SAFETY AND ANTIMICROBIAL ACTIVITIES OF HERBAL MATERIALS USED IN MANAGEMENT OF ORAL HEALTH BY TRADITIONAL MEDICAL PRACTITIONERS IN NAIROBI COUNTY, KENYA.

WAIGANJO FLORENCE WANJA (MSC.)

P97/14538/09

"A THESIS SUBMITTED IN FULFILLMENT FOR THE REQUIREMENTS OF THE AWARD OF DEGREE IN DOCTOR OF PHILOSOPHY OF KENYATTA UNIVERSITY"

OCTOBER 2013

DECLARATION

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

Signature……. ……. Date……

Waiganjo Florence Wanja

Department of Pharmacy and Complementary/ Alternative Medicine

Kenyatta University

Supervisors

"We confirm that the work reported in this thesis was carried out by the candidate under our supervision"

Signature …….Date…….

Prof. Nicholas K. Gikonyo

Department of Pharmacy and Complementary/ Alternative Medicine

Kenyatta University

Signature …….Date…….

Dr. Ruth N**.** Wanjau

Department of Chemistry

Kenyatta University

Signature……Date……

Prof. Eliud N. M. Njagi

Department of Biochemistry and Biotechnology

Kenyatta University

DEDICATION

This thesis is dedicated to my beloved husband Nelson Ngari and our children Paula Wanjiru and Ian Duncan Ndirangu.

ACKNOWLEDGEMENTS

I am grateful to almighty God who has seen me through this far.

I am greatly indebted to my supervisors Prof. Nicholas Gikonyo, Dr. Ruth Wanjau and Prof. Eliud Njagi who keenly and patiently supervised this research project, giving valuable guidance throughout the research period.

My appreciation goes to Prof. Paul Mbuthia of University of Nairobi, Kabete campus, for his assistance in histopathology work. Thanks to Mr. Kinge of Kenyatta National Hospital for haematology and biochemistry work.

My appreciation goes to Kenyatta University and Department of Pharmacy and Complementary/ Alternative Medicine for providing me with the opportunity to take my PhD studies.

I am grateful to my husband Nelson and our children; Paula Wanjiru and Ian Duncan Ndirangu for their continuous support during the research period.

I am most grateful to Kenya National Commission of Science, Technology and Innovation (NACOSTI) for providing the research funds for this work (Grant No.NCST/5/003/368).

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

DEFINITIONS OF TERMS

Active ingredients- These refer to ingredients of the herbal medicine with therapeutic activity.

Finished herbal products- They consist of herbal preparations made from one or more herbs. If more than one herb is used, the mixture herbal product can also be used.

Herbal materials- They include in addition to herbs, fresh juice, gums, fixed oils, essential oils, resins and dry powders of herbs. These materials may be processed by various local procedures such a steaming, roasting or stir baking with honey, alcoholic beverage or other materials.

Herbal medicines- Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of a plant or combinations or plant derived materials or preparations with therapeutic or other human health benefits which contains either raw or processed ingredients from one or more plants.

Herbal preparations- These are the basis for the finished herbal products and may include comminuted or powdered herbal materials or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionations, purification, concentration or other physical or biological processes.

Herbs- These include crude plant materials such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant part which may be entire, fragmented or powdered.

Traditional medicine- This is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures whether explicable or not, used in maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness

Voucher specimen- Specimen collected and preserved for future reference

ABBREVIATIONS AND ACRONYMS

ALT:	Activity of alanine aminotransferase
AST:	Activity of aspartate aminotransferase
BUN	Blood urea nitrogen
CFU	Colony forming unit
DCM	Dichloromethane
GRA:	Granulocytes
HB:	Haemoglobin
HCT:	Haematocrit
HP:	Herbal paste
HS:	Herbal suspensions
IQR:	Internal Quality control
KEBS:	Kenya Bureau of Standards
LYM:	Lymphocytes
MBC:	Minimum microbicidal concentrations
MC:	Mean cell counts
MCHC:	Mean corpusulal haemoglobin concentration
MCV:	Mean corpuscular volume
MeOH	Methanol
MIC:	Minimum inhibitory concentration
MON:	Monocytes
MPP:	Medicinal plant powder
MPV:	Mean platelet volume
NCCAM:	National Centre for Complementary and Alternative Medicine
NCCLS:	National Committee for Clinical Laboratory Standards
NEUT:	Neutrophils
OH	Oral health
OHC:	Oral health care
PCT:	Platelecrit
PCV:	Packed cell volume
PDW:	Platelet distribution width
QC:	Quality control

RDA:	Recommended daily allowance
RDW:	Red cell distribution width
SD:	Standard deviation
TXFR	Total Reflection X-ray Fluorescence
WBC:	White blood cells
WHO:	World Health Organization

ABSTRACT

The use of herbs for treatment and management of oral diseases is practiced in the developing countries including Kenya. The herbs are used in form of powders, pastes, saps, chewing sticks and seeds. They are sold in Nairobi along streets and open markets with claims of healing all oral diseases. However, safety issues in terms of microbial contaminants, levels of mineral elements profiles, toxicity, phytochemical composition and antimicrobial properties of the herbal materials in the market have not been evaluated**.** The aim of the study was to evaluate safety aspects and antimicrobial properties of herbal materials used in oral health care in Nairobi. Documentation of herbal materials was carried out by interviewing 60 herbalists using a questionnaire and through informal discussions. Investigation of safety and antimicrobial properties was carried out on 23 herbal products purchased from the herbalists. Evaluation of microbial contaminants was carried out as described by World Health Organisation. Samples were inoculated in enrichment culture medium, then subculturing in selective media, followed by microscopy and biochemical test to confirm the identity of the microbes. Elemental analysis was evaluated by use of Total X-ray Fluorescence Technique. Qualitative phytochemical constituents of herbal materials were investigated using standard methods. The antimicrobial properties were studied using disc and agar well diffusion method. Toxicity effects of herbal materials was investigated by administering 1000 mg/kg body weight of seven herbal material extracts and 3 herbal pastes on mice for twenty one days, and then biochemical, haematological parameters, relative organ weight and their histopathological changes were evaluated. Results indicated that 35 plant species were used in preparation of herbal products for management of oral conditions. *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Candida albicans* were isolated in some of the products. High levels of aluminium, phosphorous, potassium, calcium, vanadium, chromium, manganese, iron, nickel, copper, zinc, strontium and lead were observed. Major phytochemical groups were recorded in herbal powders. However, pastes and liquids lacked detectable levels of major phytochemical groups. Antimicrobial properties were reported in 78% of the herbal materials. Mortality was reported in mice treated with herbal materials of *Warbugia ugandensis, Zantoxyllum chalybeum* and *Senna didymobotrya.* The extracts of *Euclea divinorum* and herbal mixtures of *Warbugia ugandensis, Z. chalybeum* and *Terminalia brownii* showed retarded growth in mice and hypertrophy of liver and brain. There was significant ($P>0.05$) alteration of the red blood cells and neutrophils, creatine, alanine aminotransferase and thrombocytes of animals treated with some herbal extracts while severe pathological conditions on vital organs of mice was seen. The toxic effects could be due to presence of heavy metal due to poor handling by herbalists. Most of the products in the market did not meet the WHO standards. These findings may be used to sensitise the Traditional Medical Practitioners, policy makers and consumers on the safety issues and lack of antimicrobial activities in some products. This study recommends that full evaluation of safety and antimicrobial properties of herbal materials be carried out before they are released to the market.

.

CHAPTER ONE

1. INTRODUCTION

1.1 Background to the study

Plants have been used as drugs since ancient times. Traditional medicine is well known for meeting the primary health care in all parts of the world (Calixto, 2000; Samali *et al*., 2012). At present 70-90% of the population in developing countries rely largely on plant based drugs for their health care needs (Evans, 2002; Ngetich, 2005; Cunningham, 1993; Kareru *et al.*, 2007). Despite the use of herbal medicines over many centuries a relatively small number has been studied for possible medical application. One of the objectives of World Health Organisation (WHO) policy on medicinal plants is to ascertain their safety and efficacy (Ameh *et al*., 2010).

Traditional medicine has not been officially recognized in most countries, despite its popularity and continued use over many centuries (WHO, 2000). Generally the safety and efficacy data on traditional medicine are far from sufficient to meet the criteria needed to support its use world-wide. This is because there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these herbal materials.

Herbal medicines are widely perceived as being natural and free from side effects (Giveon *et al*., 2004). Nevertheless it is now well established that a number of these agents have potential to produce minor or major safety problems (De smet, 2002;

Niggermann, 2003). Some reported adverse effects following the use of herbal medicine have been associated with contamination with microorganisms and heavy metals (Ingela *et al.,* 2009). The resurgent use and interest in drugs of plant origin is due to resistance of some infective agents to allopathic drugs and the cost of the same. The need for alternative prevention and treatment options and products for oral disease that are safe, effective and economical comes from rise in disease incidence and financial constraints in developing countries (Tichy *et al.,* 1998; Badria and Zindan, 2004).

In most developing countries, government expenditure in oral health care is low and access to dental care limited (Peterson *et al.,* 2005). The situation is similar in Kenya where current oral health has been described as inadequate (Kaimenyi, 2004). Despite several antibiotics being commercially available for management of oral health, these chemical involved can alter oral microbiota and can have undesirable side effects like vomiting, diarrhea and tooth staining **(**Park *et al.,* 2003; Chung *et al*., 2006). Bacterial resistance to most if not all antibiotics used to treat oral infections has been documented (Bidault *et al.,* 2007). Other antibacterial agents used in treatment and prevention of oral diseases have been reported to be toxic. Ethanol which is commonly used as mouth wash, has been linked to oral cancer (Lachenmeier, 2008; Rodrigues, 2007). Hence the search for alternative products continues and those derived from medicinal plants used by traditional medical practitioners are considered to be good alternatives to synthetic chemicals (Prabu *et al.,* 2006).

With regard to quality, the hygiene and potential contamination of herbal products used in oral health are a concern. Most of these products are sold on pavements and in markets where the materials are often exposed to sputum, urine and feaces contravening the pharmaceutical manufacturing standards which are necessary for production and packaging of conventional medicines (Steenkamp *et al.,* 2006). A common hazard with the use of herbal medicine is the adulteration of the herbal medicine with active drugs such as corticosteroids (Ramsy, 2003) and undeclared drugs (Ernst, 2002). This is mainly because herbal medicines are not regulated as medicines in many parts of world and are freely available to everyone (Ernst, 2002).

Some plants have been reported to have toxic compounds. For example, metabolites derived from N-oxidation process of alkaloids like pyrrolizidine affects the liver leading to intense cellular alterations known as megalocytosis (Zeinsteger *et al*., 2003). Similarly an overdose of saponins has been reported to induce bloody diarrhea in laboratory animals (Diwan *et al.,* 2000). Most reports of toxic effects due to use of herbal medicines are associated with hepatotoxicity (Saad *et al.,* 2006), although reports of other toxic effects on kidney, blood and cardiovascular have been documented. However, the potential of herbal medicine to cause toxicity of liver and other organs is not well documented.

This study investigated some safety and efficacy issues of herbal products used in oral health care by traditional medical practitioners in Nairobi County.

1.2 Problem statement

In Nairobi County, the use of herbal materials for the management of oral diseases has been on the increase since there are few public hospitals which offer dental and other oral health care services. The minimum cost of basic dental procedure is approximately 10 dollars (Kenyan shillings 1000) in standard dental clinics. As such, very few people can afford dental health care offered in conventional hospitals. Many people use herbal materials prescribed by traditional medical practitioners or sold by traders of herbal products who may lack formal training in handling and processing of herbal materials. Currently there is no legal framework to regulate the quality of herbal medicine. Herbal materials are therefore prepared and sold before evaluating their safety and efficacy. With current enthusiasm on the use of herbal medicine in Kenya, quacks are feared to have invaded the practice of herbal medicine in a 'get-rich' scheme and there are possibilities of having adulterated or poor quality herbal products in the market.

The situation is worse with herbal materials used in management of oral diseases. Most of these herbal materials are prepared and sold under unhygienic conditions (Daily Nation, 2010). A number of oral health care materials are hawked when not packaged and this raises the possibility of contamination. Most of these materials are used directly without further processing (for example chewing sticks) thereby increasing the risk of disease transmission.

1.3 Justification

Oral health care facilities have not been adequate for majority of African population (Elujoba *et al.,* 2005). In Kenya, the oral health facilities and infrastructure in most health centers do not have sufficient resources (Kaimenyi, 2004). With a population of 40 million people as of 2009 national census the country has only 656 dentists, one dental hygienist, 69 dental specialists and only 130 community oral health care officers. The ratio of dentists to citizens is 1: 45,735 instead of the recommended 1:7, 000 (Kaimenyi, 2004). Majority of people will therefore continue to use herbal materials for their oral health care for a long time. The traditional healers have taken advantage of the relatively high cost of the conventional pharmaceuticals, inaccessibility of the orthodox medical services and the reservations by the public due to the prevalence of fake, substandard drugs in the market (Okunlola *et al*., 2007). With the prevalence of these herbal products in the market, it is of interest to evaluate their quality and efficacy. Indeed the issues of safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals. This could be due to lack of standards for herbal products. To maximize the potential of African traditional medicines as a source of healthcare the safety, efficacy and quality need to be assessed (Khamali, 2009).

1.4 Research questions

This study aimed at interrogating the following questions;

(i) Which are the herbal materials used in management of oral health care (OHC) in Nairobi County?

(ii) Which are the microbial contaminants in herbal materials used in OHC?

(iii) What are the levels of mineral elements in herbal materials used in OHC?

(iv) What are phytochemicals present in herbal materials used in OHC?

(v) What are the effects of herbal materials used in OHC on bacteria and fungi?

(vi) What are the pharmacological effects of herbal materials used in OHC in laboratory animals?

1.5 Research hypothesis

Herbal materials used in oral health care by traditional medical practitioners in Nairobi are not safe and are not efficacious.

1.6 General objective

To evaluate the safety and antimicrobial properties of herbal materials used in OHC in Nairobi, Kenya.

1.6.1 Specific objectives

a. To determine the herbal materials used in OHC in Nairobi County and the social conditions under which they are prescribed.

b. To evaluate the presence and levels of aluminium, phosphorous, sulphur, potassium, calcium, arscenic, titanium, vanadium, chromium, manganese,

iron, nickel, copper, zinc, bromine, rubidium, strontium and lead in the herbal materials used in OHC in Nairobi County.

c. To evaluate presence of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella spp* and *Candida albicans* in the herbal materials used in OHC.

d. To determine phytochemicals present in herbal materials used in OHC.

e. To investigate the antimicrobial activities of herbal materials used in OHC against selected bacteria and *Candida albicans*

f. To determine toxicity effects of herbal materials used in OHC by investigating the effects of

i. high oral doses of organic extracts of herbal materials on body weights of mice.

ii. herbal materials extracts on hematological and on biochemical parameters in mice.

iii. herbal extracts on the histology of vital organs in mice

1.7 Delimitation

There are several microbial contaminants of herbal materials but the study only investigated the presence and viable counts of the pathogenic microbes as recommended by the WHO. The microbes that were used in the antimicrobial test are the common bacteria and fungi associated with oral infection and those of normal micro flora of mouth. The elemental analysis included aluminium,

phosphorous, sulphur, potassium, calcium, arsenic, titanium, vanadium, chromium, manganese, iron, nickel, copper, zinc, bromine, strontium and lead which are associated with contamination and healing properties.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Oral health

Oral health is a state of being free from chronic mouth and facial pain, oral and throat cancer, oral sores, birth defects such as cleft lip and palate, periodontal (gum) disease, tooth decay and tooth loss, and other diseases and disorders that affect the oral cavity (WHO, 2008). The word oral refers to the mouth which includes the teeth, gums supporting connective tissues, ligaments and bone.

Oral health is part of total health and is essential to overall quality of life. Oropharyngeal colonization is associated with several life threatening systemic diseases (Li *et al.*, 2000; Lockhart and Durack, 1999; Loesche, 1997; Gendron *et al.,* 2000) which include heart conditions such as endorcaditis (Fowler *et al*., 2001) and pulmonary diseases (Scannapieco *et al.*, 1996). There are several psychological problems associated with having discolored, diseased or missing teeth. The victim becomes uncomfortable socializing, loses self esteem and begins a spiral of decline (Braine, 2012). In most developing countries, investment in oral health care is low. In these countries, resources are primarily allocated to emergency oral care and pain relief (Yeer and Sheiham, 2002).

There is considerable evidence linking poor oral health to chronic conditions. Diabetes is strongly associated with periodontal disease (Peterson *et al.*, 2005) while

approximately 40-50 % of people who are HIV positive have oral diseases caused by fungi, bacteria or viruses. Oral lesions strongly associated with HIV infection are pseudo membranous oral candidiasis, HIV gingivitis and periodontitis (Arendorf *et al.,* 1998). The need for oral health care and treatment of manifest oral diseases is particularly great among the disadvantaged population groups of developing countries (Holms and Stephen, 2002).

Western based medicine and dentistry have not been adequate for majority of developing countries' population (Elujoba *et al.,* 2005). This calls for search of effective, safe and economic alternative health care products to take care of the least fortunate individuals in the society. The phytomedicines offers the best, cheap and available alternative treatment for oral health.

2.2 Traditional plant based medicine

The popularity of using herbal supplements is steadily increasing among the general population of the world including the developed countries like the United States of America (USA). In 2008, it was estimated that roughly 20,000 herbal products were available in USA and in one survey; approximately 1 out of 5 adults reported having used a herbal supplement within the past year (Bent, 2008). Over 80 % of people in developing world use traditional medicines for their health care (Ngetich**,** 2005)**.** African medicinal plants play a key role in basic healthcare, particularly in rural areas due to their accessibility and affordability (Khamali, 2009). The use of medicinal plants has always been part of human culture and is widespread in Africa.

In some countries, like Ghana, government encourages the use of indigenous forms of medicine rather than allopathic drugs. Also in Nigeria, a large percentage of the population depend on herbal medicines because the commercially available orthodox medicines are expensive and out of reach to the bigger population (Lawal *et al.*, 2012).

In Kenya the high poverty levels and increased disease burden especially due to HIV/AIDS has resulted in people seeking affordable traditional medicine (Mboya, 2003; Njoroge and Kibunga, 2007). In Kenya knowledge of medicinal plants and some diseases treated by various communities is documented (Kokwaro, 2009; Beentje, 1994; Musila, 2000; Mukiama, 2005; Chelashwal *et al*., 2007; Gacathi, 2007; Njoroge and Kibunga, 2007; Ngari, 2010) and the efficacy of some medicinal plants has been investigated (Gathu, 2007; Mamo, 2007). A lot of attention has been given to killer diseases such as malaria, tuberculosis, diabetes and cancer among others. However, oral diseases have not been given much attention though they cause pain and suffering, impairment of function and reduced quality of life on individuals (Peterson *et al.*, 2005). If oral health is left unattended it is almost impossible to realize one of the major goals of Kenya Vision 2030 that emphasis good quality life to every Kenyan (Kenya Vision 2030).

Medicinal plants have been used for thousands of years in folk medicine for maintenance of oral health. The knowledge of herbal remedies has to be documented and preserved (Bhagya and Shridar, 2009) for sustainable utilization.

Research has been geared towards finding scientific evidence for the claims as to the therapeutic efficacy of African herbs by traditional healers. Most of the published and unpublished ethnomedicine data with valuable information are scattered in many documents, some of which are not easily available. Right from its beginning the recording of traditional knowledge especially medicinal uses of plants has provided many important drugs of modern day (Push-pagadan and Kumar, 2005). When people get infections they are usually treated by traditional medical practitioners who claim that their medicine is cheaper and effective than modern medicine (Rojas *et al.,* 2006). Several herbal materials and products that are claimed to heal or cure all diseases are in the market in developing world. Evidence-based verification of the efficacy of herbal medicinal products or botanicals is still frequently lacking (Bhattaram *et al.,* 2002). With the prevalence of these products in the market it would be of interest to evaluate efficacy of the products on sale. Indeed the issues of efficacy, safety and quality of these medicines have been an important concern for health authorities and health professionals.

Ethnobotanical studies have now been found to be instrumental in improving chances of discovering plants with antimicrobial activity in new drug development. In most cases the sources of these remedies are undocumented and the knowledge about them passed orally from generation to generation, hence they are under threat of disappearing with current rates of modernization (Njoroge and Bussmann, 2006).

The World Health Organisation and other bodies have stressed the need to employ traditional health care, where appropriate, to achieve the health for all, goals

(Nyunja *et al.,* 2009). This is also part of the Millennium Development Goal and vision 2030 for Kenya. Currently there is no data indicating the current quality status of herbal materials in Kenya. The objective of this study was to find out materials that are used in management of oral diseases and the conditions under which the materials are handled. Documentation of the plants used in combinations will help in highlighting and improving the value addition of the herbal products.

2.3 Elemental composition of herbal materials

Macro and micro elements play a significant role in health and diseases of human body (Hameed *et al*., 2008). Studies have shown that trace elements are important in the metabolic processes of human as well as plants (Mudassir *et al.,* 2010). Many trace elements play a significance role in the formation of active constituents which are responsible for their curative properties **(**Khan *et al.,* 2011; Balaji *et al.,* 2000). However, continuous intake of diets that are excessively high in particular elements can influence changes in functions, forms and activities of some organs (Obiajunwa *et al*., 2002). However, deficiency of these elements can lead to severe abnormalities such as anaemia in case of iron or deformities in bone and hence teeth like in case of phosphorous, calcium, zinc, strontium, vanadium and many more.

Some elements such lead and aluminum are toxic in nature and their presence in plants may be due to pollution arising from automobile and industrial activities (Rabia *et al.,* 2012). Lead induces various toxic effects in human at low doses characterized by colic, anaemia, headache, convulsions and chronic nephritis (Samali *et al.,* 2012). The maximum limit for lead in herbal materials should be 10

mg/kg while dietary intake is 3 mg/week WHO (1998). Excess amount of aluminum intake is associated with Parkinson's diseases. Iron plays an important role in heamoglobin synthesis (Strain and Cashman, 2009; Samali *et al*., 2012). The maximum permissible level of iron is 1000 µg/day (Food and Nutrition Board, NRC, 2003).

Copper is crucial in treatment of chest wounds and prevent inflammation in arthritis and similar diseases (Khan *et al.,* 2011). There are no recommended daily allowances (RDA) for copper but the suggested safe and adequate range intake is 1.5 to 3.0 mg per day (Mindell and Mundis, 2004; Strain and Cashman, (2009). Copper could be toxic depending on the dose and duration of exposure (Food and Nutrition Board NRC, 1980). The maximum permissible level of copper is 12 mg/day (Obi *et al*., 2006) and toxicity of copper is characterized by headache and vomiting, diarrhea, hemolytic anemia and liver damage, followed by coma and death (Strain and Cashman, 2009).

Phosphorous is essential for bone and tooth formation and works in conjunction with calcium. The RDA for phosphorous is 700 mg a day (Strain and Cashman, 2009). Excessive phosphorous intake when coupled with low intake of calcium may be a factor in development of osteoporosis. Calcium participates in growth and development of bones and teeth. Large doses of calcium supplements 1500 mg or more each day may result in the formation of kidney stones in susceptible people (Tull, 1996).

Zinc participates in nucleic acid metabolism (Atukorala and Wadyanatha, 1987), normal growth, brain development, behavioral responses, bone formation and wound healing (Samali *et al.,* 2012) and it has a major role major role in immunity (Shankar and Prasad, 1998; Wintergerst *et al.,* 2007). The element has anti-viral, antibacterial and anti-cancer properties (Black, 2003). Zinc plays a role in maintaining the integrity of the skin and mucosal membranes (Wintergest *et al*., 2007). The dietary limit of Zinc is 100 µg/g and it is said to be least toxic of all the heavy metals (Samali *et al.,* 2012).

A number of manganese activated enzyme play an important role in metabolism of carbohydrates, amino acids and cholesterol (Institute of Medicine, 2001). It participates in the production of collagen, a vital process in wound healing (Muszynska *et al.,* 2000). Strontium has been known to reduce incidences of tooth cavities at concentration of 6 to 10 mg/l (Gaby, 1994) and is found as trace micronutrients in plants (Rabia *et al.,* 2012).

Nickel is required for optimal growth, health skin, bone structure but the intake of high amount can cause heart diseases (Khan *et al*., 2011). Nickel exerts a potent toxic effect on peripheral tissues and on reproductive system and the maximum permissible limit is 100 µg/day (Doss and Disrupt, 2002). It causes dose related decreases in bone marrow cellularity and in granulocytomacrophage and pluripotent stem cell proliferative responses (Annan *et al.,* 2010). Rubidium resembles potassium in its' pattern of absorption and they are thought to share a transport

system (Strain and Cashman, 2009). Chromium in its trivalent form is an essential nutrient that functions in carbohydrate, lipid and nucleic acid metabolism, and there is no RDA for it (Strain and Cashman, 2009).

Botanical medicine may also take up heavy metals from other anthropogenic operations such packaging (Corns, 2003). These products are sold and packaged under conditions lacking in hygiene and sanitation (Ingela, 2009). WHO (1988) has proposed maximum daily intake or tolerable intake for some heavy metals. These provide guideline for monitoring the limit of the heavy metal for safety.

Levels of essential and toxic heavy metals in medicinal plants beyond permissible limit are a matter of great concern to public safety all over the world (Shad *et al.*, 2008). Determination of elemental compositions and elucidation in plant and related products is essential for understanding their clinical action and nutritional value (Darinka *et al.*, 2010). Medicinal plants easily get contaminated during growth, development and processing. Anthropogenic activities and geoclimatic conditions are major factors that enhance the contamination of herbal materials (Chan, 2003). Different plants have different ability to accumulate elements that is the hyper accumulators will have heavy load of metals. Since medicinal plants form the raw materials for the finished product, it is a requirement to evaluate levels of heavy metal in plants (WHO, 1998).

Several studies have been conducted to evaluate the elemental minerals composition in medicinal plants and herbal material (Ang and Lee, 2006; Mtunzi *et al.*, 2012; Ameh *et al.*, 2010; Hameed, 2008; Rabia *et al.*, 2012; Annan *et al*., 2010; Mudassir *et al.*, 2010; Khan *et al.*, 2011; Muhamed, 2007). In Kenya studies on mineral element analysis of medicinal plants are few (Bernard, 2010). The current study was designed to evaluate the mineral elemental composition of herbal material used in management of oral health.

2.3.1 Methods for elemental analysis

There are various methods that are used in determination of elemental profile of samples. X-ray Fluorescence Spectroscopy (XRF) is an analytical technique for determining the chemical composition (elements) in all kinds of materials. It can also be used to determine the thickness and composition of layers and coatings. The technique is widely used in metal, plastic, polymer, cement, oil and food industries as well as in pharmacy and research. The main principle of XRF is that when atoms are irradiated with X-rays, they emit secondary X-rays called fluorescence radiation. These fluorescence radiations are characteristic for a particular atom (element) and are of specific energy which makes it possible for qualitative and quantitative analyses. The advantage of XRF over other spectroscopy techniques is that it is fast, accurate, non-destructive (minimum sample preparation) and multi-element (all detectable elements are measured simultaneously).

In elemental analyses, two XRF methods namely, Energy Dispersive X-ray Fluorescence (EDXRF) and Total Reflection X-ray Fluorescence (TXRF) are commonly used. These methods differ in excitation geometry, sample preparation and detection limits. The current study aimed at determining elemental profile of herbal materials sold in Nairobi Kenya using Total Reflection X-ray Fluorescence (TXRF).

2.4 Microbial contaminants of herbal medicine

The presence of microbial contaminants in non-sterile pharmaceutical products can reduce the therapeutic activity of the product and has the potential to adversely affect patients taking the medicine (Nakajima *et al.,* 2005). Hence it is advisable to have some knowledge of microbial contents of all drugs including herbal medicine (Akerere and Godwin, 2002). Plants may be harvested from contaminated soil or cleaned improperly such that they may contain pathogenic microorganisms (Saad *et al.,* 2006). On the other hand fast majority of medicinal products in the informal sector are produced in crowded areas lacking in hygiene and sanitation (Khanyile *et al.,* 2009). Some infectious disease outbreaks have been associated with the use of heavily contaminated raw materials of natural origin (Enayatifand *et al.,* 2010).

Different Pharmacopeias have microbial limitation for nonsterile edible products such as powders and capsules. The limits of microbial contamination are; total aerobic bacteria 10^5 CFU/g, yeast and moulds 10^3 CFU/g while *Escherichia coli* and *Salmonella typhi* should be absent (European Pharmacopoeia, 2007). According to

Thai Pharmacopoeia (1987), *E. coli* should not be present in all the herbal products while *Staphylococcus aureus* and *S. typhi* must not be in 10 grams of herbal products taken as powdered products. Worldwide microbial contamination of herbal medicine has been reported. The occurrence of coliform in traditional medicine has been reported (Limayati, 1998). Presence of *Salmonella* species, *Klebsiella pneumonia* and *S. aureus* was detected in extracts of *Lessertia* (Govender *et al*., 2006). Bacterial toxins and fungal toxins have also been isolated from such products (Govender *et al*., 2006).

Evaluation of microbiological quality of herbal products in Thailand (Mulika *et al.*, 2003) showed that the products were found to be contaminated with *Salmonella* species, *Clostridium* species, *S. aureus* and *E. coli.* Similar studies were carried out in Saudi Arabia (Alwakeel, 2008) where pathogenic microorganisms were reported. Microbial contamination of traditional medicine known as 'Jamu gendong' was reported in Indonesia (Limayati and Junior, 1998). Halt (1998) isolated a wide spectrum of fungi including *Aspergillus, Penicillium, Rhizopus* and *Mucor* species from medicinal plants.

Microbial load of some medicinal plants sold in local markets of Bennin, Nigeria reported presence of *P. aeruginosa* and *B. subtilis* among others (Idu *et al.,* 2011). In Kaduna, studies indicated presence of pathogenic *S. typhi* in 65.7 % of herbal products analysed and *E. coli* in 58.7 % of the samples analysed (Abba *et al.,* 2009). In Eastern Romania, microbial evaluation of 8 samples of herbal products showed

high levels of *E. coli* (Poiata *et al.,* 2010). Evaluation of microbial quality of plant materials in Belgrade indicated the presence of *E. coli, Bacillus* and *Clostridia* species (Stevic *et al.,* 2012). Evaluation of microbial and fungal contaminations of six spices and herbal products was carried out in Ghana (Ahene *et al.,* 2011). In the study the spices were found to harbor aflatoxins, enterobacteria and *P. aeruginosa* among others. Investigation of pharmaceutical and microbial qualities of herbal products in Nigeria revealed presence of potential pathogenic bacteria in the products (Okunlola *et al*., 2007). In South Africa, studies have shown that herbal medicine is heavily contaminated with bacteria (Khanyile *et al.,* 2009; Enayatifard *et al.,* 2009).

There appears to be an overwhelming increase in usage of herbal products and materials in treatment and/or prevention of oral diseases in Kenya. In an attempt to enhance the acceptability of the herbal medicine by consumers, many products have been formulated into conventional dosage forms such as tablets, capsules, suspensions, solutions, pastes and powders. Most of the product vendors often have claims that their products can cure all forms of ailment (Oyetayo, 2008). Product quality is obviously the major factor that could affect the efficacy and safety of patient's or consumers (Mullika *et al*., 2003). Handling, collecting, sampling, preparation, storage and transportation of the plant material are some of stages where contamination can occur. Data on microbial contamination is limited in Kenya especially on herbal materials used in oral health. Interestingly there have been only a few reports on microbial quality of Kenya herbal products. The

objective of this study was to determine the microbial contamination of the available materials used in management of oral health.

2.5 Phytochemistry

Medicinal plants are of great importance as source of drugs to individuals and to the communities. Screening of medicinal plants for their phytochemicals, antioxidant, anticancer and antimicrobial activities is the prime concern for finding out an effective phytochemically active principle (Agbafor *et al*., 2011; Mishra and Tripathi, 2011). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al.,* 2005). The healing property of these medicinal plants is usually linked to the presence of secondary metabolites and these differ from one plant to another (Idu *et al.,* 2011).

The medicinal value of these plants is due to active ingredients such as alkaloids, tannins, flavonoids and phenolic compounds (Edeoga *et al*., 2005; Sofowora, 1982**)**. Phytochemicals are natural bioactive compounds found in plants and they are grouped in primary and secondary constituents on the basis of their functions to the plant (Krishnaiah *et al*., 2009). Primary constituents comprise mainly sugars, amino acids, proteins and chlorophyll while secondary components includes alkaloids, terpenoids and phenolic compounds (Krishnaia *et al.,* 2007) and many more such as flavonoids and tannins. Many studies in Kenya have investigated the presence of bioactive components of various plants (Gathu, 2006).

Currently there are many herbal based products in the market whose phytochemical components are not known. More over the herbalists normally use several plants in their herbal formulation. There are few studies which have attempted to verify the presence of phytochemical components in processed herbal products as claimed by the healers. Studies in Nigeria have shown that some products in the market had no herbal based components (Oyeteyo, 2008). The current study aims at investigating the phytochemical components in the plants and plant based herbal products used for oral health management in Nairobi, Kenya.

2.6 Antimicrobial activities of herbal materials

The link between oral dis**e**ases and microbial species that form part of the oral cavity micro-flora is well established (Scannapieco and Mylotte, 1996). Poor oral hygiene and periodontal disease may promote oropharyngeal colonization by potential respiratory pathogens such as *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Scannapieco and Mylotte, 1996). Furthermore, aerobes and facultative anaerobes are the most numerous components of the normal human oropharyngeal bacterial flora, and they are therefore a common cause of bacterial infections of the upper respiratory tract which have an endogenous origin such as bronchitis, sinusitis, otitis and pneumonia (Brook, 2002).

The development of dental caries or tooth decay involves acidogenic and aciduric Gram positive bacteria primarily *Streptococcus mutans, Lactobacilli* and Actinomycetes that metabolize sucrose to lactic acid (Jenkisson and Lamont, 2005).

Candida albicans belong to the family Ascomycetes and lives as a harmless commensal in many parts of the body. It causes oral candidiasis especially in immune compromised individual (Greenwood *et al*., 1997). *Staphylococcus aureus* found in nose, pharynx and lower intestine of human, is responsible for soft tissues pathology like abscesses, angular cheilitis, stomatitis, maxillary osteomylelitis and parotitis (Lee *et al.*, 2005). *Pseudomonas aeruginosa* is causative agent of ventilator associated pneumonia (Munro and Grap, 2004). Different streptococcus species constitute the majority of the oral pharyngeal microbial population known to cause tonsillitis, otitis, dental abscesses, caries and pharyngitis (Sheng *et al*., 2005). *Eteroccocus faecalis* pathogenicity ranges from life threatening diseases of immune compromised individuals to less severe conditions such as infection of obturated root canal with chronic apical periodontitis.

Most of oral diseases are caused by bacteria and antibacterial remedies are the mainstay of treatment. Lack of high efficacy with antibacterial agents, unwanted effects and resistance to these agents are drawing attention of scientists to search for new and better drugs especially from plants.

Herbal medicines hold a great promise as source of easily available effective therapy for various infections including oral health. In developing countries low income people use folk medicine for treatment of oral diseases. It is in this context that people use several plant derived preparations to cure disease. The plants are ingested as decoctions, teas or juices preparations (Gonzalez, 1980). About 10

different oral/dental conditions treatable with plants are common in traditional health practice. The ailments include toothache, decay, gingivitis, angular stomatitis, mouth ulcers, swollen tonsils, oral thrush, tonsillitis and black tongue (Hollist, 2004).

Several studies have evaluated antimicrobial properties of plant extracts. The antimicrobial and antioxidant activities of three Nigerian chewing sticks against *S. aureas, E. coli, Enteroccocus faecalis, Pseudomoonas aeruginosa, Candida albicans* and *Bacillus cerius* was investigated (Adebiyi *et al.*, 2009). In this study ethanolic extracts of herbal materials were found to be the most effective. Almas and Al-Zeid (2004) evaluated the antimicrobial effect of tooth brush and Miswaki on *S. mutans* and *lactobacilli* in Saudi Arabia. The study showed that there was a marked reduction of the *S. mutans* among the groups tested after use of chewing sticks. Comparison of the antimicrobial activity of seven different types of chewing sticks was carried using *Streptococcus faecalis, S. aureus, S. mutans* and *C. albicans* (Almas, 2001). Antimicrobial effects were only present at high concentration. Antimicrobial activity of Tanzanian chewing sticks against *S. mutans* and *C. albicans* was carried out where the findings of the study indicated that bark extracts were more effective than wood extracts (Khan *et al*., 2000).

Other studies have focused on the antimicrobial effects of specific phytochemicals on cariogenic bacteria and periodontal pathogens (Wolinsky and Sote, 1983; Almas, 2001) and inhibitory action of dental plaques formation (Wolinsky *et al*., 1996).

Sato *et al.* (2002) investigated antibacterial activity of purified phytochemicals such as flavonoids. Evidently there are no sufficient scientific studies that confirm the efficacy of these materials prescribed by healers. This study investigated *in-vitro* antimicrobial activity of these materials against pathogenic microorganisms.

2.7 Toxicity studies

Herbal medicine is believed by many people to be natural and that medication of natural origin are not toxic or dangerous (Ezejiofor *et al.,* 2008). However, herbs and herbal preparations can cause toxic adverse effects, serious allergic reactions and adverse drug interactions (Bandarayanake, 2006). As such laboratory screening including toxicity studies are important to ascertain their safety (Saha *et al.,* 2011). Several toxicological studies have been carried out on medicinal plants (Jaijoy *et al.*, 2011; Ogwang *et al*., 2008; Panunto *et al*., 2010; Ilodigwe *et al.,* 2010; Ezejiofor *et al.,* 2008; Datta *et al*., 2011). However, few studies have addressed the safety of herbal materials used in oral health.

2.7.1 Models for toxicity studies

In order to determine the effects of plant extracts, several models are used which include cell culture, tissue culture and animal models. In most of culture system, vertebrates cell cultured *in-vitro* have been grown in monolayers on artificial substrate (Saad *et al.,* 2006). However, it is known that the cells normally interact with each other and their reaction to drugs is also determined by the

microenvironment of the same. The mouse (*Mus musculus*) is the animal model most widely used to study the pathogenesis and treatment of human diseases (Cappecci, 2005). The mouse is evolutionary very close to human and its embryonic development recapitulates many aspects of human development in terms of body structure formation and gene activation (Mazzaccara *et al.*, 2008). The mouse has a short generation time which reduces the cost of animal experimentation and a large number of animals are available for study (Craigen, 2001). Germ line modifications and knock out mice have been generated for specific human disease and developmental biology studies (Cappecci, 2005). In this study Swiss white albino mice were used.

2.7.2 Parameters used to evaluate toxicity

For studies using animal models, the parameters mainly used include body weight changes, absolute organ weight, relative organ weight, haematology and biochemical parameters.

