PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL SUSCEPTIBILITY OF SELECTED PLANT EXTRACTS ON *ESCHERICHIA COLI*, *SALMONELLA TYPHI* AND *STAPHYLOCOCCUS AUREUS*

BY

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DEPARTMENT OF BIOLOGICAL SCIENCES, FACULTY OF SCIENCE, AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA

APRIL, 2014

DECLARATION

I declare that the work in this thesis entitled 'Phytochemical Analysis and Antibacterial Susceptibility of Selected Plant Extracts on *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*' has been carried out by me in the Department of Biological Sciences under the supervision of Prof. I.S. Ndams and Dr. D. Tanko. 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 this or any other institution.

Solomon Bolarinwa AYODELE	____________________	______________
Name of student	Signature	Date

CERTIFICATION

This thesis entitled "PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL SUSCEPTIBILITY OF SELECTED PLANT EXTRACTS ON *ESCHERICHIA COLI*, *SALMONELLA TYPHI* AND *STAPHYLOCOCCUS AUREUS*" by SOLOMON BOLARINWA AYODELE meets the regulations governing the award of the degree, Masters in Biology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to all who are unable to acquire formal education due to various constraints and societal challenges.

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ABSTRACT

This study screened the phytochemical composition and antibacterial susceptibility of selected plant extracts on *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. The combination of selected plant materials which is already being used as herbal mixture (Zang cure) was obtained in powdered form from a traditional medical practitioner in Jos, Plateau State, Nigeria. The plant combination included the leaves, stem barks, roots, bulbs and rhizomes of *Annogeissus leiocarpus* (Hausa: marke), *Ximenia americana* (Hausa: tsada), *Aloe vera* (Hausa: rodon daji), *Allium sativum* (Hausa: tafarnuwa), *Allium ascalonicum* (Hausa: albasa maigo), *Cymbopogon citratus* (Hausa: tsagre), *Citrus aurantifolia* (Hausa: lemun zaƙi), *Garcinia kola* (Hausa: namijin goro), *Cola acuminata* (Hausa: goro), *Strephonema pseudocola* (Hausa: kanyia), *Costus afer* (Hausa: tumfafiyar kada), *Zingiber officinale* (Hausa: citta) and *Cyperus rotundus* (Hausa: ayah). Plant materials were also collected from the field while some were purchased from markets in Zaria, Kaduna, Kafanchan and Jos to replicate the herbal mixture (Zang Cure) with a known proportion of the constituent plants. Two hundred (200 g) of the plant powder was sequentially extracted with petroleum-ether, chloroform, ethyl acetate and methanol by Soxhlet extraction method. Another one hundred (100 g) of the plant powder was extracted with distilled water to obtain the aqueous extract. Two hundred (200 g) of air-dried and pulverised mixture of the plant materials obtained from the forests and markets was also extracted with distilled water to obtain a second aqueous extract. All the extracts were subjected to standard phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites. The susceptibility test of the plant extracts on *E. coli*, *S. typhi* and *S. aureus* were done using the agar well diffusion method. Ciprofloxacin

(10 μg) was used as control. The Minimum Inhibitory Concentrations (MICs) were determined in five concentrations; 100, 50, 25, 12.5 and 6.25 mg/ml of each extract. The Minimum Bactericidal Concentrations (MBCs) of the extracts were also determined. The phytochemicals screening of the extracts revealed the presence of flavonoids, saponins, tannins, cardiac glycosides, steroids and triterpenes. The aqueous, methanolic and second aqueous extracts had no inhibitory effect on the Gram-negative bacteria, *E. coli* and *S. typhi* but showed inhibitory effects on the Gram-positive *S. aureus*, with activity increasing with increase in concentration of extracts. The second aqueous extract only showed activity on *S. aureus* at higher concentrations (50 and 100 mg/ml). The chloroform, ethyl acetate and petroleum ether extracts showed activity on all three bacteria. The extracts exhibited bacteriostatic effects on the test organisms at lower concentrations and bactericidal effects at higher concentrations. The results justifies the claim that the selected plant combination is efficacious in the management of diseases, particularly those that relate to *E. coli*, *S. typhi* and *S. aureus*, such as gastroenteritis, meningitis, diarrhoea, typhoid fever, pneumonia, boils, wound and urinary tract infections.

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ABBREVIATIONS

%- Percentage

2nd- Second

ABU- Ahmadu Bello University

ABUTH- Ahmadu Bello University Teaching Hospital

ANOVA- Analysis of Variance

APHA- American Public Health Association

AST- Antibacterial Susceptibility Test

BC- Before Christ

CAM- Complementary and Alternative Medicine

Ciproflox- Ciprofloxacin

CLED- Cysteine-Lactose-Electrolyte-Deficient

CNS- Central Nervous System

Conc- Concentration

DCA- Desoxycholate Citrate Agar

EAggEC- EnteroAggregative *E. coli*

EHEC- Enterohemorrhagic *E. coli*

EIEC- Enteroinvasive *E. coli*

EPEC- Enteropathogenic *E. coli*

ETEC- Enterotoxigenic *E. coli*

g- Gram

H_2SO_4 –Sulphuric Acid

HCl- Hydrochloric acid

kDa- Kilodaltons

LSD- Least Significant Difference

LT- Heat-liable toxins

MBC- Minimum Bactericidal Concentration

MDa- Megadaltons

mg- Milligram

MIC- Minimum Inhibitory Concentration

ml- Millilitres

mm- Millimetres

NaCl- Sodium chloride

NAFDAC- National Agency for Food and Drug Administration and Control

NaOH- Sodium hydroxide

NCCLS- National Committee on Clinical Laboratory Standards

NIPRD- National Institute for Pharmaceutical Research and Development

nm- Nanometres

NNMDA- Nigerian Natural Medicines Development Agency

SLT- Shigella-like toxins

ST- Heat-stable toxins

TM- Traditional Medicine

USA- United States of America

VT- Verotoxins

WHO- World Health Organization

µg- Microgram

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro *et al.*, 2000). Of the 252 drugs considered essential by the World Health Organization, 11% were derived from flowering plants (Rates, 2001). More than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Alagesaboopathi, 2011). Nigeria is covered with a large number of plant species, some of which have been used for centuries in folkloric medicines to diagnose, prevent and treat various ailments (El-Mahmood *et al.*, 2010). Among the diseases that have been managed successfully by traditional (herbal) medicine include malaria, epilepsy, infertility, convulsion, diarrhoea, dysentery, gonorrhoea, flatulence, tonsillitis, bacterial and fungal infections, mental illness and worm infections (Sofowora, 1996).

Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Ogundipe *et al.* (1998) asserted that plant products still remain the principal source of pharmaceutical drugs and agents used in traditional medicine. Studies have been conducted on the antimicrobial potentials of crude extract of leaves, bark, bulbs, stems and roots of various plants (Atata and Sani, 2003; Olafimihan, 2004). A number of phytotherapy manuals have stated various medicinal plants for treating infectious diseases owing to their availability and low mammalian

toxicity (Lee *et al.*, 2007). The effects of plant extracts on bacteria have been studied widely from different parts of the world (Ateb and Erdourul, 2003).

New antioxidants such as plant phenolic compounds are sought for general health maintenance (Wah Chan *et al.*, 2008). The phenolic compounds cover a very large and diverse group of chemical compounds, including flavonoids, lignins, tannins, phenolic acids, coumarins, phenols, phenylpropanoids, quinines, stilbenoids and xanthones. (Vermerris and Nicholson, 2006). Flavonoids are a large group of polyphenolic compounds, subdivided into anthocyanins, flavanols including proanthocyanidins, flavonols, dihydroflavonols, flavones, isoflavonoids, flavonones, chalcones and dihydrochalcones (Li and Beta, 2013). Flavonoid-based herbal medicines are available in different countries as antiinflammatory, antispasmodic, antiallergic, antibacterial and antifungal remedies (Rice-Euans and Parker, 2003).

Garlic (*Allium sativum* Linn.) is one of those plants that had been heavily investigated over a period of time (Cavallito and Bailey, 1994; Jabar and Al-Mossawi, 2007) and one that has already reached the market together with ginger (*Zingiber officinale*). It has been used for centuries by Egyptians to fight infections (Onyeagba *et al.*, 2006; Abubakar, 2009). According to Abubakar (2009), Jabar and Al-Mossawi (2007), the early Egyptians used it to treat diarrhoea, the ancient Greeks used it to treat intestinal and extra-intestinal diseases, while the ancient Japanese and Chinese used it to treat headache, flu, sore throat and fever. In Africa, particularly in Nigeria, garlic has been reported to be used to treat abdominal discomfort, diarrhea, otitis media and respiratory tract infections (Ankri and Mirelman, 1999; Jabar and Al-Mossawi, 2007). The phytochemical constituents of garlic have already been established by several workers (Farbman *et al.*, 1993; Cavallito and Bailey, 1994;

Ankri and Mirelman, 1999; Prodos-Rosales *et al.*, 2003). The antimicrobial properties of garlic were first described by Pasteur in 1858, and since then, research had demonstrated its effectiveness against bacteria, protozoa, fungi and some viruses (Jabar and Al Mossawi, 2007; Abubakar (2009). *Allium ascalonicum* L. (Liliaceae) is widely used along with other *Allium* as spice for food. It contains factors like potassium, calcium, and vitamin C among others (Antia, 1976). There are reports showing that ingestion of *Allium* lowers blood cholesterol and reduce the risk for coronary artery disease (Antia, 1976; Bordia *et al.*, 1996).

Aloe vera (L.) Burm.Fil (Synonym *A. brobadensis* Miller) is a cactus-like plant of the family Liliaceae. It is believed to be effective in treating constipation, stomach ailments, gastrointestinal problems, skin diseases and radiation injury because of its anti-inflammatory effect. It is also used for wound healing and burns, diabetes and also as an anti-ulcer (Arunkumar and Muthuselvam, 2009). It has been reported to contain tannins, saponins, flavonoids and terpenoids (Arunkumar and Muthuselvam, 2009). *Aloe vera* gel and leaf extracts have been shown to suppress the growth of *Streptococcus pyogenes*, *Shigella flexneri*, *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Escherichia coli* (Ferro *et al*, 2003; Arunkumar and Muthuselvam, 2009; Johnson *et al.*, 2011; Thiruppathi *et al.*, 2012).

Citrus aurantifolia Linn popularly known as lime has been used to treat ailments such as common cold, depressive illness and alcoholism. Several researchers have demonstrated the antimicrobial potentials of lime (Oboh *et al.*, 1992, Oboh and Abulu, 1997; Onyeagba *et al.*, 2006). It has been acclaimed to possess anti-inflammatory, anti-rheumatic, anti-scorbutic, anti-coagulant and anti-spasmodic properties (Taiwo *et al.*, 2007).

The ginger plant, *Zingiber officinale* (Zingiberaceae), has a biennial or perennial creeping rhizome, locally called *Citta* (Hausa) and Ginger (English). Ginger is an essential ingredient in many traditional Chinese medicines and has been used since the 4th Century BC. The Chinese take ginger for a wide variety of medical problems such as stomachache, diarrhoea, nausea, cholera, asthma, heart conditions, respiratory disorders, toothache and rheumatic complaints (Wagner and Hikino, 1965). Africans and West Indians also use ginger medically and the Greeks and Romans use it as a spice (Melvin *et al.*, 2009).

Anogeissus leiocarpus (DC) Guill and Perr family Combretaceae (Common name: Axlewood tree) has many applications in Nigeria. It is used medically as an ascaricide and for the treatment of gonorrhoea, general body pain, blood clots, asthma, cough and tuberculosis (Mann *et al.*, 2003). Information obtained from the Yorubas and South-Eastern people of Nigeria showed that the plant is also used as an antimicrobial agent against bacterial infections (Dweek, 1996). The leaves of the plant are used externally as a decoction in the eastern part of Nigeria for the treatment of skin diseases and the itch of psoriasis. The powdered bark is applied to wounds, sores, boils, cysts and diabetic ulcers with good results. The powdered bark has also been mixed with ‘green clay’ and applied as an unusual face mask for serious blackheads (Dweek, 1996). The infusion and decoctions are used as cough medicine, the pulped roots are applied to wounds and ulcers, the powdered bark is also rubbed to reduce toothache on gums, it is also used as vermifuges and the leaves decoction is used for washing and fumigation (Ibrahim *et al.*, 1997). *Anogeissus leiocarpus* is traditionally acclaimed to be effective in treating infectious wounds in man and animals (Dweek, 1996).

Traditionally, the leaves, flowers, fruit follicles and bark of *Cola acuminata* Schott and Endl (Sterculiaceae) have been used to prepare a tonic as a remedy for dysentery, cough, diarrhea, and vomiting (Ayensu, 1978). They also ease hunger and thirst, eliminate fatigue, provide energy by stimulating the muscles and nerves and enhance intellectual activity (Sundstrom, 1966; Nickalls, 1986). The *Cola acuminata* nut's primary social and economic significance lies in its being a very concentrated source of Central Nervous System (CNS) stimulants, caffeine which may help in relieving migraine (Kiple and Ornelas 2000), theobromine which act as cerebral vasodilator and is thought to relieve pain and neuralgia (Hirt and M'pia, 2001) and proanthocyanidin which is used as an antitrypanosoma compound effective against *Trypanosoma brucei* (Kubata *et al.,* 2005).

Cyperus rotundus Linn. (Family Cyperaceae) is a pestiferous perennial weed with dark green glabrous culms, arising from underground tubers (Nadkarni and Nadkarni, 1996; Kirtikar and Basu, 2001). The rhizome part of *Cyperus rotundus* is one of the oldest known medicinal plants used for treatment of dysmenorrheal and menstrual irregularities (Bhattarai, 1993). A number of pharmacological and biological activities including antidiabetic, antidiarrhoeal, cytoprotective, antimutagenic, antioxidant, antimalarial, antiinflammatory, antipyretic and analgesic activities have been reported for this plant (Raut and Gaikwad, 2006).

Costus afer Ker Gawl (Costaceae) is a tall terrestrial perennial herb which finds use in folkloric medicine as a remedy for cough, rheumatic pains, sleepiness and as a cardiotonic (Agoha, 1974; Iwu, 1982). It has also been shown to exhibit anti-arthritic activities (Iwu and Anyanwu, 1982). The phytochemical screening of *C. afer* revealed the presence of

alkaloids, flavonoids, saponins, tannins, steroids and cardiac glycosides (Odoemena *et al.*, 2008; Akpan *et al.*, 2012).

Escherichia coli is a Gram-negative, toxin producing bacterium that causes serious food poisoning in humans (Vogt and Dippold, 2005). It is a model organism for bacteria (Peter *et al.*, 1998) and extremely sensitive to antibiotics such as streptomycin or gentamycin but rapidly changing and acquiring drug resistance (Chapman *et al.*, 2002) due to overuse of antibiotics in humans (Johnson *et al.*, 2006).

Salmonella typhi is a facultatively anaerobic Gram-negative rod-shaped bacterium (Krieg and Holt, 1984) and is one of the most important food borne pathogens. If present in food, the bacteria do not affect the taste, smell or appearance of the food. Frequent hand washing, throwing out expired food, avoiding eating raw or undercooked eggs, meats, seafood or poultry are the key to preventing *Salmonella* food poisoning. Antibiotics (such as ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline) may be prescribed for moderate to severe cases of *Salmonella* food poisoning or when it occurs in a person who is at risk for complications. However, probably as a consequence of the extensive use of antibiotics, the incidence and severity of human diseases related to *Salmonella* caused by antibacterial resistant *Salmonella* is rising in many countries (Breuil *et al.*, 2000). Presence of the bacterium *Salmonella* in food and the disease *Salmonella* food poisoning and typhoid fever continue to be a major public health problem worldwide. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths. The increasingly resistance to antibiotics of food borne *Salmonella* (Breuil *et al.*, 2000) drive much of the current interest on plant antibacterial molecules.

Staphylococcus aureus is an enteric Gram-positive bacterium, capable of causing diarrhoea. It is also responsible for skin diseases (Murray *et al.*, 1998; Lansing *et al.*, 1999). Food-borne diseases are of major concern worldwide and *S. aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food (Le Loir *et al.*, 2003).

1.2 Statement of Research Problem

The wide acceptance of traditional medicine as an alternative form of healthcare and the alarming increase in the incidence of new and re-emerging infectious diseases bring about the necessity to investigate these medicinal plants. There is a continuous and urgent need to discover plants with antimicrobial activities. Moreover, since many plants are unexamined, therapeutic results have been mixed resulting to poisoning (Nostro *et al.*, 2000). Only about 20% of the world medicinal plants have been screened for pharmacological and biological activities (Reynold and Lawson, 1978; Ndukwe *et al.*, 2005, 2007). Another concern is the development of resistance of disease pathogens to the antibiotics in current clinical use (Ertürk *et al.*, 2006).

