STUDIES ON ANTIBIOTIC SUSCEPTIBILITY OF PATHOGENIC BACTERIA ISOLATED FROM LIQUID HERBAL PREPARATIONS SOLD IN MINNA METROPOLIS

BY

MUHAMMAD, TASALLAH HAUWA

DEPARTMENT OF MICROBIOLOGY
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

MARCH, 2011.

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BY

MUHAMMAD, TASALLAH HAUWA B.Sc (BUK 1995)
M.Sc/SCIEN/02236/2006-07

A THESIS SUBMITTED TO THE
POSTGRADUATE SCHOOL,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

IN PARTIAL FULFILMENT FOR THE AWARD
OF MASTERS OF SCIENCE IN
MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

SUPERVISORS:

Prof. O. S Olanitola

Dr. E. D Jatau

MARCH, 2011

DECLARATION

I declare that the work in this thesis entitled‘‘STUDIES ON ANTIBIOTIC SUSCEPTIBILITY OF PATHOGENIC BACTERIA ISOLATED FROM LIQUID HERBAL PREPARATIONS SOLD IN MINNA METROPOLIS” has been performed by me in the Department of Micrbiology under the supervision of Prof. O. S Olonitola and Dr. E. D Jatau.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

Muhammad, Tasallah Hauwa ……MARCH, 2011.

……..	……..	…….
Name of student	Signature	Date

CERTIFICATION

This thesis entitled ‘‘STUDIES ON ANTIBIOTIC SUSCEPTIBILITY OF PATHOGENIC BACTERIA ISOLATED FROM LIQUID HERBAL PREPARATIONS SOLD IN MINNA METROPOLIS’’ by Muhammad, Tasallah Hauwa meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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Prof. O.S Olonitola Date

Chairman, Supervisory Committee

---------- ----------

Dr. E. D Jatau Date

Member, Supervisory Committee

---------- ----------

Prof. O.S. Olonitola. Date

Head of Department

---------- ----------

Prof. A.A. Joshua Date

Dean, Postgraduate School

DEDICATION

This work is specially dedicated to my beloved darling husband, Alhaji Muhammad Hassan Shetima.

Also to my children:

Nana Asiya,

Ummi Farida,

Muhammad Mujaheed

&

Fatima Zahra.

ACKNOWLEDGEMENTS

All praise goes to Almighty Allah(SWT), the most High for seeing me through this research work. During the project work some people who contributed in one way or the other in ensuring a successful completion of the work deserved to be acknowledged and appreciated for their roles. I must therefore acknowledge my able supervisors, Prof. O. S Olonitola and Dr. E. D Jatau for their keen interest in my work, constant supervision, and indepth critiques of drafts. Their inputs eased the process of revision considerably. My appreciation goes to my parents, His Highness, Alhaji M. A Muregi and Hajiya Hauwa for their support and prayers. I am sincerely indebted to my husband, Alhaji M. H Shetima for his encouragement, support, patience and understanding. To my loving children for their prayers when it matters most.My sincere appreciation goes to the staff of the department of Microbiology, my Directors at the ministry of health, Minna especially Dr. B.N Kolo, my past and present heads of department, Mr. L. S Kwasu and Alhaji A.A Dangana and my colleagues at General Hospital, Minna. I wish to acknowledge Alh. S.U Katako, Dr. B. Alkali, Mr. Agada, Mal. A. A Shehu, Alh. A. M |Ladan, Mrs Oluchi E., Aliyu Maikano, Awwal, Musa, Mustapha and Usman for the assistance rendered in one way or the other during the period of this work. My brothers, sisters, in-laws and friends have been wonderful for their contributions. To all I said THANK YOU.

ABSTRACT

A total of one hundred and fifty (150) Herbal Medicinal samples were collected from 3 local governments in Minna metropolis.Bacteriological analysis of the liquid samples were carried out for the isolation of bacteria using standard methods. Pathogenic bacteria were isolated from these products, the isolates were evaluated for Total aerobic plate count, Gram's reaction, Biochemical reaction and preservatives employed. Also antimicrobial susceptibility of isolates to selected antimicrobial agents was determined by the disc diffusion method and their multiple antibiotic resistances (MAR) index calculated. Bacterial contamination was found in 67.33% of the samples. *Staphylococcus aureus* and *Escherichia coli* were detected more frequently than other species i.e *Salmonella typhi, Shigella sonnei*, *Bacillus firmus*, *Klebsiella aerogenes*, *Streptococcus faecalis* and *Pseudomonas aeruginosa.* In total, two hundred and four (204) bacteria were recorded. The aerobic plate count result showed the highest count of 10^8 in thirteen (8.7%) samples, 10^7 in fourty seven samples and no bacterial count was recorded in fourty nine (32.7%) of the samples. The effects of preservatives were determined. The Antimicrobial susceptibility of isolates showed some varying degree of resistance. At least, 98.3% of the isolates had MAR index of 0.3 and above. The bacterial isolates in this study were found to be generally resistant to the readily available cheap and often abused antimicrobial agent, which is an indication that they originate from an environment where antibiotics are frequently used.

In conclusion the study revealed that most of Herbal preparations were not sterile and may serve as source of infection to the consumers. Due to the unhygienic conditions under which these products were prepared, there is the need for the intervention of regulatory body likes the National Agency for Food and Drugs Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON) to carry out more detailed analysis of these herbal preparations.

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CHAPTER ONE

1.0 INTRODUCTION

Herbal medicinal products (HMPs) are becoming increasingly popular (Fisher and Ward, 1994; Brevoort, 1998; Eisenberg *et al.,* 1998). Herbal medicine, also called phytomedicine or botanical medicines refers to the use of any plant's part: seeds, roots, leaves, barks, flowers, fruits etc. for medicinal purposes (Chavez and Chavez, 2000). Medicine, in several developing countries, using local traditions and belief, is still mainstay of healthcare. According to Hoareau and DaSilva (1999), the World Health Organization (WHO) defined health as a state of complete physical, mental and social well being and not merely the absence of disease or infirmity.

Plants with medicinal properties popularly refers to as gift of mother nature to mankind are in use for centuries in the traditional system of medicines like Ayurveda, unani, traditional Chinese medicines etc. in India and other countries of the world for the treatment of diseases. They are considered to be effective and non-toxic. They have a vast potential but are only partly explored by modern methods (Murphy *et al*., 1996). An estimated 80% of the world population still

depends on traditional herbal medicines for their health security (Carter, 2001; WHO, 2002). In most African countries, herbal medicine is recognized as an important component of health care system especially among rural dwellers that constitutes about 70% of the population (Esimone *et al.,* 2002). In Nigeria, there appears to be an overwhelming increase in the public awareness of the usage of herbal medicinal products in the treatment and/or prevention of diseases.

This may not be unconnected with the active mass media advertisement embarked upon by the producers and marketers of the herbal medicinal products who have taken the advantage of the relatively high cost of the conventional pharmaceutical dosage forms, inaccessibility of the orthodox medical services to a vast majority of people particularly in the rural areas and the

reservations by the public due to prevalence of fake, substandard or counterfeit drugs in the market. These have placed the herbal medicinal products as a ready alternative to conventional dosage forms in the treatment of diseases. With this increased usage, the safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals (Lau *et al.,* 2003; Adeleye, *et al.,* 2005).

Herbal preparations are often perceived as being natural and therefore safe, but they are not free from adverse effects which may be due to factors such as adulteration, substitution, mis-identification, lack of standardization, incorrect preparations and contamination with pathogenic bacteria that present serious health hazards (Arias *et al.,* 1999). Although the World Health Organization advocated for the integration of herbal medicinal products into the primary health care system of developing countries (WHO, 1978; 1999), safety issues related to herbal drugs continue to be ignored by the herbalist whose methods of concocting herbal preparations for the public are usually unhygienic with the attendant microbiological hazards (Tella,1977). Accordingly, gross microbial contaminations of herbal medicinal products commonly consumed in Nigeria have been severally demonstrated (Onawunmi and Lamikanra, 1987; Lamikanra *et al.,* 1992; Esimone *et al*., 2003). On one hand, such grossly contaminated herbal medicinal products may serve as potential sources of transmission of pathogenic organisms from products to consumers. Alternatively, it may result to product spoilage (Grigo, 1976; Mendie *et al*., 1993).

This study is aimed at evaluating the potential hazards associated with consumption of liquid herbal medicines and susceptibility profile of pathogenic bacteria isolated from such products.

1.1 STATEMENT OF THE PROBLEM

Consumption of herbal medicinal product is increasingly becoming popular. Although herbal remedies are often perceived as being natural and therefore safe, consumers may be unaware of the side effects like lack of precise dosage, unhygienic conditions under which they are produced and potential contaminants e.g. pathogenic bacteria that present serious health hazards (Soforawa, 1993; Adeleye *et al.*, 2005).

1.2 JUSTIFICATION

Rising cost of orthodox medicine, emergence of drug-resistant microbial strains coupled with increasing patronage and improper use of herbal medicinal products justify decision to investigate microbial safety of these products.

Improper use of herbal medicinal products by consumers which might lead to potential side effects and/or mass infections on human justify closer look at herbal preparations.

1.3 OBJECTIVES

1. To isolate pathogenic bacteria from liquid herbal products sold in Minna Metropolis.
2. To characterize the isolates using their microscopic-biochemical properties.
3. To determine the effectiveness of the preservatives commonly employed in liquid herbal products in Minna.
4. To determine the susceptibility of isolates to antibiotics.

CHAPTER TWO

2.0 Literature Review

2.1 Traditional Medicines: Definitions

Traditional medicine can be best described as the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing or eliminating a physical, mental or social disease and which may rely exclusively on past experience and observation handed down from generation to generation, verbally or in writing (Soforawa, 1993: Firenzuoli and Gori, 2007). Bhushan (2005) reported that World Health Organization defined traditional medicines as diverse health practices, approaches, knowledge and beliefs incorporating plant, animal, and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness.

Traditional herbal medicinal products (THMP) registration dossier of 2005 described herbal preparations as any medicinal product exclusively containing active substances, one or more herbal substances or one or more herbal preparations or one or more such herbal substances in combination with one or more such herbal preparations. These preparations are obtained by subjecting herbal substances to treatments, such as extraction, distillation, expulsion, fractionation, purification, concentration or fragmentation. These include comminuted or powdered herbal substances, tinctures, extracts, essential oils, expressed juices and processed exudates.

Use of natural plant substances (botanicals) to treat and prevent illness has existed since prehistoric times and flourishes today as primary form of medicine for perhaps as much as 80% of the world's population. Over 80,000 species of plants are in use throughout the world. Along with acupuncture, herbal medicine is considered primary health care in China (Haruki, 2005).

2.2 **Classifications of Herbal Products**

As a result of classification of the herbal medicine in the compendium of Materia Medica by LI Shizhen in the Tang Dynasty [618-909], there are 1892 types recorded. Today we can briefly divide them into ten as seen in Table 2.1 below.

Herbal medicinal products can also be classified according to their taste, properties and effects as shown in Table 2.2 below.

Table 2.1: Classification of herbal medicine

Number	Classes	Examples
1	Root	Ginseng
2	Rhizome	Ginger
3	Leaf	Neem
4	Flower	Cloves
5	Seed	Castor
6	Fruit	Amla
7	Grass	Lemon grass
8	Vine	Aloe vera
9	Stem	Willow
10	Shrub	Chanca piedra

Source Kumar, N.C. (2004)

Table2. 2: Classification of herbal products based on their taste, properties and effects

Grouping	Characteristics
Six tastes	Sweet (e.g., bamboo, grapes, saffron); sour (e.g., pomegranate, hippophae, crataegus); salty (e.g., salt and several minerals); bitter (e.g., gentiana, aconite, berberis); acrid (e.g., piper, ginger, garlic); and astringent (e.g., sandalwood, terminalia, aquilegia).
Eight properties	Heavy, smooth, cool, soft, light, rough, acrid, and sharp.
Seventeen effects	Cold, hot, warm, cool, thick, thin, moist, rough, light, heavy, steady, motive, blunt, sharp, tender, dry, and soft. Disease is treated by the opposing effect; a hot disease by a cold effect, stagnant diseases by a motive effect, an accumulation by a sharp effect, a moist condition by a dry effect, etc.

Source: Liang, W. (1999)

According to Council Directive 65/65/EEC of European Commission (1998) which has been implemented into National law in all member states, herbal products are classified as medicinal products, if they claim therapeutic or prophylactic indications, and are not considered as medicinal products in so far as they do not make these claims. Products not classified as medicinal products belong in most cases to the food or cosmetic area, even though they sometimes contain plants which have pharmacological properties. For example, Sienna pods can be marketed as food in Belgium. Furthermore, there are specific categories of non-medicinal products, e.g. the so-called "therapeutic supplement products" in Austria. Such products are specifically regulated by National law and sometimes require notification to the competent National authority.

2.3 **History of Herbal Medicines**

The use of plants, plants extract or chemicals derived from plants to treat disease is therapeutic modalities which has stood the test of time (Anwannil and Atta, 2006). Herbs have been used by all cultures throughout human history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, beauty enhancers and medicines. Kossowan (1991) reported that even before the advent of western orthodox medicine, traditional medicines have been in practice.

Historically, the most important uses of herbs were medicinal and more than forty percent (40%) of prescribed medicines in the world are of plant origin (Hedberg, 1998). Prior to discovery and subsequent synthesis of antibiotics, the herb Echinacea (which comes from the plant commonly known as purple coneflower) was one of the most widely prescribed medicines in the United States. For centuries, herbalists prescribed Echinacea to fight infection. Today, research confirms that the herb boosts the immune system by stimulating the production of disease – fighting white blood cells (Barrett, 2003).

For most of his existence, man had various but limited resources for treating injuries and diseases. Separately and in combination he used any and all of the following. Magic, sorcery, prayer, music, crude operations (amputation, bleeding, trepanning), psychotherapy, physical therapy (diet, rest, exercise, first aid) and internal and external remedies prepared from plants, animals, and minerals. Of all these, plant remedies represent the most continuous and universal form of treatment, and were the basic sources of therapeutic products for professional and folk medicine from the earliest days until the twentieth century. Much of the medicinal use of plant seems to have been developed through observations of wild animal, and by trial and error that certain plants were useful for treating illness, just as he learnt that some were good to eat and others could poison and lead to death.

Viable herbal solutions in 2006 documented that the uses of plants for medicine and other purposes changed little during the middle Ages. It was documented that the early Christian church discouraged the formal practice of medicine, preferring faith healing: but many Greek and Roman writings on medicine, as on other subjects, were preserved by diligent hand copying of manuscripts in monasteries. The monasteries thus tended to become local centers of medical knowledge, and their herb gardens provided the raw materials for simple treatment of common disorders. At the same time, folk medicine in the home and village continue uninterrupted.

The continuing importance of herbs for the centuries following the Middle Ages was indicated by the hundreds of herbals published after the invention of printing in the fifteenth century. Two of such publications were *The herbal or General History of Plants* (1597) by John Gerald and *The English Physician Enlarged* (1653) by Nicholas Culpeper. These respected herbal pharmacopoeias were one of the first manuals that the layperson could use for health care, and it is still widely referred to and quoted today (Zand, 1994).

The first U.S. Pharmacopoeia was published in 1820. This volume included an authoritative listing of herbal drugs, with descriptions of their properties, uses, dosages and test of purity. It was periodically revised and became the legal standard for medical compounds in 1906. But as Western medicine evolved in 17th Century, it marked the beginning of a slow erosion of the pre-eminent position held by plants as sources of therapeutic effects.

In the early 19th Century, scientists began extracting and modifying the active ingredients from plants when methods of chemical analysis became available. Later, chemists began making their own version of plant compounds, beginning the transition from raw herbs to synthetic pharmaceuticals. Over time, the use of herbal medicines declined in favour of pharmaceuticals. Pax herbal magazine in 2010 documented that about 60% of African population use traditional medicine for their healthcare. However, despite its widespread use, traditional medicines remain unregulated. Communication between patients and traditional healthcare providers is generally poor leading to high risk in its practice.

