A STUDY OF THE QUALITY OF GINSENG CONTAINING PRODUCTS IN NAIROBI, KENYA

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Masters of Science in Pharmacognosy and Complementary Medicine

BY

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DECLARATION

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I hereby declare that this thesis is my original work and has not been presented for a degree award in any other institution.

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DEDICATION

This thesis is dedicated to my children as it demonstrates that age and poor health are not a limitation to acquiring knowledge. It is also dedicated to the Government of Kenya especially Ministry of Medical Services which is responsible for regulation of herbal medicines. Finally, I dedicate this to Kenyan researchers as a challenge that more research is required in the field of herbal medicines and that this is achievable within our national research framework.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ABC	American Botanical Council
ADH	Arginine Dihydrolase
AV. TAMC	Average Total Aerobic Microbial Count
AV. TYMC	Average Total Combined Yeast And Mould Count
BDS	Botanical Dietary Supplements
BP	British Pharmacopoeia
CDR	Committee for Drug Registration
CFU	Colony forming Units
CIT	Trisodium Citrate
DARU	Drug Analysis and Research Unit
EMB	Ethyl Methyl Blue
FDA	Food and Drug Administration
GEL	Gelatin (bovine origin)
GEP	Ginseng Evaluation Program
GLC	Gas Liquid Chromatography
cGMP	current Good Manufacturing Practices
HPLC	High Performance Liquid Chromatography
IND	Indole production test (L-tryptophane)
INN	International Non Proprietary Name
IP	Indian Pharmacopoeia
KEMRI	Kenya Medical Research Institute
KNBS	Kenya National Bureau of Statistics
KNDI	Kenya Nutritionists and Dieticians Institute

KRA	Kenya Revenue Authority
LDC	Lysine Decarboxylase
MOH	Ministry of Health
MOMS	Ministry of Medical Services
MOPHS	Ministry of Public Health and Sanitation
MSA	Mannitol Salt Agar
ODC	Ornithine Decarboxylase
OR	Odds Ratio
OTC	Over the Counter
PPB	Pharmacy and Poisons Board
QC	Quality Control
SDA	Sabouroud Dextrose Agar
SSA	Salmonella Shigella Agar
TAMC	Total Aerobic Microbial Count
TDA	Trytophane Deaminase
TK	Traditional Knowledge
TLC	Thin Layer Chromatography
TM	Traditional Medicine
TNF- α	Tumor Necrosis Factor- α
TVAMC	Total Average Aerobic Microbial Count
UK	United Kingdom
USA	United States of America
USD	United States of America Dollar
USP	United States Pharmacopoeia
UTI	Urinary Tract Infection

VP	Acetoin Production
WHO	World Health Organization
XLD	Xylose-Lysine Deoxycholate Agar

DEFINITION OF TERMS

"**Yang" effects**- Represent brightness, active, upward, hot, expanding and strong.

"Yin" effects- Represents darkness, passiveness, downward, cold, contraction and weak.

Adaptogen- A substance that stimulates adaptive mechanisms of the body, improves human health and performance on long term use without causing dependence or negative effects.

Counterfeit Medicine- WHO defines counterfeit medicine as "one which is deliberately and fraudulently mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging."

Finished herbal products- Consist of herbal preparations made from one or more herbs and may contain excipients, animal materials and minerals in addition to the active ingredients. In this study, the term "botanical dietary supplements" is used interchangeably with "finished herbal products.

General sales medicine- a medical product or pharmaceutical formulation, the use or supply of which does not require advice from a pharmacist or any other licensed person.

Herbs- Are crude plant materials such as leaves, flowers, fruits, seeds, stems, wood, barks, roots, rhizomes, or other plant parts, which may be entire, fragmented or powdered that are used as herbal medicines in the prevention or treatment of physical and mental illness in man and animals.

Herbal materials- Are herbs, fresh juices, gums, fixed oils, essential oils and/or resins that are processed by various local procedures such as steaming to be used as herbal medicines.

Herbal medicines- Are preparations or formulations from herbs or herbal material or finished herbal products used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.

Herbal preparations- Are the basis for finished herbal products and may include powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by advanced processes like extraction, fractionation, purification or concentration and together with additives they are formulated into herbal medicines.

Nutritionist- One who is registered by Kenya Nutritionists and Dieticians Institute to practice as a nutritionist in Kenya.

Over the counter medicines- A medical product or pharmaceutical formulation, the safe use of which may require advice from a pharmacist and which should be available from a pharmacy or, where pharmacy services are not available, from a licensed person.

Pharmacist- One who is registered by the Pharmacy and Poisons Board to practice as a pharmacist in Kenya.

Pharmaceutical Technologist- One who is enrolled by the Pharmacy and Poisons Board to practice as a pharmaceutical technologist in Kenya.

Pharmacovigilance- Is the science and activities relating to detection, assessment, understanding and prevention of adverse effects or any other drug related problems.

Prescription only medicines- A medical product or pharmaceutical formulation, the use or supply of which should be by or on the orders of a person permitted by law to prescribe and should be available from a pharmacist on prescription.

Quality- Is a measure of how inherent characteristics, such as bacterial contamination, labeling and levels of active ingredients, of commercial ginseng containing products comply with a set of requirements or standards including Monographs. The totality of these attributes or properties bears upon the fitness of these ginseng containing products for their intended purpose or use.

Traditional medicine- Is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the prevention, diagnosis, and management of physical and mental illness and for maintenance of well being of human beings or animals.

ABSTRACT

The use and acceptance of nutritional and herbal supplements is popular in Kenya as is the case in other developing countries. Ginseng containing products, for example, are herbal formulations that are available as hard and soft gelatin capsules, dried roots, tinctures and tea or beverage additives. These products are used based on the specific indications for which they are promoted such as, adaptogenic effects, antioxidant, anti-diabetic, anti-hypotensive, immune stimulatory and anti-cancer effects. Despite advances made in understanding medicinal and toxic properties of many herbs, the consumer today, is confronted with several unproven claims concerning the quality, efficacy and safety of herbal preparations including ginseng containing products.

The main objective of this study therefore, was to evaluate the quality of ginseng containing products and their use through retail outlets in Nairobi County, Kenya. Forty commercial products labeled as containing ginseng were obtained through a structured systematic random sampling technique from 76 retail outlets in Nairobi, Kenya. Seventy six store attendants (one from each store) were assessed for their knowledge about properties of ginseng based on their understanding of schedule or classification of ginseng containing products, conditions for which they are prescribed and side effects.

The mean knowledge score, of the attendants, on ginseng products was 3.2 ($\pm$ 1.8 SD) ranging between 0 and 8. Approximately one-third of the attendants (32.9%) had adequate knowledge on ginseng products. The highest level of education attained was not significantly associated with knowledge on ginseng products ($P=0.823$). However, the type of professional training was significantly associated with knowledge on ginseng products ($P<0.001$). An attendant with a Bachelor of Pharmacy degree (Pharmacist) was 3.11 times more likely to have adequate knowledge on ginseng products compared to an

attendant with Diploma in Pharmaceutical Technology (Pharmaceutical Technologist). The likelihood was equally high among attendants trained in other unspecified professions (3.22-folds). A trained nutritionist attendant was 16.92 times more likely to have adequate knowledge on ginseng products compared to an attendant who is a pharmaceutical technologist.

Out of 40 ginseng products 41.5% were 80 – 100 % compliant to all labeling requirements with 4.9% being fully compliant, 36.6% being 80 - <100 % compliant, 39% being between 60 – <80% and 19.5% being <60% compliant.

All the products (100%) were compliant to microbial load requirements. Mean Total Aerobic Microbial Count (AV.TAMC) was 6.5 ($\pm$ 6.0 SD) ranging between 0 and 29×10^1 while Mean Total Combined Yeast and Mould Count (AV.TYMC) was 2.3 ($\pm$ 3.1 SD) ranging between 0 and 10×10^1. Mean Total Viable Aerobic Microbial Count (TVAMC) was 8.9 ($\pm$ 7.1 SD) ranging between 0 and 29×10^1. However 22.0% of the samples did not meet pharmacopoeia requirements for absence of high-risk microbes (S*almonella* species and *Escherichia coli*).

Of the 40 samples, 90 % (36) samples were analyzed for presence of ginseng, using a Shimadzu Cs-9000 densitometer. Thirty (83.3%) of them tested positive for either Rb1 or Rg1 groups of ginsenoside(s).

From this study, it is recommended that herbal or nutritional supplements containing ginseng should be subjected to guided registration requirements and pharmacovigilance programs. Special emphasis should be put on microbial contamination, especially microbial identification, to avoid high risk microbes. Furthermore, assay for ginsenosides should be carried out to establish levels of compliance to active ingredients. It is also recommended that attendants selling these products should have basic training in herbal

and nutritional supplements and the pharmacy curriculum for Bachelor of Pharmacy degree and Diploma in Pharmaceutical Technology should be reviewed in order to empower the graduates to offer proper services in complementary medicines.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Herbal products are commonly used in developing countries for management, treatment or prevention of various diseases due to poverty and lack of access to modern medicines. It is estimated that 65-80% of populations living in these countries depend on traditional medicine (TM) for primary health care (WHO, 2005). The use and acceptance of nutraceuticals and herbal supplements is also becoming popular in the developed countries where they are marketed as Botanical Dietary Supplements (BDS) (Harkey *et al*., 2001). For example, in the United States of America the market share for herbal and nutraceutical preparations was over USD 4.2 billion in 2001 (Marcus and Grollman, 2002).

The medicinal use of herbs is deeply rooted in human history and folklore and has been incorporated into traditional medicine (TM) of most cultures. In addition, strong religious and mystical beliefs have been associated with healing properties of many herbs. These beliefs, together with the definite physiological and pharmacological effects of various herbs have been used by researchers and pharmaceutical companies to identify new lead compounds for the development of new medicines (Dubic, 1986).

Unlike conventional medicines with clearly defined active ingredients, herbal medicines may contain several active constituents with additive or synergistic effects (Evans, 2002). Consequently, if every active constituent were to be isolated, assayed and tested for biological activity, the time and financial investment would be immense and may prove to be un-economical. This is particularly true for mixtures of herbal medicine formulations containing extracts from several plants (Mukherjee *et al.,* 2002).

Kenya is rich in traditional medicine (TM) and traditional knowledge (TK) that are unique to each community. These traditional medicines are mainly formulated as decoctions and infusions made from diverse plant species found within the country (Mwangi *et al.,* 2005). Like many developing countries, Kenya faces major challenges in the development and implementation of a regulatory framework for herbal medicines (WHO, 2005).

Despite advances made in understanding medicinal and toxic properties of many herbs, consumers today, are confronted with several unproven claims concerning the efficacy and toxicity of herbal preparations. In Kenya, for example, unauthorized and unregistered conventional contraceptive medicines have been found in Chinese Herbal Clinics where they are sold as Chinese herbal contraceptives. In children of less than 3 years of age, the side effects reported were: early enlargement of breasts and uterus, swelling in the legs, pain in the muscles and difficulty in walking. Tests carried out on these products established that they contained quinesterol and levonegesterol in amounts 100 times more than the normal doses for contraception and the children were getting the drug through their mother's milk (Pharmacy and Poisons Board, 2009(a)). Ginseng crude root products are commonly stocked in these Chinese Herbal Clinics.

The use of ginseng as herbal medicine is well documented and dates as far back as 5000 years ago. The earliest written document on ginseng is *Interpretation of Creatures* written by Shi You between 48 and 33 BC (Yun, 2001). According to this document ginseng containing preparations were widely used for rejuvenation activities. North American natives used ginseng as part of their traditional medicine practice (Banthorpe, 1994). Ginseng is widely studied, with the first scientific research having been published in 1954 (Shibata *et al*, 1985). Ginseng plant contains ginsenosides which are pharmacologically

active ingredients and are unique to *Panax* species (But *et al.,* 1995). Ginsenosides are used as marker compounds for purposes of quality control of ginseng extracts (Harkey, 2001).

Currently, ginseng containing products account for a large proportion of sales for an individual herb. In the United States of America, sales of ginseng containing preparations for the year 1997 were about USD 100 million, with an estimated growth of 26% per year (Brevoort, 1998). Ginseng is formulated into herbal supplements and nutraceuticals as dried root, hard and soft gelatin capsules, tinctures, tea and as beverage additives. These formulations are based on the specific indication for which they are promoted, which include: adaptogenic effects, antioxidant, anti-diabetic, anti-hypotensive, immuno-stimulatory and anti-cancer effects.

1.2 History of Ginseng Use

Historically, the term ginseng referred to herbs from *Panax ginseng C*.A.Meyer indigenous to China and Korea (Lyons and Petrucelli, 1978). Ginseng in modern literature refers to different herbs from the family *Araliaceae* of the genus *Panax*. Common *Panax* species include *Panax ginseng C*.A.Meyer, indigenous to China and Korea also referred to as Asian ginseng; *Panax quinguefolius*, L. indigenous to North America also referred to as American ginseng, *Panax pseudo-ginseng*, Himalayan ginseng; *Panax vietnamensis*, Vietnamese ginseng; and *Panax japonicas,* Japanese ginseng (Harkey *et al.,* 2001). Most studies have focused on the Asian and American ginsengs.

Oriental ginseng from China and Korea is mentioned in one of the earliest Chinese herbal document known as, the "*Pen Tsao"* written about 2800 BC (Lyons and Petrucelli, 1978). Medical properties of ginseng were also described by Confucius around 500 BC (Dubic,

1986) and documented in first compendium of herbal knowledge for Chinese tradition, **"***Classic Herbal of Shennong***",** written around 100 AD (Dharmananda, 2002). Ginseng was given special attention by Li Shih Chen in his 52 volume encyclopedia on plants written in the sixteenth century (Dubic, 1986).

From the various literature sources, the effects of ginseng are described as: stimulation of the "yin" (the cooling or negative force) and the "yang" (the hot or positive force), a Chinese ancient concept that explains the balance in body cells and systems required to promote general health and well being. It is believed that achieving the correct balance between "yin" and "yang", the opposing forces in the body cells and systems, give vitality and good health (Dixon, 1976). Asian ginseng is thought to have more "yang" effect that heats and energizes the body. Chinese traditional medicine practitioners have prescribed Asian ginseng to counter the effects of aging, cold climate and to treat stress, asthma, hypotension, digestive disorders and anaemia. Over-all good health is thought to be achieved gradually (Barnthope, 1994; Li *et al*., 1996). However, North American ginseng is thought to have the opposite effect of Asian ginseng, that is, stimulates the "yin" more that produces cooling effect on the body. This ginseng is therefore used in warmer climates, children, elderly and those with high blood pressure and diabetes (Li *et al*., 1996).

Currently, ginseng is listed in many pharmacopoeias such as United States Pharmacopoeia (USP), British Pharmacopoeia (BP) and Chinese Pharmacopoeia. There are several ginseng containing products in the global market. Standardized ginseng extracts are available in many formulations including capsules, tablets and herb powders. Ginseng extracts have also been added into tea leaves, fruit juices, coffee, soups, soft drinks and alcohol (Kitts and Popovich, 2003).

Ginseng has long been used for medicinal conditions in East Asia and has recently become a popular complementary/alternative medicine in the Western countries as well as the rest of the developing world mainly South America. Comprehensive studies on ginseng have been carried out in East Asia, United States of America and the Western countries. However, there is no much work done in Africa and Kenya in particular. A spot check of the retail outlets in Nairobi indicates that there are many different types of ginseng containing products which are relatively expensive with prices ranging from USD 15 to 100.

1.3 Taxonomy and Cultivation of Ginseng

Ginseng is a perennial herb with characteristic branched taproot which often has two to five laterals extending from the middle of the main root in the form of a human figure {Figure 1.1(a),1.1(b) and Appendix (5) *Panax ginseng* plant and root respectively}.

Figure 1.1(a): *Panax ginseng* plant

Figure1.1 (b): *Panax ginseng* root

(Homeherbs, 2008).

The plant belongs to the family Araliaceae and species *Pana*x. The most commonly used species are *Panax ginseng* C.M. Meyer and *Panax quinquefolius L.* The crop usually starts flowering in its fourth year and the roots take 4-6 years to reach maturity. The root is the morphological part with highest concentration of active markers, the ginsenosides

(Ludwiczuki *et al*., 2006). The dry matter content of the root is about 30%. The crop is produced mainly by cultivation in Eastern Asia and United States of America (WHO Monographs, 1999).

Most problems leading to low quality roots start with poor growth conditions and can be avoided. These include inadequate soil drainage, untimely and poorly applied pesticides, and neglect of good agricultural practices, especially, during harvesting and post-harvest processes. Furthermore, diseases remain the major problem in ginseng cultivation starting from seed stratification, soil preparation prior to planting and drying of the roots (Persons, 1995). Seeding is still the major method of propagation of ginseng in spite of some success in culturing different parts of the plant (Choi *et al*., 1995).

Ginseng is an expensive crop to produce so adulteration or substitution with other cheaper products is likely to occur (Liberti and Der Marderosian, 1978). Furthermore, ginseng attracts a high turnover with global sales of more than a billion US dollars per annum (Li *et al*., 1996).

1.3.1 Asian White and Red Ginseng

Roots from the Asian ginseng, *Panax ginseng* C.A. Meyer, are often referred to as red or white ginseng depending on the drying method used for preservation. Asian white ginseng usually refers to the root of *Panax ginseng* that is air dried while red ginseng is used to describe the root that is dried through steam heating process. Steaming ginseng produces a red shade due to caramelization of sugars compared to the beige colour of air dried root. Red ginseng is produced in Korea, China, Japan and the Russian Federation. There are no major differences in terms of ginsenoside composition between the two types of ginseng. However, red ginseng is believed to be more potent (Kim *et al.,* 2000; WHO, 1999).

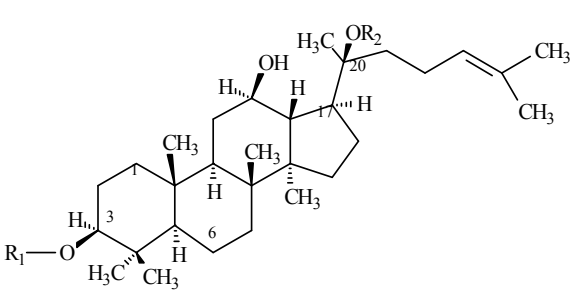
1.3.2 North American Ginseng

North American plant, *Panax quinquefolius*, is identical in shape and physical characteristics to Asian ginseng. It is typically grown in USA, Canada and Australia. China introduced the cultivation of American ginseng for both local and export markets in 1975 (Wang *et al*., 2001). Asian ginseng can be distinguished from American ginseng using the ginsenoside profile because Asian ginseng contains Rf ginsenoside while American ginseng does not (Tanaka *et al*., 1984). However, Rf Ginsenoside is often misidentified during analysis. As a result, ginsenosides ratios such as Rb1/Rb2 have been used to confirm North American ginseng (Hu and Kitts, 2001). Rb1 and Rb2 are some of the common ginsenosides found in both Asian and American ginseng plants but in different amounts. These two parameters, however, do not produce very clear distinctions between the two types of ginseng. Protein analysis and genetic markers have also been used to distinguish between ginseng species and are the definitive methods for identification of authentic ginseng samples (Cui *et al*., 2003; Wang *et al.,* 2001).

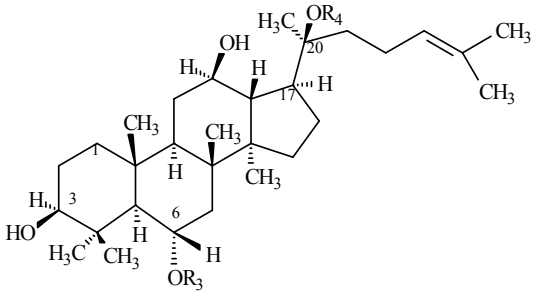
In Hong Kong, which is a world leading market for ginseng, North American ginseng is 5 to 10 times more expensive than Asian ginseng and this may lead to adulteration of the American root by unscrupulous dealers (Wang *et al.,* 2001).

1.4 Chemical Constituents of Ginseng

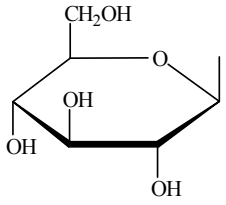
The major chemical constituents that have been demonstrated to be responsible for the pharmacological properties of ginseng are the triterpene saponins called ginsenosides. They are based on a shared common tetracyclic dammarane skeleton, a steroid nucleus having 17 carbon atoms arranged in four rings. Ginsenosides are divided into two groups; the Rg_1 and Rb_1 group (Figure 1.2).



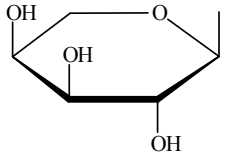
20(S)-protopanaxadiols



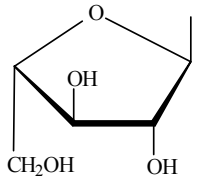
20(S)-protopanaxatriols



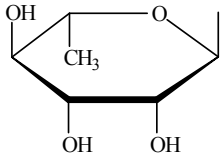
β-L-glucopyranosyl(Glc)



α-L-arabinofuranosyl(Arap)



α-L-arabinofuranosyl(Araf)



α-L-rhamnopyranosyl(Rha)

Ginsenosides sugar substitution patterns

20(S)-Protopanaxadiols			20(S)-Protopanaxatriols		
Ginsenoside	**R_1**	**R_2**	**Ginsenoside**	**R_3**	**R_4**
Rb1	Glc(β1-2)Glc	Glc(β1-6)Glc	Rg1	Glc	Glc
Rb2	Glc(β1-2)Glc	Arap(α1-6)Glc	Rf	Glc(β1-2)Glc	H
Rc	Glc(β1-2)Glc	Araf(α1-6)Glc	Re	Rha(α 1-2)Glc	Glc
Rd	Glc(β1-2)Glc	Glc			

Figure 1.2: Structures of 20(S)-protopanaxadiols and 20(S)-protopanaxatriols ginsenosides

The differences in structure arise from the type, position and number of sugar moieties (R1, R2, R3 and R4) attached by glycosidic bonds at positions C-3 and C-6 hence the classification. The Rb1 group has 20(S) - protopanaxadiol as the aglycone with a sugar moiety at position C-3. Ginsenosides in this group include Rb1, Rb2, Rc, and Rd. The Rg_1 group is also referred to as 20(S) –protopanaxatriol and consists of ginsenosides that have sugar moiety attached at C-6 on the steroid nucleus. Ginsenosides in this group include Rg1, Re, Rf, and Rg2 (Yat *et al*., 1998 and WHO Monographs, 1999). The root also contains oleanolic acid structurally based ginsenosides, Ro (Tang and Eisenbrand, 1992).

More than thirty ginsenosides have been identified from *Panax* species six of which (Rg1, Re, Rb1, Rc, Rb2 and Rd) constitute the major ginsenosides that account for over 90% of saponin content of ginseng root (Yat *et al.,* 1998). The six are normally considered reference compounds and the total composition of ginsenosides is usually expressed as percentage of one or all the six ginsenosides (Tang and Eisenbran, 1992). The ginsenosides composition of the different ginseng plant species is affected by several factors, including the region in which the crop is grown, the variety and age of ginseng plant as well as light levels (Fournier *et al*. 2003; Li and Wardle, 2002).

1.5 Pharmacological Effects of Ginseng

Ginseng has been used in traditional Chinese medicine for a long time as a general tonic and cardiotonic (Tang and Eisenbrand, 1992). These tonic effects of ginseng have led to its classification as an adaptogen that can be used to modify favorably the effects of various types of stress. According to Lee (1992), the systemic pharmacological effects of ginseng include reducing fatigue, increasing stamina and strengthening the general physical condition. It also exerts a hematopoietic action thus protecting against anaemia,

hypotension and other heart ailments. In addition, ginseng causes improved mental condition by preventing neurosis and nervous breakdown. It also increases the secretions of body fluids and thus quenches thirst. Other pharmacological effects include prevention of coughing, tuberculosis, asthma, gastroenteritis, diarrhea and constipation. Ginseng has also been shown to have antioxidant, antitumor, and immunomodulatory properties (Ni *et al*., 2010).

Laboratory and clinical studies on ginsenosides appear to support some of the traditional medical claims (But *et al*., 1995; WHO, 1999). However, findings from some other clinical studies do not corroborate some of the traditional claims (Kiefer and Pantuso, 2003).

1.5.1 Antioxidant Properties

Antioxidant activities attributed to ginseng such as free radical scavenging have been demonstrated from a number of *in vitro* studies. Ginseng extracts scavenge stable free radicals such as 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and the carbon- centered free radical 1, 2'-azo bis (2-aminopropane) dihydrochloride (AAPH) {Kim *et al.,* 2002; Kitts *et al.,* 2000}. North American ginseng has better free radical scavenging properties than Asian ginseng (Hu and Kitts, 2001).

1.5.2 Effect on Performance

Clinical studies have been carried out to determine the effect ginseng has on participants' physical performance and fatigue. These studies yielded conflicting results where in some cases there was increase in performance for the participants who resisted getting fatigued. In other cases, however, the opposite was true. In such studies participants taking ginseng and placebos did not show significant differences. This may have been as a result of poorly designed studies, lacking proper controls and using ginseng that was not

standardized. The effect of ginseng on substrate utilization, hormone production, endurance, metabolism and perception of effort during consecutive days of exhaustive exercises yielded no significant results (Wong *et al*, 2011).

1.5.3 Anti-Diabetic Effects

Animal studies have demonstrated that ginseng has anti-diabetic properties. In one such study, using mice, it was demonstrated that administration of ginseng extract from white ginseng root resulted in reduction of fasting blood sugar levels by 37% to 40% compared to the untreated group (Chung *et al*., 2001). In another study, two diabetic animal models; male KK-CAY mice and alloxan- diabetic mice were administered with 90mg/kg body weight of water extract of ginseng through intraperitoneal route. The results showed significant reduction in fasting glucose by 76% and 62% in the KK-CAY and alloxan-diabetic mice respectively (Kimura *et al*., 1999).

Ginseng has been shown, in clinical studies, to have beneficial effect in both insulin-dependent and non insulin-dependent patients (Sotaniemi *et al*., 1995). The effects include elevation of mood, improved physical performance and reduced fasting blood glucose.

1.5.4 Immune Stimulatory Activities

Some studies have shown that ginseng extracts stimulate human immune response system (Scaglione *et al.,* 1990). Administration of North American ginseng extract, of concentration 200 μg/ml, given in a placebo controlled double blind study for immunomodulatory actions revealed an increase in polymorphonuclear leucocytes, total number of T_3 and T_4 lymphocytes. Ginseng also stimulated the production of tumour necrosis factor α (TNF-α) (Zhou and Kitts, 2002). TNF-α is an important marker in early

immune response to immunomodulatory agents. Its production therefore implied that ginseng is an immune booster.

1.5.5 Impotence and Erectile Dysfunction

Studies indicate that ginseng extracts improved sperm production in men and may have some useful role in treating impotence (Owen, 1981). The ginsenosides are thought to decrease blood prolactin levels thus increasing libido.

Panax ginseng appears to be effective in the treatment of erectile dysfunction as suggested by a double blind crossover study evaluating the effect of Korean red ginseng in patients with erectile dysfunction. In tests on 45 men with erectile dysfunction, those who took *Panax ginseng* for eight weeks showed greater improvements than those given a placebo for the same time period (Bumsik et al., 2002). In an earlier study on 90 men with erectile dysfunction, 60% of the participants reported improvement in their symptoms compared with 30% of those using the placebo.

1.5.6 Psychomotor Activities

Participants who use standardized ginseng extracts have been shown to improve on their attention, processing integrated-sensory motor functions and auditory reaction time (D'Angelo *et al.,* 1986). These studies proved that ginseng was superior to placebo in improving certain psychomotor functions in healthy subjects such as mental alertness.

1.5.7 Effects on Haemolysis

Using free- radical induced human erythrocyte haemolysis model, ginsenosides were shown to exhibit different effects on haemolysis of red blood cells. However, the majority of ginsenosides had a protective effect against haemolysis after exposing cells to

free-radical haemolysis (Hosettman and Marston 1995; Liu *et al*., 2002). Some ginsenosides, like Rg3 and Rh2, increased the extent of free-radical induced haemolysis.

1.5.8 Anti-Cancer Effects

Regular consumers of ginseng have been shown to stand a lower risk of developing different type of cancers than non ginseng consumers (Yun and Choi, 1998; Yun *et al*., 2001). However, ginseng is reported to have no-specific preventive effect against the following types of cancer: human liver (Park *et al*., 2002); leukemia (Lee *et al*., 2000; Popovich and Kitts, 2002); prostate (Liu *et al*., 2000) and breast cancers (Oh *et al.,* 1999).

1.5.9 Side Effects and Contraindications of Ginseng

Ginseng products should not be used by people with hypoglycemia, high blood pressure, heart disorders, insomnia, acute asthma, nose bleeding or heavy menstruation. These products may not be used by pregnant women or nursing mothers since the effects on developing fetus or infants are not known (Balch, 2006). People with hormone-dependent illnesses such as endometriosis, uterine fibroids or cancers of the breast, ovaries, uterus, or prostate should avoid *Panax ginseng* because it may have estrogenic effects.

Ginseng generally is well tolerated, and its adverse effects are mild and reversible. Associated adverse effects include nausea, diarrhea, euphoria, insomnia, headaches, hypertension, hypotension, mastalgia, and vaginal bleeding (Kiefer and Pantuso, 2003).

Ginseng may interact with caffeine to cause hypertension and it may lower blood alcohol concentrations. It also may decrease the effectiveness of warfarin. Concomitant use of ginseng and the monoamine oxidase inhibitor, such as phenelzine, may result in manic-like symptoms. Ginseng also causes hypoglycemic activity thus caution should be exercised in the use of ginseng products in patients with diabetes. Care must be taken

when ginseng is used by diabetic persons because of possible interactions with oral hypoglycemic agents and insulin (Chan *et al.,* 2000; Kiefer and Pantuso, 2003).

1.6 Ginseng Products in Kenya

Commercially available ginseng products in the Kenyan market include those containing ginseng as the only active ingredient of the finished product or those containing mixtures of ginseng, vitamins, minerals and/or other complementary medicines such as royal jelly. Crude root preparations are also available. These formulations are imported mainly from China, the India and the Far East Asian countries.

These products are promoted as geriatric tonic for improving age related impairments of cerebral function and adaptogenic therapy during periods of stress. Other pharmacological properties for which they are promoted include adjuvant therapy for improving peripheral blood flow, physical stamina and shortening convalescence time after debilitating conditions through improved protein synthesis and body repairs.

Dealers in these products can broadly be divided into three classes: registered pharmacies where they are sold as over the counter (OTC) medicines; nutritional stores where they are treated as botanical dietary supplements (BDS) and supermarkets where they are sold as general sales products (GSP). There is no data on the total market share of ginseng trade in the Kenyan market but it suffices to presume that it contributes a substantial amount to the total share of trade in herbal medicines.

Formulations containing ginseng are either classified as foods, nutritional supplements or medicines. The use of these products, therefore, does not fall under strict regulations applied to conventional medicines by the Pharmacy and Poisons Board.

1.7 Quality Control of Herbal Products

1.7.1 Background

The natural products industry requires standard criteria for the assessment of the quality of these products for international and local markets. There are several attempts in many countries to establish quality control standards for botanical dietary supplements or herbal products. In the United States of America for example, botanical dietary supplements are currently exempted from current Good Manufacturing Practice (cGMP) requirements, do not require proof of efficacy and do not require pre- marketing approval by the FDA unless medical or drug-like claims are made. The safety of botanical dietary supplements is the responsibility of the manufacturer. The role of the FDA in safety assurance is limited to post-marketing surveillance of adverse effects (Breemen *et al.,* 2007). Therefore, the safety and efficacy of these products lack documentation, which is an area of concern for health care providers and consumers.

Kenya, like many other developing countries, faces major challenges in the development and implementation of a regulatory framework for herbal medicines (WHO, 2005). These challenges are related to regulation of the practice of herbal medicines and the products themselves. Whereas herbal products are covered by the Pharmacy and Poisons Act, Cap. 244 hence falling under the two ministries of health; Ministry of Medical Services and Ministry of Public Health, the practice fall under the Ministry of Gender, Children and Social Development. Herbal practitioners are registered and licensed by the Department of Culture in Ministry of Culture and National Heritage. The registration process for herbalists does not follow strict scientific and ethical standards observed by medical practitioners.