2.7.2.1 Body weight and absolute organ weight

Reduction in the body weight is a valuable indicator in evaluating the toxicity of preparation (Watthanachaiyingcharoen *et al.,* 2009). The loss of weight and sluggish movement exhibited by animals may result from reduced food and water intake (Ilodigwe *et al.,* 2010). It could also be attributed to presence of anti-nutritional substances such as tannins and saponins found in plants (Kahnut *et al.,*

1995). Several studies have evaluated the effect of medicinal plant on body weight of mice (Panunto *et al*., 2010; Ilodigwe *et al.,* 2010). The relative organ body weight is an index of atrophy or hypertrophy (Swanston-Flatt *et al*., 1990). An increase in organ body weight ratio is an indication of inflammation while decrease may be due to cell constriction (Moore and Dalley, 1999).

2.7.2.2 Haematology

The hematopoetic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status for both animals and humans (Adeneye *et al.,* 2006). Haematological parameters provide vital information regarding the status of bone marrow activity and intravascular effects such as haemolysis and anemia (Gregg and Voigt, 2000). Evaluation of the hemogram involves the determination of the total erythrocyte count (RBC), total white blood cell count (WBC), haematocrit (HT), haemoglobin concentration (Hb), erythrocyte indices which comprise Mean Corpususal Volume (MCV) and Mean Corpusulal Heamogloblin Concentration (MCHC). Haemoglobin is evaluated to detect anaemia and its severity and to monitor anaemic patient's response to treatment. The mean cell haemoglobin concentration gives the concentration of haemoglobin in g/l in one liter of packed cell volume (Cheesborough, 2006; Lewis *et al.,* 2001). The mean cell haemoglobin (MCH) is the amount of haemoglobin in picograms (pg) in red blood cell.

Platelets help in repairing damaged blood vessels and function to initiate the formation of blood clots (Soper *et al*., 1997). The mean platelet volume (MPV) is lower than predicted when thrombocytopenia is caused by megablastic anaemia (Lewis *et al.,* 2001). Other parameters of clinical significance include platelet distribution width (PDW) and Platelecrit (PCT). The PDW is a measure of platelet anisocytosis while PCT is an indicative of the volume of circulating platelets in a unit volume of blood. Differential white cell count provides information on the different types of white blood cells in circulating blood (Cheesbrough, 2006). A three differential cell count classify white cell into, granulocytes, lymphocytes and monocytes. The white blood cells include neutrophils, eosinophils, basophils, lymphocytes and monocytes (Simson *et al.,* 1988). An absolute increase in neutrophils can be found in metabolic disorders (Cheesbrough, 2006) while an increase in lymphocytes (lymphocytosis) can be found in lymphomas and protozoa infections. Eosinophilia can be found in allergic conditions such as hay fever and connective tissue disorders. High numbers of basophils can be found in myeloperoxidase disorders (Cheesbrough, 2006).

2.7.2.3 Biochemical parameters

Several soluble enzymes of blood plasma have been considered as indicators of hepatic dysfunction and damage (Shakoori *et al.,* 1994). Generally increment of the activities of aspartate aminotransferase (ALT), alanine amino transferance (AST) and alkaline phosphatase (ALP) is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al*., 1993) which gives an

indication on the hepatotoxic effect of herbal materials. It is known that an increase in concentration of ALT, AST and ALP in the serum directly reflects a major permeability, congestion or cell rupture (Benjamin, 1978; Pieme *et al.,* 2006; Tedong *et al.,* 2008). Alkaline phosphatase (ALP) enzyme is a sensitive biomarker to metallic salts since it is a membrane bound enzyme related to the transport of various metabolites (Lakshmi *et al*., 1991; Coleman *et al.,* 1992). The various isoenzymes of ALP are ubiquitous throughout the body, although they are mainly present in liver, bone, intestine, kidney, placenta and white blood cells (Tietz, 1976). In addition, the increase of ALP in plasma could be as a result of damage of liver cells and bile duct obstruction due to proliferation of its cells or related to the progressive liver necrosis (Newberne and Butler, 1969).

2.7.2.4 Histopathological changes in vital organ

The liver is known to be a main organ in the metabolism and detoxification of xenobiotics and therefore it is vulnerable to damage induced by a huge variety of chemicals (Solis *et al.,* 1993; Hatscheck *et al.,* 2002). Morphologically, chemical induced injury can manifest itself in several ways. The acute effects can consists of an accumulation of lipids and appearance of degenerative processes leading to death of the cell (necrosis). The necrosis process can affect small groups of cells forming focal necrosis.

Kidneys are particularly vulnerable to toxic agents given their high rate of perfusion and their ability to concentrate a range of substances in the tubular lumen

(Hatscheck *et al.,* 2002; Kumar *et al.,* 2004). The mammalian kidney is an extremely complex organ both anatomically and functionally. The primary renal function is excretion of waste products. It is also the major site for the formation of hormones that influences systematic metabolic function. A toxicological damage in the kidney could affect any or all of these functions. The effects normally reported include decreased elimination of blood urea nitrogen (BUN) or an increase in plasma creatine. Functionally, toxicity may be reported as a minor alteration capability like transient glycosuria with anuria and elevated BUN. Toxic effects of a chemical can be evaluated by several methods, but histopathology studies can provide a great deal of information about renal integrity.

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Study site

The study was carried out in Nairobi County (Appendix 1), the capital city of Kenya. The city is Kenya's principal economic and cultural centre. The important commercial and administrative areas are located in the city centre which is surrounded by residential areas. There are large markets where herbs are sold like Gikomba, Eastleigh, Ngara and Kangemi. Processed herbal products are found in herbal clinics and streets in the central business district. The above areas were used as sampling sites.

3.2 Sampling technique

The study area was divided into various strata according to the localities and product sold as described by Martin (1995). Stratified random sampling was carried out to pick traders and markets that were used in the study. A general survey to locate and estimate the traders and clinics was carried out. The sample size of participants was determined by use of sample size calculator Raosoft Inc 2004 (R). Randomly generated computer numbers were used to select the study traders, clinics and herbalists. Oral herbal products were obtained from traditional medical practitioners and from herbal clinics in Nairobi by purposive random sampling.

3.3 Sampling tool

A semi structured questionnaire (Appendix, 2) and informal discussions were used to gather ethnomedical knowledge data.

3.4 Documentation of herbal materials used in oral health

General ethnobotanical methods were used to gather information on medicinal plants and herbal materials used in management of oral health. The information gathered included plant used, part used, processing of herbal materials and administration, oral condition that people frequently complain of and the age of the clients. Biodata of the respondents were also collected which included: age, gender, level of education, number of years in practice, and source of herbal knowledge. The details of the business set up were recorded: they included locality of the enterprise, storage of the herbal products, packaging and general hygiene of the area. Visits were made to herbal clinics and herbal traders as described by Sofowora (1982); Martin (1995); Kareru *et al*. (2007); Khamali, (2009) and Nyunja *et al*. (2009). Interviews and informal discussions were held with 60 herbalist/ traders as shown on Table 1.

Table 1. Distribution of participants in the study area

Locality	Type of participants	Number of participants
Gikomba	Trader	7
Juja road	Herbalist	2
Kawangware	Herbalist	12
Kangemi	Herbalist	7
Kibera	Herbalist	5
Kenyatta University	Herbalist	1
Thika road	Herbalist	1
Eastleigh	Trader	12
Kahawa	Herbalist	2
Central business district	Hawkers	8
Central business district	Clinics	3

In some cases participants were accompanied in their plant collecting duties and observations made of the materials being collected. Where the respondents were uncomfortable with the questionnaires, discussions and informal interviews were employed and in the process information on traditional management of oral conditions were obtained. During discussions, information on combination therapy or poly herbal management of OH conditions were noted and recorded. Plants cited as useful in managing of various OH ailments during the interviews were visually identified in the field by the respondents. Voucher herbarium specimens were collected in duplicates using standard taxonomic and ethnobotanical procedures (Martin, 1995). All the important taxonomic features of the plant for identification in the herbarium were recorded. Each plant specimen collected included vital parts such as leaves, stems, flowers and fruits where available. For small herbaceous

plants, whole plants were collected. For every specimen collected the local names were recorded. The specimens were dried in the herbarium and then mounted on sheets. The collected plant materials were identified at the Jomo Kenyatta University Herbarium using the relevant local floras and other taxonomic literature (Agnew and Agnew, 1994; Beentje, 1994). Some specimens were identified at Kenyatta University Department of Pharmacy and Complementary/Alternative Medicine. Voucher herbarium specimens were deposited at Technical University of Kenya herbarium, Nairobi.

3.5 Collection of herbal materials for contamination analysis

Twenty three samples of well known herbal materials and products were purchased at random from traditional medical practitioners. The herbal products were chosen on the basis of their commercial availability and popularity of use. All herbal powders were purchased and placed in clean plastic bags at low temperatures. The samples consisted of raw plant materials, herbal mixtures, suspensions, powders and herbal tooth pastes. The collected samples were given sample codes, and the following were recorded; manufacturing and expiry date, indications, mode of application, and whether the product had Kenya Bureau of Standards (KEBS) mark of quality. The samples were stored in a refrigerator at 4 ºC pending analysis.

3.5.1 Media and isolation of pathogenic microorganisms

All media used were prepared according to the manufacturer's instructions. The required amount of medium was weighed and dissolved in distilled water, autoclaved at 121 ºC and for solid media they were dispensed in Petri dishes and slants.

3.5.2 Isolation of indicator microorganisms

The following microorganisms were used as indicators of microbial contamination; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli* and *Candida albicans* as described by Evans (2002), Enayatifard *et al.* (2009) and WHO (1998). To determine the presence of bacterial contaminants, each sample (one gram of solid samples and 1ml of liquid samples) was diluted to 100 mls by adding soybean casein digest medium and then incubated. For isolation of the *S. aureus*, a portion of the enrichment culture medium was spread on the surface of the Vogel-Johnson agar medium and manitol-salt agar which were freshly prepared according to the manufacturer's instructions. The plates were incubated at 37 ºC for twenty four hours under aerobic conditions. Colonies showing golden yellow color or colorless were considered to be *S. aureus.* The pure isolates were further subjected to gram staining for microscopy, biochemical test such catalase as well as tube and slide coagulase to confirm the identity of *S. aureus*.

A portion of the enrichment culture medium was streaked on centrimide agar medium for detection *P. aeruginosa.* To detect *E. coli* and *Salmonella* species, fluid lactose medium was added to 10 grams of the sample to make 100 mls. Fluid lactose enrichment was streaked on to differential MacConkey agar plates for *E.coli* and incubated at 44 ºC for twenty four hours. Inoculum from *E. coli* medium was then streaked on to the surface of Eosin methylene blue (EMB) agar and incubated for 24 hours. Dark colonies with metallic sheen indicated the presence of lactose fermenters. The colonies were further subjected to indole, motility, Voges Proskaur and citrate biochemical test as described by Prescott *et al.* (1999).

For detection of *Salmonella* species, 1 ml aliquot was transferred into 9 mls fluid selenite-cystine and fluid tetrationate respectively. The cultures were incubated at 35±2 ºC for 12- 24 hours and were sub cultured further on the surface of brilliant green agar and bismuth sulfite agar media respectively. The appearance of typical black and green colonies was regarded as positive for *Salmonella* species. The colonies were further then streaked on the nutrient agar slants for further biochemical identification using Triple Sugar Iron (The butt-slant).

To detect *C. albicans,* ten grams of each sample was added to Sabrounds dextrose broth to make 100 mls. The latter was incubated at 20-25 ºC for seven days. The incubated samples were examined and cultured on Sabrounds dextrose agar. In cases where microbial growth was observed, the colonies were identified by germ tube method. About 0.5 mls of human serum was placed in small test tube. Using a sterile

wire loop, the serum was inoculated with a yeast colony. The inoculated serum was incubated at 37 ºC for three hours. A drop of the serum yeast culture was transferred to a glass slide using a Pasteur's pipette. The preparations were examined using magnification of X10 and X40 objectives for sprouting yeast cells.

3.5.3 Determination of bioburden

The collected samples were subjected to the following examinations: total aerobic viable count (TAVC) by plate method, viable counts of *S. aureus*, *E. coli*, *P. aeruginosa*, and *Salmonella spp* based on the method by Enayatifard *et al.* (2009). Aerobic bacteria colony count was made by pour plate technique on soy bean casein digest agar. Ten grams of each solid sample and 10 mls of liquid samples were aseptically removed from the source and suspended in physiological saline and volume adjusted to 100 mls.

Serial dilutions were prepared so that the number of colony forming units (CFU) in Petri dishes would be less than 300. One ml aliquots in duplicates of each dilution sample were pipetted on to separate sterile Petri dishes (9 cm in diameter). Twenty ml of nutritive medium for cultivation of bacterial and Sabourauds agar for fungi was added. After solidification of the soft agar the Petri plates were incubated in duplicates at 37 ºC for 48-72 hours for bacterial counts and 25 ºC five days for fungal counts. After incubation the number of colony forming units was recorded for each plate. The number of microorganisms in each sample was evaluated by

multiplying the average number of colonies per plate by dilution factor. The counts were expressed as colony forming unit per gram (CFU/g) or 1 ml (CFU/ml) Arithmetic mean count was derived from each item having from 30-300 colonies per plate.

3.6 Determination of heavy metals using total x-ray fluorescence techniques

The elements analysed included aluminium, phosphorous, sulphur, calcium, manganese, vanadium, iron, copper, zinc, bromine, strontium and lead.

3.6.1 Sample preparation for elemental analysis

The solid, paste and liquid samples were prepared as described in the following sub section.

3.6.1.1 Liquid samples

A volume of 10 ml of each sample was pipetted into a clean vial. A volume 20 µl of 1000 µg/g Gallium stock solution was added into each sample (as internal standard) resulting into a concentration of 2 µg/g Ga in each sample. Each sample was shaken for a minute for homogenization. Aliquots of 10 µl of each sample in triplicates were pipetted onto clean quartz carrier using a micro-pipette. The carriers were then dried in an oven to evaporate the liquid.

3.6.1.2 Paste samples

Triplicate known weights (1.0 g) of each paste were placed in clean vials and 10 ml of double distilled water added and shaken for homogenization. A volume of 20 µl of 1000 µg/g Gallium stock solution was added into each sample (as internal standard) resulting into a concentration of 2 µg/g Ga in each sample. Each sample was shaken for a minute for homogenization. Aliquots of 10 µl of each sample in triplicates were pipetted onto clean quartz carrier using a micro-pipette. The carriers were then dried in an oven to evaporate the solvent.

3.6.1.3 Solid samples

Three (3) original samples from each batch or container were combined into a pooled sample and subsequently used to prepare the average sample. The average sample was prepared by "quartering" the pooled sample according to method described by WHO (1998) as follows: each pooled sample was mixed thoroughly, and constituted into a square-shaped heap. The heap was then divided diagonally into 4 equal parts. Any 2 diagonally opposite parts were taken and mixed carefully. This step was repeated as necessary until the required quantity of sample was obtained. Any material remaining was returned to the batch. The final samples were obtained from an average sample by quartering, as described above. This means that an average sample gave rise to 4 final samples. Each final sample was divided into 2 portions. One portion was retained as reference material, while the other was tested in triplicate. Triplicate known weights of each sample were placed in a clean

digestion flask. A volume of 10 ml of double distilled water and 30 mls of concentrated nitric acid were added and the sample heated to boil until the entire sample was digested. The digests were transferred into clean vials and double distilled water used to top the aliquots to 10 mls. A volume of 20 µl of 1000 mg/kg Gallium stock solution was added into each sample (as internal standard) resulting into a concentration of 2 mg/kg Ga in each sample. Each sample was shaken for a minute for homogenization. Aliquots of 10 µl of each sample in triplicates were pipetted onto clean quartz carrier using a micro-pipette. Triplicate sub-samples were prepared for each sample. The carriers were then dried in an oven to evaporate the solvent.

3.6.2 Sample spectrum acquisition and quantitative analysis

Each sample carrier was irradiated for 300 seconds using an S2 PICOFOX TXRF Spectrometer which was operated at 50 kV and a current of 1000 µA. Other instrumental conditions are described in Appendix 3. Evaluation of the measured spectra was done using S2 PICOFOX software on the basis of the chosen elements. The concentrations were calculated based on the net intensities of the analyte peak elements and that of the internal standard as per the formula described in Appendix 4. Quantitative results obtained were copied to an excel worksheet (as is the protocol of the software) and labeled as raw data. All the data in raw data worksheet was copied to edited worksheet where it was cleaned. Data in edited worksheet were further evaluated for averages and standard deviations for each set of sub-samples. Average and standard deviation functions in excel were used.

3.7 Preparation of plant samples for phytochemical analysis

The collected herbal materials were kept at room temperature away from direct sunlight in closed dry plastic bags. The sticks were cut into small pieces, dried and ground to make a powder. The powdered material was sequentially extracted using dichloromethane (DCM), for 72 hours, DCM: Methanol (1:1) and finally water. The organic extracts were concentrated by use of Rota evaporator at 40 ºC to give DCM and DCM: MeOH extracts while the water extract was subjected to freeze drying for 48 hours. The freeze dried powder was then weighed in an air tight container and stored at -20 ºC until used for bioassay. Liquid samples were partitioned into hexane to remove non polar compounds while acetone was used to remove polar compounds such as glycosides and alkaloids. The flow diagram Figure 1 shows the extraction procedure of powdered herbal materials.

3.8 Phytochemical analysis

Phytochemical analysis for major constituents of the plant extract was done using standard qualitative methods as described by various authors (Houghton and Raman, 1998; Chhetri *et al*., 2008; Roopashree *et al.,* 2008). Various groups of phytochemical components were investigated including alkaloids, glycosides, resins, phenols, amino acids, diterpenoids, flavonoids and tannins that are known to contribute to antimicrobial and toxicity properties of plants.

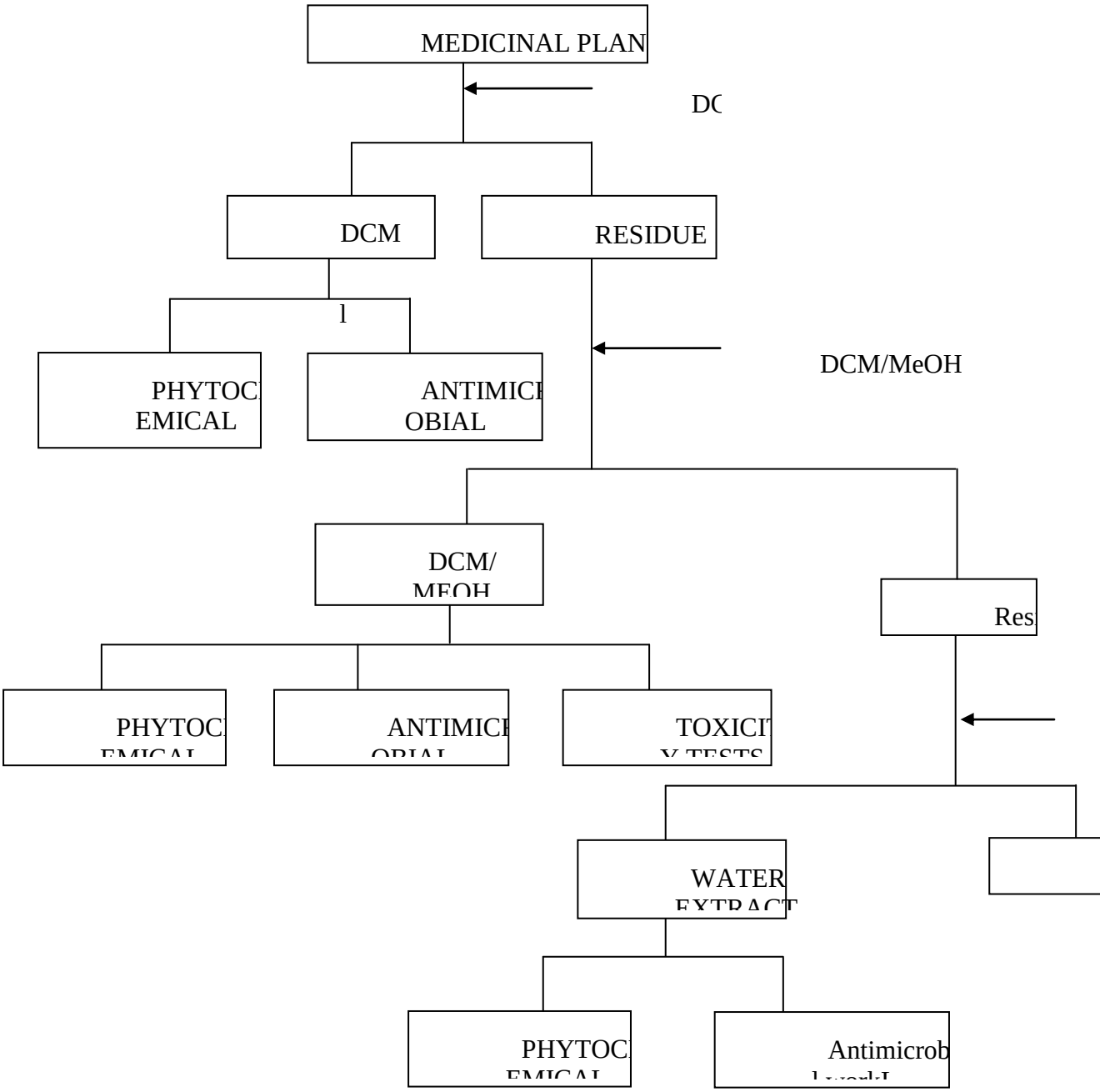


Figure **Flow diagram showi**

Figure 1 . Flow diagram showing extraction of plant materials

3.8.1 Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid (HCl) and filtered. The filtrates were used to test for the presence of alkaloids as follows;

3.8.1.1 Mayer's test

Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow colored precipitate indicated the presence of alkaloids.

3.8.1.2 Wagner's test

Filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/reddish brown precipitate indicated the presence of alkaloids.

3.8.1.3 Dragendoff's test

Filtrates were treated with Dragendoff's reagent (solution of potassium bismuth iodide). Formation of orange yellow precipitate indicated presence of alkaloids.

3.8.2 Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates using various tests.

3.8.2.1 Molisch's test

Filtrates were treated with 2 drops of alcoholic alpha-naphthol solution in a test tube and 2 ml of concentrated H_2SO_4 acid was added carefully along the sides of the test tube. Formation of a violet ring at the junction indicated the presence of carbohydrates.

3.8.2.2 Benedict's test

Filtrates were treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicated presence of reducing sugars.

3.8.2.3 Fehling's test

Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicated the presence of reducing sugars.

3.8.3 Detection of saponins

Saponins were detected as described in subsection 3.8.3.1 and 3.8.3.2

3.8.3.1 Froth test

Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of a layer of foam indicated the presence of saponins.

3.8.3.2 Foam test

Small amount of extract was shaken with little quantity of water. If foam produced persists for 1 minute it indicated the presence of saponins.

3.8.4 Detection of phytosterols

Phytosterols were detected by methods described in subsection 3.8.4.1, 3.8.4.2 and 3.8.4.3.

3.8.4.1 Salkowski's test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenoids.

3.8.4.2 Libermann Burchad's test

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added

carefully along the sides of the test tube. Formation of brown ring at the junction indicated the presence of triterpenoids.

3.8.4.3 Tshugajeu test

Extracts were treated with chloroform and filtered. Excess acetyl chloride and a pinch of zinc chloride was added, kept aside for some time till the reaction was complete and then warmed on water bath. Appearance of eosin red colour indicated the presence of triterpenoids.

3.8.5 Detection of resins

Resins were detected by Acetone water test. Extracts were treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicated the presence of resins.

3.8.6 Detection of phenols

Ferric chloride test was used to detect phenols. Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols

3.8.7 Detection of flavonoids

Flavonoids were detected as described in section 3.8.7.1 and 3.8.7.2

3.8.7.1 Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicated the presence of flavonoids.

3.8.7.2 Shinodas test

To the alcoholic solution of extracts, a few fragments of magnesium ribbon and concentrated HCl was added. Appearance of magneta color after few minutes indicates presence of flavonoids.

3.8.8 Detection of proteins

Proteins were detected through the following tests;

3.8.8.1 Xanthoproteic test

The extracts were treated with few drops of concentrated Nitric acid solutions. Formation of yellow colour indicated the presence of proteins.

3.8.8.2 Biuret test

The extracts were treated with 1 ml of 10% sodium hydroxide solution and heated. To this, a drop of 0.7 % copper sulphate solution was added. Formation of purplish violet color indicates the presence of proteins.

3.8.9 Detection of diterpenoids

Diterpenoids were detected by Copper acetate test: Extracts were dissolved in water and treated with few drops of copper acetate solution. Formation of emerald green colour indicated the presence of diterpenoids

3.9 Determination of antimicrobial activity

Disc diffusion was used to evaluate antimicrobial activities for plant extracts and the pastes while agar well diffusion was used with liquid samples.

3.9.1 Bacteria cultures

American type cultures collections (ATCC) from Kenya Medical Research Institute and clinical isolates from Kenyatta National hospital and department of Plant and microbial sciences of National University were used. They were *Staphyloccus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 6380, *Bacillus subtilis* ATCC 14579, *Candida albicans* ATCC 10231, *Esherichia coli* ATCC 25922, *S. mutans, Enterococcus faecalis*, ATCC 9790 and *Lactobacillus acidophilus.*

3.9.2 Medium preparation

The Muller Hinton medium was prepared by dissolving a weighed amount in distilled water and subjected to sterilisation in an autoclave at 121 ºC. About 20 ml of the

molten media were poured on sterile Petri dishes and allowed to set. For *Candida albicans*, Potato dextrose agar was used.

3.9.3 Inoculum preparation

The bacterial cultures were maintained on sterile nutrient agar medium made of Agar (20 g), peptone (5 g), beef extracts (3 g) and NaCl (3 g) in distilled water. They were frequently subcultured and incubated at 37 ºC for 72 hours. For assessment of efficacy, a fresh suspension of each culture was prepared in physiological saline solutions of 0.8 % NaCl in distilled water from a freshly grown agar slant. Each culture was streaked onto non inhibitory culture media and incubated overnight to obtain isolated colonies. Three to five well isolated colonies of the test organisms were selected from overnight growth using a sterile loop and emulsified in Mueller Hinton broth and Sabourands broth, respectively, and incubated overnight at 37 ºC to give a turbidity matching 0.5 McFarland turbidity ($1x10^8$ colony forming units /ml) as described by (Ayoola *et al.,* 2008). The 0.5 McFarland turbidity was prepared by adding 0.6 ml of 1% barium chloride solution to 99.4 ml of 1 % sulphuric acid solution and mixed thoroughly. For effective comparison the turbidity was liquored in similar test tubes employed to prepare the inoculums suspension. The bacteria suspension was then compared to 0.5 McFarland's standards suspensions. Respective broth was used as the diluent to adjust turbidity of the growth to match barium chloride suspension turbidity equivalent while Sabourands dextrose broth was used for *Candida albicans.* After adjusting the turbidity of the inoculum's suspension, an aliquot 100 µl of the

inoculums was spread onto Mueller Hinton agar (MHA) plates using a sterile bent glass rod (Rojas *et al.,* 2006; Ndungu, 2005).

3.9.4 Disc diffusion

Susceptibility of the test organism to crude extracts and herbal products was done according to Kirby-Bauer technique (Bauer, 1996; 1998). Using a paper punch (6 mm) filter paper discs of 6 mm diameter were obtained by cutting Whatman filter paper No. I. The discs were wrapped in aluminum foil and sterilized by autoclaving at 121 ºC for 15 minutes. One gram of dried plant extract was dissolved in 5ml in dimethylsulphoxide (DMSO) to make a final concentration of 200 mg/ml. A volume of 10 µl was titrated on the sterile paper discs. The coated discs were then laid separately on Petri dishes covered and dried in an incubator. The discs were aseptically transferred to the inoculated plates using sterile forceps. To select the appropriate positive controls from conventional antibiotic, standard antibiotics discs from Hi media, REF: 00211R-1PK100 octodiscs KGL ¼ Lot. No.0000123751 were placed on inoculated culture media. The antibiotic that displayed activities against several of the test microorganisms was used as the positive control. For negative and positive controls the paper discs were titrated with DMSO solvent and solution of septrin 200 mg a generic of Co-Trimoxazole broad spectrum antibiotic, respectively. For *C. albicans* the positive controls were set using fluconazole (200 mg). The plates were allowed to stand for about 30 minutes before incubation at 37 ºC for 18 hours as described by Pretorius *et al.* (2003).

3.9.5 Agar diffusion assay

For liquid samples, the modified agar well of diffusion assay (Perez *et al.*, 1997) was employed. The media were inoculated with the microorganism suspended in nutrient broth. Once the agar was solidified it was punched with 6 mm diameter wells using a sterile cork borer. The wells were filled with 10 µl of the test samples. Negative controls were set using sterile distilled water. Antimicrobial activity was evaluated by measuring the diameter zone of inhibition against the test organism. All the organisms that showed inhibition zone equal or greater than 7 mm were regarded as susceptible.

3.9.6 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Methods were adopted from Rajendra and Ramakrishnan (2009) and Kihara (2008). The plant extracts that showed significant antimicrobial actions was selected for determination of minimum inhibitory concentration. Stock solutions of 200 mg/ml of various plant extracts were prepared. This was double diluted to obtain various ranges of concentration ranging from 1.56 to 200 mg/ml. Equal volume of the various concentration of each extract and Mueller Hinton broth were mixed in micro-tubes to make up 0.5 ml of solution. 0.5 ml of McFarland standard of the organism suspension was added to each tube (Shahidi Bonjar, 2004).

The tubes were incubated aerobically at 37 ºC for 24 hours. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculum and the tube containing the growth medium and inoculums. The MBC was determined by sub culturing the test dilution on Mueller Hinton Agar and further incubated for 24 hours. The highest dilution that yielded no single bacterial colony was taken as the minimum bactericidal concentration (MBC) (Akinyemi *et al.*, 2005). This was carried out on some of the extracts with high antimicrobial activity and some of the highly sensitive organisms.

3.10 Toxicological studies

Toxicological studies were carried using laboratory animals as described in section 3.10.1. Only 10 of the herbal materials were evaluated for their toxicity. The rest of the products were in small quantities which were not enough to treat the animals for 21 days.

3.10.1 Experimental animals

Male albino Swiss mice 4 weeks old having a weight range of 18-24.8 g were provided by department of Zoology Kenyatta University. The animals were kept in polypropylene cages with saw dust beddings and maintained in standard laboratory conditions. All precautions were taken and animals handled according to the international biosafety guide lines.

3.10.2 Experimental design

A total of 72 male Swiss mice aged 4 weeks old of average weight 20.5 ± 0.7 g were acclimatized to the laboratory conditions for one week before commencement of the experiment. Standard pellet diet and water were given *ad libitum*. The food was provided by local manufacturers. The animals were maintained at room temperature with twelve hour day light and twelve hour of no light. At the end of one week the mice were weighed and randomly divided into 2 categories each of 36 animals. In category one the animals were randomly divided into 6 groups (control 1, MPP-4, MPP-5, MPP-6, MPP-7 and MPP-8) and in category two 36 animals were randomly divided into 6 groups (control 2, MPP-2, MPP-3, HP-1, HP-2, HP-5). Each mouse was numbered on the tail. Mice in the various groups were treated as shown in Table 2. The control groups were orally given physiological saline while the treatment groups were administered orally with 1000 mg/kg body weight of the extract for 21 days as described by Adebayo *et al.* (2010). The dosage of the extract were chosen from transition of traditional dosage using the equation below as described by Oyedemi *et al*. (2010).

$$\text{Dosage (mg/kg)} = \frac{\text{Vol of extract (ml) X concentration of the extract (mg/ml)}}{\text{Weight of the animal (kg)}}$$

The 1000 mg/kg body weight dose was selected on the basis that administration of an extract at higher dose than the therapeutic dose might be toxic. The administration of plant extract and physiological saline was done using the stomach

tubes. The animals were observed for the first 2 hours for any gross change in behavioral, neurological and any other symptom of toxicity and mortality. The animals' weights were taken after every seven days. Animals were given sufficient food and water each day.

Table 2. Herbal materials given to mice

Category	Group	Treatment
1 Herbal materials that are swallowed	Control group 1	Physiological saline
	1	MPP-4
	2	MPP-5
	3	MPP-6
	4	MPP-7
	5	MPP-8
2 Herbal materials applied atopically in the mouth cavity	Control group 2	Physiological saline
	1	MPP-2
	2	MPP-3
	3	HP-1
	4	HP-2
	5	HP-5

KEY- MPP-2 *S. didymobotrya,* MPP-3 *E.divinorum,* MPP-4 *Z. chalybeum,* MPP-5 *Warbugia ugandensis,* MPP-6 *T. brownii,* MPP-7 (a herbal mixture of *T. brownii, Z. chalybeum, W. ugandensis,* MPP-8 (a herbal mixture of *T. brownii, Z. chalybeum, W. ugandensis, A. indica).* HP-herbal paste

3.10.3 Determination of body weight

The body weight of each mouse was assessed during acclimatization period, before commencement of dosing, weekly during the dosing period and on the day of sacrifice.

3.10.4 Absolute organ weight and relative organ weight

At the end of the experimental period (4 weeks) all animals were euthanized and blood samples taken by cardiac puncture of each sacrificed animal. Blood samples were collected in K3-EDTA tubes. The blood was used to estimate

heamatological parameters. Different organs namely the heart, liver, lungs, spleen, kidneys, brain and testis were carefully dissected out, blotted dry and adipose tissue removed and weighed. Relative organ weight was determined as describe by Yakuba *et al.* (2008) using the following formula

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight X 100}}{\text{Body weight of the mouse at the day of sacrifice}}$$

Necropsy samples were collected and stored in 10% formalin. Tissues were processed using standard protocols of histopathology. The tissues were dehydrated in alcohol and embedded in paraffin cut into sections of 4-5 µm thick using a microtome and stained with haematoxylin and eosin for microscopic, observation (Saha *et al*., 2011).

3.10.5 Evaluation of heamatological parameters

The following haematological parameters: Total leukocytes counts, WBC-White blood cells, LYM-lymphocytes, HB-haemoglobin Mon–monocytes, GRA-granulocytes, RBC-red blood cells, MC-mean cell counts; MCH: mean corpuscular volume; MCHC-mean corpuscular haemoglobin concentration, RDW- red cell distribution width, HCT-haematocrit (Packed cell volume), MPV-mean platelet volume. PDW-plateletret distribution width; PCT-platelecrit were determined using a haemoanalyse Melet Schoesing Machine MS4 (France). Calibration was done using RD system CBC3D (USA). A five differential

Leucocyte counts for neutrophils, lymphocytes, granulocytes, monocytes and basophils was determined from stained blood films (Cheesbrough, 2000). Blood films stained with Geimsa stains were examined microscopically using magnification of X400 for differential WBC counts. Neutrophil (N), lymphocyte (L) and monocytes (M) absolute counts (number of cells$\times 10^9$) per litre were obtained by expressing their percent differential counts against the total WBC absolute counts (Cheesbrough, 2000).

3.10.5.1 Preparation of blood plasma

To obtain the plasma blood samples were centrifuged at 3000 rpm for ten minutes and clear plasma samples were aspirated off and stored frozen at -20º C. The collected plasma was analyzed for the following analytes: alanine aminotransferase, aspartate aminotransferase, creatine phosphokinase, lactate dehydrogenase and blood urea nitrogen.

3.10.6 Equipment used for analysis of biochemical parameters

The machine used for the sample analysis was Clinical Chemistry Autoanalyzer Olympus 640 or Olympus 400 system (Olympus Diagnostica GmbH, Hamburg, Germany). The Olympus is a discrete, Random Access Clinical Chemistry analyzer capable of performing a wide range of chemical tests in a single run.

3.10.7 Reagent preparation

All reagents for the auto analyzer machine were commercially prepared to fit the required volumes and concentration. Reagents used in Olympus auto analyzer machine were put in specific containers referred to as “reagent cartridges”. The reagent cartridges were bar-coded for the identification by the machine. User defined chemistry programme was used for those reagents, which were not bar corded.

3.10.8 Calibration of the test

To ensure that the values recovered from the plasma sample analysed were both accurate and precise, the machine performed a calibration procedure for the parameters. The purpose of the calibration procedure was to determine the relationship between measured absorbance (or in case of ion selective electrodes, voltage potential) to known concentration of these same analytes contained in calibrator solutions (such as Olympus multi-calibrator for analyzed parameters). Calibration factors were installed once the relationship was achieved.

3.10.9 Quality control materials

The assayed multisera normal was used for the quality control (QC) of the analytical work during the study. The QC multisera were supplied in lyophilised form and reconstituted as per the manufacturer’s preparation guide. For internal

quality control assessment, the prepared QC multisera was analysed daily or any other time samples for the study were being analysed.

3.10.10 Internal quality control for the studied parameters

An internal quality control (IQC) serum for specific parameter was included in each analytical session throughout the study period. Quality control results for the analyzed parameters were within the specific assigned QC range (Table 3) of target value ± 2 standard deviations (SD).

Table 3. Internal quality control for the biochemical parameters

Parameter (unit)	Assigned QC report			Study QC report		
	Session	QC range	Mean	Mean	SD	CV%
creat (µmol/L)	14	57-229	143	139	4.9	3.5
ALT (U/L)	14	19-31	25	25	3.8	16
AST (U/L)	14	21-31	26	24	3.8	16
CK (U/L)	14	158-239	199	173	8.5	4.9
LDH (U/L)	14	128-192	160	166	8.3	4.8

Key: ALT- Alanine amine transferase, AST-Aspartate aminotransferase Creat-Creatine kinase, LDH-lactate dehydrogenese SD-standard deviation and CV-Coeficient of variation

3.10.11 Analytical methods for routine biochemical parameters

The following biochemical parameters were analysed Creatine kinase, Alanine amino transferase, Lactate dehydrogenase, Aspartate aminotransferase and blood urea nitrogen.