The high cost of important conventional drugs for treating diseases and/or inaccessibility to modern health care facilities has led to reliance on traditional medicine since it is affordable and available to rural people (Kitonde *et al.,* 2014). Scientific investigations and information on the therapeutic potentials of medicinal plants are limited. This lack of scientific knowledge has restricted the use of traditional herbs as remedies to be used in conjunction with or as an alternative to orthodox medical treatment (El-Mahmood *et al.*, 2010).

1.3 Justification

The ever increasing demand for safer and cheaper herbal recipes in the developed countries has led to the extraction and development of several drugs and chemotherapeutic agents from plants as well as from traditional herbal remedies (Falodun *et al.*, 2006). There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal spices for the treatment of various diseases (Azaizeh *et al.*, 2003). The scientific literature is full of reports describing plants as the sleeping giant of the pharmaceutical industry (Smith, 1991; Michael, 2002), which when fully exploited will provide novel compounds to fight infectious diseases (Onyeagba *et al.*, 2006; El-Mahmood and Ameh, 2007; Jabar and Al-Mossawi, 2007).

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased (Vital and Rivera, 2009). In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Nascimento *et al.*, 2000) and the increase in number of drug resistant bacteria is no longer matched by discoveries of new drugs to treat variant infections (Whitman, 2008). Therefore, there is a need to source for alternative antimicrobial agents.

Also the claims of effective therapy for the treatment of various diseases by traditional herbalists worldwide have prompted interest in specific investigations of such herbal medication.

This study will however provide scientific evidence on the use of the selected plants combination which is being utilized as herbal therapy.

1.4 Aim and Objectives

The aim of this study is to screen for the secondary metabolites and antibacterial activity of a combination of medicinal plants extracts on *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*.

The specific objectives of this study are to:

1. determine the phytochemical secondary metabolites of the combination of selected plants.
2. determine the antibacterial susceptibility of *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* to the different concentrations of the selected plants combination extracts.
3. determine the minimum inhibitory concentrations (MICs) of the selected plants combination extracts on *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus.*
4. determine the minimum bactericidal concentrations (MBCs) of the selected plants combination extracts on *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus.*

1.5 Research Questions

i. What are the secondary metabolites of the selected plants combination extracts?

ii. Are the test organisms, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* susceptible to the selected plants combination extracts?

iii. Do the different concentrations of extracts have bacteriostatic effects on *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*?

iv. Do the different concentrations of extracts have bactericidal effects on *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Traditional Medicine

The World Health Organization (WHO) defines traditional medicine as health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses and maintain well-being (WHO, 2001).

It is known that many countries in Africa, Asia and Latin America use traditional medicine (TM) to meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care (WHO, 2003a).

Traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in the industrialized countries. In China, for example, traditional herbal preparations account for 30-50% of the total medicinal consumption. In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home. WHO estimates that in several African countries traditional birth attendants assist in a majority of births (Bannerman *et.al*., 1993; WHO, 2003a).

Over one-third of the population in developing countries lack access to essential medicines. The provision of safe and effective TM/CAM therapies could, thus, become a critical tool to increase access to health care (WHO, 2003a).

Despite its existence and continued use over many countries, and its popularity and extensive use during the last decade, traditional medicine has not been officially recognized in most countries. Consequently, education, training and research in this area have not been accorded due attention and support. The quantity and quality as well as the safety and efficacy of data on traditional medicine are far from sufficient to meet the criteria needed to support its use worldwide. One of the reasons for the lack of research data is due to health care policies (WHO, 2000).

Nigerians have a deep belief and reliance on the services of the traditional practitioners for their health care needs. An estimated 75% of the population still prefers to solve their health problems by consulting the traditional medical practitioners (Adesina, 2008).

2.1.1 History of herbal medicine

Herbal medicine is one of the oldest forms of healing, starting with the origin of human life. Neanderthals living 60,000 years ago in present day Iraq used plants such as Holly hock. Hippocrates in the late fifth century BC mentioned 300-400 medicinal plants (Tim, 2004). As regards to who first used it, records available show that no one knows who the first people were who used plants to make themselves feel better. In fact there's evidence that apes and other animals seek out certain types of plants when they feel ill, so the use of herbal medicine could be older than human history. According to Athanasia and Konstantinos (2003), early hominids observed animals and employed a form of empirism to determine the edibility of various plants; presumably, this led to the use of plants as medicines. Through random selection, trial and error early man acquired sufficient knowledge on herbal use of plants around him and exploited them effectively forming a

basis for the present day traditional medicinal plants used for the treatment of some diseases (Sofowora, 2006). Medicinal herbs are used by animals and humans with the apparent prophylactic effects of reducing the likelihood or severity of illness from pathogens or parasites in the future (Benjamin, 2005).

Generally, herbs are considered as plants parts, plant materials (e.g. Essential oil, exudates) used for flavour, fragrance or for medicinal purpose (Aliero, 2003). Herbal medicine sometimes called 'phytotherapy' is the use of plants, plant parts, their water, solvent extract, essential oil, gums, resins, exudates or other form of advanced product made from plant and used therapeutically to provide proactive support of various physiological systems or in a more conventional medical sense, to treat, cure or prevent a disease in animals or humans. The plant can include the fresh or dried form of herb, part, whole, chopped or powdered or an advanced form of the herb usually made via extraction by a solvent such as water, ethanol or acetone. Such advanced herbal preparations are often processed in a way that establishes relatively fixed chemical parameters, which are often called standardized extracts. These standardized chemicals may be chosen either because they are "marker compounds" specific or characteristics of a particular genus or species of plant or they are scientifically active compounds, as they contribute significantly to the preparations specific activity. Marker compounds may be active or inert and can contribute to physiological activity other than the primary intended use of the extract. Athanasia and Konstantinos (2003) explained that the word drug derived from the Dutch word "droog" meaning "to dry", originated from the use of herbal medicine, where plants were dried and used as remedies. The history of pharmacy and medicine are parallel to a significant degree to the evolution of herbal medicine. Herbal preparation is by definition chemically

complex, as it contains many naturally occurring compounds of the plant or plant part. In contrast, classic plant derived drugs such as atropine, colchicines, ephredrine, quinine, reserpine and scopolamine, are single chemical entities. Although they are extracted from plant they are isolated and purified to modern conventional drugs and this cannot technically be considered for equivalent use as the plant form (Athanasia and Konstantinos, 2003).

2.1.2 Demand for herbal drugs

Herbal drugs have increasingly been used worldwide during the last few decades as evidenced by rapidly growing global and national markets of herbal drugs. According to WHO (2003b) estimates, the demand for medicinal plants is about US $14 billion a year and by the year 2050 it would be about a trillion US dollars. Now people rely more on herbal drugs because of high price and harmful side effects of synthetic drugs and this trend is growing not only in developing countries but in developed countries too (Tor-anyiin and Shaato, 2003). A number of plants have been indicated to possess antimicrobial properties from traditional uses (National Committee on Clinical Laboratory Standards (NCCLS), 1995). Since herbs have been the source of many pharmaceutical drugs and are often approved as non- prescription medications in numerous countries around the globe (i.e. both industrialized and developing), the range of clinical applications as well as uses in self-medication using herbs is extremely wide. The example of potential clinical applications of herbs and phyto-medicines, where the herbs play either a primary or adjunct roles are;

i. Circulatory: May be used for increase peripheral circulation e.g. in peripheral arterial exclusive disease, congestive heart failure, hyperlipidemia, atherosclerosis and hypertension.

ii. Endocrinological: Used to treat symptoms of menopause and menstrual problems.

iii. Gastrointestinal: Used for the treatment of digestive upset, used as stimulant and bulk laxatives, as cholagogues hepato protectants, and for irritable bowel syndrome.

iv. Genito-urinary: Often employed as aquaretic and diuretics and for the treatment of cystitis, benign prostatic hyperplasia, vaginal infections, leucorrhea and dysmenorrhea.

v. Neurological: Used for anxiety, minor to moderate depression, insomnia and cognitive disorder like short term memory loss and demential syndrome associated with aging and early stages of Alzheimer's (Athanasia and Konstantinos, 2003).

2.1.3 Herbal medicine and their efficacy

During recent decades, different plant, derived extracts and phytochemicals have been ascribed a variety of potentially health-promoting biological activities. Plants such as *Hyptis suaveolens* have oil which is strongly aromatic and has been reported to be potent for reducing bacterial and fungal growth. The juice squeezed from the leaves, when mixed with lime juice, is drunk to cure colic, gastrointestinal disorder and when applied to forehead, alleviate headache (Akinloye, 2003). *Momordica balsamina* (Balsam apple) infusion is used for the treatment of stomach fever and yaws, a decoction taken internally for the same condition. It is similarly used for horses against intestinal disorder. In Europe the grounded fruit mixed with oil is used as a dressing for inflammatory sore and swellings,

yaws etc. The root is sometimes used as ingredient in aphrodisiac prescriptions and along with the fruit; the seeds are also used as an abortifacient as well as a remedy for urethral discharges. The root bark of *Annona senegalensis* (Hausa: gundar-daji) is boiled with natron for gastro-intestinal trouble, it is used in the treatment of veneral diseases, guinea worm and sores. *Boswellia dalziellii* (Hausa: hanu), bark is boiled in large quantity to make a wash for fever, rheumatism and gastro-intestinal troubles. *Pistia stratiotes* (Hausa: kainuwa) is applied in ulcerative conditions of the mouth and tongue and also taken internally for gastro-intestinal disorders sometimes associated with worms. *Diospyros mespiliformis* (Hausa: kanya), the leaves and fruits in cold infusion are a remedy for dysentery. *Tribulus terrestris* (Hausa: Tsaidau), whole plant are used as a diuretic and are said to be valuable for bladder troubles, while the oil prepared from the fruit are used for rheumatism. *Adansonia digitata* (Hausa: kuka), is used internally and locally applied for a variety of inflammatory conditions as a prevention against fever, dysentery and genitor-urinary conditions. *Mangifera indica* (Mango) bark and leaves have astringent properties such that a lotion and mouth-wash relieves toothache, sore gums and sore throat. An infusion of root bark is given for diarrhea and dysentery (Dalziel, 1958). *Vitex doniana* (Hausa: ɗ inya) is being used by traditional medicine practitioners in the treatment of dysentery and gastroenteritis (Kilani, 2006).

The St. John's wort was effective in treating depression (antidepressant). The pain-killer Morphine was extracted from the Opium poppy. The Aspirin was extracted from Willow-wort bark and used for the treatment of fever (Benjamin, 2005). The oil extracted from *Lavandula spp*. (Lavender flower) when inhaled is reported to have mild sedative effects; this kind of herbal medicine is referred to as aromatherapy (Athanasia and Konstantinos,

2003). The methanolic extract of stem bark of *Picralima nitida* (Apocynaceae) exhibited significant antimicrobial activity against a wide range of Gram-positive bacteria and fungi but limited activity against Gram-negative bacteria (Fakeye *et al.*, 2002). Olukemi *et al.* (2002) reported an *in vitro* antibacterial activity of the crude extract of the leaf of *Borreria verticillata* tested on *Staphylococcus aureus*, *Escherichia coli* and some bacteria isolated from the clinical specimens, namely *Proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus epidermidis*. The stem bark and leaves of *Garcinia kola* can be used for the treatment of diseases caused by *Staphylococcus aureus* (Onwukaeme and Obuekwe, 2004).

Many other plants are said to be of medical importance in treatment of conditions ranging from fever, throat, intestinal, urethral, skin and venereal diseases. It is estimated that almost 75% of the useful bioactive plants-derived pharmaceuticals used worldwide were discovered by systematic investigation of leads from traditional medicine practitioners (Okujagu *et al.*, 2005).

Herbalists believe that the use of tinctures and herbal tonic can help the body to heal itself by restoring harmony and balance and activating the body's life force. Their remedies are extracted from leaves, petals and roots, barks and plants possess a complex mixture of different compounds, a principle referred to as 'herbal synergy'. While a conventional pharmaceutical will usually be a single active ingredient, the idea of herbal synergy explains that the hundreds if not thousands of constituents of a plant extract all work together to treat an illness. For example ephedrine, an early anti-asthma drug was first isolated from the herb *Ephedra* traditionally used to treat chest complaints. One of the side effects of ephedrine is that it raises the blood pressure. Herbalist point out that among many

compounds found in the plant itself is one that lowers blood pressure so, the herbal remedy contains a compound to treat the chest, but also to counteract the side effects of the compound (Trudy, 2006). In fact numerous researches *in vitro* and *in vivo* have indicated the efficacy and effectiveness of herbs in treating one disease or the other.

Plant materials are used not only in medication but also in the control of pest and other parasites, such as mosquitoes. A compound from *Azadirachta indica* 'Azadirachtin' has adverse effect on endocrine system of beans' beetle *Epilachna variveatis* and cause sterility in the female insect and also demonstrates potency in the control of the insect in question by causing degradation in larval epidemics preventing the larvae from molting (Trudy, 2006).

2.1.4 Herbal medicine in Africa

In developing countries especially in rural context, people usually turn to traditional healers when in diseased conditions and plants of ethno botanical origin are often presented for use. Iwu (1993) pointed out that the African continent is one of the continents endowed with the richest biodiversity in the world, with an avalanche of many plants used as herbs, health food and for therapeutic purpose. This is largely due to the geographical spread spanning a landmass of approximately 216, 634, 000 hectares of closed forest areas. Over 5,000 different species of plant substances have been recognized to occur in these areas and many of them have been found to be useful in traditional medicine prophylaxis and cure of diseases. This great biodiversity therefore offer economic promise and in the rapidly emerging biotechnology industry. Nevertheless there has been little effort devoted to the development of chemotherapeutic and prophylactic agents from these plants, in view of the

emerging competitive world, therefore the evaluations of the constituents, pharmacological properties, detailed screening of bioactive substances for chemotherapeutic purpose are urgently warranted (Farombi, 2003).

In Nigeria, there is a great deal of herbal medication as a result of factors like lack of access to medical care, high cost of synthetic drugs, their side effects, development of antibiotic resistant strains of micro-organisms coupled with poverty in majority of the population (Jawetz *et al.*, 1974).

2.1.5 Traditional medicine practitioners and practice in Nigeria

Owumi (1993) posits that traditional medical practitioners acquire herbal knowledge either through inheritance or apprenticeship as a call by one or the other. In the past, many of them practiced the art as a hobby or as a form of community service with little or no financial rewards thus making the practice "pure and efficacious". Trado-medical knowledge system is well structured and organized and has survived through generations to maintain harmony between body, mind and soul within its sociocultural and religious context. The various ethnic groups in Nigeria have different traditional healthcare practitioners aside their western health care counterparts. The Yoruba call them 'Babalawo', the Igbo call them 'Dibia', while the Hausa refer to them as 'Boka'. However, different experts have emerged within their ranks including herbalists, bone-setters, psychiatrics, and birth attendants, among several others. They usually rely on vegetables, mineral substances, animal parts and certain other methods such as prayers, divinations and incantations (Owumi and Jerome, 2008).

Traditional medicine in Nigeria has come a long way. Most people believe and rely upon the services of the practitioners for the relief of physical illnesses as well as psychological and spiritual comfort. Their success is enhanced by their understanding of the personal, social, cultural and political conditions of the individuals, families and communities (Roan, 1999). Traditional medicine has impacted significantly on the lives of the people especially in the rural areas where access to orthodox medicare is minimal. Aside the lack of access, the prohibitive cost of western medications makes traditional medicine attractive (Adefolaju, 2011).

2.1.6 Challenges of traditional medical practice in Nigeria

Traditional medical practice, in spite of its popularity has been challenged on many grounds (Erinosho, 1998). One of such is that its popularity is based on the anecdotal experiences of patients. Some of the other arguments against traditional medicine include the fact that traditional medical practitioners lack the skills required for correct diagnosis of serious disorders and they are always unwilling to accept the limitations of their knowledge, skills and medicines particularly in complicated organic disorders, traditional medicine lacks standard dosage and have not been subjected to scientific verifications, the healers lack the equipment required to conduct physical examinations and even though the educated are convinced that the healers have supernatural knowledge and that this knowledge is medically useful, they have found them to be unscrupulous and dubious (Erinosho, 1998).

In a similar vein, a former Director-General of the National Agency for Food and Drug Administration and Control (NAFDAC) expressed the challenges being faced in regulating

traditional medicines (Anonymous, 2008). These include, lack of documentation, inadequate coordination of the practitioners' activities, poor communication between the practitioners and their patients, secrecy of actual contents and/or difficulty in determining actual ingredients. Furthermore, most of the claims of the traditional practitioners were said to be unsubstantiated and their post-market monitoring has been difficult. Patients were also said to have reported adverse reactions. Akinleye (2008) corroborated this when he identified some of the drawbacks of traditional medicine as incorrect diagnosis, imprecise dosage, low hygiene standards, the secrecy of some healing methods and the absence of written records about the patients.