Other challenges are related to definition and categorization, pharmacovigilance framework and post market surveillance of herbal medicines. There is also lack of knowledge and enabling legal framework on herbal medicines and the practice (WHO, 2005). Quality Control (QC) is an integral part of herbal medicines practice which ensures delivery of required quantity of quality medicament for each herbal formulation (Mukherjee, 2002).

The quality of plant raw material is determined by intrinsic and extrinsic factors (WHO, 2005; Evans, 2002, Mukherjee, 2002; Shinde *et al*., 2009). Intrinsic factors include inter/ intra-species variations and parts of plant used. Inter and intra-species variations affect the levels of secondary metabolites which are normally the active constituents. Intrinsic factors are influenced through gene and geographical variation. Because of these variations it is difficult to standardize active ingredients from plants.

Concentrations of secondary metabolites vary in different morphological parts of plants. Hence using a wrong part of the plant affects the quality of the final herbal medicine. Environmental factors such as climate, altitude and rainfall affect quality and quantities of active principles. It is important that the plants are cultivated in optimal climatic conditions (Persons *et al.,* 1995)

Good Manufacturing Practice (GMP) specifies many requirements for quality control of starting materials including correct identification of species of medicinal plant, time and procedure of harvesting, transport and storage conditions. Improper storage may lead to microbial contamination whereas excessive drying may cause loss of thermo-labile constituents (Shinde *et al.,* 2009). Other contaminants of plant raw materials for the manufacture of herbal medicines may include soil, heavy metals, pesticides and fumigants (Persons *et al*., 1995).

It is, therefore, difficult to perform quality control tests on the raw materials of herbal medicines. In the case of finished herbal medicinal products, particularly mixed herbal products, it is more difficult to determine whether all the plants or starting materials have been included (WHO, 2005).

1.7.2 Methods of Quality Control

Quality control is of paramount importance for efficacy and safety of herbal products. Quality can be defined as the status of a drug that is determined by identity, purity, content and other chemical, physical, or biological properties, or by the manufacturing processes. Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. However, for traditional medicines, the traditional methods of manufacturing such as harvesting and drying and traditional information about the identity and quality assessment of medicinal formulations should be studied and interpreted using modern technology. The modern quality control methods for herbal products are documented in recent monographs such as Pharmacopoeia of the Peoples Republic of China, United States Pharmacopeia and National Formulary, British Pharmacopeia, French Pharmacopeia and the WHO Monographs on Selected Medicinal Plants (Lee and Yuqing 2009).

Quality control methods include determination of microbial contamination or microbial load and chromatographic techniques for identification and quantification of active constituents. The quantitative determination of constituents has been made easy by recent developments in analytical instrumentation. Recent advances in the isolation, purification, and structure elucidation of naturally occurring metabolites have made it possible to establish appropriate strategies for the determination and analysis of quality and the process of standardization of herbal preparations. Thin Layer Chromatography (TLC),

High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), quantitative TLC (QTLC), and high-performance TLC (HPTLC) can determine the homogeneity of a plant extract. Infrared and Ultraviolet Visible (UV-VIS) spectrometry, Mass Spectrophotometer (MS), Gas Chromatography (GC), liquid chromatography (LC) used alone, or in combinations such as GC/MS, LC/MS, and MS/MS are powerful tools used for quality control and standardization of both the raw materials and the finished products. The results from these sophisticated techniques provide a chemical fingerprint as to the nature of chemicals or impurities present in the plant or extract (Rozylo *et al,* 2002 and Ebel *et al*, 1978).

1.7.3 Use of Marker Compounds for Quality Control

The European Medicines Agency (EMEA) defines marker compound or chemical markers as chemically defined constituents or groups of constituents of a herbal medicinal product which are of interest for quality control purposes regardless of whether they possess any therapeutic activity. Ideally, chemical markers should be unique components that contribute to the therapeutic effects of a herbal medicine. Since only a small number of chemical compounds are shown to have clear pharmacological actions, other chemical components are also used as markers. The quantity of a chemical marker can be an indicator of the quality of a herbal medicine (Songlin *et al.,* 2008). A good marker should be characteristic of or unique to the given herbal preparation and be a substance with an established chemical structure. It should also not be present in other herbs contained in the finished product when it is necessary to selectively quantify the content of one herb in a multicomponent herbal product. For the purpose of proper quality control, a selected marker should be present in sufficient quantities to develop a scientifically valid test method, be accessible to quantify with common analytical equipment, be sufficiently stable and commercially available.

Compounds with a proven therapeutic activity that have been used as markers for quality assurance include kavalactones in *Piper methysticum*, silybins/silydinins in *Silybum marianume*, piperine in *Talisadi churna* and ginsenosides in *Panax ginseng* (Shukla and Saraf, 2009; Harkey *et al*, 2001). Several surveys have been carried out on the quality of commercial ginseng containing products in the international market, particularly the USA using ginsenosides as marker compounds. In one such study, twenty five commercial ginseng samples were analyzed for levels of ginsenosides (Harkey *et al.,* 2001). This study showed that all the products were correctly identified as per botanical plant species (*Panax* spp). However, concentration of ginsenosides differed significantly by 15 to 36 fold from the labeled amounts.

1.7.4 Quality Control of Ginseng Containing Products

There are several attempts in many countries to establish quality control standards for products containing ginseng. In the United States of America, for example, the American Botanical Council (ABC) initiated, in 1994, a comprehensive study of commercial ginseng products sold throughout North America; the Ginseng Evaluation Program (GEP). This program was meant to set standards for future studies, give guidance on labeling brands and educate manufacturers on their responsibility towards ensuring safety of ginseng containing products. This arose out of the fact that ginseng containing products were classified as botanical dietary supplements following the passage of the "Dietary Supplement and Health Education Act", in 1994. These efforts led to inclusion of ginseng in the United States Pharmacopoeia.

The WHO Monograph on Selected Medicinal Plants provides for description of ginseng plant; properties of the root, which is the plant part used; purity tests; and chemical assays of ginsenosides, the active constituents. Quality control methods for the root include:

general organoleptic properties, macroscopic, transverse section and powder characteristics of the root (WHO, 1999).The finished ginseng containing formulation must also meet some specific labeling regulatory considerations in the country where they are to be marketed.

In Kenya, however, ginseng containing products are treated as nutritional supplements and scheduled together with multivitamins. These products are not subjected to strict regulatory requirements applicable to conventional medicines {Pharmacy and Poisons Board, 2010(a)}.

1.7.4.1 Detection and Analysis of Ginsenosides

Ginsenosides are unigue to *Panax* species, and are responsible for the pharmacological activity of *Panax* species. The compounds are also used as marker compounds for the quality control of ginseng (Harkey *et al*., 2001). Ginseng extracts can be prepared in several different ways for purposes of analysis. In some methods, only organic solvents are used while others employ hot water or a mixture of the two. Organic solvents reported to have been used for extraction of ginsenosides include 70% methanol (Corthout *et al*., 1999; Bonfill *et a*l., 2002), 50% methanol (Ludwiczuki *et al*., 2006) and 75% ethanol. Extraction methods have been found to have an impact on the concentration of ginsenosides in the final extract (Yong *et a*l., 2004; Ludwiczuki *et al*., 2006). There is also variation in the concentration of ginsenosides depending on the part of ginseng plant used. Ginseng root, leaf, flower, stem and seed all contain various levels of ginsenosides (Tang and Eisenbran, 1992; Ludwiczuki *et al*., 2006). The methods for isolation, identification and quantitative analysis of ginsenosides are varied as described in the literature. Methods for qualitative and quantitative analysis of ginsenosides include TLC, HPLC, GLC or Colorimetry (Lui and Staba, 1980).

Thin layer chromatography (TLC) is the standard method used to analyse many different phytochemicals including ginsenosides. Figure 1.3 illustrates analysis results of ginsenosides using TCL.

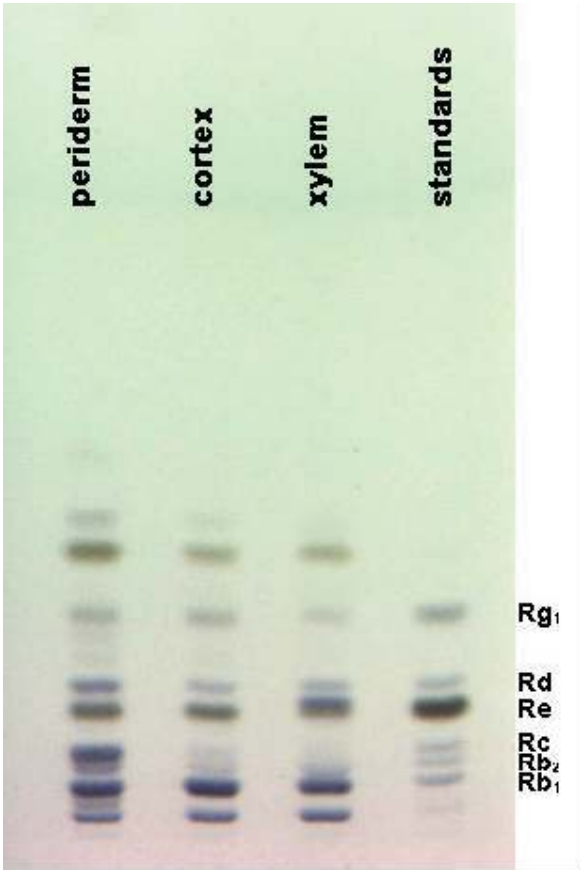


Figure 1.3: TLC profile of ginsenosides occurring in *Panax quinguefolium* Root (Ludwiczuk *et al.,* 2006**)**

Ginsenosides are named according to the retention times or distance migrated on a TLC plate. A number of different solvent systems have been used to separate ginsenosides; butyl alcohol- water- ethyl acetate (USP 2005); chloroform-methanol-ethyl-acetate-water-hexane (Ludwiczuki *et al*., 2006; Corthout *et al*., 1999) and others. Using a lower phase of a solvent system: chloroform (65ml), methanol (35ml), water (10ml) for TLC analysis, the results showed that ginsenosides generally migrated according to their polarity where the higher the polarity the less the distance of migration, R_f (Popovich and Kitts, 2003).

Ginsenosides Rb1 is found closer to the origin of the TLC plate than Rb2 (Figure 1.3). However, ginsenosides Re and Rd are the exceptions, and can reverse their order of migration when using $CHCl_3$/MeOH/H_2O solvents

Densitometry has been used to give faster scanning of TLC plates and has yielded more accurate results as shown in Figure 1.4.

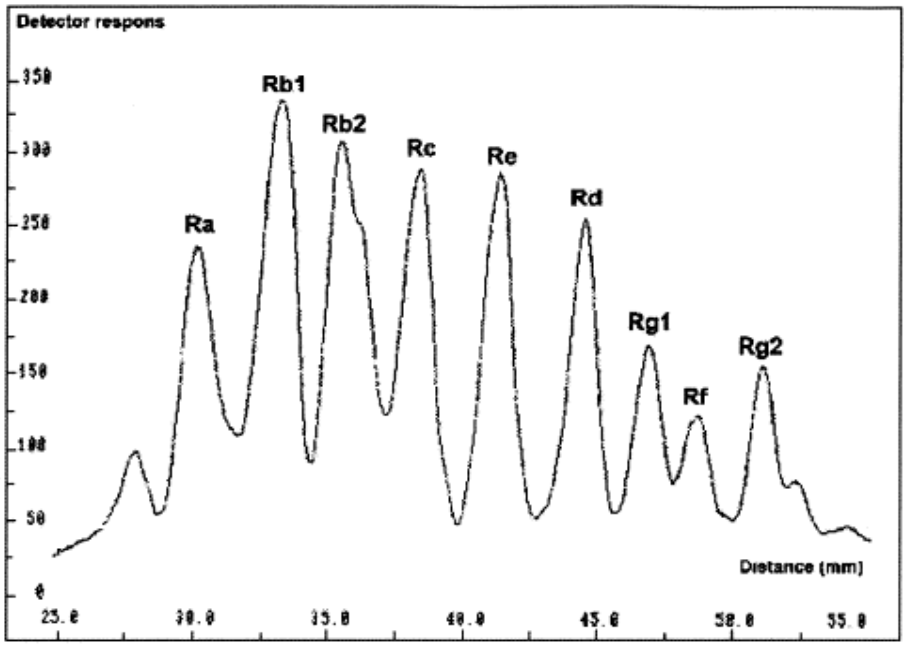


Figure 1.4: Densitogram of ginseng applied on a TLC plate (Courthout *et al.*, 1999)

Typically, ginsenosides are extracted from ginseng containing products using methanol. After the extraction of ginsenosides, a solid phase extraction (SPE) step often follows to remove interfering components before TLC analysis (Corthout *et al.,* 1999; Li *et al.,* 1996). Interfering components can also be removed by use of appropriate solvents by way of fractionation (Kwon *et al*., 2001). The solvent combination of chloroform-ethyl acetate-methanol-water is used as a mobile phase for separation of ginsenosides and p-anisaldehyde used as a detection reagent.

Other types of columns and solvent systems have been used for purification and separation of ginsenosides before densitometric analysis leading to densitograms like those in Figure 5 (Shibata *et al*., 1985; Park *et al*., 2002; Yip *et al*., 1985; Ludwiczuk *et al,* 2006).

Some researchers have used reverse phase HPLC analysis method, employing C_{18} column to determine the quantity of ginsenosides in ginseng containing products (Meier *et al.,* 1985). Typically, the mobile phase of acetonitrile-water is used for separation. This method has been successfully used to separate the main reference ginsenosides; Rb1, Rg1, Rc, Rd, Re, from the rare ginsenosides; Rg3, Rh and other aglycones.

In addition, ginsenosides have been characterized by use of LC/MS (Chan *et al.,* 2000; Haijiang *et al.,* 2003). This method has been used to distinguish between Asian ginseng and American ginseng based on identification of ginsenoside, Rf and pseudoginsenoside, F11 (Li *et al*., 2002). Scanning samples by way of MS-electrospray ionization (ESI-MS) in positive and negative ionization mode have been reported (Haijaing *et al.,* 2003; Kite *et al.,* 2003; Chan *et al.,* 2000 and Wang *et al.,* 1999).

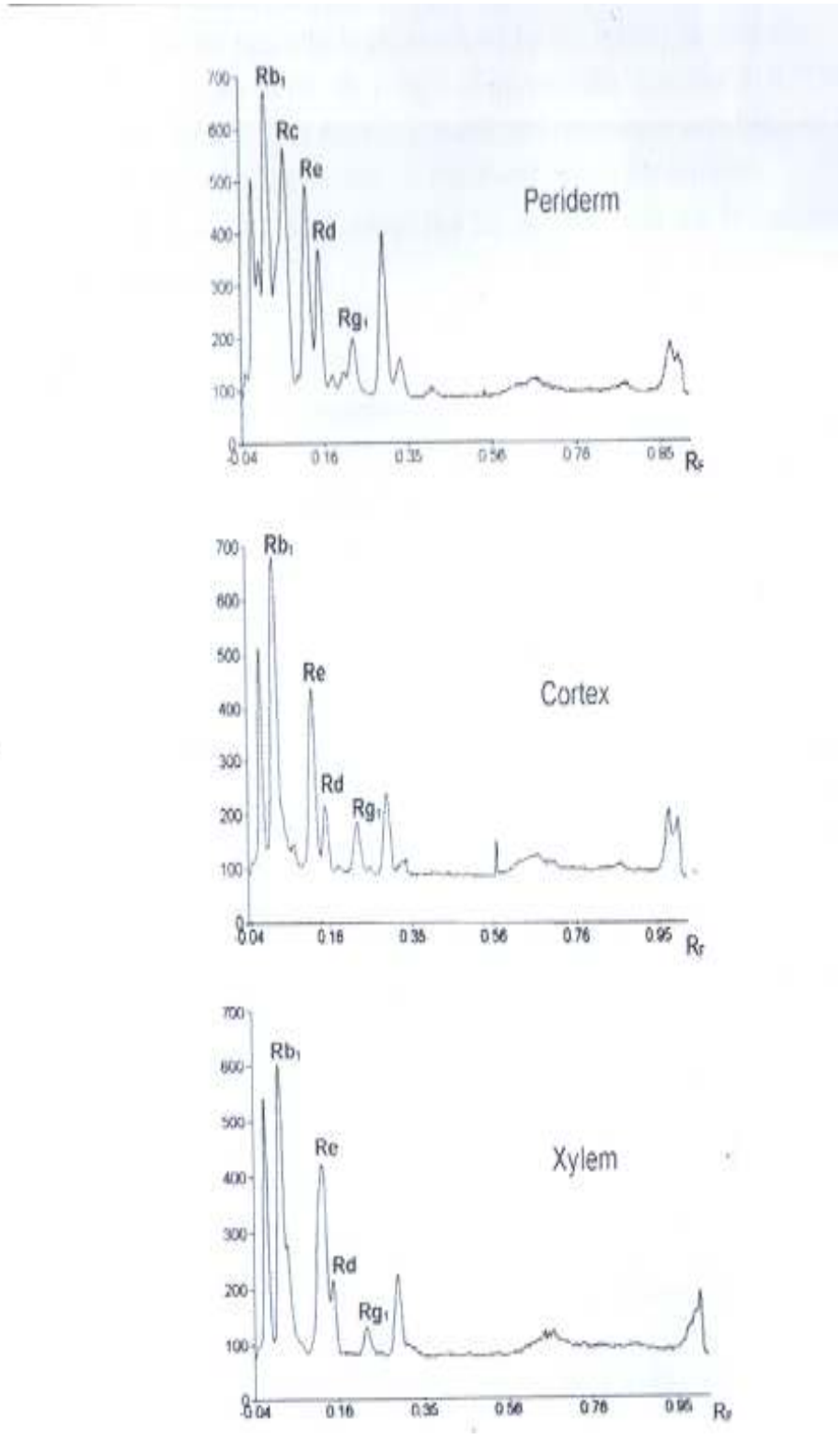


Figure 1.5: Densitograms of ginsenosides occurring in ginseng root tissues (Ludwiczuk *et al*., 2006)

1.7.4.2 Microbial Contamination of Ginseng

Most herbal products are simply washed and dried without any treatment for sterilization. There is therefore a likelihood of microbial contamination from water, soil and the environment during production and from human activities such as harvesting, drying and sorting. For these reasons, the importance of sterilization has been pointed out for quality control of crude drugs and herbal medicines. However, heat treatment is not suitable for them as it may alter the active ingredients or scent of these natural plant derivatives. Radiation treatment of <10 kGy on food has been approved by the Food and Agriculture Organization of the United Nations, International Atomic Energy Agency and World Health Organization and is widely practiced around the world. This radiation treatment has inspired some countries such as Indonesia, to apply radiation on crude drugs. However, this is not a common practice in many countries (Kimura, 1997).

The quality control of crude drugs has been at the discretion of each pharmaceutical company; therefore, microbial contamination levels vary drastically amongst herbal drugs from different manufacturers. Currently, microbial contamination on crude drugs has become an issue. Consequently quality assurance and the Good Manufacturing Practices have been instituted in some countries. Therefore, it is necessary to estimate the microbial contamination level on crude drugs at each manufacturing stage and to establish contamination assessment methods.

Commonly, microbial contamination is assessed by culture-dependent methods such as the plate count technique. However, these methods may underestimate bacterial number due to the difficulty of cultivation of starved or injured bacterial cells and by antibacterial activities in some crude drugs. In addition, the plate count method may take up to several days to detect bacteria (Amann *et al.,* 1995; Vives-Rego *et al.,* 2000).

Recent studies have revealed contamination of ginseng supplements. In one such study, ginseng supplements have been found to be contaminated with moulds, yeast and bacteria (Tournas *et al*., 2006). In the study, 46 ginseng supplements samples, including Siberian ginseng root, Chinese ginseng (herb/root), and American ginseng (root/extract), were tested for moulds and yeast contamination and the presence of bacteria. Results showed that 100% of the Siberian ginseng samples were contaminated with fungi (up to 1,400 cfu per gram) and bacteria (up to 1 million cfu per gram) (Tournas *et al*., 2006).

About 78% Chinese ginseng samples were contaminated with fungi (up to 60,000 cfu per gram) and 89% with bacteria (up to 1.2 million cfu per gram). Of the root samples, 56 % contained fungi (up to 1,400 cfu per gram) and 100 % contained bacteria (up to 680,000 cfu per gram). Out of the American ginseng root samples, 48 % contained moulds (up to 430,000 cfu per gram) and 30 % contained bacteria (up to 45,000cfu per gram). No moulds or yeasts were found in the ginseng extract but it contained bacteria (up to 1,000 cfu per gram) (Tournas *et al.,* 2006).

Most common fungi encountered in the study were *Penicillium* spp, *Rhizopus* spp, *Aspergillus* spp. and *Cladosporium* spp. Whereas the common bacterial species found in these products included *Intrasporangium* sp, *Mycobacterium* spp., *Caulobacter leidyia*, *Bifidobacterium* spp. and *Fusobacterium* spp. However, virulent disease causing bacteria like *Salmonella* and *Escherichia coli* were absent (Tournas et al., 2006).

A study of microbial contamination in concentrated Chinese medicine by Ku *et al*., (1994), where 61 semi-finished products and 20 finished products from five pharmaceutical factories were sampled, established that crude drugs such as Ginseng Radix, Glycyrrhizae Radix and Zingiber Rhizoma had serious microbial contamination. In this study, it was established that using starch as excipients yielded lesser degree of

microbial contamination. Levels of contamination also depended on granulation processes, tools and containers used and cleanliness of processing rooms and personnel

Studies have also shown potential contamination with the heavy metals such as lead, cadmium and arsenic and the pesticides such as hexachlorobenzene, quintozene and lindane. The last three are probable human carcinogens and have been banned from use on food crops throughout the world (Ku *et al.,* 1994). In another study by ConsumerLab.com, (2000) White Plains, New York, out of 22 brands of ginseng dietary supplements evaluated, eight were found to contain high levels of specific pesticides, some of which also contained toxic levels of lead. In addition, three of the eight contaminated products did not meet standards for their ginseng content, as did five other products.

1.7.4.3 Microbial Identification

Microorganisms in their living state are very difficult to visualize because they are minute, transparent and colorless when suspended in an aqueous medium. For studies and diagnostic purposes, staining, biochemical and biological methods together with light microscopy are major tools in microbial identification (Forles *et al*. 1994). Staining method explores the affinity of bacterial cells to absorb dyes or stains while biochemical and biological methods depend on biochemical characteristics of bacteria to grow on selected media.

Staining and Biochemical Methods

A stain or dye can be defined chemically as an organic compound containing a chromophore and auxochrome groups (Cappuccino and Sherman 2004). Most stains used for bacterial staining are cationic, thus on ionization, exhibit a strong affinity for the negative constituents of the cell. Staining techniques are divided into two; simple and

differential. Differential staining involves three or more chemical reagents applied sequentially to a heat fixed smear. The primary stain which is the first reagent imparts its color to all cells while the second decolorizes though it may or may not remove the primary stain based on the chemical composition of the cellular components. The final reagent called the counter stain has a contrasting color to that of the primary stain.

The gram stain is the most important type of differential stain in bacteriology which divides bacterial cells into two major groups based on their cell wall difference (Preston and Morrel 1962). Gram positive cells have a thick peptidoglycan layer while gram-negative ones have a much thinner one surrounded by outer lipid containing layers. In Gram staining, crystal violet is used as a primary stain and stains all cells purple. Gram's iodine is used as a mordant which is a substance that increases the affinity of bacterial cells for a stain thus they all appear purple-black at this point. Under 95% ethanol, Gram-negative bacteria cells will be decolorized to appear colourless while Gram-positive cells retain the purple colour. Safranine is a counter stain that lastly stains red Gram-negative cells while gram-positive cells retain the purple color of the primary stain.

Bacteria can also be identified through their biochemical characteristics that include enzymes- catalase, oxidase and decarboxylase; fermentation of sugars, capacity to digest or metabolize complex polymers and sensitivity to drugs. Identification methods that exploit bacterial biochemical characteristics include hydrogen sulphide production, indole test, phenylalanine deaminase test, nitrate reduction and methyl red test. The API 20 E system for biochemical identification of bacteria was studied and evaluated by Michael and Sharon (2002) who found out that 94.9 % of the microbes were correctly identified while 13 % were assigned unacceptable or low discrimination profile.

Differential and Selective Media

Selective media are used to isolate specific groups of bacteria by incorporating chemical substances that inhibit the growth of one type of bacteria while permitting the growth of another (Table 1.1). Differential media distinguish among morphological and biochemically related groups of microbes by incorporating chemical compounds that on inoculation and incubation produce a characteristic change in the appearance of bacteria growth or the medium surrounding the colonies thus permit differentiation. Differences may also show up as colony size, media colour, gas bubble formation and precipitate formation (Gracias and McKillip, 2004).

Table 1.1: Microbial growth on differential and selective media

S.NO	Name of the Media	Category	Target Microorganism
1	Eosin Methylene Blue	Differential	*E.coli, Enterobacter aerogenes*
2	MacConkey Agar	differential/ selective	*Gram negative enteric pathogens*
3	RCA	Selective	*Clostridium species*
4	Cetrimede Agar	Selective	*Pseudomonas species*
5	XLD Agar	Selective	*Salmonella*
6	Salmonella Shigella Agar	Selective	*Salmonella and Shigella*
7	Mannitol Salt Agar	Selective	*Staphylococci species*

Mannitol salt agar (MSA) contains a high concentration of salt, 7.5 % NaCl, which is inhibitory to the growth of most bacteria other than *Staphylococci.* It also performs a differential function by having a carbohydrate mannitol which some *Staphylococci* ferment, and phenol red, a pH indicator for detecting acid produced by mannitol

fermenting *Staphylococci* which exhibit a yellow zone surrounding their growth. Those that do not ferment mannitol will not produce color change (Sandel, 2004; Smyth and Kahlmeter, 2005).

1.8 Regulation of Herbal Medicines

The regulation of herbal medicines is characterized by large differences depending on the ethnological, medical and historical background of each country (Benzi and Ceci, 1997). The WHO *Guidelines for the Assessment of Herbal Remedies*, adopted by the International Conference of Drug Regulatory Authorities , contain the basic elements of legislation designed to assist those countries wishing to develop an appropriate legislation and registration procedure for herbal medicines used (WHO, 1991). So far, herbal medicines are regulated in United Kingdom (UK), China, India, Japan, France, Ghana and Germany.

The European guidelines for the assessment of herbal remedies follow the WHO's Guidelines for the Assessment of Herbal Remedies, which state that a substance's historical use is a valid way to document safety and efficacy in the absence of scientific evidence to the contrary. European drug regulations have made it easier to accord market authorization to natural medicines and process of registration takes less time and is cheaper. This is especially true of substances that have a long use history and can be approved under the "doctrine of reasonable certainty" (Benzi and Ceci, 1997). These products have been used since time immemorial by the respective communities hence can be assumed to be effective and safe in the form and dose so used.

In France, traditional medicines are approved by the French Pharmacopoeia Committee and licensed by the French Licensing Committee. The approval is highly dependent on ethno-pharmacology. In Germany the whole herbal product is considered as one active

ingredient which makes it simpler to define and approve the product. The German Federal Health Office regulates herbal products extracts so that potency and manufacturing processes are standardized. England generally follows the rule of prior use in relation to years of use with apparent positive effects and absence of detrimental side effects in lieu of other scientific data that the product is safe (Saito, 2000).

In Kenya, Pharmacy and Poisons Act, Cap. 244, Legal notice 192 of December, 24Th 2010 defines herbal medicines as " any medicinal substance derived from natural sources (plants, animals, soil, crude salts, minerals, vitamins) in their processed form and includes herbal preparations, ayurvedic medicines, nutraceuticals and homeopathic medicine" (Republic of Kenya, 2010). The Pharmacy and Poisons Board, in pursuant of the legal notice, has developed guidelines for registration (referred to as listing) of herbal medicines (PPB, 2010). This process of registration of herbal medicines does not follow the strict procedures followed for conventional medicines hence it is referred to as "listing". The guidelines conform to WHO guidelines for the assessment of herbal remedies and reflect the domestic level of development in the pharmaceutical sector.

Registration of conventional medicines by the Pharmacy and Poisons Board follows a procedure that is well documented and anchored in the Pharmacy and Poisons Act, Chapter, 244. In this process, an applicant is expected to provide a drug registration dossier for each drug. The dossier must contain the following information; name and address of applicant (manufacturer), nature of product to be registered (name, dosage and presentation), therapeutic classification, pharmaceutical formula of the product, names and structural formulae and specifications of active ingredients. To be also included in the dossier are specifications of raw materials used in the manufacturing process and analytical control procedures performed on active and non active materials before they

are used in the manufacturing process. In process quality control procedures and their frequencies are also documented. The dossier also contains analytical control procedures and specifications of the final product. Stability data, manufacturing method and pharmacological studies of the drug must also be availed. Other information required include, tests on physiological availability and particulars of clinical studies.

The manufacturing site must also conform to WHO current GMP requirements while the drug to be registered is subjected to laboratory quality control tests. The dossiers, GMP and laboratory reports of each product are then assessed by a team of experts, referred to as committees of drug registration (CDR), upon whose recommendations the PPB makes a decision on whether to register or decline registration of the drug. However, this process cannot be followed in the registration of herbal formulations. Name and address of manufacturer, nature of product to be registered; name, dosage and presentation and therapeutic classification are the only parameters considered for the purpose of listing of herbal medicines.

A total of 71 herbal products have been listed (Appendix 1) by PPB, 58 (81.7%) of which are imported from India. The countries of origin for the remaining 13 (18.3%) listed herbal products are distributed as shown in Figure 1.6. Over 80% of the listed herbal products originate from India because the Indian herbal products industry is highly advanced. There are more than 500,000 non-allopathic practitioners trained in more than 400 medical colleges of their respective systems of health and are registered with the official councils which monitor professionalism. The official non- allopathic systems of health include Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. In India more than 70% of the population uses herbal drugs for their health (Vaidya and Devasagayam, 2007). Indian Pharmacopoeia, 2010 has 89 monographs under Herbs and

Herbal Products, these monographs are on extracts, plant parts, tinctures, fixed oils and volatile oils.

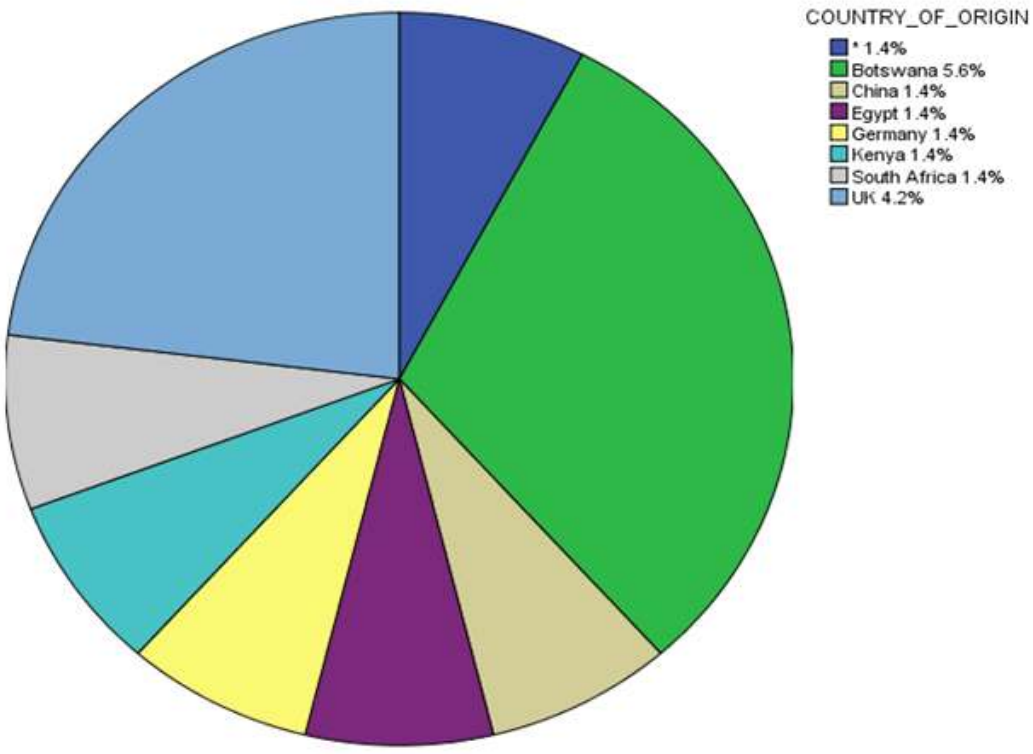


Figure 1.6: Listed herbal products in Kenya by country of origin

Historically India and Kenya have had close commercial ties in the field of pharmaceuticals. In a study carried out by the Pharmacy and Poisons Board in 2006, it was established that 85% of pharmaceuticals used in Kenya were imported. Of the total imports, India contributed 68% (PPB, 2006).