3.10.11.1 Creatine kinase

This was an enzymatic kinetic Utra violet (UV) method for quantitative determination of creatine kinase (CK). CK reversibly catalyzed the transfer of a phosphate group from creatine phosphate to adenosine diphosphate (ADP), to give creatine and adenosine triphophate (ATP) as products. The ATP formed was used to produce glucose-6-phosphate and ADP from glucose. This reaction was catalyzed by hexokinase (HK) which required magnesium ions for maximum activity. The glucose-6-phosphate (G-6-P) was oxidized by the action of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) with simultaneous reduction of the coenzyme NADP to give NADPH and 6-phosphogluconate. The sample (3 µl) was reacted with 300 µl of reagent and the change in absorbance, due to reduction of NAD was monitored at 340 nm. This change was directly proportional to the concentration of CK in the sample and was used to calculate and express concentration in U/L. The reaction took place at 37 ^{o}C for three and half minutes.

$$CK + ADP \xrightarrow{CK} Creatine + ATP$$

$$ATP + glucose \xrightarrow{HK} ADP + G\text{-}6\text{-}P$$

$$G\text{-}6\text{-}P + NADP^{+} \xrightarrow{G6P\text{-}DH} 6\text{-}phosphogluconate + ADPH + H^{+}$$

3.10.11.2 Lactate dehydrogenase

This was an enzymatic kinetic UV test for the quantitative determination of Lactate dehydrogenase (LDH). In the reaction, LDH catalyzed the oxidation of lactate to pyruvate coupled with the reduction of NAD^+ to NADH. The sample (2 µl) was reacted with 40 µl of reagent and the change in absorbance due to reduction of NAD^+ was monitored at 340 nm. This change was directly proportional to the concentration of LDH in the sample and was used to calculate and express concentration in U/L.The reaction took place at 37 ^oC for three and half minutes.

$$\text{Lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

3.10.11.3 Blood urea nitrogen

Blood Urea Nitrogen (BUN) reagent was used to measure the concentration of urea by an enzymatic rate method. In the reaction urea was hydrolyzed by urease to ammonia and carbon dioxide. Glutamate dehydrogenase (GLDH) catalysed the condensation of ammonia and α–Ketoglutarate to glutamate with the concomitant oxidation of reduced β-nicotinamide adenine dinuelotide hydride (NADH) to oxidized β-nicotinamide adenine dinucleotide (NAD^+). 3 µl of sample was reacted with 300 µl of reagent and the change in absorbance was monitored at 340 nm, due to NADH oxidation. This change was directly proportional to the concentration of BUN in the sample and was used to calculate and express concentration in mmol/L.

The reaction took place at 37 ^{o}C for one minute.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{-Ketoglutarate} + NADH + H^+ \xrightarrow{\text{GLDH}} \text{glutamate} + NAD^+ + H_2O$$

3.10.11.4 Alanine aminotransferase

The Alanine aminotransferase **(**ALT) reagent was used to measure alanine aminotransferase in the sample by an enzymatic kinetic UV rate method. In the assay reaction, the ALT catalyzed the reversible transamination of L-alanine and α-ketoglutarate to pyruvate and L-glutamine. The pyruvate then reduces to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of β-Nicotinamide Adenine Dinucleotide Hydride (reduced form) (NADH) to β-Nicotinamide Adenine Dinuleotide Hydride (NAD^+). Pyridoxal-5-phosphate was required in this reaction as a cofactor that was required for transaminase activity by binding to the enzyme using Schiff-base linkage. The sample (10 µl) was reacted with 110 µl of the reagent. The change in absorbance was monitored at 340 nm and this change was directly proportional to the activity of ALT. The activity was calculated and expressed in U/L. The reaction took place at 37 ^{o}C for three minutes. The principle of the reaction is shown in the equation below

$$\text{L-Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate L-Glutamate}$$

$$\text{Pyruvate} + NADH + H^+ \xrightarrow{\text{LDH}} \text{L actate} + NAD^+$$

3.10.11.5 Aspartate aminotransferase

Aspartate aminotransferase **(**AST) reagent was used to measure aspartate aminotransferase activity by an enzymatic kinetic UV rate method. In the reaction aspartate aminotransferase catalysed the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) with the concurrent oxidation of reduced β-nicotinamide adenine dinucleotide (NAD). The sample (10 µl) was reacted with 110 µl of the reagent. The change in absorbance was monitored at 340 nm and this change was directly proportional to the activity of Aspartate activity and was calculated and expressed in U/L. The reaction took place at 37 ^{o}C for three minutes

The principle of the reaction is shown in the equation below

$$\text{L-Aspartate} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L-Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^{+} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^{+}$$

3.10.11.6 Determination of the activity of alkaline phosphatase

The alkaline phosphatase (ALP) reagent was used to measure ALP activity by kinetic method using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction, ALP catalyzed the hydrolysis of the colorless organic phosphate ester substrate (p-nitrophenylphosphate) to the yellow colored product (p-nitrophenol and phosphate). This reaction occurred at alkaline pH of 10.3. The ratio of the sample to reagent was 1 part sample to 50 parts reagents (5µL:250µL reagent). The absorbance was

measured at 410 nm and this change was directly proportional to the activity of ALP. The machine calculated and expressed the activity in IU/L.

The reaction took place at 37 ^{0}C for three minutes (International Federation of Clinical Chemistry, 1983). The principal of the reaction is as follows:

4-NPP + $H_2O \rightarrow$ 4-NPO + Phosphate

3.11 Data analysis

Data on plant part used and oral conditions treated were presented in tables and Charts. Diameter zones of inhibitions, organ, body weight and haematological and biochemical parameters were presented as mean ±S.E.M. Data were statistically evaluated by one-way analysis of variance (ANOVA) and *t*-test using Sigma plot software.

CHAPTER FOUR

4 RESULTS

4.1 Documentation of herbal materials

Documentation of herbal materials used in the management of oral health in Nairobi was done by use of questionnaire and informal discussions. The issues addressed were (a) Biodata of the research participants such as gender, age and education level; (b) Quality control aspects - storage of herbal materials, mode of transport and the site of the business (c) Plant and plant part used (d) oral condition treated and mode of application of herbal materials (e) preparation of herbal materials.

4.1.1 Biodata

Majority of the research participants interviewed were male (72 %) (Figure. 2) while female respondents were 28 %. Most of the respondents were in age group 21-50 years while 18.5 % were above 50 years. Forty percent of participants were illiterate while 10 % had tertiary education (Figure 2). Most (93.4 %) of the participants declined to state where and how they acquired training as traditional medical practitioners.

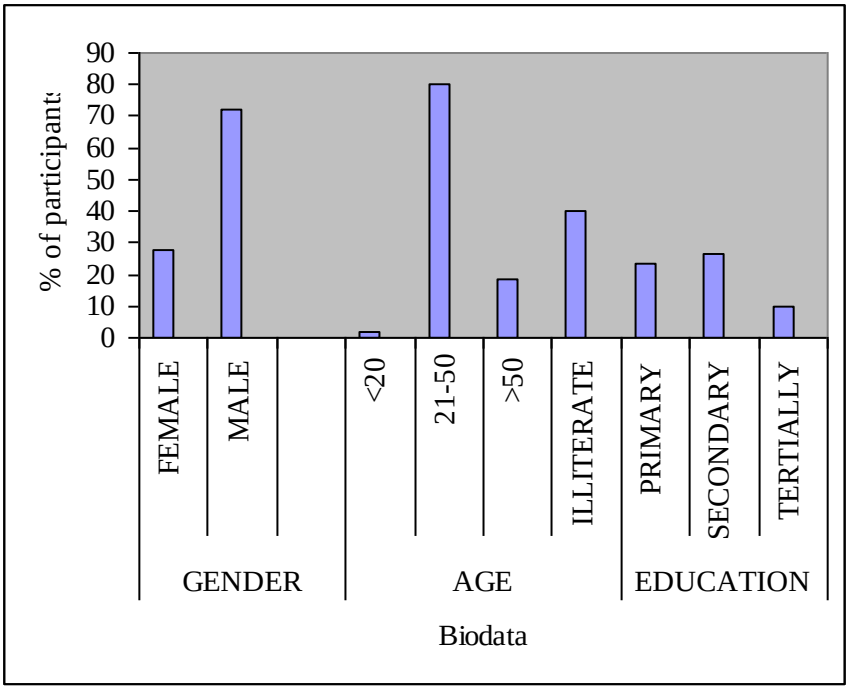


Figure 2. Biodata of the research participants

4.1.2 Quality control aspects

Ten percent of the research participants (Figure 3) had their business set up in hygienic conditions, mainly clinic. Most of the businesses (58.3 %) were located near a crowded area while 36.7 % were next to running sewer and 15 % were found near a disposal pit. In Gikomba, several traders were found along Pumwani road near Nairobi River Bridge, a crowded and polluted area. In Kangemi respondents were mainly found in the market area along Waiyaki Way near a disposal pit. Hawking of the chewing sticks in Eastleigh was concentrated in the 6th street, a crowded and dusty place. In Kibera, respondents were in Kibera Silanga area, next to Nairobi dam; an area where human waste including excreta are disposed. Majority of the practitioners use public means for the transportation of their materials. The participants from the clinic declined to give the mode of transport.

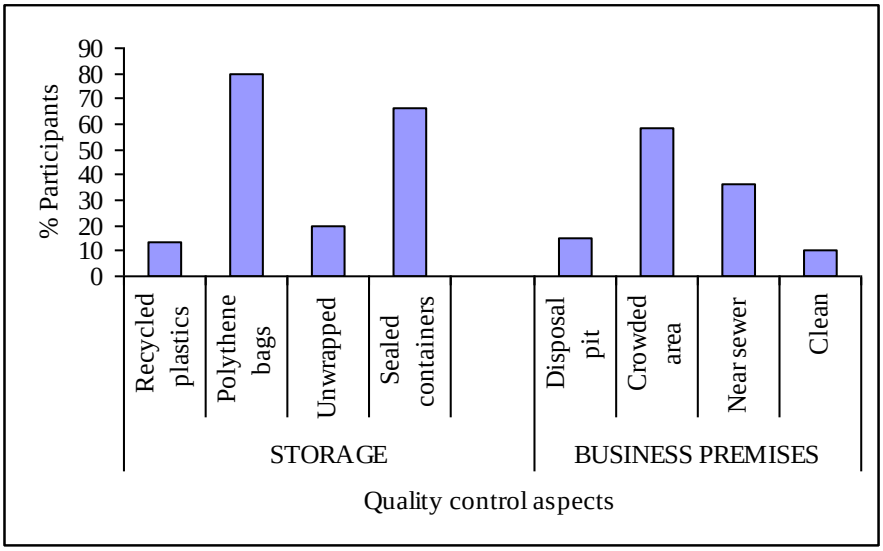


Figure 3. Storage and business premises of herbal materials

4.1.3 Plants and plant part used in preparation of herbal materials

A total of 35 plant species distributed in 24 families (Table 4 and Figure 4) were identified as being used in preparation of herbal powders, suspensions and pastes. The family with the highest number of plants was Solanaceae (14.72 %), followed by Laminaceae (11.4 %), Mimosaceae (8.6 %) while Liliacea and Asteraceae had 5.7 % each (Figure 4). All the other families had one plant each (Figure 4). *Warbugia ugandensis* was regarded as useful by 75 % of the respondents followed by *Euclea divinorum*. Other plant species such *Datura stramonium* and *Clematis hirsuta* were mentioned only once. Various plant parts were used for preparation of oral health products (Figure 5) but roots were highly utilized at 34.28 %, followed by leaves at 31.4 %, bark at 14.3 %, fruits and seeds at 8.6 % each. Plant sap was the least utilized at 2.8 %. Herbs were used as chewing sticks, seeds, herbal pastes, herbal powders, capsules, suspensions, saps, mixtures, or just unprocessed materials. About

31.4 % of the mentioned plants were used to manage toothache (Figure 6), 22.8 % for mouth ulcers, 17.1 % for tonsillitis, 11.4 % for gum bleeding, 8 % for mouth odour and 2.8 % for tooth sensitivity. Most of the plants mentioned (82.8 %) were used to manage one oral health condition. The most common mode of application is chewing (31.4 % of the plants mentioned) while 28.5 % of the plants were used in combination with others plants.

Table 4. Medicinal plants used for management of oral health in Nairobi County, Kenya

	Family	Plant species	Local name (Kikuyu)	Collection number.	Part/s used	Conditions treated	Application
1	APOCYNACEAE	*Carissa edulis* (Forssk)Vahl	Mukawa	309	Roots	Tooth ache	Chew and gurgle
2	ASCLEPIADACEAE	*Mondia whityei* (Hook.f.) Skeels	Mukombero	316	Roots	Tooth ache	Chew and gurgle
3	ASTERACEAE	*Bidens pilosa* L.	Muchege	306	Leaves	Mouth ulcers	Chew
4	ASTERACEAE	*Tagetes minuta* L.	Mubangi	311	Leaves	Tooth ache	Leaves are chewed and directed on aching tooth
5	BALANITACEAE	*Balanites aegyptiaca* (L.) Del.	Muhugu	325	Stem bark, roots	Mouth ulcers	Boil a handful of powder and drunk
6	CAESALPININCEAE	*Senna didymobotrya* Fressen	Mwinu	296	Leaves Roots	Mouth ulcers	A handful of the powder is boiled and gurgled
7	CANELLACEAE	*Warbugia ugandensis* Sprague	Muthiga	291	Stem bark	Tooth ache	Powder applied on aching tooth, Mixed with other plants (Table 5)

Table 4 (continued) Medicinal plants used for management of oral health in Nairobi County, Kenya

	Family	Plant species	Local name	Collection number.	Part used	Conditions treated	Application
8	COMBRACEAE	*Terminalia brownii* Fressen	Muuku	318	Root powder	Tooth ache, tonsillitis	Combined with other plants (Table 5)
9	CUPRESSACEAE	*Juniperus procera* Endl.	Mutarakwa	293	Bark stems	Gum bleeding	Charcoal applied on gums and teeth
10	EBENACEAE	*Euclea divinorum* Hien	Mukinyii	292	Roots	Gum bleeding	Chewing stick, place powder on tooth
11	FABACEAE	*Medicago sativa* L.	Lucern	300	Leaves	Tonsillitis	Combined with other plants (Table 5)
12	FLACOURTIACEAE	*Dovyalis abyssinica* (A. Rich) Warb	Mukambura	294	Roots	Tonsillitis	A handful of dried roots is boiled and drunk
13	LAMIACEAE	*Ajuga remota* Benth	Wanjiru wa Kieni	315	Leaves	Mouth ulcers	Chew and gurgle
14	LAMIACEAE	*Mentha piperita* L.	Mint	302	Leaves	Gum bleeding	Combined with other plants
15	LAMIACEAE	*Plectranthus barbatus* L' Herit	Muigoya	295	Stems	Mouth ulcers	Chew the stems and gurgle

Table 4 (continued) Medicinal plants used for management of oral health in Nairobi County, Kenya

	Family	Plant species	Local name	Collection number	Part used	Conditions treated	Application
16	LAMIACEAE	*Rosemarinus officinalis* L.	Rosemary	327	Leaves	Bad mouth breath	Used with five other plants to make mouth gurgles and pastes (Table 5)
17	LAURACEAE	*Persea americana* Mill.	Mukorobia	301	Seeds	Tooth ache	Mixed with stem bark of *W. ugandensis bark* powder and apply on the tooth (Table 5)
18	LILIACEAE	*Aloe secundiflora* Engler	Kiruma	307	Leaves	Mouth ulcers	Squeeze juice and apply on aching tooth
19	LILIACEAE	*Aloe vera*	Kiruma	324	Sap	Mouth ulcers and tooth ache	Used to make tooth paste
20	MELIACEAE	*Azadirachta indica* A. Juss	Mwarumbaine	319	Leaves	Tonsillitis	Combine with other
21	MIMOSACEAE	*Acacia nilotica* (L.) Del	Muruai/ Ngiruriti	305	Roots	Gum bleeding	Chew and gurgle
22	MIMOSACEAE	*Acacia seyal* Del	Mugaa	303	Bark	Gum bleeding	Chew and gurgle

Table 4 (continued) Medicinal plants used for management of oral health in Nairobi County, Kenya

	Family	Plant species	Local name	Collection number	Part used	Conditions treated	Application
23	MIMOSACEAE	*Acacia xanthophloea* Benth.	Murera	304	Stem	Tooth cleaning	Tooth brush
24	MYSINACEAE	*Myrsine africana* L.	Mugaita	299	Seeds	Bad mouth breath	Five table spoonful of powdered seeds are put in water and drunk
25	OLEACEAE	*Olea europaea* Linn.	Mutero	297	Stems	Mouth cleaning	Chew and gurgle /tooth brush
26	POLYGONACEAE	*Rumex usambarensis* (Damm.)Damm	Mugagatio	298	Leaves	Tonsillitis	Powder is mixed with powders from other five plants for mouth ulcers and tonsillitis
27	RANUNCULACEAE	*Clematis hirsuta G*uillemin &Perr	Mugaya ngundu	320	Roots	Tooth ache	Chew and gurgle
28	ROSACEAE	*Prunus africana* (Hook.f.)Kalkam	Muiri	314	Bark	Tooth sensitivity	Apply powder on aching tooth
29	RUTACEAE	*Zanthoxylum chalybeum* Engler	Mugucua	313	Roots	Tooth ache, gum problem, tonsillitis	Chew and gurgle, root powder mixed with other plants for tonsillitis (Table 5)

Table 4 (continued) Medicinal plants used for management of oral health in Nairobi County, Kenya

	Family	Plant species	Local name	Collection number	Part used	Conditions treated	Application
30	SALVADORACEAE	*Salvadora persica* Linn	Mswaki	308	Stems, roots	Cleaning of mouth	Chewing sticks
31	SOLANACEAE	*Capsicum annuum* L.	Pepper	310	Fruits	Mouth ulcers	The powder of dried fruits is mixed with other plants (Table 5).
32	SOLANACEAE	*Datura stramonium* L.	Magurukia	312	Seeds	Tooth ache	Apply a drop of cold infusion of powdered seeds In tooth cavity
33	SOLANACEAE	*Solanum aculeastrum* Dunal	Mutura	321	Fruit Roots	Tooth ache	Chew the roots, fruit are burnt and mouth smoked
34	SOLANACEAE	*Solanum incanum* L.	Mutongu	322	Fruits Root	Tooth ache	Apply juices on tooth
35	SOLANACEAE	*Withania somnifera* (L.) Dunal	Murumbae	326	Leaves	Bad mouth odour, tonsillitis	Mixed with plant powders (Table 5)

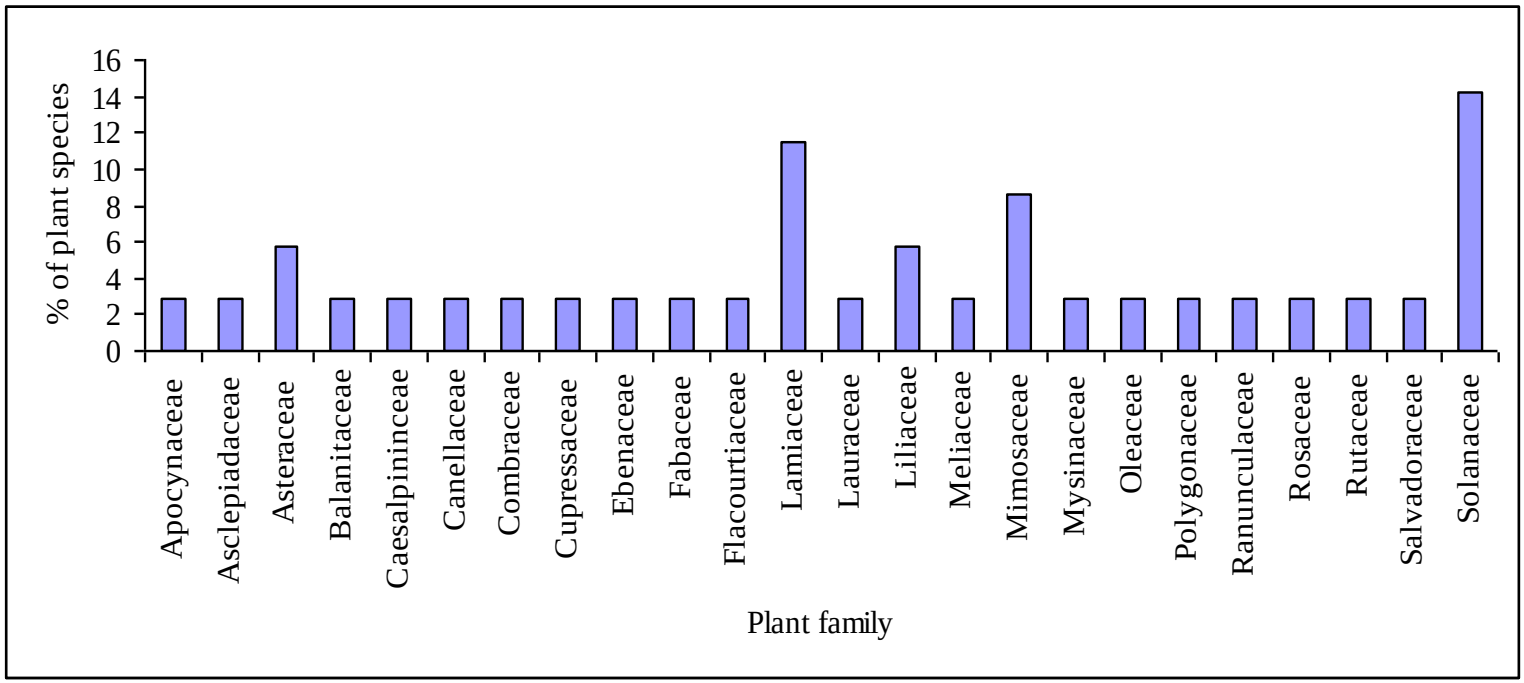


Figure 4. Distribution of plants species by families used for management of oral health condition.

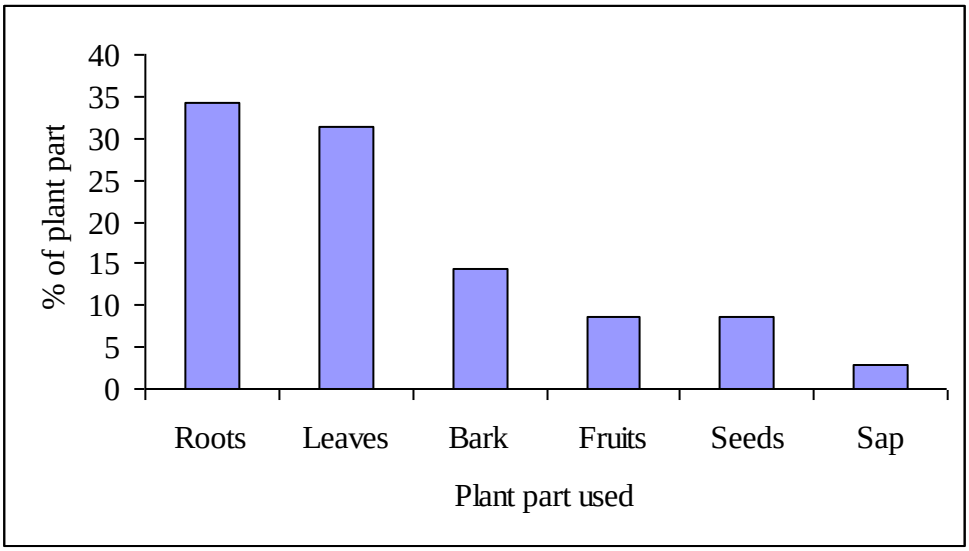


Figure 5. Plant part used in management of oral health

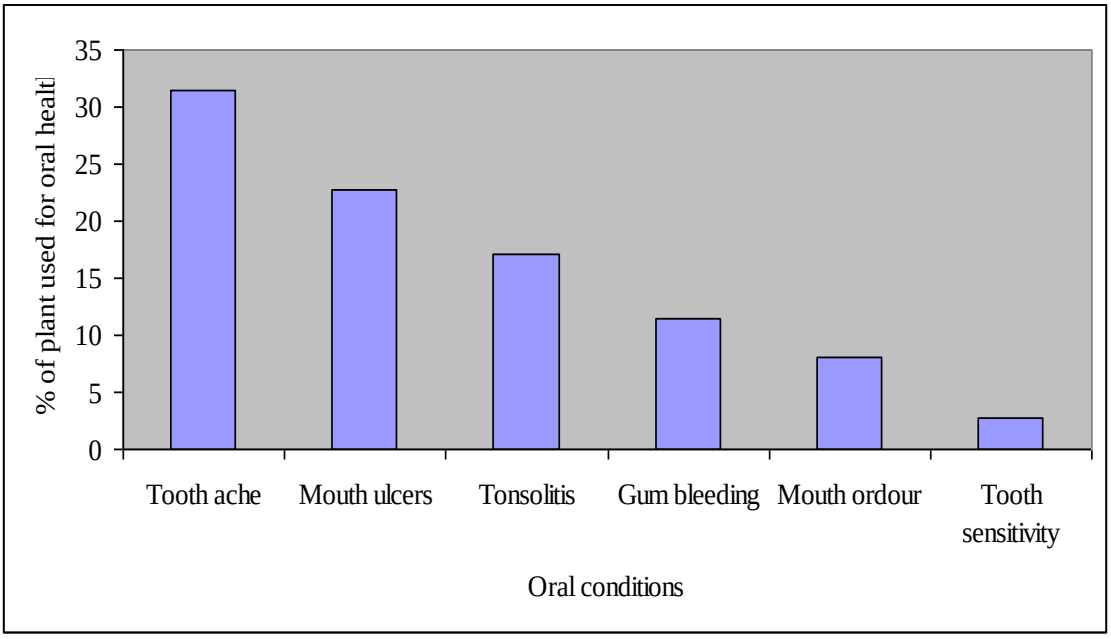


Figure 6. Percentage number of plant species used in management of oral health

4.1.4 Preparation of herbal materials

Plants were used singly or in combination for management of oral health (Table 5). Capsules were prepared by mixing W. ugandensis stem bark, M. sativa leaves, R. usamabarensis leaves, Z. chalybeum roots while antiulcers were prepared by burning different plant parts and making a fine powder. Other herbal mixtures were made by mixing powders of *W. ugandensis* stem bark, *A. indica* leaves, *Z. chalybeum* roots and *T. brownii* stem for management of several oral conditions (product MPP-7 and MPP-8). In this study, five types of herbal pastes (Table 6) and ten types of herbal suspensions (Table 7) were recorded mainly in the streets and clinics. Some of the suspensions were clearly labeled and ingredients indicated. The extracts of *Aloe vera* plants, Clove (*S. guinesse*), mint (*M. piperita),* Tea tree and Chamomile extracts (Table 6) are added to the tooth paste. The major ingredient noted was *W. ugandensis* in 60 % of herbal suspensions. Other ingredients included were *M. piperita* (mint), *R. officinallis* (Rosemary), *W. sominifera* (Table 7). Only 14 % of the products had Kenya Bureau of Statistics (KEBS) mark of quality (Table 7).

Table 5. Herbal materials used for oral health in Nairobi County, Kenya as reported by herbalists

Code	Plant and parts used	Indications	preparation
MPP-1	*Clematis hirsute* root cutting	Tooth ache	Root cuttings
MPP-2	*S. didymobotrya* leaves, roots	Mouth ulcers	Powder
MPP-3	*E. divinorum roots*	Gum bleeding	Chewing sticks
MPP-4	*Z. chalybeum* root	Tooth ache	Powder
MPP-5	*W. ugadensis* bark	Tooth ache	Powder
MPP-6	*T. brownii* root	Tooth ache tonsillitis	Powder
MPP-7	*W. ugandensis* bark, *T. brownii, Z. chalybeum*	Gum bleeding, tonsillitis, teeth problem	Powder
MPP-8	*W. ugandensis* bark, *Z. chalybeum, A. indica T. brownie*	Gum bleeding, tonsillitis, teeth problem	Powder
HM-3	*W. ugandensis* bark, *M. sativa* leaves, *R. usamabarensis,* leaves *A. indica* leaves	Tonsillitis	Capsule
HM-4	*C. annuum* fruits. *W. ugandensis*, iodine, black charcoal	Antibacterial	Powder (charcoal powder)
HM-5	*J. procera* stem *E. divinorum* roots, *C. edulis* roots	Ulcers	Charcoal

MPP- medicinal pant powder; HM- herbal mixture

Table 6. Herbal paste used for oral health in Nairobi County, Kenya as reported by herbalists

Product	ingredients	Indications	MQ	package
HP-1	*Aloe vera* gel	Antibacterial	No	Plastic tube
HP-2	*W. ugandensis* bark	Antibacterial	YES	Plastic paper bags
HP-3	Tea tree, (*Camellia sinesis*) sage (*Salvia officinalis)*, chamomile (*Matricaria chamomilla*), *Eucalyptus*	Antibacterial	YES	Plastic tube
HP-4	*S. guinesse* (Clove) oil, wintergreen oil, cinnamon (*Cinnamomum verum*), silica	Antibacterial	YES	Plastic tubes
HP-5	*W. ugandensis, M. piperita (mint)* leaves and ginger rhizome (*Zingeber officinale*), *P. american* (seeds)	Pain killer	NO	Polythene paper bags

Key: MQ: KEBS-mark of quality; HP- herbal paste

Table 7. Types of herbal suspensions used for oral health in Nairobi County, Kenya

Product	ingredients	Indications	MQ	package
HS-1	*W. ugandensis* bark extracts	Bad mouth breadth, antibacterial	YES	Air and light tight plastic container
HS-2	Herbal oil	Painkiller, antibacterial	NO	’’
HS-3	*W. ugandensis*, bark extracts	Antibacterial, heals gum disease	NO	’’
HS-4	*W. ugandensis*, *M. piperita* (mint) and *S. guinesse (*clove*)*	Antibacterial, bad mouth breath	NO	’’
HS-5	*A. vera* gel, *W. ugandensis*, *W. sominifera*	Antibacterial	NO	’’
HS-6	*W. ugandensis bark* and Rosemary leaves extracts	Antibacterial	NO	’’
HS-7	*W. ugandensis* leaves *M. piperata* (mint) leaves and *S. guinesse (*clove) oil	Antibacterial, heals gum disease	NO	’’
HS-8	*(Mweithia) sap*	Ulcers, gum bleeding	-	Plastic container
HS-9	*Aloe vera* gel *W. sominfera* and *Echenecea* species	Antibacterial, antifungal, bad mouth breath	NO	Brown dark bottle
HS-10	*W. ugandensis* bark *C. annuum* fruits extracts	Antibacterial antifungal	NO	Brown dark bottle

HS- herbal suspension MQ- KEBS mark of quality

4.2 Levels of inorganic elements in herbal materials

Presence of elements in herbal materials was determined through Total Reflection X-ray Fluorescence (TXRF).

4.2.1 Levels of elements in herbal powders

From TXRF elemental analysis, aluminum levels were highest in MPP- 3 (*E. divinorum*) (Table 8) (487.45 mg/kg) while *Z. chalybeum* (MPP-4) and MPP- 2 (*S. didymobotrya*) had more than 400 mg/kg (Table 8) each. All the other powders had aluminium below detectable levels (<16 mg/kg). The highest level of phosphorous was recorded in *T. brownii* powder (MPP-6) (2148.21 mg/kg) and the lowest amount was reported in *C. hirsuta* (MPP- 1) 527.86 mg/kg. Sulphur levels ranged from 362.0 mg/kg in *C. hirsuta* (MPP-1) to 713.87 mg/kg in *W. ugandensis* (MPP-5). High levels of potassium were recorded in *W. ugandensis* (MPP-5) (7953.97 mg/kg) and MPP-8; herbal mixtures of *W. ugandensis, Z. chalybeum, A. indica and T. brownii*) of 7513.21 mg/kg.

All the herbal powders had high levels of calcium ranging from 7087.24 mg/kg in *E. divinorum* (MP 3) to 51295.90 mg/kg in *T. brownii* (MPP- 6). Titanium was present in all the powders but in low levels compared to other elements. Vanadium was detected in powders in ranges of (4.35 mg/kg to 29.46 mg/kg) but it was not recorded in MPP-1 (*C. hirsuta*) and MPP-5 (*W. ugandensis*). Chromium was present in MPP-2 (*S. didymobotrya)* and MPP-3 (*E. divinorum*) but absent in all other powders. The highest level of manganese (87.80 mg/kg) was seen in MPP-8,

herbal mixture of *W. ugandensis, Z. chalybeum, A. indica and T. brownii*. Amount of iron varied from 247.66 mg/kg in MPP-6 (*T. brownii*) to 1204.90 mg/Kg in MPP-5 (*W. ugandensis*). Low levels of nickel (0.47-2.13 mg/kg) were reported in MPP- 8, MPP-2 (*S. didymobotrya)* and MPP-6 (*T. brownii*). The highest level of copper recorded was 8.89 mg/kg in MPP- 1 and lowest amount was 1.52 mg/ kg in MPP- 3. Zinc levels ranged from 6.49 mg/Kg to 15.36 mg/kg (MPP- 5). Sample MPP- 4 had the highest level of bromine with 4.82 mg/kg. Rubidium level varied from 16.6 mg/kg in MPP-1 to 31.68 mg/kg in MPP-5. All the samples analysed had strontium levels between 236.45 mg/kg-1168.58 mg/kg. The concentration of lead varied from 1.98 mg/kg in MPP-5 (*W. ugandensis*) to 6.2 mg/kg in MPP-6 (*T. brownii*). The amount of phosphorous, calcium, manganese, iron, copper and lead exceeds the RDA (Table 9).

4.2.2 Elemental analysis of herbal suspensions used in oral health care

Aluminium was detectable in one product only at (392.03 mg/l (Table 10). In all the other herbal suspensions, aluminium was below detectable levels or limits. Calcium was detected in all the liquid samples analysed. Manganese, copper, lead, iron, rubidium, zinc and strontium were present in all the products but in varying concentrations. Amount of iron ranged from 0.21-4.09 mg/l; copper 0.01-0.032 mg/l, zinc, 0.05-0.07 mg/l and rubidium 0.07-1.17 mg/l (Table 10). Product HS-4 had the highest level of phosphorus (549.85 mg/l). Sulphur was present in all the products apart from herbal oil (HS-2). From daily dosage estimates of herbal suspension, the products did not exceed RDA (Table 11).

4.2.3 Element analysis of herbal paste products used in oral health

Mineral elemental analysis of herbal paste products (Table 12) indicated the presence of all the investigated elements in product (HP-5), a herbal mixture of *W. ugandensis*, *P. americana* seeds and ginger. Herbal paste HP-1 had 50% of the mineral elements analysed and in addition had the highest amount of lead (13.14). Product HP-2 (*W. ugandensis* based herbal product) had the highest amount of phosphorous (70885.92 mg/kg) and strontium (286.54 mg/kg). In herbal paste HP-4, levels of vanadium, bromine, strontium and rubidium were below detectable levels. The highest elemental component recorded in product HP-3 was sulphur with 1336 mg/kg. This product had the lowest levels of calcium (30.12 mg/kg). The amount of elements likely to be taken daily was estimated from the prescribed dosage. Table 13, shows the amount of elements likely to be consumed by a person using herbal powders. Results indicate that several pastes had a mineral content that exceeded the RDA.