Traditional medicine practice in Nigeria, however, faces greater challenges in the hands of government officials who look at it with disdain and disrespect. This is a carry-over from the colonialists who "needed" to uproot this traditional medical practice for their own medical system to thrive and therefore portrayed the former as nothing more than witchcraft and fetish. Their successor, the Nigerian elite, despite the cultural background, was not better as the western propaganda had been infused to smear the historical and indigenous health care system. This is manifested in the Nigerian government's reluctance to accord traditional medicine its primate position in the healthcare delivery system. As a matter of fact, traditional medicine is practiced in Nigeria today without enabling national legislation that will regulate its practice as obtained in many parts of the world (WHO, 2011). However many states in the country have established traditional medicine boards/agencies to monitor the activities of its practitioners (Adefolaju, 2011).

2.1.7 Safety and standard of traditional medicines

The growing popularity of herbal remedies is fuelling and is to some extent fuelled by increasing scientific interest in herbal medicine (Ansari and Inamdar, 2010). WHO estimates that of the 35,000-70,000 species of plants that are used for medicinal purposes, around 5,000 have been submitted for biomedical scrutiny. Scientific evidence of efficacy is beginning to emerge from randomized controlled trials in which herbs compare favourably with placebo. Examples, St. John's wort for mild depression and of course, a number of commonly used pharmaceutical products are of botanical origin-saprin, digitoxin and quinine are three well known examples (Ekeanyanwu, 2011).

Another reason for the growing popularity of herbal medicines is that many people believe they are safer and more natural than pharmaceuticals. However, studies have shown that not all natural products are safe, some poisons are also natural (Ansari and Inamdar, 2010). Herbal medicine however natural can cause serious illness from allergy to liver or kidney malfunction to cancer and even death. In terms of carcinogenicity for example, toxicological potential of natural plant chemicals is roughly the same as that of synthetic chemicals (WHO, 2001). Most herbal products on the market today have not been subjected to drug approval process to demonstrate their safety and effectiveness. Some of them contain mercury, lead, arsenic (Kew *et al.*, 1993) and corticoids (De Smet, 1995) and poisonous organic substances in harmful amounts. Hepatic failure and even death following ingestion of herbal medicine have been reported (Chattopadhyay, 1996). A prospective study shows that 25% of the childhood blindness in Nigeria and India were associated with the use of traditional eye medicine (Harries and Cullinan, 1994). Gupta and Raina (1998) reviewed the side effects of some medicinal plants.

Perhaps the biggest problems in Nigeria with herbal medicine are a lack of standardization and of safety regulations. Standardization of herbal medicine that may contain hundreds of chemical constituents with little or no evidence indicating which might be responsible for the presumed or proven therapeutic effect is a particularly theory issue (WHO, 2001). Many users of herbal medicines consider that they are safe for human consumption, an assumption based in part on extensive prior field experience. If this concept ever had validity, it is no longer correct. In Nigeria, teas and infusions of herbs are the most popular traditional drug formulation available today. On a batch to batch basis, there must be botanical, chemical and biological standardization of products and collateral studies which would establish both the safety of the products and a demonstration of its efficacy and meaningful shelf-life (Ekeanyanwu, 2011).

2.1.8 Review of policies of traditional medicine

The Federal Republic of Nigeria is currently developing a national policy on Traditional Medicine/Complementary and Alternative Medicine (TM/CAM). Laws and regulations were issued in 1993 and revised in 1999 and a national programme is still pending. The national office was established in 1997, and is administered by the Federal Ministry of Health. The expert committee on TM/CAM was created in 1978. Nigeria has two national research institutes on TM/CAM and herbal medicines, founded in 1988 and 1992. They are the Nigerian Natural Medicines Development Agency (NNMDA) in Lagos, Nigeria and the National Institute for Pharmaceutical Research and Development (NIPRD).

Regulation of herbal medicines was introduced in Nigeria in 1993 in Decree No.15, and was revised in 1999. Herbal medicines are regulated as dietary supplements, health foods,

functional foods and as an independent regulatory category. Claims that may be made about herbal medicines include health, nutrient content and structure/function claims in accordance with the law. The Nigerian national pharmacopoeia and national monographs are in development, but no other materials are used in their place at present.

Manufacturing regulatory requirements are restricted to good hygienic practices and are enforced through checklists drawn up by the regulatory agency. Special requirements for safety assessment exist, including traditional use without demonstrated harmful effects; compliance with these requirements is ensured through animal studies to assess acute toxicity. There are currently 107 registered herbal medicines in Nigeria, but none is listed on the essential drug list. A post-marketing surveillance system is in development. In Nigeria, herbal medicines are sold without restriction by licensed practitioners (WHO, 2005).

2.2 *Escherichia coli*

Since 1885 when it was first isolated from children's faeces and described by the German bacteriologist Theodor Escherich, scientific attention has been lavished on *E. coli* to such an extent that it is probably the best understood free-living organism (Adams and Moss, 1999).

Escherichia coli has become a model organism for studying many life's essential processes partly due to its rapid growth rate and simple nutritional requirements. It can easily be grown at 37 ^{o}C either under aerobic or anaerobic conditions and is used in many different types of experiments. *E. coli* is a Gram-negative rod-shaped bacterium, with opportunistic infections of colon and other sites (Cheesbrough, 2000; Stephen, 2004).

Escherichia coli is an almost universal inhabitant of the gut of humans and other warm-blooded animals where it is the predominant facultative anaerobe though only a minor component of the total microflora. Generally a harmless commensal, it can be an opportunistic pathogen causing a number of infections such as Gram-negative sepsis, urinary tract infections, pneumonia in immunosuppressed patients, and meningitis in neonates (Adams and Moss, 1999).

2.2.1 Classification of *E. coli*

Escherichia coli is a bacterium of the phylum Proteobacteria and class Gamma Proteobacteria. It belongs to the order Enterobacteriales and family Enterobacteriaceae. The family Enterobacteriaceae includes Gram-negative, non-sporing, facultatively anaerobic rods, typically 0.3-1.0 x 1.0-6.0 µm, peritrichously flagellated or non-motile (Prescott *et al.*, 2002).

2.2.2 Biology of *E. coli*

Escherichia coli is a catalase-positive, oxidase-negative, fermentative, short, Gram-negative, non-sporing rod. Genetically, *E. coli* is closely related to the genus *Shigella*, although characteristically it ferments the sugar lactose and is otherwise far more active biochemically than *Shigella* spp. Late lactose fermenting, non-motile, biochemically inert strains of *E. coli* can however be difficult to distinguish from *Shigella*. *E. coli* is a typical mesophile growing from 7-10 ^{o}C up to 50 ^{o}C with an optimum around 37 ^{o}C, although there have been reports of some Enterotoxigenic *E. coli* (ETEC) strains growing at temperatures as low as 4 ^{o}C. It shows no marked heat resistance, with a decimal reduction time (D value) at 60 ^{o}C of the order of 0.1 min, and can survive refrigerated or frozen

storage for extended periods. A near-neutral pH is optimal for growth but growth is possible down to pH 4.4 under otherwise optimal conditions (Adams and Moss, 1999).

Most strains are capable of colonizing the intestinal mucosa of the host with the 'attaching and effacing' mechanism genetically governed by a large pathogenicity island. *E. coli* typically colonizes infants' gastrointestinal cavity and it is facultatively anaerobic, found in human colon. In this mutual relationship, *E. coli* generally is confined to human intestines, however, in a debilitated or immunosuppressed host or when the bacteria is introduced to the other tissues even in a healthy person (Center for Disease Control and Prevention, 2005). In blood agar, *E. coli* produces 1-4mm diameter colonies after overnight incubation. The colonies may appear mucoid. Some strains are haemolytic. In MacConkey agar and Cysteine-Lactose-Electrolyte Deficient (CLED) agar, *E. coli* ferments lactose producing smooth pink colonies on MacConkey agar and yellow colonies on CLED agar. Inactive strains are late or non-lactose fermenting (Cheesebrough, 2000).

2.2.3 Mode of transmission of *E. coli*

The major mode of transmission is faecal contamination of food and water. Person-person transmission has also been demonstrated (Riley *et al.*, 1983; Griffin and Tauxe, 1991). Alfredo *et al.* (2005) explained that it was discovered that drought carriage of *E. coli* 0157 by cattle and heavy rains with contamination of surface water appears to be important factors that contribute to its outbreak. The main natural reservoir is ruminant and cattle, which are considered to be the most important source of human infection with 0157 Enterohemorrhagic *E. coli* (EHEC).

2.2.4 Pathogenesis and clinical features of *E. coli*

Escherichia coli causes urinary tract infections and it is the commonest pathogen isolated from patients with cystitis, and recurring infections are common in women; infections of wounds, peritonitis, sepsis and endotoxin-induced shock, meningitis and bacteriaemia in neonates, diarrhoea, dysentery and haemorrhagic diarrhoea (Cheesbrough, 2000). The strains of *E. coli* that cause gastro-enteritis are subdivided into five groups;

i. Enterotoxigenic *E. coli* (ETEC): located in the small intestine causes travellers' diarrhoea, watery diarrhoea, vomiting, cramps, nausea and low-grade fever.
ii. Enteroinvasive *E. coli* (EIEC): in large intestine causes fever, watery diarrhoea followed by dysentery with scant blood stools.
iii. Enteropathogenic *E. coli* (EPEC): in small intestine causes infant diarrhoea with fever, nausea and vomiting.
iv. Enterohemorrhagic *E. coli* (EHEC): found in large intestine causes hemorrhagic colitis with abdominal cramps, initial watery diarrhoea, followed by grossly blood diarrhoea, little or no fever.
v. Enteroaggregative *E. coli* (EAggEC): found in small intestine causes infant diarrhoea, persistent watery diarrhoea with vomiting, dehydration and low-grade fever (Murray *et al.*, 1998).

Illness caused by ETEC usually occurs between 12 and 36 h after ingestion of the organism. Symptoms can range from mild afebrile diarrhoea to a severe cholera-like syndrome of watery stools without blood or mucus, stomach pains and vomiting. The illness is usually self-limiting, persisting for 2-3 days. In developing countries, it is a common cause of infantile diarrhoea where it can cause serious dehydration. The ingested

organism resists expulsion from the small intestine with the rapidly flowing chime by adhering to the epithelium through attachment or colonization factors in the form of fimbriae on the bacterial cell surface. These can have different morphology and be either rigid (6-7 nm diameter) or flexible (2-3 nm diameter) structures composed of 14-22 kDa protein subunits. They are mannose resistant, i.e. they mediate haemagglutination in the presence of mannose, and particular colonization fimbriae are restricted to certain O:H serotypes. They are encoded on plasmids which frequently also encode for the diarrhoeagenic toxins. Two toxin types are produced; the heat-stable toxins (ST), which can withstand heating at 100 ^{o}C for 15 min and are acid resistant, and the heat-liable toxins (LT) which are inactivated at 60 ^{o}C after 30 min and at low pH. Heat-liable toxin (LT) I bears a strong similarity to cholera toxin; it consists of five B subunits (M_r 11.5 kDa) which are responsible for binding of the toxin to the epithelial cells and an A subunit (M_r 25 kDa) which is translocated into the epithelial cell, where it activates adenylate cyclase. The subsequent increase in cyclic adenosine monophosphate (cAMP) levels then inhibits Na^+, Cl^- and water absorption by the villus cells and stimulates their loss from intestinal crypt cells thus leading to profuse watery diarrhoea. Heat-liable toxin (LT) II toxin produced by certain ETEC strains has similar biological activity to LT I but does not cross react with antiserum to LT I or cholera toxin (Adams and Moss, 1999).

Infection by EIEC results in the classical symptoms of an invasive bacillary dysentery normally associated with Shigella. Like Shigella, EIEC invades and multiplies within the epithelial cells of the colon causing ulceration and inflammation. Clinical features of EIEC infection include fever, severe abdominal pains, malaise and often watery diarrhoea which precedes the passage of stools containing blood, mucus and faecal leukocytes. Invasiveness

is determined by a number of outer membrane proteins which are encoded for on a large plasmid (≈140 MDa) (Adams and Moss, 1999).

Symptoms of EPEC infection, malaise, vomiting and diarrhoea with stools containing mucus but rarely blood, appear 12-36 h after ingestion of the organism. In infants, the illness is more severe than many other diarrhoeal infections and can persist for longer than two weeks in some cases. Pathogenesis appears to be related to the ability of EPEC strains to adhere closely to the enterocyte membrane causing cell loss from the villus tips. It is hypothesized that this causes diarrhoea by disrupting the balance between absorption and secretion in the small intestine (Adams and Moss, 1999).

Haemorrhagic colitis is typically a self-limiting, acute, bloody diarrhoea that begins with stomach cramps and watery diarrhoea after an incubation period of 3-8 days. It can be distinguished from inflammatory colitis by the usual lack of fever and absence of leukocytes in the stools. It affects mainly adults, with a peak incidence in the summer months, and can be life-threatening in the elderly. Haemolytic uraemic syndrome is characterized by three features; acute renal failure, haemolytic anaemia and thrombocytopaenia, sometimes preceded by a bloody diarrhoea. Thrombotic, thrombocytopaenic purpura is related to the haemolytic uraemic syndrome but includes fever and neurological symptoms (Adams and Moss, 1999).

Adhesion is an important factor in virulence and 0157:H7 strains have been shown to possess a 60 MDa plasmid encoding for fimbrial colonization factors which produce a similar, but not identical lesion to EPEC strains on the villus tips. EHEC strains produce the cytotoxin verotoxin (so-called because of its ability to kill vero (African green monkey kidney cells). Studies have revealed the presence of at least two toxins VT I and VT II

which because of their similarity to shiga toxin have been called shiga-like toxins, SLT I and SLT II (Adams and Moss, 1999).

2.3 *Salmonella typhi*

Salmonella was named after Daniel Elmer Salmon, an American Veterinary Pathologist who together with Theobald Smith (better known for his work on anaphylaxis) first discovered the *Salmonella* bacterium in 1885 from pigs. It is mostly transmitted through eating undercooked meat, particularly poultry (Tindall *et al.*, 2005).

2.3.1 Classification of *Salmonella typhi*

Salmonella typhi is a bacterium of the phylum Proteobacteria, class Gamma Proteobacteria, order Enterobacteriales and family Enterobacteriaceae, which includes Gram-negative, non-sporing, facultatively anaerobic rods, typically 0.3-1.0 x 1.0-6.0 µm, peritrichously flagellated or non-motile (Prescott *et al.*, 2002).

2.3.2 Biology of *S. typhi*

Salmonella are Gram-negative bacteria. In clinical laboratory, they are usually isolated on MacConkey agar or Desoxycholate Citrate Agar (DCA). Because they cause intestinal infections and are greatly outnumbered by the bacteria normally found in the healthy bowel, primary isolation requires the use of a selective medium so use of a relatively non-selective medium as CLED agar is not often practiced. The number of *Salmonella* may be so low in clinical samples that stool are routinely subjected to ‘enrichment culture’ where a small volume of stool is incubated in a selective broth medium such as selenite broth overnight. These media are inhibitory to the growth of the microbes normally found in the

healthy human bowel while allowing *Salmonella* to become enriched in numbers (Tindall *et al.*, 2005).

2.3.3 Mode of transmission of *S. typhi*

Salmonella typhi is only carried by humans, no other animal vector is known. Shedding of the *S. typhi* bacteria occurs in the stool of infected persons and also in people that are considered chronic carriers (Health Canada, 2001).

It has been reported that *S. typhi* causes typhoid fever with an estimated 16.6 million cases and 600, 000 deaths worldwide each year. *S. typhi* is transmitted through food or water that has been contaminated with faeces from either acutely infected persons, persistent excretors or from chronic asymptomatic carriers (WHO, 2003b).

2.3.4 Signs and symptoms of *S. typhi*

Salmonella typhi is responsible for typhoid fever, which may cause a sudden onset of several different symptoms, including fever, headache, nausea, constipation, diarrhoea, rose spot across the abdomen and loss of appetite. Destruction of internal organs is the leading cause of death associated with this illness (Tindall *et al.*, 2005).

Once contaminated food or water is swallowed, the bacteria multiply in the intestines and spread into the bloodstream where they are taken in by cells called ‘mononuclear phagocytes’. Phagocytes are cells of the immune system that are responsible for killing bacteria and viruses. However *S. typhi* is not deactivated by these cells after ingestion instead it is capable of multiplying within them. After multiplying *S. typhi* spills out of the cell and into the bloodstream, spreading throughout the body creating a systematic infection

and causing associated symptoms. The bacteria can move from the bloodstream into the lymphatic system and then into other tissues and major organs of the body. During the bacterial invasion into these areas the gall-bladder, liver, intestines and spleen can be most affected. Perforation of the intestinal wall causes leakage in the abdominal cavity, thus resulting in peritonitis which is a frequent cause of death from typhoid fever. Many other complications can also occur ranging from a ruptured spleen to meningitis, and even coma (Tindall *et al.*, 2005). Garba (2005) pointed out that one of the most lethal complications of typhoid fever is intestinal perforation which affects especially young men (Tindall *et al.*, 2005).