Out of the 71 listed products the number of active ingredients per product were distributed as shown in Figure 1.7 with 25 products each having more than 26 active

ingredients. This clearly illustrates the difficulty of analysis of active ingredients in finished herbal products.

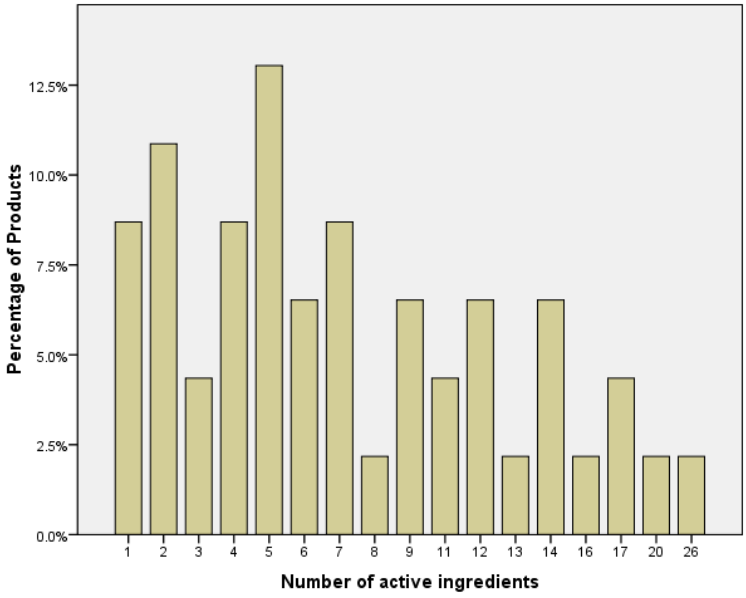


Figure 1.7: Active ingredients per listed herbal product

Therapeutic claims for the listed products were as follows: ten (14.1%) for bronco-dilatation, nine (12.7%) for anti-inflammatory activity, seven (9.9%) for adaptogenic and anti-cough properties, six (8.5%) as aphrodisiac and four (5.6%) for haematinic and anti-fungal actions respectively (Figure 1.8). There were very few products for diseases endemic in Kenya like malaria, tuberculosis and HIV/AIDS. Manufacturers of these products did not make many therapeutic claims on the labels or inserts.

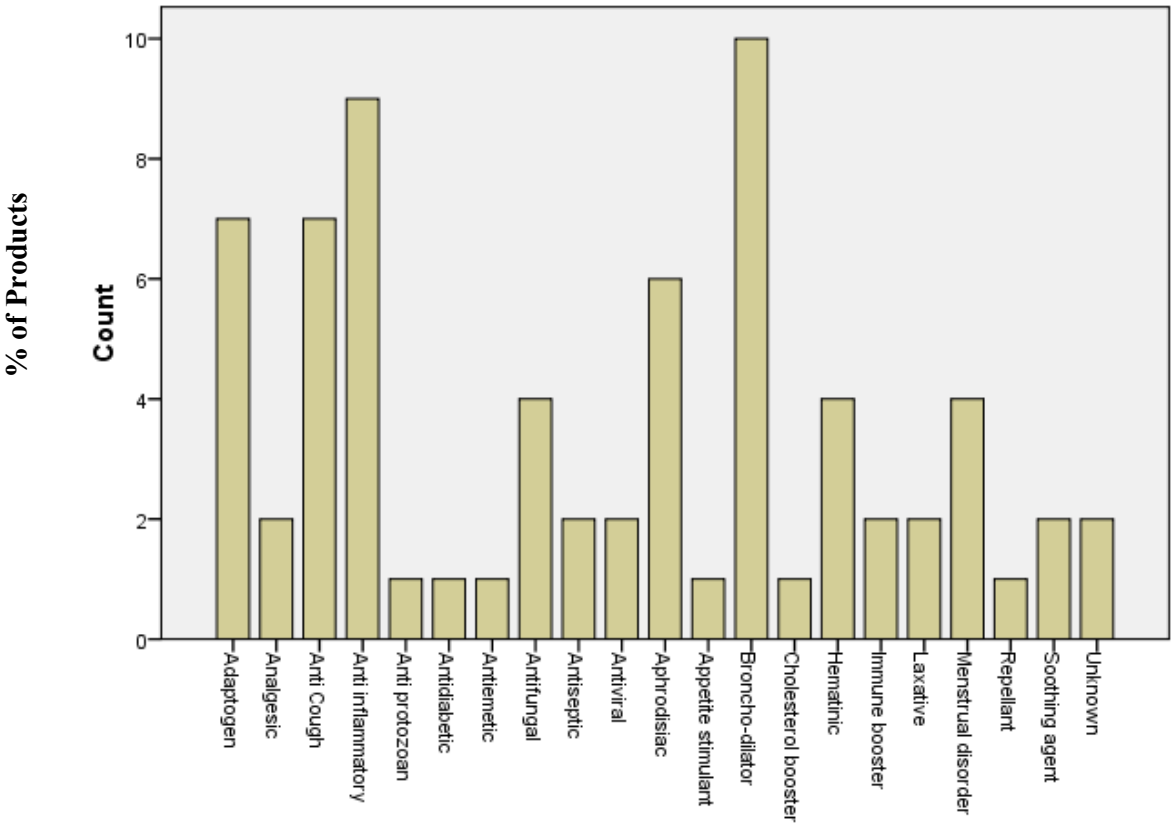


Figure 1.8: Therapeutic classification of listed herbal products

None of the listed products contained ginseng as one of the active ingredients, however, seven ginseng containing products were registered under Drug Registration Rules that apply to conventional medicines (Table 1.2). All the listed products were therapeutically classified as multivitamins and mineral supplements and each had 14 to 34 active ingredients. In spite of ginseng being one of the active ingredients, it was noted that manufacturers ignored its pharmacological effects and classified these products as multivitamins and mineral supplements.

Table 1.2: Registered ginseng containing products in Kenya

Name	Active ingredients	Country of origin	Number of active Ingredients
Ginsavit capsules	Ginseng extract 40mg + vitamins and mineral supplements	United Arab Emirates	22
Revigin capsules	Ginseng extract 42.5mg + vitamins and mineral supplements	India	19
Neovita Fortified capsules	Ginseng extract 100mg + vitamins and mineral supplements	United Kingdom	16
G-R Six capsules	Ginseng extract 40mg + Oils and Yeast	Egypt	
Ginsomine capsules	Ginseng extract 50mg + vitamins and mineral supplements	Thailand	21
Megavir capsules	Ginseng extract 3.6% + vitamins, mineral and herbal supplements	Italy	14
Vitamax capsules	Ginseng extract 40mg + vitamins and mineral supplements	Egypt	34

Pharmacy and Poisons Board, 2011

1.9 Study Justification

Herbal medicines play an important role in primary healthcare, especially for people living in developing countries. Although herbal medicines are used extensively in Kenya, they have not been investigated and their quality cannot be guaranteed. Furthermore, there are no national standards and necessary enabling policy and legislation governing their use. There is concern, therefore, among medical professionals and the general public that these products may be adulterated, are of questionable potency and that they may be toxic (Harkey *et al.,* 2001).

The medicines regulatory authority, the Pharmacy and Poisons Board, lacks capacity to evaluate applications for registration of herbal medicines both in terms of personnel with necessary skills and laboratory to ascertain quality. Applications for registration of herbal medicines, therefore, follow few steps including visual examination of commercial samples for labeling details and consideration of general information like ethno pharmacological activities, name and address of applicant (manufacturer), nature of product to be registered (name, dosage and presentation) and therapeutic classification.

Ginseng is one of the herbs that is exploited immensely for its varied pharmacological activities. Commercial products of ginseng are very expensive but readily available in the Kenyan market. Since these products are not subjected to rigorous tests that apply to conventional medicines, chances of poor quality products finding their way in the market are higher. The present study, therefore, will investigate the quality and use of ginseng containing products found in the Kenyan market and will serve as a baseline study for further research work.

This study is significant and unique because it relates to the crucial problem of quality of herbal medicines. The findings of this study are invaluable in so far as they will help the Pharmacy and Poisons Board to strengthen the regulatory structure for quality herbal medicines in Kenya. In addition, the results will be instrumental in activating the Ministry of Health to equip National Quality Control Laboratory so as to be used for assurance of quality of herbal medicines. The study will act as a catalyst for research scientists to come up with national standards for herbal products and for research institutions to train more of their personnel in quality assurance of herbal medicines. It is hoped that the findings of this study will provoke training institutions to review their

pharmacy curricula to include more content on botanical dietary supplements or herbal medicines.

1.10 Objectives of the Study

1.10.1 General Objectives

The main objective of the study was to evaluate the quality and use of ginseng containing products commercially available in Nairobi, Kenya.

1.10.2 Specific Objectives

1. To investigate the distribution of ginseng containing products in Nairobi, Kenya.
2. To assess the knowledge, about ginseng, of persons selling the ginseng containing products in the retail outlets.
3. To assess the sampled ginseng containing products for compliance to WHO labeling requirements.
4. To determine the degree of microbial contamination of the samples obtained.
5. To use densitometric method to carry out identification of ginseng in the sampled products.

CHAPTER TWO

METHODOLOGY

2.1 Introduction

The methodology for this study was divided into three sections, namely: market survey and sampling; determination of microbial contamination and profiling of ginsenosides in the samples. Market survey and sampling included study site, determination of study population, study design and administration of questionnaires. The sample size was determined according to study design while suitable questionnaires were used for the assessment of knowledge of attendants found selling ginseng containing products in the retail outlets visited. Microbial load determination covered the following areas; average total aerobic microbial count (Av. TAMC), positive microbial identification for bacteria and average total combined yeast and mould count (Av. TYMC) for fungi. Ginseng was identified by profiling ginsenosides using TLC and densitometry. Standard ginseng extract and reference standards for ginsenosides Rb1 and Rg1 were used to identify ginseng in the samples.

2.2 Market Survey and Sampling

2.2.1 Study Site

Commercial products labeled as containing ginseng were obtained through a structured systematic random sampling technique and were purchased from retail pharmacies and distribution outlets in Nairobi County, Kenya. Nairobi County is divided into nine (9) districts namely Westlands, Starehe, Langata, Madaraka, Embakasi, Kasarani, Dagoreti, Kamukunji and Njiru. These districts were used as sampling blocks. Nairobi has the highest number of registered pharmacies and herbal stores in Kenya. Therefore, the samples from these stores were deemed to be representative of ginseng products in the

Kenyan market {Ministry of Medical Services and Ministry of Public Health and Sanitation, 2010 and Pharmacy and Poisons Board, 2009(b)}. This study was carried out from January 2009 to August 2010.

2.2.2 Study Population Size or Sampling Frame

There were 339 pharmacies, 18 supermarkets and 14 nutritional stores stocking ginseng containing products in Nairobi County. These premises were distributed amongst the nine Districts surveyed as shown in Table 2.1

Table 2.1: Retail outlets stocking ginseng per district

District	Pharmacies	Supermarkets	Nutritional Stores	Total
Westlands	85	4	7	96
Starehe	87	8	3	98
Makadara	52	1	1	54
Langata	48	4	2	54
Kasarani	32	0	0	32
Dagoreti	1	1	1	3
Kamukunji	19	0	0	19
Embakasi	15	0	0	15
Njiru	0	0	0	0
Total	**339**	**18**	**14**	**371**

The population size of pharmacies was based on the data obtained from the Pharmacy and Poisons Board and analyzed based on the assumption that ginseng containing products were not stocked in un-registered pharmacies and low income neighbourhoods. This assumption was tested through a pilot study where five unregistered premises from each of the 4 slum areas in Nairobi namely, Kawangware, Kibera, Mathare and Zimmerman,

were surveyed for the presence of ginseng containing products. All the twenty premises sampled did not stock ginseng containing products.

2.2.3 Study Design

2.2.3.1 Sample Size Determination

The number or sample size of premises selected from each category (j) of outlets per district (i) was determined by the following formulae:

$$\sqrt{\mathbf{N_{ij}}} + \mathbf{1}$$

Where N was the number of outlets if they were between 4 and 300 but if the outlets were equal to 1, 2 or 3, all of them were sampled (Torbeck, 2009 and Saranadasa, 2003). There were 76 premises visited, 76 attendants interviewed and 40 products sampled.

2.2.3.2 Sampling Procedure

A wide variety of registered and un-registered ginseng containing products were found in many outlets. The outlets were categorized into three broad classes namely registered pharmaceutical outlets, herbal/nutritional shops and supermarkets. Distribution of sampled outlets was as shown in Table 2.2.

Outlets stocking ginseng containing products were selected using the systematic random sampling technique as follows: every n_{ij}^{th} (N_{ij}/n_{ij}) outlet as shown by the distribution matrix was selected based on the district (i) and the type of premises (j). Where N_{ij} =1, 2 or 3, all the outlets were sampled. The samples obtained were in different formulations including powders, hard and soft gelatin capsules and liquids. The formulations comprised of several active ingredients such as one ginseng extract, many ginseng extracts from multiple species of ginseng plants and ginseng extract(s) with multivitamins, minerals and other herbs.

Table 2.2: Sampled ginseng retail outlets per district

District	Pharmacies	Supermarkets	Nutritional Stores	Total
Westlands	10	3	4	17
Starehe	10	4	3	17
Makadara	9	1	1	11
Langata	7	4	2	13
Kasarani	6	0	0	6
Dagoreti	1	1	1	3
Kamukunji	5	0	0	5
Embakasi	4	0	0	4
Njiru	0	0	0	0
Total	**52**	**15**	**12**	**76**

A total of forty samples (40) were collected and subjected to evaluation and quality analysis during this study (Appendix 4). Out of the 40 samples, 27 (68.3%) were soft gel capsules followed by hard capsules 9 ((22.0%), tinctures 2 (4.9%), elixirs 1 (2.4%) and powder sachets 1 (2.4 %).

2.2.4 Administration of Questionnaires

Details about the premises, sampled products and attendants selling them were collected on a questionnaire administered at the time of sampling (Appendix 2). The details about the premises included the name of the premises, nature of business as well as the physical and postal address. The product details captured included the brand name, active ingredients, manufacturer, batch number and the expiry date. The educational background of the respondent and conditions for which he/she recommended the product were also entered. In addition, product features such as dosage form, colour, contents of active ingredients and labeling on the primary packets of the samples were also recorded. All

the information was obtained from questionnaires administered to the attendants selling ginseng containing commercial products in the outlets and by observing the samples.

Where there was more than one attendant in the nutritional store or supermarket, the one responsible for the section of ginseng containing products was interviewed. In the case of pharmacies, only one enrolled pharmaceutical technologist or registered pharmacist present was interviewed.

2.2.5 Knowledge Assessment of the Store Attendants

Assessment of store attendants for their knowledge about properties of ginseng was based on their scores from the questions that referred to schedule or classification of ginseng containing products, conditions for which they were prescribed and side effects (Table 2.3).

Table 2.3: **Knowledge assessment of attendants**

Category	**Score criteria**	**Maximum score**
Schedule/Classification	'2' scores for indicating 'General sales' or 'for over the counter	2
Pharmacological Effects	'1' score for each correct condition×5	5
Side Effects	'1' score' for each correct response×3	3
Total		**10**

Adding up all the attained scores in the three knowledge aspects generated the overall knowledge score. The maximum attainable overall knowledge score was 10. A mean of the percentage score was used to determine those with adequate knowledge. Anyone attaining the percentage score equal to the mean or more was considered to have adequate knowledge.

2.3 Quality Control Tests

The 40 samples in this study were subjected to three quality control tests namely: uniformity of weight, microbial load determination and identification of ginseng. Weight uniformtiy tests were applicable to the samples that were formulated as capsules and tablets. Microbial load determination was carried out on all samples. For the purpose of this study, the following microbial contamination tests were performed: average total aerobic microbial count (AV.TAMC) for bacteria, identification of aerobic bacteria that were not supposed to be present in an oral preparation, average total combined yeast and mould count (AV. TYMC) for fungi and average total viable aerobic microbial count, which was the sum of AV.TAMC and AV.TYMC. Identification of ginseng was carried out using TLC and densitometry and this only applied to tablet and capsule samples,

2.3.1 Equipments

A Mettler Toledo XP504 analytical balance (Mettler Toledo Inc. Polaris Parkway Colombus, USA) was used for weighing samples. Ultra-violet absorption of cultures in broth nutrient media was measured using spectronic 20D+ colorimeter Spectrophotometer (Cole-Parmer Instrument Company, Vernon hills, Illinois USA). Volumes of samples were measured by use of 200-1000 µl Pipetman micropipettes (Gilson inc. Middletown, USA) fitted with sterile tips

An LG refrigerator (Digitalmax Co. Ltd, Uiwang-city, South Korea) was used for storage of culture stocks and isolates at -20 °C. An All American 25 X-2 steam autoclave (Alfa Medica Co., Hempstead New York, USA) was used for sterilization. A WTB Binder incubator (Wolf Laboratories Ltd. Pocklington, U.K) was used for incubation of bacteria and fungi.

A Leica BME 13395 H2X light microscope (SEO Enterprises Inc. Lakeland Florida, USA) was used for examining stained microorganisms. Isolation, inoculation and transfer of culture samples was done in a biosafety cabinet (The Baker Company, Sanford, USA). Reflux apparatus and bath sonicator (Daihan Labotec Co. Ltd., Namyangju, Korea) were used for extraction of ginsenosides from samples and the standard ginseng extract. A Laborata 400 Rotary Evaporator (Heidolph, Schwabath, Germany) was used to reduce extracts. Silica gel Si60G glass plates (Merk, Darmastdt, Germany) were used for Thin Layer Chromatography of ginsenosides. The TLC plates were scanned using a Shimadzu Cs-9000 densitometer (Shimadzu Corp., Kyoto, Japan).

2.3.3 Materials and Reagents

All the nutrient media used in this study were standard commercial powders manufactured by Himedia Laboratories Pvt, Mumbai, India. Nutrient Agar, Nutrient Broth, MacConkey Agar, Eosin Methylene Blue (EMB) Agar, Salmonella Shigella Agar (SSA), Xylose-Lysine Deoxycholate Agar (XLD) and Mannitol Salt Agar were used for bacterial growth. Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) were used for growth of yeasts and moulds.

MacConkey Agar was used as a differential and selective nutrient media for growth of *Klebsiella pneumonia* while Eosin Methylene Blue (EMB) agar was used as a differential nutrient media for growth of *Enterobacter aerogenes* and *Escherichia coli*. Salmonella

Shigella Agar (SSA) and Xylose-Lysine Deoxycholate Agar (XLD) were used for selective growth of *Salmonella shigella* while Mannitol Salt Agar was used for selective isolation of pathogenic *Staphylococcus aureus.* Buffered Peptone Water was used as diluents for ginseng samples before they were filtered through membrane filters supported on membrane filter holders.

All the nutrient media were tested for viability using the standard micro-organisms from National Collection of Type Cultures (NCTC), London, UK. They were; *Bacillus pumilis*, NCTC 07447, *Staphylococcus aureus*, NCTC 8241, *Pseudomonas aeruginosa*, NCTC 12924, *Saccharomyces cerevisiae*, NCTC 10716, *Candida albicans*, NCPF 3179 and *Aspergillus niger*, NCPF 2275.

The reagents used for Gram staining of bacteria were; Methylene blue alkaline solution or Loffler's reagent (Fine Chemicals Ltd, New Delhi, India) and Safranin, Malachite green powder and Gram's iodine, all from Rankem (RFCL) limited, New Delhi, India. An API 20 E – Biomerieux system (Biomerieux, I'Etoile, France) was used to carry out biochemical tests for bacterial identification.

HPLC grade methanol (Merk, Darmstadt, Germany) was used in the extraction of samples. Other solvents used included chloroform and Ethyl Acetate (Lab. Tech. Chemicals, Mumbai, India), analytical grade absolute ethanol and hexane (Rankem (RFCL) Ltd, New Delhi, India).

Anisaldehyde reagent was used for spraying the TLC plates before densitometric analysis. Anisaldehyde reagent consisted of p-anisaldehyde (Riedeide, Haen AG, Germany), glacial acetic acid (Scheriau Chemie S.A, Sentmenat, Spain) and sulfuric acid (Thomas Baker Chemicals, Mumbai, India).

Standard *Radix ginseng* extract was a donation from Glaxo Smith Kline Beecham, Cairo, Egypt while ginsenoside Rb1 and Rg1 (CAS No.41753-43-9 and 110703-200726) were from Kunming Pharmaceutical Corporation, Yunnan, China.

2.4 Methods

2.4.1 Uniformity of Weight

Thirty capsule and five tablet samples were analyzed for uniformity of weight using the methods described in British Pharmacopoeia (2008). The limits of uniformity of weight for tablets and capsules given in the BP (2008) were: Not more than 2 of individual tablet/capsule masses or weights deviated from the average mass by more than 10% and none deviated by more than 20%.

From each tablet sample, twenty tablets were taken at random, weighed individually and average weight determined. For hard and soft gelatin capsules, an intact capsule was weighed and then opened without losing any part of the shell and the contents were removed as completely as possible. Soft shell capsules were washed with methanol 70% and allowed to dry until smell of methanol was no longer perceptible. The dry shell was weighed. The mass of the content in the capsule was the difference between the weight of the intact capsule and the dry empty shell.

2.4.2 Determination of Microbial Load

2.4.2.1 Preparation of Nutrient Media

A specific nutrient medium was weighed using a Mettler Toledo XP504 analytical balance and added to one liter of distilled water in a conical flask and heated to dissolve. The dissolved nutrient media was dispensed into 90 ml aliquots in culture media bottles and sterilized by autoclaving at 15 lbs pressure and 121 °C for 15 minutes. The nutrient media aliquots were cooled to about 50°C in a water bath and mixed well before being poured into sterile petri dishes. The nutrient media that were prepared following this

method included: Nutrient Agar, Nutrient Broth, Sabouraud Dextrose Agar, Sabouraud Dextrose Broth, Buffered Peptone Water, MacConkey Agar, EMB Agar and Mannitol Salt Agar. However, Salmonella Shigella Agar (SSA) and Xylose-Lysine Deoxycholate Agar (XLD) were not autoclaved since doing so would cause overheating that would destroy their selectivity. The amount of nutrient media powder dissolved in l000 ml of distilled water to prepare each nutrient medium for bacterial and fungal growth were as shown in Table 2.4. All the media prepared were tested for viability using standard reference micro-organisms.

Table 2.4: Quantity of nutrient media powder per 1000 ml of distilled water

Nutrient Media	**Amount (g) added to 1 litre of distilled water**
Nutrient Agar	28.0
Nutrient Broth	13.0
Sabouraud Dextrose Agar	65.0
Sabouraud Dextrose Broth	30.0
Buffered Peptone Water	20.0
MacConkey Agar,	51.5
EMB Agar	37.5
Mannitol Salt Agar	111.0
Salmonella Shigella Agar (SSA)	63.0
Xylose-Lysine Deoxycholate Agar (XLD)	56.6

2.4.2.2 Sample Preparation

Ginseng samples were found to be fatty soluble. Solutions of these samples were prepared as follows: 10 g or 10 ml or 10 capsules/tablets of the sample were homogenized with 5 ml of tween 80 polysorbate and warmed to 40 °C in a water bath. Using Buffered Peptone

Water, the volume of the homogenous mixture was made to 100 ml. This mixture was stirred at 40ºC for 15 minutes then passed through a Cellulose Acetate membrane filter of pore size 0.45µm mounted on a membrane filter holder. For each sample the procedure was done in duplicate yielding two 'contaminated' membrane filters that were used for bacterial and fungal growth respectively.

2.4.2.3 Inoculation and Microbial Count

All samples were analyzed for microbial contamination using membrane filtration method. About 25 ml of molten Nutrient Agar and Sabouraud's Dextrose Agar were added to different 9 cm diameter Petri-dishes at 45 °C and allowed to solidify. The petri dishes with solidified Nutrient Agar and Sabouraud's Dextrose Agar media were used to grow bacteria and fungi respectively. For each sample, the two 'contaminated' membrane filters were placed over the surfaces of the solidified Nutrient Agar media and Sabouraud's Dextrose Agar media. The plates were then incubated in an inverted position at 33 °C for bacteria and 23 °C for fungi respectively. The results were read after 48 hours for bacteria and 72 hours for fungi (Tortora *et al*., 2008). A mechanical colony counter was used to count the number of colonies. Only petri dishes that yielded between 30 and 300 colonies were evaluated. Plates with more than 300 colonies were designated as too many to count (TMTC), while those below 30 were designated as too few to count (TFTC) (Sutton, 2006).

The microbial load per ml/g/tablet/capsule was calculated by multiplying the number of cfu by the reciprocal of the dilution factor divided by the amount of inoculum which was usually 1ml (Cappuccino and Sherman, 2004). The limits were further interpreted as recommended in the British Pharmacopeia, 2010.

2.4.2.4 Isolation and Storage of Pure Cultures

Based on morphologic differences, colonies were isolated from their axenic cultures whereby form, elevation, pigmentation and size were the major distinguishing characteristics. Petri-dishes containing solidified Nutrient Agar media were divided into quadrants and sub-culturing done by the streak plate technique. The plates were then incubated in an inverted position at 33 °C and bacterial results were read after 48 hours.

The colony cultures from each quadrant on the petri-dish were transferred to the nutrient broth media using an iron loop. The colonies isolated to nutrient broth were incubated in a shaking water bath overnight and the absorbance of the broth was read using a spectrophotometer at 600 nm. They were ready for harvesting and storage once an absorbance value of 0.06 was attained. Using a sterile pipette, 30µl broth of each isolate was pipetted to 1.5ml Eppendorf tubes to which 70µl glycerol was added. Each isolate was stocked in duplicate and assigned a 3 letter code derived from the sample they were isolated and a number given in the order of isolation. The isolation date was also labeled and the temperature at which storage was done. All the isolates were stored in a deep freezer maintained at -23 °C. The stored isolates were thawed and recovered for use in the subsequent identification tests. Viability of the stored isolates was confirmed after every one month by way of streaking fungal and bacterial isolates to SDA and NA respectively and incubated under conditions earlier stated. Growth in the agar for each isolate confirmed viability.

2.4.2.5 Microbial Identification by Staining and Microscopy

Colony morphology was the major identification criteria for both bacteria and fungi. The bacterial and fungal colony morphology characteristics were as shown in Table 2.5.

Table 2.5: Colony characteristics of bacteria and fungi

Micro-organism	Colony Characteristics				
	Form	**Elavation**	**Pigmentation**	**Size**	**Texture**
Bacteria	Circular, irregular, spreading	Flat, slightly raised, markedly raised	Red, White, Pink, Colourless	Pinpoint, small, medium	Smooth, serrated, irregular
Fungi	Circular, Branched filaments, multinucleated hyaline, hyphae, sporangium and spores	Slightly raised, markedly raised	Dark, white, colourless	Small, medium, large	Moist, smooth,

For Gram staining and microscopy, a clean microscope slide was labeled with the code of the unknown organism and one loop full of its culture was applied on the slide using an inoculating loop. The smear was then heat fixed on the slide by passing it over a Bunsen flame.

The slide with the bacterial smear was held at the edge using a cloth peg over a staining basin. It was then flooded with crystal violet for 1 minute. The stain was washed off with tap water before applying Gram's iodine for one minute. The Gram's iodine was washed off with tap water before adding 95 % ethyl alcohol drop by drop until the alcohol was clear. The smear was counterstained with Safranin for about 45 seconds before it was gently washed off with tap water. The smear was subsequently blow-dried before viewing it under a microscope on the oil immersion objective lens at magnification of 45× for fungi and 100× for bacteria (KEMRI, 2003).

2.4.2. 6 Identification of Specific Bacteria by Differential and Selective Media

Staphylococcus aureus

A pure culture from Nutrient agar was sub-cultured on a plate of mannitol salt agar freshly prepared as per the manufacturer's instructions. It was incubated at 33 °C for 72 hours. Growth of white colonies surrounded by yellow zones indicated the presence of *Staphylococcus aureus* (Sandel, 2004; Smyth and Kahlmeter, 2005).

Escherichia coli

A pure culture from Nutrient agar was inoculated into Eosin Methylene Blue agar. The innoculum was incubated at 33 °C for 72 hours. Colonies with dark centers and greenish metallic sheen indicated the presence of lactose fermenters (Leininger *et al.,* 2001).

Salmonella species

A pure culture from Nutrient agar was inoculated into Salmonella Shigella agar. It was incubated at 33 °C for 48 hours. Growth of well developed red colonies with black centers was considered to be the presence of *Salmonella* spp (Health Protection Agency, 2008).

Klebsiella pneumoniae

A pure culture from nutrient agar was inoculated into MacConkey agar. It was incubated at 33 °C for 72 hours. Growth of round mucoid colonies with colorless edges was considered to be the presence of *Klebsiella pneumonia* (Bruce *et al.,* 1981).

Enterobacter aerogenes

A pure culture from nutrient agar was inoculated into Eosin Methylene Blue. It was incubated at 33 °C for 72 hours. Growth of pinkish large mucoid colonies was considered to be the presence of *Enterobacter aerogenes* (Betancourt *et al.,* 2008).

Shigella species

A pure culture from nutrient agar was inoculated into *Shigella Salmonella* agar. It was incubated at 33 °C for 72 h. Growth of mucoid colonies with black centers was considered to be the presence of *Shigella* species (Maddocks *et al.*, 2002).

All bacterial isolates; *Staphylococcus aureus, Escherichia coli, Salmonella* species *Klebsiella pneumoniae , Enterobacter aerogenes* and *Shigella species* were further subjected to gram staining and biochemical tests for confirmation.

Candida species

A pure culture isolated in Sabouraud's Dextrose broth was inoculated into Sabouraud's dextrose Agar. It was incubated at 25 °C for 5 days. Growth of small round moist, white to colorless colonies with even edges was considered to be the presence of *Candida spp*. The colonies were further subjected to microscopic tests for confirmation (Shaheen and Taha, 2006).

2.4.2.7 Bacteria Identification by API 20 E

The API 20 E strip was prepared by spraying 1 ml of distilled water into each honeycombed wells of the tray to create a humid atmosphere. The isolate code was labeled on the elongated flap of the tray. The strip was then removed from its packaging and placed in the incubation box. The inoculum was prepared by using a test tube containing 5 ml of 0.85 % sodium chloride. An isolated colony of 18 hours old from one of the honeycombed wells was added to the 5ml test tube using a pipette and carefully emulsified to achieve a homogenous bacterial suspension which was used immediately after preparation as recommended by the manufacturer.

Using a pipette, both tube and cupule of the tests Trisodium Citrate (CIT), Sodium pyruvate (VP) and Gelatin (GEL) were filled with the bacterial suspension while only the

tube was filled with bacteria for other tests. Anaerobic conditions were created in the tests Arginine Dihydrolase (ADH), Lysine Decarboxylase (LDC), Ornithine Decarboxylase (ODC), Sodium Thiosulphate (H_2S) and UREA by overlaying with mineral oil. The incubation box was closed and incubated at 37 °C for 24 hours. At the end of the incubation period, the strip was read by referring to color changes described in the literature or Appendix 3.

Some tests which required addition of specific reagents were also done. The Tryptophane Deaminase (TDA) test was done by adding 1 drop of TDA reagent then colour changes observed. The Indole production test (L-tryptophane) (IND) test was carried out by adding 1 drop of JAMES reagent then observing colour changes while the VP test was performed by adding 1 drop of VP 1 and VP 2 reagents and allowing the reaction to proceed for 10 minutes before observations were recorded.