Table 8. Elemental profiles of herbal powders used in management of oral health in Nairobi County, Kenya

Element	Herbal powders			
	MPP-1	MPP-2	MPP-3	MPP-4
Aluminium	<16	482.08±106.70	487.45±78.09	<16
Phosphorus	527.86±18.02	1026.65±31.77	600.74±61.91	1009.77± 97.90
Sulphur	362.00 ±11.27	410.72±11.28	459.61±20.59	503.27±24.86
Potassium	2994.26±46.29	3939.80±31.19	3175.78±95.09	4867.03±110.94
Calcium	2284.87±21.80	3599.70±19.97	7087.24±167.27	18387.49±423.60
Arsenic	<0.30	<0.30	<0.30	6.85±1.04
Titanium	37.25±3.67	164.89±8.71	85.73±5.64	84.79 ±2.96
Vanadium	<0.20	5.54 ±0.55	4.78±1.69	29.46±2.41
Chromium	<0.01	4.00±1.37	3.16±1.03	<0.010
Manganese	21.54±0.59	56.19±1.39	87.80±2.10	60.58±2.60
Iron	288.40±10.97	1204.90±27.2	826.88±28.82	446.82±17.30
Nickel	<0.05	0.47±0.05	<0.05	<0.05
Copper	8.89±0.07	3.83±0.08	1.52±0.18	4.47±0.08
Zinc	12.63±0.02	13.85±0.04	6.49±0.32	8.46±0.20
Bromine	0.47±0.11	31.61±0.16	1.87±0.31	4.82±0.29
Rubidium	16.66±1.19	20.89±0.89	18.61±1.21	18.04±1.38
Strontium	236.45±38.22	265.71±26.22	345.19±28.32	438.88 ±39.60
Lead	3.74±0.45	5.00±0.64	3.79±1.42	3.44±0.78

Results are expressed as mean ± stdev of three replicates per herbal powder and expressed mg/kg, Key: product code refer to Table 5

Table 8. (Continued)

Element	Herbal powders			
	MPP-5	MPP-6	MPP-7	MPP-8
Aluminium	440.30±62.52	<16	<16	<16
Phosphorus	1278.36±48.50	2148.21±103.21	1620.0±83.90	1451.23±18.10
Sulphur	713.87±53.38	471.43±6.13	603.70±44.70	631.70±11.60
Potassium	7953.97±173.20	4369.25±37.00	6221.09±0.22	7513.21 ±71.80
Calcium	21921.43±461.20	51295.90±1023.74	33678.91±1206.70	27596.32±306.8
Arsenic	<0.30	28.28±6.49	10.79±3.20	8.58±1.56
Titanium	111.99±2.76	69.28±4.07	96.84±5.20	66.20±2.79
Vanadium	4.35±1.26	21.03±1.67	20.72±8.20	15.24±6.08
Chromium	<0.10	<0.10	<0.10	<0.10
Manganese	54.94 ±1.33	46.58±1.57	51.59±2.92	82.09±2.96
Iron	561.28±20.6	247.66±11.15	437.20±23.50	356.74±12.73
Nickel	<0.05	2.13±0.93	<0.05	1.05±0.11
Copper	2.99±0.46	2.77±0.20	3.51±0.20	3.98±0.71
Zinc	15.36±0.66	7.72±0.67	11.69±0.10	10.69±0.34
Bromine	1.31±0.06	<0.02	2.92±0.31	4.37±0.17
Rubidium	31.68±1.09	16.69±2.62	25.29±2.8	23.58±1.64
Strontium	471.92±25.66	1168.58±68.82	834.70±64.1	694.88±58.22
Lead	1.98±1.33	6.20±0.25	4.71±2.48	3.42±0.41

Table 9. Amount of elements mg) taken daily from the herbal powders

Element RDA (mg)	MPP-1 *(4.10g)	MPP-2 (4.10g)	MPP-3 (2.48g)	MPP-4 (9.67g)	MPP- 5 (5.33g)	MPP-6 (7.60g)	MPP-7 (6.15g)	MPP-8 (6.15g)
Al	65	1976.51	1208.79	59.47	2346.89	121.60	98.40	98.24
P – 800	2164.21	4209.27	1489.85	9764.43	6813.66	16326.40	9962.94	8910.58
S -	1485.57	1683.93	1139.82	4866.57	3804.93	3582.83	3712.97	3878.64
K -2000	12276.47	16153.20	7875.90	47064.13	42394.66	33206.30	38259.70	46131.39
Ca- 800	9367.97	14758.81	17576.34	177807.00	116841.20	389849.5	207119.10	169441.4
Ar-	1.23	1.23	0.74	66.19	1.60	214.89	66.37	52.65
Ti-	152.70	676.05	212.60	819.10	596.91	526.49	595.57	406.44
V-	0.82	22.74	11.85	284.89	23.16	159.79	127.40	93.54
Cr-	0.41	16.40	7.82	0.97	0.53	0.76	0.62	0.62
Mn-2	88.30	230.38	217.74	585.76	292.80	353.97	317.25	504.03
Fe- 10	1182.58	4940.15	2050.66	4320.70	2991.65	1882.22	2688.69	2190.35
Ni -	0.21	1.94	0.124	0.48	0.27	16.19	0.31	6.48
Cu-1.5	36.43	15.70	3.76	43.23	15.91	21.05	21.59	24.41
Zn-12	51.76	56.79	16.10	81.76	81.87	58.63	71.86	65.64
Br -	1.91	14.80	4.64	46.61	6.98	0.15	17.96	26.83
Rb-	68.29	85.63	46.15	174.45	168.83	126.84	155.53	144.75
Sr-	969.45	1089.41	856.06	4243.97	2515.36	8881.17	5133.37	4266.53
Pb- 3*	15.31	20.48	9.40	33.22	10.53	47.08	28.97	21.00

RDA- required daily allowance *( ) Dosage determined from daily prescription from the herbalists ,* weekly maximum allowed levels

Table 10. Elements profiles of liquid herbal suspensions used in management of oral health in Nairobi County, Kenya

	Herbal suspensions				
Element	HS-1	HS-2	HS-3	HS-4	HS-5
Aluminum	< 16	< 16	< 16	< 16	< 16
Phosphorus	112.45±2.25	<3.32	4.56±0.40	549.09±7.5	420.7±8.3
Sulphur	2.18±0.23	< 2.10	3.97±0.2	4.46 ±0.28	1.14±0.03
Potassium	6.47±0.30	0.23±0.03	54.51±0.17	6.45±0.1	1.98±0.04
Calcium	4.34±0.08	0.22±0.03	5.72±0.05	5.77±0.36	4.78±0.11
Arsenic	< 0.30	< 0.30	< 0.30	< 0.30	< 0.30
Titanium	0.09±0.02	< 0.32	0.08±0.03	0.03±0	0.04±0.0
Vanadium	< 0.01	< 0.02	<0.02	0.02±0	<0.02
Chromium	0.03±0.01	< 0.11	<0.11	<0.11	0.02±0.03
Manganese	0.05±0.01	<0.05	0.79±0.00	0.08±0.01	0.09±0.00
Iron	0.75±0.12	0.21±0.05	1.14±0.01	0.55±0.02	0.48±0.01
Nickel	0.09±0.01	< 0.05	< 0.05	<0.05	< 0.05
Copper	0.01±0.0	0.02±0.00	0.03±0.0	0.013±0	0.01±0.0
Zinc	0.05±0.0	0.05±0.02	0.18±0.0	0.09±0	0.06±0.0
Bromine	0.01±0.01	< 0.023	0.03±0.0	0.02±0	0.01±0.0
Rubidium	0.03±0.00	0.21±0.09	0.23±0.0	0.07±0	0.05±0.01
Strontium	0.39±0.04	4.86±1.90	1.37±0.05	1.0±0.05	0.75±0.16
Lead	0.02±0.0	0.11±0.03	0.02±0.01	0.05±0.01	0.05±0.00

Results are expressed as mean ± standard deviation of three replicates per herbal suspension and as expressed as mg/L Key; product code Refer to table 7

Table 10 (continued)

	Herbal suspensions				
Element	HS-6	HS-7	HS-8	HS-9	HS-10
Aluminum	< 16	< 16 y	< 16	392.03±11.24	< 16
Phosphorus	3.32±0.27	24.64±0.75	11.89±0.68	179.85±0.86	<3.32
Sulphur	4.20±0.05	8.64±0.72	6.00±0.30	2336.73±48.5	4.04±0.26
Potassium	45.22±0.05	188.27±0.95	71.06±48.19	189.82±2.87	41.07±0.4
Calcium	2.96±0.11	40.19±2.49	42.59±2.88	16.48±0.47	10.36±0.1
Arsenic	< 0.30	< 0.30	< 0.30	< 0.30	< 0.30
Titanium	0.07±0.01	0.64±0.09	0.29±0.47	< 0.32	0.052±0.0
Vanadium	<0.02	< 0.02	< 0.02	< 0.02	< 0.02
Chromium	<0.11	<0.02	<0.02	0.139±0.02	<0.02
Manganese	0.74±0.02	0.74±0.01	0.1±0.02	0.86±0.34	0.34±0.01
Iron	0.96±0.04	4.09±0.12	0.60±0.34	2.21±0.06	1.85±0.05
Nickel	< 0.05	0.12±0.01	0.01±0.06	< 0.05	<0.05
Copper	0.02±0.0	0.03±0.0	0.01±0.0	0.03±0.00	0.02±0.
Zinc	0.11±0.00	0.050±0.0	0.04±0.00	0.20±0.021	0.07±0.00
Bromine	0.03±0.00	0.14±0.01	0.13±0.02	0.27±0.02	0.05±0.0
Rubidium	0.23±0.01	0.30±0.01	0.08±0.09	1.17±0.09	0.14±0.0
Strontium	2.08±0.13	3.29±0.15	0.53±1.56	8.65±1.56	2.37±0.3
Lead	0.02±0.01	0.03±0.01	0.01±0.08	0.11±0.01	0.05±0.01

Table 11. Amount of mineral elements taken daily (µg/day) from the herbal suspensions used in management of oral health in Nairobi County, Kenya

Element	RDA (mg)	HS- 1 **(10ml)**	HS- 2 **(0.15ml)**	HS- 3 **(10mls)**	HS- 4 **(10ml)**	HS- 5 **(0.15ml)**
Al	-	160	2.4	160	160	2.4
P	800	1125	0.5	45.6	5491	63.11
S	-	21.8	0	39.7	44.62	0.17
K	2000	64.7	0.04	545	64.53	0.30
Ca	800	43.4	0.03	57.2	57.75	0.72
Ars	-	3.0	3.0	3.0	3.0	0.0
Ti	-	0.91	0.01	0.79	0.3	0.01
V	-	2	0.03	2	2	0.03
Cr	-	0.32	0	0.1	0.1	0
Mn	2	0.48	0	7.93	0.69	0.01
Fe	10	7.49	0.03	11.40	5.48	0.07
Ni	-	0.94	0.01	0.5	0.08	0.01
Cu	1.5	0.1	0	0.32	0.13	0
Zn	12	0.47	0.01	1.17	0.89	0.01
Br	-	0.13	4.00	0.33	0.20	4.00
Rb	-	0.33	0.03	2.30	0.69	0.01
Sr	-	3.93	0.73	13.70	10.06	0.11
Pb	3*	0.23	0.02	0.23	0.48	0.01

RDA required daily allowance are expressed in mg per day * weekly maximum allowed levels.

( ) is the amount of herbal suspension prescribed by the herbalist
(mls) KEY: product code refer to Table 7

Table 11. (continued)

Element	RDA (mg)	HS- 6 **(0.15ml)**	HS- 7 **(0.15ml)**	HS- 8 **(10ml)**	HS- 9 **(10ml)**	HS- 10 **(10mls)**
Al	-	2.4	2.4	160	3920.3	160
P	800	0.5	3.7	119	1798.5	33
S	-	0.63	1.3	60.08	23367	40.4
K	2000	6.78	28.2	710.6	1898.2	411
Ca	800	0.44	6.03	426	164.77	104
Ars	-	0	0	3	3	3
Ti	-	0.01	0.1	2.88	0.3	0.52
V	-	0.03	0.03	2	2	2
Cr	-	0	0	0.1	1.39	0.1
Mn	2	0.11	0.11	0.99	8.58	3.36
Fe	10	0.14	0.6	6.01	22.1	18.5
Ni	-	0.01	0.02	0.13	0.5	0.5
Cu	1.5	0	0.01	0.14	0.26	0.15
Zn	12	0.02	0.01	0.38	1.98	0.68
Br	-	0	0.02	1.35	2.69	0.53
Rb	-	0.04	0.04	0.78	11.74	1.45
Sr		0.31	0.49	5.29	86.58	23.7
Pb	*3**	0	0	0.14	1.12	0.53

Table 12. Mineral elements profiles of herbal paste products used in management of oral health in Nairobi County, Kenya

	Herbal pastes				
	HP-1	HP-2	HP-3	HP-4	HP-5
Al	<16	<16	<16	<16	582.86±36.3
P	8480.40±682.70	70885.90±890	615.74±39.08	3510.86±170.1	3317±359.62
S	3660.70±522.0	168.94± 39.87	1336.32±90.26	1361.49±90.18	886.9±20.2
K	5065.60±428.60	934.25 ±23.63	26.10 ±1.78	3255.87±57.47	11162.41±55.6
Ca	278036.00±21082	57.69±2.4	30.91±1.50	125479.6±3906	3379.89±28.2
Ars	97.845±22.4	<0.3	4.23±0.53	52.077±39.09	<0.3
Ti	<0.21	8.07± 0.02	1107.66±62.79	7.60±1.224	258.79±42.6
V	<0.20	<0.2	<0.2	<0.2	10.24±1.44
Cr	<0.10	<0.1	<0.1	<0.1	7.31±0.47
Mn	11.08±2.65	5.99±0.4	2.41±0.69	116.22±2.77	233.84±4.5
Fe	123.17±7.33	52.46±3.5	90.75±6.52	L363.40±10.58	1984.2±291.05
Ni	39.44±8.43	<0.05	<0.05	16.12±0.80	0.85±0.2
Cu	<0.04	<0.04	3.24±0.16	5.91±1.08	5.54±0.52
Zn	<0.04	2.89±0.5	26.68±1.53	6.92±0.83	23.07±0.3
Br	<0.02	<0.02	<0.02	<0.02	3.41±0.41
Rb	13.14± 0.67	19.20 ±0.66	9.87±0.4	6.73±0.24	38.61±2.27
Sr	<0.03	286.54±32.95	206.6±10.9	<0.032	245.34±60.5
Pb	22.08 ±4.7	10.92±0.63	5.93±0.27	13.75±0.28	4.91±0.9

Results are expressed as mean ± standard deviation of three samples per herbal paste and are expressed as (mg/Kg)

Table 13. Amount of mineral elements taken daily (g/day) from herbal materials used in managing oral health in Nairobi County, Kenya

Element	RDA	Herbal pastes				
		HP-1 *(1.5g)	HP-2 (0.5g)	HP-3 (1.22g)	HP-4 (1.3g)	HP- 5 (0.5g)
Aluminum	-	24	8	19.52	20.8	582.86
Phosphorus	800	12720.60	35442.95	751.20	4564.12	3317
Sulfur	-	5491.05	84.47	1630.31	1769.94	886.9
Potassium	-	7598.40	467.13	31.84	4232.63	11162.41
Calcium	800	417054.0	28.85	37.71	163123.5	3379.89
Arsenic	-	146.76	0.15	5.16	67.70	0.3
Titanium	-	0.32	4.04	1351.34	9.88	258.79
Vanadium	-	0.30	0.1	0.24	0.26	10.24
Chromium	-	0.15	0.05	0.12	0.13	7.31
Manganese	2	16.62	2.99	2.94	151.09	233.84
Iron	10	184.76	26.23	110.72	472.42	1984.2
Nickel	-	59.16	0.03	0.06	20.96	0.85
Copper	1.5	0.06	0.02	3.95	7.68	5.54
Zinc	12	0.06	1.45	32.42	8.99	23.07
Bromine	-	0.03	0.01	0.02	0.03	3.41
Rubidium	-	19.76	9.6	12.04	8.75	38.61
Strontium	-	0.05	143.27	252.05	0.04	245.34
Lead	*3	33.12	5.46	7.23	17.88	4.91

RDA: required daily allowance. Results are expressed in mg per day but lead was expressed in mg/week. * **( )** is the amount of herbal paste prescribed by the herbalist (g)

4.3 Microbial contaminants in herbal materials used in oral health

The current study reports microbial contamination in some of the herbal products traded in Nairobi (Table 14). Herbal suspensions HS-1, HS-2, HS-3, HS-4, HS-5, HS-9 and HS-10 had no microbial contaminants. The same case with herbal paste HP-1, HP-2, HP-3, HP-4, and medicinal plants powders MPP-2, MPP-5 and MPP-6. *S. aureus* was not isolated from any of the products. Product MPP-4 had *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and fungal isolates. Fungal isolate from MPP-4 was confirmed to be *Aspergillus niger* and *C.albicans* by the type of spores produced. HP-5 (fine powder that is formulated in to a paste) had *E. coli* and *S. typhi.*

Table 14. Microbial contaminants isolated and identified in herbal products used for management of oral health in Nairobi County.

Product code	*E. coli*	*S. aureus*	*P.aeruginosa*	*S. typhi*	*C.albicans*	*A. niger*
MPP-1	+	-	-	+	-	-
MPP-4	+	-	+	+	+	+
MPP-5	-	-	-	-	-	-
HP-5	+	-	-	+	-	-

Key refer to Table 5 and 6. (+) present (-) absent

The purpose for evaluation of total viable count was to determine whether the herbal products meet the required microbial contaminants load as described by WHO. Total aerobic viable counts were investigated by diluting the sample and inoculating in an appropriate culture media. Incubation was done and the resulting colonies were recorded. Total aerobic viable counts were reported in 9 of the products analysed

with highest number of counts in HP-5 (Table 15). Total viable counts of *E. coli* were recorded in product MPP-1, MPP-4 and HP-5. Viable counts of *S. typhi* were present in HP-5 and MPP-4.

Table 15. Total viable counts (TVC) of microbial contaminants in herbal products used for oral health in Nairobi County

	TVC	*E. coli*	*S. aureus*	*P. aeruginosa*	*S. typhi*	Fungal counts
MPP-1	$2.10X10^5$	$1.0x\ 10^4$	0	0	0	0
MPP-2	$6.90X10^4$	0	0	0	0	0
MPP-4	$4.70X10^4$	$2.80x10^2$	0	0	$2.0X10^3$	$3.40x10^4$
MPP-5	$1.74X10^5$	0	0	0	0	0
MPP-6	$1.14\ x10^5$	0	0	0	0	0
HS-8	$3.0X10^3$	0	0	0	0	0
HS-9	$4.0X10^3$	0	0	0	0	0
HS-10	$1.13X10^5$	0	0	0	0	0
HP-2	$3.10X10^4$	0	0	0	0	0
HP-5	$2.60\ X10^6$	$2.5x10^4$	0	0	$2.40x10^4$	0

Key: product code, refer to Table 5, 6 and 7

4.4 Phytochemical composition of herbal materials used for management of oral health

The herbal powders were sequentially extracted using dichloromethane (DCM), DCM/methanol (1:1) and water solvents. The three extracts were evaluated for major phytochemical components associated with pharmacological activities such as saponins, alkaloids, flavonoids, diterpenoids, reducing sugars, amino acids, resins and tannins.

4.4.1 Yields of the extracts

The highest yield in MPP-1 (*C. hirsuta*) was recorded in water extract at 2.78%, followed by DCM extract (1.58 %) and the least was in DCM/methanol extract at (1.5 %) as shown in Table 16. MPP-2 (*S. didymobotrya)* gave highest yield in DCM/methanol extract at 3.79 % while the DCM had the lowest percentage yield (1.66 %). The extractive values of MPP-3 (*E. divinorum*) were all above 3%, with highest in DCM extracts (4.33 %). The DCM extracts of MPP-4 (*Z. chalybeum*) showed its lowest extractive yield (1.2 %) and the same powder had the highest value in water extracts (3.35 %). MPP-5 (*W. ugandensis*) gave yields of 1.39 %, 2.03 and 2.51 % in DCM, DCM/methanol and water extracts, respectively. MPP-6 showed the least extractive value in all the DCM extracts of 0.53 % while DCM/methanol and water extracts had 2.2 and 3.0 %, respectively. The polyherb product of MPP-7 had lower extractive yield in DCM:MeOH (1.48 %) and water extracts (1.72 %) compared to polyherb product of MPP-8.

Table 16. Extractive values of herbal powders used in management of oral health in Nairobi County, Kenya

	% Extractive value			
Code	Weight (g)	DCM	DCM/MeOH	water
MPP-1	330	1.58	1.50	2.78
MPP-2	330	1.66	3.79	2.19
MPP-3	300	4.33	3.17	3.0
MPP-4	500	1.20	2.50	3.35
MPP-5	500	1.39	2.03	2.51
MPP-6	500	0.53	2.2	3.0
MPP-7	500	1.71	1.48	1.72
MPP-8	500	1.39	1.54	2.02

Key: MeOH:Methanol. DCM: dichloromethane Product code: refer to Table 5

4.4.2 Phytochemicals in herbal products

The various phytochemical groups were analysed by use of standard methods as described by Chhetri *et al.* (2008) and Roopashree *et al,* (2008).

4.4.2.1 Powders

All the plant extracts contained different kinds of phytochemicals (Table 17). In MPP-1 (*C. hirsuta*), the three extracts contained triterpenoids, resins, phenols and amino acids. Alkaloids and reducing sugars were found in water extracts only while diterpenoids and tannins were present in organic extract only. The three extracts of MPP-2 (*S. didymobotrya*), contained triterpenoids, flavonoids and amino acids. Alkaloids, phenols, diterpenoids and saponins were found in water extracts only. Triterpenoids and amino acids were present in the three extracts of MPP-3 (*E. divinorum*) while the dichloromethane and water extracts contained alkaloids, reducing sugars and saponins. Both the organic and water extracts of MPP-4 (Z. *chalybeum*) contained amino acids and flavonoids. All the three extracts of sample MPP-5 (*W. ugandensis*), had saponins, triterpenoids, resins, tannins, flavonoids and amino acids. The dichloromethane/methanol extracts of MPP-6 (*T. brownii*) contained all the phytochemical groups evaluated apart from diterpenes while the water extracts lacked saponins and flavonoids. The dichloromethane extracts lacked saponins, alkaloids, triterpenoids and reducing sugars. All the extracts of herbal mixtures MPP-7 contained reducing sugars and flavonoids while the DCM/methanol extracts contained all the evaluated phytochemicals. The extracts of herbal mixture

(MPP-8) showed the presence of all the phytochemicals investigated in DCM extracts while the water extracts lacked alkaloids and diterpenoids.

4.4.2.2 Pastes and liquids

The herbal pastes HP-1, HP-2 and HP-4 were positive for saponins through froth tests as shown in Table 18. Resins were recorded in paste product HP-2. Product HP-5 tested positive for the presence of alkaloids. The same product gave a positive reaction with amino acids and diterpenoids. Phytochemicals under investigation were below detectable levels in all the suspensions/liquids (Table 18).

Table 17. Phytochemical compositions of organic and aqueous extracts of herbal powdered materials used for oral health in Nairobi County, Kenya

	MPP-1			MPP-2			MPP-3			MPP-4		
Phytochemicals	DCM/MeOH	DCM	H_2O	DCM MeOH	DCM	H_2O	DCM/MeOH	DCM	H_2O	DCM/MeOH	DCM	H_2O
Alkaloids	–	–	+	–	–	+	+	–	+	–	+	+
Reducing sugars	–	–	+	–	–	–	+	–	+	–	-	+
Saponins	+	+	–	–	–	+	+	–	+	–	+	+
Triterpenoids	+	+	+	+	+	+	+	+	+	+	–	–
Resins	+	+	+	+	+	–	+	+	–	–	+	+
Phenols	+	+	+	–	–	+	+	-	+	–	–	+
Tannins	+	+	–	+	+	–	+	+	–	–	+	+
Flavonoids	–	–	+	+	+	+	–	+	+	+	+	+
Amino acids	+	+	+	+	+	+	+	+	+	+	+	+
Diterpenoids	+	+	–	–	–	+	+	-	–	+	–	+

KEY:+ present; – absent; DCM- dichloromethane, DCM/MeOH- dichloromethane /Methanol ; H_2O- water; product code refer Table 5

Table 17. (continued)

	MPP-5			MPP-6			MPP-7			MPP-8		
Phytochemicals	DCM/MeOH	DCM	H_2O	DCM/MeOH	DCM	H_2O	DCM/MeOH	DCM	H_2O	DCM/MEOH	DCM	H_2O
Alkaloids	+	–	+	+	–	+	+	–	+	+	+	–
Reducing sugars	+	+	–	+	–	+	+	–	+	+	+	+
Saponins	+	+	+	+	–	-	+	+	+	–	+	+
Triterpenoids	+	+	+	+	–	+	+	–	+	–	+	+
Resins	+	+	+	+	+	+	+	+	–	+	+	–
Phenols	–	-	+	+	+	+	+	–	+	+	+	+
Tannins	+	+	+	+	+	+	+	–	+	+	+	+
Flavonoids	+	+	+	+	+	-	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+	+	–	+	–	+	+
Diterpenes	–	+	–	–	+	+	+	+	+	+	+	-

Table 18. Phytochemical composition of organic and aqueous extracts of herbal products used in oral health in Nairobi County, Kenya

	LIQUIDS										PASTES				
Phytochemicals	HS-1	HS-2	HS-3	HS-4	HS-5	HS-6	HS-7	HS-8	HS-9	HS-10	HP-1	HP-2	HP-3	HP-4	HP-5
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Reducing sugars	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-
Triterpenoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fats and oils	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenols	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-	-	-	-	-	-		-	-	+
Resins	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Diterpenoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Key: + present – absent. Product code refer to Table 7

4.5 Antimicrobial activity of herbal materials sold in Nairobi County

The Antimicrobial effects of the herbal materials are described in the following sections were 4.5.1.1, 4.5.1.2, 4.5.1.3 and 4.5.1.4.

4.5.1.1 Antimicrobial activity of dichloromethane/methanol extracts

The results of antimicrobial investigation of dichloromethane/methanol (DCM/MeOH), water extracts are shown in (Table 19). The DCM/MeOH extract of MPP-1 (*C. hirsuta*) had no antimicrobial activity on all the test organisms while the MPP-2 had inhibitory effects on the test organisms apart from *E. coli, S. faecalis* and *C. albicans*. MPP-3 extracts had antimicrobial activity ranging from 6.7 mm on *Lactobacillus acidophillus* to 10.8 mm on *Bacillus subtilis* but had no effects on *Candida albicans, Streptocoocus faecalis and Escherichia coli.* MPP-4 had inhibitory effects on *S. aureas* (9.38±0.85 mm), *B. subtilis* (12.25±0.52 mm), *P. vulgaris* (9.5±0.84), *Streptoccocus mutans* (10.8±0.75) and *Lactobacillus acidophillus* (12±1.68). All the test organisms were sensitive to MPP-5 and MPP-6. *S. faecalis* was resistant to both the polyherbal product of MPP-7 and MPP-8 but all the organisms showed some degree of sensitivity. Co-Trimozole and fluconazole were used as positive control for bacteria and fungi, respectively, while DMSO was used as the negative control. The zone of inhibition of the positive control ranged from 7.8 to 33.5 mm.

Table 19. Zones of inhibition (mm±s.e) against various microorganisms by DCM/MeOH extracts of herbal materials used in management of oral health in Nairobi County, Kenya (N=4)

Extract	*E. coli*	*S. aureus*	*S. faecalis*	*B. subtilis*	*P. aeruginosa*	*P. vulgaris*	*S. mutans*	*C. albicans*	*L. acidophillus*
Co-Tri moxazole	27.5±0.7	8.5±0.5	33.5±1.5	24.0±0.41	10.0	28.8±0.25	7.8±0.25	*11.3 ± 0.48	22.8±0.8
DMSO	0	0	0	0	0	0	0	0	0
MPP-1	0	0	0	0	0	0	0	0	0
MPP-2	0	11.50±1.9	0±0	16.5±1.19	6.5±0.29	**7**.0± 0.71	12.0±0 0.4	0	12.0±0
MPP-3	0	9.13±0.72	7.0±4.19	10.8±0.25	8.0±0.58	9.13±0.13	10± 0.71	0	6.7±0.48
MPP-4	0	9.38±0.85	0	12.25±0.52	0	9.5±0.84	10.8±0.75	0	12.0± 1.68
MPP-5	9.50±0.65	11.76±1.6	9.75±0.83	13.4±1.25	11.50±0.29	13±0.56	11±.0.7	10.80±0.48	10.1± 1.01
MPP-6	16.80±0.48	13.0±1.78	9.50±0.60	17±0.71	14.50±1.8	21.3±0.48	13.5±0.65	22.0± 1.7	15.0±0.41
MPP-7	1.20± 0.72	11.75±1.86	0	14.75±0.25	18.2±1.18	18.2±1.18	16.5±0.65	26±6.9	13.0± 1.1
MPP-8	10.0±0.41	9.75±0.48	0	12.80±0.85	15.75± 2.02	14.8±0.75	15±0.48	19.3±0.48	12.8±1.12

Key: product name refer to Table 5 : * for *C.albicans* the positive control was Fluconazole

4.5.1.2 Antimicrobial activity of dichloromethane extracts of herbal powders

The dichloromethane (DCM) extracts of MPP-1 and MPP-2 had no inhibitory effects on test organisms (Table 20). The extracts of MPP-3 had inhibitory effects on all the test organisms apart from *S. faecalis* and *L. acidophilus.* The extracts of MPP-5 had effects on the test organisms. The extracts of MPP-7 and MPP-8 showed marked antimicrobial activity against all the test organisms apart from *S. faecalis*. The three extracts of MPP-6 (*T. brownii*) had significant antimicrobial effects on *C. albicans*. Generally the test organisms showed decreased sensitivity to the combined extract of sample (MPP-7) and (MPP-8) in comparison to extract (MPP-6) *T. brownii*. However, the combined plant extract showed better antimicrobial properties than extract of MPP-4 and MPP-5. The zone of inhibition of Co-Trimoxazole (positive control) ranged from 7.8 mm to 33.5 mm while for fluconazole was 19.3 mm.

.

Table 20. Zones of inhibition (mm±s.e) against various microorganisms by DCM extract of herbal powders used in management of oral health in Nairobi County, Kenya

Extract	*E. coli*	*S. aureus*	*S. faecalis*	*B. subtilis*	*P. aeruginosa*	*P. vulgaris*	*S. mutans*	*C. albicans*	*L. acidophilus*
CT	27.5±0.7	8.5±0.5	33.5±1.5	24±0.41	10.0	28.8±0.25	7.8±0.25	11.3 ±0.48	22.8±0.8
DMSO (-ve control)	0	0	0	0	0	0	0	0	0
MPP-1	0	0	0	0	0	0	0	0	0
MPP-2	0	0	0	0	0	0	0	0	0
MPP-3	10.3±0.63	10.8±1.6	0	10± 0.41	10.5±0.29	13±1.7	12.5±0.96	8.25±0.75	0
MPP-4	0	8±1.12	0	0	0	0	0	0	0
MPP-5	9±0.41	13.3±1.6	9.5±0.29	18.8±0.75	18±2.1.25	10.2±0.5	14.5±1.71	30±3.33	17±0.58
MPP-6	0	16±0.91	0	13±1.44	15.5±3.7	16.5±1.55	19±0.58	16.3±1.0	9.25±1.01
MPP-7	9.25±0.48	10.8±11.26	0	15±0.82	10.7±0.25	10.8± 0.25	13.5±0.25	14.3±0.5	14.3±0.75
MPP-8	15.5±0.65	13.5±0.65	0	13.5±0.65	16.25±0.479	18.3±1.32	17± 1.5	15.8±0.75	14.4±1.4

Key: product name refer to Table 5; CT- Positive control Co-Trimoxazole

4.5.1.3 Antimicrobial activity of water extracts of herbal powders used for management of oral health

The extracts of MPP-1 and MPP-2 had no inhibitory effects on the test organisms while MPP-3 had effects only in *S. faecalis, P. aeruginosa*, and *S. mutans* (Table 21). No antimicrobial activity was reported in MPP-4. The extracts of MPP-5 had antimicrobial effect on all the test organisms apart from *S. faecalis*. MPP-6 showed activity on *S. aureus* (12.5±1.19 mm), *B. subtilis* (15.75±0.14 mm), *P. aeruginosa* (16.25±0.25 mm), *S. mutans* (16.5±0.87 mm), *C. albicans* (15.8 ± 0.56 mm), *L. acidophilus* (16±0.42 mm). MPP-7 showed antimicrobial activity against 8 of the 9 microbes tested while MPP-8 had antimicrobial activity against 6 of the 9 microbes tested. The zone of inhibition of Co-Trimoxazole ranged from 7.8 to 33.5 mm while that of fluconozole was 11.3 mm.

Table 21. Zones of inhibition (mm± s.e) against various microorganisms by water extract of herbal powders used in management of oral health, in Nairobi County, Kenya (N=4)

Extract	*E. coli*	*S. aureus*	*S. faecalis*	*B. subtilis*	*P. aeruginosa*	*P. vulgaris*	*S. mutans*	*C. albicans*	*L.a*
CT	27.5±0.7	8.5±0.5	33.5±1.5	24±0.41	10.0	28.8±0.25	7.8±0.25	11.3 ±0.48	22.8±0.8
DMSO	0	0	0	0	0	0	0	0	0
MPP-1	0	0	0	0	0	0	0	0	0
MPP-2	0	0	0	0	0	0	0	0	0
MPP-3	0	0	11.0	0	12.0 ± 1.12	0	8.6± 0.94	0	0
MPP-4	0	0	0	0	0	0	0	0	0
MPP-5	6.5±0.29	12.6±0.8	0	14.4±0.38	14.25±0.76	8± 1.23	12.8± 0.43	13±0.91	12.5±1.4
MPP-6	0	12.5±1.19	0	15.75±0.14	16.25±0.25	0±0	16.5± 0.87	15.8±0.56	16±0.42
MPP-7	9.5± 1.43	12±0	0	14.3±0.63	12.5± 0.65	12.5±0.65	15±1.08	14.3±1.5	11.2±0.95
MPP-8	17.3±0.48	15.75±1.58	0	13±1.44	12.75±0.75	4±2.30	16.5± 1.5	0	15± 0.41

Key: product name refer to Table 5; CT- Co-Trimoxazole

4.5.2 Antimicrobial activity of liquid herbal products used in management of oral health

For the liquid herbal samples, agar well diffusion was used to investigate antimicrobial properties. Samples HS-1, HS-2, HS-3, HS-4 and HS-5 showed varied antimicrobial properties to all the test organisms (Table 22). The diameter zone of inhibition of product HS-1 ranged from 9-26.5 mm, while for HS-2, 7.25-22.8 mm, HS-3 (11.3-34.5 mm) and HS-5 (19.75-36.5 mm). The product HS-4 produced zone of inhibition of 33.3 and 21.3 mm on *S. mutans* and on *Bacillus subtilis*, respectively. The same product had significant antimicrobial properties on *C. albicans.* Products (HS-6, HS-7, HS-8 and HS-9 had no antimicrobial activity. The zone of inhibition of the positive control ranged from 7.8 to 33.5 mm.

4.5.3 Antimicrobial activity of herbal paste products

The herbal paste, HP-3 and HP-4 displayed some antimicrobial activity on the test organisms while herbal paste HP-1 and HP-2 showed limited antimicrobial activity (Table 23) while that positive control ranged from 7.8 and 33.5 mm.

Table 22. Zones of inhibition (mm±s.e) against various microorganisms by liquid herbal suspensions used in management of oral health in Nairobi County, Kenya (N=4)

Test Organism	Product						
	DMSO	Co-Trimoxazole	HS-1	HS-2	HS-3	HS- 4	HS-5
E. coli	0	27.5±0.7	19.3±0.48	9.5± 1.44	11.8±0.35	33.1±0.85	25.3±0.25
S. aureus	0	8.5±0.5	12.5±0.65	10.75±1.89	11.3±0.35	20.3±1.71	20.25±1.26
S. faecalis	0	33.5±1.5	16.8±2.5	16.25±5.1	20.0±0	27.5±8.7	24.25±1.5
B. subtilis	0	24±0.41	12.0±1	14.25±3.33	34.5±2.12	21.3±0.96	21.75±2.27
P. a	0	10± 0.0	9.0±0.96	7.25±0.43	13±1.06	19.3±1.65	21.5±1.71
P. vulgaris	0	28.8±0.25	14.3±2.63	9.37±1.11	18.5±0.71	26.5±1.91	19.75±0.96
S. mutans	0	7.8±0.25	26.5±1.29	11.6±1.76	31.5±0.71	33.3±1.26	36.5±1
C. albicans	0	11.3±0.5	19.0±1.12	22.8±4.2	20.5±0.7	25±1.41	24±1.41
L. a	0	22.8±0.8	16.0±2.83	14.8±0.8	21.0±0	22.8±1.26	20±1.41

Key**:** Pa- *P. aeruginosa,* La- *L. acidophilus;* Product code: Refer to Table 7

Table 23. Zones of inhibition (mm±s.e) against various microorganisms by paste herbal products used in management of oral health in Nairobi County, Kenya (N=4)

Product	DMSO	Co-Trimoxazole	HP- 1	HP- 2	HP- 3	HP- 4	HP- 5
E. coli	0	27.5±0.7	0±0	7.5±0.7	29.3±0.6	0	20±0
S. aureus	0	8.5±0.5	20±0	11±0	18.4±0.24	11.8±2.75	11±4.9
E. faecalis	0	33.5±1.5	0	0	0	0	0
B. subtilis	0	24±0.41	22±0.7	0	18.9±0.42	13.12±2.2	7.5±0
P. a	0	10	12±0	0	20.8±0.46	11.5±2.0	0
P. vulgaris	0	28.8±0.25	0	7±0	0	12.5±3.17	0
S. mutans	0	7.8±0.25	0	10±0	16.5±1.3	12±2.3	0
C. albicans	0	11.3 ±0.48*	0	0	28.25±1.3	13±2.2	0
L.a	0	22.8±0.8	0	0	16.7±1.1	13.8±2.5	0

KEY: Pa- *P. aeruginosa*, La- *L. acidophilus;* Product code refer to Table 6 (*)- fluconazole was used as positive control

4.5.4 Activity index of herbal materials

Activity index (AI) was determined by dividing the zone of inhibition of the herbal extract with that of the positive control; (Co-Trimoxazole (septrin) and Fluconazole for *C. albicans* and results are shown in Table 24. The water extracts of MPP- 8 (herbal mixture of *T. brownii, Z. chalybeum, W. ugandensis* and *A. indica*) recorded the greatest AI (0.63) against *E. coli*. The dichloromethane extracts of MPP-6 (*T. brownii)* produced the highest AI of 1.88 against *S. aureus* and dichloromethane/methanol extract of *T. brownii* gave the best AI (0.74) against *P. vulgaris*. The dichloromethane extract of *W. ugandensis* showed the highest AI of

0.78 against of *B. subtilis.* The highest AI of 2.44 against *S. mutans* was from dichloromethane/methanol extract of *T. brownii.* The highest AI (0.27) produced against *C. albicans* was recorded in dichloromethane extract of *T. brownii.* Sample HS-4 (Table 25) produced the best AI of 1.20 against *E. coli. S. aureas* seems to be susceptible to herbal products than the positive control (Co-trimoxazole) since all samples gave an index of more than 1. The highest AI was shown by herbal suspension HS-3, HS-4 and HS-5 against *S. mutans.*

Table 24. Activity indices of extracts of herbal powders used in management of oral health in Nairobi County, Kenya

	E. coli	*S. aureus*	*E. faecalis*	*B.s*	*P. vulgaris*	*S. mutans*	*C. Albicans*	*L.a*
a.Dichloromethane/ methanol extracts								
MPP-2	0	1.35	0	0.69	0.24	1.54	0	0.53
MPP-3	0	1.07	0.2	0.45	0.32	1.28	0	0.29
MPP-4	0	1.1	0	0.51	0	1.38	0	0
MPP-5	0.35	1.38	0.3	0.56	0.45	1.41	1	0.44
MPP-6	0.61	1.53	0.3	0.71	0.74	1.73	1.9	0.66
MPP-7	0.44	1.38	0	0.61	0.63	2.12	2.3	0.57
MPP-8	0.36	1.15	0	0.53	0.51	1.92	1.7	0.56
b.Dichloromethane extract								
MPP-2	0.36	1.26	0	0.42	0.45	1.6	0.7	0
MPP-4	0	0.94	0	0	0	0	0	0
MPP-5	0.33	1.56	0.3	0.78	0.35	1.86	2.7	0.75
MPP-6	0	1.88	0	0.54	0.57	2.44	1.4	0.41
MPP- 7	0.34	1.27	0	0.63	0.38	1.73	1.3	0.63
MPP- 8	0.56	1.59	0	0.56	0.64	2.18	1.4	0.63
c. Water extract								
MPP-3	0	0	0.3	0	0	1.1	0	0
MPP-5	0.24	1.48	0	0.6	0.28	1.64	1.2	0.55
MPP-6	0	1.47	0	0.66	0	2.12	1.4	0.70
MPP-7	0.35	1.41	0	0.6	0.43	1.92	1.3	0.49
MPP-8	0.63	1.85	0	0.54	0.14	2.12	0	0.66

Key: product name refer to Table 5; *L.a- Lactobacillus acidophilus; B.s- Bacillus subtilis*

Table 25. Activity index of herbal pastes and suspensions used in management of oral health in Nairobi County, Kenya

Extract	*E. Coli*	*S. aureus*	*E.f*	*B. subtilis*	*P. vulgaris*	*S. mutans*	*C. albicans*	*L.a*
Co-Trimoxazole	1	1	1	1	1	1	1	1
HP-1	0	2.36	0	0.92	0	0	0	0
HP-2	0.27	1.29	0	0	0	1.28	0	0
HP-3	1.06	2.16	0	0.78	0	2.11	2.5	0.73
HP-4	0	1.39	0	0.54	0.43	1.54	1.15	0.60
HP-5	0.73	1.29	0	0.31	0	0	0	0
HS-1	0.70	1.47	0.5	0.5	0.5	3.4	1.68	0.7
HS-2	0.35	1.26	0.48	0.59	0.73	1.48	2.0	0.64
HS-3	0.43	1.33	0.6	1.44	0.64	4.04	1.81	0.92
HS-4	1.20	2.39	0.82	0.89	0.92	4.27	0	1
HS-5	0.92	2.38	0.72	0.91	0.69	4.68	2.12	0.88

Key: product name and code refer to Table 6 and 7; La: *Lactobacillus acidophilus, Ef-Enterococcus faecalis*

4.5.5 Minimum inhibitory concentration (MIC) of herbal extracts used for the management of oral health

The minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MBC) was determined by inoculating test organisms in serially diluted samples in micro titration wells, followed by incubation and sub culturing and the results are shown in Table 26. The least dilution where growth was recovered was taken as the MIC and the minimum concentration where growth was not recovered was taken to be MBC. The least MIC for MPP-2 was 50 mg/ml while the highest was 200 mg/ml. The MIC from MPP-3 (*E. divinorum)* and MPP-4 was 200 mg/ml in all positive cases. Extracts of MPP-6 (*T. brownii*) showed varied MIC ranging from 1.56 to 50 mg/ml. The MIC for herbal mixture MPP-8 ranged from

6.25-200 mm/l while that of MPP-7 ranged from 3.13-100 mg/ml. The MIC range for *W. ugandensis* was 6.25-12.5 mg/ml. The herbal paste HP-3 had MIC values of 200 mg/ml. For herbal suspension HS-4, the MIC value ranged from 375.5-750 mg/ml. The MIC value of septrin positive control ranged from 3.25-6.2 mg/l while that of fluconazole was 1.56.

