EVALUATION OF HEAVY METALS AND MICROBIAL CONTAMINATION IN HERBAL MEDICINES USED FOR CHRONIC ILLNESSES IN NAIROBI METROPOLIS

A thesis submitted in partial fulfilment of the requirements for the award of degree of Master of Pharmacy in Pharmaceutical Analysis of the University of Nairobi

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DEDICATION

This project is dedicated to my family:

To my husband, Farah, whose strength of will, encouragement and enthusiasm led to the completion of this work.

To my children, who persistently urged that I should not to be complacent and resilience awakens success.

To my mother, Halima Hassan, for instilling in me the virtue of hard work.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic absorption spectroscopy
AD	Alzheimer's disease
AIDS	Acquired Immune Deficiency Syndrome
API	Active pharmaceutical ingredient
ATSDR	Agency for toxic substances and disease registry
BP	British Pharmacopoeia
CAM	Complementary and alternative medicines
CFU	Colony forming units
CHM	Chinese herbal medicine
DNA	Deoxyribonucleic acid
DSHEA	Dietary supplements health education act
EDI	Estimated daily intake
EMR	Electromagnetic radiation
EU	European Union
FAO	Food and Agriculture Organization
GMP	Good manufacturing practices
HCL	Hollow cathode lamp
HIV	Human immunodeficiency virus
HMP	Herbal medicine products
IARC	International agency for research on cancer
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectroscopy

NA	Nutrient Agar
OCL	Oral component level
PCA	Principal component analysis
PCR	Polymerase chain reaction
PPB	Pharmacy and Poisons Board
Ph. Eur	European Pharmacopoeia
RVM	Rappaport Vassiliadis Medium
SD	Standard Deviation
SDA	Sabouraud's Dextrose Agar
THQ	Total Hazard Quotient
TCR	Total Cancer Risk
TVC	Total Viable Count
TMP	Traditional Medicine Practitioners
TSIA	Triple Sugar Iron Agar
USEPA	United States Environmental Protection Agency
US-FDA	United States Food and Drug Administration
USP	United States Pharmacopeia
VRBGA	Violet Red Bile Glucose Agar
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

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ABSTRACT

Introduction

The World Health Organization estimates that 70-80% of the world's population uses herbal medicines for their primary health care needs. Herbal medicines may be contaminated with toxic metals, microbes and pesticide residues among other contaminants. In spite of their popularity and immense contribution to the primary health care, many African countries lack or have insufficient quality control systems to assure the safety of these products. This study sought to evaluate marketed herbal products for two important quality parameters, heavy metals and microbial contamination, in Nairobi metropolis that comprises of Nairobi City County and sections of Kiambu, Kajiado and Machakos Counties.

Experimental

Herbal medicine samples used for the management of chronic illnesses namely diabetes, human immunodeficiency virus/acquired immune deficiency syndrome, rheumatoid arthritis, peptic ulcer disease, tuberculosis, cancer and hypertension were obtained from pharmacies, open-air markets and supermarkets within Nairobi metropolis. Heavy metals namely lead, cadmium, mercury and arsenic were quantitatively determined using inductively coupled plasma mass spectrometry. Microbial analysis was performed according to the British pharmacopeia, 2017 specifications for herbal medicines. Principal component analysis was performed in order to detect potentially harmful herbal medicines using Origin Pro 9.1 software (OriginaLab Corporation, MA, USA) while quantitative risk assessment was conducted to evaluate the potential public health risk posed by consumption of herbal medicines using target quotient and cancer risk methodology as elaborated by the United States Environmental Protection Agency.

Results and Discussion

A total of 89 herbal products were sampled for this study. A large proportion of the samples (42, 47.2%) were collected from Nairobi City County because it is the core distributive centre of the other metropolis regions. The remaining 47 samples (52.8%) were from the other three counties. Among the 89 samples, 55 (61.8%) had no both brand name and list of ingredients while 8 (9.0%) had brand name without list of ingredients. Only 26 (29.2%) samples had both brand name and list of ingredients. All the 89 samples were subjected to elemental analysis, whereby 33 (37.08%), 19 (21.35%), 3 (3.37%) and 2 (2.25%) contained lead, mercury, arsenic and cadmium, respectively, above the United States Pharmacopeia, (2018) permissible limits. A two-tailed test of significance showed no correlation between the levels of analysed metals. Analysis of variance using the F-statistic also showed that the content of the metals in the herbal products were significantly different at 0.05 level ($p=0.3093$). Risk analysis identified arsenic and mercury as being of greatest risk for non-cancerous toxicity with hazard index of greater than 1. Cancer risk (CR) and total cancer risk (TCR) higher than 10^4 were identified in some samples. Arsenic was found to be the contributor of the CR in some samples.

Eighty six samples were subjected to microbial analysis, where 14 (16.3%) had no visible growth while 72 (83.7%) exhibited growth of microorganisms and were subjected to test for specified microorganisms. Thirty nine (54.17%) of the 72 samples did not comply with the BP 2017 specifications. Twenty nine (33.72%) and 26 (36.1%) failed enumeration and test for specified micro-organisms, respectively.

Conclusion and Recommendation

The study revealed significant contamination with heavy metals and microorganisms in the herbal products. Mercury and arsenic were identified to pose the greatest non-cancer risk to consumers of herbal medicines with a two-fold higher risk in children than adults. The cancer

risk was ascribable to arsenic. Judicious use of herbal medicines in children and adults is recommended. Chronic use of these products may lead to bioaccumulation of the contaminants that may inadvertently have serious health implications. This underscores need for proper regulation of herbal products to ensure only those products that comply with standard quality specifications get to the market.

Chapter 1: INTRODUCTION

1.1 Background

Herbal medicine, herbalism or botanical medicine is an ethno- medical healthcare system based on the use of plants or plant extracts for therapy. Herbal medicine has been used by many different cultures throughout the world to maintain or restore normal physiology since ancient times (Yuan *et al.*, 2016; Adhikari and Bhusan Paul, 2018). The use of herbs for alleviating human suffering predates written human history (WHO, 1991; Barnes *et al.*, 2008). Early man explored his environment and depended on nature for a healthy life (Kosalec *et al.*, 2009). The early man learned by trial and error that some plants were suitable for food and medicines while others were poisonous (Chandira and Jayakar, 2010). Humanity continues to rely on plants and animals for their basic and curative needs to date (Kunle *et al.*, 2012). The World Health Organization (WHO) broadly defines traditional medicines to include practices, knowledge and skills indigenous to a community that are used in the maintenance of health (WHO, 2005, 2014).

Herbal medicines may be classified according to their caloric qualities or their phytochemical constituents. Herbalists aim at holistic management that utilizes all the plant constituents for their synergistic effects (Andrew, 2006). Traditional Chinese medicine, Japanese Kampo, Indian Ayurveda, the Greek medicine and Arabic Unani are some of the structured traditional medical systems (WHO, 2002, 2007; Yuan *et al.*, 2016). Herbal medicines may include crude materials, preparations or finished products. The crude plant materials can either be entire plant or plant parts which may be used wholly as powdered preparations or as extractions (Andrew, 2006; Ola *et al.*, 2013).

1.2 Processing of herbal medicines

Harvesting and collection of herbal medicine materials are done based on geographical locations, seasons and the targeted morphological parts. After collection, the materials are air dried under a shade to constituents. Tougher materials such as barks and roots are chopped into small pieces to enhance drying. The dried materials are ground to fine particles using mortar and pestle or commercial mills (Chege *et al.*, 2015), sieved to remove unwanted large materials and formulated into the desired products (Azwanida, 2015). These products may be in the form of tinctures, infusions, decoctions, macerations, poultices and compresses.

Actives are extracted from herbal materials using suitable solvents such as water, alcohols and halogenated solvents (WHO, 2007; Ola *et al.*, 2013). The extract may then be fractionated, purified and concentrated as necessary. Products containing synthetic compounds and isolated constituents from medicinal plants are not adjudged to be herbal (WHO, 1996). The cultivation and processing conditions have been shown to impact on the composition of the active principles of plant extracts (Generalić *et al.*, 2012).

1.3 Trends in use and preferences of herbal medicines

Herbal medicines form a major part of human healthcare throughout the world with an estimated annual market turnover of over 60 billion US dollars (Qurishi *et al.*, 2010). The sales of herbal medicines in the United States of America (USA) reached the four billion mark over the past two decades due to the Dietary Supplements Health Education Act (DSHEA) of 1994 (Steven and Varro, 1999). Herbal medicines have a great impact on modern medicine. Most prescription drugs have either one active ingredient from plant extracts or semi-synthetic or synthetic compounds inspired by plant isolates (Qurishi *et al.*, 2010). Despite the development of modern medicine, a large proportion of the population in both developed and developing countries still use these products (WHO, 2002; 2014). The WHO estimates that 70-80% of the

world population uses non-conventional medicines for the treatment and prevention of various ailments (Alwakeel, 2008; Dei-Tutuwa *et al.*, 2014).

Herbal medicines and traditional practitioners are sometimes the only sources of healthcare for millions of people around the world due to proximity to their homes, accessibility, affordability, cultural acceptance and trust (WHO, 2014). Herbal products have maintained their popularity due to their perceived efficacy, safety and minimal side effects (WHO, 2002; Oluwatoyin and Adebayo, 2016). At the international conference on traditional medicine for South-East Asian countries held in 2013, the WHO underscored the role of traditional medicines of proven quality, safety and efficacy in contributing to the goal of ensuring that all people have access to health care (WHO, 2014).

In Africa, traditional medicines play an important role in the healthcare of millions of people with their use differing between communities based on ethno-pathological perception of diseases and therapeutics (Fennel *et al.*, 2004). Reports indicate that about 80% of African people use traditional medicine either alone or in combination with conventional medicines (Nwoko and Mgbeahuruike, 2011).

1.4 Chemical composition of herbal medicines

Herbal medicines contain active pharmacological constituents with diverse chemical structures and biological activities (Wink, 2003). Primary plant constituents occur in large quantities in almost all plant species (Irchhaiya *et al.*, 2014). They are products of vital metabolic processes such as the Krebs cycle (Irchhaiya *et al.*, 2014). Primary metabolites include fats, proteins and carbohydrates essential for growth and development of the plant (Andrew, 2006). Secondary metabolites are biosynthetic end products of primary constituents important for plant adaptations to their environment (Demain and Fang, 2000). These secondary metabolites are important resources for the development of semi-synthetic and synthetic drugs (Bourgaud *et*

al., 2001; Yuan *et al.*, 2016). Secondary metabolites can broadly be classified into three groups as shown in Table 1.1 (Hartmann, 2007).

Table 1.1. Classification of secondary metabolites in plants

Group	Examples
Isoprenoids/Terpenes	Rubber, steroids, essential oils, carotenoids
Nitrogen containing compounds	Alkaloids, glycosides, glycosinates
Phenolic compounds	Lignin, coumarins, aflatoxins, flavonoids, tannins

Table adopted from Hartmann *et al.* (Hartmann, 2007)

1.5 Regulatory control of herbal medicines

The WHO developed guidelines for the cultivation, collection and processing of herbal medicines but insufficient attention has been given to their quality assurance and control (WHO, 2002). The European Union (EU) regulates herbal medicines as drugs for which therapeutic claims are made while those without such claims are marketed as foods (Steven and Varro, 1999; WHO, 2007).

Germany has stringent manufacturing standards for herbal medicines where their efficacy, safety and quality are monitored. In 1991, the German Ministry of Health published a draft paper for limits of heavy metals in plant products (Gasser *et al.*, 2009). The USA regulates its herbal medicines as dietary supplements hence pre-approval is not a necessity (WHO, 2005).

In 1984, the US Department of Health and Human Services established the National Investigative Committee on traditional and alternative medicine, with the aim of providing support and training (Hiral and Maheshwari, 2014). The WHO advocated for the integration of herbal medicines into the mainstream healthcare systems in developing countries (WHO, 1978,

1989). However, to be accepted in the modern medical system, quality assessment of herbal products is crucial in ensuring efficacy and safety (Kulkami *et al.*, 2010). Despite such efforts, the safety of these products continues to be ignored by herbalists (Esimone *et al.*, 2007).

The regulation of herbal medicines is done by the Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy. Regulatory provisions for herbal medicines are laid down in Drugs and Cosmetics Act 1940 and Drugs and Cosmetics Rules of 1945 (Sharma, 2011; Sahoo and Manchikanti, 2013; Sharma and Pundarikakshudu, 2019). The legal provisions relate to the import, manufacture, distribution and sale of these drugs. Additionally, government entities such as National Medicinal Plants Board of India and Quality Council of India also having a role in the quality, safety and efficacy of the herbal medicines in India (Verma, 2013) **.**

Other South East Asian countries such as Nepal, Thailand and Indonesia where the use of herbal medicines is rampant have regulatory bodies. In Indonesia, the regulation of traditional drugs is under Directorate of Traditional Drug Control domiciled under the Ministry of Health. In Nepal, herbal medicines are registered by the Department of Drug Administration, at the Ministry of Health. Similarly, western Pacific countries such as Singapore, China, Hong Kong, Macao, Fiji, Japan, Malaysia, Mongolia, New Zealand, Philippines, Republic of Korea and Australia elaborate regulatory mechanisms (Briggs, 2002).

Despite the extensive use of herbal medicines in the African continent, the regulation of herbal medicines is haphazard with some countries lacking proper regulatory framework (Malaria and Schools, 2011; Awodele *et al.*, 2014). For instance, the trade in crude indigenous herbal products is completely unregulated unless a health-claim of finished product is made for which

a drug evaluation is conducted by the Medicines Control Council before marketing (Zhang, 1998; Robinson and Zhang, 2011).

Majority of Kenyans utilize herbal medications prepared either at home or obtained from herbalists, pharmacies and supermarkets (Kibwage *et al.*, 2005). The regulations for these products and traditional medicine practice are set out in Medical Practitioners and Dentists Act (Cap. 253) of the Kenyan laws. Recognition by "own community" as a healer is the only prerequisite for registration as a practitioner in Kenya. Previous attempts by the National Drug Policy (1994) through the Pharmacy and Poisons Board (PPB) to control and standardize traditional medicines have not been fruitful due to the vast and complex nature of the constituents and lack of documented data (Kibwage *et al.*, 2005; Onyambu *et al.*, 2013).

1.6 Contamination

1.6.1 Major sources of contamination of herbal medicines

Herbal preparations may be contaminated with microbial and foreign materials during manufacturing and handling (Shaban *et al.*, 2016) which may lead to organotoxicity and fatalities. Incidences of morbidity and mortality associated with the use of herbal medicines have raised universal attention (Ahmad *et al.*, 2006). The public's, academic institutions and governments' interest in herbal medicines is growing rapidly due to increased incidences of adverse drug reactions and the economic burden of the conventional medicines (Saurabh *et al.*, 2011). Storage and transportation conditions may lead to loss of the active ingredients as well as production of inactive or toxic metabolites (Kunle *et al.*, 2012).

Heavy metal use has risen over the past decade in industries, agriculture and domestic settings, thus increasing human exposure (Sabine Martin, Ph.D. and Wendy Griswold, 2009). Exposure to these contaminants may result in mild to severe life-threatening clinical toxicities

(Vanhaelen *et al.*, 1994). Most people are chronically exposed to low levels of heavy metals, mainly through diet and water and to some extent inhalation of ambient air. Contamination or adulteration of herbal medicinal products with heavy metals may be erroneous or deliberate for economic gains (Chan, 2003).

1.6.2 Heavy metals

Heavy metals affect cellular organelles and enzymes that catalyse metabolism, detoxification and tissue repair (Chan, 2003). They cause DNA damage leading to carcinogenesis and apoptosis (Tchounwou *et al.*, 2012). The mechanisms of heavy metal-induced toxicity are not well elucidated. However, each metal possesses unique physicochemical properties that account for its specific toxicological mechanism of action (NCAPD, 2007). The heavy metals of public health significance in herbal products are cadmium, lead, mercury and arsenic (ICH, (QD3) 2009).

1.6.3 Cadmium

1.6.3.1 Chemistry, occurrence and roles

Cadmium (Cd) is found primarily as sulphide salt in ores. Cadmium may serve as a replacement for zinc at the catalytic sites on metalloenzymes. Inorganic Cd exists in the bivalent state while in an organic form as a complex with metallothionein. Plants get exposed to Cd through use of sewage sludge fertilisers on herbal gardens or from application of rock phosphate. Herbal medicines may also be contaminated with Cd from soil left on plants in cases of poor phytosanitary measures (McGeer *et al.*, 2011).

1.6.3.2 Toxic effects

Some case reports on adverse effects of cadmium have been reported in the literature (Wong *et al.*, 1993). Cadmium has slow renal clearance thus accumulates in the kidneys leading to

irreversible kidney damage (Li *et al.*, 2012). At high concentrations, cadmium affects the liver as well as vascular and immune systems (Maobe *et al.*, 2012). Because of its long half-life of 20 years, there exists a danger of bioaccumulation in the body mainly in the thyroid gland, liver, kidneys and pancreas.

1.6.3.3 Carcinogenicity

The International agency for research on cancer (IARC) periodically publishes reports on the classification of heavy metals among other agents based on their carcinogenicity. The general classification of carcinogens is shown in Table 1.2.

Table 1.2. Carcinogenicity of agents according to the IARC

Category	Implication
Group 1	Carcinogenic to humans
Group 2A	Probably carcinogenic to humans
Group 2B	Possibly carcinogenic to humans
Group 3	Not classifiable as to its carcinogenicity to humans
Group 4	Probably not carcinogenic to humans

The IARC classifies cadmium as a group 1 human carcinogen with tumours of the lungs, testicles, and prostate being reported (IARC, 2012). The postulated mechanism of carcinogenicity is through generation of cellular oxidative stress.

1.6.4 Lead

1.6.4.1 Chemistry, occurrence and roles

Lead is found at low concentrations in the earth's crust, predominantly as lead sulfide, but the widespread occurrence of lead in the environment is largely the result of anthropogenic

activity. Lead exists in two valence states of either +2 or +4. Natural lead is a mixture of four stable isotopes with differing abundances: Pb^{204} (1.4%), Pb^{206} (25.2%), Pb^{207} (21.7%) and Pb^{208} (51.7%). Similar to other heavy metals, lead exists in two forms; organic and inorganic. Organic lead occurs as ethyl lead and methyl lead.

1.6.4.2 Toxic effects

Lead complexes with other biomolecules and affects the blood, nervous, immune, renal, reproductive and cardiovascular systems (Johnson, 1998). Prenatal and early childhood lead exposure is associated with slow cognitive development (Agency for Toxic Substances and Disease Registry, 2007). Lead intoxication in adults taking Chinese herbal medicine (CHM) has been reported in literature (Wu *et al.*, 1996).

1.6.4.3 Carcinogenicity

The IARC categorizes lead as to its carcinogenicity as follows: lead (Group 2B), inorganic lead compounds (Group 2A) and organic lead (Group 3) (Table 1.2) (IARC, 2006).

1.6.5 Mercury

1.6.5.1 Chemistry and occurrence

Two forms of mercury (Hg) are recognized, namely inorganic and organic mercury. Examples of inorganic mercury include Hg vapour, mercurous Hg and mercuric Hg. Mercury vapour has a zero oxidation state chemically represented as Hg and is released from the volatile Hg liquid at ambient temperature. Mercury vapour is stable in ambient air and may linger in the atmosphere for several months. Mercury has two major oxidation states. The first oxidation state characterized by loss of one electron is referred to as mercurous mercury (Hg^{+}) while the second oxidation state that involves loss of two electrons yields mercuric ion (Hg^{2+}), sometimes referred to as mercuric mercury or divalent mercury. Mercury vapour, organic mercury and

mercurous mercury may release mercuric mercury. Divalent mercury therefore plays an important role is the toxicology of this metal.

Compounds in which the mercuric ion is covalently linked to at least one carbon atom are known as organic mercury. Examples of organic mercury include short chain alkyl CH_3 $(CH_2)_n$-Hg, phenyl-Hg and mercurial diuretics. Methyl mercury found in fish and preservatives and ethyl mercury are examples of short chain alkyl mercury.

1.6.5.2 Toxic effects

Mercury has a high affinity for thiol containing biomolecules. Both organic and inorganic mercury form complexes with reduced glutathione. Mercuric mercury also has a high affinity for selenium in its reduced form of the selenide anion, Se^{2-}. Mercuric selenide, HgSe, has a long residence time in human tissues leading to deleterious biological effects in the body.

Mercury is a neurodegenerative element that has been implicated in the causation of Alzheimer's disease (AD) through disruption of redox regulation (Mutter *et al.*, 2010). Lead and cadmium play a synergistic role in the aetiology of AD (Haley, 2007). Further, mercury may cause autism in genetically susceptible individuals (Geier *et al.*, 2008). Autistic children tend to have low levels of cysteine and glutathione necessary for mercury detoxification (Mutter *et al.*, 2005).

1.6.5.3 Carcinogenicity

Although mercury and inorganic mercury compounds are regarded by IARC as being not classifiable as to their carcinogenicity in humans (Group 3), methyl mercury and other organic mercurials are considered to be possibly carcinogenic to humans (Group 2B) (IARC, 1993).

1.6.6 Arsenic

1.6.6.1 Chemistry and occurrence

Arsenic occurs naturally in inorganic and organic forms. The trivalent metaarsenite and the pentavalent arsenate are the main inorganic forms of arsenic. Trivalent arsenic is recognized to be more toxic than the pentavalent form. The organic arsenic comprises of the methylated and thiolated arsenic metabolites. Methylated arsenicals include monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide (TMAO). Thiolated arsenicals contain As-SH and/or As=S substructures and include dimethylmonothiarsonous acid, monomethylarsonic diglutathione and arsinoglutathione. Figure 1.1 shows structures of some organic and inorganic arsenicals.

1.6.6.2 Toxic effects

The trivalent arsenic interacts with sulfhydryl groups of essential proteins in various cell types resulting in cancerous and non-cancerous effects in the body. At low doses, arsenic causes nausea and vomiting, inflammatory angiogenesis and anaemia. Long-term low exposure leads to darkening of the skin and warts on the palms and soles (Sabine Martin, Ph. and Wendy Griswold, 2009).

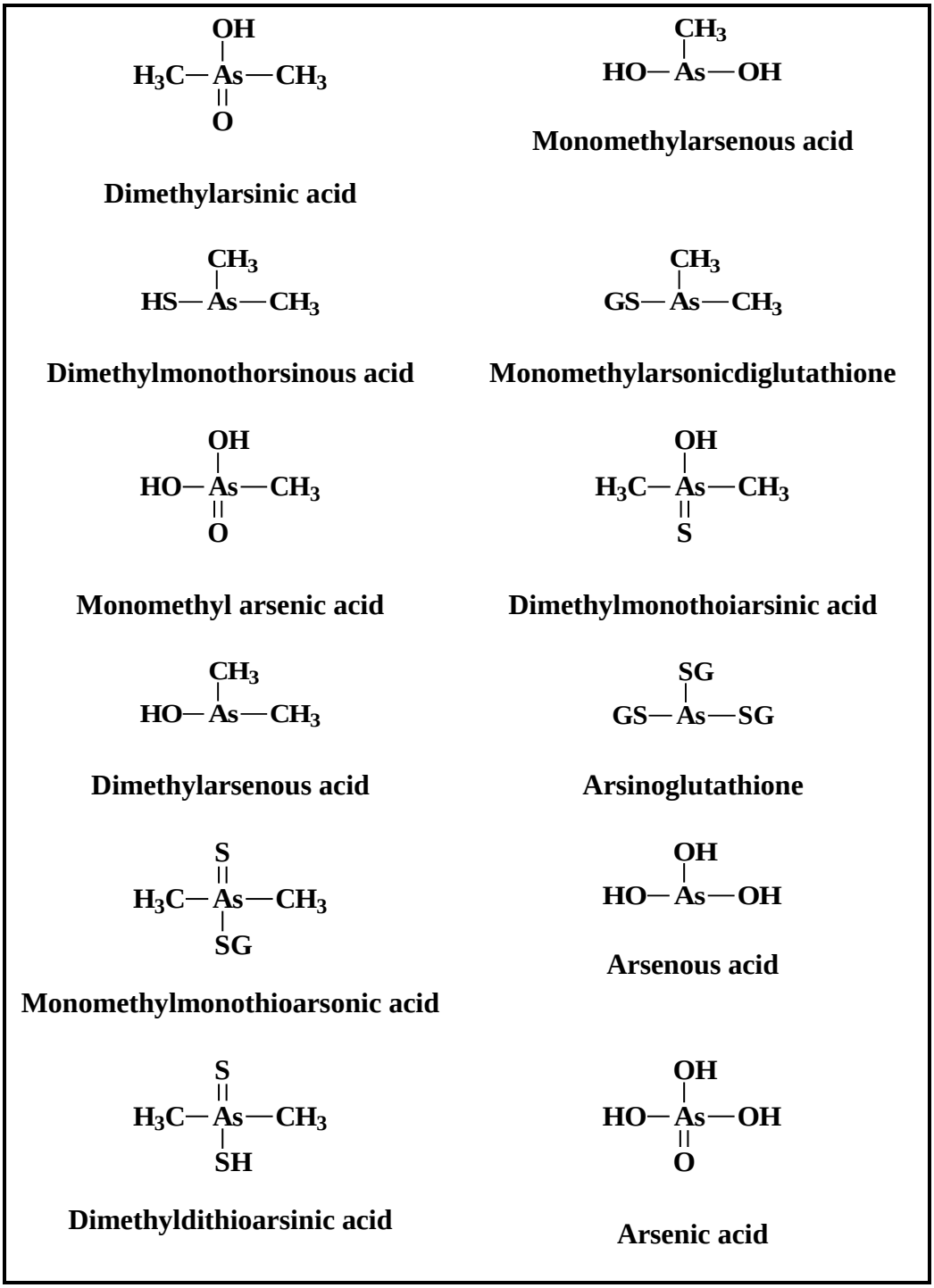

Figure 1.1. Chemical structures of some organic and inorganic arsenicals.

1.6.6.3 Carcinogenicity

The IARC classifies arsenic and inorganic arsenic compounds into Group 1 (IARC, 2012). The carcinogenic effects of arsenic are species specific. For instance in Drosophila, it does not

produce mutations while in fibroblasts and lymphocytes it leads to mutations. Amid these contradicting studies on the mutagenicity of arsenicals, arsenic is generally considered a non-genotoxic carcinogen. However, arsenic and its metabolites have been shown to induce DNA damage in various *in vivo* and *in vitro* models. Arsenic and its metabolites are capable of inducing DNA strand breaks, inhibit DNA synthesis and repair as well as retarding DNA replication. Arsenicals may also interact with DNA indirectly leading to chromosomal aberrations, sister chromatid exchanges and formation of micronuclei. The tumours of lungs, skin and liver, among other organs, have been associated with arsenic (James et al., 2015).

1.6.7 Microbial contamination

Contamination of herbal medicines with pathogenic microbes may endanger human health. The presence of microbial contaminant may lower or even inactivate the therapeutic activity of the products as well as adversely endangering lives of consumers of such products. Although herbal products administered orally are not subjected to sterility testing, determination of the microbial load is important in order to assure on safety to the consumers.

1.6.7.1 Factors influencing contamination of herbal medicines

The presence of microbes on herbals may arise from fungi, bacteria and viruses from the soil on which the plants grow or from the environmental sources such as air and water. Expectedly, environmental factors prevailing during the pre- and post-harvesting periods such as temperature, humidity and rainfall influence contamination. Other factors influencing contamination of herbals include handling practices and the storage conditions of crude and processed medicinal-plant materials. Enforcement of suitable phytosanitary measures aids to minimize contaminants. It is important also to monitor the moisture content, pH and microbiological contamination levels. Table 1.3 provides a comprehensive summary of the factors that may contribute to and/ or influence contamination of herbal medicines (Kneifel *et al.*, 2002).