A record of medicinal plants in earliest period in Nigeria is virtually not available because there was no documentation for their isolation, selection and preparation. Every fact about potent herbal plant was passed by word of mouth from generation to generation (Kochhar, 1981). Traditional practice in Nigeria is a main source of livelihood for a significant number of populations who depend on it as their main source of income. High population growth rate (2.8% annually) and poverty, coupled with dwindling economic resources in the country makes the people resort to these cheap resources for their immediate needs.

2.4 **Medicinal plants and herbal preparation**

Medicinal plants are plants that provide people with medicines-to prevent disease, maintain health or cure ailments. In one form or another, they benefit virtually everyone on earth. No exact

definition of medicinal plant is possible. There are related issues such as for nutrition, toiletry, bodily care, incense and ritual healing. FEPA (1992) reported that Nigeria has over 700 species of algae, 80 lower plants (bryophytes, etc), 150 ferns and 5,000 higher plants. About 300 plants are listed as being of medicinal value in Western Nigeria alone, and these listed plants are spread over several families including ferns, bryophytes and some fungi (Adjnahoun *et at.,* 1993). Medicinal plants are easily obtainable and can be used as extracts or as crude substances which can be further purified (Awosika, 1993). Zand (1994) reported that herbal preparations are available in varieties: including fresh, dried, in tablets or capsules, or bottled in liquid forms. They are available either singly or in mixtures formulated for specific conditions and in whatever form, herbal preparation is as good as the quality of the herbs from which it was made.

According to Bankole *et al* (2007), all plants produce chemical compounds as part of their normal metabolic activities. These are arbitrarily divided into primary metabolites, such as sugars and fats found in all plants and secondary metabolites, compounds not essential for basic function found in a smaller range of plants, some useful ones found only in a particular genus or species. Pigments harvest light, protect the organism from radiation and display colors to attract pollinators. Many common weeds, such as nettle, dandelion and chickweed have medicinal properties. The functions of secondary metabolites are varied. For example, some secondary metabolites are toxins used to deter predation, and others are pheromones used to attract insects for pollination. Phytoalexins protect against bacterial and fungal attacks. Allelochemical inhibit rival plants that are competing for soil and light.

In traditional herbal medicinal systems, herbal remedies are prepared in several standardized ways which usually vary based upon the plant utilized, and sometime, what condition is being treated. Some of these methods include: infusions, decoctions, tinctures (alcohol and water extracts), and macerations (cold-soaking). In indigenous Indian medicine systems, medicine men or shamans

generally use these same methods in addition to others. Others include preparing plants in hot baths (in which the patient is soaked in or bathed with), inhalation of powdered plants (like snuff), steam inhalation of various aromatic plants boiled in hot water and even aromatherapy. The well-trained herbalist will always thoroughly review the time-honored method in which a plant has been traditionally prepared. It holds important information for preparing an effective herbal remedy (Leslie, 2004).

Table 2.3 shows the comparative study of herbal and orthodox drugs used in various treatment categories among developed and developing countries (Ischei, 2005).

Table 2.4 Indicates the examples of some common medicinal herbs, their uses, part of plant used, forms given, possible side effects and comment (Zand, 1994).

Table 2.3 Comparative use of herbal and orthodox drugs among Developed and Developing Countries

Treatment Categories	Western Pharmacopoeia	Indigenous Pharmacopoeia
Inflammation	7	12
Dermatology	1	15
Gastrointestinal	2	15
Cancer	4	1
Obstetrics/Gynecology	14	7
Cardiovascular	10	2
Nervous System	29	10
Renal-Blood-Immune System Poisons	17	11
Antimicrobial	12	9
Others	4	16

Source: Ischei (2005)

Table 2.4: Some Medicinal Herbs and their Uses

Herbs	Medicinal Used	Part of Plant Used	How Given	Possible Side effect	Comment
Alfalfa	Tonic contains natural fluoride, helpful in preventing tooth decay.	Leaf	Tincture; Tea; Capsule	None known	
Aloevera	Topically: pain reliever, excellent for burns, sore nipples, itching of chicken pox. Internally: Relieves stomach constipation.	Pulp from inside leaf.	Liquid applied topically to affected area or taken internally.	None known	Excellent for increasing the production of breast milk
Garlic	Antibiotic, antiseptic, antihelminthic.	Clove	Fresh whole herb; capsule; liquid.	Stomach upset, contact dermatitis, flatulence.	Seeds are safe when cooked; leaves can be toxic and are not normally used.
Ginger	Aids digestion; relieves congestion; promotes perspiration and relieves fever; soothes achy muscles.	Root	Tincture; tea; bath or oil for achy muscles.	Diarrhea, nausea.	Fresh cloves may be used but odorless capsule form is more palatable for most children.
Parsley	Increases urination; helpful in treating bladder infection.	Leaf	Tea; capsule	Dizziness, headache, warmth, nausea, vomiting itching.	Use with caution during pregnancy, excessive amounts will stop milk production in nursing mothers.

Source: Zand, (1994).

2.5 Herbal Medicine in Modern Human Society

Today, the U.S Pharmacopoeia, with its reliance on herbal compounds, has been all but forgotten. Most modern physicians rely on the *Physician's Desk Reference*, an intensive listing of chemically manufactured drugs (Chong, 2003). Conversely, medicinal plants are greatly embraced by most populations in the world especially in the developing countries because they have been discovered to hide the best medicine that nature can offer to mankind (George and Pamploma-Roger, 1999). Many of pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including Opium, Aspirin, Digitalis and Quinine. The World Health Organization estimated that 4 billion people, about 80% of the world's population presently use herbal medicine for some aspect of their primary health care. Moreover, according to statistics and tables within the atlas, herbal medicine is one of the traditional, complementary and alternative medicines more likely to be popular in low-income nations (Bodeker *et al.,* 2005).

Rather than using a whole plant, pharmacologists identify, isolate, extract, and synthesize individual components, thus capturing the active properties. This can create problems, however. In addition to active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavanoids, and other substances that are important in supporting a particular herb's medicinal properties. These elements also provide an important natural safeguard. Isolated or synthesized active compounds can become toxic in relatively small doses; it usually takes a much greater amount of a whole herb, with all of its components, to reach a toxic level. Herbs are medicines, however, and they can have powerful effects. They should not be taken lightly (Chong, 2003).

The use of and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologist, Microbiologist, Botanists, and natural- products Chemists are combing the earth for phytochemicals and leads that could be developed for treatment of various diseases. In fact, according to the World Health Organization (WHO, 2002), approximately 25%

of modern drugs used in the United States of America have been derived from plants (*Traditional medicine*, 2009).

Countries in Europe, Asia, Latin America and Africa use traditional medicine to help meet their primary health needs. In China, herbal preparations account for over 30 - 50% of the total medicinal consumption. In Latin America and other industrialized regions, over 50% of the populations have used herbal preparations as alternative medicines while in Ghana, Mali, Nigeria and Zambia, the first line of treatment for malaria is the use of herbal medicine at home for 60% children with high fever resulting from malaria. Studies in Africa and North America have shown that up to 75% of people living with HIV/AIDS used traditional medicines alone or in combination with other medicine for various symptoms or conditions (WHO, 2002). Cragg (2004) explained that at least 119 chemical substances derived from 90 plant species can be considered as important drugs currently in use in one or more countries. In countries such as China and India, plants have served as the basis of sophisticated traditional medicinal systems for thousands of years.

The efficacy of many medicinal plants has been validated by scientists abroad, from Europe to the Orient. But due to modern technology, science can now identify some of the specific properties and interactions of botanical constituents. With this scientific documentation, we now know why certain herbs are effective against certain conditions. However, almost all of the current research validating herbal medicine has been done in Germany, Japan, China,

Taiwan and Russia. And for the most part, the United States Food and Drug Administration (FDA), which is responsible for licensing all new drugs (or any substances for which medicinal properties are claimed) for use in the United States, does not recognize or accept findings from across the sea. Doctors and government agencies want to see American scientific studies before

recognizing the effectiveness of a plant as medicine. Yet even though substantial research is being done in other countries, drug companies and laboratories in the United States so far have not chosen to put much money or resources into botanical research. The result is that herbal medicine does not have the same place of importance or level of acceptance in this country as it does in other countries (Chong, 2003).

Also, the rampant and prolong usage of herbal preparations world-wide as cited by the WHO and the scientific evidence from randomized clinical trials couples with the fact that herbal preparations are used unregulated and inappropriately (Sofowora, 1993) and also contain a lot of pathogenic bacteria (Adeleye *et al.,* 2005), herbal preparations have negative and dangerous effect on the health status of an individual. Elujoba *et al* (2005) reported that many medicinal plants even in the crude forms are well known in the International markets today and African countries are among the top world producers of such plants. Example, ginger (*Zhingiber officinale*) which is used as spice contains gingerol and carminative. It is an important medicinal

product well known and also produced in Nigeria.

2.6 Contaminants of herbal medicinal products

Herbal medicinal products have been found to be contaminated with pathogenic bacteria such as *Staphylococcus aureus, Escherichia Coli, Salmonella typhi, Shigella sp, Bacillus sp* amongst others that present serious health hazards (Onawunmi and Lamikanra, 1987; Lamikanra *et al.*, 1992; Soforawa, 1993; Esimone *et al.*, 2003; Oyetayo, 2008 and Abba *et al.*, 2009).

Some of these pathogenic bacteria originates from soil and adheres to parts of plants (Adeleye *et al* ., 2005) while most of them were being introduced into herbal medicinal products through processes of harvesting, drying, storage and manufacturing because of the unhygienic manners of which most herbalists prepares and handles their herbal products (Lau *et al.*, 2003; Adeleye *et al*, 2005).

Staphylococcus aureus: This is facultative anaerobic Gram positive cocci and is the most common cause of staphylococcus infection. It is frequently part of the skin normal flora found in the nose and skin (Ryan and Ray, 2004). The pathogenic Staphylococci often hemolyze blood, coagulate plasma and produce a variety of extracellular enzymes and toxins. About 20% of human populations are long term carriers of *Staphylococcus aureus. Staphylococcus aureus* can cause a range of illness from minor skin infection such as pimples, impetigo, boils, cellulitis and abscesses to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocardiatis, toxic shock syndrome (TSS), chest pain and sepsis (Whitt and Salyers, 2002). *Staphylococcus aureus* was first discovered in Aberdeen, Scotland in 1880 by the Surgeon Sir Alexander Ogston in pus from surgical abscess (Ogston, 1994). They are among the abundant pathogenic bacteria isolated from herbal medicinal preparations.

Escherichia coli: are short Gram negative rods belonging to the family, Enterobacteriaceae. They appear on eosin methylene blue (differential) media as motile, flat, non viscous colonies with metallic sheen. They produce positive tests for indole, lysine decarboxylase and mannitol fermentation and produce gas from glucose (Donnenberg, 2005).

E.coli are part of normal intestinal flora and incidentally cause disease (i.e become pathogenic) when they reach tissues outside of their normal intestinal or other less common normal flora sites. It is the most common cause of urinary tract infections particularly among females which can result in neonatal meningitis (Abbott, 2003).

Salmonella typhi: are facultative aerobic Gram negative rod shaped bacteria belonging to the family Enterobacteriaceae. They are motile by peritricious flagella and grow at temp. 2- 47^0C with rapid growth occurring between 25-43^0C (Donnenberg, 2005).

Salmonella specie are classified into serovars (serotypes) based on lipopolysaccharide (o), flagella protein (H) and sometimes the capsular (Vi) antigens. Within a serovar, there may be strains that differ in virulence. *Salmonella* are known to be pathogenic to both man and animals. *Salmonella typhi* is the main infectious cause of enteric disease in human being worldwide and most of the cases are likely to be related to food products of animal origin (Abbott, 2003). Among the host factors that contribute to resistance to salmonella infection are gastric acidity, normal intestinal microbial flora and local intestinal immunity.

Shigella sonnei: are Gram negative, non-motile, non- capsulated, facultative anaerobes but grows best aerobically. They differ from other enterobacteriaceae in that they occur exclusively as human pathogens. They are endotoxic.

Pseudomonas aeruginosa: These are Gram negative, motile, obligate aerobic rods that grow readily on many types of media at 37- 42^0C. They are oxidase positive and do not ferment glucose and lactose. Some strains hemolyse blood. They also produce the non fluorescent bluish pigment, pyocyanin which diffuses into the agar. *P.aeruginosa* is frequently present in small numbers in the normal intestinal flora and on the skin of humans and is the major pathogen of the group. They cause diseases in humans with abnormal host defenses and they are also the common causes of nosocomial infection (Kiska and Gilligen, 2003).

Bacillus firmus: These are aerobic, Gram positive spore forming bacilli. They are ubiquitous and can survive in the environment for many years. Most members are saprophytic organisms prevalent in soil, water and air and on vegetation. Bacilli are responsible for food spoilage. The presence of *Bacillus* species may be as a result of inadequate heat processing, improper handling of products and contaminated processing equipment (Frazier and Westhoff, 2003).

Klebsiella aerogenes: These are Gram negative non motile rods that show positive test for lysine decarboxylase, citrate and voges-proskauer test. *Klebsiellae* form large capsules consisting of polysaccharides (K antigens) covering the somatic (O and H) antigens and can be identified by capsular swelling tests with specific antisera. They cause pneumonia particularly in children (Abbott, 2003).

2.7 Preservatives employed in herbal preparations

Herbal medicinal preparations, if unpreserved, readily become contaminated with adventitious microorganisms leading to spoilage (Tella, 1978). Most herbal practitioners would rather prefer to employ other techniques of preservations to preserve their herbs. These include drying, carbonizing, salting, using honeys, oils and alcohols. Some others prepare their herbal remedies simply by boiling believing that there are natural preservatives inherent in the herbs.

In recent years, adequate preservation of liquid herbal preparations has increased in importance. In addition to presenting a health hazard to the users, microbial growth can cause marked effects on product stability (Hirsch *et al*., 1969; Griffith, 1986; Mendie *et al*., 1993). Non sterile pharmaceuticals have been reported to contain high levels of microbial contaminations when adequate controls were not implemented during manufacture (Ringertz and Ringertz, 1982; Okore, 1992; Onawunmi, 1999).

Preservatives are included in pharmaceutical preparations when it is necessary to combat the effects of contaminating microorganisms, which may be inherent in ingredients or introduced during the production process or during use by the patient. The quality of preservative included in any formula must be that necessary for assured protection against microbial growth (Carter, 1987;

Aulton, 1988; Underwood, 1992). For maximum protection of the consumer, the concentration of the preservative shown to be effective in the final packaged product should be considerably below the concentrations of the preservatives that may be toxic to human beings (U.S Pharmacopoeia, 1990). The selection of a preservative system must be done on an individual basis, using published information and 'in house' microbioiogical studies for guidance. Frequently, a remarkable effect of the combination of two or more preservatives is needed to achieve the desired antimicrobial effect.

2.8 Phytochemicals in Medicinal Plants

Recently there has been an explosion of research concerning the health benefit of phytochemicals in food and medicinal plants and the conclusion that, the higher the consumption of fruits and vegetables in the diet, the lower the risk of any chronic diseases. These phytochemicals which are the active constituents of plant were reported to be available from the extracts obtained from the medicinal plants growing in Nigeria (Ajibola and Motoyoshi, 1992). Phytochemicals are chemical compounds or chemical constituents formed in the plant's normal metabolic processes. The chemicals are often referred to as "secondary metabolites" of which there are several classes including alkaloids, anthraquinones, coumarins, fats, flavonoids, glycosides, gums, iridoids, mucilages, phenols, phytoestrogens, tannins, terpenes, terpenoids, ascorbic acid, folic acid and vitamin E, to mention a few (Bankole *et al.,* 2007). Extracts contain many chemical constituents, while chemicals that have been isolated from the plant are considered pharmaceutical drugs (i.e., digoxin having been isolated from the foxglove or *Digitalis lanata* plant).

