SUSCEPTIBILITY PROFILES OF SOME BACTERIA ISOLATED FROM STOOL OF DIARRHOEAL PATIENTS TO THE STEM AND ROOT BARKS EXTRACTS OF *FICUS SYCOMORUS* Linn (Moracae)

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ABSTRACT

Effective antibiotic management of diarrhoea caused by bacterial organisms faces challenge of antibiotic resistance which combines to pose serious problems. Local people often use some plants to treat diarrhoea. The efficacy of one of such plant is the subject of this investigation. Stool samples of diarrhoeal patients attending Mohammed Abdullahi Wase Specialist Hospital Kano, Nigeria were collected and the bacterial isolates were identified using standard procedures. Stem and root barks of *Ficus sycomorus* plant were extracted with aqueous and ethanolic solvents. The extracts were subjected to phytochemical screening. Fractionation of the crude extracts was also done using n-Hexane, Ethyl acetate and n-Butanol. Antibacterial activities of the extracts and fractions against the isolated bacteria were carried out using disk diffusion, agar dilution and rate of death/survival tests. Phytochemical screening of *F. sycomorus* stem and root barks revealed the presence of carbohydrates, saponins, tannins, alkaloids and flavonoids. Biochemical test results of stool isolates showed that, *Escherichia coli* constituted the majority of the isolated organisms forming up to 73.8% followed distantly by *Salmonella* and *Shigella* spp with 18.5% and 7.8% respectively. *In-vitro* antibiotic susceptibility tests showed varied susceptibility profiles of the isolated organisms to the test antibiotics and the extracts. The *F. sycomorus* extracts were observed to be significantly less active against *Salmonella* and *Shigella* spp than the reference antibiotics (gentamicin and ofloxacin) at $P < 0.05$. The ethyl acetate and n-Butanol fractions exhibited a significant inhibitory activities against the resistance *E. coli* and *Salmonella* spp stool isolates than the reference antibiotics ($P<0.05$). The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts ranged from 3.125 mg/ml to 100 mg/ml, while that of reference antibiotics ranged from 0.78-100 mg/ml respectively. Studies on the rate of kill of the ethyl acetate fraction against the isolates indicated that, the fraction had bactericidal activity against both susceptible and resistant isolates. The results therefore, support the claims of traditional healers in the use of the plant for the treatment of diarrhoea.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Medicinal Plants

Medicinal plants are therapeutic resources used by a large proportion of the population in developing countries specifically for healthcare purposes. They often serve as starting materials for drugs (Sofowora, 1993). According to World Health Organization (1997), a medicinal plant is any plant which contains in one or more of its organs, substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Furthermore, medicinal plants was also been defined as a herbal preparations produced by subjecting plant materials to extraction, purification, concentration or other physical processes which may be produced for immediate consumption or as basis for herbal products (Mume,1999).

Medicinal plants have been reported to play important role in drug development programs of the pharmaceutical industry (Baker e*t al.*, 1995). Varieties of materials derived from medicinal plants have been used for the prevention and treatment of diseases, virtually in all cultures. Medicinal plants have been used as sources of food and medicinal purposes for centuries and this knowledge have been passed from one generation to another (Adedapo, 2002). Over 40% of medicines now prescribed in the U.S. contain chemicals derived from plants (Ernst, 2011). Medicinal plants are used to derive many drugs which are used in the treatment of various types of diseases especially among the indigenous populations in developing countries of the world. It has been estimated that 80% of the people worldwide rely on medicinal plants for some aspect of primary health care (Faleyimu *et al.*, 2010). Medicinal components from plants play an important role in conventional as well as in western medicine (WHO, 2007). Although much has been achieved to this end, only less than 10% of the world flora has been chemically studied in details (Sofowara, 1993). Medicinal plants have been used for several decades for the prevention and treatment of many ailments. As at the beginning of 21st Centaury, about 170 medicinal plants were officially recognized in the United State Pharmacopoea (Inamul, 2004).

In the last twenty years in the United States, increasing public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural organic remedies, has

led to an increase in the use of medicinal plants. In Germany, about 600 to 700 plant based medicines are available and are prescribed by approximately 70% of German physicians (Faleyimu *et al.,* 2010). Medicinal plants have a variety of other uses including culinary usage. General usage differs between culinary herbs and medicinal herbs. Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional system (WHO, 1997). The leaves, roots, flowers, seeds, root bark, inner bark (cambium), berries and sometimes the pericarp or other portions of the plant might be considered in medicinal or spiritual use. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. In many cases, these substances (particularly the alkaloids) serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores (Faleyimu *et al.,* 2010).

The effects of plant extracts on bacteria have been studied by a large number of researchers in different part of the World (Erdoorul, 2002). The use of medicinal plants to treat disease is almost universal among non-industrialized and industrialized societies (Faleyimu *et al.,* 2010).

1.2 Historical Development of Herbal Medicine

Herbal medicine is the oldest method of curing diseases and infections and various plant materials have been used in different parts of the world for this purpose (Caceres *et al*., 1991). Herbal medicine has a long history, probably extending over 2000 years ago and is quite popular with many people (WHO, 2007). World Health Organization (2007) survey indicates that about 70-80% of world population rely on complementary and alternative medicines. Remedies from medicinal plants are widely used for the treatment and prevention of various diseases and often contain highly active pharmacological compounds(Akerele, 1993). Herbal medicines play an important role in home health care, health improvement, as alternative medicine and materials for medical products in many countries (WHO, 2007).

Plants had been used for medicinal purposes long before recorded history. Ancient Chinese and Egyptian papyrus writings describe medicinal uses of plants as early as 3,000 BC. Indigenous cultures (such as African and Native American) used herbs in their healing purposes, while

others developed traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used (Damery *et al.,* 2011). Researchers found that people in different parts of the world tended to use the same or similar plants for the same purposes (Manheimer *et al*., 2009).

In the early 19th Century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Later, chemists began to make their own version of plant compounds. Over time, the use of herbal medicines declined in favor of drugs. Almost one fourth of pharmaceutical drugs are derived from botanicals (Marcus, 2009).The use of herbal supplements has increased dramatically over the past 30 years. Herbal supplements are classified as dietary supplements by the U.S. Dietary Supplement Health and Education Act (DSHEA) of 1994, that means herbal supplements -- unlike prescription drugs -- can be sold without being tested to prove they are safe and effective. However, herbal supplements must be made according to good manufacturing practices (Mendes *et al.,* 2010).

Through the 20th and 21st Centuries the most commonly used herbal supplements in the U.S. include echinacea (*Echinacea purpurea* and related species), St. John's wort (*Hypericum perforatum*), ginkgo (*Ginkgo biloba*), garlic (*Allium sativum*), saw palmetto (*Serenoa repens*), ginseng (*Panax ginseng*, or *Asian ginseng;* and *Panax quinquefolius*, or *American ginseng*), goldenseal (*Hydrastis canadensis*), valerian (*Valeriana officinalis*), chamomile (*Matricaria recutita*), feverfew (*Tanacetum parthenium*), ginger (*Zingiber officinale*), evening primrose (*Oenothera biennis*), and milk thistle (*Silybum marianum*) for the treatment of various ailments including cancer therapy (Ernst, 2011).

In 2007, World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. In Germany, about 600 - 700 plant based medicines are available and are prescribed by some 70% of German physicians. In the past 20 years in the United States, public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in herbal medicine use (Faleyimu *et al.,* 2010).

Currently, even the industrialized nations are developing interest in the use of herbs. Scientific investigation into some folk remedies has provided lead to bioactive compounds and many of these compounds have been developed into drugs. Examples of such remedies include;

i. Artemisinin, an antimalarial compound obtained from Chinese medicinal herb, *Artemisia annua* (Klayman, 1991).
ii. *Ginko biloba* tree is used as an antiasthmatic and antitussive preparations (Hamburger *et al.,* 1991).
iii. *Azadirachta indica* is also used as an anti-malarial drug (Klayman,1991).

1.3 Statement of Research Problem

Pathogenic microorganisms are increasingly becoming resistant to orthodox antibiotics (Sofowara, 1993). Resistance to antimicrobial agents, particularly in hospitalized patients and the society in general is becoming a common problem nowadays. Organisms like Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Enterococci* (VRE), Multidrug Resistant *Pseudomonas* spp, *Enterobacter* spp and *Streptococcus pneumneiae* have been reported as the main culprits. The increased resistances to antibiotics have resulted in increased mortality, morbidity and longer duration of treatment worldwide. The resistances of Enterobacteriaceae such as *Salmonella, Shigella* and *E. coli* species that colonize the intestines have become a phenomenon and pose a global health problem in hospitals (Akinyemi, 1998).

In 2006, the United Nations Children's Fund (UNICEF) and the World Health Organization (WHO) reported that, generally diarrhoea remains the second leading cause of death among children under five. They further reported that, nearly one in five child death amounting to about 1.5 million each year is due to diarrhoea and that diarrhoea kills more young children than AIDS, malaria and measles combined. It has also been reported that, Pneumonia and diarrhoea are responsible for an estimated 40 per cent of all child deaths around the world each year (Njume *et al*., 2012).

A number of orthodox drugs used to treat diarrhoea are being reported ineffective due to resistance development by the causative organisms. This has resulted in the search for alternatives such as plant-derived agents.

Amongst several factors contributing to the potential use of medicinal plants with holistic approach to the health problems are lack of serious reported adverse reactions, safety, cheap and readily availabilities within a local community. Several plants are in used for the treatment of diarrhoea locally. For example, the use of *Agrimonia eupatorium* to specifically treat stomach, liver and kidney ailments has long been recognized as a traditional remedy for diarrhoea. *Berberis vulgaris* is traditionally used for the treatment of infectious diarrhoea. Its active ingredient (berberine) has been shown to inhibit the growth of bacteria in the intestine and helps the immune system function better. Berberine has also been shown to kill a wide range of other types of microorganisms, such as those that cause *Candida* (yeast) infections, viruses, and various parasites such as tapeworms and Giardia (Njume *et al*., 2012).

The extract of *Ficus sycomorus* is used in Hausa ethno medicinal medicine of Northern Nigeria for the treatment of various ailments including diarrhoea and dysentery (Wakeel *et al*., 2004). However, the widespread use of this plant locally in diarrhoea treatment has not been back up by scientific evidence, besides the pharmacological and microbiological effects reported by Ahmadu *et al*, (2007). In study by Ahmadu *et al*, (2007), it was demonstrated that, the leaves of *Ficus sycomorus* possessed anti-diarrhoeal activity by reducing intestinal motility. There is therefore, the need to explore whether the plant has inhibitory activity against some bacteria causative agents of diarrhoea and thus provide basis for the use of this plant in treating diarrhoea particularly among adults.

1.4 Justification of the Study

Medicinal plants have been used since antiquity for the prevention and treatment of various ailments. At the turn of the 21st century, approximately 170 of different medicinal plants were officially recognized in the U.S.P (Inamul, 2004). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al.,* 1996). Furthermore, the clinical evidences of safety provided by some medicinal plants and other traditional herbal therapies have no doubt contributed to a better health care system in many countries (WHO, 1997).

Several plants have been investigated for their potential use in the treatment of diarrhoeal disease; while some hold some promises, others were found to be inactive against bacterial pathogens (Wakeel *et al.,*2004). Plant-derived antimicrobial agents will help reduce incidences

of antibiotic resistance by micro-organisms and thus improve therapy and management of infections by the populace.

Generally, the use of medicinal plants in the developed and developing countries is enhanced by their accessibility, affordability, cultural and historical background (Inamul, 2004)

1.5 Aim of the Study

To establish scientific basis for the use of *Ficus sycomorus* plant in the treatment of diarrhoea.

1.6 Specific Objectives

i. Collection of stool samples from diarrhoea patients, isolation and identification of bacterial organisms from the stools using standard microbiological methods.
ii. Collection of *Ficus sycomorus* plant and extraction of its stem and root barks and fractionation of the crude extracts using different solvent systems.
iii. Phytochemical analysis of plant constituents
iv. Determination of susceptibility of the bacterial isolates to the extracts and reference antibiotics by the agar diffusion technique.
v. Determination of MIC and MBC of crude extracts as well as the fractions against bacterial isolates
vi. Determination of rate of kill study of fraction against selected isolates

1.7 Research Hypothesis

1.7.1 Null hypothesis

Ficus sycomorus stem and root barks growing in Northern Nigeria has no inhibitory activity on bacterial isolates from diarrhoeal stools.

1.7.2 Alternate hypothesis

Ficus sycomorus stem and root barks growing in Northern Nigeria has inhibitory activity on bacterial isolates from diarrhoeal stools.

1.8 Scope of the study

i. Study is limited to only some bacteria isolated from diarrhoeal stools in patients that attended Mohammed Abdullahi Wase Specialist Hospital Kano within the period of May to December, 2012.
ii. Only the aqueous and ethanol root and stem bark extracts was investigated in this study.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diarrhoea

Diarrhoea is defined as the passage of three or more loose or liquid stools per day or more frequent passage than is normal for the individual (WHO, 2009). Diarrhoeal disease is the second leading cause of death in children under five years old, and is responsible for the death of 1.5 million children every year worldwide (WHO, 2009).

Barua *et al,* (1981) reported that diarrhoea is the second major cause of morbidity among the notifiable diseases in Nigeria. According to the Federal Ministry of Health (1997), more than 300 children die every day as a result of dehydration and malnutrition caused by diarrhoea. These statistics are likely to be underestimated as many patients do not have access to the limited number of hospitals and health centers that are available (Ogunsanya *et al*., 1994). Enteric bacteria account for a substantial proportion of diarrhoea episodes worldwide (Synde an Merson d,1980). However, studies on the spread of these enteric bacteria in urban and rural areas have received only cursory attention in Nigeria.

Diarrhoea can last several days, leaving the body without the water and salts that are necessary for survival. Most people who die from diarrhoea actually die from severe dehydration and fluid loss. Children who are malnourished or have impaired immunity are most at risk of life-threatening diarrhoea (WHO, 2009). The most severe threat posed by diarrhoea is dehydration. During a diarrhoeal episode, water and electrolytes (sodium, chloride, potassium and bicarbonate) are lost through liquid stools, vomit, sweat, urine and breathing. Dehydration occurs when these losses are not replaced (Akinyemi *et al*,.1998). Diarrhoea is a syndrome that is frequently not differentiated clinically by specific etiologic agent. The use of glucose-electrolyte oral rehydration therapy (ORT) has dramatically reduced acute mortality from dehydration caused by diarrhoea. Estimates of global mortality from diarrhoea has been reported to decline from approximately 4.6 million annual deaths during the mid-1980s to the current estimate of 1.6–2.1 million. Most of these deaths occur in children under the age of 5 years and occur in developing countries (WHO, 2009).

2.1.1 Causes of diarrhoea

Diarrhoea is usually a symptom of an infection in the intestinal tract which can be caused by a variety of bacteria (*E. coli*, *Salmonella* spp, *Shigella* spp. *Campylobacter*), viruses; *Rotavirus*, *Cytomegalovirus*, *Viral hepatitis* (Joseph 1987, Alizadeh *et al*, 2007, Lorrot and Vesseur, 2007) and parasitic organisms like *Giardia lamblia*, *Entamoeba histolytica* (Sanjay *et al*., 2013). Infection is spread through contaminated food or drinking-water, or from person-to-person as a result of poor hygiene. Kain *et al*, (1987) reported that, the most frequent etiologies of diarrhoea at the community level were Enterotoxigenic *Escherichia coli* (ETEC 14%), *Rotavirus* (18%), Enteropathogenic *Escherichia coli* (EPEC 9%), and *G. lamblia* (10%). In outpatient settings, *Campylobacter* spp. and EPEC (9%) were most frequent (Pazzaglia *et al.*, 1993). In inpatient settings, *Rotavirus* (25%), EPEC (16%), and ETEC (9%) are most frequent (Huilen *et al.*, 1991). Also much more morbidity is caused by certain enteric pathogens, including *G. lamblia*, *Cryptosporidium* spp., *E. histolytica*, and *Campylobacter* spp. Conversely, enteric pathogens such as *Rotavirus*, *Salmonella* spp., and *V. cholerae* O1 and O139 seem to be important causes of mortality (WHO. 2007). Other causes are; eating local food and drinking local water during foreign travel can result in traveler's diarrhoea. Diarrhoea can also be caused by reactions to medications (including some vitamins, minerals and herbs) and ingestion of milk or dairy products by people who are lactose-intolerant. A different type of diarrhoea that results in blood in the stool, accompanied by fever or abdominal pain, could be caused by intestinal disorders such as inflammatory bowel disease or Crohn's disease (WHO. 2007).

2.1.2 Types of diarrhoea

2.1.2.1 Secretory diarrhoea

Secretory diarrhoea means that there is an increase in the active secretion, or there is an inhibition of absorption. There is little or no structural damage. The most common cause of this type of diarrhoea is a cholera toxin that stimulates the secretion of anions, especially chloride ions (Navaneethan and Giannella, 2008).

2.1.2.2 Osmotic diarrhoea

Osmotic diarrhoea occurs when too much water is drawn into the bowels. This can be the result of maldigestion (e.g. pancreatic disease or Coeliac disease), in which the nutrients are left in the lumen to pull in water. In healthy individuals, too much magnesium or vitamin C or undigested lactose can produce osmotic diarrhoea and distention of the bowel. A person who has lactose intolerance can have difficulty in absorbing lactose after high intake of dairy products. In persons who have fructose malabsorption, excess fructose intake can also cause diarrhoea (WHO, 2009).

