ANTIBACTERIAL ACTIVITY OF SELECTED MEDICINAL PLANT EXTRACTS AGAINST *ESCHERICHIACOLI* STRAINS ISOLATED FROM DIARRHOEIC STOOL OF CHILDREN IN NIGER STATE, NIGERIA

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APRIL, 2021

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P16 LSMC9038

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DOCTOR OF PHILOSOPHY (Ph.D) DEGREE IN MICROBIOLOGY

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APRIL, 2021

DECLARATION

I declare that the work in this Thesis entitled “Antibacterial Activity of Selected Medicinal Plant Extracts against *Escherichia coli* Strains Isolated from Diarrhoeic Stool of Childrenin Niger State, Nigeria” was carried out by me in the Department of Microbiology. The information obtained from literature has been duly acknowledged in the text and list of references provided. However, no part of this thesis was previously presented for another degree at any University.

IsahLegboMuhammad …… …….

Name Signature Date

CERTIFICATION

The Thesis entitled ANTIBACTERIAL ACTIVITY OF SELECTED MEDICINAL PLANT EXTRACTS AGAINST *ESCHERICHIA COLI* STRAINS ISOLATED FROM DIARRHOEIC STOOL OF CHILDREN IN NIGER STATE, NIGERIA by ISAH LEGBO MUHAMMAD, meets the regulations governing the award of Degree of Doctor of Philosophy (Ph.D) of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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Member, Supervisory Committee Signature Date

Prof. B. O. OLAYINKA …..……..…..……

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Dean, School of Postgraduate Studies Signature Date

DEDICATION

The Thesis is dedicated to the Almighty Allah who spare my life to reach this stage of academic career.

ACKNOWLEDGEMENT

My gratitude goes to Almighty Allah who made the research possible. Special thanks go to the supervisory team; Professor O. S. Olonitola, Professor J. B. Ameh and Professor B. O. Olayinka. The professors provided the needed advice from the beginning to the end of the research and most importantly constructively criticized the entire thesis.

I really appreciate the co-operation enjoyed from the laboratory technicians, Nurses and Doctors of the selected Hospitals from which samples were collected who are; Mal. Muhammad MahmoodMakusidi, Mal. Mohammed MohammedTsowa., Mr. Elijah Yisa, Mrs SalamatuUsman and Dr. Ibrahim TetengiKolo. I wish to specially thank my colleague from Ibrahim BadamasiBabangida University, Lapai who helped me to run the Nuclear Magnetic Resonance analysis on plant extracts at University of Leed in person of Dr.HussainiMajiya. May God bless him and his family. I am also indebted to MallamSarafadeenAdedeji who ran the statistical analysis for me. More grease to your elbow.

My special thanks go to parents who supported both morally and financially and alsoHajiya Fatima IyeGana, a wife who stood by me during the entire period of my research programme. Lastly, I want to thank all my siblings, cousins, nephews, nieces, friends and colleagues who supported me financially and morally to achieve this great success. May Allah bless you all ameen.

ABSTRACT

The study was carried out to determine the antibacterial activity of *Entadaafricana*, *Ptericarpuserinaceus* and *Vitexdoniana* extracts against *Escherichia coli* strains isolated from diarrhoeic stool of patients (0-5) years attending selected Hospitals in Niger State, Nigeria. The Hospitals include UmaruSanda General Hospital, Bida; General Hospital, Minna and General Hospital, Kontagora. A total of 480 stool samples were collected from diarrhoeal patients between February and October, 2014. The samples were cultured on MacConkey agar and Eosin Methylene Blue agar. The isolates obtained were Gram-stained, identified and serotyped using biochemical tests and *Escherichia coli*identification strain kits. The phytochemical screening of *Entadaafricana, Ptericarpuserinaceus*and *Vitexdoniana* leaf and stem-bark extracts was evaluated using standard qualitative methods. Acute toxicity (LD_{50}) was determined using oral administration method. The antibacterial activity of *Entadaafricana, Ptericarpuserinaceus*and *Vitexdoniana*leaf and stem-bark extracts were also evaluated using agar well diffusion method. The minimum inhibitory and minimum bactericidal concentration was evaluated using broth tube dilution and pour plating methods respectively. The work established a value of 16.9% of diarrhoeagenic *Escherichia coli* in the study area. Different strains of *Escherichia coli* associated with diarrhoeal patients that were isolated include Enteroaggregative *Escherichia coli* (n=16, 19.8%), Enterohaemorrhagic *Escherichia coli* (n=13, 16.1%), Enteroinvasive *Escherichia coli* (n=8, 9.9%), Enteropathogenic*Escherichia coli* (n=27, 33.3%) and Enterotoxigenic*Escherichia coli* (*n*= 17, 21.0%). Socio-demographic and risk factors found to be associated with diarrhoea in the study area were: age, area of domicile, occupation, educational status of parents, source of water and feeding pattern were statistically significant. The phytochemical screening of all the crude extracts were found to have anthraquinones, flavonoids, glycosides,saponins, steroids,

tannins and resins except for*Ptericarpus erinaceous*in which resins was not detected. The acute toxicity (LD_{50}) of thethree plant extracts against albino rats wasgreater than5000mg/kg body weight could be safe for use in human or higher animals. The methanolic extracts of*Entadaafricana*, *Ptericarpuserinaceus* and *Vitexdoniana* showed highest activity against *Escherichia coli* at 20mg/mL with zones of inhibition ranging between 20.00-36.00mm, 20.00-30.00mm and 24.00-38.00mm respectively.The methanolic leaf fraction of *Vitexdoniana*had the highest activity against all the tested*Escherichia coli* strain except Enteroinvasive *Escherichia coli* which had 50% susceptibility. The *Vitexdoniana* aqueous stem-bark fraction with minimum inhibitory concentration (MIC) and minimum bactericidal concentration(MBC) values of 5.0 and 10.0mg/mL emerged as the active fraction against 75% of the EAEC, while methanolic leaf fraction had MIC and MBC values of 5.0 and 10mg/mL against 77% of the EHEC. The aqueous leaf fraction had MIC and MBC values of 10mg/mL against 62.5% of the EIEC, while methanolic leaf fraction had MIC and MBC values of 5.0 and 10mg/mL against 61% and 81% of the EPEC. The methanolic stem-bark fraction exhibited highest activity with MIC and MBC values of 5.0 and 10mg/mL against 79% and 100% of the Enterotoxigenic*Escherichia coli*.The extracts of the plants could be promoted for search of new leads against enterobacterial pathogens having MIC values of 5.0-20mg/mL. The result has justified their utilization by traditional medicine practitioners for the treatment of diarrhoea and other related ailments associated with *Escherichia coli* strains.

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LISTS OF ABBREVIATION

ACRONYM **ABBREVIATIONS**

AE: Attaching effacing

ALF: Acetone leaf fraction

ASF: Acetone stem-bark fraction

cAMP: Cyclic adenosine monophosphate

DNA: Deoxyribonucleic acid

E.C:*Escherichia coli*

EAEC: Enteroaggregative *Escherichia coli*

ECP: *Escherichia coli* common pilus

EHEC: Enteroaggregative *Escherichia coli*

EIEC: Enteroinvasive *Escherichia coli*

EPEC: Enteropathogenic *Escherichia coli*

ETEC: Enterotoxigenic *Escherichia coli*

HLF: Hexane leaf fraction

HSF: Haxane stem-bark fraction

HUS: Haemolytic uremic syndrome

LT: Labile enterotoxin

MBC: Minimum Bactericidal Concentration

MIC: Minimum Inhibitory Concentration

MLF: Methanol leaf fraction

MSF: Methanol stem-bark fraction

ORT: Oral rehydration therapy

ACRONYM **ABBREVIATIONS**

OECD: Organization of economic control development

RNA: Ribonucleic acid

ST: Stable enterotoxin

WHO: World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Diarrhoea can be defined as a health condition which involves the passing out of unusually loose or liquid stool. In other words, it is the passing out of three or more loose watery stool or one bloody loose stool for a period of 24-hours (Baba *et al*., 2016). Diarrhoea is not a disease but a symptom of number of illnesses (Galadima and Kolo, 2014).

According to Guerrant *et al*. (2010), diarrhoea is of three major forms namely acute watery diarrhoea, acute bloody diarrhoea or persistent diarrhoea. Acute watery diarrhoea is that type of diarrhoea with high volume of watery stool occurring over a period of less than fourteen days. It usually results in severe dehydration if intervention measures are not sought. Acute bloody diarrhoea is diarrhoea manifested by loose or watery stools with the shedding of red blood cells. Persistent diarrhoea is usually associated with loose or watery stools with or without visible blood occurring over a period of fourteen days (Guerrant *et al*., 2010).

The immediate causes of diarrhoeal illness or gastroenteritis are often infectious in nature and involve a variety of pathogenic micro-organisms that includes bacteria, protozoa and viruses (Schiller, 2006). Among the bacterial pathogen of diarrhoeal diseases, the most commonly implicated in the endemic form of childhood diarrhoea on a global scale are strains of *Escherichia coli, Campylobacter, Shigella, Salmonella, Yersinia* and *Vibro* species (WHO, 2018).

*Escherichia coli*is the common pathogen responsible for a wide spectrum of diseases. The spectrum could range from diarrhoea, dysentery, urinary tract infection, wound infection,

peritonitis, and neonate meningitis to haemolytic *uremic* syndrome (Nataro*et al.*, 2006).*Escherichia coli* infection could be contracted through faecal-oral route, person to person in areas with poor sanitation and contaminated or non-chlorinated water has been implicated as the route of transmission (WHO, 2018).

Diarrhoeal disease is a major cause of morbidity worldwide and it is one of the leading causes of death globally. According to Kotloff *et al.* (2013)worldwide, one in ten infant mortality occurring in sub-Saharan Africa countries annually results from diarrhoeal disease during the first 5 years of life which leads to 800,000 fatalities globally. Wanke *et al.* (2013b) in another research reported that in developed countries, acute diarrhoea results in over 200,000 hospitalizations among 1.5 million patients consulting medical doctors.

World Health Organization (2013) reported that diarrhoeal diseases accounts for about 1.5 million deaths among diarrhoeal patients in the world annually. Low socio-economic status, poor hygienic condition of the people, intestinal parasitic and bacterial infections constitute a major cause of morbidity which leads to several epidemics each year (Galadima and Kolo,2014).

In Africa, the diarrhoea-specific mortality of children 0-5 years has been estimated at about 106 per 1000 (Olowe *et al.,* 2003). In addition, diarrheal illness account for an estimated 12,600 deaths each day in children less than five years of age in Asia, Africa and Latin America (Adejuyigbe *et al.,* 2004).Diarrhoeal diseases account for almost three million deaths annually in Nigeria mainly among children (0-5) years. Available reports in Nigeria indicated that more than 315,000 deaths of 0-5 year's children are recorded annually due to diarrhoea epidemics (Ifeanyi *et al.,* 2010).

Jenkin(2018) reported that enterotoxigenic *Escherichia coli*causes' watery diarrhoea due to production of plasmid mediated toxin (LT, ST) in infants and adults popularly called traveler's diarrhoea. Enteropathogenic *Esherichia coli* causes vomiting, fever and prolonged diarrhoea mainly in infants less than two years in most developing countries (Cheesbrough, 2006). Enteroinvasive *Escherichia coli* causes dysentery similar to shigellosis with symptoms of fever, colitis with blood, mucous and many pus cells in stool (Nataro *et al*., 2006). Enterohaemorrhagic *Escherichia coli* causes life threatening haemorrhagic diarrhoea (colitis) in all ages without pus cells and fever, while enteroaggregative *Escherichia coli* causes chronic watery diarrhoea and vomiting in children (Orth *et al*., 2010; Jensen *et al*., 2014)

Plants that possess therapeutic properties or exert beneficial pharmacological effect on the human body are generally designated as medicinal plant (Melkamu *et al*., 2018). Also a medicinal plant is that plant in which one or more of its organs contain active ingredients which is used for therapeutic purposes or contains active ingredients that can be used for synthesis of drugs (Tijjani *et al*., 2017).

Medicinal plants are used by traditional medicinepractioners' inform of decoction, concoction, infusion and tisane. Decoction is when a medicinal plant is prepared in cold water brought to boil and allowed to cool for about fifteen minutes (Muhammad *et al*., 2020). Concoction is a preparation of medicine in form of soup or drink usually made from many ingredients. Infusion is usually made by pouring water on a specified plant material allowing the medicine to stand for about fifteen minutes, while tisane is a tea made by either decoction or infusion (Muhammad *et al*., 2020).

Medicinal plants naturally synthesize and accumulate some secondary metabolites like alkaloids, steroids, tannins, terpenes, flavonoids, saponins, glycosides, cyanogenics, resins, lactones and

carotenoids (Melkamu *et al*.,2018). According to Abayomi (1982), medicinal plants are used in pharmaceutical industry for the production of drugs in the form of tablets, syrups, capsules and for the preparation of infusions. Plants have elaborate remarkable array of natural products many of which have antimicrobial activities (Kiyasar *et al.*, 2002).

Medicinal plants are the back bone of traditional medicine which more than 3.3 billion people in developing countries utilize on a regular basis (Tijjani *et al*., 2017). Moreso, World Health Organization (WHO), estimated that more than 80% of the world population still relies on traditional medicine for their primary health care needs (Sasidharan *et al*., 2011). In recent years, medicinal plants have represented a primary health source for the pharmaceutical industry (Melkamu *et al*., 2018). No less than 400 compounds derived from plants were reportedly used in the preparation of drugs such as vincristine and vinblastine used in the treatment of cancer, quinine and artemisimin used as antimalarial drugs (Gurib-Fakin and Ajose, 2007). The discovery of modern drugs such as quinine, vincristine, digoxin, digitoxin and artemisinin from medicinal plants signifies the huge potential that still exists for the production of more novel pharmaceuticals (Geyid *et al*., 2005).

The use of herbs in the treatment of ailments globally especially in Nigeria is an age practice. Some natural substances that have effective antimicrobial properties have been used as seasoning and they can affect the growth and metabolism of bacteria, inactivating or inhibiting their growth depending on their constitution and concentration (Shen *et al*., 2007; Nazzaro *et al*., 2009).

*Entada africana*commonly known as dorot and belongs to *Mimosaceae* Family. The plant is called *Tsawata* (Hausa), *Ogumba* (Yoruba), *Kawonuwanchi* (Nupe) (Mann *et al*., 2003). The plant was reported to be a tropical perennial plant predominant in the savannah especially the

central and eastern tropical Africa (Katende, 1995). Ethno-botanical uses include treatment of dysentery, cough, fever, wound and also as an arbotifacient (Mann *et al.*, 2003).

Ptericarpus erinaceous commonly known as African Rose tree or African treek and belongs to *Fabeceae* Family. The plant is called treek (English), *Modobiya* (Hausa),*Zanchi* (Nupe), *Apepe*(Yoruba), *Senyo or Doli* (Ghanian) (Mann *et al*., 2003). It was reported as a perennial deciduous legume tree widely distributed throughout the west and central African Savannah and dry forest. Ethno-botanical uses include treatment of dysentery, diarrhoea, fever, and as well as an arbotificent (Mann *et al*., 2003).

Vitex doniana commonly known as black plum and belongs to *Verbernaceae* Family. The plant is called *Dinya* (Hausa), *Dinchi* (Nupe), *Orisi* or *Orinla* (Yoruba), *Efifi* (Igala) and *Oliti* (Etsako). It was also reported as a deciduous evergreen tree widely found in the middle belt of Nigeria particularly Niger, Kogi, Benue and parts of savannah regions of Kaduna, Sokoto, or Kano State (Mann *et al.*, 2003).

According to the report of Arokiyaraji *et al*. (2009),*Vitex doniana* has numerous applications in traditional medicine:Leaf sap was reported to be used as an eye drop for the treatment of conjunctivitis and other eye complaints. Leaf decoction of the plant is applied externally as a galactagogue and for the treatment of headache, stiffness, measles, body rash, fever and chicken pox and also internally as body tonic and anodyne (Dash *et al*., 2005).Dash *et al*. (2005) also reported that pastes of pounded leaves and stem-bark are used for the treatment of wounds and burns, while a root decoction of the plant was reported to be administered orally to treat ankylostomiasis, rachitis, gastro-intestinal disorders and jaundice, as well as an anodyne.

Phytochemical constituents are chemical compounds that occur naturally in plant. They are the secondary metabolites (plant by-product) which act as a protective mechanism against

environmental stressors and the more environmental stressors; the more phytochemicals are produced by plants (Heneman and Zidenberg-Cherr, 2008).

1.2Statement of Research Problem

The global increase in water and food borne diseases with *Escherichia coli* strains is of growing concern and an emerging public health problem (WHO, 2013). *Escherichia coli* is an important opportunistic bacterium capable of causing a variety of diseases in human, ranging from diarrhoea to life threatening systemic infections such as haemolytic uremic syndrome (HUS) (Cheesbrough, 2006).

In Nigeria and other developing countries, diarrhoeal illness was reported as one of the major public health problems and constitutes serious causes of morbidities and mortalities (WHO, 2017). The development of resistance to antibiotics by *Escherichia coli* strains is a growing concern. Today, *Escherichia coli* strains have been confirmed to show notable resistance to drugs like tetracycline and metronidazole among many others (Okeke *et al*., 2009). This infers that in the near future, there may not be effective antibiotics with which to treat patients with serious infection. It is therefore necessary to continue the search for new and effective antibacterial agents.

The case fatality rate of children infected with antibiotic resistant strains of *Escherichia coli* was reported to be twenty-one times greater than for individual infected with other species of *Enterobacteriacea*e family (Jenkin, 2018). The problem with medicinal claims for some plants has been the insufficient scientific data on the bases of their ethno-medicinal uses and toxicological profile (Briejer *et al*., 2011).

1.3Justification for the study

Treatment of diarrhoeagenic *Escherchia coli* infection has become increasingly problematic due to the emergence of multi-drug resistant strains particularly the Enteropathogenic *Escherichia coli,* Enterohaemorrhagic *Escherichia coli,* Enteroinvasive *Echerichia coli,* Enterotoxigenic *Escherichia coli* and Enteroaggregative *Escherichia coli.* Controlling of diarrhoeagenic *Escherichia coli* strain remains a primary focus of health care system.

The epidemiological study on diarrhoeagenic *Escherichia coli* from three Hospitals in this research would also bring about the development of control programme aimed at dealing with diarrhoeagenic *Escherichia coli* strains or serovars circulating in the study area and the risk factors associated with *Escherichia coli* infections so as to understand the interaction between the pathogen, host and the environment.

The out come of the toxicity (LD_{50}) test of the plant extracts from the study is expected to reveal the level of safety of the plant and that of antibacterial susceptible pattern of diarrhoeagenic *Escherichia coli* to plant extracts is expected to give the level of antibacterial activity of the plant.

1.4 Aim and Objective of the Study

1.4.1Aim of the Study

The aim of this work was to determine the antibacterial activity of *Entada africana*, *Pteriocarpus erinaceus* and *Vitex doniana* extracts against *Escherichia coli* strains isolated from diarrhoeic stool of children (0-5) years.

1.4.2Objectives of the Study were to:

1. isolate and characterize *Escherichia coli* associated with diarrhoeic stool of children (0-5) years from three Hospitals in Niger State, Nigeria.

2. determine the socio-demographic and presdisposing factors associated with the transmission of *Escherichia coli*in the study area.

3.determine the phytochemical constituents responsible for the therapeutic potency of the leaf and stem-bark of *Entada africana, Ptericarpus erinaceus* and *Vitex doniana* extracts

4. determine the acute toxicity (LD_{50}) of crude extracts of *Entada africana, Ptericarpus erinaceus* and*Vitex doniana*.

5. determine the preliminary antibacterial activity of *Entada africana, Ptericarpus erinaceus* and *Vitex doniana* crude leaf and stem-bark extracts against *Escherichia coli*.

6. determine the antibacterial activity, minimum inhibitory concentrations and minimum bactericidal concentrations of the plant with highest potency.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Biology of *Escherichia coli*

Escherichia coli is aGram-negative, non-spore forming, facultative anaerobic and rod shaped bacterium. Bacteriological culture of the diarrhoeal stool of normal human and warm-blooded animals invariably yields *Escherichia coli* (Murray *et al.,* 2011). In 1885, Theodor Escherich discovered *Escherichia coli* in the faeces of healthy individuals and was called *Bacterium coli commune* because itwas found in colon (Eckburg*et al*., 2005). The report of Shulman *et al*. (2007) taxonomically, the specie:*Escherichia coli* belongs to genus *Escherichia* in the Family *Enterobacteriaceae*, Order Enterobacteriales, Class Gamma-proteobacteria, Phylum Proteobacteria, Kingdom Eubacteria, and Domain Bacteria.

Escherichia coli belong to a group of bacteria informally known as Coliforms that are found in the gastrointestinal tract of warm-blooded animals (Brenner *et al.*,2005; Tenaillon *et al*., 2010). Most *Escherichia coli* strainsare harmless and are also part of normal flora of the gastro-intestinal tract, therefore human (hosts) benefit from them by producing vitamin $K_2$and preventing colonization of the intestine with pathogenic bacteria (Hudault *et al.,* 2011).

Escherichia coli normally colonize an infant's gastrointestinal tract within forty hours of birth, arriving with food or water or from the individuals handling the child. In the bowel, *Escherichia coli* adhere to mucus of large intestine. It is the primary facultative anaerobes of the human gastro-intestinal tract (Todar, 2007). As long as these bacteria do not acquire genetic element encoding for virulent factor, they remain benign commensals (Evans *et al.,* 2007).

According to the report ofYu *et al.* (2014),*Escherichia coli* forms a deep red colony on MacConkey agar and produces black colonies with a greenish black metallic sheen on eosine methylated blue(EMB) agar. *Escherichia coli* are facultative anaerobes that grow by aerobic respiration or by fermentation that yields lactate, succinate, acetate, ethanol and carbon dioxide. The bacteria are lactose positive, lysine positive, Indole positive, Methyl Red positive, Vogel-Proskaeur negative and Citrate utilization negative (Forbes *et al*., 2007).

Escherichia coli comprise of five strains namely Enteropathogenic *Escherichia coli*, Enteroinvasive *Escherichia coli*, Enterotoxigenic *Escherichia coli*, Enteroaggregative *Escherichia coli* and Enterohaemorrhagenic *Escherichia coli*. *Escherichia coli* have non-pathogenic strain called Nissle (Mutafor). The mutafor strain was isolated by Alfred Nissle in 1917 during First World War and had been used to prevent and treat an assortment of gastro-intestinal disorder (flatulence) (Ulrich and Jurgen, 2009).

2.1.1 Enteropathogenic*Escherichia coli*

Enteropathogenic *Escherichia coli* (EPEC)belong to attaching and efficacy family(Araujo *et al.*, 2007).Enteropathogenic *Escherichia coli* are typically divided into two linages: strains with the EPEC adherence factor plasmid (EAF) encoded bundle-forming pilus (BFP) is referred to as typical EPEC (tEPEC), whereas atypical EPEC (aEPEC) refers to strains without the EAF(Luis and Theresa, 2020). Enteropathogenic *Escherichia coli*were the first identified diarrhoeagenic *Escherichia coli* that responsible for infant diarrhoea predominantly in children under two years in developing countries (Luis and Teresa, 2020).

Enteropathogenic *Escherichia coli* are still important cause of childhood diarrhea. Typical EPEC (tEPEC) are characterized by the presence of the intimin adhesion encoded by *eae* gene from the

locus of enterocyte effacement (LEE) pathogenicity Island and the bundle-forming pilus encoded by the *bfp* gene from EAF plasmid (Chart, 2012). Typical EPEC is exclusively related to humans; whereas samples of atypical EPEC could be detected in domestic animals, which may behave as for these bacteria (Shulman *et al*., 2007).

Diarrhoea caused by EPEC is mostly subclinical and acute, but may be moderate, severe or persistent; lead to malnutritionand can cause death particularly in children less than twelve months (Luis and Theresa, 2020). According to Jose *et al*. (2011), patients with symptomatic EPEC infection typically experience diarrhoea, vomiting, malaise and fever. The stool may contain mucuous but does not usually contain blood. Clinically, EPEC illness is characterized by vomiting, fever and watery diarrhoea without gross blood (Yang and Wang, 2014).

2.1.2 Enterotoxigenic*Escherichia coli*(ETEC)

Enterotoxigenic *Escherichia coli* strain is mostly found in humans, pigs, sheep, goat, cattle, dogs and horses (Teneja *et al*., 2006). The strain is responsible for waery diarrhoea with fever (Jenkin, 2018).Enterotoxigenic *Escherichia coli* strain uses various colonization factors (CFs) to bind enterocyte cells in the small intestine (Luis and Theresa, 2020).

Enterotoxigenic *Escherichia coli* strain can produce two proteinaceuous enterotoxins: The larger of the two proteins (LT enterotoxin) is similar to cholera toxin in structure and function (Teneja *et al*., 2006). The smaller protein (ST enterotoxin) causes cGMP accumulation in the target cells and subsequent secretion of fluid and electrolytes into the intestinal lumen (Teneja *et al*., 2006).

Enterotoxigenic *Escherichia coli* strains are non-invasive and they do not leave the intestinal lumen (Davidson *et al*., 2002). This strain is the leading bacterial cause of diarrhea in children in the developing countries as wellas the most common cause of traveler’s diarrhoea (Bourgeois *et*

al., 2016)). According to Croxen *et al*. (2013) each year, 840 million cases of ETEC are estimated to occur in developing countries and about 280 million of these cases as well as 325,000 deaths are estimated to occur in children less than five years.

2.1.3 Enteroinvasive *Escherichia coli* (EIEC)

Enteroinvasive *Escherichia coli* strains are biochemically, genetically and pathogenically related to *Shigella* species (Venkitanarayanan *et al*., 2013).These strains are only found in humans and are responsible for bloody or non-bloody diarrhea (Vieira *et al.,* 2007).

Enteroinvasive *Escherichia coli* infection causes a syndrome that is identical with shigellosis with profuse diarrhea and high fever (Lan *et al*., 2014). The most infamous member of this pathotype is strain 0157:H7, which causes bloody diarrhoea and no fever. These strains are highly invasive, and they use adhesin proteins to bind to and enter intestinal cells (Orth *et al*., 2010).

2.1.4 Enterohaemorrhagic *Escherichia coli*(EHEC)

The Enterohaemorrhagic *Escherichia coli* strains are found in humans, cattle, goats and they cause bloody diarrhoea (haemorrhagic colitis) (Rendon *et al*., 2017).The intestinal tract of domesticated cattle serves as the primary reservoir for EHEC in the United States and meat contaminated during the slaughter process (Glyces, 2007). This strain can cause haemolytic uremic syndrome and sudden kidney failure (Karmali *et al*., 2013).The EHEC uses bacterial fimbriae for attachment called *Escherichia coli* common pilus (ECP) which is moderately invasive and posses a phage-encoded Shiga toxin that can elicit an intense inflammatory response (Glyces, 2007). The most infamous member of serotype is strain 0157: H7 which

causes bloody diarrhoea without fever, haemolytic uremic syndrome and sudden kidney failure (Orth *et al*., 2010).

2.1.5 Enteroaggregative *Escherichia coli* (EAEC)

Enteroaggregative *Escherichia coli* (EAEC) strains are only found in humans and posses' fimbrae which aggregate tissues culture cells. Enteroaggregative *Escherichia coli* are non invasive and they produce a haemolysin and enterotoxin (ST) similar to that of ETEC(Haung *et al*., 2006; Weintraub, 2007). Enteroaggregative *Escherichia coli* (EAEC) are heterogenous collection of strains characterized by their autoagglutination in a "stacked brick" arrangement over the epithelium of the small intestine and sometimes in the colon (Jensen *et al.*, 2014)

Enteroaggregative *Escherichia coli*strains were first found in 1987, in childhood diarrhoea in Lima, Peru (Nataro *et al*., 1987). Since 1987, Enteroaggregative *Escherichia coli* have been recognized as agents of diarrhoea in developing and developed countries (Roche *et al*., 2010). Enteroaggregative *Escherichia coli* is most commonly found in developing countries due to less developed base and low human development compared to other countries. Countries like India, Jamaica and Mexico are the most commonly risked countries (Kalita *et al*., 2014).

The pathogenesis of Enteroaggregative *Escherichia coli* (EAEC) involves the aggregation and adherence of the bacteria to the intestinal mucosa,where they elaborate enterotoxins and cytotoxins that damage host cells and induce inflammation that results in diarrhoea (Roche *et al*., 2010).It also causes nausea, vomiting and mucoid diarrhoea in some people, abdominal cramping, pain or tenderness (Jensen *et al*., 2014).

2.2 Transmission of Diarrhoeagenic *Escherichia coli* Infection

Diarrhoeagenic *Escherichia coli*infectionoccurs through the faecal-oral route, primary via contaminated food or water (CFSAN, 2016). It could also occur through person to person contact possibly among close contacts such as families, childcare centres, and nursing homes as well as contact with animals or their environment (Trabulsi *et al*., 2012).

Escherichia coli common routes of transmission includes unhygienic food preparation, farm contamination due to manure and also irrigation of crops with contaminated grey water, faecal of pigs in crop land (Vogel *et al*., 2005).A contaminated source of food such as raw unpasteurized milk and cheese, undercooked beef and fresh produce such as sprouts, spinach and lettuce (CFSAN, 2016).Various types of animals, in particular cattle and other ruminants can be healthy carriers of human pathogenic ETEC that can be spread to human through fecal contamination (Sabin, 2006).Dairy and beef cattle are primary reservoirs of *Escherichia coli* O157:H7 and they can carry it asymptomatically and shed it in their faeces for human cntact (Orth *et al*., 2010).

2.3 Epidemiology of Diarrhoeagenic *Escherichia coli* Infection

The diarrhoea illness account for an estimated 12,600 deaths each day in children less than five years of age in Asia, Africa and Latin America (Adejuyigbe *et al.,* 2004). Studies conducted in South-Africa, Brazil and Mexico has shown that 30-40% of infant diarrhoeal cases could be attributed to Enteropathogenic *Escherichia coli* (Stenutz *et al*., 2016).

Outbreaks of illness associated with *Escherichia coli* O157:H7 have been reported throughout the hemisphere, most frequently in Canada, the United States, Japan, and United Kingdom(Orth *et al*., 2010). In Canada, infectious due to *Escherichia coli* O157:H7 appear to be more common

in the western provinces than in the east, in rural vices urban environments, and during summer as opposed to winter months (Orth *et al*., 2010).

A survey conducted shown that travelling to less developed countries is associated with higher risk for traveler's diarrhoea including *Escherichia coli* infection (Gupta *et al*., 2008). However enterotoxigenic *Escherichia coli* is the most common pathotype that cause diarrhoea among traveller's returning from most regions but other pathotypes can also cause traveler's diarrhoea. According to World Health Organization (2010), each year enterotoxigenic *Escherichia coli* causes more than 200 million cases of diarrhoea and 380,000 deaths mostly in children in developing countries. It had been estimated that each year, approximately 210 million cases and 380,000 deaths occur, mostly in children from enterotoxigenic *Escherichia coli* (Gupta *et al*., 2008).

The World Health Organizationestimated high risk areas or countries to include Africa, Asia, Middle-East, Mexico, Central and South America, while intermediate risk countries include United States, Canada, Australia, New Zealand, Japan, and countries in Northern and Western Europe (Gupta *et al*., 2008).A study was conducted in United States and also showed that strain O157: H7 was linked to the 2006 United States *Escherichia coli* outbreak, due consumption of fresh spinach (Orth *et al*., 2010).

2.4 Pathogenesis of Diarrhoeagenic *Escherichia coli* Strains

Escherichia coli possess different virulent factors that are responsible for gastrointestinal diseases (Eugene *et al*., 2007). Two important virulent factors responsible for the pathogenicity of the different *Escherichia coli* strainsinclude enterotoxin production and ability to adhere to small intestines which are coded by plasmids (Eugene *et al*., 2007). These plasmids can be transferred to other *Escherichia coli* by conjugation, thereby conferring virulence on the

recipient strain. However, gastroenteritis producing strains of *Escherichia coli* often have more than one type of virulence plasmid (Dannenberg, 1995).

Enterotoxigenic *Escherichia coli* strains produce one or both of two distinct enterotoxins, which are responsible for the diarrhoea and are distinguished by their heat stability: heat stable enterotoxin (ST) and heat labile enterotoxin (LT) (Nataro *et al*., 2017). The genes for ST and LT production and colonization factors are usually plasmid-borne and acquired by horizontal gene transfer. Stable enterotoxin (ST) binds to a glycoprotein receptor that is coupled to guanylate cyclase on the surface of intestinal epithelial cells (Teneja *et al*., 2006). Activation of guanylate cyclase stimulates the production of cycline guanosine mono-phosphate (cGMP). This leads to the secretion of electrolyte and water into the lumen of the small intestine which manifested as the watery diarrhoea, thus is the major characteristic of enterotoxigenic *Escherichia coli* infection (Qadri *et al.*, 2005).

However, labile enterotoxin (LT) binds to specific gangliosides on epithelial cells and activates membrane borne adenylate cyclase, which leads to increase production of cyclic adenosine monophosphate (cAMP) through the same mechanism employed by cholera toxin. Moreso, this leads or resulted into hyper-secretion of electrolyte and water into the intestine lumen (Luis and Theresa, 2020).

