MICROBIAL AND HEAVY METAL CONTAMINATIONS IN SELECTED HERBAL MEDICINAL PRODUCTS SOLD IN NAIROBI, KENYA

BY

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DECLARATION

This thesis is my original work and has not been presented to any other university or institution for the award of a degree or any other award.

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DEDICATION

I would like to dedicate this thesis to my beloved wife Jackline Tarus and children; Wyclif Kiprotich and Amon Kipruto. Your love, encouragement and moral support throughout my study period have finally born fruits.

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LIST OF ABBREVIATIONS/ACRONYMS

>LOD	Less than the Limits of Detection
ANOVA	Analysis of Variance
API	Analytical Profile Index
AST	Antibiotic Susceptibility Test
AAS	Atomic Absorption Spectrometer
CFU	Colony forming Units
CNS	Cetral Nervouse System
DNA	Deoxyribonucleic Acid
FAO	Food Agricultural Organization
FDA	Food and Drugs Administration
GAP	Good Agricultural Practice
GERD	Gastroesophageal Reflux Disease
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GSP	Good Supply Practice
HIV	Human immono-deficiency Virus
HMP	Herbal Medicinal Products
KEMRI	Kenya Medical Research Institute
MRSA	Methicillin-resistant *S. aureus*
NCCLS	National Commetee on Clinical Laboratory Standards
NLSI	National Laboratory Standard Institute
Pb	Lead
PCR	Polymarace Chain Reaction
PH	Probability of Hydrogen ions

PPB	Pharmacy and Poison Board
RNA	Ribonucleic Acid
SOP	Operating procedures
SPSS	Statistic Package for Social Scientist
UNAIDS	United Nations Aids
UoN	University of Nairobi
UTI's	Urinary Tract Infections
W H O	World Health Organization

ABSTRACT

Background: The use of traditional herbal medicinal products has been increasing worldwide due to the readily available raw materials and low cost compared to the synthetic industrial preparations. Medicinal herbs have been reported to be contaminated with agents and microorganisms indigenous to the soil and plants where they are grown. Confounded with poor unhygienic and handling pactices, the microbial safety and quality of these products is a public health concern. **Broad objective:** The study aimed at determining microbial and heavy metals contamination in selected herbal medicinal products sold in Nairobi, Kenya. **Methodology:** The study employed an exploratory as well as laboratory based experimental design. The herbal products were purchased from the markets, and transported to the Kenya Medical Research Institute laboratory for processing. Microbial contamination was determined using standard procedures and compared with European pharmacopoeia specifications. The levels of infestation were expressed as colony forming units. Antibiotic resistant genes were determined using polymerase chain reactions. Screening of the presence of mycotoxins was done using Envirologix Quick toxin kits. Heavy metal analysis was determined using Atomic Absorption Spectroscopy. Data was coded and analysed using SPSS and Excel computer packages. Pearson chi square test and Fisher excert test was used to determine associations between variables at 95% confidence interval ($p \leq 0.05$). **Results and Discussion:** The study sampled 138 herbal products from different market outlets. The sampled herbal products were in capsules, liquid, juice, cream/lotion, powder, and syrup formulations. Bacterial contaminated samples were 117(84.8%) while 61(44.2%) were contaminated with fungi. Products from street vendors, herbal clinics which were in powder and liquid forms had cfu/g beyond the European pharmacopeia accepted limits [bacteria $<1.0x10^3$cfu/g (47.6%) and fungi <104/105cfu/g (1.6%)]. About 15.2% of the samples had fumonisins levels beyond the accepted limits while 5.2% had aflatoxins levels beyond these limits (4ppb). These samples were from street vendors and herbal clinics, which were in powder and liquid formulations. There was no significant association between the presence of fungi and aflatoxins/fumonisin contamination in the herbal products (χ^2 test; $p>0.05$). The maximum permissible level of copper, zinc and iron were within accepted permissible limits. However, the nickel levels among some herbal products were beyond (35ppm) the recommended limits. *Bacillus, Klebsiella, Proteus, Staphylococcus, Streptomyces, Escherichia, Enterobacter, Serratia, Yersinia, Morganella, Citrobacter, Erwinia*

and *Shigella* were the bacterial genera identified. While *Aspergillus, Penicillium, Rhizomucor, Rhodotorula, Absidia, Alternaria* and *Fusarium* are among the fungal genera identified in this study. Some [35(36.5%)] bacteria isolates were resistant to one or more antibiotic tested. The phenotypic drug resistant bacteria were positive for drug resistance genes. The sources of contaminations could be soils and dust since most herbal products are prepared from roots and stem barks that are already in contact with the soil/dust. Fungi which inhabits the soil produce mycotoxins has secondary metabolites hence explaining the fumonisins and aflatoxins detection in the herbal products. Hawkers and street vendors packaged their products in bottles and plastic containers previously used for other products hence could make the product susceptible to microbial or chemical contamination due to inadequate cleaning. Handling of herbal products by street vendors could expose them to contaminations because some are not properly packaged. Those sold in the herbal clinics and supermarkets could have been contaminated during processing and handling. **Conclusion and Recommendation:** From this study, it is evident that some herbal medicinal products sold in Nairobi are highly contaminated with potential microbial pathogens including fungal toxins. Herbalist should be trained on how to apply good manufacturing practices, good harvesting practices and the safe handling and storage of herbal medicinal products. The study therefore recommends that policies be enacted to enable regulation of herbal medicinal products sold in the Kenyan market for public health reasons.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

The history of using herbs is related to that of modern medicine because many synthetic drugs listed as conventional medication were originally derived from plants. An example is the antimalarial drug quinine from *Cinchona* species (Janetzang, 1994). The high cost and inaccessibility of conventional drugs, coupled with inaccessibility to medical doctors in sub-Saharan Africa where there is a high patient to doctor ratio (40 000:1) have led to medicinal herbal products becoming more popular. Medicinal herbal products are used increasingly not only in Sub-Saharan Africa but also in developing countries in general (UNAIDS/WHO, 2009). The use of herbal medicine is also gaining popularity in developed countries (Jia and Zhang 2005) due to the growing evidence supporting the efficacy of some of the products (Langlois-Klassen *et al.,* 2007; Bii *et al.,* 2010; Korir *et al.,* 2012) and also the fact that these products are accessible and cheap. Increased use of medicinal herbs has been reported especially for HIV related complications (Bodeker *et al*., 2006; Langlois-Klassen *et al*., 2007). In fact, numerous authors, including the World Health Organization (WHO, 2007), have reported that 30% – 70% of HIV infected individuals worldwide use herbal products (Harnack *et al*., 2001; Liu *et al.,* 2005; Zhang, 2008).

Heavy metals are a known contaminant or adulterant of many herbal medicines. The Asian and Indian herbal medicines have been reported to contain high levels of arsenic, lead, cadmium and mercury (Ernst, 1998; Garvey *et al.,* 2001). The presence of heavy

metals in herbal products may be due to several possibilities such as being included intentionally for alleged curative and medicinal properties; because of accidental contamination during manufacture and when grown in soil polluted with sewage and industrial waste (Garvey *et al.,* 2001).

Most consumers and vendors believe and consider herbal products to be safe but microbial contamination in medicinal herbs is a concern, especially for the immuno-compromised individuals as a result of their lowered immunity (Ernst 1998; Keter *et al.*, 2016; Walther *et al.,* 2016). Medicinal herbs have been reported to be contaminated with microorganisms indigenous to the soil and plants where they are grown. Keter *et al.,* (2016) in a recent study reported that medicinal herbal products from Eldoret and Mombasa regions of Kenya were contaminated with pathogenic bacteria which are also multi-drug resistant. Another similar recent study has revealed that most of the liquid herbal medicinal products marketed in Mwanza city in Tanzania were contaminated with bacterial coliforms (Walther *et al*., 2016). Poor conditions during harvesting and post harvest handling of the herbs and herbal products are predisposing factors to contaminations (Farkas, 2000; Candlish, 2001). Studies have reported contamination of herbal products with *Bacillus* spp*.,* Enterobacteriaceae*, Salmonella* spp*.,* S*taphylococcus aureus*, *Penicillium spp* and *Aspergillus spp* among others (Govender *et al.,* 2006; Keter *et al.,* 2016). Moreover, elevated levels of bacterial and fungal contaminants, such as *Penicillium* spp*., Aspergillus spp* and *Fusarium spp*, have been observed in herbal drugs and spices (Candlish *et al.,* 2001). The other challenge is the presence of "adulterants" which in actual sense mean intentional or unintentional presence of undeclared

ingredients which can have negative impact on the overall product in terms of safety, efficacy and quality. This adulteration may be due to toxicity of the added undisclosed ingredients as well as microorganisms like bacteria and fungi together with their bi-products like bacterial and fungal (mycotoxins) toxins. Quality control has to be built into the whole process beginning from the selection of propagating material to the final product reaching the consumer.

In Kenya, the competent authority responsible for regulating the safety and efficacy of pharmaceutical products is the Pharmacy and Poisons Board (PPB). Products for which any medicinal claims are made must be granted marketing authorization by the PPB, which ensures that registered products are manufactured in compliance with Good Manufacturing Practices (GMP), amongst other criteria. Majority of herbal products marketed to members of the public in Kenya are not regulated by the PPB as they are categorized as traditional or cultural remedies whose mode of utilization predate modern regulation, and they thus enjoy regulatory exemption. The few manufacturers of herbal remedies, who register their products with the PPB, mostly do so in order to access the conventional medicine market through doctors' prescribing and the retail pharmacies (Onyambu *et al.,* 2013). Studies of Yesuf *et al.,* (2016) in different markets of Gondar Town, Northwest Ethiopia showed that herbal medicinal preparations sold in the study area were highly contaminated with pathogenic microorganisms with very high microbial load. More than 40% of the samples contain fecal indicator organisms. Multiple drug resistance was not uncommon and it was found that 125(83.3%) of the isolates were resistant to 2 or more antibiotics tested. The source of aflatoxin exposure to the Kenyan

population could be due to contaminated food as well as herbal products. In 2016 Yard and others in their study found that there was a widespread aflatoxin exposure across Kenya. Over three-quarters of serum specimens had evidence of recent exposure, and this exposure persisted across the spectrum of age, sex and socioeconomic status. This widespread exposure could negatively impact health throughout Kenya (Yard *et al.*, 2016). Therefore the current study evaluated mycotoxins, heavy metals and microbiological quality of herbal products marketed to the general population in Nairobi-Kenya.

1.2 Statement of the problem

Kenya being a developing nation faces various socio economic challenges including poverty, illiteracy and diseases. Due to the high levels of communicable diseases coupled with poverty, many people heavily rely on medicinal herbal products for their primary health care. Currently, there is no legal regulatory framework or policy governing the production, quality and sale of herbal medicines in Kenya. The Pharmacy and Poison Board (PPB), which is the Government drug regulatory body, does not register herbal medicinal products and herbal practitioners. Herbalists are registered under the Department of Culture and Natural Heritage in the Ministry of State for National Heritage and Culture, which has no regulatory policy on herbal products. Majority of the herbal products are marketed as food supplements and dietaries. On this note, unsuspecting public could be exposed to high levels of heavy metals, mycotoxins and disease causing microorganisms. European Pharmacopoeia specifies that organisms for instance *Escherichia coli* and *Salmonella species* should be absent from herbal products

(Gupta, 2015). Some of the microorganisms such as *Staphylococcus aureus*, *Salmonella species, Escherichia coli* and *Pseudomonas aeruginosa* are diseases causing organisms that have been isolated from herbal medicinal products. Antibiotic resistant bacteria from medicinal herbal products have also been isolated (Borges *et al.,* 2016). Fungi including *Aspergilus species*, *Penicillium species* and *Fusarium species* which are mycotoxins producers are among the contaminants present in herbal medicinal products (Stević *et al.,* 2012). When contaminated herbal products are consumed, they are likely to cause disease outbreaks. The situation could be worse if consumed by immune compromised individuals such as HIV patients, the elderly and cancer patients among others.

1.3 Justification

Herbal medicines are available for purchase from outlets ranging from herbal practitioners/clinics to food stores and retailers. A greater number of residents in Nairobi metropolis are believed to depend on traditional herbalists for their primary medicare. This is evidenced by the high number of herbal clinics and herbalists present as found by the current study. Unfortunately, there is scanty literature to support the facts that assessment of the presence of heavy metals and microbiological quality of herbal products being used in Nairobi, has been done. This calls for an urgent need for evaluation of the herbal products' purity and safety. The current study aimed to evaluate whether some herbal medicines contain heavy metals and mycotoxins and to determine whether they are contaminated with pathogenic and environmental microorganisms such as bacteria and fungi. In addition, the presense of bacteria, that are both phenotypic and genotypic drug resistant will also be determined. The study results will yield useful data that would help the herbalist and manufacturers adapt good harvesting, good

manufacturing practise and overall quality control in their practise. The data obtained would be useful to medical practitioners, the public and the Kenyan government regarding public health intervention, and that it would provide supportive evidence on the importance of policy enactment for herbal medicines regulations for public safety.

1.4 Objectives

1.4.1 General objective

To determine the presense of microbial and heavy metals contaminations in selected herbal medicinal products sold in Nairobi - Kenya

1.4.2 Specific objectives

1. To determine bacterial and fungal loads from the selected herbal medicinal products sold in Nairobi - Kenya
2. To identify the isolated bacterial and fungal contaminants in the sampled herbal medicinal products sold in Nairobi – Kenya
3. To determine the presence of fungal toxins from the herbal medicinal products sold in Nairobi - Kenya
4. To perform antibiotic susceptibility test on the isolated pathogenic bacteria organisms from the medicinal herbal products sold in Nairobi – Kenya
5. To detect the presence of antibiotic resistant genes from the isolated drug resistant bacteria from the medicinal herbal products sold in Nairobi – Kenya
6. To determine the level of heavy metal contamination from the selected herbal medicinal products sold in Nairobi - Kenya

1.5 Research hypothesis

1.5.1 Null hypothesis

1. Herbal medicinal products sold in Nairobi -Kenya are not contaminated with pathogenic microorganisms and heavy metals.
2. Herbal medicinal products sold in Nairobi -Kenya do not have bacterial and fungal loads
3. Bacterial and fungal contaminants in Herbal medicinal products sold in Nairobi - Kenya cannot be isolated and identified
4. Fungal toxins are not present in herbal medicinal products sold in Nairobi - Kenya
5. Isolated drug resistant bacteria do not contain antibiotic resistant genes
6. Herbal medicinal products sold in Nairobi – Kenya are not contaminated with heavy metals

1.5.1 Alternative hypothesis

1. Herbal medicinal products sold in Nairobi -Kenya are contaminated with pathogenic microorganisms and heavy metals.
2. Herbal medicinal products sold in Nairobi -Kenya contain bacterial and fungal loads
3. Bacterial and fungal contaminants in Herbal medicinal products sold in Nairobi - Kenya can be isolated and identified
4. Fungal toxins are present in herbal medicinal products sold in Nairobi - Kenya
5. Isolated drug resistant bacteria contain antibiotic resistant genes
6. Herbal medicinal products sold in Nairobi – Kenya are contaminated with heavy metals

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Herbal products

Herbal medicine is also referred to as herbalism or the use of herbal medicinal products. It is the study and use of medicinal properties of plants by man (Lichterman, 2004). Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and for their defence against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Lai, 2004; Tapsell *et al.,* 2006). Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus, herbal medicinal products do not differ greatly from conventional drugs in terms of their antimicrobial and pharmacological activities. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects as conventional drugs due to toxicities and contaminations (Tapsell *et al.,* 2006; Lai, 2004; Keter *et al.,* 2016).

2.2 Bacterial contaminations in herbal products

Aerobic bacteria occur naturally in plant-based products and are the most commonly used quality parameter for assessing the hygiene status of food and drug samples (Czech *et al*., 2001). The WHO aerobic plate counts limit provided for herbal products made for

internal use is 5log units/g. Coliforms are found mainly in soil, water, vegetation and the faeces of warm-blooded animals (Candlish *et al*., 2001). Previous studies have reported high coliform counts, up to 3.4×10^4 cfu/g, in other botanical products such as chamomile (Foote *et al*., 2005). In a study done in Ghana, it was observed that 50% of herbal tea and 13% of internal use remedies failed to meet the WHO requirement of less than 3 log unit/g of tea and 4log units/g for internal use, respectively. High coliform counts are an indication of poor hygiene and a lack of sanitary practices (WHO, 2007). Several studies world wide have obsereved that the herbal medicinal products were not save for human consumption due to bacterial contaminations (Keter *et al.,* 2016), Walther *et al.,* 2016; Yesuf *et al.,* 2016).

Contamination results from several sources that includes; improper cleaning procedures and open air drying of plant materials which results in contamination with soil and dust (Sharma, 2001). The presence of *E. coli* confirms faecal contamination, which is directly associated with unsanitary conditions. Because heat treatment of herbal products results in a considerable reduction in the viable counts of *E. coli* (Montville and Mathews, 2005), education is critical to prevent spread of food borne pathogens among consumers. Herbal products frequently undergo human handling as they are collected, cleaned, pooled, dried, packed and dispensed (Sharma, 2001; WHO, 2007). Some of the organisms that have been isolated from herbal medicines include enterohemorrhagic *E. coli*, *Salmonellae*, *Pseudomonas aeruginosa*, *Listeriae spp*, *Staphylococcus aureus*, *Candida albicans* and *Campylobacter jejuni* (Kneifel *et al*., 2005). Other studies on herbal products have reported the presence of antibiotic-resistant bacteria (Brown and

Jiang, 2008). Keter and others in a survey of herbal products contamination found that the samples were contaminated with both environmental and pathogenic bacteria that are capable of causing disease outbreaks (Keter *et al.*, 2016).

Other important speies of bacteria that have been isolated in herbal medicinal products include;- *Serratia spp., Erwinia spp., Yersnia spp., Morganella spp., Citrobacter spp* and *Enterobater sp.* (Onyambu, 2013; Keter *et al.*, 2016; Walther *et al*., 2016; Yesuf *et al.*, 2016). The role of *Citrobacter* species in human disease is not of great concern as that of the other coliforms. *Citrobacter freundii* and *C. diversus* have been isolated predominantly as super-infecting agents from urinary and respiratory tract infections. *Citrobacter* septicemia may occur in patients with multiple predisposing factors; *Citrobacter* are rare opportunistic nosocomial pathogens. *Citrobacter* normally cause urinary tract infections, blood stream infections, intra abdominal sepsis, brain abscesses and pneumonia including other neonatal infection, such as meningitis, neonatal sepsis, joint infection or general bacteremia. Central Nervouse System (CNS) infections are more common for infants under 2 months old than for older children or immuno compromised adult patients, but rare cases have been reported. The species *C. koseri* and *C. ferundii* cause neonatal meningitis that can lead to brain abscesses. *Citrobacter* infections can be fatal, with 33-48 % overall death rates, and 30% for neonates. Infant survivors may experience significant damage to CNS, including profound retardation, hemiparesis and seizures among others (Ryan, 2004; WHO, 2010).

Morganella morganii are commonly found in the environment and in the intestinal tracts of humans, mammals and reptiles as normal flora. Despite its wide distribution, it is an uncommon cause of community-acquired infection and is most often encountered in postoperative and other nosocomial settings. The *M. morganii* infections respond well to appropriate antibiotic therapy; however, its natural resistance to many beta-lactam antibiotics may lead to delays in proper treatment. In 2011, Kwon and others reported a case of a 65-year-old man with an infected aortic aneurysm in which the pathogen was *M. morganii*. Diagnosis requires a high index of suspicion and imaging tests (Kwon *et al*., 2011).

Serratia species normally cause several infections in human beings. *Serratia* sepsis, which may present with fever, chills, shock and respiratory distress (Zarogoulidis *et al.*, 2011). Urinary tract infections symptoms may include fever, frequent urination, dysuria, pyuria, or pain upon urination (Wu *et al.*, 2012). Patients with pneumonia may have fever, chills, productive cough (sometimes with pseudohemoptysis), hypotension, dyspnea, and/or chest pain. *Serratia* meningitis or cerebral abscesses may develop in premature children and neonates with prior sepsis. *Serratia marcescens* osteomyelitis is a common presentation of chronic granulomatous disease in infancy. Patients with *Serratia* endocarditis may present with fever, petechiae and occasionally, embolic complications (eg, stroke, arterial emboli) (Posluszny *et al.*, 2011). Patients with *Serratia* ocular infections present with keratitis or endophthalmitis. Patients with *Serratia* soft-tissue infections may have surgical scars, cellulitis, phlebitis, or skin infections. Patients with

Serratia otitis media present with earaches, hearing loss and ear discharge (Rastogi *et al.*, 2002).

Enterobacter infections can include bacteremia, lower respiratory tract infections, skin and soft-tissue infections, urinary tract infections (UTIs), endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, CNS infections, and ophthalmic infections. *Enterobacter* infections can necessitate prolonged hospitalization, multiple and varied imaging studies and laboratory tests, various surgical and nonsurgical procedures and powerful expensive antimicrobial agents. *Enterobacter* infections do not have a clinical presentation that is specific enough to differentiate them from other acute bacterial infections according to World Health Organization report (WHO, 2010).

Some members of *Yersinia* are pathogenic in humans; in particular, *Y. pestis* is the causative agent of the plague. Rodents are the natural reservoirs of *Yersinia*; less frequently, other mammals serve as the host. Infection may occur either through blood (in the case of *Y. pestis*) or in an alimentary fashion, occasionally via consumption of food products (especially vegetables, milk-derived products and meat) contaminated with infected urine or feces. The disease caused by *Y. enterocolitica* is called yersiniosis. In addition, the genus is associated with pseudoappendicitis, which is an incorrect diagnosis of appendicitis due to a similar presentation (Malekzadeh *et al.*, 2009).

2.3 Fungal contaminants

Fungal contaminations of herbal products chiefly occur during a slow drying process, because of inadequate drying or during postharvest storage if relative humidity is high and temperatures are favourable. The fungal contaminants are primarily from environmental origin, such as fungal spores found in the soil and air. Such contaminated products may cause fungal infections or other serious health complications because of mycotoxins accumulation from toxin-producing fungi such as *Aspergillus parasiticus* and *A. flavus* (Sharma, 2001). Several studies have reported the presence of mycotoxins in botanical preparations (Candlish *et al*., 2001; Lutomski, 2007; Salehei *et al.*, 2015).

Salehei *et al.*, 2015 in a study found that, powdered herbal face mask were contaminated with several saprophytic fungi. The presence of many fungi in herbal preparations may be harmful for those who used for medical care, especially those preparation applied on face skin. Gautam & Bhadauria (2009) showed that 88% of fruits and powdered samples of herbal drugs were contaminated with several species of saprophytic fungi belonging to genus Aspergillus and Penicillium. In addition, Razak illustrated *A. niger* as the most dominant fungal contaminant in herbal plants products (Razak *et al.,* 2009). On the other hand, *Fusarium* was observed as the most dominant genus in tested medicinal drugs by Stevic *et al.,* (2012). Most isolates of *Aspergillus* species such as *A. flavus*, *A. parasiticus*, *A. ochraceus*, *A. niger* and *A. fumigatus* have the ability to produce mycotoxins (Bharti & Vasudeva, 2013). Mycotoxins produced by these fungi, especially aflatoxins, are carcinogenic and cause several diseases of skin, liver, kidney, respiratory organs and nervous system (Gautam & Bhadauria, 2009).

Molds can also pose a hazard to human and animal health when they are consumed following the growth of certain mold species in herbal products and stored food. Some species produce toxic secondary metabolites, collectively termed as mycotoxins including aflatoxins, ochratoxins, fumonisins, trichothecenes, citrinin, and patulin (Salehei *et al.*, 2015). These toxic properties may be used for the benefit of humans when the toxicity is directed against other organisms; for example, penicillin adversely affects the growth of Gram-positive bacteria such as Clostridium species, certain spirochetes and certain fungal spp (Saunders, 2008).

Absidia genus, is a fungus belonging to the Mucorales group of the Zygomycetes class, it is an ubiquitous saphrophyte found in the ground which may cause zygomycosis. Although those infections have occasionally been reported in immune competent patients after accidents or trauma involving contamination through skin-ground contact, they usually occur in immune depressed hosts (Venturini *et al.,* 2002). Patients undergoing chemotherapy for cancer or leukaemia, patients with poorly controlled diabetes, particularly if ketoacidosis is present, those with iron accumulation, those undergoing chelant therapy, haemodialysis or prolonged steroid therapy, patients who are positive for human immunodeficiency virus (HIV) or who have suffered severe, extensive burns are the most vulnerable. Symptoms range from a skin infection around wounds to systemic multi-visceral involvement. Zygomycetes infection is confirmed by hyphae in tampon cultures that are refractory to antibiotic therapy and by wound secretion positivity at microscopy. Histology is not always positive for *Absidia Corymbifera*. Ribes, in a study

reported a case of mucormycosis in an apparently immune competent accident victim with multiple abdominal injuries (Ribes *et al.,* 2000).

2.4 Mycotoxins

Mycotoxin is a Latin word which refer to; "fungus toxicum" that means toxins and poison produced by fungal organisms (Mahendra *et al*., 2012). They are toxic secondary metabolite produced by organisms of the kingdom fungi, commonly known as molds (Richard, 2007a). The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops (Mahendra *et al*., 2012). One mold species may produce many different mycotoxins and the same mycotoxin may be produced by several different species (Robbins *et al*., 2000). Mycotoxicoses are examples of "poisoning by natural means" and thus are analogous to the pathologies caused by exposure to pesticides or heavy metal residues. The symptoms of mycotoxicosis depend on the type of mycotoxin; the amount and duration of the exposure; the age, health and sex of the exposed individual; and many poorly understood synergistic effects involving genetics, dietary status and interactions with other toxic insults. Thus, the severity of mycotoxin poisoning can be compounded by factors such as vitamin deficiency, caloric deprivation, alcohol abuse and infectious disease status (Turner, 2009; Salehei *et al.,* 2015). In turn, mycotoxicoses can heighten vulnerability to microbial diseases, worsen the effects of malnutrition and interact synergistically with other toxins. Even temperature treatments, such as cooking and freezing, do not destroy some mycotoxins (Robbins *et al.,* 2000; Zheng *et al.* 2017).

2.4.1 Aflatoxins

Aflatoxins are toxic metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* species of fungi in/on foods, herbal products and feeds (Walther *et al*., 2016). They are probably the best known and most intensively researched mycotoxins in the world (Korir and Bii, 2012). Aflatoxins have been associated with various diseases, such as aflatoxicosis, in livestock, domestic animals and humans throughout the world. The occurence of aflatoxins is influenced by certain environmental factors; hence, the extent of contamination will vary with geographic location, agricultural and agronomic practices and the susceptibility of commodities to fungal invasion during preharvest, storage, and/or processing periods (Finley *et al*., 1992; Zheng *et al.* 2017). Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans (Rai *et al*., 2012). In reality, absolute safety is never achieved, many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed (Richard, 2007). Fungal growth and aflatoxin contamination are the consequence of interactions among the fungus, the host and the environment. The appropriate combination of these factors determines the infestation and colonization of the substrate, and the type and amount of aflatoxin produced (Robbins *et al.*, 2000). However, a suitable substrate is required for fungal growth and subsequent toxin production, although the precise factor(s) that initiates toxin formation is not well understood. Water stress, high-temperature stress, and insect damage of the host plant are major determining factors in mold infestation and toxin production (Richard, 2007; Zheng *et al.* 2017).

Aflatoxin formation is also affected by associated growth of other molds or microbes (Korir and Bii, 2012). For example, high temperatures, prolonged drought conditions, and high insect activity favor pre-harvest aflatoxin contamination of peanuts and corn, while warm temperatures and high humidity (Heathcote and Hibbert, 1978) favor postharvest production of aflatoxins on corn and peanuts. Aflatoxin B1 has been classified as a known human carcinogen and is known to cause liver cancer in animals (Richard, 2007). Aflatoxins may also be associated with liver cell cancer in humans in Africa and Asia where peanuts are a dietary mainstay (Goldbatt, 1969). Recent research has shown a strong association between long-term dietary aflatoxin exposure in conjunction with hepatitis B (a viral infectious disease of the liver) and increased rates of liver cancer (Groopman and Eaton, 1994).

Yard and others in a study on aflatoxins levels of blood serum samples in Kenya found higher aflatoxin levels among persons who reported recently being sick or recently seeking health care. Aflatoxin exposure varied by geographic location. Serum specimens from the Eastern province had the highest exposure: the median aflatoxin adduct level (7.87 pg/mg albumin) was twofold higher than the median aflatoxin adduct level for the next highest province – the Coast province (3.70 pg/mg albumin; $p < 0.01$). Median aflatoxin levels were much lower in the other six provinces: Nairobi (2.44 pg/mg albumin), Central (2.33 pg/mg albumin), North-Eastern (1.40 pg/mg albumin), Western (1.28 pg/mg albumin), Rift Valley (0.70 pg/mg albumin) and Nyanza (<LOD). There was also considerable variation within provinces. Out of Kenya's eight provinces, four

provinces (Central, Coast, Northeastern and Western) had one district where aflatoxin was concentrated, with statistically higher levels than other districts in the same province (Yard *et al.,* (2013). Research suggests that chronic aflatoxin exposure at minute levels could stunt growth (Khlangwiset *et al.,* 2011) and impair immunity (Jiang *et al.,* 2005). Furthermore, aflatoxin is a carcinogen, and cumulative exposure to any amount increases the risk of hepatocellular carcinoma (Wu *et al.,* 2009).