2.1.2.3 Exudative diarrhoea

Exudative diarrhoea occurs with the presence of blood and pus in the stool. This occurs with inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis, food poisoning and other severe infections disease (WHO. 2007).

2.1.2.4 Motility-related diarhoea

Motility-related diarrhoea is caused by the rapid movement of food through the intestines (hypermotility). If the food moves too quickly through the gastrointestinal tract, there is not enough time for sufficient nutrients and water to be absorbed. This can be due to a vagotomy or diabetic neuropathy, or a complication of menstruation. Hyper motility can be observed in people who have had portions of their bowel removed, allowing less total time for absorption of nutrients (Navaneethan and Giannella**,** 2008).

2.1.2.5 Inflammatory diarrhoea

Inflammatory diarrhoea occurs when there is damage to the mucosal lining or brush which leads to a decreased ability to absorb the intestinal fluids. Features of all three of the other types of diarrhoea can be found in this type of diarrhoea. It can be caused by bacterial infections, viral infections, parasitic infections and bowel diseases. It can also be caused by tuberculosis, colon cancer, and enteritis (WHO, 2007).

2.1.3 Diarrhoeal disease diagnosis

According to CDC (2013), several approach were been adopted for the diarrhoea diagnosis some of which are;

2.1.3.1 Culture

A bacterial culture is used to identify bacterial species responsible for the infection. Also blood sample is used to culture bacteria that may be responsible for the infection.

2.1.3.2 Gram-stain

The stain can distinguish between the two major classes (gram-positive or gram-negative) of bacteria and aid in narrowing down what kind of infection.

2.1.3.3 Ova and Parasites

A stool sample is examined for the presence of parasites and/or their eggs.

2.1.3.4 Fecal leukocytes

Identification of the white blood cells in the stool when one has an inflammatory disease.

2.1.3.5 Serology

This method consists of drawing a blood sample and evaluating the antibodies to identify microbes with which one has recently been infected.

2.1.4 Management of diarrhoeal disease

The objective of the treatment of diarrhoea is to replace the lost fluids and the inactivation of the causative agent. This can equally be achieved by replacement of fluids and electrolytes lost through diarrhoea using a simple rehydration solution as well as inactivation of the causative organism using antibiotics. Oral Rehydration Salts (ORS), is a solution that contains specific proportions of water, salts and sugar. Diarrhoeal disease is treatable with a solution of clean water, sugar and salt, and with zinc tablets. Death can follow severe dehydration if body fluids and electrolytes are not replenished, either through the use of oral rehydration salts (ORS)

solution, or through an intravenous drip (Wilson, 2005). Several approaches are used to treat diarrhoea.

2.1.4.1 Rehydration

Rehydration with intravenous fluids in case of severe dehydration or shock and/or oral rehydration salt (ORS) solution for moderate or no dehydration. ORS is a mixture of clean water, salt and sugar, which can be prepared safely at home. ORS is absorbed in the small intestine and replaces the water and electrolytes lost in the feces**.**

2.1.4.2 Use of zinc

Zinc supplements: zinc supplements reduce the duration of a diarrhoeal episode by 25% and are associated with a 30% reduction in stool volume (WHO, 2012).

2.1.4.3 Nutrient-rich foods

The vicious circle of malnutrition and diarrhoea can be broken by continuing to give nutrient-rich foods – including breast milk – during an episode, and by giving a nutritious diet – including exclusive breast feeding for the first six months of life – to children when they are well.

2.1.4.4 Use of antidiarrhoeal agents

In infective diarrhoea cases antimicrobial therapy are instituted against the bacteria and protozoans that may be involved. These include loperamide, gentamicin, ciprofloxacin, tetracycline, metronidazole and azithromicin among others (WHO, 2012).

2.1.5 Preventive measures of diarrhoea

2.1.5.1 Practice of good personal hygiene

In developing countries, poor hygiene practice is the main reason for the high prevalence of diarrhoeal diseases. Use of clean water, flushed toilets and sanitary waste disposal habits in industrialized countries have limited the spread of diarrhoeal diseases. In addition, frequent hand

washing with soap and good cooking and dining practices are important for preventing diarrhoea diseases (CDC, 2013).

2.1.5.2 Hand washing

Frequent hand washing is the best way to control diarrhoea infection. Hand washing with soap has been shown to reduce the incidence of diarrhoeal disease by over 40 percent (CDC, 2013).

2.1.5.3 Avoidance of untreated water

Contaminated drinking water is the most common source of cholera infection. Household water treatment and safe storage systems such as chlorination and filtration in both development and developing countries reduced the number of diarrhoeal cases. It has been estimated that 88 per cent of diarrhoeal deaths worldwide are attributable to unsafe water and inadequate sanitation and poor hygiene (CDC, 2013).

2.1.5.4 Eating of completely cooked and hot food

Bacteria can survive on room temperature food for up to five days and some are not destroyed by freezing. It's best to avoid street vendor food. Raw or improperly cooked fish and seafood of any kind should also be avoided.

2.1.5.5 Health education

General public have to be educated on how to change their behaviors through community involvement, education and health-promotion activities about diarrhoeal prevention. Implementation of behavior change interventions, such as face-to-face counseling to encourage exclusive breastfeeding, sustenance of high levels of vitamin A supplementation and its effective delivery to the community with other high-impact health and nutrition interventions.

2.1.5.7 Vaccination

Vaccinations Includes; cholera, *Salmonella typhi*, *Hepatitis A* and rotavirus vaccine in national immunization programmes worldwide, which was recently recommended by the World Health Organization. Accelerating its introduction, particularly in Africa and Asia, where the rotavirus burden is greatest, should be an international priority. (CDC, 2013).

2.2 Properties of Common Pathogens of Diarrhoea

2.2.1 *Shigella* species

Shigella spp belongs to the Family Enterobacteriaceae. It is a Gram-negative, non spore forming, non-motile, rod-shaped bacteria closely related to Salmonella. It is the causative agent of human shigellosis. It is naturally found in humans and apes. The genus is named after Kiyoshi Shiga, who first discovered it in 1898. Phylogenetic studies indicate that *Shigella* is more appropriately treated as subgenus of Escherichia, and that certain strains generally considered *E. coli* – such as E. coli O157:H7 – are better placed in *Shigella* (WHO, 2012). After invasion, *Shigella* multiply intracellularly and spread to neighboring epithelial cells, resulting in tissue destruction and characteristic pathology of shigellosis. *Shigella* species are classified into four serogroups (WHO, 2012).

i. Serogroup*A*: S. dysenteriae (12 serotypes)
ii, Serogroup*B*: S. flexneri (6 serotypes)
iii. Serogroup*C*: S. boydii (18 serotypes)
iv. Serogroup*D*: S. sonnei (1 serotype)

Group *A–C are* physiologically similar. *Shigella sonnei* (group *D*) can be differentiated on the basis of biochemical metabolism assays. Three *Shigella* groups are the major disease-causing species: *S. flexneri* is the most frequently isolated species worldwide, and accounts for 60% of cases in the developing world. *Shigella sonnei* causes 77% of cases in the developed world, compared to only 15% of cases in the developing world, while *S. dysenteriae* is usually the cause of epidemics of dysentery, particularly in confined populations such as refugee camps (Thomas *et al.,* 2012).

Shigella is one of the leading bacterial causes of diarrhoea worldwide. It causes approximately 90 million cases of severe dysentery with at least 100,000 of these resulting in death each year, mostly among children in the developing world. *Shigella* infection is typically via ingestion (fecal–oral contamination) depending on age and condition of the host, less than 100 bacterial cells can be enough to cause an infection. *Shigella* causes dysentery that resulted in the destruction of the epithelial cells of the intestinal mucosa and rectum. Some strains produce enterotoxin and shiga toxin, similar to the verotoxin of E. coli O157:H7 and other verotoxin-

producing Escherichia coli. Both shiga toxin and verotoxin are associated with causing hemolytic uremic syndrome (Ram *et al.,* 2008).

Shigella species are negative for motility and are not lactose fermenters. However, *S. sonnei* can ferment lactose. They typically do not produce gas from carbohydrates (with the exception of certain strains of *S. flexneri*) and tend to be overall biochemically inert. *Shigella* should also be urea hydrolysis negative. When inoculated to a triple sugar iron (TSI) slant, they react as follows: gas and H_2s production negative, indole reactions are mixed, positive and negative, with the exception of *S. sonnei,* which is always indole negative (Cheesbrough, 2010).

2.2.2 *Escherichia coli*

Escherichia coli are the most abundant facultative anaerobic Gram-negative bacteria of the intestinal micro flora, naturally colonizing the mucous layer of the colon. Theodor Escherich first described *E. coli* in 1885, as *Bacterium coli commune,* which he isolated from the feaces of newborns (Cheesbrough, 2010). It was later renamed *Escherichia coli,* and for many years, the bacterium was simply considered to be commensal organism of the large intestine. It was not until 1935 that a strain of *E. coli* was shown to be the cause of an outbreak of diarrhoea among infants (Wakimoto *et al.,* 2004).

Escherichia coli colonize the gastrointestinal tract of most warm-blooded animals within hours or a few days after birth. The bacterium is ingested in foods or water or obtained directly from other individuals handling the infant. The human bowel is usually colonized within 40 hours of birth. Enteropathogenic *E. coli* (EPEC) and Enterohemorrhagic *E. coli* (EHEC) are highly successful pathogens that have adapted to cause infections in different hosts via related, but distinct mechanisms of transmission (Dziva *et al.,* 2004).

EPEC and EHEC cause acute gastroenteritis in humans. EPEC, the first type of *E. coli* to be associated with human disease, is a frequent cause of infantile diarrhoea in the developing world, and EHEC an emerging zoonotic pathogen, causes a wide spectrum of illnesses ranging from mild diarrhoea to severe diseases such as hemorrhagic diarrhoea in human. Strains of EHEC belonging to serogoup O157 are most commonly associated with severe human diseases (Dziva *et al.,* 2004).

Escherichia coli causes urinary tract infection, infection of wound, peritonitis, meningitis, bacteraemia in neonate, diarrhoeal disease, infantile gastroenteritis, dysentery, traveler's diarrhoea and hemorrhagic diarrhoea. Most strains of *E. coli* are indole positive, lysine decarboxylase positive, citrate and H_2S negative and are gas producers. It is a Gram negative motile rod and ferment lactose producing smooth pink colonies on MacConkey agar (Cheesbrough, 2010).

2.2.3 *Salmonella* species

Salmonellae are Gram-negative, motile rods that characteristically ferment glucose and mannose without producing gas but do not ferment lactose or sucrose. Most *Salmonellae* produce H_2S. They are aerobes and facultatively anaerobes. They are often pathogenic for humans or animals when ingested. Arizona is included in the *Salmonella* group (Cheesbrough, 2010). *Salmonella* are responsible for the causation of the following diseases;

2.2.3.1 Enteric fever (typhoid and paratyphoid)

Infection is by ingesting the organisms in contaminated food or water or contaminated hand (*S. typhi* is mainly waterborne, *S. paratyphi* is mainly food borne).

2.2.3.2 Diarrhoeal disease (Enterocolitis)

This can be caused by many *salmonella* serovers. In developing countries *S. typhimorium* and *S. enteritidis* are common causes. Common sources of infection are poultry meat and meat products, eggs and egg products. Diarrhoea, vomiting, fever, abdominal pains occur after 12-36h after eating infected food. In acute infections, blood and mucus are present in the faecal specimens (Cheesbrough, 2010).

2.2.3.2 Bacteraemia

Non-typhi salmonella (NTS), particularly *S, typhimurium* and *S. enteritidis* are common cause of bacteraemia in young children in developing countries. Organisms are usually detected in the blood and feces from 40-50% of patients during the first and second week of infection. A selective media like Deoxy Citrate Agar (DCA) and *Salmonella Shigella* Agar (SSA) are

required to isolate *Salmonella* organisms from faecal specimens. Biochemically, most Salmonella are urease, indole and lactose negative, gas production from glucose fermentation, citrate and Lysine decarboxylase (LDC) positive (Cheesbrough, 2010).

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2.2.4 *Rotavirus*

Rotavirus is the leading cause of severe dehydrating diarrhoea among children both in the United States and worldwide. It has been estimated that most children in the United States have or will be infected by 5 years of age. This very contagious virus is even resistant to good hygiene practices, including hand washing (Lorrot and Vasseur,2007).

Rotavirus was estimated to cause about 40-50 percent of all hospital admissions due to diarrhoea among children under five years of age worldwide leading to some 100 million episodes of acute diarrhoea each year that result in 350,000 to 600,000 child death (Lorrot and Vasseur,2007).

Since *Rotavirus* is very contagious, all people can get infected. Small children younger than 5 years of age, though, are at the greatest risk for severe dehydration that leads to hospitalization and sometimes death. In addition, people with weaker immune systems, such as the elderly or HIV patients, are also more likely to have severe symptoms. It was reported that, approximately 40% to 50% of hospitalizations for diarrhoea are due to *Rotavirus*, and 20 to 60 deaths due to *Rotavirus* occur each year in the United States (Lorrot and Vasseur, 2007). In developing countries, rotavirus causes more than 500,000 deaths each year in children less than 5 years of age. *Rotavirus* spreads from Person-to-person via the fecal-oral route. *Rotavirus* that is shed in stools of infected people can contaminate hands and objects and get passed to family members and close contacts. *Rotavirus* makes a toxin called "NSP4 enterotoxin," which alters the function of the intestines, causing increased fluid secretion and lactose intolerance (Lorrot and Vasseur,2007).

Rotavirus infections have been reported to be more common between November and May, coinciding with the time of year that children are in school and are more likely to be in closer contact with other children in United State (Lorrot and Vasseur,2007). In addition, the virus is commonly transmitted in close communities, such as daycare centers, nursing homes and within families. Approximately two days following exposure to the virus, symptoms typically begin

with mild fever, upset stomach, vomiting, followed by diarrhoea that last 3 to 8 days. Loss of fluids can be accompanied with dehydration (WHO, 2008). *Rotavirus* can be diagnosed by identification of the virus in the patient's stool. The most common test is the "Enzyme Immunoassay," which uses an antibody that binds specifically to the virus to detect its presence. Other tests include use of specialized microscopes (electron microscopy), protein analysis (polyacrylamide gel electrophoresis) and analysis of genetic material (PIP, 2008).

2.2.5 *Entamoeba histolytica*

Entamoeba histolytica is a protozoan parasite responsible for a disease called amoebiasis. It occurs usually in the large intestine and causes internal inflammation as its name suggests (histo = tissue, lytic = destroying). Caler and Lorenzi, (2010) reported that, fifty (50) million people are infected worldwide, mostly in tropical countries in areas of poor sanitation and in industrialized countries, most of the infected patients are immigrants, institutionalized people and those who have recently visited developing countries (Caler and Lorenzi, 2010).

Amoebiasis is diagnosed by the health care provider under a microscope by finding cysts and (rarely trophozoites) from a stool sample. The results are usually said to be negative, if *Entamoeba histolytica* is not found in three different stool samples, but it still does not necessarily mean that one is not infected because the microscopic parasite is hard to find and it might not be present in the particular samples. A blood test might also be available but is only recommended, if the health care provider believes that the infection could have spread to other parts of the body. Trophozoites can be identified under a microscope from biopsy samples taken during colonoscopy or surgery (Caler and Lorenzi, 2010).

2.3 Properties of Some Commonly Used Antidiarrhoeal Agents

2.3.1 Gentamicin

It is a member of aminoglycoside antibiotic group, which is synthesized by *Micromonospore purpura,* a genus of Gram-positive bacteria widely distributed in water and soil. Gentamicin is used in the treatment of diarrhoea diseases. It is active against many strains of Gram-positve and Gram-negative bacterial organisms. It interferes with initiation and assembly of the bacterial ribosomes, by binding to the 30S ribosomal subunit hence prevent the process of protein synthesis. Gentamicin improves nitrogen and fat absorption and hence decrease stool output. Combination of gentamicin and cholestyramine is recommended as safe and effective way of treating infants with severe and persistent diarrhoea following acute gastroenteritis (Kenneth, 2009).

2.3.2 Tetracyclines

The tetracyclines are a large family of antibiotics that were discovered as natural products of *Streptomyces species* bacteria beginning in the late 1940s. They are a group of very broad-spectrum antibiotics. They are all closely related structurally with a 4-ring carboncyclic skeleton in common and differ chemically by substituent variation on that structure. Tetracycline group include tetracycline, chlortetracycline (CTC), oxytetracycline (OTC), methacycline, demeclocycline, doxycycline and minocycline. Chlortetracycline was the first to be isolated from *Stretptomyces aureofaciens.* Oxytetracycline (OTC) and tetracycline were discovered later, which were produced by a mutant strains of *Stretptomyces aureofaciens* (Kenneth, 2009).

Tetracycline sparked the development of many chemically altered antibiotics and in doing so has proved to be one of the most important discoveries made in the field of antibiotics. It is a class of "broad-spectrum antibiotic" used to treat infections caused by Gram-positive and Gram-negative bacteria and some protozoa. It is produced semi synthetically from chlortetracycline. It blocks the binding of aminoacyl tRNA to the A site of the ribosome by binding to the bacterial 30S subunit to inhibit protein synthesis. The tetracyclines act by blocking the binding of aminoacyl tRNA to the A site on the ribosome. It is mainly bacteriostatic normally used in the treatment of cholera and other diarrhoeal diseases (Kenneth, 2009).

