommendations

The following recommendations were made from the study:

1. Training Herbalist on Safe Preparation and Storage

Training herbalist on safe preparation and storage of the herbal medicine has a potential to reduce the levels of contamination and improve safety of the medicines. Safety of herbal medicines can be improved by cleaning the plant parts properly with safe water before the grinding stage and proper storage conditions in order to reduce bacteria and fungal growth in herbal preparations.

2. Pharmacovigillance

Pharmacovigillance can help to improve safety and quality of herbal medicines. This can be preceded by registering practitioners so that it is easier to enforce regulatory systems for herbal contamination monitoring and control.

3.Quality Assurance

Herbal preparation should be subjected to the same quality assurance and certification processes like other products in the market. The Kenya Bureau of Standard should introduce standards for herbal medicines because this will help with flagging and removal of products which do not comply with the national standards.

4. Educating the Consumers

Consumers should be educated on the dangers of utilizing unsafe herbal products because this will introduce an element of accountability and participation of the consumers in monitoring and ensuring quality of herbal medicines.

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APPENDICES

Appendix 1: Questionnaire for the practitioner profile and herbal medicines

Instructions: Please tick in the relevant brackets and fill the blank spaces from the following items

Biodata of the practitioner

1. Gender Male ( ) Female ( )

2. What is your age?

Below 30 years ( ) 30 – 40 years ( )

40 – 50 years ( ) 50 – Above ( )

3. Marital status.

Single ( ) Married ( ) Widowed ( ) Divorced ( )

4. How many years have you been practicing?

Below 5 years ( ) 5 – 10 years ( )

10 – 20 years ( ) 20 – Above ( )

5. Education level

Primary ( ) Secondary ( )

Post secondary ( ) Others (specify).....

6. (i) Are you a trained professional?

Yes ( ) No ( )

(ii) If yes, what is your highest professional qualification?

Diploma ( )Graduate ( )Others (specify) ……

7. Any other source of income? Yes ( ) No ( )

8. Where do the clients come from? Urbanites ( ) Rural ( )

Product preparation and storage

9. What are the sources of raw materials?

Nairobi ( ) Beyond Nairobi ( ) Beyond Kenya ( )

10. What is the source of water for liquid preparations?

Tap water ( ) Borehole ( ) River ( ) Other specify…….

11. Method of preparation

Boiling ( ) Fermentation ( )

Distillation ( ) Others (specify).....

12. When after harvesting do you prepare the medicine?

Immediate ( ) Within a day ( )

3days ( ) 7 days ( )

More than a week ( )

13. What are the storage conditions of the product?

Refrigerator ( ) Room temperature ( )

14. What is the dosage form of the product?

Suspension ( ) Liquid ( ) Powder ( )

15. What is the measure of the dose using household items?

Spoon ( ) Bottle ( )

16. Which is the common route of administration?

Internal ( ) External ( )

17. Which is the route of administration of the medicine?

Oral ( ) Topical ( )

Intravenous ( ) Others (specify).....

Appendix 2: Catalogue of the Samples used

Solid Samples		Liquid Samples
Isabgol (Pure Psyphillium Husk)	Olugumati roots	Seven wonders
Multi cleanser	Segete seed	Cheskof (Expectorant)
Joint master	Olukumati bark	Herbal Cleanser
Alpha special	Lokumati bark	Zecuf (Hearbal Cough Remedy
Iulcers Solution	Almakutiku roots	Hoscinam special
ZANDU (Mala Sudarshau Chuma)	Omkatan	Product from Edensway Intenational
Mugutal Herbs Medicine	Okokora	Bio Herbs Product
Rayare Herbs Medicine	Osokonoi	Victory Cough Syrup
Immune guard capsules	Opoponai	Product from Edensway Intenational
Elski Kongo roots	Njaniengusero	Purifier Plus (Blood Purifier)
Olesesei blood cleanser	Okililorili	Victory Herbal Cleanser (Detoxifier)
Emukutan roots	Mwanrubaine	SAFI Natural blood purifier
Ndibiringwa Bark	Olupande	Bio Herbs (Chest Tonic)
Olesesein roots	Olusuki	Dental Care
Lurubat whole plant	Olusuki	