Table 1.3. Factors influencing contamination of herbal medicines

Intrinsic factors
Nature of plant and natural barriers
Structure of the plants
Composition of the plant in terms of antimicrobial compounds present
Intracellular microbial contamination
Extrinsic factors
Humidity
Climate
Location/position
Harvest methods
Post harvesting practices
Technological processing
Physical state
Packaging and storage conditions
Exogenous microbial contamination

Selected factors that influence microbial contamination are discussed in sections 1.6.7.2-1.6.7.5

1.6.7.2 pH

In acidic environment, there is low bacterial contamination. At neutral and in alkaline conditions, higher microbial loads are observed since the optimal pH for microbes is in the range pH 5 - 8.5.

1.6.7.3 Storage

During storage physical, chemical and biological changes may occur on herbals that are not well dried. Prolonged storage in damp and poorly ventilated areas renders herbs more susceptible to attack by toxigenic molds. Control of humidity during storage is recommended.

1.6.7.4 Extraction methods

Use of poor phytosanitary measures during extraction of herbals predisposes these medicines to microbial contamination. For instance plants that are not well cleaned may have residual dust from the environment. This coupled with cold maceration allows for multiplication of microbes. Aqueous extracts are more susceptible to microbial attacks. Hot extraction considerably minimizes heat labile microbes. However heat may destroy the actives. Therefore proper choice of solvent that maximizes on the yield but also minimizes microbial contamination is recommended.

1.6.7.5 Drying process

Post-harvest preservation of medicinal plants and conservation of medicinal qualities is dependent on the drying process. Appropriate drying helps maintain the physical, chemical and microbiological stability of herbs. Steady low temperatures that are guided by the chemical composition of the active ingredients of the medicinal plant are employed. Water activity of materials corresponds to relative humidity of the air in the vicinity of the sample. Microorganisms (fungi and bacteria) proliferate at humidity >70%. Use of high temperature during drying decreases the total aerobic microbial count since it lowers the water content. Therefore, the drying process should not only concentrate and preserve the actives but also reduce the microbial loads.

1.7 Analytical techniques

1.7.1 Elemental analysis

Analysis of metals is matrix dependent with inductively coupled mass spectrometry (ICP-MS), thermal ionization mass spectrometry (TI-MS), inductively coupled plasma optical emission spectroscopy (ICP-OES), atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES) being among the preferred techniques for elemental analysis. The ICP-MS, AES and AAS are the commonly applied techniques.

1.7.2 Inductively coupled plasma mass spectrometry

In ICP-MS, a sample is subjected to five sequential steps before detection. These steps are: ionization of the sample in the plasma, ions generated are then extracted from the plasma and focussed and transported to the mass spectrometer. The mass spectrometer separates and sorts the ions based on mass-to-charge ratio (m/z) in the mass analyser. The separated ions are then counted to quantify the amount of each element in the original sample in the detector which is usually an electron multiplier tube. Figure 1.2 shows the components of a typical ICP-MS.

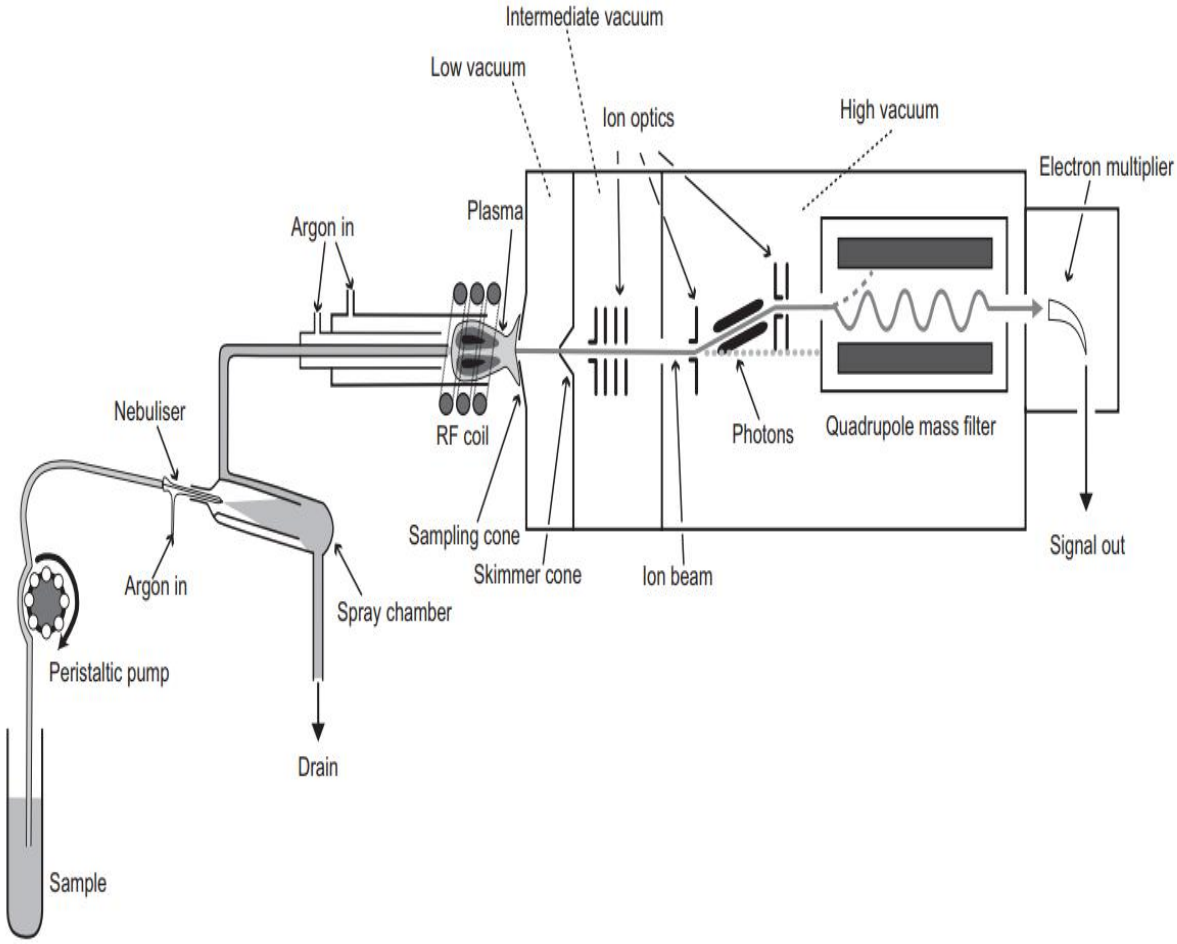

Figure 1.2. Components of an inductively coupled-mass spectrometer. (Adopted from https://www.researchgate.net).

1.7.3 Atomic absorption spectroscopy

In AAS, the sample is atomized and a beam of electromagnetic radiation (EMR) emitted from a light source, usually ultraviolet/visible is radiated through the vaporized sample. Some of the radiation is absorbed by the atoms in the sample; the amount of light absorbed is a function of the concentration of the element of interest. The unabsorbed radiation passes through a monochromator that select the required wavelength and passes into a detector.

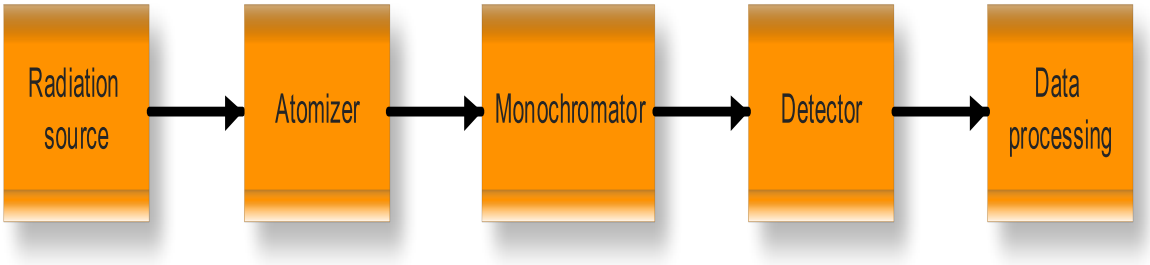

Figure 1.3. Schematic representation of the components of a typical atomic absorption spectrometer.

1.8 Regulatory framework for heavy metals in herbal medicines

Undoubtedly, heavy metals pose a hazard to public health. Therefore their content in herbal medicines must be limited. Consequently, limits for heavy metals are set for herbal medicines by health regulatory authorities. Acceptable limits are set taking into account the potential for accumulation of heavy metals in the body and reported adverse events.

In recognition of potential long-term effects of heavy metal poisoning, various regulatory authorities have prescribed limits for heavy metals as shown in Table 1.4. The WHO permissible daily intake levels for finished herbal products for lead and cadmium are set at not more than 10 mg/kg and 0.3 mg/kg, respectively. The Ph. Eur. monograph of herbal drugs recommends the following: 5 mg/kg, 0.5 mg/kg and 0.1 mg/kg, for lead, cadmium and mercury, respectively.

Table 1.4. Permissible daily intake levels for selected heavy metals by different regulatory authorities

Reference	Year	Lead (mg/kg)	Cadmium (mg/kg)	Mercury (mg/kg)	Arsenic (mg/kg)
Germany Ministry of Health	1991	5	0.2	0.1	-
World Health Organization	1999	10	0.3		-
Ph. Eur. Monograph	2007	5	4	0.1	-
Regulation (EC) 396/2005	2008			0.02	-
Ph. Eur. Monograph herbal drugs	2008	5	0.5	0.1	-
Regulation (EC) 1881/2006	2008	3.0	1.0	0.1	-
USP 41/NF 36 Monograph	2018	0.5	0.5	3	1.5

1.9 Problem statement

In order to provide high standard patient care at a minimum cost, herbal products need to be safe, effective and of high quality. Herbal products are currently not well regulated in Kenya due to the diversity of their constituents, routes of entry into the Kenyan market and insufficient surveillance mechanisms by regulatory agencies. Although herbal medicine play a considerable role in the primary health care, they may inadvertently cause serious health implications and fatalities if left unregulated. This study sought to investigate the microbial and heavy metal contamination of these products.

1.10 Study justification

The use of herbal medicine is rapidly expanding across the world. These products are either taken on their own or concomitantly with conventional medicines by a greater percentage of the world's population. Historically, the knowledge of traditional medicine was developed and

selfishly guarded within the community, then passed down from generation to generation orally. Natural products are believed to be safe and compatible with the human body, however, there are reports of serious adverse reactions linked to the use of herbal medicines worldwide. There has been an increasing concern over the safety and toxicity of natural products due to scanty traceable data on their routes of manufacture and quality assurance.

Despite the existence of herbal medicines from ancient times and their immense contribution to primary healthcare of the greater percentage of the population, most African countries do not officially recognize herbal medicines and therefore lack well established regulatory policies for these products. Ingestion of herbal medicines contaminated with heavy metals and microbes over a long period adds up to the total concentration of these contaminants in the body.

The Government of Kenya through the National Drug Policy (1994) directed the PPB to come up with quality specifications and regulatory policies to standardize herbal medicine utilization and practice. However, this was almost impossible because of lack of documented information on the efficacy, quality, safety and rational use of these products.

The results obtained from this study will provide informative data to the regulatory authorities on the microbial quality and heavy metal contamination of the herbal products in the Kenyan market and may be used to inform policy formulation to ensure adequate quality control measures are adhered to by all stakeholders.

1.11 Hypothesis

It is plausible that herbal products marketed in the diverse Nairobi metropolitan markets are contaminated with pathogenic micro-organisms and toxic heavy metals.

1.12 Objectives

1.12.1 General objective

The general aim of this study was to conduct an evaluation of herbal medicines in the diverse Nairobi metropolitan area markets for heavy metals and microbial contaminants.

1.12.2 Specific objectives

The specific objectives of this study were to:

1. Screen herbal medicines marketed in Nairobi metropolis for heavy metals lead, cadmium, arsenic and mercury.

2. Assess microbial contamination of the herbal products and characterise the microbial contaminants.

3. Carry out a quantitative risk assessment of the heavy metal contaminants in the sampled herbal medicines.

Chapter 2: LITERATURE REVIEW

2.1 Potential sources of herbal medicine contaminants

Contamination of herbal medicines can arise from environmental pollution, soil decomposition and use of fertilizers and pesticides containing arsenic and mercury (World Health Organization, 2007). Accumulation of heavy metals in plants depends on climatic factors, plant species and concentrations in air and soil (Maghrabi, 2014). External contaminants include toxic metals, microbes, pesticide residues, adulteration and misidentification while internal contaminants arise from complexity and non-uniformity of the ingredients (Zhang *et al.*, 2012). Chemical contaminants such as mycotoxins have been identified in herbs and herbal products. Biological contaminants such as bacteria, yeast, moulds, and viruses may be introduced during preparation. Toxic metals such as lead, cadmium, chromium, mercury and radioactive substances such as Cs-134 and Cs-137 are possible contaminants (Kosalec *et al.*, 2009).

2.2 Heavy metal contaminants

Heavy metals have bio-importance roles in living organisms as trace elements. Harmful effects of heavy metals are as a result of their concentrations and oxidation states (Oyaro *et al.*, 2014). The sources of heavy metals include leaching processes, chemical conversions and deposition in the earth's crust (Agency for Toxic Substances and Disease Registry, 2007; Kunle *et al.*, 2012). Environmental contamination by heavy metals occurs from both nature and human activities resulting in water and soil pollution (Hunter *et al.*, 1987; Hong *et al.*, 1996; Nriagu, 1996; Chan, 2003; Kigen *et al.*, 2012). Upon consumption of contaminated substances, the metals may interact with biomolecules such as proteins and enzymes to form biotoxic complexes (Kigen *et al.*, 2012; Mutune, *et al.*, 2014).

Pharmaceutical companies are required to follow good manufacturing practices (GMP) to ensure consistent production of safe and quality products (Food and Drug Administration, 2015). A survey conducted in south-west Nigeria in 2001, observed that small companies do not have the capacity to adhere to GMP (Okeke and Adebayo, 2001). The most affected are companies from economically challenged countries which cannot invest in facilities, machinery and qualified personnel (Oluwatoyin and Adebayo, 2016).

A study conducted in Riyadh City, Saudi Arabia, to determine heavy metal contents of 32 herbal plants showed two henna samples had lead contents of more than 1.0 ppm while the rest of the samples showed lead content of less than 1.0 ppm. Mercury content ranged from 0.630 ppm (*Lepidium sativum*) followed by 0.102 ppm (*Artemisia alba)* and then henna (0.092 ppm). *Pimpinella anisum* and mixed herbs had 0.087 ppm each. The rest of the samples had mercury contents of less than 0.08 ppm. Cadmium content was highest with *Lepidium sativum*, *Vigna radiate* and *Zingiber officinale*. The rest of the samples had cadmium contents of less than 0.02 ppm. Copper was the highest in *Cinnamomum zeylanicum* (0.284 ppm) followed by *Matricaria chamomilia* (0.282 ppm*), Carum carvi* (0.274 ppm), *Nigella sativa* (0.271 ppm), *Foeniculum vulgare* (0.267 ppm), *Achillea fragrantissima* (0.244 ppm) and *Commiphora myrrah* (0.207 ppm). The rest of the samples had copper contents less than 0.2 ppm (Alwakeel, 2008).

Nine heavy metals namely cadmium, cobalt, copper, iron, manganese, nickel, lead, zinc and mercury have been characterized in 42 Chinese herbal medicines (CHM). The samples contained higher concentrations of iron, manganese and zinc (Wong *et al.*, 1993). The concentration range of the metals was comparable to that reported in many of the East Asian vegetables and fruits. A few samples contained relatively high concentrations of toxic metals such as cadmium, lead, and mercury. This report suggested that the presence of heavy metals was probably caused by contamination during air-drying and preservation (Wong *et al.*, 1993).

A similar study conducted by Mousavi and colleagues in Iran found all the eleven samples analysed had lead and cadmium concentrations that exceeded the daily permissible levels. Cadmium was found in all the samples in the range of 0.19-1.75 µg/g. The weekly cadmium and lead intakes were estimated for each drug based on the daily recommended dose by the manufacturer. Weekly metal ingestion through consumption of these herbal drugs was calculated by multiplying the maximum recommended dose of each product by the mean levels of the metals found in the herbal formulation. The results showed that the maximum intake of the metals reached 53.43 µg/week of cadmium and 576.41 µg/week of lead in one of the analysed products (Zahra *et al.*, 2013).

The toxicity of heavy metals in both human health and the environment has captured the interest of both researchers and authorities. A survey conducted in 2015 in Dubai using microwave digestion and ASS to determine the amount of trace metals concluded that all the 78 samples analysed for heavy metal contaminants including lead, cadmium, zinc, copper and iron had levels above the FAO/WHO permissible limits. Up to 29% of the samples had cadmium levels above the permissible limit while 64% had lead content exceeding the permissible limit (Dghaim *et al.*, 2015). Comparable results were obtained in Egyptian and Iranian medicinal herbs and plants in two separate studies (Abou-Arabia and Abou-Donia, 2000; Ziarati, 2012).

A study conducted to compare heavy metal contaminants in different *Berberis* species from natural habitats and market places revealed that the market samples in India were more contaminated (Srivastava *et al.*, 2006). A study conducted between 2002 and 2007 in Germany, on 109 herbal samples found that 20 samples had higher content of cadmium while 4 samples had lead content exceeding the allowable limit (Gasser *et al.*, 2009).

Notably, herbal medicines can be a potential source of heavy metals as revealed by Maghrabi in 2013 using inductively coupled plasma atomic emission spectrophotometry (ICP-AES). The study found that all 14 commonly used herbal medicines in Saudi Arabia were contaminated with heavy metals (Maghrabi, 2014). A similar study to assess heavy metal contamination in Chinese herbal medicines marketed in Malaysia found that all the samples had detectable concentrations of manganese, copper, cadmium, iron and zinc (Ting *et al.*, 2013). A study conducted in Pakistan in 2012 by Muhamad and colleagues on 50 branded herbal products concluded that most of the products had arsenic content above the daily permissible levels (0.2 to 57µg/day (Muhamad *et al.*, 2012).

A study conducted in Kumasi, Ghana, revealed that the majority of spices were not contaminated with heavy metals except aniseed, cinnamon, ginger and pepper (Nkansah and Amoako, 2010). Idu and colleagues in 2015 carried out a study for polyherbal products marketed in Lagos, Nigeria. The study found that all the 24 products tested contained no cadmium or lead (Idu *et al.*, 2015). Contrasting results were obtained in 2011 by Chris Nwoko on selected ready-to-use herbal medicines in South East Nigeria (Nwoko and Mgbeahuruike, 2011). The study found that zinc, cadmium and lead contents in all the samples exceeded the WHO permissible limits.

Heavy metals were detected in 14 samples of commercial herbal concoctions from the South African market using inductively coupled plasma optical emission spectrophotometry (ICP-OES). The metals were above the standard limits prescribed (Okem *et al.*, 2012).

The ethanolic extract of the bark of *Detarium microcarpum* (Leguminosae family) has antibacterial activity against *Pseudomonas aeruginosa, Citrobacter freundii, Klebsiella pneumoniae* and *Staphylococcus aureus*. However, a major concern in the use of this remedy is heavy metal contamination. Atomic absorption spectroscopic analysis of five heavy metals

done on the digested stem bark, in Lagos, Nigeria found no detectable levels of lead and chromium. Manganese, iron and zinc levels were 139, 218.9 and 48.9 mg/kg respectively (Ehianeta *et al.*, 2013).

Arsenic and mercury were detected in all the 20 samples of herbal medicines in a study carried out in Lagos, while cadmium was found in 14 of the samples, eleven of which had high levels of cadmium exceeding the USP oral component level (OCL) (Adepoju- Bello *et al.*, 2012). Elemental analysis on oral health products purchased and analysed in Nairobi City County found 22 products to be contaminated with lead. Five of the products had aluminium concentrations ranging from 392.03 to 582.86 ppm while chromium concentration ranged from 0.02 to 7.31 ppm (Ngari *et al.*, 2013).

2.3 Microbial contaminants

The microbial quality of pharmaceutical products may be influenced by the environment in which they are produced and the raw materials. Growing, harvesting and manipulation methods employed cannot exclude microbial contamination of the plant material. Biological contamination includes microbes such as bacteria, spores, yeast, moulds, viruses, protozoa, insects and other organisms (Okunlola *et al.*, 2007; Araújo and Bauab, 2012). Microbial contamination usually occurs during harvesting, product handling, post-harvest and manufacturing processes by personnel infected with pathogenic microbes (WHO, 2007).

Plant materials used in herbal drug preparations are organic in nature. They contain the nutrition required for the proliferation and multiplication of microorganisms that lead to contamination, deterioration and variation in composition of the herbal products. The resulting products are of inferior quality with little or no therapeutic efficacy (Gautama *et al.*, 2009). Aseptic conditions, basic hygiene and GMP are prerequisites during processing of herbal

medicines to ensure safe, quality and consistent products reach the market (Zhang *et al.*, 2012; Ahmed *et al.*, 2016). However, the methods of producing herbal preparations are often unhygienic leading to unsafe products (Esimone *et al.*, 2007). Although oral pharmaceutical preparations are not meant to be sterile, microbial load control is mandatory. Microbial limits for pathogenic microorganisms including *E. coli*, *P. aeruginosa* and *S. aureus* as specified in the monographs should be adhered to (USP, 2013).

Microbial contaminants disrupt product stability, modify physical characteristics of the product and inactivate the active ingredients and excipients leading to decreased efficacy. The final product's flora is a summation of all the contaminants from all the possible sources (Adeola *et al.*, 2012). Many of the contaminants are bacteria and fungi. The WHO, BP and the USP specify tolerance limits for non-sterile pharmaceutical products for bacteria and fungi as 10^7colony forming units/millilitre (cfu/mL) and10^5cfu/mL, respectively (Onyambu *et al.*, 2013). Observation of basic hygiene and GMP are important in ensuring quality and safety of the herbal products (Zhang *et al.*, 2012; Ahmed *et al.*, 2016).

In an assessment for microbial quality of 32 herbal products, *Bacillus* species were the commonest contaminants of the isolated microorganisms of which *Bacillus cereus* dominated with 14 isolates (45.2%). Other microbial isolates were *Aeromonas hydrophilia, Shigella spp., Enterobacter cloacae, Staphylococcus hycus*, *Staphylococcus epidermidis*, *Acinetobacter iwoffii* and *Klebsiella.* Sensitivity testing showed that most isolated microorganisms were sensitive to amoxicillin, gentamicin, imipinem, tobramycin and trimethoprim-sulfamethoxazole. *Enterobacter cloacae* was resistant to ampicillin and cefazolin while *Aeromonas hydrophilia* showed resistance to cefotaxime and ceftazidime. *Shigella* species

exhibited resistance to cefazolin and *Escherichia coli* showed resistance to ciprofloxacin (Alwakeel, 2008).

A study conducted to assess the microbial quality of herbal solid dosage forms from the Iranian public market in 2010 confirmed that all the 20 samples evaluated failed to comply with the USP specified standards for microbial limits (Enayatifard *et al.*, 2010). Commercial oral herbal medicines in Bangladesh were evaluated for potential pathogenic microorganisms in 2013. Of the 85 liquid samples assessed, two samples were heavily contaminated with aerobic bacteria while 10 samples had heavy fungal load (Noor *et al.*, 2013). Another study conducted on seven antidiabetic herbal preparations in 2016 established that only one sample was free from bacterial and fungal contamination while the rest of the samples were contaminated with *Bacillus subtilis* (Ahmed *et al.*, 2016). A similar study found *B. subtilis* to be the predominant contaminant (Shah and Pokherel, 2012).

Twenty seven commonly used traditional Chinese patent medicines (TCPM) were investigated for fungal contamination. Three of the samples were contaminated with *Aspergillus, Eurotium, Mucor, Paescilomyces* and *Penicillium.* The genus *Aspergillus* was the commonest contaminant with three isolates, *A. foetidus, A. flavus* and *A. niger* (Chen *et al.*, 2012).

Chinese herbal medicines in Malaysia were studied to assess microbial contamination and the effect of boiling on the level of contamination. The study findings showed that the samples had microbial loads of 10^6 cfu/mL and that boiling had significant impact in reducing the level of contamination (Ting *et al.*, 2013). Microbial contamination in cosmetics during use in Lashkar and Thatipur areas in India were investigated and the results showed 100% and 68% growth of bacteria and fungi, respectively. A total of 10 bacterial species and 6 fungal species were isolated from the 25 tested samples (Dixit and Bhadauria, 2014).

In another study, dried plant materials were studied in Belgrade, Serbia for pathogenic microorganisms. A total of 40 samples from different plant species were employed for the test. The results revealed that almost all the samples of corn silk (96.8%) were contaminated with *Bacillus cereus* and *Clostridium perfringens* and all the 40 samples were contaminated with fungi. Horse tail and nettle samples were contaminated with both bacteria and fungi that exceeded the specified limits (Stević *et al.*, 2012).

In south-west Nigeria, Oluwatoyi and Lamikanra carried out a study in 2016 to assess microbial quality of 50 locally prepared and unregistered herbal oral liquids in Ille-Ife. The mean bacterial load ranged from 0 to 2.94×10^{12} cfu/mL and 0 to 3.5×10^{12} cfu/mL for fungi. Only 10 samples complied with the WHO and European Pharmacopoeia specifications of viable aerobic count (10^5 bacteria and 10^3 fungi) (Oluwatoyin and Adebayo, 2016).

Research evaluating powdered herbal preparations in Kaduna, Nigeria, found that a greater percentage of the herbal preparations was contaminated with *Salmonella typhi, Shigella spp, E. coli* and *S. aureus* (Abba *et al.*, 2009). A similar study on eight samples purchased from community pharmacies in Lagos, Nigeria by Adeola and colleagues in 2012, reported heavy loads of both bacteria and fungi (Adeola *et al.*, 2012). Rapid detection of microbial contamination by polymerase chain reaction (PCR) analysis was carried out in Ghanaian herbal medicines, where *E. coli* and *S. aureus* were detected (Dei-Tutuwa *et al.*, 2014). Similarly, microbial contamination in unripe pawpaw preparation used in the management of gastric ulcers in Ibadan, Nigeria with several bacterial species was reported (Amosu *et al.*, 2014).

In 2010, research conducted in Kenya to evaluate microbial contamination on 30 herbal products in Nairobi City County reported 67% bacterial contamination. The samples were contaminated with bacterial cultures ranging from 6×10^5 to 1.56×10^{10}cfu/mL and fungal

contamination in the range 5.30 $\times 10^4$ to 1.56 $\times 10^9$ cfu/mL. The study further found that of the 19 different types of bacteria identified, 13 (68%) were Gram negative rods while six were Gram positive rods and cocci. Presence of *Staphylococcus, Klebsiella pneumoniae, Escherichia coli, Shigella* and *Candida* species were confirmed using differential and selective media (Onyambu *et al.*, 2013). Another study conducted in 2013 on microbial quality of herbal medicines used for oral health in Nairobi City County confirmed microbial contamination of the herbal products (Ngari *et al.*, 2013). Similar evaluation on hypoglycaemic herbal preparations in Nairobi City County showed microbial and fungal contamination in both liquid and powdered products (Chege *et al.*, 2015). Evaluation of selected herbal medicinal products sold in Nairobi, Kenya for heavy metals and microbial contamination confirmed the presence of heavy metals and microbes (R. K. Korir, 2017). Similarly, evaluation of herbal products marketed for the treatment of human immunodeficiency virus (HIV) in Nairobi for microbial contamination found 96% of the samples were heavily contaminated (Kaume *et al.*, 2012).