Enteropathogenic *Escherichia coli*: These strains attach to the brush border of intestinal epithelial cells and cause a specific type of cell damage called effacing lesions. Attaching effacing (AE) lesion formation is now known to result from the delivery of specific virulent proteins into host cells, through a types 3 secretion (TTS) system (Jenkin, 2018). These virulence proteins are essential for the subversion of the intestinal epithelial host cell which signals transduction pathways (Jenkin, 2018).

The Enteropathogenic *Escherichia coli* (EPEC) virulence proteins(one homologen to Yersi TTS yop)lyse red blood cells, while cell destruction leads to the subsequent virulence (Jenkin, 2018). As a result of this pathology, the term attaching effacing (AE)*Escherichia coli* are used to describe borne Enteropathagenic *Escherichia coli* strains (Jose *et al*., 2011)). It is now known that attaching effacing *Escherichia coli*is an important cause of diarrhoea in children residing in developing countries (Jose *et al*., 2011).

Enterohaemorrhagic *Escherichia coli*: These strains carry the bacteria phage encoded genetic determinants for Shiga-like toxin (Stx-1 and Stx-2 proteins). These toxins could cause epithelial necrosis in colon leading to severe complications (Glyces, 2007). It also produces AE lesions, causing haemorrhagic colitis with severe abdominal pains and cramps followed by bloody diarrhoea. The Stx-1 and Stx-2 previously called serotoxin 1 and 2 have been implicated in the extra-intestinal disease called haemolytic uremic syndrome, a severe haemolytic anaemia leads to kidney failure and these toxins kill vascular endothelial cells (Karmali *et al*., 2013).

Enteroinvasive *Escharichia coli* strains causes diarrhoea by multiplying within the intestinal epithelial cells. The ability to invade epithelial cells is associated with the presence of a large plasmid (Lan *et al*., 2014). The strain may also produce a cytotoxin and enterotoxin. Enteroaggregative *Escherichia coli*strains adhere to epithelial cells in localised form thereby forming clumps of bacteria with a stacked brick appearance (Venkitanarayanan *et al*., 2013).

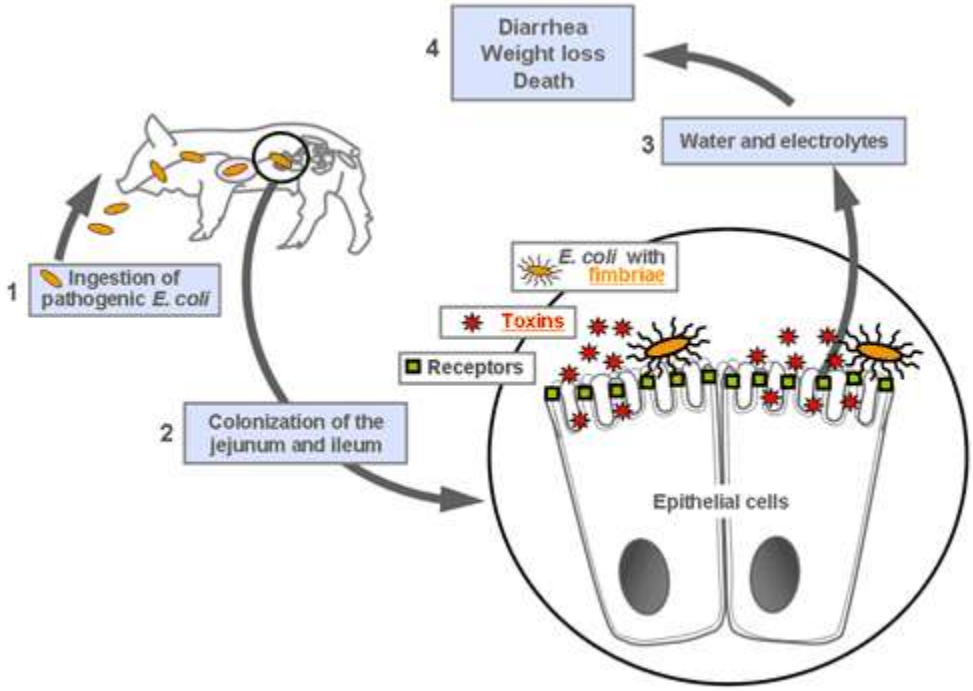


Figure 2.1 Pathogenesis of Enterotoxigenic *Escherichia coli*.

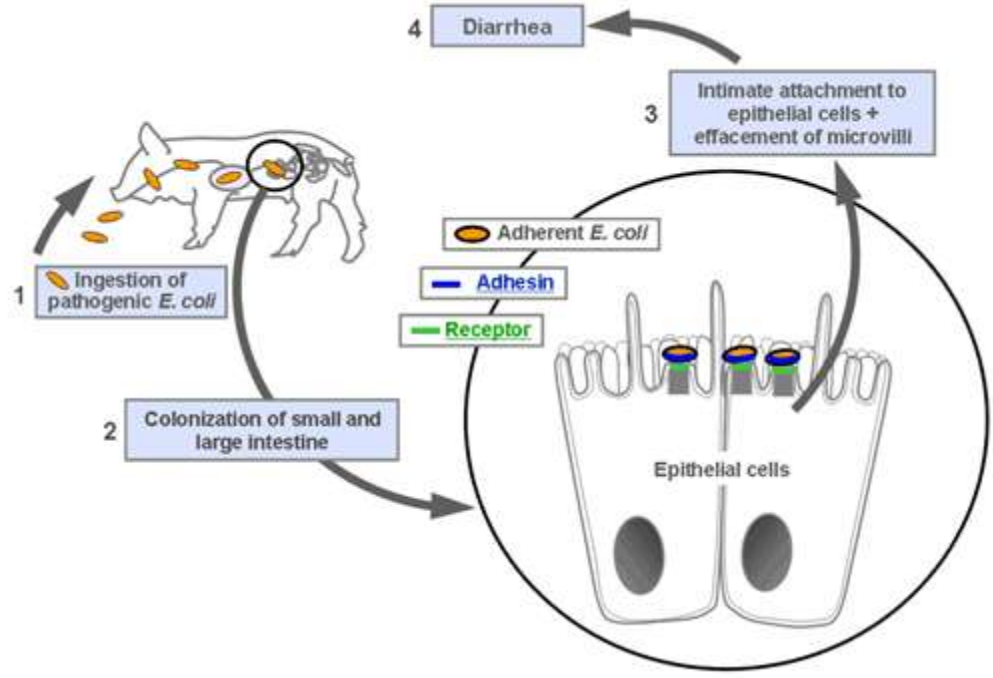
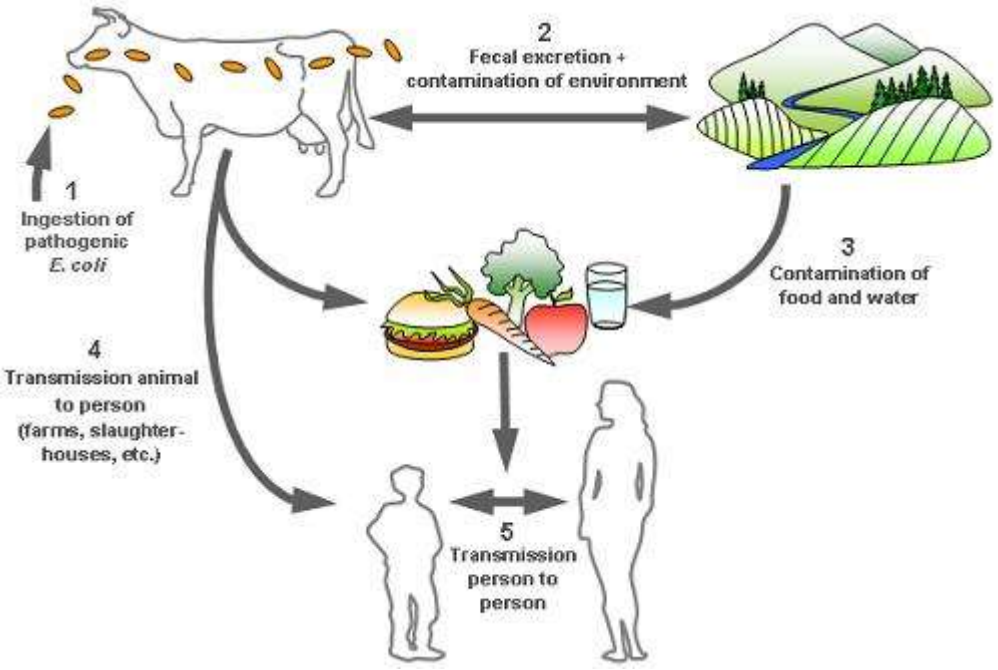
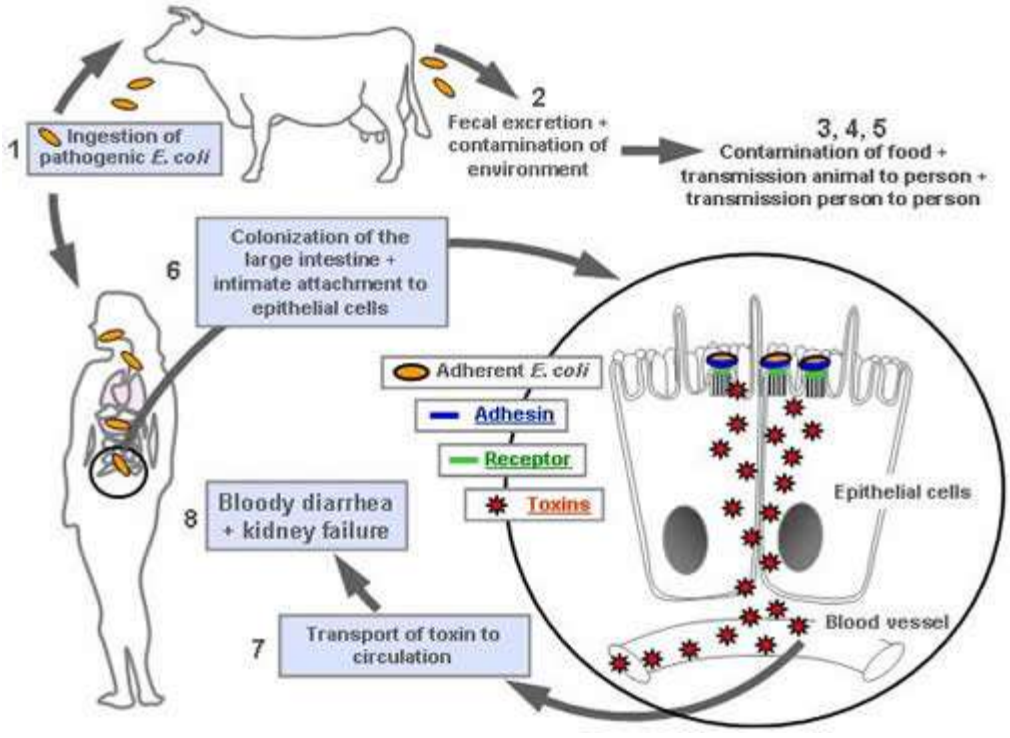


Figure 2.2 Pathogenesis of Enteropathogenic *Escherichia coli*.



(A)



(B)

Figure 2.3 Pathogenesis of pathogenic *Escherichia coli* strains through ruminants and contamination cycle.

2.5 Preventionq and Control of Diarrhoea

World Health Otganization (2009) estimated that 90% of the childhooddiarrhoea has been as aresult of poor sanitary conditions and inadequate personal, household and community hygiene. Therefore, understanding environmental and behavioural risk factors and their interactions is a pre-requisite for devising effective preventive approaches. A primary preventive intervention was reported to reduce environmental risk factors and high-risk behaviour for the whole communities by interrupting the disease transmission cycle. The cycle includes faeces, fluids, field flies, fingers, and hand washing (WHO, 2021).

Food and water were reported to be the primary sources of *Escherichia coli* infection,especially the enteropathogenic *Escherichia coli* that causes travelers' diarrhoea. Therefore, travelers should be reminded of the importance of adhering to food and water precautions. For example, contaminated food and water often pose a risk for travelers (Jose *et al*., 2011). Many of infectious diseases associated with contaminated food and water are caused by pathogens transmitted via faecal-oral route (Jose *et al*., 2011).

Travellers are also advised to select food with care; especially raw foods which are likely to be contaminated. Raw or undercooked meat, fish and shell fish can carry various intestinal systemic pathogens. In areas where hygiene and sanitation are inadequate or unknown, travellers should avoid consuming salads, uncooked vegetables, unpasteurized milk, or cheese made from unpasteurized milk (WHO, 2015). Raw fruits that are eaten unpeeled (such as straw berries) should be avoided and fruits that are eaten peeled (such as banana and mango) should be peeled by person who eats them. Produce should be washed under safe running water or soaked in water that has been purified and then rinsed with safe water (WHO, 2020).

In the case of infants and children, the safest way of preventing diarrhoea is to feed an infant less than six months with exclusive breast feeding. If the infant is fed with formula prepared from commercial powder, the powder should be reconstituted with hot water at a temperature of 70^0C(WHO, 1988). The heat kills most pathogens with which the infant formula may have been contaminated during manufacturing or through handling after opening. In many parts of the world particularly where water treatment, sanitation and hygiene are inadequate, tap water may contain disease causing agents including viruses, bacteria and parasites or chemicals contaminants such as lead (WHO, 2019).

Travellers should avoid drinking from tap water unless they are reasonably certain of its safety. Tap water is not sterile and should not be used for sinus or nasal irrigation. Beverages such as fountain drinks or other drinks made with tap water and ice drinks might not be safe for consumption as result of contamination from water (WHO, 2018).

People who may be exposed to livestock especially ruminants should be instructed about the importance of hand washing in preventing infection. However, soap and water may not be readily available at risk areas; travellers should consider taking hand sanitizer that contains at least 60% alcohol. Lastly, during *Escherichia coli* out breaks, clinicians should alert people travelling to the affected areas (Vesikari *et al.*, 1994).

2.6 Diagnosis of *Escherichia coli* Infection

For every infection to be diagnosed, microbiological result must be carefully examined in conjunction with clinical findings. However, in case of diarrhoeal patients; symptoms such as diarrhoea, vomiting, abdominal cramps, weakness of the body and dizziness are always observed. Physical examination: physical parameter such as patient's temperature may be

measured to check for fever and blood pressure and pulse can also be measured to check for signs of dehydration.

Stool culturing can also be recommended to determine causative agents and sensitivity test; to ascertain the active drugs that kill the causative agents if it is bloody diarrhoea.In stool samples, microscopy will show Gram-negative rods, with no particular cell arrangement. Then diarrhoeic stool sample would be inoculated on tomacConkey agar and incubated at 37^{0} Cfor 24 hours. On macConkey agar, deep red colonies are produced, since the organism (*Escherichia coli*) is lactose-positive, while fermentation of this sugar would cause the medium's P^{H} to drop, leading to darkening of the medium. Growth on EMB agar produces black colonies with a green metallic sheen indicates the presence of *Escherichia coli.*The biochemical characterization involved Indole, Methyl Red, Voges-Proskeur, Citrate utilization test. For *Escherichia coli;* it is Indole-positive (red ring), Methyl Red-positive (bright red) butVoges-Proskeure-negative (no change-colourless) and Citrate-negative (no change-green colour). Serotyping andPCR could provide an accurate identification of *Escherichia coli* (Chart, 2012).

2.7 Treatment of Diarrhoea

The prevention and redress of dehydration are very important in the treatment of diarrhoea because diarrhoea lead to mass loss of water; therefore enough liquid orally is administered to the petients to prevent dehydration at the beginning of diarrhoea (Franzolin *et al*., 2005). Oral rehydration salt solution (ORS) is given to patients with mild dehydration for early rehydration.Oral rehydration salt solution was reported to be a mixture of clean water, salt and sugar and when absorbed into the small intestine, replaces the water and electrolytes lost in the faeces (UNICEF, 2020).

According to the report of World Health Organization (2018), zinc supplements reduce the duration of a diarrhea episode by 25% and are associated with 30% reduction in stool volume. Rehydration with intravenous fluids in case of severe dehydration or shock, while nutrient rich foods could follow later. The vicious circle of malnutrition and diarrhea 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.

Patients with pus and bloody stool are mostly infected by invasive bacteria, which needed an effective antibacterial treatment. However, such patients are advised to consult health professional, in particular for management of persistent and bloody diarrhea. Strong antibiotic and anti-nausea drugs may be prescribed for the patient after thorough investigation.

2.8 Description of the Medicinal Plants.

2.8.1*Entada africana*

The plant is commonly known as dorot and belongs to *Mimosaceae* Family. The plant is also called *Tawatsa* (Hausa), *Ogumba (*Yoruba), *Kawonuwanchi* (Nupe) (Mann *et al*., 2003). It was repoted as a tropical perennial plant predominantly savannah especially the central and eastern tropical Africa (Katende, 1995). The tree is up to 6-8m high, usually branching low down with a wide crown. The stem-bark is grey, slightly finished off in irregular patches and the slash is red and produced gum (Mann *et al.*, 2003). The leaves are elongated, ellipsoid with rounded apex, mid ribs and nerves are distinct at both surfaces. Inflorescence consists of flowers appearing at the same time with leaves. They are usually creamy white with sweat scent and densely clustered in a spike like raceme in the leaf axils or arranged in pinnate at the end of short stalk (Beentige, 1993).

Fruits are very persistent, hanging down roughly and eventually break up. They are straight or slightly curved and thick waxy margin usually contain 10-15 elliptic flat seeds. The out coat breaks to release the brown seeds. Ethno-botanical uses include treatment of dysentery, cough, fever, wound and also as an arbotifacient (Mann *et al.*, 2003) (Plate 2.1).

Plate 2.1: Photograph of *Entada africana* plant

2.8.2 *Ptericarpus erinaceous*

The plant is commonly known as Africa Rose wood tree belonging to *Fabaceae* Family. The plant is called *Treek* (English), *Modibiya* (Hausa), *Zanchi* (Nupe), *Apepe* or *Osun chidu* (Yoruba), *Senyo* or *Doli* (Ghanian) (Mann *et al*., 2003). It was reported as a perennial deciduous legume tree and widely distributed throughout the west and central Africa savannah and dry forest (Mann *et al*., 2003).

The deciduous tree is about 8-10m high with a tall, narrow, open crown. The stem-bark of the trunk is dark grey and roughly with scales that curl up at the ends. The leaves are composite, impair pinnate with 4-5 pairs of alternated or sub opposite that are elliptic, shortly pubescent at the lower side. Inflorescences consist of golden yellow flora in lax panicle which is formed when leaves have fallen.

Flowering period is between November and February. Fruits have a special pod and when the fruit mature, papery wing all around it, which looks like a leave at a distance but with centre swelling which contain single seed (Mann *et al.,* 2003). The fruiting period is between January and March. The red heart wood is used for the preparation of the cotton dye. Ethno-botanical uses include treatment of dysentery, diarrhoea, fever, and as well as an arbotificent (Plate 2.2).

Plate 2.2: Photograph of *Ptericarpus erinaceus* plant

2.8.3 *Vitex doniana*

The plant is commonly known as black plums which belong to *Verbernaceae* Family. The plant is variously called *Dinya* (Hausa), *Orisi* or *Onilan* (Yoruba), *Efifi* (lgala) and *Oliti* (Etsako). *Vitex doniana* is a deciduous evergreen tree usually 4-8m high, occasionally up to 15m high with a dense rounded crown. The plant is widely distributed in tropical Africa and south east African countries including Uganda, Kenya and Tanzania. It was also reported to be found in the middle belt of Nigeria particularly Niger, Kogi, Benue and part of savannah regions of Kaduna, Sokoto and Kano State (Mann *et al*., 2003).

The plant bark is light grey with numerous vertical fissures. Branchlets are not hairy and leaves are composite, digitate with five leaflets that are oval or elliptic and alternated at the base. The leaflet is 5-20cm long, 4-10cm broad and petiolated. Inflorescence is made up of auxiliary cymes with small white fluorescence sometime bearing dark purple spots. Fruits are drupes usually green but black at maturity. The fruit ripens between May and August and consist of a thin exocarp, the edible mesocarp (pulp) and a thick endocarp (Burkill, 1995). Ethno-botanical uses include treatment of stomach and rheumatic pains, inflammatory disorder, diarrhoea and dysentery indicating that the leaves may possess anti- inflammatory and analgesic properties among others (Mann *et al*., 2003) (Plate 2.3).

Plate 2.3: Photograph of *Vitex doniana* plant

2.9 Phytochemical Constituents

Phytochemicals are chemical compounds that occur naturally in plant which includes phenolic, terpenoids, alkaloids, essential oil constite, lectins polypeptide and polyacetylenes (Molyneux *et al*., 2017). They are the secondary metabolites (plant by-product) which act as a protective mechanism against environmental stressors. The more environmental stressors, the more phytochemicals are produced by plant (Heneman and Zidenberg-Cherr, 2008). However, phytochemical constituents found in fruit and vegetable are called phyto-nutritive and they vary with growing condition (Brown and Arthur, 2011). Some phytochemical constituents are responsible for colouration and other organoleptic properties such as deep colour of blue Berries and smell of Garlic (Sneader, 2010). Some phytochemical compounds with physiological properties may be elements rather than complex organic molecules for example selenium which is abundant in many fruit and vegetable (Brown and Arthur, 2011).

There are many as 4, 000 different phytochemical constituents (Molyneux *et al*., 2017).Phytochemicals are divided into two classes namely phyto-anticipins and phyto-alexins. Molecules that are present in an inactive form, example glycosides belong to phyto-anticipins, while phyto-alexins are large group of chemically diverse molecules including simple phenylpropanoides derivatives, alkaloids, glycosteroids, flavonoids, sulphur products, terpenes and polyketides (Tapas*et al*., 2008). The same molecule can be a phyto-alexin or a phyto-anticipin in different organs of the same plant and example are terpenoids, quinones and tannins (Abreu *et al*., 2012).

There are several phytochemical classes with antimicrobial properties. However, the medical community does not recognize them as therapeutic agents. This is mainly explained because the majority of phytochemicals have weak spectra of activities (Tapas *et al*., 2008). The

concentration of the phytochemical constituents required is too high to be clinically significant(Molyneux *et al*., 2017).

2.9.1 Alkaloids

Alkaloids are a class of basic, naturally occurrimg organic compounds that containat least one nitrogen atom or atoms (amino or amido in some cases)in their structures (Andreas, 2009).This group also include some related compounds with neutral and even weakly acidic properties (Manske, 2005). However, some synthetic compounds of similar structure may also be termed as alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulfur and other elements such as bromine and phosphorous (Plemenkov, 2011)

Alkaloids such as morphine, strychnine, atropine, colochicine, ephedrine, quinine and nicotine are mostly found in herbaceous plants, but some occur in woody plants (Robert, 2008). Normally, alkaloids are produced from the *Apocinaseae* and *Solanaceae* families. Alkaloids commonly concentrated in particular organs such as the leaves, stem-bark and roots.Alkaloids are produced generally by many plant species, mainly by flowering plants and animals such as frog and lizard (Robert, 2008). Plants produce and store many organic compounds like amino acids, proteins, carbohydrates, fats and alkaloids which are usually regarded as secondary metabolites. They are stored in each part of the plants such as leaves, stem-bark, root and fruits of the plant but in different amount (Qius *et al*., 2014). Alkaloids are classified based on chemical structures namely true alkaloids, proto-alkaloids, polyamine alkaloids, pseudo-alkaloids and peptide and cyclopeptide (Andreas, 2009).True alkaloids contain nitrogen in the heterocycle and originate from amino acids and examples are atropine, nicotine and morphine (Fattorusso *et al*., 2008). This group also includes some alkaloids that besides the nitrogen

heterocycle contain terpene (evonine), peptide fragment (ergotamine), while conine and coniceine are example of piperidine alkaloids (Dewick, 2012).

Proto-alkaloids contain nitrogen but not nitrogen heterocycle and also originate from amino acids and examples are adrenaline, mescaline and ephedrine (Plemenkov, 2011). Polyamine alkaloidsare derivatives of putrescine, spermidine and spermine.Pseudo-alkaloids are alkaloid-like compounds that do not originate from amino acids (Aniszewski, 2007). This group includes terpene and steroid-like alkaloids (Plemenkov, 2011) as well as purine-like alkaloids such as caffeine, theobromine, theacrine and theophylline (Aniszewski, 2007).

Alkaloids have diverse physiological effects such as antibacterial, antimitotic, anti-inflamatory, analgesic, local anesthetic, hypnotic, psychotropic and antitumor activity (Chisholm, 2015). Natural alkaloids are used for the development of antimalaria agents (Quinine and Chloroquine), anticancer agents (taxal, vinblastine and vincristine) and promoting blood circulation in the brain (vincamine) (Aniszewski, 2007).The solutions of alkaloids are intensely bitter with pharmacological application as anaesthetics and central nervous system stimulants (Madziga *et al*., 2010).Alkaloids such as squalamine (polyamine alkaloids) were reported to acts through a detergent-like mechanism of action against Gram-negative bacteria leading to disruption of their outer membrane (Cushnie *et al*., 2014).

Alkaloids such as pergulamine and tylophorinidine have effect on bacterial cell division by inhibiting an enzyme called dihydrofolate reductase which is responsible for the production of purine and pyrimidine precursors for biosynthesis amino acids, RNA and DNA (Ome-Kalu *et al*., 2015).

2.9.2 Anthraquinones

Anthraquinones are also called anthracenediones or dioxoanthracenes and are important members of the quinone family. Anthraquinones are polycyclic aromatic hydrocarbon derived from anthracene or phthalic anhydride (Vogel, 2012).They constitutes a large structural variety of compounds among the polyketide group. Anthraquinones are structurally built from an anthracene ring with a keto group on position 9, 10 as the basic core and different functional groups such as –OH, -CH3, -OCH3, -CH2OH, -CHO and –COOH may substitute at various positions (Brito, 2007).

Anthraquinones and their derivatives rea produced as secondary metabolites in plants, lichens, insects and higher filamentous fungi (Kar, 2007). These compounds could also be found at lower amounts in other types of vegetables and herbs such as cabbage, lettuce, beans, and peas and occur in a free form or as glycosides (Milazzo and Horneber, 2015). These glycosides are formed when one or more sugar molecules, mostly glucose or rhamnose are bound to the aglycone by an O-glycoside linkage to a hydroxyl group (Lindhorst, 2007).

Examples of anthraquinones are emodin, physcion, chrysophanol, alizarin, quinolizarin, rhein, hypercin and protohypercin. Several anthraquinones has been reported to inhbit the replication of viruses or even directly kill enveloped or un-enveloped strains (Cushnie *et al*., 2014).

Anthraquinones act by inhibiting two enzymes involved in bacterial DNA synthesis;(topoisomerase II and DNA gyrase) which block DNA replication thereby impair cell division (Ome-Kalu *et al*., 2015).

2.9.3 Glycosides

Glycosides are generally defined as the condensation products of sugars (including polysaccharides) with varieties of organic hydroxyl (occasionally thiol compounds) invariably monohydrate in characters of carbohydrates example hemiacetal entity of carbohydrate (Brito, 2007). These compounds have been reported to be colourless, water soluble and found in the cell sap (McNaught and Wilinson, 2019). Chemically, glycosides contain a carbohydrate (glucose) and non-carbohydrate part (aglycone or genin) (Lindhorst, 2007).

Glycosides are classified based on the glycosidic bond lies below or above the plane of the cyclic sugar molecule thus alpha and beta glycosides (Lindhorst, 2007). These compounds are also classified according to the chemical nature of the aglycone; alcoholic glycosides, cardiac glycosides, anthraquinone, coumarin, chromne, cyanogenic, flavonoid phenolic, saponin, steviol, iridoid and thioglycosides (Kar, 2007).

Salicin is an example of alcoholic glycoside which has been found in the genus Salix. Salicin is reported to be converted into salicylic acid in the body, which is closely related to aspirin, thus has analgesic, antipyretic and anti-inflammatory effect (Milazzo and Horneber, 2015).

Cardiac glycosides act on the muscles, while anthracene glycosides are used for the treatment of skin diseases as well as purgative for the treatment of diarrhoea. Chalcone glycosides are used as anticancer (Sarker and Nahar, 2007). Cynogenic glycosides are used as flavouring agents in many preparations. Amygdalin has been used in the treatment of gastric cancer and as well as cough suppressant in various preparations (Gleadow and Moller, 2014).

The glycosides have been reported to inhibit bacterial protein synthesis by binding with high affinity to the A-site on the 16s ribosomal RNA of the 30s ribosome (Cushnie *et al*., 2014).

2.9.4 Flavonoids

These are group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure a range of C15 aromatic compounds (Kar, 2007). The compounds are repoetedly derived from parentcompounds known as flavans (Delage, 2015). Over 4,000 flavonoids are known to exist and some of them are pigments in higher plants (Nic *et al*., 2009).

Flavonoids have ability to act as powerful antioxidants which can protect the human body for free radicals scavenging activities (Aniszewski, 2007). Examples of flavonoids are luteolin and catechisn which are better antioxidant than nutrient antioxidant such as vitamin C, E and beta carotene (Delage, 2015). They also possess anti-inflammatory activity, enzyme inhibition, antimicrobial, oestrogenic, anti-allergic, vascular and cytotoxic anti tumour activity (Ravishankar*et al*., 2013).

2.9.5 Saponins

Saponins are derived from *Saponaria vaccaria* (*Quillaja saponaria*), a plant with abundant saponin and was once used as soap (Kar, 2007). Structurally, they are glycosides, sugars attached to anotherorganic molecule, usually a steroids building block (Hostetmann and Marston, 2005).There are two major groups of saponin and these include steroid saponins and triterpene saponins. Saponins are water and fat soluble and insoluble in ether, and like glycosides on hydrolysis and gives aglycones (Kar, 2007). Some examples of these chemicals are glycyrrhizin and licorice flavoring; quillaia, bark extract used in beverage; and sequalene, a biological precursor to cholesterol that has been used as a vaccine adjuvants (Hostetmann and Marston, 2005).

The compounds are reportedly derived their name from the soapwort plant known as Saponaria which belongs to the*Caryophyllaceae*family (Hostetmann and Marston, 2005). Saponins have been found in the unripe fruit of *Mamikara zapota*, Soapberry and horse chestnut (Yucekutiu *et al*., 2008).These compounds are extremely poisonous and can cause haemolysis of blood therefore known to cause fish and cattle poisoning (Foerster, 2006).

Many saponins acts as antimicrobial agents by inhibiting mould growth and protect plants from insect attack (Murthy *et al*., 2010). According to the report of Lorent *et al*. (2014), saponins have shown in membrane permeabilising, immuno-stimulant, hypocholesterolaemic and anticarcinogenic properties. Saponin acts as antioxidant, antifungal and antiviral activity and reported tohave ability to kill protozoan and molluscs.

Saponins were reported to act by altering the permeability of the cell membrane and hence exerting toxicity on all organized tissues. They exert some bacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (Lacaille-Dubois and Wangner, 2010).

2.9.6 Steroids

Steroid is a class of natural or synthetic organic compounds characterized by a molecular structure of 17 carbon atoms arranged in a four rings.A steroid is also defined as a biologically active organic compound with four rings arranged in a specific configuration (Lednicer, 2011). Steroids have two principal biological functions: as important components of cell membranes which alter membrane fluidity and as signaling molecule (Victor, 2001).

These compounds are reportedly found in plants, animals and fungi and all are mabufactured in cells from sterols lanosterol (opisthokonts) or cycloartenol (plants). Lanosterol and cycloartenol

are reportedly derived from the cyclization of the triterpene squalene (Moss, 2014). Steroids are forms of sterols with a hudroxyl group at position three and a skeleton derived from cholestane (Hill *et al*., 2015).

Steroids are classified based on their chemical composition (Zorea, 2014)). Examples of this classification include cholestanes (cholsterol), cholanes (cholic acid), pregnanes (progesterone), androstanes (testosterone)and estranes (estradiol) (Edgreen and Stenczyk, 2009).

Plant steroids (steroid glycosides) are referred to as cardiac glycosides and one of the naturally plant phytochemicals (Firn, 2010). The cardiac glycosides are basically steroids with an inherent ability to afford a specific and powerful action mainly on the cardiac muscles when administered through injection into man or animal. Steroids (anabolic steroids) have been reported to promote nitrogen retention in osteoporosis and in animals with wasting illness (Hanson, 2010).

Steroids have been reported to have synergistic effect with saponins by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (Lacaille-Dubois and Wangner, 2010).

2.9.7 Tannins

Tannins are phenolic compounds of high molecular weight widely distributed among plant flora. Tannins are found in the root, bark, stem and outer layer of plant tissue. Tannins are soluble in water and alcohol, and capable of converting skin into leather. They are acidic in reaction and that is attributed to the presence of phenolics or carboxylic group (Kar, 2007).

Plant extracts containing tannin are reportedly used as astringents against diarrhoea, as diureticand against stomach and duodenal tumours (Haslam, 2017), also anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceutical (Gao and Chen, 2012). Tannins are used

in the dye stuff industry as caustic for cartonic dyes. They are also used in the production of inks (iron gallate ink). They are used to clarify wine, beer, fruit juices and as coagulants in rubber production (Gyamfi and Aniya, 2002). Tannins are polyphenol with pronounced autolytic enzymes of microbial metabolism such as proteolytic macerating enzymes (Obase *et al*., 2011).