Alkaloids contain a ring with nitrogen. Many alkaloids have dramatic effects on the central nervous system. Caffeine is an alkaloid that provides a mild lift but the alkaloid in datura cause severe intoxication and even death.

Glycosides consist of a glucose moiety attached to an aglycone. The aglycone is a molecule that is bioactive in its free form but inert until the glycoside bond is broken by water or enzymes. This

mechanism allows the plant defer the availability of the molecule to an appropriate time, similar to a safety lock on a gun. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by herbivore.

Terpenoids are built up from terpene building blocks. Each terpene consists of two paired isoprenes. The names monoterpenes, sesquiterpenes, diterpenes and triterpenes are based on the number of isoprene units. The frangrance of rose and lavender is due to monoterpenes. The carotenoids produce the reds, yellows and oranges of pumpkin, corn and tomatoes.

Phenolics contain phenol rings. The anthocyanin that gives grapes their purple colour, the isoflavones, the phytoestrogens from soy and the tannins that give tea its astringency are phenolics (Tyler and Foster, 1999).

Of the numerous phytochemicals (such as alkaloids, tannins, flavonoids and terpenes) present in active extracts, tannins and flavonoids are thought to be responsible for anti-diarrhoeal activity by increasing colonic water and electrolyte reabsorption. Others act by inhibiting intestinal motility. Some of the active ingredients are potentially toxic, while others, though not yet classified as nutrients, their substances are known for the prevention and treatment of health conditions, including cancer, heart disease, diabetes, and high blood pressure (Palombo, 2006). About 1,123 phytochemicals have been used to treat diabetes and out of 295 traditionally used plants screened in cell cultures, 81% were potentially anti diabetic and 1/3 may be dangerous. However many of the phytochemicals are hypoglycemic because they are toxic to our system (Duke, 2005).

2.9 Method of testing Antibacterial Activity of Medicinal Plants Extracts

The antibacterial activity of the medicinal plant extracts have been investigated against wide range of bacteria (Taura *et al*., 2004). In view of this, a number of antibacterial screening tests and procedures were developed to test crude extracts of plants against both gram-positive and gram-

negative bacteria. Antibacterial susceptibility testing may be performed reliably by either dilution or diffusion methods and the choice of methodology may be based on factors such as relative ease of performance, cost, flexibility and use of automated or semi-automated devices to facilitate testing and the perceived accuracy of the methodology (Jorgensen, 1993). Both the dilution and diffusion methods are reproducible and are used in microbiology laboratory to determine the degree of resistance among the other methods. Disk diffusion test is technically simple and very reproducible, the reagents provide category results that are easily understood and it is flexible regarding the selection of antimicrobial agent used for testing. The only limitation to this test method is the spectrum of organisms for which it has been standardized (Yolker *et al*., 2005).

2.10 Quality of Herbal Preparations

Herbal medicinal products are known as complex mixtures which originate from biological sources, great care is necessary to guarantee constant and adequate quality. The World Health Organization (WHO, 2002) survey indicated that about 70-80% of the world population particularly in developing countries rely on non-conventional medicines mainly of herbal origins for their primary healthcare. This is because herbal medicines are accessible and cheap (Sofowora, 1993). Therefore, the quality and safety of herbal preparations are also of great concerned. WHO (2002) explained that quality is the basis of reproducible, efficacy and safety of herbal drugs, and to ensure the reliability and repeatability of any research on herbal medicines, the quality of the plant materials or preparations must be determined.

Bauer (1998) showed that the quality criteria for drugs are based on a clear scientific definition of the raw materials and it is difficult to establish comprehensive quality criteria for the herbal drugs due to several aspects that exist with it, but in order to improve the identity and purity which determine the quality of the herbal drugs, the type of preparation, sensory features, moisture, ash, physical constants, solvent residual adulteration, biological activity, microbiological contamination

as well as foreign materials such as heavy metals, pesticide residuals, aflatoxins and radioactivity are to be tested. Wongwiwat *et al* (2005) reported the contamination of aflatoxins in herbal medicinal products. Also adulteration of herbal drugs with synthetic drugs is a potential serious problem which needs to be addressed by adequate regulatory measures. One report from Taiwan suggested that 24% of the samples analyzed were contaminated with at least one conventional pharmacological compound (Ernst, 2002). Medicinal plants were reported to be associated with a broad variety of microbial contaminants, which are represented by bacteria, fungi and viruses. Inevitably, the microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparation (Wolfgang *et al.,* 2002).

2.10.1 Bacteriological Quality of Herbal Medicinal Preparations

Microbial quality of herbal preparations is another important issue and of great concern because 80% of the world population use them as an alternative to cure diseases (WHO 2002). Traditional herbalists in Nigeria use various herbal preparations to treat various types of ailments, including diarrhea, urinary tract infection, typhoid fever and skin diseases (Sofowora, 1994). Most of the herbal preparations are used in different forms and normally carry a large number of various kinds of microbes originating from soil and are normally adhered to leaves, stem, flowers, seeds and root of the herbs (Adeleye *et al*., 2005). As one of the potential herbal contaminations, the level of microbial contamination tends to be much higher than synthetic materials (De Smart, 1999).

Arias *et al* (1999) evaluated the bacteriological quality of medicinal herbs most commonly used in Costa Rica and concluded that the results showed poor bacteriological quality of the herbs used as medicines. In other studies pathogenic bacteria such as *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* were also found to be potential contaminants of herbal medicinal preparations (Erich *et al*., 2001; Okunlola *et al.,* 2007).

The British pharmacopeia takes into account that the processing of crude herbs can reduce the number of bacteria by allowing a higher contamination in herbal remedies to which boiling water is added before use. It also specified that total viable aerobic count for bacteria and enterobacteria should not be greater than 10^5 and 10^3 per gram respectively, but the pharmacopeia indicates the limit for *E. coli* to be not greater than 10^2 per gram of the sample. The herbal remedies to which boil water is not added before use, the bacterial limit for total viable aerobic count should not be greater than 10^5 per gram (Anonymous, 2003). National Agency for Food and Drugs Administration and Control (NAFDAC) recommended that the total absence of pathogenic bacteria from the herbal preparation is better (NAFDAC SOP, 2000).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Study Area(s) and Sample Collection

The sample size was determine by using the formula: $n = pq /(E/1.96)^2$ and a total of 150 samples were purchased from Minna metropolis (Minna central market, old kasuwan Gwari market, new kasuwan Gwari market, Bosso and Chanchaga markets as well as herbalist dwellers within the vicinity) for analysis.

All the samples collected from the sites were analyzed in Bacteriology department of National Veterinary Research Institute, General Hospital Minna, and Zalika Fadilil–Lah (ZFL) Laboratory Minna.

3.2 Bacteriological Analysis

The pathogenic bacteria for this research work were isolated from the liquid herbal medicinal preparations using Mannitol salt agar (MSA), Levine's eosin-methylene blue agar, Salmonella – Shigella agar (SSA), Cetrimide Agar, Blood agar (BA), MacConkey agar (MCA) and Nutrient agar (NA). Each of the samples collected was replicated on each of these agars and into some broth as the need arose in order to increase the chances of isolating the pathogenic bacteria from each of the samples collected.

3.3 Preparation of Media

3.3.1 Mannitol salt agar (MSA)

Media was prepared according to manufacturer's instruction. 111g of MSA was weighed and dissolved into 1000ml distilled water and allowed to soak for 10mins. It was swirled to mix and sterilized by autoclaving at 121^0C for 15 minutes. The sterile media were allowed to cool to temperature of 50^0C and dispensed into sterilized petri dishes following aseptic techniques (Cheesbrough, 2002). The sterility of the prepared media was checked by incubation of the plates

at 37^0C for 24hrs. The sterile agar plates were stored before use in the refrigerator at 4^0C in sealed plastic bags.

3.3.2 Levine's eosin methylene blue agar

Media was prepared according to manufacturer's instruction. 36g of Eosin methylene blue agar (modified) was weighed and dissolved into 1000ml distilled water and mixed thoroughly. It was then heated with frequent agitation and boiled for one minute. It was sterilized by autoclaving at 121^0C for 15 minutes. The agar was allowed to cool to 45^0C, agitated gently and dispensed into sterilized petri dishes following aseptic techniques (Holt- Harris and Teague, 1916). The sterility of the prepared media was checked by incubation of the plates at 37^0C for 24hrs. The sterile agar plates were stored before use in the refrigerator at 4^0C in sealed plastic bags.

3.3.3 Salmonella-Shigella agar (SSA)

60g of SSA was weighed and dissolved into 1000ml distilled water and allowed to soak for 10mins. It was swirled to mix and bring to boil. The sterile media were allowed to cool to temperature of 50^0C and dispensed into sterilized petri dishes following aseptic techniques (Cheesbrough, 2002). The sterility of the prepared media was checked by incubation of the plates at 37^0C for 24hrs. The sterile agar plates were stored before use in the refrigerator at 4^0C in sealed plastic bags.

3.3.4 Cetrimide agar (CA)

45.3g of CA (Agar Medium N) and 10ml. of glycerol were suspended into 1000ml distilled water. It was heated with frequent agitation and boiled for one minute to completely dissolve the medium. It was then sterilized by autoclaving at 121^0C for 15 minutes. The sterile media were allowed to cool to temperature of 50^0C and dispensed into sterilized petri dishes following aseptic techniques (Lowbury *et al*., 1955). The sterility of the prepared media was

checked by incubation of the plates at 37^0C for 24hrs. The sterile agar plates were stored before use in the refrigerator at 4^0C in sealed plastic bags.

3.3.5 Blood agar (BA)

14g of NA was weighed and dissolved into 500ml distilled water and allowed to soak for 10mins. It was swirled to mix and sterilized by autoclaving at 121^0C for 15 minutes. The sterile media was transferred to a 50^0C water bath. The agar was allowed to cool to 50^0C and 25ml of sterile defibrinated blood was added aseptically, mixed gently and dispensed into sterilized petri dishes (Cheesbrough, 2002). The sterility of the prepared media was checked by incubation of the plates at 37^0C for 24hrs. The sterile agar plates were stored before use in the refrigerator at 4^0C in sealed plastic bags.

3.3.6 MacConkey agar (MCA)

Media was prepared according to manufacturer's instruction. 52g of MacConkey agar was weighed and dissolved into 1000ml distilled water and allowed to soak for 10mins. It was swirled to mix and sterilized by autoclaving at 121^0C for 15 minutes. The sterile media were allowed to cool to temperature of 50^0C and dispensed into sterilized petri dishes following aseptic techniques (Cheesbrough, 2002). The sterility of the prepared media was checked by incubation of the plates at 37^0C for 24hrs. The sterile agar plates were stored before use in the refrigerator at 4^0C in sealed plastic bags.

3.3.7 Nutrient agar (NA)

28g of NA was weighed and dissolved into 1000ml distilled water and allowed to soak for 10mins. It was swirled to mix and sterilized by autoclaving at 121^0C for 15 minutes. The sterile media were

allowed to cool to temperature of 50^0C and dispensed into sterilized petri dishes following aseptic techniques (Cheesbrough, 2002). The sterility of the prepared media was checked by incubation of the plates at 37^0C for 24hrs. The sterile agar plates were stored before use in the refrigerator at 4^0C in sealed plastic bags.

3.4 Total Aerobic Plate Count and Identification of Microorganisms

3.4.1 Total Aerobic Plate Count

A ten-fold serial dilution of the bacterial suspension was made. A series of 9 McCartney bottles containing 9ml of sterile peptone water each were placed on a sterilized bench. With a sterile delivery pipette, 1ml of the collected sample was transferred into the first bottle of diluents (10^{-1}), with another fresh sterile 1ml pipette; 1ml from the first dilution was transferred into the second bottle of diluents (10^{-2}) dilution. This was done until 10^{-10} dilution was achieved. 0.1ml was then pipette from 10^{-8} – 10^{-9} dilution onto the surface of two petri dishes containing 20ml of a liquefied and sterile plate count agar (PCA) and once spread evenly with a sterile glass spreader.

The plates were incubated for a maximum of 48hrs at 37^0C. Finally the counting of the bacterial colonies was done using the Stuart Digital colony counter. After incubation, the number of colonies was chosen because this range was considered statistically significant. Hence if they are less than 30 colonies a plate, it could be due to error in dilution which may have drastic effect on the final count, likewise if there are more than 300 colonies on a plate, it could be due to poor isolation and colonies may have grown together (B1OL 230 Lab manual, Lab 4). In determining the colony forming units (CFUs), the number of colonies counted on a plate was multiplied by the dilution factor to obtain the colony forming units (CFUs).

The number of CFUs per ml of sample = the number of colonies x the dilution factor of the plate counted.

3.4.2 Isolation of *Staphylococcus aureus*

The samples were cultured on 7.5% NaCl enriched Mannitol Salt Agar (MSA) by streaking. The inoculated media were incubated at 37^0C for 24hrs under aerobic conditions. The media plates were then examined for growth, colony morphology and any change in the medium. Pure cultures were obtained by sub-culturing from a typical and well isolated colony on 7.5% NaCl enriched Mannitol Salt Agar (MSA).

Identification of *Staphylococcus aureus* was done by gram staining technique according to Green berge *et al* (1992). These findings were further subjected to biochemical identification using catalase and coagulase tests.

3.4.3 Isolation of *Escherichia coli*

Isolation of *Escherichia coli* was done by streaking the sample onto the surface of Eosin Methylene Blue (EMB) agar and incubated for 24hrs at 44^0C. The media plates were then examined for growth, colony morphology and any change in the medium. Pure cultures were obtained by sub-culturing from a typical and well isolated colony on Eosin Methylene agar.

Identification of *Escherichia coli* was done by gram staining technique. These findings were further subjected to biochemical identification using indole, citrate utilization, triple sugar iron, lysine decarboxylate and urease test.

3.4.4 Isolation of *Salmonella typhi*

Inoculums from the liquid herbal preparations were streaked onto the surface of Salmonella-Shigela Agar (SSA). The plates were incubated at 37^0C for 24hrs. The media plates were then examined for growth, colony morphology and any change in the medium. Pure cultures were obtained by sub-culturing from a typical and well isolated colony on Salmonella-Shigella agar.

Identification of *Salmonella typhi was* done by gram staining technique. The biochemical reactions carried out on *Salmonella typhi* include indole, citrate utilisation, triple sugar iron agar (TSI), lysine decarboxylate and urease test.

3.4.5 Isolation of *Shigella sonnei*

Isolation of *Shigella sonnei* was achieved by streaking the sample onto the surface of Salmonella-Shigella agar which was incubated at 37^0C for 24hrs. The media plates were then examined for growth, colony morphology and any change in the medium. Pure cultures were obtained by sub-culturing from a typical and well isolated colony on Salmonella-Shigella agar.

The bacterial isolates were identified with gram staining techniques and suitable biochemical test such as Indole, triple sugar iron (TSI), motility, lysine decarboxylase and urease test.

3.4.6 Isolation of *Pseudomonas aeruginosa*

Inoculums from the liquid herbal preparations were taken using a sterilized wire loop. The inoculum was streaked onto the surface of Cetrimide agar which was incubated at 37^0C for 24hrs under aerobic condition. The media plates were then examined for growth, colony morphology and any change in the medium. Pure cultures were obtained by sub-culturing from a typical and well isolated colony on Cetrimide agar.

Gram staining and suitable biochemical tests such as nitrate reduction, gluconate oxidation and gelatin liquefaction tests.

3.4.7 Isolation of *Bacillus firmus*

Isolation of *Bacillus firmus* was achieved by streaking the sample onto the surface of MacConkey agar (MCA) and incubated for 24hrs at 37^0C. The media plates were then examined for growth, colony morphology and any change in the medium. Pure cultures were obtained by sub-culturing from a typical and well isolated colony on Blood agar. The colonies were identified by gram staining technique and further biochemical tests such as motility, gelatin liquefaction and lecithinase tests to confirm *Bacillus firmus*.