2.4 *Staphylococcus aureus*

The staphylococci were first described by the Scottish surgeon, Sir Alexander Ogston as the cause of a number of pyogenic (pus forming) infections in humans. In 1882, he gave them the name staphylococcus (Greek: staphyle, bunch of grapes; coccus, a grain or berry), after their appearance under the microscope (Adams and Moss, 1999).

Staphylococci are Gram-positive spherical bacteria that occur in microscopic (1µm in diameter) clusters resembling grapes (because staphylococci divide into two planes). The configurations of the cocci help to distinguish staphylococci from streptococci, which are slightly oblong cells that usually grow in chains (because they divide in one plane only). They are non spore-forming and non-motile organisms which grow readily on most bacteriologic media under aerobic or micro aerobic conditions with their optimum growth temperature being 37 ^{o}C. The young culture of *Staphylococcus* spp. stain strong Gram-

positive but on aging many cells become Gram-negative. This is considered as 'Gram variation' (Brooks *et al.*, 2002).

Bacteriological culture of the nose and skin of normal humans invariably yields Staphylococci. In 1884, Rosenbach described the two pigmented colony types of Staphylococcus and proposed the appropriate nomenclature: *Staphylococcus aureus* (Yellow) and *Staphylococcus epidermidis* (White) (Chambers, 2001; Menichetti, 2005). The *S. aureus* and *S. epidermidis* are significant in their interactions with humans, *S. aureus* colonizes mainly the nasal passages but it may be found regularly in most other anatomical locales. *S epidermidis* is an inhabitant of the skin. It lives completely harmless on the skin and in the nose of about one third of the normal healthy people. This is referred to as colonization or carriage. *S. aureus* can cause actual infection and disease, particularly if there is an opportunity for the bacteria to enter the body e.g. via a cut or an abrasion (Kenneth, 2006).

2.4.1 Classification of *Staphylococcus aureus*

Staphylococcus aureus is a bacterium belonging to the phylum Firmiates, class Bacilli, order Bacillales and family Staphylococcaceae (Prescott *et al.*, 2002; Tindall *et al.*, 2005).

2.4.2 Morphology and test for *Staphylococcus aureus*

S. aureus forms a fairly large yellow colony on rich medium and often haemolytic on blood agar, facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. The bacteria are catalase-positive and oxidase-negative. *S. aureus* can grow at a temperature range of 15 to 45 ^{o}C and at NaCl concentrations as high as 15 percent. Nearly all strains of *S. aureus* produce the enzyme coagulase and should be

considered a potential pathogen. The catalase test is important in distinguishing streptococci (catalase-negative) from staphylococci, which are vigorous catalase-producers. The test is performed by adding 3% Hydrogen peroxide to a colony on an agar plate or slant. Catalase-positive culture produces oxygen and bubble at once. The test should not be done on blood agar because blood itself contains catalase. Coagulase is an extracellular protein, which binds to prothrombin in the host to form a complex called staphylothrombin. The protease activity characteristic of thrombin is activated in the complex, resulting in the conversion of fibrinogen to fibrin. Coagulase is a traditional marker for identifying *S. aureus* in the clinical microbiology laboratory. However it is speculated that the bacteria could protect themselves from phagocytic and immune defenses by causing localized clotting (Kenneth, 2006).

2.4.3 Characteristics of *S. aureus*

- Gram positive, cluster-forming coccus
- Non-motile, non-spore-forming facultative anaerobes
- Fermentation of glucose produces mainly lactic acid
- Ferment Mannitol (distinguishes from *S. epidermidis*)
- Catalase positive
- Coagulase positive
- Golden-yellow colony on agar
- Normal flora of human as found in nasal passages, skin and mucus membranes
- Pathogen of humans; causes a wide range of supportive infections as well as food poisoning and toxic syndrome.

2.4.4 Pathogenesis of *S. aureus* infections

S. aureus is of clinical importance; it is one of the common causes of opportunistic infections in the hospital and community where it causes a variety of pus forming infections and toxinoses in humans (Eady and Cove, 2003). It causes superficial skin lesions such as boil styles and furunculosis, more serious infections such as pneumonia, mastitis, phlebitis, meningitis and urinary tract infections and deep seated infections such as osteomyelitis and endocarditis. It is also a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with individual medical devices. It causes food poisoning by releasing enterotoxin in food, and toxic shock syndrome by release of super tigers into bloodstream (Eady and Cove, 2003).

Staphylococcus aureus pathogenic capacity is due to the combined effect of intracellular factors and toxins together with the invasive properties of the strain. At one end of the disease spectrum is staphylococcal food poisoning attributable solely to the ingestion of preformed enterotoxin (Brooks *et al.*, 2002). Food poisoning by *S. aureus* is characterized by a short incubation period, typically 2-4 h. Nausea, vomiting, stomach cramps, retching and prostration are the predominant symptoms, although diarrhoea is also often reported, and recovery is normally complete within 1-2 days (Adams and Moss, 1999).

Though frequently described as enterotoxins, the *S. aureus* toxins are strictly neurotoxins. They elicit the emetic response by acting on receptors in the gut which stimulate the vomiting centre in the brain via the vagus and sympathetic nerves. If these nerves are severed then vomiting does not occur. It is not known how the toxin induces diarrhoea but it has been shown not to stimulate adenylate cyclase activity (Adams and Moss, 1999).

2.4.5 Virulence factors and tissue invasion of *S. aureus*

For the majority of diseases caused by *S. aureus*, pathogenesis is multifactorial, so it is difficult to determine precisely the role of any given factor. However, there are correlations between strains isolated from particular disease and expression of particular virulence determinants, which suggest their role in a particular disease.

Human staphylococcal infections are frequent, but usually remain localized at the port of entry by the normal host defence. The port may be a hair follicle, but usually it is a break in the skin which may be a minute needle stick or a surgical wound. Foreign bodies, including sutures are readily colonized staphylococci, which may make infections difficult to control. Another port of entry is the respiratory tract. Staphylococcal pneumonia is a frequent complication of influenza. The localized host response to staphylococcal infection is inflammation, characterized by an elevated temperature at the site, swelling, and accumulation of pus and necrosis of tissue. Around the inflamed area a fibrin clot may form, walling off the bacteria leukocytes as a characteristic pus-filled boil or abscess. More serious infections of the skin may occur such as furuncles or impetigo. Localization of the bone is called osteomyelitis. Serious consequences of staphylococcal infections occur when the bacteria invade the bloodstream. Resulting septicemia may be rapidly fatal; a bacteriamia may result in seeding other internal abscesses, other skin lesions or infections in the lung, kidney, heart, skeletal, muscles or meninges (Chambers, 2001; Brooks *et al.*, 2002; Menichetti, 2005).

2.5 Culture Media

A medium (plural: media) is any solid or liquid preparation made specifically for the growth, storage or transport of bacteria. When used for growth, the medium generally supplies all necessary nutrients. Before use, a medium must be sterile, i.e. it must contain no living organisms (Singleton, 1997).

A liquid medium may be used in a test tube (which is stoppered by a plug of sterile cotton wool, or which has a simple metal cap) or in a glass, screw-cap bottle. Most solid media are jelly-like materials which consist of a solution of nutrients, 'solidified' by agar (a complex polysaccharide gelling agent obtained from certain seaweeds). A solid medium is commonly used in a plastic Petri dish. The medium, in a molten (liquid) state, is poured into the Petri dish and allowed to set; a Petri dish containing the solid medium is called a plate (Singleton, 1997).

Media (agar and broth) chosen for the test have been manufactured and tested specifically for antibacterial susceptibility test (AST) and other sensitivity tests. The Mueller-Hinton agar (CM 337) was originally formulated to grow pathogenic *Neisseria* and it was adopted for use in the Bauer-Kirby test later, following agreement between culture media manufacturers and representations of the National Committee on Clinical Laboratory Standards (NCCLS). Mueller-Hinton medium is now produced which meet the NCCLS specifications; therefore the Mueller-Hinton medium suitable for AST will carry a statement on the label that meets NCCLS standard (Ching, 2006).

Bridson (1995) explained that Petri dishes were used with Mueller-Hinton agar (5-6mm depth). NCCLS recommends Mueller-Hinton agar due to the fact that it results in good

batch to batch reproducibility; it is low in sulfonamide, trimethroprim and tetracycline inhibitors, it results in satisfactory growth of most bacteria pathogens and large amount of data has been collected concerning susceptibility tests performed with this medium (WHO, 2003b).

2.6 Sterilization and Autoclaving

2.6.1 Sterilization

Sterilization may be defined as the statistically complete destruction of all microorganism including the most resistant bacteria and spore. By employing steam sterilization techniques, a high level of sterility can be achieved and the most popular piece of equipment used in laboratories and hospitals is the steam sterilizer or autoclave (APHA, 1992; Astell, 2006).

2.6.2 Autoclaving

Steam can sterilize at lower temperatures (for shorter time duration) than those used in a hot-air oven. At normal atmospheric pressure, steam has a temperature of only 100 ^{o}C - a temperature at which some endospores can survive for long periods- but, when under pressure, steam can reach higher temperatures suitable for sterilization; in fact, there is a definite relationship between the pressure and temperature of pure steam, i.e. steam containing no air: the higher the pressure, the higher the temperature. When sterilizing with steam, items to be sterilized are placed inside a strong, metal, gas-tight chamber (an autoclave) (Singleton, 1997).

Autoclaves are used for numerous medical and laboratory applications which include sterilizing instruments (wrapped and unwrapped in bottles such as prepared paper discs). The following can be autoclaved; media preparation, utensils and laboratory equipments (plates, bottles, glass rods, micropipette etc.) and distilled water (APHA, 1992; WHO, 2003b).

2.7 Methods and Solvents of Extraction

2.7.1 Methods of extraction

Conventional extraction of plant material often involves for example steam distillation or various solid-liquid extraction procedures relying on organic solvents (Maarit *et al.*, 2002).

The galenical extraction procedures yield preparations which, according to the extraction method used, are known as ‘extracts’. There are various methods used for extraction of plant constituents for screening for their medicinal uses or antibacterial effect. These include; infusions, maceration, percolation, digestion and decoction.

Maceration Process: The plant material in suitable state of comminution (liquid, dry or soft) are placed in a closed vessel with the extracting solvent, and allowed to stand for seven days, with occasional stirring. The mixture is strained, and the marc pressed to remove retained solution. The liquids are combined, and any precipitated solid removed, either by decanting the clear fluid after a period of standing or by filtration.

Percolation Process: The plant material in suitable state of comminution, are moistened with the solvent and penetration of the material ensured by leaving it to stand for four hours in a closed container. The mass is then packed into a cylindrical percolator and solvent

added until the material is saturated. The mixture is allowed to macerate for 24 hours. By opening the tap of the percolator, the mixture is collected with the addition of more solvent, until either the drug is exhausted or the required volume of solution has been collected. The marc is pressed, and the expressed liquid mixed with the percolate and filtration or decantation is carried out.

Digestion: The raw material is packed in a suitable solvent with gentle heating for about an hour, after which filtration is carried out (Maarit *et al.*, 2002).

2.7.2 Solvents of extraction

Different solvents are used for extraction of the active constituents of plants, some of which include water, methanol, ethanol, chloroform, ether and acetone. The following served as criteria for selecting the solvent to be used for extraction; It should be cheap and affordable, non-toxic, stable (physically and chemically inert), could remove the desired active constituent, readily available, not having unpleasant odour (Brian and Turner, 1975). Water is almost universally the solvent used to extract activity. Dried plants can be ingested as teas (plants steeped in hot water) or rarely, tinctures (plants in alcoholic solutions) or inhaled via steam from boiled suspensions of the parts. Dried plant parts can be added to oils or petroleum jelly and applied externally. Poultices can also be made from concentrated teas or tinctures (Cowan, 1999).

The wide variety of compounds which plant material may yield and the composition of the extract will depend largely on the solvent used. The solvents of extraction were ranked in the order methylene dichloride, methanol, ethanol and water (Cowan, 1999).

The crude products of extraction can be used in disc diffusion and broth dilution assays to test for the antifungal and antibacterial properties and in a variety of assays to screen for antiviral activity (Cowan, 1999).

Preparations which can be used medicinally in place of the powdered drug include tinctures, infusions, and liquid extracts and these require extraction processes which will separate the active compounds from the plant tissues. Development of the extraction procedures will result in the ultimate separation of the constituents as crystalline isolates.

The degree of separation (from the cellular material of both) between pharmacologically active and inactive compounds depends upon their relative solubility in the solvent used. Certain liquid extract preparations if crude drugs demonstrate either a higher activity than expected from the known concentration of recognized constituents, or produce a modified, and sometimes more acceptable pharmacological response. These variations can be attributed to the synergistic, potentiating or antagonistic effects of compounds present which are not normally recognized as potent constituents (Brian and Turner, 1975; Cannell, 1998).

Other factors which must not be overlooked include the availability of the constituent in the tissue, in terms of accessibility to the extractants and the effect of the actual extraction system on the constituents required and on related compounds. Such economic matters as the cost of the solvent and the time necessary to carry out the extraction process must also be considered (Cannell, 1998).

2.7.3 Products of plant extraction

Alkaloids: The name derived from the word alkaline, originally used to describe any nitrogen-containing base. Alkaloids are usually derivatives of amino acids and many have a bitter taste. They are found as secondary metabolites in plants. Many alkaloids can be purified from crude extracts by acid-base extraction. While many alkaloids are poisonous, some are used medicinally as analgesics (pain relievers) or anaesthetics, particularly morphine and codeine, and for other uses. The nitrogenous compounds which occur in plants may be optically active and are basic in nature forming salts with plant acids. They may also combine with sugars to form glycoalkaloids and with the alcohol to form esters. The solubilities show considerable variation and depend upon whether they are present as the salt or as the free base. As a general rule, it can be said that as salts they are soluble in water and insoluble in organic solvents and as the free base, they are insoluble in water and soluble in organic solvents (Brian and Turner, 1975). Chemically, the alkaloids are complex nitrogenous bases.

Flavonoid: such as flavanones and anthocyanins, occur both in the free-state and as glycosides. They are widely distributed in nature and are frequently found in their highest concentration in association with essential oils. Extraction procedures may therefore be designed in two parts, the first to remove the essential oil and the second to separate the flavonoid.

Saponins: are the glycosides of 27 carbon atom steroids, or 30 carbon atom titerpenoids in plants. They are found in various parts of the plant such as leaves, stems, roots, bulbs, blossom and fruits. Saponins dissolve in water to form a stable soapy froth and this is

thought to be due to their amphiphilic nature. They are also characterized by their bitter taste and their ability to haemolyze red blood cells. The plant glycosides has basic nucleus either of steroidal or triterpenoidal type. The most important use of these compounds is that they can be converted to medicinally important steroid-like Vitamin D, Cardiac glycosides, Corticoids, Sex hormones, Oral contraceptives and Duiretics etc. These glycosides have common basic properties that can be used as basis for their detection in drugs. All known triterpenoid and steroidal saponins are haemolytic in nature and lower the surface tension in water. Triterpenoids occur as glycosides as well as free triterpene. Steroidal saponins are never found as free sapogenins in plant materials. The ability of saponins to produce frothing in aqueous solution and haemolysed red blood cells was used as screening test for these compounds (Cannell, 1998; Sofowora, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of Plant Materials

A combination of selected plant powder, a herbal mixture called Zang cure was obtained from a traditional medical practitioner in Jos, Plateau State, Nigeria.

The plant combination included the leaves, stem barks, roots, bulbs and rhizomes of the following plant species; *Annogeissus leiocarpus* (Hausa: marke), *Ximenia americana* (Hausa: tsada), *Aloe vera* (Hausa: rodon daji), *Allium sativum* (Hausa: tafarnuwa), *Allium ascalonicum* (Hausa: albasa maigo), *Cymbopogon citratus* (Hausa: tsagre), *Citrus aurantifolia* (Hausa: lemun zaƙi), *Garcinia kola* (Hausa: namijin goro), *Cola acuminata* (Hausa: goro), *Strephonema pseudocola* (Hausa: kanyia), *Costus afer* (Hausa: tumfafiyar kada), *Zingiber officinale* (Hausa: citta) and *Cyperus rotundus* (Hausa: ayah).

Plant materials were also collected from the field and some purchased from markets in Zaria, Kaduna, Kafanchan and Jos. They were identified and authenticated at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria. These plant materials were collected to replicate the herbal mixture (Zang Cure) with a known proportion of the constituent plants.

3.2 Source of Test Organisms

Clinical isolates of *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* were obtained from the Medical Microbiology Laboratory, Ahmadu Bello University Teaching

Hospital (ABUTH), Zaria. The isolates were maintained on nutrient agar and stored at 4 ^{o}C until required.