Doxycycline is one of the members of tetracyclines group which has been found clinically effective in the treatment of a variety of infections caused by susceptible strains of Gram-positive and Gram-negative bacteria and certain other micro-organisms. It is used in the treatment of

gastrointestinal disorders, such as abdominal pain, stomatitis, anorexia, nausea, vomiting, diarrhoea, dyspepsia and rarely dysphagia, oesophagitis and oesophageal ulceration have been reported in patients receiving doxycycline. A significant proportion of these occurred with the hydrochloride salt in the capsule. Doxycycline acts by blocking the binding of aminoacyl tRNA to the A site of the ribosome by binding to the bacterial 30S subunit to inhibit protein synthesis (Kenneth, 2009).

2.3.3 Sulphonamides

Suphonamides such as cotrimaxazole are synthetic antimicrobial agents. It is a mixture of sulphamethoxazole and trimethoprim. Both of them in combination have bactericidal as well as bacteriostatic activity against a wide range of Gram-negative and Gram-positive bacteria and some protozoan. It is use in the treatment of urinary tract infections (UTI), respiratory tract infections (RTI), brucellosis, enteric infections cause by *Salmonella*, *Shigella species*, cholera, AIDS associated diarrhoea, typhoid fever and paratyphoid fever. It is also used in the treatment of amoebic dysentery, pneumonia and otitis media. Sulphonamide acts competitively by inhibiting the incorporation of para amino benzoic acid into dihydropteric acid which leads to formation of false folate analogues (Hugo and Russells, 2007*).*

2.3.4 Nitroimidazole

The imidazoles comprise a large and diverse group of compounds with properties encompassing antibacterial and antiprotozoal (metronidazole), antifungal (clotrimazole, miconazole, ketoconazole, econazole) and antihelmintic (mebendazole) activity (Hugo and Russells,2007*).*

Metronidazole inhibits the pathogenic protozoa. A very low concentration being effective against the protozoa *Trichomonas vaginalis, entamoeba histolytica* and *Giardia lamblia.* It is also used to treat bacterial vaginosis caused by *Gardinella vaginalis*. Metronidazole is administered orally or through infussion. Metronidazole was first introduced into clinical medicine in 1959 for the treatment of *Trichomonas vaginalis* infections and it has been subsequently used for invasive amebiasis and giardiasis (Cohen *et al*., 2010). Metronidazole is one of the mainstay drugs for the treatment of anaerobic infections and the treatment of choice for most patients with mild to moderate *Clostridium difficile*-associated diarrhoea. It is approved by the US Food and Drug Administration (FDA) for the treatment of anaerobic and protozoal infections. Metronidazole

exerts its antimicrobial effects through the production of free radicals that are toxic to the microbe (Lofmark *et al.*, 2010). Recent studies have documented its in-vitro bactericidal activity against anaerobic bacteria. Metronidazole appears to be efficacious in clinical anaerobic infections and two groups have reported its use prophylactically in gynecological and colonic surgery (Lofmark *et al.*, 2010). Metronidazole is selective for anaerobic bacteria due to their ability to intracellularly reduce metronidazole to its active form. This reduced metronidazole then covalently binds to DNA, disrupt its helical structure, inhibiting bacterial nucleic acid synthesis and resulting in bacterial cell death (Cohen *et al*., 2010).

The limitation of these diverse antimicrobial agents is the exclusive activity against anaerobic microorganisms (Hugo and Russells, 2007*).*

2.3.5 Fluoroquinolones

Ciprofloxacin is the member of fluoroquinolones. However, the fluoroquinolones was developed from quinolones by the introduction of fluorine substituent at C-6, which led to a considerable increase in potency and spectrum of activity compared with first generation such as nalixidic acid. The second generation quinolones are known as fluoroquinolones examples of which are; ciprofloxacin and norfloxacin. Ciprofloxacin has broad spectrum of activity against both Gram-negative and Gram-positive bacteria. Fluoroquinolones are antibiotic drugs used in the treatment of many infections caused by bacteria in the human body. Among other infections, these drugs are used to treat urinary tract infections, joint and bone infections, and respiratory infections, such as tuberculosis, pneumonia, and bronchitis. Ciprofloxacin may be used in the treatment of organisms resistant to other antibiotics. These drugs directly act on the DNA of bacteria, killing them. Bacterial DNA is essential in the multiplication of bacteria inside the body. It can also be used in conjunction with a β-lactam or aminoglycosides (Hugo and Russells, 2007).

2.4 The Plant *Ficus Sycomorus*

The *F. sycomorus* plant has several names depending on the locality. It is called *subula* in Arabic, *wild fig*, *Strangler fig*, *Sycomore*, *Sycomore fig* and *Bush fig* in English, *Figuier sycomore* in French, *Baure* in Hausa, *Iwere-jeje* in Yoruba, *Oju ologbo* in Igbo, *Burum* in Kanuri and *Ibbi* in Fulfulde.

2.4.1 Botanical description

The *F. sycomorus* belong to the Mulberry Family, Moracae, which consists of about forty (40) genera and over one thousand and four hundred (1,400) species of trees, shrubs and herbs often with milky latex juices (Adeshina *et al.*, 2010). *F. sycomorus* is a large, semi-deciduous spreading savannah tree, up to 21m (max. 46m), occasionally buttress. Bark on young stems is pale green with a soft powdery covering but on older stems, is grey-green, fairly smooth with scattered grey scales and pale brown patches where scales have fallen off. Flowers are unisexual, cycle and greenish. Ficus is the Latin name for fig, derived from the Persian 'fica'. In Greek 'syka' means fig. The species name comes from the Greek, 'sykamorea' (Tengnas *et al.,* 1993).

2.4.2 Natural habitat

Ficus sycomorus is a common savannah tree that grows in high water table areas, often found along water courses such as streams and rivers, swamps and waterholes. The Sycamore fig is susceptible to frost but can withstand some cold. It is found in rain forests especially along edges and in clearings, riverine forests, woodland, evergreen bush land left as single trees in farmland and occasionally seen as single trees on rocky outcrops (Beentje,1994).

2.4.3 Geographical distribution

Ficus sycomorus is a native of several countries in Africa such as Angola, Benin, Botswana, Burundi, Cameroon, Congo, Cote d'Ivoire, Democratic Republic of Congo, Djibouti, Egypt, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Israel, Kenya, Lesotho, Madagascar, Mozambique, Namibia, Nigeria, Rwanda, Saudi Arabia, Senegal, Somalia, South Africa, Sudan, Tanzania, Uganda, Zambia and Zimbabwe. It is also found in Syrian Arab Republic (Abbiw,1990).

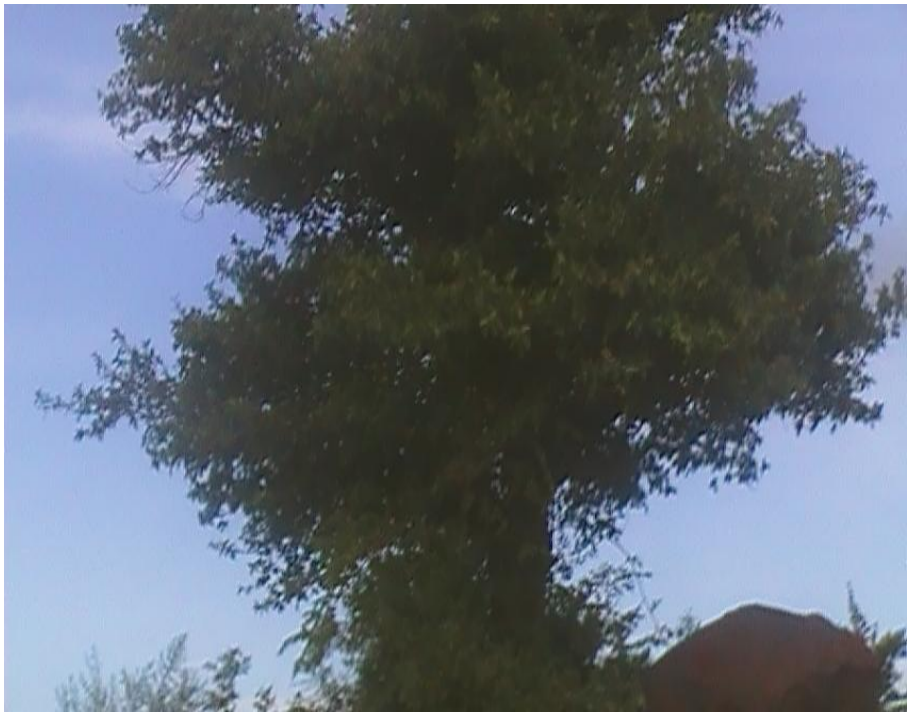

plate I: *F. sycomorus* whole tree in its natural habitat (This study as source)

Plate: II *F. sycomorus* leaves and fruit (This study as source)

2.4.4 Reproductive biology

In southern Africa, flowering and fruiting occur throughout the year, with a peak from July to December (Beentje,1994). Small wasps which develop in some of the flowers and live symbiotically inside the syconium, pollinate the unisexual flowers. Bats achieve seed dispersal (Beentje,1994).

2.4.5 General uses of the plant

i. The mature fruits are eaten fresh or dried and stored for later use. Fruit can also be used for the preparation of an alcoholic beverage. Leaves are used in soups and groundnut dishes. The bark is chewed together with kola nut. In Ghana, the wood ash is commonly used as a salt substitute. Leaves are a much-sought fodder with fairly high nutritive value (9% crude protein and 7 mj/kg net energy). They are valuable fodder in overstocked semi-arid areas where the trees occur naturally. Fruits are eaten by livestock, wild animals and birds (Abbiw,1990).

ii. The tree can be used as firewood and for making charcoal by many people throughout Africa and use a piece of dry wood from this tree as the base block when starting a fire by the friction method (Tengnas *et al.,* 1993).

iii. Inner part of the root used as weaving fiber and a strong rope can be made from the inner bark (Abbiw,1990).

iv. The wood is creamy brown, has a fairly uniform structure and is very light (air-dry 510 kg/m^3), soft to moderately hard, tough, strong, easy to work, finishes smoothly and holds nails firmly. It is not very durable and is easily attacked by termites. Mainly used for making mortars and pestles, drums, stools, doors, beehives, dugout canoes, carvings and for house building (Beentje, 1994).

2.4.6 Ethno medicinal uses

The active principles of many drugs found in plants are secondary metabolites. These secondary metabolites which constitute important source of pharmaceutical drugs have been isolated from different parts of plants. Some of these compounds have been reported to be present in the *Ficus* species such as tannins, saponins, flavonoids, steroids, anthraquinone glycosides and reducing sugars (are a diverse group of pharmaceuticals used in the treatment of epileptic seizures). *F. sycomorus* have been reported to possess anti-diarrhoeal activities. Sedative and anticonvulsant

properties of this plant have also been reported (Olusesan *et al.,* 2010). The plant stem bark is used in treating tuberculosis and the plant also reported to have antibacterial activity (Mohamed *et al.,* 2013) The extract of this plant is used in Hausa ethnomedicinal medicine of Northern Nigeria for the treatment of various ailments such as diarrhoea, chest pain, and other chest conditions, dysentery, cough, convulsive disorders and pain relief (Wakeel *et al*., 2004). Latex from the root is used traditionally in curing tuberculosis disease (Kwari and Sandabe, 2000). *F. sycomorus* has also been reported to be used in the treatment of snake bites, jaundice, cold, coughs and throat infections (Pravin, 2006). Aqueous stem bark extract is used for the treatment of a variety of ailments such as mental illness, diarrhoea and pain relief (Wilson, 2005). The aqueous root bark extracts induced 50% anaesthesia at 30 mg/ml on rabbit compared with xylocaine (Pravin, 2006).

2.4.7 Other uses

i. Wild fig can be used for riverbank stabilization, shade or shelter. The tree gives useful shade and is common at market places, where people gather under it for many social functions (Tengnas *et al.*, 1993).
ii. Shed leaves form a valuable litter improving the nutrient status, infiltration rate and water-holding capacity of the soil (Tengnas *et al.,* 1993).
iii. It is an important tree planted for ornamental purposes near temples, roadsides, wells and community places such market centers in rural areas. The yellowish bark shows at an early stage, contrasting well with the green leaves. A popular species to grow as a bonsai (Friis, 1992).
iv. Usually intercropped with bananas as an under storey (Friis, 1992).
v. The sycamore fig is widely valued for spiritual and sacred purposes. Such use can be traced back to ancient Egypt, and it is often mentioned in the Holy Bible (Orwa *et al.,* 2009).

2.5 Phytochemistry of *Ficus Sycomorus*

In the study by Zaku *et al,* (2009), the aqueous extract of the leaves, stem-bark and root-bark of *Ficus sycomorus* were reported to contained tannins, alkaloids, reducing compounds, saponins,

flavonoids, glycoside, steroid, terpenoids and anthracenoside. Adeshina *et al*, (2010) and Ahmadu *et al*, (2007) also reported the presence of these compounds in *Ficus sycomorus*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment

The following under listed equipment were used in the study;

Refrigerator, manufactured by NAPCO Model 630 Portland Oregon, U.S.A., Ambient Incubator Model-630, Natural Appliance: Aheinicke Company Portland Oregon U.S.A., Autoclave Adelphi MFG Co Ltd. U.K., Electronic weighing balance model-PA313, Ohaus, U.S.A., Water bath Gallenkamp, U.K., Oven, Model 4144262/27 Bairdand Tatlock (London) Limited, UK.

3.1.2 Reagents

The following solvents used in this study were products of Sigma Aldrich Missouri, USA; Ethanol (Absolute), Ethyl acetate, n-butanol and hexane. Tween 80 was a product of BDH, England.

3.1.3 Bacteriological media

The following under listed media used in this study were all products of Oxoid Ltd, England;Salmonella Shigella Agar (SSA) , MacConkey Agar (MA), Eosin Methylene Blue (EMB), Mueller Hinton Agar (MHA), Nutrient Broth (NB), Nutrient Agar (NA), Triple Sugar Iron Agar (TSIA), Citrate Agar (CA), Urea Agar (UA).

The culture media were reconstituted in suspensions according to manufacturer's instructions. They were sterilized by autoclaving at 121^0C for 15 minutes and kept in refrigerator at (4^0C) until ready for use.

3.1.4 Reference antibiotics

Gentamicin injection of strength 80mg/ml and ofloxacin tablet 250 mg, both products of Hovid Pharmaceuticals company, Malaysia were used as reference antibiotics.

3.2 Methodology

3.2.1 Plant collection and treatment

The plant samples of *Ficus sycomorus* Linn (stem and root barks) were collected at Karnaya town, Dutse Local Government Area of Jigawa State, Nigeria with the assistance of Mallam Alhassan Karnaya a traditional herbalist in the area. The collected plant materials were identified and a voucher specimen numbers 953 was deposited at the Herbarium Unit of the Department of Biological Science, Ahmadu Bello University, Zaria. The fresh plant samples collected were air dried at room temperature and reduced into powder using mortar and pestle in the laboratory as described by Mukhtar and Tukur (2001).

3.2.2 Extraction procedures

3.2.2.1 Ethanol extraction

Maceration method of extraction as described by Fatofe *et al,* (1993) was adopted in this study. Five hundred grams (500 g) for each of stem and root barks powdered materials was weighed and transferred into separating funnel with cotton wool placed at the lower end of the funnel. One thousand (1000 ml) of 70% ethanol was added to the powder and left for 24 hours. Thereafter, it was decanted. The procedure was repeated with another 1000 ml to ensure complete extraction of the active ingredient. The extracts were filtered and evaporated to dryness on a water bath at 60^0C. The dried extracts were then weighed and stored in tightly closed bottles in a refrigerator at 4^0C until required.

3.2.2.2 Water extraction

For the water extraction, decoction procedure was used. Five hundred grams (500 g) of each of the stem and root barks powder was weighed and soaked in 1000mls of distilled water in a beaker and heated to boil. It was left to cool and then filtered using sterile filter paper (Whatman No.1) into a clean conical flask. The filtrate was evaporated on a water bath at 60^oC. The extracts were then stored in a refrigerator (Fatofe *et al,* 1993).

3.2.2.3 Fractionation of crude extracts

Ten grams (10 g) of ethanolic extracts was suspended in 250 ml of distilled water and partitioned with hexane (3 x 250 ml), followed by ethyl acetate (3 x 250 ml) and n-buthanol (3 x 250 ml) to get the hexane, ethyl acetate and n-butanol fractions respectively (Aliyu, 2006). The

same procedure was used for the fractionation of the aqueous extract. The fractions were kept in suitable containers until required for use.

3.2.3 Phytochemical screening of plant extracts

Phytochemical screening of plant extracts of *F. sycomorus* which included test for tannins, alkaloids, glycosides, saponins, flavonoids, proteins, resin balsams and carbohydrates respectively were carried out according to the method of Evans (2002).

3.2.3.1 Test for carbohydrates

(i) Molisch Test

Few drops of Molisch reagent were added to the extract in a test tube. One (1) ml of concentrated sulphuric acid was added to flow down the side of the inclined tube, so that the acid forms a layer beneath the aqueous solution without mixing with it. The appearance of reddish brown color indicates a positive test.

(ii) Fehling's Test for Reducing Sugar

Five (5) mls of Fehling's solution A and B in the ratio of 1:1were added to 2 ml of test extract in a test tube. The resultant mixtures were boiled for a few minutes. The appearance of brick red precipitate indicates a positive test.