3.4.8 Isolation of *Klebsiella aerogenes*

Inoculums from the liquid herbal preparation were taken using a sterilized wire loop. The inoculums were streaked on MacConkey agar plate which was incubated at 37^0C for 24hrs under aerobic condition. Pure cultures were obtained by sub-culturing from a typical and well isolated colony on MacConkey agar.

Gram reaction and biochemical test using indole, methyl red, voges-prokauer, citrate utilization, lysine decaboxylase and gluconate oxidation tests were further carried out to identify *Klebsiella aerogenes.*

3.4.9 **Isolation of *Streptococcus faecalis***

Isolation of *Streptococcus faecalis* was achieved by streaking the sample onto the surface of Blood agar (BA) and incubated for 24hrs at 37^0C. The media plates were then examined for growth, colony morphology and any change in the medium. Pure cultures were obtained by sub-culturing from a typical and well isolated colony on Blood agar.

Identification of *Streptococcus faecalis* was done by gram staining technique. These findings were further subjected to biochemical identification using catalase, motility and gluconate oxidation tests.

3.5 Gram Staining

The method of Green berge *et al* (1992) was adopted in identification of bacteria Gram reaction (Gram positive and Gram negative). Bacteria smear was prepared on a clean grease-free slide, air dried and heat fixed. The slide was flooded with crystal violet for 1 minute, decanted and rinse with water. Gram's iodine (mordant) was applied for 30 seconds and rinsed. Acetone was used in decolorizing and washed immediately then counter stained with neutral red for 1 minute. It was

then rinsed with water, blotted carefully and air dried. Finally the preparations were observed under the microscope using oil immersion objectives (x100).

3.6 Biochemical Analysis

3.6.1 Catalase Test

The method of Matsen (1980) was adopted in carrying out catalase test. A drop of 3% hydrogen peroxide was placed on a clean grease-free slide using a sterile wire loop and a loopful of pure culture of the isolate was added and emulsified. Formation of effervescence indicates a positive test while absence of effervescence indicates a negative test.

3.6.2 Coagulase Test

The method of Sonnerwith (1980) was adopted in carrying out coagulase test. A drop of distilled water was placed on a clean glass slides and a thick suspension of bacteria isolate was made on the slide, then a loopful of citrated lumen plasma was added and the slide was rocked (tickled back and forth) for a minute. A positive test showed an immediate clumping of bacterial suspension while a negative test showed no clumping of the cells.

3.6.3 Indole Test

A test tube containing tryptic soy broth was inoculated with the test organism and incubated, at 37^0C for 24hrs. 0.5ml of kovacs indole reagent was added to the tube containing the amino acid tryptone and shaken gently. Formation of a red colour in the surface layer after 1 minute indicates a positive result.

3.6.4 Methyl Red Test

Glucose phosphate medium was prepared and inoculated with the test organism, then incubated at 37^0C for 48hrs. 0.04% methyl red indicator was added. Formation of a red color indicated a positive reaction while a yellow color indicates a negative result.

3.6.5 Voges- Proskauer Test

Glucose phosphate medium was prepared as was done for methyl red. 3ml of creatine was added followed by 1ml of KOH and mixed thoroughly. Appearance of a pink color after 48hrs indicated a positive result.

3.6.6 Citrate Utilisation Test

Simmons citrate agar was prepared and inoculated with the test organism by streaking the agar slant and stabbing the butt using sterile wire needle, incubated at 37^0C and examined daily (24-72hrs) for growth. Color change from pale green to blue indicated a positive result.

3.6.7 Triple Sugar Iron agar Test

The test was done to identify enteric bacteria by their ability to attack glucose, lactose or sucrose and to liberate sulfides from sodium thiosulfite. Triple sugar Iron agar was prepared according to manufacturer's specification. Pure colonies of the test organism from nutrient agar plate were streaked on the slant and the butt was stabbed as well and incubated at 37^0C for 24hrs. Color change was observed and recorded.

For *Shigella sonnei* the presumptive colonies on TSI slants were inoculated into prepared urea slant and incubated at 37^0C for 3hrs. Absence or change of color suggested the presence of *Shigella sonnei*.

Ability of the organism to grow along the line of the stabbed region indicated a positive motility test and absence of organism along the stabbed region indicate a negative test.

3.6.8 Lysine decarboxylase Test

The test was done to differentiate bacteria that decaboxylate or deaminate the amino acid lysine.

A sterile slant of lysine decarboxylase broth was prepared according to manufacturer's specifications. Pure bacterial colonies from nutrient agar plate were streaked on the slant and butt was stabbed as well. They were then layered with sterile paraffin oil and incubated at 35^0C for 24hrs. Color change was observed and recorded.

3.6.9 Motility Test

The motility medium was prepared and inoculated with the test organism by making a fine stab with a needle to a depth of 2cm short of the tube. This was incubated at 37^0C for 24hrs. The tube was then examined and recorded.

3.6.10 Urease Test

Urea agar was prepared and dispensed into bijou bottles. The test organism was inoculated and the urea slants were incubated at 37^0C for 24hrs. Development of a bright pink colour indicates a positive reaction.

3.6.11 Nitrate reduction Test

The test organisms were inoculated in 5ml Nitrate broth, Durham tubes were inserted and incubated for 24hrs. Gas production was observed. To those that there was no gas production, 10 drops of Sulphanilic acid in 5N acetic acid and a pinch of zinc granule were added. A red colour develops around the granule within two minutes and eventually colours the broth if nitrate is present.

3.6.12 Gluconate oxidation Test

Gluconate broth was prepared and 5ml were dispensed into bijou bottles. The test organisms were inoculated into the 5ml gluconate broth and incubated for 24hrs. 1ml of Benedict solution was added, they were placed in boiling water bath for 15mins. A reddish brown precipitate develops within 10mins in a positive test.

3.6.13 Gelatin liquefaction Test

Gelatin agar was prepared, inoculated with test organisms by streaking and incubated at 37^0C for 24hrs. The plate was then flooded with acid mercuric chloride solution. A positive reaction is indicated by a definite zone of clearing around the streak.

3.7 Antibacterial Susceptibility Test

Susceptibility tests were performed following the M2-A6 disc diffusion method recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1997) using nutrient agar. Isolates were grown overnight on nutrient agar and suspended in sterile physiological saline (0.9% w/v NaCl) to obtain an equivalent of 0.5 McFarland turbidity standards. A sterile, non-toxic cotton swab was dipped into the standardized innocula and used to spread the entire surface of Mueller Hinton agar plates (NCCLS, 2002).

Antibiotic discs (PS003G-VE, polytes Laboratory, Enugu, Nigeria) were placed aseptically on the surface of the agar plates using sterilized forceps and thereafter incubated at 37^0C for 24hrs. The antibiotics screened include the following: cloxacillin (CXC), 5µg; tetracycline (TET), 50µg; erythromycin (ERY), 5µg; ciproxin [a brand of ciprofloxacin by Bayer, Nigeria] (CPX), 10µg; augmentin (AUG), 30µg; chloramphenicol (CHI), 10µg; cotrimoxazole (COT), 50µg and gentamycin (GEN), 10µg. Zone of inhibition produced after the incubated periods were measured in millimeters using calibrated ruler.

3.8 Determination of Multiple Antibiotic Resistance (MAR) Index

The multiple antibiotic resistance (MAR) index is determined for each isolate by dividing the number of antibiotics to which the organism is resistance by the number of antibiotics tested (Krumperman, 1983; Paul *et al*., 1977).

MAR index= Number to which organism is resistance/ Total number of antibiotic tested.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of hygiene practices on liquid herbal preparations sold in Minna Metropolis

Of one hundred and fifty samples, eighty nine (59.3%) were prepared without washing while sixty one (40.7%) was washed before preparation (Table 4.1). This information was obtained through the fillings of questionnaire (Appendix IV) served on the participants.

4.2 Distribution of bacterial isolates in the herbal preparations in Minna Metropolis

A total of two hundred and four (204) strains of bacteria were isolated from three local government of Minna Metropolis (Table 4.2). The number and percentages of isolated pathogenic bacteria in decreasing order were: *Staphylococcus aureus* 60(40.0%), *Escherichia coli* 55(36.7%), *Salmonella typhi* 33(22%), *Shigella sonnei* 19(12.7%), *Bacillus firmus* 15(10.0%), *Klebsiella aerogenes* 13(8.7%), *Streptococcus faecalis* 5(3.3%) and *Pseudomonas aeruginosa* 4(2.7%).

4.3: Distribution of bacterial isolates in the herbal preparations in Chanchaga Local Government

Figure 4.1 shows the distribution of bacterial isolates in the herbal preparations in Chanchaga Local Government. It is observed that *Staphylococcus aureus* 14(23.3%) was the most frequently isolated pathogenic bacteria while *Pseudomonas aeruginosa* 1(1.7%) was the least isolated.

Table 1 Effect of hygiene practices on liquid herbal preparations sold in Minna Metropolis.

Samples	Number contaminated	% contaminated
Washed	61	40.7
Not washed	89	59.3
Total	150	100

Table 2 Distribution of bacterial isolates in the herbal preparations in Minna Metropolis

Bacterial isolates	Numbers Positive	percentages Negative	Total
Staph aureus	60 (40.00)	90 (60.00)	150 (100)
Escherichia coli	55 (36.67)	95 (63.33)	150 (100)
Salmonella typhi	33 (22.00)	117 (78.00)	150 (100)
Shigella sonnei	19 (12.67)	131 (87.33)	150 (100)
Bacillus firmus	15 (10.00)	135 (90.00)	150 (100)
Klebsiella aerogenes	13 (8.67)	137 (91.33)	150 (100)
S. faecalis	5 (3.33)	145 (96.67)	150 (100)
P. aeruginosa	4 (2.67)	146 (97.33)	150 (100)

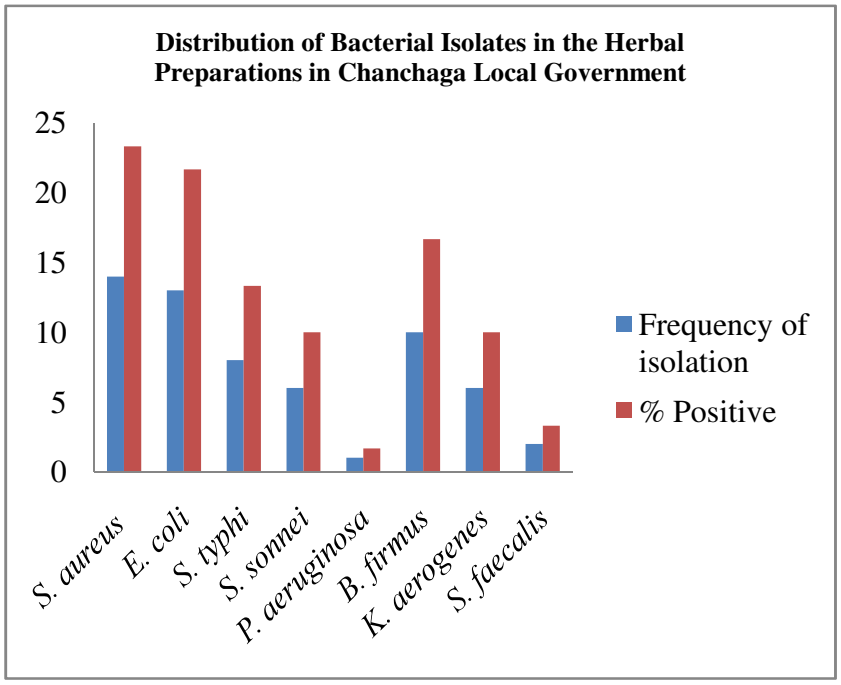

Figure 1: Distribution of bacterial isolates in the herbal preparations in Chanchaga Local Government

4.4 Distribution of Bacterial Isolates in the Herbal Preparations in Bosso Local Government

The result obtained in figure 4.2 shows the frequency of isolated pathogenic bacteria in decreasing order: *Staphylococcus aureus* 25(35.71%), *Escherichia coli*, 23(32.86%), *Salmonella typhi* 12(17.14%), *Shigella sonnei* 6(8.57%), *Klebsiella aerogenes* 3(4.29%) and *Bacillus firmus* 1(1.43%).

4.5 Distribution of bacterial isolates in the herbal preparations in Minna West Local Government

The result obtained in figure 4.3 also showed that *Staphylococcus aureus* 21(28.38%) was the most frequently isolated pathogenic bacteria from herbal preparations followed by *Escherichia coli* which is 19(25.67%) while *Pseudomonas aeruginosa* and *Streptococcus faecalis 3(4.05%)* were the least isolated.

4.6 The total aerobic bacterial content of liquid herbal preparations

Many types of bacteria were isolated and identified, with varying isolation levels from 150 samples.

The gram-positive cocci

Staphylococcus aureus: were isolated from sixty (60) liquid herbal samples. The average rate of isolation from all samples was 29.41% (Table 4.3).

Streptococcus faecalis; were isolated from five (5) liquid herbal samples. The average rate of isolation from all samples was 2.45%.

The gram-positive rods

Bacillus firmus: were isolated from 15 liquid herbal samples. The average of isolation from all samples was 7.35%.

The gram-negative rods were isolated from 124 herbal samples. The average rate of isolation from all samples was 60.79%. The named species are *Escherichia coli***,** *Salmonella typhi, Shigella sonnei, Pseudomonas aeruginosa* and *Klebsiella aerogenes.*

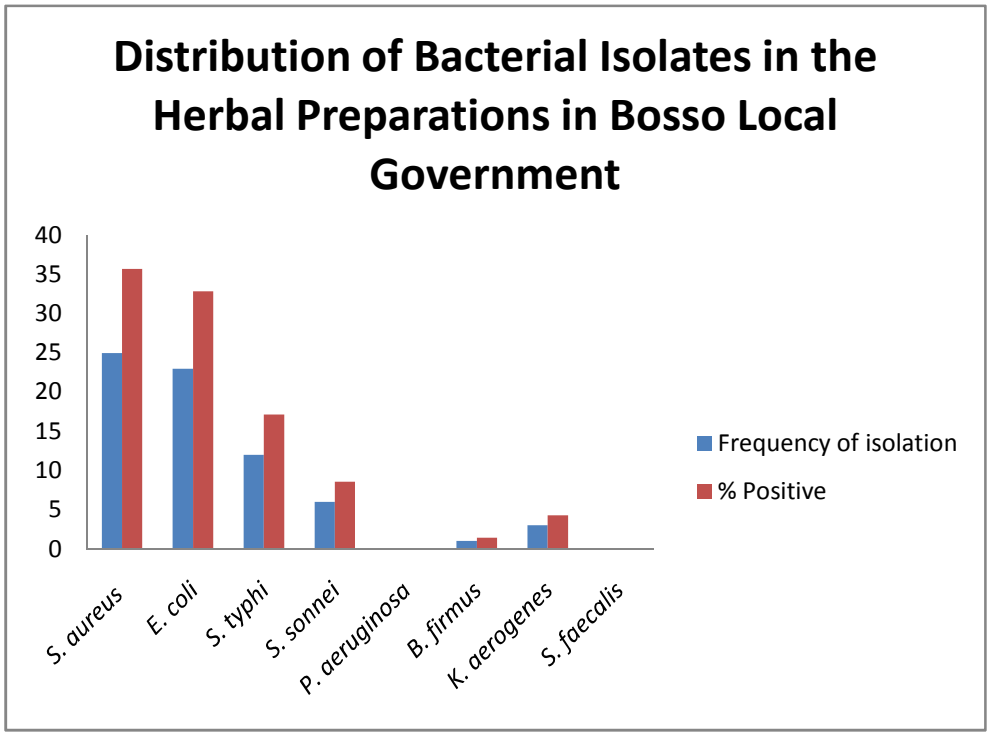

Figure 2: Distribution of bacterial isolates in the herbal preparations in Bosso Local Government

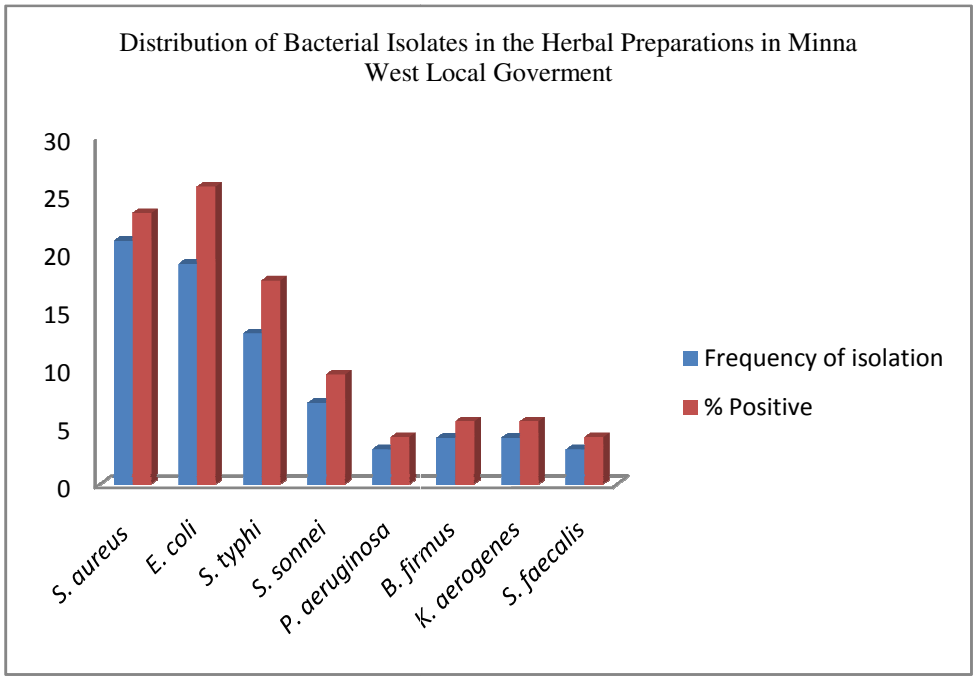

Figure 3: Distribution of Bacterial Isolates in the Herbal Preparations in Minna West Local Government.