Table 26. Minimum inhibitory concentration (mg/ml) of selected herbal materials used in management of oral health in Nairobi County, Kenya

	C. albicans	*E. coli*	*S. aureus*	*B. subtilis*	*E.f*	L.a	*P. vulgaris*	P.a	*S. mutans*
MPP-2	-	-	200	50	-	200	200	-	200
MPP-3	200	-	200	200	-	200	200	-	200
MPP-4	-	-	200	200	-	200	200	200	-
MPP-5	6.25	12.5	6.25	6.25	6.25	6.25	6.25	6.25	6.25
MPP-6	25	25	25	50	1.56	1.56	6.25	25	1.56
MPP-7	100	100	100	100	-	3.13	12.5	50	6.25
MPP-8	200	50	100	6.25	-	6.25	25	50	12.5
HP-3	-	-	200	200	-	200	200	200	200
HS-4	750	750	750	375	375	375	375	375	375
*+ve	1.56	6.25	6.25	6.25	3.25	25	6.25	-	3.25

*Co trimoxazole for bacteria and fluconazole for fungus

Key: product code Table 5, 6 and 7 ; Pa- *P. aeruginosa*, La- *L. acidophilus*, *Ef -Enterococcus faecalis*

4.5.6 Minimum microbiocidal concentration (MBC) of extracts from herbal materials used for management of oral health care

The MBC for MPP-6 (*T. brownii)* ranged from 3.13 to 100 (Table 27) while that of herbal mixture MPP-8 varied from 12.5->200 mg/ml. *W. ugandensis* displayed a MBC value of 12.5-25 mg/ml while that of MPP-3 (*E. divinorum*) was more than 200 mg/ml. The herbal paste HP-3 had MBC of over 200 mg/ml. Fluconazole showed more than MBC of 1.56 while cotrimoxazole had the lowest MBC of 6.25 and a maximum of 100 mg/ml.

Table 27. Minimum microbicidal concentration (mg/ml) of selected herbal materials used in management of oral health in Nairobi County, Kenya

	C. albicans	*E. Coli*	*S. aureus*	*B. subtilis*	*Ef.*	L.a	*P. vulgaris*	P.a	*S. mutans*
MPP-3	>200	-	>200	>200	-	>200	>200	-	>200
MPP-4	-	-	>200	>200	-	>200	>200	>200	>200
MPP-5	12.5	25	12.5	12.5	12.5	12.5	12.5	12.5	12.5
MPP-6	50	50	50	100	3.13	3.13	12.5	50	3.13
MPP-7	200	100	200	200	-	6.25	6.25	6.25	6.25
MPP-8	>200	100	200	12.5	-	12.5	50	100	25
HP-3	-	-	>200	>200	-	>200	>200	>200	>200
HS-4	1500	1500	1500	750	750	750	750	750	750
*control	>1.56	25	50	100	6.25	50	100	-	6.25

Key: L.a- *Lactobacillus acidophilus*, P.a-*P.aeruginosa*, *Ef- Enterococcus faecalis*, *positive control-Co-Trimoxazole and fluconazole; Product code refer to Table 5, 6 and 7.

4.6 Effect on body weight of mice treated with extracts of herbal materials used in management of oral health

Growth rate was determined by weighing the animals on weekly basis for twenty one days and results of percentage body weight gain are shown in Figure 7. Oral administration of animals with *W. ugandensis* (MPP-5) and *T.brownii (*MPP-6) showed increased growth rate compared to control group in week one while the extracts of MPP-7 (*W.ugandensis, Z. chalybeum, T. brownii*) MPP-8 (*W. ugandensis, Z. chalybeum, T. brownii* and *A. indica* and MPP-4 (*Z. chalybeum*) showed negative growth rate. A similar pattern was observed week 2 (day 14) although the percentage body weight gain in MPP-5 was slightly lower than that recorded in week one. In week 3 (Day 21), all the treatment groups gave lower percentage body weight gain compared to control apart from organic extracts of MPP-6.

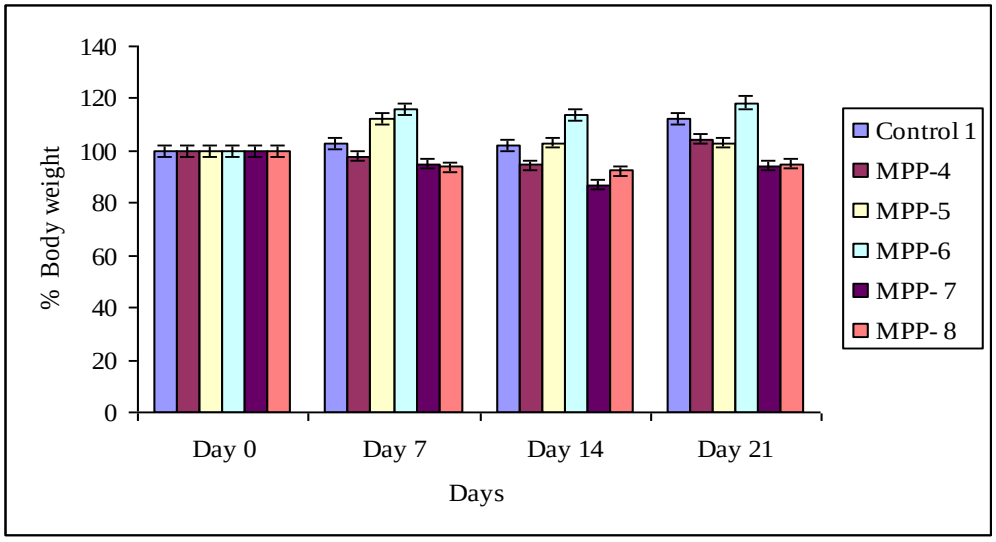


Figure 7. Percentage body weight gain in mice treated with 1000 mg/kg body weight of dichloromethane /methanol extracts of herbal powders

Animals treated with herbal (HP-1) had reduced growth rate by day seven while the other treatment groups compared well with the control. On week 2 (day 14) all the treatments groups had positive growth rate but that of MPP-2 and MPP-3 extract had normal growth rate comparable to the control. Decreased weight gain was reported in animals treated with extracts of *S. didymobotrya* (MPP-2) (Figure 8).

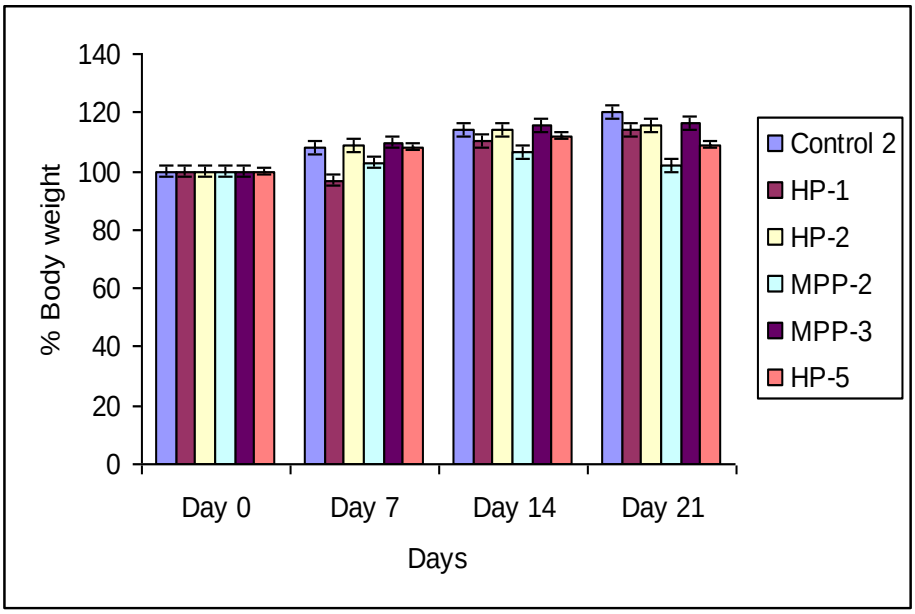


Figure 8. Percentage body weight gain in mice treated with 1000 mg/kg body weight herbal paste used in management of oral health in Nairobi County, Kenya

Generally the treatment groups showed negative growth rate in week three compared to control.

4.7 Effects on absolute organ weight of mice treated with herbal plant extracts used for oral health

At the end of experiments, mice were killed and the organ weights determined by dissecting out all the organs and weighing them. Results are recorded in Table 28. In category 1, oral administration of MPP-4 (*Z. chalybeum*) extract to animals significantly (P<0.05) (P=0.0494) atrophied the brain and kidney organs (P=0.011). The extract of *W. ugandensis* (MPP-5) and *T. brownii* (MPP- 6) did not have any significant effect on all the organs studied. Animals treated with extracts of herbal mixture of MPP-7 (*T. brownii*, *Z. chalybeum* and *W. ugandensis*) significantly (P<0.05) lowered the weight of brain, liver, kidney and heart while the weight of lungs, spleen and testis were not significantly affected by the treatment. The extract of MPP-8 decreased the weight of kidney but there was no significant effect on other organs.

In category 2, animals that were treated with extracts of MPP-2 (*S. didymobotrya*), were observed to have significantly reduced (P<0.05) (P=0.005) brain and kidney (P=0.0047) compared to control 2. Animals that received MPP-3 (*E. divinorum*) extracts had significantly (P<0.05) (P=0.04) enlarged testis, kidney (P=0.046) compared to control. The spleen weight (0.154) was lower than that of the control but within the normal range. Animal treated with Aloe based tooth paste (HP-1) showed significantly enlarged kidney (0.417g) while the rest of organs were not affected. The herbal paste (HP-2) had no significant effects on either of the organs. The herbal paste (HP-1) significantly reduced the weight of brain (P<0.05) (P=0.04)

and kidney (P= 0.004) compared to control. Animals treated with herbal paste HP-5 had significantly hypertrophied kidney (P<0.05) (P=0.026) compared to control.

4.7.1 Relative organ weight index in mice treated with herbal materials used internally in the management of oral health care

The relative organ weight was obtained by dividing the absolute organ weight with final body weight and multiplying by one hundred. Results indicate that mice treated with *W. ugandensis* (MPP-5) (Figure 9) had higher relative brain weight while mice treated with MPP-7 had the least relative brain weight. All the others showed some degree of reduction (Figure 9). Significant reduction of relative organ weight of liver weight was seen in animals treated with MPP-4 *(Z. chalybeum*) and MPP-7 (poly herbal product of *W. ugandensis, T. brownii* and *Z. chelybeum*). Mice treated with MPP-4, MPP-6, MPP-7 and MPP-8 showed reduced relative kidney weight compared to control. The relative organ weight of lungs was not affected in all treatments. All the treatment group showed reduction in spleen compared to control. Testicular organs of all treatment groups gave higher relative organ weight compared to control apart from animals treated with MPP-4. Slight reduction in relative organ weight of heart tissue was recorded in animals treated with MPP-4 and MPP-6.

Table 28.The effect of oral administration of 1000 mg/kg body weight of dichloromethane/methanol extracts on mice organ weight (N=6)

		ORGAN						
Ca	GROUP	BRAIN	LIVER	KIDNEY	LUNGS	SPLEEN	TESTIS	HEART
1	CONT. 1	0.41±0.00	1.65±0.15	0.44±0.02	0.20±0.01	0.39±0.16	0.21±0.02	0.13±0.06
	MPP-4	0.38±0.01*	1.36±0.16	0.36±0.02*	0.21±0.01	0.22±0.02	0.17±0.02	0.12±0.01
	MPP-5	0.42±0.02	1.46±0.13	0.43±0.03	0.21±0.012	0.17±0.01	0.26±0.01	0.12±0.01
	MPP-6	0.398±0.01	1.71±0.12	0.45±0.03	0.22±0.01	0.19±0.03	0.23±0.01	0.12±0.01
	MPP-7	0.261±0.02*	1.06±0.20*	0.26±0.02*	0.18±0.02	0.13±0.01	0.22±0.01	0.12±0.06*
	MPP-8	0.378±0.01	1.48±0.08	0.33±0.03*	0.22±0.02	0.13±0.01	0.22±0.02	0.12±0.01
2	CONT. 2	0.40±0.07	1.42±0.09	0.36±0.01	0.23±0.11	0.16±0.019	0.17±0.02	0.11±0.00
	MPP-2	0.341±0.01*	1.18±0.12	0.36±0.02	0.12±0.02*	0.14±0.02	0.20 ±0.01	0.10±0.01
	MPP-3	0.39±0.01	1.48±0.06	0.41±0.01*	0.27±0.02	0.15±0.11	0.23±0.02*	0.10±0.00
	HP-1	0.34±0.01*	1.60±0.09	0.45±0.02*	0.2±0.01	0.14±0.01	0.20±0.01	0.11±0.01
	HP-2	0.37±0.02	1.38±0.1	0.37±0.02	0.17±0.02	0.15±0.02	0.20±0.01	0.12±0.02
	HP-5	0.39±0.02	1.25±0.03	0.42±0.02*	0.24±0.01	0.15±0.01	0.22±0.02	0.11±0.05

Results are expressed as means ± standard error mean (SEM). n=6 *P<0.05 significantly different from control

Key; product name refer to Table 5 and Table 7; Ca - category, CONT; control

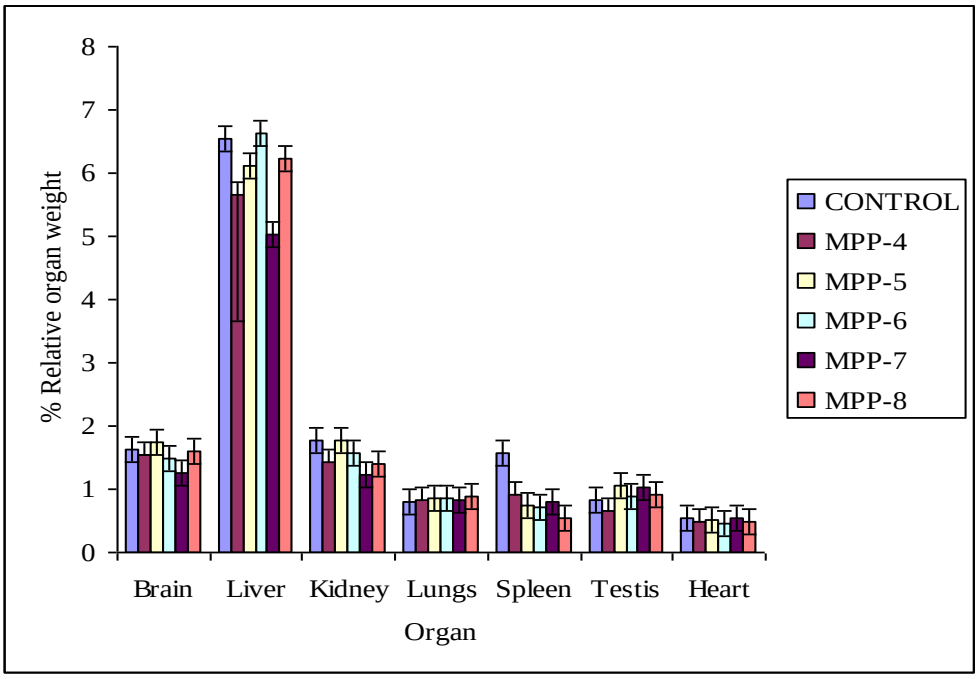


Figure 9. Relative organ weight of mice treated with 1000 mg/kg body weight of dichloromethane/ methanol extract of plant powders used in management of oral health in Nairobi County, Kenya

4.7.2 Relative organ weight index in mice treated with herbal materials used to clean externally to clean mouth

Mice that received HP-1 and HP-5 gave a lower relative brain index compared to control (Figure 10). The relative organ weight of the liver was higher in HP-1, HP-2 and MPP-3 compared to control but reduced in MPP-2 and HP-5. Higher relative weight of kidney was noted in the entire treatment group. Animals treated with extracts MPP-3 showed higher relative organ weight of lungs compared to control. Slight reduction in relative organ weight of spleen was indicated in animals treated with HP-5, HP-1 and MPP-2. Higher relative weight of testis was noted in the

entire treatment group. The relative organ weight of the heart was slightly higher in animals treated with MPP-2.

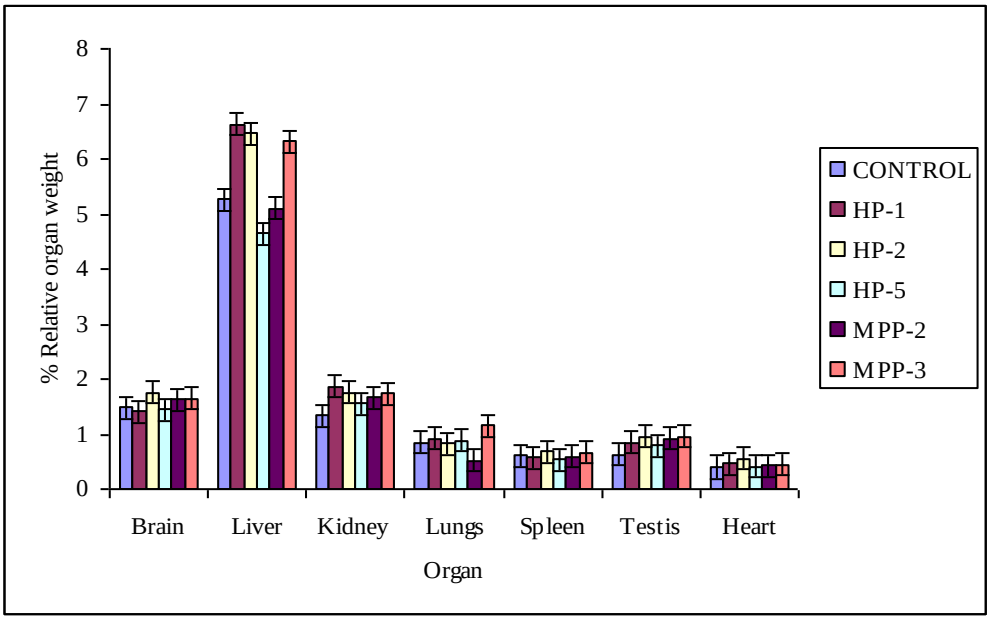


Figure 10. Relative organ weight of mice treated with 1000 mg/kg body weight of herbal materials used in management of oral health in Nairobi County, Kenya

4.8.1 The effect of on red cell indices of mice treated with 1000 mg/kg body weight of herbal materials

The end point red cell indices were evaluated by use of automated heamoanalyser and the results are shown in Table 29. Treatment of mice with extracts of MPP-4 had no significant effect on any of the red cell indices. Animals treated with organic extract of MPP-5 (*W. ugandensis*) significantly ($P<0.05$) ($P=0.018$) lowered haematocrit counts (HCT) (17.8), and significantly at ($P<0.05$) ($P=0.039$) raised mean cell volume (MCV). Other parameters were not significantly lowered thrombrocytes (THR) (120.67), red blood cells RBC (3.69), packed cell volume

compared to equivalent mean values of the control group 1. Mice that received extracts of MPP-6 (*T. brownii*) had red blood cells, haematocrit and haemoglobin levels significantly increased ($P<0.05$) compared to control group. Similar pattern was observed with animals receiving herbal extracts of MPP-7. Oral administration of organic extracts of herbal mixture of MPP-8 (*W. ugandensis, Z. chalybeum, T. brownie* and *A. indica*) significantly raised ($P<0.05$) red blood cells -RBC (7.31), and haemoglobin (HB) levels (11.25), HCT, (mean cell volume) and MCV. Generally all haematological parameters were raised in this group. The rest of analytes were almost the same with that of the control. In category 2, treatment with extracts of MPP-2 (*S. didymobotrya*) had no significant effects on red cell parameters compared to the control group 2. Product HP-1 showed significant increase of RDW compared to control. The MCHC levels were not altered by any of the product.

Table 29. The effect of oral administration of 1000 mg/kg body weight of different plant extracts and herbal products on some end point haematological parameters in mice (N=6)

Category	ANALYTE	RBC (M/mm3	MCV (fl)	HCT%	MCH (pg)	MCHC(g/dl	RDW	HB (g/dl)
1	Control 1	6.03±0.29	44.61±0.92	26.91±1.60	13.46±0.47	30.28±0.62	12.87±0.78	8.18±0.58
	MPP-4	6.17±0.46	44.02±2.06	27.14±2.5	13.54±0.52	31±0.71	13.64±0.99	8.41±0.81
	MPP-5	3.69±0.68	48.77±1.4*	17.8±2.68*	24.2±6.55	49.5±17.00	11.2±0.50	8.5±2.51
	MPP-6	7.65±0.30*	47.72±1.47	36.44±1.63*	15.16±0.72	31.82±0.86	11.3±0.55	11.66±0.77*
	MPP-7	9.78±1.13*	47.77±6.32	46.88±6.32*	17.1±2.76	35.67±4.87	10.97±0.78	17.33±4.74*
	MPP-8	7.31±0.36*	47.88±0.40*	34.93±1.53*	15.38±0.34*	32.2±0.47	10.65±0.25	11.25±0.45*
2	Control 2	6.18±0.33	55.55±6.46	35.67±12.95	11.55±0.99	22.93±2.75	15.87±1.8	6.42±0.0.7
	MPP-2	4.17±0.33	47.62±1.3	19.9±I.8	13.14±0.22	27.82±0.77	13.94±1.46	5.54±0.53
	MPP-3	4.89±0.19	48.17±1.55	23.4±1.01	13.88±0.36	29.03±1.14	13.77±1.06	6.78±0.18
	HP-1	5.16±0.57	49.48±1.62	25.48±3.01	12.98±0.23	26.55±1.18	13.42±1.18*	6.72±0.7
	HP-2	5.13±0.72	49.75±0.44	25.56±3.7	13.23±0.3	26.66±0.77	15.86±1.79	6.8±0.95
	HP-5	5.18±0.34	46.5±0.88	24.12±1.93	13.0±0.17	28.13±0.76	12.72±1.01	6.73±0.37

Results are expressed as means ± standard error mean (SEM). *P<0.05 significantly different from control

RBC-red blood cells, MCV-mean cell volumes; MCH: mean cell haemoglobin, MCHC- mean corpuscular haemoglobin concentration, RDW- red cell distribution width, HCT-haematocrit

4.8.2. The effect of oral administration of 1000 mg/kg body weight of plant extracts and herbal products on platelet indices

Very few parameters of platelets were significantly altered by the various treatment (Table 30) that is the platelet distribution width (PDW) in animals treated with *W. ugandensis* was significantly raised (P<0.05) while thrombocytes in animals treated with *E. divinorum* was significantly (P<0.05) lowered.

Table 30. The effect of oral administration of 1000mg/kg body weight of different plant extracts and herbal products on some end point platelets parameters in mice

Category	ANALYTE	Thrombocytes (m/mm3	MPV (fl)	PCT (%)	PDW
1	Control 1	232.0±68.71	7.17±0.36	0.16±0.04	6.98±0.3
	MPP-4	247.2±84.2	7.56±0.26	0.16±0.06	6.97±0.23
	MPP-5	120.66±16.38	7.67±0.12	0.09±0.01	9.35±0.35*
	MPP-6	324.8±57.7	6.54±0.29	0.23±0.04	6.86±0.41
	MPP-7	381.0±82.17	6.73±0.45	0.26±0.06	7.4±0.44
	MPP-8	248.33±30.5	6.55±0.23	0.13±0.03	6.1±0.54
2	Control 2	215.17±27.84	7.17±0.0.55	0.12±0.04	7.08±0.3
	MPP-2	134.0±11.06	7.38±0.24	0.1±0.01	7.96±1.06
	MPP-3	179.17±12.03*	7.55±0.23	0.14±0.01	6.88±0.49
	HP-1	210.0 ±28.0	7.27±0.2	2.16±0.02	7.58±0.27
	HP-2	215.12±27.83	7.63±0.28	0.16±0.02	8.18±0.88
	HP-5	187.8±14.83	7.02±0.1	0.14±0.01	7.53±0.3

Results are expressed as means ± standard error mean (SEM). *P<0.05 significantly different from contro, Key; MPV-mean platelet volume, PDW-platelets distribution width; PCT-plateletcrit

4.8.3 The effect of oral administration of 1000 mg/kg body weight of plant extracts on differential leukocyte counts

The absolute leukocytes results are recorded in (Table 31). Mice treated with dichloromethane/methanol extracts of MPP-4 (*Z. chalybeum*) showed high levels of

white blood cell counts. However this treatment had no significant effect on the other lymphocytes, neutrophils, basophils, eosinophils and monocytes. Animals administered with organic extract of MPP-5 (*W. ugandensis*) significantly ($P<0.05$) lowered levels of white blood cells, lymphocytes, neutrophils and eosinophils. Extracts of MPP-6, MPP-7 and MPP-8 significantly ($P<0.05$) increased levels of neutrophils. Animals given herbal paste HP-1 showed high levels of eosinophils compared to the control and the monocytes were much lowered.

Table 31. The effect of oral administration of 1000 mg/kg body weight of different plant extracts and herbal products on differential leukocyte counts in mice

CATEGORY	TREATMENTS	ANALYTE (10^9m/l)					
1	GROUPS	WBC	Lymphocytes	Neutrophils	Eosinophils	Monocytes	Basophils
	Control 1	6.94±0.58	4.07±0.36	2.13±0.17	0.41±0.04	0.32±0.03	0.09±0.01
	MPP-4	6.72±0.55	4.03±0.40	2.02±0.18	0.37±0.03	0.34±0.04	-
	MPP-5	3.46±1.77*	1.85±0.96*	1.18±0.59*	0.24±0.12*	0.18±0.08	0.01±0.0
	MPP-6	7.32±0.55	4.18±0.35	2.34±0.13*	0.44±0.04	0.36±0.05	-
	MPP-7	8.31±1.90	4.96±1.48	2.58±0.40*	0.40±0.06	0.34±0.06	0.03±0.03
	MPP-8	7.24±0.94	4.29±0.58	2.15±0.24*	0.42±0.06	0.35±0.06	0.01±0.0
2	Control 2	4.05±0.73	2.18±0.41	1.13±0.23	0.4±0.05	0.28±0.06	0.07±0.02
	MPP-2	3.43±0.71	2.13±0.42	0.74±0.13	0.30 ±0.07	0.17±0.04	0.09±0.05
	MPP-3	4.41±0.42	2.85±0.27	0.99±0.10	0.39±0.04	0.14±0.02	0.04±0.01
	HP-1	5.28±0.71	3.36±0.43	1.19±0.16	0.51±0.06	0.17±0.06	0.05±0.01
	HP-2	4.36±0.72	2.71±0.48	0.99±0.15	0.37±0.06	0.24±0.03	0.06±0.01
	HP-5	4.15±0.39	2.65±0.22	0.97±0.10	0.31±0.02	0.18±0.04	0.05±0.01

Results are expressed as means ± standard error mean (SEM). *$P<0.05$ significantly different from control, WBC –white blood cells; product code Refer to Table 5, 6 and 7.

4.9 The effect of oral administration of 1000 mg/kg body weight of plant extracts on biochemical parameters

The biochemical parameters were determined from blood plasma using Autoanalyzer Olympus 640 or Olympus 400 system (Olympus Diagnostica GmbH, Hamburg, Germany) and results are shown in Table 32. In this experiment only three biochemical parameters were evaluated due to the small amount of plasma that was obtained. Animals treated with extract of MPP-4 (*Z. chalybeum*) had normal values of blood urea nitrogen (BUN) 6.05, as compared to the control but LDH was significantly higher ($P<0.05$), almost three fold (Table 32). Mice treated with MPP-5 (*W. ugandensis)* had BUN level much lower (4.8) compared to control and lower levels of AST (120.5) were observed. Animals that received extracts of MPP-7 showed significant low levels AST compared to control.

The animals treated with aloe based herbal paste HP-1 (Table 33), had blood urea nitrogen (BUN) of 9.38, Aspartate aminotransferase **(**AST) levels of 27.16, Alanine aminotransferase **(**ALT), 34.5, Alkaline phosphatase (ALP) 200.7 and creatine kinase (CK) of 217.2 which was slightly higher than the control. Animals that received HP-2 showed significant ($P<0.05$) lower LDH compared to control Animals that were administered with MPP-2 showed significant ($P<0.05$), lower LDH ($P=0.043$) compared to control. Extracts of MPP-3 had significant ($P<0.05$) ($P=0.017$) higher ALT and CK ($P=0.015$) but lower LDH levels compared to control. Animals in group HP-5 showed significantly lower LDH ($P<0.05$) ($P=0.004$) compared to control in category 2.

Table 32. The effect of oral administration of 1000 mg/kg body weight of different plant extracts on some end point biochemical parameters in mice

ANALYTE	BUN	AST	LDH
CONTROL	7.18±2.46	283.5±7	716.0±79
MPP-4	6.05±1.34	272.5±31.8	2723.0±151*
MPP-5	4.8±1.96	120.5± 6.3	1607.0±73
MPP-6	4.05±3.7	93.0±46	732.0±2.8
MPP-7	4.23±0.25	104.25±17.1*	432.0±16
MPP-8	9.98±2.71	150.5±10.6	2300.0±398

Results are expressed as means ± standard error mean (SEM). *$P<0.05$ significantly different from control. KEY: ALT- Alanine aminotransferase; AST - aspartate aminotransferase; LDH-lactate dehydogenase BUN- Blood urea nitrogen, product code – refer to Table 5

Table 33. The effect of oral administration of 1000 mg/kg body weight of different herbal products on biochemical parameters of mice

ANALYTE	BUN	AST	ALT	ALP	LDH	CK
CONTROL	9.1±0.9	30.83±6.89	30.83±3.73	166.68± 24.19	1726.66±95.9	128.65±24.8
MPP-2	7.96±0.64	33.4±7.13	31.6±5.81	172.4 ±44.19	1228.5±153.18*	187.63±30.4
MPP-3	8.95±0.49	37.33±3.7	45.83±3.70*	234.16± 30. 19	1350.16±131.10*	235.83±26.46*
HP-1	9.38±0.88	27.16±8.24	34.5±6.73	200.7± 32.9	1286.16±151.07	217.2±33.9
HP-2	10.10±1.15	48.83±6.0	42.83±7.24	248.80±40.93	1622.67±96.50	588.50±292.49
HP-5	9.48±0.67	38.83±11.77	39.5±10.70	216.83±63.29	1138.5±125.6*	168.5±42.86

Results are expressed as means ± standard error mean (SEM). *P<0.05 significantly different from, AST - aspartate aminotransferase; ALT- alanine aminotransferase; LDH-lactate dehydogenase, ALP- Alkaline phosphatase CK- creatine Kinase Product code – Refer to Table 5 and 6

4.10 Effects of oral administration of herbal materials on histopathology of vital organs

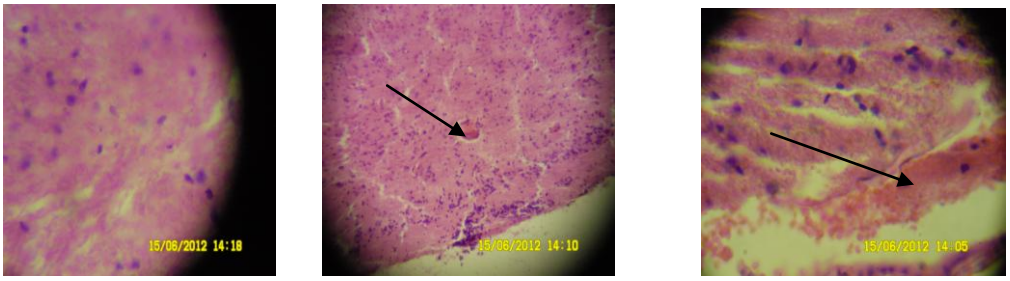
The mortality and pathological conditions observed during the study are recorded in Table 34. The highest mortality reported was 50% in animals treated with MPP-5 (*W. ugandensis*). Only one animal died (16.6 %) in mice treated with extracts of MPP-2 (*S. didymobotrya*) and MPP-4 (*Z. chalybeum*). Sinusoidal congestion was common in animals treated with extracts of MPP-5 (*W. ugandensis*) with 33% of mice in this group showing the condition. Alterations of hepatocytes architecture was high in animals treated with MPP-4 (*Z. chalybeum*) and in animals treated with MPP-6 (*T. brownii*). Mild nephrosis was observed in all the treatments group apart from animals treated with *Z. chalybeum.*

Table 34. Percentage prevalence of pathological conditions in mice treated with extracts of herbal products

		Herbal extracts									
Organ	Pathological Conditions	MPP - 2	MPP - 3	MPP - 4	MPP - 5	MPP - 6	MPP - 7	MPP - 8	HP-1	HP-2	HP-5
Liver	Mortality	16.6	0	16.6	50	0	0	0	0	0	0
	Sinusoid	20	33.3	17	33	0	0	16.6	0	0	100
	Hepatocytes	60	66.7	50	0	50	0	33.3	50	33.3	50
Kidney	Nephrosis	60	50	0	33	16.6	33	16.6	50	33.3	50
	Blood vessel Congestion	40	50	0	33	16.6	0	16.6	16.7	0	66.7
Lungs	Aletactasis	0	33.3	0	0	0	0	0	50	16.7	100
	Emphysema	0	33.3	0	0	0	0	0	50	16.7	66.7
	Sinusoid	60	50	0	0	0	0	0	50	16.7	16.7
	Blood vessel congestion	20	33.3	0	0	0	0	0	50	33.3	66.7
Spleen	Sinusoid	60	66.7	0	0	0	0	0	0	16.7	100
	More Red pulp	20	33.3	33.3	0	16.6	0	0	50	33.3	66.7
	More White Pulp	20	16.7	0	33.3	16.6	0	16.6	0	0	0
	Heamatin	0	50	0	33.3	33.3	0	16.6	0	0	50
Brain	Heamorrage	0	0	0	0	0	33	0	0	0	50
	Plasma cells	0	16.7	0	66	33.3	33	50	0	0	100
Heart	Myelination	0	0	0	0	33.3	17	0	16.7	0	0
	Macrophage	0	0	0	0	33.3	17	0	16.7	0	0
Testis	Reduced sperms	40	0	0	0	0	0	0	50	0	0
Intestine	Sloughing	40	0	0	0	0	0	0	33.3	16.7	0

4.10.2.1 Effects of herbal treatment on brain tissues

The brain tissue in most of animals showed normal cellular architecture as similar to the control (Plate 1 A). Administration of the animals with herbal MPP-7, showed brain heamorrage (Plate 1 B) blood capillary congestion, destruction of the brain membrane and reduced number of neurons. Accumulation of plasma cells was seen in brain of animals treated with *W. ugandensis.*

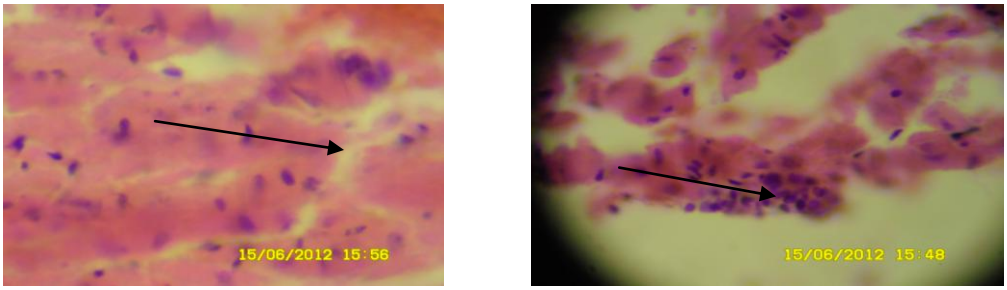


A B C

Plate 1. A. Histological section of brain from a mouse treated with normal Physiological saline for 21 days showing intact tissues. B. Histological section of the brain of the mouse treated with herbal mixtures showing blood vessel congestion (arrow) Mg X100. C. Histological section of brain showing severe congestion of blood vessel (arrow) (Mg X400).

4.10.2.2 Effects of herbal treatment on heart tissues

Heart tissues from the control group showed intact muscles with no accumulation of macrophages (Plate 2A). The heart tissue of mice treated with dichloromethane/ methanol extracts of MPP-7 herbal mixture of *T. brownii, Z. chalybeum* and *W. ugandensis* showed accumulation of macrophage cells and myocardial degeneration (Plate 2 B).

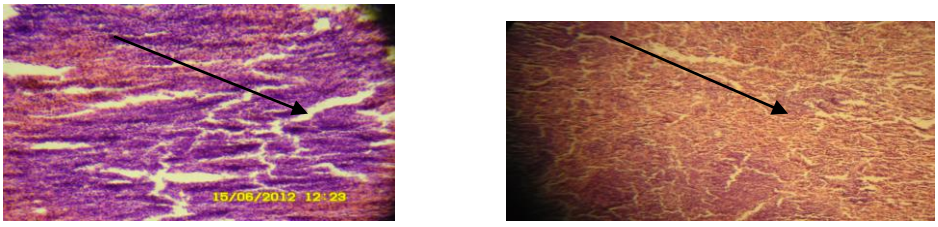


A. **B.**

Plate 2. A. Histological section of heart tissues of a mouse treated with normal physiological saline showing intact mycordial tissues (arrow). B. Histological section of heart tissues of mouse treated with herbal extracts of MPP-7 showing macrophage aggregation and degeneration of heart muscle (arrow) (Mg X400**).**

4.10.2.3 Effects of herbal treatment on spleen

Spleen from the control control group showed well defied follicles containing germinal centres. The white and red palp had proportional distribution (Plate 3 A). while those treated with *W. ugandensis* herbal mixture (HP-5), showed lymphocytic necrosis (Plate 3 B).



A. B.

Plate 3.A Histological section of spleen of a mouse treated with physiological saline for 21 days showing intact lymphoid tissues (arrow) (Mg X400). B**.** Histological section of spleen of mouse treated with product HP-5 (*W.ugandensis* herbal mixture) showing reduction of lymphoid population (arrow) (Mg X100)

4.10.2.4 Effects of herbal treatment on liver

The liver cells in the mice in the control group characterized normal hepatic cells, with distinct nuclei, normal eosinophilic cytoplasm with normal sinusoids (Plate 4). Under the microscopic examination, the liver of mice treated with plant extract of MPP-6- *T. brownii* showed abnormal cellular architecture with nucleated formation mostly around the central veins. Congestion and sinusoid haemorrage was observed. However animals treated with MPP-2 *(S. didymobotrya*) showed hepatosis with pyknosis and reduced cytoplasm, denser nucleus, severe congestion, fibrin deposition and general disruption of hepatocytes anatomy. The livers of the animals treated with MPP-3 (*E. divinorum*) root extracts were characterized by hydrophobic degeneration and cellular boundary are lacking (Plate 4).