3.2.3.2 Test for Anthraquinone

A small portion of extracts /fractions was shaken with 10 mls of chloroform and filtered. Five (5)mls of 10% ammonia solution was added to the filtrate and stirred. Absence of colour change indicates absence of anthraquinone.

3.2.3.3 Test for sponins

Two (2) mls of plant extracts and 10 mls of distilled water were added into a test tube and shaken vigorously for 30 seconds. The tube was allowed to stand vertically for 30 minutes. Formation of foam indicates a positive result.

3.2.3.5 Test for steroids

Two drops of plant extracts, a drop of H_2SO4 and 2-3 drops of anhydride were mixed in a test tube and shaken. Appearance of blue black indicates the presence of steroid.

3.2.3.4 Test for tannins

A small quantity of the extract was boiled and filtered. Two drops of ferric chloride was added to the filtrate. Formation of a blue black or dirty green precipitate was taken as evidence for the presence of tannins.

3.2.3.5 Test for alkaloids

One gram of the extract was stirred with 1% aqueous hydrochloric acid on a water bath and filtered. The filtrate was divided into three portions.

To the first portion, few drops of Dragendroff reagent was added and observed for the formation of orange to brownish precipitate.

To the second portion, one drop of Mayer reagent was added and observed for formation of white to yellowish or cream color precipitate.

To the third portion, one drop of Wagner reagent was added. A brown or reddished brown precipitate is taken as positive reaction.

3.2.3.6 Test for flavonoids (Shinoda test)

To an alcoholic solution of the extract, three pieces of Magnesium chips was added, followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

3.2.4 Stool samples collection and ethical clearance

Ethical approval was solicited and given by the Hospitals Management Board Kano (See Appendix I), after which the stool samples were collected from diarrhoea patients at the Mohammed Abdullahi Wase Specialist Hospital, Kano.

3.2.5 Isolation and identification of bacteria from stool

For the isolation of bacteria from stool samples collected from diarrhoea patients, the protocol described by Cheesbrough (2010) was adopted.

Growth on culture plates was initially identified by colony morphology and Gram staining. Further identification was carried out using standard biochemical tests (Cheesbrough, 2010).

3.3 Purification of the Isolates

3.3.1 Gram staining

The procedure described by Cheesebrough (2010) was adopted. A smear of the test organism was prepared on a sterile slide and heat fixed. The smear was flooded with crystal violet and allowed to stand for 60 seconds. The slide was then washed with water. The slide was then flooded with iodine solution and allowed to stand for 60 seconds after which it was washed again with water. The slide was then decolorized with alcohol by adding in a drop-wise manner and then rinsed with water for few seconds. The smear was counterstained with safaranin for 2 minutes and then washed with clean water. The back of the slide was wiped clean and placed on a rack for the smear to air-dry before viewing it under the microscope.

3.3.2 Eijkman test

This test shows the ability of an organism to ferment lactose at 45^0C and produce acid and gas. Lactose broth media was prepared according to manufacturer´s specification. Overnight cultures of the test organisms were taken using a sterile wire loop and aseptically inoculated into a test tube containing lactose broth and an inverted Durham tubes. It was then incubated at 44.5^0C for 18- 24 hrs. Production of acid and gas in the Durham tubes indicates a positive result.

3.3.3 Indole test

The test was carried out as described by Cheesebrough (2010). Test organisms were inoculated into bijou bottles containing 3 mls of sterile trypton water and incubated for 24 hours. Thereafter, 2-3 drops of xylene was added in to the bijou bottles followed by 0.5 ml of Kovac's reagent. This was shaken gently. Appearance of red colour on the surface layer within two minutes indicates the presence of *E. coli.*

3.3.4 Triple sugar iron test for *Salmonella* and *Shigella* species

Overnight cultures of the test organisms were taken using sterile inoculating loop streaked on the slope and stabbed onto the butt of TSIA. The cultures were then incubated at 37^{o}C for 24 hours. Presence of yellow butt and red slope indicates presence of either *Shigella* spp or *Salmonella* spp. Citrate test was later carried out to differentiate between the two organisms (Cheesebrough, 2010).

3.3.5 Methyl Red / Voges – Proskauer test

Two (2 ml) aliquot from overnight culture broth of the organisms was aseptically transferred in two separate tubes. Thereafter, reagents were added to the tubes. In tube one, three drops of methyl red reagent were added and observed immediately. Red colour formation indicates a positive result. While no colour change indicates negative MR result.

In tube 2, 0.6 ml of VP reagent A was added to the tubes and shaken gently for one minute to oxygenate the medium, after which 0.2 ml of VP reagent B was added and the tube shaken gently for another one minute. The tubes were then placed in a test tube rack and kept for 20 minutes. Red colour formation at the surface in 10-20 minutes indicates a positive VP, while unchanged after 20 minutes indicates negative VP result.

3.3.6 Citrate test

This was carried out as described by Cheesbrough (2010). Overnight cultures of the test organisms were streaked on the slope and stabbed on to the butt of Simon citrate agar. The cultures ware then incubated at 37^{0}C for 24 hours. Formation a bright blue coloration indicates a positive result, while unchanged of the initial colour of the media indicates a negative result.

3.3.7 Urea test

Test organisms were inoculated into urea agar and incubated at 37^{0}C for 24 hours. Formation of a pink-red coloration indicates positive test (Cheesbrough, 2010).

3.3.8 Inoculum preparation

Eighteen hour (18 h) broth cultures of test organisms were suspended in sterile Mueller Hinton broth. It was standardized according to CLSI (2008) by adding gradually sterile 1% normal

saline and comparing its turbidity with McFarland standard of 0.5 (sulphuric acid 99.04% plus barium chloride 0.6%) which is approximately 1.0 x 10^8 cfu/ml.

3.4 Preparation of Stock Solution

Using serial dilution technique, stock solution was prepared of which 1g of solid plant extracts were dissolved in 10 ml of normal saline to produce a stock of 100 mg/ml suspension. Further serial dilution was employed to get other concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.13 mg/ml, 1.56 mg/ml, and 0.78 mg/ml) (Hena *et al.,* 2010).

3.4.1 Anti-bacterial activity screening

The agar diffusion method adopted by Adeshina *et al,* (2010) was used to determine the antibacterial activity of the crude plant extracts and fractions. Twenty microlitre (20 µl) of standardized overnight culture containing approximately 10^6 cfu/ml were inoculated into molten sterile Mueller-Hinton agar at 45°C. The bacterial agar mixture was allowed to set and dry at room temperature. Wells of 6 mm diameter were bored in the agar aseptically in each plate. One drop of molten agar was used to seal the bottom of the bored hole, so that the extract will not sip beneath the agar. Each of the well was filled with 100 µl of the different concentrations (25 and 50 mg/ml) of the plant extracts with aid of sterile Pasteur pipette. Positive control was standard antibiotic discs of gentamicin (10 µg) and ofloxacin (5 µg) while distilled water served as a negative control. Plates were allowed for 1 h pre-diffusion at room temperature after which they were incubated at 37^0C for 24 hours. Diameters of zones of inhibition were measured to the nearest millimeter (mm). The test was done in duplicate and the mean zones of inhibition diameters were calculated.

3.4.2 Determination of minimum inhibitory concentration (MIC)

The protocol adopted by Ogbonnia *et al,* (2008) was used in this study. This involved two fold serial dilutions of the stock solution of the extracts/fractions to obtain concentrations between 100 to 0.38 mg/ml. Two (2 ml) portions of each dilution was mixed with 18 ml double strength Mueller Hinton Agar and poured into Petri dishes. Sterile filter paper discs of 6.00 mm diameter were aseptically placed on Mueller Hinton Agar surfaces. Standardized inoculum of the isolate

were immediately added to the discs in volumes of 20 µl. Twenty (20 µl) of sterile distilled water was added to the sterile paper disc as a negative control. The plates were left at ambient temperature for 15 minutes to allow excess pre- diffusion of organism prior to incubation at 37^0C for 24 hours.

The lowest concentration that did not show any visible growth around the paper disc when compared to the control was considered as the Minimum Inhibitory Concentration. Gentamicin and ofloxacin were used as standard antibiotics in the study.

3.4.3 Determination of minimum bactericidal concentration (MBC)

This was determined by using sterile forceps to place the filter paper discs that did not show any growth from the MIC plates into sterile Nutrient broth containing inactivating agent 5% v/v Tween 80) as described by Ehinmidu (1993). This was incubated at 37^0C for 24 hours. The Minimum Bactericidal Concentration was considered as those tubes that did not show any turbidity (Aboaba *et al*., 2006). The above methods were carried out in duplicates with the reference antibiotics (gentamicin and ofloxacin).

3.4.4 Determination of rate of kill

One (1) ml of standardized overnight culture of selected strains of test organisms(approximately $1x10^6$ cfu/ml) that had been judged as susceptible and resistant based on the MBC result were added to 9 ml each of *Ficus sycomorus* extracts and mixed thoroughly at room temperature and kept inside digital water bath at 37^0C. At different time intervals (0.5, 1, 2, 4, 6, 12 and 24 hours), 1ml test organism – extract admixture was taken and tenfold dilution protocol performed with sterile normal saline containing 5% Tween 80. These dilutions were then plated out in duplicates on sterile molten Mueller – Hinton agar supplemented with 5% tween 80. The solidified agar plates were then incubated at 37^oC for 42 hours and colonies observed were counted. Same procedure was done for the gentamicin as the standard antibacterial agent. A plot of bacterial count versus time was later done to compare the survival rate and subsequently, the rate of kill against the isolates (Adeshina *et al.,* 2010).

3.5 Statistical Analysis

Gentamicin and ofloxacin were used as reference antibiotics to which the activities of the extracts were compared using ANOVA. $P < 0.05$ was considered significant and $P > 0.05$ not significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Extraction and Fractionation of *Ficus Sycomorus*

The percentage yield of the plant extracts is as shown in Table 4.1. Ethanol solvent produced higher percentage yields of extracts for stem bark (ESBE) of 12.0% and root bark (ERBE) of 8.4% compared with water, for the stem bark (6.6%) and root bark (3.2%) for ASBE and ARBE respectively . Ethanolic extracts were brownish, while the aqueous solvents produced chocolate colour.

4.2 Phytochemical Screening of *Ficus Sycomorus*

Phytochemical screening revealed the presence of several chemical constituents in the various extracts and fractions as shown in Table 4.2. All the extracts revealed the presence of carbohydrates, flavonoids, alkaloids, saponins and tannins. Anthroquinone was not detected.

4.3 Antibacterial Assay of the Crude Extracts and Fractions of *Ficus Sycomorus*

The extracts and fractions showed varying degree of inhibition against the test organisms at the various concentrations. The diameter of zones of inhibition were classified into three categories; resistant (those with zone diameters less than 13 mm), intermediate (those with zone diameters of 13-14 mm) and susceptible (those with zone diameters of at least 15 mm), based on the CLSI (2008) categorization for the reference antibiotic (gentamicin).

Data presented in Table 4.3, shows the proportion of the *E. coli* isolates with diameters of zones of inhibition within the different categories. About 72.5% of *E. coli* isolates were found to be resistant to ESBE as against 100% for ASBE, 96.1% and 98% for ERBE and ARBE respectively. Proportions of resistant to gentamicin and ofloxacin were considerably lower 9.8% for gentamicin and 43.1% for ofloxacin.

Table 4.1: Physical Characteristics and Yield of Extracts *Ficus sycomorus*

Extracts	**Colour**	**Percentage Yield (%)**
ESBE	Brown	12.0
ERBE	Dark brown	8.4
ASBE	Chocolate	6.6
ARBE	Chocolate	3.2

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract,

ARBE = Aqueous Root Bark Extract

EAF= Ethyl Acetate Fraction,

n-BF= n-Butanol Fraction

Table 4.2: Distribution of Secondary Metabolites of the *Ficus sycomorus* Plant Extracts

		Extracts					
S/NO.	**Secondary Metabolites**	**ESBE**	**ERBE**	**ASBE**	**ARBE**	**n-BF**	**EAF**
1	Carbohydrate	+	+	+	+	+	+
2	Flavonoids	+	+	+	+	+	+
3	Alkaloids	+	+	+	+	+	+
4	Anthroquinone	-	-	-	-	-	-
5	Saponnins	+	+	+	+	+	+
6	Tannins	+	+	+	+	+	+

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

n-BF= n-Butanol Fraction

EAF= Ethyl acetate Fraction

+ = Presence of Secondary Metabolite

- = Absence of Secondary Metabolite

Table 4.3: Diameter of Zones of Inhibition of 25 mg/ml Extracts and Standard Antibiotics on *E. coli* (n=51) Stool Isolates

	Percentage of isolates with Diameter of zones of inhibition		
Extracts/Drugs	**≤12 mm (R)**	**13-14 mm (I)**	**≥15 mm (S)**
ESBE	72.5	9.8	15.7
ASBE	100	0.0	0.0
ERBE	96.1	3.90	0.0
ARBE	98.0	0.0	2.0
GENTAMICIN (µg)	9.8	0.0	90.2
OFLOXACIN (µg)	43.1	5.9	50.0

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

The performance of the test extracts and reference antibiotics is depicted in Table 4.4. As shown in this table, 72.5% of *E. coli* isolates were found to be resistant to ESBE as against 100% for ASBE and 96% for ERBE and ARBE and compared with 9.8% for gentamicin and 43.1% for ofloxacin. Only an increment of 10% was observed for the number of organisms that had increased susceptibility at 50mg/ml strength.

The inhibitory activities of the extracts against *Salmonella* organisms isolated from diarrhoea stool are presented in Tables 4.5- 4.6. Table 4.5 shows the percentage distribution of diameters of zones of inhibition produced by the different extracts and the reference antibiotics against the *Salmonella* spp isolates. Among the extracts, ASBE exhibit a higher inhibitory against *Salmonella* spp with 47.8% of the isolates being susceptible and only 13.0% resistant to it. ARBE exerted the least activity, having 87.0% of the organisms being resistant.

Data presented in Table 4.6 represents the percentage distribution of diameters of zones of inhibition produced by different extracts against *Salmonella* spp at 50 mg/ml. ASBE and ESBE yielded higher activity against the organisms with 72.3% and 60.9% susceptible respectively, which are comparable to those of reference antibiotics. It is to be noted that, none of the *Salmonella* isolates were resistant to the extracts at this higher concentrations, whereas, up to one fifth of them were not susceptible to the reference antibiotics.

Results of the antibacterial activity of the extracts against diarrheagenic *Shigella* isolates are presented in Tables 4.7-4.8.Table 4.10 shows the percentage distributions of zones of inhibition produced by different test extracts against *Shigella* spp at 25 mg/ml. ESBE, ERBE, ASBE and ARBE exerted a high activity effecting inhibition for up to 80%, 80%, 60% and 60% of *Shigella* isolates respectively. Only 20% of the isolates were resistant to ASBE and ARBE at the test concentrations. None of the isolates was resistant to ESBE and ERBE extracts.

At higher concentration, all the five *Shigella* isolates were readily susceptible to the four different extracts (Table 4.8).

Table 4.4: Diameter of Zones of Inhibition of 50 mg/ml Extracts and Standard Antibiotics on *E. coli* (n=51) Stool Isolates

	% Diameter of zones of inhibition (mm)		
Extracts/Drugs	**≤12 mm (R)**	**13-14 mm (I)**	**≥15 mm (S)**
ESBE	72.5	2.0	25.5
ASBE	100	0.0	0.0
ERBE	96.0	2.0	2.0
ARBE	96.1	0.0	3.9
GENTAMICIN (µg)	9.8	0.0	90.2
OFLOXACIN (µg)	43.1	5.9	51.0

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

Table 4.5: Diameter of Zones of Inhibition of 25 mg/ml Extracts and Standard Antibiotics on *Salmonella* spp. (n=23) Stool Isolates

	% Diameter of zones of inhibition (mm)		
Extracts/Drugs	**≤12 mm (R)**	**13-14 mm (I)**	**≥15 mm (S)**
ESBE	47.8	26.1	26.1
ASBE	13.0	39.1	47.8
ERBE	52.2	21.7	26.1
ARBE	87.0	4.3	8.7
GENTAMICIN (µg)	21.7	4.3	73.9
OFLOXACIN (µg)	21.7	17.4	61.0

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

Table 4.6: Diameter of Zones of Inhibition of 50 mg/ml Extracts and Standard Antibiotics on *Salmonella* spp. (n=23) Stool Isolates

	% Diameter of zones of inhibition (mm)		
Extracts/Drugs	**≤12 mm (R)**	**13-14 mm (I)**	**≥15 mm (S)**
ESBE	0.0	39.1	60.9
ASBE	0.0	21.7	72.3
ERBE	34.8	34.8	30.4
ARBE	87.0	0.0	13.0
GENTAMICIN (µg)	21.7	4.3	73.9
OFLOXACIN (µg)	21.7	17.4	60.9

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

Table 4.7: Diameter of Zones of Inhibition of 25 mg/ml Extracts and Standard Antibiotics on *Shigella* spp (n=5) Stool Isolates

	% Diameter of zones of inhibition (mm)		
Extracts/Drugs	**≤12 mm (R)**	**13-14 mm (I)**	**≥15 mm (S)**
ESBE	0.0	20.0	80.0
ASBE	20.0	20.0	60.0
ERBE	0.0	20.0	80.0
ARBE	20.0	20.0	60.0
GENTAMICIN (µg)	0.0	0.0	100
OFLOXACIN (µg)	0.0	0.0	100

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

Table 4.8: Diameter of Zones of Inhibition of 50 mg/ml Extracts and Standard Antibiotics on *Shigella* spp (n=5) Stool Isolates

	% Diameter of zones of inhibition (mm)		
Extracts/Drugs	**≤12 mm (R)**	**13-14 mm (I)**	**≥15 mm (S)**
ESBE	0.0	0.0	100
ASBE	0.0	0.0	100
ERBE	0.0	0.0	100
ARBE	0.0	0.0	100
GENTAMICIN (µg)	0.0	0.0	100
OFLOXACIN (µg)	0.0	0.0	100

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

Plate III, shows the susceptibility pattern of reference antibiotics and aqueous stem bark extract (ASBE) of *F. sycomorus* against one of the susceptible *Salmonella* spp. As shown in the plate, ofloxacin (5 µg) produced a wider zone of inhibition against the isolate followed by the crude ethanolic stem bark crude extract of *F. sycomorus* plant (25 and 50 mg/ml). and gentamicin (10 µg).