2.4.2 Fumonisins

Fumonisins are environmental toxins produced mainly by the molds *Alternaria, Fusarium moniliforme* (*F. verticillioides*), *F. proliferatum*, and several other *Fusarium* species that grow on agricultural commodities in the field or during storage. These mycotoxins have been found worldwide, primarily in corn. More than ten types of fumonisins have been isolated and characterized. Of these, fumonisin B1 (FB_1), B2 (FB_2), and B3 (FB_3) are the major fumonisins produced. The most prevalent of these mycotoxins in contaminated corn is FB_1, which is believed to be the most toxic (Thiel *et al*., 1992; Musser and Plattner, 1997; Do *et al.,* 2015). The levels of fumonisins in raw products are influenced by environmental factors such as temperature, humidity, drought stress, and rainfall during pre-harvest and harvest periods. For example, high levels of fumonisins are associated with hot and dry weather, followed by periods of high humidity (Shelby *et al*., 1994). Fumonisin levels in raw products are also influenced by storage conditions. For example, optimal growth of fumonisin-producing mold that leads to increased levels of fumonisin can also occur when the moisture content of harvested raw corn during storage is 18-23 percent (Bacon and Nelson, 1994; Bokhari and Aly, 2013). High levels of fumonisins may also occur in raw corn that has been damaged by insects

(Bacon and Nelson, 1994). However, corn hybrids genetically engineered with genes from the bacterium *Bacillus thuringiensis* (*Bt* corn) that produce proteins that are toxic to insects, specifically the European corn borer, have been found to be less susceptible to *Fusarium* infection and contain lower levels of fumonisins than the non-hybrid corn in field studies (Munkvold *et al.*, 1999; Do *et al.,* 2015).

2.5 Mycotoxins contaminations in herbal products

The risk of mycotoxins to humans is primarily from the consumption of contaminated mouldy foodstuffs. Some toxins can also be absorbed by inhalation or skin contact. The danger of mycotoxins is due to their chronically toxic properties of carcinogenicity, necrogenicity, mutagenicity and teratogenicity (Yard *et al.,* 2013). Acute poisoning with symptoms like vomiting and diarrhoea are only observed in toxins, which belong to the group of the trichothecenes. The mycotoxins are absorbed by animals via fodder/feeds and partly transmitted to animal products/foodstuffs (meat, eggs, milk). As a matter of principle, foodstuffs matured with mould fungi such as cheese and cooked meat as well as enzymes generated with mold fungi, vitamins and many more can be affected. The most important of the about 300 known mycotoxins are aflatoxins, citrinin, patulin, ochratoxin A, fumonisin, trichothecene (deoxynivalenol, nivalenol, T2-toxin, HT2-toxin) and Zearalenon. Most studies on herbal medicines have not found excessive levels of mycotoxin (Hitokoto *et al.,* 1978). However, researchers in India and Egypt have reported significant levels of mycotoxins contamination (Kumari *et al.,* 1989). This could be as a result of high heat and humidity in tropical regions which favour the development of fungal growth if inadequate drying procedures are used (Walther *et al*., 2016).

2.6 Heavy metals contamination on herbal products

Heavy metals are a known contaminant or adulterant of many herbal medicines. The Asian and Indian herbal medicines have been reported to contain high levels of arsenic, lead, fluoride cadmium and mercury (Ernst, 1998; Garvey *et al.,* 2001). The presence of heavy metals in traditional medicines and herbal products could be due to several possibilities such as heavy metals contamination during manufacture like grinding weights or lead-releasing containers or other manufacturing utensils. In addition, medicinal herbs may contain heavy metals when grown in heavily polluted soil. Several studies have reported unacceptable levels of heavy metals in some herbal raw materials and products (Dunbabin *et al.,* 1992; Markowitz *et al.,* 1994; Garvey *et al.,* 2001; Rai *et al.,* 2001). A survey of 34 samples of some common Indian herbs found that the concentrations of lead (Pb) and cadenium (Cd) were beyond the permissible WHO limits for most of the samples (Rai *et al*., 2001). The presence of toxic metals in herbal preparations can pose clinically relevant problems depending on the heavy metal in question (Dunbabin *et al.,* 1992; Markowitz *et al.,* 1994; DeSmet, 1995; Zayas *et al.,* 1996; DeSmet, 2002). Some preliminary work at CTMDR, KEMRI observed varying amounts of heavy metals contamination in some herbal materials with high amount of lead and cadmium (Keter *et al.,* 2013). Mukundi in a study on Bacteria, Aflatoxins And Fluoride Levels In Locally Processed Herbal Medicines From Nairobi County, Kenya found that 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 (Mukundi, 2015).

Clinical manifestations of metal poisoning have been well characterized (Harbison, 1998); but heavy metal poisoning has decreased because of improved industrial hygiene and environmental controls. Sometimes the signs and symptoms of such poisoning are likely to go undetected. However, if metal poisoning is identified, the true source may be wrongly associated with environmental occupational exposures but not medicament (Smitherman and Harber, 1991; Rai *et al*., 2001). Failure to establish the true cause of exposure means that the patient continues taking the metal-containing medication. Thus, the screening of herbal medicines for efficacy and safety has been recommended to protect the public (Chang, 1995; DeSmet, 2002). The manifestations of cadmium poisoning include nephrotoxicity, aminoaciduria, glycosuria and tubular necrosis while lead poisoning usually include haemolytic anaemia, blue line on gums, lead encephalopathy, peripheral neuritis, constipation, colic, fits, chronic nephritis and hypertension (Williams, 1982; Keter *et al.,* 2013). Clinical features in arsenic poisoning include intense thirst and severe gastroenteritis among others. Ataxia, excessive salivation, scanning speech and hatter's shakes are some of the mercury poisoning manifestations. Copper is relevant to humans because it is both essential and toxic depending on the dose and duration of exposure. Acute copper poisoning results in gastrointestinal disturbances whereas chronic exposure results in liver damage (Williams, 1982; Bremmer, 1998; DeSmet 2002).

Clinical features of arsenic poisoning include intense thirst, abdominal pain and vomiting. Severe gastroenteritis and circulatory collapse may occur. Nephrotoxicity, aminoaciduria, glycosuria and tubular necrosis are some of the manifestations of

cadmium poisoning and it has been noted to occur in levels even below the 7 µg/kg (Satarug and Moore, 2004; Keter *et al.,* 2013). Some of the lead poisoning clinical symptoms include; haemolytic anaemia, blue line on gums, lead encephalopathy, peripheral neuritis, constipation, colic, fits, chronic nephritis and hypertension.

Nickel is a widely used heavy metal, which exert a potent toxic effect on peripheral tissues as well as on the reproductive system (Das and Dasgupta, 2002; Keter *et al.,* 2013). Nickel causes skin rashes, myocardial infarction, nausea, vomiting and respiratory illness. Chronic selenium poisoning in people is characterized primarily by loss of hair and changes in fingernail morphology (Yang *et al.,* 1998). Liver dysfunction, skin reactions, gastrointestinal disturbances, fever, shaking chills, lethargy, headache and cough are symptoms associated with zinc poisoning (Fischer *et al.,* 1984; Keter *et al.,* 2013).

2.7 Quality control of herbal products

Quality of the herbs is influenced by the storage conditions (Snowden, 1992; Lisiewska *et al.,* 1997; Keter *et al.,* 2016). Due to improper storage of medicinal herbs, wide variety of mycoflora becomes activated. Usually plant and plant products are stored in tin, plastic, jute and wooden buckets, boxes and sacs. These containers will activate the growth of aerophiles and anaerobic bacteria as well as yeast and molds (Mycopath, 2006). Bauer *et al.* (1998) demonstrated that the quality criteria for herbal drugs are based on a clear scientific definition of the raw materials. It is difficult to establish comprehensive quality criteria for herbal drugs due to 'professional secrecy' of herbalists. In order to improve

the purity and safety of the products, observation of basic hygiene during preparation, standardization of some physical characteristic such as moisture content, heavy metals, pH and microbiological contamination levels are desirable. Previous studies have confirmed the presence of potential contaminants in herbal preparations (De-Smet, 2002; Das and Dasgupta, 2002; Walther *et al*., 2016). The contaminants that present serious health hazard are pathogenic bacteria such as *Salmonella spp, Escherichia coli, Staphylococcus aureus, Shigella spp* and other Gram positive and Gram negative strains of bacteria (Erich *et al.,* 2001; Wolfgang *et al.,* 2002; Adeleye *et al.,* 2005; Okunlola *et al.,* 2007; Keter *et al.,* 2016; Walther *et al*., 2016).

2.8 Antibiotic susceptibility test

Antibiotic sensitivity is the susceptibility of bacteria to antibiotics. Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*. Testing for antibiotic sensitivity is often done by the Kirby-Bauer method. Small wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth (WHO, 2011). Other methods to test antimicrobial susceptibility include the Stokes method, E-test (also based on antibiotic diffusion). The agar and broth dilution methods for determining Minimum Inhibitory Concentration are also AST methods in use (CLSI, 2015). Ideal antibiotic therapy is based on determination of the aetiological agent and its relevant antibiotic sensitivity. Empiric treatment is often started before laboratory microbiological reports are available when treatment should not be delayed

due to the seriousness of the disease. The effectiveness of individual antibiotics varies with the location of the infection, the ability of the antibiotic to reach the site of infection and the ability of the bacteria to resist or inactivate the antibiotic (Silbergeld *et al.*, 2008). Some antibiotics actually kill the bacteria (bactericidal), whereas others merely prevent the bacteria from multiplying (bacteriostatic) so that the host's immune system can overcome them. Muller Hinton agar is most frequently used agar media in antibiotic susceptibility test (CLSI, 2015).

2.9 Antibiotic resistance

Resistance is an inevitable consequence of antibiotic use; the more antibiotics are used, the more bacteria will develop resistance. In recent years, scientists have begun to understand at the molecular level the sophisticated mechanisms that enable bacteria to fend off or neutralize antibiotics (WHO, 2011). Antibiotic resistance is recognized as a growing problem that poses a major threat to the continued effectiveness of antibiotics used to treat human and veterinary illnesses. Further exacerbating the problem, pharmaceutical companies are developing fewer new antibiotics to replace those that are no longer effective (Silbergeld *et al.,* 2008). Numerous studies have documented direct transference of antibiotic-resistant bacteria from animals to humans. Researchers have found that when antibiotics were administered to animals to treat infections, the prevalence of antibiotic-resistant *E. coli* and *Campylobacter* bacteria also increased in humans (Levy *et al.*, 1976; Smith *et al.*, 2010). Other studies have confirmed that antibiotic-resistant *Campylobacter, Salmonella typhimurium* DT 104 and *Salmonella* Newport have moved from animals to humans through foods of animal origin (Smith *et*

al., 2010; Ribot *et al.,* 2002). Reflecting the fact that bacteria can develop resistance to numerous antibiotics at the same time. A group of related antibiotic-resistant *Salmonella* Newport strains is resistant to most available antimicrobial agents approved for the treatment of salmonellosis, particularly in children (Gupta *et al.,* 2003). The human health consequences of resistant pathogens include more serious infections duration of illness, increased frequency of bloodstream infections (bacteremia), greater likelihood of hospitalization, increased mortality and frequency of treatment failures (Angulo *et al.,* 2004). Health-care costs increase with longer hospital stays and the need for more expensive antibiotics to fight resistant pathogens (Caroline *et al.,* 2013). The antibiotics used to treat resistant pathogens can be more toxic, with more serious side effects in the patients (FDA, 2003; WHO, 2011). Akosua *et al.,* (2017) in a study on antibiotic resistance of Campylobacter found that high levels of resistance were expressed against most of the antibiotics. Faecal and carcass strains showed resistance range of 97–100% to erythromycin which is consistent with work in Nigeria and Spain, where resistance of 81–82.6% to erythromycin has been described (Sáenz *et al.,* 2001; Okunlade *et al.,* 2015), but lower rates have been reported in Ethiopia (60.3%) and USA (55%) (Abamecha *et al.,* 2015; Noormohamed *et al.,* 2013).

Yesef and others in a study in Ethiopia found that multiple drug resistance was common among the bacterial isolates from herbal products. They found that 131(87.3%) of the isolates were resistant to ampicillin, 95(63.3%) resistant to amoxicillin clavulanic acid (augmentin), 92(61.3%) to amoxicillin, and 30(57.7%) to penicillin (Yesef *et al.,* 2016). Adeleye *et al.,* (2005), reported that most of the isolates were resistant to ampicillin,

penicillin, cotrimoxazole, and gentamicin. A study conducted in Saudi Arabia showed that bacterial isolates of Shigella spp., Enterobacter spp., *Escherichia coli, Staphylococcus spp*., and *Klebsiella spp*. were sensitive to amoxicillin and gentamicin (Esimone *et al.,* 2007).

2.10 Molecular PCR based technuques

Primer-directed amplification of DNA, were first used in PCR to amplify cognate regions present at very low levels in the genome, has extended DNA analysis to regions adjacent to sequenced DNA segments, to unknown, and even to the study of RNA expressed sequences (Karlin and Brendel, 2011). Amplified genes are often responsible for drug resistance in cultured cells apart from genes responsible for antibiotic resistance, and this has strengthened the demand for molecular characterization for quick identification of these genes (Kumar *et al*., 2006). The accurate and rapid diagnosis of antibiotic resistance genes in the treatment of bacterial infections is extremely important in preventing the spread of diseases.

PCR-based molecular techniques are often preferred for determination of antibiotic resistance genes (Woodford, 2005). Considering the marked importance of pathogenic bacteria, characterizing the different strains isolated from herbal medicinal products is necessary. This is crucial in studying antimicrobial resistant genes (Duijkeren *et al.,* 2003). Different molecular markers throughout genomic DNA are now being used for the detection of various genes such as Shiga Toxin (Stx) – producing *E. coli* (Stx – EC), using polymerase chain reaction (Osek and Gallein, 2002). Moreover, the role of

plasmids in conferring antimicrobial resistance gene by transforming plasmid preparations from different isolates into *E. coli* wild type, MG1655 has been reported (Osek and Gallein, 2002). Studies also show that virulent strains of *Salmonella enteric* serovar Typhi causing typhoidal fever posses the Vi capsular antigen. Thus, the DNA sequence encoding the Vi antigen, pertaining to the ViaB region is useful in developing DNA based diagnostic tests for *Salmonella enteric* serovah Typhi (Hashimoto *et al.,* 1995). Rapid detection of MRSA by PCR for *mecA* gene coding for methicillin resistance via penicillin binding protein 2a(pBp2a) is well established in some *E. coli* isolates. This PCR has become the "gold standard" for detection of methicillin resistance because methicillin (oxacillin) resistance is often heterogeneously expressed *in vitro* (Hashimoto *et al.,* 1995). Therefore, in this study PCR was used in order to determine the genes responsible for antibiotic resistance in phenotypic resistant bacteria.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study sites

The study was undertaken in Nairobi, the capital and largest city in Kenya, governed by the County Government of Nairobi. Nairobi has several herbal clinics especially in the high densely populated areas. However, herbal products/drugs are also sold in nutrition stores, pharmacies/chemists, supermarkets, local retailers and hawkers, among other outlets (Appendix 1 is the map of Nairobi County).

3.2 Study design

The study employed an exploratory as well as laboratory based experimental design. The exploratory part of the study involved visiting different markets to explore what herbal products were present and then identifying the products for purchase. Experimental design involved laboratory analysis of the collected herbal products. 'Mystery shopper' approach was employed during the purchase of the herbal products hence minimised biasness of information by the vendors.

3.3 Sample collection

During sample collection, the herbal products were purchased at the market price. They were packaged in containers especially of 1kg for the powders and 1litre for the liquids. The liquid unpackaged herbal products were collected in sterile containers while the powdered products were kept in sterile brown envelopes for transportation to the laboratory for processing and analysis. The formulations of the selected samples,

indication and locality where applicable were recorded. The samples were assigned unique codes for use in laboratory assays, data analysis, report writing and presentation of the results. The microbial and mycotoxins contamination were determined at the KEMRI, Centre for Microbiology Research laboratories. Heavy metal analysis was determined at the Mines and Geology Laboratories in the industrial area of Nairobi City.

3.4 Sample size

Due to high number of these unregulated products with many different pharmacological claims, appropriate number of representative samples was selected. Surveys into the quality of conventional drugs in the Kenyan market indicated a general failure rate of about 5-17% (Wafula *et al.*, 2017). Therefore, the study estimated that, approximately 10% of the herbal products could not meet the quality standards. Using the Fischer *et al.*, (1998) formulae: $N = Z^2_{1-a} [P (1-P)]/ D^2$

Where,

N = sample, Z = standard error from the mean at 1.96, P = prevalence (percentage rate of quality failure), D = absolute precision (at 5 %), a = level of significance (at 5 %).

Hence,

$$N = \frac{1.96^2_{1-0.5} [0.10 (1- 0.10)]}{0.05^2}$$

N = 138.3

At 5 % precision, a minimum of 138 herbal products were sampled in this study.

3.5 Sampling method

Vendors of herbal products in Nairobi City formed the sampling frame. The vendors were selected through purposive sampling method and there after snowballing methods was applied. The study employed stratified sampling technique. The stratum were hawkers/roadside vendors, supermarket/shops, herbal clinics, wholesaler/manufacturers/retail and pharmacies/chemist. Since herbal products were not uniform in each stratum, the samples were purchased per vendor to get an appropriate representative number of samples within the study area.

3.6 Microbial analysis

3.6.1 Determination of microbial contamination

Microbiological parameters of interest were; total viable counts, total mould count, total enterobacterial and their count (cfu-Colony forming units) (WHO, 1996; British Pharmacopoeia, 2007). Briefly, 1 gm of herbal powder of each herbal product was aseptically suspended in 1 ml of sterile distilled water, mixed thoroughly and sieved aseptically. Appropriate serial dilutions were made and 0.1 ml of the dilution transferred aseptically to sterile petri dish/plates containing culture media; Sabouraud's Dextrose Agar (Oxoid, UK) supplemented with chloramphenicol to inhibit bacterial contamination for fungal determination. Plate count agar, manital salt agar, salmonella shigella agar, mackonkey agar and nutrient agar were used for bacteria determination. Triplicate plates of each sample were incubated at 30^{o}C for 3-7 days and examined daily for fungal growth while bacteria were incubated at 37^{o}C for 12-18 hours. After incubation, CFU were determined, distinct colonies were sub-cultured. In case of liquid formulations, the

initial concentrations were identified then serial dilution carried out and sample transferred into the culture media and incubated accordingly. Identification of the isolates was carried out macroscopically, microscopically through staining and biochemical tests (Abba *et al*., 2009; WHO, 2007). Microbial contamination was compared with pharmacopoeia and WHO specified limits (WHO, 1996; British Pharmacopoeia, 2007).

3.6.2 Identification of bacteria and fungal contaminants

Colonies that grew on the media were sub cultured on nutrient agar and Sabaroud dextrose agar until pure cultures were obtained. Pure cultures were identified using biochemical techniques and analytical profile index (API) 20E kit where necessary (bioMérieux). Reactions were read according to a standard reading table/chart and identification was done using the analytical profile index book. Cultural and morphological characteristics were used to identify the fungal isolates. Fungal cells were stained using lacto-phenol cotton blue for microscopic identification (Larone, 2014).

3.6.3 Disk diffusion method for antibiotic susceptibility test

Antibiotic susceptibility tests were performed using disc diffusion method. Growth medium, usually Mueller-Hinton agar, was first evenly seeded throughout the plate with the isolate of interest that had been emulsified at a 0.5 macFalant standard concentration. Commercially prepared disks (SXT-Sulphamethoxazole/trimethoprim- 30µg, CAZ-Ceftazidime-30µg, C-Chloramphenical-30µg, CTX-Cefotaxime-30µg, CIP-Ciprofloxacin-30µg, CN- Gentamicin-30µg, PRL- Piperacilin-30µg and NOR – Norfloxacin-30µg) each pre-impregnated with a standard concentration of a particular antibiotic, was evenly dispensed and lightly pressed onto the agar surface. The test antibiotic diffuses outward from the disks, creating a gradient of antibiotic concentration

in the agar such that the highest concentration is found close to the disk with decreasing concentrations further away from the disk. After an overnight incubation, the bacterial growth around each disc was observed. If the test isolate is susceptible to a particular antibiotic, a clear area of "no growth" was observed around that particular disk (CLSI, 2016).

The zone around an antibiotic disk that has no growth is referred to as the zone of inhibition since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. This zone was measured in millimetres (mm) and compared to a standard interpretation chart used to categorize the isolate as susceptible/sensitive, intermediately susceptible or resistant (NLSI, 2006). American type culture collection (ATCC) strains were used for quality control (*E. coli* ATCC no. 258790 and *Staphyloccocus aureus* ATCC no. 398764).

3.6.4 Determination of antibiotics resistant genes from drug resistant bacteria

The isolated and identified bacteria confirmed to be phenotypically resistant to the panel of antibiotics tested were sub-cultured into brain heart infusion broth at 37^{o}C overnight to obtain freshly growing isolates. Total DNA were isolated from 5 ml of a broth culture grown overnight. Briefly, after incubation, bacterial cells were harvested by centrifugation at 3000 RPM/× g for 10 minutes; the cell pellet were re-suspended in phosphate-buffered saline with 100 µg of lysostaphin per ml and incubated at 37°C for 30 min. The phenol/chloroform extraction method was used for nucleic acid extraction and the DNA was precipitated in 1 ml 70 per cent ethanol. The DNA precipitate was

dissolved in 50 µl of TE buffer [10 mM Tris chloride-1 mM EDTA (pH 8.0)] and stored at -20°C until processing (Strommenge *et al.,* 2003).

The PCR amplification was performed in a 25 µl reaction mixture (2.5 ml of 10x reaction buffer without $MgCl_2$; 200 µM of each deoxynucleoside triphospate, 2 mM $MgCl_2$; 2.5 pmol of each primer and approximately 2 – 4 µl of template DNA) and brought up to a 25 µl final volume with sterile DNA/RNA free distilled water. In order to reduce the formation of nonspecific extension products, "hot-start" protocol was adapted. The PCR reactions was hot started for 5 min at 95°C and placed on ice, and 2 µl of Taq polymerase was added. Reaction mixtures were subjected to 30 PCR cycles (95°C for 2 min, 1 min at 54°C and 1 min at 72 °C). A final elongation step at 72°C for 7 min was applied in a thermal cycler (Strommenge *et al.,* 2003). Table 3.1 shows the genes of interest that were investigated in this study.

Table 3.1: Primers name and sequences of antibiotic resistance genes

Gene	Drug	Primer name	Primers sequence 5'------3’	Size (bp)
aacA-*aphD*-	CN	aacA-*aphD* - F	TAA TCC AAG AGC AAT AAG GGC	277
		aacA-*aphD*- R	GCC ACA CTA TCA TAA CCA CTA	
Bla_{CMY}	CAZ	Bla_{cmy} - F	GACAGCCTCTTTCTCCACA	205
		Bla_{cmy} - R	TGGAACGAAGGCTACGTA	
CTX-M	CXT	CTXM1– F3	GAC GAT GTC ACT GGC TGA GC	499
		CTXM1- R2	AGC CG C CGA CGC TAA TAC A	
gyrA	NOR/ CIP	GYRA - F	ATG TCA GAC AAT CAA CAA CAA GC	574
		GYRA – R	ACA TTC TTG CTT CTG TAT AAC GC	
SulA	SXT	*SulA* - F	AC TGC CAC AAG CCG TAA	360
		SulA - R	GTC CGC CTC AGC AAT ATC	

Key: bp- Base pairs, R- Reverse, F- Forward, 5’ – five prime, 3’ – three prime

3.7 Determination of aflatoxins and fumonisins

Screening of the presence of aflatoxins and fumonisins were done using Envirologix Quick Tox^{TM} Kit (Envirologix Inc, USA) following the manufacturer’s instructions. Briefly, the toxins were extracted from the sample using 70% methanol, shaken for 2 minutes before incubating for five minutes. Approximately, 100 µl of the top layer were drawn and put in a small vial and then mixed with 100µl buffer solution and mixed well before applying the strip and incubating for five minutes. The two lines in the rapid strip

indicate a positive test while one line indicates a negative test. The strips were then placed in a Quick Scan Machine-reader and the machine read the concentrations of the toxins and displayed the results on a computer screen in parts per billion (ppb). Two standards with 0.0025 ppb levels of aflatoxins and 0.004ppb of fumonisins were included in the analysis for quality control purposes.

3.8 Heavy metals determination

3.8.1 Procedure for digestion of herbal products for heavy metal assays

Known amounts of each sample (2.5 grams) were weighed into conical flasks. Twenty millimitres of concentrated nitric acid (HNO_3) was then added to each sample, and heated under low temperature until they dissolved. The solution was allowed to cool and then filtered into a 100ml volumetric flask and topped with distilled water. The filtrate was used for the determination of the various heavy metals by the atomic absorption spectroscopy (Garvey *et al.*, 2001; Obi *et al.*, 2006; WHO, 2007).

3.8.2 Full assay procedure for heavy metal analysis

Known volume (0.1 gms) of the samples were measured into 100 ml plastic bottles. One millimetre of quaregia (1:3 HNO_3: HCL) was added, followed by 3 mls of hydrofluoric (HF) acid. This was left to digest overnight. Fifty millimetres of Boric acid was then added, and the mixture incubated for 1 hour. After the one hour incubation, 46 mls of distilled water was used to top the mixture to make a total of 100 mls. The samples were then stored as they waited reading using Atomic Absorption Spectrometer (AAS).

3.8.3 Assays of heavy metals in liquid sample

A volume of 25mls of the liquid samples was measured into 250ml beakers. About 5mls of concentrated nitric acid was added to each sample. Digestion was done on the hotplate until all the dissolved plants materials were digested and the final volume was 10mls. The solution was allowed to cool and then filtered into a 50ml volumetric flask and topped with distilled water. The filtrate was then used for the determination of the various heavy metals by the atomic absorption spectroscopy (Obi *et al.,* 2006; WHO, 2007). Levels of safe exposure for the various elements were calculated and compared with the limits that have been set by the health authorities (FAO/ WHO, 1993).

3.8.4 Determination of pH

Concentration of the hydrogen ion, also known as pH, is the degree of acidity-alkalinity of a substance. It is measured using a pH meter, which reads from 1-14. The pH meters are convenient and their main purpose is to determine the acid or base content of substances by measuring the pH, or concentration of hydrogen ions. The pH readings are categorized as follows; strong acids - pH 1-3; weak acids -pH 4-6; neutral-7; weak bases-pH 8-10 and finally strong bases-pH 11-14. The pH of 138 samples was tested and categorized as strong acids, weak acids, neutral, weak bases and strong bases. A volume of 5 gms of samples were weighed into 100 ml beakers. About 75 ml of distilled water was added and the suspension was stirred vigorously with a magnetic stirrer. It was then covered with a cover glass and allowed to stand for several hours preferably overnight. It was then stirred immediately before pH determination.

3.9 Statistical analysis

Statistic package for social scientist (SPSS) was used for all statistical analysis (SPSS version 20). Ms excel was also used to analyse microbial, mycotoxins and pH values. A significance level of 0.05 was used for all the tests. The microbial contamination data was analysed statistically and results compared with the European pharmacopoeial requirements (European Pharmacopoeia, 2007). The total microbial count was determined and compared with the limits. The mycotoxins concentration in parts per billion (ppb) and heavy metals in parts per million (ppm) as well as sample pH was analysed statistically using Pearson Chi square test and Fisher excert test. The degree of variability of microorganisms was evaluated and tabulated. Phenotypic resistant and genotypic resistance were compared accordingly.

3.10 Approval for the study

Permission to carry out the study was sought from University of Nairobi's Board of Postgraduate Studies and the Department of Medical Microbiology, School of Medicine. Ethical clearance was obtained from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UoN EREC) ref No: KNH-ERC/A/284 P421/07/2014 (Appendix 2). This project was nested within a bigger survey, which was sponsored by the National Commission for Science Technology and Innovation (NACOSTI) ref No: NCST/RCD/ST&I/WS/3rd CALL/104 and was cleared by KEMRI Scientific and Ethical Research Unit (SERU) ref No. 30982.

CHAPTER FOUR

4.0 RESULTS

4.1 Microbial loads in the herbal products

4.1.1 Sampled herbal products

A total of 138 samples of herbal products were collected from different outlets in Nairobi. The outlets were; - street vendors/hawkers, herbal clinics, supermarkets, chemist/pharmacy, health food stores and manufacturers/wholesalers. The sampled herbal products were in different formulations such as; capsules, liquid, juice, cream /lotion, powder and syrups. Herbal products from the following formulations were sampled; 2(1.4%) capsules, 16(11.6%) liquids, 4(2.9%) creams, 2(1.4%) juices, 106(76.9%) powders and 8(5.8%) syrups (Figure 4.1).

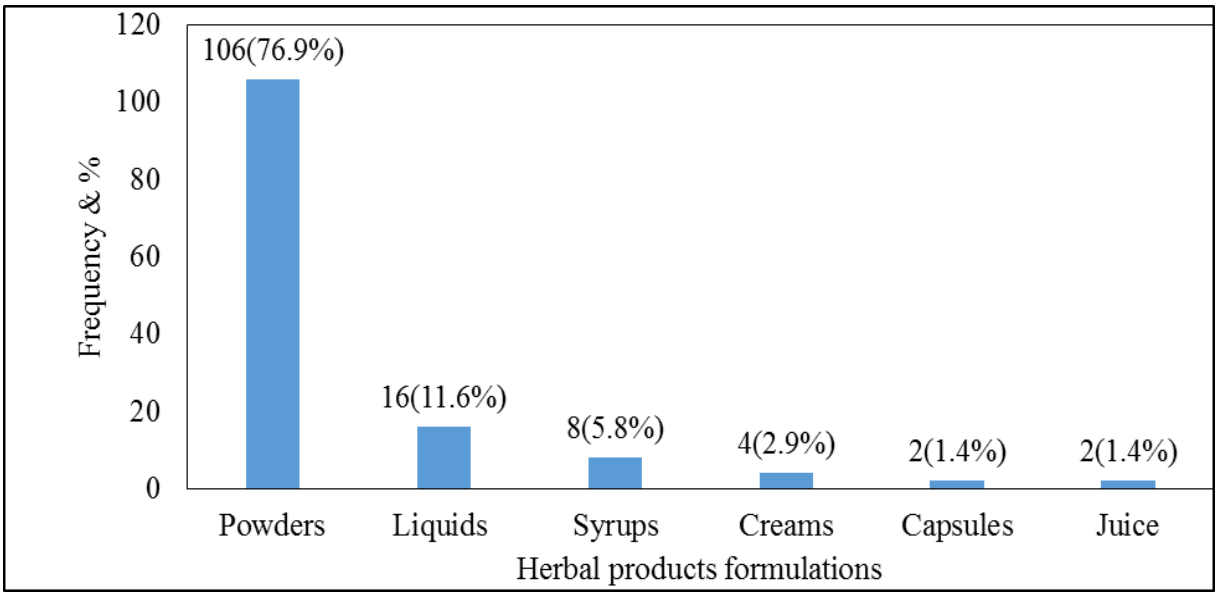

Figure 4.1: Herbal products formulations collected in the study (n=138)
X- axis represent herbal products formulations, Y-axix represents frequecncy and percentages in brackets of the sampled herbal products.