Chapter 3: EXPERIMENTAL

3.1 Study location

This study on the evaluation of herbal medicines for heavy metals and microbial contaminants was carried out at the National Quality Control Laboratory (NQCL) and Kenya Plant Health Inspectorate Service (KEPHIS) laboratories in Nairobi, Kenya.

3.2 Herbal products samples and sampling plan

Samples of herbal products comprising tablets, capsules, liquids and powder dosage forms were obtained from pharmacies, supermarkets, herbal clinics and open air markets within Nairobi Metropolis. A total of 89 herbal products for oral administration used for the management of chronic illnesses were sampled purposively from these outlets in both formal and informal settlements in the metropolis. The chronic illnesses targeted are asthma, Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV-AIDS), tuberculosis (TB), hypertension, diabetes, rheumatoid arthritis, peptic ulcer disease and cancer.

Nairobi City County was selected as the centre of the study because it is a major commercial centre and a central distributive point for most commercial herbal products. It serves as a pivot point that governs product distribution patterns in the satellite towns of the metropolitan region and Kenya at large. Therefore, the results of this study may be extrapolated to herbal products in the wider Kenyan market.

The sampling area was divided into four main loci namely Nairobi, Machakos, Kiambu and sections of Kajiado, encompassing both formal and informal settlement regions. Where one sample material was not enough for both elemental and microbiological analysis, additional samples of the product were purchased.

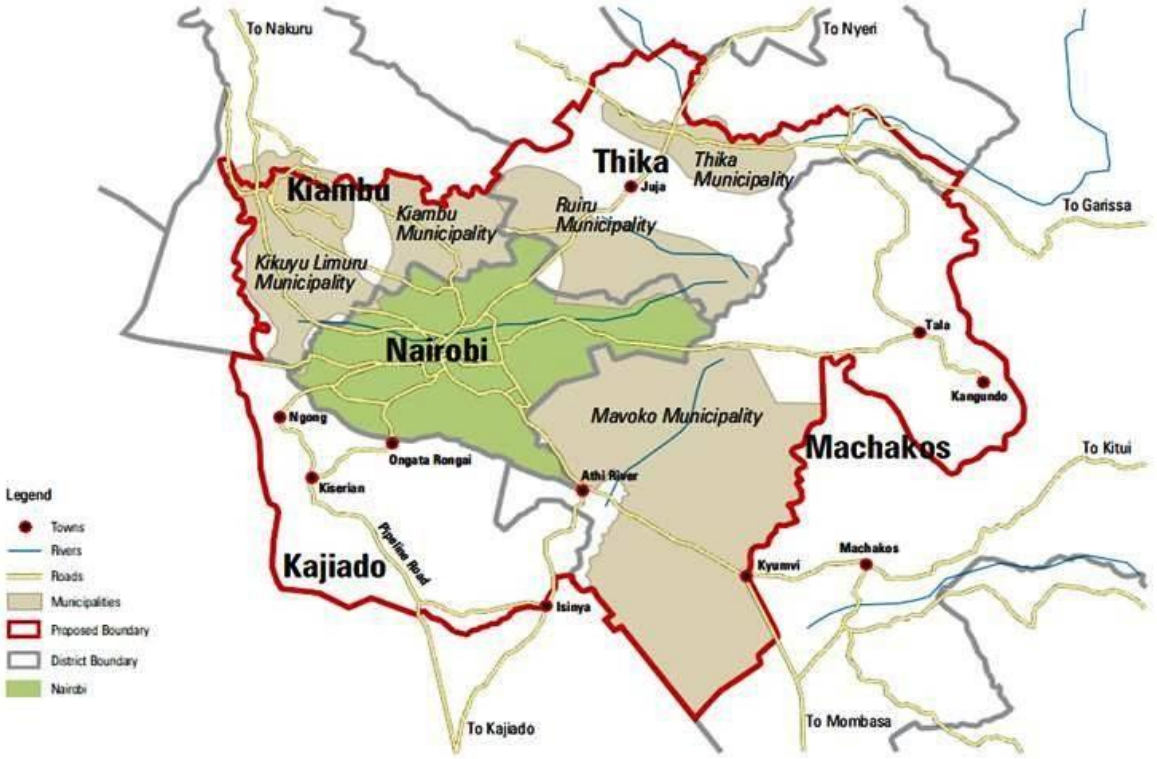

Figure 3.1. A Map of Nairobi Metropolis showing the study regions

(Map adopted from UN-Habitat 2010).

3.3 Sample size determination

The Cochran formula (Equation **1)** for categorical data was used for sample size calculation.

$$\boldsymbol{n = \left[\frac{Z}{1-\frac{\alpha}{2}}\right]^2 / d^2 \times p \times q} \qquad \textbf{Equation 1}$$

Where *Z* is 1.96 which is the Z score at α for a two tail hypothesis test, alpha (α) is the level of significance set at 0.05, *d* is the limit of precision (accepted margin of error) of the estimated prevalence of contaminated herbal products with heavy metals and microbes, p is the estimated prevalence of contaminated products obtained from previous studies (10%) (R. K. Korir, 2017) and q is 1-p.

Accordingly,

$$n = \frac{[1.96]^2}{0.05^2} \times 0.1 \times 0.9 = 138$$

Although the study projected to utilise 138 samples, only 89 samples were obtainable due to limited number of products in the market and also because of logistical issues that arose during this study.

3.4 Elemental analysis

3.4.1 Inductively coupled mass spectrometric analysis

Elemental analysis of cadmium, lead, arsenic and mercury in the selected herbal products was carried out using an Agilent 7900a ICP-MS interfaced with massHunter® version 4.3 software (Agilent, CA, USA). The Agilent 7900a was equipped with a standard Nickle sampling and skimmer cones with 1.0 mm and 0.4 mm orifice diameters, respectively, a standard glass nebulizer, a quartz spray chamber chilled at 2 °C and a quartz torch with 2.5 mm injector. An Agilent ASX-520 auto sampler was used to deliver the samples, contained in 50 mL vials to the ICP-MS. The system included a 4th generation collision/reaction cell and an octopole reaction system (ORS) which provided optimized removal of polyatomic interference using Helium (He) mode. Before each experiment, the inductively coupled plasma mass spectrometry (ICP-MS) was tuned using an aqueous multi-element standard solution of Li, Sc, Ge, Rh, In, Tb, Lu and Bi all prepared in 10% HNO_3 for consistent sensitivity. Operating conditions of the ICP-MS analysis are summarized in Table 3.1.

Table 3.1. Operating conditions for inductively coupled mass spectrometer

Plasma conditions	Flow rate
Radio frequency power supply	1.55 Kw
Plasma argon	15 L/min
Auxiliary argon	0.9 L/min
Nebulizer argon	0.9 L/min
Collision gas (He)	3.9 mL/mm

3.4.2 Reagents, solvents and materials

Pro-analytical metal grade nitric acid, hydrochloric acid, and perchloric acid from Sigma (Sigma-Aldrich, Taufken, Germany) were used for sample digestion. All solutions were prepared with analytical reagent-grade chemicals and ultrapure water.

3.4.3 Sample preparation

3.4.3.1 Microwave assisted digestion

Herbal products material (0.5 g) was digested in 9 mL HNO_3 acid (67-69%), 1 mL HCl and 4.0 mL $PCLO_4$ using a multiwave 300 single reaction chamber microwave digester (Anton paar, Australia) equipped with 16 high pressure quartz vessels with a capacity of 80 mL. The temperature and pressure were ramped to 240 °C and 150 bar, respectively. The samples were then allowed to cool in the microwave after which they were quantitatively transferred into a 250 mL volumetric flask, filled to the mark using de-ionized water and subjected to ICP-MS analysis.

3.4.3.2 Preparation of standard stock and working solutions

Multi-stock standard solutions of 10 ppm containing the target elements (lead, cadmium, arsenic and mercury) were used to prepare a series of working standards of 0, 10, 20, 30, 50

and 100 µg/L. Using a calibrated micropipette 0, 100, 200, 300, 500 and 1000 microliter were measured into 200 mL volumetric flask and made to volume using 5% HNO_3.

3.4.4 Calibration and determination of unknown concentration of analyte

3.4.4.1 Working curve method

Calibration was carried out to determine instrument signal response to changes in concentration. Working standard solutions of known concentrations and in increasing concentrations of multi-element solution containing each element of interest was used. Calibration curve of response (counts per second) versus concentration was plotted and concentrations of unknown analyte established from the calibration curve.

3.4.4.2 Standard addition technique

Several aliquots of the sample solution of equal volume were measured into test tubes and known amounts of standards of increasing concentrations added. Each solution to which standard addition has been made and one solution of the analyte were analysed and the response signal plotted as a function of the concentration of added standard. The linear plot was extrapolated to the concentration axis whereby the point of intercept corresponds to the concentration of the analyte in the sample solution.

3.4.5 Quality assurance

Daily quality assurance procedures were conducted and evaluated according to set internal quality controls for validity of test results. Solutions consisting of a blank, working standards and samples were analyzed in triplicate. A multi-element standard solution of 10 ppm comprising of the examined heavy metals was employed to prepare a working standard curve. The calibration curves for all the samples were built on 5 different concentrations (0, 10, 20, 30, 50 and 100 ppb). All the elements in the samples were within the linear range of calibration

curves and above the established lower linearity limit. A relative standard deviation (RSD) of 5% was considered adequate for this analysis.

3.4.6 Calculations

The recovery range of spiked samples was determined and considered satisfactory if found to be in the range 60%-130%. The coefficient of variation (CV) for the metal concentrations was calculated using data from the triplicate sample assays as a measure of dispersion. In addition, the accuracy of the procedure was tested based on the analysis of spiked samples. The obtained results from spiked samples were 96.9, 92.6, 99.4 and 99.2% for As, Cd, Hg and Pb respectively. The concentration of metals in the herbal medicinal products was calculated using Equation **2**.

Element (µg/kg) = Reading × Dilution ÷ Weight of sample in gram **Equation 2**

3.5 Risk assessment for heavy metals

3.5.1 Exposure assessment

A quantitative health risk assessment of the heavy metals in herbal medicines was conducted using point estimate. Exposure assessment of heavy metals was computed from daily ingestion amount (recommended dosages) and physiological bodyweight of 60 kg for adults and 32.7 kg for children (US EPA, 2003). The estimated daily intake (EDI) was computed using Equation **3**.

$$EDI = (C \times E_F \times E_D \times D_{IR}) \div (W_{AB} \times T_A) \quad \text{Equation 3}$$

Where, C is the concentration of the heavy metal E_F is exposure frequency (365 days per year); E_D is exposure duration (length of use of medicine); D_{IR} is the daily dose per person (mg/kg/day), W_{AB} is the average body weight (60 kg for adults and 32.7 kg for children), T_A is

the average exposure time for non-carcinogens usually 365 days/year for 30 years (i.e., T_A =10,950 days) (US EPA, 2003).

Both the non-cancer and cancer risks of the heavy metals in herbal medicines were then quantitatively estimated.

3.5.2 Non-cancer risk

The target hazard quotient (THQ) used to estimate the non-carcinogenic risk level due to individual heavy metal exposure was adopted. The human health risk from consuming metal-contaminated herbal medicines was calculated using the THQ as per USEPA Region III Risk-Based Concentration Table (USEPA 2011). Equation **4** was used for estimating THQ.

$$\mathbf{THQ = EDI \div R_{fD}} \qquad \textbf{Equation 4}$$

The reference doses for oral ingestion of heavy metals were derived from benchmark doses (BMD) and uncertainty factors. Table 3.2 gives the reference doses of heavy metals used for calculation of THQ (Harmanescu *et al.*, 2011).

Table 3.2. Oral reference doses of heavy metals

Metal	Oral reference dose (mg/kg/day)
As	0.0003
Cd	0.001
Pb	0.0035
Hg	0.0005

The interactive or additive effects of heavy metals to the non-cancer risks are premised on the fact that two or more metals share similar mechanisms in producing toxicity. For this purpose, the sum of all the THQs christened hazard index (HI) was computed using Equation **5**.

$$\mathbf{HI = THQ\ (Hg) + THQ\ (Cd) + THQ\ (As) + THQ\ (Pb)} \qquad \textbf{Equation 5}$$

Where HI is hazard index; THQ (Hg), THQ (Cd), THQ (As), THQ (Pb) are target hazard quotients of Hg, Cd, As and Pb, respectively.

A HI and THQ below 1 implies no significant risk whereas a value greater than 1 signifies risk for health.

3.5.3 Cancer risk

The cancer risk due to the heavy metals in the herbal medicines was computed using the target cancer risk (TR) methodology laid down by the USEPA (USEPA, 2015). The methodology is elaborated in USEPA Region III Risk-Based Concentration Table (USEPA 2011). The model for estimating TR is given in Equation **6**.

$$\mathbf{TR = (E_F \times E_D \times D_{IR} \times C_F \times C_M \times C_{PS_o}) \div (W_{AB} \times T_A)} \qquad \textbf{Equation 6}$$

where T_R is the target cancer risk, C_M is the metal concentration in medicine (μg/g), D_{IR} is the daily dose per person (g/day), C_{PSo} is the carcinogenic potency slope, oral (mg/kg/day), W_{AB} is the average body weight, C_f is conversion factor and T_A is the average exposure time for carcinogens (365 days/year for 64.4 years for Kenyan males and 68.9 for females, i.e. 23506 and 25148 days respectively), E_F and E_D are defined in equation 3. The life expectancy of males and females are 64.4 and 68.9 years in Kenya respectively (Cohen, 2011).

For purposes of risk assessment, a CR or TCR < 10^{-6} is considered to be negligible while CR or TCR > 10^{-4} is considered unacceptable by most international regulatory agencies(U.S EPA 2011a, Guney *et al.*, 2010).

3.6 Microbial analysis

3.6.1 Instrumentation and media

A weighing balance (Mettler Toledo Excellence, Ohio, USA) was used to weigh samples and reagents while an autoclave (LTE TouchClave-Lab K 300, Greenfield, UK) was used for sterilization of media and glassware.

Microbial analysis was carried out in class II biosafety cabinet (TopSafe 1.2, Siziano, Italy) in an isolated room to minimize the risk of contamination during sample dilution and plating. Two calibrated incubators (WTB-Binder GmbH, Tuttlingen, Germany), set at 30-37 °C and 20-25 °C, respectively were used for incubation to determine bacterial and fungal contamination respectively. A ProtoCOL 3 automated colony counter/zone reader (Symbiosis, Cambridge, UK) and a Leica DM 750 binocular microscope equipped with an ICC50 camera (Leica Microsystems, New Jersey, US) were used for counting CFUs and Gram staining, respectively.

Different media were used at different stages of analysis and prepared according to the manufacturers' instructions. A negative control sample was inoculated concomitantly to assure absence of contaminants introduced during the test.

Buffered peptone water, nutrient agar, Sabouraud´s dextrose agar, MacConkey broth and agar, Rappaport Vassiliadis medium (RVM), Enterobacteriaceae enrichment (EE) broth-Mossel, xylose lysine deoxycholate (XLD) agar, violet red bile glucose(VRBG) agar, urease and triple sugar iron (TSI)agar used were from HiMedia (Mumbai, India).

3.6.2 Sample preparation and enumeration test

The sample stock solutions were prepared by dissolution of herbal product in buffered peptone water. For solid powdered samples, ten grams of the powder were dissolved in 100 mL of the diluent. For tablets and capsules, a quantity equivalent of 10 g was used. For liquid samples,

10 mL was mixed with 90 mL diluent to make 100 mL. The sample stock solutions were further subjected to ten-fold serial dilutions in 100 mL media bottles up to appropriate dilutions as per the monograph.

Solutions from each dilution were inoculated by pour plate technique (Ifeanyi *et al.*, 2014), in duplicate. One millilitre of the solution was pipetted into sterile Petri dishes. A 25 mL aliquot of molten Nutrient agar (NA) and Sabouraud's Dextrose agar (SDA) for bacterial and fungal enumeration respectively, were added to respective Petri dishes, swirled to homogeneity and left on the bench to solidify for one hour. The Petri dishes were separately incubated for 5 days at 30-37 °C and 20-25 °C for bacteria and fungi enumeration, respectively.

3.6.3 Sub-culturing and purification

After enumeration, discrete colonies from NA petri dishes were aseptically transferred into test tubes of MacConkey broth, RVM and EE broth incubated at 35 °C for 18-24 h and observed for growth (turbidity and/or colour change). Discrete colonies from the above media were then inoculated into MacConkey agar, XLD agar, and VRBG agar, respectively, by streak plate method.

3.6.4 Characterisation

Purified colonies obtained from the sub-culturing step were examined macroscopically for colony morphological characteristics, microscopically for Gram reaction and cell morphological properties. The isolates were further subjected to biochemical tests (urease and triple sugar iron).

3.6.4.1 Test for specified micro-organisms

Samples that exhibited microbial growth on NA were sub-cultured on selective and differential media namely MacConkey broth and agar, Rappaport Vassiliadis medium (RVM), Enterobacteriaceae enrichment broth (Mossel), xylose lysine deoxycholate (XLD), violet red

bile glucose (VRBG) agar, triple sugar iron (TSI) agar and urea agar slant. Characteristic colonies on these media presumptively indicated presence of the target bacteria**.**

3.6.5 Determination of viable counts

After incubation, the total viable count (TVC) of contaminating microbes in the samples was determined. The TVC was determined based on the assumption that contaminants are homogeneously mixed, and when plated, each cell grew into a colonial mass of cells that could then be counted using a colony counter. It was assumed that colonies growing on nutrient agar are bacterial whereas those on SDA were fungal. Colonies were enumerated from the least dilute sample to the most dilute solution. Bacterial colonies that were more than 250 in number on a plate were regarded as too numerous to count (TNTC), as were those plates with more than 50 fungal colonies. The total number of contaminating microorganisms was the average of replicate plates multiplied by the dilution factor.

3.7 Data analysis and interpretation

3.7.1 Determination of limits of measurements

The limit of detection (LoD) and limit of quantitation (LoQ) were determined from the calibration curves of each of the metals obtained using the corresponding standards as shown in Section 4.2. The LoD and LoQ are three and ten times the standard error, respectively.

3.7.2 Descriptive statistics

The mean, standard deviation (SD), minimum and maximum content of heavy metals were computed using Origin Pro 9.1 software (OriginLab Corporation, MA, USA). The average concentration of each heavy metal was used for further calculations. Student's t-test was employed for statistical analysis of heavy metal concentrations in the herbal medicines and performed using Origin Pro 9.1 software (OriginLab Corporation, MA, USA). A p-value of

0.05 was used to indicate a significant difference in the heavy metal content in the herbals sampled.

3.7.3 Compendial comparison

The levels of heavy metals and microbes in the samples were assessed against the British Pharmacopoeia 2017 specifications for herbal medicines. Samples that did not meet the set criteria were adjudged unsafe for human consumption.

3.7.4 Principal component analysis

Principal component analysis (PCA) was used to identify possible outlier samples and also to check on the quality of data. In addition to showing relationships between metal concentrations in the samples, PCA was also used to explain variance and covariance in the content of heavy metals in the herbal medicines. The PCA was performed using Origin Pro 9.1 software (OriginLab Corporation, MA, USA).

Chapter 4: RESULTS AND DISCUSSION

4.1 Herbal products sampled

A total of 89 herbal products comprising 38 powders, 13 capsules, 17 tablets and 21 liquid formulations were collected from pharmacies, herbal clinics and retail outlets in Nairobi Metropolis. Detailed sample information is provided in Table S1 (Appendix 1). Fifty five (61.8%) of the 89 products sampled did not bear a brand name or list of ingredients on the product label and were majorly obtained from herbalists. This underscores the secrecy of the practice of herbal medicine. Without this information, the patients are only able to fill the prescriptions from the herbalists whom they first consulted. This may disadvantage the patients considering that in the event that the practitioner is not available such patients may not be able to access the medicines. Additionally, 8 (9.0%) of the sampled products did not have list of ingredients but had brand name included. This omission denies the patient the right to information as enshrined in consumer protection protocols. Twenty six (29.2%) samples had both brand name and list of ingredients Fifty nine (66.3%) herbal products were obtained from herbalists with the rest from manufacturers. A high number of samples (42, 45%) were collected from Nairobi City County because it serves as the core distributive centre of the region. A majority of the herbal products sampled (27, 30%) were indicated for the management of rheumatoid arthritis. The sampling distributions are shown in Figures 4.1 and 4.2.

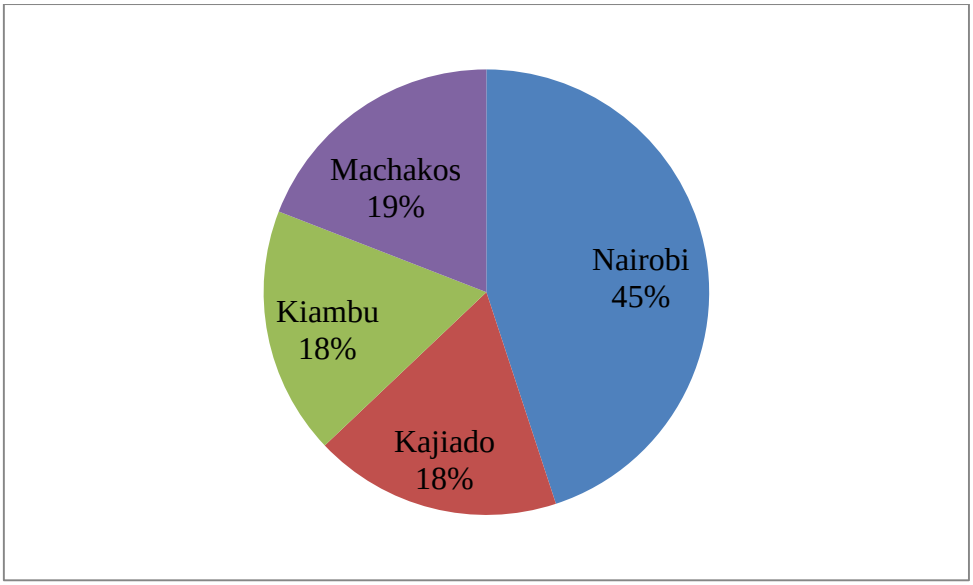

Figure 4.1. Proportion of samples collected per county in Nairobi Metropolis.

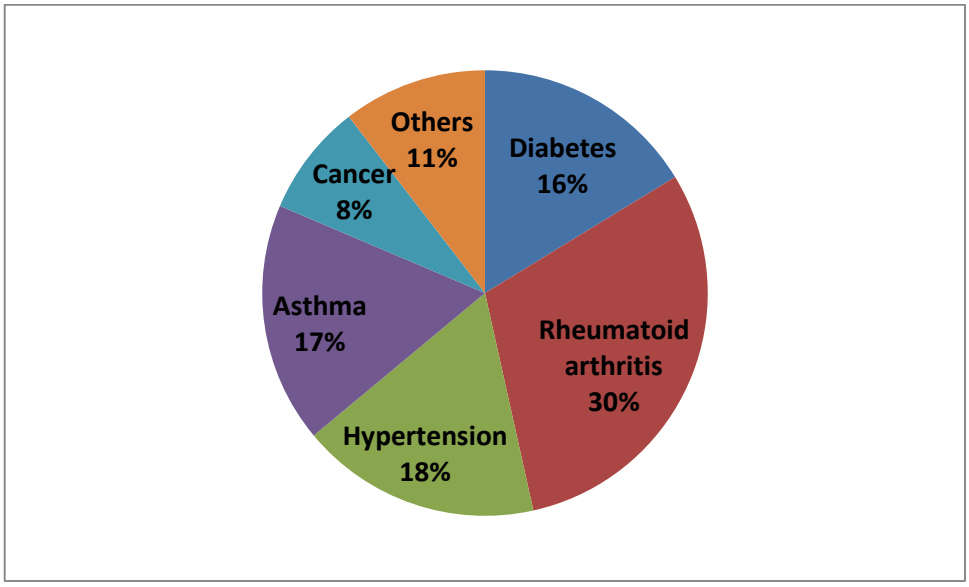

Figure 4.2. Proportion of samples collected per indication.

4.2 Calibration and limits of measurements

The linearity of detector response to the concentration of analytes was established by plotting calibration curves of the metals determined. Using six data points calibration, the coefficients of determination (R^2) for each metal were > 0.99 for the whole data range considered. The plot was based on three replicate measurements. Figures 4.3-4.6 show the calibration plots for As, Cd, Hg and Pb. The limits of detection and quantification for the heavy metals (Table 4.1) were determined using the slope methodology as elaborated by the International Conference on Harmonization guidelines (ICH, 2005).

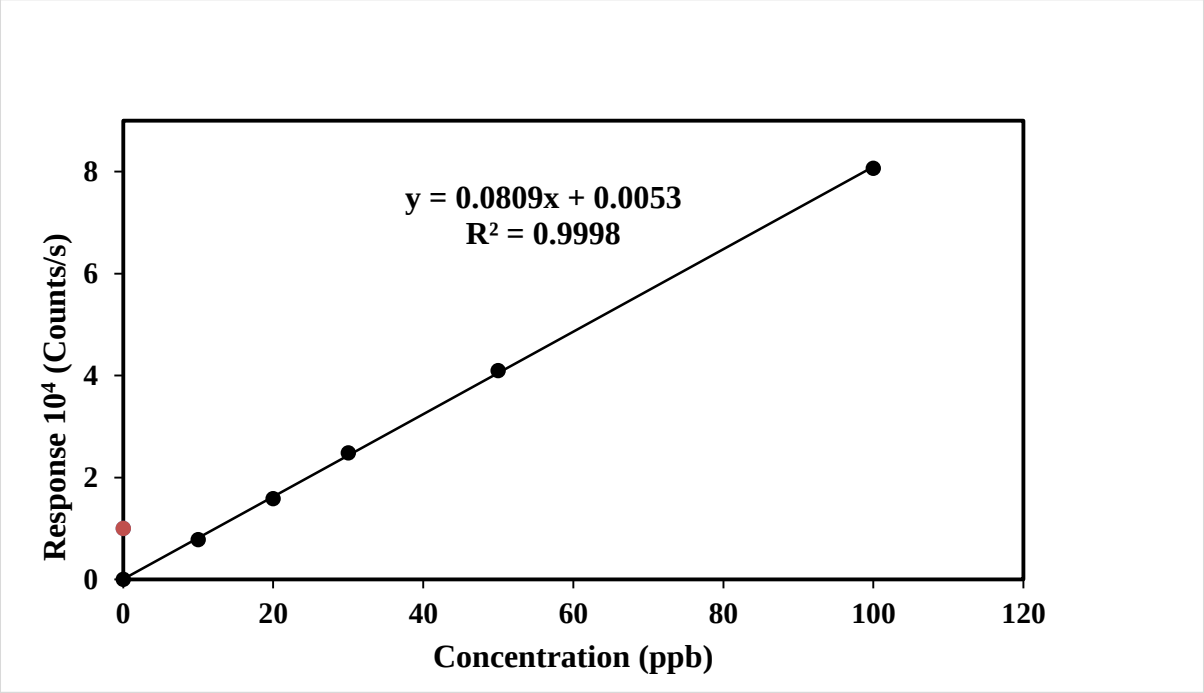

Figure 4.3. Calibration curve for arsenic

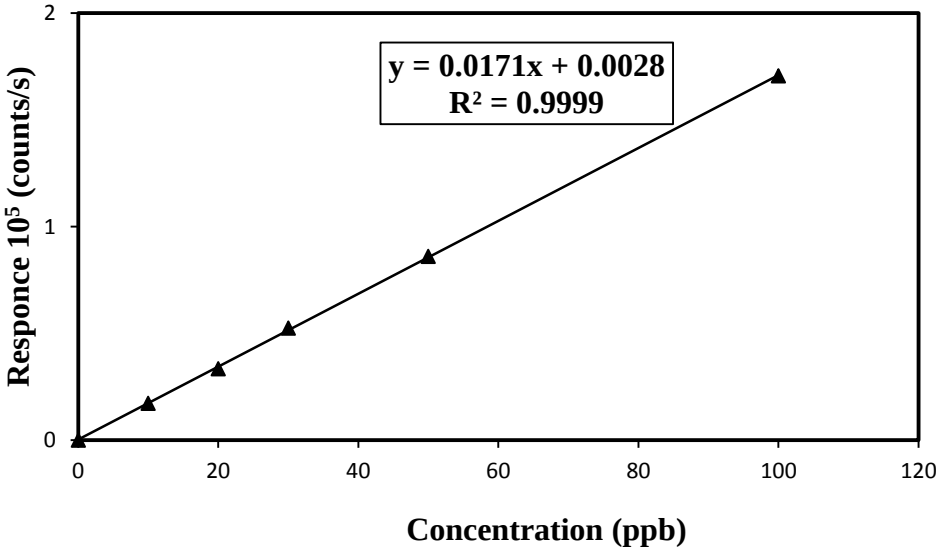

Figure 4.4. Calibration curve for cadmium.