Plate IV, show the resistant and susceptibility pattern of reference antibiotics and ethyl acetate fraction (EAF) of *F. sycomorus* extract against one of the resistant *Salmonella* spp . As shown in the plate, ethyl acetate fraction exhibited higher antibacterial activity which produced a wider diameter of zones of inhibition against the isolates at the concentration of 50 and 25 mg/ml, whereas the isolate was completely not susceptible to the two reference antibiotics (gentamicin (10 µg and ofloxacin 5 µg).

Plate III: The Susceptibility of reference antibiotics and Aqueous Stem Bark Extract (50 and 25 mg/ml) of *F. sycomorus* against one of the susceptible *Salmonella* spp

KEY:

ESBE= ethanolic stem bark extract

O= ofloxacin (5 µg)

G= gentamicin (10 µg)

Plate IV: Susceptibility of the reference antibiotics and Ethyl acetate fraction (EAF) of *F. sycomorus* extract (50 and 25 mg/ml) against one of the resistant *Salmonella* spp

KEY;

ESBE= Ethanolic stem bark extract,

EAF= Ethyl acetate fraction,

GEN= gentamicin (10 µg)

OFX= ofloxacin (5 µg)

C= control.

Table 4.9 shows the mean diameters of zones of inhibition exerted by 25 and 50 mg/ml concentrations of the different extracts and standard antibiotics (gentamicin 10 µg and ofloxacin 5 µg) against the *Shigella* spp isolates. As presented in the table, it showed that, as the concentration of the extracts increased, the diameter of zones of inhibition against *Shigella* by all the extracts also increased.

The percentage distribution of susceptibility pattern of the test isolates against different concentrations of gentamicin is presented in Table 4.13, which showed that, none of the test organisms was inhibited at 0.78 mg/ml concentration. However, at 1.56 mg/ml concentration, all the *Shigella* isolates, and 47.1% of *E. coli* were inhibited by the extract, compared with only 4.3% of the *Salmonella* spp isolates at the same MIC value. Generally, gentamicin was more inhibitory against *Shigella* spp, followed by *E. coli*. *Salmonella* isolates were the least susceptible, with only 51.8% of them being inhibited at 25.0 mg/ml.

The data presented in Table 4.11 shows the percentage distribution of susceptibility pattern of the test isolates at different concentrations of ofloxacin. As indicated in the table, the drug exerts different potencies against the three test organisms. At MIC value of 0.78 mg/ml, 80% of *Shigella* and 56.5% of *Salmonella* spp isolates were inhibited, whereas only 20.2 % of the *E. coli* isolates were inhibited at the same concentration. Thus, the extract generally exerted higher inhibitory activities against *Shigella* isolates.

Table 4.15 shows the percentage distribution of susceptibility patterns of *E. coli*, *Salmonella* spp. and *Shigella* spp isolates at different concentrations of ethanolic stem bark extracts. As shown in the table, the extract was able to exert 100% and 95.7% inhibition against *Shigella* and *Salmonella* spp isolates respectively at 50 mg/ml concentration and even at 100 mg/ml concentration, the extract could only cause a 31.4.6% inhibition against *E. coli* isolates.

Table 4.9: Mean Diameters of Zones of Inhibition of Extracts and Standard Antibiotics on *Shigella* spp. Stool Isolates

	Diameter of zones of Inhibition (mm) at concentration of;	
Antibacterial Agents	**25 mg/ml**	**50 mg/ml**
ESBE	14.0±2.8	16.0±2.7
ASBE	14.4±2.4	17.9±0.8
ERBE	15.6±1.2	17.6±1.1
ARBE	15.6±1.2	17.4±1.3
GENTAMICIN (10 µg)	23.2±6.4	23.2±4.5
OFLOXACIN (5 µg)	17.8±1.0	17.8±0.9

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

Table 4.10: Susceptibility of Isolates to Different Concentrations of Gentamicin

	% Distribution		
MIC Values (mg/ml)	***E. coli*** **(n =51)**	***Salmonella*** **spp. (n = 23)**	***Shigella*** **spp. (n =5)**
0.78	0.0	0.0	0.0
1.56	47.1	4.3	100
3.12	13.7	17.4	0.0
6.25	9.8	13.0	0.0
12.5	7.8	17.4	0.0
25.0	3.9	0.0	0.0
50.0	5.9	26.4	0.0
>50.0	11.8	21.7	0.0

Table 4.11: Susceptibility of Isolates to Different Concentrations of Ofloxacin

MIC **Values (mg/ml)**	**% Distribution**		
	E. coli **(n=51)**	***Salmonella* spp. (n=23)**	***Shigella* spp. (n=5)**
0.78	20.2	56.5	80.0
1.56	3.9	13.1	0.0
3.12	23.9	0.0	20.0
6.25	5.9	8.7	0.0
12.5	0.0	0.0	0.0
25.0	0.0	0.0	0.0
50,0	2.0	8.7	0.0
>50.0	43.1	13.0	0.0

Table 4.12: Susceptibility of Isolates to Different Concentrations of Ethanolic Stem Bark Extract

	% Distribution		
MIC Values (mg/ml)	***E. coli*** **(n=51)**	***Salmonella*** **spp. (n=23)**	***Shigella*** **spp. (n=5)**
0.78	0.0	0.0	0.0
1.56	0.0	0.0	0.0
3.12	0.0	0.0	0.0
6.25	0.0	4.3	0.0
12.5	0.0	21.7	20.0
25.0	0.0	39.1	20.0
50.0	9.8	30.4	60.0
100	21.6	4.3	0.0

Table 4.13, presents the percentage distribution of susceptibility pattern of *E. coli*, *Salmonella* spp. and *Shigella* spp. isolates at different concentrations of aqueous stem bark extracts. As shown in the table, *E. coli* was not inhibited at all the tested concentrations (0.78-100 mg/ml), as against 78.3% of isolates of *Salmonella* spp. at MIC of 50 mg/ml, and 100% of *Shigella* isolates at 6.25 mg/ml.

The percentage distribution of the *E. coli*, *Salmonella* and *Shigella* spp. at different MIC values of ethanolic root bark extract is presented in Table 4.14. As shown in the table, only 5.9% of *E. coli* isolates were inhibited at the MIC value of 100 mg/ml, whereas at MIC value of 50 mg/ml, up to 60.8%of *Salmonella* isolates were inhibited. The five *Shigella* isolates had MIC value of 6.25 mg/ml. Generally, the extract was more active against the *Shigella* isolates.

Data presented in Table 4.15, shows the percentage distributions of susceptibility patterns of *E. coli*, *Salmonella* and *Shigella* spp. at different MIC values of the aqueous root bark extract. *E. coli* is still the least susceptible among the test organisms with only 3.9% of the 51 isolates being inhibited at 50 mg/ml concentration. Only 26.1% of *Salmonella* isolates had MIC value of 100 mg/ml, while all the *Shigella* spp isolates were inhibited at 25 mg/ml.

Table 4.16, shows the percentage distribution of resistant *E. coli* and *Salmonella* spp. at different MIC values, n-Butanol fraction inhibited all the *E. coli* at MIC value of 25 mg/ml, whereas all the *Salmonella* spp. were inhibited at MIC value of 6.25 mg/ml. However, the ethyl acetate fraction inhibited all the *E. coli* isolates at MIC value of 6.25 mg/ml, whereas all the *Salmonella* spp. were inhibited at even MIC value of 1.56 mg/ml.

The susceptibility profiles of the ethyl acetate and n-butanol fractions of the ESBE against selected resistant isolates of *E. coli* and *Salmonella* is shown in Table 4.17. As shown in the table, among the 6 *E. coli* resistant isolates, only two isolates (No. 55 and 67) which were not susceptible to ofloxacin and gentamcin were inhibited by the fractions. The diameters of zones of inhibition were significantly higher than the corresponding crude extracts. The four selected *Salmonella* isolates, which were also resistant to gentamicin and ofloxacin were also susceptible to the inhibitory action of the fractions. Generally, the ethyl acetate fraction gave higher diameters of zones of inhibition against the selected test organisms.

Table 4.13: Susceptibility of Isolates to different concentrations of Aqueous Stem Bark Extract

	% Distribution		
MIC Values (mg/ml)	***E. coli*** **(n=51)**	***Salmonella*** **spp. (n=23)**	***Shigella*** **spp. (n=5)**
0.78	0.0	0.0	0.0
1.56	0.0	0.0	0.0
3.12	0.0	0.0	0.0
6.25	0.0	0.0	100
12.5	0.0	0.0	0.0
25.0	0.0	26.1	0.0
50.0	0.0	52.2	0.0
100	0.0	21.7	0.0

Table 4.14: Susceptibility of Isolates to different concentrations of Ethanolic Root Bark Extract

	% Distribution		
MIC Values (mg/ml)	***E. coli*** **(n=51)**	***Salmonella*** **spp (n=23)**	***Shigella*** **spp (n=5)**
0.78	0.0	0.0	00
1.56	0.0	0.0	0.0
3.12	0.0	0.0	0.0
6.25	0.0	0.0	100
12.5	2.0	0.0	0.0
25.0	0.0	8.7	0.0
50.0	2.0	52.1	0.0
100	3.9	8.7	0.0

Table 4.15: Susceptibility of Isolates to different concentrations of Aqueous Root Bark Extracts

	% Distribution		
MIC Values (mg/ml)	***E. coli*** **(n=51)**	***Salmonella*** **spp (n=23)**	***Shigella*** **spp (n=5)**
0.78	0.0	0.0	0.0
1.56	0.0	0.0	0.0
3.12	0.0	0.0	0.0
6.25	0.0	0.0	40.0
12.5	0.0	0.0	20.0
25.0	0.0	0.0	40.0
50.0	3.9	0.0	0.0
100	0.0	26.1	0.0

Table 4.16: Susceptibility of Some Resistant Isolates to ESBE Fractions (mg/ml)

	% Distribution			
	n-BF.		EAF	
MIC Values	***E. coli*** **(n=6)**	***Sal.* spp.** **(n=4)**	***E. coli*** **(n=6)**	***Sal.* spp.** **(n=4)**
0.78	0.0	0.0	0.0	0.0
1.56	0.0	0.0	0.0	0.0
3.12	3.3	15.0	16.7	25.0
6.25	16.7	60.0	66.7	75.0
12.5	33.3	25.0	16.7	0.0
25.0	I6.7	0.0	0.0	0.0
50.0	30.0	0.0	0.0	0.0

KEY;

n-BF =n-Butanol Fraction,

EAF =Ethyl Acetate Fraction,

ESBE= Ethanolic Stem bark Extract

Table 4.17: Diameter of Zones of Inhibition of some selected resistant *Escherichia coli* and *Salmonella* spp isolates against n-butanol, Ethyl acetate Fraction of ASBE and reference Antibiotics

		% Diameter of Zones of Inhibition (mm) at different fractions and concentrations					
		n-BF		EAF		GEN	OFL
Organisms	**Isolate no.**	**25 mg/ml**	**50 mg/ml**	**25 mg/ml**	**50 mg/ml**	**10 µg**	**5 µg**
***E. coli* (n=6)**	7	0	0	10	12	14	0
	53	0	0	0	0	0	0
	55	20	21	21	24	0	0
	63	0	0	0	0	12	0
	65	0	0	0	0	12	0
	67	20	20	21	22	0	0
***Sal.* spp (n=4)**	44	23	26	24	28	0	0
	64	16	16	26	27	0	0
	81	16	16	24	25	0	0
	84	18	20	24	25	0	0

KEY: ASBE =Aqueous Stem bark Extracts
NBF =n-Butanol Fraction
EAF =Ethyl Acetate Fraction
GEN=Gentamicin
OFL=Ofloxacin

The susceptibility profiles of the ethyl acetate and n-butanol fractions of the ESBE against some *E. coli* and *Salmonella* resistant isolates are shown in Table 4.18. Among the six *E. coli* resistant three isolates, (No. 53, 55 and 67) which were not susceptible to gentamicin and ofloxacin were all inhibited by the two fractions. Furthermore, the diameters of zones of inhibition were significantly higher than the corresponding crude extracts. Also, among the four (4) selected *Salmonella* spp isolates three (3) of which were also resistant to the reference antibiotics (gentamicin and ofloxacin) were also highly susceptible to the inhibitory action of the fractions of the crude extracts. Generally, the ethyl acetate fraction gave higher diameters of zones of inhibition.

Data presented in Table 4.19 showed that, the extracts were largely bacteriostatic unable to kill all the *E. coli* isolates at concentration as much as 100 mg/ml.

The distribution of the isolates at different minimum bactericidal concentration of the test extracts against *Salmonella* spp isolates is depicted in Table 4.20. The data in the table showed that, at 100 mg/ml concentration of ESBE and ASBE, 69.5% and only 17.6% of the *Salmonella* spp isolates were killed respectively. Similarly, the ethanol and aqueous root bark extract exerted bactericidal activity on only 26% of the isolates at 100 mg/ml concentration.

Data presented in Table 4.21, represent the percentage distribution of Minimum Bactericidal Concentration of *F. sycomorus* extracts against *Shigella* isolates. As shown in the table, at 100 mg/ml concentration of ESBE, ERBE and ARBE only 60%, 40% and 40% of the isolates were killed respectively. In contrast bactericidal activity of ASBE was exerted against all the isolates at concentration greater than 100 mg/ml.

Table 4.18: Diameter of Zones of Inhibition of some selected resistant E*scherichia coli* and *Salmonella* isolates against n-Butanol, Ethyl acetate fractions of ESBE and reference Antibiotics

		% **Diameter of Zones of Inhibition (mm) at different fractions and concentrations**					
		n-BF		EAF		GEN	OFL
Organisms	**Isolate no.**	**25 mg/ml**	**50 mg/ml**	**25 mg/ml**	**50 mg/ml**	**10 µg**	**5 µg**
E. coli (n=6)	7	0	0	13	15	12	0
	53	16	18	17	18	0	0
	55	18	19	20	21	0	0
	63	13	13	15	16	14	0
	65	13	15	14	16	12	0
	67	17	19	19	21	0	0
Sal. Spp *(n=4)*	44	12	15	17	20	20	0
	64	16	19	18	22	0	0
	81	18	21	20	23	0	0
	84	17	19	22	26	0	0

KEY:

ESBE= Ethanolic Stem bark Extract, NBF =n-Butanol Fraction, EAF =Ethyl Acetate Fraction, GEN=Gentamicin, OFL=Ofloxacin

Table 4.19: Percentage distribution of *E. coli* isolates at different Minimum Bactericidal Concentration of the *Ficus sycomorus* (n=51)

	Proportion of Isolates			
MBC values mg/ml	**ESBE**	**ASBE**	**ERBE**	**ARBE**
0-25	0.0	0.0	0.0	0.0
26-50	0.0	0.0	0.0	0.0
51-100	3.9	0.0	2.0	0.0
>100	100	100	98.0	96.1

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

Table 4.20: Percentage distribution of *Salmonella* spp. isolates at different Minimum Bactericidal Concentration of the *Ficus sycomorus* extracts (n=23)

	Proportion of Isolates			
MBC values mg/ml	**ESBE**	**ASBE**	**ERBE**	**ARBE**
0-25	4.3	0.0	0.0	0.0
26-50	17.4	13.0	13.0	0.0
51-100	47.8	4.3	13.0	26.1
>100	30.4	82.6	74.0	73.9

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

Table 4.21: Percentage distribution of *Shigella* spp. isolates at different Minimum Bactericidal Concentration of the *Ficus sycomorus* extracts (n=5)

	Proportion of Isolates			
MBC value mg/ml	ESBE	ASBE	ERBE	ARBE
0-25	0.0	0.0	0.0	0.0
26-50	40.0	0.0	20.0	0.0
51-100	20.0	0.0	20.0	40.0
>100	40.0	100	60.0	60.0

KEY:

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

Fig. 4.1: compares the antibacterial activities between the crude extracts of *Ficus sycomorus* plant with that of reference antibiotics (gentamicin and ofloxacin) against E. *coli* isolates. As depicted in the figure, there is a statistically significant differences between the activity of the two antibiotics and the four different crude extracts (ESBE, ASBE, ERBE and ERBE) i.e. $P<0.05$.

A comparism of antibacterial activities between the two standard antibiotics and that of crude extracts of *F. sycomorus* plant against the *Shigella* spp. stool isolates is depicted in Fig. 4.2: As shown, a statistically significant difference was observed between their activities ($P< 0.05$).