There were 74(53.6%) samples from the street vendors/hawkers, 34(24.6%) from herbal clinics, 19(13.8%) from supermarkets/shops, 7(5.1%) from the manufacturers/ wholesalers and 2(1.4%) each from chemists and health food stores, respectively (Figure 4.2). Appendix 3 shows herbal products displayed in the shelfs of an herbal clinic as well as in the bench awaiting analysis.

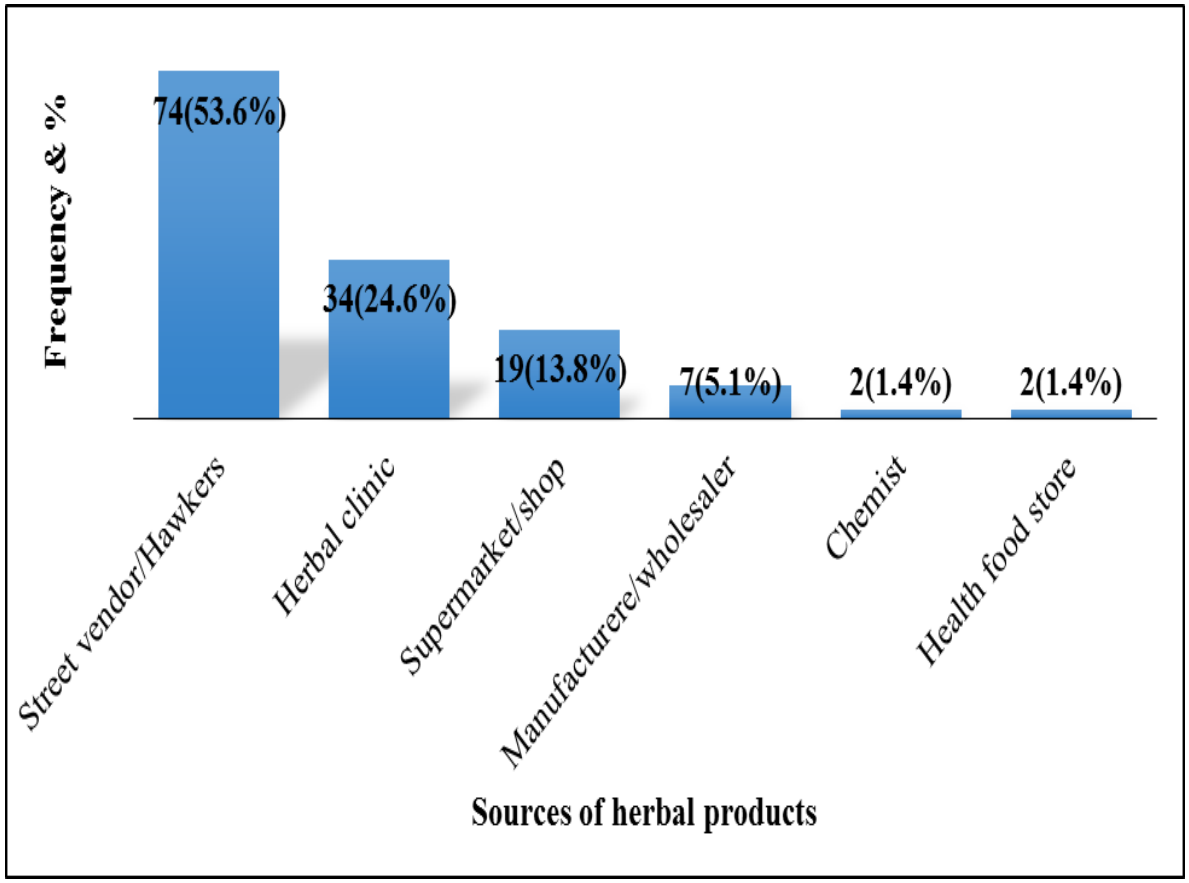

Figure 4.2: Herbal products outlets/source (n=138)
X- axis represent herbal products sources where they were collected, Y-axix represents frequecncy and percentages in brackets of the sampled herbal products.

4.1.2 Bacterial contaminations (loads) in terms of colony forming units

Out of the 138 samples, 117(84.8%) were contaminated with bacteria while 21(15.2%) were not contaminated. The contaminating bacteria were aerobic, coli-forms and other pathogenic bacteria. Among the 117 contaminated samples 61(4.2%) had bacterial colony

forming units ranging from 1-1000, while 56(40.6%) had more than 1000 bacterial colony forming units. All the products from chemist/pharmacy were not contaminated. Among the products from the manufacturer, 2(2.8%) had between 1-1000 bacteria cfu while 5(71.4%) had more than 1000 bacterial cfu. Five (14.7%) products from herbal clinics were not contaminated, 14(41.2%) had bacterial cfu ranging from 1-1000 while 15(44.1%) were contaminated beyond 1000 bacterial cfu. Eleven products from the street vendors/hawkers were not contaminated, 33(44.6%) had bacterial cfu from 1-1000, while 30(40.5%) had bacterial cfu above 1000. Among the products from the supermarkets, 2(10.5%) were not contaminated, 11(57.9%) had bacterial cfu beoween 1-1,000, while 30(40.5%) had bacterial cfu above 1000. Appendix 4 is a table showing recommended microbial limits for herbal drugs (values in CFU/g).

In terms of formulations, all the capsules were contaminated with bacterial cfu range from 1-1,000. Among the liquids, 4(25.0%) were not contaminated, 6(37.5%) had cfu range from 1-1000 and beyond 1,000, respectively. All the juices were not contaminated while 2(50.0%) creams/lotions had cfu range of 1-1,000 and beyond 1,000, respectively. Nine (8.7%) of the powders were sterile, 48(45.0%) had bacterial contamination ranging from 1-1,000cfu while 49(46.2%) had bacterial cfu above 1,000. Four (50.0%) of the syrups were not contaminated, 3(37.5%) had bacterial cfu ranging from 1-1,000 while 1(12.5%) had bacterial cfu above 1,000. The samples from different formulations were not uniform. There was significant associations between (χ^2 test; $p<0.05$) powders and liquids formulation with bacterial contaminations (Table 4.1). Plate 4.1 is culture plates of bacterial colonies.

Table 4.1: Bacterial contamination in terms of source and formulations

Source	**Contamination Level in cfu**			**n/freq**	χ^2
	0	**1-1000**	**> 1000**		
Chemist	2 (100.0%)	0(0.0%)	0(0.0%)	2	
Health Food Store	1(50.0%)	1(50.0%)	0(0.0%)	2	
Herbal Clinic	5 (14.7%)	14(41.2%)	15(44.1%)	34	0.001
Manufacturer/ Wholeseller	0(0.0%)	2(28.6%)	5(71.4%)	7	0.157
Street vendor/ Hawker	11(14.9%)	33(44.6%)	30(40.5%)	74	0.001
Supermarket/ Shop	2(10.5%)	11(57.9%)	6(31.6%)	19	0.001
Total	21(15.2%)	61(44.2%)	56(40.6%)	138	
Formulations	**Contamination Level in cfu**			**n/freq**	χ^2
Capsules	0(0.0%)	2(100.0%)	0(0.0%)	2	
Liquid	4(25.0%)	6(37.5%)	6(37.5%)	16	0.001
Juice	2(100.0%)	0(0.0%)	0(0.0%)	2	
Cream /Lotion	2(50.0%)	2(50.0%)	0(0.0%)	4	0.083
Powder	9(8.5%)	48(45.3%)	49(46.2%)	106	0.001
Syrup	4(50.0%)	3(37.5%)	1(12.5%)	8	0.180
Total	21(15.2%)	61(44.2%)	56(40.6%)	138	

Key: 0-No contamination, 1-1000- Bacterial cfu range, >1000-fungal cfu more than 1000, n-number of samples per category, χ^2- Pearson chi square test.

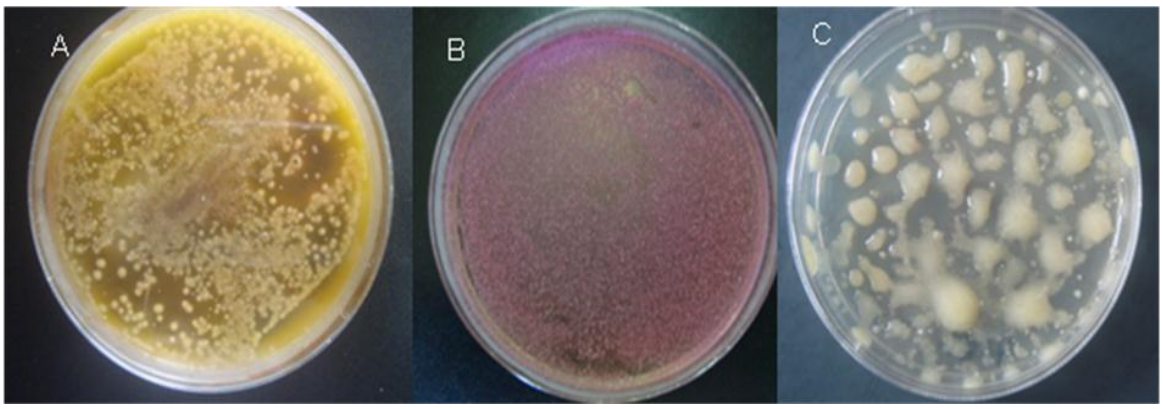

Plate 4.1: Culture plates of bacterial colonies (A, B and C)
A is a salmonella shigella agar with lactose fermenting bacteria with some colonies being dark centred due to hydrogen sulphide production, B- is a MacConkey plate with pink colonies, C- is a plate count showing environmental bacteria which are very large and mucoid. These wre obtained from samples collected from herbal clinics in form of powders.

4.1.3 Fungi Contamination

Out of 138 herbal products, 61(44.2%) samples were contaminated with fungi. The total molds and yeast counts were presented in colony forming units. All the samples from the chemist, health food stores, 1(14.3%) from manufactures/wholesalers, 37(50.0%) from street vendors/hawkers and 13(68.4%) from the supermarkest/shops were sterile (no contamination). Eleven (32.4%) samples from herbal clinics, 6(85.7%) from wholesalers/manufacturers, 34(45.9%) from street vendors/hawkers and 6(31.6%) from supermarkets/shops had fungal cfu ranging from 1-105. One sample (2.9%) from herbal clinics and 3(4.1%) from street vendors/hawkers had fungal cfu above 105. There was significant association (χ^2 test; p=0.001) between herbal products from street vendors/hawkers and herbal clinics with fungal contaminations.

Regarding formulations, all juices, 1(50.0%) capsule, 15(93.85) liquids, 50(47.25) powders and 7(87.5%) syrups had no fungal contaminations. Two creams/lotions (50.0%), 52(50.0%) powders and one capsule and syrup respectively had fungal cfu range of 1-150. Only powders [4(3.8%)] had cfu above 105. There was significant association (χ^2 test; p=0.001) between powders and liquid formulations with fungal contaminations (Table 4.2). Plate 4.2 is culture plates of fungi colonies.

Table 4.2: Fungal contamination in terms of source and formulations

Source	**Fungal contamination level**			**n/Freg**	χ^2
	0	**1-105**	**>105**		
Chemist	2(100.0%)	0(0.0%)	0(0.0%0	2	
Health Food Store	2(100.0%)	0(0.0%)	0(0.0%)	2	
Herbal Clinic	22(64.7%)	11(32.4%)	1(2.9%)	34	0.001
Manufacturer/ Wholeseller	1(14.3%)	6(85.7%)	0(0.0%)	7	0.080
Street vendor/ Hawker	37(50.0%)	34(45.9%)	3(4.1%)	74	0.001
Supermarket/ Shop	13(68.4%)	6(31.6%)	0(0.0%)	19	0.100
Total	77(55.8%)	57(41.3%)	4(2.9%)	138	
Formulations		**Fungal contamination level**		**n/Freg**	χ^2
Capsules	1(50.0%)	1(50.0%)	0(0.0%)	2	
Liquid	15(93.8%)	1(6.3%)	0(0.0%)	16	0.001
Cream/lotions	2(50.0%)	2(50.0%)	0(0.0%)	4	0.080
Juice	2(100.0%)	0(0.0%)	0(0.0%)	2	
Powder	50(47.2%)	52(50.0%)	4(3.8%)	106	0.001
Syrup	7(87.5%)	1(12.5%)	0(0.0%)	8	0.105
Total	77(55.8%)	57(41.3%)	4(2.9%)	138	

Key: 0-No contamination, 1-105- Fungal cfu range, >105-fungal cfu more than 105, χ^2 – Pearson Chi square test, n-number of samples per category

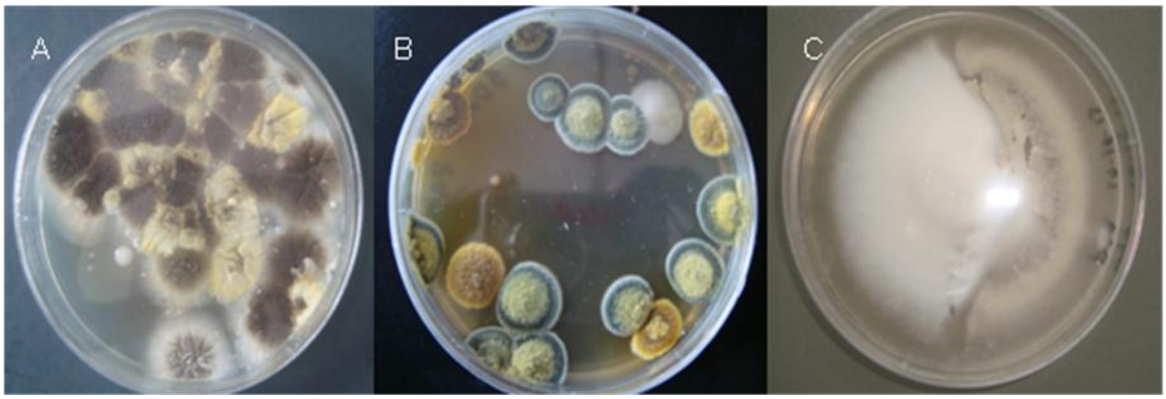

Plate 4.2: SDA culture plates of fungi colonies (A, B and C)
A shows *Aspergillus niger* and some yeast, B-*Penicillium* species and *Aspergillus* spp, *C-Absidia corymbifera*. These were obtained from samples from street vendors inform of liquids.

4.2 Identification of the contaminating microorganisms from the herbal products

4.2.1 Bacterial contaminants

The bacterial contaminants isolated from the herbal products collected in Nairobi were grouped into 13 genera; Bacillus, Klebsiella, Proteus, Staphylococcus, Streptomyces, Escherichia, Enterobacter, Serratia, Yersnia, Morganella, Citrobacter, Erwinia and Shigella (Appendix 5). The isolated bacteria were identified to species level using cultural and morphological characteristics. The following bacteria were isolated and identified in this study; *Enterobacter aerogens, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Proteus penneri, Serratia fonticola, Serratia marcescens, Serratia rubidaea, Streptomyces spp., Citrobacter diversus, Erwinia chrysanthemi, Morganella morganii, Shigella sonnei, Bacillus spp, Bacillus anthracoides, Staphylococcus aureus* and *Yersinia enterocolitica.* They were also grouped in terms of gram staining reaction. Out of the 13 genera of bacteria 3 were gram positives

(Streptomyces, Bacillus and Staphylococcus) (Table 4.3). Plate 4.3 are gram reactions of bacterial isolates.

Table 4.3: Bacterial pathogens isolated from herbal products in Nairobi (n=233)

Genus	Organism	Gram reaction	Frequency (%)
Citrobacter	*Citrobacter diversus*	Gram -ve	3 (1.29)
Enterobacter	*Enterobacter aerogens, Enterobacter cloacae*	Gram -ve	22 (9.44)
Streptomyces	*Streptomyces spp.*	Gram +ve	74(31.76)
Bacillus	*Bacillus anthracoides, Bacillus spp.*	Gram +ve	64(27.47)
Erwinia	*Erwinia chrysanthemi*	Gram -ve	1 (0.42)
Escherichia	*Escherichia coli*	Gram -ve	7 (3.0)
Morganella	*Morganella morganii*	Gram -ve	2 (0.86)
Klebsiella	*Klebsiella pneumoniae*	Gram -ve	4 (1.72)
Proteus	*Proteus penneri*	Gram -ve	25 (10.73)
Serratia	*Serratia fonticola, Serratia marcescens, Serratia rubidaea*	Gram -ve	14 (6.01)
Shigella	*Shigella sonnei*	Gram -ve	1 (0.43)
Staphylococcus	*Staphylococcus aureus*	Gram +ve	5 (2.15)
Yersnia	*Yersnia enterocolitica*	Gram -ve	11 (4.72)
			233 (100.0)

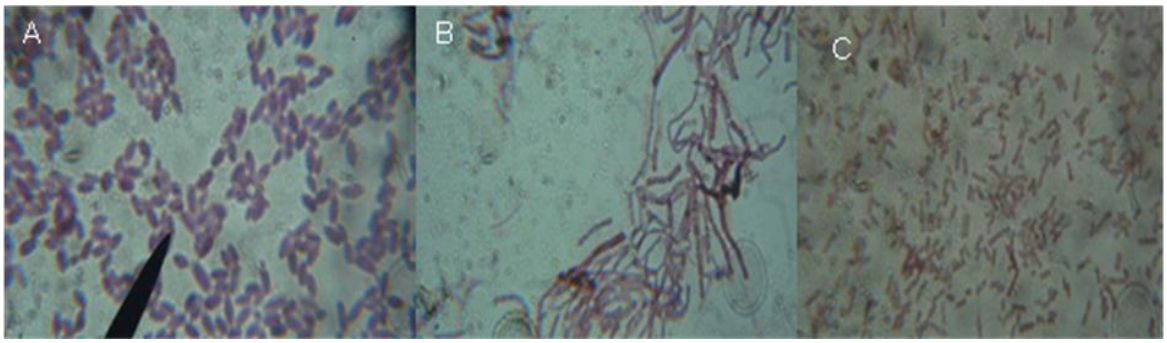

Plate 4.3: Gram stain reactions of bacteria isolated from herbal products (A, B and C)

Gram reactions of some isolates as seen in oil immersion (x100 objective of a microscope). A- Gram negative rods, B-Gram negative large rods, C-Gram negative short rods

4.2.2 The spectrum of fungi isolated from the herbal products

The following fungi were isolated and identified from the herbal products; *Absidia corymbifera, Alternaria alternate, Aspergillus candidus, A. flavus, A. fumigatus, A. glaucus, A. nidulans, A. niger, A. parasiticus, A. ustus, A. versicolor, Candida glabrata, Penicillium spp., P. verrucosum, Rhizomucor pusillus, Rhizopus arrhizus, Fusarium spp* and *Rhodotorula glutinis*. The fungi were grouped into three categories namely; Filamentous, dimorphic and yeasts (Table 4.4). Plate 4.4 are microscopic presentation of fungi.

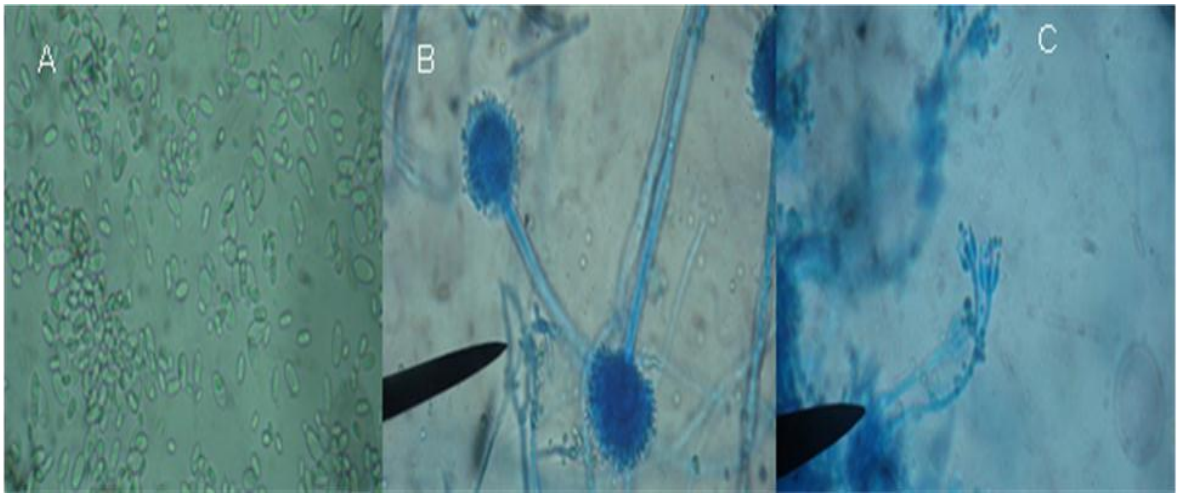

Plate 4.4: Microscopic presentation of *Candida glabrata* (A), *Aspergillus* spp (B) and *Penicillium* spp (C)
A- *Candida* species (yeast), B- *Aspergillus* species and C- *Penicillium* species under a microscope (x40 objective).

Table 4.4: The Genus and specific epithets of the fungi isolated from HMP

Genus	Species	Description	Freguency (%)
Absidia	*Absidia corymbifera*	Filamentous	4 (2.8)
Alternaria	*Alternaria alternata*	Dimorphic	4 (2.8)
Aspergillus	*Aspergillus candidus, A. flavus, A. fumigatuss, A. glaucus, A. nidulans, A. niger, A. parasiticus, A. ustus, A. versicolor*	Filamentous	62 (43.4)
Candida	*Candida glabrata*	Yeast	13 (9.1)
Fusarium	*Fusarium moliniform, F. oxysporum, F. solani, Fusarium spp.*	Filamentous	25 (17.5)
Penicillium	*Penicillium spp*	Filamentous	23 (16.1)
Rhizomucor	*Rhizomucor pusillus*	Filamentous	2 (1.4)
Rhizopus	*Rhizopus arrhizus*	Filamentous	4 (2.8)
Rhodotorula	*Rhodotorula glutinis*	Yeats	6 (4.2) 143 (100.0)

4.3 Antibiotic susceptibility test of the isolated pathogenic bacteria

Out of the 233(100%) bacterial isolates, only 96(41.2%) bacteria were tested for susceptibility to the commonly used antibiotics. *Streptomyces* and *Bacillus* species (137[58.8%]) were excluded because they are not commonly isolated from clinical specimen. These two genera of bacteria are considered environmental contaminants. Most of the isolated bacterial were generally sensitive to the panel of antibiotics tested in this study. The results showed that 35(36.5%) bacteria were resistant to at least one of the antibiotic tested.

4.3.1 Susceptibility test of isolated bacteria to Sulphamethoxazole/ trimethoprim

Majority [89(92.1%)] of the isolated bacteria were sensitive, 1(1.0%) was intermediate while 6(6.3%) were resistant to sulphamethoxazole/ trimethoprim. All the seven *E. coli* and 2(100.0%) *Shigella sonnei* isolates were sensitive to SXT. Three *Klebsiella*

pneumonia isolates were sensitive while one was resistant to this drug. All the [5(100%)] isolated *Staphylococcus aureus* and *Serratia* species were sensitive to SXT (Table 4.5). Plate 4.5 shows drug susceptibility test.

Table 4.5: Susceptibility of the isolated bacteria to Sulphamethoxazole/ trimethoprim

Isolates	**Susceptibility pattern n (%)**			
	S	I	R	T
Citrobacter diversus	1(33.3)	0 (0.0)	2(66.7)	3(100)
Enterobacter aerogens	8(100)	0 (0.0)	0(0)	8(100)
Enterobacter cloacae	13(92.9)	0 (0.0)	1(7.1)	14(100)
Erwinia chrysanthemi	1(100)	0 (0.0)	0(0)	1(100)
Escherichia coli	7(100)	0 (0.0)	0(0)	7(100)
Klebsiella pneumonia	3(75)	0 (0.0)	1(25)	4(100)
Morganella morganii	1(50)	0 (0.0)	1(50)	2(100)
Proteus penneri	24(96)	1 (100.0)	0(0)	25(100)
Serratia fonticola	3(100)	0 (0.0)	0(0)	3(100)
Serratia marcescens	4(100)	0 (0.0)	0(0)	4(100)
Serratia rubidaea	7(100)	0 (0.0)	0(0)	7(100)
Shigella sonnei	2(100)	0 (0.0)	0(0)	2(100)
Staphylococcus aureus	5(100)	0 (0.0)	0(0)	5(100)
Yersnia enterocolitica	10(90.9)	0 (0.0)	1(9.1)	11(100)
Total	**89(92.7)**	**1 (1.0)**	**6(6.3)**	**96(100)**

Key: S-Sensitive, I-Intermediate, R-Resistant, T-Total, (n)-Frequency, (%)-percentage

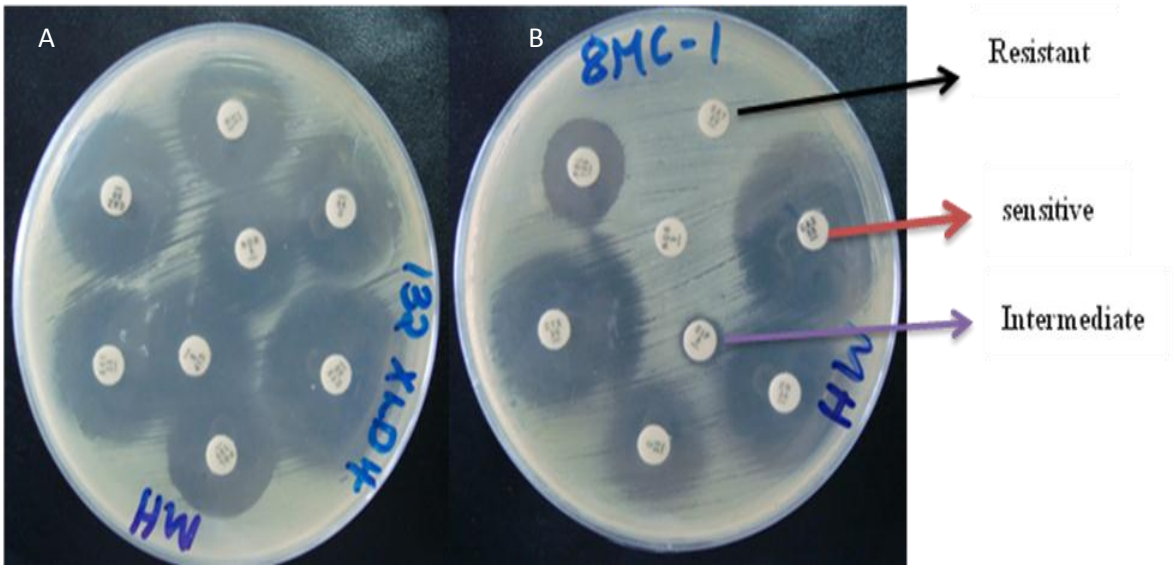

Plate 4.5: Drugs susceptibility test of some isolates (A-*S. aureus and* B- *P. penneri*)
A shows a pathogen (*Staphylococcus aureus*) susceptible to all antibiotics tested, while B shows (*Proteus penneri*) various degree of susceptibilities (sensitive, intermediary sensitive and resistant, respectively).

4.3.2 Susceptibility test of isolated bacteria to Ceftazidime

Table 4.6 shows that 52(53.2%) isolates were sensitive, 13(13.8%) were intermediate and 31(33.0%) were resistant to ceftazidime. All the isolated *E. coli* and *Shigella sonnei* were sensitive to ceftazidime. The five (5) *Staphylococcus aureus* isolates were intermediary sensitive.

Table 4.6: Susceptibility patterns of bacteria isolated from HMPs in Nairobi to Ceftazidime

Bacterial isolates	Susceptibility pattern n (%)			
	S	I	R	T
Citrobacter diversus	3 (100.0)	0 (0.0)	0 (0.0)	3(100.0)
Enterobacter aerogens	6 (75.0)	1 (12.5)	1 (12.5)	8 (100.0)
Enterobacter cloacae	7 (50.0)	0 (0.0)	7 (50.0)	14 (100.0)
Erwinia chrysanthemi	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)
Escherichia coli	7 (100.0)	0 (0.0)	0 (0.0)	7 (100.0)
Klebsiella pneumoniae	1 (50.0)	1 (50.0)	0 (0.0)	2 (100.0)
Morganella morganii	5 (55.6)	0 (0.0)	4 (44.4)	9 (100.0)
Proteus penneri	11 (50.0)	2 (9.1)	9 (40.9)	22 (100.0)
Serratia fonticola	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
Serratia marcescens	1 (50.0)	1 (50.0)	0 (0.0)	2 (100.0)
Serratia rubidaea	1 (25.0)	0 (0.0)	3 (75.0)	4 (100.0)
Shigella sonnei	2 (28.6)	2 (28.6)	3 (42.8)	7 (100.0)
Staphylococcus aureus	0 (0.0)	5 (100.0)	0 (0.0)	5 (100.0)
Yersnia enterocolitica	7 (63.6)	1 (9.1)	3 (27.3)	11 (100.0)
Total	**52 (53.2)**	**13 (13.8)**	**31 (33.0)**	**96 (100.0)**

Key: S-Sensitive, I-Intermediate, R-Resistant, T-Total, (n)-Frequency, (%)-percentage

4.3.3 Susceptibility test of isolated bacteria to Cefotaxime

Susceptibility profile of the isolated bacteria to cefotaxime showed that 50(50.1%) were sensitive, 13(13.5%) were intermediary sensitive while 33(34.4%) were resistant to CTX. Most [11(50.0%)] of the isolated *Proteus penneri* were resistant to cefotaxime. Six (42.9%) isolated *Enterobacter cloacae* and 3(33.3%) *Klebsiella pneumonia* were resistant while all the *E. coli* and *Shigella sonnei* were sensitive (Table 4.7).