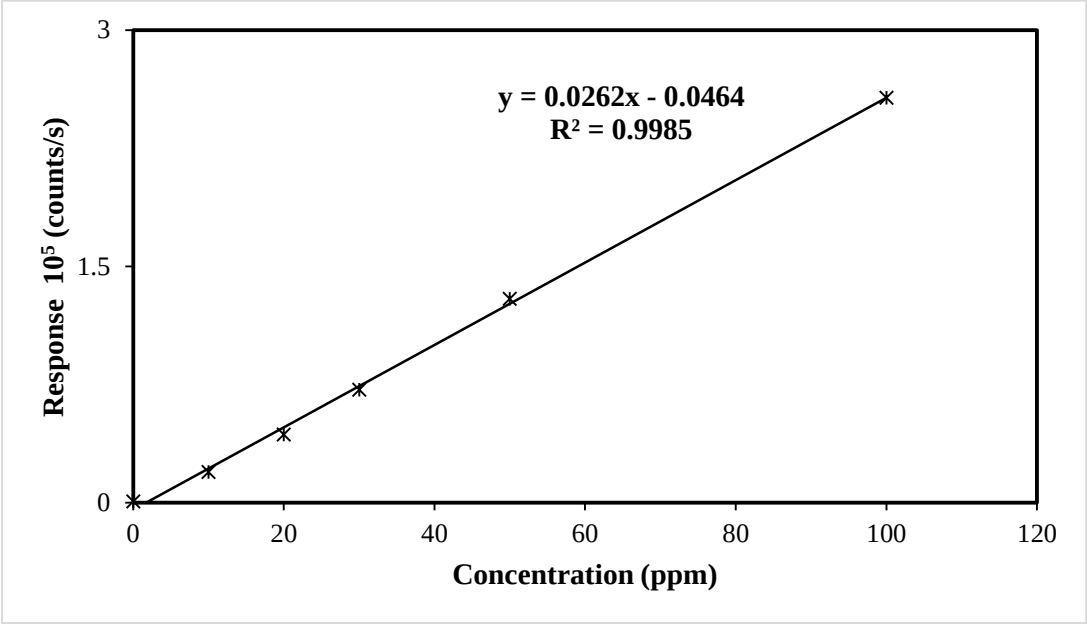

Figure 4.5. Calibration curve for mercury

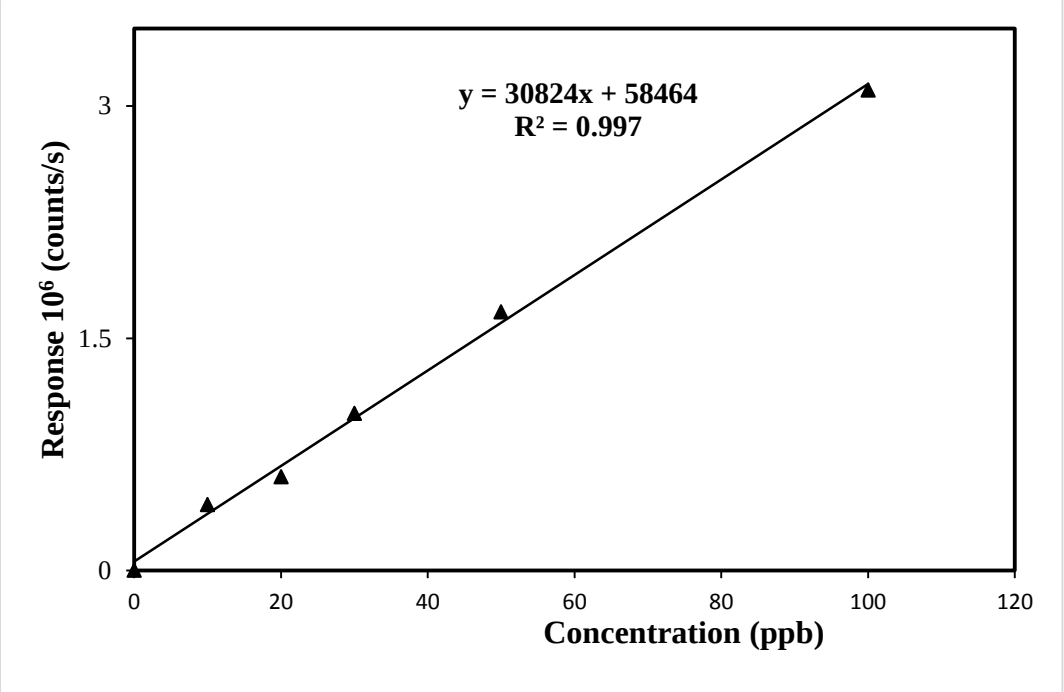

Figure 4.6. Calibration curve for lead.

Table 4.1. Calibration coefficients, limits of detection and limits of quantification

Element	Molecular mass	R^2	LOD (ppb)	LOQ (ppb)
As	75	0.9998	1.671798	5.572661
Cd	111	0.9999	1.363076	4.543587
Hg	201	0.9985	4.726475	15.75492
Pb	208	0.9969	6.703868	22.34623

4.3 Heavy metal content of analysed samples

The analytical results on the content of heavy metals are given in Table 4.2. The concentration of As, Hg, Cd and Pb in the herbal medicines varied widely possibly due to differences in their origins and processing.

Table 4.2. Content of selected heavy metals in samples of herbal medicines used for management of chronic diseases as determined using ICP-MS

Sample code	Concentration (mg/kg)			
	Arsenic	**Cadmium**	**Mercury**	**Lead**
CA180389	0.251 (1.14)	0.218 (1.60)	0.227 (1.25)	0.787 (4.20)
CA180390	0.091(1.42)	0.248 (2.53)	0.158 (1.54)	0.514 (1.88)
CA180391	0.150 (0.86)	0.280 (1.05)	0.289 (7.0)	0.403 (4.60)
CA180392	0.093 (3.01)	0.192 (2.20)	0.101 (1.51)	0.185 (5.46)
CA180393	0.062 (3.61)	0.203 (5.50)	0.127 (9.60)	0.366 (3.57)
CA180394	0.146 (1.71)	0.184 (1.14)	0.058 (1.79)	0.332 (2.98)
CA180395	0.078 (1.20)	0.193 (1.09)	0.012 (0.92)	0.269 (2.24)
CA180396	0.159 (1.76)	0.224 (1.56)	0.140 (2.14)	0.971(1.26)
CA180397	0.054 (2.67)	0.237 (6.90)	0.134 (3.17)	0.232 (5.71)
CA180398	0.107 (1.66)	0.158 (1.03)	0.078 (3.12)	0.223 (2.46)
CA180399	0.136 (2.76)	0.186 (1.87)	0.209 (1.03)	0.872 (2.56)
CA180400	0.095 (3.77)	0.220 (1.93)	0.101 (2.11)	0.526 (4.86)
CA180401	0.058 (2.47)	0.167 (4.60)	0.049 (2.76)	0.268 (1.83)
CA180402	0.035 (1.09)	0.148 (1.31)	0.022 (3.01)	0.294 (4.64)
CA180403	0.113 (0.11)	0.184 (0.69)	0.071 (4.3)	0.163 (5.52)
CA180404	0.054 (0.59)	0.158 (1.21)	0.042 (3.48)	0.169 (3.39)
CA180405	0.109 (2.61)	0.220 (1.29)	0.028 (5.3)	0.223 (3.53)
CA180406	0.140 (1.01)	0.200 (0.42)	0.006 (5.81)	0.693 (1.22)
CA180407	0.025 (0.90)	0.153 (0.94)	0.037 (1.39)	0.158 (9.92)
CA180408	0.173 (0.94)	0.216 (0.62)	0.042 (1.32)	0.535 (2.35)
CA180409	0.074 (0.83)	0.166 (3.08)	0.012 (1.20)	0.192 (5.96)
CA180410	0.136 (1.20)	0.176 (3.08)	0.017 (3.87)	0.194 (4.03)
CA180411	0.138 (1.36)	0.145 (2.13)	ND	0.291 (6.72)
CA180412	0.070 (0.45)	0.170 (2.54)	0.020 (3.12)	0.349 (2.66)
CA180413	0.027 (0.62)	0.170 (1.66)	0.013 (1.49)	0.173 (6.61)
CA180414	0.253 (2.35)	0.535 (0.75)	0.014 (1.18)	0.654 (10.02)
CA180415	0.185 (1.76)	0.276 (5.00)	ND	0.425 (2.05)
CA180416	0.087 (2.57)	0.238 (1.12)	ND	0.418 (2.87)
CA180417	0.301 (8.64)	0.237 (1.82)	ND	0.559 (1.84)

Sample code	Concentration (mg/kg)			
	Arsenic	Cadmium	Mercury	Lead
CA180419	0.037 (1.67)	0.187 (1.74)	ND	0.083 (7.69)
CA180420	0.229 (1.95)	0.274 (1.24)	ND	0.406 (2.73)
CA180421	0.045 (3.42)	0.169 (2.15)	ND	0.172 (6.09)
CA180422	0.016 (4.33)	0.307 (3.81)	ND	0.133 (7.58)
CA180423	ND	0.165 (1.53)	ND	0.688 (5.06)
CA180424	0.006 (1.73)	0.227 (1.16)	ND	0.169 (4.28)
CA180425	0.157 (1.59)	0.264 (1.26)	0.001 (2.64)	0.404 (3.63)
CA180426	0.115 (3.04)	0.160 (5.59)	0.005 (0.83)	0.295 (3.65)
CA180427	0.550 (5.94)	0.269 (2.88)	3.768 (3.90)	1.533 (2.19)
CA180428	0.161 (3.11)	0.189 (4.97)	0.107 (9.30)	0.501 (2.00)
CA180429	0.412 (1.21)	0.236 (1.20)	0.031 (3.93)	1.928 (1.44)
CA180430	2.783 (3.54)	1.075 (0.96)	0.048 (1.89)	3.324 (3.69)
CA180431	0.426 (1.47)	0.239 (8.15)	0.048 (1.38)	1.055 (3.07)
CA180432	0.220 (1.98)	0.200 (3.3)	0.030 (3.20)	0.755 (5.44)
CA180433	0.937 (9.15)	0.228 (1.22)	0.030 (8.30)	1.466 (1.65)
CA180434	0.214 (8.81)	0.236 (4.35)	0.107 (2.42)	2.185 (1.23)
CA180435	226.726 (1.61)	0.480 (2.74)	18221.93 (2.30)	0.711 (3.72)
CA180436	0.397 (3.59)	0.256 (6.28)	600.980 (9.50)	2.635 (1.76)
CA180437	0.208 (6.18)	0.191 (1.25)	86.505 (3.00)	4.864 (4.01)
CA180438	0.715 (7.80)	0.238 (1.95)	38.667 (6.20)	0.996 (5.14)
CA180439	4.038 (3.68)	0.269 (9.66)	134.645 (0.50)	0.671 (1.88)
CA180440	0.787 (4.47)	0.256 (7.60)	19.549 (3.60)	0.890 (3.45)
CA180441	0.344 (1.57)	0.216 (1.37)	13.626 (1.33)	1.154 (5.19)
CA180442	0.671 (2.32)	0.212 (1.46)	10.327 (9.40)	0.200 (3.59)
CA180443	1.215 (0.29)	0.322 (6.86)	7.972 (6.10)	0.250 (3.84)
CA180444	0.239 (1.94)	0.199 (5.09)	6.566 (3.90)	0.342 (5.01)
CA180445	1.242 (3.59)	0.352 (1.02)	5.649 (7.70)	0.185 (0.94)
CA180446	0.789 (5.56)	0.203 (6.00)	4.750 (5.60)	0.144 (6.55)
CA180447	0.126 (4.64)	0.169 (2.07)	4.295 (1.03)	0.203 (4.30)
CA180448	0.369 (5.89)	0.179 (5.24)	3.881 (1.21)	1.051 (7.48)
CA180449	0.544 (1.34)	0.221 (7.51)	3.600 (8.50)	0.764 (1.46)
CA180450	0.105 (5.97)	0.457 (7.19)	3.304 (2.80)	0.333 (5.63)

Sample code	Concentration (mg/kg)			
	Arsenic	Cadmium	Mercury	Lead
CA180451	0.140 (2.51)	0.221 (2.75)	3.102 (2.13)	0.627 (3.35)
CA180452	0.270 (2.90)	0.419 (5.28)	2.866 (5.10)	0.562 (2.54)
CA180453	0.126 (2.52)	0.212 (1.53)	2.526 (0.81)	0.625 (5.73)
CA180454	0.043 (1.43)	0.184 (1.41)	2.235 (7.32)	0.875 (1.92)
CA180455	0.089 (8.06)	0.147 (8.03)	2.351 (6.48)	0.118 (4.03)
CA180456	0.107 (8.81)	0.183 (1.24)	2.099 (3.90)	0.257 (1.55)
CA180457	0.014 (0.65)	0.168 (4.39)	1.893 (9.28)	0.074 (3.92)
CA180458	0.002 (1.73)	0.147 (6.39)	1.647 (5.20)	0.063 (1.88)
CA180459	0.006 (1.00)	0.205 (1.07)	1.592 (7.06)	0.062 (7.31)
CA180460	0.033 (1.08)	0.196 (1.12)	2.146 (3.62)	0.093 (3.19)
CA180461	0.027 (7.05)	0.183 (1.48)	1.506 (5.31)	0.150 (7.44)
CA180462	0.016 (1.20)	0.180 (1.36)	1.404 (2.62)	0.092 (1.37)
CA180463	0.004 (2.29)	0.162 (1.85)	1.385 (6.76)	0.106 (3.97)
CA180464	0.021 (1.73)	0.172 (8.41)	1.373 (6.49)	0.109 (1.06)
CA180465	0.014 (1.07)	0.156 (1.14)	1.301 (4.46)	0.096 (1.72)
CA180466	0.023 (5.67)	0.173 (9.87)	1.337 (0.82)	0.222 (6.85)
CA180467	0.126 (2.27)	0.165 (2.49)	1.415 (4.49)	5.591 (2.83)
CA180468	0.008 (2.41)	0.255 (3.68)	1.277 (4.75)	0.157 (6.12)
CA180469	0.016 (5.72)	0.153 (2.97)	1.359 (1.20)	0.215 (9.41)
CA180470	0.099 (6.25)	0.155 (6.80)	1.481 (1.18)	0.238 (6.88)
CA180471	0.140 (2.58)	0.145 (1.16)	1.360 (8.09)	0.285 (5.36)
CA180472	0.025 (0.91)	0.166 (9.72)	1.408 (9.07)	0.138 (1.05)
CA180473	0.109 (1.82)	0.189 (4.46)	1.320 (3.56)	0.195 (4.78)
CA180474	0.016 (5.73)	0.159 (1.81)	1.157 (7.78)	0.175 (7.05)
CA180475	0.056 (6.18)	0.204 (1.15)	1.404 (5.46)	0.310 (4.25)
CA180476	0.912 (1.70)	0.248 (1.44)	0.955 (9.16)	1.376 (3.57)
CA180477	0.148 (1.10)	0.199 (2.94)	1.153 (0.66)	0.351 (5.60)

% RSD is given in parenthesis, ND- not detected. For purpose of further statistical± analysis, n.d. was substituted with zero to enable computation of summary statistics.

The mean content of the heavy metals were: As 2.858±24.15 mg/kg; Cd 0.227±0.15 mg/kg; Hg 246.378±2062.95 mg/kg and Pb 0.646±0.92 mg/kg with the ranges being: As 0.002-

226.726 mg/kg, Cd 0.145-1.075 mg/kg, Hg 0.001-18221.930 mg/kg and Pb: 0.062-5.591 mg/kg. The relative standard deviation of determination of heavy metals was found not to be higher than 15% and this was considered adequate for this study (Table 4.2).

Overall, Hg concentration was significantly higher than As ($p < 0.01$), Pb ($p < 0.01$) and Cd ($p < 0.01$). The content of Hg was at least thousand orders of magnitude higher than that of Cd, while As was over ten and four times higher than Cd and Pb, respectively (Figure 4.7). Mercury was detected in 78 (87.6%) out of 89 samples analysed while arsenic was detected in all samples except one (98.9%). The relatively high concentration of Hg in the analysed samples could be attributed to accumulation from several sources including processing procedures.

The content of Pb in samples analysed was in the range 0.062-5.591 mg/kg which was less than 10 ppm limit set by WHO and thus the samples of herbal medicines complied with the WHO specifications. However, one sample (CA180467) had 5.591 ppm of Pb which is slightly above the 5 ppm limit set by the European Pharmacopoeia. The sample was therefore adjudged not to meet the Eur. Ph. specifications for Pb in herbal drugs. Although majority met the criteria for Pb levels, long term use could ultimately lead to bioaccumulation and attendant toxicities.

A majority of herbal medicine samples were contaminated with Pb. The least contaminant was found to be mercury. The number of samples contaminated for each metal is shown in Figure 4.8. Asthma herbal medicines were also found have the highest content of lead (Figure 4.9).

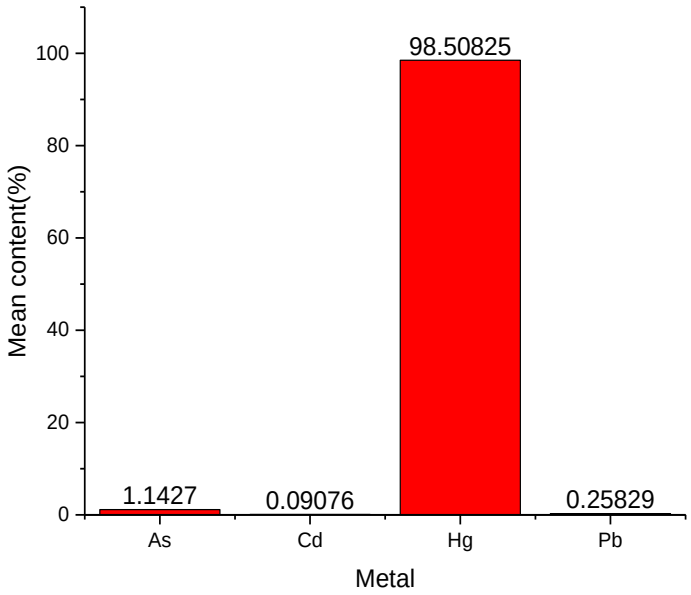

Figure 4.7. Relative mean contents of heavy metals in herbal medicines.

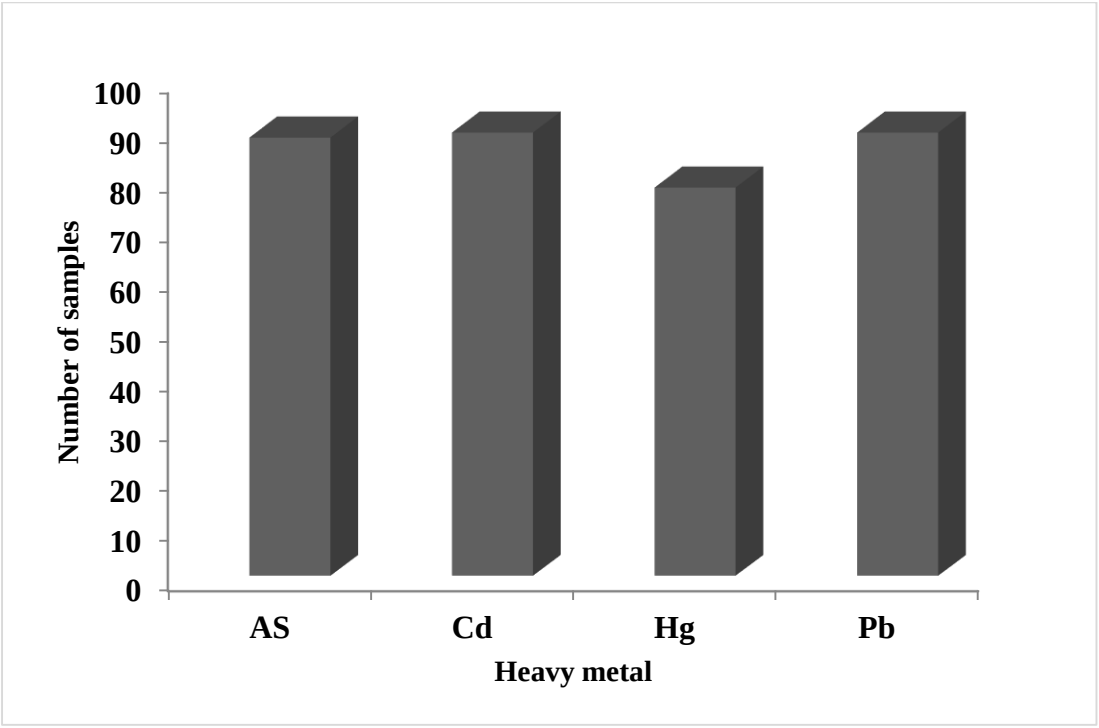

Figure 4.8. Number of samples contaminated with each heavy metal.

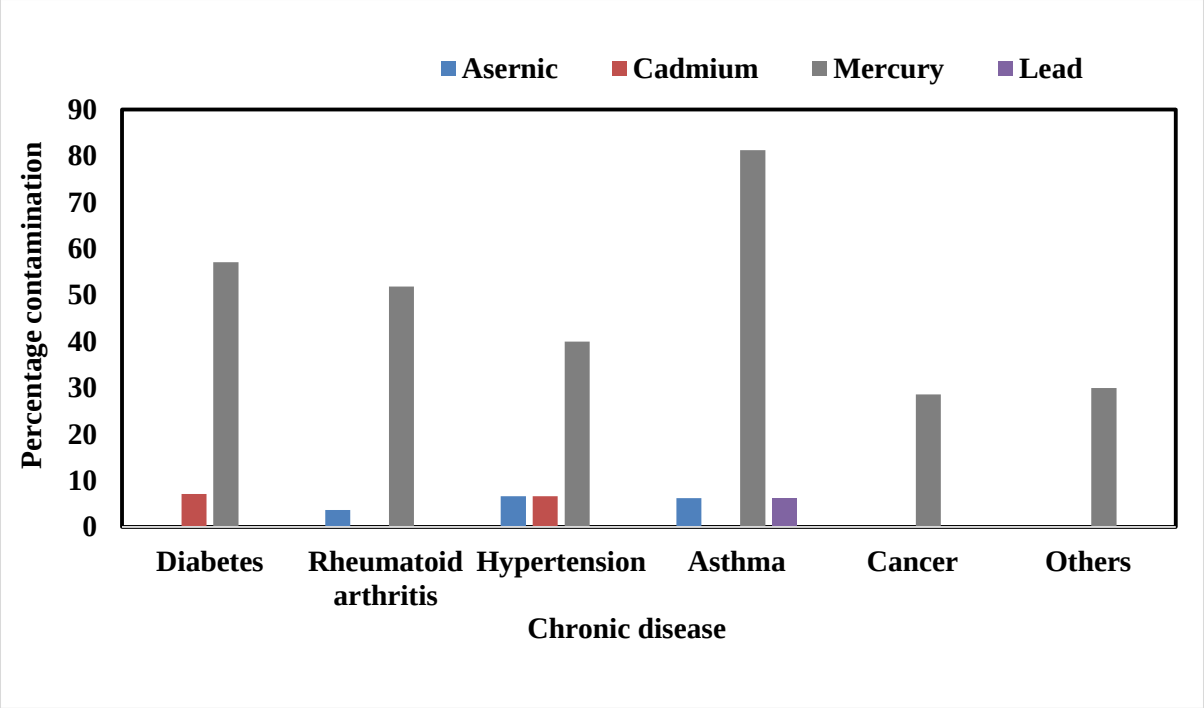

Figure 4.9. Proportion of non-complaint samples per chronic disease.

With a mean content of 0.227 mg/kg, the occurrence of cadmium was the lowest among the four metals analysed across all the herbal medicines (Table 4.3). A study conducted in Saudi Arabia reported concentrations of Cd of up to 0.1 mg/kg (Maghrabi, 2014). This study found a two-fold higher contamination of herbals with Cd. A similar study conducted on herbal medicines in Kenya in 2017 using AAS did not detect cadmium (Richard Kipserem Korir, 2017). This is probably due to the higher sensitivity of ICP-MS used in the present study compared to AAS. Although the main sources of human exposure to Cd remain cigarette smoke, welding and contaminated food and beverages, continuous monitoring of Cd in herbal medicines is recommended.

Table 4.3. Descriptive statistics of samples of herbal medicines analyses

Metal	Concentration ((mg/kg)			
	Mean±SD	**Median**	**Minimum**	**Maximum**
As	2.858±24.15[a]	0.126	0.002	226.726
Cd	0.227±0.15	0.200	0.145	1.075
Hg	246.378±2062.95[a]	1.310	0.001	18221.930
Pb	0.646±0.92[a]	0.332	0.062	5.591

[a]The high SD values observed were due to outlier samples that disproportionately contained higher amounts of heavy metals and thus distorted the dispersion in the data.

Using a two-tailed test of significance, no correlation among the analysed metals was found (Table 4.4). Nevertheless, the levels of As and Hg were found to be correlated although the level of significance was zero. Using paired t-test, the mean metal content of As and Cd were found not to be significantly different at the 0.05 level with p=0.3093. Similarly the concentration of Hg and Pb were found to be statistically different. Analysis of variance using the F-statistic also showed that the content of the metals in the herbal products were significantly different at 0.05 level.

Table 4.4. Pearson correlations between the contents of the heavy metals in herbal medicines analysed

		As	**Cd**	**Hg**	**Pb**
As	Pearson Correlation	1.000	0.248	0.999	0.014
	Significance	0.000	0.020	0.000	0.899
Cd	Pearson Correlation	0.248	1.000	0.240	0.293
	Significance	0.020	0.000	0.034	0.005
Hg	Pearson Correlation	0.999	0.240	1.000	0.016
	Significance	0.000	0.034	0.000	0.889
Pb	Pearson Correlation	0.014	0.293	0.016	1.000
	Significance	0.899	0.005	0.889	0.000

The comparative mean content of heavy metals used to manage chronic conditions is shown in Table 4.5. The content of arsenic was highest in products used to manage asthma (mean = 14.413 mg/kg). The average content of As in products used to treat chronic conditions was as follows: rheumatoid arthritis (0.278 mg/kg), hypertension (0.304 mg/kg), cancer (0.244 mg/kg) and others (0.234 mg/kg). Oral intake of herbal products for asthma may therefore predispose the consumer to higher load of arsenic. The mean content of Hg in products used to treat diabetes, rheumatoid arthritis and asthma were 12.107, 34.429 and 1141.071 mg/kg respectively. Mercury in any of its forms is considered toxic. The content was above the 2 ppm limit set by US EPA for drinking water. Generally, all the samples were above the 0.1 ppm limit by European Pharmacopoeia. Tables 4.5-4.10 give concentrations of heavy metals in herbal medicines per category of chronic condition.