The oxidase test was carried out by using oxidase discs (Himedia laboratories, Mumbai, India) to detect oxidase production by microorganisms. This was done by touching and spreading a well-isolated colony on oxidase disks then reaction observed within 10 seconds at 25 °C. Change in colour was observed and if none after 60 seconds the test was considered negative.

Identification was obtained with a numerical profile which was interpreted by use of a result sheet that was supplied with the API 20 E kit. In the sheet, the tests are separated into groups of 3 and a value 1, 2 or 4 was indicated for each. By adding together the values corresponding to positive reactions within each group, a 7-digit profile number was obtained for the 20 tests. The oxidase reaction constituted the 21st test and had a value of 4 if positive. Identification was performed with the analytical profile index by looking up at the numerical profile in the list of profile (Appendix 3 - KEMRI CMR).

2.4.3 TLC Analysis of Ginseng Samples and Standards

2.4.3.1 Tablets

There were five samples formulated as tablets. Twenty tablets of each sample were crushed to a fine powder from which powder equivalent to about 100 mg of ginseng extract was dissolved in 20 ml portion of mixture of methanol-distilled water (8:2). This mixture was extracted for 30 minutes in a bath sonicator thermostated at 55ºC. The extraction process was done three times each with 20 ml portion of mixture of methanol and distilled water (8:2). The combined extract was evaporated to dryness under vacuum at 55º C and the residue was dissolved in a 5 ml methanol. There were two 5 ml preparations for each sample.

2.4.3.2 Hard gelatin capsules

Eight samples were extracted using this method. Contents of twenty hard gelatin capsules were crushed to a powder. Powder equivalent to about 100 mg ginseng extract was put in a conical flask and extracted with 3× 20 ml portion of a mixture of methanol and water(4:1) for 30 minutes in a bath sonicator thermostated at 55ºC. The combined extract was evaporated to dryness under vacuum at 55º C and the residue was dissolved in 5 ml of methanol.

2.4.3.3 Soft gelatin capsules

Twenty two samples were extracted under this method. For each sample, the contents of 20 soft gel capsules were stirred to a homogenous mixture. An accurately weighed portion equivalent to about 100 mg of total ginseng extract from a homogenized mixture was dissolved in 30 ml mixture of hexane-methanol-water (20:15:10) contained in a separating funnel (USP, 2008). The lower layer of the mixture was collected while the upper layer was washed with three-15 ml mixture of methanol and water (3:2). The

washings were combined with the lower layer evaporated to dryness under vacuum at 50ºC. The residue was dissolved in 5 ml methanol.

2.4.3.4 Powdered Asian ginseng

One product in this category was analyzed for identification of ginseng. A gram of powdered ginseng root was transferred to a 25 ml flask fitted with a reflux condenser to which 10 ml of a mixture of methanol-water (7:3) were added. This was heated under reflux for 15 minutes. The resultant mixture was cooled, filtered and diluted with methanol to 10 ml.

2.4.3.5 Radix ginseng extract

A gram of *Radix ginseng* extract powder was added to 10 ml of a mixture of methanol and water (7:3) and heated under reflux for 15 minutes. The mixture was filtered and the filtrate was dissolved in 10 ml of methanol.

2.4.3.6 Reference Ginsenoside Rb1 and Rg1

Solutions of Rb1 and Rg1 standard substance were prepared in methanol at a concentration of 400 µg/ml.

2.4.3.7 TLC Analysis of ginseng samples

Thin layer chromatographic separation of ginsenosides was performed on analytical pre-coated silica gel Si 60G glass plates using as mobile phase: chloroform- methanol-ethylacetate- water-hexane (20:22:60:8:4 v/v) as described by Ludwiczuki *et al*.,(2006). The mobile phase was allowed to migrate to a distance of 150mm and after development, the plates were dried and sprayed with *p*-Anisaldehyde reagent and heated at 106ºC for 10 minutes (USP, 2007). Qualitative densitometric evaluation was performed at wavelength of 366 nm using a Cs-9000 Shimadzu densitometer.

The R_f values of the ginsenosides were calculated and R_x values were determined relative to standard ginsenosides Rb1 and Rg1. The methods adopted for identification of

protopanaxadiols and the protopanaxatriols were as contained in USP, 2008. Accurate measures of 20 µl of samples, standard extract of radix ginseng root powder and reference standard ginsenosides Rb1 and Rg1 were spotted.

2.5 Data Analysis

Quantitative data from the field and the laboratory was coded and double entered into a computer database designed using MS-Access version 2010 application. Exploratory data techniques were used at the initial stage of analysis to uncover the structure of data and identify outliers or unusually entered values. Data cleaning and validation was performed in order to achieve a clean dataset that was then exported into a Statistical Package format (SPSS). Back up files were stored in CDs and/or flash discs, this was done regularly to avoid any loss or tampering. All the questionnaires were secured for confidentiality.

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) statistical software (Nie, Bent and Hull; New York, USA). Descriptive statistics such as proportions were used to summarize categorical variables while measures of central tendency were used for continuous variables. Graphs and summary tables were used in data presentation. Pearson chi-square was used to test for the association between categorical variables. Odds ratio (OR) with corresponding 95% confidence interval were used to test for the strength of the association.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Results

This chapter organizes, presents and explains the data collected and laboratory findings established in this study. Data on quality of ginseng containing products was obtained from an evaluation of 40 samples and assessment of 76 store attendants from nine Districts of Nairobi County, Kenya. This chapter focuses on the assessment of attendants for their knowledge about ginseng and evaluation of the samples for compliance to WHO labeling requirements for market authorization. Furthermore, the samples were also assessed for their compliance to pharmacopoeial requirements including microbial contamination and identification of ginseng.

3.2 Assessment of Store Attendants' Knowledge

3.2.1 Sample Size of Store Attendants

Table 3.1 shows number of premises sampled per 8 Districts where ginseng stores were found.

Table 3.1: Premises sampled per district

Districts	No. of outlets	Percentage
Dagoreti	3	3.9
Embakasi	4	5.3
Kamukunji	5	6.6
Kasarani	6	7.9
Langata	13	17.1
Makadara	11	14.5
Starehe	17	22.4
Westlands	17	22.4
Total	**76**	**100**

Seventy six (76) premises were sampled from eight (8) districts of Nairobi as follows: from Westlands, 17 (22.4%), Starehe 17 (22.4%), Langata 13 (17.1%), Makadara 11 (14.5%), Kasarani 6 (7.9%), Kamukunji 5(6.6%), Embakasi 4 (5.3%) and Dagoreti 3 (3.9%). This is shown in Table 3.1. There were large numbers of premises stocking ginseng containing products in Westlands and Starehe Districts

Of the 76 stores stocking ginseng containing products, 14.5% were nutritional stores, 17.1% supermarkets and 68.4% pharmacies respectively (Table 3.2). A total of 76 store attendants, one from each premises, were assessed by analyzing the information about each one of them captured on the questionnaires.

Table 3.2: Distribution of stores by type

Type of store	**Number**	**%**
Nutritional stores	11	14.5
Pharmacies	52	68.4
Supermarkets	13	17.1
Total	76	100

Studies have shown that most people residing in Westlands and Starehe districts are medium and high income earners (Mitullah, 2003). In Kenya, the poverty index is 52.2% and income disparities are evident as 35% of the national income is controlled by 10% of the population while the masses live below the poverty line defined as Ksh. 2,200 a month (KNBS, 2007).

It appears that the traders wanted to capture the markets of the well to do social class residing in the two districts. Going by pricing index, it was noted that the cheapest product went for Ksh. 1200 (USD 15) and the highest Ksh.8, 300 (USD 100) for one

packet containing thirty dosage units. A supermarket chain had a store in low income location that did not stock ginseng containing products, while its up market stores stocked them.

3.2.2 Education Level of the Store Attendants

The education level of the store attendants was as illustrated in Figure 3.1. Out of 76 attendants that were interviewed, 48 (63.2%) had attained diploma education whereas 18 (23.7%) had university education. Ten attendants (13.2%) reported to have only attained secondary school education.

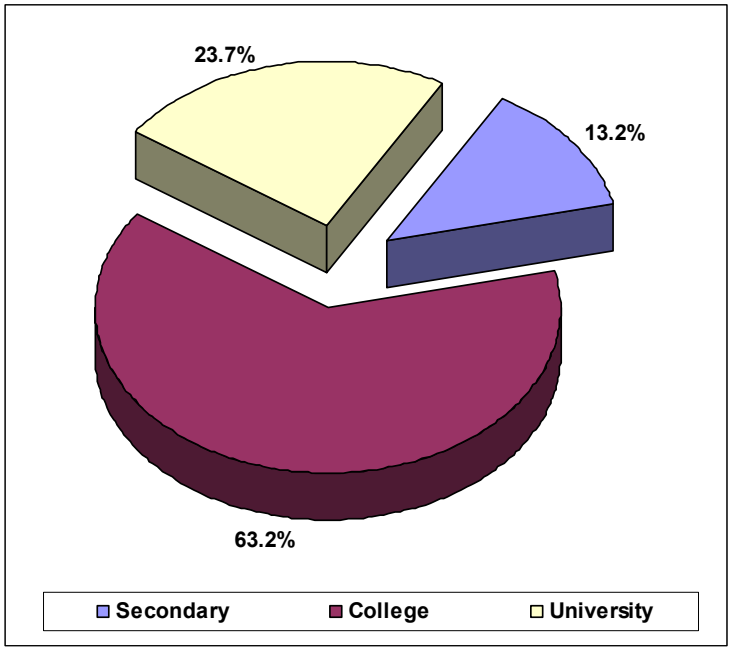


Figure 3.1: Highest level of education attained by the store attendants

Out of 76 attendants interviewed, 10 (13.2%) were registered pharmacists, 33 (43.4%) enrolled pharmaceutical technologists and 20 (26.3%) nutritionists. Other professions accounted for 3 (3.9%) while 10 (13.2%) of the attendants had no professional training (Figure 3.2). Of the 52 pharmacies, 39 were owned by pharmacists whereas 13 were owned by pharmaceutical technologists. A majority of pharmacies owned by pharmacists had one or more pharmaceutical technologists employed to dispense and attend to clients.

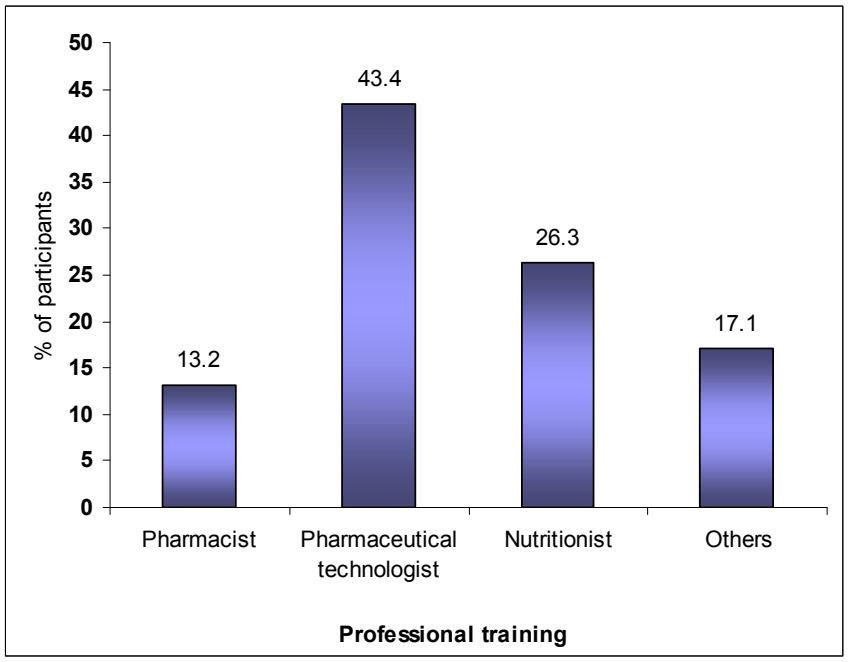


Figure 3.2: Distribution of attendants by professional training

3.2.3 Attendants' Knowledge of Ginseng products

Assessment of attendants on their knowledge about ginseng containing products was based on three aspects namely: classification, indications or pharmacological effects and side effects associated with ginseng products. Out of the 76 attendants 2 (2.6%) classified ginseng products as prescription only while the same number did not know where to classify the products they were selling. In addition, 25 (33%) classified them as over the counter only while a majority 40 (52.6%) classified them as general sales only. Combination classification was reported by 7 (9.2%) of the attendants out of which 1 (1.3%) classified them as over the counter as well as general sales and 6 (7.9%) as prescription, over the counter and general sales. The mean knowledge score on ginseng products was 3.2 ($\pm$ 1.8 SD), range from 0 - 8. Approximately one-third of the attendants (32.9%) had adequate knowledge.

3.2.4 Effects of Attendants Attributes on Knowledge about Ginseng

The relationship between knowledge on ginseng products and selected attributes of the store attendants was analyzed as presented in Tables 3.3**.**

Table 3.3: Effects of attendants attributes on knowledge about ginseng

Education Variables	Knowledge about ginseng							
	Adequate (n=25)		Inadequate (n=51)		Odds Ratio	95% Confidence Interval		
	N	%	N	%		Lower	Upper	P value
Highest level								0.823
University	7	38.9	11	61.1	1.48	0.28	7.74	
Diploma College	15	31.3	33	68.8	1.06	0.24	4.68	
Secondary	3	30.0	7	70.0	Reference			
Professional training								**<0.001**
Nutritionist	14	70.0	6	30.0	16.92	4.10	69.77	**<0.001**
Pharmacist	3	30.0	7	70.0	3.11	0.56	17.17	
Others	4	30.8	9	69.2	3.22	0.67	15.56	
Pharm. technologist	4	12.1	29	87.9	Reference			

Highest level of education attained was not significantly associated with knowledge on ginseng product (P=0.823). The proportion of attendants with adequate knowledge was lowest among those with secondary school education (30.0%) followed by diploma education (31.3%) and highest among those with university education (38.9%). An attendant with diploma education was 1.06 times more likely to have adequate knowledge on ginseng products compared to one with secondary school education. The likelihood was higher in those with university education (Odds ratio, OR=1.48)).

The type and level of professional training significantly impacted on knowledge about ginseng products (P≤0.001). The proportion of attendants with adequate knowledge was lowest among the pharmaceutical technologists (12.1%) followed by pharmacists

(30.0%), other unspecified professions (30.8%) and highest among nutritionists (70.0%). A pharmacist was 3.11 times more likely to have adequate knowledge on ginseng products compared to a Pharmaceutical Technologist. The likelihood was equally high among attendants trained in other unspecified type of professions (OR=3.22). A nutritionist attendant was 16.92 times more likely to have adequate knowledge on ginseng products compared to a pharmaceutical technologist. The other professions included persons trained on the job, especially those who were not pharmaceutical technologists, nutritionists or pharmacists.

3.2.5 Feedback on Use of Ginseng Products

Table 3.4 shows attendants' responses on feedback from users of ginseng.

Table 3.4: Feedback from users of ginseng products

Feedback response		**Number of Responses**	**Percentage**
Receipt status	Received	44	58.0
	Not received	24	31.5
	Not indicated	8	10.5
Total		**76**	**100**
Description	Somewhat useful	3	6.8
	Useful	28	63.6
	Very useful	13	29.6
	Not received	24	Nil
	Not indicated	8	Nil
Total		**76**	**100**

Of the attendants interviewed, 58% affirmed that they received feedback from their customers indicating that they benefited from using ginseng containing products. While

6.8% of the feedbacks indicated that the products were somewhat useful, the majority (63.6%) indicated that they were useful and 29.5% indicated that they were very useful

3.3: Assessment of Label Information of Ginseng Samples

3.3.1: Sample Size of Ginseng Products

Out of 76 outlets visited, 40 unique ginseng products were identified and a sample of each taken for laboratory evaluation (Appendix 4). The distribution of the products per district is shown in Table 3.5.

Table 3.5: Sampled ginseng containing products per district

District	Number of samples	Percentage
Westlands	14	35.0
Embakasi	2	5.0
Langata	4	10.0
Makadara	5	12.5
Kamukunji	2	5.0
Kasarani	1	2.5
Dagoretti	3	7.5
Starehe	9	22.5
Total	**40**	**100**

3.3.2: Evaluation of Label Information of Samples

The WHO guidelines for registration of herbal medicines require that every herbal formulation must be correctly labeled in clearly legible indelible letters in English or any other language as determined by respective National Medicines Regulatory Authority (NMRA). The label should state the name of the product, method of administration, shape and colour of dosage form, list of active ingredients, date of manufacture and expiry, address of manufacturer and batch number {Pharmacy and Poisons Board, 2010(b)}.

The guidelines further specify that a literature insert for the patient or prescriber be included in the commercial pack stating the indications and recommended dose where applicable. Further, storage conditions or handling precautions may be stated. Directions for use and warning or precautions that may be necessary should also be included in the literature insert {Pharmacy and Poisons Board, 2010 (b)}.

Of the forty samples, 7 (17.1%) were labeled as containing ginseng alone while 33 (82.9%) contained ginseng, vitamins and minerals. Further, 22 (55%) of the samples had manufacturing and expiry dates correctly labeled. All the samples had correct labels for batch number, dosage form and manufacturer's address. About 13 (32%) were labeled as containing ginseng root extract standardised to contain a stated percentage of ginsenosides while 21 (53.7%) were labeled as containing a specified amount of ginseng root extract without specifying the level of ginsenosides. Samples labeled as ginseng without quantities of either ginseng root extract or ginsenosides being specified accounted for 14.6%. Only 14 (34.1%) of products were correctly labeled for active ingredients (Table 3.6).

Table 3.6: Compliance to labeling requirements

Label characteristics	**Number of samples**	**Percentage**
Manufacturing and expiry date	22	55.0
Batch number	40	100
Patient information literature	16	40.0
Active ingredients	14	35.0
Dosage form	40	100
Manufacturers' address	40	100

Most labeling errors were observed with regard to active ingredients (65.9%), followed by lack of patient information literature (61.0%) and failure to indicate manufacturing or expiry date (46.3%). All the samples had batch numbers, dosage forms and manufacturers' addresses clearly indicated.

Of the 40 identified products 17 (41.5%) were 80 – 100 % compliant to all labeling requirements constituted by 2 (4.9%) that were fully compliant and 15 (36.6%) that were 80 - <100 % compliant (Figure 3.3).

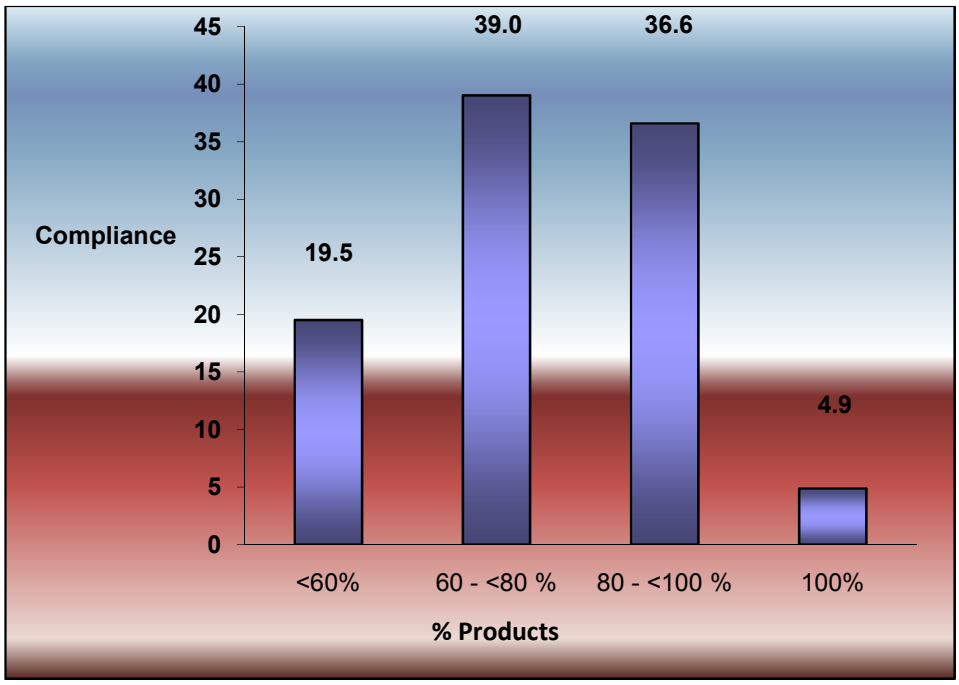


Figure 3.3 Compliance to Labeling Requirements Using all Characteristics

3.3.3: Number of Active Ingredients per Sample/Product

The number of active ingredients per sample was as illustrated in Table 3.7. Out of 40 samples 15% had active ingredients ranging from one to four. There were 7.3% of samples that had one active ingredient (ginseng), 7.3% had two and 12.2% had five. Those with more than one active ingredients contained either several species of ginseng or a mixture of ginseng and other herbs, minerals and vitamins.

Table 3.7: Number of active ingredients per sample

Number of ingredients	Number of samples	Percentage
1-4	6	15.0
5-8	8	20.0
9-12	3	7.5
13-16	5	12.5
17-20	5	12.5
21-24	7	17.5
25-28	2	5.0
29-43	4	10.0
Total	**40**	**100**

3.3.4: Country of Origin of the Samples

Samples were also analyzed with respect to the country of origin and the results were as illustrated in Figure 3.4.

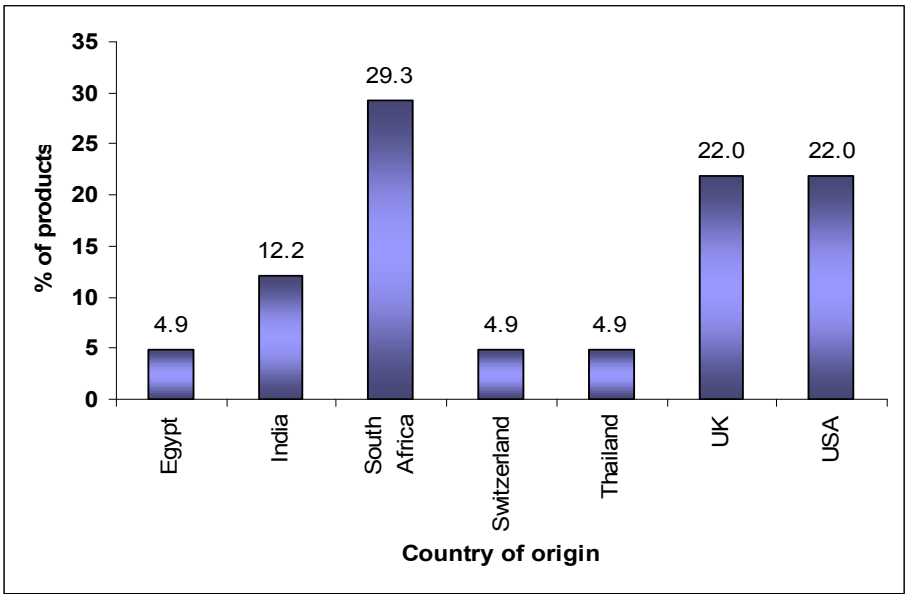


Figure 3.4: Distribution of ginseng containing products by country of origin

Most of the samples originated from South Africa followed by UK, USA and India respectively. This is unlike the case for herbal medicines that were submitted to the Pharmacy and Poisons Board for registration or listing where over 80% originated from India (Appendix **1).**

Analysis of compliance with WHO labeling requirement was done by country of origin as shown in Table 3.8.

Table 3.8: Conformity to WHO labeling requirement by country of origin

Labeling variables	Country of origin							P value
	Egypt (n=2)	India (n=4)	South Africa (n=12)	Switzer-land (n=2)	Thailand(n=2)	UK (n=9)	USA (n=9)	
Man. & Exp. Dates								
Present	2	4	10	1	2	3	0	0.002
Not present	0	1	2	1	0	6	9	
Patient Information Literature								
Present	0	4	8	1	2	1	0	0.002
Not present	2	1	4	1	0	8	9	
Active ingredients								
Present	0	0	2	1	0	5	6	0.050
Not present	2	5	10	1	2	4	3	
Overall compliance								
Low (<60 %)	0	1	1	1	0	2	3	
Medium (60 - <80 %)	2	0	3	0	0	5	6	0.015
High (80 - <100 %)	0	4	7	0	2	2	0	
Full (100%)	0	0	1	1	0	0	0	

There was a significant difference in distribution of products that met minimum labeling requirements by dates of manufacturing and expiry between countries of origin (P=0.002). All the sampled ginseng containing products originating from Egypt and Thailand met minimum specifications for labeling of manufacture and expiry dates. However, all the samples from the USA did not meet labeling requirements for the manufacturing and expiry dates. Compliance with the batch numbers and dosage forms requirements were observed for all the sampled products (100%).

Distribution by presence of patient information literature was significantly different between countries of origin (P=0.002). All the sampled ginseng containing products originating from Thailand met the specifications for patient information literature while those from the USA and Egypt did not meet the requirements for patient information literature.

Distribution by correct labeling of active ingredients was marginally different between countries of origin (P=0.050). Correct labeling of active ingredients was observed in a number of products originating from countries like the UK (55.6%) and USA (66.7%). Of the samples from Switzerland, 50% met labeling specifications while only 16.7% from South Africa satisfied the requirements. Complete non-compliance was observed in products originating from Egypt, India and Thailand.

Assessment of the overall compliance using all characteristics revealed an interesting out come. There was a significant difference in distribution of overall compliance by all labeling requirements between countries of origin (P=0.015). Out of all the sampled products only 8.3% of those originating from South Africa and 50.0% of those originating from Switzerland fully complied with the five labeling requirements. High compliance to the five labeling requirements was observed in products from India (80.0%), South Africa

(66.6%), Switzerland (50.0%), Thailand (100%) and the UK (22.2%). All the sampled products from Egypt and the USA scored medium or low in compliance.

3.4: Uniformity of Weight

All the thirty capsule and four tablet samples met the BP (2008) requirements for uniformity of weight.

3.5: Microbial Load

3.5.1: Total Aerobic Microbial Count (AV.TAMC)

The limits of aerobic bacterial contamination given in the USP (2008) are: Average Total Aerobic Microbial Count (AV.TAMC) not more than 10^5 bacteria for dried or powdered botanicals and not more than 10^4 for powdered botanical extracts or nutritional supplements with botanicals.

The results of the Mean Total Aerobic Microbial Count (AV.TAMC) for the samples tested were as shown in Table 3.9.

Table 3.9: Average total aerobic microbial count (AV.TAMC) of the samples

AV.TAMC(cfu/ml, gm) ($n\times10^1$)	Number of samples	Percentage
0	4	10.0
1-2	9	22.5
2-4	7	17.5
4-6	2	5.0
6-8	2	5.0
8-10	8	20.0
10-12	3	7.5
12-14	2	5.0
Above 14	3	7.5
Total	**40**	**100**

The Mean Total Aerobic Microbial Count (AV.TAMC) for the samples tested was 6.5 (± 6.0 SD) ranging between 0 and 29×10^1. Approximately 10% of the 40 samples did not show any growth for aerobic bacteria while 41.4% samples had a colony count of 1.0×10^1 to 4.0×10^1. This means that none had a colony count of above 40cfu/g or 40cfu/ml. Twenty nine samples (70.7%) contained an average total aerobic microbial load count of 1.0×10^1 to 1.0×10^2 cfu/ ml. Eight (19.5 %) of the samples had an average total microbial load count of between 1.0×10^2 cfu/ ml and 2.9×10^2 cfu/ml (Table 3.9). Typical bacterial colonies were as shown in Appendix 6.

All the 40 (100%) samples in this study met the USP (2008) specifications for Total Aerobic Microbial Count.

3.5.2: Total Combined Yeast and Mould Count (AV.TYMC)

The limits of fungal contamination given in the USP (2008) are: Average total yeast and mould count (AV.TYMC) not more than 10^3 fungi for dried or powdered botanicals and AV.TYMC not more than 10^3 fungal for powdered botanical extracts or nutritional supplements with botanicals.

The average total combined yeast and mould count (AV.TYMC) for the 40 ginseng products was 2.3 (± 3.1 SD) ranging between 0 and 10×10^1. Typical fungal colonies were as shown in Appendix 7. Seventeen (41.5%) samples did not show any fungal/mould growth while twenty four (56.3%) samples contained between 1.0×10^1 to 9.0×10^1 cfu/ ml of average total combined yeast and mould count (AV.TYMC). One sample (2.4%) was contaminated to an extent of 1.0×10^2 cfu/ mg average total combined yeast and mould count (Table 3.10). All the 40 (100%) samples under the study met the USP (2005) specifications for Total Combined Yeast and Mould Count. Table 3.10 summarizes the results of the mean total combined yeast and mould count (AV.TYMC) for the 40 ginseng products in this study.

Table 3.10: Average total combined yeast and mould count of the samples

AV.TYMC (cfu/ml, gm) ($n \times 10^1$)	No. of Samples	Percentage
0-1	15	37.5
1-2	13	32.5
2-4	2	5.0
4-6	5	12.5
6-8	2	5.0
8-10	3	7.5
Total	**40**	**100**

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3.5.3: Total Viable Aerobic Microbial Count (TVAMC)

The limits for Total Viable Aerobic Microbial Count (TVAMC) were not provided for in the British and US Pharmacopeia. However, the limits of bacterial and fungal contamination given in the Indian Pharmacopeia (IP) of 2008 were: Total Viable Aerobic Microbial Count (TVMC) which is the sum of fungal and bacterial load not more than 10^2 bacteria and fungi per 1g or 1ml for preparations meant for oral administration containing materials of natural origin

The Mean Total Viable Aerobic Microbial Count (TVAMC) for the 40 samples was 8.9 (+ 7.1 SD) ranging between 0 and 29×10^1. Four (9.8%) samples did not show any contamination by either aerobic bacteria or moulds and yeast. However, this did not mean that they were sterile since the samples under this study were not subjected to microbial growth tests under anaerobic conditions. Twenty three samples (56.1%) contained between 0 to 9×10^1 cfu/ml, gm total viable aerobic microbial count (TVAMC) while

eighteen (43.9%) were contaminated at the level of $1.0x10^2$ to 2.9×10^2 cfu/ml or 2.9×10^2 cfu/gm TVAMC. These results are summarized in Table 3.11.

Table 3.11: Total viable microbial count of the samples

TVAMC (cfu/ml, mg) ($n\times10^1$)	**Number of samples**	**% samples**
0	4	10.0
1-4	10	25.0
5-9	8	20.0
10-14	8	20.0
15-19	8	20.0
>19	2	5.0
Total	**40**	**100**

Out of the 40 samples, 61.0% complied with the IP (2008) specifications for Total Viable Aerobic Microbial Count.

3.5.4: Compliance to Microbial Load Standards by Country of Origin

Analysis of compliance to microbial standards was done by country of origin as shown in Table 3.12. Compliance to Average Total Aerobic Microbial Count (AV.TAMC) and Average Total Combined Yeast and Mould Count (AV.TYMC) was observed in all the sampled products from each country of origin. Distribution of compliance by Total Viable Aerobic Microbial Count (TVAMC) requirement was not significant between different countries of origin (P=0.063). Products from Switzerland were 100% compliant to this requirement. High compliance was also observed with products originating from India (80.0%) and the USA (88.9%). While low compliance was recorded for products originating from South Africa (50.0%) and the UK (55.6%). Products originating from Egypt and Thailand were not compliant.

Table 3.12: Compliance to microbial standards by country of origin

Compliance Variables (cfu/ml,mg)	Country of origin							
	Egypt (n=2)	India (n=5)	South Africa (n=12)	Switzer-land (n=2)	Thai-and (n=2)	UK (n=9)	USA (n=9)	P value
AV.TAMC								
Compliant	2	5	12	2	2	9	9	-
AV.TYMC								
Compliant	2	5	12	2	2	9	9	-
TVAMC								
Compliant	0	4	6	2	0	5	8	0.063
Non-compliant	2	1	6	0	2	4	1	

3.6: Positive Microbial Identification

3.6.1: Gram Staining and Biochemical Characterization

Gram Staining

A total of 121 isolates were initially tagged with the codes RMI-IS 1 to RMI-IS 121 of which 118 isolates were found to be viable. Of the 118 viable isolates, 86 (72.8%) were confirmed to be bacteria, 18 (15.3%) were fungus and 14 (11.9%) colonies were treated as anaerobic isolates due to their growth characteristics. Out of the 86 aerobic bacterial isolates 61 (70.9%) appeared negative to Gram's staining, 16 (18.6%) positive and 9 (10.4%) were neither negative nor positive. Non response to gram stain indicates that the isolates were likely to be anaerobic bacteria.