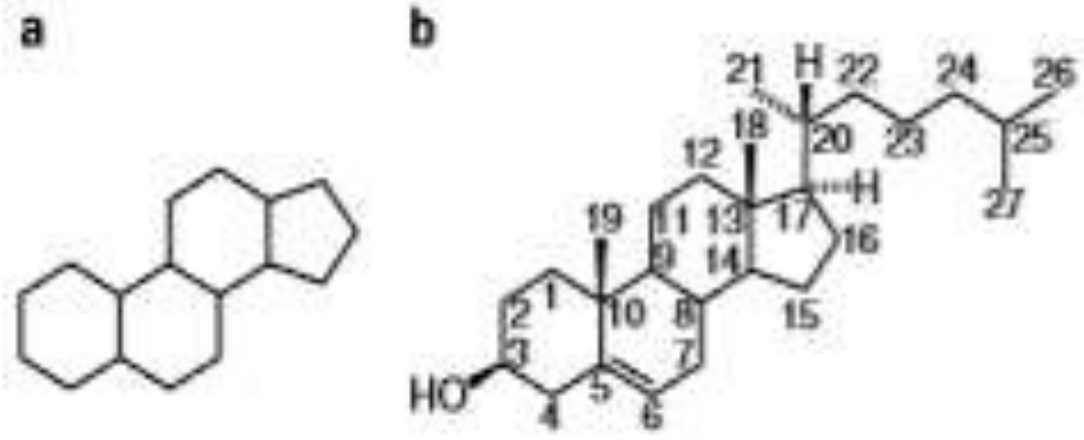


Figure 2.4Structure of Steroids: (a) Perhydricyclopentenophenanthrene (b) cholesterol and steroid locants.

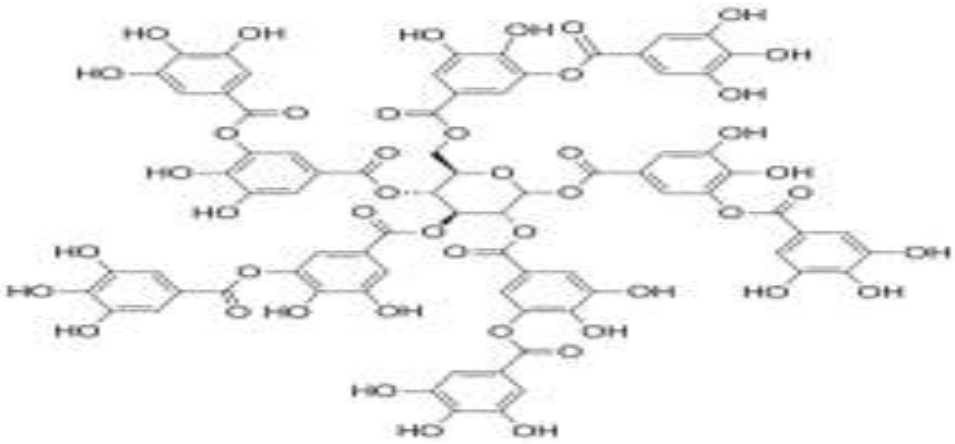


Figure 2.5Structure ofTannin (tannic acid).

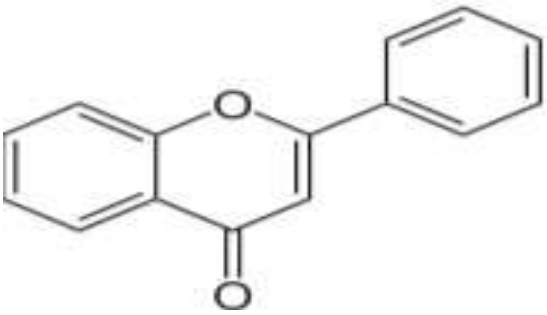


Figure 2.6 Structure of Anthraquinone.

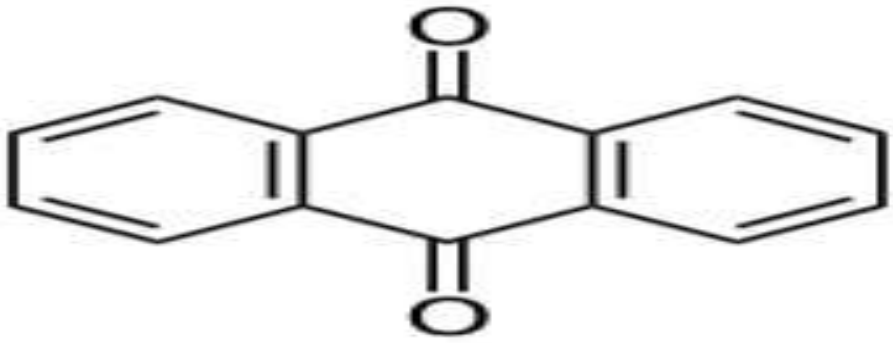


Figure 2.7Structure of Saponin.

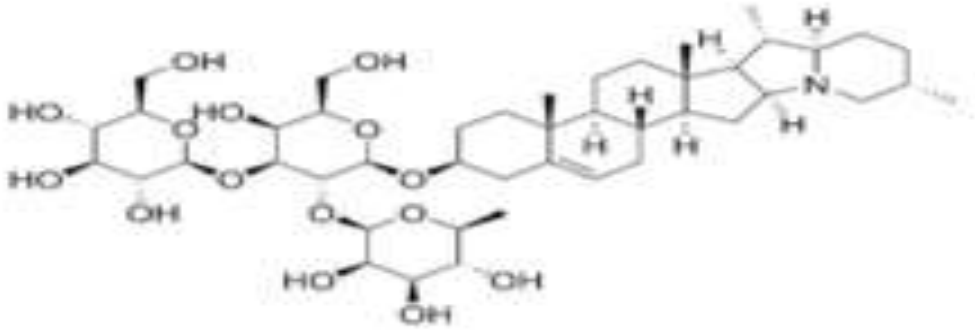


Figure 2.8Structure of Flavonoids (2phenyl-1,4-benzopyrone).

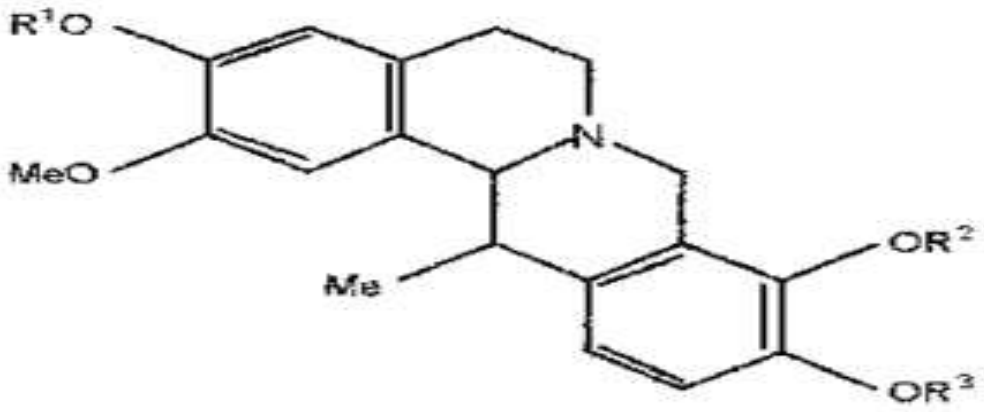


Figure 2.9Structure of Alkaloid.

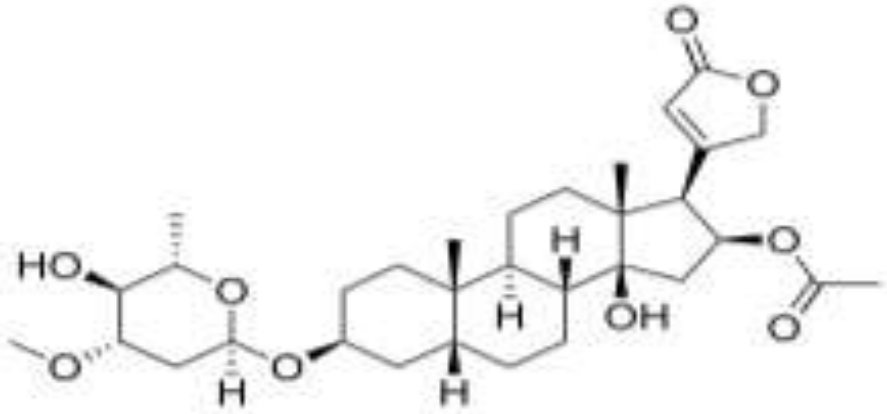


Figure 2.10Structure of Glycoside (Oleandrin).

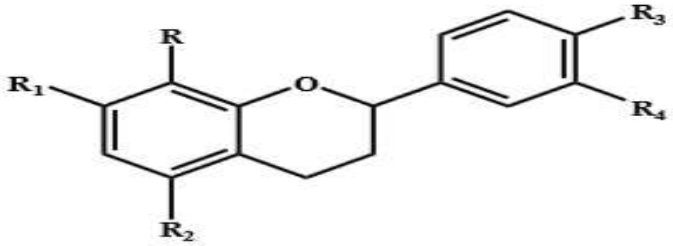


Figure 2.11Structure of Resin.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area as Source of the Study Population

The study was carried out in Niger State, Nigeria. Niger State is located in the central part of Nigeria (middle belt) and is the largest state in the country. The state lies on latitude $08^0$to $11^{0.}30^1$ North and longitude $03^0.30^1$ to $07^0.40^1$ east. It has a land mass of seventy-six thousand three hundred and sixty-three kilometer square (76,363km^2) with a population of four million eighty-two thousand five hundred and fifty-eight (4,082,558). The state has twenty-five (25) local government areas and is divided into three senatorial zones namely Zone A (Niger South), Zone B (Niger East) and Zone C (Niger North) with headquarter located at Bida, Minna and Kontagora respectively. The selected Hospitals include Etsu Umaru Sanda General Hospital, Bida, (Niger South Senatorial zone), General Hospital, Minna (Niger State East Senatorial zone) and General Hospital, Kontagora (Niger State North Senatorial zone) where diarrhoeal cases are reported. Below is the map ofNigeria showing Niger State and map of Niger State showing the sampling sites (Fig 3.1a, b and c) (Niger State Ministry of Land and Housing, 2020).

3.2 Ethical Approval

Ethical approval was obtained from the authorities of Niger State Hospitals Management Board, Minna, Niger State; before the commencement of sample collection (Appendix II).

3.3 Study Design

The study was cross-sectional and Hospital-based and involved sample collection from both male and female (infant and young children) patients.

STUDY AREA

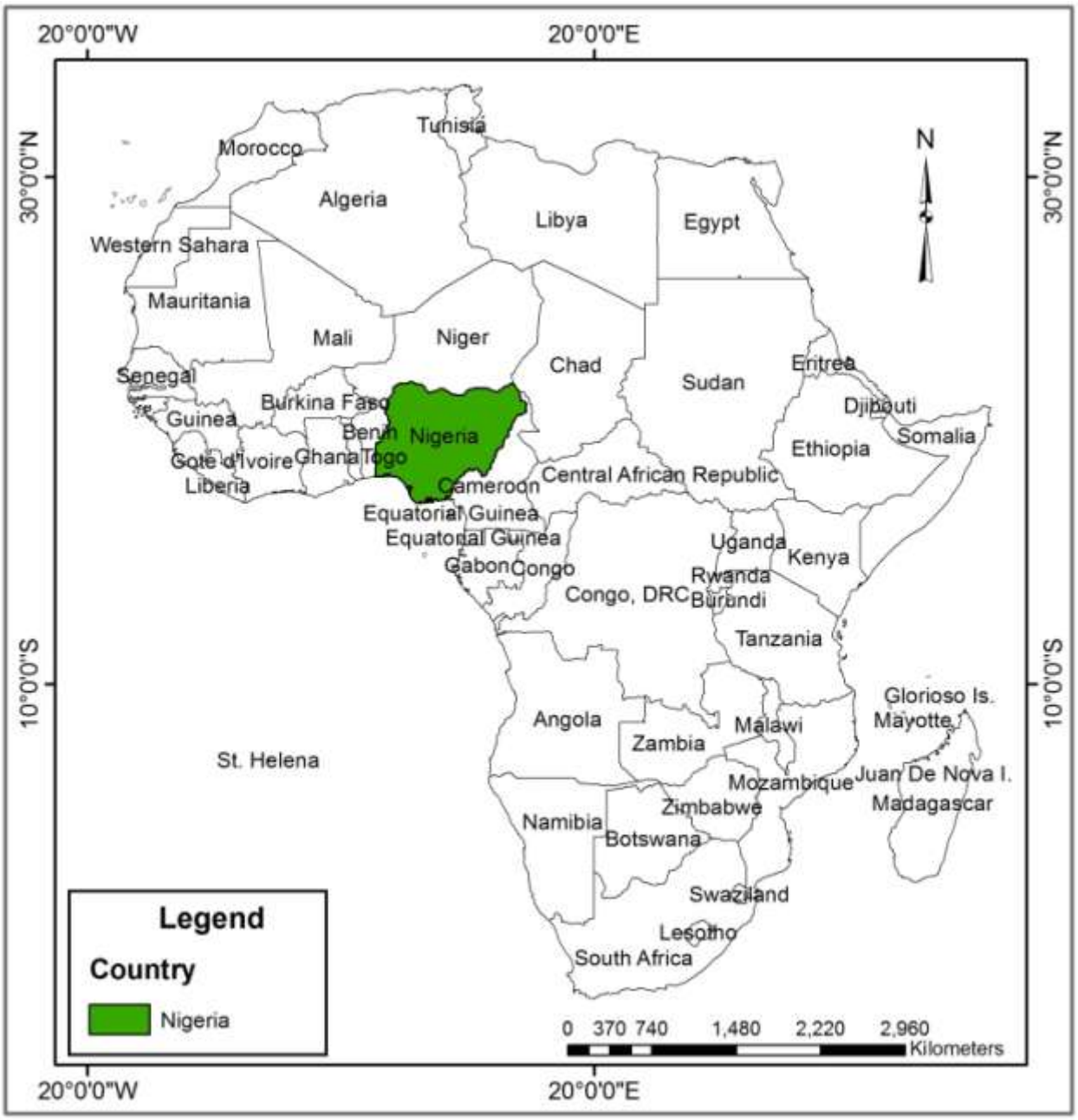


Fig. 3.1a:Map of Africa showing Nigeria

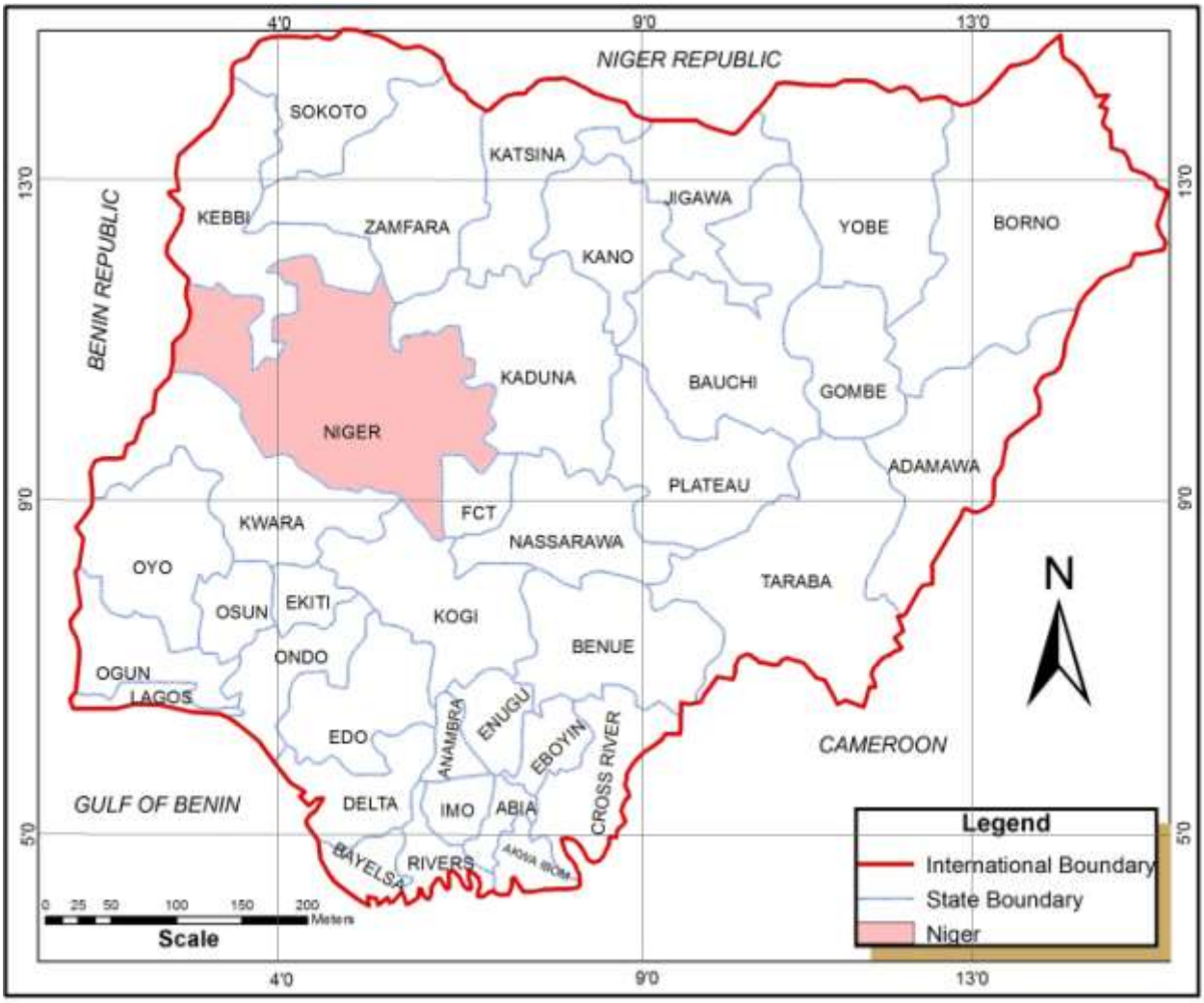


Fig. 3.1b: Map of Nigeria showing Niger State

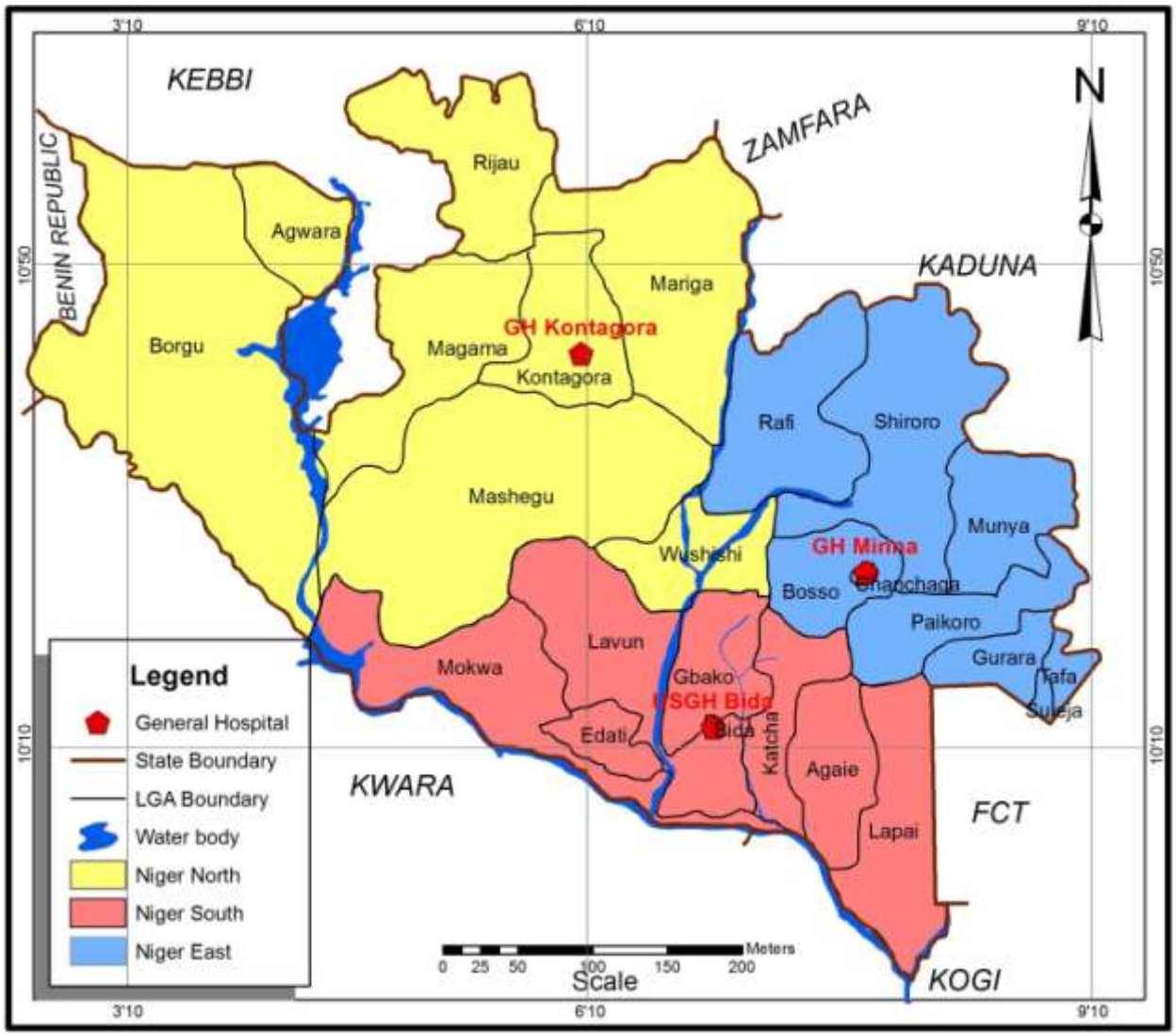


Fig. 3.1c: Map of Niger State showing sampling site

3.4 Sample Size

The sample size of four hundred and eighty (480) was obtained using the formula below:

$$N=\frac{Z^2PQ}{L^2}$$

N=Sample size

Z=95% confidence interval (1.96)

P=Known prevalence of infection= 15% (0.15) (Iwalokun, 2008)

Q=1-P = 1-0.15= 0.85

L=allowable error =0.05

$$N=\frac{(1.96)^2\ (0.15)\ (0.85)}{(0.05)^2}$$

$$N=\frac{(3.8416)\ (0.1275)}{0.0025}$$

$$N=0.\frac{489804}{0.0025}$$

N=195.9216

N=196

Arising from the formula, four hundred eighty (480) samples were used for the research to have enough isolates.

3.5 Study Participants

These comprised of patients (infants and young children) aged 0-5 years presenting with diarrhoea. However, informed consent was obtained from parents of patients concerned.

3.6 Inclusion Criteria: patients (infants and young children) aged 0-5 years of both sexes having diarrhoea in 2-4 weeks preceeding sample collection.

3.7 Exclusion Criteria: patients aged above 5-years of both sexes who had diarrhoea but whose parents did not give consent, and children who had been on antimicrobial drugs.

3.8 Collection and Authentication of Plant Material

The leaves and stem-bark of *Entada africana*, *Ptericarpus erinciceus* and *Vitex doniana* were collected in July, 2014 around Ibrahim Badamasi Babangida University, Lapai, Niger State. All the plants were taxonomically identified and authenticated by Dr. Adebola of Biological Sciences Department, Ibrahim Badamasi Babangida University Lapai, Niger State. The specimen with vouchernumber: EA/00245, PE/00246 and VD/00247 were deposited at the Departmental herbarium respectively.

3.9 Administration of Questionnaire to Examine Socio-demograhic/Risk factors

At the respective Hospitals, arrangement was made with medical personnel for the administration of the structured questionnaire to the enrolled subjects. Clinical data for each patient were collected such as age, sex, appearance of the stool, area of domicile, sources of water, feeding pattern, occupation and educational status of parents using structured questionnaire to identify the possible socio-demographic factors that are associated with a patient's risk for diarrhoeagenic *Escherichia coli* infection.

3.10 Collection of Stool Samples

A total of four hundred and eighty (480) stool specimens were collected into wide mouth sterile screw cap bottles from three selected Hospitals in Niger State, one from each senatorial zone.

The stool samples were collected from the three Hospitals earlier mentioned weekly (160 diarrhoeal stool samples from each Hospital).Each sample was clearly labeled and registered with patient's sex, age and sample appearance. All stool samples were initially stored at Medical Microbiology Laboratory of selected Hospitals before transporting them to Ibrahim Badamasi Babangida University multi-purpose laboratory Lapai, Nigeria for final analysis.

3.11 Stool Analysis

3.11.1 Isolation of *Escherichia coli* from theDiarrhoeic Stool Samples

Each sample was homogenized with swab stick and about 0.5mL was introduced into MacConkey agar and then incubated at 37^0C for 24 hours (Cheesbrough, 2000). Colony with pink red was sub-cultured on EMB agar plate and incubated at 37^0C for 24 hour. Colony with green metallic sheen confirmed *Escherichia coli* isolates.

3.11.2Gram staining

Heat-fixed smears of the isolates were prepared on clean glass slides using the inoculating wire loop by emulsifying two colonies of the isolate in a drop of normal physiological saline. The heat-fixed smears were flooded with crystal violet solution for 60 seconds and rinsed under running water, and air-dried. This was followed by addition of Lugol's iodine for 60 seconds. The smears were also rinsed and dried. Few drops of 95% ethanol were then added to the slides, and rinsed with distilled water. The smears were counterstained with a few drops of safranin, rinsed with distilled water and air-dried. The appearance of pink colour of the cell indicated a positive result (Gram -), while a purple colour indicated negative result (Gram +) (Cheesbrough, 2000).

3. 11.3 Biochemical Tests for Identification of *Escherichia coli*

The isolates were subjected to the following preliminary tests to identify them before further serotyping them to strain level using O-specific *E. coli* Antisera kits.

3.11.3.1Catalase test

A drop of 6% hydrogen peroxide was placed on a glass slide and a colony of the test isolates was emulsified in hydrogen peroxide with a flamed nichrome wire loop. Immediate bubbling and frothing on the slide indicated positive result, while no bubbling and frothing showed negative result (Cheesbrough, 2000).

3.11.3.2Oxidase test

A drop of Oxidase reagent was placed on the left side of the filter papers. The test isolates were placed on the drop of oxidase reagent on the filter paper. The presence of purple colour indicated positive result and colourless indicated negative result (Cheesbrough, 2000).

3.11.3.3Citrate utilization test

The test organism was inoculated unto the slant surface of Simmon's citrate agar with an inoculating needle. The butt of the medium was also stabbed. The test tube was incubated at 37^0C for 24 hours. Lack of colour change from the original green colour of the agar was indicative of negative result, while blue colour indicated positive result (Cheesbrough, 2000).

3.11.3.4Indole test

A colony of the test organism was inoculated into peptone water and incubated at 37^0C for 24 hours. About 0.5mL of Kovac's reagent was added into the overnight broth culture. A red ring at the surface reagent layer within 10 minutes was recorded as a positive result, whilea yellow color indicated a negative result (Cheesbrough, 2000).

3.11.3.5Methyl red/Voges Proskauer test

A single broth containing glucose and peptone was used for the test. The broth was inoculated with the test organism and incubated at 37^{o}C for 24 hours. The incubated broth was further divided into two tubes. To the first tube, 3 drops of methyl red were added. The red colour indicates positive result and orange colour indicates negative result. To the second tube, 2 drops of 40% KOH and 0.6 mL of alpha-naphthol solution was added and a pink colour indicated positive results, while no color change indicated the negative result (Cheesbrough, 2000).

3.11.3.6Serotyping

Species identified as *Escherichia coli* biochemically were serotyped by slide agglutination tests using O specific antisera (Biotech., UK) as described by Chart, (2012). A loopful of 4% physiological saline was placed on a sterile glass slide. A small amount of growth of culture was then picked, emulsified and mixed by titling the slide to and fro for about 30 seconds. The result from the mixture was observed for agglutination against dark background. Those slideswithout agglutination were considered for the serotyping. However, a loopful of the specific test antiserum was added, mixed and then examined for agglutination within 1-2 minutes (Chart, 2012).

3.12 Preparation of Plant Sample

The leaves and stem-bark of *Entada africana* (Dorot*), Ptericarpus erinaceous* (Africa rose treek) and *Vitex doniana* (Black plum) were rinsed with distilled water, cut into pieces with a sterile scalpel and air-dried in the laboratory for three weeks at room temperature (22^{O}C) in a shaded area. The dried plant materials were grinded into particles by the aid of a clean mortar and pestle. The grinded particles were sieved with mesh sieve size (0.26mm) to obtain a fine powder.

3.12.1 Extraction of Pulverised Plant Sample

Extraction of plant samples was carried out using Soxhlet extractor. Acetone, water, hexane and methanol were used as solvents of extraction. Methanol being a polar solvent has capacity of extracting hydrophilic compounds, while hexane (non polar) has capacity of extracting lipophilic compounds.

A hundred gram (100g) of the powdered leaves of each plant (*Entada africana, Ptericapus erinaceu*s and *Vitex doniana*) were weighed and wrapped in a plain paper and placed in a Soxhlet extractor and extracted with water, acetone, hexane and methanol. The extraction was done until solvent in the Soxhlet turned colourless. The filtrate was transferred into Porcelain dish and then allowed to dry. The extract obtained was labeled, weighed and kept for further analysis. The same procedure was followed for stem-bark extraction.

3.12.2 Fractionation of Plant Extracts

After preliminary antibacterial activity testing, the plant extracts with highest potency was considered for further fractionation using Tijjani *et al*. (2017) method.

The most active plant extract (*Vitex doniana* methanol leaf extract)(12.5g) was fractionated on a silica gel column chromatography usingsolvents such as hexane, acetone, methanol and distilled water. Three hundred grams (300g) silica gel 60-120 mesh was packed manually into a column (75cm x 3cm). The silica gel was allow hydrating in n-hexane and filled into the column with bedding height of 60cm and then allowed to settle and packed. The methanol leaf extract of *Vitex doniana*was then dissolved in 9 mL of n-hexane, mixed with silica gel subsequently applied on the top of the column.

The methanol leaf extract of *Vitex doniana* was gradiently and successively eluted with n-hexane, acetone, and methanol 100% and aqueous at a flow rate of 1mL/min. Each eluents fraction (200mL aliquots) was collected. The collected fractions were concentrated to dryness using rotatory evaporator at 65^{0}C and were subjected later to thin layer chromatography analysis by making slurry of silica gel 60G Merck, Germany on 20cm x 20cm x 7cm x 5cm pre-coated silica gel Thin layer chromatography plates. Fractions with similar Rf values from the same solvents system were pooled together. The same procedure was followed for methanolic stem-bark extracts. The phytochemical testing of all the fractions was performed using qualitative methods of Ogukwe *et al*.(2004), Danlami, (2009) and Hassan *et al*. (2005).

3.12.3 Preliminary Phytochemical Screening of Plant Extracts

The method of Hassan *et al.* (2005) and El-Mahmood and Doughari (2009) were used to detect the presence of phytochemical constituents. The crude aqueous, acetone, hexane and methanolic extracts were subjected to phytochemical screening for alkaloids, anthraquinones, flavonoids, glycosides, resin, saponins, steroid and tannins.

3.12.3.1Alkaloids

To 3ml of extract, 1mL of 1% of HCl was added. The mixture was treated with two drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids. Absence of white precipitate indicated negative result (Ogukwe *et al*., 2004).

3.12.3.2Anthraquinones

To 4mL of extract, 4mL of 100% ammonia solution was added. Pink violet or red colour in the ammonical layer (lower layer) indicated the presence of anthroquinones, while yellow colour indicated negative result (Danlami, 2009).

3.12.3.3Flavonoids

To 1mL of extract, 3 drops of ammonia solution was added. Half (0.5) mL of conc. HCl was further added to the mixture. A pale brown coloration indicated the presence of flavonoids, while white colour indicated a negative result (Odebiyi and Sofowora, 1978).

3.12.3.4Glycosides

To 1 mL of the extract, 2 mL of acetic acid was and added and then cool in an ice bath at (4^0c). To the mixture, 1 mL of conc. H_2SO_4 was added drop wise. Oil layer formed on top of the solution indicated the presence of glycosides, while absence of oil layer formation indicated a negative result (Odebiyi and Sofowora, 1978; Ogukwe *et al*., 2004).

3.12.3.5Resins

To 5mL of extract, 5 mL of copper acetate solution was added. The mixture was shaken vigorously and the allowed to separate. A reddish brown precipitate indicated the presence of resins, while a pale blue indicated a negative result (El-Mahmood and Doughari, 2009).

3.12.3.6Saponins

To 2mL of the extract, 5 drops of olive oil was added. The mixture was vigorously shaken, a stable emulsion forms in the extract indicated the presence of saponins, while absence of emulsion form indicated a negative result (Hassan, 2005).

3.12.3.7Steroids

To 1mL of extract, 1mL of conc.H_2SO_4 was added. A red coloration indicated the presence of steroids, while a yellow colour indicated a negative result (Hassan *et al*., 2005).

3.12.3.8Tannins

Two drops of 5% $FeCl_3$ was added to 1mL of extract and a dirty green precipitate indicated presence of tannins, while absence of green precipitate indicated a negative result (Ogukwe *et al*., 2004).