Table 4.7: Susceptibility patterns of isolated bacteria from HMPs in Nairobi to Cefotaxime

Bacterial isolates	Susceptibility pattern n (%)			
	S	I	R	T
Citrobacter diversus	0 (0.0)	2 (66.7)	1 (33.3)	3 (100.0)
Enterobacter aerogens	7 (87.5)	0 (0.0)	1 (12.5)	8 (100.0)
Enterobacter cloacae	7 (50.0)	1 (7.1)	6 (42.9)	14 (100.0)
Erwinia chrysanthemi	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)
Escherichia coli	7 (100.0)	0 (0.0)	0 (0.0)	7 (100.0)
Klebsiella pneumoniae	1 (50.0)	0 (0.0)	1 (50.0)	2 (100.0)
Morganella morganii	6 (66.7)	0 (0.0)	3 (33.3)	9 (100.0)
Proteus penneri	10 (45.5)	1 (4.5)	11 (50.0)	22 (100.0)
Serratia fonticola	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)
Serratia marcescens	1 (50.0)	1 (50.0)	0 (0.0)	2 (100.0)
Serratia rubidaea	1 (25.0)	0 (0.0)	3 (75.0)	4 (100.0)
Shigella sonnei	3 (42.8)	2 (28.6)	2 (28.6)	7 (100.0)
Staphylococcus aureus	0 (0.0)	5 (100.)	0 (0.0)	5 (100.0)
Yersnia enterocolitica	6 (54.5)	1 (0.1)	4 (36.4)	11 (100.0)
Total	50 (52.1)	13 (13.5)	33 (34.4)	96 (100.0)

Key: S-Sensitive, I-Intermediate, R-Resistant, T-Total, (n)-Frequency, (%)-percentage

4.3.4 Susceptibility test of isolated bacteria to Gentamicin

A total of 94(97.9%) isolated bacteria were sensitive to gentamicin while 2(2.1%) were resistant with no intermediates. Only 1(33.3%) *Citrobacter diversus* and 1(4.0%) *Proteus penneri* were resistant to this drug (Table 4.8).

Table 4.8: Susceptibility pattern of bacteria isolated from HMPs in Nairobi to Gentamicin

Bacterial isolates	Susceptibility pattern n (%)			
	S	I	R	T
Citrobacter diversus	2(66.7)	0 (0.0)	1 (33.3)	3(100)
Enterobacter aerogens	8(100)	0 (0.0)	0 (0.0)	8(100)
Enterobacter cloacae	14(100)	0 (0.0)	0 (0.0)	14(100)
Erwinia chrysanthemi	1(100)	0 (0.0)	0 (0.0)	1(100)
Escherichia coli	7(100)	0 (0.0)	0 (0.0)	7(100)
Klebsiella pneumoniae	4(100)	0 (0.0)	0 (0.0)	4(100)
Morganella morganii	2(100)	0 (0.0)	0 (0.0)	2(100)
Proteus penneri	24(96)	0 (0.0)	1 (4.0)	25(100)
Serratia fonticola	3(100)	0 (0.0)	0 (0.0)	3(100)
Serratia marcescens	4(100)	0 (0.0)	0 (0.0)	4(100)
Serratia rubidaea	7(100)	0 (0.0)	0 (0.0)	7(100)
Shigella sonnei	2(100)	0 (0.0)	0 (0.0)	2(100)
Staphylococcus aureus	5(100)	0 (0.0)	0 (0.0)	5(100)
Yersnia enterocolitica	11(100)	0 (0.0)	0 (0.0)	11(100)
Total	94(97.9)	0 (0.0)	2 (2.1)	96(100)

Key: S-Sensitive, I-Intermediate, R-Resistant, T-Total, (n)-Frequency, (%)-percentage

4.3.5 Susceptibility test of isolated bacteria to Chloramphenicol

Among all the isolates tested for drug susceptibilities, 90(93.8%) were sensitive to chloramphenicol, 1(1.0%) was intermediate while five (5.2%) were resistant to this drug. One (33.3%) *Citrobacter diversus,* 2(100.0%) *Morganella morganii,* 2(18.2%) *Yersinia enterocolitica* were resistant to chloramphenicol. However, only one (9.1) *Yersinia enterocolitica* was intermediary sensitive to chloramphenicol (Table 4.9).

Table 4.9: Susceptibility patterns of the isolated bacteria to Chloramphenical

Bacterial isolates	Susceptibility pattern n (%)			
	S	**I**	**R**	**T**
Citrobacter diversus	2(66.7)	0 (0.0)	1 (33.3)	3(100)
Enterobacter aerogens	8(100)	0 (0.0)	0 (0.0)	8(100)
Enterobacter cloacae	14(100)	0 (0.0)	0 (0.0)	14(100)
Erwinia chrysanthemi	1(100)	0 (0.0)	0 (0.0)	1(100)
Escherichia coli	7(100)	0 (0.0)	0 (0.0)	7(100)
Klebsiella pneumoniae	4(100)	0 (0.0)	0 (0.0)	4(100)
Morganella morganii	0(0)	0 (0.0)	2 (100.0)	2(100)
Proteus penneri	25(100)	0 (0.0)	0 (0.0)	25(100)
Serratia fonticola	3(100)	0 (0.0)	0 (0.0)	3(100)
Serratia marcescens	4(100)	0 (0.0)	0 (0.0)	4(100)
Serratia rubidaea	7(100)	0 (0.0)	0 (0.0)	7(100)
Shigella sonnei	2(100)	0 (0.0)	0 (0.0)	2(100)
Staphylococcus aureus	5(100)	0 (0.0)	0 (0.0)	5(100)
Yersnia enterocolitica	8(72.7)	1 (9.1)	2 (18.2)	11(100)
Total	90(93.8)	1 (1.0)	5 (5.2)	96(100)

Key: S-Sensitive, I-Intermediate, R-Resistant, T-Total, (n)-Frequency, (%)-percentage

4.3.6 Susceptibility test of isolated bacteria to Piperacillin

Drug susceptibility test of the isolated bacteria to piperacillin showed that, 93(96.9%) were sensitive, two (2.1%) were intermediary sensitive while one (1.0%) was resistant. However, one (33.3%) *Citrobacter diversus* and one (7.1%) *Enterobacter cloacae* were intermediary sensitive. Only one (12.5%) *Enterobacter aerogens* was resistant to piperacillin (Table 4.10).

Table 4.10: Susceptibility profile of bacteria isolated from HMPs in Nairobi to Piperacillin

Bacterial isolates	Susceptibility pattern n (%)			
	S	I	R	T
Citrobacter diversus	2(66.7)	1 (33.3)	0 (0.0)	3(100)
Enterobacter aerogens	7(87.5)	0 (0.0)	1 (12.5)	8(100)
Enterobacter cloacae	13(92.9)	1 (7.1)	0 (0.0)	14(100)
Erwinia chrysanthemi	1(100)	0 (0.0)	0 (0.0)	1(100)
Escherichia coli	7(100)	0 (0.0)	0 (0.0)	7(100)
Klebsiella pneumoniae	4(100)	0 (0.0)	0 (0.0)	4(100)
Morganella morganii	2(100)	0 (0.0)	0 (0.0)	2(100)
Proteus penneri	25(100)	0 (0.0)	0 (0.0)	25(100)
Serratia fonticola	3(100)	0 (0.0)	0 (0.0)	3(100)
Serratia marcescens	4(100)	0 (0.0)	0 (0.0)	4(100)
Serratia rubidaea	7(100)	0 (0.0)	0 (0.0)	7(100)
Shigella sonnei	2(100)	0 (0.0)	0 (0.0)	2(100)
Staphylococcus aureus	5(100)	0 (0.0)	0 (0.0)	5(100)
Yersnia enterocolitica	11(100)	0 (0.0)	0 (0.0)	11(100)
Total	93(96.9)	2 (2.1)	1 (1.0)	96(100)

Key: S-Sensitive, I-Intermediate, R-Resistant, T-Total, (n)-Frequency, (%)-percentage

4.3.7 Susceptibility test of isolated bacteria to Norfloxacin

Susceptibility profile of isolated bacteria to norfloxacin drug showed that 89(93.7%) bacterial isolates were sensitive, 4(4.2%) were intermediary sensitive while 2(2.1%) were resistant to this drug. Only two (66.75%) *Citrobacter diversus* isolates were resistant to norfloxacin. One (12.5%) *Enterobacter aerogens*, 1(7.7%) *Enterobacter cloacae* and 1(4.0%) *Yersinia enterocolitica* were intermediary sensitive to norfloxacin (Table 4.11).

Table 4.11: Susceptibility patterns of bacteria isolated from HMPs in Nairobi to Norfloxacin

Bacterial isolates	Susceptibility pattern n (%)			
	S	I	R	T
Citrobacter diversus	1(33.3)	0 (0.0)	2 (66.7)	3(100)
Enterobacter aerogens	7(87.5)	1 (12.5)	0 (0.0)	8(100)
Enterobacter cloacae	12(92.3)	1 (7.7)	0 (0.0)	13(100)
Erwinia chrysanthemi	1(100)	0 (0.0)	0 (0.0)	1(100)
Escherichia coli	7(100)	0 (0.0)	0 (0.0)	7(100)
Klebsiella pneumoniae	4(100)	0 (0.0)	0 (0.0)	4(100)
Morganella morganii	2(100)	0 (0.0)	0 (0.0)	2(100)
Proteus penneri	24(96)	1 (4.0)	0 (0.0)	25(100)
Serratia fonticola	3(100)	0 (0.0)	0 (0.0)	3(100)
Serratia marcescens	4(100)	0 (0.0)	0 (0.0)	4(100)
Serratia rubidaea	7(100)	0 (0.0)	0 (0.0)	7(100)
Shigella sonnei	2(100)	0 (0.0)	0 (0.0)	2(100)
Staphylococcus aureus	5(100)	0 (0.0)	0 (0.0)	5(100)
Yersnia enterocolitica	10(90.9)	1 (9.1)	0 (0.0)	11(100)
Total	89(93.7)	4 (4.2)	2 (2.1)	95(100)

Key: S-Sensitive, I-Intermediate, R-Resistant, T-Total, (n)-Frequency, (%)-percentage

4.3.8 Susceptibility test of isolated bacteria to Ciprofloxacin

Ninety-four (96.9%) isolated bacteria were sensitive to ciprofloxacin while 2(2.1%) isolates were resistant to this drug. There was no intermediary resistance/sensitive. Only one of each *Citrobacter diversus* (33.3%) and *Yersinia enterocolitica* (9.1%), respectively, were resistant to ciprofloxacin (Table 4.12).

Table 4.12: Susceptibility pattern of bacteria isolated from HMPs in Nairobi to Ciprofloxacin

Bacterial isolates	Susceptibility pattern n (%)			
	S	I	R	T
Citrobacter diversus	2(66.7)	0 (0.0)	1 (33.3)	3(100)
Enterobacter aerogens	8(100)	0 (0.0)	0 (0.0)	8(100)
Enterobacter cloacae	14(100)	0 (0.0)	0 (0.0)	14(100)
Erwinia chrysanthemi	1(100)	0 (0.0)	0 (0.0)	1(100)
Escherichia coli	7(100)	0 (0.0)	0 (0.0)	7(100)
Klebsiella pneumoniae	4(100)	0 (0.0)	0 (0.0)	4(100)
Morganella morganii	2(100)	0 (0.0)	0 (0.0)	2(100)
Proteus penneri	25(96)	0 (0.0)	0 (0.0)	25(100)
Serratia fonticola	3(100)	0 (0.0)	0 (0.0)	3(100)
Serratia marcescens	4(100)	0 (0.0)	0 (0.0)	4(100)
Serratia rubidaea	7(100)	0 (0.0)	0 (0.0)	7(100)
Shigella sonnei	2(100)	0 (0.0)	0 (0.0)	2(100)
Staphylococcus aureus	5(100)	0 (0.0)	0 (0.0)	5(100)
Yersnia enterocolitica	10(90.9)	0 (0.0)	1 (9.1)	11(100)
Total	94(96.9)	0 (0.0)	2 (2.1)	96(100)

Key: S-Sensitive, I-Intermediate, R-Resistant, T-Total, (n)-Frequency, (%)-percentage

The prevalence of resistance was eveidenced among some bacteria. The isolated bacteria showed resistance to one, two, three and four antibiotics, respectively. The following isolates were resistant to only one antibiotic; *Morganella morganii* (MM2) was resistant to chloramphenical, *Enterobacter cloacae* (EC3) isolate was resistant to ceftazidime and *Proteus penneri* (PP20) was resistant to cefotaxime. Four isolates were resistant to three antibiotics in this study. The isolates were *Citrobacter diversus* (CD1) resistant to CN, CTX and NOR, *Morganella morganii* (MM1) resistant to SXT, C and CTX, *Enterobacter aerogens* (EA2) resistant to CAZ, CTX and PRL and *Klebsiella pneumonia* (KP2) resistant to SXT, CAZ and CTX. Two isolates exhibited resistance to four antibiotics. The isolates were *Citrobacter diversus* (CD2) resistant to SXT, C, NOR and CIP and *Enterobacter* cloacae (EC5) was resistant to SXT, CAZ, CTX and NOR. Twenty-six isolates were resistant to two antibiotics. Most of the isolates 22(62.9%) were

resistant to both CAZ and CTX. These resistant bacteria were isolated from herbal products sourced from; herbal clinics, street vendors and supermarkets and were formulated into powders, liquids and a few creams as shown in Table 4.13.

Table 4.13: Antibiotic resistant profiles of isolated bacteria from HMPs in Nairobi

No.	Isolate	Code	Drug resistant	Source	Form
1	*C. diversus*	CD1	CN, CTX and NOR	Supermarkets	Powder
2	*C. diversus*	CD2	SXT, C, NOR and CIP	Street vendor	Powder
3	*E. cloacae*	EC2	CAZ and CTX	Street vendor	Liquid
4	*E. cloacae*	EC3	CAZ	Street vendor	Liquid
5	*E. cloacae*	EC4	CAZ and CTX	Street vendor	Powder
6	*E. cloacae*	EC5	SXT, CAZ, CTX & NOR	Street vendor	Cream
7	*E. cloacae*	EC8	CAZ and CTX	Supermarket	Powder
8	*E. cloacae*	EC9	CAZ and CTX	Herbal clinic	Liquid
9	*E. cloacae*	EC10	CAZ and CTX	Street vendor	Powder
10	*E. aerogens*	EA2	CAZ, CTX and PRL	Herbal clinic	Liquid
11	*E. chrysanthemi*	ERC1	CAZ and CTX	Street vendor	Powder
12	*K. pneumoniae*	KP2	SXT, CAZ and CTX	Herbal clinic	Powder
13	*M. morganii*	MM1	SXT, C and CTX	Street vendor	Powder
14	*M. morganii*	MM2	C	Street vendor	Powder
15	*P. penneri*	PP4	CAZ and CTX	Herbal clinic	Powder
16	*P. penneri*	PP8	CN and CTX	Supermarket	Powder
17	*P. penneri*	PP9	CAZ and CTX	Herbal clinic	Powder
18	*P. penneri*	PP10	CAZ and CTX	Herbal clinic	Cream
19	*P. penneri*	PP11	CAZ and CTX	Herbal clinic	Liquid
20	*P. penneri*	PP12	CAZ and CTX	Street vendor	Powder
21	*P. penneri*	PP13	CAZ and CTX	Herbal clinic	Liquid
22	*P. penneri*	PP17	CAZ and CTX	Street vendor	Powder
23	*P. penneri*	PP18	CAZ and CTX	Street vendor	Powder
24	*P. penneri*	PP20	CTX	Supermarket	Powder
25	*P. penneri*	PP21	CAZ and CTX	Street vendor	Powder
26	*P. penneri*	PP22	CAZ and CTX	Street vendor	Powder
27	*S. marcescens*	SM1	CAZ and CTX	Street vendor	Liquid
28	*S. marcescens*	SM2	CAZ and CTX	Herbal clinic	Powder
29	*S. rubidaea*	SR4	SXT and CTX	Street vendor	Powder
30	*S. rubidaea*	SR6	CAZ and CTX	Herbal clinic	Liquid
31	*S. rubidaea*	SR7	CAZ and CTX	Street vendor	Liquid
32	*Y. enterocolitica*	YE3	C and CIP	Herbal clinic	Powder
33	*Y. enterocolitica*	YE 6	CAZ and CTX	Herbal clinic	Powder
34	*Y. enterocolitica*	YE7	CAZ and CTX	Street vendor	Powder
35	*Y. enterocolitica*	YE8	SXT and C	Street vendor	Powder

Key: SXT-Sulphamethoxazole/trimethoprim, CAZ-Ceftazidime, C-Chloramphenical, CTX-Cefotaxime, CIP-Ciprofloxacin, CN- Gentamicin, PRL- Piperacilin, NOR-Norfloxacin

4.4 Detection of resistant genes from the isolated drug resistant bacteria

In this study, 35 isolates that were resistant to one or more antibiotics tested were subjected to PCR to determine the presence of antibiotic resistant genes. The following bacteria were tested for resistance genes: - one isolate of *Enterobacter aerogens* (EA2), *Erwinia chrysanthemi* (ERC1) and *Klebsiella pneumonia* (KP2), respectively. Two isolates of *Citrobacter diversus* (CD1 and CD2), *Morganella morganii* (MM1 and MM2) and *Serratia marcescens* (SM1 and SM2), respectively. Three isolates of *Serratia rubidaea* (SR4, 6 and 7) and four isolates of *Yersinia enterocolitica* (YE3, 6, 7 and 8). Seven isolates of *Enterobacter cloacae* (EC 2, 3, 4, 5, 8, 9 and EC 10) and twelve isolates of *Proteus penneri* (PP4, 8, 9, 10, 11, 12, 13, 17, 18, 20, 21 and 22),

All the bacteria that were resistant to the antibiotics tested were found to contain antibiotic resistant genes. There was direct association between phenotypic and genotypic drug resistant among the bacteria. Most of the isolates were genotypically resistant to CTX and CAZ and were found to contain genes coding for CTX and CAZ resistance. Plate 4.6 shows DNA fragments for isolates that were resistant to CTX.

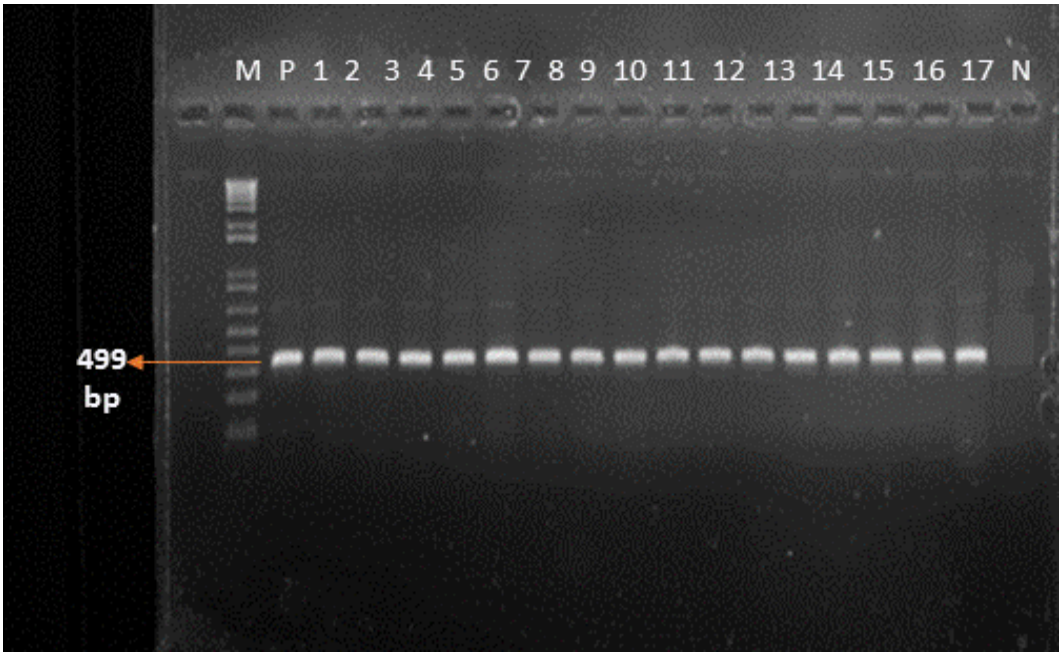

Plate 4.6: Isolates positive for CTX-M gene coding for CTX resistant with 499bp
[Lane M - is a molecular marker with different bands denoting different molecular weights (each bands stands for 150 base pairs - pb), P- Positive control, N- Negative control, 1- PP4 *Proteus penneri*, 2- MM1 (*Morganella morganii*), 3- SR4 (*Serratia rubidaea*), 4 -YE 6 (*Yersinia enterocolitica*), 5 - PP8 (*Proteus penneri*), 6- SR6 (*Serratia rubidaea*), 7- EA2 (*Enterobacter aerogens*), 8 - SM1 (*Serratia marcescens*), 9 -SM2 (*Serratia marcescens),* 10 - PP9 (*Proteus penneri*), 11 - PP10 (*Proteus penneri*), 12 - PP11 (*Proteus penneri),* 13- KP2 (*Klebsiella pneumonia*), 14 - SR7 (*Serratia rubidaea),* 15 -PP12 (*Proteus penneri),* 16-EC2 (*Enterobacter cloacae)* and 17-EC8 (*Enterobacter cloacae*)].

Plate 4.7 shows DNA fragments for isolates that had genes coding for CAZ resistance.

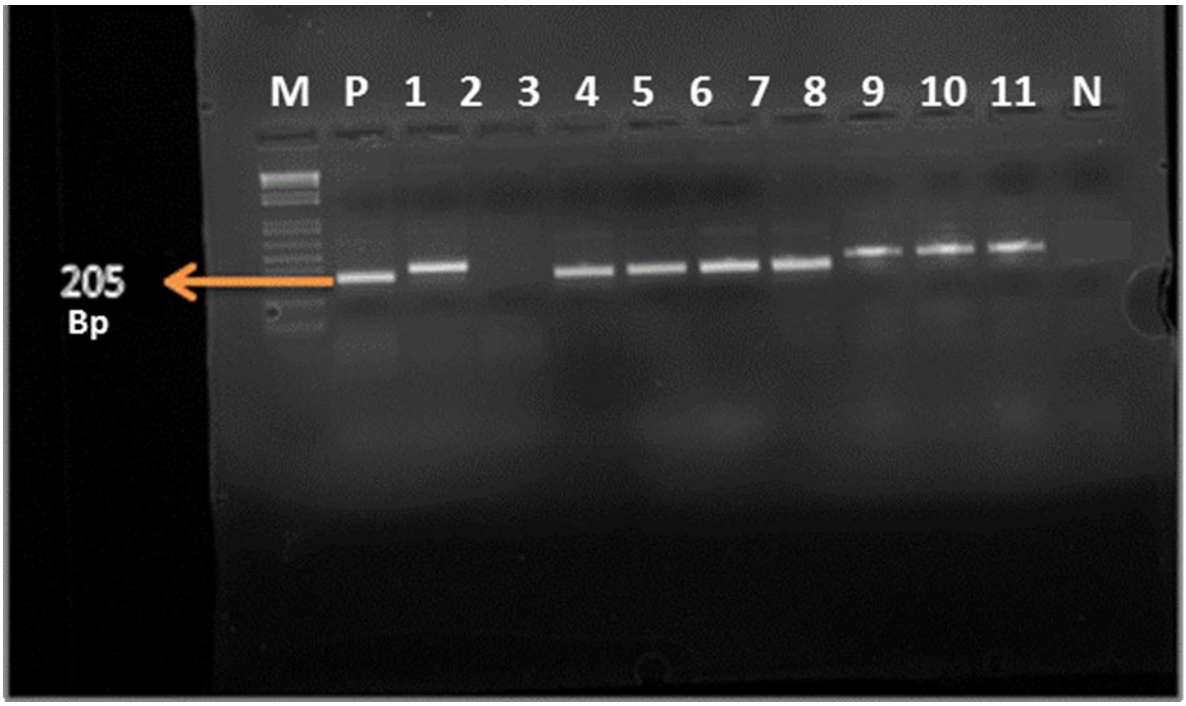

Plate 4.7: Isolates with Bla CMY gene coding for CAZ resistant with 205bp
[Lane M is the molecular marker of 50 bp. Bla CMY gene has 205 base pairs, P- positive control and N – negative control. Sample 3 does not have the gene of interest, 1-EC3 (*E. cloacae*), 2-EC4 (*E. cloacae*), 4-EC5 (*E. cloacae*), 5-EC8 (*E. cloacae*), 6-EC9 (*E. cloacae*), 7-EC10 (*E. cloacae*), 8-EA2 (*E. aerogens*), 9-ERC1 (*E. chrysanthemi*), 10-KP2 (*K. pneumonia*) and 11-PP4 (*P. penneri*)].

Plate 4.8 shows DNA fragments of isolate using multiplex PCR.

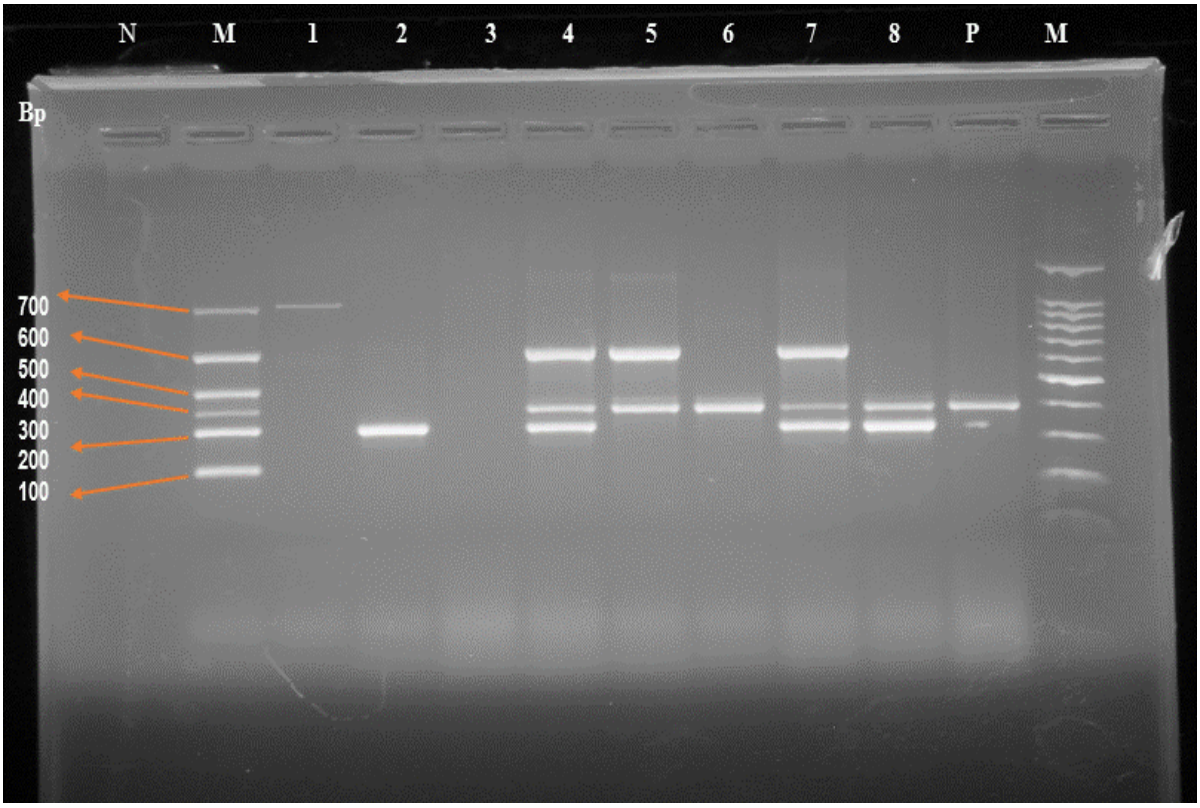

Plate 4.8: Multiplex PCR showing presence resistant genes of the selected bacterial isolates

{Lane M is a molecular ladder of 100 bp; **N** is a negative control while lane **P** is a positive control of *Klebsiella pneumonia* (ATCC 12816) which is resistant to SXT. Lane **1, 2, 3, 4, 5, 6, 7** and **8** are the genotypic drug resistant genes. **Lane 1** – bacteria resistant to NOR, **Lane 2**- isolate resistant to CAZ, **Lane 3** – Does not have DNA fragments, **Lane 4** - isolate resistant to CTX, CAZ and SXT, **Lane 5** – Bacteria resistant to CN, and SXT, **Lane 9**- Resistant bacteria to SXT **Lane 7**- isolates resistant NOR, CTX and SXT, **Lane 8** – Bacteria resistant to CTX and CAZ and}.

4.5 Mycotoxins contamination of the herbal products

4.5.1 Determination of aflatoxins from the herbal products

The herbal products were contaminated with aflatoxin to varying degrees. They were detected in 74(53.6%) samples while in 64(46.4%) samples; their levels were below the limit of detection. All the samples from the chemist had aflatoxin levels below the limit of detection (<LOD). Among the aflatoxins contaminated samples 67(48.6%) had aflatoxin contamination range of 0.001-4.00ppb. These samples were; 2(100.0%), 2(28.6%) from health food stores and manufacturers/wholesalers, respectively, 22(64.7%) were from herbal clinics, 34(45.9%) were from street vendors/hawkers while 7(36.8%) were from supermarkest. Only 7(5.1%) samples had aflatoxins levels above 4.0ppb. These samples were; 4(11.8%) from herbal clinics and 3(4.1%) from street vendors. There was significant association (χ^2 test; p=0.001) between herbal products sourced from herbal clinics and street vendors/hawkers with aflatoxins contaminations. Data analysis showed that there was significant association between samples from herbal clinics and street vendors/hawkers (χ^2 test; p=0.000) with aflatoxins contaminations.