3.3 Preparation of Extracts

Each of the plant parts collected was air-dried, pulverised in a wooden mortar and weighed on an electronic weighing balance. They were then mixed in proportions of 50 g each of *Annogeissus leiocarpus* bark, *A. leiocarpus* leaves, *Ximenia americana* leaves, *Allium sativum*, *Cymbopogon citratus*, *Garcinia kola*, *Zingiber officinale*, 40 g of *Cyperus rotundus* rhizomes and 30 g of *Aloe vera*, to obtain a 420 g of mixture of plant materials.

Two hundred (200 g) of the Zang powder was sequentially extracted in petroleum-ether, chloroform, ethyl acetate and methanol by Soxhlet extraction method while one hundred (100 g) of the powder was extracted in distilled water to obtain the aqueous extract.

Two hundred (200 g) of the mixture of plant materials was extracted with distilled water to obtain a second aqueous extract.

All extracts were filtered through Whatman (No.1) filter paper and concentrated over a water bath using a rotary-vacuum evaporator to recover the solvents. The extracts were then air-dried at room temperature. The crude solid extracts were stored at 4 ^{o}C until required for antibacterial activity studies.

3.4 Phytochemical Screening of Extracts

All the extracts were subjected to standard phytochemical qualitative screening for secondary metabolites as described by Trease and Evans (1989), Sofowora (1993) and Harborne (1998).

3.4.1 Test for carbohydrates

3.4.1.1 *Molisch's test*

Three drops of Molisch Reagent was added to a 0.1 g of each extract in a test tube and 1ml of concentrated Sulphuric acid was allowed to run down the side of the test tube to form a layer. Purple to violet colour at the interface indicates the presence of carbohydrates.

3.4.1.2 *Fehling's test*

To 2 ml of each extract, 5 ml of Fehling's solution A and B in the ratio of 1:1 was added and the mixture boiled for three minutes. A brick red precipitate indicates the presence of free reducing sugar.

3.4.2 Test for cardiac glycosides (Kella-Killiani test)

Half (0.5 g) of each extract was dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45 degree, 1 ml of concentrated Sulphuric acid was added down the side. A purple-coloured ring at the interface indicates cardiac glycosides.

3.4.3 Test for free anthraquinones derivatives

3.4.3.1 *Test for free anthraquinones (Borntrager's test)*

Ten (10 ml) of Benzene was mixed with 0.2 g of each extract and filtered. Five (5 ml) of 10% ammonia solution was added to the filtrate and stirred. The production of a pink-red or violet colour indicates the presence of free anthraquinones.

3.4.3.2 *Test for combined anthracene (Modified Borntrager's test)*

Five (5 ml) of 10% Hydrochloric acid was boiled with 0.2 g of each extract for 3 min. This hydrolyses the glycoside to yield aglycone which is soluble in hot water only. The solution was filtered hot; the filtrate was cooled and extracted with 5 ml of Benzene. The benzene layer was filtered off and shaken gently with half of its volume with 10% ammonia solution. A rose-pink or cherry red colour indicates combined anthracene.

3.4.4 Test for saponins (Frothing test)

Half (0.5 g) of each extract was dissolved in 10 ml of distilled water and then shaken vigorously for 30 seconds and allowed to stand for 30 minutes. A honey comb-like froth formed for more than 30 minutes indicates saponin.

3.4.5 Test for steroids

Two (2 ml) of acetic anhydride was added to 2 ml of each extract in a test tube. 1ml of concentrated Sulphuric acid was added down the side of the tube. A colour change was observed immediately and later. Blue-green colour indicates steroids.

3.4.6 Test for terpenoids

Half (0.5 g) of each extract was dissolved in 2 ml of Chloroform and 3ml of concentrated H_2SO_4 were added to form a lower layer. A reddish brown colour at the interface indicates the presence of terpenoids.

3.4.7 Test for flavonoids

3.4.7.1 *Shinoda test*

Half (0.5 g) of each extract was dissolved in 2 ml of 50% methanol in the heat. Metallic magnesium and four drops of concentrated HCl were added. A red or orange colour indicates the presence of flavonoids aglycones.

3.4.7.2 *Sodium hydroxide test*

Five drops of aqueous NaOH was added to 5 ml of each extract, a yellow colouration shows the presence of flavonoid.

3.4.8 Test for tannins

3.4.8.1 *Lead sub-acetate test*

Three drops of lead sub-acetate solution were added to 2 ml of each extract. A coloured precipitate indicates tannins.

3.4.8.2 *Ferric chloride test*

Half (0.5 ml) of each extract was dissolved in 10 ml of distilled water, and then filtered. Two drops of Ferric chloride solution were added to the filtrate. Formation of a blue-black precipitate indicates hydrolyable tannins and green precipitate indicates the presence of condensed tannin.

3.4.9 Test for alkaloids

3.4.9.1 *Mayers Test*

Two drops of the Mayers' Reagent were added to 2 ml of the extract in a test tube, cream precipitate indicates alkaloids.

3.4.9.2 *Dragendorff's Test*

Two drops of the Dragendorff's Reagent were added to 2 ml of each extract. A rose red precipitate indicates the presence of alkaloids.

3.4.9.3 *Wagner's Test*

Two drops of the Wagner's Reagent were added to 2 ml of each extract. A brown/reddish brown precipitate indicates the presence of alkaloids.

3.4.10 Test for steroidal glycosides

Ten (10 ml) of each extract was evaporated to dryness in a test tube on a boiling water bath. The residue was dissolved in 0.5 ml Chloroform. The solution was transferred to a dried test tube and 2 ml of concentrated Sulphuric acid was added with a pipette from the bottom (Liebermann-Burchard's reaction). At the separating level, the two liquid were separated by a reddish brown or violet brown ring formed the superior layer being bluish green or violet for the presence of steroids and triterpenes.

3.5 Preparation of Culture Media

The media used were Mueller Hinton agar and Nutrient broth. The media were prepared according to manufacturer's instruction. Thirty five (35 g) of medium was mixed with one litre of distilled water in a screw cap container and autoclaved at 121 °C for 15 minutes. The medium was later dispensed into 90 mm sterile agar plates and left to set. The agar plates were incubated for 24 hours at 37 °C to confirm sterility.

3.6 Antibacterial Activity of the Plant Extracts

3.6.1 Susceptibility test

The susceptibility test of the plant extracts was carried out using the agar well diffusion method (Irobi *et al.*, 1994). Clinical isolates of *E. coli*, *S. typhi* and *S. aureus,* were inoculated separately on the surface of Mueller Hinton agar plates by surface spreading using a sterile cotton swab and each bacterium evenly spread over the entire surface of agar

plate to obtain a uniform inoculum. Five wells of 6 mm diameter and 5 mm depth were made on the solid agar on each plate using a sterile glass borer and numbered for the different concentrations of extracts. 1g of each extract was dissolved in 10 ml of distilled water to obtain a 100 mg/ml concentration. Serial dilution was employed to obtain the other concentrations, 50, 25, 12.5 and 6.25 mg/ml. The different concentrations of each extract were then dispensed into their respective wells on the inoculated plates. 10 μg ciprofloxacin was used as control since it is a broad spectrum antibiotic. The set up was incubated for 24 hours at 37 ^{o}C. All the tests were run in triplicates. After incubation, the zones of inhibition were measured in millimeters (mm) using a transparent ruler. Oxoid (1985) standard susceptibility range was used to classify zones of inhibition as either sensitive (> 10 mm) or resistant (≤ 10 mm).

3.6.2 Minimum Inhibition Concentration (MIC) evaluation

MIC of each extract was determined according to the method described by Cheruiyot *et al.* (2009). The MICs were evaluated on plant extracts that showed antibacterial activity in the agar well diffusion assay. It was performed for the five concentrations of each extract (100, 50, 25, 12.5 and 6.25 mg/ml) employing doubling dilutions of plant extract in nutrient broth up to the fifth dilution. One millilitre (1 ml) of the nutrient broth was put in a test tube and equal amount (1 ml) of the extract was added to the first test tube and serial dilution was done with the last 1 ml being discarded. Each organism was separately suspended in 5 ml of nutrient broth and incubated overnight, after which 0.1 ml was added to all the test tubes and the preparation incubated at 37 ^{o}C for 18 hours. MICs were recorded as the lowest concentration of extract that did not show any visible growth of inoculated microorganism after overnight incubation (Andrew, 2001).

3.6.3 Minimum Bactericidal Concentration (MBC) evaluation

The MBCs of the extracts were determined using the method described by Adegboye *et al.* (2008). Samples were taken from tubes with no visible growth in the MIC assay and sub-cultured onto freshly prepared nutrient agar medium and later incubated at 37 ^{o}C for 48 hours. The MBCs were taken as the lowest concentration of extract that did not allow any bacterial growth on the surface of the agar plates.

3.7 Statistical Analysis

All values obtained were expressed as means ± standard error. Data obtained were subjected to analysis of variance (ANOVA) to determine the significant differences between the concentrations of extracts and the zones of inhibition of the bacteria. Least Significant Difference (LSD) test was used to compare the treatment means where significant. P value of < 0.05 was considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Constituents of the Extracts

The results of the phytochemical screening of the extracts revealed that carbohydrates, flavonoids, cardiac glycosides, steroids and triterpenes were confirmed present in all the extracts from Petroleum ether, Chloroform, Ethyl Acetate, Methanolic and Aqueous extracts. Saponins and tannins were confirmed present in all other extracts but absent in the Petroleum ether extract. The tests however revealed the absence of anthraquinones and alkaloids in all extracts (Table 4.1).

4.2 Intra-Extract Comparisons

4.2.1 Antibacterial activity of the aqueous extract

The antibacterial activity of the aqueous extract is presented on Table 4.2. All concentrations of the aqueous extract did not show activity on *E. coli* and *S. typhi* i.e. the organisms were not susceptible at 6.25, 12.5, 25, 50 and 100 mg/ml concentrations of the aqueous extract. There was no significant difference in the mean zones of inhibition of *E. coli* and *S. typhi* for each concentration of the aqueous extract. *E. coli* and *S. typhi* however showed significant differences with *S. aureus* for each of the concentrations.

There was no significant difference in the activity of the varying concentrations on *E. coli* and *S. typhi* but there were significant differences in the mean zones of inhibition (activity) of the varying concentrations of the aqueous extract on *S. aureus*. The concentrations also showed statistically significant differences ($P<0.05$) in their activity on *S. aureus* compared

Table 4.1: Phytochemical Constituents of the Extracts

	Extracts				
Constituents	**Aqueous**	**Methanolic**	**Ethyl Acetate**	**Chloroform**	**Petroleum Ether**
Carbohydrates	+	+	+	+	+
Anthraquinones	-	-	-	-	-
Flavonoids	+	+	+	+	+
Saponins	+	+	+	+	-
Tannins	+	+	+	+	-
Alkaloids	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+
Steroid & Triterpenes	+	+	+	+	+

Key: + Presence - Absence

Table 4.2: Zones of Inhibition (Mean ± Standard Error) of Bacteria in Aqueous Extract

	Zone of Inhibition (mm)		
Conc. (mg/ml)	***E. coli***	***S. typhi***	***S. aureus***
6.25	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	10.67 ± 0.67^{fb}
12.50	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	12.33 ± 0.33^{eb}
25.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	16.67 ± 0.67^{db}
50.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	18.33 ± 0.33^{cb}
100.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	20.00 ± 0.00^{b}
Ciproflox (10µg)	38.00 ± 0.00^{a}	10.00 ± 0.00^{a}	45.00 ± 0.00^{a}

Mean values with different superscripts in the same column are significantly different. *P<0.05*

with the control (ciprofloxacin). The highest mean zone of inhibition (20 mm) of *S. aureus* by the aqueous extract was recorded at 100 mg/ml and the lowest (10.67 mm) was recorded at 6.25 mg/ml (Table 4.2).

4.2.2 Antibacterial activity of the methanolic extract

All the concentrations of the methanolic extract did not show any activity on *E. coli* and *S. typhi* but they showed activity on *S. aureus*. There was no significant difference in the mean zones of inhibition of *E. coli* and *S. typhi* for each concentration of the methanolic extract. *E. coli* and *S. typhi* however showed significant differences with *S. aureus* for each of the concentrations (Table 4.3).

The highest mean zone of inhibition (19 mm) of *S. aureus* by the methanolic extract was recorded at 100 mg/ml while the lowest (11.67 mm) was at 6.25 mg/ml. There was no statistically significant difference ($P \geq 0.05$) in the mean zones of inhibition of *S. aureus* by the methanolic extract at all concentrations, though they differ significantly with the control (ciprofloxacin).

4.2.3 Antibacterial activity of the chloroform extract

There was no activity of the chloroform extract at the concentrations 6.25, 12.5 and 25 mg/ml on *E. coli* and *S. typhi*. There was also no activity on *S. typhi* at 50 mg/ml. *S. aureus* was not susceptible to the chloroform extract at 6.25 and 12.5 mg/ml. There were no significant differences in the activity of the chloroform extract at concentrations 6.25, 12.5 and 100 mg/ml on all three test organisms (Table 4.4).

Table 4.3: Zones of Inhibition (Mean ± Standard Error) of Bacteria in Methanolic Extract

Conc. (mg/ml)	Zone of Inhibition (mm)		
	E. coli	***S. typhi***	***S. aureus***
6.25	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	11.67 ± 0.67^{dfe}
12.50	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	13.33 ± 0.33^{eb}
25.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	16.00 ± 1.00^{db}
50.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	18.33 ± 0.33^{bc}
100.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	19.00 ± 0.00^{b}
Ciproflox (10µg)	38.00 ± 0.00^{a}	10.00 ± 0.00^{a}	45.00 ± 0.00^{a}

Mean values with different superscripts in the same column are significantly different. *$P<0.05$*

Table 4.4: Zones of Inhibition (Mean ± Standard Error) of Bacteria in Chloroform Extract

	Zone of Inhibition (mm)		
Conc. (mg/ml)	***E. coli***	***S. typhi***	***S. aureus***
6.25	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{e}
12.50	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{e}
25.00	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	11.67 ± 0.33^{db}
50.00	12.33 ± 0.33^{cb}	0.00 ± 0.00^{b}	14.00 ± 0.00^{cb}
100.00	16.67 ± 0.67^{b}	17.33 ± 0.67^{a}	16.00 ± 0.00^{b}
Ciproflox (10μg)	38.00 ± 0.00^{a}	10.00 ± 0.00^{a}	45.00 ± 0.00^{a}

Mean values with different superscripts in the same column are significantly different. *$P<0.05$*

The mean zones of inhibition of *E. coli* by the chloroform extract at all the concentrations were significantly different from that of the control, the highest (16.67 mm) being at 100 mg/ml and the lowest (12.33 mm) at 50 mg/ml. There was no statistically significant difference in the mean zones of inhibition of *E. coli* by the chloroform extract at 50 and 100 mg/ml.

The mean zone of inhibition (17.33 mm) of *S. typhi* at 100 mg/ml of the chloroform extract was significantly different with that of the control.

There was no statistically significant difference in the mean zones of inhibition of *S. aureus* at 25, 50 and 100 mg/ml of the chloroform extract, but they differ significantly with that of the control. The highest mean zone of inhibition (16 mm) was recorded at 100 mg/ml and the lowest (11.67 mm) at 25 mg/ml (Table 4.4).

4.2.4 Antibacterial activity of the ethyl acetate extract

Salmonella typhi was not susceptible to the Ethyl acetate extract at 6.25, 12.5 and 25 mg/ml. It was however susceptible at 50 and 100 mg/ml with lower (17.33 mm) and higher (17.67 mm) mean zones of inhibition respectively, but not significantly different with that of the control (10 mm). *E. coli* and *S. aureus* were susceptible to all the concentrations of the Ethyl acetate extract. There was no significant difference in the mean zones of inhibition of *E. coli* with the varying concentrations, though they differ significantly with the control. The highest mean zone of inhibition (24.33 mm) of *E. coli* was recorded at 100mg/ml while the lowest (12.67 mm) was at 6.25 mg/ml (Table 4.5).

There was no statistically significant difference in the mean zones of inhibition of *S. aureus* with the varying concentrations of the ethyl acetate extract, though they differ significantly

Table 4.5: Zones of Inhibition (Mean ± Standard Error) of Bacteria in Ethyl Acetate Extract

	Zone of Inhibition (mm)		
Conc. (mg/ml)	***E. coli***	***S. typhi***	***S. aureus***
6.25	12.67 ± 0.67^{fb}	0.00 ± 0.00^{b}	11.00 ± 0.00^{fb}
12.50	16.67 ± 0.67^{eb}	0.00 ± 0.00^{b}	13.00 ± 0.00^{eb}
25.00	18.33 ± 0.33^{db}	0.00 ± 0.00^{b}	15.33 ± 0.67^{db}
50.00	21.33 ± 0.67^{cb}	17.33 ± 0.67^{a}	17.67 ± 0.33^{cb}
100.00	24.33 ± 0.33^{b}	17.67 ± 0.33^{a}	19.33 ± 0.67^{b}
Ciproflox (10µg)	38.00 ± 0.00^{a}	10.00 ± 0.00^{a}	45.00 ± 0.00^{a}

Mean values with different superscripts in the same column are significantly different. $P<0.05$

with the control. The highest mean zone of inhibition (19.33 mm) of *S. aureus* was recorded at 100 mg/ml and the lowest (11 mm) at 6.25 mg/ml.