3.13 Experimental Animals (Albino Rats)

Young Albino rats (male) with an average body weight (70 – 130g) were used for this study. They were obtained from the Animal House Unit of the Department of Biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger State. They were housed in a polypropylene cage and maintained at 30-37ºC for 12 hours' dark/light cycle. The animals were acclimatized to laboratory condition for 7 days prior to experiment. They were fed with standard mash and water ad libitum. For hygienic purpose, the litter to the cage was cleaned twice a week.

3.14 Acute Toxicity Studies (Determination of LD_{50})

The method of OECD, (2001) and Hassan *et al.* (2007) were used for out acute toxicity study of the plant extracts. Water (aqueous) extract of *Entada africana* leaf extract (1000, 2000, 3000, 4000 and 5000 mg/kg) was administered to six (6) groups, (six Wister albino rat per group) of rats (one after the other at a grace observation of 48 hours) in single oral dose by using a feeding needle. The control group received distilled water. The same procedure was followed with aqueous *Ptericarpus erinaceus* and *Vitex doniana* leaf and stem-bark extracts respectively.

Observation of toxic symptoms were made and recorded systematically at one, two and six hours after administration. The number of survivors was noted after 48 hours for each animal (rat). The toxicological effect was assessed on the basis of mortality, which was expressed as LD_{50} and was calculated using the limit test or fixed dose.

3.15Determination of Antibacterial Activity of the Plant Extracts

Before antibacterial susceptibility testing was carried out, the isolates were sub-cultured unto fresh nutrient agar slants and incubated at 37^{o}C for 24 hours. Broth suspension of the 24 hour culture was standardized to 0.5 McFarland standards.Eighteen milliliter (18 mL) of molten sterile Mueller-Hilton agar waspoured into sterile Petri plate and was allowed to solidify. The standardized suspension was used to inoculate the surface of agar plates using sterilized swab. A standard cork borer of (6mm diameter) was used to punch five wells aseptically into the agar and each well was filled with 0.2mL of the extract and diffusion time of 45 minutes was allowed. The inoculated agar plates were then incubated at 37^{o}C for 24 hours. The antibacterial activities were evaluated by measuring zones of inhibition using transparent metric ruler (Junaid *et al*., 2006).

3.16 Determination of Minimum Inhibitory Concentration (MIC) of the Extracts

The broth tube dilution method was adopted for the study. The stock solution of each fraction was first prepared by weighing 0.4g of each fraction into the 10mL of Mueller-Hinton broth to make the concentration of the stock to 40mg/mL. The sterile capped test tubes were numbered 1-6. Approximately 2.0 mLs of extract stock solutions (40mg/mL) was placed into first test tube, and 2.0mL of sterile Mueller-Hinton broth was added to all other tubes. Exactly 2.0mL was transferred from the first test tube that contains the extract and the broth to the second tube. The content of the second tube was mixed, using another sterile syringe and needle, 2.0mL was again transferred to the third tube. The dilution continued to tube 5, using a sterile syringe and needle at each stage of dilution. Exactly 2.0mLwas removed fromtube 5 and discarded, while tube 6 contained the broth and inoculum only which served as the negative control. To all the test tubes, 0.1 mLof the bacterial suspension (inoculum) that conformedto 0.5 Mcfarland standardswas

added. All the tubes were incubated at 37^{o}C for 24 hours. The tubes were examined for turbidity. The tube with the highest dilution without growth was the MIC of the extracts.

3.17 Determination of Minimum Bactericidal Concentraction (MBC) of the Extracts

The minimum bactericidal concentration was determined by subculturing the contents of the tubes that showed no growth in minimum inhibitory concentraction tubes on to extract free Mueller-Hinton agar plates, incubated at 37^{0}C for 48 hours and then examined for bacterial growth. The Mueller-Hinton agar plate with the highest dilution of extract (lowest concentration) that showed no growth was taken as the minimum bactericidal concentraction (MBC) of the extract.

3.18 Data Analysis

The chi-square (χ^2) test of association was used to identify if association exists between the occurrences of *Escherichia coli* strains in diarrhoea and socio-demograhic factors. Data on descriptive statistics such as percentages and chi-square were analyzed using statistical package for social sciences (SPSS 20).

CHAPTER FOUR

4.0: RESULTS

Out of 480 stoolsamples screened for*Escherichia coli*, eighty-one (81) samples were positive indicating overall prevalence of 16.9% (Figure 4.1). The distribution of *Escherichia coli* among diarrhoeal patients enrolled in selected Hospitals in Niger State is shown in Table 4.1. The findings revealed that the highest prevalence of 21.3%was found among patients attending General Hospital, Kontagora followed by General Hospital, Minna with the prevalence of 17.0%, while the patients attending Umaru Sanda Ndayako General Hospital, Bidahad the lowest prevalence of 12.5%. However, the difference was not statistically significant (χ^2=6.000; 0.293).

Different strains of *Escherichia coli* isolated from stool samples ofdiarrhoeal patients included Enteteroaggregative *Escherichia coli* (n=16; 19.8%), Enterohaemorrhagic *Escherichia coli* (n=13; 16.0%), Enteroinvasive *Escherichia coli* (n=8; 9.9%), Enteropathogenic *Escherichia coli*(n=27; 33.3%) and Enterotoxigenic *Escherichia coli*(n=17; 21.0%) (Table 4.2).

The result of occurrence of *Escherichia coli* analyzed according to socio-demographic characteristics of the study subjects is shown in Table 4.3. *Escherichia col*i isolates were isolated in all age groups with the highest prevalence of 21.8% recorded in age group of 1-2 years, followed by children in age group of 0-1 year with prevalence of 16.2%, while the lowest prevalence of 11.9% and 12.8% was recorded in age group of 2-3 and 4-5 years respectively. The result showed statistically significant with age association of *Escherichia coli* (χ^2=25.000; 0.001).

The prevalence of diarrrhoeagenic *Escherichia coli* in relation to sex in this study showed a higher prevalence of 18.0% among female as compared to the male counterpart with prevalence

of 15.6%. However, there was no association between the *Escherichia coli* infectionand sex (χ^2=5.000; 0.153). Area of domicile and the prevalence of diarrhoeagenic *Escherichia coli* infection were found to be statistically associated (χ^2=20.000; 0.002), with those children who reside in rural areas having the higher prevalence of 18.6%, while children who reside in urban areas had a lower prevalence of 13.3%.

The distribution of *Escherichia coli* with respect to mother's occupation revealed that the highest prevalence of 19.2% was recorded among children whose mothers were housewives, while children whose mothers were civil servants had a prevalence of 9.5%. The association between the *Escherichia coli* infectionand the occupation of mother was statistically significant (χ^2=26.000; 0.000) (Table 4.3).

The distribution of *Escherichia coli* in relation to mother's educational status in the study revealed a prevalence of 25.6% among children whose mothers had no formal education, while children whose mothers had only primary education showed 10.7% prevalence. The lowest prevalence of 1.00% was obtained in children whose mothers had tertiary education. The study indicated a statistically significant association between *Escherichia coli* infection and educational status of the mothers (χ^2=23.850; 0.001). The distribution of *Escherichia coli* in relation to father's educational status also revealed a prevalence of 24.7% among children whose father's had no formal educaton, while children whose fathers had tertiary education recorded a lowest prevalence of 11.7%. The study showed a statistically significant association between *Escherichia coli* infection and educational status of father (χ^2=20.000; 0.002) (Table 4.3).

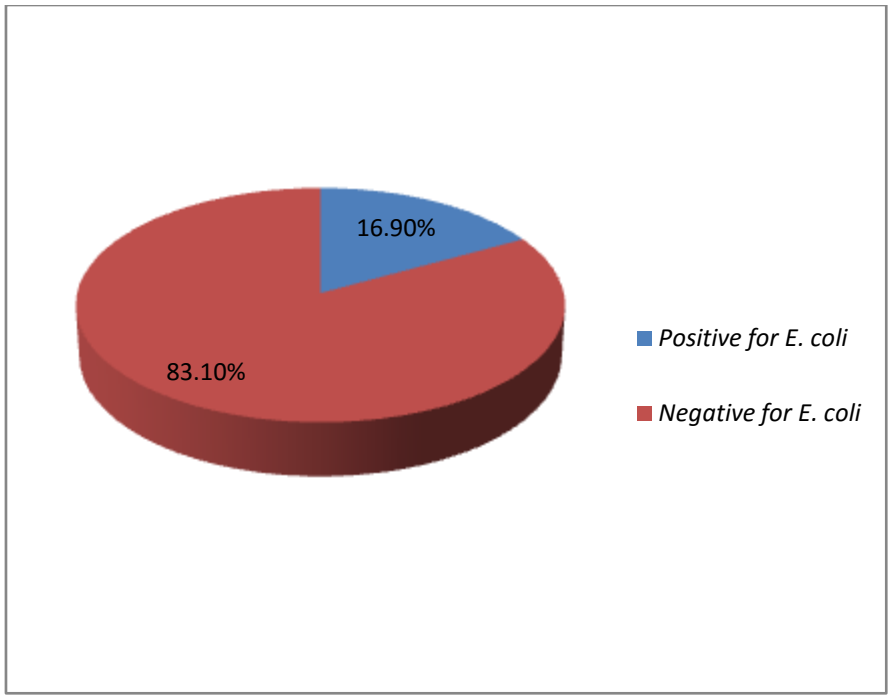


Figure 4.1: The prevalence of *E. coli* isolates from diarrhoeal patients attending selected Hospitals in Niger State, Nigeria.

Table 4.1: Occurrence of *Escherichia coli*among Diarrhoeal Patients Attending Selected Hospitals in Niger State, Nigeria (N=160)

Hospital	No. of positive	Occurrence rate (%)	Chi-square (χ^2)	P-value
USGHB	20	12.5	6.000	0.293
GHM	27	16.9		
GHK	34	21.3		
Total	81	16.9		

Key: USGHB=Umaru Sanda General Hospital, Bida; GHM=General Hospital, Minna GHK=General Hospital, Kontagora; %=Percent; χ^2=Chi-square.

Table 4.2: Distribution of *Escherichia coli* Serotypesamong Children 0-5 Years in Selected Hospitals in the Study Area.

DEC strains n=81	Number of strains	Percentage
Enteroaggregative *Escherichia coli* (EAEC)	16	19.8
Enterohaemorrhagic*Escherchiacoli* (EHEC)	13	16.0
Enteroinvasive *Escherichia coli* (EIEC)	08	09.9
Enteropathogenic *Escherichia coli* (EPEC)	27	33.3
Enterotoxigenic *Escherchia coli* (ETEC)	17	21.0
Total	81	100.0

Key: DEC=Diarrhoeagenic *Escherichia coli*

Table 4.3: Prevalence of *Escherichia coli* infectionin Relation to Some Socio-Demographic Characteristics of Children 0-5 years with Diarrhoea.

Socio-demographic characteristics	Subjects enrolled	No of Positive isolates	Prevalence (%)	Chi-square(χ^2)	Degree of freedom (df)	P-value
Age (year)						
0-1	135	22	16.2	25.000	4	0.001
1-2	165	36	21.8			
2-3	74	10	13.5			
3-4	59	07	11.9			
4-5	47	06	12.8			
Total	480	81	16.9			
Sex						
Male	225	35	15.6	5.000	1	0.153
Female	255	46	18.0			
Total	480	81	16.9			
Area of domicile						
Rural	323	60	18.6	20.000	1	0.002
Urban	157	21	13.3			
Total	480	81	16.9			
Mother's occupation						
Civil servant	116	11	9.5	26.000	1	0.000
House wife	364	70	19.2			
Total	480	81	16.9			
Mother's educ. Status						
Non-formal	215	55	25.6	23.850	3	0.001
Primary	150	16	10.7			
Secondary	113	08	8.0			
Tertiary	02	02	1.00			
Total	480	81	16.9			
Father's educ. Status						
Non-formal	81	20	24.7	20.150	3	0.002
Primary	110	19	17.3			
Secondary	195	31	15.9			
Tertiary	94	11	11.7			
Total	480	81	16.9			

The association between the source of water and the prevalence of *Escherichia coli* infection was also examined. Children who drink from tap water had a prevalence of 35.1% followed by those who drink stream water with a prevalence of 22.0%, while those who use well water and bore-hole water had prevalence of 13.3 and 7.4% respectively. The association between the source of water and the prevalence of *Escherichia coli* infection was statistically significant (χ^2=25.000; 0.001) (Table 4.4).

The association between feeding pattern and the prevalence of *Escherichia coli* infection was examined.Children who used formula milk with other foods showed a prevalence of 44.3% followed by children who feed on the other foods had prevalence of 14.7%.While children who had breast milk and formula milk with prevalence of 9.0%. No isolate was recovered from exclusively breast fed children and the difference was found to be statistically significant (χ^2=21.205; 0.002) (Table 4.4).

The preliminary phytochemical screening of *Entada africana* leaf extracts revealed that the acetone leaf extract contained alkaloids, anthraquinones, flavonoids, glycosides, resins and steroids, while the aqueous leaf extract contained alkaloids, flavonoids and glycosides. The hexane leaf extract contained alkaloids, flavonoids and glycosides, while methanol leaf extract contained all the phytochemical constituents considered except steroids (Table 4.5). As for the stem-bark, acetone stem-bark extractcontained alkaloids, anthraquinones, flavonoids, steroids and tannins, the aqueous stem-bark extract contained alkaloids, anthraquinones, flavonoids and saponins.The hexane stem-bark extract contained all phytochemical constituents except resins, while the methanol stem-bark contained alkaloids, anthraquinones, resins, saponins and steroids (Table 4.5).

Table 4.4: Prevalence of *Escherichia coli* in Relation to Some Risk Factors Among 0-5 year Children with Diarrhoea.

Risk factor	Subjects enrolled	No Positive isolates	Prevalence (%)	Chi-square (χ^2)	Degree of freedom (df)	P-value
Source of water						
Bore hole	135	10	7.4	25.000	3	0.001
Stream water	59	13	22.0			
Tap water	74	26	35.1			
Well water	165	22	13.3			
Total	480	81	16.9			
Feeding pattern						
Exclusive breast feeding	10	00	0.00	21.204	3	0.002
Breast and formula milk	201	18	9.00			
Formula milk and other food	79	35	44.3			
Other food only	190	28	14.7			
Total	480	81	16.9			

Table4.5: Phytochemical Constituents of Leaf and Stem-Bark Crude Extracts of *Entada africana.*

	Phytochemical constituents							
Extract	Alkaloids	Anthraquinones	Flavonoids	Glycosides	Resins	Saponins	Steroids	Tannins
EAALE	+	+	+	+	+	-	+	-
EAQLE	+	-	+	+	+	-	-	-
EAHLE	+	-	+	+	-	-	-	-
EAMLE	+	+	+	+	+	+	-	+
EAASBE	+	+	+	-	-	-	+	+
EAQSBE	+	+	+	-	-	+	-	+
EAHSBE	+	+	+	+	-	+	+	+
EAMSBE	+	+	-	-	-	-	+	+

Key: EAALE=*Entada africana* acetone leaf extract; EAASBE= *Entada africana* acetone stem-bark extract; EAHLE= *Entada africana* hexaneleaf extract; EAHSBE= *Entada africana* hexane stem-bark extract; EAMLE=methanol leaf extrract; EAMSBE= *Entada africana* methanol stem-bark extract; EAQLE=*Entada africana* aqueous leaf extract; EAQSBE= *Entada africana* aqueous stem-bark extract; + = Present; - = Absent.

The phytochemical screening of *Ptericarpus erinaceus* leaf extracts indicated the presence of alkaloids; flavonoids and steroids in the acetone leaf extract; while alkaloids, flavonoids and saponins were found to be in aqueous leaf extract. However, hexane leaf extract contained alkaloids, flavonoids and steroids, while methanol leaf extract contained all the phytochemical constituents tested for except saponins (Table 4.6).

As for the *Ptericarpus erinaceus* stem-bark, acetone stem-bark contained anthraquinones, flavonoids, saponins, glycosides and steroids, the aqueous stem-bark extract contained anthraquinones, glycosides, saponins and steroids. The hexane stem-bark extract contained alkaloids, anthraquinones, glycosides, saponins and steroids. Finally, methanol stem-bark extract contained anthraquinones, glycosides, saponins, tannins and steroids (Table 4.6).

The preliminary phytochemical screening of *Vitex doniana* leaf extracts indicated the presence of anthraquinones, saponins and steroids in aqueous leaf extract. Hexane leaf extract contained alkaloids, anthraquinones, resins, saponins and steroids, while methanol leaf extract indicated the presence of anthraquinones, glycosides, saponins, tannins and steroids.

As for the stem-bark extract, the acetone stem-bark contained anthraquinones, flavonoids, glycosides, saponins and tannins, the aqueous stem-bark contained all phytochemicals tested except resins. The hexane stem-bark extract contained only anthraquinones and tannins, while the methanol stem-bark extract contained all phytochemical constituents tested except steroids and saponins (Table 4.7).

Table4.6: Phytochemical constituents of Leaf and Stem-Bark Crude Extracts of *Ptericarpus erinaceus.*

	Phytochemical constituents							
Extract	Alkaloids	Anthraquinones	Flavonoids	Glycosides	Resins	Saponins	Steroids	Tannins
PEALE	+	-	+	-	-	-	+	-
PEQLE	+	-	+	-	-	+	-	-
PEHLE	+	-	+	-	-	-	+	-
PEMLE	+	+	+	+	+	-	+	-
PEASBE	-	+	+	-	-	+	+	-
PEQSBE	-	+	-	+	-	+	+	-
PEHSBE	-	+	-	+	-	+	+	-
PEMSBE	+	+	-	+	-	+	+	+

Key: PEALE=*Ptericarpus erinaceus* acetone leaf extract; PEASBE=*Ptericarpus erinaceus* acetone stem-bark extract; PEHLE=*Ptericarpus erinaceus* hexaneleaf extract; PEHSBE= *Ptericarpus erinaceus* hexane stem-bark extract; PEMLE=*Ptericarpus erinaceus* methanol leaf extract; PEMSBE=*Ptericarpus erinaceus* methanol stem-bark extract; PEQLE=*Ptericarpus erinaceus* aqueous leaf extract; PEQSBE=*Ptericarpus erinaceus* aqueous stem-bark extract; + = Present; - = Absent.

Table4.7: Phytochemical Constituents of Leaf and Stem-Bark Crude Extracts of *Vitex doniana.*

	Phytochemical constituents							
Extract	Alkaloids	Anthraquinones	Flavonoids	Glycosides	Resins	Saponins	Steroids	Tannins
VDALE	-	+	-	-	-	+	+	-
VDQLE	-	-	+	-	-	+	+	-
VDHLE	+	-	-	-	+	+	+	-
VDMLE	-	+	-	+	-	+	+	+
VDASBE	-	+	+	+	-	+	-	+
VDQSBE	+	+	+	+	-	+	+	+
VDHSBE	-	+	-	-	-	-	-	+
PEMSBE	+	+	+	+	+	+	-	+

Key: VDALE=*Vitex doniana* acetone leaf extract; VDASBF=*Vitex doniana* acetone stem-bark extract; VDHLE=*Vitex doniana* hexaneleaf extract; VDHSBE=*Vitex doniana* hexane stem-bark extract; VDMLF=*Vitex doniana* methanol leaf extrract; VDMSBE=*Vitex doniana* methanol stem-bark extract; VDQLF=*Vitex doniana* aqueous leaf extract; VDQSBE=*Vitex doniana* aqueous stem-bark extract; + = Present; - = Absent.

The result of acute toxicity on albino rat at 3000mg/kg body weight for *Entada africana* aqueous leaf and stem-bark extracts produced no mortality after 48 hours of observation. Oral administration of low doses of 1000-3000mg/kg produced no obvious toxicity. However, higher doses of 4000-5000mg/kg caused slow movement indicating weakness of the animals (Table 4.8). The acute toxicity test of *Ptericarpus erinaceus* aqueous leaf and stem-bark and that of *Vitex doniana* leaf and stem-bark extracts showed no toxic effect at 1000-4000mg/kg but exhibited behavioural change like slow movement indicating weakness of the body at 5000mg/kg (Tables 4.9 and Table 4.10).

The results of preliminary antibacterial activity of crude leaf extract of *Entada africana* are presented in Table 11. *Entada africana* showed that six *Escherichia coli* isolates were resistant to acetone leaf extract, while four isolates were susceptible with zones of inhibition which ranged from 8.00mm-11.00mm at 5.0mg/mL concentration. At concentration of 10mg/mL, all *Escherichia coli* isolates were susceptible to acetone leaf extract with zones of inhibition which ranged from 9.0mm-15.00mm. At concentration of 20mg/mL, all *Escherichia coli* isolates were susceptible with zones of inhibition which ranged from 11.00mm-25.00mm in which *Escherichia coli* coded 186 recorded a zone of inhibition of 25.00mm.

All the *Escherichia coli* isolates were susceptible to *Entada africana* aqueous leaf extractwith zones of inhibition ranged between 12.00mm and 24.00mm at 20mg/mL, while at 5.0mg/mL, *Escherichia coli* isolates coded 149 and 445 were resistant to the extract. However, *Escherichia coli* coded 003 had the highest zone of inhibition (24.00mm) and *Escherichia coli* coded 445 had the lowest zone of inhibition (12.00mm).

The *Escherichia coli* coded 003, 010 and 166 respectively were fairly susceptible to hexane leaf extract with zones of inhibition which ranged from 8.00 mm-9.00 mm and 10.00 mm-11.00 mm at concentration of 10 mg/mL and 20 mg/mL respectively.

The remaining isolates were resistant to hexane leaf extract at 5.0-20 mg/mL concentrations.

All *Escherichia coli* isolates were susceptible to methanol leaf extract at 5.0-20 mg/mL concentrations but at 20 mg/mL concentration, *Escherichia coli* coded 003 and 268 showed highest zones of inhibition of 36 mm and 34 mm respectively (Table 4.11).

The results of antibacterial activity of *Entada africana* stem-bark extract against some *Escherichia coli* isolates are presented in Table 4.12. The hexane stem-bark extract had lowest activity at 10 mg/mL with zones of inhibition ranging from 8.00 mm-10.00 mm, while at 20 mg/mL, eight isolates were susceptible with zones of inhibition which ranged between 10.00 mm-12.00 mm except *Escherichia coli* isolates coded 360 and 445 that were resistant to the extract. At 20 mg/mL, methanol stem-bark extract inhibited all *Escherichia coli* isolates with zones of inhibition which ranged between 22.00 mm-36.00 mm, followed by aqueous stem-bark extract that inhibited all the tested *Escherichia coli* isolates with zones of inhibition which ranged between 14.00 mm-22.00 mm.

Table 4.8: Acute Toxicity(LD_{50}) of Crude Aqueous Leaf and Stem-Bark Extracts of *Entada africana* against Albino Rat.

Plant extract	Group	Albino Rat	Weight of animals (g)	Dosage (mg/kg)	Vol. of extract administered (g/mL)	Mortality
Leaf	Grp A	A1	182	1000	0.2	None
		A2	185	2000	0.4	"
		A3	190	3000	0.6	"
		A4	210	4000	0.8	"
		A5	220	5000	1.1	"
		Control	220	0.00	1.0mL dw	"
Stem-bark	Grp B	B1	184	1000	0.2	None
		B2	192	2000	0.4	"
		B3	196	3000	0.6	"
		B4	200	4000	0.8	"
		B5	205	5000	1.0	"
		Control	208	0.00	1.0mL dw	"

Key: LD_{50}=Median Lethal Dose; Dw= Distilled water; g = gram;ml=mililire; kg=Kilogram; mg= Miligram; Vol=Volume.

Table 4.9: Acute Toxicity(LD_{50}) of Crude Aqueous Leaf and Stem-Bark extracts of *Ptericarpus erinaceus* against Albino rat.

Plant extract	Group	Albino Rat	Weight of animals (g)	Dosage (mg/kg)	Vol. of extract administered (g/mL)	Mortality
Leaf	Grp C	C1	180	1000	0.2	None
		C2	185	2000	0.4	"
		C3	202	3000	0.6	"
		C4	208	4000	0.8	"
		C5	210	5000	1.1	"
		Control	215	0.00	1.0mLdw	"
Stem-bark	Grp D	D1	188	1000	0.2	None
		D2	190	2000	0.4	"
		D3	192	3000	0.6	"
		D4	195	4000	0.8	"
		D5	204	5000	1.0	"
		Control	205	0.00	1.0mL dw	"

Key: LD_{50}=Median Lethal Dose; dw=Distilled water; g=gram; mL=mililire; kg=Kilogram mg=Miligram; vol=Volume

Table 4.10:Acute Toxicity (LD_{50}) of Crude Aqueous Leaf and Stem-Bark Extracts of *Vitex doniana* against Albino Rat.

Plant extract	Group	Albino Rat	Weight of animals (g)	Dosage (mg/kg)	Vol. of extract administered (g/mL)	Mortality
Leaf	Grp E	E1	185	1000	0.2	None
		E2	190	2000	0.4	"
		E3	192	3000	0.6	"
		E4	195	4000	0.8	"
		E5	202	5000	1.0	"
		Control	230	0.00	1.0mL dw	"
Stem-bark	Grp F	F1	192	1000	0.2	None
		F2	195	2000	0.4	"
		F3	200	3000	0.6	"
		F4	215	4000	0.9	"
		F5	220	5000	1.1	"
		Control	230	0.00	1.0mLdw	"

Key: LD_{50}=Median Lethal Dose; dw=Distilled water; g=gram; mL=mililire; kg=Kilogram mg=Miligram; vol=Volume

Table 4.11: Preliminary Antibacterial Activity of *Entada africana* Crude Leaf Extracts against *Escherichia coli*Isolates.

Code	Zone of Inhibition(mm) and Concentration of crude extracts (mg/mL)											
	EAALE			EAQLE			EAHLE			EAMLE		
	5.0	10	20	5.0	10	20	5.0	10	20	5.0	10	20
EUS003	10.00	15.00	22.00	9.00	14.00	24.00	0.00	9.00	10.00	14.00	22.00	36.00
EUS010	0.00	10.00	13.00	8.00	12.00	16.00	0.00	8.00	10.00	8.00	12.00	18.00
EUS149	0.00	9.00	11.00	0.00	8.00	12.00	0.00	0.00	0.00	9.00	12.00	16.00
GHM166	11.00	15.00	25.00	10.00	13.00	20.00	0.00	9.00	11.00	10.00	14.00	25.00
GHM220	0.00	10.00	14.00	9.00	13.00	18.00	0.00	0.00	0.00	9.00	13.00	20.00
GHM268	10.00	14.00	20.00	10.00	14.00	20.00	0.00	0.00	0.00	14.00	21.00	34.00
KGH349	0.00	11.00	16.00	8.00	11.00	16.00	0.00	0.00	0.00	12.00	17.00	28.00
KGH360	0.00	11.00	15.00	8.00	14.00	18.00	0.00	0.00	0.00	10.00	14.00	20.00
KGH399	0.00	9.00	12.00	8.00	12.00	16.00	0.00	0.00	0.00	8.00	12.00	16.00
KGH445	8.00	11.00	15.00	0.00	8.00	12.00	0.00	0.00	0.00	8.00	10.00	14.00

Key: EUS=Etsu Umaru Sanda General Hospital, Bida; GHM=General Hospital, Minna; KGH=General Hospital, Kontagora; EAALE=*Entada africana* acetone leaf extract; *E. coli*=*Escherichia coli*; EAQLE= *Entada africana* aqueous leaf extract; EAHLE=*Entada africana* hexane leaf extract; EAMLE= *Entada africana* methanol leaf extract; 0.0=No zone of inhibition.

Table 4.12: Preliminary Antibacterial Activity of *Entada africana* Crude Stem-Bark Extracts against *Escherichia coli*Isolates.

Code	Zone of Inhibition (mm) and Concentration of crude extracts (mg/mL)											
	EAASBE			EAQSBE			EAHSBE			EAMSBE		
	5.0	10	20	5.0	10	20	5.0	10	20	5.0	10	20
EUS003	9.00	14.00	22.00	11.00	15.00	22.00	0.00	9.00	12.00	12.00	17.00	26.00
EUS010	8.00	11.00	15.00	8.00	11.00	16.00	0.00	0.00	0.00	9.00	14.00	20.00
EUS149	9.00	15.00	17.00	9.00	13.00	20.00	0.00	8.00	10.00	10.00	14.00	22.00
GHM166	8.00	10.00	15.00	8.00	14.00	18.00	0.00	0.00	0.00	10.00	15.00	23.00
GHM220	9.00	15.00	17.00	9.00	12.00	19.00	0.00	0.00	0.00	9.00	13.00	20.00
GHM268	8.00	11.00	16.00	11.00	15.00	16.00	0.00	8.00	11.00	10.00	14.00	22.00
KGH349	0.00	8.00	12.00	8.00	10.00	14.00	0.00	0.00	0.00	11.00	15.00	22.00
KGH360	8.00	10.00	16.00	8.00	12.00	16.00	8.00	10.00	12.00	10.00	15.00	24.00
KGH399	9.00	12.00	16.00	9.00	12.00	18.00	0.00	8.00	10.00	11.00	16.00	26.00
KGH445	8.00	11.00	16.00	11.00	14.00	20.00	0.00	0.00	0.00	13.00	19.00	36.00

Key: EUS=Etsu Umaru Sanda General Hospital, Bida; GHM=General Hospital, Minna; KGH=General Hospital, Kontagora; EAASBE=*Entada africana* acetone stem-bark extract; *E. coli=Escherichia coli*; EAQSBE= *Entada africana* aqueous stem-bark extract; EAHSBE=*Entada africana* hexane stem-bark extract; EAMSBE= *Entada africana* methanol stem-bark extract; 0.0=No zone of inhibition.

Table 4.13 shows the results of antibacterial activity of *Ptericarpus erinaceus* leaf extracts against selected *Escherichia coli* isolates. All *Escherichia coli* isolates were resistant to hexane leaf extract at various concentrations. Acetone leaf extract inhibited all tested isolates at concentration of 20mg/mL with zones of inhibitionwhich rangedfrom 14.00mm-20.00mm. At concentration of 10mg/mL, all tested *Escherichia coli* isolates were susceptible with zones of inhibition ranging from 9.00mm-13.00mm and at 5.0mg/mL concentration, the two isolates coded 010 and 349 were not susceptible to acetone leaf extract.

For methanol leaf extract, all tested *Escherichia coli* isolates were susceptible at 5.0mg/mL with zones of inhibition which ranged from 8.0mm-11.00mm. At concentration of 10mg/mL, all test *Escherichia coli* isolates were susceptible with zones of inhibition which ranged between 11.00mm-15.00mm and at concentration of 20mg/mL, all *Escherichia coli* isolates were inhibited with zones of inhibition ranging from 16.00mm-22.00mm (Table 4.13).

Table 4.14 shows the results of preliminary antibacterial activity *Ptericarpus erinaceus* stem-bark extract. Acetone stem-bark extract fairly inhibited the growth of *Escherichia coli* isolates with zones of inhibition which ranged from 8.00mm-9.00mm at 5.0mg/mL concentration except *Escherichia coli* coded 349. At concentration of 10mg/mL, all tested *Escherichia coli* isolates were susceptible with zones of inhibition rangng from 8.00mm-15.00mm and at 20mg/mL concentration, all *Escherichia coli* isolates were susceptible with zones of inhibition ranging from 12.00mm-22.00mm. In the same vein, all isolates tested were susceptible to aqueous stem-bark extract with zones of inhibition ranging from 8.00mm-11.00mm, 10.00mm-15.00mm and 14.00mm-22.00 mm at 5.0, 10 and 20 mg/mL concentrations respectively. For hexane stem-bark extract, five out of ten *Escherichia coli* isolates tested, were fairly susceptible with zones of inhibition ranging from 8.00mm-10.00mm and 10.00mm-12.00mm at 10 and 20mg/mL

concentration respectively. Methanol stem-bark extract indicated high antibacterial activity against the *Escherichia coli* isolates with zones of inhibition ranging from 20.00mm-26.00mm at 20mg/mL and low antibacterial activity at 5.0mg/mL with zones of inhibition ranging from 9.00mm-12.00mm, while *Escherichia coli* isolates showed moderate antibacterial activity at 10mg/mL concentration with zones of inhibition ranging from 13.00mm-17.00mm (Table 4.14).

The results of preliminary antibacterial activity of *Vitex doniana* crude leaf extracts indicated that two *Escherichia coli* isolates coded 186 and 445 were resistant to acetone leaf extract at 5.0 mg/mL concentration, while the remaining isolates were susceptible with zones of inhibition ranging from 8.00 mm-10.00 mm. At 10 and 20 mg/mL concentration, *Escherichia coli* isolates were susceptible to acetone leaf extract with zones of inhibition ranging between 8.00 mm-14.00 mm and 10.00 mm-22.00 mm respectively (Table 4.15).

Aqueous leaf extract inhibited all tested *Escherichia coli* isolates except *Escherichia coli* coded 149, while at 5.0, 10 and 20 mg/mL concentration, all tested isolates were susceptible to aqueous leaf extract with zones of inhibition ranging from 8.00 mm-14.00 mm, 9.00 mm-18 mm and 14.00 mm-24.00 mm respectively. Three *Escherichia coli* isolates coded 186, 268 and 445 were resistant to hexane leaf extract and the remaining *Escherichia coli* isolates were susceptible to hexane leaf extract with zones of inhibition ranging from 8.00 mm-15.00 mm and 10.00 mm-23.00 mm at 10 and 20 mg/mL concentration respectively. At 5.0mg/mL, all tested isolates were susceptible to methanol leaf extract with zones of inhibition which ranged from 9.00 mm-14.00 mm, while at 10 and 20 mg/mL concentration, all the tested *Escherichia coli* isolates were susceptible with zones of inhibition which ranged from 10.00 mm-23.00 mm and 14.00 mm-36.00 mm respectively (Table 4.15).