Regarding the formulations, one (6.3%) liquid and six (5.8%) powders had aflatoxins levels above 4.0ppb totaling to seven. Seven (43.8%) liquids, 2(66.7%) creams/lotion, 48(45.2%) powders and 7(87.5%) syrups had aflatoxins levels below the limit of detection. Samples with aflatoxins ranging from 0.001-4.00 were; 2(100.0%) capsule and juices, respectively, 2(33.3%) creams/lotion, 52(49.0%) powders and 1(12.5%) syrups. There was significant association (χ^2 test; p=0.001) between powders and liquids formulations with aflatoxins contaminations (Table 4.14).

Table 4.14: Association between aflatoxin contamination and herbal products

Source	**Aflatoxicin Contamination Level (ppb)**			**n/Freq**	χ^2
	<LOD	**0.001-4.0**	**>4.000**		
Chemist	2(100.0%)	0(0.0%)	0(0.0%)	2	
Health Food Store	0(0.0%)	2(100.0%)	0(0.0%)	2	
Herbal Clinic	8(23.5%)	22(64.7%)	4(11.8%)	34	0.001
Manufacturer/ Wholeseller	5(71.4%)	2(28.6%)	0(0.0%)	7	1.000
Street vendor/ Hawker	37(50.0%)	34(45.9%)	3(4.1%)	74	0.001
Supermarket/ Shop	12(63.2%)	7(36.8%)	0(0.0%)	19	1.000
Total	64(46.4%)	67(48.6%)	7(5.1%)	138	
Formulations	**Aflatoxicin Contamination Level (ppb)**			**n/Freq**	χ^2
Capsules	0(0.0%)	2(100.0%)	0(0.0%)	2	
Liquid	7(43.8%0	8(50.0%)	1(6.3%)	16	0.001
Cream/lotion	2(66.7%)	2(33.3%)	0(0.0%)	3	.100
Juice	0(0.0%)	2(100.0%)	0(0.0%)	2	
Powder	48(45.2%)	52(49.0%)	6(5.8%)	106	0.001
Syrup	7(87.5%)	1(12.5%)	0(0.0%)	8	1.000
Total	64(46.4%)	67(48.6%)	7(5.1%)	138	

Key: LOD-Limit of detection, 0.001-4.00-fumonisin range, >4.000- fumonisin more than 4.0ppb, n-Number of samples per category, χ^2 - Chi square test

4.5.2 Relationship between fungi and aflatoxins

Table 4.15 showed that, 44(59.5%) samples that were not contaminated with fungi were positive for aflatoxins. Thirty four (53.1%) fungal contaminated samples were also

positive for aflatoxins. There was no significant association between the presence of fungi and aflatoxins contamination among the herbal products (χ^2 test; p=0.779).

Table 4.15: Association between fungal contamination and the presense of aflatoxins

Samples	Aflatoxins Detection			Statistics		
Contaminatio n	>LOD (n) (%)	With toxins (n) (%)	Total (n) (%)	χ^2	df	p
No Fungi	30 (40.5)	44 (59.5)	74 (100.0)	0.078	1	0.779
With Fungi	30 (46.9)	34 (53.1)	64 (100.0)			
Total	60 (43.5)	78 (56.5)	138 (100.0)			

Key: χ^2 - Pearson Chi-square Value, df – Degree of freedom, p-value, ppb-parts per billion, (n)-freguency, (%) - percentage

4.5.3 Determination of fumonisins from the herbal products

Sixty-four (46.4%) samples had fumonisins levels below the limits of detection. All samples from chemists, 9(26.5%) from herbal clinics, 4(57.1%) from manufacturers/wholesalers, 38(51.4%) from street vendors/hawkers and 11(57.9%) from supermarkets had fumonisins levels below the limits of detection (LOD). All samples from health food stores, 16(47.1%) from herbal clinics, 3(42.9%) from manufacturers/wholesalers, 26(35.1%) from street vendors/hawkers and six (31.6%) from supermarkets had fumonisins ranging from 0.001-4.0ppb. Nine (26.5%) samples from herbal clinics, 10(13.5%) from streets vendors/hawkers and two (10.5%) from the supermarkets had fumonisins levels above 4.0ppb. Data analysis showed that there was

significant association between samples from herbal clinics, street vendors/hawkers and supermarkets/shops with fumonisins contaminations (χ^2 test; p=0.001).

Regarding formulations, 2(100.0%) capsules, 5(31.3%) liquids, 43(41.3%) powders and one cream/lotion, juice and syrups, respectively had fumonisins contamination ranging from 0.001-4.0ppb. Two (12.5%) liquids and 19(17.3%) powders had fumonisins levels above 4.0ppb. There was significant association between liquids and powders formulations with fumonisins contaminations (χ^2 test; p=0.001) as shown in Table 4.16.

Table 4.16: Fumonisins contamination of HMPs sourced from Nairobi

Sources & formulations	**Fuminosin Contamination (ppb)**			**n/Freq**	χ^2
	<LOD	**0.001-4.000**	**>4.000**		
Chemist	2(100.0%)	0(0.0%)	0(0.0%)	2	
Health Food Store	0(0.0%)	2(100.0%)	0(0.0%)	2	
Herbal Clinic	9(26.5%)	16(47.1%)	9(26.5%)	34	0.001
Manufacturer/ Wholeseller	4(57.1%)	3(42.9%)	0(0.0%)	7	1.000
Street vendor/ Hawker	38(51.4%)	26(35.1%)	10(13.5%)	74	0.001
Supermarket/ Shop	11(57.9%)	6(31.6%)	2(10.5%)	19	0.001
Total	64(46.4%)	53(38.4%)	21(15.2%)	138	
Formulations	**Fuminosin Contamination (ppb)**			**n/Freq**	χ^2
Capsules	0(0.0%)	2(100.0%)	0(0.0%)	2	
Liquid	9(56.3%)	5(31.3%)	2(12.5%)	16	0.001
Cream/lotion	2(66.7%)	1(33.3%)	0(0.0%)	3	.100
Juice	1(50.0%)	1(50.0%)	0(0.0%)	2	
Powder	44(41.3%)	43(41.3%)	19(17.3%)	106	0.001
Syrup	7(87.5%)	1(12.5%)	0(0.0%)	8	1.000
Total	64(46.4%)	53(38.4%)	21(15.2%)	138	

Key: LOD-Limit of detection, 0.001-4.00-fumonisin range, >4.000- fumonisin more than 4.0ppb, n-Number of samples per category, χ^2 - Chi square test

4.5.4 Relationship between fungi and fumonisin

Table 4.17 shows that 36(47.4%) samples were not contaminated with fungi yet were positive for fumonisins contamination. Thirty eight (62.3%) samples were contaminated with fungi and positive for fumonisins. There was no significant association between fungal contamination and the presence of fumonisins (χ^2 test; p=0.081).

Table 4.17: Relationship between fungi and fumonisin contaminations

Samples	**Fumonisins**			**Statistics**		
Fungal Contamination	LOD (n) (%)	Detected (n) (%)	Total (n) (%)	χ^2	df	p
Not contaminated	38 (52.6)	38 (47.4)	76 (100.0)	3.035	1	0.081
Contaminated	23 (37.7)	39 (62.3)	62 (100.0)			
Total	61 (46.0)	77 (54.0)	138 (100.0)			

4.6 Determination of heavy metal contaminations in the herbal products

Lead, arsenic, cadmium, mercury and uranium were not detected in this study. However, copper, zinc, nickel and iron were detected in various quantities among some herbal products. Copper contaminations were detected in 109(79.1%) herbal products out of 138(100%). Among the herbal products, the lowest detectable copper values were 0.04ppm and the highest were 17.72ppm. Copper was not detected in all products from the chemist, 10(29.4%) from herbal clinics, 14(18.9%) from street vendors/hawkers and three (15.8%) from the supermarkets, respectively. Two (100%) products from health food store, 23(67.6%) from herbal clinics, 7(100%) from manufacturer/wholesale, 49(66.2%) from street vendor/ hawker and 15(78.7%) from supermarket/shop had copper contamination ranging from 0.04 to 8.0 ppm. Eleven (14.9%) samples from street vendors/hawkers, 1(2.9%) from the herbal clinics and one [1(5.3%)] from the

supermarkets had copper values above 8.1ppm. There was no significant association (F test; p=0.196) between samples from different source and copper contamination.

Regarding formulations, Copper was not detected in all (100%) capsules, (100%) creams and 19(17.9%) powders. Thirteen (12.4%) powdered samples had copper values above 8.1ppm. The following formulations had copper contamination between 0.04-8.0ppm; 12(75.0%) liquids, 2(100%) juice, 49(66.2%) powders and 7(87.5%) syrups. Statistical analysis showed that there was no significant association (F test; p=0.185) between herbal products from different sources/formulations and copper detection/contaminations (Table 4.18). Figure 4.3 is a graph showing absorption integration for Copper (Cu) standard.

Table 4.18: Copper contamination among the HMPs sourced from Nairobi

Sources products	**Copper contamination in PPM**				**Statistics**
	ND	**0.04 – 8.0**	**8.1 – 20.0**	**n**	**F, p**
Chemist	2(100.0)	0		2	7.35, 0.196
Health food store		2(100)		2	
Herbal clinic	10(29.4)	23(67.6)	1(2.9)	34	
Manufacturer/Wholesale		7(100)		7	
Street vendor/ Hawker	14(18.9)	49(66.2)	11(14.9)	74	
Supermarket/ Shop	3(15.8)	15(78.7)	1(5.3)	19	
Total	29	96	13	138	
Formulations	**Copper contamination in PPM**			**n**	**statistics**
Capsules	2(100)			2	11.31, 0.185
Liquid	4(25)	12(75.0)		16	
Cream	4(100)	0		4	
Juice		2(100.0)		2	
Powder	19(16.3)	74(71.1)	13(12.4)	106	
Syrups	1(12.5)	7(87.5)		8	
Total	29	96	13	138	

Key: χ^2 **–** Pearson Chi-square Value, df – Degree of freedom, p-value, ppm-parts per million, (n)-freguency, (%)- percentage, ND-Not detected

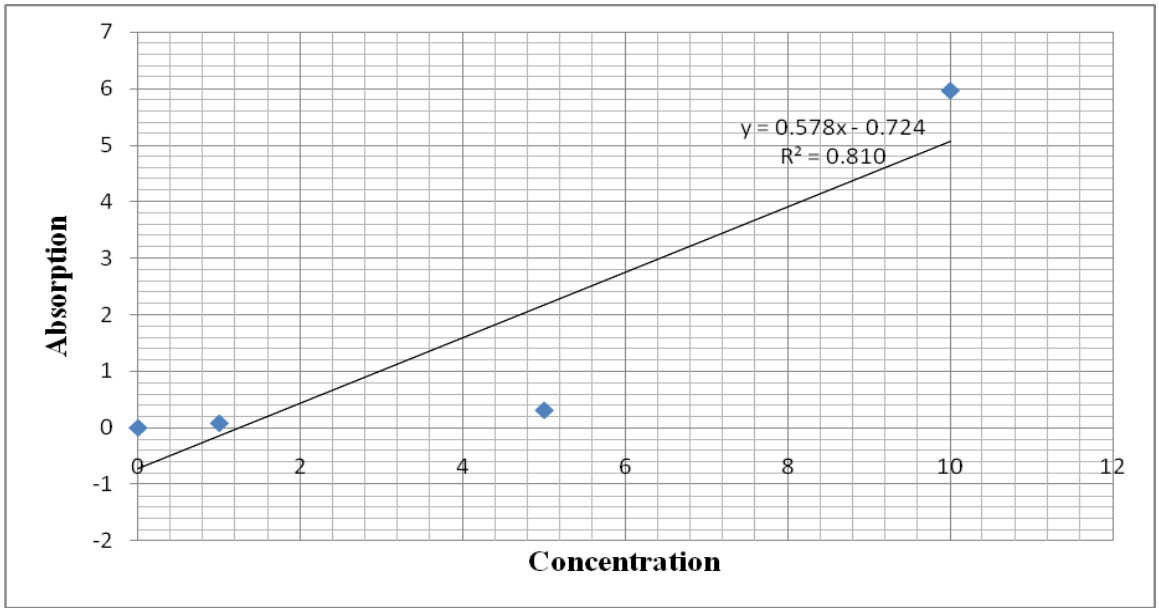

Figure 4.3: A graph showing absorption integration for Copper (Cu) standard
A graph of copper standards, three standards were tested and plotted accordingly. X-axis is the concentration while y-axis is the absorption spectrum.

Zinc micronutient was detected in 135(97.8%) of the herbal products. The lowest detectable value was 2.36 ppm and the highest was 29.88 ppm. In this study, 66(47.8%) of the samples had zinc values ranging from 0-10.0ppm, 57(41.3%) samples had zinc ranging from 10.1-20.0-10.0ppm. Twelve (8.7%) had zinc values above 20.1ppm. Four samples each from herbal clinics, street vendors/hawkers and supermarkets, respectively are among the twelve, which had zinc values above 20.1ppm. Among the formulations, 1 capsule and 11(10.5%) powders had zinc values above 20.1ppm (Table 4.19). There was no significant association between herbal products from different sources/formulations and zinc contamination in this study (F test; p=0.08 and 0.28). Figure 4.4 is a graph showing absorption integration for Zinc (Zn) standard.

Table 4.19: Zinc contamination among the HMPs sourced from Nairobi

Source	**Zinc contamination in PPB**					**Statistics**
	ND	**0-10**	**10.1-20**	**20.1 -30**	**n**	**F, p**
Chemist	2(100)				2	6.3, 0.28
Health food store		2(100)			2	
Herbal clinic		17(50.0)	13(38.2)	4(11.8)	34	
Manufacturer/		6(85.7)	1(14.3)		7	
Wholesale						
Street vendor/ Hawker	1(1.4)	38(51.4)	31(41.9)	4(5.4)	74	
Supermarket/ Shop		3(15.8)	12(63.2)	4(21.0)	19	
Total	3	66	57	12	138	
Formulations	**Zinc contamination in PPB**				**n**	**Statistics**
	ND	**0-10**	**10.1-15**	**20.1 -25**		**F, p**
Capsule		1(50)		1(50)	2	14.27, 0.08
Liquid	2(12.5)	12(75.0)	2(12.5)		16	
Cream		4(100)			4	
Juice		1(50)	1(50)		2	
Powder	1(0.96)	39(37.5)	54(51.0)	11(10.5)	106	
Syrups		8(100.0)			8	
Total	3	66	57	12	138	

Key: χ^2 **-** Pearson Chi-square Value, df – Degree of freedom, p-value, ppm-parts per million, (n)-freguency, (%)- percentage, ND-Not detected

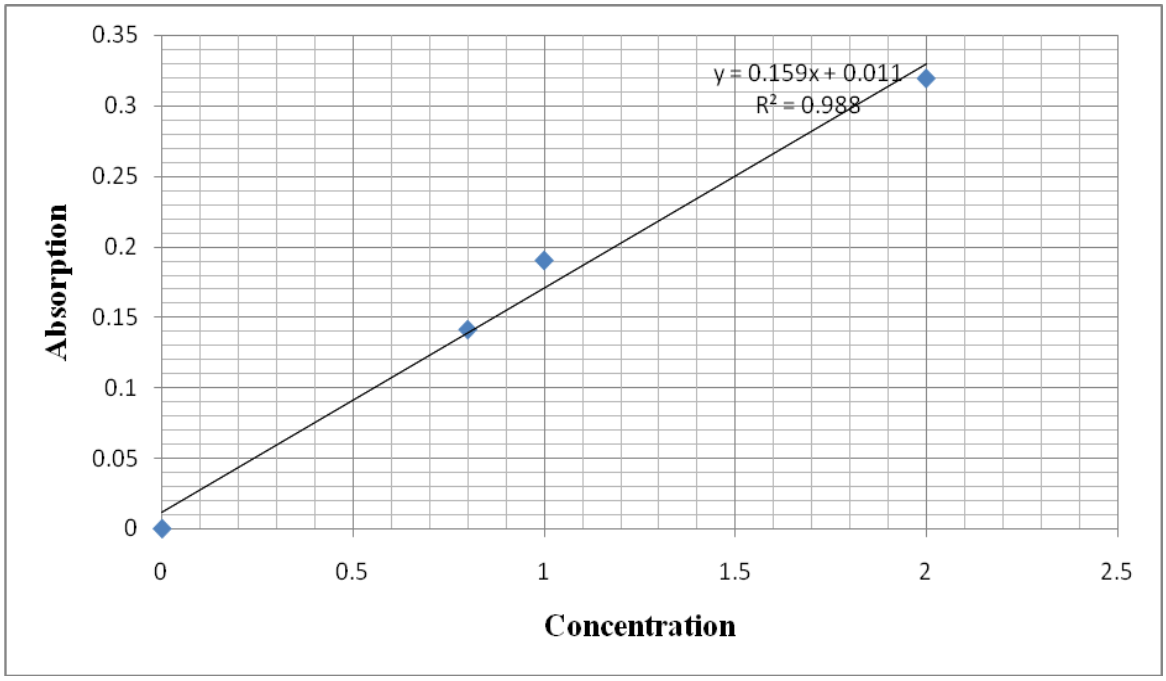

Figure 4.4: A graph showing absorption integration for Zinc (Zn) standard
A graph of zinc standards, three standards were tested and plotted accordingly. X-axis is the concentration while y-axis is the absorption spectrum.

Table 4.20 shows that nickel was detected in 117(84.8%) herbal products out of the total sample size of 138(100.0%). Nickel was detected in varying degrees among the herbal products. About 42(30.5%) samples had nickel ranging from 0-20.0ppm, 80(58.1%) samples had nickel ranging from 10.1-20.0ppm, 16(11.6%) samples had nickel ranging from 20.1-40.0ppm and 36(26.1%) samples had nickel ranging from 30.1-40.0ppm while one (0.7%) sample had nickel contamination above 40.1ppm. Only one sample from street vendors/hawkers, which was in powdered form, had nickel values above 40.1ppm. Nickel levels should not go beyond the accepted limits of 35ug/gm (equivalent to 35ppm) according to health authoriries. There was no significant association between the herbal products from different sources/formulations and nickel contamination in this study (F

test; p=0.29and 0.08). Figure 4.5 is a graph showing absorption integration for Nickel (Ni) standard.

Table 4.20: Nickel contamination among the HMPs sourced fromNairobi

Source	**Nickel contaminations in PPB**				**n**	**Statistics**
	ND	0-20.0	20.1-40	40.1-50		F, p
Chemist	2(100)				2	6.11,
Health food store		2(100)			2	0.29
Herbal clinic	5(14.7)	26(76.5)	3(8.8)		34	
Manufacturer/ Wholesale		5(71.4)	2(28.6)		7	
Street vendor/ Hawker	11(14.9)	32(43.2)	30(40.5)	1(1.4)	74	
Supermarket/ Shop	3(15.8)	15(79.0)	1(5.3)		19	
Total	21	80	36	1	138	
Formulations	**Nickel contaminations in PPB**				**n**	**Statistics**
Formulation	ND	0-10	20.1-30	40.1-50	Total	F, p
Capsules	1(50)	1(50)			2	0.08,
Liquids	5(31.3)	10(62.5)	1(6.25)		16	14.3
Cream	4(100)				4	
Juice		1(50.0)	1(50.0)		2	
Powder	11(8.7)	68(65.4)	16(25.0)	1(0.96)	104	
Syrups			8(100.0)		8	
Total	21	80	16	1	138	

Key: χ^2 - Pearson Chi-square Value, df – Degree of freedom, p-value, ppm-parts per million, (n)-freguency/number of samples per category, (%)- percentage, ND-Not detected

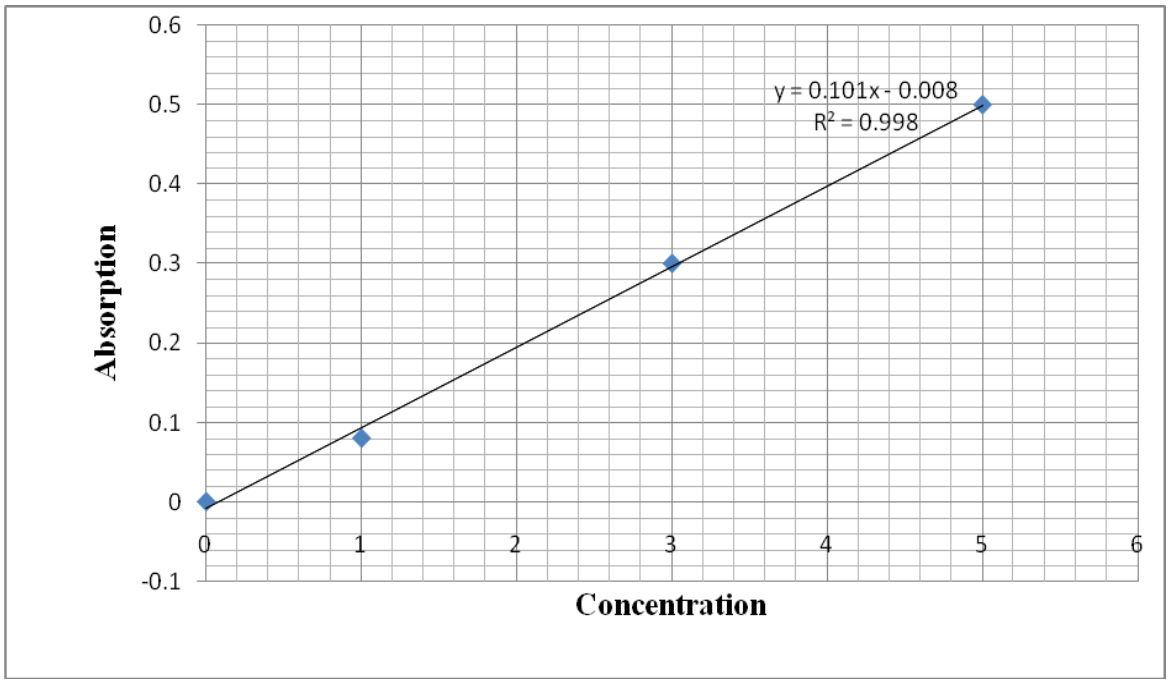

Figure 4.5: A graph showing absorption integration for Nickel (Ni) standard
A graph of nickel standards, three standards were tested and plotted accordingly. X-axis is the concentration while y-axis is the absorption spectrum.

Iron was detected in 120(87.0%) herbal products in varrying degrees. Twenty-nine (21.0%) herbal products were not contaminated with iron. Ninty six (69.6%) samples had iron ranging from 0.4-8.0ppm while 13(9.4%) samples had iron range above 8.1ppm. About 11(14.9%) samples from street vendors/hawkers and 1 sample each from herbal clinics and supermarkets, respectively had iron levels above 8.1ppm totalling to 13 samples. All the 13 samples with 8.1ppm and above iron levels were powders (Table 4.21). There was no significant association between herbal products samples in terms of sources/formulations and iron contamination (F test; p=0. 21 and 0.19). Figure 4.6 is a graph showing absorption integration for Iron (Fe) standard.

Table 4.21: Iron contamination among the HMPs sourced from Nairobi

Sources	**Iron contaminations in PPB**				**Statistics**
	ND	**0.04 - 8.0**	**8.1 - 20.0**	**n**	**F, p**
Chemist	2(100)	0		2	7.35, 0.21
Health food store		2(100)		2	
Herbal clinic	10(29.4)	23(467.6)	1(2.9)	34	
Manufacturer/Wholesale		7(100)		7	
Street vendor/ Hawker	14(18.9)	49(66.2)	11(14.9)	74	
Supermarket/ Shop	3(15.8)	15(78.7)	1(5.3)	19	
Total	29	96	13	138	
Formulations	**Iron contaminations in PPB**			**n**	**Statistics**
Capsules	2(100)			2	11.3, 0.19
Liquid	4(25)	12(75.0)		16	
Cream	4(100)	0(0.0)		4	
Juice		2(100.0)		2	
Powder	19(16.3)	74(71.1)	13(12.46)	106	
Syrups	1(12.5)	7(87.5)		8	
Total	29	96	13	138	

Key: χ^2 **-** Pearson Chi-square Value, df – Degree of freedom, p-value, ppm-parts per million, (n)-freguency/number of samples per category, (%) - percentage, ND-Not detected

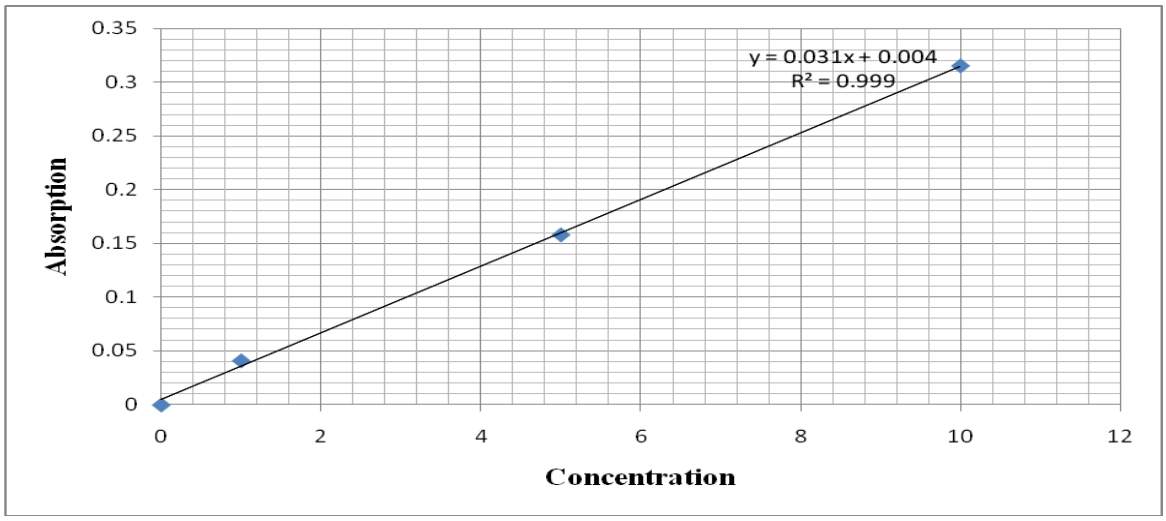

Figure 4.6: A graph showing absorption integration for Iron (Fe) standard
A graph of iron standards. Three standards were tested and plotted accordingly. X-axis is the concentration while y-axis is the absorption spectrum.

4.6.1 pH of the herbal products

The pH of 138 samples was tested. In total 3(2.2%) products were found to be strong acids, 109(79.0%) were weak acids, 6(4.3%) were neutral and 20(14.5%) were weak bases (Figure 4.7).

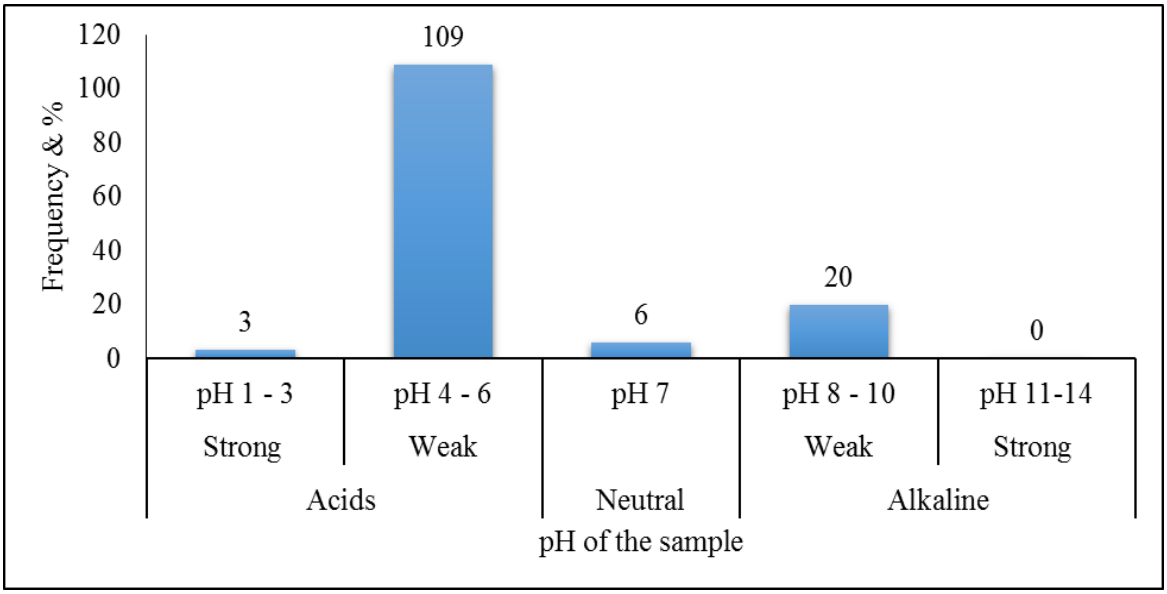

Figure 4.7: pH of the HMPs sourced from Nairobi

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMEDNDATIONS

5.1 Discussion

5.1.1 Herbal products sources and formulations

In this study, 138(100%) herbal medicinal products were collected from different sources in Nairobi. Majority of the products purchased from the street vendors/ hawkers were semi processed and raw materials while those from other sources were processed and packaged in well-labelled containers. The findings of this study on packaging of the products are similar to those found by Kaume *et al.,* (2012) in their study on microbial contamination of herbs marketed to HIV-infected people in Nairobi who reported similar formulations and packaging of herbal products. Hawkers and street vendors packaged their products in bottles and plastic containers previously used for other products hence could make the product susceptible to microbial or chemical contamination due to inadequate cleaning. The semi processed products and raw materials were packaged in nylon papers and recycled bottles (plastic and glass). Keter *et al.,* (2016) in a similar study observed that the main packaging material among the hawkers/street vendors was nylon paper bags and previously used plastic containers.

The study observed that there are many vendors of herbal products in Nairobi an indication of the presence of ready market for medicinal herbs. A study by WHO, (2007) on survey of herbal medical practitioners observed that in the last two decades, the numbers of herbal medical practitioners in Kenya have increased hence concurring with the findings of the current study. Majority of the vendors were hawkers who sell their

products in the streets, buses and corridors. Processed herbal medicines and food supplements were sold in chemists, herbal clinics and health food stores. Manufacturers and wholesaler were found to be stocking both processed and semi processed herbal products in large quantities. Wylie and Nowicki, (2005) observed that, the process of harvesting, drying, storage, handling and the soil influence the bacteriological quality of raw material which in turns affects the entire quality of the herbal preparation and the final finished products. However, these processes were not observed in the current study since the products were purchased at the market.