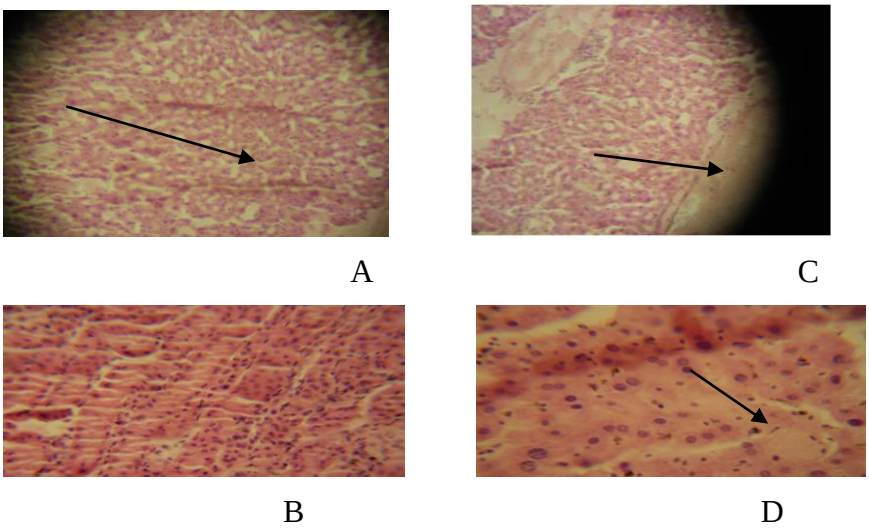
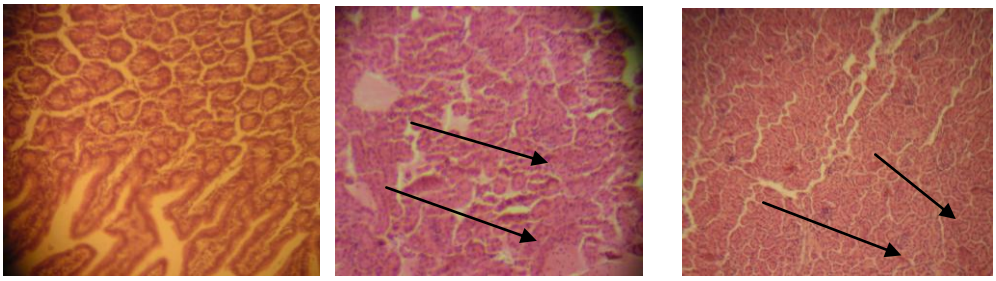


Plate 4. A and B. Histological section of liver of a mouse treated with normal physiological saline for 21 days showing intact hepatocytes (Mg X100 and 400 respectively). C. Histological section of mouse treated with extracts *S. didymobotrya* showing hepatososis with severe congestion in the central vein (arrow) (Mg X100). D. Histological section of mouse treated with extracts *E. divinorum* showing massive destruction of hepatocytes (arrow) (MgX400)

4.10.2.5 Effects of herbal treatment on kidney

Kidney from the control group displayed normal renal architecture with normal glomeruli, proximal tubules and collecting ducts (Plate 5 A). Mice treated with root extracts of *E. divinorum* showed mild nephritis with no proper partitions of cells (Plate, 5). Similar situation was seen in mice in treated with herbal mixture MPP-7 and MPP-2 (*S. didymobotrya*) root extracts.

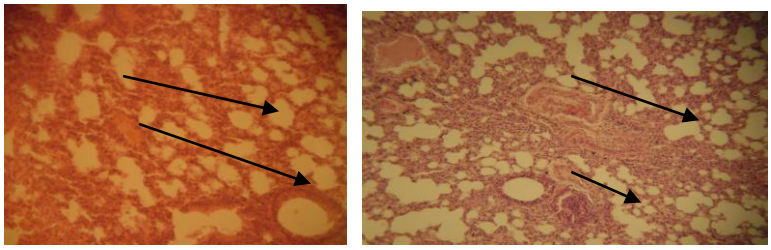


A **B** **C**

Plate 5. A. Histological section of kidney of mouse treated with normal physiological saline (Mg X 100) with a normal architecture. B. Histological section from kidney of mouse treated with root extracts of *E. divinorum* showing diffuse degeneration of tubular epithelium (arrow) and general nephritis and eosinophilic caste (Mg X 400). C. Histological section from the kidney of mouse treated with herbal extracts of MPP-7 showing mild nephritis (arrow) (Mg X 400**).**

4.10.2.6 Effects of herbal treatment on lungs

Lung tissue from the satellite group showed fine alveolar sac structure and well defined ducts (Plate 6). Mice that were treated with aloe paste HP-1, displayed atelectasis and alveolar emphysema (Plate 6 B).

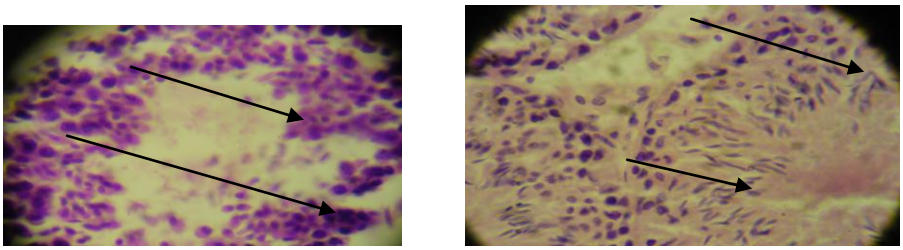


A **B**

Plate 6. A. Histological section of lungs from mouse treated orally with normal physiological saline for (21) days. The air sacs (alveolar tissues) are observable (arrow). B. Histological section of lungs from mouse treated with aloe based tooth paste showing congestion of blood vessel (Arrow).

4.10.2.7 Effects of herbal treatment on testicular tissues

Mice from the control had their testicular organs with normal somniferous tubules showing different stages of germinal cells with spermatozoa and many spermatogonia. Mice treated MPP-2 (*S. didymobotrya*) had decreased spermatogonia (Plate 7).



A

B

Plate 7**.** A**.** Histological section of testicular tissues of mouse treated with normal physiological saline for (21) days showing normal population of spermatogonia (arrow) Mg X 400. B**.** Histological section of the testicular tissues of mouse treated with dichloromethane, methanol extracts of MPP-2 *S. didymobotrya* showing decreased spermatogonia (arrow) Mg X 400.

CHAPTER FIVE

5 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

Gender division of labour among subsistence communities is common (Voeks, 2007). The current findings indicates that majority of the respondents were men (78%). This contradicts the finding in South Africa where 74 % of plant harvesters, street vendors and traditional healers were women and only 26 % were men (Donald and Cocks, 2002). Studies in different parts of world show that women have better knowledge of plants than men (Begossi *et al.*, 2002). In this study the dominance of men in the trade could be due to the difficulties involved in the sourcing and transportation of herbal materials. Women are more likely to be involved in management of house hold chores.

Ethnobotanical researchers have frequently lamented the little or no interest among young members of communities to assimilate and pass on the medicinal plant legacy of previous generation (Begossi *et al*., 2002; Voeks and Nyawa, 2001). On the contrary, the current study shows that most of the respondents were in age group of 21-50 years. The involvement of the youthful group in herbal trade could be due to lack of employment but not necessarily due to herbal knowledge. The inclusion of the youth in the herbal trade is a sure way of preserving the traditional knowledge and cultural set up of the society. Voeks (2007) indicates that the most pressing threat to the knowledge and existence of medicinal plants in tropical regions appears

to be culture changes. The findings of this work alleviate such fears since youth are more involved in herbal trade. The high level of illiteracy (40 %) recorded here agrees with earlier findings in South Africa and Ethiopia (Giday *et al*., 2009). Most of the traditional knowledge is passed orally from generation to generation hence no need of formal education.

In Africa and other parts of the world, studies show that herbal materials are prepared under unhygienic condition lacking in sanitation facilities (Oyetayo, 2008; Stevic *et al.*, 2012). The finding of the current study agrees with these facts since only 10 % of the premises were located in hygienic condition. The mode of transport and storage of herbal material is likely to compromise quality through contamination. Most of these products were stored in recycled containers including paint tins increasing the possibility of heavy metal contamination. The storage of the products in polythene bags that are heat labile is likely to alter the active chemical components of the herbal powders in the market.

The results of the current investigation show that a large number of plants (35 species) are traditionally used in management of oral health care. A similar number of plants have been reported in different parts of India although they are different species (Jose *et al.,* 2011). A lower figure has been recorded by Badgujar (2008) for Maharashtra in India.

Various plant parts are harvested for the preparation of herbal materials. The harvesting of roots and stem bark threatens plant survival. Harvesting plant medicines from bark is known to be the most destructive method of harvesting particularly because debarked trees rarely survive (Zschocke *et al*., 2000).

The ethnomedicinal uses of several plants recorded here agree with their pharmacological work and traditional uses elsewhere. The use of *C. edulis* to relieve tooth ache agrees with Iwu (1993) while its use in management of arthritis, sorcery, typhoid, ulcers and anti diarrhoeal conditions by Nandi community is documented by Jemto *et al.* (2010) and for painkilling by Njoroge and Bussman (2007). Pharmacological studies on *Balanites aegyptiaca* root extracts demonstrated strong anti Candida activity (Runyoro *et al.*, 2006) and antibacterial activity (Karmegen *et al.,* 2008), a property that probably explains why the species is appropriate for management of mouth ulcers as reported in this study. The use of *Bidens pilosa* in treatment of sore throat in Rwanda (Alfonse *et al*., 2010) and management of wound and cuts in South Africa (Boer *et al.*, 2005) agrees with the current uses in oral health. These properties may be attributed to the anti-ulcerogenic properties (Geissberger and Sequin, 1991) and antimicrobial properties as recorded by Rojas *et al*. (2006) and Oliveira *et al*. (2004).

The pain relieving activities of *T. minuta* can be adduced to presence of camphor (Ghiasvand *et al.,* 2011) which has analgesic and anti-infective, antipruritic properties (Budavari, 1989). *W. ugandensis* is highly valued for its medicinal

properties (Akwatulira *et al*., 2011). *W. ugandensis* has been recorded as a useful pain killer in tooth ache (Kokwaro, 2009; Beentje, 1984; Iwu, 1993; Gacathi, 2007; Wamwala *et al.,* 2006), antimalaria (Kokwaro, 2009, Kiringe, 2006), antituberclosis (Wube *et al*., 2005) and treatment of HIV opportunistic infections (Kayombo *et al*., 2007).

Euclea divinorum is sold all over Kenya as a tooth brush (Maundu *et al*., 1999) an indication of its popularity among many communities in Kenya. Antiperiodontopathic bacteria activity of this species (Homer *et al.,* 1990) and management of bacterial diseases such as gonorrhea (Balemie *et al*., 2004) has been documented. The anti inflammatory and antimicrobial properties of *Medicago sativa* (Doss *et al*., 2011) are likely to be basis of its use in management of tonsillitis. *Z. chalybeum* is used to manage chronic joint pains by the Kambas of Kenya (Wambugu *et al.,* 2011) indicating that it might be having some analgesic properties hence its use in tooth ache relief. The wound healing (Nayak *et al.,* 2008) and antibiotic properties (Olaeta *et al*., 2007) of *P. americana* are qualities that would make it useful in management of mouth ulcers.

The use of tissues of *Capsicum* species in oral herbal formulations corroborates with findings by Iwu (1993) where the extracts of the fruit are employed in various formulations like mouth gurgle to manage laryngitis. The ethnobotanical uses of *A. nilotica* recorded agree with Meena *et al*. (2006), where tender twigs are used as tooth brush. The antimicrobial property of stem bark extracts against some oral

bacteria such as *S. viridians, S. aureus* and *E. coli* has been recorded (Ali *et al.*, 2012; Kalaivani and Mathew, 2010; Banso *et al*., 2009) thus validate its use in oral health.

Phytochemical analysis of *M. africana* seeds revealed the presence of several kinds of secondary metabolites (Abbhi *et al*., 2011) justifying its ethnomedicinal uses. Some of the plants used to manage tooth ache in this study have been recorded elsewhere to have similar application. *C. hirsuta* is used as a pain killer by Kikuyus of Kenya (Gachathi, 2007) while its anti-inflammatory activities have been reported by Morgan (1981) and Xu *et al.* (1996). *S. incanum* has been documented as useful for tooth ache management (Mwonjoria *et al.*, 2011; Kokwaro, 2009; Beentje, 1994). Fruits of *D. stramonium* are used for tooth ache, tonsillitis and sore throat (Van Wyke *et al.*, 1997). The leaves are smoked to treat headache, although the plant is reported to be very poisonous (Beentje, 1994). The same species contains hysocyamine and protein albumin which are responsible for narcotic, antispasmodic and anodyne (Ali, 1993). Investigation on safe dosage need to be carried out to avoid poisoning since many herbalists are not trained.

Polyherb therapy is common in herbal practice but very few studies have so far recorded the use of different plants in combinations to prepare herbal materials. *W. ugandensis* and *Z. chelybeum* are used in formulation of a wound management product in Uganda (Ogwang *et al.*, 2008) while *T. brownii* is used combined with other plants to manage various conditions by Mbeere people (Kareru *et al*., 2007).

The use of polyherbal concoctions needs investigation with respect to bioassay and phytochemistry. Synergism of these combinations needs thorough investigations. In Thailand herbal combination are used to manage Oral mucosal diseases (OMDs), such as recurrent aphthous stomatitis (RAS) that affects about 0.5-60% of the population (Jurge *et al.,* 2006; Zain, 2000). The methanolic extracts of *P. africana* haves demonstrated antimicrobial activity against bacteria and fungi (Bii *et al.*, 2010). Combination of pure compounds isolated from *J. procera* with Isonicotinic acid hydrazide (INH) has demonstrated synergistic activity against mycobacterium species (Mossa *et al.,* 2004). The extracts of *D. abyssinica* have been shown to inhibit the growth of fungal strains (Geyid *et al*., 2005). The antimicrobial properties of *M. piperita* (peppermint) plant against *E. coli, S. aureas* and *C. albicans* has been demonstrated (Pramilla *et al.,* 2012). The analgesic properties of *A. remota* have been reported (Makonnen *et al*., 2003). Organic extracts of *P. barbatus* were reported to posses anti-inflammatory and antimicrobial properties (Matu and Van Staden, 2003; Runyoro *et al.,* 2006). This trend is common in Kenya as well as other countries.

The current study reveals inadequate labeling and lack of KEBS mark of quality implying the low standards of products in the market and failure of the product to meet the WHO standards. This agrees with earlier studies carried out on documentation of herbal products in other parts of the world where labeling is not clearly done (Bandaranayake, 2006).

The mineral elements play vital role in many physiological reactions and their deficiency or excess can affect human health (Ekinci *et al*., 2004). In the current study the high levels of aluminum reported pose a health hazard to the consumers. The levels exceed the maximum allowed levels of 0.2 mg/kg (Alwakeel, 2008). The dosage from these medicinal plants should be monitored to avoid toxicity. Aluminium as a metal when present in food, water and soil can induce individuals to suffer from Aluminium toxicity. It is believed that Alzheimer disease is related to Aluminium toxicity. The presence of aluminium could be attributed to environmental pollution in the region where plants were harvested, processed and stored.

The presence of lead in all the products raises concern on the overall quality of herbal products. All the herbal powders exceeded the RDA of 3 mg/week and this is likely to produce brain damage (Samali *et al*., 2011) leading to malfunctioning of both the brain and kidney (Haider, 2004). The levels of lead (1.98-13.75 mg) reported here are much higher compared to those recorded by Ameh *et al.* (2010) and Samali *et al.* (2011) which were below 1 mg. The recorded difference could be due to different ecological condition of the plants or the handling. The levels detected in all the products did not however, exceed the recommended maximum content of 10 mg/gram of dried herbal products. The presence of this element in herbal materials may be due to environmental pollution arising from automobile and industrial activities (Rabia *et al.*, 2012) or poor packaging especially using recycled paint containers.

The high levels of potassium (2994.26-7953.97 mg/kg) reported here agree with literature as documented by Rabia *et al.* (2012). The high potassium levels in plant samples make them suitable sources of potassium, although most of the herbal products exceeded the RDA of 2 g/day (Strain and Cashman, 2009) meaning that the consumers may be at risk of potassium poisoning. The bioavailability studies should be undertaken to determine the actual amount of potassium available. The current levels of potassium are much higher than amount reported by Rabia *et al*. (2012) and Khan *et al*. (2011) which were below 12 mg/g.

Results of the current study indicate high levels of calcium in herbal powders (2284.87-51295.90 mg/Kg) compared to levels reported in medicinal plants (22-109 ppm) in Pakistan (Ata *et al*., 2011), however the results agree with those of Muhammad (2007), where the average levels of calcium reported was 22490.99 ppm. Herbal suspensions had low levels of calcium probably due to the small amount of raw materials that are used to prepare herbal suspensions. Excess use of the herbal powders may result to calcification of soft tissues and also deficiency syndromes associated with other elements.

The amount of iron reported (247.66-1204.9 mg/Kg) is much higher than that reported by Muhammad (2007) of 147.91-540.0 ppm and that of Samali *et al.,* (2011) which ranged from 0.00-5.96 µg/g. The requirement of iron is 20 mg/day for an adult and 10 mg/day for a child, implying that people utilizing the medicinal plant analysed have a good source of Iron. Prolonged daily consumption of this plant is

likely to give elevated levels which are toxic to human. The plant samples can be used to improve iron status of processed foods. Zinc is necessary for bone formation, and wound healing (Samali *et al.*, 2011). The presence of zinc in these products may be responsible for their healing properties. The differences in levels of zinc-observed in plant powder and pastes can be attributed to the processing where a lot of zinc could have been oxidized. The levels reported in this study (4.37-15.36mg/Kg) are much lower than those reported in Ghana 43.5-495.0 µg/g (Annan *et al*, 2010). The highest daily intake of 71.16 µg is much lower than the estimated daily intake which is between (10,000 µg and 20,000 µg/ day (Food and nutrition board, 1980). This shows that the samples analysed are safe for consumption and they can provide the daily requirement of zinc.

High levels of manganese reported could be attributed to its abundance in the soil. The maximum safe and adequate daily dietary intake is 11000 µg/g (Russel, 2001) meaning that herbal powders materials are not safe for consumption. The range of manganese content of (21.5 to 87.8 mg/kg) compares well with levels reported in herbal teas (7.4-86.67) in Nigeria (Samali *et al*., 2011) but the reported levels are much lower than that recorded by Annan *et al*. (2010) of 556 µg /g to 1455 µg.

The high levels of strontium (236.45 mg to 1168.58 mg/kg) in herbal powders justify their use in management of dental problems (Gaby, 1994). *T. brownii* (MPP-6) powder (1168.58 mg/kg) can be a good source of strontium and it can be incorporated in pastes to help control dental caries. Strontium has been reported in

medicinal plants powders of Quetta (Mudassir *et al.*, 2010). Nickel has been associated with degenerative effects on hepatic tissues (Das *et al*., 2006) and respiratory cancer. In this study only 5 (21.7%) of the products had detectable levels of nickel. Similar finding were reported in Ghana by Annan *et al.* (2010). Lack of detectable levels of nickel in various samples probably is due to the fact that plants do not require it or its scarcity in soil.

Chromium plays a major role in regulation of insulin (Manhan and Escott, 2008). The chromium levels recorded here ranges from 0.03 to 7.31 mg/kg while those recorded by Samali *et al*. (2011) ranged from 0.00-2.63 µg/g. The concentration of copper in herbal powders that ranged from 1.5 mg/kg to 8.89 mg/kg is much lower than that reported by Annan *et al.* (2010) of 8.0 to 114.5 mg/g. The difference can be explained by sources of samples. In Kenya, few studies have evaluated levels of mineral elements of herbal preparation. The current study shows presence of high levels of mineral elements in herbal medicine in the market implying an impending danger to the consumer. The United States Pharmacopoeia proposes a limit of 20 µg/g for total content of heavy metals in herbal extracts meaning that majority of herbal suspensions and herb pastes do not meet this criterion.

The presence of microbial contaminants in herbal medicine depends on several environmental factors and this exerts an important impact on the overall quality of herbal products and preparations (Bandayanake, 2006). The microbes isolated and identified in this work such as *E. coli, P. aeruginosa, S. typhi* and *C. albicans* are

some of the microorganisms that present serious health hazard (Arias *et al.,* 1999; Erlich *et al.,* 2001; Wolfgang *et al.,* 2002; Adeleye *et al.,* 2005). The presence of *E. coli* may be due to poor method of handling, of herbal products and faecal contamination since the bacteria is an intestinal flora of humans and other animals (Okunlola *et al.,* 2007). As Stevic *et al.* (2012) observed, contamination with *E. coli* may be as result of the habitat proximity of settlement and animals that contaminate the herbs with urine and feaces, which was evident during the study as most of the products were poorly packaged and the unhygienic condition of the herbal business set up. This was so in Gikomba market, in Nairobi where herbal goods were traded next to Nairobi River that is heavily polluted. Consumers of these products risk infection associated with *E. coli* like diarrhoea especially in children (Chesborough, 2000). Microbial limits of *E. coli* must not be more than 50 colonies (Mulika *et al.,* 2003). The high levels of *E. coli* reflect the poor handling and harvesting techniques of medicinal plants. The significance of feacal bacteria is that their presence indicates possibility of having other harmful bacteria in the product (Forest, 2004). There is urgent need for establishing certain minimum hygiene practices in the preparation of herbal remedies.

Isolation of a gram negative *P. aeruginosa* from herbal materials raises deep health concerns. Edaphic factors are the probable source of the isolate as the bacteria is primarily a soil bacterium, reflecting poor cleaning and harvesting of herbal materials. Proper cleaning of the materials is recommended before utilization to eliminate the contaminants. Salmonella species detected in some samples are

causative agents of various infections like salmonella food poisoning a major problem in the world affecting many countries (Greenwood *et al.*, 1997). Plants cells could be possible sources of contaminants since salmonella species in plant cells can successfully evade all the defense mechanism (Enayatifard *et al.*, 2010).

The presence of the fungi contaminants shows the possibility of poor storage conditions. This requires further investigation since some common species of fungi produce toxins like aflatoxins. According to the WHO (2000), aflatoxins in herbal drugs can be dangerous to health even if they are absorbed in minute amount. Aflatoxins producing fungi sometimes build up during storage.

Presence of pathogenic microbes in these products poses a major health risk to the immune compromised and diabetic patients who normally have ulcers in their oral cavity. The wounds in the mouth can acquire a secondary bacterial infection further worsening their situation. In most countries, there is no universal regulatory system that ensures the safety and activity of phytopharmaceuticals (Bhattaram *et al.*, 2002). This was reflected by lack of KEBS mark of quality in most of the products (Table 4). The product's packaging need improvement to reduce chances of microbial infection. Most products sold in sealed containers had no microbial contaminants at all. This can be explained by the good hygiene packing or probably presence of bacteriostatic substance that would have killed possible microbial contaminants. Investigation of possible antimicrobial adulterants is suggested. The isolation of the pathogens from herbal products in other parts of the world has been reported.

Microbial load of some medicinal plants sold in local markets of Benin, Nigeria reported presence of *P. aeruginosa* and *B. subtilis* among others (Idu *et al.,* 2011). In Kaduna, studies indicated presence of pathogenic *S. typhi* in 65.7% of herbal products analysed and *E. coli* in 58.7% of the samples analysed (Abba *et al.,* 2009). Evaluation of microbial quality of plant materials in Belgrade indicated the presence of *E. coli, Bacillus* and *Clostridia* species (Stevic *et al.,* 2012). Evaluation of microbial and fungal contaminations of six spices and herbal products was carried out in Ghana (Ahene *et al.,* 2011). In the study the spices were found to harbour aflatoxins, nitrobacteria and *P. aeruginosa* among others. In South Africa studies have shown that herbal medicine is heavily contaminated with bacteria (Enayatifard *et al.,* 2009).

The plant kingdom has enormous reservoir of biologically active compounds with various chemical structures and protective or disease preventive properties known as phytochemicals (Abbhi *et al*., 2011). The current phytochemical work on herbal powders leads to the observation of many potentially pharmacologically active secondary metabolites such as alkaloids, steroids, flavonoids, tannins, terpenoids and many others, justifying their use in ethnomedicine. The presence of many phytochemicals in the powder of *W. ugandensis* corroborates with previous finding (Mbwambo *et al*., 2009).

Lack of key secondary metabolites in the liquids and pastes questions their herbal basis. The finding agrees with earlier work in Nigeria where evaluation of two herbal

products revealed lack of these components (Okunlola *et al.*, 2007). This observation contradicts studies in South Africa that showed the herbal products in the market had all the phytochemical compounds investigated (Mtunzi *et al.*, 2012). Lack of secondary metabolites could be attributed to poor processing leading to disintegration of phytochemicals or may be the levels of the secondary metabolites were very low or a fake product.

The problem of antibiotic resistance has necessitated the need for a continued search for new antimicrobial compounds (Sibanda and Okah, 2007) and plants can be a good alternative (Pretorius *et al*., 2003; Moreillion *et al*., 2005). The antimicrobial properties of *W. ugandensis* can be attributed to the many phytochemicals and some elements present in this powder. Studies have demonstrated that antimicrobial properties of natural products can be enhanced by the addition of metal ions (Sivasankaran and Selwin, 2008). The powder of *W. ugandensis* was found to have zinc, copper, and aluminium and these elements have been reported to reduce the water coliform (Varkey, 2010). The variation of antimicrobial effects of the three extracts can be explained on the basis of the extracting solvent as observed by (Idris *et al*., 2009).

The antibacterial activity reported in this study agrees with the findings of those described by various authors (Kioy *et al*., 1990; Lunde and Kubo 2000; Wube *et al*., 2005; Ngure *et al.*, 2009; Opiyo *et al*., 2011; Olila *et al.*, 2001). The antimicrobial activity displayed by MPP-6 (*T. brownii)* against majority of test organisms can be

correlated to the phytochemical constituents found in this plant. The current findings concurs with previous work carried out by Mbwambo *et al*., (2009) where activity against *E. coli, P. aeruginosa, K. pnemoniae B. anthracis* and a fungi, *C. albican* was reported. The high antimicrobial activity observed against *E. coli* (Kareru *et al.*, 2007; Zakaria *et al.*, 2007) contradicts the current finding where *E. coli* showed minimal susceptibility. The reduced antibacterial activity against *E. coli* could be due to development of resistance mechanism towards the plant extract or different ecological areas. From the total viable counts reported from this plant, it is clear that the raw powder cannot kill the microorganisms and therefore extraction of the material with appropriate solvent is necessary.

The reduced antibacterial activity against most of the test organism in the water and dichloromethane extracts of *Z. chalybeum* studies partially corroborates with findings by Olila *et al.* (2001) where the crude extracts as well as purified compounds lacked antibacterial activity against *E. coli* and *S. aureus*. Variation in the current study with that of Olila *et al.* (2001) can be attributed to ecological factors that are known to contribute to the types and amounts of secondary metabolites. The findings on antibacterial activities are in agreement with investigation on the plant extracts as indicated by Matu and Van Stande (2003). Recent studies show that use of extracts in combinations of two or more plants exhibit effective antimicrobial activity against a wide range of microorganisms including drug resistant bacteria (Karmegam *et al*., 2008). In this study, investigation of synergistic antimicrobial activity of herbal mixture extracts of *T. brownii, W.*

ugandensis, Z. chalybeum and *A. indica* (MPP-8) resulted to antagonism. In the studies reporting synergy, the crude extracts or purified compounds are normally combined after extraction in a predetermined ratio. In the current study the herbal powders were combined before extraction, and then macerated. This would be the appropriate methods for checking synergy in herbal materials since the herbalists normally combine their preparation before extracting. The higher antimicrobial activity (*Terminalia brownii*) in comparison to its herbal mixture with *Z. chalybeum*, *W. ugandensis* (MPP-7), and *A.indica*, indicates lack of additive effects. This can be explained on the basis of the many phytochemical components found in the plant extracts and their mixtures.

The presence of tannins in *E. divinorum* can contribute to its antimicrobial and anti ulcer properties as plant containing tannins are used to treat non specific inflammation of mouth, throat and slightly injured skin (Westernderp, 2006). The low antimicrobial activity could be due to low amount of tannins. Combination therapy should be sought to address the resistance seen in *C. albicans* and *L. acidophilus.* The moderate antimicrobial properties in dichloromethane/methanol extracts of *S. didymobotrya* bark agrees with Korrir *et al.* (2012), where low antimicrobial activity was found.

The findings of the current work reveal antimicrobial activity in some of the herbal products although they had no detectable phytochemicals in them. It is suspected that this antimicrobial activity can be due to adulterants which should be

investigated. Lack of antimicrobial properties in some products confirms the latest concern of Pharmacy and poisons board of Kenya on the efficacy of herbal materials in the market. The current findings agree with a study evaluating antimicrobial activity of 14 products where 10 products showed antimicrobial activity against the test bacteria while 6 of them showed activity against *C. albicans* (Lee *et al.*, 2005).

The reasonable zones of inhibition displayed by some products can be attributed to stated ingredients on the label which included clove which is used as anadyne for dental emergencies (Prashar *et al.,* 2006). Toxicity and possible adulterants needs to be investigated before it is incorporated fully in the primary oral health care systems. The observed zones of inhibition in herbal pastes HP-1, HP-2 and HP-3 can be attributed to the different herbal extracts found in these pastes. From the label on the product HP-2, it has different herbal extracts such as *S. aromaticum* oil and cinnamon. The antimicrobial properties of mint (Saeed *et al.*, 2006; Pramilla *et al.*, 2012; Elhoussine *et al.*, 2010) and clove are well known (Nunez *et al.*, 2001; Babu *et al.*, 2011; Ayoola *et al.*, 2008).

The ability of the herbal extracts to inhibit the growth of the test organisms indicates lack of antimicrobial resistance of these organisms to the active ingredients in the extracts. This significance cannot be over emphasized with the recent trend of high percentage of multidrug resistance to the present day antibiotics (Styers *et al*., 2006; Komolafe and Adegoke, 2008). Additional studies are needed to determine anti plaque, anticaries and antimycotic properties of the herbal materials used in (OH).

The use of herbal medicine has received a great attention as alternative to synthetic pharmaceutical products, leading to an increase in their demand. As such experimental screening method including thorough toxicity study is important to ascertain their safety (Saha *et al.*, 2011). The toxic signs are shown by the abnormal appearance of respiration pattern, color of body surfaces, frequency and nature of movement, both involuntary and voluntary (Chan, 2003; Auletta 2002). The current investigation reveals lethal toxicity; allergic reactions and poor growth rate effects on laboratory animals treated with *Z. chelybeum* extracts and in some case death was reported for *W. ugandensis*.

Reduction in the body weight is a valuable indicator in evaluating the toxicity of substance (Watthanachaiyingcharoen *et al.*, 2009). The retarded growth recorded in all treatment groups might have resulted from reduced food and water intake as argued by Ilodigwe *et al.* (2010). In case of organic extracts, reduced growth can be attributed to presence of anti nutritional substances such as tannins and saponins found in these plants (Kahnut *et al.*, 1995), components reported to cause nutritional mal-absorption (Conning, 1993). The negative growth rate in MPP-8, shows that the extracts imposed an acute fluid loss, proteolysis, lipolysis and hence the body weight loss (Albert and Zimmet, 1998). The slow growth rate could also be attributed to high levels of iron in the various products (447.2, Table 7) which is known to cause hemochromatosis, characterized by weakness, weight loss among others. The overall

lack of remarkable increase in body weight in various groups indicates the inability of these plants to control muscle wasting and induction of adiposeness.

Extracts of *W. ugandensis* were reported to be highly toxic to VERA cells (Olila *et al.,* 2001). The lethality effect at the cellular level could also be the cause of reported mortality of animals. The elemental analysis shows high levels of manganese and iron which could be the cause of mortality. The phytochemicals of this plant powder are also likely to indjuce lethality and mortality. Records indicate that all parts of *W. ugandensis* are edible and that leaves, bark, young shoots and fruits are used in curries while roots are used for soup (Kokwaro, 2009; Mbuya *et al*., 1994), suggesting that the plant is safe to human, however, a history of traditional usage is not always a reliable guarantee of safety since it is difficult for traditional practitioners to detect or monitor delayed effects (mutagenicity), rare adverse effects arising from long term use (Ernst, 1998). The contradictory results of toxicity is probably because, the TMPS patients often chewed leaves or roots or bark or prepared tea by boiling plant parts to extract the active principles compared to the modern technology where organic solvents are used. In addition, historical documentation of toxicity from herbal remedies may be poor because patients may not have the knowledge of identifying the difference between disease and toxicity due to treatment.

The reported increase in relative brain weight in extracts MPP-5 (*W. ugandensis*) and HP-5 (herbal paste) may be due to inflammatory process which leads to accumulation of fluid in the intercellular space as explained by Moore and Dalley

(1999). Significant decrease of brain index as seen in mice treated with (herbal mixture) MPP-7 could have been due to constriction of brain due to heamolysis as seen in Plate 1. Increase in the relative liver organ weight in animals treated with *E. divinorum* (MPP-3) may be due to increase in functional ability of the organ as indicated by Ashafa *et al.* (2009). This implies that extracts are increasing metabolic rate leading to enlargement of the liver.

Haematological parameters provide vital information regarding the status of bone marrow activity and intravascular effects such as haemolysis and anaemia (Gregg and Voigt, 2000). The significant rise in levels of RBC, HCT and HB in mice treated with MPP-6 shows that the extracts increased the rate of erythropoiesis, meaning that the plant can be used to activate bone marrow in cases of anaemic situation. The raised heamatocrit is an indication of haemo concentration which may be due to increased RBC mass (Nwinuka *et al*., 2008). The higher values of RBC and associated parameters may be suggestive of polycythemia (American Diabetes Association, 2000). The raised values observed in treatment with MPP-8 (herbal mixture) may be due to inclusion of *A.indlica* since the leaf extract had demonstrated some heamopoetic activity, the observation could be due to protective effects of tannins and flavanoids which are known to stabilise erythrocytes membranes. Lack of significant reduction in HB and RBC shows that there was no reduction or disturbance in the erythropoisis in bone marrow (Panda *et al*., 1975). The increase of mean volume ration (MCV) observed in treatment groups by extracts of MPP-5 (*W. ugandensis*) could be an indication of large red blood cells. Enlarged RBC are

not able to transport oxygen to various cells and this may result to hypoxia and eventually death of the animals. This fact may help to explain the observed mortality in this treatment group. Administration of extracts *A. indica* (a blood purifier) plant extract showed improvement effect on almost all blood parameters. The observations corroborated with the report of Sharma and Pandey (2010) where inclusion of some blood purifier plants (Kirtikar and Basau, 1993) improves blood parameters. These plants possibly act by stimulating liver and spleen which remove defective and damaged RBCs from peripheral blood circulation.

Platelet aggregation plays a pivotal role in the physiopathology of thrombotic diseases. Moreover, platelet activity may play a major role in the development as well as in the stability of atherosclerotic plaques (Adebayo *et al*., 2010). The anti platelet activity observed in animals treated with extracts of MPP-3 *(E. divinorum*) could be due to presence of flavonoids. Flavonoids have been shown to act at the blood platelet level by preventing platelet activity-related thrombosis (Harnafi and Amrani, 2007). The extract may be useful in management of cardiovascular diseases.

The decreased counts of white blood cell (WBC) reported in animals treated with MPP-5 (*W. ugandensis*) probably show immunosuppressive activity. The current findings disagrees with the report by Swenson and Reece (1993), who reported that toxic plants cannot have normal response to foreign bodies or stress associated with the chronic toxicity studies**.** In clinical practice an increase in neutrophils in the blood (neutrophil 'leucocytosis' or 'neutrophilia') is a common accompaniment to infection and tissue injury. The significant rise of neutrophils in treatment with

MPP-7 (Herbal mixture) and MPP-8 (herbal mixture) could be due to tissue injury and this may be responsible for the reduced growth observed in these groups. The reduced neutrophils in the blood (neutropenia) seen in MPP-5 could be due to infection as result of decreased count of white blood cells. The drugs may be responsible for neutropenia.

An increase in concentration of Alaline aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) in the serum directly reflects a major permeability, congestion or cell rupture (Benjamin, 1978; Pieme *et al.,* 2006; Tedong *et al.,* 2008). The remarkable increase of ALT enzyme in mice treated with *E. divinorum* root extracts could be associated with hepatocellular necrosis an argument supported by histopathological studies (Plate 4B). Prolonged use of this plant may result to possible liver damage but since the plant extract are taken in very small quantity pronounced liver damage may not occur. The significant decrease in AST in animals treated with MPP-5 and MPP-7 may be due to severe prolonged intoxication as a result of severe damage to liver making most of cells to be non-functional. The destruction could be due to various phytochemicals and elements contained in these plant extracts. The significant rise in LDH could be associated with destruction of several body organs as this enzyme is found in all body parts. Majority of animals showed degeneration of various tissues. The effects of these products may not be obvious on liver enzymes as these products are not normally swallowed, but the labeling does not give direction on the usage of the

paste. This would be dangerous to children who tend to swallow the product during the cleaning of the teeth.

The normal blood urea nitrogen reported in all treatment groups showed that plant extracts had no effects on kidney function as the amount of urea is a test of renal function (Cheesborough, 2005). The remarkable increase of Creatine Kinase (CK) observed in animals treated with MPP-3 may be due to destructive effects of plant extract on the heart, brain and skeletal muscle as indicated by Mayne, (1994). The increased level of CK observed in animals treated with HP-1 shows the toxic effects of the extract on the skeletal muscles as the other organs seem to be intact as indicated in the Table 28. The various isoenzymes of ALP are ubiquitous throughout the body, although they are mainly present in liver, bone, intestine, kidney, placenta and white blood cells (Tietz, 1976). The high levels of enzyme reported in various groups could be associated with destruction of various tissues of heart, liver and mild nephrosis in various groups (Table, 34).

5.2 Conclusions

From the results and discussions above, the following conclusions can be made**.**

a. Herbal materials are widely used in Nairobi to manage oral health conditions. They are used in form of chewing sticks, powders, pastes and suspensions. Polyherb mixtures are used to prepare powders and

suspensions. This implies that the use of herbs is an accepted alternative medicine in Nairobi, the capital city of Kenya.

b. Herbal materials especially powders have very high levels of toxic elements such as aluminium, lead, copper that could be a health hazard to the consumers

c. Some herbal products such as MPP-1, MPP-4 and HP-5 sold in Nairobi are contaminated with pathogenic bacteria *E. coli* and *S. typhi* and their utilisation may lead to infection

d. Phytochejmicals in all the liquid and paste herbal products used in management of oral conditions were below detectable levels. This suggests that they could be adulterated with mineral elements.

e. Herbal suspensions HS-1, HS-2, HS-3, HS-4 and HS-5 used in Nairobi have antimicrobial properties while HS-6, HS-7, HS-8 and HS-9 had no antimicrobial activity. This implies that some herbal products in the market are not effective as advertised and some may be counterfeight.

f. (i) The herbal materials MPP-2, MPP-7 and MPP-8 gave negative growth in mice while herbal materials, MPP-2, MPP-4 and MPP-5-had lethal effects on mice. This implies that prolonged use of these herbal materials by children may retard growth.