Table 4.13: Preliminary Antibacterial Activity of *Ptericarpus erinaceus* Crude Leaf Extracts against *Escherichia coli.*

Code	Zone of Inhibition(mm) and Concentration of crude extracts (mg/mL)											
	PEALE			PEQLE			PEHLE			PEMLE		
	5.0	10	20	5.0	10	20	5.0	10	20	5.0	10	20
EUS003	10.00	13.00	20.00	8.00	14.00	18.00	0.00	0.00	0.00	11.00	15.00	22.00
EUS010	0.00	9.00	13.00	0.00	9.00	12.00	0.00	0.00	0.00	9.00	12.00	16.00
EUS149	8.00	11.00	16.00	8.00	10.00	14.00	0.00	0.00	0.00	19.00	13.00	20.00
GHM166	9.00	11.00	15.00	8.00	10.00	14.00	0.00	0.00	0.00	9.00	12.00	18.00
GHM220	8.00	10.00	14.00	0.00	10.00	13.00	0.00	0.00	0.00	8.00	11.00	18.00
GHM268	8.00	10.00	15.00	8.00	10.00	14.00	0.00	0.00	0.00	9.00	12.00	18.00
KGH349	0.00	9.00	13.00	0.00	9.00	12.00	0.00	0.00	0.00	9.00	11.00	16.00
KGH360	9.00	11.00	15.00	8.00	10.00	14.00	0.00	0.00	0.00	10.00	14.00	20.00
KGH399	8.00	11.00	16.00	8.00	11.00	14.00	0.00	0.00	0.00	10.00	13.00	20.00
KGH445	8.00	10.00	14.00	8.00	9.00	12.00	0.00	0.00	0.00	8.00	12.00	16.00

Key: EUS=Etsu Umaru Sanda General Hospital, Bida; GHM=General Hospital, Minna; KGH=General Hospital, Kontagora; PEALE=*Ptericarpus erinaceus* acetone leaf extract; *E. coli=Escherichia coli*; PEQLE= *Ptericarpus erinaceus* aqueous leaf extract; EPHLE= *Ptericarpus erinaceus* hexane leaf extract; PEMLE= *Ptericarpus erinaceus* methanol leaf extract; 0.0=No zone of inhibition.

Table 4.14: Preliminary Antibacterial Activity of *Ptericarpus erinaceus* Crude Stem-Bark Extracts against *Escherichia coli* isolates.

Code	Zone of Inhibition(mm) and Concentration of crude extracts (mg/mL)											
	PEASBE			PEQSBE			PEHSBE			PEMSBE		
	5.0	10	20	5.0	10	20	5.0	10	20	5.0	10	20
EUS003	9.00	14.00	22.00	11.00	15.00	22.00	0.00	9.00	12.00	12.00	17.00	26.00
EUS010	8.00	11.00	15.00	8.00	11.00	16.00	0.00	0.00	0.00	9.00	14.00	20.00
EUS149	9.00	12.00	18.00	9.00	13.00	20.0	0.00	8.00	10.00	10.00	14.00	22.00
GHM166	8.00	10.00	15.00	8.00	10.00	18.00	0.00	0.00	0.00	10.00	15.00	23.00
GHM220	9.00	12.00	17.00	9.00	15.00	19.00	0.00	0.00	0.00	9.00	13.00	20.00
GHM268	8.00	11.00	16.00	9.00	11.00	16.00	0.00	8.00	11.00	10.00	14.00	22.00
KGH349	0.00	8.00	12.00	8.00	10.00	14.00	0.00	0.00	0.00	11.00	15.00	20.00
KGH360	8.00	10.00	16.00	8.00	12.00	16.00	8.00	10.00	12.00	10.00	15.00	24.00
KGH399	9.00	15.00	16.00	9.00	12.00	18.00	0.00	8.00	10.0	11.00	16.00	26.00
KGH445	8.00	11.00	16.00	11.00	14.00	20.0	0.00	0.00	0.00	13.00	19.00	30.00

Key: EUS=Etsu Umaru Sanda General Hospital, Bida; GHM=General Hospital, Minna; KGH=General Hospital, Kontagora; PEALE= *Ptericarpus erinaceus* acetone stem-bark extract; *E. coli=Escherichia coli*; PEQLE=*Ptericarpus erinaceus* aqueous stem-bark extract; PEHLE= *Ptericarpus erinaceus* hexane stem-bark extract; PEMLE= *Ptericarpus erinaceus* methanol stem-bark extract; 0.0=No zone of inhibition

Table 4.15: Preliminary Antibacterial Activity of *Vitex doniana* Crude Leaf Extracts against Diarrhoeagenic *Escherichia coli* isolates.

Code	Zone of Inhibition(mm) and Concentration of crude extracts (mg/mL)											
	VDALE			VDQLE			VDHLE			VDMLE		
	5.0	10	20	5.0	10	20	5.0	10	20	5.0	10	20
EUS003	10.00	14.00	20.00	10.00	18.00	24.00	8.00	11.00	18.00	14.00	24.00	38.00
EUS010	8.00	11.00	17.00	11.00	14.00	21.00	9.00	11.00	15.00	12.00	16.00	24.00
EUS149	9.00	12.00	19.00	0.00	9.00	12.00	0.00	8.00	10.00	10.00	14.00	21.00
GHM166	0.00	9.00	14.00	10.00	14.00	23.00	8.00	10.00	15.00	8.00	10.00	14.00
GHM220	9.00	11.00	16.00	10.00	15.00	23.00	8.00	10.00	14.00	11.00	15.00	24.00
GHM268	10.00	14.00	22.00	11.00	16.00	26.00	0.00	0.00	12.00	14.00	22.00	36.00
KGH349	8.00	11.00	17.00	10.00	14.00	22.00	11.0	15.00	23.00	13.00	19.00	33.00
KGH360	9.00	12.00	16.00	12.00	16.00	27.00	9.00	11.00	24.00	12.00	18.00	30.00
KGH399	9.00	11.00	16.00	11.00	14.00	20.00	8.00	9.00	11.00	10.00	14.00	21.00
KGH445	0.00	8.00	10.00	8.00	10.00	14.00	0.00	8.00	10.00	9.00	13.00	19.00

Key: EUS=Etsu Umaru Sanda General Hospital, Bida; GHM=General Hospital, Minna; KGH=General Hospital, Kontagora; VDALE=*Vitex doniana* acetone leaf extract; *E. coli=Escherichia coli*; VDQLE= *Vitex doniana* aqueous leaf extract; VDHLE= *Vitex doniana* hexane leaf extract; VDMLE= *Vitex doniana* methanol leaf extract; 0.0=No zone of inhibition.

Table 4.16 shows the results of preliminary antibacterial activity of *Vitex doniana* crude stem-bark extract. From the results, all the *Escherichia coli* isolates were susceptible to acetone stem-bark extract at 5.0 mg/mL-20 mg/mL concentration tested. At 5.0 mg/mL, all *Escherichia coli* isolates were susceptible to the extract with zones of inhibition ranging from 8.00 mm-11.00 mm and at 10mg/mL concentration with zones of inhibition ranging from 11.00-17.00 mm, while at 20 mg/mL concentration, the zones of inhibition ranged berween 17.00 mm-26.00 mm.

As for the aqueous stem-bark extract, it inhibited the growth of *Escherichia coli* isolates at various concentrations (5.0, 10, 20mg/mL). Zones of inhibition ranging from 10.00mm-15.00mm and 12.00mm-21.00mm were recorded at 5.0mg/mL and 10mg/mL concentration respectively. At concentration of 20mg/mL, all the *Escherichia coli* isolates were susceptible with zones of inhibition which ranged from 18.00mm-36mm.

The hexane stem-bark extract inhibited all tested *Escherichia coli* isolates except that coded 445 at 20mg/mL and 10mg/mL concentration respectively.*Escherichia coli* coded 360, 399 and 445 were resistant to hexane stem-bark extract, while five *Escherichia coli* coded 003, 010, 186 and 268 were susceptible with zones of inhibition ranging from 8.00mm-9.00mm. The methanol stem-bark extract inhibited all tested *Escherichia coli* with zones of inhibition ranging from 12.00mm-15.00mm at5.0mg/mL and zones of inhibition ranging from 15.00mm-22.00mm were recorded at 10mg/mL concentration, while all tested *Escherichia coli* isolates were susceptible to *Vitex doniana* stem-bark extract with zones of inhibition ranging from 24.00mm-38mm at 20mg/mL concentration (Table 4.16).

Table 4.16: Preliminary Antibacterial Activity of *Vitex doniana* Crude Stem-Bark Extracts against *Escherichia coli* isolates.

Code	Zone of Inhibition (mm) and Concentration of crude extracts (mg/ml)											
	VDASBE			VDQSBE			VDHSBE			VDMSBE		
	5.0	10	20	5.0	10	20	5.0	10	20	5.0	10	20
EUS003	11.0	17.00	26.00	15.00	21.00	36.00	9.00	13.00	18.00	15.00	22.00	36.00
EUS010	11.0	15.00	24.00	14.00	17.00	27.00	8.00	10.00	14.00	12.00	18.00	28.00
EUS149	10.0	14.00	21.00	11.00	15.00	22.00	0.00	9.00	12.00	12.00	17.00	26.00
GHM160	9.00	12.00	17.00	9.00	13.00	19.00	8.00	11.00	15.00	11.00	15.00	24.00
GHM220	8.00	14.00	18.00	11.00	16.00	25.00	8.00	11.00	16.00	11.00	15.00	25.00
GHM268	10.00	14.00	19.00	11.00	15.00	24.00	8.00	10.00	14.00	10.00	17.00	27.00
KGH349	9.00	11.00	15.00	9.00	12.00	18.00	0.00	9.00	10.00	11.00	15.00	24.00
KGH360	8.00	11.00	16.00	11.00	16.00	28.00	0.00	0.00	0.00	14.00	22.00	38.00
KGH399	9.00	11.00	17.00	10.00	15.00	24.00	0.00	0.00	0.00	14.00	20.00	34.00
KGH445	10.00	14.00	22.00	12.00	16.00	26.00	0.00	0.00	0.00	14.00	22.00	36.00

Key: EUS=Etsu Umaru Sanda General Hospital, Bida; GHM=General Hospital, Minna; KGH=General Hospital, Kontagora; VDASBE= *Vitex doniana* acetone stem-bark extract; *E. coli=Escherichia coli*; VDQSBE=*Vitex doniana* aqueous stem-bark extract; VDHSBE= *Vitex doniana* hexane stem-bark extract; VDMSBE=*Vitex doniana* methanol stem-bark extract; 0.0=No zone of inhibition.

The results of susceptibility pattern of *Escherichia coli* strains to *Vitex doniana* fractions are presented in Table 4.17. The susceptibility pattern of the *Escherichia coli* strains to various fractions of *Vitex doniana* showed that 94% (n=15/16), 88% (n=14/16) and 81.30% (n=13/16) of the Enteroaggregative *Escherichia coli* (EAEC) strains were highly susceptible to methanol leaf fraction, aqueous stem-bark fraction and acetone leaf fraction respectively. On the other hand, methanol stem-bark fraction, aqueous leaf fraction and hexane leaf fraction were active against 75% (n=12/16), 75% (n=12/16) and 69% (n=11/16) of the Enteroaggregative *Escherichia coli*, whileacetone stem-bark fraction was active against 50% (n=8/16) of the Enteroaggregative *Escherichia coli*.

In the case of Enterohaemorrhagic *Escherichia coli* (EHEC) strains:100%(n=13/13) were highly susceptible to the methanol leaf and stem-bark fraction respectively, while92% (n=12/13) weresusceptible to the acetone stem-bark and aqueous leaf fractions. However, hexane leaf fraction and acetone stem-bark fraction were active against 77% (n=10/13), 46.2% (n=6/13) and 38.5% (n=5/13) of the Enteroinvasive *Escherichia coli* (EIEC) respectively.

In the case of Enteroinvasive *Escherichia coli*: 75% (n=6/8) were susceptible to acetone leaf fraction, while 63% (n=5/8) of the Enteroinvasive *Escherichia col*i were also susceptible to hexane leaf and stem-bark fraction respectively. Moreso, methanol leaf and aqueous leaf fraction were active against 50% (n=4/8) of the Enteroinvasive *Escherichia col*i, while acetone stem-bark fraction was less active against 38% (n=3/8) of the Enteroinvasive *Escherichia coli*.

For Enteropathogenic *Escherichia coli*: 100% (n=27/27) of them were highly susceptible methanol leaf fraction, while 96% (n=26/27), 93% (n=23/26), 89% (n=24/27) and 85% (n=23/27) were susceptible hexane leaf fraction, methanol stem-bark fraction, aqueous leaf

fraction and acetone leaf fraction respectively. Moreso, aqueous stem-bark fraction and acetone stem-bark fraction were active against 82% (n=12/27) and 59% (n=11/27)against Enteropathogenic *Escherichia coli* respectively.

Eight eight percentage (88%) (n=15/17)of Enterotoxigenic *Escherichia coli* were susceptible to acetone leaf fraction, hexane leaf and aqueous stem-bark fraction respectively, while 82% (n=14/17) Enterotoxigenic *Escherichia coli* were also susceptible to methanol stem-bark fraction and aqueous leaf fraction respectively. Methanol leaf fraction was active against 100% (n=17/17) Enterotoxigenic *Escherichia coli* (EPEC) strains, while 53% (n=9/17) Enterotoxig*ic Escherichia coli* were susceptible to acetone stem-bark fraction.

Table 4.17: The Percentage Susceptibility Pattern of *Escherichia coli* Strains to *Vitex doniana* Fractions.

Plant fraction	EAEC (n=16)	EHEC (n=13)	EIEC (n=8)	EPEC (n=27)	ETEC (n=17)
VDALF	13 (81.30)	12 (92.00)	06 (75.00)	23 (85.00)	15 (88.00)
VDASBF	08 (50.00)	05 (39.00)	03 (38.00)	16 (59.00)	09 (53.00)
VDHLF	11 (69.00)	10 (77.00)	05 (63.00)	26 (96.00)	15 (88.00)
VDMLF	15 (94.00)	13 (100.0)	04 (50.00)	27 (100.0)	17 (100.0)
VDMSBF	12 (75.00)	13 (100.0)	05 (63.00)	25 (93.00)	14 (82.00)
VDQLF	12 (75.00)	12 (92.00)	04 (50.00)	24 (89.00)	14 (82.00)
VDQSBF	14 (88.00)	09 (69.00)	03 (38.00)	22 (82.00)	15 (88.00)

Key: EAEC=Enteroaggregative *Escherichia coli*; EHEC Enterohamorrhagic *Escherichia coli* strains Enterohamorrhagic *Escherichia coli* strains Enterohamorrhagic *Escherichia coli* strains Enterohamorrhagic *Escherichia coli* strains =Enterohaemorrhagenic *Escherchia coli* EIEC=Enteroinvasive *Escherchia coli*; EPEC=Enteropathogenic *Escherichia coli*; ETEC=Enterotoxigenic *Escherchia coli;* VDALF=*Vitex doniana* acetone leaf extract; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* stem-bark fraction; - =No inhibition.

The results of minimum inhibitory concentration (MIC)and minimum bacterial concentration (MBC) of*Vitex doniana* fractionsagainst Enteroaggregative *Escherichia coli* strains are presented in Table 4.18.*Vitex doniana*acetone leaf fraction (VDALF) had MIC value of 5.0mg/mL against25% (n=4/16) of the Enteroaggregative *Escherichia coli* strains, MIC of 10mg/mL against 43%(n=7/16) of the Enteroaggregative *Escherichia coli* strains and MIC of 20mg/mL against 12.5% (n=2/16) of the Enteroaggregative *Escherichia coli* strains respectively. However, Acetoneleaf fraction had MBC of 10mg/mL and 20 mg/mL against 43.8% (n=7/16) and 31.3% (n=5/16)of the Enteroaggregative *Escherichia coli* strains rrespectively.

Vitex doniana acetone stem-bark fraction had MIC of 10mg/mL and 20mg/mL against 37.5% (n=6/16) and 6.3% (n=1/16) of the Enteroaggregative *Escherichia coli* strains. On the other hand, acetone stem-bark fraction had MBC of 10mg/mL and 20 mg/mL against 25% (n=4/16) and 12.5% (n=2/16) of the Enteroaggregative *Escherichia coli* strains respectively.Hexane leaf fraction had MIC of 5.0mg/mL, 10 mg/mL and 20mg/mL against 12.5% (n=2/16), 31.3%(n=5/16) and 18.8% (n=3/16) of the Enteroaggregative *Escherichia coli* strains respectively. The same fraction had MBC value of 10mg/mL and 20mg/mL against 31.3% (n=5/16) of the Enteroaggregative *Escherichia coli* strains respectively.

Vitex doniana methanol leaf fraction had MIC of 5.0mg/mL, 10mg/mL and 20mg/mL against 50% (n=8/16), 18.8% (3/16) and 6.3% (1/16) of the Enteroaggregative *Escherichia coli* strains respectively. The same methanol leaf fraction had MBC of 10mg/mL and 20mg/mL against 56.3% (n=9/16) and 12.5% (n=2/16) Enteroaggregative *Escherichia coli* strains respectively.*Vitex doniana* methanol stem-bark fraction had MIC of 5.0mg/mL and 10mg/mL against 43.8%, (n=7/16) and 18.8% (3/16)of the Enteroaggregative *Escherichia coli* strains respectively.

On the other hand, methanol stem-bark fraction had MBC of 5.0 mg/mL and 10 mg/mL against 12.5% (n=2/16) and 50% (n=8/16) of the Enteroaggregative *Escherichia coli* strains respectively. Aqueous leaf fraction of *Vitex doniana* had MIC of 5.0 mg/mL, 10 mg/mL and 20 mg/mL against 6.3% (n=1/16), 43.8% (n=7/16) and 25% (n=4/16) of the Enteroaggregative *Escherichia coli* strains respectively, while the same fraction had MBC of 5.0 mg/mL, 10 mg/mL and 20mg/mL against 6.3% (n=1/16), 31.3% (n=5/16) and 37.5% (n=6/16) of the Enteroaggregative *Escherichia coli* strains respectively. *Vitex doniana* aqueous stem-bark fraction had MIC value of 5.0 mg/mL and 10 mg/mL against 75% (n=12/16) and 25% (n=4/16) of the Enteroaggregative *Escherichia coli* strains respectively. However, the same fraction had MBC value of 5.0 mg/mL and 10mg/mL against 25% (n=4/16) and 75% (n=12/16) of the Enteroaggregative *Escherichia coli* strains respectively.

The results of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of *Vitex doniana* fractionsagainst Enterohaemorrhagic *Escherichia coli* strains are presented in Table 4.19. *Vitex doniana* acetone leaf fraction (VDALF) had MIC value of 5.0 mg/mL against 46.2% (n=6/13) of the Enterohaemorrhagic *Escherichia coli* strains and MIC 10 mg/mL against 26.7% (n=4/13) of the Enterohaemorrhagic *Escherichia coli* strains respectively. However, Acetone leaf fraction had MBC of 10mg/mL against 76.9% (n=10/13) of the Enterohaemorrhagic *Escherichia coli* strains.

Vitex doniana acetone stem-bark fraction had MIC of 5.0mg/mL and 10mg/mL against 30.7% (n=4/13) and 30.7% (n=4/13) of the Enterohaemorrhagic *Escherichia coli* strains respectively. On the other hand, acetone stem-bark fraction had MBC of 5.0 mg/mL and 10 mg/mg/mL

against 7.7% (n=1/13) and 38.5% (n=5/13) of the Enheamorrhagic *Escherichia coli* strains respectively.

(n=2/13) of the Enterohaemorrhagic *Escherichia coli* strains respectively. The same fraction had MBC of 5.0mg/mL and 10mg/mL against 7.7% (n=1/13) and 69.2% (n=9/13) of the Enterohaemorrhagic *Escherichia coli* strains respectively (Table 4. 19).

Vitex doniana methanol leaf fraction had MIC of 5.0mg/mL and 10mg/mL against 76.9%, (n=10/13) and 23.1% (3/13) of the Enterohaemorrhagic *Escherichia coli* strains respectively. The same methanol leaf fraction had MBC of 5.0mg/mL and 10mg/mL against 23.1% (n=3/13) and 76.9% (n=10/13) of the Enterohaemorrhagic*Escherichia coli* strains respectively. *Vitex doniana* methanol stem-bark fraction had MIC of 5.0mg/mL and 10mg/mL against 61.5%, (n=8/13) and 38.5% (5/13)of the Enterohamorrhagic*Escherichia coli* strains respectively. On the other hand, methanol stem-bark fraction had MBC of 10mg/mL and 20mg/mL against 92.5% (n=12/13) and 7.7% (n=1/13) of the Enterohamorrhagic *Escherichia coli* strains respectively.

Aqueous leaf fraction of *Vitex doniana* had MIC of 5.0mg/mL and 10mg/mL against 53.8% (n=7/13) and 26.7% (n=4/13) of the Enterohaemorrhagic*Escherichia coli* strains respectively, while the same fraction had MBC of 5.0mg/mL and 10mg/mL against 7.7% (n=1/13) and 84.6% (n=11/13) of the Enterohaemorrhagic*Escherichia coli* strains respectively. *Vitex doniana* aqueous stem-bark fraction had MIC value of5.0mg/mL and 10mg/mL against 15.4% (n=2/13) and 46.2% (n=6/13) of the Enterohaemorrhagic*Escherichia coli* strains respectively. However, the same fraction had MBC value of 10mg/mL against 61.5% (n=8/13)of the Enterohaemorrhagic*Escherichia coli* strains respectively (Table 19).

Table 4.18: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentraction (MBC) of *Vitex doniana* Fractions against Enteroaggregetive *Escherichia coli* isolates (n=16).

Vitex doniana	MIC (mg/mL)			MBC (mg/mL)		
Fraction	5.0	10.0	20.0	5.0	10.0	20.0
VDALF	4(25.0)	7(43.8)	2(12.5)	-(0.0)	7(43.8)	5(31.3)
VDASBF	-(0.0)	6(37.5)	1(6.3)	-(0.0)	4(25.0)	2(12.5)
VDHLF	2(12.5)	5(31.3)	3(18.8)	-(0.0)	5(31.3)	5(31.3)
VDMLF	8(50.0)	3(18.8)	1(6.3)	-(0.0)	9(56.3)	2(12.5)
VDMSBF	7(43.8)	3(18.8)	-(0.0)	2(12.5)	8(50.0)	-(0.0)
VDQLF	1(6.3)	7(43.8)	4(25.0)	1(6.3)	5(31.3)	6(37.5)
VDQSBF	12(75.0)	4(25.0)	-(0.0)	4(25.0)	12(75.0)	-(0.0)

Key: VDALF=*Vitex doniana* acetone leaf fraction; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* aqueous stem-bark fraction; - =No inhibition; MIC=Minimum inhibitiory concentration; MBC=Minimum bactericidal concentration.

Table 4.19: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBCs) of *Vitex doniana* Fractions against Enteroheamorrhagic *Escherichia coli* isolates (n=13).

Vitex doniana	MIC (mg/mL)			MBC (mg/mL)		
Fraction	5.0	10.0	20.0	5.0	10.0	20.0
VDALF	6(46.2)	4(30.8)	-(0.0)	-(0.0)	10(76.9)	-(0.0)
VDASBF	4(30.8)	2(15.4)	-(0.0)	1(7.7)	5(38.5)	-(0.0)
VDHLF	8(61.5)	2(15.4)	-(0.0)	1(7.7)	9(69.2)	-(0.0)
VDMLF	10(76.9)	3(20.2)	-(0.0)	3(20.2)	10(76.9)	-(0.0)
VDMSBF	8(61.5)	5(38.5)	-(0.0)	-(0.0)	12(92.3)	1(7.7)
VDQLF	7(53.8)	4(30.8)	-(0.0)	1(7.7)	11(84.6)	-(0.0)
VDQSBF	2(15.4)	6(46.2)	-(0.0)	-(0.0)	8(61.5)	-(0.0)

Key: VDALF=*Vitex doniana* acetone leaf fraction; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* aqueous stem-bark fraction; - =No inhibition; MIC=Minimum inhibitiory concentration; MBC=Minimum bactericidal concentration.

The results of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of *Vitex doniana* fractionsagainst Enteroinvasive *Escherichia coli* strains are presented in Table 4.20. *Vitex doniana* acetone leaf fraction (VDALF) had MIC value of 5.0mg/mL against 25% (n=2/8) of the Enteroinvasive *Escherichia coli* strains, MIC 10 mg/mL against 62.5% (n=5/8) of the Enteroinvasive *Escherichia coli* strains and MIC of 20mg/mL against 12.5% (n=1/8) of the Enteroinvasive *Escherichia coli* strains respectively. However, Acetone leaf fraction had MBC of 10mg/mL and 20mg/mL against 75% (n=6/8) and 25% (n=2/8) of the Enteroinvasive*Escherichia coli* strains respectively.

Vitex doniana acetone stem-bark fraction had MIC of 5.0mg/mL and 10mg/mL against 25% (n=2/8) and 12.5% (n=2/8) of the Enteroinvasive*Escherichia coli* strains respectively. On the other hand, acetone stem-bark fraction had MBC of 10 mg/mL against 37.5% (n=3/8) of the Enteroinvasive *Escherichia coli* strains. Hexane leaf fraction had MIC of 5.0mg/mL and 10mg/mL against 12.5% (n=1/8) and 50% (n=4/8) of the Enteroinvasive*Escherichia coli* strains respectively. The same fraction had MBC of 10mg/mL against 62.5% (n=5/8) of the Enteroinvasive*Escherichia coli* strains.

Vitex doniana methanol leaf fraction had MIC of 5.0mg/mL, 10mg/mL and 20mg/mL against 12.5%, (n=1/8), 25% (n=2/8) and 12.5% (n=1/8) of the Enteroinvasive *Escherichia coli* strains respectively. The same methanol leaf fraction had MBC of 10mg/mL and 20mg/mL against 25% (n=2/8) of the Enteroinvasive *Escherichia coli* strains respectively. *Vitex doniana* methanol stem-bark fraction had MIC of 5.0mg/mL and 10mg/mL against 37.5% (n=3/8) and 25% (n=2/8) of the Enteroinvasive *Escherichia coli* strains respectively. On the other hand methanol stem-bark fraction had MBC of 5.0mg/mL, 10mg/mL and 20mg/mL against 12.5% (n=1/8), 37.5% (n=3/8) and12.5% (n=1/8) of the Enteroinvasive *Escherichia coli* strains respectively.

Aqueous leaf fraction of *Vitex doniana* had MIC of 5.0 mg/mL and 10 mg/mL against 12.5% (n=1/8) and 37.5% (n=3/8) of the Enteroinvasive *Escherichia coli* strains respectively, while the same fraction had MBC of 10 mg/mL against 50 (n=4/50) of the Enteroinvasive *Escherichia coli* strains. *Vitex doniana* aqueous stem-bark fraction had MIC value of 10 mg/mL against 37.5% (n=3/8) of the Enterohaemorrhagic *Escherichia coli* strains. However, the same fraction had MBC value of 10 mg/mL against 37.5% (n=3/8) of the Enteroinvasive *Escherichia coli* strains (Table 4.20).

Table 4.20: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentraction (MBC) of *Vitex doniana* Fractions against Enteroinvasive *Escherichia coli* (n=8).

Vitex doniana	MIC (mg/mL)			MBC (mg/mL)		
Fraction	5.0	10.0	20.0	5.0	10.0	20.0
VDALF	2(25.0)	5(62.5)	1(12.5)	-(0.0)	6(75.0)	2(25.0)
VDASBF	2(25.0)	1(12.5)	-(0.0)	-(0.0)	3(37.5)	-(0.0)
VDHLF	1(12.5)	4(50.0)	-(0.0)	-(0.0)	5(62.5)	-(0.0)
VDMLF	1(12.5)	2(25.0)	1(12.5)	-(0.0)	2(25.0)	2(25.0)
VDMSBF	3(37.5)	2(25.0)	-(0.0)	1(12.5)	3(37.5)	1(0.0)
VDQLF	1(12.5)	3(37.5)	-(0.0)	1(12.5)	4(50.0)	-(0.0)
VDQSBF	-(0.0)	3(37.5)	-(0.0)	-(0.0)	3(37.5)	-(0.0)

Key: VDALF=*Vitex doniana* acetone leaf fraction; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* aqueous stem-bark fraction; - =No inhibition; MIC=Minimum inhibitiory concentration; MBC=Minimum bactericidal concentration.

The results of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of *Vitex doniana* fractionsagainst Enteropathogenic *Escherichia coli* strains are presented in Table 4.21. *Vitex doniana* acetone leaf fraction (VDALF) had MIC value of 5.0mg/mL against 26% (n=7/27) of the Enteropathogenic*Escherichia coli* strains, MIC 10 mg/mL against 48.1% (n=13/27) of the Enteropathogenic*Escherichia coli* strains and MIC of 20mg/mL against 7.4% (n=2/27) of the Enteropathogenic*Escherichia coli* strains respectively. However, Acetone leaf fraction had MBC of 10mg/mL and 20mg/mL against 63% (n=17/27) and 11.1% (n=3/27) of the Enteropathogenic*Escherichia coli* strains respectively.

Vitex doniana acetone stem-bark fraction had MIC of 5.0mg/mL and 10mg/mL against 22.2% (n=6/27) and 33.3% (n=9/27) of the Enteropathogenic*Escherichia coli* strains respectively. On the other hand, acetone stem-bark fraction had MBC of 5.0mg/mL, 10mg/mL and 20mg/mL against 3.7% (n=1/27), 37% (n=10/27) and 14.8% (n=4/27)of the Enteropathogenic*Escherichia coli* strains. Hexane leaf fraction had MIC of 5.0mg/mL, 10mg/mL and 20mg/mL against 25.9% (n=7/27), 59.3% (n=16/27)and 3.7% (n=1/27) of the Enteropathogenic*Escherichia coli* strains respectively.

The same fraction had MBC of 5.0 mg/mL, 10 mg/mL and 20 mg/mL against 11.1% (n=3/27), 63% (n=17/27) and 14.8% (n=4/27) of the Enteropathogenic *Escherichia coli* strains respectively. *Vitex doniana* methanol leaf fraction had MIC of 5.0 mg/mL, and 10 mg/mL against 55.6% (n=15/27) and 44.4% (n=12/27) of the Enteropathogenic *Escherichia coli* strains respectively. The same methanol leaf fraction had MBC of 5.0 mg/mL, 10 mg/mL and 20 mg/mL against 3.7% (n=1/27), 88.9% (n=24/27) and 7.4% (n=2/27) of the Enteropathogenic *Escherichia coli* strains respectively.

Vitex doniana methanol stem-bark fraction had MIC of 5.0 mg/mL, 10 mg/mL and 20 mg/mL against 66.7% (n=18/27), 22.22% (n=6/27) and 3.7%% (1/27) of the Enteropathogenic *Escherichia coli* strains respectively. On the other hand methanol stem-bark fraction had MBC of 5.0 mg/mL, 10 mg/mL and 20 mg/mL against 3.7% (n=1/27), 81.5%% (n=22/27) and 7.4% (n=2/27) Enteropathogenic *Escherichia coli* strains respectively.

Aqueous leaf fraction of *Vitex doniana* had MIC of 5.0 mg/mL, 10 mg/mL and 20 mg/mL against 18.5% (n=5/27), 63% (n=17/27) and 3.7% (n=1/27) of the Enteropathogenic *Escherichia coli* strains respectively, while the same fraction had MBC of 10 mg/mL and 20 mg/mL against 66.7 (n=18/27) and 18.5% (n=5/27) of the Enteropathogenic *Escherichia coli* strains. *Vitex doniana* aqueous stem-bark fraction had MIC value of 5.0 mg/mL and 10 mg/mL against 37% (n=10/27) of the Enterohaemorrhagic *Escherichia coli* strains respectively. However, the same fraction had MBC of 5.0 mg/mL and 10 mg/mL and 20 mg/mL against 3.7% (n=1/27), 55.6% (n=15/27) and 14.8% (n=4/27) of the Enteropathogenic *Escherichia coli* strains respectively (Table 4.21).

Table 4.21: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentraction (MBC) of *Vitex doniana* Fractions against Enteropathogenic *Escherichia coli* (n=27).

Vitex doniana	MIC (mg/mL)			MBC (mg/mL)		
Fraction	5.0	10.0	20.0	5.0	10.0	20.0
VDALF	7(25.9)	13(48.1)	2(7.4)	-(0.0)	17(63.0)	3(11.1)
VDASBF	6(22.2)	9(33.33)	-(0.0)	1(3.7)	10(37.0)	4(14.8)
VDHLF	7(25.9)	16(59.2)	1(3.7)	3(11.1)	17(63.0)	4(14.8)
VDMLF	15(55.6)	12(44.4)	-(0.0)	1(3.7)	24(88.9)	2(7.4)
VDMSBF	18(66.7)	16(59.2)	1(3.7)	1(3.7)	22(81.5)	2(7.4)
VDQLF	5(18.5)	17(63.0)	1(3.7)	-(0.0)	18(66.7)	5(18.5)
VDQSBF	10(37.0)	10(37.0)	-(0.0)	-(0.0)	15(55.6)	4(14.8)

Key: VDALF=*Vitex doniana* acetone leaf fraction; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* aqueous stem-bark fraction; - =No inhibition; MIC=Minimum inhibitiory concentration; MBC=Minimum bactericidal concentration.