Table 4.5. Comparative mean content of heavy metals used to manage chronic conditions

Disease	Mean heavy metal content (mg/kg)			
	Arsenic	Cadmium	Mercury	Lead
Diabetes	0.280	0.229	12.107	1.059
Rheumatoid arthritis	0.278	0.205	34.429	0.467
Hypertension	0.304	0.278	1.896	0.671
Asthma	14.413	0.239	1141.071	0.672
Cancer	0.244	0.197	1.327	0.455
Others	0.226	0.199	1.384	0.519

Table 4.6. Content of selected heavy metals in samples of herbal medicines used for diabetes

Sample code	Concentration (mg/kg)				Dosage form	Collection County
	Arsenic	Cadmium	Mercury	Lead		
CA180412	0.070	0.170	0.020	0.349	Powder	Nairobi
CA180414	0.253	0.535	0.014	0.654	Powder	Kiambu
CA180421	0.045	0.169	ND	0.172	Powder	Nairobi
CA180427	0.550	0.269	3.768	1.533	Tablet	Kajiado
CA180431	0.426	0.239	0.048	1.055	Tablet	Nairobi
CA180432	0.220	0.200	0.030	0.755	Tablet	Kajiado
CA180434	0.214	0.236	0.107	2.185	Tablet	Nairobi
CA180437	0.208	0.191	86.505	4.864	Tablet	Machakos
CA180438	0.715	0.238	38.667	0.996	Tablet	Nairobi
CA180440	0.787	0.256	19.549	0.890	Tablet	Kiambu
CA180448	0.369	0.179	3.881	1.051	Capsule	Nairobi
CA180457	0.014	0.168	1.893	0.074	Liquid	Kiambu
CA180461	0.027	0.183	1.506	0.150	Liquid	Kajiado
CA180462	0.016	0.180	1.404	0.092	Liquid	Kajiado
Mean	**0.280**	**0.229**	**12.107**	**1.059**		
Minimum	**0.014**	**0.168**	**0.014**	**0.074**		
Maximum	**0.787**	**0.535**	**86.505**	**4.864**		

Among the 14 herbal products used to manage diabetes, 8 (57.1%), (CA180427, CA180437, CA180438, CA180440, CA180448, CA180457, CA180461 and CA180462) had Hg levels above 0.1 ppm set by the European Pharmacopoeia (Table 4.6). All the samples complied with the WHO and European Pharmacopoeia limits for Pb and USP specification for arsenic, as well as WHO specification of 0.3 ppm for Cd except sample CA180414 that contained 0.535 ppm. All the samples complied with USP specification of 1.5 ppm for arsenic. A repeat analysis on samples CA180437, CA180438 and CA180440 gave repeatable results of 87.092, 38.926 and 20.134 mg/kg Hg, respectively.

Table 4.7. Content of selected heavy metals in samples of herbal medicines used for rheumatoid arthritis

Sample code	Concentration (mg/kg)				Dosage form	Collection county
	Arsenic	Cadmium	Mercury	Lead		
CA180390	0.091	0.248	0.158	0.514	Powder	Nairobi
CA180392	0.093	0.192	0.101	0.185	Powder	Kajiado
CA180399	0.136	0.186	0.209	0.872	Powder	Kiambu
CA180400	0.095	0.220	0.101	0.526	Powder	Nairobi
CA180401	0.058	0.167	0.049	0.268	Powder	Machakos
CA180402	0.035	0.148	0.022	0.294	Powder	Nairobi
CA180403	0.113	0.184	0.071	0.163	Powder	Machakos
CA180404	0.054	0.158	0.042	0.169	Powder	Kiambu
CA180406	0.140	0.200	0.006	0.693	Powder	Nairobi
CA180408	0.173	0.216	0.042	0.535	Powder	Nairobi
CA180416	0.087	0.238	ND	0.418	Powder	Kajiado
CA180417	0.301	0.237	ND	0.559	Powder	Kiambu
CA180419	0.037	0.187	ND	0.083	Powder	Machakos
CA180424	0.006	0.227	ND	0.169	Tablet	Kiambu
CA180428	0.161	0.189	0.107	0.501	Tablet	Nairobi
CA180436	0.397	0.256	600.980	2.635	Tablet	Nairobi
CA180439	4.038	0.269	134.645	0.671	Tablet	Kiambu
CA180447	0.126	0.169	4.295	0.203	Capsule	Nairobi
CA180449	0.544	0.221	3.600	0.764	Capsule	Kajiado
CA180452	0.270	0.419	2.866	0.562	Capsule	Nairobi
CA180453	0.126	0.212	2.526	0.625	Capsule	Nairobi
CA180455	0.089	0.147	2.351	0.118	Capsule	Kajiado
CA180463	0.004	0.162	1.385	0.106	Liquid	Kiambu
CA180464	0.021	0.172	1.373	0.109	Liquid	Machakos
CA180469	0.016	0.153	1.359	0.215	Liquid	Kajiado
CA180474	0.016	0.159	1.157	0.175	Liquid	Kajiado
CA180476	0.912	0.248	0.955	1.376	Powder	Nairobi
Mean	**0.301**	**0.207**	**28.059**	**0.500**		
Minimum	**0.004**	**0.147**	**0.006**	**0.083**		
Maximum	**4.038**	**0.419**	**600.980**	**2.635**		

ND- not detected

Among the 27 herbal products used to treat rheumatoid arthritis, 16 (59.25%) samples, CA180390, CA180399, CA180417, CA180424, CA180436, CA180439, CA180447, CA180449, CA180452, CA180453, CA180455, CA180463, CA180464, CA180469, CA180474 and CA180476 contained Hg above the 0.1 ppm limit set by the European Pharmacopoeia (Table 4.7). Notably, samples CA180436 and CA180439 had extremely high levels of mercury, 600.98 mg/kg and 134.645 mg/kg, respectively. Similarly, a repeat test carried out on these two samples had comparable results of 597.894 and 140.176 mg/kg, respectively. All the 27 samples complied with the specifications for Cd, lead and arsenic except sample CA180439 with arsenic content of 4.038 mg/kg and 3.997mg/kg for the first and second tests, respectively.

Table 4.8. Content of heavy metals in herbal medicines used to treat hypertension

Sample code	Concentration (mg/kg)				Dosage form	Collection county
	Arsenic	Cadmium	Mercury	Lead		
CA180396	0.159	0.224	0.140	0.971	Powder	Kiambu
CA180397	0.054	0.237	0.134	0.232	Powder	Nairobi
CA180398	0.107	0.158	0.078	0.223	Powder	Nairobi
CA180409	0.074	0.166	0.012	0.192	Powder	Nairobi
CA180415	0.185	0.276	ND	0.425	Powder	Kajiado
CA180420	0.229	0.274	ND	0.406	Powder	Nairobi
CA180422	0.016	0.307	ND	0.133	Powder	Machakos
CA180429	0.412	0.236	0.031	1.928	Tablet	Kiambu
CA180430	2.783	1.075	0.048	3.324	Tablet	Kajiado
CA180441	0.344	0.216	13.626	1.154	Capsule	Machakos
CA180451	0.140	0.221	3.102	0.627	Capsule	Nairobi
CA180459	0.006	0.205	1.592	0.062	Liquid	Nairobi
CA180465	0.014	0.156	1.301	0.096	Liquid	Nairobi
CA180468	0.008	0.255	1.277	0.157	Liquid	Machakos
CA180472	0.025	0.166	1.408	0.138	Liquid	Nairobi
Mean	**0.304**	**0.278**	**1.896**	**0.671**		
Minimum	**0.006**	**0.156**	**0.012**	**0.062**		
Maximum	**2.783**	**1.075**	**13.626**	**3.324**		

ND- not detected

Of the fifteen samples indicated for the management of hypertension, 6 (40%) of the samples namely, CA180441, CA180451, CA180459, CA180465, CA180468 and CA180472 contained Hg above the 0.1 ppm limit set in the European Pharmacopoeia. The levels of Pb and Cd were within the permissible limits set by the WHO and European Pharmacopoeia except sample CA180430 had Cd content of 1.075 mg/kg (Table 4.8). Arsenic contents were within the USP limit of 1.5 mg/kg except sample CA180430 (2.783 mg/kg).

Table 4.9. Content of heavy metals in herbal medicines used to treat asthma

Sample code	Concentration (mg/kg)				Dosage form	Collection county
	Arsenic	Cadmium	Mercury	Lead		
CA180391	0.150	0.280	0.289	0.403	Powder	Machakos
CA180407	0.025	0.153	0.037	0.158	Powder	Nairobi
CA180423	ND	0.165	ND	0.688	Powder	Kajiado
CA180435	226.726	0.480	18221.930	0.711	Tablet	Nairobi
CA180443	1.215	0.322	7.972	0.250	Capsule	Nairobi
CA180445	1.242	0.352	5.649	0.185	Capsule	Nairobi
CA180446	0.789	0.203	4.750	0.144	Capsule	Machakos
CA180450	0.105	0.457	3.304	0.333	Capsule	Machakos
CA180454	0.043	0.184	2.235	0.875	Liquid	Kajiado
CA180456	0.107	0.183	2.099	0.257	Liquid	Kiambu
CA180460	0.033	0.196	2.146	0.093	Liquid	Nairobi
CA180467	0.126	0.165	1.415	5.591	Liquid	Nairobi
CA180470	0.099	0.155	1.481	0.238	Liquid	Nairobi
CA180471	0.140	0.145	1.360	0.285	Liquid	Kiambu
CA180473	0.109	0.189	1.320	0.195	Liquid	Kiambu
CA180477	0.148	0.199	1.153	0.351	Powder	Machakos
Mean	**14.413**	**0.239**	**1141.071**	**0.672**		
Minimum	**0.025**	**0.145**	**0.037**	**0.093**		
Maximum	**226.726**	**0.480**	**18221.930**	**5.591**		

ND- not detected

Sixteen samples used to manage asthma were analysed where, 14 (87.5%) had Hg levels above the 0.1 ppm limit set by the European pharmacopoeia (Table 4.9). Sample CA180435 contained extremely high levels of both As and Hg, 226.73 and 18221.93 mg/kg, respectively. The contents of Pb and Cd in the herbal medicines used to treat asthma were within the permissible limits set by the WHO and European Pharmacopoeia. A second test carried out on this sample gave comparable results of 227.909 mg/kg and 18226.220 mg/kg for As and Hg, respectively.

Two out of the 7 (28.57%) samples indicated for the management of cancer contained Hg above the 0.1 ppm limit set by the European Pharmacopeia and WHO. The levels of Pb and Cd were within the limits specified by the WHO and European Pharmacopoeia as well as arsenic as per the USP 2018 specifications (Table 4.10).

Table 4.10. Content of heavy metals in herbal medicines used to manage cancer

Sample code	Concentration (mg/kg)				Dosage form	Collection county
	Arsenic	Cadmium	Mercury	Lead		
CA180395	0.078	0.193	0.012	0.269	Capsule	Nairobi
CA180410	0.136	0.176	0.017	0.194	Powder	Nairobi
CA180411	0.138	0.145	ND	0.291	Powder	Nairobi
CA180425	0.157	0.264	0.001	0.404	Tablet	Kajiado
CA180433	0.937	0.228	0.030	1.466	Tablet	Nairobi
CA180444	0.239	0.199	6.566	0.342	Capsule	Kajiado
CA180466	0.023	0.173	1.337	0.222	Liquid	Machakos
Mean	**0.244**	**0.197**	**1.327**	**0.455**		
Minimum	**0.023**	**0.145**	**0.001**	**0.194**		
Maximum	**0.937**	**0.264**	**6.566**	**1.466**		

ND- not detected

Table 4.11. Content of heavy metals in herbal medicines used to treat other chronic conditions

Sample code	Concentration (mg/kg)				Disease	Dosage form	Collection county
	Arsenic	Cadmium	Mercury	Lead			
CA180418	0.723	0.269	ND	2.441	TB	Tablet	Nairobi
CA180394	0.146	0.184	0.058	0.332	HIV	Powder	Kajiado
CA180442	0.671	0.212	10.327	0.200	HIV	Capsule	Machakos
CA180426	0.115	0.160	0.005	0.295	Memory	Tablet	Nairobi
CA180458	0.002	0.147	1.647	0.063	Memory	Liquid	Nairobi
CA180389	0.251	0.218	0.227	0.787	TB	Tablet	Nairobi
CA180393	0.062	0.203	0.127	0.366	TB	Tablet	Nairobi
CA180413	0.027	0.170	0.013	0.173	PUD	Powder	Machakos
CA180405	0.109	0.220	0.028	0.223	PUD	Powder	Kiambu
CA180475	0.056	0.204	1.404	0.310	TB	Powder	Nairobi
Mean	**0.216**	**0.199**	**1.384**	**0.519**			
Minimum	**0.002**	**0.147**	**0.005**	**0.063**			
Maximum	**0.723**	**0.269**	**10.327**	**2.441**			

ND- not detected, TB- tuberculosis, HIV/AIDs- Human immunodeficiency Virus/Acquired immune deficiency syndrome, PUD- peptic ulcer disease

Four out of the 10 (40.0%) samples used to manage other chronic conditions, HIV/AIDS, memory loss and TB were found to contain high amount of Hg. The concentrations were 10.327, 1.647, 1.404 and 0.227 mg/kg for samples CA180442, CA180458, CA180475 and CA180389, respectively. These concentrations were above the 0.1 ppm set by the WHO and European Pharmacopoeia. All the samples complied with the European pharmacopoeia, WHO and USP 2018 specifications for Cd, Pb and As, respectively (Table 4.11).

4.4 Principal component analysis

Principal component analysis is a multivariate analytical technique that enables reduction of data dimensions while at the same time detecting outlier samples. The outliers are either inherently divergent samples or a result of poor measurements. In order to determine the number of principal components that account for variation in the data, a scree plot was constructed (Figure 4.10). From the plot, 2 principal components were adjudged adequate as they accounted for more than 83.4% of the data.

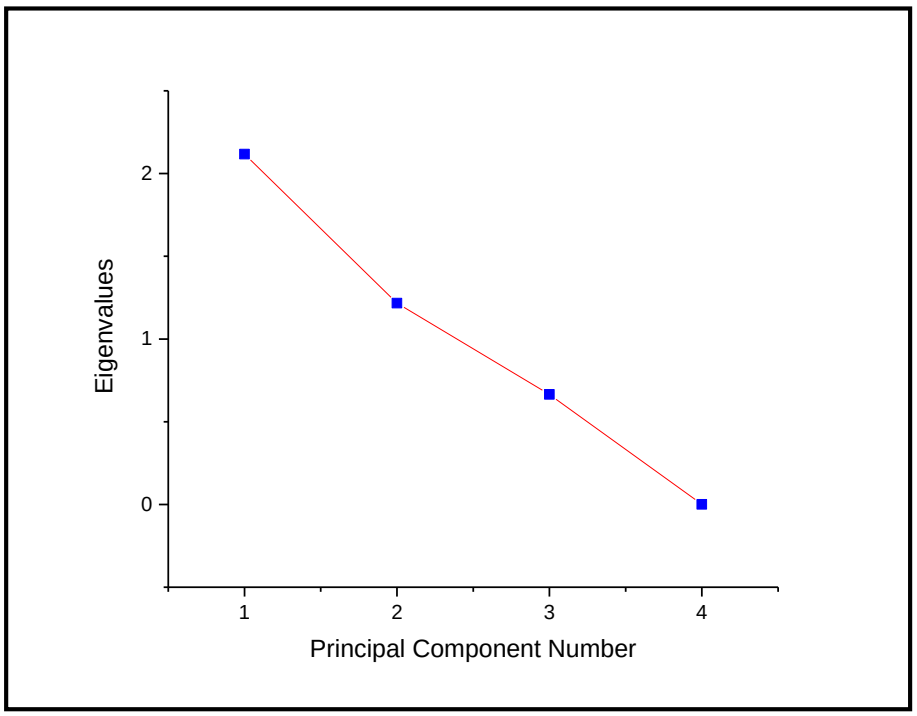

Figure 4.10. Scree plot showing number of principal components adequately explaining sample differences in heavy metal content.

In a bid to point out outlier samples, scores plot were constructed using principal components 1 and 2. The scores plot (Figure 4.11) showed 4 samples as being outliers (CA180435, CA180436, CA180437 and CA180467). One sample (CA180435) was extremely high in mercury content while the other three (CA180436, CA180437 and CA180467) contained high

amount of lead compared with the rest of the samples. A loading plot explaining the differences in the metal content is shown in Figure 4.12.

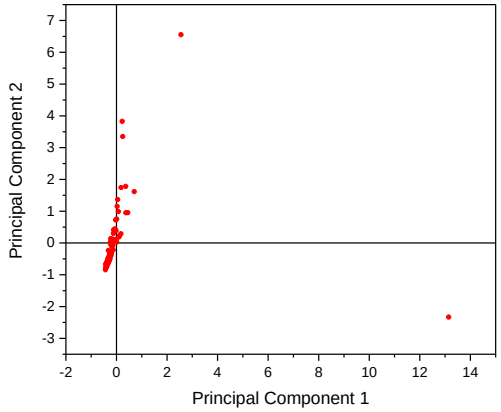

Figure 4.11. Scores plot for all samples

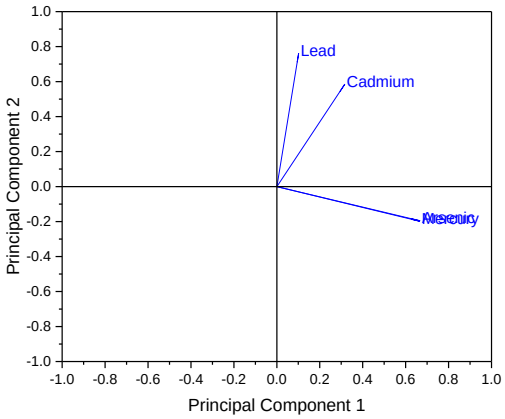

Figure 4.12. Loading plot explaining the divergent samples

Since lead and mercury had high influence on the PCA model, further divergence was investigated using cadmium and arsenic. The scores plot only detected two samples that were divergent from the rest (Figures 4.13 and 4.14). One sample was high in cadmium (CA180430) while the other contained relatively higher concentration of arsenic (CA180435).

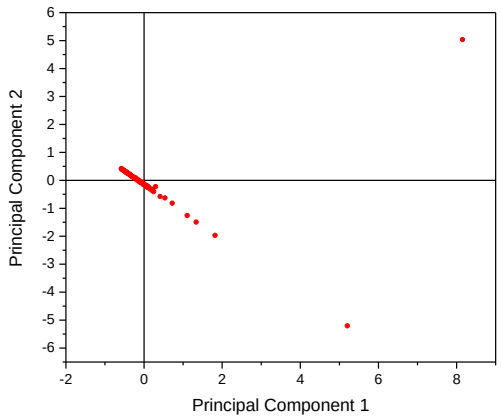

Figure 4.13. Scores plot for arsenic and cadmium in the samples of herbal medicines

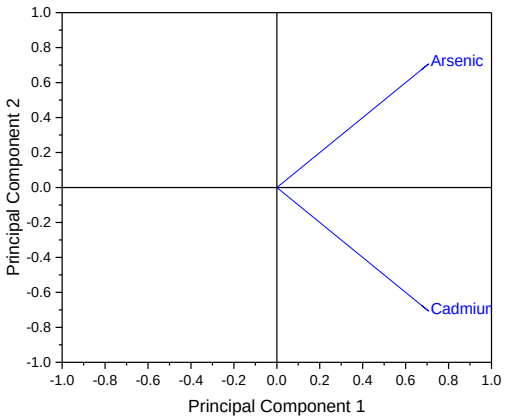

Figure 4.14. Loading plot explaining the divergence in content of arsenic and cadmium

4.5 Risk assessment for heavy metal contamination

4.5.1 Estimated daily intake

The estimated daily intake (EDI) of the heavy metals was estimated from the exposure to the analysed herbal products for a duration of 30 years which is usually set by US EPA for heavy metals. Individual scenarios were calculated for adults and children since their body weights differ. The EDI values calculated for each of the 89 herbal products using Equation **3** are given in Table 4.12.

Table 4.12. Estimated daily intake of heavy metals in herbal medicines for adults and children

Sample code	Arsenic ($\times 10^{-5}$)		Cadmium ($\times 10^{-5}$)		Mercury ($\times 10^{-5}$)		Lead ($\times 10^{-5}$)	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child
CA180389	2.51	4.61	2.2	4	2.3	4.2	7.9	14.4
CA180390	0.91	1.66	2.5	4.6	1.6	2.9	5.1	9.4
CA180391	1.5	2.76	2.8	5.1	2.9	5.3	4	7.4
CA180392	0.93	1.7	1.9	3.5	1	1.9	1.9	3.4
CA180393	0.62	1.13	2	3.7	1.3	2.3	3.7	6.7
CA180394	1.46	2.68	1.8	3.4	0.6	1.1	3.3	6.1
CA180395	0.78	1.44	1.9	3.5	0.1	0.2	2.7	4.9
CA180396	1.59	2.91	2.2	4.1	1.4	2.6	9.7	17.8
CA180397	0.54	0.98	2.4	4.3	1.3	2.5	2.3	4.3
CA180398	1.07	1.97	1.6	2.9	0.8	1.4	2.2	4.1
CA180399	1.36	2.49	1.9	3.4	2.1	3.8	8.7	16
CA180400	0.95	1.74	2.2	4	1	1.8	5.3	9.7
CA180401	0.58	1.06	1.7	3.1	0.5	0.9	2.7	4.9
CA180402	0.35	0.64	1.5	2.7	0.2	0.4	2.9	5.4
CA180403	1.13	2.08	1.8	3.4	0.7	1.3	1.6	3
CA180404	0.54	0.98	1.6	2.9	0.4	0.8	1.7	3.1
CA180405	1.09	2	2.2	4	0.3	0.5	2.2	4.1
CA180406	1.4	2.57	2	3.7	0.1	0.1	6.9	12.7
CA180407	0.25	0.45	1.5	2.8	0.4	0.7	1.6	2.9
CA180408	1.73	3.17	2.2	4	0.4	0.8	5.3	9.8
CA180409	0.74	1.36	1.7	3	0.1	0.2	1.9	3.5
CA180410	1.36	2.49	1.8	3.2	0.2	0.3	1.9	3.6
CA180411	1.38	2.53	1.5	2.7	0	0	2.9	5.3
CA180412	0.7	1.28	1.7	3.1	0.2	0.4	3.5	6.4
CA180413	0.27	0.49	1.7	3.1	0.1	0.2	1.7	3.2
CA180414	2.53	4.65	5.4	9.8	0.1	0.3	6.5	12
CA180415	1.85	3.4	2.8	5.1	0	0	4.2	7.8

Sample code	Arsenic ($\times 10^{-5}$)		Cadmium ($\times 10^{-5}$)		Mercury ($\times 10^{-5}$)		Lead ($\times 10^{-5}$)	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child
CA180416	0.87	1.59	2.4	4.4	0	0	4.2	7.7
CA180417	3.01	5.52	2.4	4.3	0	0	5.6	10.3
CA180418	7.23	13.26	2.7	4.9	0	0	24.4	44.8
CA180419	0.37	0.68	1.9	3.4	0	0	0.8	1.5
CA180420	2.29	4.19	2.7	5	0	0	4.1	7.5
CA180421	0.45	0.83	1.7	3.1	0	0	1.7	3.2
CA180422	0.16	0.3	3.1	5.6	0	0	1.3	2.4
CA180423	0	0	1.6	3	0	0	6.9	12.6
CA180424	0.06	0.11	2.3	4.2	0	0	1.7	3.1
CA180425	1.57	2.87	2.6	4.8	0	0	4	7.4
CA180426	1.15	2.12	1.6	2.9	0.1	0.1	2.9	5.4
CA180427	5.5	10.09	2.7	4.9	37.7	69.1	15.3	28.1
CA180428	1.61	2.95	1.9	3.5	1.1	2	5	9.2
CA180429	4.12	7.56	2.4	4.3	0.3	0.6	19.3	35.4
CA180430	27.83	51.06	10.7	19.7	0.5	0.9	33.2	61
CA180431	4.26	7.82	2.4	4.4	0.5	0.9	10.5	19.4
CA180432	2.2	4.04	2	3.7	0.3	0.5	7.6	13.9
CA180433	9.37	17.19	2.3	4.2	0.3	0.6	14.7	26.9
CA180434	2.14	3.93	2.4	4.3	1.1	2	21.9	40.1
CA180435	2267.26	4160.11	4.8	8.8	182219.3	334347.3	7.1	13
CA180436	3.97	7.29	2.6	4.7	6009.8	11027.2	26.4	48.3
CA180437	2.08	3.82	1.9	3.5	865.1	1587.3	48.6	89.2
CA180438	7.15	13.11	2.4	4.4	386.7	709.5	10	18.3
CA180439	40.38	74.08	2.7	4.9	1346.5	2470.6	6.7	12.3
CA180440	7.87	14.44	2.6	4.7	195.5	358.7	8.9	16.3
CA180441	3.44	6.31	2.2	4	136.3	250	11.5	21.2
CA180442	6.71	12.32	2.1	3.9	103.3	189.5	2	3.7
CA180443	12.15	22.3	3.2	5.9	79.7	146.3	2.5	4.6
CA180444	2.39	4.38	2	3.6	65.7	120.5	3.4	6.3

Sample code	Arsenic ($\times 10^{-5}$)		Cadmium ($\times 10^{-5}$)		Mercury ($\times 10^{-5}$)		Lead ($\times 10^{-5}$)	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child
CA180445	12.42	22.79	3.5	6.5	56.5	103.6	1.8	3.4
CA180446	7.89	14.47	2	3.7	47.5	87.2	1.4	2.6
CA180447	1.26	2.31	1.7	3.1	42.9	78.8	2	3.7
CA180448	3.69	6.76	1.8	3.3	38.8	71.2	10.5	19.3
CA180449	5.44	9.98	2.2	4.1	36	66.1	7.6	14
CA180450	1.05	1.93	4.6	8.4	33	60.6	3.3	6.1
CA180451	1.4	2.57	2.2	4.1	31	56.9	6.3	11.5
CA180452	2.7	4.95	4.2	7.7	28.7	52.6	5.6	10.3
CA180453	1.26	2.31	2.1	3.9	25.3	46.3	6.3	11.5
CA180454	0.43	0.79	1.8	3.4	22.3	41	8.7	16.1
CA180455	0.89	1.62	1.5	2.7	23.5	43.1	1.2	2.2
CA180456	1.07	1.97	1.8	3.4	21	38.5	2.6	4.7
CA180457	0.14	0.26	1.7	3.1	18.9	34.7	0.7	1.4
CA180458	0.02	0.04	1.5	2.7	16.5	30.2	0.6	1.2
CA180459	0.06	0.11	2	3.8	15.9	29.2	0.6	1.1
CA180460	0.33	0.6	2	3.6	21.5	39.4	0.9	1.7
CA180461	0.27	0.49	1.8	3.4	15.1	27.6	1.5	2.7
CA180462	0.16	0.3	1.8	3.3	14	25.8	0.9	1.7
CA180463	0.04	0.08	1.6	3	13.9	25.4	1.1	1.9
CA180464	0.21	0.38	1.7	3.1	13.7	25.2	1.1	2
CA180465	0.14	0.26	1.6	2.9	13	23.9	1	1.8
CA180466	0.23	0.42	1.7	3.2	13.4	24.5	2.2	4.1
CA180467	1.26	2.31	1.6	3	14.1	26	55.9	102.6
CA180468	0.08	0.15	2.6	4.7	12.8	23.4	1.6	2.9
CA180469	0.16	0.3	1.5	2.8	13.6	24.9	2.1	3.9
CA180470	0.99	1.81	1.5	2.8	14.8	27.2	2.4	4.4
CA180471	1.4	2.57	1.5	2.7	13.6	25	2.9	5.2
CA180472	0.25	0.45	1.7	3	14.1	25.8	1.4	2.5
CA180473	1.09	2	1.9	3.5	13.2	24.2	1.9	3.6

Sample code	Arsenic ($\times 10^{-5}$)		Cadmium ($\times 10^{-5}$)		Mercury ($\times 10^{-5}$)		Lead ($\times 10^{-5}$)	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child
CA180474	0.16	0.3	1.6	2.9	11.6	21.2	1.8	3.2
CA180475	0.56	1.02	2	3.7	14	25.8	3.1	5.7
CA180476	9.12	16.74	2.5	4.6	9.5	17.5	13.8	25.2
CA180477	1.48	2.72	2	3.6	11.5	21.1	3.5	6.4

4.5.2 Non-cancer risk

The non-cancer risk of heavy metals which basically estimates the probability of organ damage for each of heavy metals was computed by dividing the EDI with the oral reference doses. Table 4.13 gives a summary of the total hazard quotients (THQs) for adults and children. Risk analysis identified arsenic and mercury as being of greatest risk for non-cancerous toxicity. The level of toxicity differed between children and adults owing to differences in the body weights. Arsenic content of sample CA180430 posed risk to the children only while samples CA180435 and CA180439 contained As at levels that posed risk for health to both children and adults. In all cases the risk of arsenic was 2-fold higher for children than in adults. The risk was on the magnitude of hundreds.