There was no significant difference in the mean zones of inhibition of *E. coli* and *S. aureus* for each concentration of the ethyl acetate extract. *E. coli* and *S. aureus* however showed significant differences with *S. typhi* for each of the lower concentrations, 6.25, 12.50 and 25 mg/ml. There was no significant difference in the mean zones of inhibition of all three test organisms at each of 50 and 100 mg/ml concentrations of the ethyl acetate extract (Table 4.5).

4.2.5 Antibacterial activity of the petroleum ether extract

Salmonella typhi and *Staphylococcus aureus* were not susceptible to the petroleum ether extract at 6.25 mg/ml. *S. aureus* was not susceptible at 12.5 mg/ml. *E. coli* was however susceptible at all the concentrations. There was no statistically significant difference in the susceptibility of the three test organisms to the varying concentrations of the petroleum ether extract. The increase in concentration of the petroleum ether extract also had no significant effect on each of the test organism (Table 4.6).

The highest mean zone of inhibition (23.33 mm) of *E. coli* was recorded at 100 mg/ml and the lowest (16 mm) at 6.25 mg/ml. The highest mean zone of inhibition (23 mm) of *S. typhi* was at 100 mg/ml and the lowest (20.33 mm) at 12.5 mg/ml. The least mean zone of inhibition (13.67 mm) of *S. aureus* was at 25 mg/ml and the highest (15 mm) was recorded at 100 mg/ml of the petroleum ether extract (Table 4.6).

Table 4.6: Zones of Inhibition (Mean ± Standard Error) of Bacteria in Petroleum Ether Extract

	Zone of Inhibition (mm)		
Conc. (mg/ml)	***E. coli***	***S. typhi***	***S. aureus***
6.25	16.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
12.50	18.33 ± 0.33	20.33 ± 0.67	0.00 ± 0.00
25.00	20.33 ± 0.33	20.00 ± 0.00	13.67 ± 0.33
50.00	22.00 ± 0.00	21.67 ± 0.33	14.67 ± 0.33
100.00	23.33 ± 0.33	23.00 ± 0.00	15.00 ± 0.00
Ciproflox (10µg)	38.00 ± 0.00	10.00 ± 0.00	45.00 ± 0.00

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4.2.6 Antibacterial activity of the second aqueous extract

Escherichia coli and *Salmonella typhi* were not susceptible to all the concentrations of the second aqueous extract. *S. aureus* was however susceptible at the higher concentrations (50 and 100 mg/ml), with the highest mean zone of inhibition (17 mm) at 100 mg/ml (Table 4.7).

There were no significant differences in the activity of the second aqueous extract at concentrations 6.25, 12.5 and 25 mg/ml on all three test organisms. There were also no significant differences in the activities of the second aqueous extract at concentrations 50 and 100 mg/ml on *E. coli* and *S. typhi*, but they differ significantly with *S. aureus* (Table 4.7).

4.3 Inter-Extract Comparisons

4.3.1 Activity at 6.25 mg/ml

Escherichia coli was not susceptible to 6.25 mg/ml concentration of the aqueous, methanolic, chloroform and second aqueous extracts. It was however susceptible to 6.25 mg/ml concentration of the ethyl acetate and petroleum ether extracts, with no statistically significant differences between the extracts. The highest mean zone of inhibition (16 mm) was recorded for petroleum ether (Table 4.8).

Salmonella typhi was not susceptible to all the extracts at 6.25 mg/ml concentration.

Staphylococcus aureus was not susceptible to 6.25 mg/ml concentration of chloroform, petroleum ether and second aqueous extracts. It was susceptible to 6.25 mg/ml concentration of the aqueous, methanolic and ethyl acetate extract with the highest mean

Table 4.7: Zones of Inhibition (Mean ± Standard Error) of Bacteria in Second Aqueous Extract

Conc. (mg/ml)	Zone of Inhibition (mm)		
	E. coli	*S. typhi*	*S. aureus*
6.25	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
12.50	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
25.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
50.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	10.33 ± 0.33^{a}
100.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	17.00 ± 0.00^{a}
Ciproflox (10µg)	38.00 ± 0.00^{a}	10.00 ± 0.00^{b}	45.00 ± 0.00^{a}

Mean values with different superscripts on the same row are significantly different. $P<0.05$

Table 4.8: Activity of 6.25 mg/ml Concentration of Extracts on Bacteria

	Zone of Inhibition (mm)		
Extract	***E. coli***	***S. typhi***	***S. aureus***
Aqueous	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	10.67 ± 0.67^{a}
Methanolic	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	11.67 ± 0.67^{a}
Chloroform	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Ethyl Acetate	12.67 ± 0.67^{a}	0.00 ± 0.00^{b}	11.00 ± 0.00^{a}
Petroleum ether	16.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
2nd Aqueous	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Ciproflox	38.00 ± 0.00^{a}	10.00 ± 0.00^{b}	45.00 ± 0.00^{a}

Mean values with different superscripts on the same row are significantly different. *P<0.05*

zone of inhibition (11.67 mm) recorded for the methanolic extract. There were however no statistically significant differences in the mean zones of inhibition of *S. aureus* at 6.25 mg/ml of all the extracts (Table 4.8).

The activities of 6.25 mg/ml of the aqueous and methanolic extracts did not differ significantly on *E. coli* and *S. typhi*, though there is a significant difference on *S. aureus*. The activities of 6.25 mg/ml chloroform and second aqueous extracts were not significantly different for all three test organisms. The activity of 6.25 mg/ml of the ethyl acetate extract did not differ significantly on *E. coli* and *S. aureus*, though there was a significant difference on *S. typhi*. The activity of 6.25 mg/ml of the petroleum ether extract did not differ significantly on *S. typhi* and *S. aureus*, though there was a significant difference on *E. coli* (Table 4.8).

4.3.2 Activity at 12.5 mg/ml

At 12.5 mg/ml, *E. coli* was only susceptible to the ethyl acetate and petroleum ether extracts, with no statistically significant difference in mean zones of inhibition. The highest mean zone of inhibition of *E. coli* at 12.5 mg/ml concentration was recorded for the petroleum ether extract (18.33 mm). *S. typhi* was only susceptible to the petroleum ether extract. *S. aureus* was susceptible to the aqueous, methanolic and ethyl acetate extracts, with the highest mean zone of inhibition (13.33 mm) recorded for the methanolic extract and the least (12.33 mm) for the aqueous extract. There was no significant difference in the mean zone of inhibition of *S. aureus* for 12.5 mg/ml concentration of all the extracts (Table 4.9).

Table 4.9: Activity of 12.5 mg/ml Concentration of Extracts on Bacteria

	Zone of Inhibition (mm)		
Extract	***E. coli***	***S. typhi***	***S. aureus***
Aqueous	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	12.33 ± 0.33^{a}
Methanolic	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	13.33 ± 0.67^{a}
Chloroform	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Ethyl Acetate	16.67 ± 0.67^{a}	0.00 ± 0.00^{b}	13.00 ± 0.00^{a}
Petroleum ether	18.33 ± 0.33^{a}	20.33 ± 0.67^{a}	0.00 ± 0.00^{b}
2nd Aqueous	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Ciproflox	38.00 ± 0.00^{a}	10.00 ± 0.00^{b}	45.00 ± 0.00^{a}

Mean values with different superscripts on the same row are significantly different. $P<0.05$

The activities of 12.5 mg/ml of the aqueous, methanolic and petroleum ether extracts did not differ significantly on *E. coli* and *S. typhi*, though there was a significant difference on *S. aureus*. The activities of 12.5 mg/ml chloroform and second aqueous extracts were not significantly different for all three test organisms. The activity of 12.5 mg/ml of the ethyl acetate extract did not differ significantly on *E. coli* and *S. aureus*, though there was a significant difference on *S. typhi*. The activity of 12.5 mg/ml of the petroleum ether extract did not differ significantly on *E. coli* and *S. typhi*, though there was a significant difference on *S. aureus* (Table 4.9).

4.3.3 Activity at 25 mg/ml

Escherichia coli was not susceptible to 25 mg/ml of the aqueous, methanolic, chloroform and second aqueous extracts. It was however susceptible to the ethyl acetate and petroleum ether extracts, with no statistically significant differences. The highest mean zone of inhibition (20.33 mm) of *E. coli* at 25 mg/ml concentration of extract was recorded for the petroleum ether extract.

At 25 mg/ml, *S. typhi* was only susceptible to the petroleum ether extract; with a mean zone of inhibition of 20 mm. *S. aureus* was susceptible to all other extracts but the second aqueous extract. The highest mean zone of inhibition (16.67 mm) of *S. aureus* was recorded for 25 mg/ml concentration of the aqueous extract. There was however no statistically significant differences in the mean zone of inhibition of *S. aureus* at 25 mg/ml concentrations of the aqueous, methanolic, chloroform, ethyl acetate and petroleum ether extracts (Table 4.10).

Table 4.10: Activity of 25 mg/ml Concentration of Extracts on Bacteria

	Zone of Inhibition (mm)		
Extract	***E. coli***	***S. typhi***	***S. aureus***
Aqueous	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	16.67 ± 0.67^{b}
Methanolic	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	16.00 ± 1.00^{cb}
Chloroform	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	11.67 ± 0.33^{fb}
Ethyl Acetate	18.33 ± 0.33^{cb}	0.00 ± 0.00^{b}	15.33 ± 0.67^{db}
Petroleum ether	20.33 ± 0.33^{b}	20.00 ± 0.00^{a}	13.67 ± 0.33^{eb}
2nd Aqueous	0.00 ± 0.00^{d}	0.00 ± 0.00^{b}	0.00 ± 0.00^{g}
Ciproflox	38.00 ± 0.00^{a}	10.00 ± 0.00^{a}	45.00 ± 0.00^{a}

Mean values with different superscripts in the same column are significantly different. $P<0.05$

The activities of 25 mg/ml petroleum ether and second aqueous extracts were not significantly different for all three test organisms. The activities of 25 mg/ml of the aqueous, methanolic and chloroform extracts did not differ significantly on *E. coli* and *S. typhi*, though there were significant differences on *S. aureus*. The activity of 25 mg/ml of the ethyl acetate extract did not differ significantly on *E. coli* and *S. aureus*, though there was a significant difference on *S. typhi* (Table 4.10).

4.3.4 Activity at 50 mg/ml

At 50 mg/ml, *E. coli* was only susceptible to the chloroform, ethyl acetate and petroleum ether extracts, with the highest mean zone of inhibition (22 mm) for the petroleum ether extract and the lowest for the chloroform extract (12.33 mm). There were no significant differences in the mean zones of inhibition of *E. coli* at 50 mg/ml concentration of the chloroform, ethyl acetate and petroleum ether extracts (Table 4.11). They however differ significantly ($P < 0.05$) with 50 mg/ml concentration of the aqueous, methanolic and second aqueous extracts.

S. typhi was only susceptible to the ethyl acetate and petroleum ether extracts at 50 mg/ml concentration, with no statistically significant difference in mean zones of inhibition. The higher mean zone of inhibition (21.67 mm) was recorded for the petroleum ether extract (Table 4.11).

S. aureus was susceptible to all the extracts at 50 mg/ml concentration. There were no statistically significant differences in the mean zones of inhibition of *S. aureus* at 50 mg/ml concentration of all the extracts (Table 4.11).

Table 4.11: Activity of 50 mg/ml Concentration of Extracts on Bacteria

	Zone of Inhibition (mm)		
Extract	***E. coli***	***S. typhi***	***S. aureus***
Aqueous	0.00 ± 0.00^{e}	0.00 ± 0.00^{b}	18.33 ± 0.33^{b}
Methanolic	0.00 ± 0.00^{e}	0.00 ± 0.00^{b}	18.33 ± 0.33^{b}
Chloroform	12.33 ± 0.33^{db}	0.00 ± 0.00^{b}	14.00 ± 0.00^{eb}
Ethyl Acetate	21.33 ± 0.67^{cb}	17.33 ± 0.67^{a}	17.67 ± 0.33^{cb}
Petroleum ether	22.00 ± 0.00^{b}	21.67 ± 0.33^{a}	14.67 ± 0.33^{db}
2nd Aqueous	0.00 ± 0.00^{e}	0.00 ± 0.00^{b}	10.33 ± 0.33^{fb}
Ciproflox	38.00 ± 0.00^{a}	10.00 ± 0.00^{a}	45.00 ± 0.00^{a}

Mean values with different superscripts in the same column are significantly different. $P<0.05$

The activities of 50 mg/ml ethyl acetate and petroleum ether extracts were not significantly different for all three test organisms. The activities of 50 mg/ml of the aqueous, methanolic and second aqueous extracts did not differ significantly on *E. coli* and *S. typhi*, though there were significant differences on *S. aureus*. The activity of 25 mg/ml of the chloroform extract did not differ significantly on *E. coli* and *S. aureus*, though there was a significant difference on *S. typhi* (Table 4.11).

4.3.5 Activity at 100 mg/ml

Escherichia coli was only susceptible to the chloroform, ethyl acetate and petroleum ether extracts at 100 mg/ml concentration. There were no statistically significant differences in the mean zones of inhibition of *E. coli*, the highest (24.33 mm) recorded for the ethyl acetate extract. *S. typhi* was also susceptible only to the chloroform, ethyl acetate and petroleum ether extracts. The mean zones of inhibition were also not significantly different, the highest (23 mm) being for the petroleum ether extract and the lowest (17.33 mm) for the chloroform extract (Table 4.12).

Staphylococcus aureus was susceptible to all the extracts at 100 mg/ml concentration, the highest mean zone of inhibition (20 mm) recorded for the aqueous extract and the lowest (15 mm) for the petroleum ether extract. There were no statistically significant differences in the mean zones of inhibition of *S. aureus* at 100 mg/ml of all the extracts (Table 4.12).

The activities of 100 mg/ml chloroform, ethyl acetate and petroleum ether extracts were not significantly different for all three test organisms. The activities of 100 mg/ml of the aqueous, methanolic and second aqueous extracts did not differ significantly on *E. coli* and *S. typhi*, though there were significant differences on *S. aureus* (Table 4.12).

Table 4.12: Activity of 100mg/ml Concentration of Extracts on Bacteria

	Zone of Inhibition (mm)		
Extract	***E. coli***	***S. typhi***	***S. aureus***
Aqueous	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	20.00 ± 0.00^{a}
Methanolic	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	19.00 ± 0.00^{a}
Chloroform	16.67 ± 0.67^{a}	17.33 ± 0.67^{a}	16.00 ± 0.00^{a}
Ethyl Acetate	24.33 ± 0.33^{a}	19.67 ± 0.33^{a}	19.33 ± 0.67^{a}
Petroleum ether	23.33 ± 0.33^{a}	23.00 ± 0.00^{a}	15.00 ± 0.33^{a}
2nd Aqueous	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	17.00 ± 0.00^{a}
Ciproflox	38.00 ± 0.00^{a}	10.00 ± 0.00^{b}	45.00 ± 0.00^{a}

Mean values with different superscripts on the same row are significantly different. $P<0.05$

4.4 Minimum Inhibition Concentrations (MICs) of the Extracts

The MIC was 25 mg/ml for the chloroform extract against *E. coli* and 12.5 mg/ml for both the ethyl acetate and petroleum ether extracts as shown on Table 4.13.

The MIC for the ethyl acetate extract against *S. typhi* was 25 mg/ml and 12.5 mg/ml for both the chloroform and petroleum ether extract (Table 4.13).

The MICs for the aqueous, chloroform and ethyl acetate extracts against *S. aureus* was recorded as 12.5 mg/ml; 6.25 mg/ml for methanolic, 25 mg/ml for petroleum ether and 50 mg/ml for the second aqueous extracts (Table 4.13).

4.5 Minimum Bactericidal Concentrations (MBCs) of the Extracts

The MBC of the chloroform extract against *E. coli* was 50 mg/ml and 25 mg/ml for both ethyl acetate and petroleum ether extracts (Table 4.14).

The MBC of the ethyl acetate extract against *S. typhi* was recorded as 50 mg/ml and 25 mg/ml for both chloroform and petroleum ether extracts (Table 4.14).

The MBCs of the aqueous, chloroform and ethyl acetate extracts against *S. aureus* were recorded as 25 mg/ml; 12.5 mg/ml for the methanolic extract and 50 mg/ml for the ethyl acetate extract. The MBC for the second aqueous extract was not within the range of the concentrations used for this study (Table 4.14).