The results of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of *Vitex doniana* fractionsagainst Enterotoxigenic *Escherichia coli* strains are presented in Table 4.22. *Vitex doniana* acetone leaf fraction (VDALF) had MIC value of 5.0mg/mL and 10mg/mL against 23.5% (n=4/17) and 64.7% (n=11/17) of the Enterotoxigenic *Escherichia coli* strains respectively. However, Acetone leaf fraction had MBC of 10mg/mL and 20mg/mL against 58.8% (n=10/17) and 29.4% (n=5/17) of the Enterotoxigenic *Escherichia coli* strains respectively.

Vitex doniana acetone stem-bark fraction had MIC of 5.0mg/mL, 10mg/mL and 20mg/mL against 17.6% (n=3/17), 29.9% (n=5/17) and 6.0% (n=1/17) of the Enterotoxigenic *Escherichia coli* strains respectively. On the other hand, acetone stem-bark fraction had MBC of 10mg/mLand 20mg/mL against 41.2% (n=7/17) and 11.8% (n=2/17)of the Enterotoxigenic *Escherichia coli* strains. Hexane leaf fraction had MIC of 5.0mg/mL and 10mg/mL against 5.9% (n=1/17) and 82.4% (n=14/17) of the Enterotoxigenic *Escherichia coli* strains respectively. The same fraction had MBC of 10mg/mL and 20mg/mL against 64.7% (n=11/17) and 23.5% (n=4/17) Enterotoxigenic*Escherichia coli* strains respectively.

Vitex doniana methanol leaf fraction had MIC of 5.0mg/mL and 10mg/mL against 40.7% (n=11/17) and 36% (n=6/17) of the Enterotoxigenic *Escherichia coli* strains respectively. The same methanol leaf fraction had MBC of 10mg/mL against 100% (n=17/17) Enterotoxigenic *Escherichia coli* strains.*Vitex doniana* methanol stem-bark fraction had MIC of 5.0mg/mL and 10mg/mL against 82.4% (n=14/17) of the Enterotoxigenic *Escherichia coli* strains respectively. On the other hand methanol stem-bark fraction had MBC of 5.0mg/mL and 10mg/mL against 29.4% (n=5/17) and 52.9% (n=9/17) of the Enterotoxigenic *Escherichia coli* strains respectively.

Aqueous leaf fraction of *Vitex doniana* had MIC of 5.0mg/mL, 10mg/mL and 20mg/mL against 29.4% (n=5/17), 41.2% (n=7/17) and 11.8% (n=2/17) of the Enterotoxigenic *Escherichia coli* strains respectively, while the same fraction had MBC of 10mg/mL and 20mg/mL against 52.9% (n=9/17) and 29.4% (n=5/17) of the Enterotoxigenic *Escherichia coli* strains. *Vitex doniana* aqueous stem-bark fraction had MIC value of 5.0mg/mL,10mg/mL and 20mg/mL against 64.7% (n=11/17), 17.6% (n=3/17) and 5.9% (n=1/17) of the Enterotoxigenic *Escherichia coli* strains respectively. However, the same fraction had MBC of 5.0mg/mL, 10mg/mL and 20mg/mL against 5.9% (n=1/17), 76.5% (n=13/17) and 5.9% (n=1/17) of the Enterotoxigenic *Escherichia coli* strains respectively (Table 4.22).

Table 4.22: Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentraction (MBCs) of *Vitex doniana* Fractions against Enterotoxigenic *Escherichia coli* (n=17).

Vitex doniana	MIC (mg/mL)			MBC (mg/mL)		
Fraction	5.0	10.0	20.0	5.0	10.0	20.0
VDALF	4(23.5)	11(64.7)	-(0.0)	-(0.0)	10(52.9)	5(18.5)
VDASBF	3(17.6)	5(18.5)	1(5.9)	-(0.0)	7(41.2)	2(11.8)
VDHLF	1(5.9)	14(82.4)	-(0.0)	-(0.0)	11(64.7)	4(23.5)
VDMLF	11(64.7)	16(94.1)	-(0.0)	-(0.0)	17(100)	-(0.0)
VDMSBF	14(82.4)	-(0.0)	-(0.0)	5(18.5)	9(52.9)	-(0.0)
VDQLF	5(18.5)	7(41.2)	2(11.8)	-(0.0)	9(52.9)	5(18.5)
VDQSBF	11(64.7)	3(17.6)	1(5.9)	1(5.9)	13(76.5)	1(5.9)

Key: VDALF=*Vitex doniana* acetone leaf fraction; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* aqueous stem-bark fraction; - =No inhibition; MIC=Minimum inhibitiory concentration; MBC=Minimum bactericidal concentration.

CHAPTER FIVE

5.0: DISCUSSION

Diarrhoea may be contracted through ingestion of contaminated food or drinking of un-potable water (Iruka and Okeke, 2009). These contaminants are mostly bacteria, viruses and Protozoa and are capable of causing inflammation of gut resulting in diarrhoea among other symptoms of gastro-intestinal illness.

This study revealed the 16.9% prevalence of*Escherichia coli* infection among diarrhoeal patients. This shows that diarrhoeagenic *Escherichiacoli*could be responsible for childhood diarrhoea. The low prevalence of diarrhoeagenic *E. coli*(16.9%) among diarrhoea cases in this study may be as a result of pathogens like *Entamoeba histolytica, Giardia lambilia;Salmonella* species, *Campylobacter* species and *Rotavirus* that were not investigated which might be responsible for diarrhoea.

The prevalence of 16.9% is similar to the findings of Kandakai-Oluwakemi *et al.* (2009) who reported a prevalence of 15.0% in a similar study carried out in Gwagwalada, Abuja, Nigeria and 19.37% prevalence reported in Minna, Nigeria (Kolo *et al.*, 2014). This finding is also similar to the report of Onanuga*et al.* (2014) who documented a prevalence of 21% in a study conducted in Abuja, FCT, Nigeria and 22% of prevalence in a study carried out in Ile- Ife, Nigeria on isolation and evaluation of *Escherichia coli* in diarrhoeal stool samples from children in Ile –Ife (Olaniran *et al.*, 2015).

This finding is also similar to the findings of Nfongel *et al.* (2018) who reported a prevalence of 19.13% on the risk analysis and antibiogram spectrum of *Escherihia coli* 0157:H7 serotype from children stool in households across Cross-River State, Nigeria.

The finding is however contrary to that of Kingsley *et al*. (2017) who reported a lower prevalence of 9.33% in Calabar South Local Governments, Cross River State, Nigeria on the prevalence and antibiotic susceptibility pattern of *Escherichia coli* in children 0-60 months. The study also disagrees with the findings of Casmir *et al.* (2015) who documented a low prevalence of 12.8% from the study carried out on diarrhoea in FCT, Abuja.

Contrary to our findings, Ogunsaya *et al.* (1994) reported a higher prevalence of 98.4% in Lagos, Nigeria and Samuel *et al.* (2009) reported a prevalence of 53.8% in Leon, Nicaragua. A higher prevalence of 89.4% of diarrhoeagenic *Escherichia coli*was also reported from a similar study conducted in Benin, Nigeria (Ogbu *et al.,* 2008) and 62.8% obtained in a study carried out in the FCT, Abuja, Nigeria by Ifeanyi *et al.* (2010) among children who presented with diarrhoea.

The differences in prevalence of *Escherichia coli* infection observedin the three Hospitals; General Hospital Kontagora is the only referral Hospital in that senatorial zone and therefore, receives more acute diarrhoeal cases than the other two hospitals. The higher prevalence of 21.25% could be due to the significant difference in infrastructural development, educational level, behavioural pattern of people living in that area of the study, The season of sample collection there (rainy season) may also be a contributing factor.

Frequency of diarrhoeagenic *Escherichia coli* strain in this study showed that Enteropathogenic *Escherichia coli*(EPEC); 27(33.3%) was the most prevalent etiology of gastroenteritis in this area. This was followed by Enterotoxigenic *Escherichia coli* (ETEC) 17(21.0%), Enteroaggregative *Escherichia coli* (EAEC) 16(19.8%), Enterohaemorrhagic *Escherichia coli*(EHEC) 13(16.1%) and Enteroinvasive *Escherichia coli* (EIEC)8(9.9%).

This study indicated that Enteroaggregative *Escherichia coli*(EAEC) had a prevalence of 19.8% which is similar to the findings of Kandakai-Oluwakemi *et al.* (2009) who reported a prevalence of 20% in a study carried out at National Hospital, Abuja, Nigeria and 19.50% prevalence reported in Gwagwalada, Nigeria (Onanuga*et al.*, 2014). It is also similar to the findings of Galadima and Kolo (2014) who documented a prevalence of 19.0%and 20% in Minna, Nigeria and in Tehran, Iran respectively for Enteroaggregative *Escherichia coli* strains.

This finding is contrary to the reports of other previous studies carried out in other locations such asBauchi, Nigeria (Iliyasu *et al.*, 2016) with prevalence of 25.10% and Gwagwalada, FCT, Nigeria (Onanuga*et al.*, 2014) with a prevalence of 34.40%. In a study carried out in Egypt among children with acute diarrhoea, the prevalence of Enteroaggregative *Escherichia coli* has been reported to be 30.71%. Contrary to our finding, Jafari *et al.* (2009) reported a low prevalence of 8.2% for Enteroaggregative *Escherichia coli* among studied cases. Pobst *et al.* (2013) also reported a low prevalence of 10.2% of Enteroaggregative *Escherichia coli* in Swizerland and Benjamin (2016) reported a prevalence of 13.6% for Enteroaggregative *Escherichia coli* strains in Dar-Salam, Tanzania.

The finding is however contrary to that of some studies conducted in other locations such as Bauchi, Nigeria (Iliyasu *et al*., 2016) with prevalence of 24.15% and in Gwagwalada Abuja, Nigeria (Onanuga*et al.*, 2014) with a prevalence of 31.10% for Enterohaemorrhagic *Escherichia coli* strains. Casmir *et al.* (2015) documented a low prevalence of 1.5% for EHEC strain. A similar study was carried out in Bahir Dar Salam, Tanzania with a prevalence of 2.7%. This study is also contrary to the findings of other previous studies carried out in other town such as Calabar, River State, Nigeria (Benjamin, 2016) with a prevalence of 1.1% and Gwagwalada, Nigeria (Onanuga*et al.*, 2014) with a prevalence of 1.60%.

Based on our findings, EPEC had the highest prevalence of 33.33% compared to the other strains recovered. This finding is in line with that of a study conducted in Bauchi, Nigeria (Iliyasu *et al*., 2016) with a prevalence of 36.60%. This study is also contrary to the finding of other previous studies carried out in other locations such as Gwagwalada, Nigeria (Onanuga*et al.,* 2014) with prevalence of 15.0% and in Tunisia (Casmir *et al.*, 2015) with a prevalence of 4.5%. Similar result has been reported with high prevalence of 35% in Ifakara, Tanzania (Benjamin *et al.,* 2016)

This study recorded a prevalence of 20.99% for ETEC serotype. It is similar to the result of Onanuga*et al.*(2014) in Abuja, Nigeria who reported 19.8% prevalence for ETEC among children with diarrhoea. However, in a study carried out in Bauchi, (Iliyasu *et al*., 2016) reported 34.50% prevalence for these diarrhoeagenic *Escherichia coli* strains among studied cases. A similar study in Tunisia also reported 9.1% prevalence for ETEC strains (Benjamin, 2016). ETEC was the second serotype detected in our study and one of the most important causes of childhood diarrhoea.

The distribution of diarrhoegenic *Escherichia coli* among various childrenin this study showed that children in age group of 1-2 years had the highest prevalence of 21.82%. The reasons may be attributed to the fact that during this period, children are weaned, start moving around and indiscrimately putting few contaminated toys and other things into their mouth. This could also be attributed to the beginning of environmental exposure and increased introduction of solid foods to children whose immune system is still developing. Findings in this study coincide with the reports published by King *et al*. (2003), Longstreeteh *et al*. (2006), Kondakai – Oluwakemi *et al*. (2009) and Onanuga*et al.* (2014) who observed that the highest incidence of gastroenteritis occurred in children of 1-2 years.

However, children of age group of 0-1 years had prevalence of 16.23% for diarrhoeagenic *Escherchia coli.* This could be attributed to fact that only few mothers practice exclusive breast feeding in the study area, others feed their children with formula milk and other food constituents.This finding agrees with the previous studies conducted in Abuja and Minna (Kondakai-Oluwakemi *et al*., 2009; Galadima and Kolo, 2014).

Children within age group of 3-5 years recorded low prevalence of 11.86% for diarrhoeagenic *Escherichia coli*infection.The reason for low recovery of diarrhoeagenic *Escherichia coli* could be attributed to ability to adhere to hygiene practice and development of immunity or loss of reception for some specific adhesion molecules. This finding is contraryto the reports ofAyrikin *et al*. (2014); Galadima and Kolo (2014); Onanuga*et al*. (2014) and Kingsley *et al.* (2017) documented the prevalence of 2.10%, 2.05%, 2.02% and 2.0% respectively. The finding is contrary to reports published by several authors, Requa *et al*. (1990), Olanipeokun (1999) and Kandakai-Oluwakemi *et al*. (2009) that the highest incidence of gastroenteritis in children was found among children of 48 months above.

The prevalence of diarrhoeagenic *Esherichia coli* in relation to sex in this study showed a higher prevalence of 18.04% in female as compared to the male counterparts with prevalence of 15.56%. However, there was no statistically significant association between the prevalence of *Escherichia coli* and sex. (χ^2= 5.000;P=0.1530). Higher prevalence in females observed in the present study might be possibly due to chance as the difference was not statistically significant. Therefore, most cases of diarrhoea are not sex specific since they have or possess the same immune cells. This finding agrees with the previous studies which documented diarrhoeagenic *Escherichia coli* occurrence being higher in females than males (Ayrikin *et al.*, 2014; Galadima and Kolo, 2014; Olaniran, 2015). A similar study was also conducted in Bauchi Nigeria with

higher prevalence of 18.2% for female and 15.2% for male (Iliyasu *et al*., 2016). This finding agrees with the previous studies which documented diarrhoeagenic *Escherichia coli* infection in females than males (Onanuga*et al*., 2014). However, many studies have documented lower prevalence of diarrhoegenic *Escherichia coli* in females than in males which disagree with our findings (Ayrikin *et al*., 2014; Olaniran *et al*., 2015). Other study in Bhair-Darr however reported higher prevalence of 27.5% in males than in females with prevalence rate of 20.9% (Ayinkin *et al*., 2014).

Area of domicile and the prevalence of diarrheagenic *Escherichia coli* infection was found to be statistically significant (χ^2= 20.000; P=0.002), with those children who reside in rural area having the highest prevalence of 18.58%, while children whose parents reside in urban area had a lowest prevalence of 13.34%. The reason of high prevalence of *Escherchia coli* infection with children residing in rural area could be attributed to fact that majority of rural dwellers lack access to treated water or chlorinated pipe borne water and medical facilities. They also have less access to enlightenment campaign that will educate them on how to practice exclusive breast feeding and hygienic preparation of food for the children.

This finding is similar to the report of Galadima andKolo(2014) who documented a higher prevalence of 15.0% and 18.0% respectively with children who reside in rural areas. Studies by Kandakai – Oluwakemi *et al*. (2009) and Olaniran *et al.* (2015) in Abuja and Ile Ife, Nigeria reported the highest prevalence of 22% respectively in children residing at urban area.

Mother's occupation and prevalence of *Escherichia coli* infection was found to be statistically significant (χ^2=26.000; P=0.000). Children whose mothers are house wives had higher prevalence of 19.20%, while children whose mothers are civil servant had lowest prevalence of 9.48%. The

report of this study shows that working class mothers should have learnt enough personal hygiene, practices exclusive breast feeding, possible attending immunization program and vaccinate her children when necessary. However, house wife may not have access to enlightenment campaign that will educate them on how exclusively breast feed and hygienic preparation of food for children are done.

The relationship between mother's educational status and the number of *Escherichia coli* isolates was investigated. The prevalence of diarrhoeagenic *Escherichia coli* infection was found to be highest (25.55%) among children whose mothers had no-formal education and lowest (1.00%) among the tertiary education category. The difference in *Eschericha coli* infection prevalence among the different levels of mother's education was found to be statistically significant (χ^2=23.87; P<0.05). The high number of infection among children whose mothers had non-formal education is due to lack of education as well as their poor knowledge of sanitation and food hygiene related practices. This result is in agreement with the report of Galadima and Kolo (2014) that stated that illiteracy of mothers is a predisposing factor that contributes to infants and young children acquiring the infection.

The result is similar to the finding of Kandakai-Oluwakemi *et al.* (2009) who documented a high prevalence of 29.20% among children whose mothers had no-formal education and lowest prevalence of 8.20% among children with mother's who had tertiary school education. Contrary to our findings Akingbade *et al*. (2014) and Olaniran *et al*. (2015) reported a high prevalence of 66.67% and 44.40% among children whose mothers had received secondary school education respectively. A similar study was conducted in Bhair-Dar town which disagrees with our findings with highest prevalence of 19.2% among children whose mother had secondary school education (Ayrinkin *et al*., 2014). Also, this study disagrees with the findings of Olaniran*et al.*

(2015) who documented high occurrence of *Escherichia coli* infection in children whose mothers had tertiary education, while low occurrence was found among children whose mothers had secondary education.

The relationship between father's educational status and the number of *E. coli* isolates. The prevalence of *E. coli* infection was found to be highest (24.69%) among children whose fathers had non-formal education, while lowest prevalence of 11.70% was found among children whose fathers had tertiary education. This could be attributed to fact that an educated father would put preventive measures in place and also could quickly rush the child to any health care center once he notices any sign and symptom of infection.

The association between sources of water and the number of *Escherchia coli* isolates was also examined. Children who drink from tap water had prevalence of 35.14% followed by those who drink from stream water with prevalence of 22.03%, while those who drink from well water and bore-hole water had prevalence of 13.33% and 7.41% respectively. The reason may be attributed to improper treatment of drinking water processing (sedimentation, filtration and chlorination) or post contaminated of water during piping to various houses. This finding is similar to report of Olaniran *et al.* (2015) who documented highest prevalence of 24% among children who drink tap water, and least prevalence was found to be among children that drinks rain water. Similar finding from study conducted in Bahir Dar, Ethiopia, reported highest prevalence rate of 41% among children who drink tap water, while lowest prevalence of 0.7% was recorded among children that drinks from hands dug well.

The association between feeding pattern and the prevalence of *Escherichia coli* examined. Children who had formula milk with other food had prevalence of 44.30% followed by children

whofeed on other foods only had prevalence of 14.74%, while children who feed on breast milk and formula milk had lowest prevalence of 8.96%. No isolate was recovered from exclusively breast fed children and the difference was found to be statistically significant (χ^2=21.20; P=0.002). The reason may be attributed to non-compliance to exclusive breast feeding practices recommended by World Health Organization (WHO, 1988). Breast milk has been reported to contain high level of immunoglobulin A (Ig.A) antibodies against any strain of bacteria.This result is similar to 49.15%, 54.3% and 55.3% reported by (Kandakai-Oluwakemi *et.al*, 2009, Akingbade *et al.,* 2014 and Olaniran *et.al.,* 2015) respectively. However, higher prevalencewas reported from other Nigerian studies (Kolo *et al.,* 2014, Onanuga*et al.,* 2014 and Casmir *et al.,* 2015). Similar findings from studies conducted in Abuja reported higher prevalence of 61.5% among children who had mixed feeding (Ifeanyi *et al*, 2015).

From this work, the phytochemical screening of *Entada africana* leaf extracts indicated the presence of glycosides, alkaloids and flavonoids as the phytochemical constituent's common to all the extracts. This could be attributed to the facts that these phytochemical constituents are the ones mostly found in the photosynthetic part of the plant such as leaves, and also in the vegetables,fruits, grains and seeds. This work is similar to the findings of Agbaku *et al*. (2015) who reported the presence of flavonoids, glycosides and alkaloids in their studies of phytochemical analysis of ethanolic leaf extract of *Entada africana.* The work is also in conformity with the findings of Abu *et al*. (2017) who reported the presence of cardiac glycosides, flavonoids and saponins in their studies of phytochemical screening and toxicological studies of aqueous, acetone and ethanolic leaf extracts of *Entada africana.*

This result is however contrary to findings of Adisa *et al*. (2015) who reported the presence of flavonoids, glycosides, alkaloids and anthraquinones in their studies on the antibacterial activity

and phytochemical analysis of*Entada africana* leaf extracts as well as to that of Abu *et al.* (2017) who reported the presence of alkaloids, anthraquinones and glycosides in their studies on the evaluation of phytochemical constituents and antimicrobial activity of *Entada africana* leaf and stem-bark extracts. This could be attributed to geographical area and season of plant collection because the more the environmental stressor, the more phytochemical constituents are produced. Shuaibu and Abdullahi (2016)investigated the phytochemical constituents, antiplasmodial and toxicological studies of *Entada africana* leaf extracts and reported the presence of protein, balsam, flavonoids, alkaloids, glycosides, saponnins and tannins which is contrary to findings in this study. The reason is also attributed to geographical location and time of collection which was March and in Tangaza town, Sokoto State.

The phytochemical screening of *Entada africana* stem-bark extracts revealed the presence of anthraquinones, saponins, steroids, alkaloids and tannins. The reason may be as a result of anthraquinones, resins, alkaloids and tannins reported to be found in the stem-bark, rhizome and bulb of the plant. Presence of flavonoids could be attributed to shift of phytochemical constituents in response to the seasonal change. The study agrees with the findings of Patrick *et al*. (2016) who reported the presence of alkaloids, flavonoids, tannins, saponins, steroids and phenol in their studies on *in-vitro* antioxidant activity and phytochemical evaluation of aqueous and methanol stem-bark extract. The work is however contrary to the findings of Abdullahi *et al*. (2012) who reported the presence of flavonoids, alkaloids, glycosides, saponnins, steroids, terpenes, carbohydrates, balsam and tannins in their studies on the evaluation of some medicinal plants from Nupe land for their *in-vitro* antitrypanosomal activity of *Entada africana* extracts.

Comparatively, stem-bark of *Entada africana* contained more phytochemical constituents than leaf extracts. This could be attributed to the season of the plant collection which was the dry

season (heat period), in which plant shed their leaves and therefore could be minimizing metabolic cost by retieving the metabolites to the stem-barks and the roots or rhizomes for senescing leaf tissues and transporting constituents to the stem and underground parts.

In the phytochemical screening of *Ptericarpus erinaceus* leaf extracts, the methanolic leaf extract contained all the phytochemical constituents tested for except saponins. The reason may be as a result of solvent used. Methanol being a polar solvent has ability to extract both hydrophilic and some lipophilic compounds. Also flavonoids and alkaloids detected in all the leaf extracts are reported to be organ specific under normal environmental condition. This result is contrary to the findings of Onwuliri *et al*. (2006) who reported the presence of only tannins, saponins, flavonoids, glycosides and balsam in the leaf extracts in their studies on phytochemical, toxicological and histopathological analysis of some medicinal plants in Nigeria. This could be attributed to the season of plant collection and geographical area. The report is also contrary to the findings Haizhom *et al*. (2008) who documented the presence of only cyanogenic glycosides and maltol glycosides in their phytochemical analysis of glycosides in leaf extracts of *Ptericarpus erinaceus.* The report is also contrary to the findings of Onwuliri *et al*. (2007) who reported the presence of tannins, saponins, terpenes and steroids, balsam and phenol in their studies on phytochemical, toxicological and histopathological studies of some medicinal plants in Nigeria.

From the study, it was observed that *Ptericarpus erinaceus* stem-bark extracts contained anthraquinones, saponins, steroids and glycosides. The reason may be as a result of season of plant collection. The findings is however contrary to findings of Musa (2006) who reported the presence of steroids, glycosides, saponins, tannins, carbohydrates, proteins, protein and amino acids in his study on some pharmacological studies of the ethanol stem-bark of *Ptericarpus*

erinaceus. The report is also contrary to the findings to Patrick *et al*. (2016) who found saponins, phenols, alkaloids, flavonoids, steroids and tannins in their studies on *in-vitro* anti-oxidant activity and phytochemical evaluation of aqueous and methanol extracts of *Ptericarpus erinaceus.* The reason may be the water and methanol used as extractants. The two solvents are polar and can extract hydrophilic compounds whereas methanol on the other hand can extract both hydrophilic and some lipophilc compounds, thus they are called broad spectrum solvents.

The result is in conformity with the findings of Ajayi *et al*. (2017) who also reported that *Ptericarpus erinaceus* extracts contained cardiac glycosides, flavonoids, alkaloids, saponins, tannins and carbohydrates in their studies on the anti-diabetic effect of methanolic leaf extract of *Ptericarpus erinaceus* in Streptozotocin induced diabetic rats.

Abdullahi *et al*. (2012) investigated the phytochemical constituents of leaf extract of *Ptericarpus erinaceus* and reported the presence of alkaloids, tannins, glycosides, saponins, terpenes, steroids, balsam and carbohydrates which is contrary to findings in this study.

The phytochemical screening of *Vitex doniana* leaf extracts revealed the presence of anthraquinones, steroids and saponins. This could be attributed to the season of plant collection and also due to phytochemicals shift. Also, trace element of alkaloids, flavonoids, glycosides and resins observed may be attributed to different extractants used for extraction. This disagrees with the findings of Dauda *et al.* (2011) who reported the presence of cardiac glycosides, flavonoids, anthraquinoes, steroids and resin in their studies on phytochemical and *in-vitro* antibacterial investigation of *Vitex doniana* leaf, stem-bark and root extracts. This also disagrees with the findings of Agbolafor and Nwuchukwu (2011), who reported the presence of anthraquinones, saponins, flavonoids and terpenoids and tannins in their studies of phytochemical analysis and antioxidant properties of leaf extracts of *Vitex doniana* and *Mucuna*

pruiens. The findings is as well contrary to the report of Ferguson (2001) who documented the presence of resins, tannins and saponins in his study on phytochemical and antimicrobial assessment of hexane leaf extract of *Vitex doniana.* This could be attributed to hexane used as extractant which is capable of extracting lipophilic compounds only.The finding however agrees with that of Dawang (2015) who reported the presence of flavonoids, cardiac glycosides, steroids and resin in his studies of constituents and toxicological analysis of *Vitex doniana* leaf extracts.

This study revealed the presence of anthraquinones, tannins, flavonoids and glycosides in the stem-bark extracts of *Vitex doniana.*The reason may be attributed to the period of plant collection. This report is similar to the findings of Emmanuel *et al*. (2015) who reported the presence of anthraquinones, tannins, flavonoids, glycosides and alkaloids in their studies on phytochemical and antimicrobial screening of the stem-bark extracts of *Vitex doniana.* It also agrees with the findings of Ali *et al*. (2017) who reported the presence of anthraquinones, tannins, glycosides and phenol, in their studies on the assessment of antibacterial activity and phytochemical screening of *Vitex doniana* extracts on clinical isolates of *Salmonella* Typhi.

However, this is contrary to the findings of Olusola *et al*. (1997) who reported the presence of alkaloids, anthraquinones, saponins, tannins, resins and glycosides in their studies on the effect of *Vitex doniana* stem-bark extract on blood pressure. This could be attributed to ethyl acetate used as extractant, being polar aprotic solvent which is capable of extracting hydrophilic and lipophilic compounds.

The result of acute toxicity test (LD_{50}) of*Vitex doniana* extracts on the albino rats in the present study indicated that all albino rats tested survived at 5000mg/kg body weight. No mortality was recorded and one of the toxicological indices accepted for the determination of the safety of drug/substances/extract is lethal dose of 50% (LD_{50}), which is the amount of acute dose required

to kill half of the test population (Dawang, 2015). However, since there was no death recorded during acute toxicity experiment by oral administration, then it is evidence that the median lethal dose (LD_{50}) is greater than 5000mg/kg weight (Assab*et al.*, 2011),this implies that *Vitex doniana* extract is safe (non-toxic). The result in this study agrees with the finding of Dawang (2015) who reported that all albino rats tested against *Vitex doniana* ethanolic leaf extract survived at 5000mg/kg. This is also in conformity with the findings of Abdulrahman *et al*. (2007).

The result of acute toxicity of aqueous leaf and stem-bark extract of *Ptericarpus erinaceus* showed that, all test albino rats survived at LD_{50} of 5000mg/ kg of body weight. However, *Ptericarpus erinaceus* leaf extract at 4000mg/kg body weight, the animals showed slow movement but did not die, while stem-bark extract of *Ptericarpus erinacus* at 5000mg/kg body weight exhibited slow movement (weakness of the body). None of the albino rats died, which shows that the plant is safe for consumption but may have little adverse effect when taken in excess. The result in this study is supported by the findings of Onwuliri *et al.* (2007) who documented LD_{50} of greater than 5000mg/kg in his study. It is also in comformity with the findings of Haizhom*et al*. (2008).

The result of acute toxicity (LD_{50}) of the *Entada africana* extracts against the albino rats in the present study indicated that all albino rats tested survived at 5000mg/kg body weight. This is an indication of safety of the plant.The result in this study is in conformity with Shuaibu and Abdullahi (2016) and Patrick *et al*. (2016) who reported that *Entada africana* leaf extract had LD_{50} of greater than 5000mg/kg body weight.

The results of preliminary antibacterial activity of *Entada africana* crude leaf extracts indicated that the ten *Escherichia coli*isolates tested were susceptible to acetone leaf extract at 10 and 20mg/mL concentration with zones of inhibition ranging from 9.00mm-15.00mm and 11.00mm-

22.00mm respectively. This finding is contrary to the report of Yusuf *et al*. (2019), where all *Escherichia coli* isolates tested were susceptible, with zones of inhibition ranging from 5.00mm-9.00mm and 6.00mm-19.00mm at 10 and 20mg/mL concentration, and similar to what was reported by Kwuje *et al*. (2017) with zones of inhibition ranged from 5.00mm-18.00mm at 20mg/mL concentration. The reason may be attributed to different phytochemical constituents detected in acetone leaf extract.

In a related development, a study carried out on phytochemical analysis, antibacterial and antioxidant activities of *Entada africana* stem-bark extract on *Echerichia coli* isolatesshowed that these *Echerichia coli* isolates were susceptible to methanol stem-bark extract with zones of inhibition ranging from 8.40mm-11.00mm at 5.0 mg/mL (Kwuje *et al*., 2017) which is similar to the finding in the present study. The study is also similar to the findings of Olarenwajuand Ahmed (2018) who reported that methanol stem-bark extract of *Entada africana*inhibited the growth of *Echerichoa coli*isolates with zones of inhibition which ranged from 9.00mm-12.00mm at 5.0 mg/mL. This could be attributed to the fact that methanol being polar solvent was able to extract all phytochemical constituent tested except steroids. Tannin as one of the phytochemical constituent detected was reported to have potent inhibitor of many hydrolytic enzyme used by pathogens. However, there are contrary reports to this, for instance Marthe *et al*. (2014) reported zones of inhibition of 8.00mm and 14.00mm at 20 and 40mg/mL concentration. This could be attributed to flavonoids and glycosides that were detected. Flavonoid was reported to have abilty to achieve complex formation with extracellular soluble proteins and as well as cell wall of bacteria. Some lipid soluble flavonoids can penetrate bacteria cell and cause the disruption of cell membrane (Cushnie and Lamb, 2008).

The methanol stem-bark extracts of *Entada africana* significantly inhibited the growth of the tested *Escherichia coli* isolates with zones of inhibition which ranged from 9.00mm-13.00mm, 13.00mm-19.00mm and 20.00mm-36.00mm at 5.0, 10 and 20mg/mL concentrations respectively. The reason may not only be attributed to genetic factor but also to agroclimatic conditions since the plant was harvested during the dry and heat season, while phytochemical constituents might have migrated to stem-bark and root-bark organs. This study is contrary to the findings of Yusuf *et al*. (2019) who reported zones of inhibition which ranged from 14.00mm-17.00mm exhibited by *Escherichia coli*isolates at 50mg/mL of*Entada africana* methanol stem-bark extracts.

The result of *Ptericarpus erinaceus* leaf extracts revealed that all tested *Escherichia coli* isolates were susceptible to acetone leaf extract at 10 mg/mL with zones of inhibition ranging from 9.00mm-13.00mm, and at 20 mg/mL with zones of inhibition ranging from 13.00mm-20.00mm. The result is contrary to the findings of Abdullah *et al*. (2012)who reported zones of inhibition ranging from 7.00mm-16.00mm at 50 mg/mL of *Ptericarpus erinaceus* acetone leaf extract and at 100 mg/mL with zones of inhibition ranging from 12.00mm-22.00mm and also to what was reported by Prtrick *et al*.(2016) with zones of inhibition ranging from 6.00mm-15.00mm and 10.00mm-22.00mm at 20 and 40mg/mL concentration. The reason may be attributed to less concentration of extract used in our study.