Similarly, for Hg some samples only posed risk to children and not adults. These samples included CA180427 and CA180447-552. However, samples CA180435-446 had THQs higher than 1 with some samples presenting a ten-fold risk for non-cancer toxicity (Table 4.13). The risks due to Hg in children were 2-fold those of adults. This underscores the vulnerability of children to toxic effects of metals in herbal medicines. This calls for greater caution and judicious use of herbals in the paediatric population.

Table 4.13. Target hazard quotients of heavy metals in herbal medicines

Sample code	Arsenic		Cadmium		Mercury		Lead	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child
CA180389	0.084	0.154	0.022	0.040	0.045	0.083	0.022	0.041
CA180390	0.030	0.055	0.025	0.046	0.032	0.058	0.015	0.027
CA180391	0.050	0.092	0.028	0.051	0.058	0.106	0.012	0.021
CA180392	0.031	0.057	0.019	0.035	0.020	0.037	0.005	0.010
CA180393	0.021	0.038	0.020	0.037	0.025	0.046	0.010	0.019
CA180394	0.049	0.089	0.018	0.034	0.012	0.021	0.009	0.017
CA180395	0.026	0.048	0.019	0.035	0.002	0.004	0.008	0.014
CA180396	0.053	0.097	0.022	0.041	0.028	0.051	0.028	0.051
CA180397	0.018	0.033	0.024	0.043	0.027	0.049	0.007	0.012
CA180398	0.036	0.066	0.016	0.029	0.016	0.029	0.006	0.012
CA180399	0.045	0.083	0.019	0.034	0.042	0.077	0.025	0.046
CA180400	0.032	0.058	0.022	0.040	0.020	0.037	0.015	0.028
CA180401	0.019	0.035	0.017	0.031	0.010	0.018	0.008	0.014
CA180402	0.012	0.021	0.015	0.027	0.004	0.008	0.008	0.015
CA180403	0.038	0.069	0.018	0.034	0.014	0.026	0.005	0.009
CA180404	0.018	0.033	0.016	0.029	0.008	0.015	0.005	0.009
CA180405	0.036	0.067	0.022	0.040	0.006	0.010	0.006	0.012
CA180406	0.047	0.086	0.020	0.037	0.001	0.002	0.020	0.036
CA180407	0.008	0.015	0.015	0.028	0.007	0.013	0.005	0.008
CA180408	0.058	0.106	0.022	0.040	0.008	0.015	0.015	0.028
CA180409	0.025	0.045	0.017	0.030	0.002	0.004	0.005	0.010
CA180410	0.045	0.083	0.018	0.032	0.003	0.006	0.006	0.010
CA180411	0.046	0.084	0.015	0.027	0.000	0.000	0.008	0.015
CA180412	0.023	0.043	0.017	0.031	0.004	0.007	0.010	0.018
CA180413	0.009	0.016	0.017	0.031	0.003	0.005	0.005	0.009
CA180414	0.084	0.155	0.054	0.098	0.003	0.005	0.019	0.034
CA180415	0.062	0.113	0.028	0.051	0.000	0.000	0.012	0.022
CA180416	0.029	0.053	0.024	0.044	0.000	0.000	0.012	0.022

Sample code	Arsenic		Cadmium		Mercury		Lead	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child
CA180417	0.100	0.184	0.024	0.043	0.000	0.000	0.016	0.029
CA180418	0.241	0.442	0.027	0.049	0.000	0.000	0.070	0.128
CA180419	0.012	0.023	0.019	0.034	0.000	0.000	0.002	0.004
CA180420	0.076	0.140	0.027	0.050	0.000	0.000	0.012	0.021
CA180421	0.015	0.028	0.017	0.031	0.000	0.000	0.005	0.009
CA180422	0.005	0.010	0.031	0.056	0.000	0.000	0.004	0.007
CA180423	0.000	0.000	0.016	0.030	0.000	0.000	0.020	0.036
CA180424	0.002	0.004	0.023	0.042	0.000	0.000	0.005	0.009
CA180425	0.052	0.096	0.026	0.048	0.000	0.001	0.012	0.021
CA180426	0.038	0.071	0.016	0.029	0.001	0.002	0.008	0.015
CA180427	0.183	0.336	0.027	0.049	0.754	1.383	0.044	0.080
CA180428	0.054	0.098	0.019	0.035	0.021	0.039	0.014	0.026
CA180429	0.137	0.252	0.024	0.043	0.006	0.011	0.055	0.101
CA180430	0.928	1.702	0.107	0.197	0.010	0.018	0.095	0.174
CA180431	0.142	0.261	0.024	0.044	0.010	0.018	0.030	0.055
CA180432	0.073	0.135	0.020	0.037	0.006	0.011	0.022	0.040
CA180433	0.312	0.573	0.023	0.042	0.006	0.011	0.042	0.077
CA180434	0.071	0.131	0.024	0.043	0.021	0.039	0.062	0.115
CA180435	75.575	138.670	0.048	0.088	3644.386	6686.947	0.020	0.037
CA180436	0.132	0.243	0.026	0.047	120.196	220.543	0.075	0.138
CA180437	0.069	0.127	0.019	0.035	17.301	31.745	0.139	0.255
CA180438	0.238	0.437	0.024	0.044	7.733	14.190	0.028	0.052
CA180439	1.346	2.469	0.027	0.049	26.929	49.411	0.019	0.035
CA180440	0.262	0.481	0.026	0.047	3.910	7.174	0.025	0.047
CA180441	0.115	0.210	0.022	0.040	2.725	5.000	0.033	0.060
CA180442	0.224	0.411	0.021	0.039	2.065	3.790	0.006	0.010
CA180443	0.405	0.743	0.032	0.059	1.594	2.925	0.007	0.013
CA180444	0.080	0.146	0.020	0.036	1.313	2.410	0.010	0.018
CA180445	0.414	0.760	0.035	0.065	1.130	2.073	0.005	0.010

Sample code	Arsenic		Cadmium		Mercury		Lead	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child
CA180446	0.263	0.482	0.020	0.037	0.950	1.743	0.004	0.008
CA180447	0.042	0.077	0.017	0.031	0.859	1.576	0.006	0.011
CA180448	0.123	0.225	0.018	0.033	0.776	1.424	0.030	0.055
CA180449	0.181	0.333	0.022	0.041	0.720	1.321	0.022	0.040
CA180450	0.035	0.064	0.046	0.084	0.661	1.212	0.010	0.017
CA180451	0.047	0.086	0.022	0.041	0.620	1.138	0.018	0.033
CA180452	0.090	0.165	0.042	0.077	0.573	1.052	0.016	0.029
CA180453	0.042	0.077	0.021	0.039	0.505	0.927	0.018	0.033
CA180454	0.014	0.026	0.018	0.034	0.447	0.820	0.025	0.046
CA180455	0.030	0.054	0.015	0.027	0.470	0.863	0.003	0.006
CA180456	0.036	0.066	0.018	0.034	0.420	0.770	0.007	0.013
CA180457	0.005	0.009	0.017	0.031	0.379	0.695	0.002	0.004
CA180458	0.001	0.001	0.015	0.027	0.329	0.605	0.002	0.003
CA180459	0.002	0.004	0.020	0.038	0.318	0.584	0.002	0.003
CA180460	0.011	0.020	0.020	0.036	0.429	0.788	0.003	0.005
CA180461	0.009	0.016	0.018	0.034	0.301	0.552	0.004	0.008
CA180462	0.005	0.010	0.018	0.033	0.281	0.515	0.003	0.005
CA180463	0.001	0.003	0.016	0.030	0.277	0.508	0.003	0.006
CA180464	0.007	0.013	0.017	0.031	0.275	0.504	0.003	0.006
CA180465	0.005	0.009	0.016	0.029	0.260	0.477	0.003	0.005
CA180466	0.008	0.014	0.017	0.032	0.267	0.491	0.006	0.012
CA180467	0.042	0.077	0.016	0.030	0.283	0.519	0.160	0.293
CA180468	0.003	0.005	0.026	0.047	0.255	0.469	0.004	0.008
CA180469	0.005	0.010	0.015	0.028	0.272	0.499	0.006	0.011
CA180470	0.033	0.060	0.015	0.028	0.296	0.544	0.007	0.012
CA180471	0.047	0.086	0.015	0.027	0.272	0.499	0.008	0.015
CA180472	0.008	0.015	0.017	0.030	0.282	0.517	0.004	0.007

Sample code	Arsenic		Cadmium		Mercury		Lead	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child
CA180473	0.036	0.067	0.019	0.035	0.264	0.484	0.006	0.010
CA180474	0.005	0.010	0.016	0.029	0.231	0.425	0.005	0.009
CA180475	0.019	0.034	0.020	0.037	0.281	0.515	0.009	0.016
CA180476	0.304	0.558	0.025	0.046	0.191	0.350	0.039	0.072
CA180477	0.049	0.091	0.020	0.036	0.231	0.423	0.010	0.018

Additionally, the interactive effects of these heavy metals in contributing to negative health were estimated by summing up all the THQs for individual metals. The hazard indices (HIs) were separately calculated for children and adults due to differences in body weights (Table 4.14).

Samples CA180427, CA180435-438, CA180447, CA180453-455 and CA180487 gave HI greater than 1 and thus were identified to pose health risk with sample CA180487 only posing risk to children. In all instances the risk to children was higher than to adults. The magnitude of the risk posed by sample CA180435 was in thousand-folds as shown by the HI values of 3720.03 and 6825.743 in adults and children, respectively (Table 4.14).

Table 4.14. Hazard index of heavy metals in herbal medicines

Sample code	Hazard Index	
	Adult	Child
CA180389	0.173406	0.318176
CA180390	0.101299	0.18587
CA180391	0.147458	0.270565
CA180392	0.075616	0.138744
CA180393	0.076629	0.140604

Sample code	Hazard Index	
	Adult	Child
CA180394	0.08819	0.161817
CA180395	0.055504	0.101841
CA180396	0.130974	0.240319
CA180397	0.074926	0.137479
CA180398	0.073482	0.134829
CA180399	0.130664	0.239751
CA180400	0.088772	0.162884
CA180401	0.05342	0.098019
CA180402	0.039381	0.072259
CA180403	0.075095	0.13779
CA180404	0.046759	0.085797
CA180405	0.070357	0.129095
CA180406	0.087727	0.160967
CA180407	0.035378	0.064915
CA180408	0.10299	0.188973
CA180409	0.049199	0.090274
CA180410	0.071914	0.131952
CA180411	0.06882	0.126274
CA180412	0.054171	0.099396
CA180413	0.033448	0.061373
CA180414	0.159351	0.292388
CA180415	0.1015	0.186238
CA180416	0.064557	0.118454
CA180417	0.139873	0.256648
CA180418	0.33762	0.619487
CA180419	0.033438	0.061355
CA180420	0.115196	0.211368
CA180421	0.036884	0.067677
CA180422	0.039987	0.073371

Sample code	Hazard Index	
	Adult	Child
CA180423	0.036122	0.066279
CA180424	0.029595	0.054303
CA180425	0.090425	0.165917
CA180426	0.063928	0.1173
CA180427	1.007508	1.848639
CA180428	0.108267	0.198656
CA180429	0.222231	0.407764
CA180430	1.139749	2.091282
CA180431	0.2057	0.37743
CA180432	0.120973	0.221969
CA180433	0.383115	0.702963
CA180434	0.178822	0.328114
CA180435	3720.03	6825.743
CA180436	120.4294	220.9714
CA180437	17.52849	32.16236
CA180438	8.023838	14.72264
CA180439	28.32094	51.96503
CA180440	4.223113	7.748832
CA180441	2.894463	5.310941
CA180442	2.316208	4.249923
CA180443	2.038708	3.740748
CA180444	1.422554	2.610192
CA180445	1.584196	2.906782
CA180446	1.237342	2.270352
CA180447	0.92345	1.694404
CA180448	0.946991	1.737599
CA180449	0.945228	1.734364
CA180450	0.75102	1.378019
CA180451	0.707181	1.29758

Sample code	Hazard Index	
	Adult	Child
CA180452	0.721102	1.323122
CA180453	0.586168	1.075537
CA180454	0.5048	0.926238
CA180455	0.517864	0.950209
CA180456	0.481121	0.88279
CA180457	0.402261	0.738093
CA180458	0.346681	0.636112
CA180459	0.342612	0.628645
CA180460	0.462478	0.848584
CA180461	0.33263	0.610331
CA180462	0.307027	0.563352
CA180463	0.297637	0.546122
CA180464	0.301642	0.553472
CA180465	0.283357	0.519922
CA180466	0.298597	0.547885
CA180467	0.500994	0.919255
CA180468	0.288188	0.528785
CA180469	0.298786	0.548232
CA180470	0.351456	0.644873
CA180471	0.341416	0.626451
CA180472	0.31039	0.569522
CA180473	0.324797	0.595958
CA180474	0.257864	0.473145
CA180475	0.328624	0.60298
CA180476	0.559192	1.026039
CA180477	0.309841	0.568516

4.5.3 Cancer risk

Although the cancer risk (CR) attributable to a metal is the probability of contracting cancer over a lifetime of 70 years, there was no need to use years reflective of the life expectancy in Kenya as the risk would be marginally different since the life expectancy in Kenya is 64.4 years and 68.9 years for males and females, respectively. Table 4.15 gives results of the cancer risk assessment for heavy metals in herbal medicines used to manage chronic conditions in Kenya.

Table 4.15. Cancer risk of heavy metals in herbal medicinal products

Sample code	Cancer risk			Total cancer risk
	Arsenic	Cadmium	Lead	
CA180389	3.97E-07	1.42E-05	1.15E-06	1.58E-05
CA180390	1.01E-05	4.05E-06	1.87E-07	1.43E-05
CA180391	7.41E-06	4.55E-06	1.47E-07	1.21E-05
CA180392	3.54E-05	3.13E-06	6.75E-08	3.85E-05
CA180393	1.03E-05	3.3E-06	1.33E-07	1.38E-05
CA180394	2.65E-05	3.0E-06	1.21E-07	2.96E-05
CA180395	0.000179	3.14E-06	9.79E-08	0.000182
CA180396	2.74E-05	3.65E-06	3.54E-07	3.14E-05
CA180397	1.42E-05	3.86E-06	8.44E-08	1.81E-05
CA180398	6.02E-05	2.57E-06	8.11E-08	6.29E-05
CA180399	1.38E-05	3.03E-06	3.18E-07	1.71E-05
CA180400	0.014575	3.59E-06	1.92E-07	0.014579
CA180401	2.56E-05	2.71E-06	9.77E-08	2.84E-05
CA180402	1.34E-05	2.41E-06	1.07E-07	1.59E-05
CA180403	4.59E-05	3E-06	5.93E-08	4.9E-05
CA180404	0.00026	2.57E-06	6.14E-08	0.000262
CA180405	5.06E-05	3.59E-06	8.13E-08	5.42E-05
CA180406	2.21E-05	3.25E-06	2.52E-07	2.56E-05
CA180407	4.32E-05	2.49E-06	5.75E-08	4.57E-05

Sample code	Cancer risk			Total cancer risk
	Arsenic	Cadmium	Lead	
CA180408	7.81E-05	3.52E-06	1.95E-07	8.18E-05
CA180409	1.54E-05	2.7E-06	6.98E-08	1.81E-05
CA180410	7.98E-05	2.87E-06	7.07E-08	8.28E-05
CA180411	5.07E-05	2.36E-06	1.06E-07	5.32E-05
CA180412	8.08E-06	2.76E-06	1.27E-07	1.1E-05
CA180413	2.37E-05	2.76E-06	6.28E-08	2.65E-05
CA180414	3.5E-05	8.71E-06	2.38E-07	4.39E-05
CA180415	6.75E-06	4.49E-06	1.55E-07	1.14E-05
CA180416	9.0E-06	3.87E-06	1.52E-07	1.3E-05
CA180417	1.73E-05	3.86E-06	2.04E-07	2.14E-05
CA180418	8.08E-06	4.38E-06	8.89E-07	1.33E-05
CA180419	2.78E-06	3.05E-06	3.02E-08	5.86E-06
CA180420	5.69E-06	4.46E-06	1.48E-07	1.03E-05
CA180421	6.88E-06	2.75E-06	6.28E-08	9.69E-06
CA180422	9.27E-07	5.0E-06	4.84E-08	5.97E-06
CA180423	1.32E-07	2.68E-06	2.51E-07	3.07E-06
CA180424	3.97E-07	3.7E-06	6.16E-08	4.16E-06
CA180425	2.12E-06	4.3E-06	1.47E-07	6.57E-06
CA180426	1.72E-06	2.6E-06	1.07E-07	4.43E-06
CA180427	1.06E-06	4.38E-06	5.58E-07	6.0E-06
CA180428	2.65E-07	3.08E-06	1.83E-07	3.53E-06
CA180429	1.32E-06	3.84E-06	7.02E-07	5.87E-06
CA180430	9.27E-07	1.75E-05	1.21E-06	1.96E-05
CA180431	1.46E-06	3.89E-06	3.84E-07	5.73E-06
CA180432	8.08E-06	3.25E-06	2.75E-07	1.16E-05
CA180433	5.3E-07	3.71E-06	5.34E-07	4.78E-06
CA180434	1.06E-06	3.84E-06	7.96E-07	5.7E-06
CA180435	6.36E-06	7.82E-06	2.59E-07	1.44E-05
CA180436	9E-06	4.17E-06	9.6E-07	1.41E-05

Sample code	Cancer risk			Total cancer risk
	Arsenic	Cadmium	Lead	
CA180437	1.59E-06	3.11E-06	1.77E-06	6.47E-06
CA180438	7.02E-06	3.87E-06	3.63E-07	1.13E-05
CA180439	1.06E-06	4.38E-06	2.44E-07	5.68E-06
CA180440	3.57E-06	4.17E-06	3.24E-07	8.07E-06
CA180441	5.87E-05	3.52E-06	4.2E-07	6.26E-05
CA180442	9.53E-06	3.46E-06	7.27E-08	1.31E-05
CA180443	0	5.24E-06	9.11E-08	5.33E-06
CA180444	0	3.24E-06	1.25E-07	3.36E-06
CA180445	0	5.73E-06	6.74E-08	5.8E-06
CA180446	0	3.3E-06	5.26E-08	3.35E-06
CA180447	0	2.75E-06	7.4E-08	2.82E-06
CA180448	0	2.92E-06	3.83E-07	3.3E-06
CA180449	0	3.6E-06	2.78E-07	3.88E-06
CA180450	0	7.44E-06	1.21E-07	7.56E-06
CA180451	0	3.6E-06	2.28E-07	3.83E-06
CA180452	0	6.82E-06	2.05E-07	7.03E-06
CA180453	0	3.46E-06	2.28E-07	3.69E-06
CA180454	0	3.0E-06	3.19E-07	3.32E-06
CA180455	0	2.4E-06	4.31E-08	2.44E-06
CA180456	0	2.98E-06	9.36E-08	3.08E-06
CA180457	0	2.73E-06	2.7E-08	2.76E-06
CA180458	0	2.4E-06	2.3E-08	2.42E-06
CA180459	0	3.33E-06	2.25E-08	3.36E-06
CA180460	0	3.19E-06	3.39E-08	3.22E-06
CA180461	0	2.98E-06	5.45E-08	3.04E-06
CA180462	0	2.94E-06	3.37E-08	2.97E-06
CA180463	0	2.63E-06	3.87E-08	2.67E-06
CA180464	0	2.79E-06	3.98E-08	2.83E-06
CA180465	0	2.54E-06	3.48E-08	2.57E-06

Sample code	Cancer risk			Total cancer risk
	Arsenic	Cadmium	Lead	
CA180466	0	2.82E-06	8.07E-08	2.91E-06
CA180467	0	2.68E-06	2.04E-06	4.72E-06
CA180468	0	4.16E-06	5.7E-08	4.22E-06
CA180469	0	2.49E-06	7.81E-08	2.57E-06
CA180470	0	2.52E-06	8.66E-08	2.61E-06
CA180471	0	2.36E-06	1.04E-07	2.47E-06
CA180472	0	2.7E-06	5.03E-08	2.75E-06
CA180473	0	3.08E-06	7.09E-08	3.15E-06
CA180474	0	2.59E-06	6.38E-08	2.65E-06
CA180475	0	3.32E-06	1.13E-07	3.43E-06
CA180476	0	4.05E-06	5.01E-07	4.55E-06
CA180477	0	3.24E-06	1.28E-07	3.37E-06

As shown in Table 4.15, CR and TCR higher than 10^{-4} were identified in samples CA180395, CA180400 and CA180404. The CR and TCR for the potentially toxic samples were as follows; sample CA180395 (CR-1.8E-04, TCR – 1.82E-04), sample CA180400 (CR- 1.458E-04, TCR-1.458E-04) and sample CA180404 (CR- 2.6E-04, TCR-2.62E-04). Arsenic was found to be the contributor of the CR in all the three samples. Sample CA180400 poses the greatest risk for cancer.

4.6 Microbial analysis

4.6.1 Total viable aerobic count and moulds / yeast counts

Among the 89 herbal medicine collected, 86 were subjected to microbial analysis. The 86 samples comprised 13 capsule, 21 liquid, 35 powder and 17 tablet formulations while the remaining three were in insufficient quantities for microbial enumeration and characterization. Nutrient agar media

was used to enumerate total bacteria while SDA was utilised for enumeration and identification of total fungi.

Fourteen samples (16.3%) had no growth while 72 (83.7%) exhibited visible growth and were subjected to further microbial analysis. Twenty nine (33.7%) samples did not meet the microbial enumeration test. Out of the 13 capsule formulations collected, 7 (53.8%) did not comply with the B.P. (2017) specifications for microbial enumeration while 8 (38.1%) out of the 21 liquid herbal products also failed the enumeration test. Similarly, 11 (31.4%) out of 35 powder and 3 (17.6%) out of 17 tablet formulations failed to meet the B.P. (2017) specifications for microbial enumeration (Table 4.15). The microbial loads were as shown in Tables 4.16-4.19.

Table 4.16. Total average microbial counts of capsule-formulated herbal medicines

Sample code	Absolute count on NA	Absolute count on SDA	Average aerobic count (10^7cfu/mL)	Average fungi count (10^5cfu/mL)	Inference
CA180441	6.50E+06	1.60E+06	0.65	16.00	**Not complied**
CA180442	5.00E+01	9.00E+02	0.00	0.01	**Complied**
CA180443	1.80E+04	1.00E+04	0.00	0.10	**Complied**
CA180444	3.00E+06	6.50E+05	0.30	6.50	**Not complied**
CA180445	1.00E+05	5.00E+02	0.01	0.01	**Complied**
CA180446	ND	ND	ND	ND	**Complied**
CA180447	ND	ND	ND	ND	**Complied**
CA180448	6.20E+06	3.20E+05	0.62	3.20	**Complied**
CA180449	7.00E+06	1.12E+06	0.70	11.20	**Not complied**
CA180450	1.00E+05	1.00E+04	0.01	0.10	**Complied**
CA180451	1.40E+06	5.30E+05	0.14	5.30	**Not complied**
CA180452	3.20E+06	1.23E+05	0.32	1.23	**Complied**
CA180453	3.30E+06	6.60E+05	0.33	6.60	**Not complied**

NA- nutrient agar, SDA- Sabouraud's Dextrose Agar, cfu- colony forming units, ND- not detected.

Four (30.8%) out of the 13 capsule-formulated medicines complied with the BP (2017) specifications for enumeration test (Table 4.16) while 13 (61.9%) out of the 21 liquid-formulated herbals complied with the enumeration test (Table 4.17). Majority of the liquid formulations were freshly boiled and had minimal microbial growth because heat denatures bacteria and fungi while most of the prepacked liquids failed enumeration test.

Table 4.17. Total microbial counts on liquid-formulated herbal medicines

Sample code	Absolute count on NA	Absolute count on SDA	Average aerobic count (10^5cfu/mL)	Average fungi count (10^3cfu/mL)	Inference
CA180454	4.30E+03	1.10E+02	0.04	0.11	**Complied**
CA180455	ND	2.00E+01	ND	0.02	**Complied**
CA180456	1.14E+04	4.40E+02	0.11	0.44	**Complied**
CA180457	6.10E+06	1.46E+04	61.00	14.6	**Not complied**
CA180458	ND	1.00E+01	ND	0.00	**Complied**
CA180459	ND	ND	ND	ND	**Complied**
CA180460	3.09E+04	1.57E+03	0.31	1.57	**Complied**
CA180461	5.00E+05	5.00E+03	5.00	5.00	**Complied**
CA180462	2.50E+06	4.00E+03	25.00	4.00	**Not complied**
CA180463	2.26E+04	3.25E+03	0.02	3.25	**Complied**
CA180464	2.50E+06	2.45E+04	25.0	24.50	**Not complied**
CA180465	1.85E+04	1.10E+05	0.19	110.00	**Not complied**
CA180466	3.30E+03	1.00E+01	0.03	0.00	**Complied**
CA180467	2.00E+04	9.50E+03	0.20	9.50	**Not complied**
CA180468	3.00E+04	8.00E+02	0.30	0.80	**Complied**

Sample code	Absolute count on NA	Absolute count on SDA	Average aerobic count (10^5cfu/mL)	Average fungi count (10^3cfu/mL)	Inference
CA180469	2.09E+04	6.70E+03	0.21	6.70	**Not complied**
CA180470	9.00E+04	3.00E+02	0.90	0.30	**Complied**
CA180471	3.00E+04	9.00E+03	0.30	9.00	**Not complied**
CA180472	1.61E+04	7.80E+03	0.16	7.80	**Not complied**
CA180473	1.16E+04	4.42E+02	0.12	0.44	**Complied**
CA180474	4.00E+02	0.00E+01	0.00	ND	**Complied**

NA=Nutrient agar; SDA=Sabouraud,'s Dextrose Agar; Cfu= Colony forming units, ND- not detected.