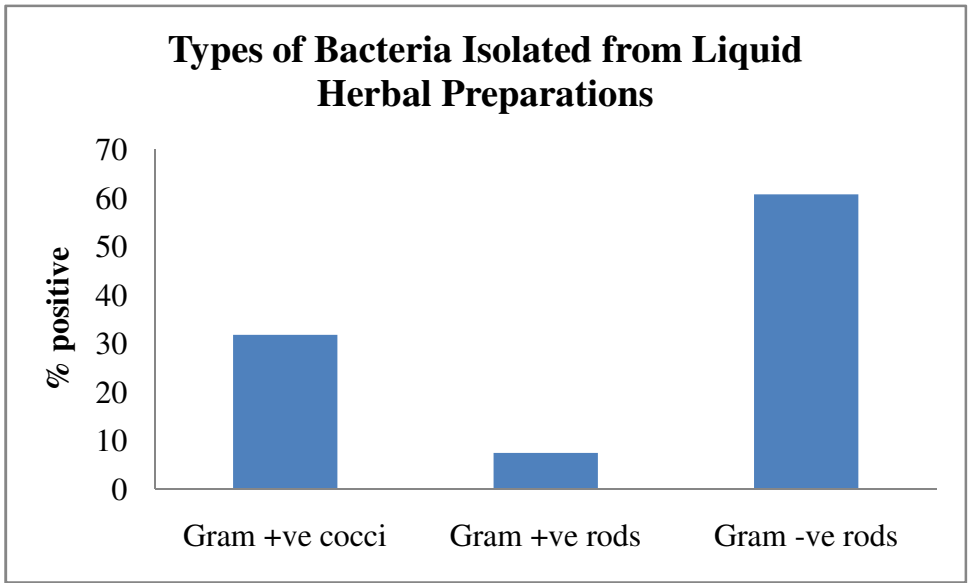

Figure 4: Types of bacteria isolated from Liquid herbal preparations.

4.7 Distribution of bacteria count of herbal medicinal products

The distribution of bacterial count of the herbal preparation is presented in Table 4.4. Results showed that 47(31.3%) samples indicated aerobic plate count of 10^7, 17(11.3%) showed the bacterial count of 10^3 and 1(0.7%) showed aerobic plate count of 10^6cfu/ml.

4.8 Total bacterial count of some herbal preparations sampled in Minna Metropolis based on source of preparation

Table 4.5 showed the mean and standard deviation of bacteria count of herbal medicinal products as observed through the source plant used as shown in appendix B. The aerobic plate count of all the parts used varied from 1.2×10^7 to 1.0×10^8cfu/ml with the standard deviation between the ranges of 2.2×10^7 to 1.4×10^8.

4.9 Effect of preservative on frequency occurrence of pathogenic bacteria in herbal Preparations

The effect of preservatives on frequency of occurrence of pathogenic bacteria in liquid herbal preparations is presented in Table 4.6. It was observed that twenty out of twenty five samples that were preserved by Kanwa (potash) were contaminated with pathogenic bacteria; eleven out of eighteen that was preserved by lime and thirty three out of fifty that were preserved by boiled water were also contaminated.

Table 3 Distribution of bacteria count of herbal medicinal products

Aerobic plate count (cfu/ml)	Number of Samples	Percentage (%)
0	49	32.7
10^3	17	11.3
10^4	23	15.3
10^5	Nil	0
10^6	1	0.7
10^7	47	31.3
10^8	13	8.7
Total	150	100

Table 4 Total bacterial count on some herbal preparations sampled in Minna Metropolis based on source of preparations

Source of product	Number of positive samples	Mean count	Standard Deviation
Leaves	20	1.9×10^7	2.2×10^7
Stem	22	3.5×10^7	4.5×10^7
Roots	19	1.0×10^8	1.4×10^8
Whole plant	20	1.2×10^7	4.1×10^7
Combined	20	5.1×10^7	4.9×10^7

Table 5 Effect of preservative on frequency occurrence of pathogenic bacteria in herbal preparations

Preservative used	Number of Sample	Number of Sample contaminated	% contaminated
Kanwa	25	20	19.80
Jan Kanwa	14	9	8.91
Lime	18	11	10.89
Lemon	6	4	3.96
Cafra	17	10	9.90
Salt	4	3	2.97
Refrigerated	5	4	3.96
Boiling	50	33	32.67
No Preservative	11	7	6.94
Total	150	101	100

Table 6 Antibiotic Susceptibility of *Staphylococcus aureus* isolates from Liquid Herbal Products

S/N	Antibiotics	Disk Content (µg)	Susceptibility		
			Number (%) Resistance	Number (%) Intermediate	Number (%) Susceptible
1	Cloxacillin	5	60 (100)	0 (0)	0 (0)
2	Tetracycline	50	31 (51.7)	15 (25)	14 (23.3)
3	Erythromycin	5	35 (58.3)	23 (38.4)	2 (3.3)
4	Ciproxin	10	13 (21.6)	16 (26.7)	31 (51.7)
5	Augmentin	30	3 (5)	0 (0)	57 (95)
6	Chloramphenicol	10	43 (71.7)	1 (1.7)	16 (26.7)
7	Cotrimoxazole	50	32 (53.3)	10 (16.7)	18 (30)
8	Gentamycin	10	14(23.3)	4 (6.7)	42 (70)

Table 7 Antibiotic Susceptibility of *Escherichia coli* isolates from Liquid Herbal Products

S/N	Antibiotics	Disk Content (µg)	Susceptibility		
			Number (%) Resistance	Number (%) Intermediate	Number (%) Susceptible
1	Cloxacillin	5	52 (94.6)	2 (3.6)	1 (1.8)
2	Tetracycline	50	27 (49.1)	10 (18.2)	18 (32.7)
3	Erythromycin	5	28 (51)	22 (40)	5 (9)
4	Ciproxin	10	8 (14.5)	13 (23.6)	34(61.8)
5	Augmentin	30	48 (87.3)	5 (9.01)	2 (3.6)
6	Chloramphenicol	10	42 (76.4)	3 (5.5)	10 (18.2)
7	Cotrimoxazole	50	32 (58.2)	7 (12.7)	16 (29.1)
8	Gentamycin	10	11(20)	0 (0)	44 (80)

Table 8 Antibiotic Susceptibility of *Salmonella typhi* isolates from Liquid Herbal Products

S/N	Antibiotics	Disk Content (µg)	Susceptibility		
			Number (%) Resistance	Number (%) Intermediate	Number (%) Susceptible
1	Cloxacillin	5	31(93.9)	0 (0)	2 (6.1)
2	Tetracycline	50	19 (57.6)	9 (27.3)	5 (15.1)
3	Erythromycin	5	11(33.3)	22 (66.7)	0 (0)
4	Ciproxin	10	6 (18.2)	2 (6.1)	25 (75.7)
5	Augmentin	30	27(81.8)	2 (6.1)	4 (12.1)
6	Chloramphenicol	10	27 (81.8)	1(3)	5 (15.1)
7	Cotrimoxazole	50	21(63.6)	6 (18.2)	6 (18.2)
8	Gentamycin	10	7 (21.1)	1(3)	25 (75.7)

Table 9 Antibiotic Susceptibility of *Shigella sonnei* isolates from Liquid Herbal Product

S/N	Antibiotics	Disk Content (µg)	Susceptibility		
			Number (%) Resistance	Number (%) Intermediate	Number (%) Susceptible
1	Cloxacillin	5	19 (100)	0 (0)	0 (0)
2	Tetracycline	50	13 (68.4)	5 (26.3)	1(5.3)
3	Erythromycin	5	8 (42.1)	10 (52.6)	1(5.3)
4	Ciproxin	10	11 (57.8)	2 (10.5)	6 (31.5)
5	Augmentin	30	16 (84.2)	3 (15.8)	0 (0)
6	Chloramphenicol	10	11(57.9)	1(5.3)	7 (36.8)
7	Cotrimoxazole	50	12 (63.2)	2 (10.5)	5 (26.3)
8	Gentamycin	10	7 (36.8)	0 (0)	12 (63.2)

Table 10 Antibiotic Susceptibility of *Pseudomonas aeruginosa* isolates from Liquid Herbal Products

S/N	Antibiotics	Disk Content (µg)	Susceptibility		
			Number (%) Resistance	Number (%) Intermediate	Number (%) Susceptible
1	Cloxacillin	5	4 (100)	0 (0)	0 (0)
2	Tetracycline	50	3 (75)	1(25)	0 (0)
3	Erythromycin	5	2 (50)	2 (50)	0 (0)
4	Ciproxin	10	2 (50)	0 (0)	2 (0)
5	Augmentin	30	4 (100)	0 (0)	0 (0)
6	Chloramphenicol	10	2 (50)	1 (25)	1 (25)
7	Cotrimoxazole	50	2 (50)	0 (0)	2 (50)
8	Gentamycin	10	2 (50)	0 (0)	2 (50)

Table 11 Antibiotic Susceptibility of *Bacillus firmus* isolates from Liquid Herbal Products

S/N	Antibiotics	Disk Content (µg)	Susceptibility		
			Number (%) Resistance	Number (%) Intermediate	Number (%) Susceptible
1	Cloxacillin	5	15(100)	0 (0)	0 (0)
2	Tetracycline	50	7 (46.7)	2 (13.3)	6 (40)
3	Erythromycin	5	10 (66.7)	4 (26.6)	1(6.7)
4	Ciproxin	10	7 (46.7)	5 (33.3)	3 (20)
5	Augmentin	30	15 (100)	0 (0)	0 (0)
6	Chloramphenicol	10	13 (86.7)	0 (0)	2 (13.3)
7	Cotrimoxazole	50	12(80)	1 (6.7)	2 (13.3)
8	Gentamycin	10	2 (13.3)	0 (0)	13(86.7)

Table 12 Antibiotic Susceptibility of *Klebsiella aerogenes* isolates from Liquid Herbal Products

S/N	Antibiotics	Disk Content (µg)	Susceptibility		
			Number (%) Resistance	Number (%) Intermediate	Number (%) Susceptible
1	Cloxacillin	5	13 (100)	0 (0)	0 (0)
2	Tetracycline	50	8(60.7)	3 (23.1)	2 (15.2)
3	Erythromycin	5	6 (46.2)	6 (46.2)	1(7.6)
4	Ciproxin	10	5(38.4)	4 (30.8)	4 (30.8)
5	Augmentin	30	12 (92.4)	1(7.6)	0 (0)
6	Chloramphenicol	10	11(84.8)	0 (0)	2 (15.2)
7	Cotrimoxazole	50	9 (69.3)	1(7.6)	3(23.1)
8	Gentamycin	10	3 (23.1)	0 (0)	10 (76.9)

Table 13 Antibiotic Susceptibility of *Streptococcus faecalis* isolates from Liquid Herbal Products

S/N	Antibiotics	Disk Content (µg)	Susceptibility		
			Number (%) Resistance	Number (%) Intermediate	Number (%) Susceptible
1	Cloxacillin	5	5 (100)	0 (0)	0 (0)
2	Tetracycline	50	2(40)	1(20)	2 (40)
3	Erythromycin	5	5 (100)	0 (0)	0 (0)
4	Ciproxin	10	3 (60)	2(40)	0 (0)
5	Augmentin	30	4 (80)	1(20)	0 (0)
6	Chloramphenicol	10	3 (60)	0 (0)	2 (40)
7	Cotrimoxazole	50	3 (60)	0 (0)	2 (40)
8	Gentamycin	10	3 (60)	0 (0)	2 (40)

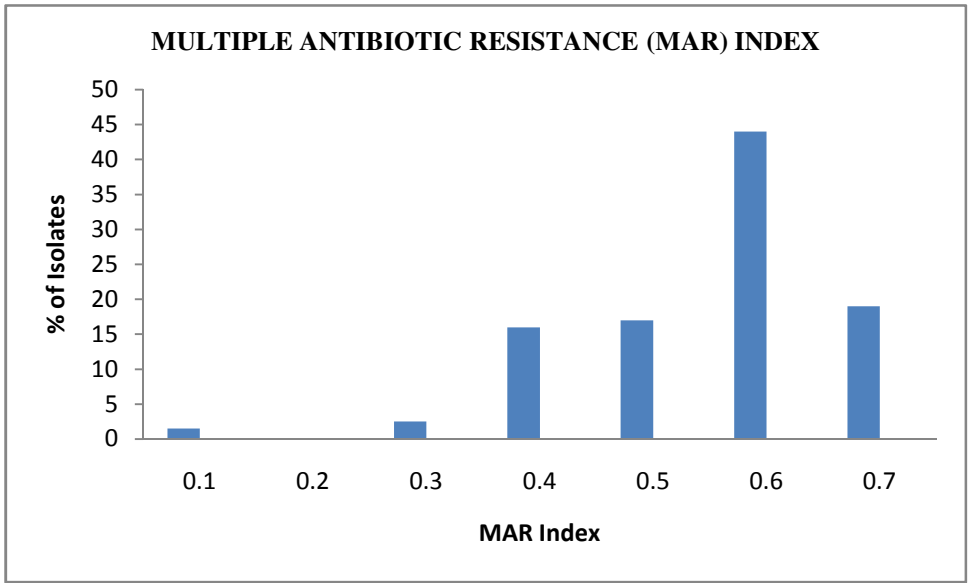

Figure 5: MULTIPLE ANTIBIOTIC RESISTANCE (MAR) INDEX

CHAPTER FIVE

5.0 DISCUSSION

All the liquid herbal medicinal preparations used in this study with the exceptions for those samples without the manufacturing and expiry dates indicated, were within their shelf life at the time of investigation. Ninety (60.0%) of the herbal products had their manufacturing and expiry dates stated while only 65(43.3%) of the samples have been registered by NAFDAC. This is contrary to the prohibition of the manufacturer, advertisement, sale and distribution of herbal medicinal products in Nigeria without proper registration by NAFDAC (Herbal Medicines and Related Products (Registrations) Regulations, 2004). The European Agency for the Evaluation of Medicinal Products (EMEA) and World Health Organization (WHO) have stated that the quantity of the herbal drug should be given as a range corresponding to a defined quantity of the constituent with known therapeutic activity and if constituent(s) responsible for the therapeutic activity are unknown, the quantity of the herbal drugs should not be given (EMEA, 1998; WHO, 1993). Furthermore, the dosage form, therapeutic indications and expiry dates should be stated. However, 69(46%) of the samples did not have their contents stated even though their therapeutic claims were indicated on the containers.