Biochemical Characterization by Differential and Selective Media and API 20 E

The test for specified microbes, carried out using differential and selective media, showed growth of *Escherichia coli* and *Enterobacter aerogenes* in Eosin Methylene Blue (EMB), *Pseudomonas* species in Cetrimide agar, *Salmonella* species in Salmonella Shigella Agar (SSA) and *Staphylococci* species in Mannitol Salt Agar (MSA). The bacteria, *Aeromonas hydrophyla*, *Proteus penneri* and *Klebsiella* species were diagnosed by API 20 E. This is shown in Table 3.13.

Table 3.13: Numerical profiles of the microbes identified by API 20 E

ISOLATE CODE	API CODE	IDENTIFICATION
RMI IS – 5	7247124	*Aeromonas hydrophila, V. cholera (Presumptive ID)*
RMI IS – 28	3243024	*Salmonella species*
RMIIS – 39	3207124	*Aeromonas hydrophyla*
RMIIS – 72	0026020	*Proteus penneri*
RMIIS – 77	7304552	*Salmonella species*
RMIIS – 90	6304112	*Salmonella species*
RMIIS – 92	0234020	*Proteus penneri*
RMIIS – 101	7404512	*Salmonella species*
RMIIS – 126	6204552	*Klebsiella species*

Adopted from biomeriux vitek,

3.6.2: Positive Identifications for Microbial Contaminants

The microbial contaminants profiled from the 40 ginseng containing samples are as shown in Figure 3.5. Twenty-five (61.0%) samples were contaminated with one or more bacteria and fungi. The contaminants included high risk microbes *Salmonella* spp and *E.coli* that are pathogenic and should be absent in all oral formulations. Others were risky microbes that form part of the normal flora in man but can cause diseases if products are

contaminated above the recommended levels especially in immune-compromised persons.

Salmonella spp was the most frequently isolated bacteria from 19.5% (8) of the samples followed by *Pseudomonas and Staphylococci* spp. The other contaminants were *Escherichia coli* 7.3% (3), *Aeromonas hydrophyla* 4.8% (2), *Proteus penneri* 4.8% (2*)* and *Klebsiella* species 2.4% (1). *Rhizopus* spp and *Fusarium* spp formed majority of the perfect fungal isolates from 9.7 %(2 samples for each) of the samples followed by *Candida albicans* 2.4 % (1). The high level of contamination with Salmonella indicated that the raw materials were not handled in a hygienic manner.

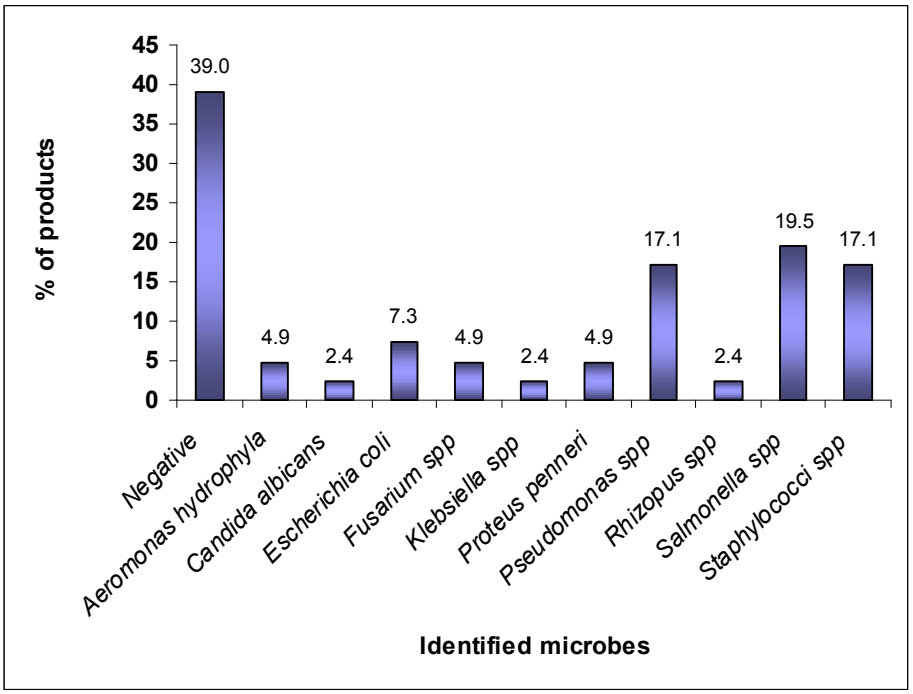


Figure 3.5: Occurrence of microbes in the sampled products

Figure 3.6 presents the distribution of samples by status of isolated microbes. A total of 9 (22.0%) samples did not meet pharmacopoeia requirements for absence of the high-risk microbes, S*almonella* species and *Escherichia coli* (USP, 2005). Of the 40 samples, 39%

were contaminated with risky microbes or contaminants that could be harmful in oral preparations if they exceeded pharmacopeial limits.

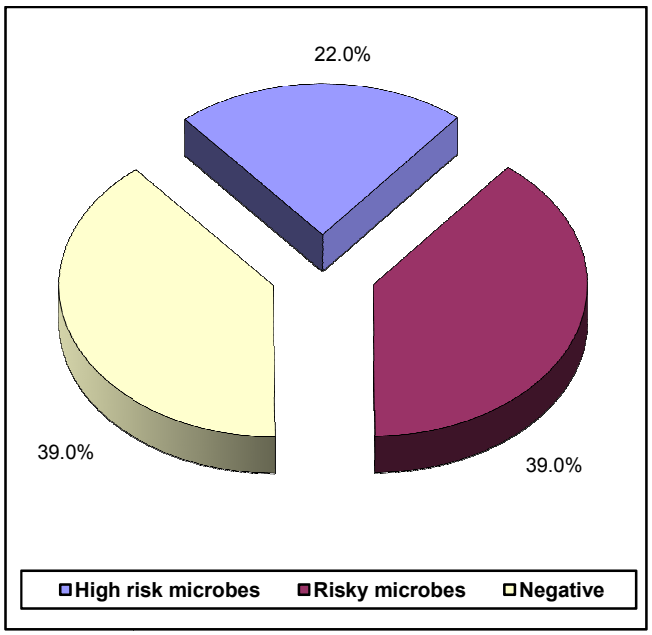


Figure 3.6: Status of microbes identified in the sampled products

Analysis of status of identified microbes was done by country of origin as shown in Table 3.14. Distribution of status of identified microbes between countries of origin was not significantly different (P=0.304). Microbial isolates were found in samples from every country. However, high-risk microbes were found in samples originating from South Africa (33.3%), Thailand (50.0%), UK (33.3%) and USA (11.1%).

Table 3.14: Status of identified microbes by country of origin

Status of microbes	Egypt (n=2) %	India (n=5) %	South Africa (n=12) %	Switzer-land (n=2) %	Thailand (n=2) %	UK (n=9) %	USA (n=9) %	P value
High risk	0	0.	33.3	0	50	33.3	11.1	0.304
Risky	100	60	16.7	100	50	22.2	44.4	
Negative	0	40	50.0	0	0	44.4	44.4	

3.7 Identification of Ginseng

3.7.1 Tablet Samples

Five out of 40 samples were tablet formulations. The five samples were labeled as containing specified quantities of ginseng. Four were labeled as containing specific levels of ginseng extract while one sample was labeled as having a stated amount of ginseng root. The labeling did not indicate the species of ginseng plant.

Chromatograms and densitograms for all tablet samples were similar to those of standard ginseng extract hence they probably had ginseng (Figure 3.7).

Two samples had densitogram peaks with R_f values similar to that of standard ginsenoside Rb1 (Appendix 28 and 39) and two other samples had densitograms with peaks that had R_f values similar to that of standard ginsenoside Rg1 (Appendix 22 and 26).

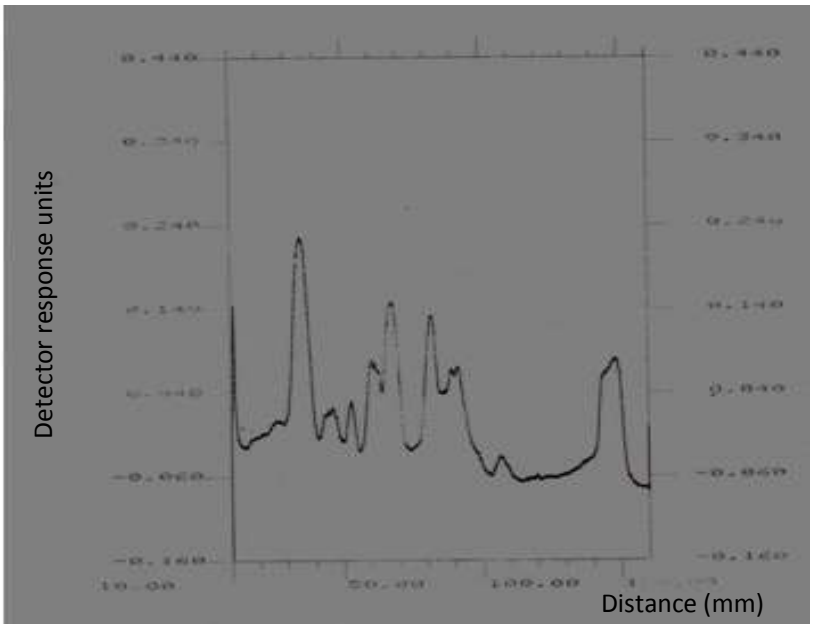


Figure 3.7: Densitogram of standard ginseng extract

Densitograms and chromatograms of standard ginsenosides Rb1and Rg1 were as shown in Figure 3.8.

Although larger peaks are expected for standard substances, the peaks in this case were small. This may have arisen out of the fact the densitometeric conditions were not optimized for the standard ginsenosides because the standards were supplied in very small quantities.

However, the peaks were good enough since the identification of ginsenoside was dependant on position of the peaks or R_f values. This would have been different if it were a quantitative experiment. The spots for the standard ginsenosides Rg1 and Rb1 were at R_f values of 0.53 and 0.29 respectively.

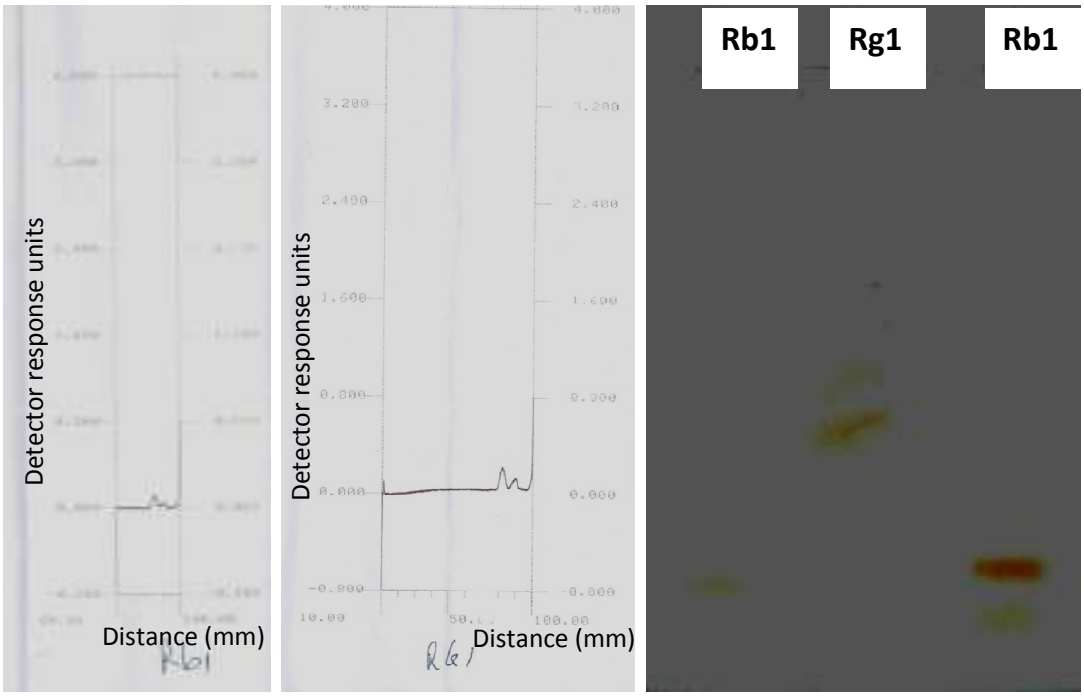


Figure 3.8: Densitograms and Chromatograms of standard ginsenosides Rb1 and Rg1

Typical TLC chromatograms for the 5 tablet samples were as illustrated in Figure 3.9.

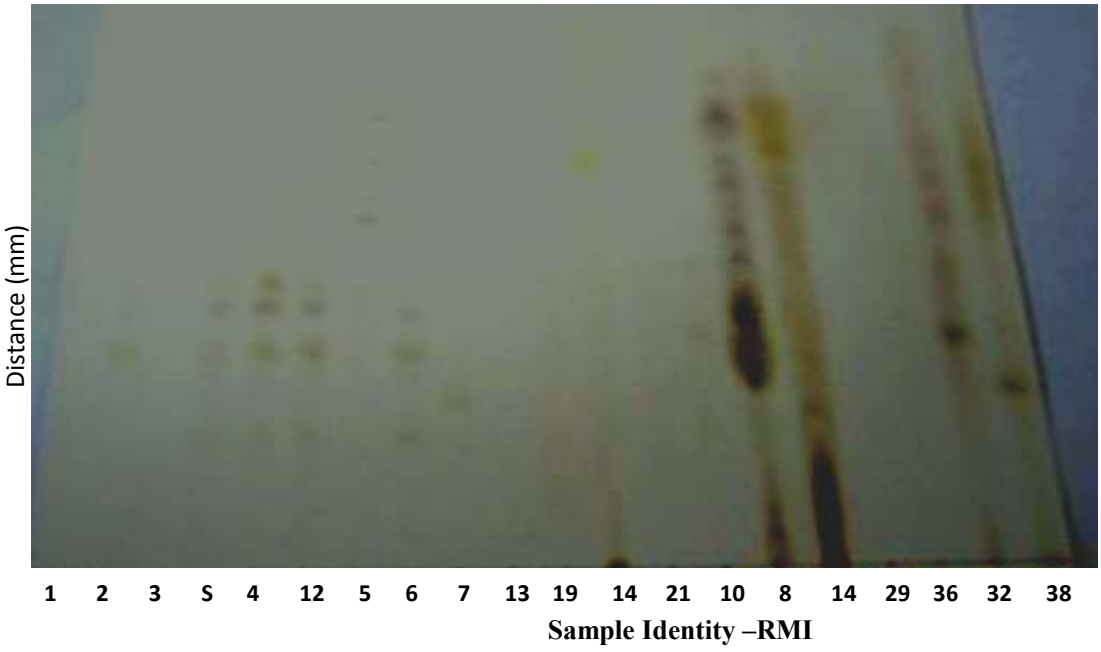


Figure 3.9: Typical Chromatograms of some samples and the standard ginseng extract-(s)

The densitogram for sample RMI 12 (Figure 3.10) had similar peaks to those of the standard ginseng extract (Figure 3.7).

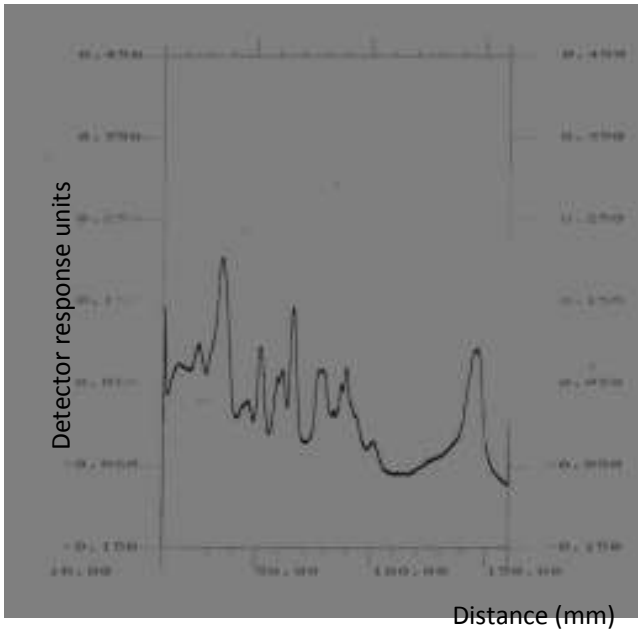


Figure 3.10: Densitogram of RMI 12

The R_f and Rx values for the ginsenosides present in the sample indicated that ginsenoside Rg1 was present. Each tablet was labeled as containing 42.5 mg of ginseng extract and several vitamins and minerals. The sample originated from India and was correctly labeled in terms of name and address of manufacturer, manufacturing date and batch number. The sample also met pharmacopoeial standards for microbial load but did not meet requirements for patient information literature and expiry date.

In addition, R_x values for the ginsenosides were calculated based on R_f values of ginsenosides Rg1 and Rb1. R_x values were used to confirm presence of ginsenosides Rg1 and Rb1 in the samples. The R_f and R_x values of the tablet samples were as shown in Table 3.15.

Table 3.15: R_f and R_x values of ginsenosides of the tablet samples

Sample ID (RMI)	R_f Values×100	Rx Values based on reference ginsenosides Rg1 and Rb1	
		Rg1	Rb1
12	17, 18, 38, 44, 54, 63	0.32, 0.33, 0.71, 0.83, 1.01, 1.18	0.58, 0.62, 1.31, 1.51, 1.86, 2.17
16	10, 15, 22, 49, 50, 63	0.18, 0.28, 0.41, 0.92, 0.94, 1.18	0.34, 0.51, 0.75, 1.68, 1.72, 2.17
20	35, 57	0.66, 1.07	1.20, 1.96
24	15, 21, 29, 45, 59	0.28, 0.39, 0.54, 0.84, 1.11	0.51, 0.72, 1.00, 1.55, 2.03
39	8, 21, 31, 32, 45, 65	0.15, 0.39, 0.58, 0.60, 0.84, 1.22	0.27, 0.72, 1.06, 1.10, 1.55, 2.24
STD Rg1	53	1.00	1.86
STD Rb1	29	0.54	1.00
Std ginseng extract	14, 16, 45, 53 ,59, 66	0.26, 0.30, 0.84, 1.00, 1.11, 1.24	0.48, 0.55, 1.55, 2.03, 2.27

STD=Standard, ID=Identity

3.7.2: Hard Gelatin Capsules Samples

Of the eight hard gelatin samples, six (75%) had chromatograms and densitograms similar to those of standard ginseng extract and showed presence of ginsenosides Rb1 and Rg1. Two (25%) samples did not show any spots on the TLC chromatograms or peaks on the densitograms hence a likelihood of them being falsified or counterfeit products. The R_f and R_x values for the six hard gelatin capsule samples were as illustrated in Table 3.16.

Appendices 11, 12, 13, 15, 33 and 39 are densitograms for samples RMI 4, 5, 6, 8, 30 and 38 respectively. Samples RMI 19 and 21 had no spots on the TLC chromatograms and R_f values corresponding to those of standard ginseng extract and this was taken to indicate absence of ginseng.

Table 3.16: R_f and R_x values of ginsenosides of the hard gelatin capsule samples

Sample Identity (RMI)	R_f Values×100	R_x Values based on reference ginsenosides Rg1 and Rb1	
		Rg1	Rb1
4	19, 30, 40, 49, 57, 65	0.35, 0.56, 0.75, 0.92, 1.07,1.22	0.65, 1.03, 1.37, 1.68, 1.96, 2.24
5	16, 26, 46, 56	0.30, 0.49, 0.86, 1.06	0.55, 0.89, 1.58, 1.93
6	16, 26, 40, 57	0.30, 0.49, 0.75, 1.07	0.55, 0.89, 1.37, 1.96
8	18, 25, 37, 44, 55, 63	0.33, 0.47, 0.69, 0.83, 1.03, 1.18	0.62, 0.86, 1.27, 1.51, 1.89, 2.17
19	No spots	N/A	N/A
21	No spots	N/A	N/A
30	21, 30, 37, 45, 66	0.39, 0.56, 0.69, 0.84, 1.24	0.72, 1.03, 1.27, 1.55, 2.27
38	16, 26, 52, 59	0.30, 0.49. 0.98, 1.11	0.55, 0.89, 1.79, 2.03

The label for RMI-19 indicated that each capsule contained 150 mg of *Panax ginseng* root standardized to 5% ginsenosides. In addition, the label stated that each capsule contained 140 mg of *Paullinia cupana* seed, 100 mg of *Camellia sinensis* leaf, 75 mg *Eleutherococcus senticosus* root, 50 mg of *Rhodiola rosea* root, 50 mg of *Glycyrrhiza glabra* root, 50mg *Centella asiatica*, 25mg *Ilex paraquariensis* leaf, 12.5 mg *Capsicum annuum* and 15 mg Octacosanol from spinach and several minerals and vitamins.

This sample comprised of many herbs that had several active ingredients hence the difficulty in isolating and identifying ginsenosides. This sample is also likely to have been falsely labeled thus qualifying to be a counterfeit.

3.7.3: Soft Gelatin Capsules Samples

There were 23 samples in this category but only 19 were found to have ginseng with the R_f and R_x values illustrated in Table 3.17.

Table 3.17: R_f and R_x values of ginsenosides in the soft gelatin capsule samples

Sample Identity (RMI)	R_f Values×100	R_x Values based on reference ginsenosides Rg1 and Rb1	
		Rg1	Rb1
1	22, 33, 37, 45	0.41, 0.62, 0.69, 0.84	0.75, 1.13, 1.27, 1.55
2	26, 38, 44	0.49, 0.71, 0.83	0.89, 1.31, 1.51
3	18, 31, 41, 56, 60	0.33, 0.58, 0.77, 1.05, 1.13	0.62, 1.06, 1.41, 1.93, 2.06
7	16, 17, 39, 45, 57	0.30, 0.32, 0.73, 0.84, 1.07	0.55, 0.58, 1.34, 1.55, 1.96
9	16, 36, 41, 53	0.30, 0.67, 0.77, 1.00	0.55, 1.24, 1.41, 1.82
10	39, 44, 49, 56, 64	0.73, 0.83, 0.92, 1.20	1.34, 1.51, 1.68, 1.93, 2.20
11	48, 77	0.90, 1,45	1.65, 2.65
13	No spots	N/A	N/A
14	15, 21, 24, 38, 45, 56, 62	0.28, 0.39, 0.45, 0.71, 0.84, 1.05, 1.16	0.51, 0.72, 0.82, 1.31, 1.55, 1.93, 2.13
17	12, 40	0.22, 0.71	0.41, 1.37
18	36	0.67	1.24
23	21, 27, 36	0.39, 0.50, 0.67	0.72, 0.93, 1.24
26	26, 46	0.49, 0.86	0.89, 1.58
27	No spots	N/A	N/A
28	66, 86	1.24, 1.62	2.27, 2.96
29	No spots	N/A	N/A
31	43, 67, 78, 86	0.81, 1.26, 1.47, 1.62	1.48, 2.31, 2.68, 2.96
32	21, 34, 41, 68	0.39, 0.64, 0.77, 1.28	0.72, 1.06, 1.41, 2.34
33	28, 31, 36, 56, 86	0.52, 0.58, 0.67, 1.05, 1.62	0.96, 1.06, 1.24, 1.93, 2.96
34	10, 15, 41, 61, 71	0.18, 0.28, 0.77, 1.15, 1.33	0.34, 0.51, 1.41, 2.10, 2.44
35	10, 19, 43	0.18, 0.35, 0.81	0.34, 0.65, 1.48
36	No spots	N/A	N/A
40	21, 30, 32, 37, 45, 66, 68	0.39, 0.56, 0.60, 0.69, 0.84, 1.24, 1.28	0.72, 1.03, 1.10, 1.27, 1.55, 2.27, 2.34

Presence of ginseng in the samples was demonstrated by comparing the profiles of the samples to that of standard ginseng extract and confirmed by having peaks with R_f values corresponding to those of standard ginsenosides Rb1 and Rg1. The TLC chromatograms for 4(17.4%) samples did not show any spots and the corresponding densitograms did not have any peaks. The TLC chromatograms and densitograms for 19(82 .6%) samples exhibited similar profiles to that of standard ginseng extract and reference ginsenosides Rg1 and Rb1 respectively. Denistograms of the samples are as shown in the Appendix chapter.

3.7.4: Occurrence of Ginsenosides in the Analyzed Samples

Of the 36 samples analyzed for identification of ginsenosides, 30 (83.3%) were positive for ginseng. Figure 3.11 presents the distribution of samples by ginsenosides.

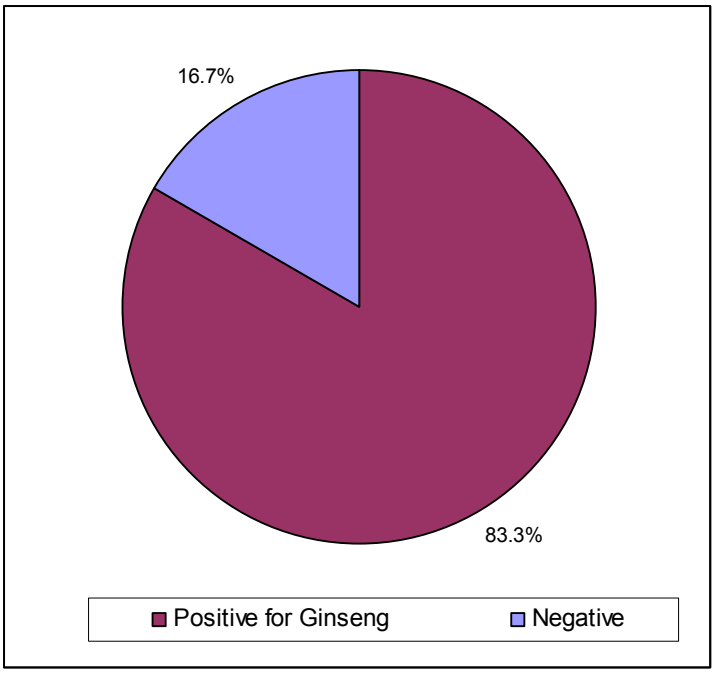


Figure 3.11: Presence of ginsenosides in the samples

Presence of ginsenosides was established by comparing ginsenoside profiles of the samples to that of the standard ginseng extract. The ginseng profiles of the samples were

also compared to the corresponding TLC chromatograms and densitograms of standard ginsenosides Rb1 and Rg1. In this study, confirmed ginsenosides were those whose reference standards were available hence the R_f values were ascertained. Of the 36 samples, 26 (72.2%) had confirmed ginsenosides Rb1 and Rg1. Broadly, 19 (52.7%) samples were identified to have the Rb1 ginsenoside, 24 (66.6%) contained the Rg1 ginsenosides and 17 (47.2%) had both Rb1 and Rg1.

Further, 4 (11.1%) samples showed densitograms similar to that of the standard ginseng extract (Figure 3.7) but did not show peaks corresponding to ginsenosides Rb1 or Rg1. This indicates presence of the other ginsenosides other than Rb1 or Rg1. Another observation was that 7 (19.4%) samples had Rg1 but no Rb1 while 2 (5.5%) samples only had Rb1 ginsenosides. The distribution of ginsenosides in the 36 samples was as illustrated in Figure 3.12.

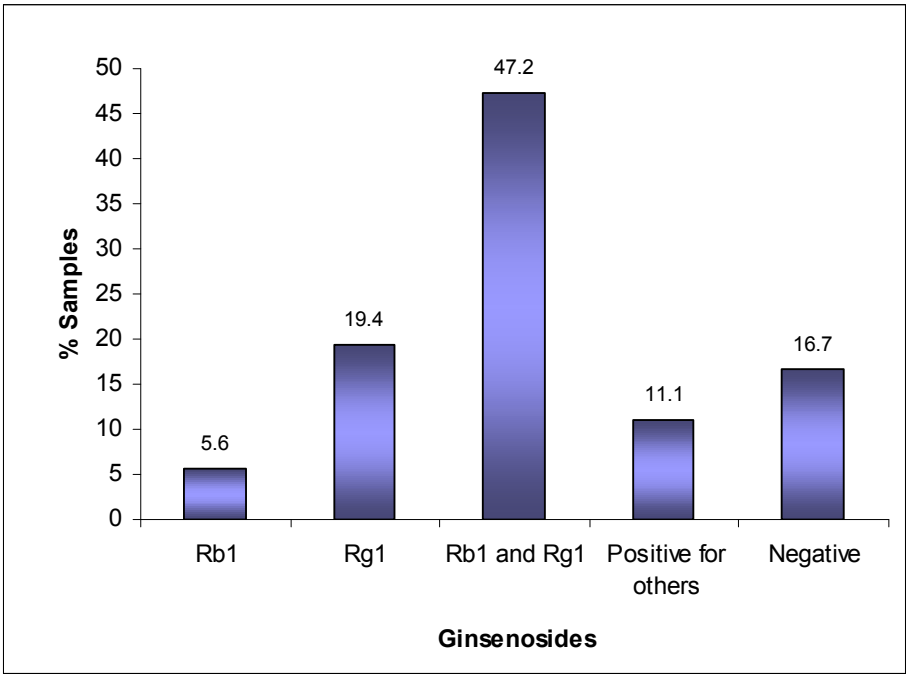


Figure 3.12: Distribution of Rg1 and Rb1 ginsenosides in the samples

Profiling of ginsenosides in the 40 samples in this study mirror the work done by Courthout *et al.,* (1999) who established R_f values for the 8 common ginsenosides as illustrated in Table 3.18. Though the experimental conditions for the two studies were different, the results had a lot of similarities.

Table 3.18: R_f values of 8 ginsenoside (Courthout *et al.*, 1999)

Ginsenoside group	Ginsenoside	R_f×100 value
Protopanaxadiols(Rb1 Group)	Rb1	30
	Rb2	35
	Rc	39
	Rd	49
protopanaxatriols(Rg1 Group)	Rg1	53
	Re	43
	Rf	56
	Rg2	60

3.8: Discussion

In this study, commercial ginseng containing products from Nairobi County were sampled. This decision was made out of the realization that Nairobi is the commercial city of Kenya. All the big super market chains, nutritional stores, medical stores and pharmacies have their head quarters in the Nairobi. It was, therefore, inferred that the sampling results on the distribution of ginseng products are generalisable to all urban areas in Kenyan.

The results of the survey in Nairobi County illustrated that there was a large number of premises stocking ginseng containing products in Westlands and Starehe Districts. Majority of the inhabitants of these two districts were medium and high income earners with a significant number of foreigners (Mitullah, 2003). Of the eight stores of a leading supermarket chain sampled, it was established that only the one in Githurai slums did not

stock any ginseng products. Githurai is an area in Nairobi County that is largely inhabited by low income earners (Mitullah, 2003). This finding showed that ginseng products were preferentially consumed by medium and high income earning population of Nairobi County. The samples were purchased at various prices ranging from 12 to 100 USD per monthly dose.

The assumption had been that ginseng containing products were not stocked in un-registered pharmacies and in stores found in low income neighbourhoods. The study was also limited to ginseng containing products of unique batch numbers. Same products with different batch numbers were treated as distinct different products.

Persons selling ginseng containing products in the outlets were broadly classified as pharmacists, pharmaceutical technologists, nutritionists and others who were none of the three categories. One of the specific objectives for this study was to establish how much knowledge they had about ginseng containing products. Their knowledge was tested through questionnaires that referred to the classification or scheduling, pharmacological uses and side effects of ginseng products.