Table 4.13: Minimum Inhibitory Concentrations (MICs) of Extracts

	MICs (mg/ml)		
Extract	***E. coli***	***S. typhi***	***S. aureus***
Aqueous	-	-	12.5
Methanolic	-	-	6.25
Chloroform	25	12.5	12.5
Ethyl Acetate	12.5	25	12.5
Petroleum ether	12.5	12.5	25
Second Aqueous	-	-	50

- No activity

Table 4.14: Minimum Bactericidal Concentrations (MBCs) of Extracts

	MBCs (mg/ml)		
Extract	***E. coli***	***S. typhi***	***S. aureus***
Aqueous	-	-	25
Methanolic	-	-	12.5
Chloroform	50	25	25
Ethyl Acetate	25	50	25
Petroleum ether	25	25	50
Second Aqueous	-	-	>100

- No activity

The plates I-XVIII show the zones of inhibition of the extracts and control (ciprofloxacin) on the three test organisms. The clear area around the wells or discs indicates the region where the bacterial growth has been inhibited by the action of the extract. The absence of the clear zones shows that the extract is not active on the bacterium (Plates II, III, VII and IX). The wells are numbered 1, 2, 3, 4 and 5 to indicate the 100, 50, 25, 12.5 and 6.25 mg/ml concentrations respectively.

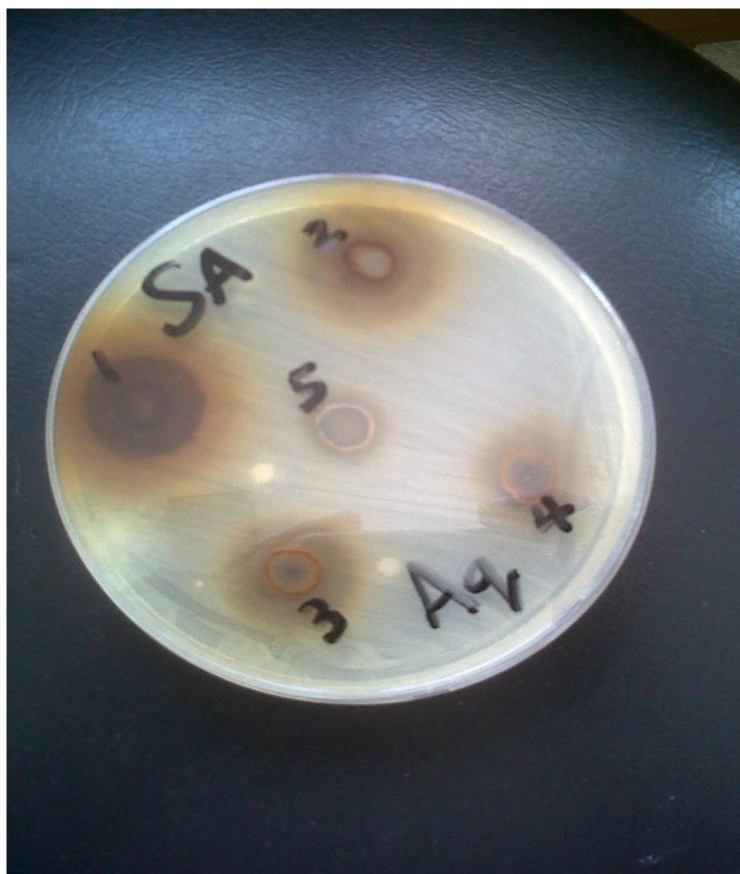

Plate I: Zones of inhibition of aqueous extract against *Staphylococcus aureus*

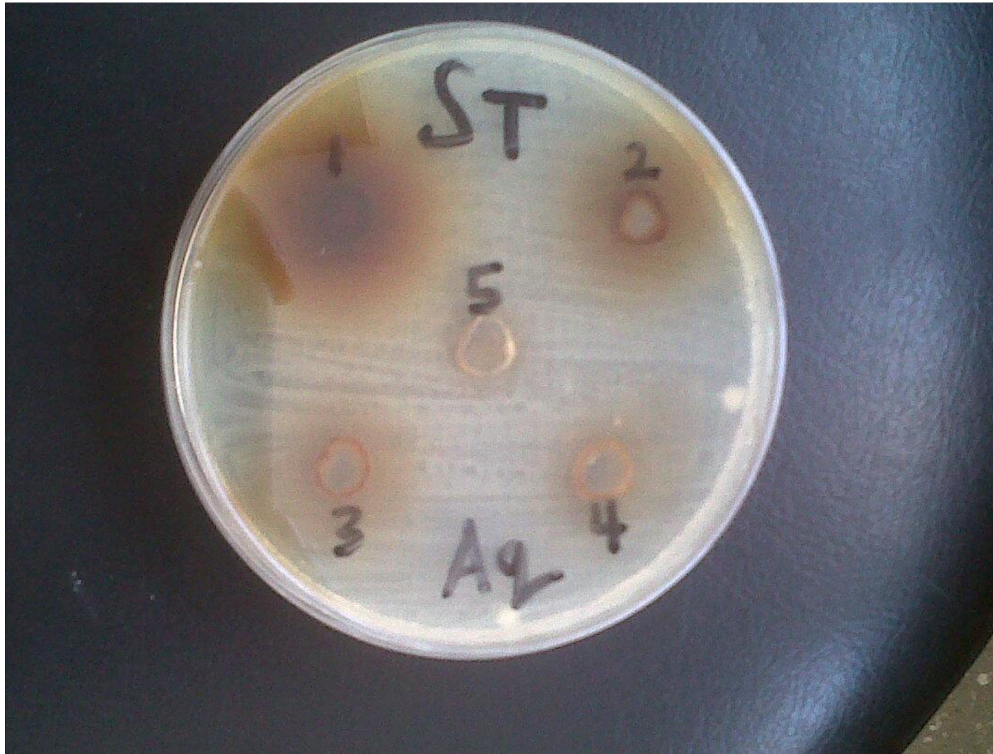

Plate II: Zones of inhibition of aqueous extract against *Salmonella typhi*

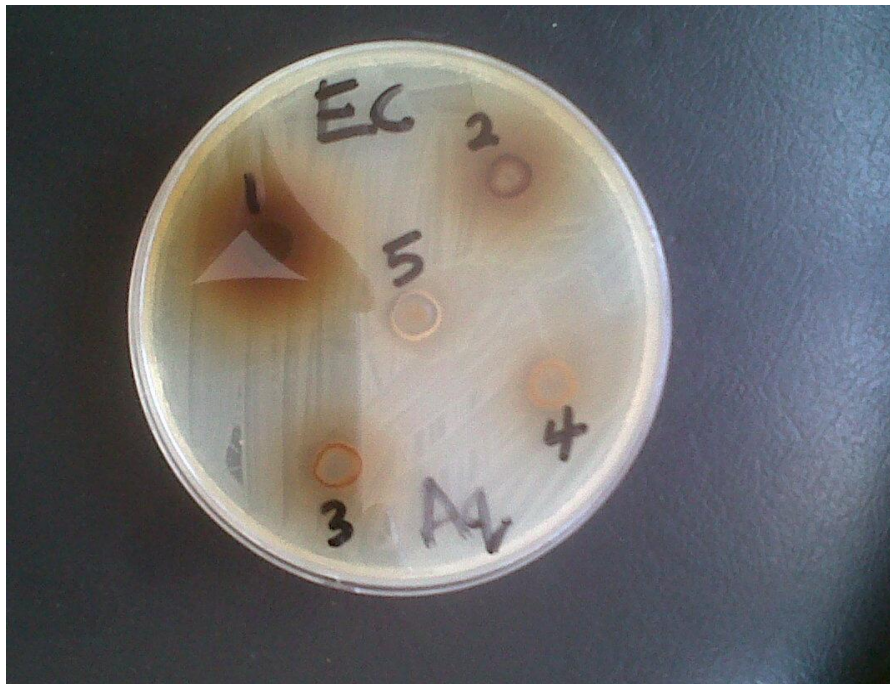

Plate III: Zones of inhibition of aqueous extract against *Escherichia coli*

Plate IV: Zones of inhibition of ethyl acetate extract against *Escherichia coli*

Plate V: Zones of inhibition of ethyl acetate extract against *Staphylococcus aureus*

Plate VI: Zones of inhibition of ethyl acetate extract against *Salmonella typhi*

Plate VII: Zones of inhibition of methanolic extract against *Escherichia coli*

Plate VIII: Zones of inhibition of methanolic extract against *Staphylococcus aureus*

Plate IX: Zones of inhibition of methanolic extract against *Salmonella typhi*

Plate X: Zones of inhibition of chloroform extract against *Salmonella typhi*

Plate XI: Zones of inhibition of chloroform extract against *Staphylococcus aureus*

Plate XII: Zones of inhibition of chloroform extract against *Escherichia coli*

Plate XIII: Zones of inhibition of petroleum ether extract against *Staphylococcus aureus*

Plate XIV: Zones of inhibition of petroleum ether extract against *Salmonella typhi*

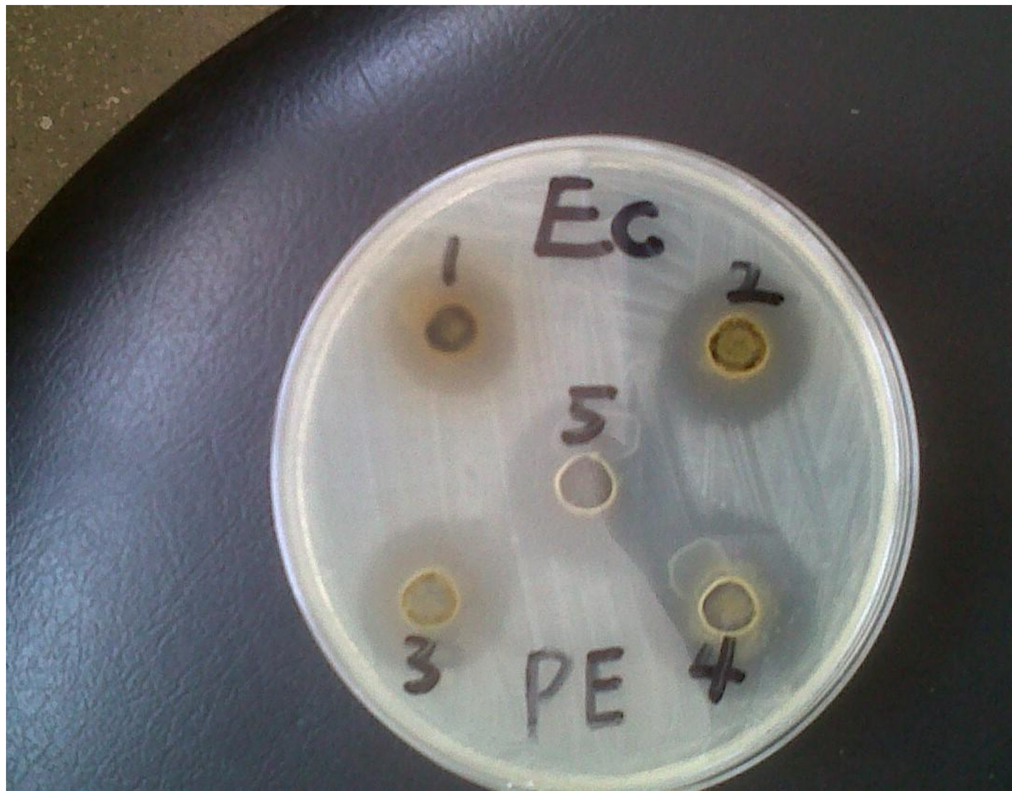

Plate XV: Zones of inhibition of petroleum ether extract against *Escherichia coli*

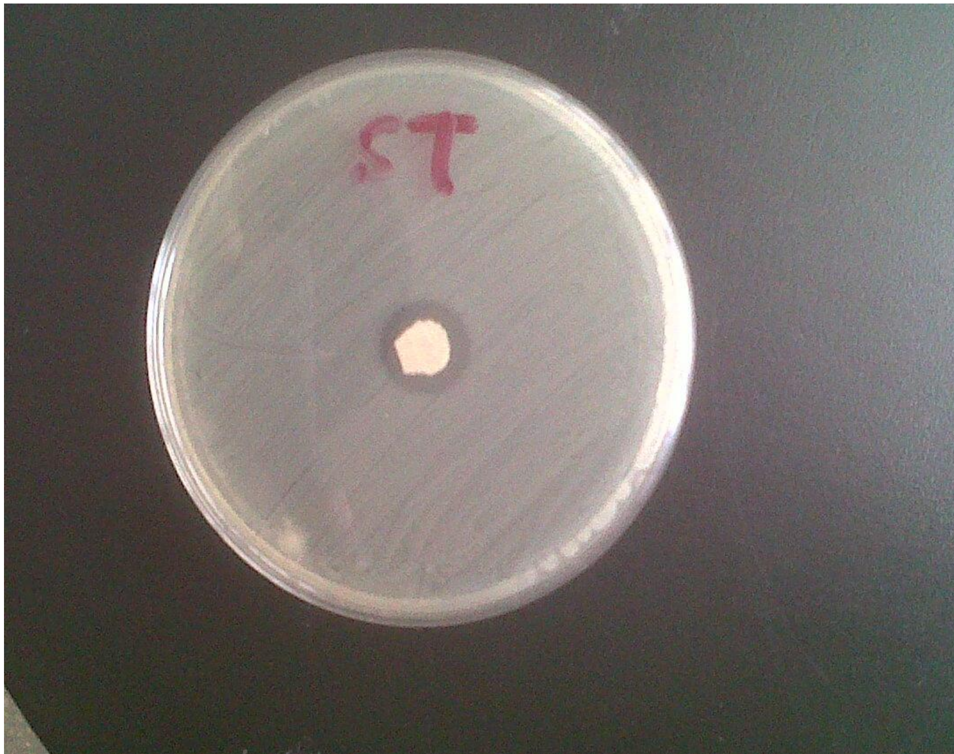

Plate XVI: Zones of inhibition of the control (Ciprofloxacin) against *Salmonella typhi*

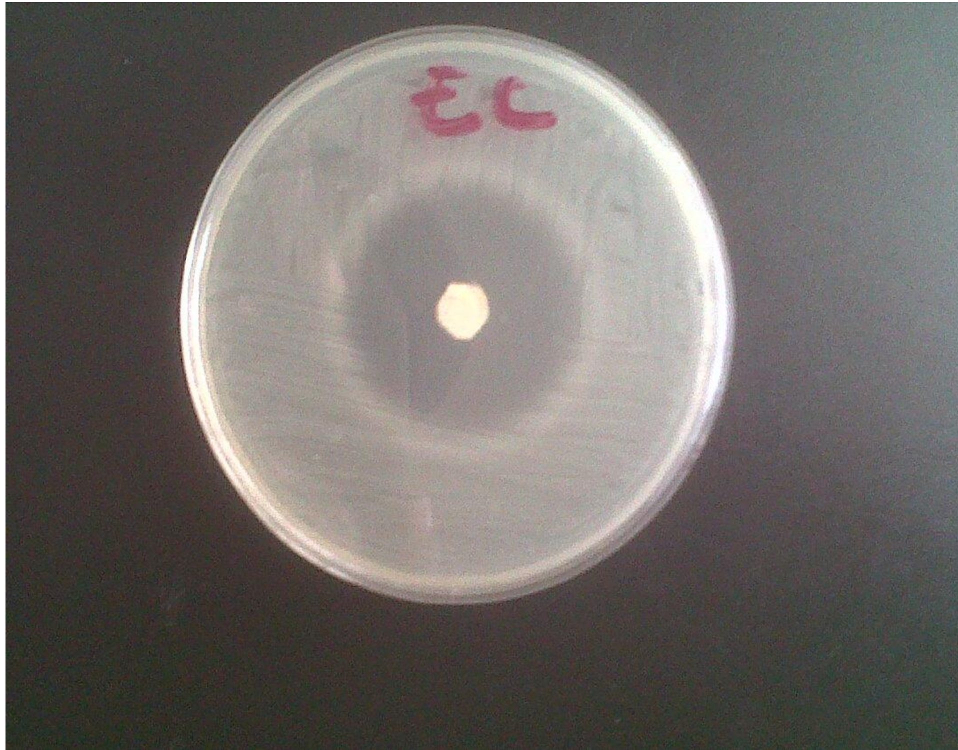

Plate XVII: Zones of inhibition of the control (Ciprofloxacin) against *Escherichia coli*

Plate XVIII: Zones of inhibition of the control (Ciprofloxacin) against *Staphylococcus aureus*

CHAPTER FIVE

5.0 DISCUSSION

The phytochemicals detected in the extracts (flavonoids, saponins, tannins, cardiac glycosides, steroids and triterpenes) have been associated with antimicrobial activities (Reynolds and Dweck, 1999; Aliyu *et al.*, 2008). The secondary metabolites could be responsible for antibacterial activity of the plant extracts. Flavonoids have been shown to exhibit anti-inflammatory, antiangionic, anti-allergic effects, analgesic and antioxidant properties (Hodek *et al.*, 2002). Just *et al.* (1998) revealed inhibitory effect of saponins on inflamed cells. Tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of the cell protein synthesis (Dharmananda, 2003). Cardiac glycosides have been reported in naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Yukari *et al.*, 1995) and as a novel cancer therapeutic agent (Robert *et al.*, 2008).