5.1 Distribution of liquid herbal medicinal preparations.

Herbal medicinal products usually contain bacteria from soil and atmosphere. The presence of microbial contaminants in these products can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect the patient taking the medicines (Frazier and Westhoff, 2003; Nakajima *et al.,* 2005).The high microbial content obtained in this study is in conformity with a similar work carried out in Kano, Nigeria (Shamsudeen *et al*, 2008). Distributions of bacterial isolates in the herbal medicinal products are presented in Table 2 and figures 1 – 3. The results in this study are in agreement with other authors (Esimone *et al.*, 2007;

Oyetayo, 2008; Abba *et al*., 2009) that found similar number of bacteria in different herbal products. The presence of large number of pathogens in this study may be due to the raw materials (which may be shabbily collected with no known aseptic method), water (which may be inadequately or not treated) and methods of preparations used. Other possible sources of contaminations may be the personnel(s) or manufacturer(s) that could introduce the bacteria during processing and also the sanitary condition of the environment where the herbal products were produced. Hence all the methods involve in the process of manufacturing herbal medicinal products such as harvesting, drying, storage, handling and the soil influence the bacteria quality of raw material which in turn affects the entire quality of the herbal preparations. On the other hand, the result was in contrary to the work of Okunlola *et al* (2007) who do not isolate *Pseudomonas aeruginosa* in their work.

5.2 Total bacteria count of liquid herbal preparations

The aerobic plate count is an indication of the sanitary conditions under which the medicinal products were being prepared. The limits of bacterial contamination given in European pharmacopoeia as reported by Okunlola *et al* (2007) are: total aerobic bacteria (10^5cfu/ml), Enterobacteria and other Gram negative organisms (10^3cfu/ml). *Escherichia coli* and *Salmonella* should be absent. The herbal products under this study did not meet these specifications in most cases. Figure 4 and tables 3 – 4 showed bacteria count of herbal preparations. The samples were contaminated to varying degrees with pathogenic bacteria and the limits of bacterial count in some samples are beyond that given in European pharmacopoeia. Forty seven (31.3%) out of 150 herbal preparations showed bacterial count of 10^7cfu/ml, thirteen (8.7%) showed count of 10^8cfu/ml. The high microbial content was not in agreement with Uraih, 2004 acceptable standard of MPN value of 10/ml. Of concern also is the level of contamination of herbal medicinal preparations by pathogenic Gram negative bacteria. One hundred and twenty four (60.79%) of the samples were

contaminated by one or more gram negative bacteria such as *E. coli*, which is an intestinal bacterium and is an indicator for faecal contamination, *Salmonella typhi, Shigella sonnei., Klebsiella aerogenes* and *Pseudomonas aeruginosa*.

5.3 Effects of preservatives on frequency of pathogenic bacteria in herbal preparations

The use of preservatives in liquid herbal medicines is necessary, although the herbalist sees the incorporation of preservatives in their preparation as adulteration. This should not be so, as adequate preservative is essential not only to prevent deterioration of the product, but also to stop the proliferation of contaminating pathogens (Esimone *et al.*, 2007). It is apparent that these preparations proved a suitable environment for the growth/survival of microorganisms. However, these contaminated liquid preparations were also preserved to varying extent by all the preservatives used. The USP (1990) requires that for the preservative to be effective in the product examined, the concentrations of viable bacteria be reduced to minimal of the initial concentration by the 14^{th} day. The effect of preservative on the frequency of occurrence of pathogenic bacteria in herbal medicinal preparations is presented in Table 4.5. The result is contrary to that obtained by (Esimone *et al.*, 2007). This negative results against the preservatives used might be due to the methods of preparation and the hygiene nature of both the manufactures and the environment under which these liquid herbal medicines were being produced.

5.4 The zone of inhibition of the antibiotics to the bacterial isolates

The response of bacterial isolates to antibiotics showing zones of inhibitions for each antibiotic is shown in appendix C and Table 6 - 13. The bacteria isolates were sensitive to fluoroquinolone (ciprofloxacin) and aminoglycosides (gentamycin). Also susceptibility was recorded against tetracycline and chloramphenicol in some of the isolates. Chloramphenicol is a useful antimicrobial and has stood the test of time. It has been known to be the drug of choice in the

treatment of Salmonellosis and has also been shown by the result obtained, to be very effective on all the bacteria isolates with the highest bactericidal activity on *Salmonella typhi.*

The isolates also showed wide resistance to penicillins, especially augmentin (amoxicillin-clavulanic acid combination) and cloxacillin, suggesting that they could be producers of penicillinases. Similar resistance to erythromycin by most of the contaminants was also observed. More worrisome is the resistance to trimethoprin-sulphamethoxazole (cotrimoxazole) observed especially among the Gram-negative isolates. *S. aureus* can originate from handlers, as its habitat is human skin. The number of *Streptococcus faecalis* found was very few. These results are in agreement with other authors that found a similar number of these bacteria in different Pharmaceuticals (Devleeschouwer and Dony, 1979; Garcia-Arribas *et al*., 1983). These gram-positive cocci are found mainly in raw materials, air and water and on human beings and like *Bacillus spp.*, they can survive in the environment and thus contaminate the medicaments. Resistance to antimicrobial agents had been reported by many researchers (Power, 1998; Esimone *et al.,* 2007). Work by French and Phillips (1997) had also showed that increasing resistance to antibiotics is a consequence of selective pressure, warranted by antimicrobial therapy which tends to select resistant bacteria to survive and spread in environment.

5.5 Multiple antibiotic resistance (MAR) Index

The result of this study is in conformity with previous observations, Olayinka *et al* (2004); Wenzel and Edmond (1998) that most bacterial isolates of herbal medicinal products are resistant to a large number of commonly prescribed antibiotics. The MAR index gives an indirect suggestion of probable source(s) of an organism. According to previous workers, MAR index of >0.2 indicates that an organism must have originated from an environment where antibiotics are often used.

5.6 CONCLUSION

In conclusion, the study revealed that most of the herbal preparations were not sterile and may serve as source of infection to the consumers. Also coupled with the unhygienic conditions under which these products were being prepared and handled, there is need for regulatory agency, NAFDAC and SON to carry out more detailed and regular analysis of these herbal preparations to prevent the unsuspecting and uninformed customers from buying what may aggravate their ailment.

The high rate of resistance to antimicrobial agents of strain isolated from these herbal preparations may indicate a widespread antibiotic resistance among micro-organisms from different sources. It is therefore mandatory that herbal medicines should not be taken indiscriminately and that current good manufacturing practices (cGMPs) must be observed by these herbal practitioners in the production of the medicines**.**

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APPENDIX I

S/NO.	Total bacterial count (cfu/ml)	*S. aureus*	*E. coli*	*S. typhi*	*S. sonnei*	*P. aeruginosa*	*B. firmus*	*K. aerogenes*	*S. faecalis*
1.	1.5×10^7	+	-	-	-	-	+	+	-
2.	-	-	-	-	-	-	-	-	-
3.	2.4×10^3	-	-	-	-	-	-	+	-
4.	3.1×10^3	-	+	-	-	-	+	+	-
5.	-	-	-	-	-	-	-	-	-
6.	-	-	-	-	-	-	-	-	-
7.	1.2×10^7	+	-	-	-	-	-	-	-
8.	2.25×10^4	+	-	-	-	-	-	-	-
9.	2.7×10^3	-	-	-	-	-	+	+	-
10.	1.8×10^4	-	-	-	-	+	-	-	-
11.	5.0×10^7	+	-	+	+	-	-	-	-
12.	2.0×10^7	+	-	-	-	-	-	-	-
13.	3.0×10^7	-	+	-	-	-	-	-	-
14.	6.0×10^7								
15.	1.5×10^4	-	+	+	-	-	-	-	-
16.	2.0×10^7	-	-	-	+	-	-	-	-
17.	4.0×10^7	-	-	+	-	-	-	-	-
18.	3.0×10^7	-	+	-	-	-	-	-	-
19.	3.5×10^7	+	-	-	+	-	-	-	-
20.	-	-	-	-	-	-	-	-	-
21.	-	-	-	-	-	-	-	-	-
22.	-	-	-	-	-	-	-	-	-
23.	7.5×10^7	+	+	+	-	-	-	-	-
24.	1.1×10^3	-	-	-	-	-	-	+	-
25.	-	-	-	-	-	-	-	-	-
26.	-	-	-	-	-	-	-	-	-
27.	2.2×10^3	-	-	-	-	-	+	-	-
28.	1.6×10^4	+	-	-	-	-	+	-	+
29.	-	-	-	-	-	-	-	-	-
30.	-	-	-	-	-	-	-	-	-
31.	1.3×10^3	-	-	-	-	-	+	-	-
32.	2.7×10^3	+	-	-	-	-	+	-	-
33.	-	-	-	-	-	-	-	-	-
34.	-	-	-	-	-	-	-	-	-
35.	-	-	-	-	-	-	-	-	-
36.	1.55×10^4	+	+	-	+	-	-	-	-
37.	7.5×10^7	+	+	+	+	-	-	-	-
38.	1.0×10^7	-	+	-	-	-	-	-	-
39.	5.5×10^7	-	+	+	-	-	-	-	-
40.	-	-	-	-	-	-	-	-	-
41.	1.5×10^8	+	+	+	+	-	-	-	-
42.	8.5×10^7	-	+	+	-	-	-	-	-
43.	1.0×10^3	-	-	-	-	-	+	-	-
44.	1.8×10^4	+	-	-	-	-	-	-	+
45.	1.8×10^4	-	+	-	-	-	-	-	-
46.	2.0×10^3	-	-	-	-	-	+	+	-
47.	-	-	-	-	-	-	-	-	-
48.	1.6×10^3	-	-	-	-	-	+	-	-
49.	2.05×10^4	+	-	-	-	-	-	+	-
50.	-	-	-	-	-	-	-	-	-
51.	-	-	-	-	-	-	-	-	-
52.	2.5×10^7	-	-	+	-	-	-	-	-

53.	-	-	-	-	-	-	-	-	-
54.	4.0×10^7	+	+	-	+	-	-	-	-
55.	2.0×10^7	-	+	-	-	-	-	-	-
56.	1.0×10^7	-	+	-	-	-	-	-	-
57.	4.0×10^7	+	+	-	-	-	-	-	-
58.	8.5×10^7	+	-	+	-	-	-	-	-
59.	1.3×10^8	+	+	-	-	-	-	-	-
60.	4.5×10^7	+	-	+	-	-	-	-	-
61.	-	-	-	-	-	-	-	-	-
62.	8.5×10^7	+	+	+	+	-	-	-	-
63.	-	-	-	-	-	-	-	-	-
64.	1.0×10^8	+	+	+	-	-	-	-	-
65.	9.0×10^7	+	-	+	-	-	-	-	-
66.	9.5×10^7	+	+	-	+	-	-	-	-
67.	6.0×10^7	+	+	-	-	-	-	-	-
68.	1.0×10^7	+	-	-	-	-	-	-	-
69.	-	-	-	-	-	-	-	-	-
70.	1.0×10^8	+	+	+	-	-	-	-	-
71.	5.0×10^7	+	+	-	-	-	-	-	-
72.	-	-	-	-	-	-	-	-	-
73.	1.8×10^8	+	+	+	+	-	-	-	-
74.	6.0×10^7	+	+	-	-	-	-	-	-
75.	-	-	-	-	-	-	-	-	-
76.	1.05×10^4	+	-	+	-	-	-	-	-
77.	1.2×10^8	+	+	+	-	-	-	-	-
78.	6.5×10^8	-	-	+	-	-	-	-	-
79.	-	-	-	-	-	-	-	-	-
80.	1.5×10^8	+	+	+	-	-	-	-	-
81.	1.1×10^7	-	+	-	-	-	-	-	-
82.	-	-	-	-	-	-	-	-	-
83.	-	-	-	-	-	-	-	-	-
84.	1.75×10^4	+	+	+	+	-	-	-	-
85.	-	-	-	-	-	-	-	-	-
86.	3.0×10^7	-	+	-	-	-	-	-	-
87.	-	-	-	-	-	-	-	-	-
88.	8.5×10^7	+	+	-	-	-	-	-	-
89.	5.5×10^7	+	-	-	-	-	-	-	-
90.	-	-	-	-	-	-	-	-	-
91.	1.5×10^7	+	-	-	-	-	-	-	-
92.	-	-	-	-	-	-	-	-	-
93.	5.0×10^7	+	-	+	+	-	-	-	-
94.	1.8×10^4	-	+	-	-	-	+	-	-
95.	3.2×10^3	+	+	-	-	-	-	-	-
96.	-	-	-	-	-	-	-	-	-
97.	1.5×10^4	-	+	-	-	-	-	+	-
98.	$2.0 \text{ X } 10^4$	+	-	-	-	-	-	+	-
99.	-	-	-	-	-	-	-	-	-
100.	-	-	-	-	-	-	-	-	-
101.	3.7×10^3	+	+	-	-	-	-	-	-
102.	2.25×10^4	-	+	-	-	-	+	-	-
103.	1.7×10^4	-	-	-	-	-	-	+	-
104.	1.5×10^4	-	-	-	-	-	-	-	+
105.	2.0×10^4	+	-	-	-	-	+	-	-
106.	-	-	-	-	-	-	-	-	-
107.	2.2×10^4	+	-	-	-	-	-	-	-

108.	-	-	-	-	-	-	-	-	-
109.	4.0×10^3	-	+	-	-	-	-	+	-
110.	3.2×10^3	-	+	-	-	-	+	+	-
111.	-	-	-	-	-	-	-	-	-
112.	2.2×10^3	+	-	-	-	-	-	-	-
113.	1.3×10^3	+	-	-	-	-	-	-	-
114.	-	-	-	-	-	-	-	-	-
115.	1.2×10^3	-	-	-	-	-	-	-	+
116.	1.45×10^4	+	+	+	+	-	-	-	-
117.	1.8×10^8	-	+	+	+	-	-	-	-
118.	-	-	-	-	-	-	-	-	-
119.	-	-	-	-	-	-	-	-	-
120.	1.15×10^4	+	+	+	-	-	-	-	-
121.	1.0×10^8	-	+	+	-	-	-	-	-
122.	-	-	-	-	-	-	-	-	-
123.	2.5×10^7	-	-	-	-	+	-	-	-
124.	3.0×10^7	-	+	-	+	-	-	-	-
125.	2.5×10^7	+	-	-	-	-	-	-	-
126.	6.0×10^7	+	+	-	-	-	-	-	-
127.	-	-	-	-	-	-	-	-	-
128.	7.0×10^7	+	+	+	-	-	-	-	-
129.	-	-	-	-	-	-	-	-	
130.	6.5×10^7	-	+	-	-	+	-	-	+
131.	1.5×10^8	+	+	+	-	-	-	-	-
132.	-	-	-	-	-	-	-	-	-
133.	-	-	-	-	-	-	-		-
134.	6.0×10^7	+	-	+	-	-	-	-	-
135.	1.5×10^7	+	-	-	-	+	-	-	-
136.	2.5×10^7	+	-	-	-	-	-	-	-
137.	7.5×10^7	+	-	-	-	-	-	-	-
138.	-	-	-	-	-	-	-	-	-
139.	1.85×10^4	+	+	+	+	-	-	-	-
140.	-	-	-	-	-	-	-	-	-
141.	1.45×10^4	+	+	+	+	-	-	-	-
142.	1.0×10^7	-	-	+	-	-	-	-	-
143.	3.5×10^7	+	-	-	+	-	-	-	-
144.	-	-	-	-	-	-	-	-	-
145.	1.7×10^8	+	+	+	-	-	-	-	-
146.	1.05×10^4	+	+	+	+	-	-	-	-
147.	1.0×10^8	+	+	+	-	-	-	-	-
148.	-	-	-	-	-	-	-	-	-
149.	-	-	-	-	-	-	-	-	-
150.	3.1×10^6	-	+	-	-	-	+	+	-