A majority (52.6%) classified them as general sales food supplements. Indeed, this was the most appropriate classification. However, for attendants in pharmacies and nutritional stores a classification of 'over the counter' would suffice. Attendants who gave either of the two answers were 32.9%. The registered ginseng containing products were scheduled as multivitamins by the the Pharmacy and Poisons Board. This scheduling was wrong and was not supported by any official legal instrument. In the United States of America, ginseng containing products are classified as botanical dietary supplements following the passage of the "Dietary Supplement and Health Education Act" of 1994 (Breemen *et al.,*

2007). The case in Kenya was similar to that in Canada where several efforts to develop a comprehensive schedule for herbal products had not been successful.

This study brought out the fact that most personnel advising the public on use of ginseng products had poor understanding of pharmacological activities of ginseng. Mean knowledge score of the 76 store attendants was 3.2 (± 1.8 SD) ranging between 0 and 8. It was established that a trained nutritionist attendant was about 17 times more likely to have adequate knowledge on ginseng products compared to one trained as a pharmaceutical technologist or as pharmacist. Although nutritionists scored highly, most of them had undergone in-service training. It is suggested from the results of this study that the Bachelor of Pharmacy curriculum and Diploma in Pharmaceutical Technology syllabus be reviewed to strengthen training on clinical aspects of specific herbal products. This study exposed the difficulties that would arise when regulation of herbal products is instituted by the Pharmacy and Poisons Board. Herbal products were not scheduled while those who were supposed to practice herbal medicines had little understanding of what the products entailed. There were risks pertaining to the then prevailing situation since ginsenosides are phytoestrogens (steroidal glycosides) and their safety in infants and pregnant women has not been studied (Osaki and Kennelly, 2003). Another worrying trend was that there were no clear recommended daily nutritional doses both for adults and children.

Forty samples were assessed for quality by way of compliance with WHO labeling requirements, microbial load as per pharmacopeia standards and identification of ginseng. Out of 40 identified samples 17 (41.5%) were 80 – 100 % compliant to all WHO labeling requirements for market authorization. The high level of non conformity may have arisen out of the fact that different countries followed different labeling regimes. The European guidelines for the assessment of herbal remedies followed up on WHO's Guidelines for

the Assessment of Herbal Medicines while the USA, Canada, China, India, Germany, Britain and France had country specific guidelines that had different directives on labeling. In Germany, for example, the whole herbal product is considered as one active ingredient (Benzi and Ceci 1997; Saito, 2000). In the UK, The Traditional Herbal Medicines Registration Scheme requires that relevant products are accompanied by the necessary information for its safe use, such as safety warnings and contraindications. It is therefore important to note that not all the samples complied with their country of origin labeling requirements. In the absence of proper scheduling and poor understanding of ginseng containing products by the professionals involved in dispensing them, the chances of misuse are likely to be higher. This was worsened by the fact that 61% of the sampled products did not have patient information literature. Furthermore, all the sampled products had no labels that indicated storage conditions yet some were soft gel capsules and others contained jellies that are susceptible to spoilage at higher temperatures.

Another important aspect of this study was to establish the level of contamination and identify microbes that were not supposed to be present in ginseng containing products prepared for oral use. Contamination by *Salmonella* Species and *E. coli,* in whatever amounts, would render these products unfit for human consumption. There was 100% compliance to microbial load, however, 22.0% of the samples did not meet pharmacopoeial requirements of absence of high-risk microbes, S*almonella* species and *Escherichia coli* (USP NF, 2005). *Salmonella* species was the most frequently isolated bacteria from 19.5% of the samples while *Rhizopus* and *Fusarium* spp formed majority of the perfect fungal isolates from 9.7 % of the samples. *Escherichia coli* and *Salmonella* are normally associated with infections such as diarrhea, urinary tract infections (UTI), pneumonia and typhoid fever (Lessnau and Bronze, 2010).

The presence of several bacteria and fungi in the analyzed samples in this study may be due to the methods of their preparation or the equipment and materials used in preparing the ginseng containing samples. Other possible sources of contaminants are the personnel(s) that could introduce the microbes when handling the raw materials during processing. Therefore, the process of harvesting, drying, storage, handling and the soil influence the microbial contamination of raw material which in turns affects the entire quality of the finished herbal preparation. Thus manufacturers should ensure the highest possible level of hygiene during manufacturing so as to maintain correct quality of the final herbal preparations (Abba *et al.,* 2009).

Gram negative organisms which were the majority of isolates in this study are pathogenic and majority are associated with feacal contamination (Adenike *et al*. 2007). Presence of *E. coli* may indicate a possible presence of harmful disease-causing organisms since the bacteria constitute the intestinal flora of humans and other animals, and are therefore used as indicator organisms and as an index of possible contamination by human pathogens. The significance of faecal bacteria is that if these specific bacteria are present then other harmful microorganisms may also be present, such as *Salmonella* (Abba *et al.,* 2009).

Staphylococcus aureus isolated and identified in this study is a member of the normal flora. However, higher contamination by these bacteria has been associated with a number of complications especially in immune-compromised individuals. It disables the immune system, destroying tissues and releases exotoxins which cause gastroenteritis, toxic shock syndrome and scalded skin syndrome (Gladwin and Trattler 2004). It has also been associated with pneumonia, osteomyelitis, acute bacterial endocarditis, septic arthritis and urinary tract infections.

Klebsiella pneumonia, a Gram negative lactose fermenting bacterium found in the normal flora of the mouth is ranked second to *E. coli* for urinary tract infections in older people and also an opportunistic infection of people with weakened immunity. It is also a common cause of pneumonia. *Pseudomonas aeruginosa* also a widespread microorganism in soil, water and plants is pathogenic to humans and a common cause of nasal-comical infections such as pneumonia and also UTI. Though rare to healthy individuals, it is life threatening to immuno-compromised people (Lessnau and Bronze, 2010).

Fusarium is also a fungus common in soils and plants. Though most of its species are harmless saprobes and abundant members of the soil microbial community, it has been associated with mycotoxins at lower temperatures; it can also cause allergies and asthma in immunocompromised individuals (WHO, 1999). *Rhizopus* spp. is frequently found in house dust, soil, fruits, nuts and seeds. Exposure to large numbers of this fungus has been reported as cause of respiratory complications. It is also an opportunistic pathogen for immunocompromised individuals. People with diabetes, malnutrition and severe burns are also at risk. The fungus *Candida* spp. also isolated and identified in this study is the cause of many infections. *Candida albicans* causes mouth, skin, vaginal and penile infections. In rare occasions, it has been traced in the blood stream causing infections to vital organs especially in leukemia and HIV/ Aids (Dugdale, 2009).

These findings agreed with those of another study by Tournas *et al*., (2006), who analyzed forty six samples, and concluded that most ginseng products were contaminated with moulds and yeast as well as aerobic bacteria. A Study of microbial contamination in concentrated Chinese medicine by Ku *et al*., (1994) established that crude drugs such as Ginseng Radix, Glycyrrhizae Radix and Zingiber Rhizoma had serious contamination. This study also corroborates the findings of Esimone *et al.,* (2007) and Dnyaneshwar and

Bhushan (2005) who found *Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli, Salmonella spp.*, and *Pseudomonas aeruginosa* as major bacterial contaminants of herbal medicines sold and marketed in Nigeria.

Out of 36 samples that were analyzed for presence of ginseng, 83.3% were positive for either Rb1 or Rg1 group of ginsenosides. Broadly, 19 (52.7%) samples were identified to have the Rb1 ginsenoside, 24 (66.6%) contained the Rg1 ginsenoside and 17(47.2%) had both Rb1 and Rg1. These results demonstrated that similar investigations can be done within our local setting and with the resources available. Secondly, it was noteworthy that the results obtained in this study were similar to those found in other studies elsewhere. In one such study in the USA, twenty five commercial ginseng samples were analyzed for levels of ginsenosides (Harkey *et al.,* 2001). This study showed that all the products were correctly identified as per botanical plant species (*Panax* spp); however, concentration of ginsenosides differed significantly by 15-36 folds from the labeled amounts.

Further, the R_f values for ginsenosides contained in some of the samples in this study were similar to those obtained in another one by Courthout *et al*., (1999) though under different experimental conditions (Table 3.18).

This study was limited to identification because of lack of reference standards for all the eight common ginsenosides. Furthermore, there were no columns for a solid phase extraction (SPE) step coming immediately after the extraction of ginsenosides. A solid phase extraction (SPE) step would have removed interfering components before densitometric analysis (Corthout *et al.,* 1999; Li *et al.,* 1996). However, this study was able to show that 5(13.9%) samples did not contain any ginsenosides. These results were critical because ginsenosides are thought to be the active ingredients hence their chemical

standardization guarantees pharmacological activity of ginseng products (Harkey *et al.,* 2001). Absence of ginsenosides meant that these samples were counterfeits.

Whereas ginseng if used as unprocessed herb may be safe and effective as shown in numerous studies, ginseng containing products formulated together with vitamins, minerals and other herbs, as was the case in this study, should go through a registration process under a Drug Regulatory Authority. A registration process where the manufacturing plants and finished ginseng containing product must conform to cGMP requirements. For market authorization, submissions should include the manufacturing methods and analytical control procedures performed on raw materials used in the manufacturing process. Other than using this information to grant market authorization, medicine regulatory authorities will be able to carry out pharmacovigilance activities.

In the UK for instance, most herbal medicines remained as unlicensed herbal remedies up to the year 2011 by which all were to be registered. There were no quality standards for unlicensed herbal remedies. The same applies to South Africa where there is only a draft policy on African Traditional Health. There are no prescribed quality standards for herbal medicines. In the USA, herbs are defined as dietary supplements, and manufacturers can therefore produce, sell, and market herbs without first demonstrating quality, safety and efficacy, as is required for pharmaceutical drugs. The case in Thailand is different since there is proper regulation of herbal products but compliance to current GMP requirements is voluntary. The study did not establish the registration status of the samples that originated from Thailand. It is therefore important that quality standards of herbal medicines be enforced by PPB to safeguard public healthy.

3.9 Conclusion

This study established that ginseng products were found in medium to high income neighborhoods of Nairobi County, especially Westlands and Starehe Districts and that they were relatively expensive with a unit pack costing 15 to 100 USD. These findings agreed with other studies conducted elsewhere such as those reported by Liberti and Der Marderosian (1978), Wang *et al.,* (2001). It was suggested that these products should be regulated to avoid counterfeiting and adulteration.

The study also showed that ginseng containing products were poorly understood by the attendants at selling points and were not scheduled by the Pharmacy and Poisons Board. About 40% of the samples were 80 – 100 % compliant to all WHO labeling requirements for market authorization. A majority of samples (59.5%) failed to comply with WHO labeling requirements for market authorization. A significant number (22%) was contaminated by *Salmonella* species and *E. coli*, bacteria that must not be present in oral formulations. This study also revealed that 83.3% were positive for ginseng while 16.7% of the samples did not contain ginseng.

Lack of regulation for ginseng products is likely to expose the public to some health risks, a situation that is worsened by the fact that the attendants in the facilities had a poor understanding of the pharmacological properties and side effects of ginseng. The present study was significant and unique because it concerns quality and use of herbal medicines in Kenya. The findings of this study are invaluable to policy makers and the Pharmacy and Poisons Board as a stimulant to strengthen the regulatory framework for the quality and practice of herbal medicines in Kenya.

3.10 Recommendations

From the foregoing, it is discernible that further studies need to be conducted to establish the distribution and use of processed herbal products in Kenya and their value of trade.

This study also provides us with an opportunity to re-examine training curriculum for the professional cadres involved in the practice of herbal medicines especially for pharmaceutical technologists and pharmacists. With respect to quality of ginseng containing products, more research for assay of ginsenosides, dissolution profile and disintegration of some dosage forms, contamination by heavy metals and pesticides residues is needed. The study affirms the need for regulation of herbal medicines so that their quality, safety and efficacy can be assured. Herbal medicines require regulation with respect to product development, quality control of finished products and their safety. Meanwhile, the study has brought out the need to strengthen pharmacovigilance activities in order to safeguard the public against poor quality herbal products.

REFERENCES

Abba D., Inabo H.I., YakubuS.E. and Olayeni S Olonitola O.S.(2009): Contamination of Herbal Medicinal Products Marketed in Kaduna Metropolis with Selected Pathogenic Bacteria. *Afr. J. Trad. Compl. Alt. Med*., 6(1): 70–77.

Adenike O., Adewoyin A. and Oluwatoyin 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.

Amann R.I., Ludwig W. and Schleifer K.-H., (1995): Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.,* 59: 143–169.

Andrew M., (2000): The use of 16SrDNA methods in soil microbial ecology. *Braz. J. Microbiol.,* 31: 77-82.

Balch, P., (2006): Prescription for Nutritional Healing, New York, NY: Penguin Group. ISBN-10-1583332367, ISBN-13-1583332368.

Banthorpe D.V., (1994): Terpenoids. Natural Products: Their Chemistry and Biological Significance, Mann, J., Ed., Longman Scientific and Technical: Harlow, Essex, England, 1994: 289-359.

Benzi G. and Ceci A., (1997): Herbal Medicines in European Regulation. *Pharmacol. Res.,* 35: 355-362.

Betancourt D.A., Loveless T.M., Brown J.W. and Bishop P.E., (2008): Characterization of Diazotrophs Containing Mo-Independent Nitrogenases, Isolated from Diverse Natural Environments. *Appl. Environ. Microbiol*, 74 (11):3471-3480.

Biomeriex Vitek, Inc, 20 E analytical profile index, enterobacteriaceae and other gram-negative bacteria, 10th edition.

Bonfill M, Casals I, Palazon J, Mallol A and Morales C., (2002): Improved high perfomance liquid chromatographic determination of ginsenosides in Panax ginseng-based pharmaceuticals using a diol column. *Biomed.Chromatogr.* 16: 68-72.

Breemen R.B., Harry H.S., Fong and Farmsworth, N.M., (2007): The Role of Quality Assurance and Standardization in the Safety of Botanical Dietary Supplements. *Chem. Res. Toxicol.*, 20 (4): 577-582.

Brevoort P., (1998): The booming US botanical market-a new overview. *Herbalgram*, 44: 33-46.

British Pharmacopeia, (2008): The stationery office, London, 995-997. ISBN-13: 9780113227501, ISBN-10: 0113227507

Bruce S.K., Schick D.G., Tanaka L., Jimenez E.M., and Montgomerie J.Z**.,** (1981): Selective medium for isolation of *Klebsiella pneumoniae*. *J. Clin. Microbiol.*, 13(6):1114-1116.

Bumsik H., Young H. J., Jun H.H., Yeul K.I. N. and Tai Y. A., (2002): A Double-Blind Crossover Study Evaluating the Efficacy of Korean Red Ginseng in Patients with Erectile Dysfunction: A Preliminary Report. *J. Urology,* 168 (5): 2070-2073.

But P.P.H., Hu S.Y. and Cao, H., (1995): The ginseng plant; products and quality. P. 44-49. In: W.G. Bailey, C. Whitehead, J.T.A. Proctor, and J.T. Kyle, Proc. Int. ginseng conf. Vancouver1994, Canada.

Cappuccino and Sherman (2004): Microbiology, a Laboratory Manual; Pearson Education Inc. Sate University of New York.

Chan T.W.D., But P.P.H., Cheng S.W., Kwok I.M.Y., Lau F. W. and Xu H.W., (2000): Differentiation and Authentication of *Panax ginseng*, *Panax quinquefolius*, and Ginseng Products by Using HPCL/MS. *J. Anal. Chem.*, 72 (10): 2329-2329.

Choi K.T., Park J.C., and Ahn I.O., (1995): Characteristics of the growth and suspension of ginsenosides in the suspension-cultured cells of the Korean ginseng (*Panax ginseng*, C.A.Meyer), Proc. Int. Ginseng Conf, Vancouver 1994, Canada, 259-269.

Chung S.H., Choi C.G., and Park S.H., (2001): Comparisons between white ginseng radix and root for anti-diabetic activity and mechanism in KKAy mice. *Arch. Pharm. Res.* 24(3): 214-218.

ConsumerLab, (2000): Independent tests of ginseng. http://www.consumerlab.com/index.asp

Corthout J., Naessens T., Apers S. and Vlientinck A.J., (1999): Quantitative Determination of Ginsenosides from Panax ginseng roots and ginseng preparations by Thin Layer Chromatography-Densitometry. *J. Pharm. Biomed. Anal.,* 21**:** 187-192.

Cui X., Lo C., Yip K., Dong T. and Tsim K., (2003): Authentication of *Panax notoginseng* by 5s-Rrna spacer domain and random amplified polymorphic DNA (RAPD) analysis. *Planta Med*. 69: 584-586.

Cui Y., Shu X.O., Gao Y.T., Cai H., Tao M.H.and Zheng, W., (2006): Association of Ginseng Use with Survival and Quality of Life among Breast Cancer Patients. *Am. J. Epidemiol.,* 7**:** 645-653.

D'Angelo L., Grimaldi R., Caravaggi M., Marcoli M., Perucca E., Lecchini S., Frigo G.M., and Crema A., (1986): A double-blind, placebo-controlled clinical study on the effect of a standardized ginseng extract on psychomotor performance in healthy volunteers. *J. Ethnopharmacol.***,** 16 (1):15-22.

Dharmananda S., (2002): The Nature of Ginseng: From Traditional Use to Modern Research. *Internet Journal of the Institute for Traditional Medicine and Preventive Health Care*. http://www.itmonline.org/arts/ginsengnature.htm

Dixon P., (1976): Ginseng; Duckworth & Co: London. ISBN-10-071561007H.

Dnyaneshwar W. and Bhushan P. (2005): Botanicals: Quality and regulatory issues. *J. Sci. Ind. Res.* 64: 83-92.

Dubic M.A., (1986): Historical perspective on the use of herbal preparations to promote health. A paper presented to the 69th Annual Meeting of the Federation of American Societies for Experimental Biology, Anaheim, C.A. April 21-26, 1985.

Dugdale D.C., **(**2009): Candida Esophagitis. www.healthscout.com/ency/6813/main.html

Ebel S., Gigalke H.J. and Voelkl S. (1978): AMDHPTLC Analysis of Medicinal Plants.Proceedings of 4th International Symposium of Instrumental HPTLC, Selvino/Bargamo, Italy, 113.

Esimone C.O., Ibezima E.C. and Oleghe P.O., (2007): Gross microbial contamination of herbal medicinal products marketed in Mid Western Nigeria. *Int. J. Mol. Med. Adv. Sci 3(2): 87-92.*

European Medicine Agency Inspection, (2006): Guidelines on Quality of Herbal Medicinal Products, 14-17.

European pharmacopoeia (2005): 5th Edition, council of Europe, European Directorate for the Quality of Medicines (EDQM), Strasbourg.

Evans W.C., (2007): Saponins, cardioactive drugs and other steroids. Trease and Evans, 15TH Edition, Elservier Ltd, London, 295-297.

Forles B. A., Sahm D. F. and Weissfield A. S., (1994): Balley and Scott's diagnostic Microbiology 10th edition. Elsevier Ltd, ISBN-978-0-323-08330-0

Fournier A.R., Proctor J.T., Gauthier L., Khanizadeh S., Belanger A. and Doras M., (2003): Understanding effect of light on root ginsenosides in forest grown *Panax quinquefolius. Phytochemistry*, 63: 777-782.

Fuzzati N., Gabetta B., Jayakar K., Pace R. and Peterlongo F., (1999): Liquid chromatography-electrospray mass spectrometric identification of ginsenosides in Panax ginseng roots. *J. Chromatogr. Anal.*, 854: 69-79.

Gladwin M. and Trattler B., (2004): Clinical microbiology made ridiculously simple, 3rd edition, Medmaster Publishers. ISBN-10: 094-0780496, ISBN-13: 978-0940780491.

Gracias S.K. and McKillip J.L., (2004): A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Can. J. Microbiol.,* 50 (11): 883-890.

Haijiang Z.W., Yongjiang W. and Yiyu C**.,** (2003): Analysis of SHENMAI Injection by HPLC/MS/. *J. Pharm. Biomed. Anal.***,** 31: 175-183.

Harkeys M.R., Henderson G.L., Gershwin M.R., Stern J.S and Hackman, R.M., (2001): Variability in commercial ginseng products: an analysis of 25 preparations .*Am. J. Clin. Nutr.,* 73 (6): 1106-1106.

Health Protection Agency UK, (2009): Salmonella Reference Unit. www.hpa.org.uk

Hiai S., Oura H., Hamanaka H. and Odaka Y., (1975): A colour reaction of panaxadiol with vanillin and sulphuric acid. *Planta Med*. 28: 363-369.

Homeherbs (2008): Asian Ginseng (Panax ginseng) Medicinal, Historical and Botanical Information http://www.panax-ginseng.co.uk.

Hosettman K. and Marston A., (1995): Analysis and Isolation. Chemistry and Pharmacology of Natural Products; Cambridge University Press: New York, 122-174.

Hu S. and Kitts D.D., (2001): Free radical scavenging capacity as related to antioxidant activity and ginsenoside composition of Asian and North American ginseng extracts. *J. Am. Oil Chem. Soc.*, 78: 249-255.

KEMRI, (2003): Pelvic Inflammatory Disease (PID) Laboratory manual, KEMRI.

Kenya National Bureau of Statistics (KNBS), 2007: Basic report on well-being in Kenya: Based on Kenya Integrated Household Budget Survey-2005/2006.

Kiefer D. and Pantuso T., (2003): Panax Ginseng. *Am. Fam. Physician*, 68(8): 1539-1542.

Indian Pharmacopoeia, (2008): A publication of the Government of India, Delhi, 9-10.

Kim W.Y., Kim J.M., Han S.B., Lee S.K., Kim N.D., Park M.K., Kim C.K. and Park J.H, (2000): Steaming of ginseng at high temperature enhances biological activity. *J. Nat. Prod.*, 63: 1702-1704.

Kim Y.K., Guo Q. and Packer L., (2002): Free radical scavenging activity of red ginseng aqueous extracts. *Toxicology*, 172: 149-156.

Kimura L., Nakashima N., Sugihara Y., Fu-jun C. and Kimura M., (1999): The antihyperglyceamic blend effect of traditional Chinese medicine byakko-ka-ninjin-to on alloxan and diabetic KK-CA(y) mice. *Phytother. Res.*, 13: 484-488.

Kimura S., (1997): Use of the radiation method for sterilization of herbal medicines. *J. Antibiot. Japan,* 25: 475–479.

Kite G.C., Howes M.J., Leon C.J. and Simmonds M.S., (2003): Liquid chromatography/mass spectroscopy of malonyl- ginsenosides in the authentication of ginseng. *Rapid Commun. Mass Sp.*, 17: 238-244.

Kitts D.D. and Hu C., (2001): Efficacy and safety of ginseng. *Public Health Nutr.,* 3: 473-485.

Kitts D.D. and Popovich D.G. (2003): Ginseg: In Performance functional foods, Watson, D., Ed., Woodhead Publishing Ltd., New York, 78-88.

Kitts D.D., Wijewickreme A.N. and Hu C., (2000): Antioxidant properties of a North American ginseng extract. *Mol. Cell. Biochem.,* 203: 1-10.

Kombo D.K. and Tromp D.L.A., Proposal and Thesis Writing, Pauline Publications Africa, Nairobi, 2006.

Krochmal R., Hardy M., Boweman S., Wang Q.L., Elashoff R.M. and Heber D., (2004): Phytochemical Assay of Commercial Botanical Dietary Supplements. *Evid-based Comp. Alt.* 3:305-313.

Ku Y.R., Chou L.M., Jang C.F., Liu, Y.C. Lin, J.H. and Wen, G.C., (1994): Study of microbial contamination in concentrated Chinese medicine. *J. Food and Drug Anal.,* 2(1): 49-62.

Kwon S.W., Han S.B., Park I.H., Kim J.M., Park M.K. and Park J.H., (2001): Liquid chromatographic determination of less polar ginsenosides in processed ginseng. *J. Chromatogr. A,* 921: 335-339.

Lee F.C., (1992): Facts about Ginseng, the Elixir of Life. Hollym Intern. Corp. Elizabeth, New Jersey.

Lee J. and Yuqing Z., (2009): Current evaluation of millennium phytomedicine-Ginseng: Etymology, Pharmacognosy, Phytochemistry, Market and Regulation. *Curr. Med. Chem*.16: 2475-2484.

Lee S.J., Ko W.G., Kim J.H., Sung J.H., Moon C.K. and Lee B.H., (2000): Induction of apoptosis by a novel intestinal metabolite of ginseng saponin via cytochrome c-mediated activation of capase-3 protease. *Biochem. Pharmacol.,* 60: 677-685.

Leininger D.J., Roberson J.R and Elvinger F., (2001): Use of eosin methylene blue agar to differentiate *Escherichia coli* from other gram-negative mastitis pathogens. *J. Vet. Diagn. Invest.,* 13: 273-275.

Lessnau K. and Bronze M.S (2010): *Pseudomonas aeruginosa* Infections. http://emedicine.medscape.com/article/226748.

Li T.S, and Wardle D. (2002): Seasonal fluctuations of leaf and weight and ginsenoside contents of 2, 3 and 4 year old American ginseng plants. *Hort. Technology*, 12: 229-231.

Li T.S., Mazza G., Cotrell A.C. and Gao L., (1996): Ginsenosides in Roots and Leaves of American Ginseng. *J. Agr. Food Chem.*, 44: 717-720.

Liberti L., and Der Marderosian, A., (1978: Evaluation of Commercial Ginseng Products. *J. Pharm. Sci,*, 67: 27-38.

Liu W.K., Xu S.K. and Che C.T., (2000): Anti-proliferative effect of ginseng saponins on human prostate cancer cell lines. *Life sci.*, 67: 1297-1306.

Liu Z.Q., Luo X.Y., Sun Y.X., Chen Y.P. and Wang Z.C., (2002): Can ginsenosides protect human erythrocytes against free-radical-induced hemolysis? *Biochem. Pharmacol.* 1572: 58-66.

Ludwiczuk A., Chmielewska E.W. and Wolski T., (2006): Localisation of ginsenosides in *Panax quinquefolium* root tissues. *Acta Agrobot*, 59: 7-15.

Lui J.H. and Staba E.J., (1980): The ginsenosides of various ginseng plants and selected products. *J. Nat. Prod.*, 43: 340-346.

Lyons A. and Petrucelli J.R., (1978): Medicines: An Illustrated History, H.N. Abrams: Baldwin, New York, USA, 604-607.

Maddocks S., Olma T and Chen S., (2002): Comparison of CHROMagar Salmonella Medium and Xylose-Lysine-Desoxycholate and Salmonella-Shigella Agars for Isolation of *Salmonella* Strains from Stool Samples. *J. Clin. Microbiol.*, 40 (8): 2999-3003.

Marcus D.M. and Grollman, A.P., (2002): Botanical Medicines—the need for new regulations. *New Engl. J. Med.,* 31(25):2073-6.

Meier B., Meir-Bratsch A., Dallenbach-Tolke K. and Sticher O., (1985): Quantitative analysis of ginseng by HPLC. In Advances in Chinese Material Research, Chang H.M., Yeung H.W., Tso W.W., Koo A. Edition; World Scientific Publication Co. Singapore, 471-484.

Michael J. J and Sharon A, L., (2002): Bacterial Identification for Publication. *J. Clin. Microbiol.* 40: 6, 1887-1891.

Ministry of Medical Services and Ministry of Public Health, (2010): Session Paper on National Pharmaceutical Policy, Gov. Printers.

Mitullah W., (2003): The challenge of slums. A Case study of Nairobi. UN Habitat Gliobal Report on Human Settlement. Londres, Earthscan.

Mugenda M.O., Mugenda A.G., (2003): Research Methods, Quantitative and Qualitative Approaches, African Center for Technological Studies, Nairobi.

Mukherjee P.K., (2002): Quality Control of Herbal Drugs, Business Horizons, New Delhi, 2.

Mwangi J. W., Mungai N.N., Thoithi G.N. and Kibwage, I.O., (2005): Traditional herbal medicine in national healthcare in Kenya. *East Cent. Afr. J. Pharm.Sci.,* 8 (2): 22-26.

Ni W.,Zhang X., Wang B., Chen Y., Han H., Fan Y., Zhou Y. and Tai G., (2010): Antntitumor activities and immunomodulatory effects of Ginseng neutral polysaccharides in combination with 5- Fluorouracil. *J. Med. Food*, 13 (2): 270 – 277.

Oh M., Choi Y.H., Choi S., Chung H., Kim K., Kim S.I. and Kim. D.K., (1999): Anti-proliferative effects of ginsenosides Rh2 on MC-7 human breast cancer cells. *Int. J. Oncol.*, 14: 869-875.

Osaki A.L and Kennelly E.J., (2003): Phytoestrogens: A review of the present state of research. *Phytother. Res.,* 17 (8): 845-869.

Owen R.T., (1981): Ginseng: A pharmacological profile. *Drugs of today*, 17:343–351.

Park I.H., Piao L.Z., Kwon S.W., Lee Y.J., Cho S.Y., Park M.K. and Park J.H., (2002): Cytotoxic dammarane glycosides from processed ginseng. *Chem. Pharm. Bull.,* 50: 538-540.

Persons W.C., (1995): American ginseng farming in its woodland habitat 78-83. Int. Ginseng Conf.Vancouver, 1994.

Pharmacy and Poisons Board (2006): Data on Pharmaceutical Trade in Kenya for 2005. www.pharmacyboardkenya.org

Pharmacy and Poisons Board (2009(a)): Public Alert on '*Herbal* Contraceptives'. www.pharmacyboardkenya.org.

Pharmacy and Poisons Board (2009(b)): Lists of registered premises for pharmacists and pharmaceutical Technologists. www.pharmacyboardkenya.org.

Pharmacy and Poisons Board (2010 (a)): Registration of Herbal and Complementary Medicines; Guidelines for Submission of Applications. www.pharmacyboardkenya.org.

Pharmacy and Poisons Board (2010 (b)): Registration of Drugs: Guidelines to Submission of Applications. www.pharmacyboardkenya.org

Pharmacy and Poisons Board (2011): List of Registered Medicines. www.pharmacyboardkenya.org

Popovich D.G. and Kitts D.D., (2002): Structure-function relationship exists for ginsenosides in reducing cell proliferation and inducing apoptosis in human leukemia (THP-1) cell line. *Arch. Biochem. Biophys*., 406: 1-8.

Popovich D.G. and Kitts D.D., (2003): Bioactive properties of ginseng and ginsenosides constituent. *Mol. Cell. Biochem*, 1: 137-149.

Preston N.W. and Morrel A., (1962): Reproductive results with Gram stain. *J. Pathol Bacteriol.* 84: 241-243.

Quiming N.S., Denola N.L., Soliev A.B., Saito Y.O. and Jino K., (2007): High performance liquid chromatographic separation and quantitative analysis of

ginsenosides using a polyvinyl alcohol-bonded stationary phase. *Chromatographia,* 66 (1): 5-11.

Rózylo J.K., Zabinska A., Matysiak J., and Niewiadomy A.J., (2002): HPTLC Method in Physicochemical Studies of a New Group of Antimycotic Compounds. *J. Chromatogr. Sci.,* 40: 581.

Saito H., (2000): Regulation of herbal medicines in Japan. *Pharmaco. Res*. 41, 5: 515-519.

Sandel M., (2004): Virulence and recovery of Staphylococcus aureus relevant to the food industry using improvements on traditional approaches. *Food Control*, 15 (1): 5-10.

Sang-Hyun P., Sangryeol R. and Dong-Hyun K**.,** (2010): Improved Selective and Differential Medium for Isolation of *Escherichia coli* O157:H7. *J. Clin. Microbiol.,* 41 (10): 839-841.

Saper B.R, Fletcher W.S, and Eamranond P., (2010): Overview of herbal medicines and dietary supplements. Up To Date. http://www.uptodate.com.

Saranadasa H., (2003): "The Square Root of N Plus One Sampling Rule: How Much Confidence Do We Have?" *Pharm. Technol.*, 27 (5): 50.

Scaglione F., Ferrara F., Dugnani S.,Falchi M., Santoro G., and Fraschini F., (1990): Immunomodulatory effects of two extracts of *Panax ginseng* C. A. Meyer. *Drugs Exp. Clin.* Res., 16: 537-542.

Shaheen M.A. and Taha M., (2006): Species identification of candid isolates obtained from oral lesions of hospitalized and non hospitalized patients with oral candiasis. *Egypt. Dermatol. Line J*., 2 (1): 1-11.