Aqueous leaf extract of *Ptericarpus erinaceus* inhibited the growth of *Echerichoa coli* isolates with zones of inhibition ranging from 9.00mm-13.00mm and 13.00mm-20.00mm at 10 and 20mg/mL concentration respectively. The reason could be attributed to geographical location and season of harvesting. This finding is similar to the report of Musa *et al*. (2006) who documented zones of inhibition ranging from 13.00mm-16.00mm at 10 mg/mL of aqueous leaf extract andat

20mg/mL concentration with zonesof inhibition ranging from 16.00mm-19.00mm. Contrary to the report of Onwuliri *et al*. (2007) who documented zones of inhibition ranging from 10.00mm-12.00mm at 10 mg/mL of *Ptericarpus erinaceus* aqueous leaf extract and at 20 mg/mL with zone of inhibition ranging from 16.00mm-18.00mm.

The methanol leaf extract of *Ptericarpus erinaceus* indicated that at 5.0, 10 and 20mg/mL concentrations, all tested *Escherichia coli* isolates were highly susceptible with inhibition zones ranging from 8.00mm-19.00mm, 11.00mm-15.00mm and 16.00-22.00mm respectively. This finding also contradict the report of Ajayi *et al.*(2017) who documented zones of inhibition ranging from 5.00mm-8.00mm, 10.00mm-12.00mm and 11.00mm-18.00mm at 25ug/mL, 50ug/mL and 75ug/mL for methanol leaf extract of *Ptericarpus erinaceus*. The reason may be attributed to variation of concentration.

The antibacterial activity of stem-bark extracts of*Ptericarpus erinaceus* indicated that, all tested *Echerichoa coli* isolates were moderately susceptible to acetone stem-bark with zones of inhibition ranging from 8.00mm-9.00mm and 8.00mm-15.00mm at 5.0mg/mL and 10mg/mL concentration and highly susceptible with zones of inhibition ranging from 12.00mm-22.00mm at 20mg/mL concentration. The finding is contrary to the report of Abdullahi *et al*. (2012) who documented that methanol stem-bark extract inhibited the growth of *Echerichoa coli* isolates with zones of inhibition ranging from 5.00mm-9.00mm and 8.00mm-10.00mm at 50mg/mL and 100mg/mL concentrations respectively. This could be attributed to concentration of extract adopted for the study.Aqueous stem-bark extract of *Ptericarpus erinaceus* inhibited all tested*Echerichoa coli* isolates with zones of inhibition ranging from 9.00mm-14.00mm and 12.00mm-18.00mm at 10 and 20mg/mL concentrations respectively. The result is contrary to the findings of Musa *et al.* (2016) who reported that aqueous stem-bark inhibited the growth of

Echerichoa coli isolates with zonesof inhibition ranging from 3.00mm-5.00mm, 5.00mm-10.00mm and 10.00mm-17.00mm at 5.0, 10 and 20mg/mL concentration respectively. The reason may be as result of geographical location and season of plant collection.

The methanolstem-bark extract of *Ptericarpus erinaceus*inhibited all tested *Echerichoa coli* isolates with zones of inhibition ranging from 9.00mm-13.00mm, 13.00mm-19.00mm and 20.00mm-30.00mm at 5.0, 10 and 20 mg/mL concentration respectively. The study is contrary to the report of Ajayi *et al*. (2017) who also documented that methanol stem-bark inhibited the growth of *Echerichoa coli* isolates with zones of inhibition ranging from 8.00mm-11.00mm, 10.00mm-14mm and 12.00mm-18.00mm at 25mg/mL, 50mg/mL and 75mg/mL concentrations respectively.

The result of antibacterial activity of *Vitex doniana* acetone leaf extract showed that eight out of the ten *Escherichia coli* isolates were susceptible at 5.0mg/mL with zones of inhibition ranging from 8.00mm-10.00mm, while at 10 and 20mg/mL concentration, all tested *Escherichia coli* isolates were susceptible with zones of inhibition ranging from 8.00mm-14.00mm and 10.00mm-20.00mm respectively. The result is contrary to the finding of Ejikwe and Uzoeto (2010) who documented a zone of inhibition of 17.30mm at 20 mg/mL for acetone leaf extract of *Vitex doniana*. This finding is similar to what was documented by James *et al*. (2014) and Ali *et al*. (2017) that acetone leaf extract of *Vitex doniana* at 20 and 40 mg/mL had zones of inhibition of 13.00mm and 33.00mm respectively. This could be attributed to ecological and seasonal variation.

Aqueous *Vitex doniana* leaf extract indicated that, all *Escherichia coli* isolates except *Escherichia coli* coded 149 were susceptible with zones of inhibition ranging from 8.00mm-

12.00mm, 10.00mm-18.00mm and 12.00mm-27.00mm at 5.0, 10 and 20mg/mL concentration. This finding contradicts the report of Ejikene and Uzoeta (2010) who documented a zone of inhibition of 5.10mm at 2.0mg/mL concentration for aqueous *Vitex doniana* leaf extract. A contrary finding was reported by Osuaagwu *et al*. (2013) who documented a zone of inhibition of 15.00mm at 2.0mg/mL concentration, while Ali *et al.* (2017) also reported zones of inhibition of 15.00mm, 20.00mm and 27.00mm at 50mg/mL, 100mg/mL and 150mg/mL concentration for aqueous *Vitex doniana* leaf extract which is contrary to our findings.

The methanol leaf extract of *Vitex doniana* revealed that all *Echerichia coli* isolates tested were susceptible with zones of inhibition ranging from 9.00mm-14.00mm, 10.00mm-23.00mm and 14.00mm-38.00mm at 5.0, 10 and 20mg/mL concentration respectively. The result is contrary to the findings of Ejikeme and Uzoeto (2010) and Osuagwu *et al*. (2013) who reported a zone of inhibitions of 14.60mm, and 14.00mm respectively at concentration of 2.0mg/mL for methanol leaf extract of *Vitex doniana*. A contrary finding was also reported by Ali *et al*. (2017) with zones of inhibition of 19.00mm, 23.00mm and 27.00mm respectively at 50, 100 and 150mg/mL concentration and James *et al*. (2014) also documented a zone of inhibition of 33.00mm at 100mg/mL concentration. The reason may be attributed to the method of extraction and harvest condition of the plant.

The results of antibacterial activityof *Vitex doniana* stem-bark indicated that all tested *Escherichia coli* isolates were susceptible to acetone stem-bark extract with zones of inhibition ranging from 8.00mm-11.00mm, 11.00mm-17.00mm and 15.00mm-26.00mm at concentration of 5.0, 10 and 20mg/mL respectively. The study is contrary to the finding of James *et al*. (2014) and Ali *et al*. (2012) who reported the zones of inhibition of 27.00mm at 100mg/mL

concentration and 18.00mm, 19.00mm and 20.00mm at 50, 100, 150mg/mL concentration respectively. This could be attributed to different concentrations of extract used.

The aqueous extract stem-bark revealed that, all *Escherichia coli* isolates tested were susceptible with zones of inhibition ranging from 9.00mm-15.00mm, 12.00mm-21.00mm and 18.00mm-36.00mm at 0.5, 1.0 and 2.0mg/mL concentrations respectively. This could be attributed to phytochemical constituents (bioactive shift). Contrary to this finding, James *et al*.(2014) documented a zone of inhibition of 22.00mm at 100mg/mL concentration and Osuagwu *et al*. (2013) also reported inhibition zone of 20.00mm at 2.0mg/mL concentration.

The result of methanol stem-bark of *Vitex doniana* also indicated that, all the tested *Echerichia coli* isolates were susceptible with zones of inhibition ranging from 4.00mm-9.00mm, 9.00mm-16.00mm and 18.00mm-32.00mm at 5.0, 10 and 20mg/mL concentration respectively. This may be attributed to concentration of extract used. This may be complimented with the fact that stem-bark of medicinal plant has been reported to generally show high antimicrobial activity than the leaf extracts (Yusuf *et al*., 2019).This finding is contrary to the report of Ali *et al*. (2017) who documented that*Escherichia coli* isolates were inhibited with zones of inhibition of 13.00mm 17.00mm and 20.00mm at 50, 100 and 150mg/mL concentration respectively and Osuagwe *et al*.(2013) also reported inhibition zone of 28mm at 2.0mg/mL concentration.

From the result of MIC and MBC test of *Vitex doniana* fractions against Enteroaggregative *Escherchia coli*. Isolates of Enteroaggregative *Escherchia coli*were susceptible to the fractions at MIC of 5.0mg/mL especially aqueous stem-bark fraction having inhibitory effect against 75% isolates.The reason may be as a result ofanthraquinonespresent in the fraction. It was reported that anthraquinonesact by inhibiting two enzymes involved in bacterial DNA synthesis;

topoisomerase II and DNA gyrase which block DNA replication thereby impair cell division (Ome-Kalu *et al*., 2015).

Resistance exhibited by some of the Enteroaggregative *Escherchia coli* strains to *Vitex doniana* fractions might be due to some mutant EAEC strains that possess genes called *gyrA, gyrB, ParC*[1]and *Par*C[2] whichare capable of altering their efflux pump to unspecific compounds or substrates (Cushnie and Lamb, 2005).

Vitex doniana fractions had an MIC of 5.0 and 10mg/mL against Enteroheamorrhagic *Escherichia coli*. The *Vitex doniana* methanol leaf fraction had an MIC of 5.0mg/mL against 76.9% (n=10/13) of the Enteroheamorrhagic *Escherichia coli.* This could be attributed to the presence of tannins in the methanol leaffraction.Tannins are polyphenol with pronounced autolytic enzymes of microbial metabolism such as proteolytic macerating enzymes (Obase *et al*., 2011).

The result indicated that 53.85% (n=7/13) of the Enteroheamorrhagic *Escherichia coli*were resistant to the methanol leaf fraction. This might be due to some mutant strains that possess gene that rendered the fractions inactive. It was reported that some mutant EHEC and EPEC possess an enzyme called Beta-lactamase enzyme and this contributed to high level of resistance to phytochemical constituents such as flavonoids, tannins and antibiotics like ampicillin and penicillin (Cushnie and Lamb, 2005; Ome-Kalu *et al*., 2015).

The result indicated that*Vitex doniana*methanol stem-bark fraction had an MIC of 5.0mg/mL, while acetone leaf fraction had an MIC of 10.0mg/mL against 37.5% (n=3/8) and 62% (n=5/8) of theEnteroinvasive*Escherichia coli*.The inhibitory effect of the fractionsagainst Enteroinvasive*Escherichia coli*might be due to the presence of saponins and terpenoids in the

Vitex doniana methanol stem-bark and *Vitex doniana* acetone leaf fraction respectively. Saponins might act by altering the permeability of the cell membrane and hence exerting toxicity on all organized tissues. They exert some bacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (Lacaille*et al*., 2010).

However, some strains of Enteroinvasive*Escherichia coli*were resistant to saponins. This may be attributed to the fact that mutant EIEC possess an enzyme that is capable of altering mode of action of terpenoids that act on beta-hydroxyl acyl-acyl carrier protein dehydrates activities, thus the RNA synthesis will not be impaired (Cushnie and Lamb, 2005).

Methanol stem-bark and aqueous leaf fractions of *Vitex doniana*had an MIC of 5.0mg/mL and 10.0mg/mL against 66.7% (n=18/27) and 63% (n=17/27) of the Enteropathogenic*Escherchia coli.*The reason may be as a result of glycosides and flavonoids detected in the methanol stem-bark and aqueous leaf fractions respectively. Flavonoids have been reported to form complexes with both extracellular and soluble proteins as well as bacterial cell wall (Cowan, 1999). The glycosides have been also reported to inhibit protein synthesis by binding with high affinity to the A-site on the 16s ribosomal RNA of the 30s ribosome (Cushnie *et al*., 2014).

However, some strains of Enteropathogenic *Escherichia coli* were resistant to phytochemical constituent detected in the fractions. It was reported that some mutant EPEC possess an enzyme called Beta-lactamase enzyme and this contributed to high level of resistance to phytochemical constituents such as flavonoids, tannins and antibiotics like ampicillin and penicillin (Cushnie and Lamb, 2005; Ome-Kalu *et al*., 2015).

Vitex doniana methanol leaf fraction had MIC and MBC values of 5.0mg/mL respectively against 82.4% (n=14/17) and 15.8%(n=5/17) of the Enterotoxigenic *Escherichia coli*, while

methanol leaf fraction had MIC value of 5.0mg/mL against 94.1% (n=16/17)of the Enterotoxigenic *Escherichia coli*. This might be attributed to the presence of alkaloids and flavonoids in the fraction respectively. According to Ome-Kalu *et al*. (2015), alkaloids such as pergulamine and tylophorinidine have effect on cell division by inhibiting dihydrofolate reductase which is responsible in the production of purine and pyrimidine precursors for amino acids, RNA and DNA biosynthesis. Flavonoids on the other have been reported to form complexes with both extracellular and soluble proteins as well as bacterial cell wall (Cowon, 1999).

However, some strains of Enterotoxigenic *Escherichia coli* were resistant to phytochemical constituents detected in *Vitex doniana* fractions. This may be due to mutation that might have occurred within some strains of Enterotoxigenic *Escherichia coli*.

The result of antibacterial susceptibility study of the fractions against the *Escherichia coli* strains in this study indicated that they had activity against almost all the *Escherichia coli*strains. This activity is attributed to phytochemical constituents that were found present in the plant extract which have been reported to have activity against bacteria. Anthraquinones were reported to inhibit DNA gyrase thereby preventing DNA synthesis, Saponins might act by altering the permeability of the cell membrane and hence exerting toxicity on all organized tissues. They exert some bacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (Lacaille*et al*., 2010). Steroids have been reported to have synergistic effect with saponins (Hanson, 2010; Madziga *et al*., 2010). Tannins are polyphenol with pronounced autolytic enzymes of microbial metabolism such as proteolytic macerating enzymes (Obase *et al*., 2011).

CHAPTER SIX

6.0: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Altogether, eighty-one (81) isolates of *Escherichia coli* were obtained from the study giving a prevalence of 16.9%. The *Escherichia coli* detected from diarrhoeic stool of children in this study area included Enteroaggregative *Escherichia coli* (EAEC) 19.8%, Enterohaemorrhagic *Escherichia coli* (EHEC) 16.0%, Enteroinvasive *Escherichia coli* (EIEC) 9.4%, Enteropathogenic*Escherichia coli* (EPEC) 33.3%, Enterotoxigenic*Escherichia coli* (ETEC) 21.0%.

The socio-demographic and risk factor found to be associated with diarrhoeagenic *Eschericheria coli* infection in this study area were age, area of domicile, educational status of parents, source of water and feeding habits are statistically ($P<0.05$) significant.

All the crude extracts of the plants were found to have anthraquinones, flavonoids, glycosides, saponins, steroids, tannins and resins except for *Ptericarpuserinaceus* in which resins was not detected.

The lethal dose (LD_{50}) of all the plants' extracts on albino rats was greater than 5000mg/kg, indicating that the plants are safe for consumption.

The methanolic crude extracts of *Entada africana*, *Ptericarpus erinaceus* and *Vitex doniana* showed highest activity against *Escherichia coli* at 20mg/mL with zones of inhibition ranging between 20.00-36.00mm, 20.00-30.00mm and 24.00-38.00mm respectively.

The methanolic leaf fraction of *Vitex doniana* had the highest activity against all the tested *Escherichia coli* strain except Enteroinvasive *Escherichia coli* which had 50% susceptibility.

The *Vitex doniana* aqueous stem-bark fraction with MIC and MBC values of 5.0 and 10.0mg/mL emerged as the active fraction against 75% of the EAEC, while methanolic leaf fraction had MIC and MBC values of 5.0 and 10mg/mL against 77% of the EHEC. The aqueous leaf fraction had MIC and MBC value of 10mg/mL against 62.5% of the EIEC, while methanolic leaf fraction had MIC and MBC values of 5.0 and 10mg/mL against 61% and 81% of the EPEC. The methanolic stem-bark fraction exhibited highest activity with MIC and MBC values of 5.0 and 10mg/mL against 79% and 100% of the Enterotoxigenic *Escherichia coli*.

6.2 Recommendation

1. Public awareness should be encouraged upon, particularly on personal hygiene and effective sewage disposal.
2. Proper treatment of drinking water should be encouraged, especially among rural dwellers who have limited acess to chlorinated pipe-borne water.
3. Conduct research to develop and test new diarrhoea prevention and control strategies in this study area.
4. Further study is needed to purify, characterize and elucidate the structure of bioactive compounds present in the fractions which were responsible for the antibacterial activity with a view to supplement conventional drug development.

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APPENDIX I

Structured questionnaire for a study, to determine the anti-bacterial activity of *Entada africana, Ptericarpus erinaceous* and *Vitex doniana* extracts on *Escherichia coli* isolated from diarrhoeal stool of children (0-5) years.

NAME:

DATE:

SERIAL number:

HOSPITAL number:

Age:

Gender: male ( ) female ( )

Appearance of stool: Watery ( ), Bloody ( ), Mucus ( ), None ( ).

Number of stool per day: Once ( ), Twice ( ), Many ( ).

Source of water: Well Water ( ), Stream/River ( ), Bore hole ( ), Tap water ( ).

Area of domicile: Rural ( ), Urban ( )

Occupation of parents: Mother; House wife ( ), Civil servant ( ).

Father; Civil servant ( ) Farmer ( ), Painter ( ), Welder ( ).

Mechanics ( ), Carpenter ( ), Bricklayer ( ).

Driver ( ).

Ecaduactional status of parents: Mother; Primary ( ), Secondary ( ), Tertiarry ( )

Father; Primary ( ), Secondary ( ), Tertiarry ( )

Feeding habits: Exclusive breast feeding ()

Breast feeding with formula milk ()

Formula milk with other foods ()

Other foods ( )

APPENDIX II:Ethical letter from Ministry of Health, Minna

MINISTRY OF HEALTH
NIGER STATE GOVERNMENT OF NIGERIA
Block 'C' First Floor, Abdul-Kareem Lafene Secretariat Complex, Paiko Road, P.M.B. 57, Minna, Niger State.
e-mail: ngsmohmx@yahoo.com

28th February, 2014

Mohammed Isah Legbo,
Department of Microbiology,
Faculty of Science,
Ahmadu Bello University, Zaria.

ETHNICAL APPROVAL

The Niger State Ministry of Health has given approval for the implementation of your research protocol titled:
Antibacterial Activity of Entada Africana, Ptericarpus Erinaceus and Vitex doniana Extracts Against Escherichia Coli Strains Isolated from Diarrheal Stool of Children less than five years.

You are required to submit periodically, a review of the study to this committee. On completion of the study, a final full review must be submitted to this committee. Any challenge in the course of the studies must be brought to the notice of the committee within seven (7) days. This committee must be informed before your research finding is published.

Thank you.

Dr. Ibrahim Tefengi Kolo
Deputy Director Medical Services and Training

APPENDIX III:

Morphological and Physiological Properties of the Test Isolates

S/N	Code of Isolates	Colony	Shape	G/Stain	Oxidase	Catalase	Indole	Methyl Red	Voges Prokauer	Citrate	Isolates
1	EUS003	Round, Convex Green Metallic Sheen	Rod	-	-	+	+	+	-	-	*E. coli*
2	EUS010	"	"	-	-	+	+	+	-	-	*E. coli*
3	EUS015	"	"	-	-	+	+	+	-	-	*E. coli*
4	EUS024	"	"	-	-	+	+	+	-	-	*E. coli*
5	EUS033	"	"	-	-	+	+	+	-	-	*E. coli*
6	EUS047	"	"	-	-	+	+	+	-	-	*E. coli*
7	EUS054	"	"	-	-	+	+	+	-	-	*E. coli*
8	EUS063	"	"	-	-	+	+	+	-	-	*E. coli*
9	EUS074	"	"	-	-	+	+	+	-	-	*E. coli*
10	EUS094	"	"	-	-	+	+	+	-	-	*E. coli*
11	EUS103	"	"	-	-	+	+	+	-	-	*E. coli*
12	EUS118	"	"	-	-	+	+	+	-	-	*E. coli*
13	EUS126	"	"	-	-	+	+	+	-	-	*E. coli*
14	EUS140	"	"	-	-	+	+	+	-	-	*E. coli*
15	EUS149	"	"	-	-	+	+	+	-	-	*E. coli*
16	EUS080	"	"	-	-	+	+	+	-	-	*E. coli*
17	EUS133	"	"	-	-	+	+	+	-	-	*E. coli*
18	EUS180	"	"	-	-	+	+	+	-	-	*E. coli*

19	GHM156	“	“	-	-	+	+	+	-	-	*E. coli*
20	GHM165	“	“	-	-	+	+	+	-	-	*E. coli*
21	GHM169	“	“	-	-	+	+	+	-	-	*E. coli*
22	GHM181	“	“	-	-	+	+	+	-	-	*E. coli*
23	GHM186	“	“	-	-	+	+	+	-	-	*E. coli*
24	GHM198	“	“	-	-	+	+	+	-	-	*E. coli*
25	GHM201	“	“	-	-	+	+	+	-	-	*E. coli*
26	GHM208	“	“	-	-	+	+	+	-	-	*E. coli*
27	GHM214	“	“	-	-	+	+	+	-	-	*E. coli*
28	GHM220	“	“	-	-	+	+	+	-	-	*E. coli*
29	GHM224	“	“	-	-	+	+	+	-	-	*E. coli*
30	GHM227	“	“	-	-	+	+	+	-	-	*E. coli*
31	GHM231	“	“	-	-	+	+	+	-	-	*E. coli*
32	GHM237	“	“	-	-	+	+	+	-	-	*E. coli*
33	GHM241	“	“	-	-	+	+	+	-	-	*E. coli*
34	GHM245	“	“	-	-	+	+	+	-	-	*E. coli*
35	GHM252	“	“	-	-	+	+	+	-	-	*E. coli*
36	GHM263	“	“	-	-	+	+	+	-	-	*E. coli*
37	GHM268	“	“	-	-	+	+	+	-	-	*E. coli*
38	GHM276	“	“	-	-	+	+	+	-	-	*E. coli*
39	GHM280	“	“	-	-	+	+	+	-	-	*E. coli*
40	GHM289	“	“	-	-	+	+	+	-	-	*E. coli*
41	GHM290	“	“	-	-	+	+	+	-	-	*E. coli*

42	GHM296	“	“	-	-	+	+	+	-	-	*E. coli*
43	GHM298	“	“	-	-	+	+	+	-	-	*E. coli*
44	KGH303	“	“	-	-	+	+	+	-	-	*E. coli*
46	KGH312	“	“	-	-	+	+	+	-	-	*E. coli*
47	KGH314	“	“	-	-	+	+	+	-	-	*E. coli*
48	KGH318	“	“	-	-	+	+	+	-	-	*E. coli*
49	KGH321	“	“	-	-	+	+	+	-	-	*E. coli*
50	KGH324	“	“	-	-	+	+	+	-	-	*E. coli*
51	KGH327	“	“	-	-	+	+	+	-	-	*E. coli*
52	KGH328	“	“	-	-	+	+	+	-	-	*E. coli*
53	KGH330	“	“	-	-	+	+	+	-	-	*E. coli*
54	KGH332	“	“	-	-	+	+	+	-	-	*E. coli*
55	KGH335	“	“	-	-	+	+	+	-	-	*E. coli*
56	KGH349	“	“	-	-	+	+	+	-	-	*E. coli*
57	KGH354	“	“	-	-	+	+	+	-	-	*E. coli*
58	KGH360	“	“	-	-	+	+	+	-	-	*E. coli*
59	KGH365	“	“	-	-	+	+	+	-	-	*E. coli*
60	KGH372	“	“	-	-	+	+	+	-	-	*E. coli*
61	KGH375	“	“	-	-	+	+	+	-	-	*E. coli*
62	KGH381	“	“	-	-	+	+	+	-	-	*E. coli*
63	KGH387	“	“	-	-	+	+	+	-	-	*E. coli*
64	KGH394	“	“	-	-	+	+	+	-	-	*E. coli*
65	KGH397	“	“	-	-	+	+	+	-	-	*E. coli*

66	KGH399	“	“	-	-	+	+	+	-	-	*E. coli*
67	KGH402	“	“	-	-	+	+	+	-	-	*E. coli*
68	KGH406	“	“	-	-	+	+	+	-	-	*E. coli*
69	KGH411	“	“	-	-	+	+	+	-	-	*E. coli*
70	KGH418	“	“	-	-	+	+	+	-	-	*E. coli*
71	KGH431	“	“	-	-	+	+	+	-	-	*E. coli*
72	KGH437	“	“	-	-	+	+	+	-	-	*E. coli*
75	KGH445	“	“	-	-	+	+	+	-	-	*E. coli*
76	EUS464	“	“	-	-	+	+	+	-	-	*E. coli*
77	GHM466	“	“	-	-	+	+	+	-	-	*E. coli*
78	KGH472	“	“	-	-	+	+	+	-	-	*E. coli*
79	KGH477	“	“	-	-	+	+	+	-	-	*E. coli*
80	KGH478	“	“	-	-	+	+	+	-	-	*E. coli*
81	KGH480	“	“	-	-	+	+	+	-	-	*E. coli*

APPENDIX IV:

Antibacterial Activity of *Vitex doniana* Leaf and Stem-Bark Fractions.

Code	Isolates	Concentration of *Vitex doniana* leaf and stem-bark fractions (2.0mg/mL)						
		Zones of inhibition (mm)						
		ALF	ASF	HLF	MLF	MSF	QLF	QSF
EUS 080	EAEC	32	24	26	34	38	16	36
EUS 103	EAEC	29	00	32	27	26	24	28
EUS 149	EAEC	20	00	32	24	32	26	34
GH 180	EAEC	32	19	30	38	40	26	36
GHM208	EAEC	32	00	26	32	28	42	26
GHM214	EAEC	20	00	24	34	30	20	38
GHM 227	EAEC	18	24	00	32	30	16	38
GHM 245	EAEC	32	20	18	34	34	00	32
GHM 252	EAEC	24	23	00	32	34	32	36
GHM290	EAEC	20	26	00	38	36	26	36
KGH 307	EAEC	12	12	26	30	28	20	38
KGH 324	EAEC	00	00	32	21	00	00	24
KGH 375	EAEC	00	00	00	18	00	00	00
KGH 399	EAEC	16	00	18	22	12	14	26
KGH 442	EAEC	00	00	00	00	00	00	00
GHM 469	EAEC	22	12	14	20	00	14	32
EUS 010	EHEC	26	00	32	30	34	34	32
EUS 047	EHEC	34	00	35	32	36	32	00
EUS 063	EHEC	00	28	00	31	30	28	24

EUS 094	HEC	34	36	00	28	28	00	30
GHM 156	EHEC	38	38	34	38	26	28	31
GHM 186	EHEC	38	00	26	36	36	32	26
GHM 201	EHEC	00	00	00	38	28	32	00
GHM 263	EHEC	28	34	32	32	28	24	26
KGH 314	EHEC	27	26	32	22	20	33	00
KGH 332	EHEC	32	00	34	30	34	32	32
KGH 381	EHEC	22	26	34	38	34	24	00
KGH 445	EHEC	24	30	42	30	34	36	26
EUS 458	EHEC	36	00	38	30	38	36	38
EUS 024	EIEC	28	00	00	36	26	28	27
EUS 126	EIEC	25	26	24	25	30	26	28
GHM198	EIEC	28	36	22	00	34	26	00
GHM241	EIEC	16	00	00	16	18	00	00
KGH 411	EIEC	30	32	36	30	36	34	24
KGH 431	EIEC	00	00	00	00	00	00	00
KGH 475	EIEC	00	00	28	00	00	00	00
KGH 480	EIEC	16	00	22	00	00	00	00
EUS 033	EPEC	32	30	32	38	36	28	30
EUS 054	EPEC	26	24	00	34	34	26	18
EUS 140	EPEC	36	30	12	28	26	30	24
GHM169	EPEC	24	00	60	22	28	30	00
GHM 23	EPEC	26	34	34	32	36	28	26

GHM 268	EPEC	28	40	42	28	28	32	24
GHM 276	EPEC	30	30	26	24	00	28	00
GHM 289	EPEC	28	26	40	18	30	32	00
GHM296	EPEC	26	24	32	22	36	30	25
GHM 298	EPEC	30	22	22	32	38	24	36
KGH 303	EPEC	27	00	30	25	24	21	28
KGH 318	EPEC	18	00	22	20	30	24	34
KGH 321	EPEC	30	00	28	36	38	24	36
KGH 328	EPEC	20	16	24	26	32	26	22
KGH 330	EPEC	18	00	22	30	34	18	38
KGH 335	EPEC	16	00	24	32	32	28	30
KGH 349	EPEC	32	18	16	32	32	00	32
KGH 354	EPEC	20	32	24	30	36	22	30
KGH 360	EPEC	00	22	20	30	34	24	36
KGH 372	EPEC	00	18	26	38	34	36	20
KGH 402	EPEC	00	00	24	24	00	00	00
KGH 406	EPEC	22	24	24	26	21	00	00
KGH411	EPEC	30	20	00	30	32	00	00
KGH 418	EPEC	00	00	32	28	26	28	00
KGH 442	EPEC	20	00	21	32	30	22	20
EUS 452	EPEC	18	00	22	32	26	20	24
GHM466	EPEC	20	00	20	32	16	18	20
EUS 003	ETEC	18	00	00	30	34	18	32

EUS 015	ETEC	22	00	28	28	00	16	34
EUS 074	ETEC	31	34	26	24	38	30	32
EUS 118	ETEC	24	00	22	30	36	22	34
EUS 133	ETEC	26	26	22	32	36	32	30
GHM165	ETEC	28	26	22	30	34	18	22
GHM181	ETEC	34	28	22	28	34	00	00
GHM220	ETEC	32	33	20	32	42	00	00
GHM224	ETEC	20	16	22	22	34	20	32
GHM231	ETEC	00	00	24	34	38	24	36
KGH 312	ETEC	22	18	26	36	34	30	30
KGH 327	ETEC	20	00	20	30	32	16	24
KGH 365	ETEC	00	00	22	24	00	00	22
KGH 387	ETEC	18	28	26	36	38	30	38
KGH 394	ETEC	18	00	20	30	34	27	32
KGH 437	ETEC	22	00	00	29	00	24	16
KGH 418	ETEC	33	36	30	26	42	32	34

APPENDIX V:

The Percentage Weight of *Entada africana* Extract Yield after Extraction.

Plant Sample	Solvent	Weight of Plant sample(g)	Weight of extract (g)	Percentage of extract(%)	Colour of extract	Texture of extract
Entada africana Leaf	Acetone	100	20.44	20	Greenish	Gummy
	Aqueous	100	23.52	24	Greenish	Smooth
	Hexane	100	16.16	16	Green	Gummy
	Methanol	100	28.36	28	Green	Smooth
Entada africana stem-bark	Acetone	100	23.24	23	Brownish	Gummy
	Aqueous	100	25.44	25	Brownish	Smooth
	Hexane	100	17.80	18	Brownish	Smooth
	Methanol	100	40.56	41	Brownish	Smooth

Key: g=Gram, %=Percentage

APPENDIX VI:

The Percentage Weight of *Ptericarpus erinaceus* Extract Yield after Extraction

Plant Sample	Solvent	Weight of Plant Sample(g)	Weight of extract (g)	Percentage of extract(%)	Colour of extract	Texture of extract
Ptericarpus erinaceus Leaf	Acetone	100	31.68	32	Greenish	Smooth
	Aqueous	100	20.88	21	Greenish	Smooth
	Hexane	100	19.58	20	Green	Smooth
	Methanol	100	32.03	32	Green	Smooth
Ptericarpus erinaceus stem-bark	Acetone	100	20.24	20	Pale Brown	Gummy
	Aqueous	100	23.36	23	Brown	Coarse
	Hexane	100	31.40	31	Brown	Brown
	Methanol	100	25.56	26	Brown	Smooth

Key: g=Gram, % = Percentage

APPENDIX VII:

The Percentage Weight of *Vitex doniana* Extract Yield after Extraction.

Plant Sample	Solvent	Weight of plant sample(g)	Weight of extract (g)	Percentage of extract (%)	Colour of extract	Texture of extract
Vitex	Acetone	100	31.68	32	Greenish	Smooth
doniaana	Aqueous	100	20.88	21	Greenish	Smooth
Leaf	Hexane	100	19.58	20	Green	Smooth
	Methanol	100	32.03	32	Green	Smooth
Vitex	Acetone	100	20.24	20	Pale Brown	Coarse
doniana	Aqueous	100	23.36	23	Brown	Coarse
stem-bark	Hexane	100	31.40	31	Brown	Gummy
	Methanol	100	25.56	26	Brown	Smooth

g=Gram, %= Percentage

APPENDIX:VIII

Calculation for limit or fixed dose.

Test limit dose chosen, 3000mg/kg (OECD, 2001)

What volume of 5% extract should be given to albino rat of body weight (185g) at 3000mg/kg concentration?

5% of extract contained 5g or 5000mg

Therefore 5g or 5000mg should be dissolving in 100mL.

5000mg/100mL=50mg/mL.

5% of extract =50mg/mL.

Step1: Weight of animal = 185g

Dosage 3000mg/kg

3000mg/1000g

Then = Xmg/185g

Hence = Xmg 185x3000/1000

555000/100

555mg/185g

Hence, 3000mg/kg = 555mg/185g

555/1000 = 0.555

= 0.6g

Therefore 0.6g was dissolve in 1mL (0.6g/mL) to administer to the animal.