The results for the antibacterial screening have shown that though at varying concentrations, all the extracts have antibacterial activity on at least one of the three test organisms; *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. The results of the inhibition of bacterial growth have shown that the extracts are active at high concentration and inactive at very low concentrations. Thus the study suggests that the inhibition of bacterial growth activity of the extracts is dose dependent.

The aqueous, methanolic and second aqueous extracts had no inhibitory effect on the Gram-negative bacteria, *E. coli* and *S. typhi* but showed inhibitory effects on the Gram-positive *S. aureus*, with activity increasing with increase in concentration of extracts. The second aqueous extract only showed activity on *S. aureus* at higher concentrations (50 and 100 mg/ml). The chloroform, ethyl acetate and petroleum ether extracts showed activity on all three bacteria. This reveals that the non-polar

organic solvents (Petroleum ether, Chloroform and Ethyl Acetate) exhibited greater antibacterial activity than the polar solvents (water and methanol). This may be due to the better solubility of the bioactive agents in the non-polar solvents. This corroborates the report of El-Mahmood *et al.* (2010) that the extractability of phytoconstituents differs from solvent to solvent depending on their polarities. It also reveals that the extracts were more active against the Gram-positive bacteria. This corroborates an earlier report by Jigna and Sumitra (2006) but is at disparity with the report by Doughari *et al.* (2007), where a greater activity of extracts was observed against Gram-negative bacteria than the Gram-positive bacteria. The activity of the extracts against both Gram-negative and Gram-positive bacteria tested may indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms.

Since the traditional herbal remedy preparation use water as the extractant, it is a paradox that the aqueous extracts were less active in this study. It is possible that the aqueous crude extracts may contain antimicrobial constituents insufficient for efficacy in our study and which may explain why large amounts of the decoctions must be drunk by the patients. Jigna and Chanda (2007) and Kitonde *et al.* (2014) found out that, aqueous extracts showed little or no antimicrobial activity in contrast to those made using organic solvents. Yinegar *et al.* (2008) reported that the success in traditional medicines may be due to administration of the extracts in large quantities and over a long period of time.

The MIC of the chloroform extract was highest for *E. coli* (25 mg/ml) than for *S. typhi* and *S. aureus* (12.5 mg/ml), suggesting that the chloroform extract is more efficacious on *S. typhi* and *S. aureus*. The MIC of the ethyl acetate extract was highest for *S. typhi*

(25 mg/ml) than for *E. coli* and *S. aureus* (12.5 mg/ml), suggesting that the ethyl acetate extract is more efficacious on *E. coli* and *S. aureus*. The higher MIC of the petroleum ether extract (25 mg/ml) was also recorded for *S. aureus* compared to 12.5 mg/ml for *E. coli* and *S. typhi*, suggesting that the petroleum ether extract is more efficacious on *E. coli* and *S. typhi*. The highest MIC of extract on *E. coli* was recorded for the chloroform extract (25 mg/ml), suggesting that the ethyl acetate and petroleum ether extracts were more efficacious on *E. coli*. The highest MIC of extract on *S. typhi* was recorded for the ethyl acetate extract (25 mg/ml), suggesting that the chloroform and petroleum ether extracts were more efficacious on *S. typhi*. The highest MIC of extract on *S. aureus* was recorded for the second aqueous extract (50 mg/ml) and the lowest MIC recorded for the methanolic extract (12.5 mg/ml). This suggests that the methanolic extract is more efficacious against *S. aureus* than the other extracts.

A similar trend to the MICs of extracts was also observed for the MBCs of extracts on the test organisms, which depicts the lowest concentration of extract that did not allow any bacterial growth on the surface of the agar plates (i.e. the lowest concentration that kills all the bacteria). It was however observed that MICs of the extracts were lower than the MBCs, suggesting that the extracts were bacteriostatic at lower concentrations and bactericidal at higher concentrations. This supports the report of Aliyu *et al.* (2008), which evaluated the antibacterial activity of 12 medicinal plants used in Northern Nigerian traditional medicine against 25 hospital isolates of methicillin resistant *Staphylococcus aureus* (MRSA) using disc diffusion and broth dilution assays. According to Noumedem *et al.* (2013), an extract is bactericidal when the ratio MBC/MIC ≤ 4 and bacteriostatic when this ratio is >4. It therefore appeared that all except the second aqueous extract were bactericidal against the susceptible

bacteria. The second aqueous extract did not show a bactericidal effect within the concentrations used for this study.

These observations are likely to be the result of the differences in the cell wall structure between Gram-negatives and Gram-positive bacteria, with Gram-negative outer membrane acting as a barrier to many environmental substances, including antibiotics (Tortora *et al.*, 2001). According to Nester *et al.* (2004), the cell walls of Gram-negative bacteria (*E. coli and S. typhi*), contain an outer membrane and lipid bilayer embedded with proteins and porins (carrier proteins). These proteins allow passage of certain small molecules or ions either into or out of the cell periplasm. The active compounds may not be able to pass into the cells, making them inactive. The size of the porin channel particularly determines the size of the molecule that can pass through it and thus, the outer membrane serves as a barrier to the passage of many molecules and excludes many toxic compounds, and hence less sensitive to many extracts. However, the Gram-positive bacterium (*S. aureus*) has a relatively thick membrane consisting of layers of peptidoglycan, but regardless of its thickness, peptidoglycan is fully permeable to many substances including sugars, ions, and amino acids, and thus sensitive to most extracts.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The secondary metabolites detected in the extracts from the combination of plant materials include flavonoids, cardiac glycosides, steroids and triterpenes, which are present in all the extracts. Tannins and saponins were absent in the petroleum ether extract but present in all other extracts.

Escherichia coli and *Salmonella typhi* were not susceptible to the aqueous, methanolic and second aqueous extracts within the concentration range for this study. *Staphylococcus aureus* was susceptible to all the extracts. The extracts of the non-polar organic solvents (petroleum ether, chloroform and ethyl acetate) exhibited greater activity than those of the polar solvents (water and methanol). The petroleum ether extract was the most active that could compete favourably with the conventional antibiotic (ciprofloxacin) at 25 mg/ml and higher concentrations. The activity of the extracts on the test organisms increased with increase in concentration.

The extracts exhibited bacteriostatic effects on the test organisms at lower concentrations and bactericidal effects at higher concentrations. All but the second aqueous extract were bactericidal against the susceptible bacteria. The second aqueous extract did not show a bactericidal effect within the concentration range of this study.

This research may serve as a scientific basis and lend credence to the claim by the traditional medicine practitioner that the extracts from the selected plants combination is a useful herbal remedy, with a broad spectrum antibacterial activity against Gram-

positive and Gram-negative bacteria. It justifies the claim that the selected plant combination is efficacious in the management of diseases particularly related to *E. coli*, *S. typhi* and *S. aureus*, such as gastroenteritis, meningitis, diarrhoea, typhoid fever, pneumonia, boils, wound and urinary tract infections. It also concludes that the potency of the herbal mixture is dependent on the solvent of extraction and the dose administered, which may vary with target organisms or infections.

6.2 Recommendations

1. Experiments should be carried out at higher concentrations of the aqueous and methanolic extracts to assess their activity on *E. coli* and *S. typhi*.
2. The extracts should be tested on other micro-organisms to ascertain their activity on other disease-causing agents.
3. Further research can be conducted using laboratory animals to test the efficacy of the extracts *in vivo* and to address the limiting issue of dosage.
4. Research should be carried out using bioassay guided fractionation to identify, isolate and characterize the bioactive components of the plant extracts.
5. It is necessary to determine the toxicity of the bioactive constituents and their side effects, thereby establishing their pharmacokinetics.
6. The ministry of health should make it mandatory that all herbal products be subjected to scientific verifications before being sold as remedy.

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APPENDICES

APPENDIX I: Set-up of Soxhlet apparatus for the extraction process

APPENDIX II: Evaporotary apparatus set-up for the concentration of extract and recovery of solvent

APPENDIX III: Experimental set-up showing minimum inhibitory concentration determination

Appendix IV: ANOVA for Zones of Inhibition of Bacteria in Aqueous Extract

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
6.25	3	10.67	3.55667	37.9496
12.5	3	12.33	4.11	50.6763
25	3	16.67	5.55667	92.6296
50	3	18.33	6.11	111.996
100	3	20	6.66667	133.333
Ciprofloxacin	3	93	31	343
E. coli	6	38	6.33333	240.667
S. typhi	6	10	1.66667	16.6667
S. aureus	6	123	20.5	156.651

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Concentration of extract	1685.09	5	337.017	8.7574	0.00202	3.32583
Test organisms	1154.33	2	577.167	14.9977	0.00098	4.10282
Error	384.837	10	38.4837			
Total	3224.26	17				

Appendix V: ANOVA for Zones of Inhibition of Bacteria in Methanolic Extract

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
6.25	3	11.67	3.89	45.3963
12.5	3	13.33	4.44333	59.2296
25	3	16	5.33333	85.3333
50	3	18.33	6.11	111.996
100	3	19	6.33333	120.333
Ciprofloxacin	3	93	31	343
E. coli	6	38	6.33333	240.667
S. typhi	6	10	1.66667	16.6667
S. aureus	6	123.33	20.555	151.364

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Concentration of extract	1674.51	5	334.903	9.07663	0.00176	3.32583
Test organisms	1161.61	2	580.803	15.7411	0.00081	4.10282
Error	368.972	10	36.8972			
Total	3205.09	17				

Appendix VI: ANOVA for Zones of Inhibition of Bacteria in Chloroform Extract

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
6.25	3	0	0	0
12.5	3	0	0	0
25	3	11.67	3.89	45.3963
50	3	26.33	8.77667	58.4696
100	3	32.67	10.89	89.0563
Ciprofloxacin	3	93	31	343
E. coli	6	67	11.1667	225.15
S. typhi	6	10	1.66667	16.6667
S. aureus	6	86.67	14.445	272.248

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Concentration of extract	2027.05	5	405.409	7.46228	0.0037	3.32583
Test organisms	528.567	2	264.283	4.86461	0.03345	4.10282
Error	543.278	10	54.3278			
Total	3098.89	17				

Appendix VII: ANOVA for Zones of Inhibition of Bacteria in Ethyl Acetate Extract

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
6.25	3	23.67	7.89	47.3863
12.5	3	29.67	9.89	76.7263
25	3	33.66	11.22	96.6663
50	3	56.33	18.7767	4.91853
100	3	61.33	20.4433	12.0185
Ciprofloxacin	3	93	31	343
E. coli	6	131.33	21.8883	78.1459
S. typhi	6	45	7.5	75.0116
S. aureus	6	121.33	20.2217	156.478

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Concentration of extract	1130.03	5	226.007	5.40496	0.01151	3.32583
Test organisms	743.285	2	371.643	8.88786	0.00605	4.10282
Error	418.147	10	41.8147			
Total	2291.46	17				

Appendix VIII: ANOVA for Zones of Inhibition of Bacteria in Petroleum Ether Extract

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
6.25	3	16	5.33333	85.3333
12.5	3	38.66	12.8867	125.55
25	3	54	18	14.0889
50	3	58.34	19.4467	17.1396
100	3	61.33	20.4433	22.2496
Ciprofloxacin	3	93	31	343
E. coli	6	137.99	22.9983	60.8093
S. typhi	6	95	15.8333	81.5462
S. aureus	6	88.34	14.7233	270.284

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Concentration of extract	1090.56	5	218.113	2.2425	0.12978	3.32583
Test organism	242.09	2	121.045	1.24451	0.32912	4.10282
Error	972.632	10	97.2632			
Total	2305.29	17				

Appendix IX: ANOVA for Zones of Inhibition of Bacteria in Second Aqueous Extract

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
6.25	3	0	0	0
12.5	3	0	0	0
25	3	0	0	0
50	3	10.33	3.44333	35.5696
100	3	17	5.66667	96.3333
Ciprofloxacin	3	93	31	343
E. coli	6	38	6.33333	240.667
S. typhi	6	10	1.66667	16.6667
S. aureus	6	72.33	12.055	309.754

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Concentration of extract	2210.5	5	442.099	7.07426	0.0045	3.32583
Test organisms	324.865	2	162.433	2.59917	0.12332	4.10282
Error	624.941	10	62.4941			
Total	3160.3	17				

Appendix X: ANOVA for Activity of 6.25mg/ml Concentration of Extracts on Bacteria

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Aqueous	3	10.67	3.55667	37.9496
Methanolic	3	11.67	3.89	45.3963
Chloroform	3	0	0	0
Ethyl acetate	3	23.67	7.89	47.3863
Petroleum ether	3	16	5.33333	85.3333
2nd aqueous	3	0	0	0
Ciprofloxacin	3	93	31	343
E coli	7	66.67	9.52429	204.257
S typhi	7	10	1.42857	14.2857
S aureus	7	78.34	11.1914	253.217

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Extracts	2094.24	6	349.04	5.6884	0.00525	2.99612
Test organisms	381.811	2	190.906	3.11124	0.08155	3.88529
Error	736.32	12	61.36			
Total	3212.37	20				

Appendix XI: ANOVA for Activity of 12.5mg/ml Concentration of Extracts on Bacteria

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Aqueous	3	12.33	4.11	50.6763
Methanolic	3	13.33	4.44333	59.2296
Chloroform	3	0	0	0
Ethyl acetate	3	29.67	9.89	76.7263
Petroleum ether	3	38.66	12.8867	125.55
2nd aqueous	3	0	0	0
Ciprofloxacin	3	93	31	343
E coli	7	73	10.4286	216.099
S typhi	7	30.33	4.33286	63.6489
S aureus	7	83.66	11.9514	253.977

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Extracts	2119.53	6	353.255	3.91484	0.02118	2.99612
Test organisms	227.545	2	113.773	1.26085	0.31841	3.88529
Error	1082.82	12	90.2349			
Total	3429.89	20				

Appendix XII: ANOVA for Activity of 25mg/ml Concentration of Extracts on Bacteria

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Aqueous	3	16.67	5.55667	92.6296
Methanolic	3	16	5.33333	85.3333
Chloroform	3	11.67	3.89	45.3963
Ethyl acetate	3	33.66	11.22	96.6663
Petroleum ether	3	54	18	14.0889
2nd aqueous	3	0	0	0
Ciprofloxacin	3	93	31	343
E coli	7	76.66	10.9514	225.627
S typhi	7	30	4.28571	61.9048
S aureus	7	118.34	16.9057	186.056

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Extracts	2045.31	6	340.885	5.1376	0.00784	2.99612
Test organisms	558.016	2	279.008	4.20502	0.04131	3.88529
Error	796.213	12	66.3511			
Total	3399.54	20				

Appendix XIII: ANOVA for Activity of 50mg/ml Concentration of Extracts on Bacteria

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Aqueous	3	18.33	6.11	111.996
Methanolic	3	18.33	6.11	111.996
Chloroform	3	26.33	8.77667	58.4696
Ethyl acetate	3	56.33	18.7767	4.91853
Petroleum ether	3	58.34	19.4467	17.1396
2nd aqueous	3	10.33	3.44333	35.5696
Ciprofloxacin	3	93	31	343
E coli	7	93.66	13.38	213.638
S typhi	7	49	7	87.8196
S aureus	7	138.33	19.7614	132.254

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Extracts	1806.08	6	301.013	4.5368	0.01253	2.99612
Test organisms	569.989	2	284.995	4.29537	0.03918	3.88529
Error	796.191	12	66.3492			
Total	3172.26	20				

Appendix XIV: ANOVA for Activity of 100mg/ml Concentration of Extracts on Bacteria

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Aqueous	3	20	6.66667	133.333
Methanolic	3	19	6.33333	120.333
Chloroform	3	50	16.6667	0.44223
Ethyl acetate	3	63.33	21.11	7.8052
Petroleum ether	3	61.33	20.4433	22.2496
2nd aqueous	3	17	5.66667	96.3333
Ciprofloxacin	3	93	31	343
E coli	7	102.33	14.6186	227.035
S typhi	7	70	10	102.706
S aureus	7	151.33	21.6186	109.685

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Extracts	1668.65	6	278.108	3.44795	0.03233	2.99612
Test organisms	479.086	2	239.543	2.96982	0.08958	3.88529
Error	967.909	12	80.659			
Total	3115.64	20				