Shibata S., Tanaka O., Shoji J. and Saito H., (1985): Chemistry and Pharmacology of Panax. In Economic and Medicinal Plant Research, Wagner H, Edition; Academic Press: London; Orlando, FLA., 217-284.

Shinde V.M., Dwalwal K., Potdar M., Mahadik K.M.,(2009): Application of quality control principles to herbal drugs. *Int. J. Phytochem.,* 1: 4-8.

Shukla S.S. and Saraf S., (2009): Development of spectrophotometric fingerprinting method for *Talisadi churna. East Cent. Afr.J. Pharm. Sci.,* 12: 52-54.

Smyth R.W and Kahlmeter G., (2005): Mannitol Salt Agar-Cefoxitin Combination as a Screening Medium for Methicillin-Resistant *Staphylococcus aureus. J.Clin. Microbiol.,* 43 (8): 3797–3799.

Songlin L., Quanbin H., Chunfeng Q., Jingzheng S., Chuen L. C. and Hongxi X., (2008): Chemical markers for the quality control of herbal medicines: an overview. *Chin. Med.,* 3 (7): 1-23.

Sotaniemi E.A., Haapakoski E., and Rautio A., (1995): Ginseng therapy in non-insulin-dependent diabetic patients. *Diabetes Care* 18: 1373–1375.

Sundra- Rao and P.S.S., Richard J., (2006): Introduction to Biostatistics and Research Methods, 4th Edition, Printice Hall of India Private LTD. New Delhi.

Sutton S., (2006): Microbiology Network-Counting Colonies: Dedicated to improvement of regulatory science and compliance**.** info@microbiol.org-(585)**:** 594-8273.

Tanaka O. and Kasai R. (1984): Saponins of ginseng and related plants. In the Progress of organic natural products, Herz, W., Grisebach, H., Kirby, G.W., Tamm, C.H., Eds; Springer-Verag: New York, 2-75.

Tang W. and Eisenbrand G. (1992): Chinese Drugs of Plant Origin, Vol. 91, Springer: Berlin 711-737.

Torbeck L., (2009): Is the square root of (N) + 1 a statistically valid scheme? *Pharm. Technol.,* 33 (10): 128.

Tortora J.G, Funke B.R, Case C.L and Al-Mukhtar T., (2008): Microbiology: An Introduction, 10th Ed. Benjamin/ Cummings-ISBN-13: 978-0-321-55007-1 ISBN-10: 0-321-55007-2. San Francisco, California, USA.

Tournas V.H., Katsoudas E. and Miracco E.J., (2006): Moulds, yeasts and aerobic plate counts in ginseng supplements. *Int. J. Food Microbiol.,* 108 (2):178-181.

USP/NF, (2012): United States Pharmacopeia/ National Formulary, Twinbrook Parkway, Rockville. 1174-1188

Vaidya A.D.B. and Devasagayam T.P.A., (2007): Current Status of Herbal Drugs in India: An Overview. *J. Clin. Biochem. Nutr.,* 41 (1): 1–11.

Vives-Rego J., Lebaron P. and Von Caron G.N., (2000): Current and future applications of flow cytometry in aquatic microbiology. *FEMS Microbiol. Rev.,* 24: 429–448.

Wang J., Ha W., Ngan F., But P. and Shaw (2001): Application of sequence characterized amplified region analysis to authenticate *Panax* species and their adulterants. *Planta Med*. 67: 781-783.

Wang X., Sakuma T., Asafu-Adjaye G.K. and Shiu G.K., (1999): Determination of ginsenosides in plant *extracts from Panax ginseng* and *Panax quinguefolius* L. by LC/MS. *Anal. Chem.,* 71: 1579-1584.

WHO, (1991): (WHO/TRM/91.4):Guidelines for the Assessment of Herbal Medicines. Geneva.

WHO, (1998): Quality Control Methods for Medicinal Materials, Geneva, 22. http;//www.who.int/edicines/services/expertcommittee/pharmprep/QAS05-131Rev1-QCM Methods-med-Plant Material UpdateSep05.pdf.

WHO, (1999): Monograph on selected medicinal plants, Vo. 1, Geneva.

WHO, (2005): National Policy on Traditional Medicine and Regulation of Herbal Medicines- Report of a WHO Global Survey.

Wong F, Ping C and Amit B., (2011): Effects of acute supplementation of Panax ginseng on endurance running in hot and humid environment. *Indian J. Med. Res.,* 133, 1: 96-102.

Yat P.N., Arnason J.T. and Awang D.V.C., (1998): An improved extraction procedure for rapid quantitative high performance liquid chromatographic estimation of the main eleutheroside(B and E) in Eleutherococcus senticocus. *Phytochem. Anal.*, 9: 291-295.

Yip T.T., Lau C.N., But P.P. and Kong Y.C., (1985): Quantitative analysis of ginsenosides in fresh Panax ginseng. *Am. J. Chin. Med.,* 13: 77-88.

Yong L., Ming C., Gui C., Zheng W. and Zhi H.,(2004): Ruggedness/robustness evaluation and system suitability test on United States Pharmacopeia XXVI assay ginsenosides in Asian and American ginseng by high performance liquid chromatography. *J. Pharm. Biomed. Anal,* 3: 1083-1091.

Yun T.K., (2001): Brief introduction of *Panax ginseng* C.M. Meyer. *J. Kor. Med. Sci.,* 16: 3-5.

Yun T.K. and Choi S.Y., (1998): Non –organ specific cancer prevention of ginseng: a prospective study in Korea. *Int. J. Epidemiol.,* 27**:** 359-364.

Yun T.K., Choi S.Y., and Yun H.Y., (2001): Epidemiological study on cancer prevention by ginseng; are all kinds of cancer preventable by ginseng? *J. Kor. Med. Sci.*, 16: 19-27.

Zhou D. and Kitts D.D., (2002): Peripheral blood mononuclear cell production of NTF-alpha in response to North American ginseng stimulation. *Can. J. Physiol. Pharmacol.*, 80: 1030-1033.

APPENDICES

Appendix 1: Listed Herbal Products in Kenya

List adapted and modified from Pharmacy and Poisons Board (Kenya), 2011

NAME	COMPOSITION (No. of active ingredients)	THERAPEUTIC CLASSIFICATION	COUNTRY OF ORIGIN
Encof cough syrup	12	Cough syrup	India
Antarth soft gel	Several	Anti inflammatory	India
Spermon soft gel	Several	Skin ointment	India
Vigomax capsules	16	sexual dysfunction	India
Atochol tablets	1	Cholesterol booster	India
Healthy betas tablets	1	Anti-arthelo sclerotic	India
Guava syrup	3	Cough syrup	Egypt
Zecuf herbal cough remedy	11	Cough syrup	India
Zecuf lozenges	4	Cough lozenges	India
Malbet capsules	7	Anti protozoan	India
Burnactil cream	6	Tropical antiseptic	India
Moov ointment	4	Analgesic ointment	India
Krack SR cream	9	Antiseptic cream	India
Moscand mosquito repellant jelly	2	Repellant	Not stated
Indiaga for men capsules	7	Aphrodisia	South Africa
Stamiforte capsules	Several	Multivitamin/ nutritional suppliment	India
Hemon capsules	9	Hematinic	India
Hemo-perfecta tablets	17	Hematinic	India
Dermoluconil tablets	4	Not stated	India
Enzoy powder	3	Aphrodisia	Kenya
Sylovac – sf powder	2	Laxative	India
Zoom tablets	11	Aphrodisiac	India
Superliv concentrate premix	12	Procects from Mycotoxins and aflatoxins	India
Sylovac – sf powder	2	Laxative	India
Nikoff cough syrup	13	Cough Syrup	India

NAME	COMPOSITION (No. of active ingredients)	THERAPEUTIC CLASSIFICATION	COUNTRY OF ORIGIN
Haleezy tablets	6	for Bronchial asthma	India
Haleezy syrup	7	For Bronchial asthma	India
Paedritone Drops	1	Not stated	India
Aptizooom syrup	8	For anorexia	India
Livomyn syrup	1	Hepatoprotective	India
Livomyn tablets	17	Hepato-protective	India
Gum tone powder	20	For Gingivitis	India
Kofol syrup	14	Anti –inflammatory Broncho-dilator	India
M2 tone tablets	26	Menstrual disorder	India
M2 Tone syrup	Several	Menstrual disorder	India
Addyzoa capsules	Several	Menstrual disorder	India
Evanova capsules	Several	Peri menopausal syndrome	India
Gum tone gel	Several	Antifungal (Gingivitis)	India
Femiforte tablets	Several	Leucorrhoea Vaginitis	India
Arthrella tablets	Several	Rheumatoid arthritis	India
Hyponidd tablets	Several	Antidiabetic	India
Streroak premix	5	Immuno- modulator Antistress Adaptogen	India
Toxiroak premix	9	Mineral & toxin binder	India
Apdyl-h syrup	4	Antitussive	India
Femix herbal capsules	2	Sexual disorders in Women	India
Manix capsules	12	General restorative tonic	India
Kofol lozenges (regular)	5	Cough and sore throat	India

NAME	COMPOSITION (No. of active ingredients)	THERAPEUTIC CLASSIFICATION	COUNTRY OF ORIGIN
Kofol lozenges (honey & lemon)	5	Cough and sore throat	India
Kofol lozenges (pineapple)	5	Cough and sore throat	India
Hapenz syrup	6	Appetive stimulant	India
TIB tablets	Several	Anti- Retroviral	China
Arthrella ointment	Several	Masculoskeletal disorders	India
Kofol rub	Several	Nasal decongestant	India
Arshonyt ointment	Several	For Haemorroids	India
Arshonyt forte tablets	Several	For Haemorroids	India
Honitus syrup	Several	Antitussive	India
Canova liquid	Several	Analgesic Anti-inflammatory Antiviral Antiemetic	Botswana
Olbas oil drops	Several	Inhalant decogestant	UK
Olbas pastilles	Several	Soothing agent	UK
Kalmis tablets	Several	Stress Irritability Strains	UK
Nexacof tablets	14	Cough remedy	India
Imnohans	2	Immune enhancer	India
Nokof syrup	Several	Cough suppressant	India
Hormotex pills	Several	Aphrodisiac	Germany
Emami Mentho plus balm	7	Muscle relaxant & Analgesic	India

Appendix 2: Questionnaire

1. Premises Details

a) Name: ……

b) Nature of business: ……

c) Registration status: …….....

d) Physical address: …….

e) Postal Address: ……

2. Personal Details of the Attendant

a) Highest level of Education attained:

Primary □ Secondary □ College □ University□

b) Relevant Professional Training (If any): ……

c) Enrolment/Registration No. (If any): ……..

3. Product Details

a) Name……

b) Active Ingredient(s): ……

c) Manufacturer…… ….

d) Address…….....

e) Manufacturing Date…….

f) Expiry Date……

g) Batch No…….....

h) Formulation Details……

4. Product Classification

Please tick [√] the option that best fits the sell, control and use of the product in this outlet

a) 0=Prescription only…… □

b) 2=Over the counter…… □

c) 2=General sales…….. □

d) 0=All the above…….. □

5. Do you have any information about what this product contains?

a) 1=Yes …….□ b) 2=No……. □

6. Indications as Understood by the Attendant: Please choose the option that best describes the indications or the pharmacological effects of ginseng

a) Reduce fatigue, increase stamina and strengthen the general body conditions
b) Promotes formation of red blood cells and improves blood circulation
c) Promotes immunity and has ant-diabetic properties
d) All the above; a), b) and c)

7. (i) Does this product have any side effects?

a) 1=Yes …….□ b) 2=No……. □

(ii) If the answer to 7(i) above is yes, please choose the option that best describes the side effects

a) Causes dizziness, loss of libido or sexual desire and anaemia
b) Causes cancer if used for a long time
c) May lower blood sugar, increase blood pressure or increase lack of sleep
d) Is an immune suppressant, causes congestion and vaginal bleeding

8. (i) Do you receive any feedback from users of this product?

a) 1=Yes …….□ b) 2=No……. □

(ii) If the answer to 8(i) above is yes, please choose the option that best describes the user feedback:

a) 1=The product was not useful at all…… □
b) 2=The product was hardly useful…… □
c) 3=The product was somewhat useful…….. □
d) 4=The product was useful……... □
e) 5=The product was very useful…… □

Appendix 3: Interpretation of Chemical Reactions of Bacteria using API 20 E Strip

Tests	Active ingredients	Qty (mg/cup)	Reactions/ Enzymes	Results	
				Negative	Positive
ONPG	2-nitrophenile-βD-galactopyranoside	0.223	β-galactosidase (ortho NitroPhenyl-βD-galactopyranosidase)	Colorless	yellow (1)
ADH	L-arginine	1.9	Arginine DiHydrolase	Yellow	red/orange (2)
LDC	L-lysine	1.9	Lysine DeCarboxylase	Yellow	red/ orange(2)
ODC	L-ornithine	1.9	Ornithine DeCarboxylase	Yellow	red/orange(2)
CIT	trisodium citrate	0.756	CITrate utilization	pale-green/yellow	blue-green/ blue (3)
H_2S	sodium thiosulphate	0.075	H_2S production	colorless/ grayish	black deposit/ thin line
URE	Urea	0.76	UREase	Yellow	Red/ orange (2)
TDA	L-tryptophane	0.38	Tryptophane DeAminase	TDA/ immediate	
				Yellow	reddish brown
IND	L-tryptophane	0.19	INDole production	JAMES/immediate	
				colorless, pale-green/yellow	pink
VP	Sodium pyruvate	1.9	Acetoin production (voges proskauer)	VP 1 + VP 2 / 10 min	
				Colorless	Pink/red (5)
GEL	Gelatin (bovine origin)	0.6	GELatinase	no diffusion	diffusion of black pigment
GLU	D-glucose	1.9	Fermentation / oxidation (GLUcose) (4)	Blue / blue-green	Yellow / yellowish-green
MAN	D-mannitol	1.9	Fermentation / oxidation (MANnitol) (4)	Blue / blue-green	Yellow

Tests	Active ingredients	Qty (mg/cup)	Reactions/ Enzymes	Results	
				Negative	Positive
INO	Inositol	1.9	Fermentation / oxidation (INOsitol) (4)	Blue / blue-green	Yellow
SOR	D-sorbitol	1.9	Fermentation / oxidation (SORbitol) (4)	Blue / blue-green	Yellow
RHA	L-rhamnose	1.9	Fermentation / oxidation (RHAmnose0 (4)	Blue / blue-green	Yellow
SAC	D-sucrose	1.9	Fermentation / oxidation (SACcharose) (4)	Blue / blue-green	Yellow
MEL	D-melibiose	1.9	Fermentation / oxidation (MELibiose (4)	Blue / blue-green	Yellow
AMY	Amygdalin	0.57	Fermentation / oxidation (AMYgdalin) (4)	Blue / blue-green	Yellow
ARA	L-arabinose	1.9	Fermentation / oxidation (ARAbinose) (4)	Blue / blue-green	Yellow
OX	Oxidase	0.18	Cytochrome- Oxidase	No color change	Deep purple blue

(1) A very pale yellow should also be considered positive.

(2) An orange color after 36-48 h incubation must be considered negative.

(3) Reading made in the cupule (aerobic).

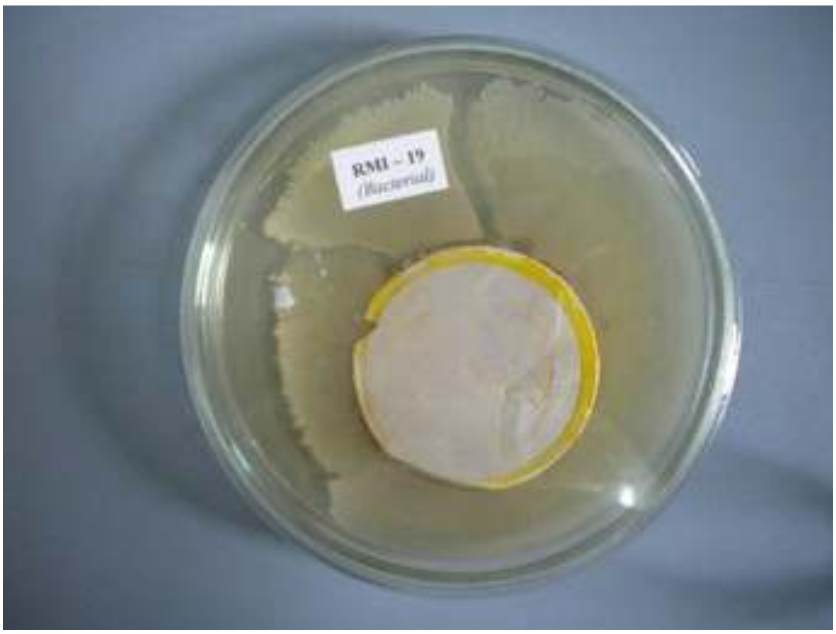
(4) Fermentation begins in the lower portion of the tubes, oxidation begins in the cupule.

(5) A slightly pink color after 10 minutes should be considered negative.

Table copied from biomerieux http://www.biomeriux.com

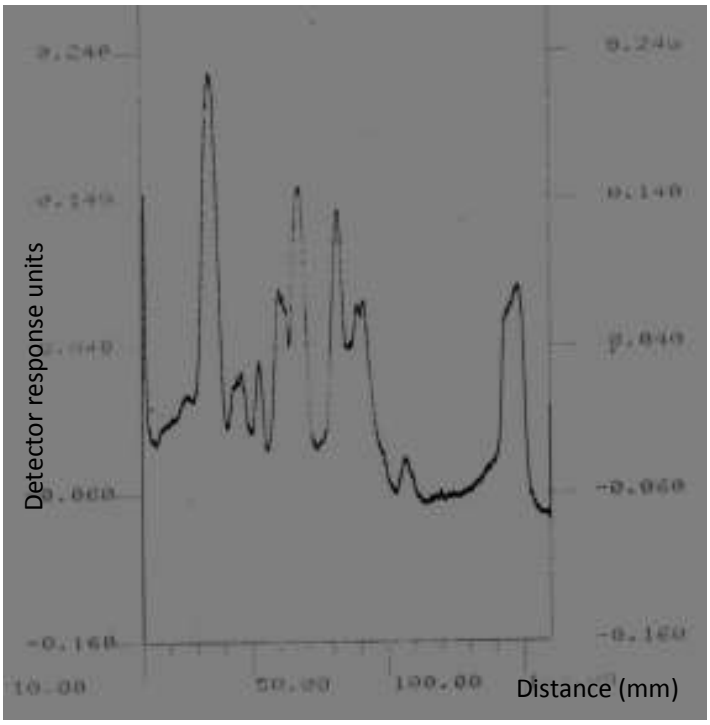
Appendix 4: Some Samples used in this Study

Appendix 5: Image of a Typical Ginseng Root used in this Study

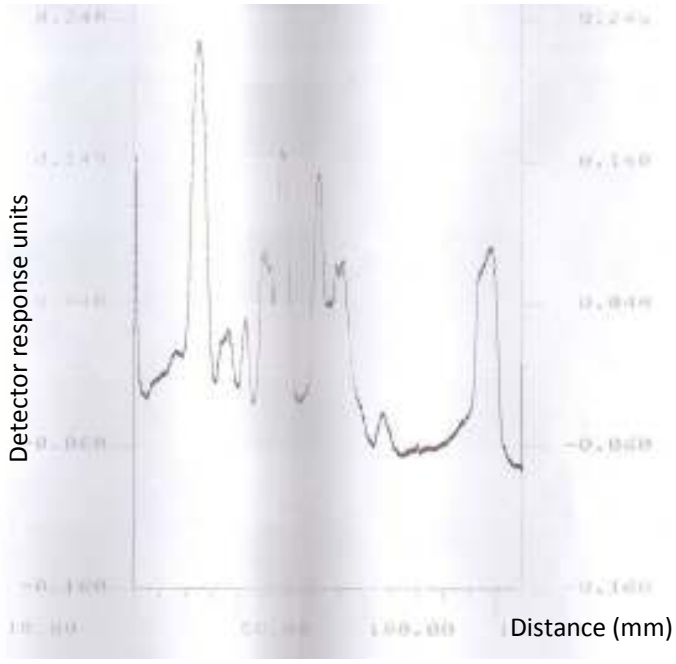


Appendix 6: Typical Bacterial Colonies of RMI-19

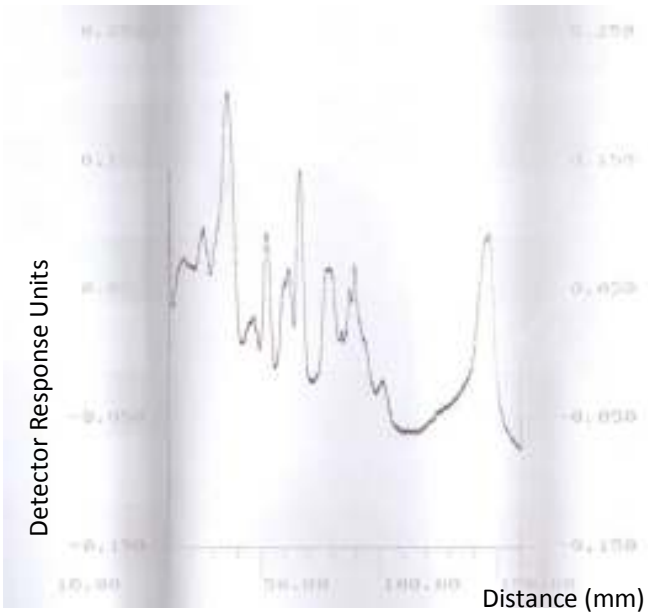
Appendix 7: Typical Fungal Colonies of RMI 37