Eleven (31.4%) of the 35 powder formulated samples and 3 (17.6%) out of 17 tablet-formulated samples did not comply with the BP (2017) specification for enumeration test (Tables 4.18 and 4.19).

Table 4.18. Total microbial counts on powder formulated herbal medicines

Sample code	Absolute count on NA	Absolute count on SDA	Average aerobic count (10^7cfu/mL)	Average fungi count (10^5cfu/mL)	Inference
CA180389	3.00E+03	1.30E+03	ND	0.01	**Complied**
CA180390	4.00E+05	1.00E+04	0.04	0.10	**Complied**
CA180391	3.00E+04	1.00E+04	0.00	0.10	**Complied**
CA180392	2.00E+05	1.00E+05	0.02	1.00	**Complied**
CA180393	1.00E+04	1.00E+03	0.00	0.01	**Complied**
CA180394	1.00E+07	2.00E+04	1.00	0.20	**Complied**
CA180395	9.00E+06	6.00E+05	0.90	6.00	**Not complied**
CA180396	2.00E+04	5.00E+03	ND	0.05	**Complied**
CA180397	2.60E+04	3.00E+05	ND	3.00	**Complied**
CA180398	6.00E+05	ND	0.06	ND	**Complied**
CA180399	1.00E+07	6.00E+05	1.00	6.00	**Not complied**
CA180400	ND	ND	ND	ND	**Complied**

Sample code	Absolute count on NA	Absolute count on SDA	Average aerobic count (10^7cfu/mL)	Average fungi count (10^5cfu/mL)	Inference
CA180401	5.40E+06	6.00E+06	0.54	60.00	**Not complied**
CA180402	1.00E+05	2.00E+04	0.01	0.20	**Complied**
CA180402	1.00E+05	2.00E+04	0.01	0.20	**Complied**
CA180403	1.00E+05	1.00E+04	0.01	0.10	**Complied**
CA180404	4.00E+05	1.00E+03	0.04	0.01	**Complied**
CA180405	3.22E+02	2.00E+05	ND	2.00	**Complied**
CA180406	5.00E+03	1.00E+05	0.00	1.00	**Complied**
CA180407	2.80E+08	5.00E+05	28.00	5.00	**Not complied**
CA180408	1.00E+05	1.80E+03	0.01	0.02	**Complied**
CA180409	3.00E+07	6.00E+06	3.00	60.00	**Not complied**
CA180410	7.00E+05	1.00E+05	0.07	1.00	**Complied**
CA180411	1.00E+06	2.00E+03	0.10	0.02	**Complied**
CA180412	6.00E+05	1.37E+07	0.06	137.00	**Not complied**
CA180414	2.00E+06	6.00E+05	0.20	6.00	**Not complied**
CA180415	5.00E+05	1.30E+05	0.05	1.30	**Complied**
CA180416	1.00E+07	1.70E+06	1.00	17.00	**Not complied**
CA180417	2.00E+07	1.40E+04	2.00	0.14	**Complied**
CA180418	7.00E+05	2.00E+02	0.07	0.00	**Complied**
CA180419	1.40E+06	8.00E+05	0.14	8.00	**Not complied**
CA180420	4.00E+03	1.00E+05	0.00	1.00	**Complied**
CA180421	5.00E+07	1.00E+06	5.00	10.00	**Not complied**
CA180422	4.00E+05	6.00E+05	0.04	6.00	**Not complied**
CA180423	7.30E+02	7.00E+01	0.00	0.00	**Complied**
CA180431	4.00E+05	2.50E+04	0.04	0.25	**Complied**

NA=Nutrient agar; SDA=Sabouraud's Dextrose Agar; Cfu=Colony forming units, ND- not detected.

Table 4.19. Total microbial counts on tablet formulated herbal medicines

Sample code	Absolute count on NA	Absolute count on SDA	Average aerobic count (10^4cfu/mL)	Average fungi count (10^2cfu/mL)	Inference
CA180424	ND	ND	ND	ND	**Complied**
CA180425	ND	1.30E+01	ND	0.13	**Complied**
CA180426	ND	ND	ND	ND	**Complied**
CA180427	ND	ND	ND	ND	**Complied**
CA180428	1.00E+04	ND	1.00	ND	**Complied**
CA180429	ND	ND	ND	ND	**Complied**
CA180430	ND	ND	ND	ND	**Complied**
CA180431	ND	ND	ND	ND	**Complied**
CA180432	ND	ND	ND	ND	**Complied**
CA180433	ND	ND	ND	ND	**Complied**
CA180434	1.00E+04	1.00E+03	1.00	10.00	**Not complied**
CA180435	ND	ND	ND	ND	**Complied**
CA180436	ND	ND	ND	ND	**Complied**
CA180437	1.00E+04	ND	1.00	ND	**Complied**
CA180438	4.50E+05	1.30E+03	4.50	13.00	**Not complied**
CA180439	9.00E+01	ND	0.01	ND	**Complied**
CA180440	3.00E+04	1.00E+03	3.00	10.00	**Not complied**

NA=Nutrient agar; SDA=Sabouraud's Dextrose Agar Cfu= Colony forming units, ND- not detected.

4.6.2 Microbial contaminants characterised

4.6.2.1 Microbial contaminants identified

MacConkey agar and broth are selective and differential media that support growth of *Salmonella spp, E. coli* and most of the bile tolerant bacteria. The selective action of these media was due to the formation of neutral red and bile salts which are inhibitory to most species of Gram positive bacteria. The media appeared purple on preparation and after incubation

turbidity and colour change (light purple) signified growth. Clear transparent colonies with or without dark centres indicate non-lactose fermenters while pink or red colonies are expected for lactose fermenters. The BP (2017) recommends use of MacConkey agar for sub culturing and identification of *E. coli*. Figure 4.15 shows mixed colonies of Lactose and Non-Lactose fermenters on MacConkey Agar plate.

Figure 4.15. Petri dish showing mixed colonies of Lactose and Non-Lactose fermenters on MacConkey Agar.

Rappaport Vassiliadis Medium is a selective enrichment media for *Salmonellae* that contains Malachite green that inhibits growth of other bacteria. The media has low pH of 5.0-5.4 and high osmotic pressure in which *Salmonella* thrives. Medium appeared blue on preparation and turbidity after incubation signified growth.

Enterobacteriaceae Enrichment (EE) broth (Mossel) is a selective and differential media for Enterobacteriaceae. Brilliant green and ox bile in the media inhibit growth of Gram positive bacteria. In this media, lactose is replaced with dextrose to allow for identification of late lactose fermenters. The media on preparation appeared dark green and turned light green to yellow after incubation indicating growth.

The selective and differential medium, xylose lysine deoxycholate (XLD) agar, enabled identification of *Salmonella spp.* Sodium deoxycholate is the selective agent that inhibits growth of Gram positive microorganisms. Salmonella rapidly fermented xylose with production of hydrogen sulphide resulting in the formation of colonies with black centres. Figure 4.16 show growth of *Salmonella spp*. on XLD agar. Moreover, *Salmonella spp*. produce hydrogen sulphide and carbon dioxide that lead to blackening and cracking of Triple Sugar Iron media and cracks in the medium (Figure 4.16).

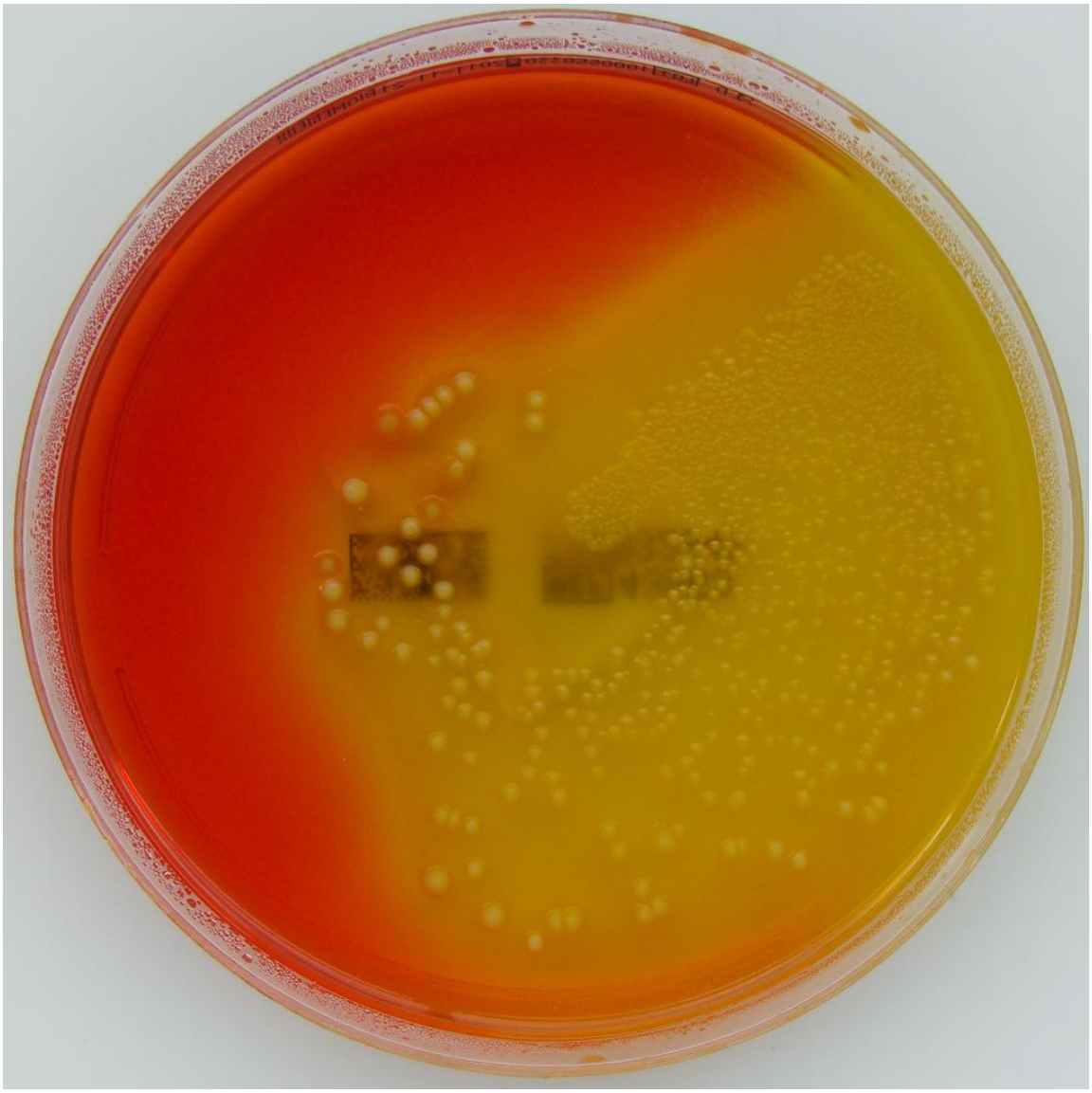

Figure 4.16. Petri dish showing growth of *Salmonella spp* on XLD agar

Triple sugar iron agar is recommended for identification of *Salmonella* species. The media contains glucose, lactose and sucrose as fermentable carbohydrates, hydrogen sulphide indicator system and phenol red pH indicator. Depending on the carbohydrate fermented the by-products lead to pH change that further changes the colour of the medium to either different or same colours in the butt and the slant. Some microorganisms produce hydrogen sulphide and carbon dioxide that lead to blackening of the medium and cracks in the medium respectively. Figure 4.17 show growth of *Salmonella spp*. on Triple Sugar Iron Agar.

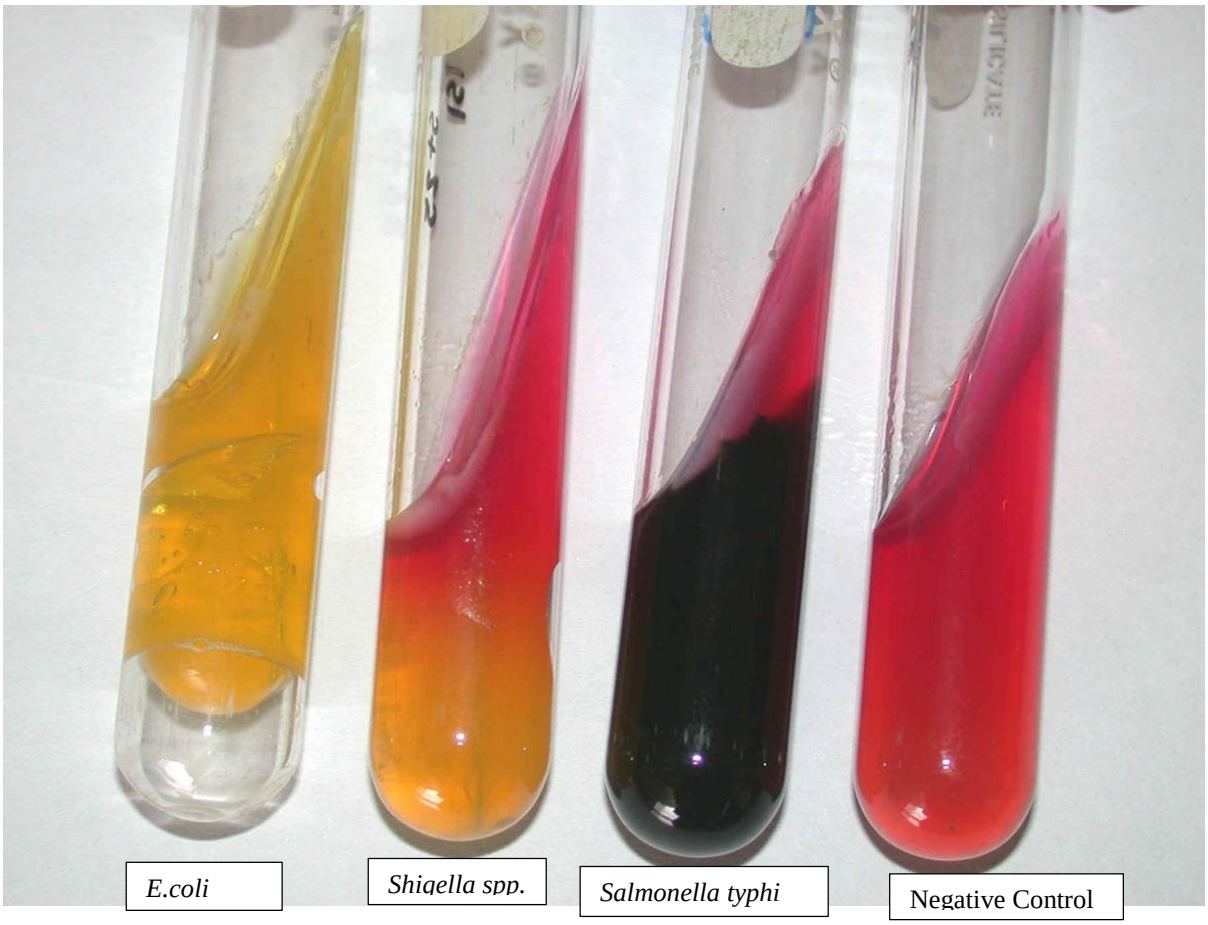

Figure 4.17. Growth of *E. coli* and *Salmonella spp*. on Triple Sugar Iron Agar

Violet Red Bile Glucose Agar (VRBGA), a selective medium for Enterobacteriaceae especially the bile tolerant Gram negative bacteria. Selectivity is due to bile salts and crystal violet that inhibit Gram positive bacteria. The media appeared reddish purple on preparation. Distinct pink colonies signified growth of bile tolerant micro-organisms. Glucose fermenting strains produce red colonies in the presence of neutral red. Figure 4.18 shows growth of bile tolerant bacteria on VRBGA.

Figure 4.18. Culture of bile tolerant bacteria on Violet red bile glucose agar.

Out of the 86 samples subjected to enumeration test 72 (83.7%) had CFU(s) and were further subjected to characterization tests. A total of 26 (36.1%) out of the 72 samples had the target pathogenic microorganisms. Some samples had more than one target microorganism with four samples testing positive for *Escherichia coli*, *Salmonella* species and bile tolerant Enterobacteriaceae. Three samples were found to have *Salmonella* and bile tolerant Enterobacteriaceae while four had *E. coli* and bile tolerant Enterobacteriaceae. A total of 41 isolates were identified comprising 17 (41.5%) bile tolerant, 14 (34.1%) *E. coli* and 10 (24.4%) *Salmonella* species. The proportion of sample contamination was 65.4% (bile tolerant), 53.8% (*E. coli*) and 38.5% (*Salmonella* spp.). Figure 4.16 gives the proportion of micro-organisms in the 72 samples subjected to test for specified microorganisms. Overall 39 (45.3%) samples failed to comply with the BP (2017) specifications for microbial analysis by failing either enumeration, test for specified micro-organisms or both. Ten samples complied with the specifications for enumeration but were found not to meet those of the test for specified micro-

organisms. Appendix 2 summarises the characterisation of microbes in the herbal products sampled.

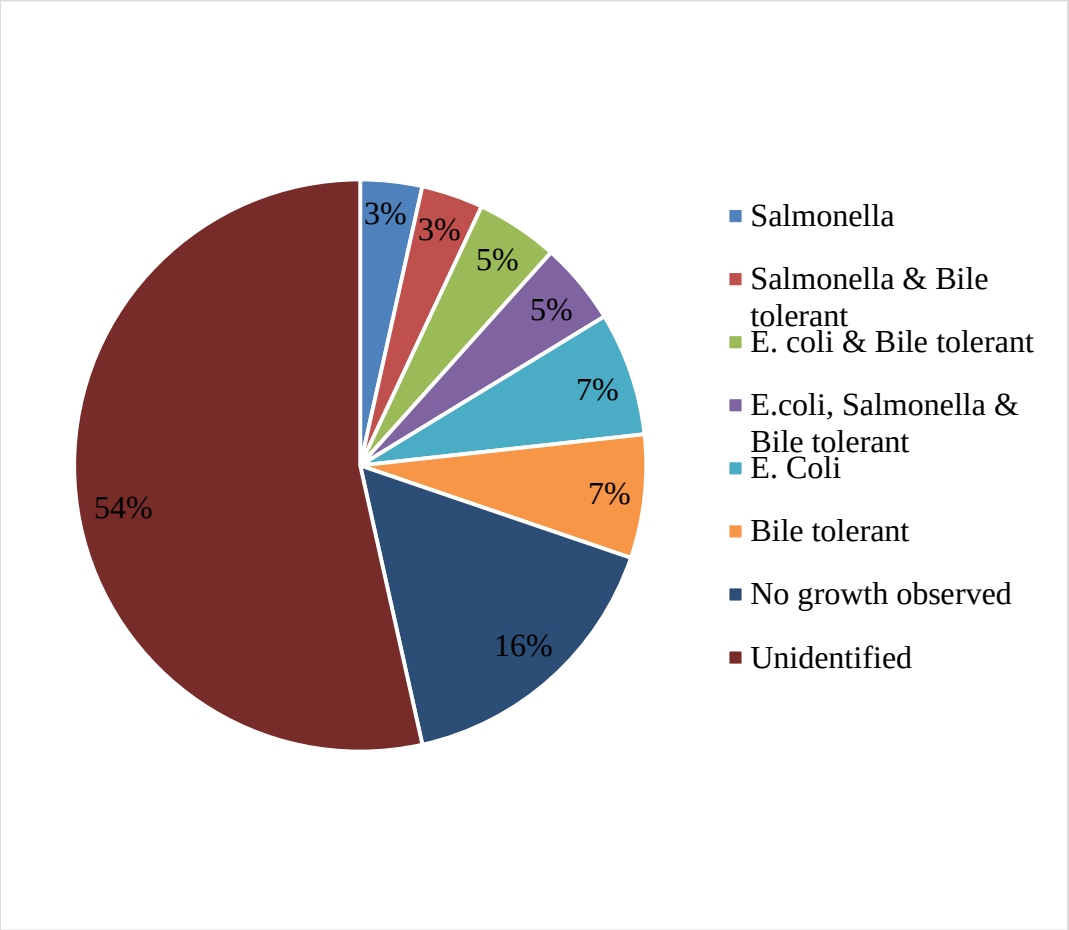

Figure 4.19. Proportion of micro-organisms in the herbal products.

4.6.2.2 Biochemical tests

Biochemical tests were carried out on 20 samples whose growth characteristics were suggestive of *Salmonella* or *E. coli*. Triple sugar iron (TSI) agar and urease tests were carried out and the results tabulated in Table 4.20.

Table 4.20. Biochemical test results using Triple sugar iron agar and urea agar slant

Sample ID	TSI				Urease	Microorganism
	Slant	**Butt**	**H_2S**	Gas		
CA180399	Yellow	Yellow	-	+	-	*E. coli*
CA180401	Yellow	Yellow	-	+	-	*E. coli*
CA180402	Red	Yellow	+	-	-	*Salmonella typhi*
CA180410	Red	Yellow	+	-	-	*Salmonella typhi*
CA180411	Yellow	Yellow	-	+	-	*E. coli*
CA180412	Yellow	Yellow	-	+	-	*E. coli*
CA180414	Yellow	Yellow	-	+	-	*E. coli*
CA180434	Red	Yellow	+	-	-	*Salmonella typhi*
CA180441	Yellow	Yellow	-	+	-	*E. coli*
CA180456	Yellow	Yellow	-	+	-	*E. coli*
CA180461	Yellow	Yellow	-	+	-	*E. coli*
	Red	Yellow	+	-	-	*Salmonella typhi*
CA180462	Red	Yellow	+	-	-	*Salmonella typhi*
CA180463	Yellow	Yellow	-	+	-	*E. coli*
	Red	Yellow	+ (Ring)	-	-	*Salmonella paratyphi A*
CA180464	Yellow	Yellow	-	+	-	*E. coli*
	Red	Yellow	+	-	-	*Salmonella typhi*
CA180466	Yellow	Yellow	-	+	-	*E. coli*
CA180467	Red	Yellow	+	-	-	*Salmonella typhi*
CA180468	Yellow	Yellow	-	+	-	*E. coli*
CA180471	Yellow	Yellow	-		-	*E. Coli*
CA180474	Yellow	Yellow	-	+	-	*E. coli*
	Red	Yellow	+ (Ring)	-	-	*Salmonella paratyphi A*
CA180498	Red	Yellow	+	-	-	*Salmonella typhi*

Fourteen of the isolates were *E.coli*, 8 *Salmonella typhi*, and 2 *Salmonella paratyphi* A. Four liquid samples, CA180461, CA180463, CA180464 and CA180474 had both *E. coli* and either *Salmonella typhi* or *Salmonella paratyphi* A.

Chapter 5: CONCLUSION AND RECOMMENDATIONS

5.1 Heavy metal contamination

Microwave sample preparation provided an efficient, versatile and clean sample preparation for multi-element analytical techniques such as ICP-MS in contrast with open-vessel digestion. Some of the benefits of microwave assisted digestion include reproducible digestion and with reduced preparation time, high analytical throughput is achievable. The low detection limits and multi-element capability of ICP-MS makes it an attractive option for elemental analysis. The combination of chemometrics with ICP-MS enabled rapid detection of potentially hazardous constituents of the herbal medicines with ease.

Concentrations of As, Hg, Cd and Pb in all analysed herbal medicines varied widely, possibly due to differences in origins and the processing the medicines are subjected to. The mean content of the heavy metals were: As: 2.858±24.15 mg/kg; Cd: 0.227±0.15 mg/kg; Hg: 246.378±2062.95 mg/kg and Pb: 0.646±0.92 mg/kg with the ranges being: As: 0.002-226.726 mg/kg, Cd: 0.145-1.075 mg/kg, Hg: 0.001-18221.930 mg/kg and Pb: 0.062-5.591 mg/kg. Overall, Hg concentration was significantly higher than As ($p < 0.01$), Pb ($p < 0.01$) and Cd ($p < 0.01$). The content of Hg was thousand orders of magnitude higher than other metals probably due to accumulation from several sources including processing procedures. Mercury was detected in 87.6% of the samples analysed while the content of Pb was in the range 0.062-5.591 mg/kg which was less than 10 ppm limit set by WHO and thus the samples of herbal medicines complied with the WHO specifications for Pb content.

Apart from diet, exposure of humans to heavy metals arises from natural weathering of heavy metal rich geological forms into the environment, pesticide use, mining, manufacturing, burning of fossil fuels, wielding, cigarette smoke and incineration, among other anthropogenic sources.

5.2 Risk assessment

Mercury and arsenic were identified to pose the greatest non-cancer risk. The risk was 2-fold higher in children than adults. This calls for judicious use of herbal medicines in children. However, care should also be taken even for adult consumers. The cancer risk was only ascribable to As. Other metals were found to present negligible risk for cancer in the herbal products sampled.

Three samples were identified to pose cancer risk since they had cancer risk (CR) and total cancer risk (TCR) indices that were higher than 10^{-4}. Arsenic was found to be the contributor of the CR in all the three samples.

5.3 Microbial contaminants

A total of 39 (45.3%) samples failed to comply with the BP (2017) specifications for enumeration and test for specified micro-organisms in herbal products for oral use. Fourteen (16.3%) samples showed no growth while 72 (83.7%) had CFUs. Twenty six (36.1%) of the 72 samples had the objectionable micro-organisms namely bile tolerant Enterobacteriae, *E. coli* and *Salmonella* spp while 46 (63.9%) had growth of other microorganisms. A total of 41 isolates were identified in this study. The isolates comprised of 17 (41.5%) bile tolerant, 14 (34.1%) *E. coli* and 10 (24.4%) *Salmonella* species. The occurrence of the target microorganisms in the 26 samples was 65.4% (bile tolerant), 53.8% (*E. coli*) and 38.5% (*Salmonella spp*.).

5.4 Study limitations

One of the limitations of this study was the small number of samples analysed due to lack of accessibility and unwillingness of the herbalist to sell some products for some chronic conditions such as cancer. This is because the regulation of herbal medicines is poor with no product listing by governmental agencies taking place. This study mainly focused on selected

heavy metals and objectionable microorganisms, therefore, there is a possibility that there are other contaminants in the analysed herbal medicines which were not identified.

5.5 Recommendations

Considering the wide variability in heavy metal content and microbial contamination of herbal medicines in this study, it is not plausible to prescribe uniform guidelines for evaluation of these products. Permissible levels of contaminants ought to remain as set but continuous market surveillance should be encouraged. Adoption of hazard analysis and critical control points (HACCP) should guide the industry to reduce risks such as those posed by heavy metals and microorganisms. Further study to ascertain possible adulteration of herbal products with heavy metals and conventional medicinal products is recommended.

From the analysis there was high level of microbial contamination in the herbal products sampled, therefore, close monitoring and regulation is recommended. Further investigations are recommended for the large number of the unidentified contaminants which were out of scope of the monograph specifications. Antimicrobial sensitivity testing using standard of care antibiotics to identify resistant strains is recommended.