APPENDIX II

SAMPLE	SOURCE/PART USED	LOCATION	MODE OF PREPARATION	PRESERVATIVE	DOSAGE (Taken)	THEURAPEUTIC CLAIMS
1	Leaves	Chanchaga	Infusion	Red Potash	Orally 3x daily	Fever
2	Leaves	Chanchaga	Infusion	Lime,	Half cup	Rheumatic fever, Revitalise energy
3	Leaves	Chanchaga	Maceration		Orally	Menstrual pain
4	Leaves	Chanchaga	Decoction	Potash	Orally	Cough
5	Leaves	Chanchaga		Lemon		Malarial fever
6	Leaves	Chanchaga	Maceration	Lime		
7	Leaves	Chanchaga	Strong decoction	Red potash	Twice daily	Stomach pain, laxative, pneumonia, skin infection.
8	Leaves	Chanchaga	Decoction		Thrice daily.	Cough, Epilepsy.
9	Leaves	Chanchaga	Infusion		Drink and apply topically thrice daily.	Worm expeller, Chicken pox and convulsion in children.
10	Leaves	Chanchaga	Decoction		A cup once daily.	Ulcer, Purgative.
11	Leaves	Bosso	Decoction	Cafra	Orally	Asthma and Bronchial problem
12	Leaves	Bosso	Infusion	Salt	Half tumbler at a time	Impotence and suppressed menstruation
13	Leaves	Bosso	Decoction	Salt	Thrice daily	Abdominal pain
14	Leaves	Bosso	Decoction		Once daily	Breast cancer, fever
15	Leaves	Bosso	Decoction		Once daily	Oedema
16	Leaves	Bosso	Decoction		Once daily	Purgatives, vermifuge, diarrhea and cardiac arrest
17	Leaves	Bosso	Maceration		Once	Purgative, liver problem
18	Leaves	Bosso	Decoction		Daily	Cancer
19	Leaves	Bosso	Decoction	Lemon	Often as tea	Diuretic, antirheumatic and antidiarrhoea
20	Leaves	Bosso	Decoction	Cafra	Often as tea	Diabetes
21	Leaves	Minna West	Decoction		Orally	Antidote for snakebite

22	Leaves	Minna West	Decoction	Cafra	Thrice daily	Cough, fever and diarrhea
23	Leaves	Minna West	Decoction		Orally	Malaria
24	Leaves	Minna West	Strong decoction		Daily	Increase fertility, preserve pregnancy
25	Leaves	Minna West	Strong decoction		Orally	Analgesic
26	Leaves	Minna West	Infusion	Lime	Once daily	Typhoid fever
27	Leaves	Minna West	Decoction	Lemon	Thrice daily	Typhoid fever, malaria
28	Leaves	Minna West	Strong decoction	Potash	Often	Teething
29	Leaves	Minna West	Maceration		Orally	Analgesic
30	Leaves	Minna West	Infusion	Lime	Half cup	Menstrual pain
31	Stem bark	Chanchaga	Decoction		Daily	Malaria fever, asthma, diabetes and dysentery
32	Stem bark	Chanchaga	Infusion	Lime	Thrice daily	Lower abdominal pain
33	Stem bark	chanchaga	Strong decoction	Lime	Thrice daily	Menstrual pain
34	Stem bark	Chanchaga	Decoction	Potash	Daily	C ough, tuberculosis
35	Stem bark	Chanchaga	Infusion	Cafra	Once daily	Oxytosic [induce labour]
36	Stem bark	Chanchaga	Decoction	Lime	Once daily	Pile
37	Stem bark	Chanchaga	Strong decoction	Red potash	Daily	Dysentry, diarrhea
38	Stem bark	Chanchaga	Maceration	Lime	Once daily	Stomach upset and pile
39	Stem bark	Chanchaga	Decoction	Potash	Thrice daily	Menstrual pain
40	Stem bark	Chanchaga	Infusion	Refridgeration	Thrice daily	Cough, sore throat and heart burn
41	Stem bark	Bosso	Decoction	Lime	Daily	Pneumonia
42	Stem bark	bosso	Decoction		Twice daily	Dysentry, sore throat and vermifuge
43	Stem bark	Bosso	Infusion	Potash	Thrice daily	Dizziness, stomach pain and vomiting
44	Stem bark	Bosso	Strong decoction	Potash	Twice daily	Diarrhea,dysentry and pile
45	Stem bark	Bosso	Decoction	Lime	Often	Chest pain and ulcer
46	Stem bark	Bosso	Maceration	Refridgeration	Once daily	stomach upset, worm
47	Stem bark	Bosso	Infusion	Cafra	Once daily	Convulsion and dysentery

48	Stem bark	Bosso	Decoction	Potash	Often	Abdominal disorder
49	Stem bark	Bosso	Strong decoction	Potash	When necessary	Sore throat and cough
50	Stem bark	Bosso	Maceration	Refridgeration	Twice daily	Pneumonia, laxative
51	Stem bark	Minna West	Maceration		Orally	Jaundice, cough
52	Stem bark	Minna West	Infusion	Refridgeration	Thrice daily	General body pain
53	Stem bark	Minna West	Strong decoction	Red potash	Thrice daily	Nausea and vomiting
54	stem bark	Minna West	Decoction	Potash	Daily	Stimulant and appetizer
55	stem bark	Minna West	Strong decoction	Red potash	Half cup once daily	Cough, diarrhea, pile
56	stem bark	Minna West	Decoction	Red potash	Twice daily	Dysentry
57	Stem bark	Minna West	Strong decoction	Potash	Twice daily	Ulcer, heart burn
58	Stem bark	Minna West	Infusion		Often	Nausea, vomitting and stomach upset
59	Stem bark	Minna West	Decoction	Potash	Thrice daily	Teething remedy
60	Stem bark	Minna West	Decoction	Cafra	Thrice daily	Fever, cancer,dysentry, boils, wounds and ulcer
61	Root	Chanchaga	Infusion	Salt	Half tumbler at a time	Anti cancer,anti spoison,intestinal ulcer
62	Root	Chanchaga	Decoction	Red potash	Once daily	Gonorrhoea
63	Root	Chanchaga	Decoction		Once daily	Hernia, gonorrhea, stomach upset
64	Root	Chanchaga	Infusion		Once daily	Ulcer,purgative, abortifacient and laxative
65	Root	Chanchaga	Decoction		Once daily	Gonorrhoea
66	Root	Chanchaga	Maceration	Refridgeration	Thrice daily	Lower abdominal pain
67	Root	Chanchaga	Decoction	Refridgeration	When necessary	Cough
68	Root	Chanchaga	Decoction	Cafra	Once daily	Abortifacient and rheumatism
69	Root	Chanchaga	Decoction		Ocassionally	Purgative, tonic

70	Root	Chanchaga	Decoction		Thrice daily	Abscess
71	Root	Bosso	Decoction	Cafra	Regularly	General disease condition
72	Root	Bosso	Decoction	Cafra	Orally	Asthma, cough,cold and dysentery
73	Root	Bosso	Decoction		A tumbler full daily	Fever, measles and cancer
74	Root	Bosso	Decoction		When necessary	Headach and body pain
75	Root	Bosso	Decoction		Orally	Headach, rheumatism and diabetes
76	Root	Bosso	Strong decoction	Potash	Thrice daily	TYphoid fever and pile
77	Root	Bosso	Infusion	Lime	Thrice daily	Yellow fever, typhoid fever and malaria
78	Root	Bosso	Infusion	lemom	Orally	Stomach and menstrual pain
79	Root	Bosso	Decoction		Twice daily	Ulcer, heart burn and pile
80	Root	Bosso	Maceration		Once	Stomach upset
81	Root	Minna West	Decoction	Cafra	Orally	Liver disease
82	Root	Minna West	Strong decoction	Potash	Often	Sore throat and cough
83	Root	Minna West	Decoction		Twice daily	Fever, cough and stomach ache
84	Root	Minna West	Strong decoction	Red potash	Orally	Sterility
85	Root	Minna West	Decoction	Red potash	Once daily	Gonorrhoea
86	Root	Minna West	Infusion	Lime	Thrice daily	Lower abdominal pain
87	Root	Minna West	Decoction		Twice daily	Fever , cough and stomach ache
88	Root	Minna West	Decoction		When necessary	Dysentry, fever and headache
89	Root	Minna West	Maceration		Often	Malaria and fever
90	Root	Minna West	Infusion	Lime	Orally	General disease condition
91	Whole plant	Chanchaga	Decoction	Cafra	Orally	Fever
92	Whole herb	Chanchaga	Poultice		Once	Antihelminthic
93	Whole plant	Chanchaga	Decoction	Red potash	Orally	Mystic

94	Whole plant	Chanchaga	Decoction		Orally	Fever
95	Whole plant	Chanchaga	Decoction		Regurlarly	General disease condition
96	Whole plant	Chanchaga	Infusion	Potash	Orally	Convulsion and dysentery
97	Whole plant	Chanchaga	Infusion	Lime	Orally	Abortion
98	Whole plant	Chanchaga	Decoction	lemom	Orally	Stomach pain
99	Whole plant	Chanchaga	Decoction		Orally	Fever
100	Whole plant	Chanchaga	Infusion	Cafra	wash the affected eye	Eye
101	Whole plant	Bosso	Decoction	Potash	Orally	Measles
102	Whole plant	Bosso	Decoction		Orally	Ulcer
103	Whole herb	Bosso	Decoction		Orally	Disrupted menstrual cycle
104	Whole herb	Bosso	Maceration		Orally	Dizziness
105	Whole plant	Bosso	decoction	Potash	Orally	Cough
106	Whole herb	Bosso	Decoction		Once daily	Malaria
107	Whole herb	Bosso	Infusion	Cafra	Once daily	Gonorrhoea, abortifacient
108	Whole plant	Bosso	Decoction	Potash	Orally	Trypanosomiasis, measles
109	Whole plant	Bosso	Decoction	Cafra	Orally	Stomach pain
110	Whole plant	Bosso	Decoction		Orally	General antidote
111	Whole plant	Minna West	Decoction	Lemon	Orally	Urinary tract infection
112	Whole plant	Minna West	Infusion		One teaspoon daily	Pile and sterility
113	Whole plant	Minna West	Decoction	Cafra	Orally	General health condition of children
114	Whole plant	Minna West	Decoction		Daily	Fever and measles
115	Whole plant	Minna West	Infusion	Lime	Orally	Liver ailment, dysuria bladder, kidney and lung disease
116	Whole plant	Minna West	Decoction		Orally	Analgesic

117	Whole plant	Minna West	Decoction		Orally	Stomach pain
118	Whole plant	Minna West	Strong decoction	Red potash	Twice daily	Fever,diarrhea and impetigo
119	Whole plant	Minna West	Strong decoction	Cafra	Regurlarly	Cough, rheumatism and general analgesic
120	Whole plant	Minna West	Decoction		Oncedaily	Dysentry
121	Leaves and flower	Chanchaga	Decoction		Orally	Asthma and bronchial problems
122	Leaves and stem bark	Chanchaga	Strong decoction	Red potash	Twice daily	Veneral disease, st
123	Leaves and stem bark	Chanchaga	Decoction	Potash	Often	Malaria fever, tonic and laxative
124	Bark and seed	Chanchaga	Strong decoction		Once daily	Syphilis, malaria fever, vermifuge, antihelminthic and abortifacient
125	Stem bark and leaves	Chanchaga	Strong decoction	Potash	Twice daily	Gonorrhea and syphilis
126	Root and leaves	Chanchaga	Decoction		Several times	Fever, ulcer, cough, general body pain, jaundice and joint dislocation
127	Leaves,stem and root bark	Chanchaga	Strong decoction		Thrice daily	Cancer, tuberculosis and abortifacient
128	Fresh stem bark,leaves and fruit	Chanchaga	Decoction	Potash	Often	Tonic
129	Fresh stem bark,leaves and fruit	Chanchaga	Cold infusion		Once daily	Jaundice, cough,yellow fever and general body pain
130	Leaves,seeds and root	Chanchaga	Cold infusion		Half cup twice daily	Malaria fever, diabetes, gonorrhea, syphilis and antihelminthic
131	Roots, barks and seeds	Bosso	Decoction		Once	Gonorrhea
132	Leaves and twigs	Bosso	Strong decoction	Potash	Thrice	Cold symptoms in children
133	Stem and root bark	Bosso	Decoction		Thrice daily	Cough, diarrhea, stimulant purgative and sore throat
133	Roots and leaves	Bosso	Maceration	Potash	Once daily	Purgative and asthma
135	Fruits and leaves	Bosso	Decoction		Once	Purgative and liver problem
136	Leaves and stem bark	Bosso	Decoction		Bath	Paralysis and body weakness

137	Leaves and stem	Bosso	Strong decoction	Potash	Daily	Teething remedy
138	Leaves and stem	Bosso	Decoction	Lime	Orally	Spleen infection
139	Roots and leaves	Bosso	Decoction	Potash	Thrice daily	Gonorrhea and jaundice
140	Leaves and stem bark	Bosso	Decoction		Orally	Anti-malaria and inflammatory
141	Leaves,dry stem bark and fruits	Minna West	Maceration		Once daily	Asthma, wounds, fever and toothache
142	Stem and leaves	Minna West	Strong decoction	Lime	Orally	Male sterility
143	Leaves and stem bark	Minna West	Decoction	Red potash	Often	Fevrer,stomach pain and ulcer
144	Leaves and stem bark	Minna West	Decoction		Daily	Oedema, dysentry and epilepsy
145	Leaves and stem bark	Minna West	Strong decoction		Orally and bath	Growth promoter in children
146	Leaves,stem bark and roots	Minna West	Strong decoction	Potash	Twice daily	Dysentry, sore throat and vermifuge
147	Leaves and roots	Minna West	Decoction	Lime	Orally	Measles,chicken pox, smallpox, veneral disease and a stimulant
148	Young roots and bark	Minna West	Decoction		Twice daily	Asthma and delayed ossifaction of anterior fontanelle
149	Roots and leaves	Minna West	Decoction	Potash	Thrice daily	Fever, aphrodisiac and dermatomycosis
150	Bark and leaves	Minna West			Orally	Typhoid fever and pyresxia

APPENDIX III

The zone of inhibition of the antibiotics to the bacterial isolates and multiple antibiotic resistance indexes