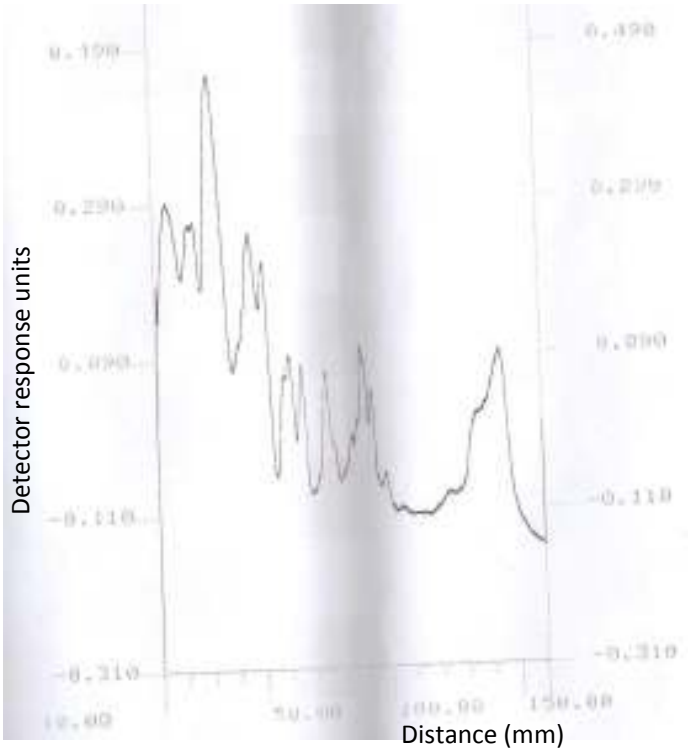
Appendix 8: TLC Densitogram for RMI 3



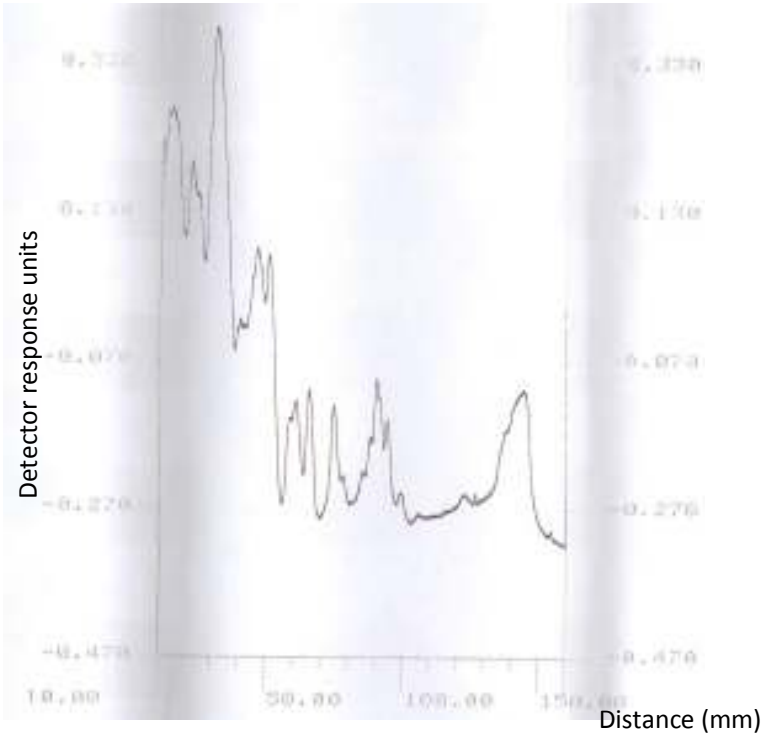
Appendix 9: TLC Densitogram for RMI 4



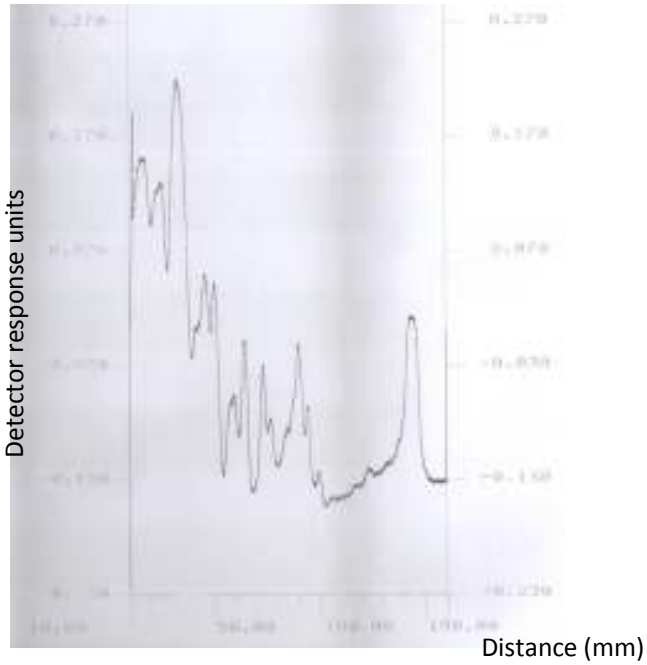
Appendix 10: TLC Densitogram for RMI 5



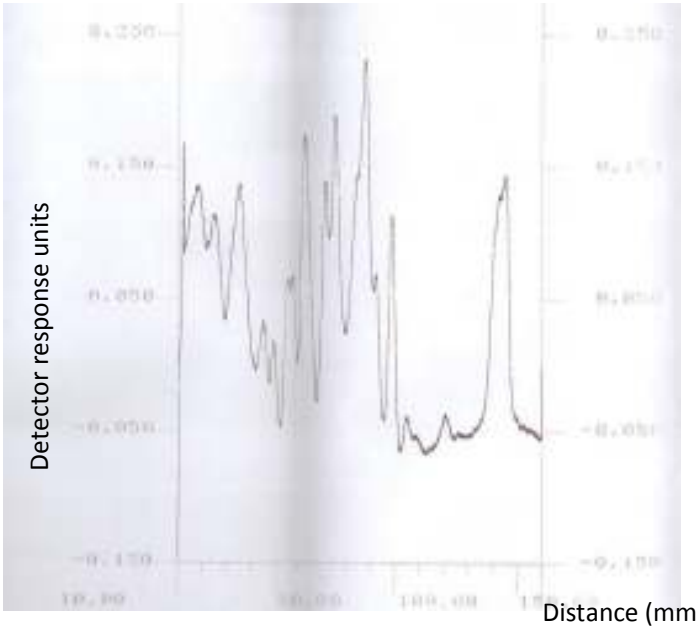
Appendix 11: TLC Densitogram for RMI 6



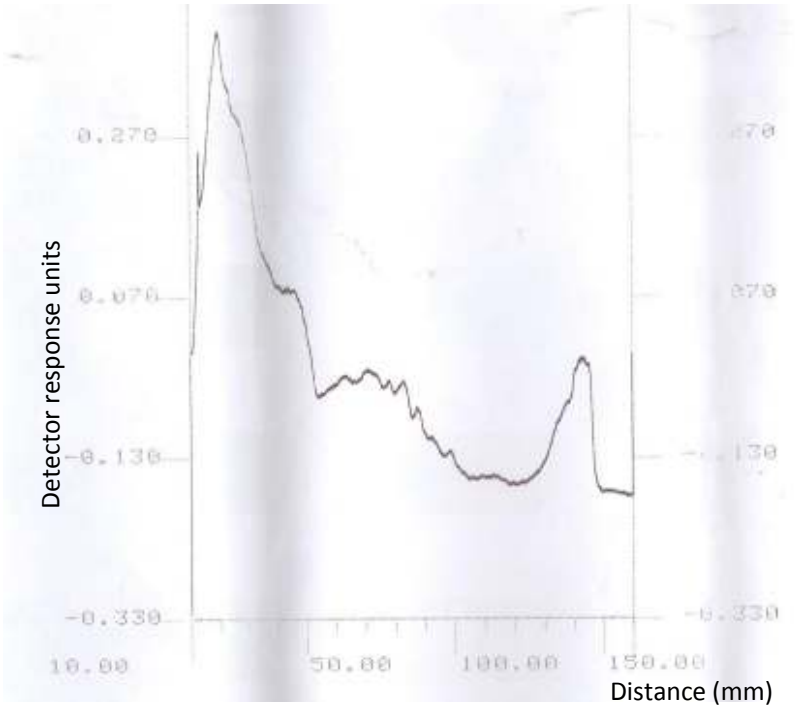
Appendix 12: TLC Densitogram for RMI 7



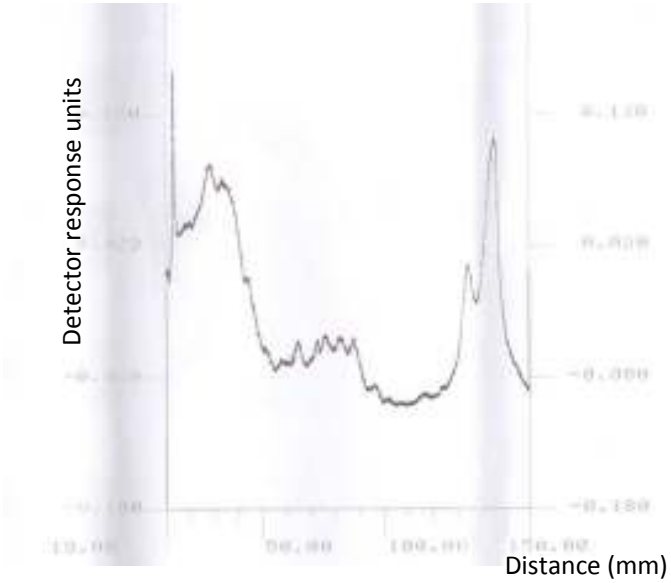
Appendix 13: TLC Densitogram for RMI 8



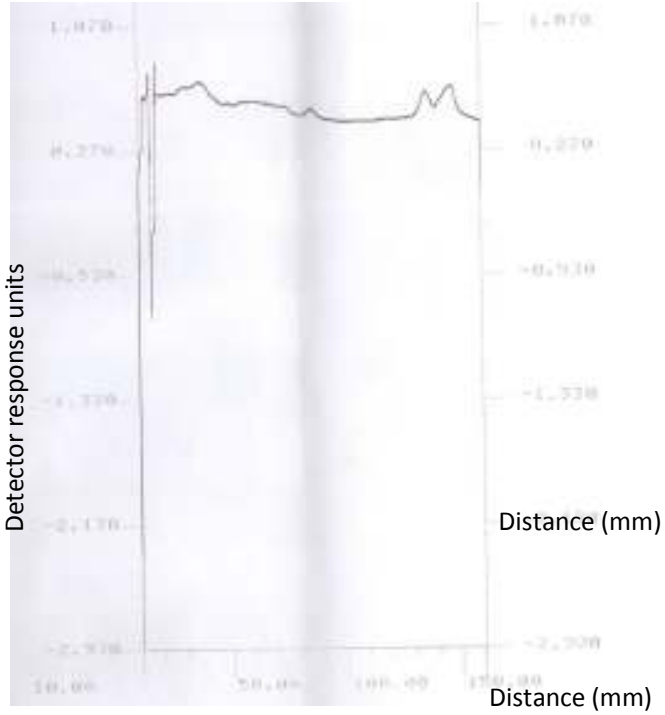
Appendix 14: TLC Densitogram for RMI 9



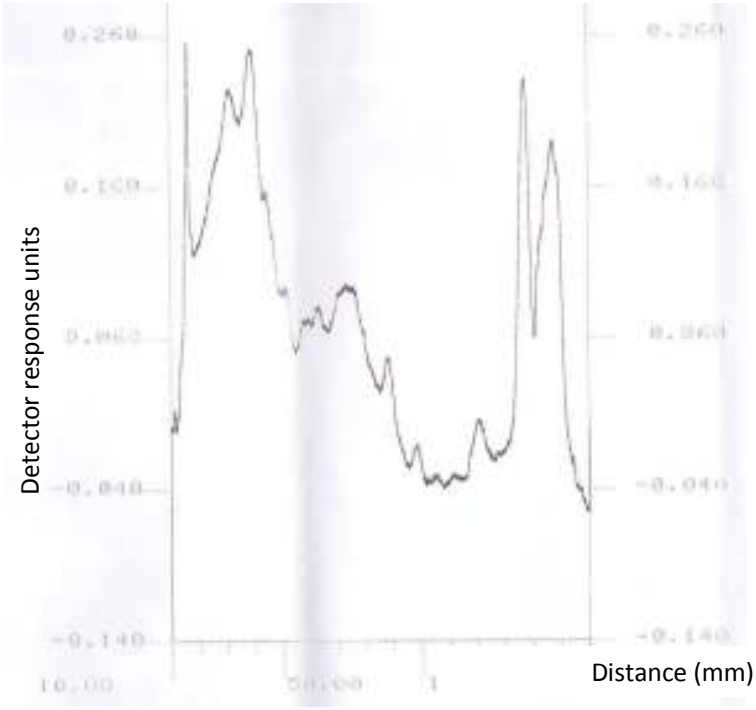
Appendix 15: TLC Densitogram for RMI 10



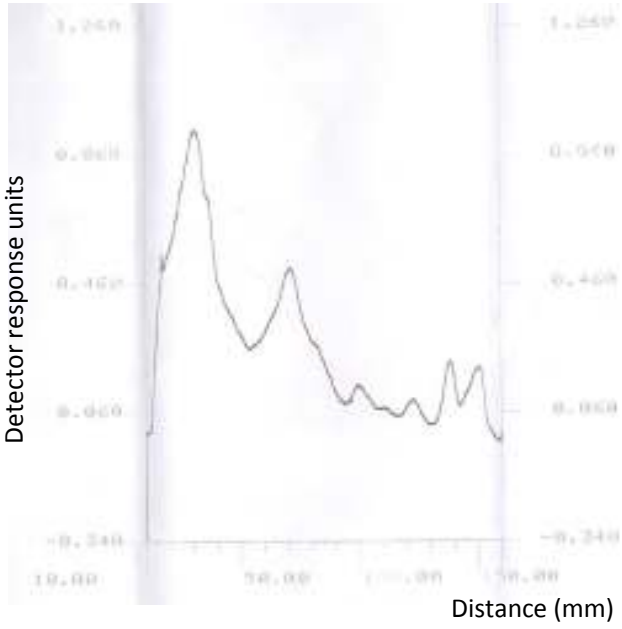
Appendix 16: TLC Densitogram for RMI 11



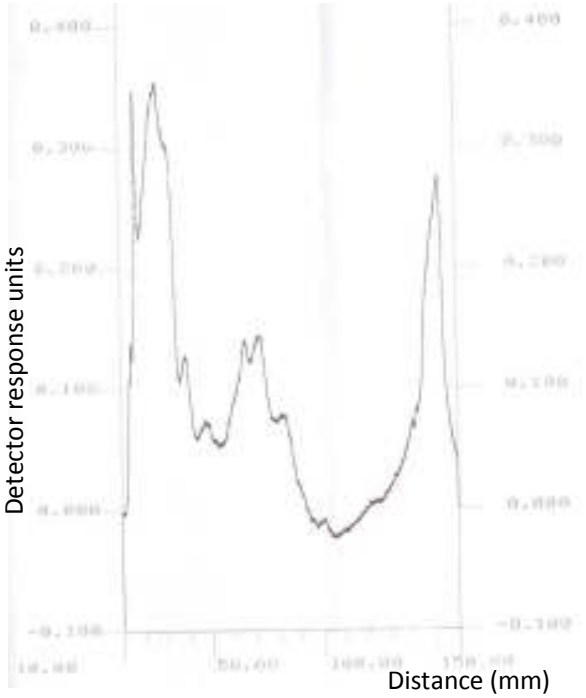
Appendix 17: TLC Densitogram for RMI 13



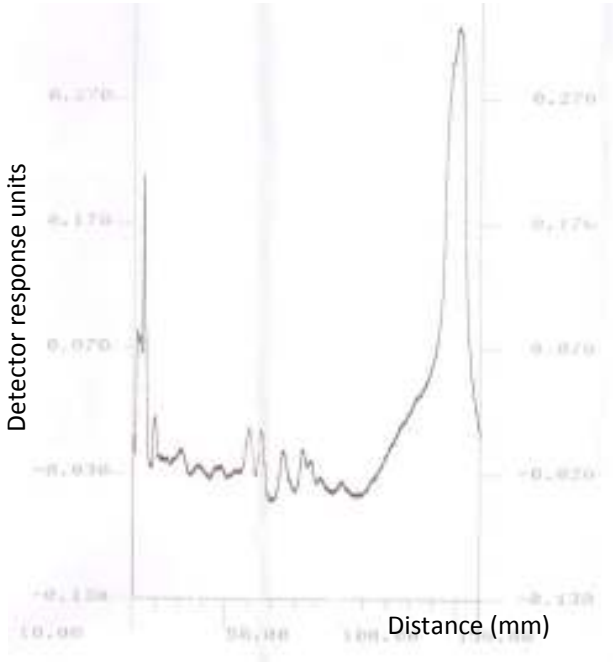
Appendix 18: TLC Densitogram for RMI 14



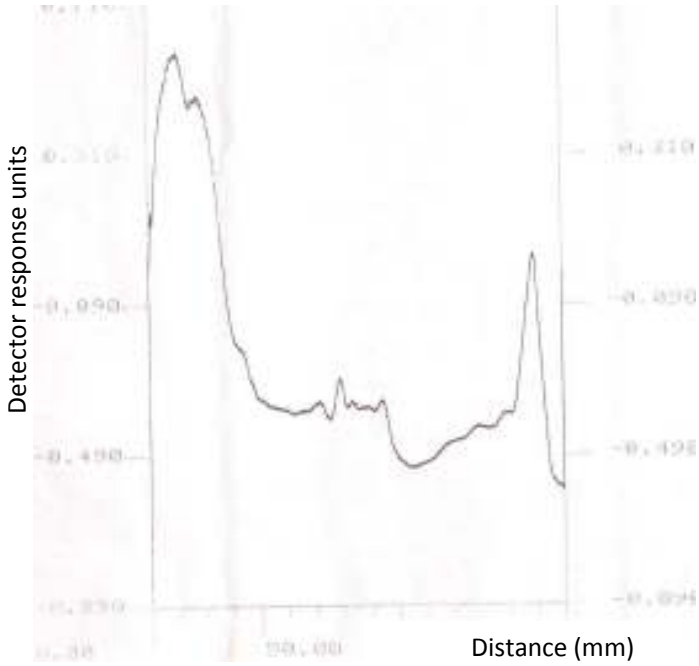
Appendix 19: TLC Densitogram for RMI 16



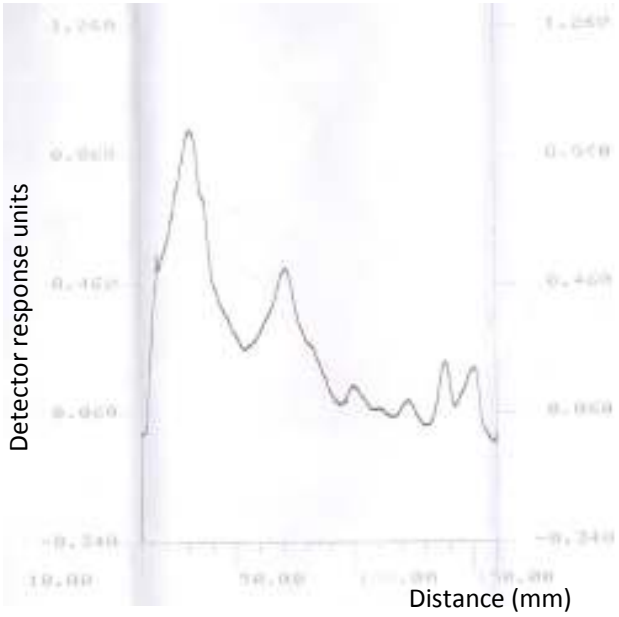
Appendix 20: TLC Densitogram for RMI 17



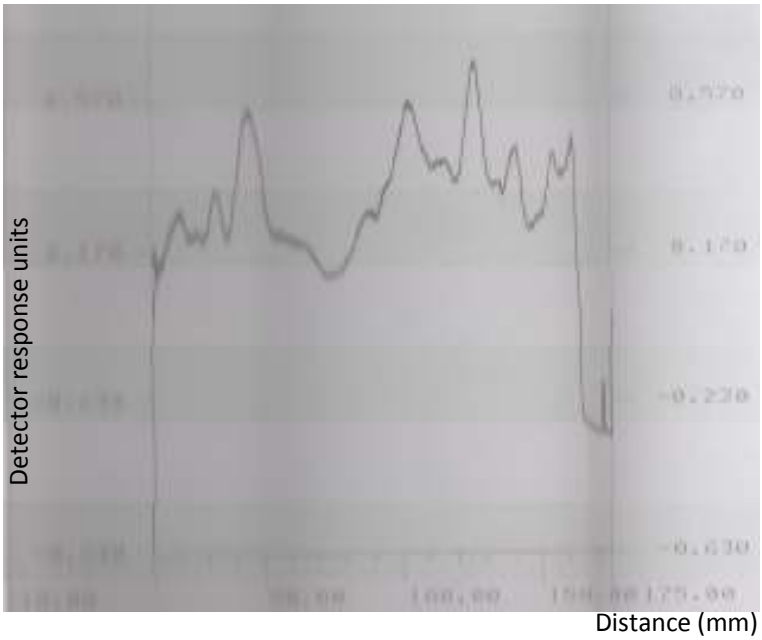
Appendix 21: TLC Densitogram for RMI 18



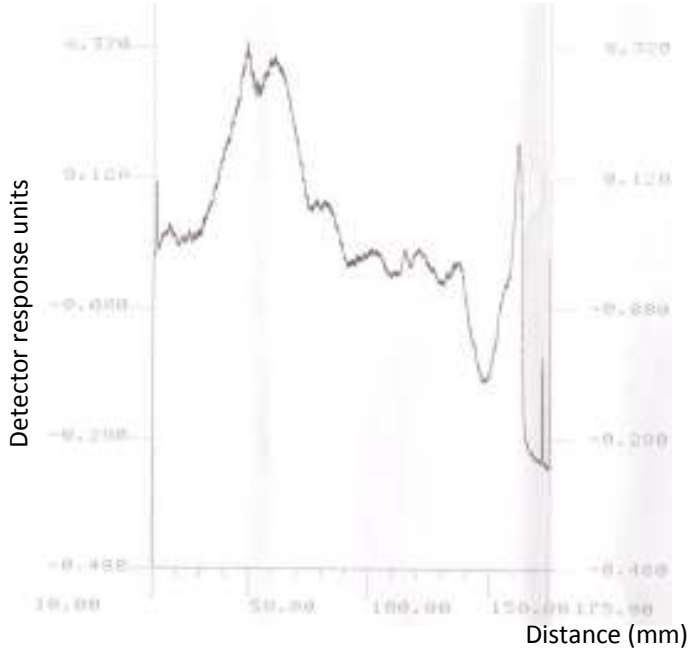
Appendix 22: TLC Densitogram for RMI 20



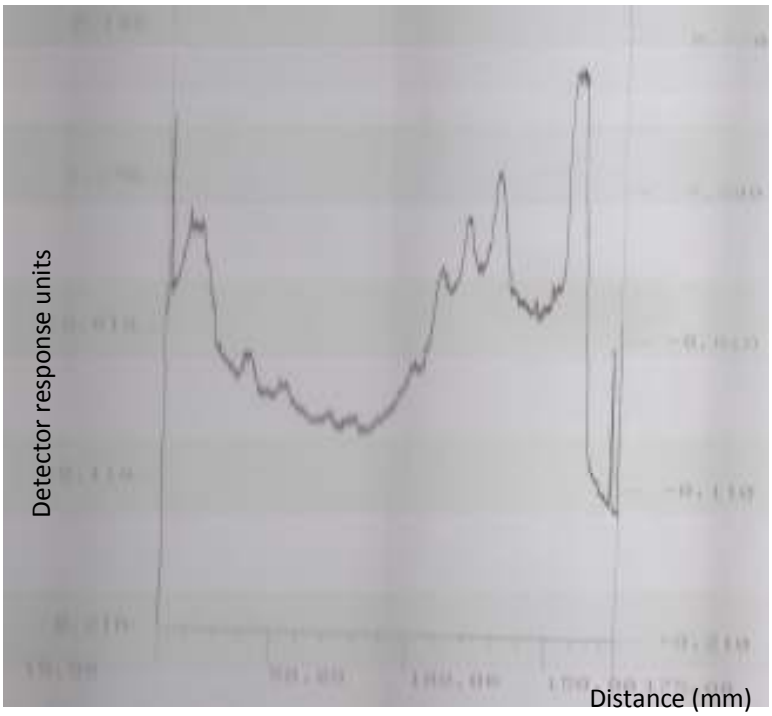
Appendix 23: TLC Densitogram for RMI 23



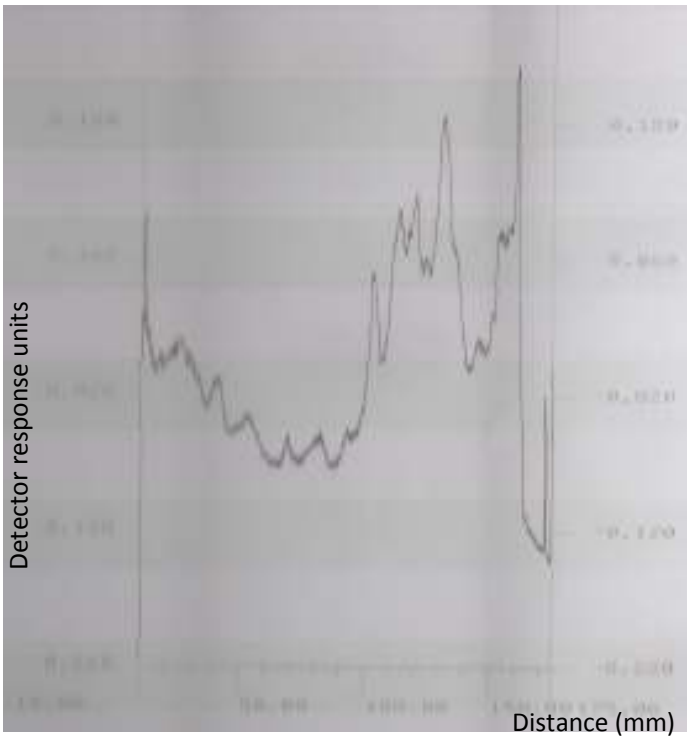
Appendix 24: TLC Densitogram for RMI 24



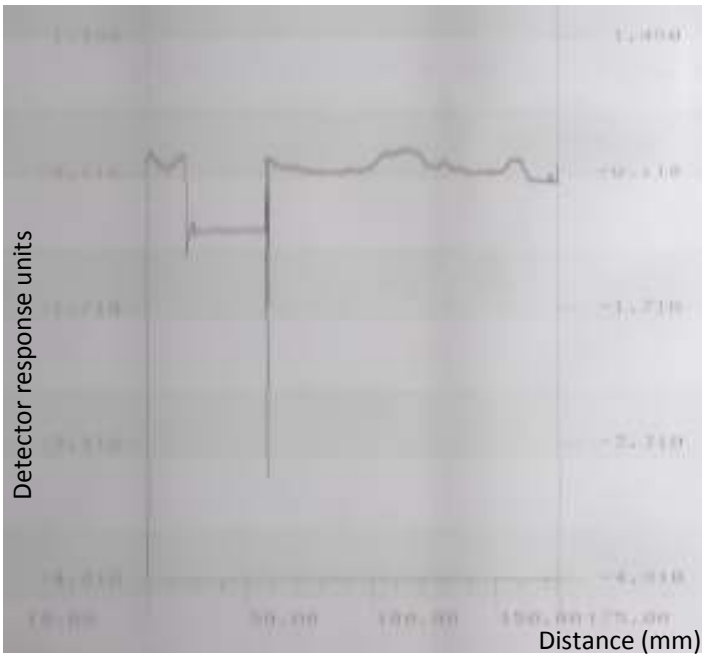
Appendix 25: TLC Densitogram for RMI 26



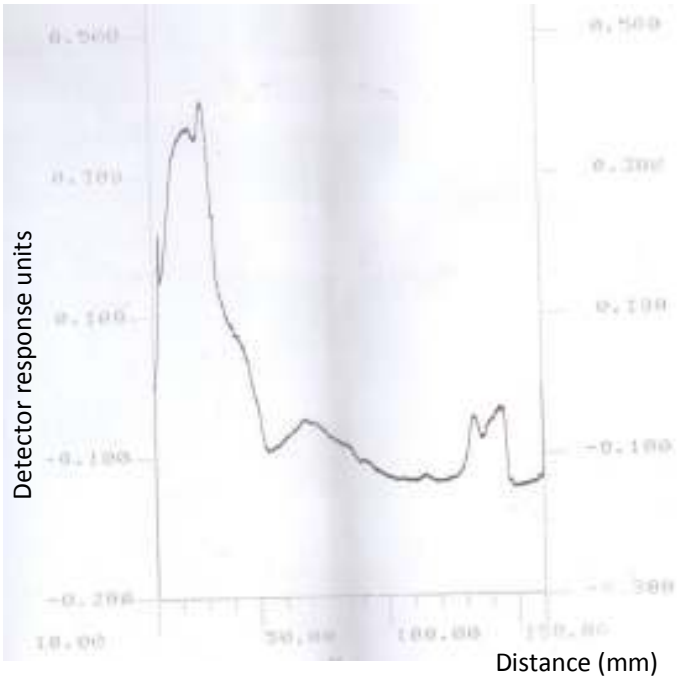
Appendix 26: TLC Densitogram for RMI 27



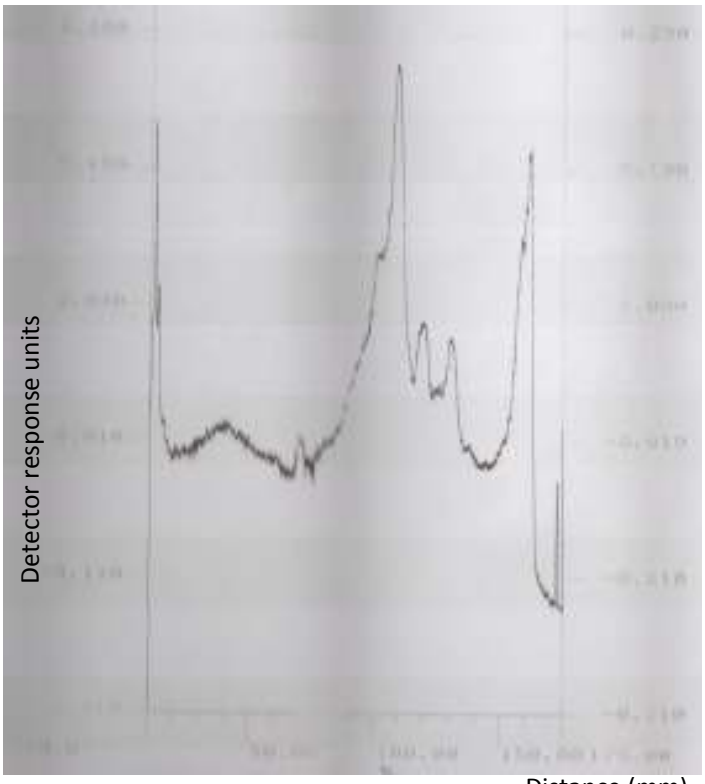
Appendix 27: TLC Densitogram for RMI 28



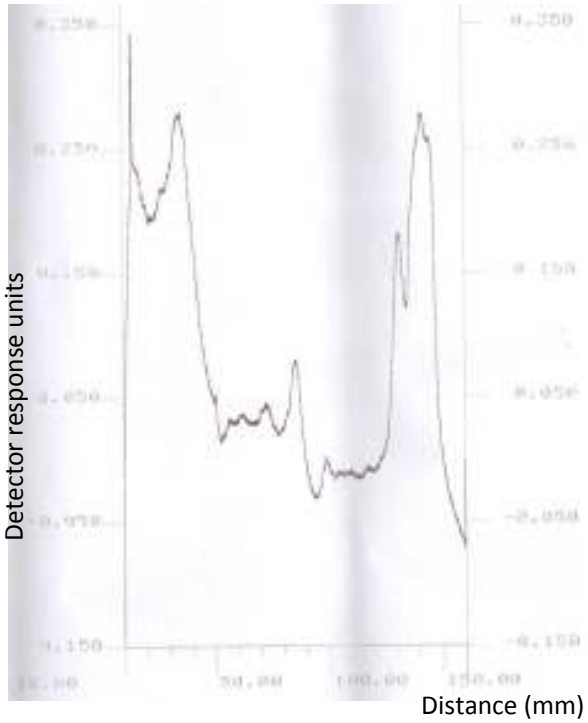
Appendix 28: TLC Densitogram for RMI 29



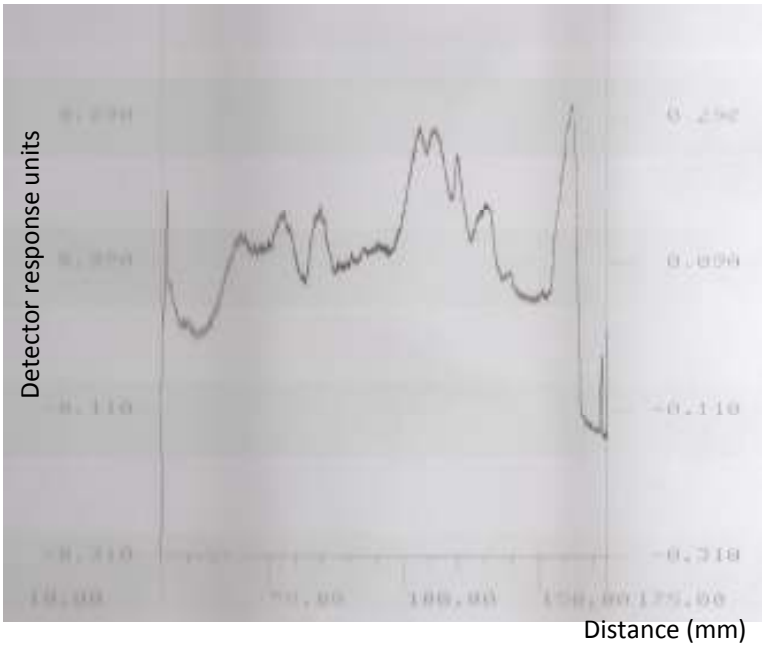
Appendix 29: TLC Densitogram for RMI 30



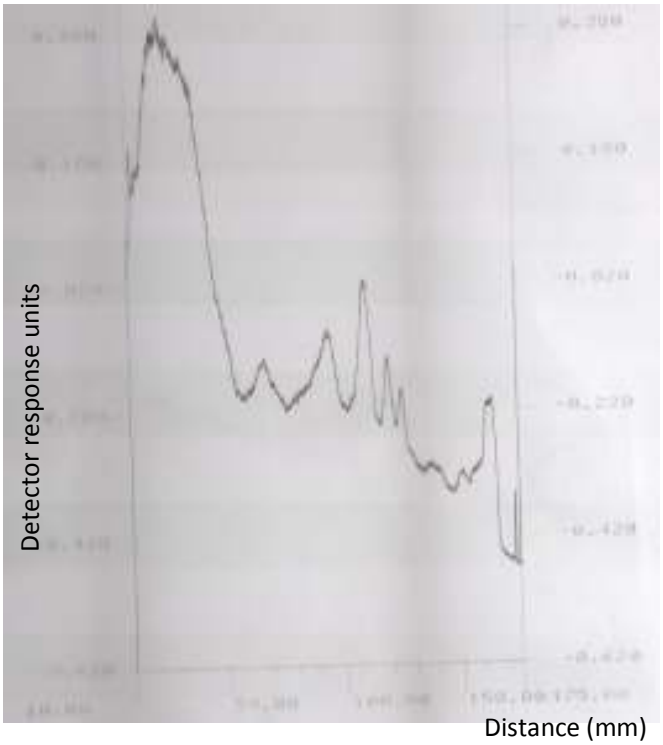
Appendix 30: TLC Densitogram for RMI 31



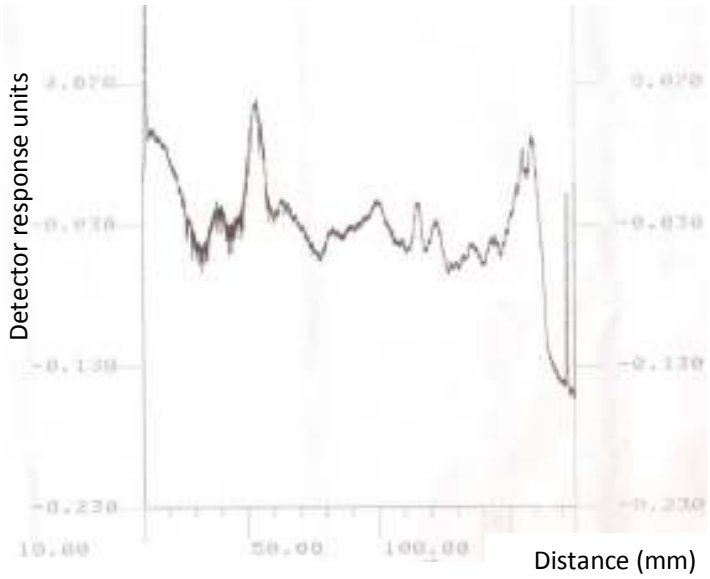
Appendix 31: TLC Densitogram for RMI 32



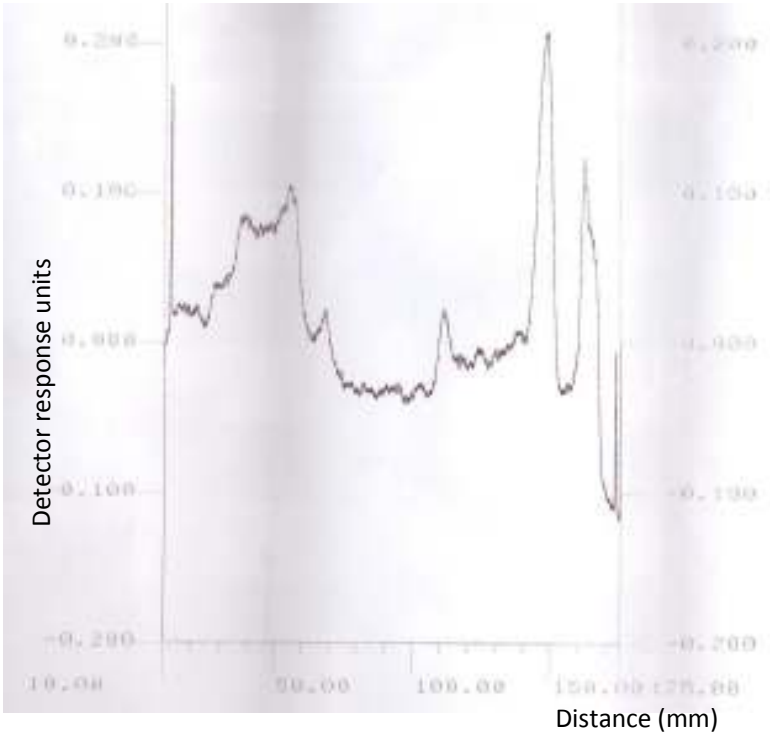
Appendix 32: TLC Densitogram for RMI 33



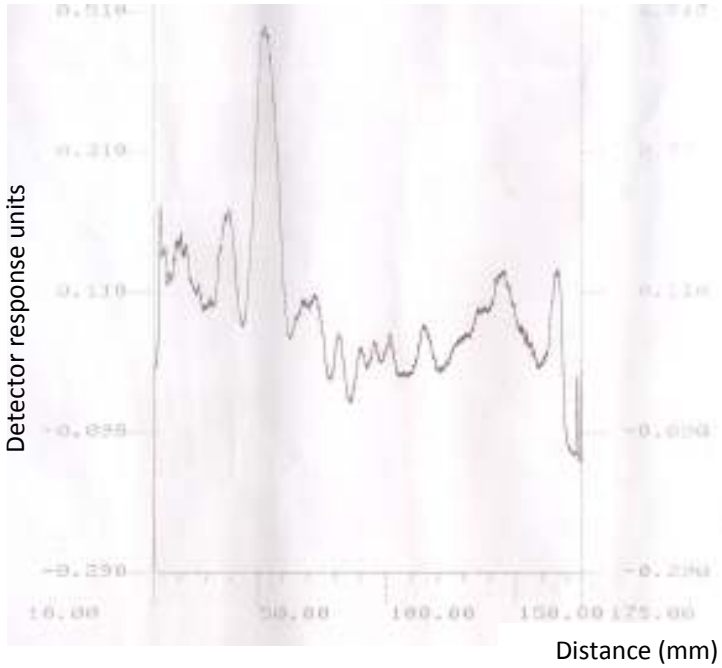
Appendix 33: TLC Densitogram for RMI 34



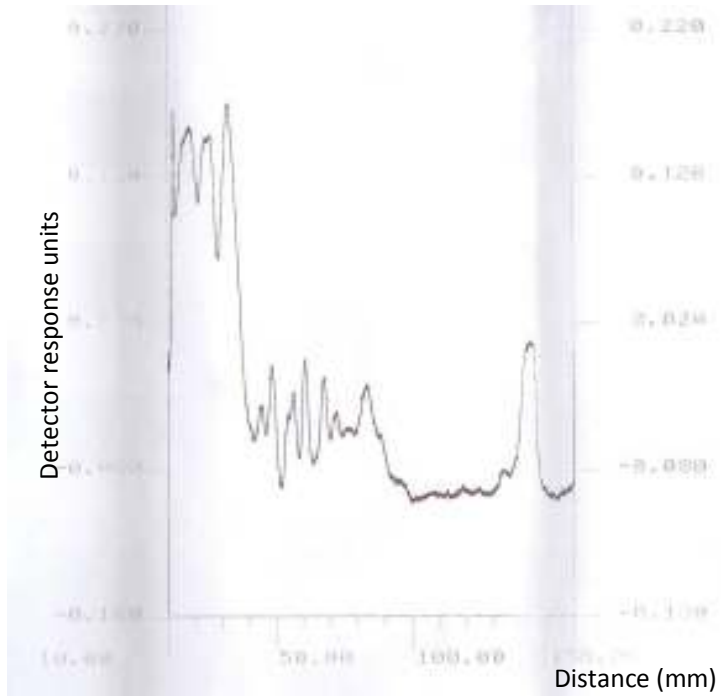
Appendix 34: TLC Densitogram for RMI 35



Appendix 35: TLC Densitogram for RMI 38



Appendix 36: TLC Densitogram for RMI 39



Appendix 37: TLC Densitogram for RMI 40