5.1.2 Microbial contamination in the herbal products

5.1.2.1 Bacteria load

In this study, 117(84.8%) samples were contaminated with aerobic, coli-forms and pathogenic bacteria. The sources of these contaminations could be soils and dust since most herbal products are prepared from roots and stem barks that are already in contact with the soil/dust. Unprocessed herbal products contain microorganism indigenous to the soil. Products that were fully processed, packaged and properly labelled which were sourced from chemist were not contaminated. The microbial quality of the Herbal Medicinal Products (HMP) is influenced by the environment, handling procedures and quality of the raw materials used during formulation. Raw and semi processed products were dispensed in un-hygienic conditions among the herbalist thus explaining the high contamination level.

Among the herbal products, 56(40.6%) had more than $1.0x10^3$ cfu which is beyond the European pharmacpoeia (2007) permissible limits. The limits of bacterial contamination given in European pharmacopoeia (2007) as reported by Okunola *et al*, (2007) and EHIA, (2011), are total aerobic bacteria 10^5 cfu/g, *Enterobacteria* and other Gram-negative 10^3 cfu/g; while *E. coli* and *Salmonella* species should be absent. Onyambu *et al.,* (2013) and Keter *et al.,* (2016) reported similar findings in a study in Kenya. In that most herbal medicine samples recorded microbial loads ranging from $3.00x10^6$ to $1.56x10^{10}$ cfu/ml, levels exceeding by far the recommended llimits. The present study found that the microbial load of the sampled products varied considerably with different species of bacteria. Of concern is the level of contamination of the products by Gram negative organisms which are considered pathogenic. Walther *et al.,* (2016) in a similar study found that most of the liquid herbal produts marketed in Mwanza city were contaminated with bacterial coliforms.

Among the products, 44.1% from herbal clinics and 40.5% from street vendors/hawkers and six from supermarkets were contaminated beyond $1.0x10^3$ cfu. These products were in form of liquids and powders. Analysis showed that there was significant association between herbal products from herbal clinics, street vendors/hawkers and supermarkets (χ^2 test; $p<0.05$) and bacterial contamination. Products dispensed by the street vendors/hawkers and herbal clinics were bearing the greatest burden of contamination as well as a few from the supermarkets. These could be because the herbal products sold in the street were exposed to dust hence prone to contamination. Handling of herbal products by street vendors could expose them to contaminations because some were not

properly packaged. Those sold in the herbal clinics and supermarkets could have been contaminated during processing and handling. Raw plant materials contain environmental bacteria hence the high contaminants observed from herbal products sourced from hawkers since they were selling raw and semi processed products. Contamination may result from several sources, including improper cleaning procedures and open air-drying of plant materials. This leads to contamination with soils and dust. Herbal products are prepared from roots and stem barks that are already in contact with the soil thus explaining the high contaminations rate observed in the current study.

It is probable that unhygienic practices in the processing of HMPs, which can potentially contaminate these products, may have been responsible for these contaminations. Contaminations from such practices have previously been reported in studies done in Dar es salaam, Tanzania and South Africa (Govender *et al.*, 2006; Temu-Justin *et al.*, 2011). Keter *et al.*, (2016) had similar observation regarding herbal products contaminations and supposed sources of contaminations.

Processing of herbal products into; syrups, creams and capsules normally reduces the rate of bacterial contamination. Some of these products are mixed with buffers stabilizers and preservatives hence reduction of contaminants. Nakajima *et al*., (2005) in a study found that the presence of bacterial contaminant in non-sterile herbal medicinal products could reduce or even inactivate the therapeutic activity of the product and has potential to affect the patient taking the medication adversely. Therefore, since the microbial quality of the HMP are influenced by packaging, handling, environment and quality of the raw

materials used during formulation, the processing of these products should ensure that the bacterial load is minimised to safety level. The raw materials, packaging, handling, processing and finished products should not contain bacterial contaminants to maintain appropriate quality, safety and efficacy of the products.

5.1.2.2 Fungi loads

Fungi are either filamentous molds or yeasts. Fungi contaminations and their resultants loads were determined and presented as spore/colony forming units per gram (cfu/g) for both molds and yeasts in this study. Molds are ubiquitous, and mold spores are a common component of household and workplace dust. However, when mold spores are present in large quantities, they become potential contaminants to food, herbal products and drinks. Molds are a health hazard to humans, potentially causing allergic reactions and respiratory problems not to mention the health hazards.

In this study, fungal contamination was observed in 44.2% of the samples. Plants materials are prone to molds infestation when stored in humid conditions with high temperatures. Generally, herbal drugs are considered free from side effects but the poor practices of their harvesting, collection, transportation and storage often lead to extensive fungal growth and accumulation of toxins. Fungal and mycotoxin contaminations are the major cause of decline of market value of herbal drug raw materials (Turner, 2009). Such contamination degrades the quality of raw materials and the medicinal value of the formulated herbal drugs. Synthetic chemical preservatives have been prescribed to control different post-harvest fungal contaminations (Robbins *et al.,* 2000) but due to

their residual and mammalian toxicities, herbal pharmaceutical industries need some safer chemicals as preservatives during post-harvest processing of herbal raw materials.

Generally, the fungal colony forming units were lower as compared to bacterial cfu/g in this study, and ranged from 1cfu/g to 500cfu/g. Infact, most (93.4%) of the fungi contaminated samples had cfu/g ranging from 1cfu/g to 100cfu/g. One sample from herbal clinics and three samples from street vendors/hawkers had fungal cfu above 105 and were powders. The recommended limits for European pharmacopoeia for molds and yeast are 105/104 cfu/g: The first value represents herbal medicinal products to which boiling water is added before use while the second value represents herbal medicinal products to which boiling water is not added before use (European Pharmacopoeia, 2007). In the present study, the first value is applicable to powders while the second values is applicable to the other formulations since they are consumed directly without further processing. In total four contaminated samples did not meet the European pharmacopoeia specified limits of fungi contamination. The results of the current study in terms of fungal colony forming units contradicts the results found in a similar study by Onyambu *et al.*, (2013); their samples were more contaminated. Bokhari & Aly (2013) in a similar study found that herbal tea samples including Sage, different flowers, Chrysanthemum and Cardamom were contaminated with fungi hence concur with the results of the current study on fungal contamination.

5.1.3 Identification of the microorganisms from the herbal products

5.1.3.1 Identification of the isolated bacteria

Identification of the various bacterial organisms in the herbal medicinal products was meant to isolate the bacteria with clinical importance. In the present study, bacterial isolated were grouped into 13 genera namely; Bacillus, Klebsiella, Proteus, Staphylococcus, Streptomyces, Escherichia, Enterobacter, Serratia, Yersinia, Morganella, Citrobacter, Erwinia and Shigella. These bacterial were both gram negatives and gram positives with the former being majority. Herbal products from all the sites except the chemist were contaminated with varying degrees of these bacteria. Again all the formulations carried similar burden of bacteria contaminations. Medicinal products designed for the purpose of chemotherapeutic and pharmacological benefits should be effective against the target medical condition. Several factors could compromise this goal, one of them being possible contamination with pathogenic and non-pathogenic microorganisms (Okunlola *et al*., 2007; Keter *et al.,* 2016). Apart from possible microbial degradation of the active constituents contained in the herbal preparations, the presence of these contaminating microorganisms could constitute a source of infection and serious health risk to the consumers of the herbal preparations; who were probably already overwhelmed by the serious medical conditions for which the herbal drugs were initially indicated (Bowler *et al.*, 2001). Lau *et al*., (2003) in a study reported that some infectious disease outbreaks have been associated with the use of heavily contaminated raw materials of natural origin. In similar studies, bacterial contaminants that present serious health hazards such as *Salmonella spp, E. coli, Staphylococcus spp, Shigella spp* and other gram positive and gram negative strains were isolated (Ogunshe *et al*, 2006;

Adenike *et al.*, 2007; Abba *et al*, 2009; Nordmann *et al.*, 2011). Keter *et al.* (2016) in a similar study in Eldoret and Mombasa isolated the same organisms except *Salmonella* species. Pathogenic Gram-negative bacteria such as *Shigella, Proteus* and *E. coli* that are expected to be absent in herbal products were positive indications of the risks posed by the use of herbal preparations under investigation.

Soil bacteria formed the bulk of the isolates. The bacteria were *Streptomyces sp*p [74(53.6%] and *Bacillus anthracoides* [64(46.4%] which are indication of environmental contamination. Moss *et al*., (2003), in a study described *Streptomyces* spp., as usually saprophytic to humans, can cause local cutaneous fistulized nodules known as actinomycetoma or mycetoma. Severe invasive infections have seldom been reported among the bacteria in this genus, but most cases infections have occurred in immune compromised patients (Bauer *et al.,* 2008). According to a study by Grierson (1928), *B. anthracoides* is pathogenic to guinea-pigs and mice under experimental conditions and would appear to occupy a position between the virulent *B. anthracis* and the non-pathogenic members of the group of aerobic sporing bacilli, such as *B. subtilis, B. mesentericus*. The clinical spectrum of infections caused by *Bacillus* species in human beings, include self limiting food poisoning, localized infections related to trauma (e.g. ocular infections), deep seated soft tissue infections and systemic infections (e.g. meningitis, endocarditis, osteomyelitis, and bacteremia). Fulminant eye infections are widely recognized complications of non-anthrax *Bacillus* infections most commonly *B. cereus*. The various species implicated in serious infections include *B. cereus*, *B. subtilis*, *B. sphaericus*, *B. alvei*, *B. laterosporus*, *B. licheniformis*, *B.*

megaterium and *B. pumilus*. The presence of *Bacillus* species recovered in the present study may be because of inadequate heat processing, improper handling of products and contaminated processing equipment. Keter *et al.,* (2016) in a similar study found *Streptomyces spp* and *Bacillus anthracoides* being the most contaminating bacterial in herbal products purchased from Mombasa and Eldoret towns in Kenya.

The most important isolated bacteria that are of clinical importance were *Shigella sonnei, Klebsiella pneumoniae, Proteus penneri, Staphylococcus aureus,* and *Escherichia coli* in the current study. Frazier and Westhoff (2003) also isolated bacteria of clinical importance from herbal products but the bacteria consisted of *Bacillus* species, *Salmonella sp.* and *E. coli*. *Salmonella* species was not isolated from the present study. Shukla *et al.,* (2004), Walther *et al.,* (2016) and Keter *et al.,* (2016) in similar studies reported a high recovery rate of similar bacteria of clinical importance from indigenous herbal medications. Danladi *et al.,* (2008) in their study on herbal preparations found similar results. A similar study by Onyambu *et al.,* (2013) on microbial quality of unregulated herbal medicinal products in Kenya found that majority of the samples was contaminated with bacteria of clinical importance.

About 0.43% of *Shigella*, species were isolated in this study. *Shigella* species causes an infectious disease called Shigellosis. Most people who are infected with *Shigella* develop diarrhea, fever and stomach cramps starting a day or two after they are exposed to the bacteria. The diarrhea is often bloody. Shigellosis usually resolves in 5 to 7 days

according to WHO, (2010) report. Walther *et al.,* (2016) and Keter *et al.,* (2016) in similar studies isolated different species of *Shigella* in herbal products.

In the current study, 25(10.73%) isolates of *Proteus* species were isolated. *Proteus* species are most commonly found in the human intestinal tract as part of normal flora. *Proteus* species frequently cause nosocomial infections of the urinary tract, surgical wounds and lower respiratory tract. Less frequently, *Proteus* species cause bacteremia, most often in elderly patients according to WHO (2010) report. *Proteus mirabilis* causes 90% of *Proteus* infections and can be considered a community-acquired infection. *Proteus vulgaris* and *Proteus penneri* are easily isolated from individuals in long-term care facilities and hospitals and from patients with underlying diseases or compromised immune systems. Patients with recurrent infections, those with structural abnormalities of the urinary tract, those who have had urethral instrumentation and those whose infections were acquired in the hospital have an increased frequency of infection caused by *Proteus*. Onyambu (2013) and Keter *et al.,* (2016) in similar studies in Kenya isolated *Proteus* species in herbal medicinal products.

Escherichia coli were also isolated from seven (5.1%) sample in this study. These indicate potential faecal contamination since it is found mostly in faeces of warm-blooded animals including human beings. *Escherichia coli* is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI) and traveler's diarrhea, among other clinical infections such as neonatal meningitis and pneumonia. A previous study reported high

coli-form counts, up to 3.4×10^4 cfu/g, in other botanical products such as chamomile (Foote *et al*., 2005). Bonkoungou *et al*., (2013) in a study found that 75% of the samples were contaminated with *E. coli* and other enteric-pathogens. Previous studies in Kenya and Nigeria (Okunlola *et al.,* 2007; Temu-Justin *et al.,* 2011; Onyambu *et al.,* 2013; Keter *et al.,* 2016) found similar results regarding the high prevalence of enteric bacteria including *E. coli*. Hence concurs with the results of the current study which showed that the samples were contaminated with *E. coli*. Studies of Marcelo & Taís (2012) on microbial quality of medicinal plant materials noted that although enterobacteria can be found in nature, this family possesses some indicative value towards faecal contamination. Moreover, the indicator species denoting recent feacal contamination (*E. coli*) accounted for 7.3% of all bacterial coli-forms isolated in this study. This is contrary to 65.3% reported in Nigeria (Okunlola *et al.,* 2007). The variation in the bacterial isolates could be related to the sources of the raw materials and solvents used to prepare them as well as the extent of environmental contamination level. The presence of enterobacteria and *E. coli* reflect the situation regarding faecal contamination (Czech *et al.*, 2001). Kosalec *et al.,* (2009) noted that *E. coli* together with other group of coliforms can be taken as an indicator for undesirable hygiene conditions, although this conclusion has to be related to the magnitude of viable count measured. The high contamination rates of herbal medicinal products with enteric bacteria were also reported by studies from Kaduna-Nigeria, Nairobi – Kenya, Dhaka – Bangladesh and recently in Mwanza city (Okunlola *et al.,* 2007; Khanom *et al.,* 2013; Onyambu *et al.,* 2013; Clementine *et al.,* 2016).

Seven isolates of *Staphylococcus aureus* were isolated in this study. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis and food poisoning. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. The *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis. It is still one of the five most common causes of hospital-acquired infections and is often the cause of postsurgical wound infections (Senok *et al.,* 2009; Hoffman, 2012). Aside from the enormous cost measured in human life, nosocomial infections prolong the duration of hospitalization by an average of 4 days and increase the cost of medical care and are responsible for increased mortalities according to WHO (2013). Kosalec *et al.,* (2009) argued that *Staphylococcus aureus* is not a common contaminant of plant materials and relatively rarely found in nature. However, this organism was isolated from some samples in the current study and thus contamination could provide amount of enterotoxin produced by *S. aureus*, depending on the specific nature of the individual as described by Kosalec *et al.,* (2009) in a study.

In the present study, several isolates of *Klebsiella pneumonia* were isolated from herbal products. Keter *et al.,* (2016) also isolated *K. pneumonia* in herbal medicinal products sourced from Mombasa and Eldoret Kenya. Klebsiella infections refer to several different types of healthcare-associated infections that are caused by the *Klebsiella species* of

bacteria, including pneumonia; bloodstream infections; wound or surgical site infections and meningitis. Healthy people usually do not get Klebsiella infections. However, people who are hospitalized and receiving treatment for other conditions may be susceptible to these infections. In healthcare settings, people who require long courses of antibiotics and/or devices such as ventilators (breathing machines) or intravenous (vein) catheters are at the highest risk for Klebsiella infections. These infections are often treated with antibiotics, although some *Klebsiella* species of bacteria may be resistant to certain types of antibiotics (Shahab, 2013; Wen-Liang & Yin-Ching, 2013).

Other important genera of bacteria isolated in this study were; *Serratia spp., Erwinia spp., Yersnia spp., Morganella spp., Citrobacter spp* and *Enterobater sp.*. Keter *et al.,* (2016) isolated similar gram-negative entero bacteria in their study. Although these bacteria are pathogenic to immuno-compromised individuals, the claims in the herbal products labels and also from herbalist in this study revealed that the products are dispensed to all the peoples including; healthy people as supplements, the sick, old, small children and HIV/AIDS patients as medications. Therefore, these contaminating bacteria could further endanger lives of immuno-compromised individuals. As opportunistic pathogens, these bacteria take advantage of weakened host defenses to colonize and elicit a variety of disease states.

5.1.3.2 Identification of the isolated fungi

The fungal isolates from all the samples in this study were categorised into moulds and yeast. Fungus belonging to six genera were identified; Aspergillus, Absidia, Candida, Penicillium, Rhizomucor and Rhodotorula. The genus Aspergillus was the most dominant

genus recovered followed by Fusarium and Penicillium, respectively. These observations are in agreement with reports by other researchers (Freire *et al.,* 2000; Elshafie *et al.,* 2002; Mandeel, 2005; Clement *et al.,* 2016; Keter *et al.,* 2016).

Samples from the chemist and health food stores were sterile while those from supermarkets, street vendors/hawkers and wholesalers were contaminated with fungi to varying degrees. All the formulations were also contaminated with fungi to varying degrees. The presence of a wide range of environmental fungi indicates that considerable improvements could be made during post-harvest storage of the raw materials. Strains of *Aspergillus flavus, A. niger, Fusarium* and *Penicillium* species were the most dominant and frequently isolated among the molds. These results approximate with previous reports that showed Aspergillus were the main contaminant recovered from different herbal and spices samples (Mandeel, 2005). Aspergillus and Fusarium are the two major genera reported to produce mycotoxins (Rodriguez-Amaya and Sabino, 2002; Biso *et al.,* 2017). The presence of fungus such as *Penicillium* spp, *Candida* spp and *Aspergillus* spp are of additional concern. Lin *et al.,* (2001) and Bateman *et al.* (2002) in their studies alluded that, both *Penicillium* spp and *Aspergillus* spp are associated with food poisoning and may be responsible for infections particularly in immunocompromised individuals. *Penicillium* spp has been isolated from solids and liquid herbal products in a study by Esimone *et al*. (2007). In addition *Penicillium* spp produces the mycotoxin, ochratoxin A, which is both nephrotoxic and carcinogenic (Burge, 1989).

The prevalence of the genus Aspergillus was 62(43.4%) in this study. Aspergillus associated infections are rare and life-threatening infection in immunosuppressed patients. It has nonspecific clinical manifestations that often mimic other disease entities especially in patients who have extensive comorbidities. *Aspergillus* species cause serious disease in humans and animals. Vallabhaneni *et al.,* (2017) reported that immune compromised hospitalized patients were infected by aspergilosis. The most common species causing allergic disease are *A. fumigatus* and *A. clavatus*. Aspergillus can cause neonatal infections (Cloherty, 2012). The *A. fumigatus* (the most common species) infections are primary pulmonary infections and can potentially become a rapidly necrotizing pneumonia with a potential to dissemination. Biso *et al.,* (2017) in a study found that, among patients predisposed to aspergillosis, cancer is the most common underlying medical condition (44%), followed by bone marrow transplant (25%), solid-organ transplant (13%), HIV/AIDS (3.8), autoimmune (2%), and systemic steroid use (3.5%).

Penicillium species was the third (23 [16.1%]) predominant genera of fungi isolated in this study. *Penicillium* causes penicilliosis in human beings. Keter *et al.,* (2016) in a similar study also isolated *Penicillium* species from herbal medicinal products. Lo and others in a study in 2000 showed that there is a high incidence of penicilliosis in AIDS patients in South East Asia; 10% of patients in Hong Kong get penicillosis as an AIDS-related illness. Cases of *P. marneffei* human infections (penicillosis) have also been reported in HIV-positive patients in Australia, Europe, Japan, the UK and the U.S. All the patients, except one (Lo *et al.,* 2000), had visited Southeast Asia previously. Although

both the immunocompetent and the immunocompromised can be infected, it is extremely rare to find systemic infections in HIV-negative patients. Patients commonly complain with symptoms and signs of infection of the reticuloendothelial system, including generalized lymphadenopathy, hepatomegaly and splenomegaly. The respiratory system is commonly involved as well; cough, fever, dyspnea and chest pain may be present, reflecting the probable inhalational route of acquisition. Approximately one-third of patients may also exhibit gastrointestinal symptoms, such as diarrhea (Cloherty, 2012).

Several studies (Verma & Heffeman, 2008; Bassiri *et al*, 2010; Moreno & Arenas, 2010) have revealed that onychomycosis are not only caused by dermatophytes but occasionally by nondermatophytic fungi including, *Aspergillus* spp., *Fusarium* spp., and *Acremonium* spp. Gianni *et al.*, (2000) noted that in the past, molds were being regarded as saprophytic or opportunistic fungi and were ignored. Recently, due to an an increase in the number of immune compromised cases and environmental changes, more attention has been given to this wide, but generally non-pathogenic group of fungi. Onychomycosis caused by nondermatophytic molds is becoming increasingly prevalent even in healthy people. This apparent emergence might be an artefact of improved diagnostic techniques or increased awareness that these fungi are potential etiologic agents (Gupta *et al.*, 2003a). Other studies (Giann & Romano, 2004; Romano *et al.*, 2005; Gupta *et al.*, 2008) have reported onychomycosis cases caused by *Aspergillus* species worldwide. Surjushe *et al.*, (2007) in a study reported onychomycosis caused by *Aspergillus niger*.

The prevalence of Fusarium species in this study was 25(17.5%). Keter *et al.,* (2016) in a similar study isolated Fusarium species in herbal medicinal products. Fusarium species are ubiquitous and may be found in the soil, air and on plants. In humans, *Fusarium* species can also cause disease that is localized, focally invasive or disseminated. The pathogen generally affects immunocompromised individuals with infection of immunocompetent persons being rarely reported. Localized infection includes septic arthritis, endophthalmitis, osteomyelitis, cystitis and brain abscess. In these situations, relatively good response may be expected following appropriate surgery and oral antifungal therapy. Disseminated infection occurs when two or more noncontiguous sites are involved. The species most commonly involved include *Fusarium solani*, *Fusarium oxysporum* and *Fusarium moniliforme*. The diagnosis of Fusarium infection may be made on histopathology, gram stain, mycology, blood culture or serology. Portals of entry of disseminated infection include the respiratory tract, the gastrointestinal tract and cutaneous sites. The skin can be an important and an early clue to diagnosis since cutaneous lesions may be observed at an early stage of the disease and in about seventy-five cases of disseminated Fusarium infection. Typical skin lesions may be painful red or violaceous nodules, the center of which often becomes ulcerated and covered by a black eschar (Gupta *et al.,* 2000; Gupta *et al.,* 2003b). Taj-Aldeen (2017) in his study reported the emergence of multidrug resistant Fusarium species among the study participants. Fusarium species has also been isolated in diabetic foot infections.

In the current study, the prevalence of *Candida* species was 13(9.1%). Similarly, Keter *et al.,* (2016) isolated *Candida* species in herbal medicinal products. Candida infections

(candidiasis) are the most prevalent opportunistic fungal infection on humans and, as such, a major public health problem. In recent decades, candidiasis has been associated with *Candida* species other than *Candida albicans*. Moreover, biofilms have been considered the most prevalent growth form of Candida cells and a strong causative agent of the intensification of antifungal resistance. Silva *et al.,* (2017) in a study in Portugal found that *Candida* Species Biofilms' were resistant to antifungal drugs which is a rare case globally. Candidiasis is a fungal infection due to any type of the *Candida species*. When it affects the mouth, it is commonly called thrush (James *et al.,* 2006). Signs and symptoms include white patches on the tongue or other areas of the mouth and throat. Other symptoms may include soreness and problems swallowing. When it affects the vagina, it is commonly known as yeast infection (Senses-Ergul *et al.,* 2006). Signs and symptoms include genital itching, burning and sometimes a white "cottage cheese-like" discharge from the vagina. Less commonly, the penis may be affected, resulting in itchiness. Very rarely, the infection may become invasive spreading throughout the body, resulting in fevers along with other symptoms depending on the parts of the body affected (Miceli *et al.,* 2012).

Alternaria is a genus of ascomycete fungi. *Alternaria* species are known as major plant pathogens. In the current study, only 4(2.8%) isolates of Alternaria were isolated. They are also common allergens in humans, growing indoors and causing hay fever or hypersensitivity reactions that sometimes lead to asthma. They readily cause opportunistic infections in immunocompromised people such as AIDS patients. The spores are airborne and found in the soil and water. They are also found in doors and on

domestic objects, (Venturini *et al.,* 2002; Mosquera *et al.,* 2016). Many human health disorders can be caused by these fungi, which grow on skin and mucous membranes, including on the eyeballs and within the respiratory tract. Allergies are common, but serious infections are rare, except in people with compromised immune systems (Tournas *et al.,* 2006). However, species of this fungal genus are often prolific producers of a variety of toxic compounds. Brás *et al.,* (2015) reported a clinical case of a liver-transplanted patient suffering acutaneous co-existent infection with *A. alternata* as well as *A.infectoria*.The first case of cutaneous concomitant infection due to those two species reported not only in Portugal but also world wide.The patient was treated with surgical excision of the lesions and oral itraconazol without relapse.

Rhodotorula species are ubiquitous saprophytic yeasts that can be recovered from many environmental sources. The prevalence of *Rhodotorula* in this study was 6 (4.2%). This yeast has a strong affinity for plastic, having been isolated from various medical equipments, such as dialysis equipment, fibre-optic bronchoscopes and other environmental sources, including shower curtains, bathtubs and toothbrushes according to studies of Heras-Vazquez *et al.,* (2003).

Zygomycetes are opportunistic pathogens capable of causing acute infections in humans. Among them, some of the predominant organisms causing disease are *Rhizopus* spp., *Mucor* spp. and *Absidia* spp. These funguses were isolated in the current study. Despite the introduction of several new antifungal drugs over the past decade, options for the treatment of zygomycosis remain limited. Amphotericin B is the drug of choice and has

been the only effective agent. It is associated with improved outcome diagnosis in human and animal zygomycosis, (Mosquera *et al.,* 2016) although morbidity and mortality remain high. Bassetti & Bouza (2017) and Vallabhaneni *et al.*, (2017) in different studies reported that Mucormycosis is a rare, but increasingly prevalent disease that occurs mainly in patients with uncontrolled diabetes mellitus, immunocompromised individuals or previously healthy patients with open wounds contaminated with Mucorales.

The risk of the presence of microorganisms in a pharmaceutical product depends on the finality of the use, its nature and its potential damage to the consumers. Although high fungal loads may be due to the natural origin of these products, they indicate the potential for spoilage and mycotoxin contamination (Mandeel, 2005). On the basis of this results, the microbiological quality of the herbal products are influenced to varying degrees by the microbial levels of the starting raw materials though it was not confirmed in the current study, probably the production method and the production environment. High counts of harmful microorganisms such as *Aspergillus flavous, Fusarium, Candida* and *Penicillium* may affect the human health and drug quality, and hence it is necessary to improve plant material quality and to establish better hygienic conditions during production of herbal medicine. Some of these environment related factors can be controlled by implementing standard operating procedures (SOP) leading to Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Supply Practice (GSP) and Good Manufacturing Practice (GMP) for producing these medicinal products from herbal or natural sources (Chan, 2003).

5.1.4 Antibiotic susceptibility test of the isolated pathogenic bacteria

Determination of antibiotic susceptibility patterns on the identified pathogenic bacteria was done and the results compared to a standard interpretation chart (CLSI, 2016) used to categorize the isolate as sensitive (S), intermediate (I), resistant (R) (Wayne, 2004). Majority of the isolates were sensitive [61(63.5%)] to piperacilin -PRL, ciprofloxacin CIP, norfloxacine -NOR, cefotaxime- CTX, gentamicin -N, sulphamethox/ trimethoprim –SXT and ceftazidime –CAZ antibiotics. The results concur with a study done by Alwakee, (2008) on microbial contaminants of herbal medicine where he found that most (75%) of the bacteria isolated were sensitive to the panel of antibiotic tested. In the current study, 36.5% of the isolated bacteria were resistant.

Multiple drug resistance was common in the present study. Yesuf *et al.,* (2016) and Keter *et al.,* (2016) in similar studies in Ethiopia and Kenya, respectively found that multidrug resistance was also a common problem among the pathogen isolated from medicinal herbal products hence concurs with the results of the current study. Testing bacterial pathogens for their responses to chemotherapeutic agents is common practice in clinical and food microbiology (Adenike *et al.,* 2006). The result of other studies does not concur with this study, since they observed a relatively high level of resistance to the commonly used antibiotics (Angulo *et al.,* 2004; Ryan *et al.* 2005; Adenike *et al.,* 2006; De-Waal *et al*., 2013). The reason could be that the bacteria tested in this study were environmental while those from the other studies were bacterial recovered from clinical specimen.

Drug susceptibility test of the isolated bacteria to sulphamethoxazole/ trimethoprim showed that, most [89(92.1%)] bacteria were sensitive. All the *E. coli, Staphylococcus aureus, Serratia* species and *Shigella sonnei* isolates were sensitive to SXT. Keter *et al.,* (2016) in a similar study in Eldoret and Mombasa reported that some entero bacteria were resistant to sulphamethoxazole/ trimethoprim hence concurs with the results of the current study. Sang *et al.,* (2012) in a study on traveler's diarrhea found conflicting results in that all *E. coli* isolates from the study displayed resistance to sulphamethoxazole /trimethoprim. The *E. coli* isolated in this study were sensitive to SXT while those isolated in the other study were resistant because they were isolated from patients while the current study isolated bacteria from the herbal products. Environmental isolates are not commonly resistant to drugs because they are not exposed constantly to antibiotics. In previous studies, this antimicrobial agent was more effective as CIP in the therapy of traveller's diarrhoea (Harumi *et al.*, 2001; DuPont, 2010). The resistance of enteric pathogens to currently used antimicrobial agents has increased the world over as a result of the widespread use of antimicrobials/antibiotics.