Appendix 3: SPSS Cross Tabs Results (IBM SPSS Statistics Version 20)

Education and Klebsiella Cross Tabulation							**Education and E.Coli Cross Tabulation**				**Education and Aflatoxins Cross Tabulation**				
			Klebsiella				***E. Coli***				**Aflatoxins**				
			No	**Yes**	**Total**	**Pearson Chi Square (p)**	**No**	**Yes**	**Total**	**Pearson Chi Square (p)**	**No**	**Yes**	**Total**	**Pearson Chi Square (p)**	
Education	**No**	Count	27	2	29	**0.035**	29	0	29	**0.321**	11	9	9	29	**0.599**
		% within Education	93.1 %	6.9%	100.0 %		100.0 %	0.0%	100.0 %		37.9 %	31.0 %	31.0 %	100.0%	
	Yes	Count	91	30	121		117	4	121		49	45	27	121	
		% within Education	75.2 %	24.8 %	100.0 %		96.7%	3.3%	100.0 %		40.5 %	37.2 %	22.3 %	100.0%	
	Total	Count	118	32	150	**Not Significant**	146	4	150	**Not Significant**	60	54	36	150	**Not Significant**
		% within Education	78.7 %	21.3 %	100.0 %		97.3%	2.7%	100.0 %		40.0 %	36.0 %	24.0 %	100.0%	

Professional Training and *Klebsiella* Cross Tabulation							Professional Training and *E. Coli* Cross Tabulation				Professional Training and Aflatoxins Cross Tabulation			
			Klebsiella			Pearson Chi Square (p)	*E. Coli*			Pearson Chi Square (p)	Aflatoxins			Pearson Chi Square (p)
			No	Yes	Total		No	Yes	Total		No	Yes	Total	
Professional Training	No	Count	81	24	105	0.486	101	4	105	0.184	42	21	105	0.143
		% within Professional Training	77.1%	22.9%	100.0%		96.2%	3.8%	100.0%		40.0%	20.0%	100.0%	
	Yes	Count	37	8	45		45	0	45		12	15	45	
		% within Professional Training	82.2%	17.8%	100.0%		100.0%	0.0%	100.0%		26.7%	33.3%	100.0%	
	Total	Count	118	32	150	Not Significant	146	4	150	Not Significant	54	36	150	Not Significant
		% within Professional Training	78.7%	21.3%	100.0%		97.3%	2.7%	100.0%		36.0%	24.0%	100.0%	

Highest Training and Klebsiella Cross Tabulation							Highest Training and E.Coli Cross Tabulation				Highest Training and Aflatoxins Cross Tabulation			
			Klebsiella			Pearson Chi Square (p)	*E. Coli*			Pearson Chi Square (p)	Aflatoxins			Pearson Chi Square (p)
			No	Yes	Total		No	Yes	Total		No	Yes	Total	
Highest Training	No	Count	76	24	100	0.260	96	4	100	0.152	39	21	100	0.392
		% within Highest Training	76.0%	24.0%	100.0%		96.0%	4.0%	100.0%		39.0%	21.0%	100.0%	
	Yes	Count	42	8	50		50	0	50		15	15	50	
		% within Highest Training	84.0%	16.0%	100.0%		100.0%	0.0%	100.0%		30.0%	30.0%	100.0%	
	Total	Count	118	32	150	Not Significant	146	4	150	Not Significant	54	36	150	Not Significant
		% within Highest Training	78.7%	21.3%	100.0%		97.3%	2.7%	100.0%		36.0%	24.0%	100.0%	

Material Source and Klebsiella Cross Tabulation							Material Source and E. Coli Cross Tabulation				Material Source and Aflatoxins Cross Tabulation			
			Klebsiella				*E. Coli*				Aflatoxins			
			No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)
Material Source	Beyond Kenya	Count	39	11	50	0.888	50	0	50	0.152	18	12	50	1.000
		% within Material Source	78.0%	22.0%	100.0%		100.0%	0.0%	100.0%		36.0%	24.0%	100.0%	
	Beyond Nairobi	Count	79	21	100		96	4	100		36	24	100	
		% within Material Source	79.0%	21.0%	100.0%		96.0%	4.0%	100.0%		36.0%	24.0%	100.0%	
	Total	Count	118	32	150	Not Significant	146	4	150	Not Significant	54	36	150	Not Significant
		% within Material Source	78.7%	21.3%	100.0%		97.3%	2.7%	100.0%		36.0%	24.0%	100.0%	