(ii) The herbal materials MPP-2, MPP-4, MPP-7, and HP-1 significantly atrophied the brain. This shows that the use of these products may interfere with functioning of the brain

(iii) The herbal materials MPP-5 significantly lowered heamatocrit while the herbal materials MPP-3 significantly raised alanine aminotransferase and creatine kinase compared to control implying that the use of product (MPP-3) may lead to destruction of liver and kidney.

(iv) The herbal material MPP-2, MPP-3, MPP-5, and HP-2 used in management of oral health resulted to necrotic changes on kidney, liver lungs and lungs respectively. This implies that prolonged use may result to respective organ failure

The overall conclusion of the study is that some of the herbal materials used in management of oral health lack antimicrobial activity while others are not safe for human use.

5.3 Recommendations

Based on the data collected and analyzed, and from the conclusions drawn from the study, a number of recommendations can be made.

a. The traditional medical practitioners should be sensitised on good harvesting practice to reduce the microbial and heavy metal contaminants in the herbal materials.

b. The Pharmacy and Poisons Board of Kenya should put in place regulatory mechanism to ensure safe handling of herbal products by traditional medical practitioners. All the herbal products should undergo thorough evaluation of toxicity before releasing them to the market

c. The Ministry of Health should encourage and educate consumers of herbal materials safe use of herbal products in the market.

d. The ministry of Health of Kenya should develop a policy on the integration of some of the effective herbal products used in Oral health care into the mainstream health care.

5.4 suggestions for further research

a. Investigation of safety and efficacy of herbal products for management of other chronic conditions such diabetes and malaria should be conducted

b. Evaluation of chemical composition of efficacious products with undetectable levels of phytochemical constituents should be carried out.

c. The value chain of the practice of traditional herbal medicine in Kenya should be researched on and enhanced.

d. The pharmacognostic characteristics of commonly used herbal materials in Kenya and the East African region should be established and documented.

REFERENCES

Abba, D., Inabo, H. I., Yakubu, S. E. and Olonitola, O. S. (2009). Contamination of herbal medicinal products marketed in Kaduna metro polis with selected pathogens. *Afri J Tradit Complement Altern Med* **6(1):** 70-77.

Abbhi, V., Joseph, L. and George, M. (2011). Phytochemical analysis of fruits extract of *Myrsine Africana, Inter kJ of pharm and pharmaceutical sci* **3(4):** 427-430.

Adebayo, A. H., Abolaji, A. O., Opata, T.K., and Adegbenro, I.K. (2010). Effects of ethanolic leaf of *Chrysophyllum albidum* G. on biochemical and haematological parameters of albino wistar rats. *Afri J of Biotechnol* **9(14):** 2145-2150.

Adebiyi, A. O., Koekemor, T., Adebiyi, A., Smith, N., Baxter, E., Naude, R.J. and Venter, M. (2009). Antimicrobial and antioxidant activities of crude extracts of two Nigerian chewing sticks. *Pharm. Bio* **47**: 320-327.

Adeleye, I. A., Okogi, G. and Ojo, E. O. (2005). Microbial contamination of herbal preparation in Lagos, Nigeria *J health popul nutr* **23(3):** 296-297.

Adeneye, A. A., Ajagabonna, O. P., Adeleke, T. I., and Bello, S. O. (2006). Preliminary toxicity and phytochemical studies of the stem bark of aqueos extract of *Musanga cecropoides* in rats. *J. Ethnopharmacol* **105**: 374-379.

Agbafor, K. N., Akubugwo, E. I., Ogbashi, M. E., Ajah, P. M. and Ukwandu, C. C (2011). Chemical and antimicrobial properties of leaf extracts of *Zapoteca portoricensis*, *Res J. Med.Plant*, **5**: 605-612.

Agnew, A. D. and Agnew, S. (1994). Upland Kenya wild flowers. Second Edition, Nairobi pp 44-296.

Ahene, R. E., Odaitten, G. T. and Owusu, E. (2011). Fungal and bacterial contaminants of six spices and spice products in Ghana**.** *Afri J of Enviro Sci and Tech.* **5(9):** 633-640.

Akerere, J. O. and Godwin, C. U. (2002). Aspects of microbial contamination of tablets dispensed in hospitals and community pharmacies in Benin City, Nigeria**.** *Trop.J. Pharm. Res*. **1 (1):** 23-28.

Akinyemi, K. O., Oladapo, O., Okwara, C. E., Ibe, C. C. and Fasure, K. A. (2005). Screening of crude extracts of six medicinal plants used in southwest Nigerian unorthodox medicine for antimethicillin resistant *Staphylococcus aureus* activity. *BMC Comp and Alt Med,* 5:6.

Akwatulira, F., Gwali, F., Ssegawa, P., Okullo, J. B., Tummwebazei, S. B., Mbwambo, J. R. and Muchugi, A. (2011). Vegetative propagation of *Warbugia Ugandensis* Sprague, An important medicinal plant tree species in eastern Africa**.** *J of Med. Plants Res* **5(30):** 6615-6621.

Alberti, K.G. and Zimmet, P.Z. (1998). Definition Diagnosis and classification of Diabetes Mellitus and its complications part 1: Disguises and classification of Diabetes Mellitus provisional Report of WHO consultations. *Diabet. Med*., **15**: 539-553.

Alfonse, N.I., Bigendako, M., Fawcet, K. and Yansheng, G. U. (2010). Ethnobotaniac study around volcanoes national park**,** Rwanda**.** *New York Sci J* **3(5):** 37-49.

Ali, A., Akhtar, N., Khan, A., Khan, M.S., Rasul, A., Zaman-Shahiquz, Khalid, N., Waseem, K., Mahood, T. and Ali, L. (2012). *Acacia nilotica*: A plant of multipurpose medicinal uses. *J of Med Plants Res* **6(9):**1492-1496.

Ali, M. (1993). Text book of pharmacognosy, CBS publishers and distributors pp 298-300.

Aliyu, A. B., Musa, A. M., Oshanimi, J. A., Ibrahim, H. A. and Oyewale (2006). Phytochemical analysis and mineral elements composition of some medicinal plants of Northern Nigeria**.** *Nigr. J. Pharm. Sci.* **7:** 119-125.

Almas, K. (2001). The antimicrobial effects of seven different types of Asian chewing sticks *Odonto- Stomatologie Tropicale* **95:** 17-20.

Almas, K. and Al-Zeid, Z. (2004). The immediate antimicrobial effects of a toothbrush and Miswaki on cariogenic bacteria. A clinical study. *J. Contemporary dent Practice***, 5(1):**1-8.

Alwakeel, S. S. (2008). Microbial and heavy metal contamination of herbal medicines. *Res J of microbio* **3(12)**: 683-691.

Ameh, S. J., Tarfa, F., Abdulkareem, M.T., Ibe, M.C., Onanugu, C. and Obodozie, O. O. (2010). Pysiochemical analysis of the aqueos extract of six Nigerian medicinal plants, *Tro J Pharm Res* **9(2):** 119-125.

American Diabetes Association, (2000). Nutrition Recommendation and principles for people with diabetes mellitus, clinical practice recommendations.*Diabet. Care*, **23**: 543-546.

Ang, H. and Lee, K. L. (2006). Contamination of mercury in tongkat *Ali hitam* herbal preparations. *Food Chem. Toxicol* **44**: 1245-1250.

Annan, K., Kojo, A. I., Cindy, A. N Samuek and Tunkugmen, M. M. (**2010)**. Profile of heavy metals in some medicinal plants from Ghana commonly used as component of herbal formulations. *Pharmacognosy Res* **2(1):** 41-44.

Arendorf, T. M., Bredekamp, B., Cloete, C. A. and Sauer, G. (**1998).** Oral manifestations of HIV infection in 600 South Afhrican patients. *J of oral Patho and Med* **27**:176-179.

Arias, M. L., Chaves, C. and Alfaro, D. (1999). Microbiological analysis of some herbal infusions used as medicine. *Rev.Biomed* **10 (1):** 1-6.

Ashafa, A. T., Yakubu, M. T., Grierson, D. S. and Afolayan, A. J. (2009). Effects of aqueous extract from the leaves of *Chrysocoma ciliata* L. on some biochemical parameters of Wistar rats. *Afr. J. Biotechnol.* **8**: 1425- 1430.

Ata, S., Farooq, F. and Javed, S. (2011). Elemental profile of 24 common medicinal plants of Pakistan and their direct link with Traditional use**.** *J of Med Plant Res* **5(26):** 6164-6168.

Atukorala, T. M. and Waidyanatha, U. S. **(1987**). Zinc and copper content of some common foods. *J Nat Sci Coun*.**15**: 61–69.

Auletta, C. S. (2002). Acute, Subchronic and Chronic Toxicology. In: Derelanko, M.J., Hollinger, M.A., (editors). Handbook of Toxicology. USA: CRC Press Inc. pp 69-126.

Ayoola, G. A, Lawore, F. M, Adelowotan, T., Aibinu. E., Adenipekun, E., Coker, H. A., and Odugbemi, T. O (2008). Chemical analysis and antimicrobial activity of the essential oil of *Syzigium aromaticum* (Clove). *Afri J of Micro Res* **2:**162-166.

Babu, A. J., Sundari, R., Indumathi, J., Srujan, R. V. and Sravanthi, M. (2011). Study on the antimicrobial activity and minimum inhibitory concentration of Essential Oils of species**.** *Vet World* **4(7):** 311-316.

Badgujar, S. B., Mahajan, R.T. and Kosalge, S. B. (2008). Traditional practice for oral health care in Nandurbar district of Mahashtra, India**,** *Ethnobotanical leaflets* **12:** 1137-1144.

Badria, F. A, and Zidan, O. A. **(2004**). Natural products for dental caries prevention. *J Med Food* **7**: 381-384.

Balemie, K., Kelbessa, E. and Asfaw, Z. (2004). Indigenous medicinal plant utilization management and threats in Fentalle area, Eastern Shewa, Ethiopia**.** *Ethiopian J Biol Sci* **3**: 37-58.

Bandaranayake, W. M. (2006). *Quality control, screening, toxicity and regulation of herbal drugs.* Wiley-VCH Verlag GmbH &Co. KGA, Weinheim**.**

Banso, A. (2009). Phytochemical and antibacterial investigation of the bark extract**s** of *Acacia nilotica. J. Med. Plants Res* **3**: 82-85.

Bauer, A. W., Kirby, W. M. and Sherris, J. C. (1996). Antibiotic susceptibility testing by standardized single disk method, *Am.J. Clin. Pathol.* **45**: 493-496.

Bauer, R. (1998). Quality criteria and standardization of phytopharmaceuticals: Can acceptable drug standards be achieved? *Drug Inf J.* **32**: 101–110.

Bhattaram, V. A., Graefe, V., Kohlert, C., Veit, M. and Derendorf, H. (2002). Pharmaceutical and bioavailability of herbal medicine . *Phytomedicine* **9(3):** 1-33.

Beentje, H. J. (1994). Kenyan Trees, Shrubs and Lianas. National Museums of Kenya, Nairobi

Begossi, A., Hanazaki, N. and Tamashiro, J. Y. (2002). Medicinal plant in the Atlantic forest (Brazil) Knowledge, use and conservation. *Human Eco.* **54**:90-102.

Benjamin, M. N. (1978). Outline of Veterinary Clinical Pathology. University Press, lowa, USA pp. 229-232.

Bent, S. (2008). Herbal medicine in the United States: review of efficacy, safety and regulation. *J Gen Intern Med* **23**: 854-859.

Bernard, B. K. (2010). Assessment of trace elements content in the foodstuff commonly consumed in some parts of Rift valley in Kenya, Unpublished, Msc thesis University of Nairobi.

Bhagya, B. and Sridhar, K. R. (2009). Ethnobiology of coastal sand dune legumes of Southwest coast of India. *Indian J of Trad Knowledge* **8(4**):611-620.

Bhattaram, V. A., Graefe, U., Kohlet, C., Veit, M. and Derendorf, H. (2002). Pharmacokinetics and bioavailability of herbal medicinal product. *Pytomedicine* **9 (3):** 1-33.

Bidault, P., Chandad, F. and Grenier, D. (2007) Risk of bacterial resistance associated with systemic antibiotic therapy in peridiontology. *J can Dent Assoc* **73**: 721-725.

Bii, C., Korir, K.R., Rugutt, J. and Mutai, C. (2010). The potential of *Prunus africana* for the control, treatment and management of common fungal and bacterial infection *J. Med. Plants Res***. 4:** 995-998.

Black, R. E. (2003). Zinc deficiency, infectious diseases and mortality in the developing world**.** *J Nutr* **(133):**1485S-1489S.

Boer, H., Kool, A., Broberg, A., Mziray, W. R., Herdberg, I. and Levenfors, J. J. (2005). Antifungal and antibacterial activity of some herbal remedies from Tanzania. *J of Ethnopharmacol* **96**: 461-469.

Braine, T. (2012). More oral health care needed for aging population**.** *Bull. of WHO*

Brook, I. (2002). Anaerobic bacteria in upper respiratory tract and other head and neck infections. *Ann Otol Rhinol Laryngol* **111(5**): 430-440.

Budavari, S. (1989). The Merck index. An encyclopidia of chemicals, drugs and biologicals**.** Merck and Co. Inc, Rahway N.J.USA.

Calixto, J. B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz. J. Med. Biol. Res.* **33**: 179-189.

Cappecci, M. R. (2005). Gene targeting in mice:fuctional analysis of the mammalian genome for the twenty first century. *Nat Rev Genet* **6:** 507-512.

Chan, K. (2003). Some aspects of toxic contaminants in herbal medicines. *Chemosphere,* **52:** 1361-1371.

Cheesbrough, M. (2005). District Laboratory Practices in Tropical Countries, part 1. Low price edition, pp. 310-313.

Cheesbrough, M. (2006). District laboratory practice in tropical countries**.** Part 2. 2nd Ed. Cambridge. pp. 268-347.

Chelashwal, J. M., Kamau, F. N. and Gatheri, G. W. (2007). A survey of some medicinal plants sold in Nairobi. *East Afri. J. Bot.* **1 (1**): 46-63.

Chhetri, H., Yogol, S. H., Sherchan, J., Anupa, K. C. and Mansoor, P. T. (2008). phytochemical and antimicrobial evaluation of medicinal plants of Nepal. *J of Sci, Engineering and Tech* **1:** 49-54.

Chung, J. Y., Choo, J. H., Lee, M. H. and Hwang, J. K. (**2006)**. Anticariogenic activity of maccelignan isolated from *Myristica fragrans* (nutmeg) against *Streptococus mutans. Phytomedicine* **13**:261-266.

Coleman, J. E. (1992). Structure and mechanism of alkaline phosphatase. *Annu. Rev. Biophys. Biomol. Structure* **21**: 441-483.

Conning, D. M. (1993). Experimental Toxicology: The Basic Issues. Anderson, D. and Conning, D.M. Eds. 2nd ed; University Press, London pp 1-3.

Corns, C. M. (**2003**). Herbal remedies and clinical biochemistry. *Ann Clin Biochem* **40** (**Pt 5**):489-507.

Craigen, W. J. (2001). Mouse models of human genetic disorders. In. Scriver C.R. Beudet, A.L.,Valle, D., Sly, W.S. (eds) The metabolic and molecular baisi of inherited diseases 8th ed Mc Graw Hill PP. 379-415**.**

Cunningham, A. B. (1993). African medicinal plants, people and plant working paper, UNESCO PP. 1-50.

Daily Nation February 8th (2010). Be warned herbal prescription could be harmful to your health

Darinka, G. Tatjana, K. P., Katerina, B. and Trajce, S. (2010). Some toxic and essential metals in medicinal plants growing in R. Macedonia American–*Eurasian J.Toxic. Sci* **2 (1):** 57-61.

Das, K. K., Gupta, A. D., Dhundasi, S. A., Patil, A. M., Das, S. N. and Ambekar, J. G. (2006). Effect of L-ascorbic acid on nickel-induced alterations in serum lipid profiles and liver histopathology of rats. *J Basic Clin Physiol Pharmacol* **17**: 29-44.

Datta, P. K., Diwakar, B. T., Viswanatha, S., Murthy, K. N. and Naidu, K. A. (2011). Safety evaluation studies on Garden cress (*Lepidium sativum L*.) seeds in Wistar rats *Intern J of Applied Res in Nat Products* **4(1):** 37-43.

De Smet, P.A. (2002). Herbal remedies. *N Engl J. Med* **347 (25):** 2046-2056.

Diwan, F. H., Abdel-Hassan, I. A. and Mohammed, S. T. (2000). Effect of saponin on mortality and hitopathological changes in mice. *Eastern Mediterranean Health J* **6**: 345-351.

Dold, A. P. and Cocks, M. L. (2002). The trade in medicinal plants in the Eastern Cape Province, South Africa. *S Afr J of Sci* **98**: 589-597.

Donald, A. P. and Cocks, M. L. (2002). The trade in medicinal plant in Eastern cape Province, *South Afr J of Sci***: 98:** 11-12.

Doss, A., Parivuguna, V., Vijayasanthi, M. and Surendran, S. (2011). Antibacterial and phytochemical analysis *of Medicago sativa* against some microbial pathogens *Indian J of Sci and Techn* **4 (5):** 550 -552.

Edeoga, H. O., Okwu, D. E. and Mbaebre, B. O. (2005). Phytochemical, constituent of some Nigerian Medicinal Plants. *Afr J Biotechnol* **4**: 685-688.

Ekinci, N., Ekinci, R., Polat, R. and Budaka, G. (2004). Analysisi of trace elements in medicinal plants with energy dispersive X ray fluorescence *J Radional. Nucl. Chem***. 260:**127

Elhoussine, D., Zineb, B. and Boukir, A. (2010). Antibacterial activity and chemical composition of the leaf essential oil of *Mentha rotundifolia* from Morroco**,** *EJEAF chem***. 9(1):** 19-28.

Elujoba, A., Odeleye, O. and Ogunyemi, C. M. (2005). Traditional medicine development for medical and dental primary health care delivery system in Africa**.** *Afri. J. Trad. CAM* **2 (1):** 46-61.

Enayatifard, R. Asgarirad, H. and Kazemi, S. (2010). Microbial quality of some herbal solid dosage forms. *Afri J of Biotech* **9(11**):1701-1705.

Erlich, C., Wolfgang, K. and Briggette, K. (2001). Microbiological status of commercially available medicinal herbal drugs. A screening study**.** *Planta Med.* **67:**263-269.

Ernst, E. (1998). Harmless herbs? A review of the recent literature. *Am J Med.* **104**:170- 178.

Ernst, E. (2002). Toxic heavy metals and undeclared drugs in Asian herbal medicine, *Trends Pharmacol Sci* **23**: 136-139.

European Pharmacopoiea, (2007). *Directorate for the quality of medicine of the council of Europe*.5th Strasbourg**,** France

Evans, W. C. (2002). Trease and Evans, Pharmacognosy,15th, Edn. WB. Sauders company, London

Ezejiofar, N. A., Maduagqunwn, Onyiaaroh, V. I., Hussaini, D. C. and Orissakwe, O. E. (2008). Multiple organ toxicity of Nigerian herbal supplement (U and D, sweet bitter) in male Albino rats**.** *Pak J. Pharm Sci* **21 (4**): 426-429.

Food and Nutrition Board NRC. **(1980)**. Washington, DC: National Academy of Sciences; NRC Recommended dietary allowances. 9th edn**.**

Forest, J. (2004). Feacal Coliforms. Vol. 36. University of Iowa Hygienic Laboratory Manual;pp. 2-4.

Fowler, E. B., Breault, L. G. and Cuenin, M. F. (2001). Periodontal disease and its association with systemic disease. *Mil Med.* **166**:85-89.

Gaby, A. R. (1994). Preventing and Reversing Osteoporosis, Prima Publishing, Rocklin, CA.

Gacathi, F. N. (2007). Kikuyu botanical dictionary. A guide to plant names, uses and cultural values. Tropical Botany

Gathu, L. W. (2007). Evaluation of Antimicrobial activity of some plants used in central Kenya, MSc. Thesis, Kenyatta University, unpublished.

Geissberger, P. and Sequin, U. (1991). Constituents of *Bidens pilosa* L. do the components found so far explain the use of this plant in traditional medicine? *Acta Trop***. 48:** 251-261.

Gendron, R., Grenier, D. and Maheu-Robert, L. (2000). The oral cavity as a reservoir of bacterial pathogens for focal infections. *Microbes Infect.***2**:897-906.

Geyid, A., Abebe, D., Debella, A., Makonnen, Z,. Aberra, F., Teka, F., Kebede, T., Urga, K., Yersaw, K., Biza, T. Mariam, B.H. and Guta, M. (2005). Screening of some medicinal plants of Ethiopia for their antimicrobial properties and chemical profile *J. ethnopharmacol* **97(3) :** 421-427.

Ghiasvand, A. R., Nasseri, M., Farsizaeh, S., Meshkatalsadat, M. H., Shadabi, S. and Borzoei, M. (2011). Chemical characterization of cultivated *Tagetes minuta* L. by use of ultra assisted Head Space SPME and GC-MS *Chromatographia* **73**: 1031-1035.

Giday, M., Asfaw, Z., Elmqvist, T. and Woldu, Z. (2003). An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. *J.Ethnopharmacol*. **85:** 43-52.

Giveon, S., Liberman, Klang, N. and Kahan, E. (2004). Are people whose use natural drugs aware of their potentially harmful side effects and reporting to family physician? *Patients Educ Couns* **5**: 5-11**.**

Gonzalez, J. (1980). Medicinal plants in Colombia. *J ethnopharmacol* **2**: 43-47.

Govender, S. D.du Plesssis- Stoman, Downing and van de Venter, M. (2006). Traditional herbal medicine; microbial contamination, consumer safety and need for standards. *S.Afri. J.Sci* **102:** 253-255.

Greewood, D., Slack, R. C. and Peutherer, F. J. (1997). Medical microbiology. Church hill Livingstone

Gregg, L. and Voigt, D. V. (2000). Anaemies and and polychythenias. In: heamatlogy techniques and concepts for veterinary technicians. USA. IOWA state University press pp 95-101.

Haider, S., Naithani, V., Barthwal, J. and Kakkar, P. (2004). Heavy metal content in some therapeutically important medicinal plants. *Bull. Environ. Contam. Toxicol.* **72:**119-127.

Halt, M. (1998). Molds and mycotoxins in herb tea and medicinal plants. *European J of Epidemiology* **(14)**: 269-274.

Hameed, I., Dastagir, G. and Hussain, F. (2008). Nutritional and elemental analysis of some selected medicinal plants of the family polygonaceae**.** *Pak. J. Bot***. 40(6):** 2493-2502.

Harnafi, H. and Amrani, S. (2007). Review Article Flavonoids as Potent Phytochemicals in Cardiovascular Diseases Prevention. *Phcog. Rev*. **1:** 193-202.

Hatschek, W., Rousseaux, C. and Wallig, M. (2002). Handbook of Toxicologic Pathology. Vol1 2nd ed. Academic Press, New York. p. 187-362.

Hollist, N. O. (2004). A collection of traditional Yoruba oral and dental medicaments, Olubena, Ibadan

Holms, H. K. and Stephen, L. X. (**2002).** Oral lesions of HIV infection in developing countries. *Oral Diseases*: **8** (Suppl **2**): 40-43.

Homer, K. A., Manjai, F. and Beighton, D. (1990). Inhibition of protease activities of periodontopathic bacteria by extracts of plants used in Kenya as chewing sticks (mswaki): *Arc Oral Biol*. **35(6):**421-424.

Houghton, P. J. and Raman, A. (1998). Laboratory handbook for the fractionation of natural extract,**.** Chapman and Hall pp 155-167.

Idris, I. S., Ndukwe, G. I. and Gimba, C. E. (2009). Preliminary phytochemical screening and antimicrobial activity of *P.american* (Avacado Pear**)** *Bajopas* **2(1):** 173-176.

Idu, M., Erhabor, J. O. and Idele, S. O. (2011). Microbial load of some medicinal plants sold in local market of Bennin city, Nigeria**.** *Int. J. Med. aroma. Plants***, 1(3):** 272-277.

Ilodigwe, E. E., Akah, P. A and Nworu, C. S. (2010). Evaluation of the acute and sub chronic toxicities of ethanol leaf extract of *Spathodea campunalata. P*. Beauv. *International J of Applied Res in Nat products*. **3(2):**17-21.

Ingela, J., Johnson, K. A., Gerden, B. and Hagg, M. D. (2009). Spontaneously reported adverse reactions in association with complementary and alternative medicine substances in Sweden, *Pharmacoepidemiology and drug safety* **18**: 1039-1047.

Institute of Medicine (2001). Dietary reference intakes for vitamin A, Vitamin K, arsenic, boron, chromium, cupper, iodine, iron, manganese, molybedenum, nickel, silicon, vanadium and *zinc*. National academy Press, Washington.

Iwu, M. M. (1993). Hand book of African medicinal plants**.** CRC Press pp.25

Jaijoy, K., Soonthornchareonnon, N., Lertprasertsuke, N., Panthong, A. and Sireeratawong, S. (2011). Acute and chronic oral toxicity of standardised water extracts from the fruit of *Phyllanthus emblica* Linn. *Intern J of Applied Res in Nat Products* **3(1):** 48-58.

Jemuto, P., Mutai, C., Ouma, G., Lukhoba, C., Nyamaka, R. and Manani, S. (2010). Ethnobotanical survey and propagation of some endangered medicinal plants from South Nandi district of Kenya**.** *J Animal and Plant Sci* **8 (3):** 1016-1043.

Jenkinsson, H. F. and Lamont, R. J. (2005). Oral microbial communities in sickness and health. *Trends Microbial* **13:** 589-595.

Jose, M., Bhagya, B. and Shautaraun, M. (2011). Ethnomedicinal herbs used in oral health and hygiene in Coastal Dakshina Kannada. *J Oral Health Comm.Dent*. **5(3**):119-123.

Jurge, S., Kuffer, R., Scully, C. and Porter, S. R. (2006). Mucosal disease series. Number VI.Recurrent Aphthous Stomatitis. *Oral Dis* **12**:1-21.

Kahnut, A. M., Probstle, H., Rimpter, R. and Heinrich, R. (1995). Biological and pharmacological activities and further constituents of *Hyptis suaveolons*. *Planta medica* **6**: 227-237.

Kaimenyi, J. T. (2004). Oral health in Kenya**.** *Inter dent J* **54:** 378-382.

Kalaivani, T. and Mathew, L. (2010). Free radical scavenging activity from leaves of *Acacia nilotica . J. Med. Plants Res* **3:** 082-085**.**

Kareru, P. G., Kenji, G. M., Gacanja, A. N., Keriko, J. O. and Mungai, G. (2007). Traditional medicine among the Embu and Mbeere people of Kenya. *Afri. J.trad. CAM* **4(1):** 75-86.

Karmegan, N., Karuppusamy, M., Prakash, M., Jayakumar, M. and Rajasekar, K. (2008). Antibacterial potency and synergistic effect of certain plant extracts against food –borne diarrheal bacteria *Biomed Pharm Sci* **2:** 88-93.

Kayombo, E. J., Uiso, F. C., Mbwambo, Z. H., Mahunnah, R. L., Moshi, M. J. and Mgonda, Y. (2007). Experience of initiating collaboration of traditional healers in managing HUV and AIDS in Tanzania. *J of Ethnobio and Ethnomed* **3**, 6.

Kenya Vision 2030 (2007). Government of Kenya**.** pp. 95

Khamali, H.H. (2009). Medicinal plant in East Africa. Challenges and constraints. *J.ethnobotanical leaflets* **13**: 364-369.

Khan, N. M., Ngassapa, O. and Matee, M.I. (2000). Anti microbial activity of Tanzanian chewing sticks against oral pathogenic microbes. *Pharma. Bio***. 38 (3):** 235-240.

Khan, K. Y., Khan, M. A., Niamat, R., Munir, M., Fazala, H., Mazari, P., Seema, N., Bashir, T., Kanwal, A. and Ahmed, N. (2011) Elemental content analysis of plants of genus ficus using atomic absorption spectrometer. *Afri.J .of Pharm.and Pharmaco*. **5(3):** 317-321**.**

Khanyile, Z. C., Singh, N., Smith, M., Shode, F. O., Mngomezulu, S. and Dewir, Y. H. (2009). Comparative assessment of bacterial contamination in commercial herbal products of *Lessertioa futescens*. *American-Eurasian J. Agric. & Enviro. Sci*. **5(4)**: 494-499.

Kihara M. W. (2008). In vitro evaluation of antibacterial activity crude aqueous extract of *Azadirachta indica* (neem) leaves MSc. Thesis, Kenyatta University. Unpublished

Kioy, D., Gray, A. I. and Waterman, P. G. (1990). A comparative study of the stem bark drimane sesquiterpenes and leaf volatile oils of *W. ugandensis* and *W. stuhlimannii. Phytochemi* **29**: 3535-3538.

Kiringe, J. W. (2006). A survey of ethnomedicinal health remedies used by the Maasai of Southern Kajiando District, Kenya *Ethnobotany Res and Appl***. 4:** 061-073.

Kirtikar, K. R. and Basu, B. D. (1993). Indian Medicinal Plants. 1st,ed Lalit Mohan Basu, Allahabad, India, pp: 77.

Kokwaro, J. O. (2009). Medicinal plants of East Africa (3rd ed**)**. Kenya Literature Bureau Nairobi, Kenya p 67-300.

Komolafe, A. O. and Adegoke, A. A. (2008). Incidence of bacterial septicaemia in Ile-Ife Metropolis, Nigeria. *Malaysian J. of Microbio* **4(2)**: 51- 61.

Korrir, R. K., Mutai, C. Kiiyukia, C. and Bii, C. (2012). Antimicrobial activity and safety of two medicinal plants traditionally used in Bomet District of Kenya, *Res J of Med Plant* **6:** 370-382**.**

Krishnaiah, D., Devi, T., Bono, A. and Sarbatly, R. (2007). Phytochemical antioxidants for health and medicine – A move towards nature. *Biotech. Mol. Bio. Rev***. 1(4)** :97-104**.**

Krishnaiah, D., Devi, T., Bono, A. and Sarbatly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. *J of Med Plant Res* **3(2**): 67-72.

Kumar, V., Abbas, A. K. and Fausto, N. (2004). Robbins & Cotran Pathologic Basis of Disease, 7th edn. Philadelphia: Elservier Saunders, New York. p. 831-981.

Lachenmeier, D. W. (**2008).** Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. *J Occup Med Toxicol* **3**: 26

Lakshmi, R. R., Kundu, E. T. and Mansuri, A. P. (1991). Mercuric chloride induced inhibition of acid and alkaline phosphatase activity in the kidney of Mudskipper; *Boleopthalmus dentatus*. *Acta. Hydrochin. Hydrobiol*. **3**: 341-344.

Lawal, H. O., Etatuvie, S. O. and Fawehinmi, A. B. (2012) Ethnomedicinal and Pharmacological properties of *Morinda lucida J of Nat Products* **5**: 93-99.

Lee, J. W., Obrien, C. N., Grindly, A. J., Parper, A. M., Shaver-Weaver, K. A. and Zhao, X. (2005). Effects of trivalent vaccine against *Staphylococcus aureas* mastitis, lymphocytes subpopulations antibody production and neutrophil phagocytosis. *Can J Vet Rest* **69(1):** 11-18.

Lewis, S. M., Bain, B. J and Bates, I. (2001). Practical haematology**.** Churchill Livingstone

Li, X., Kolltveit, K.M., Tronstad, L. and Olsen, I. (**2000**). Systemic diseases caused by oral infection. *Clin Microbial Rev.* **13**: 547-558.

Limayati, D. A and Junior, B. L. (1998). Jamu Gendong, a kind of traditional medicine in Indonesia; the microbial contamination of its raw material and its end products. *J. Ethnopharmacol* **63**: 201-208.

Lockhart, P and Durack, D. T. (1999). Oral microflora as a cause of endocarditis and other distant site infections. *Infect Dis Clin North Am.***13**: 833-850,

Loesche, W. J. (1997). Association of the oral flora with important medical diseases. *Curr Opin Periodon* **4**: 21-28.

Lunde, C. S. and Kubo, I. (2000). Effect of polygodial on the mitochondrial ATPase of *Sacharomyces cerevisiae*. *Antimicrobial Agents Chemotherapy* **44:** 1943–1953.

Makonnen, E., Debella, A., Abebe, D. and Teka, F. (**2003**). Anagestic properties of some Ethiopian medicinal plants in different models of nociception in mice. John Wiley and sons.

Mamo, A. (2007). Effects of common medicinal plants extracts against common opportunistic bacteria isolated from septic herpetic lesions among HIV and AIDS patients, MSc thesis unpublished, Kenyatta University

Mahan, L.K. and Escott, S. (2008). Krauses food and Nutrition therapy. Saunders Elservier pp. 103- 138.

Martin, G. J. (1995). A method manual. Chapman and Hall, London

Matu, E.N and van Staden, J. (2003). Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya**.** *J.Ethnopharmacol* **87:** 35-41.

Maundu, P. M., Ngugi, G. W. and Kabuye, H. S. (1999). Traditional food plants of Kenya. National museums of Kenya**,** Nairobi

Mayne, P.D. 1994. Clinical Chemistry in diagnosis and treatment 6th edition. Arnold, **London** pp 304.

Mazzaccara, C., Labrun, G., Cito, G., Scarfo, M., Felice, M., Pastore, L. and Saccheti, L. (2008). Age related reference Intervals of the main biochemical and heamatological parameters in C57BL/6J, 129S/EV and C3H/HeJ mouse stains Plosone **3**: 11 (e3772): 1-6.

Mboya, T.O. (2003). The department of standards and regulatory services: Background information. Proceedings in the second National congress on Quality improvement of primary health care. Medical Research and traditional medicine, Nov. 24-28.

Mbuya, L.P., Msanga, H.P., Ruffo, C.K., Birnie, A. and Tengnäs, B. (1994). Useful trees and shrubs for Tanzania. Identification, propagation and Management for Agricultural and Pastoral Communities. *Technical Handbook* No.6 pp. 510-511.

Mbwambo, Z. H., Erasto, P., Innocent, E. and MaSimba, P. J. (2009). Antimicrobial and cytotoxic activities of fresh leaf extracts of *Warburgia ugandensis Tanzanian J of Health Res* **11 (2):**75-78.

McClellan, W. M., Knight, D. F., Karp, H. and Brown, W. W. (1997) Early detection and treatment of renal disease in hospitalized diabetic and hypertensive patients: important differences between practice and published guidelines. *Am J of Kidney Dis*, **29**: 368-375.

Meena, P. D., Kaushik, P., Shukla, S., Soni, A.K., Kumar, M. and Kumar, A (2006). Anticancer and antimutagenic properties of *Acacia nilotica* (Linn.) on 7, 12-dimethylbenz(a) anthracene-induced skin papillomagenesis in Swiss albino mice. *Asian Pac. J Can. Prev* **7**: 627-632.

Mindell, E. and Mundis, H. (2004). Vitamin bible; updated information on nutraceutical, herbs, alternative therapies and antiaging supplements , Warner book group pp, 13-17 and 56- 77.

Mishra, B. B. and. Tripathi, S. P (2011). Repellent activity of plant derived essential oils against *Sitophilous oryzae* (Linnaeus) and *Tribolium castaneum* (Herbst). *Singapore J. Sci Res*. **1:** 173-178.

Moore, K.L. and Dalley, A.F. (1999). **Clinical Oriented Anatomy** (4th Edition). Lippincot Williams and Williams; a Woller Klumner Corporation, Philadelphia. pp. 263-271.

Moreillion, P., Que, Y.A. and Glauser, M.P. (2005). *Staphylococcus aureus* (including staphylococcus Toxic shoch). In Principles and practice of infectious disease (Ed). Mandella, G.L., Bennett, J.E., Dolin, R. 6th Ed. Churchill livingstone, Pennyslyvania.

Morgan, G. R. (**1981)**. Sugar Bowls (*Clematis hirsutissima*): A horse restorative of the Nez Perces. *J. Ethnopharmarcol* **4**: 117-120.

Mossa, J. S., El-Feraly and Muhammad, I. (2004). Antimycobacterial constituents from *Juniperous procera, Ferula communis* and *Plumbago zeylanica* and their in vitro synergistic activity with isonicotic acid hydrazide**.** *Pytother Res***: 18 (11):** 934-937.

Mtunzi, F., Nkwe, J., Modise, J., Sipala, A. and Dikio, E. (2012). Phytochemical analysis and heavy metals composition of some medicinal plants products in South Africa *J Nat. Pro***. 5:** 21-26.

Mudassir, A., Zaid, A., Shaheen, G., Jehan, N,. Mansoor, A. and Yousafzai, A. (2010). Elemental composition of some arid environment fodder and medicinal plants of Quetta, Balochistan**.** *J .Chem. Soc. Pak***. 32 (1):**71-77.

Muhammad, A. R. (2007). Elemental composition of medicinal flowers *Pak. J. Bot* **39 (7):** 2541-2552.

Mukiama, T. K. (2005). Medicinal plants of Kenya. Agricultural Research Institute.

Mullika, T., Paojinda, P., Narknoppmanee, N. and Rungreangyingyyod, L. (2003). Evaluation of microbiological quality of herbal products in Thailand, *Thai J. of phytopharma**cy*** **10(2):** 37-47.

Munro, C. L. and Grap, M. J. (2004). Oral health and care in the intensive care unit. state of science. *Am J Cri Care* **13:** 25-34.

Musila, W. M. (2000). A preliminary survey of the medicinal plants used by Kamba traditional midwifes in Mwingi District, Kenya. UNESCO people and plant programme. Nairobi.

Muszynska, P., Palka, J. and Gorodkiewicz, E. (2000). The mechanism of daunorubicin induced inhibition of prolidase activity in human skin fibroblast and its implication to impaired collagen biosynthesis. *Exp. Toxcol. Pathol.* **52(2**): 149-155.

Mwonjoria, J. K., Kariuki, H. N. and Waweru, F. N. (2011). The antiseptive antipyrectic of *Solunum incanum* (Linneus) in animal models. *Intern of Pharmacol* **2 (1):** 22-26.

Nakajima, K., Nonaka, K., Yamamoto, K., Yamaguchi, N., Tani, K. and Nasu, M. (2005). Rapid monitoring of microbial contamination on herbal medicines by fluorescent staining method. *Lett. Applied Microbial* **40(2):** 128-132.

Navarro, C. M., Montilla, A., Martin, J. J. and Utrilla, P. M. (1993). Free radicals scavenger and antihepatotoxic activity of rosmarinus. *Plant Med*., **59:** 312-314.

Nayak, B. S., Raju, S. S. and Chalapathi, R. A. (2008). Wound healing activity of *Persea americana* (avocado) fruit. A preclinical study on rats *J Wound Care* **17:** 123-126.

Ndungu, P. W. (2005). Bacterial and Rota virus causes of diarrhoea in children under five years and genetic basis of Antibiotic resistance, unpublished Msc Thesis, Kenyatta University

Newberne, P. M. and Butler, W. H. (1969). Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals a review. *Cancer Res*., **29**: 236-250.