Susceptibility of the isolated bacteria to ceftazidime showed that 53.2% were sensitive. All the isolated *E. coli* and *Shigella sonnei* were sensitive to CAZ. Fereshteh *et al.* (2009) in a study observed that, more than 90% of *Shigella* isolates were susceptible to ceftazidime hence his findings concur with the results of the current study. Sader *et al.,* (2017) in a study in US found that the bacteria were resistant to ceftazidime. However, Hariharan *et al.,* (2015) found that all *E. coli* and majority of *Klebsiella* and *Enterobacter* were resistant to ceftazidime and sensitive to ceftazidime combined with tazobactam

suggesting that they were ESBL producers. Generally the isolates tested in the current study were sensitive to ceftazidime alone because they were environmental isolates meaning that they have not been constantly exposed to antibiotics.

Slightly more than half (50.1%) of the isolated bacteria were sensitive to cefotaxime while (34.4%) were resistant in the current study. Studies of Hussain *et al.,* (2012) and Giannoula *et al.,* (2013) also found that cefotaxime, were moderately sensitive against most *Enterobacteriaceae* isolates. Noguchi *et al.,* (2017) found that the prevalence of cefotaxime resistance was very high in different geographic regions among the entero bacteria. A study from Spain revealed that 9.7, 12.5 and 29.1% of third-generation cephalosporin resistance in bloodstream infections were caused by *E. coli, K. pneumoniae* and *Enterobacter* spp., respectively (Chopra *et al.,* 2015). In the SENTRY program study from the United States of America, the prevalence of third-generation cephalosporin-resistant *Enterobacteriaceae* that caused bacteremia was 6.4% (Castanheira *et al.,* 2010). In the Asia-Pacific region, approximately 10% of *Enterobacteriaceae* were phenotypically positive for ESBL production (Sheng *et al.,* 2013).

Majority (97.9%) of the isolated bacteria in this study were sensitive to gentamicin while only 2.1% were resistant. This shows that the drug is good for the isolated bacteria and could be recommended for the treatment of ailments caused by these bacteria. The efficacy of gentamycin may be related to the mode of its administration which has limited its abuse and misuse. Studies of Ayalu *et al.,* (2011) and Keter *et al.,* (2016) concur with

the current study, in that they reported high level of sensitivity of *Shigella spp* and other entric bacteria to gentamicin. Previous studies in different parts of the world have reported high sensitivity of enteric bacteria to gentamicin (Ashenafi *et al.,* 1985; Roma *et al.,* 2000) including a report from Kenya (Brooks *et al.,* 2006). However, Tiruneh (2009) reported moderate resistance of 25% of all the enteric pathogens isolated and tested. Magwenzi *et al.,* (2017) in a study in Zimbabwe found that there were high rates of carriage of ESBL and gentamicin resistant bacteria on admission to hospital and high rates of acquisition for the same resistance types during the inpatient period.

Most (93.8%) of the isolated bacteria in this study were sensitive to chloramphenical. Two *Morganella morganii and Yersnia enterocolitica,* respectively and one *Citrobacter diversus* were resistant to this drug. Keter *et al.,* (2016) found that the bacteria isolated from herbal medicine were generally sensitive to chloramphenicol. *Shigella* isolates showed high levels of resistance to chloramphenicol according to a study, in Kenya by Sang *et al.,* (2013). Studies of Daniel *et al.,* (2012) also reported that resistance to chloramphenicol was significantly higher in bacteria isolated from cattle feaces than in human. These two studies contradict the findings of the current study because *Shigella* isolates were sensitive to chloramphenical. This may be because the current study tested *Shigella* isolates from herbal product while the other studies were dealing with clinical and veterinery isolates. Clinical and veterinery isolates becomes resistant to common antibiotics due to constant exposure. It can also be true to say that Shigella isolates in the current study was not the resistant strain. Chiyangi *et al.,* (2017) in a study in Zambia

found that all the organisms isolated exhibited high level drug resistance to chloramphenicol, including resistance to multiple drugs.

Reaction of piperacilin to the isolated bacteria showed that 96.9% of the bacteria were sensitive to the drug. Similarly Keter *et al.,* (2016) in a similar study, had the same observation. This result was in conformance with those observed by NagKumar *et al.* (2015) who reported high (73.7%) bacterial sensitivity to piperacillin. Similarly, Mohammed *et al.,* (2016) showed that susceptibility of bacterial isolates to pipracillin was 74.3%. However, the results were in discordance with those obtained by Meradji *et al* (2015) who reported the resistance rate of isolates to piperacillin was 36.25%. The reason of difference in susceptibility test of bacteria to piperacillin could be due to geographical difference.

Generally norfloxacin was effective against the isolated bacteria. Susceptibility test showed that 93.7% bacteria were sensitive to norfloxacin. A study by Maji *et al.,* (2016) in India showed that gram positive isolates were sensitive to norfloxacin and other antibiotics. The two drugs norfloxacin and ciprofloxacin belong to the same class of quinolones but their reactions to the bacteria in this study were different. Chiyangi *et al.,* (2017) in a study showed that all the pathogens were sensitive to norfloxacin.

Among the isolated bacteria 96.9% were sensitive to ciprofloxacin while 2.1% were resistant though the results are almost similar to that of norfloxacin. In the current study, one isolate of *Citrobacter diversus* and *Yersinia enterocolitica*, respectively were

resistant to ciprofloxacin. Keter *et al.*, (2016) in a similar study found that all the bacteria isolates were sensitive to ciprofloxacin. Several studies in the world have recommended ciproflaxocine as an effective therapy for diarrhoea ailments against enteric bacteria (Harumi *et al.,* 2001; Sang *et al.,* 2006; Sang *et al.,* 2012; Baron *et al.,* 2016) hence concurs with the result of the current study. In contrast Ogbolu *et al.,* (2012) on enteric bacteria reported that using the E-test, 88.9% of bacterial isolates were resistant to ciprofloxacin, 92.6% were resistant using broth microdilution, 96.3% were resistant using agar dilution and 72.2% were resistant using disc diffusion methods. However, the current study did not utilize Etest for drug susceptibility tests. Studies of Ogbolu *et al.,* (2012) showed high resistant because they were clinical isolates and also due to geographical difference with the current study and the previous one as discussed above.

The widespread and often indiscriminate use of antibiotics has created drug-resistant Gram-negative bacilli that readily acquire multiple resistances through transmission of drug resistance plasmids (R factors). Also, development of new surgical procedures, health support technology, and therapeutic regimens has provided new portals of entry and compromised many host defenses. The prevalence of resistance was noted among some bacteria in this study. The bacteria showed single drug resistant, multidrug resistance as well as extended drug resistance. Some isolates were resistant to one drug, two drugs, three and four drugs, respectively. Most of these drug resistant bacteria were recovered in herbal products sourced from herbal clinics and street vendors/hawkers. They were presented in liquid and powder formulations.

Morganella morganii (MM2) was resistant to chloramphenical, *Enterobacter cloacae* (EC3) isolate was resistant to ceftazidime and *Proteus penneri* (PP20) was resistant to cefotaxime. Some were resistant to four anitibiotices; *Citrobacter diversus* (CD1) was resistant to CN, CTX and NOR, *Morganella morganii* (MM1) resistant to SXT, C and CTX, *Enterobacter aerogens* (EA2) resistant to CAZ, CTX and PRL and *Klebsiella pneumonia* (KP2) resistant to SXT, CAZ and CTX. Keter *et al.,* (2016) in a similar study observed that some of the isolated bacteria were resistant to one drug, two drugs, three drugs and four drugs hence concurs with the current study. Elsewhere clinical isolates has been reported to be resistant to more than one antibiotic by several studies (Schaberg and Zerros, 1986; Ryan *et al.* 2002; Adenike *et al.,* 2006; Richard, 2007b; Sang *et al.,* 2012). Hariharan *et al.,* (2015) in a study observed that a notable percentages of the isolates were multi-drug resistant hence concurs with the current study. Similar resistance pattern among Indian Enterobacteriaceae clinical isolates have been widely reported by Sarma *et al.,* (2011).

5.1.5 Detection of resistant genes from the isolated drug resistant bacteria

Multidrug resistant was a concern because some bacteria, which cause human diseases in Kenya, were noted to be resistant to more than one antibiotic tested. These bacteria were also found to contain resistant genes. There was direct association between the phenotypic resistance and genotypic resistance in this study. These bacteria isolates, which contained drug resistant genes, were sourced from street vendors/hawkers and were formulated into powders and liquids. These bacteria might have acquired the resistant gene from the environment. Ronald *et al.,* (2002) in a study found that bacteria

with intrinsic resistance to antibiotics are found in nature. Such organisms may acquire additional resistance genes from bacteria introduced into soil or water and the resident bacteria may be the reservoir source of widespread resistant organisms found in many environments. In their study, they found that the most common resistant organisms belonged to the following genera: Citrobacter, Enterobacter, Pseudomona and Serratia, hence concurring with the results of the current study.

Environmental bacteria communities, including *E. coli* have been associated with recognized antibiotic-resistant gene pools that have been transferred into normal human and animal flora, where they exert strong selective pressure for the emergence and spread of resistance (Kinge *et al.,* 2010; Alves *et al.,* 2014). Multiple antibiotic resistance index (MARI) has been used to estimate health risk associated with the spread of drug resistance in an environment. Molecular analysis of antibiotic resistance gene reveals the presence of 5 genes conferring resistance to different classes of antibiotics in the current study. Genes conferring resistance to CAZ and CTX were mostly detected; suggesting that resistance to this class of drug may be genetically mediated because of long-term exposure. Momtaz *et al.,* (2012) in a similar study, reported high rate of detection of resistant genes conferring resistance to CAZ and CTX. Some of the isolates in this study were found to contain multiple resistant genes conferring resistance to two or more different classes of antibiotics. The report of antibiotic-resistant genes found in the bacterial isolates in the current study might serve as a pointer to the possible presence of other antibiotic drug-resistant genes conferring resistance to other classes of antibiotics that were not targeted. This finding are in line with other reports on the detection of

multiple antibiotic-resistance gene in some commensal and pathogenic strains of bacteria including *E. coli* (Bailey *et al.,* 2010; Karczmarczyk *et al*., 2011).

In a study by Adeyemi *et al.,* (2015) on antibiotic resistance profiles of bacterial isolates cultured from HIV seropositive patients, observed that about 90.9% of the two *Pseudomonas* species were multi-drug resistant to more than 3 classes of antibiotics among seropositive patients. The expression of the efflux pump has been implicated in resistance of bacteria species to beta lactam antibiotics. These proteins transport the antibiotics from within the cell to the external environment. This multi-drug system has been shown to provide resistance to a very wide range of compounds in Gram negative bacilli (Poole, 2004), coupled with a low permeability of the outer membrane (Mesaros *et al.,* 2007), and a remarkable ability to acquire further resistance mechanisms to multiple groups of antimicrobial agents, including β-lactams, aminoglycosides and fluoroquinolones (Bubonja-Sonje *et al.,* 2015). It is notable that many of the resistant mechanisms are often present simultaneously, thereby conferring multi-resistant properties to the organism (Mc-Gowan, 2006).

In bacteria, antimicrobial resistance is facilitated through their ability to quickly adapt to new environments and along with their ability to replicate very quickly comes the aptitude to mutate their DNA acquired from other drug resistant bacteria (Stohr, 2000). Therefore, the acquisition of resistance may be due to chromosomal mutations or mobile genetic elements like plasmids that are often capable of transfer from one strain of organism to another, even across the species barrier. The ability of transposons to

integrate into either conjugative plasmids or the organisms' chromosome enhances the transferability of a given resistant determinant. This process is a natural phenomenon exacerbated by the abuse, overuse and misuse of antimicrobials in the treatment of human illness and in animal husbandry, aquaculture and agriculture (Lexchin, 2000; Stohr, 2000). The importance of surveying resistant environmental strains is that under favourable situations, they may transfer their resistance plasmids to pathogens (Ugwu *et al*., 2009). If such organisms are present in medicaments, such as herbal medicinal products they could behave as opportunist pathogens and initiate an infection, particularly in immuno-compromised patients as well as lead to transfer of antibiotic resistance traits to hitherto sensitive microorganisms co-habiting within the consumers of those products. Given the increasing rate of development of resistant bacteria strains, the main challenge is to slow the rate at which resistance develops and spreads. In order to decrease the spread of resistance among antibiotics, physicians, pharmacists, researchers and consumers alike need to be more aware of the selective pressures driving these bacteria to decrease their susceptibility (Gershman, 1997).

These selective pressures include the abuse, overuse and misuse of antimicrobials in therapy, improperly manufactured and mishandled HMPs (Lexchin, 2000; Esimone *et al*., 2007) as well as other numerous socioeconomic factors that govern the development of multi-drug resistant bacteria strains (Toebe, 2001). In such circumstances, a collective and concerted effort towards the prevention of development of resistant bacteria strains through rational antimicrobial use policy, right practices and intensive research leading to

novel and alternative drugs therapies would help put under check the emergence of multiple drug resistant bacterial strains.

5.1.6 Mycotoxin determination from the herbal products

5.1.6.1 Determination of aflatoxins from the herbal products

Aflatoxin contaminations were determined among the sampled products in this study. The results showed that the herbal products were contaminated with aflatoxins to varying degrees. The general safety limits for total mycotoxins in herbal products should not exceed 4 ppb (µg/kg). Most (53.6%) samples were contaminated with aflatoxins although a few (5.16%) had aflatoxins levels beyond 4ppb hence were not safe for human consumptions. Four (11.8%) samples from herbal clinics and three (4.1%) from street vendors had aflatoxins levels above 4.0ppb. Salehei *et al.,* (2015) in a similar study in Iran isolated fungi which are associated with aflatoxin production in herbal medicinal products. These samples from herbal clinics and street vendors/hawkers were contaminated with aflatoxins levels above the FAO accepted limits in the current study. These contaminated samples were in form of powders and liquids formulations. Aflatoxins are secondary metabolites of some moulds, which include highly toxic, mutagenic or teratogenic compounds. Several environmental factors influence mycotoxin production, but temperature and humidity are considered the most critical (Simsek *et al.,* 2002).

Aflatoxins are produced by the genus *Aspergillus* which includes *A. flavus* and *A. parasiticus*. Sometimes the fungus is damaged by adverse conditions especially during

processing of the products. However mycotoxins are not affected by processing because they are heat stable. The stability of mycotoxins during food processing is affected by many factors, including: the moisture of the product, the toxin concentration and its location and the presence of additives. These factors should therefore be considered with respect to each type of processing (e.g. milling, roasting, canning, oil extraction) when estimating the fate of the mycotoxin. In the current study, the highest level of aflatoxins reported was 7.14ppb in 5.9% of the samples.

Aflatoxins were detected in 59.5% of the samples and yet they were free of fungi contamination while some (53.1%) of fungi contaminated samples had aflatoxins. In this case aflatoxins were not detected in all the fungi contaminated samples likewise to the samples which were free from fungi contamination, aflatoxins were detected in some. For the samples free from fungi yet aflatoxins were detected could be because fungi were destroyed during herbal products processing. The study found that there was no significant association between ($p<0.05$) the presence of fungi and aflatoxins contamination. The occurrence of aflatoxins in herbal medicines as observed in the present study has been reported previously (Hitokoto *et al.,* 1978; Kneifel *et al.,* 2002; Sewaram *et al.,* 2006; Adriana *et al.,* 2006; Pavlovic *et al.,* 2006; Alwakeel, 2008). The aflatoxins pose great concern over consumer safety. Adriana *et al.,* (2006) in a study on fungal contamination of herbal products found that 49% of the isolates produced high levels of aflatoxins. Alwakeel, (2009) in a similar study reported the occurrence of toxigenic mycoflora and mycotoxins in medicinal plants and herbal products. The effects of mycotoxins especially aflatoxins on various organs have been reported in both

experimental animals and man (Alwakeel, 2009). Impaired liver, kidney and brain function are well known consequences of ingesting even minute quantities of aflatoxins.

Some studies claim that exposure to high levels of aflatoxins can lead to neurological problems and in some cases may lead to death (Kneifel *et al.,* 2002; Pavlovic *et al.,* 2006; Sewaram *et al.,* 2006; Alwakeel, 2008). Since they are generally regarded as indestructible in all contaminated consumable items, they cannot be removed or destroyed, so prevention is the only real way forward. Aflatoxins are both hepatotoxic and hepatocarcinogenic (Orsi *et al,* 2007). Prolonged exposure, (especially daily home exposure), may be particularly harmful (Ezekwesili *et al.,* 2014). Yard and others in a study in 2007 on aflatoxin exposure found that there was widespread aflatoxin exposure across Kenya. Over three quarters of serum specimens they investigated had evidence of recent exposure, and this exposure persisted across the spectrum of age, sex and socioeconomic status. This widespread exposure could negatively impact health throughout Kenya (Yard *et al.,* 2013). Maize and other cereals which are prone to fungal investation are staple food in Kenya. These funguses are capable of producing aflatoxins and other fungal toxins as secondary metabolites hence putting the consumers at a risk. When food and herbal products containing aflatoxins are consumed by the general population, their health is put at risk.

Although not all fungi produce aflatoxins, *Aspergillus flavus* and *A. parasiticus* are the two major genera reported to produce toxigenic aflatoxins (Tassaneeyakul *et al.,* 2004; Riba *et al.,* 2008; Korir & Bii, 2012). Samples in the current study were contaminated

with saprophytic fungi, pathogenic/mycotoxin producing fungi as well as pathogenic yeasts. Several environmental factors are reported to influence aflatoxins production, but temperature and humidity are considered to be the most critical according to a study by Simsek *et al.,* (2002). The prevalent weather conditions of Nairobi County where the herbal products were sampled from, is a predisposing factor for fungal growth which eventually produce aflatoxins and other mycotoxins as secondary metabolites.

5.1.6.2 Determination of fumonisins from the herbal products

Fumonisins are mycotoxins produced by fungi of the genus *Fusarium*. *Fusarium oxysporum, Fusarium moliniform, Fusarium solani* and *Fusarium spp* were isolated in this study and are associated with fumonisins contamination among the samples. Samples had fumonisins levels in varying degrees. Twenty-one samples had fumonisins levels above 4.0ppm hence beyond accepted limits. Nine samples from herbal clinics, ten from streets vendors/hawkers and two from the supermarkets had fumonisins levels above 4.0ppb. These products were in form of liquids and powders. Bokhari & Aly, (2013) in a similar study in Saudi Arabia found similar results. The presence of fumonisins and fungal contamination among the samples showed that 47.4% of the samples were free from fungal contamination yet they had fumonisins contamination. Some (62.3%) were contaminated with both fungi and fumonisins. Statistical analysis revealed that there was no significant association (χ^2 test; p=0.081) between fungal contamination and fumonisin detection among the samples interms of sources/formulations.

According to Dragan *et al.,* (2001), the prevalence of *Fusarium* and the subsequent production of fumonisins are enhanced by warm climates and under drought conditions,

factors that cannot be controlled, although growers and consumers should be made aware that high concentrations of fumonisins are to be expected under such conditions. Insect damage also affects the accumulation of fumonisins, and both the prevalence and degree of insect damage are significantly correlated with the concentrations of the toxins. Maintenance of rigorous insect control may assist in reducing fumonisin contamination. Herbal products are made from plants products ranging from leaves, root and stem barks, seeds and ponds of fruits. Seeds and fruit ponds are prone to insect damages, which is a risk factor of fumonisins contamination. The strategies for coping with mycotoxins after harvest include inhibition of fungal growth by methods including chemical and physical means, natural products and biological control; segregation; and removal or destruction of existing mycotoxins by physical means, chemical treatment, adsorption, or biological degradation. Different methods can be combined to achieve a synergistic effect, expressed as enhanced activity. The safe moisture content for natural product is 14–15%. Several studies on laboratory animals (Charles *et al.,* 2000; Carlson *et al.,* 2001; Van-der-Westhuizen *et al.,* 2001) have associated fumonisins with several cancers of the liver and the kindney. The toxins are known to be nephrotoxic. Two cellular modes of action for the toxicity and carcinogenicity of fumonisin B_1 have been proposed that are well supported by results obtained in vivo. In both hypotheses, altered lipid metabolism is the initial biochemical mechanism. In one hypothesis, the initial biochemical lesion is presumed to be inhibition of ceramide synthase (Merrill *et al.,* 2001; Riley *et al.,* 2001) and in the other, the biochemical lesion is attributed to disruption of the *Delta* 6 desaturase and cyclooxygenase metabolic pathways (Gelderblom *et al.,* 2001). In both hypotheses, it is assumed that other initial sites of action could contribute to the observed

cellular responses. The two invoke similar cellular mechanisms, to the extent that fumonisin B_1-induced imbalances in the rates of cell death and proliferation in target tissues are considered to contribute to cancer development (Howard *et al.,* 2001).

In both liver and kidney, fumonisin B-induced toxicity is often characterized; initially by increased apoptotic and oncotic necrosis, regeneration and in the case of liver, bile-duct hyperplasia (Bhandari *et al.,* 2001). Experimental evidence for synergistic interactions between aflatoxin B_1 and fumonisin B_1 (Gelderblom *et al.,* 1999; WHO, 2000a; Carlson *et al.,* 2001) and between aflatoxin B_1 and nivalenol (Ueno *et al.,* 1992; Cohen *et al.,* 2000) in inducing hepatic cancer in rats was reported. According to WHO (2000b) an association has been established between the occurrence of the *Fusarium verticillioides*, *Fusarium moniliforme* and other Fusarium mycotoxins on maize and the incidence of oesophageal cancer in various regions of the world. In this study Fusarium producing mycotoxins were detected hence, the products are not safe for human consumption to some degree. Salehei *et al.,* (2015) in Iran in a similar study observed that different species of Fusarium were present in herbal medicinal products hence concurs with the results of the current study.

5.1.7 Determination of heavy metal contaminations in the herbal products

Heavy metals are natural constituents of the earth's crust, but indiscriminate human activities have drastically altered their geochemical cycles and biochemical balance. This results in accumulation of metals in plant parts having secondary metabolites, which is responsible for a particular pharmacological activity. In this study lead, arsenic,

cadmium, mercury and uranium were not detected in the herbal products examined. Lead is one of the most toxic heavy metals which have no nutritive value. Progressive exposure to lead results in a decrease in the performance of the nervous system and affects renal clearance (Salawu *et al.,* 2009).

Inorganic lead is carcinogen and may cause miscarriage in pregnant women. Nkansah *et al.,* (2016) in a similar study in Ghana found that, the levels of lead recorded were very low. Sarpong and Boateng (2013) reported similar results from different samples of unregistered herbal preparations marketed in and around Kumasi, with the levels of Pb below the WHO limits. Because of its ability to bioaccumulate in biological tissues, patients who use medicinal herbs with even low concentrations of Pb over a long period of time might be at risk of chronic Pb toxicity and should be monitored for any signs of lead poisoning. Plants are particularly efficient at absorbing Pb from the soil and are reported to retain up to 7%. Soil naturally contains up to 50 mg/kg of Pb, although this is significantly higher in rocky (igneous) soils (Sarpong & Boateng, 2013).

In the current study, copper, zinc, nickel and iron were detected in some herbal products. Keter *et al.,* (2013) found herbal products contained lead, cadmium, copper and arsenic but in levels that were within the Food and Agricultural Organization/ World Health Organization (FAO/WHO) provisional tolerable intake (FAO/WHO, 1993). Prolonged exposure to heavy metals such as copper, lead, nickel, and zinc can cause deleterious health effects in humans.

Copper is relevant to humans because it is both essential and toxic depending on the dose and duration of exposure. According to a study by Williams, (1982), acute copper poisoning results in gastrointestinal disturbances such as salivation, epigastric pain, nausea, vomiting and diarrhoea, all of which are probably due to the irritant effect of copper on the mucosa, whereas chronic effects from long-term overexposure results in liver damage (Bremmer, 1998). In this study, copper contamination was detected in 79.1% herbal products. There was no significant association between the type of herbal products and copper contamination (F test; $p=0.05$). Eleven (14.9%) samples from street vendors/hawkers, one sample from the herbal clinics and supermarkets, respectively had copper values above 8.1ppm. All these samples with copper values above 8.1ppm were powders. The maximum permissible level of copper is 12,000 μg per gram for daily intake (NRC, 1989) whereas in this study the levels were below 20ppm which is equivalent to 20μg per gram for herbal samples hence they are within the permissible limits.

Presences of essential micronutrients such as Zinc (Zn), was noted among the herbal products in this study. Zinc mocronutient was detected in 99.3% samples. There was no significant association ($p<0.05$) between the herbal products samples and the presence of zinc. The Zinc daily-recommended intake is between 10 and 20 mg per gram per day (NRC, 1989) therefore; the herbal product in this study might supplement the daily intake if taken for a long time especially for chronic infections like diabetes. Zinc is an essential element; it plays an important role in growth and has a recognized action in more than 300 enzymes by participating in their structure or their catalytic and regulatory action.

Zinc deficiency causes growth retardation and hypogonadism. Zinc deficiency may also affect the bone metabolism and gonadal function (Rai *et al*., 2001). A similar study by Keter *et al.* (2013) found similar results in that; there were low levels of Zinc of 8 µg per gram in their herbal products. Nkansah *et al.,* (2016) in a similar study in Ghana found that all the 15 medicinal plant samples analysed had some degree of zinc concentration hence concurs with the current study. Other studies in India and Pakistan by Rathore & Mohit, (2013), and Ansari *et al.,* (2004), respectively reported that their medicinal herbal samples contained zinc levels to some degree.

According to the agency for toxic substances and disease registry (ATSDR), chronic exposure to nickel is commonly associated with allergic reactions in humans, especially skin rashes, which appear at the site of contact. Animal data showed blood, stomach, liver and kidney disorders (ATSDR, 2008). Majority (84.8%) of the samples in this study had nickel at varrying degreses. Majority (53.6%) of samples with nickel had levels ranging from 10.1 – 40ppm. One sample from street vendors/hawkers, which was in powder form, had nickel values beyond 40.1ppb. There were no significant association ($p<0.05$) between the herbal medicinal products and nickel detection in this study. Nickel was not detected in the tested samples in a similar study by Ekeanyanwu *et al.,* (2013). Some samples in this study were higher (40.0ppm equivalents to 40ug/g) than the nickel permissible limits by various authors which is 35µg/g according to Forstner & Wittman, (1981) and 25 -35µg/g according to (Anke, 1995).

Iron is a micronutients required by the body in trace amounts and herbal products/supplements can be a very good source of iron. Iron is an essential element for human growth and development and an essential component of haemoglobin (Abou *et al.*, 2000). It facilitates the oxidation of carbohydrates, proteins and fats to control body weight, which is a very important factor is diabetes management. Iron is necessary for the formation of haemoglobin and also plays an important role in oxygen and electron transfer in the human body (WHO, 2010). Low iron content causes gastrointestinal infection, nose bleeding and myocardial infection. Iron has several key functions in human body including oxygen supply, energy production and immunity. Iron overdose is associated with symptoms of dizziness, nausea and vomiting, diarrhea, joints pain, shock and liver damage. Iron toxicity has an adverse effect on various metabolic functions and cardiovascular system (Martin & Griswold, 2005). Most (87.0%) samples in this study had iron micronutrients in varrying degrees. Eleven samples from street vendors/hawkers and one sample each from herbal clinics and supermarkets, respectively had iron levels above 8.1ppm totalling to 13 samples. All the 13 samples with 8.1ppm and above iron levels were powders. There was no significant association between the HMP from different sources and formulations and iron detection in this study (F test; p=0.05). Rania *et al.*, (2015) in a study observed that the range of iron was between 81.25 and 1101.22 mg/kg. Nkansah *et al.*, (2016) in a similar study in Ghana found that all the samples contained iron with a mean concentration was 4.66 ± 0.02 mg/kg. The WHO limit for iron in medicinal herbs has not been established yet (WHO, 2010). The result of the current study shows a wide variation of iron in different herbal samples. These results

are comparable to values of iron found in Egyptian spices and medicinal plants with wide iron variations (Abou *et al.,* 2000).

The use of herbal medicinal products is not generally expected to expose the population to significantly high heavy metal contaminants. Although it is generally believed that herbal and natural products are safer than the synthetic or modern medicines, some indigenous herbal products contain heavy metals as essential ingredients. However, it should be understood that the heavy metal content of herbal medicines when taken daily especially for chronic infections adds to the burden originating from food (WHO, 2007). Several studies have revealed that herbal remedies contained high levels of heavy metals sufficient to cause adverse health effect when regularly taken as recommended (Schicher, 1983; Ernst, 1998; Koh & Woo, 2000; Garvey *et al*, 2001). Thus it is critical that heavy metal contamination is minimized. Heavy metals disrupt metabolic functions in two ways; they accumulate and thereby disrupt function in vital organs and glands such as the heart, brain, kidneys, bone and liver among others. They displace the vital nutritional minerals from their original place, thereby, hindering their biological function. It is, however, impossible to live in an environment free of heavy metals. There are many ways by which these toxins can be introduced into the body such as consumption of foods, beverages, skin exposure, inhaled air and herbal products as evidenced in this study

5.1.7.1 pH of the samples

The pH of 138 samples was tested and categorized as strong acids, weak acids, neutral, weak bases and strong bases. Majority (79.0%) of the samples were weak acids, 4.3%

were neutral while 14.5% were weak bases. About 2.2% were strong acids. Acids have a bitter or sore test hence explaining why most of the herbal products are normally bitter when tasted. The pH of the solvents and other materials used for formulation can influence/determine the final pH of an herbal product. Previouse studies on herbal medicinal products have not determined the pH of the samples. Expired products tend to ferment because of contamination with bacteria and yeast and their pH can change say from alkaline to acidic due to fermentation. The human body needs to take in the right amount of acidic and alkalizing nutrients to maintain a healthy pH balance. The recommended daily intake should be 20% acidic and 80% alkalizing according to Theodore, (2014). The author noted that if acid/alkaline balance is not maintained; the body has to compensate by; robbing minerals from the bones, joints, muscles, gallbladder and mucosal lining of the digestive track. When the body robs calcium from the bones one can develop a weak back. If left untreated, this can develop into osteoporosis (low bone density). Robbing calcium and sodium from the joints can cause them to start to lock up, get stiff, sore and/or crack. After some time, the immune system often attacks acid which may settle in the joints leading to "arthritis". Minerals robbed from the muscles, ligaments and tendons increase the likelihood of an injury or tear. Acid deposited in the muscles can cause fibromyalgia, soreness and/or stiffness. Minerals robbed from the heart muscle can cause weakening, arrhythmia and angina. If the body needs to rob minerals from the lining of the intestinal track, or if the body tries to eliminate acid through the intestine, one develops pain in the colon, colitis, diarrhea, Crohn's or irritable bowel (Theodore, 2014). Natural sodium is often robbed from the lining of the oesophagus. If enough erosion happens, it can prevent the sphincter muscle

at the top of the stomach from closing properly, resulting in heartburn, acid reflux or Gastroesophageal Reflux Disease (GERD). Damage to the esophagus can cause difficulty in swallowing or a narrowing of the esophagus pipe.