Source of Water and Klebsiella Cross Tabulation							Source of Water and E. Coli Cross Tabulation				Source of Water and Aflatoxins Cross Tabulation			
			Klebsiella				*E. Coli*				Aflatoxins			
			No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)
Source of Water	River	Count	15	0	15	0.034	15	0	15	0.499	3	6	15	0.227
		% within Source of Water	100.0%	0.0%	100.0%		100.0%	0.0%	100.0%		20.0%	40.0%	100.0%	
	Tap Water	Count	103	32	135		131	4	135		51	30	135	
		% within Source of Water	76.3%	23.7%	100.0%		97.0%	3.0%	100.0%		37.8%	22.2%	100.0%	
	Total	Count	118	32	150	Significant	146	4	150	Not Significant	54	36	150	Not Significant
		% within Source of Water	78.7%	21.3%	100.0%		97.3%	2.7%	100.0%		36.0%	24.0%	100.0%	

Extract Preparation Boiling and Klebsiella Cross Tabulation							Extract Preparation Boiling and E. Coli Cross Tabulation				Extract Preparation Boiling and Aflatoxins Cross Tabulation			
			Klebsiella				*E. Coli*				Aflatoxins			
			No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)
Boiling	No	Count	76	24	100	0.260	96	4	100	0.152	42	18	100	0.24
		% within Boiling	76.0%	24.0%	100.0%		96.0%	4.0%	100.0%		42.0%	18.0%	100.0%	
	Yes	Count	42	8	50		50	0	50		12	18	50	
		% within Boiling	84.0%	16.0%	100.0%		100.0%	0.0%	100.0%		24.0%	36.0%	100.0%	
	Total	Count	118	32	150	Not Significant	146	4	150	Not Significant	54	36	150	Not Significant
		% within Boiling	78.7%	21.3%	100.0%		97.3%	2.7%	100.0%		36.0%	24.0%	100.0%	

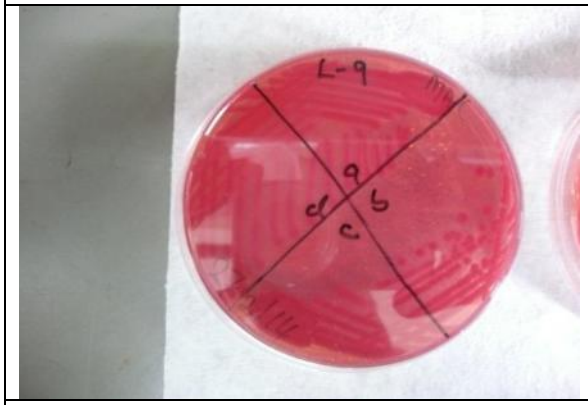
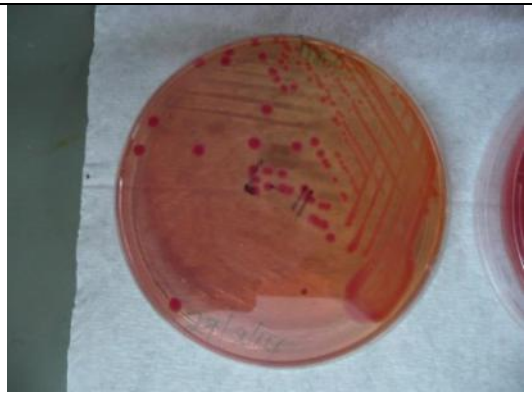
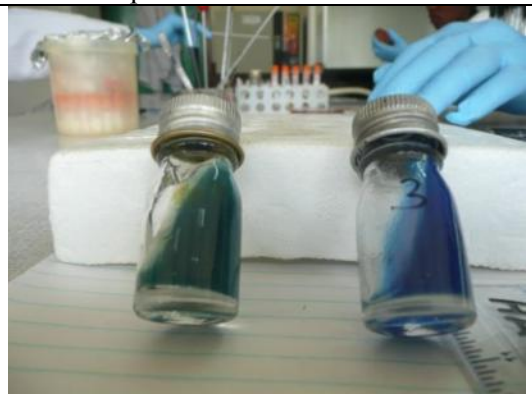
Extract Preparation Distillation and Klebsiella Cross Tabulation							Extract Preparation Distillation and E. Coli Cross Tabulation				Extract Preparation Distillation and Aflatoxins Cross Tabulation			
			Klebsiella				*E. Coli*				Aflatoxins			
			No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)
Distillation	No	Count	113	32	145	0.236	141	4	145	0.707	51	36	145	0.355
		% within Distillation	77.9%	22.1%	100.0%		97.2%	2.8%	100.0%		35.2%	24.8%	100.0%	
	Yes	Count	5	0	5		5	0	5		3	0	5	
		% within Distillation	100.0%	0.0%	100.0%		100.0%	0.0%	100.0%		60.0%	0.0%	100.0%	
	Total	Count	118	32	150	Not Significant	146	4	150	Not Significant	54	36	150	Not Significant
		% within Distillation	78.7%	21.3%	100.0%		97.3%	2.7%	100.0%		36.0%	24.0%	100.0%	