Fig. 4.4: compares the antibacterial activities between the crude extracts of *Ficus sycomorus* plant with that of reference antibiotics (gentamicin and ofloxacin) against *Salmonella* spp. isolates. As depicted in the figure, there is no statistically significant differences between the activity of the two antibiotics and the three different crude extracts of ESBE, ASBE and ERBE respectively ($P>0.05$).

A comparism of antibacterial activities of the n-Butanol fraction, ethyl acetate fraction and that of reference antibiotics against one of the resistant *E. coli* stool isolates is shown in Fig. 4.5. As depicted in the figure, there is a statistical significant differences between the activities of reference antibiotics and that of the fractions of ethanolic stem bark extract ($P<0.05$).

Fig. 4.6: compares the antibacterial activities of the n-Butanol fraction, ethyl acetate fraction and that of reference antibiotics against one of the resistant *Salmonella* spp. stool isolates. As shown in the figure, a statistically significant difference was observed between the activity of the fractions and the reference antibiotics ($P<0.05$).

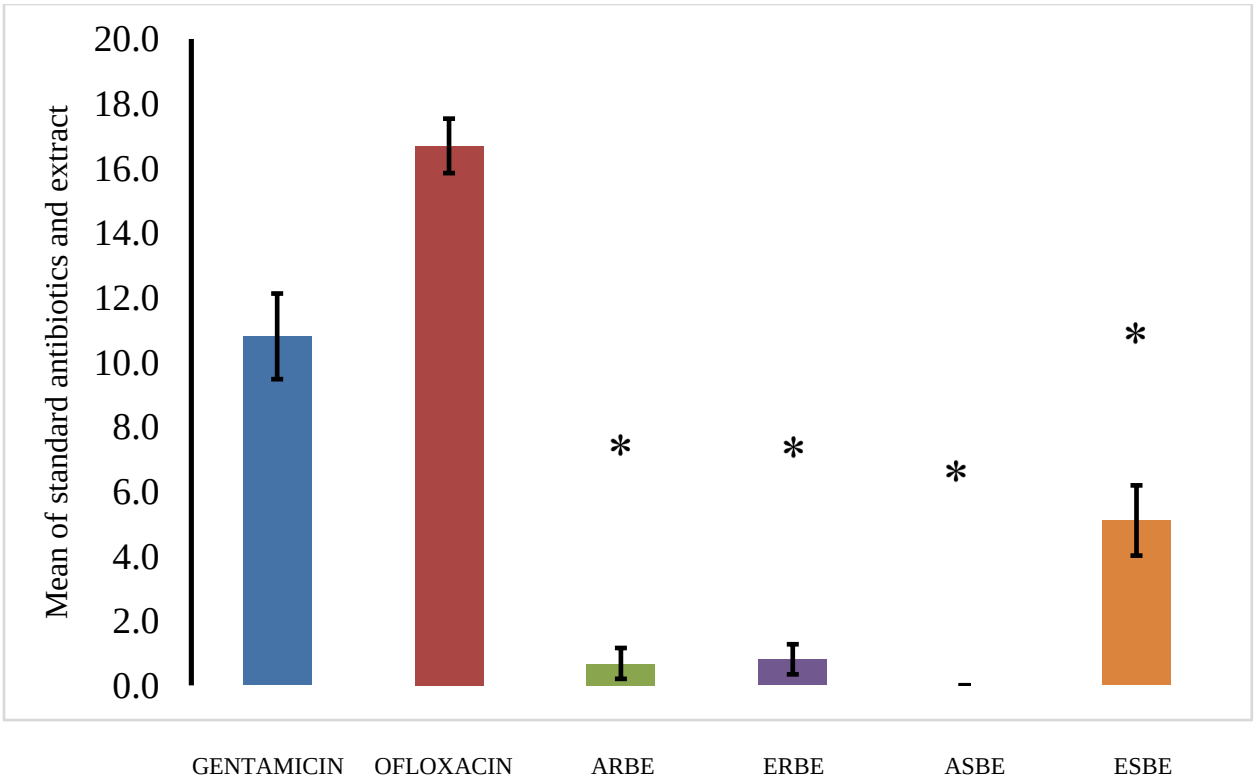

Fig. 4.1: comparison of the mean Diameter of Zones of Inhibition of 50 mg/ml crude extracts, Gentamicin (10 µg) and Ofloxacin (5 µg) on *Escherichia. coli* stool isolates (P<0.05)

KEY:

* = statistically significant difference

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

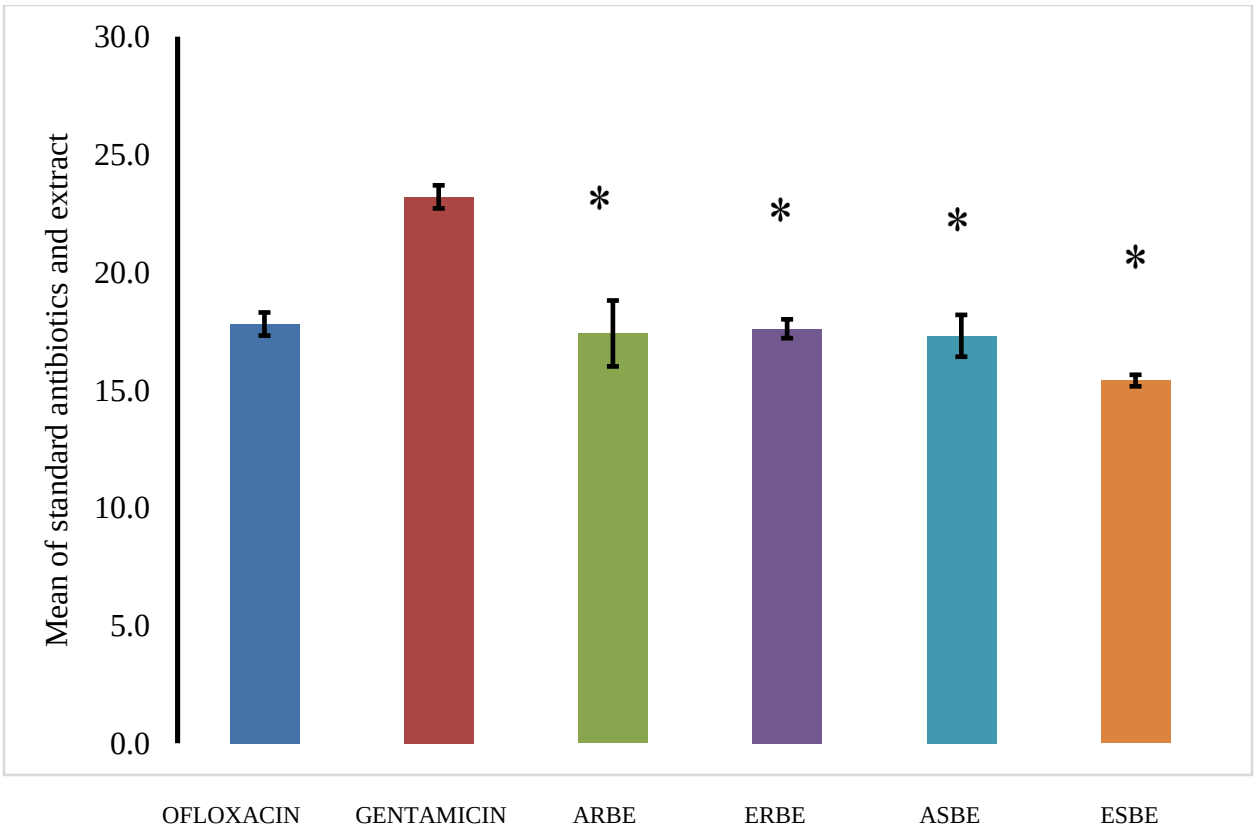

Fig. 4.2: Comparison of the Mean Diameters of Zones of Inhibition of 50 mg/ml crude extracts, Gentamicin (10 µg) and Ofloxacin (5 µg) on *Shigella* spp. stool isolates ($P<0.05$)

KEY:

* = statistically significant difference

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

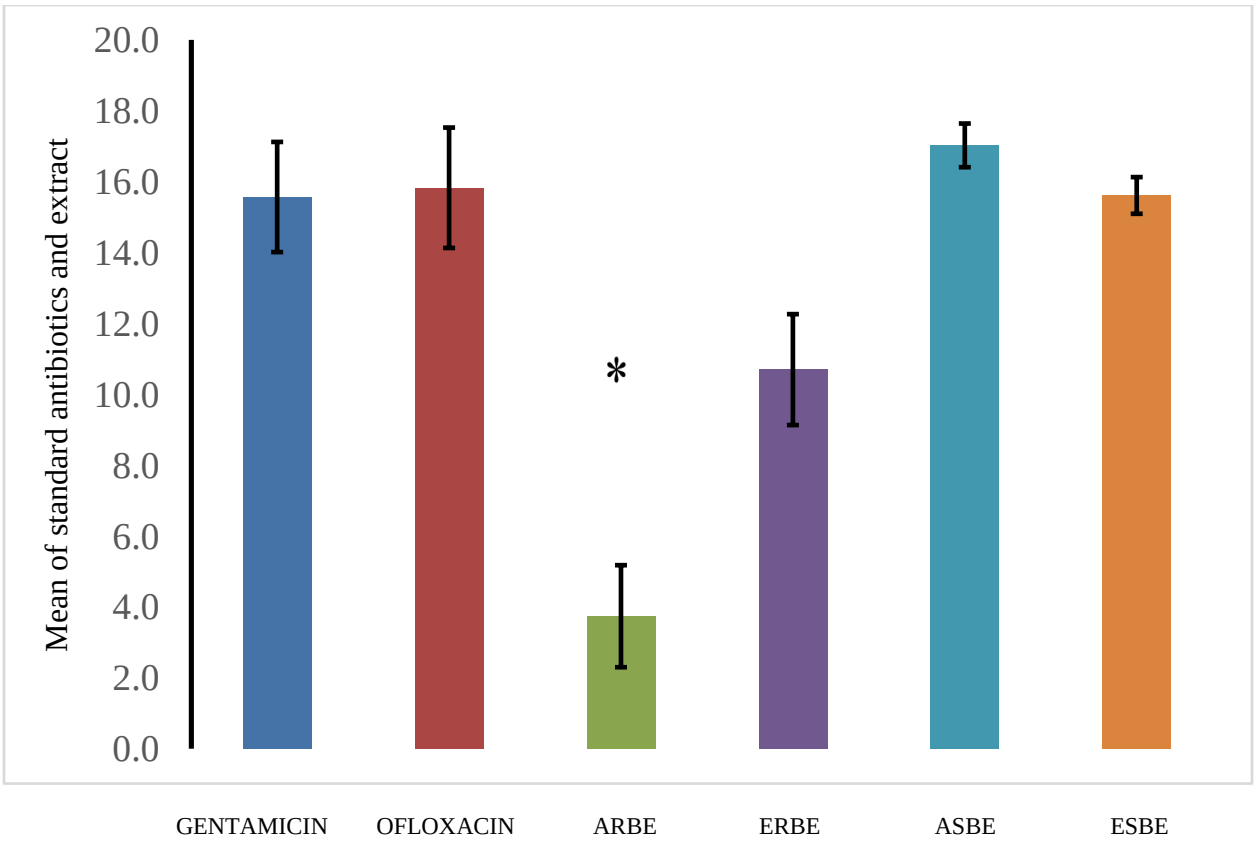

Fig Gentamicin (10 µg) and Ofloxacin (5 µg) on *Salmonella* spp. stool isolates (P>0.05) 4.3: Comparison of the Mean Diameter of Zones of Inhibition of 50 mg/ml crude extracts

KEY:

* = statistically significant difference

ESBE = Ethanolic Stem Bark Extract

ERBE = Ethanolic Root Bark Extract

ASBE = Aqueous Stem Bark Extract

ARBE = Aqueous Root Bark Extract

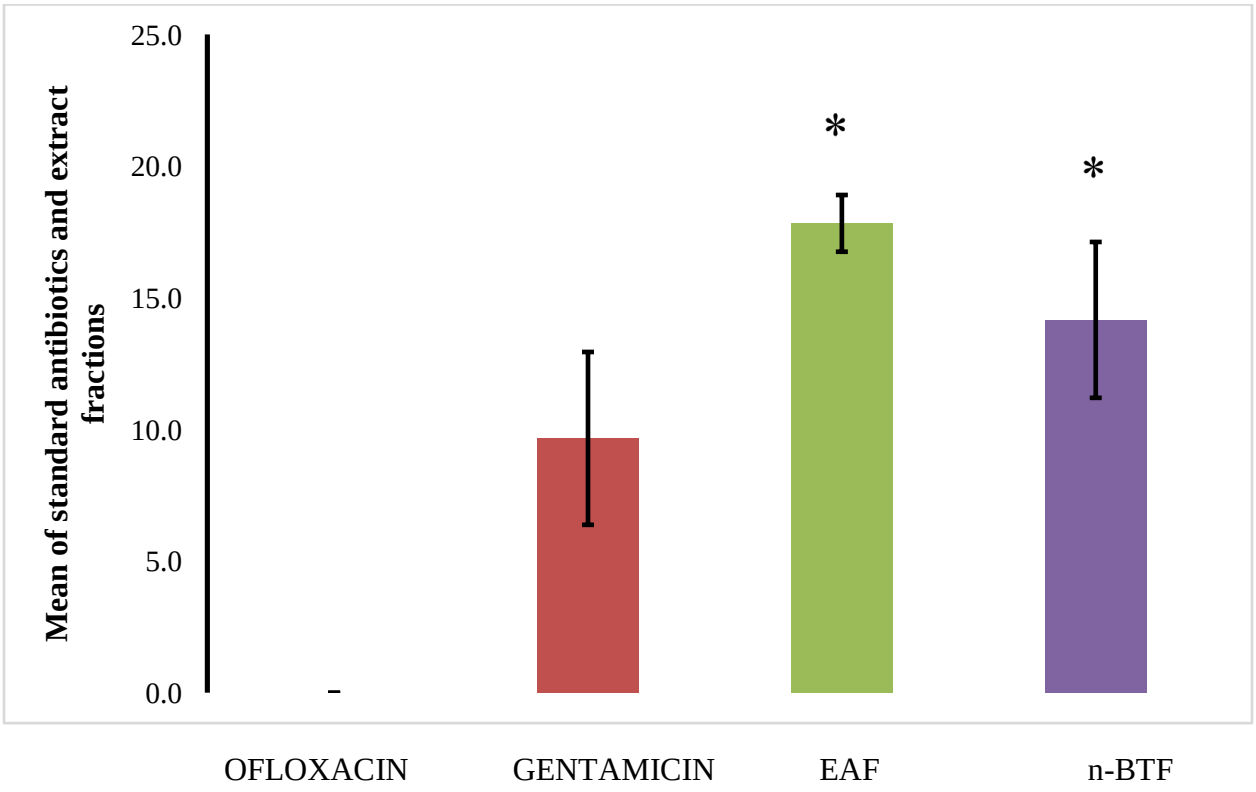

Fig. 4.4: Comparison of the Mean Diameter of Zones of Inhibition of 50 mg/ml each of n-Butanol, Ethyl acetate extracts fractions of ESBE, Gentamicin (10 µg) and Ofloxacin (5 µg) on resistant *E. coli* stool isolates ($P<0.05$)

KEY:

* = statistically significant difference

EAF= Ethyl acetate Fraction

n-BTF= n-Butanol Fraction

ESBE = Ethanolic Stem Bark Extract

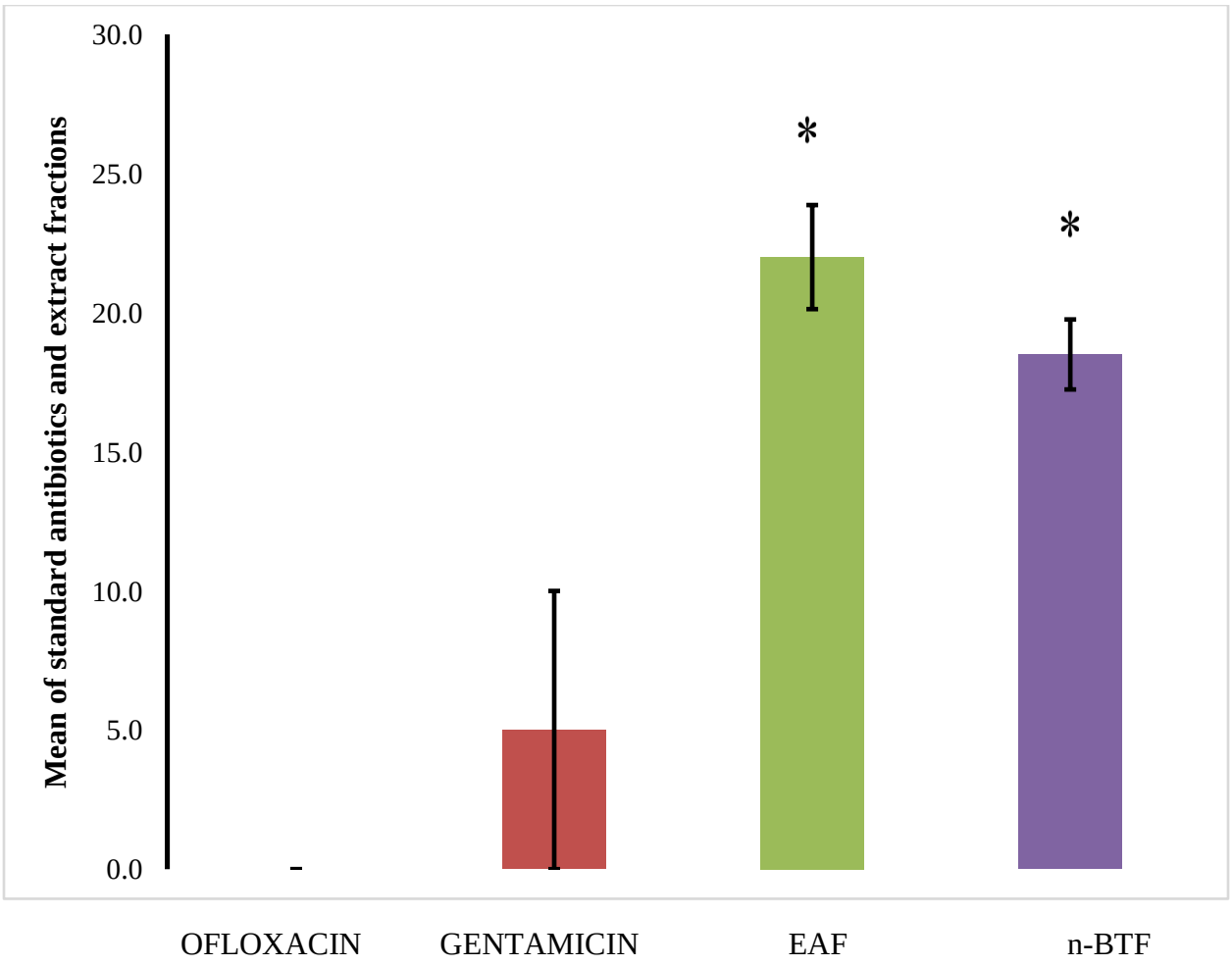

Fig. 4.5: Comparison of the Mean Diameter of Zones of Inhibition of 50 gm/ml of each n-Butanol, Ethyl acetate extracts fractions of ESBE, gentamicin (10 µg) and ofloxacin (5 µg) on resistant *Salmonella* spp. stool isolates ($P<0.05$)

KEY:

* = statistically significant difference

EAF= Ethyl acetate Fraction

n-BTF= n-Butanol Fraction

ESBE = Ethanolic stem bark Extract

4.6 Rate of kill Studies on the Survival of Organisms to Ethyl acetate Fraction and Gentamicin Concentrations

The growth curves showed that, the test organisms are able to grow in the media. Ethyl acetate fraction and gentamicin shows a varying degree of inhibitory activities against the selected isolates. Generally, it was observed that, there was an initial slow decrease in cell population followed by rapid cell death in cell population in the curves.

The survival/death curves of the susceptible *E. coli* isolate on exposure to EAF and gentamicin concentrations is shown in Fig. 4.6. As shown, in both the EAF and gentamicin, there was an initial slow decrease in cell population in the first 6hrs, followed by rapid cell death at 12hrs contact time between the agents and the *E. coli* isolates.

Fig.4.7: shows the survival curves of the resistant *E. coli* isolate on exposure to EAF and gentamicin concentrations. However, for the gentamicin, it was observed that, there was no decrease in the cell population from zero to 24hrs contact time between the gentamicin and the organism. Whereas, the EAF effect initial slow decrease in the cell population in the first 12hrs, and subsequently followed by a complete cell death after period of 24hrs.

Fig. 4.8 shows the growth curves of the susceptible *Salmonella* spp. isolate on exposure to EAF and gentamicin concentrations. As depicted in this figure, a slow and gradual decrease in cell population was observed with both EAF and gentamicin over a period of 6hrs, after which a rapid and complete killed in cell population were observed by the EAF and gentamicin at 12 and 24hrs respectively.

The survival curves of the resistant *Salmonella.* spp. isolate on exposure to EAF and gentamicin concentrations are shown in Fig.4.9. As shown, gentamicin was not effects any decrease in the cell population from zero to 24hrs contact time against *Salmonella* isolates. Whereas, the EAF exhibited initial slow decrease in the cell population in the first 12hrs and subsequently followed by complete cell death after period of 24hrs.

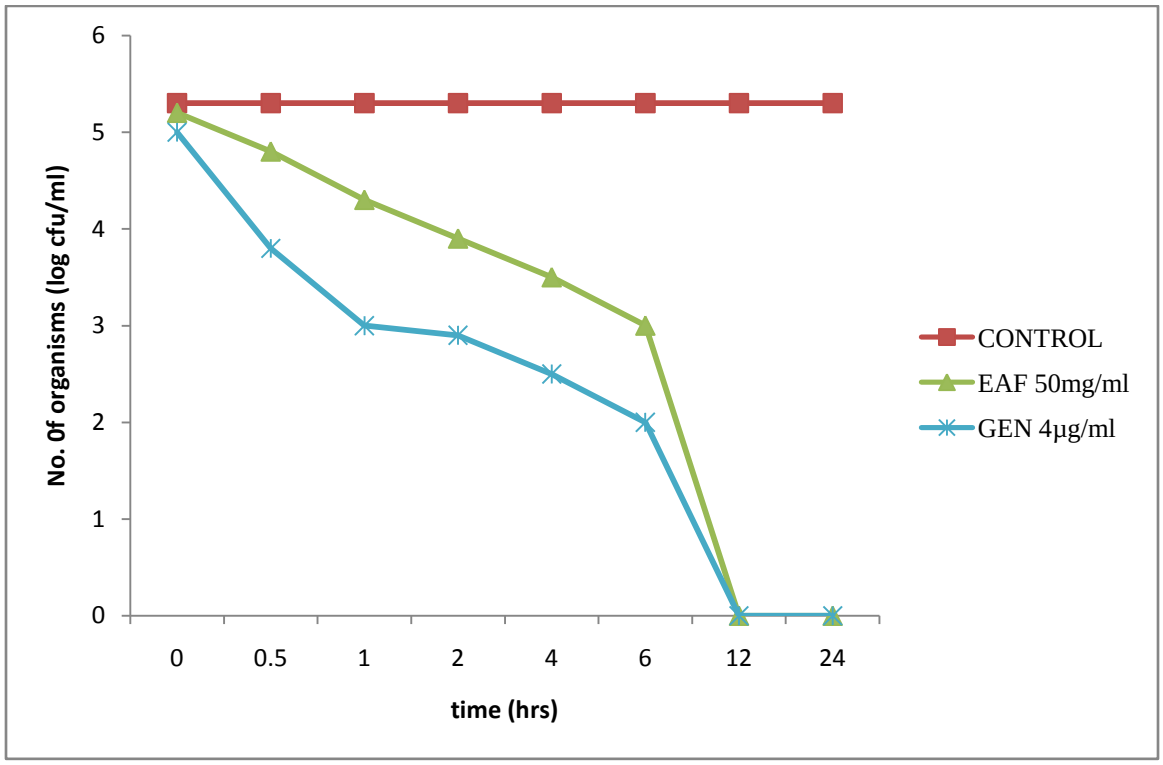

Fig.4.6: Survival curve of a susceptible *Escherichia coli* isolates on exposure to Ethyl acetate fraction and gentamicin

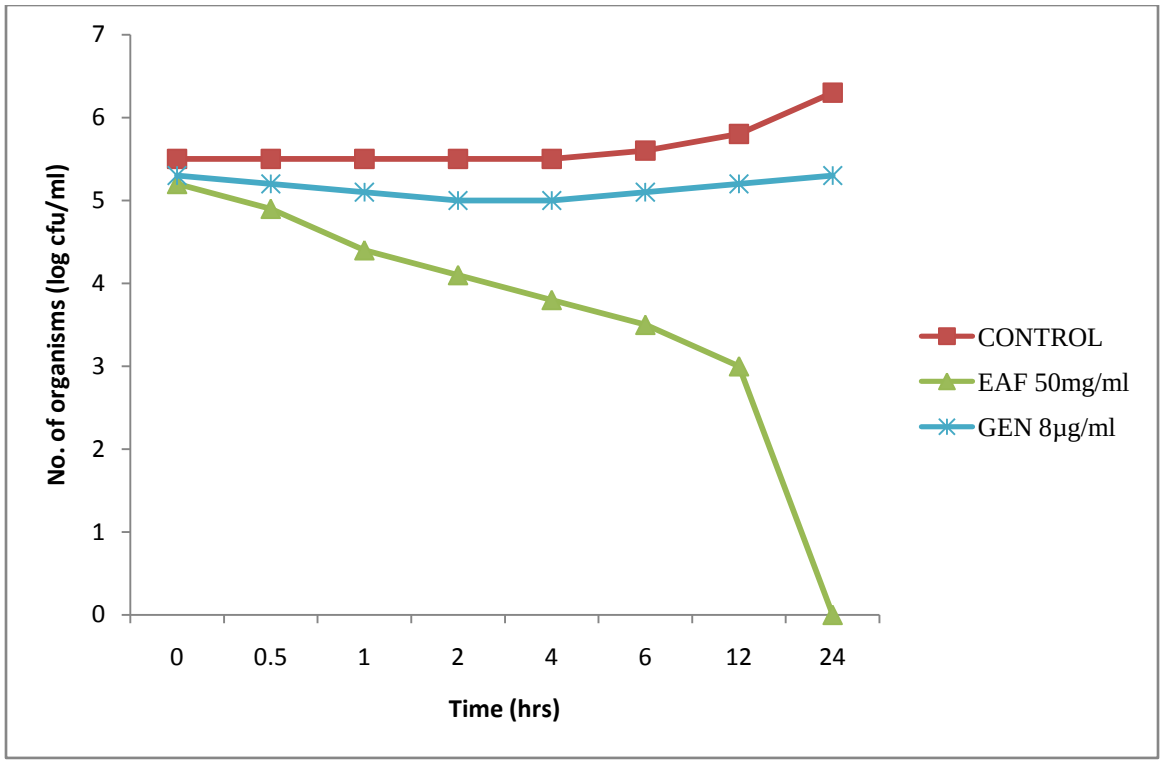

Fig. 4.7: Survival curve of a resistant *E. coli* isolate on exposure to Ethyl acetate fraction and gentamicin

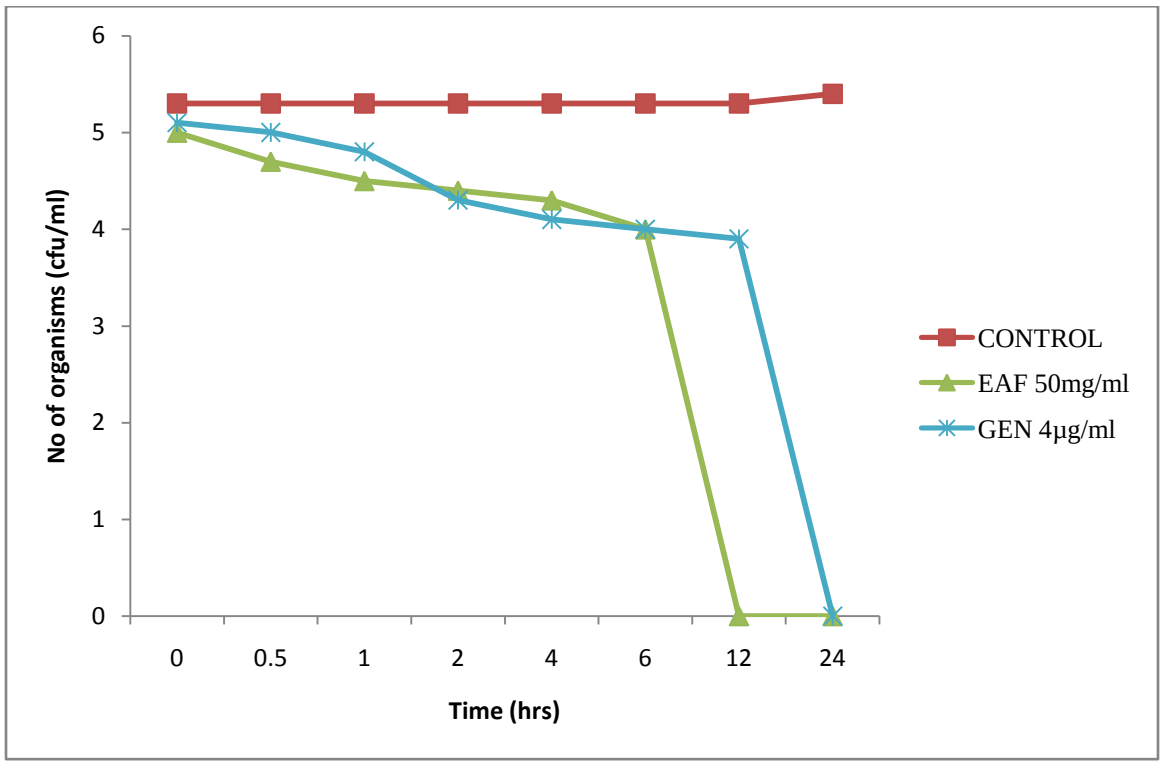

Fig. 4.8: Survival curve of a susceptible *Salmonella* spp isolate on exposure to Ethyl acetate fraction and gentamicin

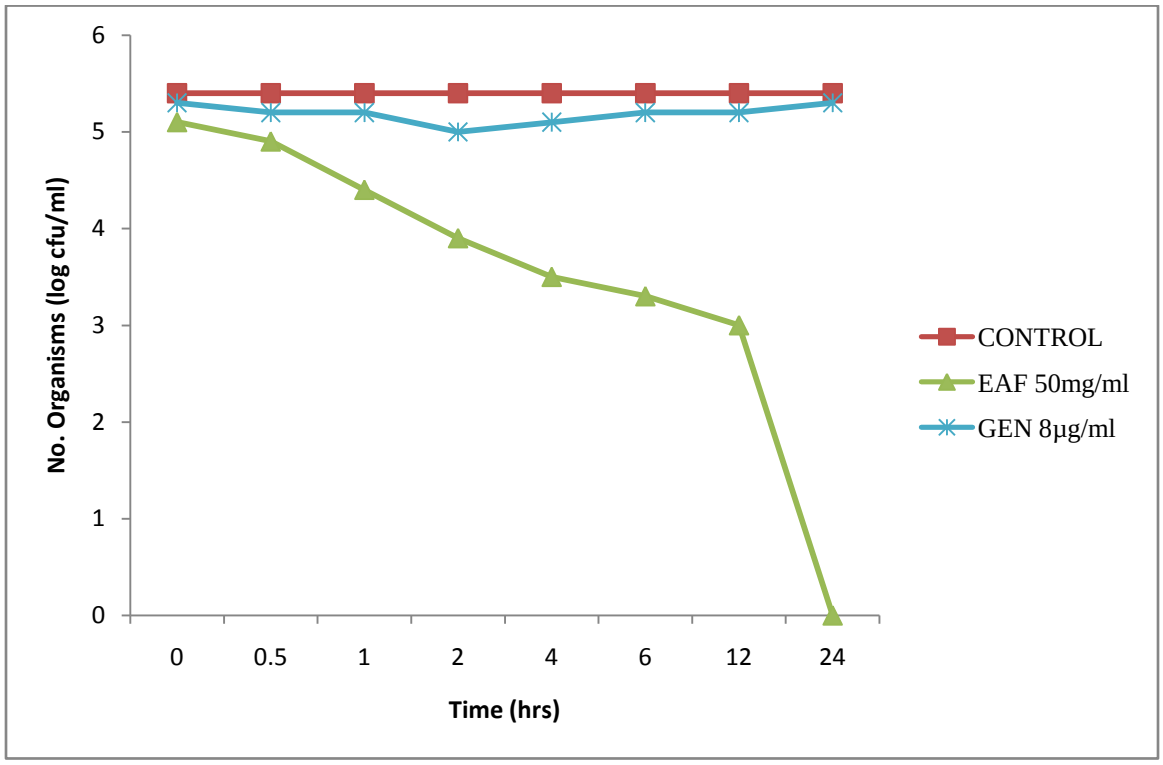

Fig.4.9: Survival curve of a resistant *Salmonella* spp isolate on exposure Ethyl acetate fraction and gentamicin

CHAPTER FIVE

5.1 Discussion

The indiscriminate use of antibiotics has led to the development of multi-drug resistant micro-organisms creating immense clinical problems in the treatment of infectious diseases including those caused by enteric bacteria (Akinyemi, 1998). Therefore, there is a need to develop alternative antimicrobial agents for the treatment of these infectious diseases. A non-antibiotic approach to the treatment and prevention of these infections includes the application of medicinal plants. For example, plant essential oils and extracts which have been used in traditional medicine to improve the quality of healthcare (Iwu *et al.*,1999). Plants are potential sources of novel antimicrobial compounds especially against bacterial pathogens (Chirag *et al*., 2012). The main objective of the present study is to evaluate the ability of the extract of *Ficus sycomorus* plant to inhibit the growth of pathogenic bacteria isolated from stool of diarrhoea patients.

The phytochemical screening of the *Ficus sycomorus* plant showed that, different solvents produced different amount of extracts, with Ethanolic stem bark extract producing a greater yield than water extract. The differences in the percentage yield might be due to the polarity of the solvents (Hassan *et al.,* 2007). The less polar solvents yielded greater quantities of the extracts compared with the more polar solvent such as water. Although, the n-butanol fraction had more yield than the ethyl acetate fraction which was exhibited less antibacterial activity when compared. This is an indication that, the ethyl acetate was able to elute more of the active ingredient than n-butanol.