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APPENDICES

Appendix 1. Composition, indications and collection sites of herbal products analysed

Code	Name of product	Composition	Manufacturer	Dosage form	Indication (s)	Collection site	Mfg & Exp. Dates	Dosage	Price (KShs)
CA180389	Not indicated	Not indicated	Herbalist	Powder	TB	Nairobi	-	1 tsp*2	600
CA180390	Not indicated	Not indicated	Herbalist	Powder	RA	Nairobi	-	1 tsp*2	650
CA180391	Not indicated	Not indicated	Herbalist	Powder	Asthma	Machakos	-	1 tsp*3	400
CA180392	Not indicated	Not indicated	Herbalist	Powder	RA	Kajiado	-	1 tsp*3	7040
CA180393	Not indicated	Not indicated	Herbalist	Powder	TB	Nairobi	-	1 tsp*3	1400
CA180394	Not indicated	Not indicated	Herbalist	Powder	HIV	Kajiado	-	1 tbs*3	800
CA180395	Not indicated	Not indicated	Herbalist	Powder	CA	Machakos	-	1 tbs*3	930
CA180396	Not indicated	Not indicated	Herbalist	Powder	HTN	Kiambu	-	1 tbs*2	600
CA180397	Not indicated	Not indicated	Herbalist	Powder	HTN	Nairobi	-	1 tsp*2	1200
CA180398	Ngetwa	Not indicated	Traditional Medicine Research	Powder	HTN	Nairobi	-	2 tsp*3	2100
CA180399	Not indicated	Not indicated	Herbalist	Powder	RA	Kiambu	-	1 tsp*3	1230
CA180400	Not indicated	Not indicated	Herbalist	Powder	RA	Nairobi	-	1 tsp*3	300
CA180401	Not indicated	Not indicated	Herbalist	Powder	RA	Machakos	-	1 tsp*2	600
CA180402	Not indicated	Not indicated	Herbalist	Powder	RA	Nairobi	-	1 tsp*2	1000

Code	Name of product	Composition	Manufacturer	Dosage form	Indication (s)	Collection site	Mfg & Exp. Dates	Dosage	Price (KShs)
CA180403	Not indicated	Not indicated	Herbalist	Powder	RA	Machakos	-	1 tsp*2	600
CA180404	Turmeric	*Curcuma longa*	Herbalist	Powder	RA	Kiambu	-	1 tsp*3	700
CA180405	Scayenne powder	Not indicated	Herbalist	Powder	HTN	Kiambu	-	1 tsp*2	450
CA180406	Antex Natural herbs	Bitter gourd, ginger, garlic, basil, celery turmeric	Herbalist	Powder	RA	Nairobi	12/2018	1 tsp*2	1500
CA180407	Not indicated	Not indicated	Herbalist	Powder	Asthma	Nairobi	-	1 tsp*2	1000
CA180408	Not indicated	Not indicated	Herbalist	Powder	RA	Machakos	-	1 tsp*2	1200
CA180409	Not indicated	Not indicated	Herbalist	Powder	HTN	Nairobi	-	1 tsp*2	860
CA180410	Not indicated	Not indicated	Herbalist	Powder	Cancer	Nairobi	-	1 tsp*4	1500
CA180411	Not indicated	Not indicated	Herbalist	Powder	Cancer	Nairobi	-	1 tsp*3	1700
CA180412	Not indicated	Not indicated	Herbalist	Powder	Diabetes	Nairobi	-	1 tsp*3	2000
CA180413	Not indicated	Not indicated	Herbalist	Powder	Ulcers	Machakos	-	1 tsp*2	1500
CA180414	Mphage II	Not indicated	KAM Herbs	Powder	Diabetes	Kiambu	-	1 tsp*2	2690
CA180415	Kamidopa	Not indicated	Pure natural herbs	Powder	HTN	Kajiado	2027	1 tsp*2	2500
CA180416	Not indicated	Not indicated	Herbalist	Powder	RA	Kajiado	-	1 tsp*2	1500
CA180417	Kam Septillin	Not indicated	KAM herbs	Powder	RA	Kiambu	-	1 tbs*3	3100

Code	Name of product	Composition	Manufacturer	Dosage form	Indication (s)	Collection site	Mfg & Exp. Dates	Dosage	Price (KShs)
CA180418	Not indicated	Not indicated	Herbalist	Powder	TB	Nairobi	-	1 tsp*2	1200
CA180419	Not indicated	Not indicated	Herbalist	Powder	RA	Machakos	-	1 tsp*2	3200
CA180420	Not indicated	Not indicated	Herbalist	Powder	HTN	Nairobi	-	1 tsp*3	1900
CA180421	Not indicated	Not indicated	Herbalist	Powder	Diabetes	Nairobi	-	1 tsp*2	860
CA180422	KAM Detoxx	Not indicated	Kam Herbs	Powder	HTN	Machakos	-	1 tsp*3	1700
CA180423	Immun Strong	Colostrum	BF Suna	Powder	Asthma	Kajiado	11/2019	1 sachet daily	3500
CA180424	Shallaki	Boswellia	Himalaya	Tablets	RA	Kiambu	05/2017 – 05/2019	1 tab*2	2300
CA180425	Liv compound	*Phyllanthus nituri, Edipta alba*	Alarsin	Tablets	cancer	Kajiado	03/2017 – 02/2020	1 tab*2	2290
CA180426	Siberian ginseng	Ginseng	Senticosus	Tablets	Vitality	Nairobi	2017-2019	1 tab*1	2000
CA180427	Sundasham	Not indicated	Enami Ltd	Tablets	Diabetes	Kajiado	07/2016-2019	1 tab *2	1700
CA180428	Alluretic	Azadmacha indica, *Embica officinalis,* beberis, Aristata	Alarsin	Tablets	RA	Nairobi	10/2017 – 09/2022	1 tab*2	2400
CA180429	Kalonji	*Nigella sativa, Zingiber officinale*	Top treatments	Tablets	HTN	Kiambu	2019	2 tabs*2	3800

Code	Name of product	Composition	Manufacturer	Dosage form	Indication (s)	Collection site	Mfg & Exp. Dates	Dosage	Price (KShs)
CA180430	Microcycle	*Radix sativa*, netogiseng, *Bemeolum syntheticum*	BF suma	Tablets	HTN	Kajiado	08/2017-08/2019	2 tabs*2	360
CA180431	Not indicated	Not indicated		Tablets	Diabetes	Nairobi	-	2 tabs*2	1750
CA180432	Muscalt forte	Guduchi extract, Lodhira extract, siadinkuchila	Amil pharmaceuticals	Tablets	Diabetes	Kajiado	-	2 tabs*3	1620
CA180433	Not indicated	Not indicated	Herbalist	Tablets	Cancer	Nairobi	-	2 tabs*2	2720
CA180434	Panvelley forte	Karvasmamenj, pauciflorum, yasthimodu	Panvelley herbal products	Tablets s	Diabetes	Nairobi	Exp. 2019	2 tabs*1	3200
CA180435	Zandu	*Cassia angustifolia, Embilica officinalis, Terminora chebula, Terminora bellerica*	Imami Ltd	Tablets	asthma	Nairobi	09/2016 - 09/2018	2 tabs*2	1600
CA180436	Rumalaya	*Tinospora cardifolia*, Rribulusterrestrs AlpiniaGalanga,	Himalaya	Tablets	RA	Nairobi	2017-2020	2 tabs*2	2300

Code	Name of product	Composition	Manufacturer	Dosage form	Indication (s)	Collection site	Mfg & Exp. Dates	Dosage	Price (KShs)
CA180437	Tripralaguggul	*Terminalia achebula, Comphora wightii, Terminalia beleria*	Emami Ltd	Tablets	TB	Machakos	2019	2 tabs*3	3100
CA180438	Not indicated	Not indicated	Herbalist	Tablets	Diabetes	Nairobi	-	2 tabs*2	2700
CA180439	Not indicated	Not indicated	Herbalist	Tablets	RA	Kiambu	-	1 tab*2	900
CA180440	Not indicated	Not indicated	Herbalist	Tablets	Diabetes	Kiambu	-	1 tab2	1200
CA180441	Not indicated	Not indicated	Herbalist	Capsules	HTN	Machakos	-	2 caps*2	700
CA180442	Not indicated	Not indicated	Herbalist	Capsules	HIV	Machakos	-	2 caps*2	400
CA180443	Not indicated	Not indicated	Herbalist	Capsules	Cancer	Kajiado	-	2 caps*3	1200
CA180444	Not indicated	Not indicated	Herbalist	Capsules	Cancer	Kajiado	-	2 caps*2	1360
CA180445	Spirulina	Cyanobacteria	Herbalist	Capsules	Asthma	Nairobi	-	2016 - 2020	3600
CA180446	Not indicated	Not indicated	Herbalist	Capsules	Asthma	Machakos	-	1 cap*3	3100
CA180447	Not indicated	Not indicated	Herbalist	Capsules	RA	Nairobi	-	1 caps*2	620
CA180448	Zubex	Asphaltum, *Curcuma longa*, *Eugenia jambolana*, Mika Murakab	Qarshi industries Ltd	Capsules	Diabetes	Nairobi	-	1 cap*3	750

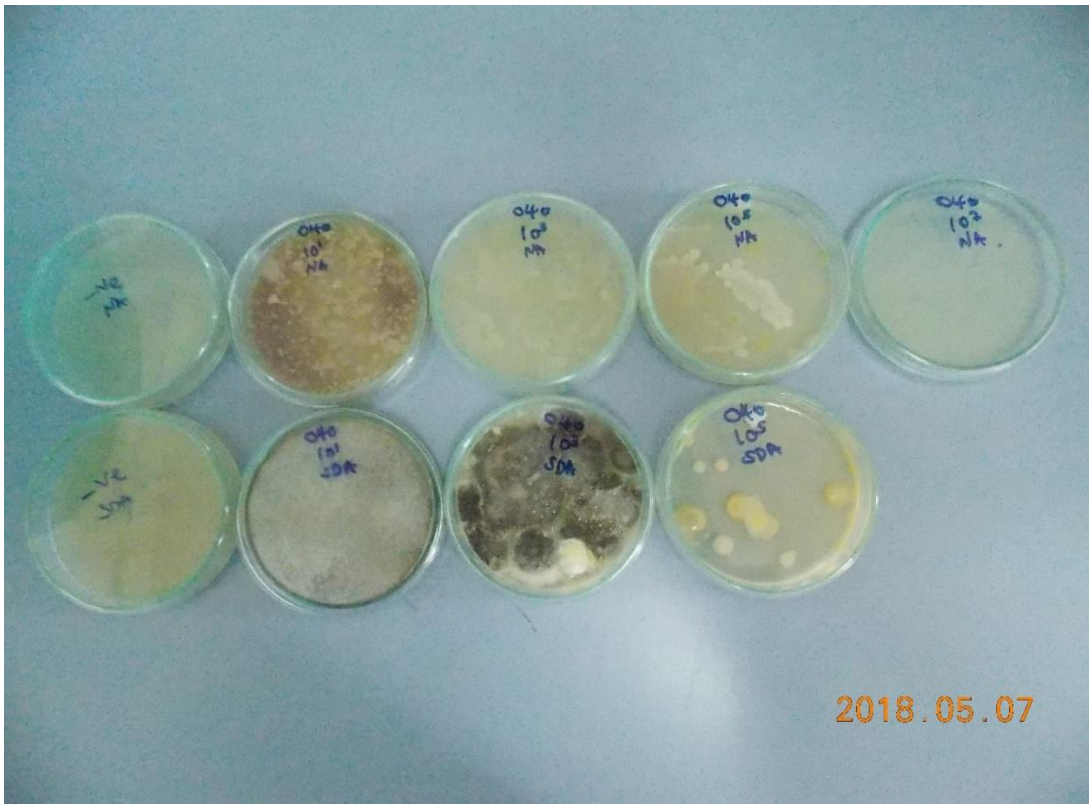
Code	Name of product	Composition	Manufacturer	Dosage form	Indication (s)	Collection site	Mfg & Exp. Dates	Dosage	Price (KShs)
CA180449	Good care	*Withania somnifera, Momordica charantia, Terminalia arjuna*	Goodcare Pharma	Capsules	Diabetes	Kajiado	-	1 cap*2	810
CA180450	Not indicated	Not indicated	Herbalist	Capsules	Asthma	Machakos	-	2 caps*3	950
CA180451	Not indicated	Not indicated	Herbalist	Capsules	HTN	Nairobi	-	1 caps*3	1100
CA180452	Not indicated	Not indicated	Herbalist	Capsules	RA	Nairobi	-	2 caps*3	2500
CA180453	Not indicated	Not indicated	Herbalist	Capsules	RA	Nairobi	-	1 caps*3	3240
CA180454	Not indicated	Not indicated	Herbalist	Capsules	Asthma	Kajiado	-	1 tsp*3	720
CA180455	Not indicated	Not indicated	Herbalist	Capsules	RA	Nairobi	-	1 tsp*2	800
CA180456	Not indicated	Not indicated	Herbalist	Capsules	Asthma	Kiambu	-	I tbs*2	600
CA180457	Not indicated	Not indicated	Herbalist	Capsules	Asthma	Kiambu	-	1 tbs*3	1720
CA180458	Ginkgo biloba	*Ginkgo biloba*	FLP Ltd	Capsules	Memory enhancer	Nairobi	2017-2020	1 tab *1	1680
CA180459	Not indicated	Not indicated	Herbalist	Capsules	HTN	Nairobi	-	1 cap*3	1100
CA180460	Not indicated	Not indicated	Herbalist	Liquid	Asthma	Nairobi	-	1 cap*3	2490
CA180461	Not indicated	Not indicated	Herbalist	Liquid	Diabetes	Kiambu	-	1 tsb*3	2200
CA180462	Not indicated	Not indicated	Herbalist	Liquid	Diabetes	Kajiado	-	2 tsp*3	1650
CA180463	Not indicated	Not indicated	Herbalist	Liquid	RA	Kiambu	-	1 tsp*3	1760
CA180464	Not indicated	Not indicated	Herbalist	Liquid	RA	Machakos	-	2 tsp*2	1200

Code	Name of product	Composition	Manufacturer	Dosage form	Indication (s)	Collection site	Mfg & Exp. Dates	Dosage	Price (KShs)
CA180465	Not indicated	Not indicated	Herbalist	Liquid	HTN	Nairobi	-	2 tsp*3	1800
CA180466	Constirelax	Fructooligosaccharide, *Redix astragali*	BF Suna	Liquid	Cancer	Machakos	2018	2 tbs*3	4000
CA180467	Victory syrup	Eucalyptus oil, Soya oil, Cammomile	Victory Nutritional clinic	Liquid	Asthma	Nairobi	-	1 tsp*3	340
CA180468	Extra Detox	Not indicated	Antex Natural Herbs	Liquid	HTN	Machakos	2019	1 tsp*2	750
CA180469	Dawa osha	Milk thistle, apple cider, Dandelion	Shine herbal	Liquid	RA	Kajiado	-	1 tsp*2	340
CA180470	Spasmokof	Eucalyptus, Menthol, cane honey	Glory herbs	Liquid	Asthma	Nairobi	-	1 tsp*2	520
CA180471	Chest tonic	Frish Moss, Lungwort, Eucalyptus	Shine herbal	Liquid	Asthma	Kiambu	2020	1 tsp*2	390
CA180472	Moring pure	Moringa	Amaze Herbal	Liquid	HTN	Nairobi	2020	1 tab*3	515
CA180473	Chestkof	Eucalyptus, Menthol, Chamomile, Elecampane	Glory herbs	Liquid	Asthma	Kiambu	2018	1 tsp*3	450
CA180474	Herbalex	Not indicated	Antex Natural	Liquid	RA	Kajiado	2020	1 tsp*3	750

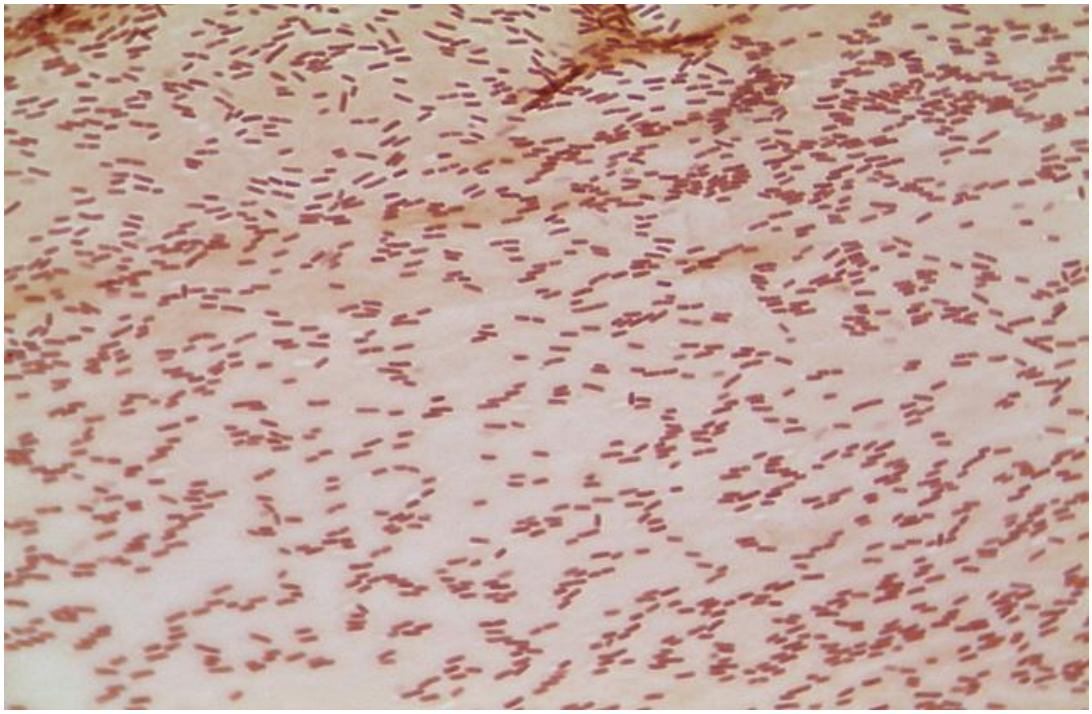
Code	Name of product	Composition	Manufacturer	Dosage form	Indication (s)	Collection site	Mfg & Exp. Dates	Dosage	Price (KShs)
CA180475	Not indicated	Not indicated	Herbalist	Powder	TB	Nairobi	-	1 tsp*2	600
CA180476	Not indicated	Not indicated	Herbalist	Powder	RA	Nairobi	-	1 tsp*2	650
CA180477	Not indicated	Not indicated	Herbalist	Powder	Asthma	Machakos	-	1 tsp*3	400

RA- rheumatoid arthritis, TB- tuberculosis, HTN – hypertension, HIV/AIDS – Human immunodeficiency virus /Acquired immunodeficiency syndrome

Appendix 2. Petri dishes showing growth of bacteria and fungi



Appendix 3: Gram negative rods visualised using light miscrocopy



Appendix 4. Characterisation of microbial contaminants

Sample code	Formulation	Mac Broth	RVM	EE broth	Mac Agar	XLD Agar	VRBGA	G. Stain	Morphology	Summary	Inference
CA180389	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180390	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180391	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180392	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180393	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180394	Powder	growth	Growth	growth	Small Clear Colonies	No growth	Pink Mucoid Colonies	Gram negative	Bacilli	Bile tolerant	**Not complied**
CA180395	Powder	growth	Growth	growth	Pink Mucoid colonies	Clear Mucoid Colonies	clear colonies with pink centers	Gram negative	Bacilli	*E. coli*	**Complied**
CA180396	Powder	growth	Growth	growth	No growth	No growth	No growth	N/A	N/A	Not identified	**Complied**
CA180397	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180399	Powder	growth	Growth	growth	Pink Mucoid Colonies	Clear Mucoid Colonies	Pink Mucoid Colonies	Gram negative	Bacilli	*E. coli*/ bile tolerant	**Not complied**

Sample code	Formulation	Mac Broth	RVM	EE broth	Mac Agar	XLD Agar	VRBGA	G. Stain	Morphology	Summary	Inference
CA180400	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180401	Powder	Growth	Growth	Growth	Clear colonies	Clear Mucoid Colonies	No growth	Gram negative	Bacilli	*E. coli*	**Not complied**
CA180402	Powder	Growth	Growth	Growth	Clear Mucoid colonies	Clear Mucoid colonies	Pink Colonies	Gram negative	Bacilli	Salmonella bile olerant	**Complied**
CA180403	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180404	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180405	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180406	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180407	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180408	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180409	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Not complied**
CA180410	Powder	growth	Growth	growth	Clear Mucoid Colonies	Clear Mucoid colonies	Pink colonies	Gram negative	Bacilli	Salmonella	**Complied**

Sample code	Formulation	Mac Broth	RVM	EE broth	Mac Agar	XLD Agar	VRBGA	G. Stain	Morphology	Summary	Inference
CA180411	Powder	Growth	Growth	Growth	Pink Mucoid Colonies	No growth	No growth	Gram negative	Bacilli	E coli	**Not complied**
CA180412	Powder	Growth	Growth	Growth	Pink Mucoid Colonies	Clear Mucoid colonies	Pink Mucoid Colonies	Gram negative	Bacilli	*E. coli*/ bile tolerant	**Not complied**
CA180413	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180414	Powder	growth	Growth	growth	Clear Mucoid colonies	Clear Mucoid colonies	Pink Mucoid Colonies	Gram negative	Bacilli	*E. coli*/ bile tolerant	**Complied**
CA180415	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180416	Powder				Pink colonies	Clear colonies	Pink Mucoid Colonies	Gram negative	Bacilli	Bile tolerant	**Not complied**
CA180417	Powder	growth	Growth	growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180418	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180419	Powder				Small Clear Colonies	Small Clear colonies	Pink colonies	Gram negative	Bacilli	Not identified	**Complied**
CA180420	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**

Sample code	Formulation	Mac Broth	RVM	EE broth	Mac Agar	XLD Agar	VRBGA	G. Stain	Morphology	Summary	Inference
CA180421	Powder	Growth	Growth	Growth	Small Clear Colonies	No growth	Pink Mucoid Colonies	Gram negative	Bacilli	Bile tolerant	**Not complied**
CA180422	Powder	Growth	Growth	Growth	Clear Mucoid Colonies	Clear Colonies	Clear Mucoid Colonies	Gram negative	Bacilli	Bile tolerant	**Not complied**
CA180423	Powder	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180424	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180425	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180426	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180427c	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180428	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180429	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180430	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180431	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180432	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**

Sample code	Formulation	Mac Broth	RVM	EE broth	Mac Agar	XLD Agar	VRBGA	G. Stain	Morphology	Summary	Inference
CA180433	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180434	Tablet	Growth	Growth	Growth	Non Mucoid Pink colonies	Clear Mucoid colonies	Clear Mucoid colonies	Gram negative	Bacilli	Salmonella	**Not complied**
CA180435	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180436	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180437	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180438	Tablet	Growth	Growth	Growth	Clear Colonies	Clear Colonies	Pink Colonies	Gram negative	Bacilli	Not identified	**Complied**
CA180439	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180440	Tablet	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180441	Capsule	Growth	Growth	Growth	Clear colonies	Clear Mucoid Colonies	No growth	Gram negative	Bacilli	E coli	**Complied**
CA180442	Capsule	No Growth	No Growth	No Growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180443	Tablet	No Growth	No Growth	No Growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**

Sample code	Formulation	Mac Broth	RVM	EE broth	Mac Agar	XLD Agar	VRBGA	G. Stain	Morphology	Summary	Inference
CA180444	Capsule	Growth	Growth	Growth	No growth	Clear Mucoid Colonies	No growth	Gram negative	Bacilli	Salmonella	**Not complied**
CA180445	Capsule	Growth	Growth	Growth	Small Clear Mucoid Colonies	No growth	Pink Mucoid Colonies	Gram negative	Bacilli	Bile tolerant	**Not complied**
CA180446	Capsule	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180447	Capsule	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180448	Capsule	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180449	Capsule	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180450	Capsule	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180451	Capsule	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180452	Capsule	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Not complied**
CA180453	Capsule	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Not complied**
CA180454	Liquid	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**

Sample code	Formulation	Mac Broth	RVM	EE broth	Mac Agar	XLD Agar	VRBGA	G. Stain	Morphology	Summary	Inference
CA180455	Liquid	Growth	Growth	Growth	Small Clear Colonies	Small Clear Colonies	Pink colonies	Gram negative	Bacilli	Not identified	**Complied**
CA180456	Liquid	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180457	Liquid	Growth	Growth	Growth	Small Clear Colonies	Small Clear Colonies	Pink colonies	Gram negative	Bacilli	Not identified	**Complied**
CA180458	Liquid	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180459	Liquid	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180460	Liquid	Growth	Growth	Growth	Small Clear Colonies	Small Clear Colonies	Pink colonies	Gram negative	Bacilli	Not identified	**Complied**
CA180461	Liquid	Growth	Growth	Growth	Pink Mucoid/ Clear Colonies	Clear Mucoid Colonies	Pink Mucoid/clear Colonies	Gram negative	Bacilli	*E. coli/ Salmonella*/ bile tolerant	**Not complied**
CA180462	Liquid	Growth	Growth	Growth	Clear mucoid/ pink colonies	Clear Mucoid Colonies	Pink Mucoid Colonies	Gram negative	Bacilli	Salmonella/ bile tolerant	**Complied**
CA180463	Liquid	Growth	Growth	Growth	Pink Mucoid/ Clear Colonies	Clear Mucoid Colonies	Pink Mucoid/clear Colonies	Gram negative	Bacilli	*E. coli*/ Salmonella/ bile tolerant	**Not complied**

Sample code	Formulation	Mac Broth	RVM	EE broth	Mac Agar	XLD Agar	VRBGA	G. Stain	Morphology	Summary	Inference
CA180464	Liquid	Growth	Growth	Growth	Pink Mucoid/ Clear Colonies	Clear Mucoid Colonies	Pink Mucoid/clear Colonies	Gram negative	Bacilli	E. coli/ Salmonella /bile tolerant	**Not complied**
CA180465	Liquid	Growth	Growth	Growth	Small Clear Colonies	Small Clear Colonies	Pink colonies	Gram negative	Bacilli	Not identified	**Complied**
CA180466	Liquid	Growth	Growth	Growth	Pink Mucoid Colonies	Clear colonies	Clear Mucoid Colonies	Gram negative	Bacilli	Bile tolerant	**Not complied**
CA180467		Growth	Growth	Growth	Clear Mucoid Colonies	Clear Mucoid Colonies	Pink Colonies	Gram negative	Bacilli	Salmonella / bile tolerant	**Not complied**
CA180468	Liquid	Growth	Growth	Growth	Clear colonies	Clear Mucoid Colonies	Pink Mucoid Colonies	Gram negative	Bacilli	E. coli/ bile tolerant	**Not complied**
CA180469	Liquid	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180470	Liquid	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180471	Liquid	Growth	Growth	Growth	Pink Mucoid Colonies	No growth	No growth	Gram negative	Bacilli	E. coli	**Not complied**
CA180472	Liquid	Growth	Growth	Growth	Small Clear Mucoid Colonies	Clear small colonies	Pink colonies	Gram negative	Bacilli	Not identified	**Complied**

Sample code	Formulation	Mac Broth	RVM	EE broth	Mac Agar	XLD Agar	VRBGA	G. Stain	Morphology	Summary	Inference
CA180473	Liquid	No growth	No growth	No growth	N/A	N/A	N/A	N/A	N/A	N/A	**Complied**
CA180474	Liquid	Growth	Growth	Growth	Pink Mucoid/ Clear Colonies	Clear Mucoid Colonies	Pink Mucoid/clear Colonies	Gram negative	Bacilli	*E. coli*/ Salmonella /bile tolerant	**Not complied**

Mac broth-MacConkey broth, RVM-Rappaport vassiliadis salmonella enrichment broth, EE broth-Enterobacteria enrichment broth, Mac Agar-MacConkey Agar, XLD-Xylose, Lysine, deoxycholate Agar, VRBGA- violet red bile glucose Aga