Extract Preparation Grinding and Klebsiella Cross Tabulation							Extract Preparation Grinding and E. Coli Cross Tabulation				Extract Preparation Grinding and Aflatoxins Cross Tabulation			
			Klebsiella				*E. Coli*				Aflatoxins			
			No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)
Grinding	No	Count	42	8	50	0.260	50	0	50	0.152	12	18	50	0.024
		% within Grinding	84.0%	16.0%	100.0%		100.0%	0.0%	100.0%		24.0%	36.0%	100.0%	
	Yes	Count	76	24	100		96	4	100		42	18	100	
		% within Grinding	76.0%	24.0%	100.0%		96.0%	4.0%	100.0%		42.0%	18.0%	100.0%	
	Total	Count	118	32	150	Not Significant	146	4	150	Not Significant	54	36	150	Significant
		% within Grinding	78.7%	21.3%	100.0%		97.3%	2.7%	100.0%		36.0%	24.0%	100.0%	

Storage time before Preparation and Klebsiella Cross Tabulation							Storage time before Preparation and E. Coli Cross Tabulation				Storage time before Preparation and Aflatoxins Cross Tabulation			
			Klebsiella				*E. Coli*				Aflatoxins			
			No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)	No	Yes	Total	Pearson Chi Square (p)
Storage time before Preparation	No	Count	70	15	85	0.208	81	4	85	0.076	24	27	85	0.016
		% within Storage time before Preparation	82.4%	17.6%	100.0%		95.3%	4.7%	100.0%		28.2%	31.8%	100.0%	
	Yes	Count	48	17	65		65	0	65		30	9	65	
		% within Storage time before Preparation	73.8%	26.2%	100.0%		100.0%	0.0%	100.0%		46.2%	13.8%	100.0%	
	Total	Count	118	32	150	Not Significant	146	4	150	Not Significant	54	36	150	Significant
		% within Storage time before Preparation	78.7%	21.3%	100.0%		97.3%	2.7%	100.0%		36.0%	24.0%	100.0%	

Storage Conditions and *Klebsiella* Cross Tabulation							Storage Conditions and *E. Coli* Cross Tabulation			
			Klebsiella				*E. Coli*			
			No	**Yes**	**Total**	**Pearson Chi Square (p)**	**No**	**Yes**	**Total**	**Pearson Chi Square (p)**
Storage Conditions	Refrigerator	Count	2	3	5	**0.054**	5	0	5	**0.863**
		% within Storage Conditions	40.0%	60.0%	100.0%		100.0%	0.0%	100.0%	
	Room Temperature	Count	111	29	140		136	4	140	
		% within Storage Conditions	79.3%	20.7%	100.0%		97.1%	2.9%	100.0%	
	Total	Count	118	32	150	**Almost**	146	4	150	**Not**
		% within Storage Conditions	78.7%	21.3%	100.0%	**Significant**	97.3%	2.7%	100.0%	**Significant**

Appendix 4: Research Pictures

Some of the Solid Samples	Herbal Product	Liquid Sample
		
Culture Plate 1	Culture Plate 2	Solid Samples
		
Culture Plate 3	Culture Plate 4	Laboratory Procedure