All the solvent used in extraction shows the presence of; carbohydrates, tannins, alkaloids flavonoids and saponnins. The presence of these constituents have also been reported in *F. sycomorus* obtained from Sokoto and Maiduguri, Nigeria (Hassan *et al.*, 2006; Sandabe *et al.*, 2006) which have been previously reported as having antimicrobial activities (Hassan *et al.*, 2006; Ahmadu *et al.*, 2007; Kubmarawa *et al.*, 2007) Anthraquinone was absent in the solvents.

Geographical location has been reported to influence the chemical constituents of plant extracts of the same genus found in different environments. This may explain the absence of anthroquinone in *F. sycomorus* obtained in Dutse, Jigawa State,

Nigeria as against the findings of Adeshina *et al.*, (2010) on the same *F. sycomorus* collected in Zaria, Kaduna State, Nigeria. Similarly, the presence of alkaloids, flavonoids, and the absence of anthraquinone in all the extracts of *F. sycomorus* plant have also been documented in Jos and Abuja, Nigeria (Ahmadu *et al.*, 2007). It also confirms findings made by Abdullahi *et al.*, (2003).

These secondary metabolites have been variously reported to possess appreciable inhibitory activities against various organisms. For example, Saponins, flavonoids and tannins are well known to possess antimicrobial activities (Akiyama *et al.*, 2001). Flavonoids reported to be synthesized by plants in response to microbial infection have been shown to have antimicrobial activities (Kujumgiev *et al*., 1999). The presence of flavonoids in the *F sycomorus* plant could be responsible for its antidiarrhoeal activity (Bylka *et al.*, 2004). Tannins have also been reported to possessed antidiarrhoeal activities (Wangensteen *et al*., 2013).

Analysis of antibacterial activity of the plant extracts showed that, a higher percentage of the *E. coli* isolates were resistant to the action of crude extracts of *F. sycomorus* plant but susceptible to the reference antibiotics. This is in contrast to the *Salmonella* and *Shigella* spp isolates which were largely susceptible to the action of ethanolic stem bark and aqueous stem bark extracts. This is in agreement with the findings of Olusesan *et al*, (2010) who reported that, the ethanolic stem bark extract of *Ficus sycomorus* have antibacterial activity against *Salmonella* spp.

The stem bark extracts exhibited a higher antibacterial activity against the isolates than the aqueous root bark extracts. Furthermore, ethanolic extracts was also found to be more active than aqueous extracts in this study. This might be due to the polarity properties of the different solvents. Hassan *et al,* (2007) reported that, different solvent extracts of some plants to have different pharmacological properties. He further reported that, organic stem bark extracts of *F. sycomorus* exhibit a higher antimicrobial activity than root bark extracts. The variation in level of activity among the extracts could be due to the difference in solubility of active ingredient in each solvent on one hand and to the constitutional or structural variability of the tested organisms on the other hand (Adewunmi *et al.*, 2001).

The MIC results are similar to those reported by Olusesan *et al*, (2010). Generally, the ethyl acetate fraction gave higher diameters of zones of inhibition against the

selected resistant test organisms. The higher activity of the ethyl acetate fraction might be due to the presence of tannins which have been reported to exert antibacterial activity against a large number of bacteria due to their ability to complex with extracellular and soluble proteins such as enzymes involved in active transport across the cell membrane (Tsuchia *et al.,* 1996).

The MBC results is similar to those reported by Lorentz (2008) and showed that, the extracts were largely bacteriostatic, unable to kill all the *E. coli* isolates at concentration as much as 100 mg/ml, while a higher proportion of *Salmonella* and *Shigella* spp. were inhibited by the action of ESBE. This signifies that, ethanolic extract exhibit a higher antibacterial activity than its corresponding aqueous extract. This is in agreement with the report of Hassan *et al,* (2007).

Analysis of biocidal activity of *Ficus sycomorus* fractions showed an initial slow decreased in cell population in the first 1-6 hours of contact between the organisms and the ethyl acetate fraction followed by rapid cell death in cell population. Similar trend has been reported by Adeshina *et al,* (2010). This might be due to the action of tannin preceding the flavonoids action i.e. coagulation of the cell wall protein and then the release of cytoplasmic poison (Adeshina *et al.,* 2010). Garret and Brown (2001) reported that, there was no single concentration of an antimicrobial agent at which all cells in a suspension will be killed instantaneously. Killing of cells occur chiefly as a function of time within a range of concentrations and this probably explains the increased lethal activity of higher concentration of these antimicrobial agents above the minimal biocidal concentration.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The study assessed the susceptibility profiles of some bacteria isolated from stool of diarrhoea patients to the stem and root barks extracts of *Ficus sycomorus* Linn in tertiary institution at Mohammed Abdullahi Wase Specialist Hospital Kano, Nigeria revealed that:

- The commonly implicated organism among various stool sample analyzed was also the *E. coli* followed by *Salmonella* and *Shigella* spp in decreasing frequencies.
- Most of the isolates of *E. coli* were not susceptible to the crude extracts of *F. sycomorus* plant.
- Isolates of *Salmonella* spp were most susceptible organisms to the extracts and reference antibiotics followed by *Shigella* spp .
- Stem bark extract exerts a significant inhibitory activity against the tested organisms isolated from diarrhoea stool than the root bark of the plant.
- Ethyl acetate and n-Butanol fractions of *F. sycomorus* exhibited a significant antibacterial activity against both the resistant isolates of *Salmonella* and *E. coli.*

6.2 Conclusion

- Extracts of *F. sycomorus* particularly ethyl acetate fraction possess a significant antibacterial activities that can be used in the treatment of diarrhoea infections
- The study therefore, explained the rationale for the use of extract of *F. sycomorus* by herbalist in the treatment of diarrhoeal infections.

6.3 Recommendations

i. Step should be taken to encourage cultivation and conservation of the *F. sycomorus* plant for its medicinal uses . .

ii. Long term toxicity studies should be evaluated on the extracts before use.

iii. The extracts especially EAF should be formulated into appropriate dosage form (tabs and suspension) for use in the treatment of diarrhoeal infections.

iv. Extensive research in the area of isolation and characterization of the active principles of these plants are required so that better, safer and cost effective drugs for treating bacterial infections can be developed.

6.4 Contribution to Knowledge

i. Ethyl acetate fractions of the root and stem bark extracts of *F. sycomorus* plant possess more significant inhibitory activities against diarrhoeal bacterial isolates compared with the water and ethanolic extracts.

ii. *E. coli* and *Salmonella* spp diarrhoeal isolates resistant to the traditional antibiotics were readily susceptible to the inhibitory activities of the ethyl acetate fractions.

iii. Result of this study read credence to the usefulness of this plant as an antidiarrhoeal agent by the herbalists.

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APPENDIX I

KANO STATE
HOSPITALS MANAGEMENT BOARD
BOARD HEADQUARTERS
P.M.B 3540, POST OFFICE ROAD, KANO

HOD Records.
Pls allow him to conduct research as approved

HMB/GEN/488/II

13/04/2012
(22/05/1433AH)

ABDULLAHI MUSA
Department of Pharmaceutical Science,
Ahmadu Bello University, Zaria.

PROVISIONAL ETHICAL CLEARANCE

Sequel to your application to conduct research titled "SUSCEPTIBILITY PROFILE OF SOME BACTERIA CAUSING DIARRHOEA ISOLATED FROM STOOL TO THE STEM AND ROOT BARKS EXTRACTS OF Ficus sycomorus linn" in MMSH and MAWSH. In light of the above, I am mandated to convey provisional clearance to proceed on your study based on the following conditions.

I. That you should liase with the management of the facility of your focus for appropriate guidance.

II. That any publication related to the study should be brought to the knowledge of the ethical committee for approval.

III. That a copy of your finding should be submitted for documentation, record and final approval, please.

Best wishes.

NAZIR AHMED YA'U
SENIOR ASSISTANT SECRETARY
FOR: EXECUTIVE SECERTARY.

APPENDIX II

Phytochemical Analysis of *Ficus sycomorus*

TEST	APPEARANCE	INFERENCE			
		ESBE	ASBE	ERBE	ARBE
1.FLAVONOIDS a. Shinoda test	Appearance of an orange, pink or red to purple color was obtained	+	+	+	+
2. ALKALOID a. Dragendoff reagent	Formation of yellow to brown precipitate	+	+	+	+
b. Mayer's reagent	Formation of white to yellowish or Green color precipitate	+	+	+	+
c. Wagner's reagent	Formation of brown or reddish brown precipitate	+	+	+	+
3.TANNINS Ferric Chloride	Blue mixture was obtained in ethanol and ethyl acetate. Dirty green coloration was obtained in water extract.	+	+	+	+
4.SAPONIN Frothing test	Frothing which persisted for 15 minutes	+	+	+	+
5.CARBOHYDRATE TEST a. Molisch test	Appearance of reddish color is observed	+	+	+	+
b. Fehling's test for reducing sugar	Appearance of brick red precipitate	+	+	+	+
6. ANTHRAQUINONES Free anthraquinones	Absence of pink, red or violet colour in the ammoniacal layer is observed	-	-	-	-

APPENDIX III

Death/Survival of Susceptible *E. coli* Isolates on exposure to Ethyl acetate Fraction and Gentamicin

Extract/Drug	Viable count or organisms at different time interval (in hours)							
	Zero	0.5	I	2	4	6	12	24
CONTROL	2000	2000	2000	2000	2000	2000	2000	2000
EAF(50 mg/ml)	2000	1864	1800	1764	1600	1500	0	0
GEN(4 µg/ml)	2000	1746	1600	1600	1324	1224	0	0

KEY:

EAF=Ethyl acetate Fraction

GEN=Gentamicin,

APPENDIX IV

Death/Survival of Resistant *Echerichia coli* Isolate on Exposure to Ethyl Acetate Fraction and Gentamicin

Extract/Drug	Viable count or organisms at different time interval (in hours)							
	Zero	0.5	I	2	4	6	12	24
CONTROL	3500	3500	3500	3500	3500	3500	3500	3500
EAF (50 mg/ml)	3500	3224	3000	2824	2700	2624	2424	0
GEN (8 µg/ml)	3500	3500	3500	3624	3664	3700	3754	3774

KEY:

EAF=Ethyl acetate Fraction

GEN=Gentamicin

APPENDIX V

Death/Survival of Susceptible *Salmonella* spp. Isolates on Exposure to Ethyl Acetate Fraction and Gentamicin

Extract/Drug	Viable count or organisms at different time interval (in hours)							
	Zero	0.5	I	2	4	6	12	24
CONTROL	3500	3500	3500	3500	3500	3500	3600	3654
EAF (50 mg/ml)	3500	3500	3000	2824	2700	2626	2424	0
GEN(4 µg/ml)	3500	3500	3500	3624	3664	3700	3754	3774

KEY:

EAF=Ethyl acetate Fraction

GEN=Gentamicin

APPENDIX VI

Death/Survival of Resistant *Salmonella* spp. Isolates on Exposure to Ethyl Acetate Fraction and Gentamicin

Extract/Drug	Viable count or organisms at different time interval (in hours)							
	Zero	0.5	I	2	4	6	12	24
CONTROL	3000	3000	3000	3000	3000	3000	3000	3000
EAF (50 mg/ml)	3000	3000	2840	2724	2626	2500	2424	0
GEN (8 µg/ml)	3000	3000	2800	3000	3064	3054	3200	3254

KEY: EAF=Ethyl acetate Fraction, GEN=Gentamicin, MIC=Minimum Inhibitory Concentration

APPENDIX VII

BIOCHEMICAL TEST RESULTS OF THE ISOLATES

Sam. No.	Lab no.	Sex	Age	Sample	Urea	VP	M.R.	CITRATE	INDOL	FERMT.	Slope	Butt	H_2s	Gas	CAT. TEST	LAC. TEST	G.S.	ORG ISLTD
1	436	M	18M	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
2	433	M	3Y	NO GROWTH														
3		M	6M	stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
4	335	F	2Y	NO GROWTH														
5	441	M	2Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Absent	positive	Negative	GNRB	*Sal.* Spp.
6	442	M	2M	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
7	443	F	13M	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
8	446	M	IY	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Present	positive	Negative	GNRB	*Sal.* Spp.
9	447	M	8M	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
10	450	F	1.5Y	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
11	452	F	2Y	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
12	444	M	24Y	Stool	NO GROWTH													
13	455	M	6M	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
14	453	M	16M	Stool	NO GROWTH													
15	454	M	31Y	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
16	460	F	60Y	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
17	461	M	2Y	Stool	NO GROWTH													
18	462	M	2M	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
19	464	F	20M	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
20	465	F	11Y	Stool	NO GROWTH													
21	467	F	19Y	Stool	NO GROWTH													
22	473	M	9M	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
23	474	M	4Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Sal.* Spp.
24	475	M	30Y	Stool	NO GROWTH													
25	476	F	1Y	Stool	Neg.	Neg.	positive	Negative	positive	LF	Red	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
26	480	M	1Y	Stool	Neg.	Neg.	positive	Negative	positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
27	481	M	1Y	Stool	Neg.	Neg.	positive	Negative	positive	LF	Red	yellow	Absent	Present	positive	positive	GNRB	*E. coli*

28	486	M	1Y	Stool	NO GROWTH													
29	488	M	3M	Stool	NO GROWTH													
30	494	M	10M	Stool	NO GROWTH													
31	507	F	3Y	Stool	NLF	Neg.	positive	Negative	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Shig* spp
32	503	M	3Y	Stool	NO GROWTH													
33	515	M	2Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
34	516	M	3M	Stool	Neg.	Neg.	positive	Negative	Negative	LF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Shig* spp
35	517	F	2M	Stool	Neg.	Neg.	positive	Negative	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Shig* spp
36	518	F	9M	Stool	Neg.	Neg.	positive	Negative	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Shig* spp
37	519	M	20y	Stool	NO GROWTH													
38	522	F	1Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
39	520	M	2Y	Stool	NO GROWTH													
40	516	F	65Y	Stool	NO GROWTH													
41	527	M	7Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
42	531	F	6M	Stool	NO GROWTH													
43	536	F	3M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
44	537	F	2Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Present	positive	Negative	GNRB	*Sal.* Spp.
45	543	M	7M	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Present	positive	Negative	GNRB	*Sal.* Spp.
46	544	F	8M	Stool	NO GROWTH													
47	552	F	2Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
48	555	M	5M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
49	557	F	3Y	Stool	NO GROWTH													
50	559	M	4M	Stool	NO GROWTH													
51	567	M	1M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
52	580	M	1.5Y	Stool	Neg.	Neg.	positive	Negative	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Shig* spp
53	579	M	2Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
54	583	M	1Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
55	399	F	4M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
56	400	M	9M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
57	409	M	1Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
58	411	M	2Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Red	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
59	412	M	1Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
60	413	M	31Y	Stool	NO GROWTH													
61	414	F	IY	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
62	415	F	1Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Red	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
63	516	F	2Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*

64	517	M	5M	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Sal.* Spp.
65	518	M	4M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Red	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
66	523	M	40y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
67	554	M	10M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
68	525	F	1Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
69	534	M	35Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Sal.* Spp.
70	537	M	1Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Present	positive	Negative	GNRB	*Sal.* Spp.
71	538	M	1y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
72	546	F	2Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Present	positive	Negative	GNRB	*E. coli*
73	545	F	1Y	Stool	NO GROWTH													
74	551	M	5M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
75	552	F	7Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
76	553	F	26Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Absent	positive	Negative	GNRB	*Sal*. Spp.
77	554	M	31Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Sal.* Spp.
78	548	F	27Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Sal.* Spp.
79	549	M	7Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Absent	positive	Negative	GNRB	*Sal.* Spp.
80	556	F	1OM	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
81	559	F	2Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Absent	positive	Negative	GNRB	*Sal.* Spp.
82	557	F	3Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Present	positive	Negative	GNRB	*Sal.* Spp.
83	563	M	10Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Sal.* Spp.
84	565	M	3Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Absent	positive	Negative	GNRB	*Sal.* Spp.
85	566	F	1M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
86	567	M	8M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
87	569	F	1Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
88	590	M	10M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
89	574	F	20M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Red	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
90	575	F	5M	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Red	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
91	582	F	2Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
92	586	M	2Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Sal.* Spp.
93	602	F	18M	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Sal.* Spp.
94	598	F	4Y	Stool	NO GROWTH													
95	603	M	27Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
96	606	F	1Y	Stool	NO GROWTH													
97	605	M	5Y	Stool	NO GROWTH													
98	607	F	1M	Stool	NO GROWTH													
99	610	F	3Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Red	yellow	Absent	Present	positive	positive	GNRB	*E. coli*

100	612	F	3Y	Stool	Neg.	Neg.	positive	Negative	Positive	LF	Yellow	yellow	Absent	Present	positive	positive	GNRB	*E. coli*
101	613	F	7Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Present	positive	Negative	GNRB	*Sal.* Spp.
102	614	F	6Y	Stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Absent	Absent	positive	Negative	GNRB	*Sal.* Spp.
103	615	F	9Y	stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Absent	positive	Negative	GNRB	*Sal.* Spp.
104	616	F	9Y	stool	Neg.	Neg.	positive	Positive	Negative	NLF	Red	yellow	Present	Absent	positive	Negative	GNRB	*Sal.* Spp.

KEY;

LF =LACTOSE FERMENTER.
NLF = NON LACTOSE
FERMENTER

GNRB = GRAM NEGATIVE ROD BACILLI
G. S. = GRAM STAIN