Isolates no	CXC	TET	ERY	CPX	AUG	CHI	COT	GEN	MARI
1a	0	11	0	26	0	21	21	22	0.5
1f	0	0	0	18	0	0	0	19	0.8
1g	0	0	0	21	16	6	0	19	0.6
3g	0	0	0	16	0	0	20	18	0.6
4b	0	20	22	30	0	0	28	22	0.4
4f	0	0	17	14	0	12	21	20	0.6
4g	0	18	20	18	13	0	0	24	0.5
7a	0	18	0	28	11	0	0	19	0.6
8a	0	12	0	0	0	0	20	17	0.8
9f	0	20	6	20	0	0	14	23	0.5
9g	0	14	18	24	6	18	11	21	0.3
10e	0	0	20	0	0	0	18	22	0.6
11a	0	11	0	21	14	0	21	18	0.6
11c	0	10	21	28	24	20	0	14	0.4
11d	0	16	20	24	14	18	13	22	0.4
12a	14	0	6	10	0	0	0	16	0.8
13b	0	20	0	21	6	0	11	14	0.6
14a	7	14	0	26	0	22	0	22	0.5
14b	0	20	0	18	0	23	21	25	0.4
15b	0	20	0	26	0	0	0	23	0.6
15c	10	6	0	24	11	0	20	18	0.6
16d	0	0	0	0	10	12	0	0	0.8
17c	0	0	0	24	10	20	0	0	0.8
18b	0	11	26	14	0	0	10	20	0.6
19a	6	18	18	30	0	0	14	15	0.4
19d	10	20	22	6	0	0	20	0	0.6
23a	0	0	20	18	14	0	0	17	0.6
23b	0	0	17	26	6	0	0	0	0.8
23c	0	18	0	24	11	0	0	0	0.8
24g	0	18	0	18	0	0	0	21	0.6
27f	0	14	22	23	0	0	0	22	0.6
28a	0	22	0	21	16	0	0	20	0.5
28f	0	20	0	16	0	0	0	18	0.6
31f	0	17	18	22	0	12	0	20	0.4
32a	0	0	22	28	0	0	26	0	0.6
32f	0	14	23	22	0	20	30	21	0.4
36a	0	0	0	20	23	0	0	22	0.6
36b	0	0	18	32	0	16	23	18	0.4
36d	0	11	21	25	19	21	24	22	0.1
37a	0	0	18	28	0	0	0	16	0.6
37b	14	0	14	14	0	13	17	14	0.4

37c	0	0	21	25	19	21	24	22	0.3
37d	0	0	18	28	0	0	0	16	0.6
38b	0	0	14	14	0	13	17	14	0.6
39b	0	0	19	28	0	0	0	22	0.6
39c	19	21	17	14	0	23	12	25	0.1
41a	0	0	29	24	0	0	31	15	0.5
41b	0	0	11	20	18	0	0	18	0.6
41c	0	21	15	23	11	18	0	18	0.4
41d	0	16	0	16	0	21	20	0	0.5
42b	19	17	0	20	0	23	12	0	0.4
42c	0	20	0	25	0	22	20	21	0.4
43f	0	20	0	22	0	0	0	15	0.6
44a	0	17	0	26	0	21	22	14	0.4
44h	0	19	0	16	0	18	21	0	0.5
45b	0	16	16	18	0	21	0	22	0.4
46f	0	16	0	28	0	0	0	23	0.6
46g	0	11	17	10	0	12	0	14	0.8
48f	0	0	0	30	0	6	0	12	0.8
49a	0	11	0	20	0	21	21	22	0.5
49g	14	22	28	20	0	24	0	28	0.4
52c	20	0	20	22	24	14	0	22	0.4
54a	0	14	10	16	0	0	14	14	0.5
54b	0	15	19	22	13	0	0	19	0.4
54d	0	0	19	0	11	0	0	17	0.8
55b	0	12	0	24	0	20	14	20	0.5
56b	6	0	20	20	14	0	14	23	0.4
57a	0	14	18	24	6	18	11	21	0.5
57b	0	0	20	21	0	18	22	0	0.5
58a	11	20	0	22	14	0	13	17	0.6
58c	10	12	21	0	24	21	18	19	0.4
59a	0	16	20	28	14	18	13	22	0.1
59b	14	0	6	28	0	10	0	16	0.6
60b	0	20	0	24	6	0	11	14	0.6
60d	7	14	0	0	0	22	0	22	0.8
62a	0	18	0	20	0	23	21	25	0.4
62b	0	0	0	22	10	12	6	0	0.8
62c	0	20	0	21	0	11	10	23	0.6
62d	10	6	0	21	0	20	16	18	0.5
62h	0	16	10	17	0	21	20	12	0.4
64a	11	17	0	20	10	23	12	0	0.6
64b	0	20	0	25	0	22	20	21	0.4
64c	6	18	18	26	0	0	14	15	0.4
65a	10	20	22	26	0	0	20	0	0.5
65c	0	0	20	18	6	0	14	19	0.6
66a	0	11	0	30	0	21	21	22	0.5
66b	0	20	0	21	6	23	0	11	0.5

66d	0	18	18	24	10	20	11	23	0.4
67a	0	16	20	26	14	18	10	20	0.4
67b	0	0	0	28	0	6	0	12	0.8
68a	0	11	17	22	0	12	0	14	0.6
70a	0	0	0	21	6	18	19	14	0.5
70b	0	12	17	22	6	12	0	24	0.6
70c	7	14	0	28	0	22	0	22	0.6
71a	0	20	0	18	0	23	21	25	0.4
71b	0	20	0	26	0	0	0	23	0.6
73a	10	6	0	23	11	0	20	18	0.6
73b	0	0	0	28	10	12	0	0	0.8
73c	0	0	0	21	10	20	0	0	0.8
73d	0	11	26	14	0	0	10	20	0.6
74a	6	18	18	22	0	0	14	15	0.4
74b	10	20	22	26	0	0	20	0	0.5
76a	0	0	20	18	14	0	0	17	0.6
76c	0	0	17	26	6	0	0	0	0.8
77a	0	18	0	24	11	0	0	0	0.8
77b	0	18	0	18	0	0	0	21	0.6
77c	0	14	22	23	0	0	0	22	0.6
78a	0	22	0	0	16	0	0	20	0.6
80a	0	20	0	16	0	0	0	18	0.6
80b	0	13	21	25	0	0	0	22	0.6
80c	0	17	18	22	0	12	0	20	0.5
81b	0	0	22	28	0	0	26	0	0.6
84a	0	20	0	6	0	0	0	23	0.8
84b	10	6	0	28	11	0	20	18	0.6
84c	0	0	0	26	10	12	0	0	0.8
84d	0	0	0	0	10	20	0	0	0.8
86b	0	11	26	14	0	0	10	20	0.6
88a	6	18	18	28	0	0	14	15	0.4
88b	10	20	22	26	0	0	20	0	0.5
89a	0	0	20	18	14	0	0	17	0.6
91a	0	0	17	26	6	0	0	0	0.8
93a	0	18	0	24	11	0	0	0	0.8
93c	0	18	0	18	0	0	0	21	0.6
93d	0	14	22	23	0	0	0	22	0.6
94b	0	22	0	21	16	0	0	20	0.6
94f	0	20	0	16	0	0	0	18	0.6
95a	10	6	0	10	21	13	0	19	0.5
95b	6	18	14	30	0	0	14	15	0.6
97b	0	16	0	16	18	21	19	10	0.3
97g	0	0	17	26	6	0	0	0	0.8
98a	19	17	0	0	23	20	12	12	0.4
98g	0	11	13	8	0	0	6	18	0.8
101a	0	24	0	11	6	11	16	16	0.6

101b	7	14	0	22	0	22	0	22	0.6
102b	0	20	0	18	0	23	21	25	0.4
102f	0	20	0	6	0	0	0	23	0.8
103g	10	6	0	30	11	0	20	18	0.6
104h	0	0	0	22	10	12	0	0	0.8
105f	0	0	0	0	10	20	0	0	0.8
107a	0	11	26	14	0	0	10	20	0.6
109b	6	18	18	24	0	0	14	15	0.4
109g	10	20	22	26	0	0	20	0	0.5
110b	0	0	20	18	14	0	0	17	0.6
110f	0	0	17	26	6	0	0	0	0.8
110g	0	18	0	24	11	0	0	0	0.8
112a	0	18	0	18	0	0	0	21	0.6
113a	0	14	22	23	0	0	0	22	0.6
115h	0	22	0	28	16	0	0	20	0.5
116a	0	20	0	16	0	0	0	18	0.6
116b	0	13	21	25	0	0	0	22	0.6
116c	0	17	18	22	0	12	0	20	0.5
116d	0	0	22	28	0	0	26	0	0.6
117b	0	6	0	12	11	0	21	24	0.8
117c	0	16	14	20	0	0	0	19	0.6
117d	0	14	17	12	21	16	0	21	0.5
120a	0	21	22	15	0	22	14	17	0.3
120b	0	14	24	14	11	12	18	0	0.4
120c	6	18	18	0	0	0	14	15	0.5
121b	10	20	22	24	0	0	20	0	0.5
121c	0	0	20	18	14	0	0	17	0.5
123e	0	0	17	26	6	0	0	0	0.6
124b	0	18	0	24	11	0	0	0	0.8
124d	0	18	0	18	0	0	0	21	0.6
125a	0	14	22	23	0	0	0	22	0.6
126a	0	22	0	0	16	0	0	20	0.6
126b	0	20	0	16	0	0	0	18	0.6
128a	0	13	21	25	0	0	0	22	0.6
128b	0	17	18	22	0	12	0	20	0.5
128c	0	0	22	28	0	0	26	0	0.6
130b	0	20	0	26	0	0	0	23	0.6
130e	10	6	0	30	11	0	20	18	0.5
130h	0	0	0	22	10	12	0	0	0.8
131a	0	0	0	0	10	20	0	0	0.8
131b	0	11	26	14	0	0	10	20	0.6
134a	10	20	22	6	0	0	20	0	0.6
134c	0	0	20	18	14	0	0	17	0.5
135a	0	0	17	26	6	0	0	0	0.8
135e	0	18	0	24	11	0	0	0	0.8
136a	0	18	0	18	0	0	0	21	0.6

137a	0	14	22	23	0	0	0	22	0.6
139a	0	22	0	0	16	0	0	20	0.6
139b	0	20	0	16	0	0	0	18	0.6
139c	0	13	21	25	0	0	0	22	0.6
139d	0	17	18	22	0	12	0	20	0.5
141a	0	0	22	28	0	0	26	0	0.6
141b	0	20	0	28	0	0	0	23	0.6
141c	10	6	0	21	11	0	20	18	0.6
141d	0	0	0	21	10	12	0	0	0.8
142c	0	20	0	22	0	0	0	23	0.6
143a	10	6	0	0	11	0	20	18	0.8
143d	0	0	0	25	10	12	0	0	0.8
145a	0	0	0	0	10	20	0	0	0.8
145b	0	11	26	24	0	0	10	20	0.6
145c	6	18	18	21	0	0	14	15	0.4
146a	10	20	22	6	0	0	20	0	0.6
146b	0	0	20	18	14	0	0	17	0.5
146c	0	0	17	26	6	0	0	0	0.8
147a	0	18	0	24	11	0	0	0	0.8
147b	0	18	0	18	0	0	0	21	0.6
147c	0	14	22	23	0	0	0	22	0.6
150b	0	22	0	25	16	0	0	20	0.5
150f	0	20	0	16	0	0	0	18	0.6
150g	0	13	21	25	0	0	0	22	0.6

Keys:

CXC = Cloxacillin

TET = Tetracycline

ERY = Erythromycin

CPX = Ciproxin

AUG = Augmentin

CHI = Chloramphenicol

COT = Cotrimoxazole

GEN = Gentamycin

APPENDIX IV

QUESTIONAIRE: Based on the Research topic:

"STUDIES ON ANTIBIOTIC SUSCEPTIBILITY OF PATHOGENIC BACTERIA ISOLATED FROM LIQUID HERBAL PREPARATIONS SOLD IN MINNA METROPOLIS"

1. NAME OF PRODUCTS……..

2. TYPE OF PACKAGING/CONTAINER USE……..

3. DOSAGE/ DURATION OF USE…….

4. ANY SPECIAL INSTRUCTION REGARDING THE PRODUCT……..

5. COMPOSTION OF THE PRODUCT……

6. NAFDAC REGISTRATION……

7. WAS THE COMPOSITION (i.e Leaves, roots, barks e.t.c)

   WASHED BEFORE MIXING. YES…… OR NO……..

8. IS ANTIBIOTICS ADDED TO THE HERBAL PEPARATIONS?

   YES……..OR NO…….

9. IF YES WHAT TYPE OF ANTIBIOTICS

   A …….
   B …….

10. METHOD OF PREPARATION (i.e. Decoction, Infusion and Maceration)……

11. TYPES OF PRESERVATIVES USED

    A ……..
    B ……..

12. THERAPEUTIC CLAIMS

    A ……..
    B ……..

13. SHELF-LIFE: DOES IT HAVE EXPIRY DATE?

    YES…….. OR NO……

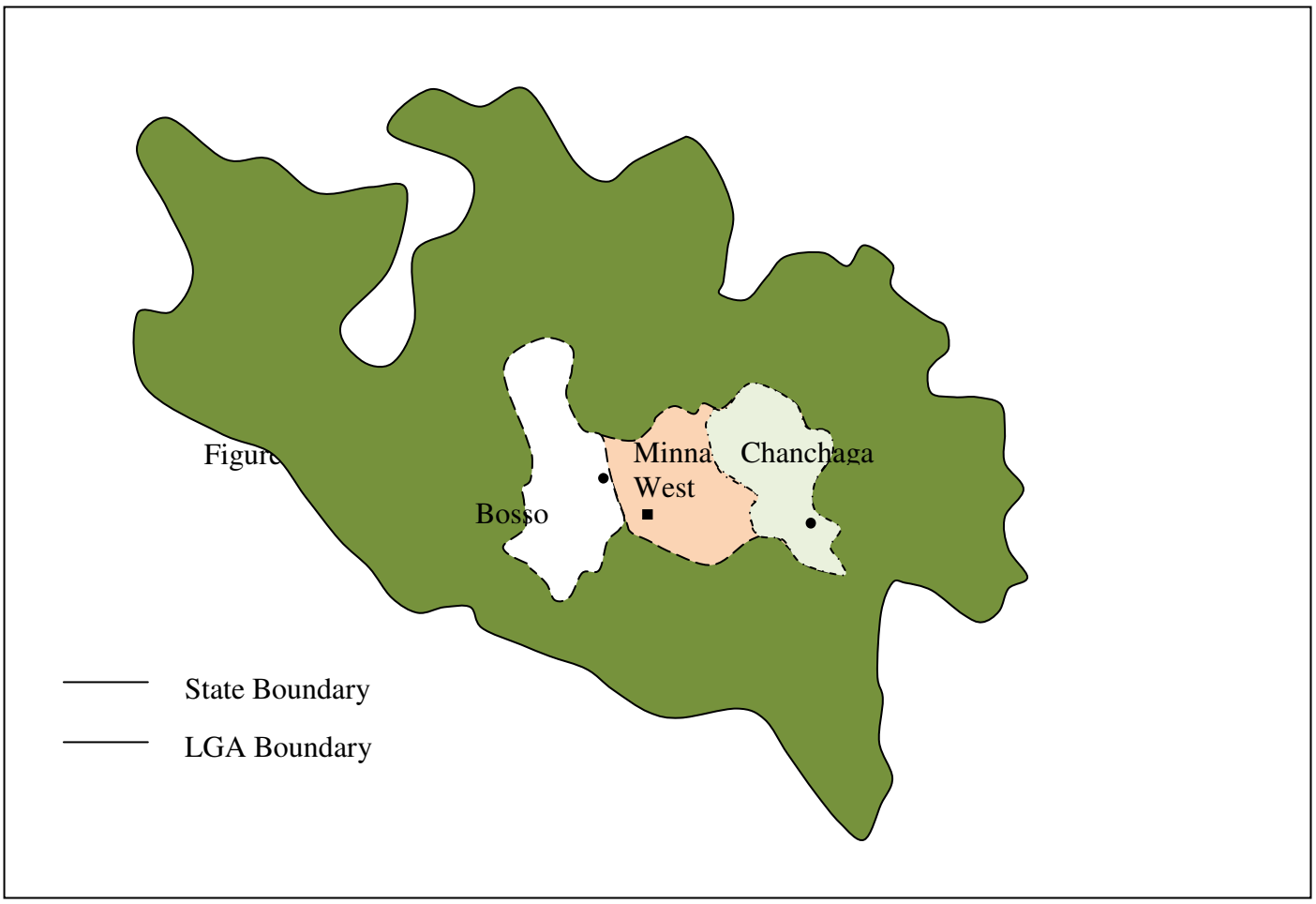

Figure 6: Map of Niger state showing the case study.