For protection, the body will try to eliminate acid through the bowel, kidneys, skin and respiratory system. Eliminating through the lungs (lower respiratory system) can cause asthma. If acid settles in the sinuses (upper respiratory system), the body will try to dilute it with mucus, which can result in post nasal drip or sinus problems. Eliminating through the urinary system can cause blood in the urine and/or bladder irritation, cystitis and later on kidney damage. It can also increase the likelihood of developing UTI's (Urinary Tract Infections). When the body's pH is acidic for a long period of time, it can start changing the blood's pH from an ideal 7.41 to a more acidic 7.35, which can cause irritation and inflammation in the arteries. In response to this, the body will line the arteries with cholesterol to protect the arteries from the pH imbalance (Theodore, 2014).

When the body gets to the point that it can no longer rob minerals safely, the body will commission the liver to produce ammonia in order to help neutralize the acid leading to alkalinity or a pH above 7.0. Often the liver loses its ability to produce ammonia and then cells are bathed in acidic fluid. The body uses enzymes to break down abnormal cells in the body. Enzymes cannot work in an acidic medium. Therefore, the body cannot protect itself from the growth of abnormal cells. It has been concluded that acid feeds cancer cells. More accurately, it protects cancer cells from enzymes used by the immune

system. Without the enzymes to break up the cancer cells, the body can not eliminate them (Theodore, 2014).

5.2 Conclusion

1. From this study, it is evident that herbal medicinal products sold in Nairobi are highly contaminated with microbes that are potential pathogens. Products from street vendors, herbal clinics which were in powder and liquid forms had microbial loads in terms of cfu/g beyond the European pharmacopeia accepted limits [bacteria $<1.0x10^3$cfu/g (47.6%) and fungi <104/105cfu/g (1.6%)].

2. The enteric bacterial counts were detected in higher numbers and some of the members recovered in this study like *E. coli*, *Shigella* species and *Yersinia spp* are established human pathogens. When coliforms contaminated herbal products are consumed, they could lead to a very serious outbreak of diarrhea diseases. *Staphyloccocus aureus* and other pathogenic bacteria were also recovered. The genera Streptomyces was isolated in almost all the herbal products which imply that the products were contaminated with the soils. Among fungi isolated, the presence of the genera Aspergillus, Fusarium and Penicillium was greater than other genera from the studied herbal products. Most of the identified fungi are associated with mycotoxins production.

3. Samples were contaminated with fumonisins and aflatoxins at varying degrees. About 15.2% of the samples had fumonisins levels beyond the accepted limits while 5.2% had aflatoxins levels beyond this limits (4ppb) hence were not safe for human consumptions.

Herbs from street and herbal clinics, which were powders and liquids, were highly contaminated (beyond 4.0ppb). There was no significant association between the presence of fungi and aflatoxins/fumonisin contamination in the herbal product (χ^2 test; p=0.779 and χ^2 test; p=0.081, respectively).

4. Majority of the isolates were sensitive [61(63.5%)] to the panel of antibiotic tested in this study. The bacteria showed some resistance (single drug resistant, 2 drugs, and 3 antibiotics as well 4 drugs resistance). *Citrobacter diversus* (CD1) was resistant to CN, CTX and NOR, *Morganella morganii* (MM1) resistant to SXT, C and CTX, *Enterobacter aerogens* (EA2) resistant to CAZ, CTX and PRL and *Klebsiella pneumonia* (KP2) resistant to SXT, CAZ and CTX. Herbs from street vendors and herbal clinics, which were in powder and liquid formulations, had drug resistant bacteria.

5. There was direct association between the phenotypic resistance and genotypic resistance in this study. The wide use of antimicrobials might determine co-selection processes that ensure persistence of these genes in bacterial communities. In this study, aacA-*aphD* gene coding for gentamicin resistant, Bla_{CMY} gene code for Ceftazidime, gyrA gene codes for NOR resistant, CTX-M genes coding for CTX resistance and SulA gene codes for Sulphamethoxazole/Trimethoprim resistance were detected.

6. Copper, zinc, nickel and iron were detected in some herbal products. The maximum permissible level of copper, zinc and iron were within accepted permissible limits. However, the nickel levels among some herbal products were beyond 35ppm, which is

recommended by FAO/WHO. The product with high level of nickel was from street vendors/hawkers inform of powder formulation. Different herbal products had different pH range. Majority (79.0%) were weak acids. These products when consumed daily may lead to problems associated with acidity such as heartburn, acid reflux or gastroesophageal reflux disease among others.

5.3 Recommendations

- Due to the high number of bacterial and fungal contaminations among the samples in this study, there is an urgent need to have specific educative programs, policies and regulations addressing herbal products safety which are specifically focused on prevention of microbial contaminations, so as to prevent the possibility of these pathogens to be involved in deadly invasive infections. The policy should guide on quality control measures and safe handling practices to reduce the health risks associated with microbial contamination.
- From this study large coli-forms and other pathogenic bacteria and fungi were identified therefore necessitating the need to assess similarities between these pathogens and those implicated in causing infections. The study also recommends other researchers to focus on parasitic and viral contaminations in order to determine their prevalence in herbal products contamination.
- Other heavy metals of interest should be determined among herbal products in order to conclude on their safety. Metals like aluminum, fluoride, tin and other trace elements should be investigated and their effects on the overall PH of the final products should be noted.

- There were varieties of molds isolated in this study, which are associated with mycotoxins production, the current study only focused on aflatoxins and fumonisins and therefore other mycotoxins should be investigated so as to ensure overall fungi toxins safety among the herbal products.
- Antibiotics susceptibility profile should be expanded to cover all the classes of antibiotics in order to determine the range of resistance.
- Further studies should focus on sequencing bacterial that were found to be genotypic resistance in order to determine their relatedness. Since the current study focused on chromosomal genes, mobile resistant genes should be determined. This is because bacteria sharing the same environment can transfer these mobile genes to antibiotic sensitive bacteria through plasmids and transposons.
- Herbalists should be trained on how to apply Good Manufacturing Practices, good harvesting practices and the safe handling and storage of herbal medicinal products.

5.4 Limitations of the study

- Sampling was not uniform in all the sites/sources and formulations which brought problems during analysis as comparison could not be achieved effectively
- The study was carried out in Nairobi hence the results cannot be generalised to other regions of Kenya.

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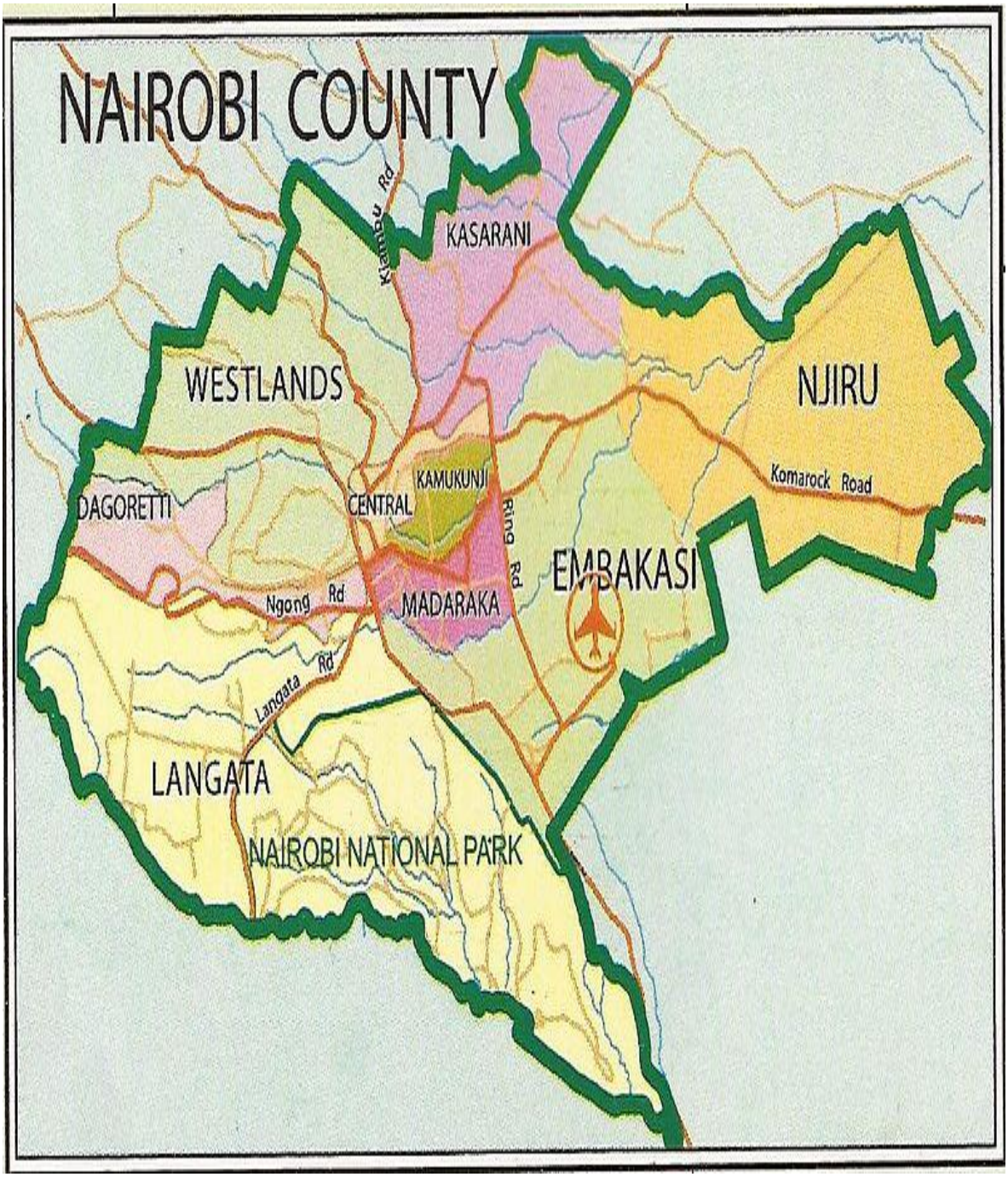
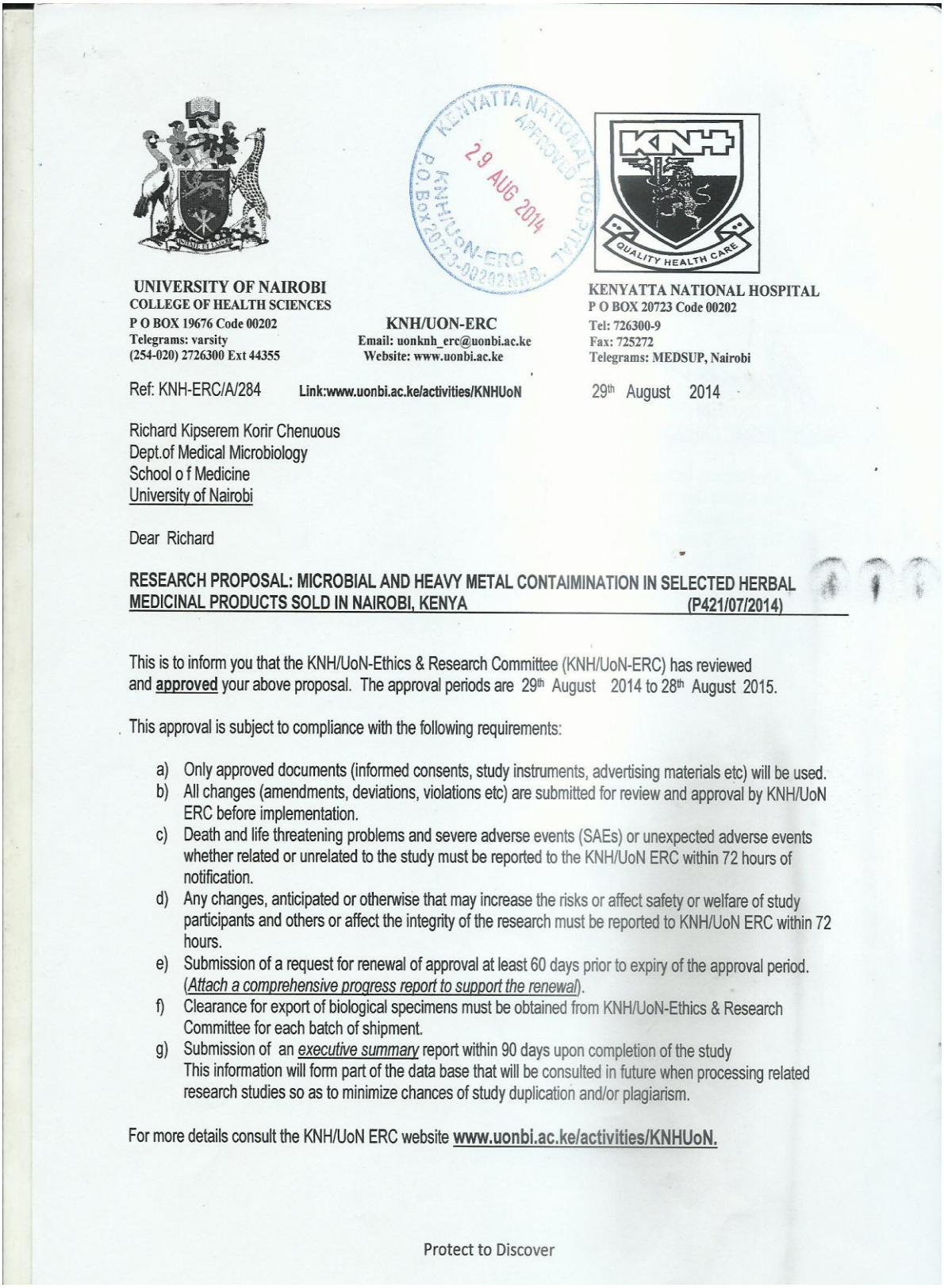
APPENDICES

Appendix 1: Map of Nairobi County



Appendix 2: Ethical clearance letter



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Ref: KNH-ERC/A/284 Link:www.uonbi.ac.ke/activities/KNHUoN 29th August 2014

Richard Kipserem Korir Chenuous
Dept.of Medical Microbiology
School o f Medicine
University of Nairobi

Dear Richard

RESEARCH PROPOSAL: MICROBIAL AND HEAVY METAL CONTAIMINATION IN SELECTED HERBAL MEDICINAL PRODUCTS SOLD IN NAIROBI, KENYA (P421/07/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 29th August 2014 to 28th August 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

Protect to Discover

Appendix 3: Herbal products displayed in shelves

(A) Shows herbal products displayed in shelves in an herbal clinic while (B) shows samples of some herbal products displayed in the laboratory bench awaiting analysis.

Appendix 4: Recommended microbial limits for herbal drugs (values in CFU/g)

Contaminants	**USA**	**EU**	**WHO**	**Brazilian**
Aerobic bacteria	$10^5/10^4/10^2$	$10^7/10^5$	$*/10^7/10^5$	$10^7/10^5/10^4$
Mold and yeast	103/102/10	105/104	$10^5/10^4/10^3$	$10^4/10^3/10^2$
Enterobacteria and other Gram negative bacteria	$10^3/*/*$	$*/10^3$	$*/10^4/10^3$	$10^4/10^3/10^2$
E. coli	Absent	10^3/absent	$10^4/10^2/10$	absent
Salmonella sp.	Absent	*/absent	*/absent/absent	absent

Key: * - Limits are not specified, X/y/z - First/second/third category or value respectively.

The most widely accepted guideline is that recommended by WHO and European Pharmacopeia for total count of microorganisms in plant materials, for instance WHO procedures has drawn the following specifications for products for oral use: 10^4 aerobic bacteria/g or ml, 10^2 fungi/g and absence of *Salmonella* spp, *Shigella spp*, *E. coli* and *S. aureus.* However, the European Pharmacopeia indicates detection of other indicators of increased risk for oral administration, such as *P. aeruginosa, B. cereus, Enterobacter* spp, *C. albicans, A. parasiticus* and *A. flavus.* The identification of high microbial loads is indicative of the possibility of potentially pathogenic microorganisms (European Pharmacopeia, 2007).

Appendix 5: Profiles of isolated bacteria

a) Isolated bacteria from different formulations

Organism	Caps (n) (%)	Liquid (n) (%)	Cream (n) (%)	Juice (n) (%)	Powder (n) (%)	Syrup (n) (%)	Total (n) (%)
Citrobacter diversus	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)	2 (100.0)
Enterobacter aerogens	0 (0.0)	2 (25.0)	0 (0.0)	0 (0.0)	6 (75.0)	0 (0.0)	7 (100.0)
Enterobacter cloacae	0 (0.0)	3 (22.2)	0 (0.0)	0 (0.0)	11 (77.8)	0 (0.0)	9 (100.0)
Erwinia chrysanthemi	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
Escherichia coli	0 (0.0)	3 (40.0)	0 (0.0)	0 (0.0)	4 (40.0)	0 (0.0)	5 (100.0)
Morganella morganii	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	1 (100.0)
Klebsiella pneumoniae	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	3 (75.0)	0 (0.0)	4 (100.0)
Proteus penneri	0 (0.0)	2 (5.9)	2 (5.9)	2 (5.9)	19 (82.4)	0 (0.0)	17 (100.0)
Serratia fonticola	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	1 (100.0)
Serratia marcescens	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	3 (66.7)	0 (0.0)	3 (100.0)
Serratia rubidaea	0 (0.0)	3 (20.0)	0 (0.0)	0 (0.0)	4 (80.0)	0 (0.0)	5 (100.0)
Shigella sonnei	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	1 (100.0)
Staphylococcus aureus	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	2 (100.0)
Yersnia enterocolitica	0 (0.0)	2 (33.3)	0 (0.0)	0 (0.0)	9 (66.7)	0 (0.0)	3 (100.0)
Total	0 (0.0)	18 (11.6)	3 (2.2)	2 (1.4)	71 (75.4)	0 (0.0)	96 (100.0)

b) Islated bacteria from different sources of herbal products

Organism	Herbal C (n) (%)	Manufacturer/ W (n) (%)	Street vendor/ H (n) (%)	Supermarket / S (n) (%)	Total (n) (%)
Citrobacter diversus	0 (0.0)	0 (0.0)	2 (50.0)	1 (50.0)	2 (100.0)
Enterobacter aerogens	3 (42.9)	0 (0.0)	3 (42.9)	2 (14.3)	7 (100.0)
Enterobacter cloacae	2 (22.2)	0 (0.0)	7 (55.6)	4 (22.2)	9 (100.0)
Erwinia chrysanthemi	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
Escherichia coli	1 (20.0)	0 (0.0)	5 (80.0)	1 (100.0)	5 (100.0)
Morganella morganii	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	1 (100.0)
Klebsiella pneumoniae	3 (75.0)	0 (0.0)	1 (25.0)	0 (0.0)	4 (100.0)
Proteus penneri	8 (23.5)	0 (0.0)	12 (52.9)	5 (23.5)	17 (100.0)
Serratia fonticola	1 (50.0)	1 (100.0)	0 (0.0)	1 (50.0)	1 (100.0)
Serratia marcescens	2 (66.7)	0 (0.0)	2 (33.3)	0 (0.0)	3 (100.0)
Serratia rubidaea	2 (20.0)	0 (0.0)	5 (80.0)	0 (0.0)	5 (100.0)
Shigella sonnei	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	1 (100.0)
Staphylococcus aureus	0 (0.0)	0 (0.0)	2 (100.0)	3 (100.0)	2 (100.0)
Yersnia enterocolitica	6 (33.3)	0 (0.0)	4 (33.3)	1 (33.3)	3 (100.0)
Total	28 (24.6)	1 (5.1)	48 (53.6)	18 (13.8)	95 (100.0)

c) Isolated Fungi from samples of different sources

Organism	Herbal C (n) (%)	Manufacturer/ W (n) (%)	Street vendor/ H (n) (%)	Supermarket/ S (n) (%)	Total (n) (%)
Absidia corymbifera	1 (25.0)	0 (0.0)	3 (75.0)	0 (0.0)	4 (100.0)
Alternaria alternata	0 (0.0)	0 (0.0)	1 (25.0)	3 (75.0)	4 (100.0)
Aspergillus	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
Aspergillus candidus	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	2 (100.0)

Aspergillus flavus	5 (18.8)	2 (12.5)	11 (62.5)	1 (6.2)	17 (100.0)
Aspergillus fumigatus	0 (0.0)	0 (0.0)	4 (80.0)	1 (20.0)	5 (100.0)
Aspergillus glaucus	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
Aspergillus nidulans	3 (37.5)	0 (0.0)	5 (62.5)	0 (0.0)	8 (100.0)
Aspergillus niger	2 (15.4)	0 (0.0)	11 (84.6)	0 (0.0)	13 (100.0)
Aspergillus parasiticus	0 (0.0)	1 (20.0)	3 (60.0)	1 (20.0)	5 (100.0)
Aspergillus ustus	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	2 (100.0)
Aspergillus versicolor	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
Candida glabrata	2 (15.4)	0 (0.0)	10 (76.9)	1 (7.7)	13 (100.0)
Fusarium moliniform	0 (0.0)	0 (0.0)	2 (50.0)	2 (50.0)	4 (100.0)
Fusarium oxysporum	4 (66.7)	0 (0.0)	2 (33.3)	0 (0.0)	6 (100.0)
Fusarium solani	2 (28.6)	1 (14.3)	4 (57.1)	0 (0.0)	7 (100.0)
Fusarium spp.	2 (25.0)	0 (0.0)	6 (75.0)	0 (0.0)	8 (100.0)
Penicillium marneffei	7 (38.9)	1 (5.6)	10 (55.5)	0 (0.0)	18 (100.0)
Penicillium verrucosum	0 (0.0)	4 (80.0)	1 (20.0)	0 (0.0)	5 (100.0)
Rhizomucor pusillus	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (100.0)
Rhizopus arrhizus	0 (0.0)	1 (25.0)	2 (50.0)	1 (25.0)	4 (100.0)
Rhodotorula glutinis	1 (16.7)	2 (33.3)	3 (50.0)	0 (0.0)	6 (100.0)
Total	32 (22.9)	13 (9.3)	84 (60.0)	11 (7.8)	143 (100.0)

d) Isolated Fungi form samples of different formulations

Organisms	Capsules (n) (%)	Liquid (n) (%)	Cream (n) (%)	Powder (n) (%)	Syrup (n) (%)	Total (n) (%)
Absidia corymbifera	0 (0.0)	0 (0.0)	0 (0.0)	4 (75.0)	0 (0.0)	4 (100.0)
Alternaria alternata	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	4 (100.0)
Aspergillus	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
Aspergillus candidus	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)
Aspergillus flavus	1 (6.3)	0 (0.0)	0 (0.0)	11 (87.4)	1 (6.3)	17 (100.0)
Aspergillus fumigatus	0 (0.0)	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)	5 (100.0)
Aspergillus glaucus	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
Aspergillus nidulans	1 (12.5)	0 (0.0)	0 (0.0)	7 (87.5)	0 (0.0)	8 (100.0)
Aspergillus niger	0 (0.0)	1 (7.7)	0 (0.0)	11 (84.6)	1 (7.7)	13 (100.0)
Aspergillus parasiticus	0 (0.0)	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)	5 (100.0)
Aspergillus ustus	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)
Aspergillus versicolor	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
Aspergiluss flavus	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	2 (100.0)
Candida glabrata	1 (7.7)	0 (0.0)	0 (0.0)	12 (92.3)	0 (0.0)	13 (100.0)

Fusarium moliniform	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	4 (100.0)
Fusarium oxysporum	0 (0.0)	0 (0.0)	1 (16.7)	5 (83.3)	0 (0.0)	6 (100.0)
Fusarium solani	1 (14.3)	0 (0.0)	0 (0.0)	5 (71.4)	1 (14.3)	7 (100.0)
Fusarium spp.	0 (0.0)	0 (0.0)	0 (0.0)	7 (87.5)	1 (12.5)	8 (100.0)
Penicillium marneffei	0 (0.0)	0 (0.0)	1 (5.6)	17 (94.4)	0 (0.0)	18 (100.0)
Penicillium verrucosum	0 (0.0)	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)	5 (100.0)
Rhizomucor pusillus	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	2 (100.0)
Rhizopus arrhizus	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)	0 (0.0)	4 (100.0)
Rhodotorula glutinis	0 (0.0)	0 (0.0)	1 (16.7)	5 (83.3)	0 (0.0)	6 (100.0)
Total	4 (2.9)	1 (0.7)	4 (2.9)	126 (89.2)	5 (3.6)	143 (100.0)

E) Baterials isolates for sensitivity

Organism	**Total (n) (%)**
Citrobacter diversus	2 (100.0)
Enterobacter aerogens	7 (100.0)
Enterobacter cloacae	9 (100.0)
Erwinia chrysanthemi	1 (100.0)
Escherichia coli	5 (100.0)
Morganella morganii	1 (100.0)
Klebsiella pneumoniae	4 (100.0)
Proteus penneri	17 (100.0)
Serratia fonticola	1 (100.0)
Serratia marcescens	3 (100.0)
Serratia rubidaea	5 (100.0)
Shigella sonnei	2 (100.0)
Staphylococcus aureus	2 (100.0)
Yersnia enterocolitica	3 (100.0)
Total	96 (100.0)

Appendix 6: Abstract published in East African Health Research Journal

Multidrug-Resistant Bacterial Isolates Recovered from HerbalMedicinal Products Sold in Nairobi, Kenya

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ABSTRACT
Background: Medicinal herbs have been reported to be contaminated with microorganisms indigenous to the environment. These microbes become a threat when they harbour drug-resistant traits.
Objective: The aim of this study was to evaluate phenotypic and genotypic drug-resistant traits of bacteria isolated from herbal medicinal products in Nairobi, Kenya.
Methods: We employed an exploratory as well as laboratory-based experimental design. Herbal products were purchased from markets and transported to Kenya Medical Research Institute laboratories for processing and analysis. Microbial contamination and antibiotic susceptibility were determined following standard methods. Antibiotic-resistant genes were determined using polymerase chain reaction. Data were coded and analysed accordingly. **Results:** We collected 138 samples of herbal products in the form of liquids, powders, capsules, creams/lotions, and syrups. In total, 117 samples (84.8%) were contaminated with bacteria and 61 (44.2%) were contaminated with fungi. Bacillus, Klebsiella, Proteus, Staphylococcus, Streptomyces, Escherichia, Enterobacter, Serratia, Yersinia, Morganella, Citrobacter, Erwinia, and Shigella were the bacterial genera identified. Most of the isolated bacteria were generally sensitive to the panel of antibiotics tested, although a few (35 [36.5%]) were resistant; more than half of these were resistant to more than 1 of the antibiotic agents we tested. **Discussion:** We found an association between phenotypic and genotypic drug resistance among the drug-resistant bacteria. This study makes it evident that herbal medicinal products sold in Nairobi are contaminated with drug-resistant bacteria. **Conclusions:** The results show that herbal medicinal products are a potential source of dissemination of multidrugresistant bacteria. There is an urgent need for specific education programmes, policies, and regulations that address herbal products' safety to prevent the possibility of these pathogens being involved in deadly invasive infections.

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Appendix 7: Abstract published in Food Science and Quality Management Journal

Occurrence of Aflatoxins and Fumonisins Contamination in Herbal Medicinal Products Sold in Nairobi, Kenya

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Abstract

Aflatoxins and fumonisins are referred to as Mycotoxins. They are secondary metabolites of some moulds which are highly toxic, mutagenic or teratogenic compounds. These substances are not formed by all mould species but are characteristic of their producers. The aim of this study was to determine the occurrence of Aflatoxin and Fumonisins contamination in herbal medicinal products sold in Nairobi Kenya. The study was undertaken in Nairobi, the capital and largest city in Kenya. Nairobi has several herbal clinics, especially in densely populated areas. This study employed an exploratory as well as laboratory-based experimental design to sample 138 herbal medicinal products. The sample were in different preparations, which included liquids, powders, capsules, creams/lotions, and syrups. Screening of the presence of aflatoxins and fumonisins were done using Envirologix Quick Tox™ Kit following the manufacturer's instructions. Fumonisins and aflatoxins concentration in parts per billion (ppb) was analyzed statistically using Pearson Chi square test at 95% confidence interval. Contaminations were presented in form of frequencies and percentages. Aflatoxins were detected in 74(53.6%) samples while fumonisins were detected in 75(54.3%). Four (11.8%) samples from herbal clinics and 3(4.1%) from street vendors in form of powders and liquids had aflatoxins levels above 4.0ppb. Nine (26.5%) samples from herbal clinics, 10(13.5%) from streets vendors/hawkers and two (10.5%) from the supermarkets in form of powders and liquids had fumonisins levels above 4.0ppb. There was no significant association (c^2 test; $p>0.05$) between detection of fungi from an herbal product and the occurrence of mycotoxins. *Aspergillus flavus* and *A. parasiticus* isolated in this study were associated with occurrence of aflatoxins while the *Fusarium* isolated were responsible for the presence of fumonisins among the herbal products. We conclude that herbal products investigated were contaminated with fumonisins and aflatoxins in varying degrees. Some were contaminated beyond the accepted limits. There were many genera of molds isolated in this study, which are associated with mycotoxins production, but the current study only focused on aflatoxins and fumonisins and therefore other mycotoxins should be investigated so as to ensure overall fungi toxins safety among the herbal products.

Key words: aflatoxin, fumonisins, contamination, herbal medicinal products, Aspergillus, Fusarium, Nairobi.
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