Ngari, F. W. (2010). Forest plants used in Ragati, Mt. Kenya: Their taxonomy, exploitation, economic values and conservation status. MSc Thesis, VDM pp. 27-33.

Ngetich, A. K. (2005). Indigenous knowledge, Alternative medicine and Intellectual property rights concerns in Kenya. Proceedings in the General assembly, Maputo, Mozambique, 6-10.

Ngure, P. K., Tonui, W. K., Ingonga, J., Mutai, C. H., Kigondu, E., Nganga, E., Rukunga, Z. and Kimutai, A. (2009). *In vitro* anti leishmanial activity of extracts of *Warbugia ugandensis* (Canellaceae), a Kenyan medicinal plant**.** *J of Med Plants Research* **3:** 61-66.

Niggemann, B. and Gruber, C. (2003). Side effects of complementary and alternative Medicine. *Allergy* **58**: 707-716.

Njoroge, G. and Bussmann, R. W. (2006). Traditional management of ear and throat, *J of ethnomedicine and ethnobotany* **2:** 54

Njoroge, G.N. and Kibunga, J.W. (2007). Herbal medicine acceptance, sources and utilisation for diarrhoea management in a cosmopolitatan area Thika, Kenya. *Afri. J. Ecol* **45** (suppli**.** 1**): ** 65-70.

Nunez, L., D'Aquino, M. and Chirefe, J. (2001). Antifungal properties of clove oil (*Eugenia caryphylata*) in sugar solution**.** *Brazillian J of Microbio* **32:** 123-126.

Nwinuka, N. M., Monanu, M. O. and Nwiloh, B. I. (2008). Effects of aqueous extract of *Mangifera indica* L. (mango) stem bark on haematological parameters of normal albino rats. *Pak. J. of Nutr* **7(5**): 663-666.

Nyunja, A. R., Onyango, J. C. and Erwin, B. (2009). The Kakamega forest medicinal plants Resources and their utilization by adjacent Luhya community. *Medwell J.* **4 (3):** 82-90.

Obi, E., Akunyili, D. N., Ekpo, B. and Orisakwe, O. E. (**2006)**. Heavy metal hazards of Nigerian herbal remedies. *Sci Total Environ*. **369**: 35–41.

Obiajunwa, E. I., Adebajo, A. C. and Omobuwajo, O. R. (2002). Essential and trace elements contents of some Nigerian medicinal plants**.** *J Radioanalytical Nuclear Chem***., 252 (3):** 473-476.

Ogwang, P. E., Tumusiime, R, Agwaya, M., Mugisha, G., Nambatya, K. G., Galiwango, B. and Waako, P. (2008). Repeat-dose effects of *Zanthoxylum*

chalybeum root bark extract: A traditional medicinal plant used for various diseases in Uganda *Afri J of Pharm and Pharmacol*. **2 (6):** 101-105.

Okunlola, A., Babatunde, Adewoyin, A and Odeku, A. (2007). Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in south western Nigeria. *Trop J Pharm Res* **6(1):** 661-670.

Olaeta, J.A., Schwartz, M., Undurraga, P. and Contreas, S. (2007). Use of Hass avocado (*Persea americana* Mill) seed as a processed product. Proceedings of the VI World Avocado Congress: 1–8.

Olila, D., Odyeki, O. and Asibo, J. O. (2001). Antibacterial and antifungal activities of extracts of *Z. chalybeum* and *W. Ugandensis*, Ugandan medicinal plants**,** *Afri.Health Sci.* **1(2**): 66-72.

Oliveira, F. Q., Andrade-Neto, V., Krettli, A. U. and Brandão, M. G. (2004). New evidences of antimalarial activity of *Bidens pilosa* roots extract correlated with polyacetylene and flavonoids. *J. Ethnopharmacol* **93(1**): 39-42.

Opiyo, S. A., Manguru, L. O., Owour, P. O., Ateke, E. M. and Lemmen, P. (2011). 7ά acetylugandensolide and antimicrobial properties of Warbugia ugandensis extracts and isolates against Sweet potatoes pathogens *Phytochem letters* **4(2**): 161-165.

Oyedemi, S. O., Bradley, G. and Afolayan, A. J. (2010). Toxicological effects of the aqueous stem bark extract of *Strychnos henningsii* Gilg in Wistar rats. *J Nat Pharm*;**1**:33-39.

Oyetayo, V. O. (2008). Microbial load and antimicrobial property of two Nigerial herbal remedies. *Afri J Tradit complement Med* **5(1**): 74-78.

Panda, P. C., Shankar, M., Sreenavasa, M. and Murti, I. (1975). Effect of Aflatoxin on the hematological pictures of albino rats and guinea pigs. *Indian J. Exp. Biol.* **13:** 569-570.

Panunto, W., Jaijoy, K., Lerdvuthisopon, N., Lertprasertsuke, N., Jiruntanat, N., Soonthornchareonnon, N. and Sireeratawong, S. (2010). Acute and chronic studies of water extract from dried fruits of *Terminalia chebula Rezt*. in rats *Inter J of Applied Res in Nat Products* **3(4):** 36-43.

Park, K. M, You, J. S., Lee, M.H., Hwang, J. K. and Kuwanon, G. (**2003).** An antibacterial agent from the root of *Morus alba* against oral pathogens. *J Ethnopharmaco* **84**:181-185.

Perez, C., Pauli, C. and Basevque, P. (1997). An antibiotic assay by the agar well diffusion method. *Acta Biologie et Medicine Experimetallis* **15:**113-115.

Peterson, P. E., **Bourgeois, D., Ogawa, H., Estupian-Day, S. and Ndiaye, C**. (**2005).** The global burden of oral diseases and risks to oral health. *Bull. World Health Organ* **83**: 661-669.

Pieme, C. A., Pendlap, V. N., Nkegoum, B., Taziebou, C. L., Tekwu, E. M., Etoa, F. X. and Ngongang, J. (**2006)**. Evaluation of acute and sub acute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L) Roxb Ceasalpiniaceae.*Afri. J. Biotechnol.* **5(3)**: 283-289.

Poiata, A., Christina, T., Elvira, G., Aprotosoaie, C., Ursula, S. and Monica, H. (2010). The microbial level contamination in dried plant materials evaluated by the standard plate count**.** *Natura Montenegara, Podgorica* **9(3):** 883-887.

Prabu, G. R., Gnanamani, A., Sadulla, S. and Guaijaverin (**2006).** A plant flavanoid used as a potential ant plaque agent against *streptococcus mutans. J Appl Microbial* **101**: 487-95.

Pramilla, D. M., Xavier, R., Marimuthu, K., Kathiresan, S., Khoo, M. L, Senthilkumar, M., Sathya, K. and Sreeramanan, S. (2012). Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermit **(***Mentha piperita:* Lamiaceae**)** *J. Med Plants Res* **6(2):** 331-335.

Prashar, A., Locke, I.C and Evans, C.S. (2006). Cytotoxicity of clove (*Syzygium aromaticum*) oil and its major components to human skin cells. *Cell Prolif.*, **39:** 241-248.

Prescott, M. L., Harley, J., Donald, P. and Klein, A. (1999). Antimicrobial Chemotherapy. Microbiology 2nd edition, Brown publishers, U.S.A

Pretorius, J. C., Magana, S. and Zietsman, P. C. (2003). Growth inhibition of plant pathogenic bacteria and fungi by extracts from selected South African plant species**.** *S Afri J of Bot* **20:** 188-192.

PushPagadan, P. and Kumar, B. (2005). Ethnobotany, CBD, WTO and Biodiversity Act of India**.** *Ethnobotany* **17:** 2-12.

Rabia, N., Khan, A., Khan, K. Y., Ahmad, M., Ali, B., Mazari, M. and Ahmad, A. (2012). Elemental content of some ethnomedicinal Ziziphus Linn. species using atomic absorption spectroscopy technique**.** *J of Applied Pharm, Sci.* **2(3):** 96-100.

Rajendran, N. K. and Ramakrishnan, J. (2009). *In vitro* evaluation of Antimicrobial activity of crude extracts of medicinal plants against multidrug resistant pathogens**.** *Bibda* **2 (2):** 97-101.

Ramsy, H., Goddard, W., Gill, S. and Moss, C. (2003). Herbal creams used for atopic eczma in Birmingham, UK illegally contain potent corticosteroids. *Arch Dis Child* **88**: 1032-1033.

Rodrigues, F., Lehmann, M., do Amaral, V.S., Reguly, M.L. and de Andrade, H.H. (2007). Genotoxicity of three mouth wash products, Cepacol, Periogard, and Plax, in the Drosophilla wing spot test *Environ Mol Mutagen* **48**: 644-649.

Rojas, T., Ochoa, V. J., Ocampo, S. A. and Monoz, J. F. (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric

medicine: a possible alternative in the treatment of nosocomial infections. Complementary and alternative Medicine *Biomedcentral.com/1472-6882/6/2*.

Roopashree, T. S., Raman, D., Shobha, R.H. and Narendra, C. (2008). Antimicrobial activity of ant psoriatic herbs: *Cassia tora, Momordica charantia* and *Calendula officinallis. Inter J of applied Res in Nat products* **1(3):** 20- 28.

Runyoro, D. K., Matee, M. I., Ngassapa, O. D., Joseph, C. C. and Mbwambo, Z. H. (2006). Screening of Tanzanian medicinal plants for Anti-Candida activity**.** *BMC complementary Alte Med* **30:** 6-11.

Russell, R. M. (2001). New micronutrient dietary reference intakes from the National Academy of Sciences. *Nutr Today* **36**: 163–171.

Saad, B., Azaizeh, H., Hijleh, G. A. and Said, O. (2006). Safety of traditional Arab herbal medicine. *eCAM* **3(4):**433-439.

Saeed, S., Naima, A. and Tariq, P. (2006). In vitro antibactrials activity of peppermint *Pak. J. Bot.* **38 (3):** 869-872.

Saha, P., Mazumder, U.K. and Haldar, P.K. (2011). Acute and sub chronic toxicity of *C. maxima* aerial parts. *Inter J of Res in Pharm and biomedical sci* **2 (2**): 634-639.

Samali, A., Rukaiyatu, A., Kirim and Musatafa, K.B. (2012). Qualitative evaluation of some herbal teas commonly consumed in Nigeria. *Afri. J. Pharm. Pharmacol* **6(6):** 384-388.

Sato, M., Tanaka, H., Fujiwara, S., Hirata, M., Yamaguchi, R. and Etoh, H. (2002). Antibacterial property of isoflavanoids isolated from *Erythrina variegate* against cariogenic oral bacteria. *Phytomedicine* **9**: 427-433.

Scannapieco, F. A. and Mylotte, J. M. (1996). Relationships between periodontal disease and bacterial pneumonia. *J Periodontol*, **67(10 Suppl**): 114-122.

Shad, A. K., Lajbar, K., Iqbal, H., Khan, B. M. and Naveed, A. (**2008)**. Profile of heavy metals in selected medicinal plants. *Pak. J. Weed Sci. Res.*, **14 (1-2):** 101-110.

Shahidi Bonjar, G. H. (2004). Evaluation of Antibacterial properties of Iranian Medicinal plants against *Micrococcus aureus, Serratia marcescens, Klebsiella pneunomiae* and *Bordella bronchoseptica*. *Asian J. of Sci* **3** (1): 82-86.

Shakoori, A. R., Butt, U., Riffat, R. and Aziz, F. (1994). Hematological and biochemical effects of dental administered for two month on the blood and liver of rabbits. *Zeitschrift fuer Angewandte Zoologie* **80**: 165-180.

Shankar, A. H. and Prasad, A. S. (1998). Zinc and Immune function: the biological basis of altered resistance to infection**.** *American J of clinical nutrition* **68**: 447-463.

Sharma, V. and Pandey, D. (2010). Beneficial effects of *Tinospora cordifolia* on blood profile in male mice exposed to lead. *Toxicol. Int*. **17**: 8-11.

Sheng, J. Y., Huang, Z. W. and Liu, Z. (2005). A comparison of the activities of membrane-bound, proton translocating ATPases between Streptococcus mutans fluoride-resistant and their parent strains. *Shanghai Kou Qiang Yi Xue* **14 (1):** 71-73.

Sibanda, T. and Okah, A.I. (2007). The challenges of overcoming antibiotic resistance plant as potential sources of antimicrobial and resistance modifying agents. *Afr J. of Biotech* **6 (25):** 2886-2896.

Simson, E., Ross, D. W. and Kochelar, W. D. (1988). An atlas of automated blood cytochemical haematology**.** Technicon, Tarry town.

Singh, G., Sharma, P. K., Dudhe, R. and Singh, S. (2010). Biological activities of *Withania sominifera Annals of Biol Res* **1(3):** 56-63.

Singleton, P. (1999). Bacteria in Biology, Biotechnology and medicine (4th edition) John Wiley and sons Ltd. New York.

Sivasankaran, N. M. and Selwin, J. R. (2008). Synthesis and characterization of Co (II), Ni (II), Cu (II), and Zinc (II) complexes of tridentate Schiiff base derived from vanillin and DL-α-aminobutyric acid**.** *Spectrochim Acta* **70:** 749-753.

Sofowora, A. (1982). Medicinal plants and traditional medicine in Africa. John Wiley and Sons pp. 125k

Solis, P., Wright, C., Anderson, M., Gupta, M. and Phillipson, J. (1993). A micro well cytotoxicity assay using *Artemia salina* (brine shrimp). *Planta Medica* **59**: 250-252.

Soper, R. (1997). Biological science**.** Cambridge pp. 375-417.

Steenkamp, P. A., Harding, N. M. Van Heerden, F.R. and Van Wyk, B.E. (2006). Identification of atractyloside by LC-ESI-MS in alleged herbal poisoning *Foresnsic Sci Inter*. **163**: 81-92.

Stevic, T., Pavlovic, S., Stankovic, S. and Savikin, K. (**2012**). Pathogenic microorganisms of medicinal herbal drugs. *Arc. Biol. Sci*.64 (**1):** 49-58.

Strain, J. J. and Cashman, K. D. 2009. Mineral and trace elements. In: Gibney, M.J., Lanham-New, S.A., Cassidy, A. and Vorsten, H.H. editors. Introduction to Human Nutrition, 1st edition, Wiley-Blackwell Publishers; The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK; 188-237.

Styers, D., Sheetian, D. J., Hogan, P. and Sahm, D. F. (2006). Laboratory based surveillance of current antimicrobial resistance pattern and trends among *S. aureus*, *Ann. Clin Microb. Antimicrob* 5:2.

Swanston-Flatt, S. K., Day, C., Bailey, C. J. and Flatt, P. R. (1990). Traditional plant treatments for diabetes: Studies in normal and streptozotocin diabetic mice. *Diabetologia* **33**: 462-464.

Swenson, M. J. and Reece W. O. (1993). Duke's Physiology of Domestic Animals. 11th ed. Comstock Publishing Associates, Ithaca, New York, U.S.

Tedong, L., Dzeufiet, P. D., Dimo, T., Asongalem, E. A., Sokeng, S. N., Flejoy, J., Callard, P. and Kamtchouing, P. (2008). Acute and sub chronic toxicity of *Anacardium occidentale* Linn Anacardiaceae) leaves hexane extract in mice. *Af. J. Trad. Comp. Alt. Med*. **4(2**): 140-147.

Thai Pharmacopieian Volume 1 part 1. (1987). Bangkok: Department of medical sciences 467-488. The United States Pharmacopieae 26th revision *Asian Ed. Toronto (***2003)**:2006-2011.

Tichy, J. and Novak, J. (1998). Extraction, assay, and analysis of antimicrobials from plants with activity against dental pathogens (*Streptococcus* sp.). *J.Alter Complement Med* **4**: 39-45.

Tietz, N. W. (1976). Fundamentals of Clinical Chemistry. 2nd Edn., W.B. Saunders, Philadelphia, pp: 878-878.

Tull, A. (1996). Food and nutrition Oxford University press ,pp.25

Van Wyk, B., Oudshoorm and Gericke, N. (1997). Medicinal plants of South Africa. Briz publications, Pretoria p, 21-25.

VanVonderen, M. G., Klinkenberg-Knot, E. C. and Craanen, M. E. (**2000**). Severe gastrointestinal symptoms due to lead poisoning from Indian traditional medicine. *Am. J. G.* **95**: 1591.

Varkey, A. J. (2010). Antibacterial properties of some metals and alloys in combating coliforms in contaminated water. *Sci Res and Essays* **5(24)**: 3834-3839.

Voeks, R. A. (2007). Are women reservoirs of traditional plant knowledge? Gender, ethnobotany and globalization in northeast Brazil. *Singapore J. of tropical Geog* **28**: 7-20.

Voeks, R. A. and Nyawa, S. (2001). Forgetting the forest: Assessing medicinal plant erosion in Eastern Brazil. *Econ. Bot* **58 (suppl.):** 294-306.

Wambugu, S. N., Mathiu, P. M., Gakuya, D. W. Kanui, T. I. and Kabasa, J. D. (2011). Medicinal plants used in management of chronic joint pain in Machakos and Makueni counties**.** *Kenya J. of ethnopharmacol* **137(2):** 945-955.

Wamwala, N. L., Oballa, P. and Gicheru, J. (2006). Genetic variation of *Warbugia ugadensis* in Kenya and implications for its cultivation**,** Kenya Forestry Research Institute (KEFRI), Nairobi.

Watthanachaiyingcharoen, R., Kantimanee, P., Apichat, P. and Narisa, K. (2009). Acute toxicity test of medicinal plants and herbal remedies of aphthous ulcer. *J Health Res* **23(4**): 169-174.

Westendarp, H. (2006). Effects of tannins in animal nutrition. *Dtsh Tieraztl Wochenschr* **113 (7):** 264-268.

Wintergest, E. S., Maggani, S. and Horning, D.H. (2007). Contributions of selected vitamins and trace elements to immune function.*Ann. Nutr. Metab* **51:** 301-323.

Wolfgang, K., Erich, C. and Briggette, K (2002). Microbial contamination of medicinal plants – A review**.** *Planta Medica* **68:**5-15.

Wolinsky, L. E. and Sote, E. O. (1983). Inhibiting effects of aqueous extracts of eight Nigerian chewing sticks on bacterial properties favouring plaque formation. *Caries Res* **17**: 253-58.

Wolinsky, L. E., Mania, S., Nachnani, S. and Ling, S. (1996). The inhibiting properties of aqueous *Azadrirachata indica* (neem) extract upon bacterial properties influencing in vitro plaque formation. *J. Dent, Re***. 75:** 816-822.

World Health Organisation (1998). Quality Control Methods for Medicinal Plant Materials, WHO Geneva Switzerland. Materials, WHO Geneva Switzerland, p. 128.

World Health Organisation, (2000). General guide lines for the methodologies on research and evaluation of traditional medicine**,** Geneva

Wube, A. A., Bucar, F., Gibbons, S. and Asres, K. (2005). Sesquiteroenes from *Warbugia ugandensis* and their antimycobacterial activity**.** *Phytochemistry* **66**: 2309-2315.

Xu, R. W., Zhao, J., Xu, J., Shao, B. and Qin, G. (1996). Studies on bioactive saponins from Chinese medicinal plants. *Adv. Exp. Med. Biol*. **404:** 371-382.

Yakubu, M. T., Akanji, M. A.and Oladiji, A. T. (2008). Alterations in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia argrestis* stem. *Res. J. Med. Plant*. **2**: 66-73.

Yeer, R. and Sheiham, A. (2002). The burden of restorative dental treatment for children in third world countries. *International Dental J* **52**: 1-9.

Zain, R. B. (2000). Oral recurrent aphthous ulcers/stomatitis: prevalence in Malaysia and an epidemiological update. *J Oral Sci.* **42**:15-19.

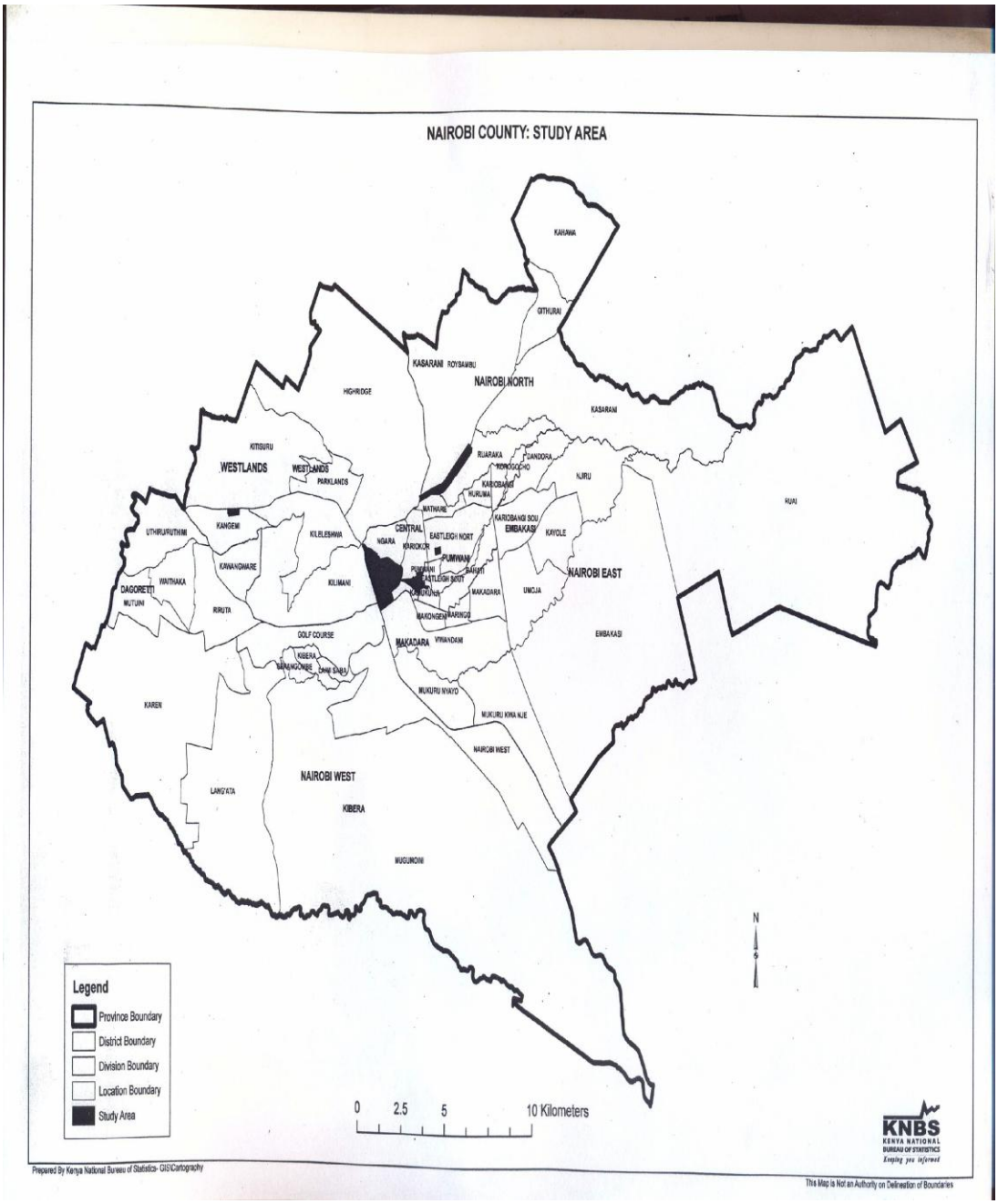
Zakaria, H. M., Mainen, J. M., Pax, J. M., Modest, C. K. and Ramadhani, S. (2007). Antimicrobial activity and brine shrimp toxicity of *Terminalia brownii* roots and stem. *J of comp and Altern Med* **1**: 7-9.

Zeinsteger, P., Romero, A., Teibler, P., Montenegro, M., Rios, E. M. Acosta, O. and Jorge, N. (2003). Toxicity of volatile compounds of *Senacio gisebachil* baker (margarita) flowers, in mice. *Revistade Investigaciones Agropecuarias*, **32:** 125-136.

Zschocke, S., Rabe, T., Taylor, J. L., Jager, A.K. and van Staden, J. (2000). Plant part Substitution a way to conserve endangered medicinal plants**?** *J of Ethnopharmacol* **71:** 281-292.

LIST OF APPENDICES

Appendix 1 Study site



Appendix 2. Questionare

Name of the researcher……

Institution……

Serial No……

Location of the informant……

Date …….

Biodata

1. Date
2. Locality…….
3. Name of the informant.....
4. a. Sex M [ ] F [ ]

   b. Age? Teenager [ ] 50yrs [ ] above 50 yrs [ ]
5. Level of education: Primary [ ] secondary [ ] others
6. How many languages do you speak? Vernacular [ ] Kiswahili [ ] English
7. How long have you been practicing? Less than one [ ] 5yrs [ ] 10 years [ ] others specify……
8. Do you have any formal training? Yes [ ] No [ ]

Diseases treated

9. a. What oral diseases do your clients normally complain off? Bad mouth ordour [ ] Gum bleeding [] tooth ache [ ] mouth ulcer [ ] tonsillitis [ ] any other.....

b. What are the ages of your clients? Less than 10years [ ] teenagers middle aged [ ] above 50years [ ]

Plant/ plants and plant part used to

A. Bad mouth odour

10. Name the medicinal plant/herbal materials used in treatment of above oral diseases

Vernacular name…….

Family…….

Genus ……

Species……

Common name……

Part used: roots [ ] Barks [ ] seeds [ ] stems [ ] flowers [ ] sap [ ] others specify ……..

Do you use one plant or a combination……no [ ] yes [ ]

If yes state

(b) Plant-

Vernacular name……..

Family……

Genus ……

Species……

Common name……..

Part used: roots [ ] Barks [ ] seeds [ ].stems [ ] flowers [ ] sap [ ] others specify ……..

B. Gum bleeding

Name the medicinal plant/herbal materials used in treatment of above oral diseases

Vernacular name…….

Family…….

Genus ……

Species……

Common name……

Part used: roots [ ] Barks [ ] seeds [ ] stems [ ] flowers [ ] sap [ ] others specify ……

Do you use one plant or a combination……no [ ] yes [ ]

If yes state

Vernacular name……..

Family……

Genus ……

Species……

Common name……..

C. Bad mouth odour

Name the medicinal plant/herbal materials used in treatment of above oral disease

Vernacular name…….

Family…….

Genus ……

Species……

Common name……

Part used: roots [ ] Barks [ ] seeds [ ].stems [ ] flowers [ ] sap [ ] others specify ……..

Do you use one plant or a combination……no [ ] yes [ ]

If yes state

Vernacular name……..

Family……

Genus ……

Species……

Common name……..

D. Mouth ulcers

Name the medicinal plant/herbal materials used in treatment of above oral diseases

Vernacular name…….

Family…….

Genus ……

Species……

Common name……

Part used: roots [ ] Barks [ ] seeds [ ].stems [ ] flowers [ ] sap [ ] others specify

Do you use one plant or a combination……no [ ] yes [ ]

If yes state

Vernacular name……..

Family……

Genus ……

Species……

Common name……..

E. Tonsillitis

Name the medicinal plant/herbal materials used in treatment of above oral disease

Vernacular name…….

Family…….

Genus ……

Species……

Common name……

Part used: roots [ ] Barks [ ] seeds [ ].stems [ ] flowers [ ] sap [ ] others specify

Do you use one plant or a combination……no [ ] yes [ ]

If yes state

Vernacular name……..

Family……

Genus ……

Species……

Common name……..

Usage /Form and Dosage

11. In which form do you use medicinal plant? Powder [ ] infusions [ ] others specify

12. If extracts/liquids are used what solvent do you use to extract? [ ]

Water [ ] milk [ ] alcohol [ ] others specify …….

13. If powder-

a. Do you clean the plant material before grinding? Yes [ ] No [ ]

b. Do you remove the cork? Yes [ ] No [ ]

c. How do you dry the plant material? Under the shade [ ] under the sun [ ] use fire [ ] others specify ……

14. How is the medicine applied/ used? Chewing [ ] apply powder [ ] boil and gaggle [ ] boil and swallow [ ]

15. What dosage do you use? One tea spoonful [ ] 2 teaspoonfuls [ ] 3 teaspoonfuls [ ] others specify

16. How many times is the medicine taken per day? once [ ] twice [ ] thrice [ ]

17. What is the duration of treatment? One day [ ] three days [ ] one week [ ] others specify ……

18. Do you use other material in your herbal preparation apart from medicinal plants yes [ ] no [ ]

19. If yes which materials milk [ ] honey [ ] alcohol [ ] preservatives [ ]others specify

Source of the herbal material

20. How do you source medicinal plants? Self [ ] Buy [ ] if buying
21. If you buy medicinal plants, how do you identify the product? Taste [ ].smell [ ] others [ ]

b. Do you process before use? Yes [ ] No [ ]

c. If yes, what process? Cutting [ ] cleaning [ ] grinding [ ]

Quality assurance

22. Is herbal material cultivated [ ] wild harvested [ ]

23. How do you transport your products? Public means [ ] private means [ ] others specify …..

24. How do you store you medicinal plants? In translucent bags [ ] plastic bottles [] tins [] others specify…….

25. Do you give instruction for storage of prescribed herbal material? No [ ] Yes [ ] Not sure [ ]
26. Do your products have manufacturing and expiry date…Yes [].…../ No…….
27. How long is the shelf life? Less than a year [ ] One year [ ] Two years [ ] others specify.
28. How do you determine expiry date?……
29. Is the supply of medicinal plant always sufficient?.....

Locality of the herbalist

30. How is the area where the trader/ herbalist is located crowded [ ] slums [ ] near disposal pits [ ] sewer [ ]

Appendix 3 Instrumental conditions for elemental analysis

No.	Element	Line	Energy/keV	Cycl.	Net	Backgr.	Sigma	Chi
1	Al	K12	1.486	500	33	3809	87	1.8
2	Si	K12	1.74	500	10153	3726	133	2.18
3	P	K12	2.01	500	2221	3689	98	1.46
4	S	K12	2.309	500	3344	3528	102	1.01
5	Cl	K12	2.622	500	1068	3331	88	4.21
6	Ar	K12	2.958	500	2028	3151	91	7.03
7	K	K12	3.314	500	101938	2913	328	4.41
8	Ca	K12	3.692	500	109116	2603	338	1.43
9	Sc	K12	4.093	500	1	2222	67	8.02
10	Ti	K12	4.512	500	3291	1989	85	1.45
11	V	K12	4.953	500	200	1942	64	1.95
12	Cr	K12	5.415	500	111	1952	63	1.45
13	Mn	K12	5.9	500	3657	1966	87	1.34
14	Fe	K12	6.405	500	63200	1916	259	1.48
15	Co	K12	6.931	500	1	1829	60	1.77
16	Ni	K12	7.48	500	76	1779	60	0.94
17	Cu	K12	8.046	500	3168	1794	82	0.68
18	Zn	K12	8.637	500	5176	1875	94	1.13
19	Ga	K12	9.251	500	14130	1917	134	1.97
20	Ga	L1	1.098	500	894	4197	96	5.29
21	As	K12	10.543	500	1	2089	65	13.51
22	As	L1	1.282	500	1	3868	88	0.92
23	Se	K12	11.224	500	1	2209	66	1.55
24	Se	L1	1.379	500	1	3818	87	1.11
25	Br	K12	11.924	500	281	2416	72	1.6
27	Rb	K12	13.396	500	11596	2891	132	219.01
28	Rb	L1	1.692	500	724	3676	90	2.34
29	Sr	K12	14.165	500	160201	3028	408	14401.37
30	Sr	L1	1.806	500	484	3681	89	2.07
31	Pb	L1	10.551	500	1724	1920	75	15.49
32	Pb	M1	2.342	500	353	3499	86	0.96

Appendix 4 Formula for calculating elemental concentration

$$C_x = \frac{Nx / Sx}{Nis / Sis} xCis$$

Where, C_x ---- Concentration of the analyte

C_{is} ---- Concentration of the internal standard

N_x----- Net intensity of the analyte

N_{is}----- Net intensity of the internal standard

S_x------ Relative sensitivity of analyte

S_{is}------Relative sensitivity of internal standard

Appendix 5 Haematological normal values

Parameters	Values
PCV (%)	35.1-45.4
Hb (g/dL)	11.0-15.1
RBC (x 10^6/μL)	6.36-9.42
MCH (pg)	14.1-19.3
MCHC (g/dL)	30.2 – 34.2
MCV (fL)	45.4 – 60.3
WBC (x 10^3/L)	1.8-10.7
Neutrophil (%)	6.6-38.9
Lymphocytes (%)	55.8-91.6
Monocytes (%)	0.0-7.5

Appendix 6 Some plants identified in the study

M. whytei

T. brownii

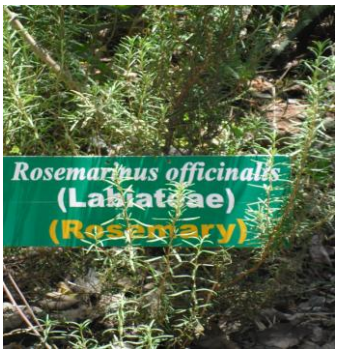
A. xanthophloea

A. nilotica

B. aegyptica

A. secondiflora

W. ugandensis



R. officinalis

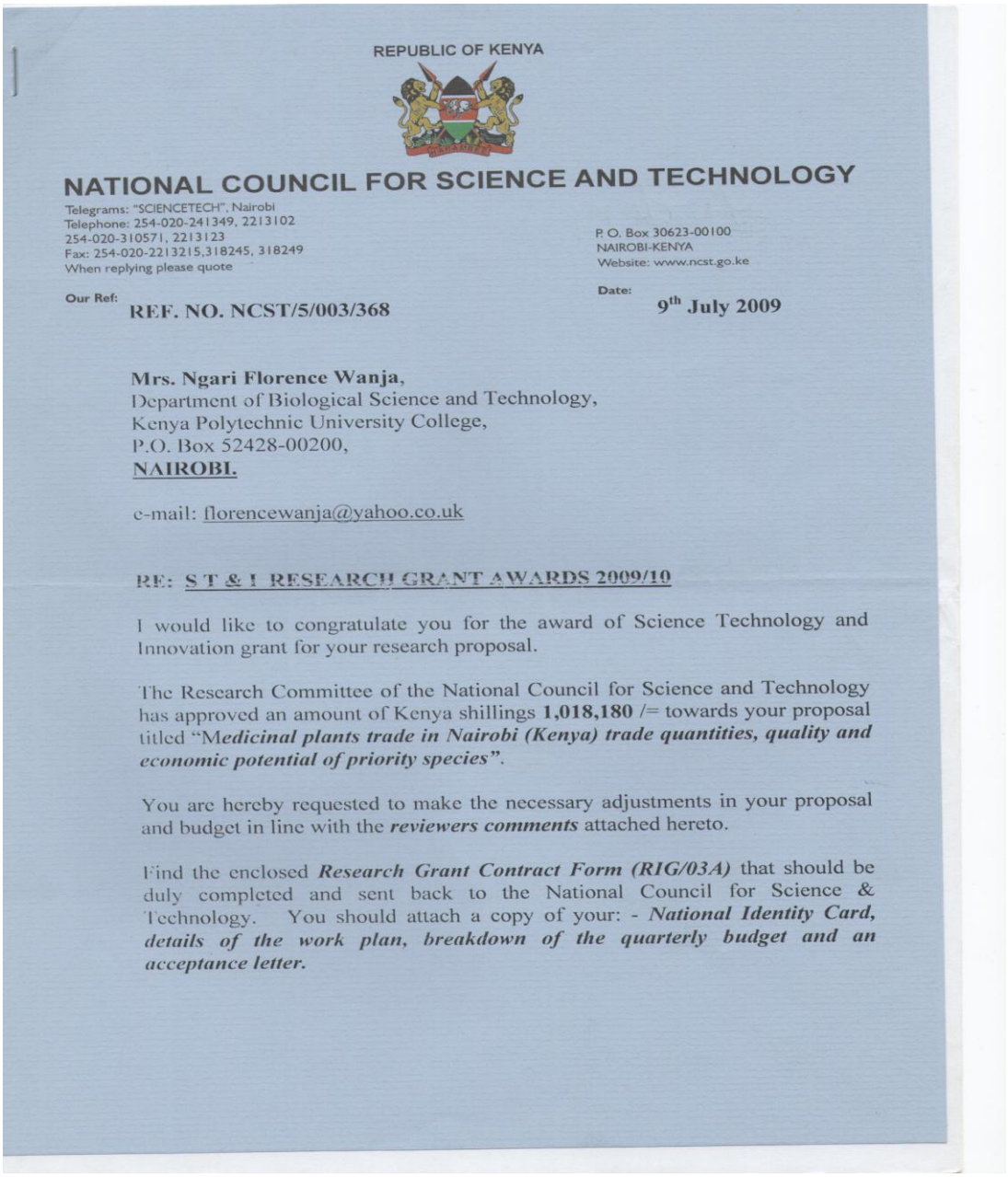
D. abyssinica

A. vera

M. piperita

E. divinorum

Appendix 7 Research grant letter



REPUBLIC OF KENYA

NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telegrams: "SCIENCETECH", Nairobi
Telephone: 254-020-241349, 2213102
254-020-310571, 2213123
Fax: 254-020-2213215,318245, 318249
When replying please quote

P. O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref: **REF. NO. NCST/5/003/368**

Date: **9th July 2009**

Mrs. Ngari Florence Wanja,
Department of Biological Science and Technology,
Kenya Polytechnic University College,
P.O. Box 52428-00200,
NAIROBI.

e-mail: florencewanja@yahoo.co.uk

RE: S T & I RESEARCH GRANT AWARDS 2009/10

I would like to congratulate you for the award of Science Technology and Innovation grant for your research proposal.

The Research Committee of the National Council for Science and Technology has approved an amount of Kenya shillings **1,018,180** /= towards your proposal titled "***Medicinal plants trade in Nairobi (Kenya) trade quantities, quality and economic potential of priority species***".

You are hereby requested to make the necessary adjustments in your proposal and budget in line with the ***reviewers comments*** attached hereto.

Find the enclosed ***Research Grant Contract Form (RIG/03A)*** that should be duly completed and sent back to the National Council for Science & Technology. You should attach a copy of your: - ***National Identity Card, details of the work plan, breakdown of the quarterly budget and an acceptance letter.***

Appendix 8 Research permit

PAGE 2

THIS IS TO CERTIFY THAT:
Prof./Dr./Mr./Mrs./Miss/Institution
Florence Wanja Waiganjo
of (Address) Kenyatta University
P.O.Box 43844-00100, Nairobi.
has been permitted to conduct research in

Location
District
Province

Nairobi

on the topic: Safety and antimicrobial activities of herbal materials used in management of oral health by Traditional Medical Practitioners in Nairobi.

for a period ending: 31st March, 2013.

Applicant's Signature

PAGE 3

Research Permit No. *NCST/RCD/14/013/102*
Date of issue *14th February, 2013*
Fee received *KSH. 2,000*

Secretary
National Council for
Science &Technology