APPENDIX: IX

Phytochemical Analysis of Methanolic Leaf and Stem-Bark Fractions of *Vitex doniana*.

Fraction	ALD	ATQ	GCD	FVD	RSN	SPN	STD	TNS	TPD
VDALF (A)	-	-	-	-	-	+	-	+	_
VDHLF (B)	-	-	-	-	-	+	-	-	-
VDMLF (C)	-	+	-	+	-	-	-	+	-
VDQLF (D)	-	-	-	+	-	+	-	-	-
VDASF (E)	-	-	+	-	-	-	-	+	-
VDHSF (F)	-	-	-	-	-	-	-	-	-
VDMSF (G)	-	-	-	+	-	+	-	+	-
VDQSF (H)	-	+	+	-	-	-	+	-	-

Key:ALD=Alkaloids.
ATQ = Anthraquinones.
GCD = Glycosides.
FVD = Flavonoids.
RSN = Resins.
SPN = Saponins.
STD = Steroids.
TNS = Tannins.
TPD = Terpenoids**.**

APPENDIX: X

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *Vitex doniana* Leaf and Stem-Bark Fractions

Codes	Isolates	Concentration of extracts (mg/mL)													
		VDALF		VDASBF		VDHLF		VDMLF		VDMSBF		VDQLF		VDQSBF	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
EUS 080	EAEC	5.0	10	10	10	10	10	5.0	5.0	5.0	5.0	2020		5.010.0	
EUS 103	EAEC	5.0	10	-	-	5.0	10	10	10	10	10	10	10	5.0	5.0
EUS 149	EAEC	10	20	-	-	10	10	5.0	5.0	5.05.0		10	10	5.0	10
EUS 180	EAEC	5.0	10	10	20	5.0	10	10	10	5.0	10	10	10	5.0	10
GHM 208	EAEC	10	10	-	-	10	10	5.0	10	5.0	10	5.0	5.0	10	1.0
GHM 214	EAEC	10	20	-	-	-	-	5.0	10	5.0	10	10	20	5.0	5.0
GHM 227	EAEC	10	20	10	10	-	-	5.0	10	5.0	10	20	20	5.0	5.0
GHM 245	EAEC	5.0	10	10	20	10	20	5.0	10	10	10	-	-	5.0	10
GHM 252	EAEC	10	10	1.0	10	-	-	5.0	10	10	10	10	10	5.0	10
GHM 290	EAEC	10	20	10	10	-	-	5.0	10	-	-	-	-	5.0	10
KGH 307	EAEC	20	40	20	40	20	20	10	10	-	-	-	-	5.0	5.0

KGH 324	EAEC	-	-	-	-	20	20	-	-	40		-	-	10	10
KGH 375	EAEC	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KGH 399	EAEC	20	20	-	-	10	20	20	40	-	-	-	-	10	10
KGH 442	EAEC	-	-	-	-	-	-	-	-	-	-	-	-	10	10
KGH 469	EAEC	10	10	-	-	20	20	-	-	-	-	20	20	5.0	10
EUS 010	EHEC	-	-	-	-	5.0	10	5.0	10	5.0	10	10	10	5.0	10
EUS 047	EHEC	5.0	10	-	-	5.0	10	5.0	10	5.0	10	5.0	10	-	-
EUS 063	EHEC	-	-	10	10	-	-	5.0	10	5.0	10	5.0	10	10	10
EUS 094	EHEC	5.0	10	5.0	10	-	-	10	10	10	10	-	-	5.0	10
GHM 156	EHEC	5.0	10	5.0	5.0	5.0	10	5.0	5.0	10	10	10	10	10	10
GHM 186	EHEC	5.0	10	-	-	10	10	5.0	10	5.0	10	5.0	10	10	10
GHM 201	EHEC	-	-	-	-	-	-	5.0	5.0	10	10	10	10	-	-
GHM 263	EHEC	10	10	5.0	10	10	10	5.0	10	10	10	10	10	10	10
KGH 314	EHEC	10	10	10	10	5.0	10	10	10	10	20	5.0	10	-	-
KGH 332	EHEC	5.0	10	-	-	5.0	10	5.010		5.0	10	5.0	5.0	20	40
KGH 381	EHEC	10	10	5.0	10	5.0	10	5.0	5.0	5.0	10	10	10	-	-

KGH 445	EHEC	10	10	-	-	5.0	5.0	5.0	10	5.0	10	5.0	10	10	10
EUS 458	EHEC	5.0	10	-	-	5.0	10	10	10	5.0	10	5.0	10	10	10
EUS 024	EIEC	10	10	-	-	-	-	5.0	10	10	10	10	10	10	10
EUS 126	EIEC	10	10	10	10	10	10	10	10	5.0	10	10	10	10	10
GHM 198	EIEC	10	10	5.0	10	10	10	-	-	5.0	10	-	-	-	-
GHM 241	EIEC	20	20	-	-	-	-	2.0	20	10	20	5.0	10	-	-
KGH 411	EIEC	5.0	10	5.0	10	5.0	10	10	20	5.0	5.0	-	-	10	10
KGH431	EIEC	10	10	-	-	-	-	-	-	-	-	-	-	-	-
KGH 475	EIEC	10	20	-	-	10	10	-	-	-	-	-	-	-	-
KGH480	EIEC	5.0	10	-	-	10	10	-	-	-	-	10	10	5.0	10
EUS 033	EPEC	10	10	5.0	10	5.0	5.0	5.0	5.0	5.05.0		10	10	10	20
EUS 054	EPEC	10	20	10	10	-	-	5.0	5.0	5.0	5.0	5.0	10	10	10
EUS 140	EPEC	10	20	5.0	10	5.0	10	5.0	10	10	10	5.0	10	-	-
GHM 169	EPEC	5.0	10	-	-	-	-	10	10	5.0	5.0	10	10	10	10
GHM 023	EPEC	10	10	5.0	10	5.0	10	10	10	5.0	5.0	5.0	10	10	10
GHM 268	EPEC	10	20	5.0	5.0	5.0	5.0	5.0	10	-	-	10	10	10	10

GHM 276	EPEC	20	40	5.0	10	5.0	5.0	10	10	5.0	5.0	5.0	10	-	-
GHM 289	EPEC	-	-	10	10	10	10	10	10	5.0	5.0	10	10	-	-
GHM 296	EPEC	-	-	10	10	5.0	10	10	20	10	10	10	10	10	10
GHM 298	EPEC	20	20	10	10	5.0	10	10	10	5.0	10	10	10	5.0	10
KGH 303	EPEC	-	-	-	-	10	10	5.0	10	5.0	5.0	10	10	5.0	10
KGH 318	EPEC	10	10	-	-	10	10	10	10	5.0	5.0	10	2.0	5.0	10
KGH 321	EPEC	10	10	-	-	5.0	10	10	20	5.0	5.0	10	10	10	10
KGH 328	EPEC	5.0	40	20	20	10	10	10	10	5.0	5.0	-	-	5.0	5.0
KGH 330	EPEC	-	-	-	-	10	10	5.0	10	5.0	5.0	10	10	5.0	10
KGH 335	EPEC	5.0	10	-	-	10	10	5.0	10	5.0	5.0	10	10	5.0	10
KGH 349	EPEC	5.0	10	10	20	10	10	5.0	10	5.0	5.0	5.0	1.0	5.0	10
KGH354	EPEC	5.0	10	5.0	10	10	10	5.0	10	5.0	5.0	-	-	5.0	10
KGH 360	EPEC	-	-	10	10	20	20	5.0	10	-	-	-	-	5.0	10
KGH 372	EPEC	10	10	10	20	10	10	5.0	10	10	20	-	-	10	20
KGH 402	EPEC	10	10	-	-	10	20	5.0	10	5.0	10	10	10	-	-
KGH 406	EPEC	5.0	10	10	10	10	10	10	10	10	10	10	10	-	-

KGH 411	EPEC	10	10	10	20	10	10	10	10	5.0	10	10	20	-	-
KGH 418	EPEC	10	10	-	-	10	10	10	10	10	10	10	20	-	-
KGH 442	EPEC	5.0	10	-	-	-	-	5.0	10	5.0	10	10	20	10	20
EUS 452	EPEC	10	10	-	-	10	20	5.0	10	10	10	20	20	10	10
GHM 466	EPEC	10	10	-	-	10	10	5.0	10	20	20	10	20	10	20
EUS 003	ETEC	10	20	-	-	-	-	5.0	10	-	-	10	20	5.0	10
EUS 015	ETEC	10	10	-	-	10	10	10	10	5.0	5.0	20	20	5.0	10
EUS 074	ETEC	5.0	10	5.0	10	10	10	10	10	5.0	10	5.0	10	5.0	10
EUS 118	ETEC	10	10	-	-	10	10	5.0	10	5.0	10	10	10	5.0	10
EUS 133	ETEC	10	10	10	10	10	10	5.0	10	5.0	10	5.0	10	5.0	10
GHM 165	ETEC	10	10	10	10	10	10	5.0	10	5.0	10	10	20	10	10
GHM 181	ETEC	5.0	10	10	10	10	10	10	10	5.0	5.0	-	-	-	-
GHM 220	ETEC	5.0	10	5.0	10	10	20	5.0	10	5.0	10	-	-	-	-
GHM 224	ETEC	10	20	20	20	10	10	10	10	5.0	10	10	20	5.0	10
GHM 231	ETEC	-	-	-	-	10	20	5.0	10	-	-	10	10	5.0	10
KHG 312	ETEC	10	10	10	20	10	10	5.0	10	5.0	5.0	5.0	10	5.0	10

KGH 327	ETEC	10	20	-	-	10	20	10	10	5.0	10	20	20	10	10
KGH 365	ETEC	-	-	-	-	10	10	5.0	10	5.0	5.0	-	-	10	10
KGH 387	ETEC	10	20	10	10	10	10	5.0	10	5.0	10	5.0	10	5.0	5.0
KGH 394	ETEC	10	20	-	-	10	20	5.0	10	5.0	10	10	10	5.0	10
KGH 437	ETEC	10	10	-	-	-	-	5.0	10	-	-	10	10	20	20
KGH 418	ETEC	5.0	10	5.0	10	5.0	10	10	10	5 .0	5.0	5.0	10	5.0	10

Key: EAEC=Enteroaggregative *Escherchia coli*; EHEC=Enterohaemorrhagic *Escherichia coli*; EIEC=Enteroinvasive *Escherichia coli*; EPEC=Enteropathogenic *Escherichiacoli*; ETEC=Enterotoxigenic Escherichia coli, USGHB=Umaru Sanda General Hospital, Bida; GHM=General Hospital, Minna; KGH=Kontagora General Hospital; MIC=Minimum Inhibitory Concentration; MBC=Minimum Bactericidal Concentration; mg=Milligram; mL=milliliter; - = No inhibition.

APPENDIX: XI

Table 1: Case Profile of Diarrhoeal Patients Attending Selected Hospitals in Niger state, Nigeria.

S/N	Hospital Number	Gender	Age (Month)	Appearance of stool	Source of water	Area of Domicile	Occupation of parents		Educational status of parents	
							Mother	Father	Mother	Father
1	EUS001	Male	49-60	Watery	Well water	Rural	House wife	Farmer	No-formal	No-formal
2	EUS002	Female	25-36	Mucous	Bore hole	Urban	House wife	Civil servant	No-formal	Tertiary
3	EUS003	Female	0-12	Bloody	Stream	Urban	House wife	Civil servant	No-formal	Tertiary
4	EUS004	Female	0-12	Bloody	Stream	Rural	House wife	Civil servant	Primary	Secondary
5	EUS005	Male	37-48	Watery	Stream	Rural	House wife	Farmer	No-formal	No-formal
6	EUS006	Male	13-24	Mucus	Well water	Urban	House wife	Carpenter	No-formal	Primary
7	EUS007	Male	49-60	Bloody	Bore hole	Urban	Civil servant	Bricklayer	Secondary	Secondary
8	EUS008	Male	25-36	Mucus	Bore hole	Urban	Civil servant	Painter	Secondary	Secondary
9	EUS009	Female	0-12	Watery	Well-water	Rural	House wife	Painter	Secondary	Secondary
10	EUS010	Female	13-24	Bloody	Stream	Rural	House wife	Painter	Secondary	Secondary
11	EUS012	Male	25-36	Watery	River	Rural	House wife	Farmer	Primary	No-formal

12	EUS013	Female	49-60	Bloody	Well-water	Rural	House wife	Bricklayer	No-formal	Secondary
13	EUS014	Male	13-24	Watery	Well-water	Rural	House wife	Painter	No-formal	Primary
14	EUS015	Male	37-48	Bloody	Bore-hole	Urban	Civil servant	Civil servant	Secondary	Tertiary
15	EUS016	Female	0-12	Watery	Stream	Rural	House wife	Bricklayer	No-formal	No-formal
16	EUS017	Female	25-36	Mucus	Bore-hole	Urban	House wife	Carpenter	Primary	Secondary
17	EUS018	Male	37-48	Mucus	Stream	Rural	House wife	Farmer	No-formal	No-formal
18	EUS019	Male	49-60	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
19	EUS020	Male	13-24	Bloody	Bore-hole	Rural	House wife	Farmer	No-formal	No-formal
20	EUS021	Male	0-12	Watery	Well-water	Rural	House wife	Carpenter	No-formal	No-formal
23	EUS023	Female	0-12	Bloody	Well	Rural	House wife	Farmer	No-formal	No-formal
24	EUS024	Male	49-60	Mucus	Borehole	Rural	Civil servant	Civil servant	Tertiary	Tertiary
25	EUS025	Female	13-36	Mucus	Well	Rural	House wife	Carpenter	No-formal	Primary
26	EUS026	Male	0-12	Water	Well	Rural	House wife	Brick Layer	No-formal	Secondary
27	EUS027	Male	0-12	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
28	EUS028	Male	37-48	Water	Stream	Rural	House wife	Farmer	No-formal	No-formal
29	EUS029	Female	25-35	Bloody	Stream	Rural	House wife	Farmer	No-formal	Primary

30	EUS030	Male	49-60	Bloody	Stream	Rural	House wife	Carpenter	No-formal	Secondary
31	EUS031	Male	13-24	Bloody	Well	Rural	House wife	Painter	Primary	Secondary
32	EUS032	Male	13-24	Mucus	Well	Rural	House Wife	Farmer	No-formal	No-formal
34	EUS034	Male	0-12	Water	Stream	Rural	Civil servant	Brick layer	Secondary	Secondary
35	EUS035	Male	13-24	Water	Stream	Rural	House wife	Farmer	No-formal	No-formal
36	EUS036	Female	37-48	Bloody	Well	Rural	House wife	Farmer	No-formal	No-formal
37	EUS037	Male	49-60	Water	Borehole	Rural	Civil servant	Driver	Secondary	Secondary
38	EUS038	Male	13-24	Water	Well	Rural	House wife	Farmer	No-formal	No-formal
39	EUS039	Male	13-24	Bloody	Well	Rural	House wife	Farmer	No-formal	No-formal
40	EUS040	Male	13-24	Mucus	Well	Rural	House wife	Farmer	No-formal	No-formal
41	EUS041	Male	0-12	Water	Stream	Rural	House wife	Farmer	No-formal	Primary
42	EUS032	Female	37-48	Bloody	Stream	Rural	Civil servant	Civil servant	Secondary	Tertiary
43	EUS043	Male	25-36	Water	River	Rural	House wife	Painter	No-formal	No-formal
44	EUS044	Male	13-24	Bloody	Well	Urban	House wife	Brick layer	Primary	Secondary
45	EUS045	Female	0-12	Water	Well	Rural	House wife	Farmer	No-formal	No-formal

46	EUS046	Female	0-12	Water	Borehole	Urban	Civil servant	Civil servant	Secondary	Tertiary
47	EUS047	Male	25-36	Bloody	Well water	Rural	House wife	Farmer	No-formal	No-formal
48	EUS048	Male	13-24	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
49	EUS049	Male	0-12	Water	Stream	Rural	House wife	Wielder	No-formal	Secondary
50	EUS050	Female	25-36	Water	Well	Rural	House wife	Carpenter	No-formal	No-formal
51	EUS051	Female	37-48	Blood	Borehole	Rural	House wife	Driver	No-formal	Secondary
52	EUS052	Female	49-60	Blood	Borehole	Urban	Civil servant	Civil servant	Secondary	Tertiary
53	EUS053	Female	37-48	Mucus	Well	Rural	House wife	Farmer	No-formal	No-formal
54	EUS054	Male	25-36	Mucus	Well	Rural	House wife	Farmer	No-formal	No-formal
55	EUS055	Male	13-24	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
56	EUS056	Male	0-12	Water	Stream	Rural	House wife	Farmer	Primary	No-formal
57	EUS057	Male	49-60	Bloody	Stream	Rural	House wife	Farmer	No-formal	Primary
58	EUS058	Male	37-48	Mucus	Well	Rural	House wife	Carpenter	No-formal	Primary
59	EUS059	Female	25-36	Mucus	Well	Rural	Civil servant	Civil servant	Secondary	Tertiary
60	EUS060	Female	25-36	Bloody	Stream	Rural	House wife	Civil servant	No-formal	Secondary
61	EUS061	Female	13-24	Watery	Stream	Rural	House wife	Farmer	No-formal	No-formal

62	EUS062	Male	0-12	Watery	Stream	Rural	House wife	Farmer	No-formal	Primary
63	EUS063	Male	0-12	Bloody	Well	Rural	Civil servant	Brick layer	No-formal	No-formal
64	EUS064	Male	0-12	Bloody	Well	Rural	House wife	Farmer	No-formal	Primary
65	EUS065	Female	13-24	Watery	Stream	Rural	House wife	Farmer	No-formal	Primary
66	EUS066	Female	1.3-24	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
67	EUS067	Female	25-36	Mucus	Stream	Rural	House wife	Carpenter	No-formal	Primary
68	EUS068	Female	25-36	Mucus	Stream	Rural	House wife	Farmer	No-formal	No-formal
69	EUS069	Male	39-48	Bloody	Well	Urban	Civil servant	Brick Layer	Secondary	Secondary
70	EUS070	Female	25-36	Watery	Well	Rural	House wife	Farmer	Secondary	Primary
71	EU5071	Male	13-24	Watery	Well	Rural	House wife	Farmer	No-formal	Primary
72	EU5072	Female	0-12	Bloody	Well	Rural	Civil servant	Civil servant	Secondary	Tertiary
73	EU5073	Female	0-12	Watery	Well-water	Rural	House wife	Farmer	No-formal	No-formal
74	EU5074	Female	13-24	Bloody	Stream	Rural	House wife	Carpenter	No-formal	No-formal
75	EU5075	Female	25-36	Mucus	Well water	Rural	House wife	Driver	Secondary	Secondary
76	EU5076	Male	25-36	Mucus	Well-water	Rural	House wife	Farmer	No-formal	No-formal
77	EU5077	Female	49-60	Mucus	Well-water	Urban	House wife	Mechanic	No-formal	Secondary

78	EUS078	Male	49-60	Watery	Bore hole	Urban	Civil servant	Brick layer	Secondary	Secondary
79	EU5079	Female	89-49	Watery	Bore hole	Urban	House wife	Brick Layer	Primary	Primary
80	EU5080	Male	25-36	Watery	Bore hole	Rural	Civil servant	Brick Layer	Tertiary	Tertiary
81	EU5081	Male	13-24	Bloody	Bore hole	Rural	House wife	Farmer	Primary	Secondary
82	EU5082	Male	0-12	Watery	Well-water	Rural	Civil servant	Driver	Secondary	Secondary
83	EU5083	Female	0-12	Mucus	Stream	Rural	Civil servant	Farmer	No-formal	No-formal
84	EU5084	Female	13-24	Mucus	Well	Urban	Civil servant	Civil Servant	Secondary	Tertiary
85	EUS085	Female	25-36	Bloody	Borehole	Urban	House wife	Mechanic	No-formal	Primary
86	EUS086	Female	13-24	Mucus	Well-water	Rural	House wife	Farmer	No-formal	No-formal
87	EUS087	Male	0-12	Watery	Bore-hole	Rural	House wife	Farmer	Primary	Primary
88	EUS088	Male	13-24	Watery	Borehole	Rural	Civil servant	Mechanic	Secondary	Secondary
89	EUS089	Male	25-36	Bloody	Well water	Urban	Civil servant	Brick Layer	Primary	Secondary
90	EUS090	Male	13-24	Mucous	Well water	Rural	House wife	Carpenter	Secondary	Tertiary
91	EUS091	Male	13—24	Watery	Well water	Rural	House wife	Painter	No-formal	Primary
92	EUS092	Female	0-12	Mucus	Stream	Rural	House wife	Farmer	No-formal	No-formal
93	EUS093	Male	37-48	Bloody	Stream	Rural	House wife	Farmer	No-formal	Primary

94	EUS094	Female	40-60	Bloody	Borehole	Urban	Civil servant	Civil servant	Secondary	Tertiary
95	EUS095	Female	49-60	Mucus	Well water	Urban	Civil servant	Civil servant	Secondary	Tertiary
96	EUS096	Male	37-48	Watery	Stream	Rural	House wife	Farmer	No-formal	Primary
97	EUS097	Female	49-60	Watery	Well water	Urban	House wife	Civil servant	No-formal	Secondary
98	EUS098	Male	25-36	Bloody	Well water	Urban	House wife	Civil servant	Primary	Tertiary
99	EUS099	Male	0-12	Watery	River	Rural	House wife	Farmer	No-formal	No-formal
100	EUS100	Male	13-24	Water	Well water	Rural	House wife	Farmer	No-formal	No-formal
101	EUS101	Male	37-48	Bloody	Bore hole	Urban	Civil servant	Civil servant	Tertiary	Tertiary
102	EUS102	Male	0-12	Watery	Well water	Urban	Civil servant	Civil servant	Secondary	Tertiary
103	EUS103	Female	37-48	Watery	Stream	Rural	House wife	Brick layer	No-formal	Secondary
104	EUS104	Female	13-24	Mucous	Stream	Rural	House wife	Painter	No-formal	Primary
105	EUS105	Male	49-60	Water	Well water	Rural	House wife	Carpenter	Primary	Secondary
106	EUS106	Male	25-36	Watery	Borehole	Rural	House wife	Farmer	No-formal	Primary
107	EUS107	Female	25-36	Watery	Borehole	Urban	House wife	Mechanic	No-formal	Primary
108	EUS108	Male	37-48	Mucus	Well water	Urban	Civil servant	Civil servant	Tartary	Tertiary
109	EUS109	Female	49-60	Watery	Well water	Rural	House wife	Farmer	No-formal	No-formal

110	EUS110	Male	13-24	Watery	Well water	Rural	House wife	Farmer	No-formal	No-formal
111	EUS111	Female	13-24	Watery	Stream	Rural	House wife	Farmer	Primary	Secondary
112	EUS112	Female	0-12	Bloody	Stream	Rural	House wife	Carpenter	No-formal	Primary
113	EUS113	Male	0-12	Bloody	Well water	Urban	House wife	Brick layer	No-formal	Secondary
114	EUS114	Male	25-36	Watery	Well water	Rural	House wife	Farmer	No-formal	No-formal
115	EUS115	Female	13-24	Bloody	Stream	Urban	House wife	Carpenter	No-formal	Secondary
116	EUS116	Female	49-60	Bloody	River	Rural	House wife	Farmer	No-formal	No-formal
117	EUS117	Male	37-48	Watery	Stream	Urban	House wife	Carpenter	No-formal	Secondary
118	EUS118	Female	13-24	Mucus	Well water	Urban	House wife	Farmer	Secondary	No-formal
119	EUS119	Female	0-2	Watery	Well water	Rural	House wife	Painter	No-formal	No-formal
120	EUS120	Male	13-24	Watery	Stream	Urban	House wife	Carpenter	Secondary	Primary
121	EUS121	Male	0-12	Watery	Stream	Rural	House wife	Farmer	No-formal	Secondary
122	EUS122	Female	13-24	Bloody	Stream	Rural	House wife	Civil servant	No-formal	Tertiary
123	EUS123	Male	29-60	Bloody	Borehole	Urban	Civil servant	Civil servant	Tertiary	Tertiary
124	EUS124	Male	25-36	Watery	Well water	Urban	House wife	Civil servant	No-formal	Tertiary
125	EUS125	Female	37-48	Watery	Well water	Rural	House wife	Farmer	No-formal	No-formal

126	EUS126	Male	13-24	Watery	Stream	Urban	House wife	Farmer	No-formal	No-formal
127	EUS127	Female	13-24	Watery	Well-water	Rural	House wife	Farmer	No-formal	No-formal
128	EUS128	Male	0-12	Watery	Stream	Rural	House wife	Driver	No-formal	Primary
129	EUS129	Female	0-12	Watery	Stream	Urban	House wife	Carpenter	No-formal	Secondary
130	EUS130	Male	13-24	Bloody	Well-water	Urban	Civil servant	Civil servant	Secondary	Primary
131	EUS131	Female	25-36	Watery	Well water	Rural	House wife	Farmer	No-formal	Primary
132	EUS132	Female	13-24	Watery	Well water	Rural	House wife	Farmer	No-formal	Primary
133	EUS133	Female	13-24	Watery	Well water	Urban	House wife	Brick Layer	No-formal	Secondary
134	EUS134	Female	0-12	Watery	Bore hole	Rural	House wife	Painter	No-formal	No-formal
135	EUS135	Male	49- 60	Mucus	Bore hole	Rural	House wife	Farmer	No-formal	Primary
136	EUS136	Female	37- 48	Watery	Well water	Rural	House wife	Carpenter	No-formal	Secondary
137	EUS137	Female	0-12	Watery	River	Rural	Civil servant	Driver	Secondary	Secondary
138	EUS138	Female	13-24	Watery	Stream	Rural	House wife	Farmer	No-formal	No-formal
139	EUS139	Female	25-36	Mucus	Well water	Rural	House wife	Farmer	No-formal	No-formal
140	EUS140	Female	49- 60	Watery	Bore hole	Urban	Civil servant	Civil servant	Tertiary	Tertiary
141	EUS141	Female	37-48	Watery	Bore hole	Urban	House wife	Mechanic	No-formal	Primary

142	EUS142	Female	13- 24	Bloody	Well water	Rural	House wife	Farmer	No-formal	No-formal
143	EUS143	Female	13- 24	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
144	EUS144	Male	13- 24	Watery	Stream	Urban	House wife	Brick layer	Primary	Primary
145	EUS145	Male	0-12	Watery	Well water	Rural	House wife	Farmer	No-formal	No-formal
146	EUS146	Male	0-12	Watery	Bore hole	Urban	Civil servant	Civil servant	Secondary	Secondary
147	EUS147	Female	13- 24	Bloody	Bore hole	Rural	House wife	Painter	No-formal	Secondary
148	EUS148	Female	13-24	Mucus	Well-water	Urban	House wife	Brick layer	No-formal	No-formal
149	EUS149	Female	0-12	Watery	Stream	Rural	House wife	Painter	No-formal	Secondary
150	EUS150	Male	13-24	Mucus	Stream	Rural	House wife	Farmer	No-formal	No-formal
151	GHM151	Female	0-12	Bloody	Well	Rural	House wife	Farmer	No-formal	Primary
152	GHM152	Female	13-24	Watery	Well	Urban	Civil servant	Civil servant	Secondary	Tertiary
153	GHM153	Female	0-12	Watery	Well	Urban	House wife	Carpenter	No-formal	Secondary
154	GHM154	Male	13-24	Mucus	Well	Urban	Civil servant	Civil servant	Primary	Tertiary
155	GHM155	Female	25-36	Bloody	Stream	Rural	House wife	Famer	No-formal	No-formal
156	GHM156	Female	37-48	Bloody	Stream	Rural	Civil servant	Civil servant	Secondary	Tertiary
157	GHM157	Male	37 -48	Mucus	Well	Rural	House wife	House wife	No-formal	Primary

158	GHM158	Female	25-36	Mucus	Stream	Urban	House wife	Civil servant	No-formal	Tertiary
159	GHM159	Female	13-24	Watery	Bore-hole	Rural	House wife	Painter	Primary	Secondary
160	GHM160	Female	49-60	Watery	Bore-hole	Urban	Civil servant	Driver	Secondary	Secondary
161	GHM161	Female	37-48	Bloody	Bore-hole	Rural	House wife	Farmer	No-formal	No-formal
162	GHM162	Female	49-60	Bloody	Bore-hole	Urban	House wife	Painter	No-formal	Primary
163	GHM163	Female	25-36	Watery	Bore-hole	Rural	Civil servant	Mechanic	Secondary	Secondary
164	GHM164	Male	13-24	Mucus	Stream	Rural	House wife	Farmer	No-formal	No-formal
165	GHM165	Female	13-24	Bloody	Stream	Urban	House wife	Mechanic	No-formal	Secondary
166	GHM166	Male	13-24	Bloody	Well	Urban	House wife	Painter	No-formal	No-formal
167	GHM167	Female	13-24	Watery	Well	Rural	House wife	Farmer	No-formal	Secondary
168	GHM168	Female	0-12	Bloody	Well	Urban	Civil servant	Driver	Secondary	Secondary
169	GHM169	Female	25 -36	Mucus	River	Rural	House wife	Driver	No-formal	Secondary
170	GHM170	Male	13-24	Mucus	River	Unban	House wife	Carpenter	Primary	Secondary
171	GHM171	Male	37-48	Bloody	Well	Rural	House wife	Farmer	No-formal	No-formal
172	GHM172	Female	49-60	Watery	Borehole	Urban	Civil-servant	Brick Layer	Secondary	Secondary
173	GHM173	Female	0-12	Mucus	Well	Rural	House wife	Farmer	No-formal	Primary

174	GHM174	Male	0-12	Bloody	Well	Rural	House wife	Carpenter	Primary	Primary
175	GHM175	Female	0-12	Watery	Stream	Urban	House wife	Carpenter	No-formal	No-formal
176	GHM176	Female	0-12	Bloody	Stream	Rural	Civil servant	Civil servant	Secondary	Secondary
177	GHM177	Female	13-24	Mucus	Borehole	Rural	Civil servant	Driver	Primary	Secondary
178	GHM178	Female	0-12	Watery	Borehole	Urban	Civil servant	Mechanic	Secondary	Secondary
179	GHM179	Male	13-24	Watery	Well	Urban	House wife	Painter	No-formal	No-formal
180	GHM180	Male	25-36	Bloody	Well	Rural	House wife	Farmer	No-formal	Primary
181	GHM181	Male	25-36	Bloody	Borehole	Rural	House wife	Mechanic	Primary	Secondary
182	GHM182	Male	13-24	Bloody	Stream	Rural	House wife	Farmer	Primary	Primary
183	GHM183	Male	13-24	Watery	Stream	Urban	House wife	Carpenter	No-formal	Primary
184	GHM184	Male	40-160	Bloody	Well	Urban	Civil servant	Brick layer	Secondary	Secondary
185	GHM185	Female	49-60	Bloody	Well	Rural	House wife	Farmer	No-formal	No-formal
186	GHM186	Female	37-48	Mucus	Borehole	Rural	House wife	Driver	No-formal	Secondary
187	GHM187	Female	25-36	Watery	Well	Rural	House wife	Famer	Primary	No-formal
188	GHM188	Male	49-60	Bloody	Stream	Rural	House wife	Brick layer	No-formal	Secondary
189	GHM189	Male	13-24	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal

190	GHM190	Male	13 -24	Watery	Wall	Rural	House wife	Farmer	No-formal	No-formal
191	GHM191	Female	49 -60	Mucus	Borehole	Urban	Civil servant	Bricklayer	No-formal	Primary
192	GHM192	Male	37-48	Blood y	Well	Urban	Civil servant	Civil servant	Secondary	Secondary
193	GHM193	Female	25 -36	Watery	Well	Rural	House wife	Carpenter	No-formal	Primary
194	GHM194	Female	13-24	Watery	Well	Rural	House wife	Farmer	No-formal	No-formal
195	GHM195	Male	0-12	Watery	Borehole	Urban	House wife	Brick layer	No-formal	No-formal
196	GHM196	Male	0-12	Bloody	Borehole	Rural	House wife	Civil servant	No-formal	Tertiary
197	GHM197	Male	13-24	Bloody	Stream	Rural	House wife	Farmer	No-formal	Primary
198	GHM198	Female	0-12	Mucus	Stream	Rural	House wife	Farmer	No-formal	No-formal
199	GHM199	Female	25-36	Watery	Well	Rural	House wife	Farmer	No-formal	No-formal
200	GHM200	Mle	13-24	Watery	Well	Rural	House wife	Farmer	No-formal	No-formal
201	GHM201	Female	36-48	Bloody	Borehole	Urban	Civil servant	Driver	Secondary	Secondary
202	GHM202	Male	25-36	Mucus	Well	Urban	House wife	Driver	No-formal	No-formal
203	GHM203	Male	13-24	Mucus	Well	Urban	House wife	Mechanic	No-formal	Primary
204	GHM204	Female	13-24	Watery	Borehole	Urban	House wife	Printer	No-formal	No-formal
205	GHM205	Female	10-12	Bloody	Borehole	Rural	House wife	Farmer	No-formal	Secondary

206	GHM206	Female	0-12	Watery	Well	Rural	Civil servant	Civil servant	Secondary	Tertiary
207	GHM207	Female	0-12	Well	Well	Rural	House wife	Farmer	No-formal	Primary
208	GHM208	Male	49-60	Stream	Stream	Rural	House wife	Driver	No-formal	Primary
209	GHM209	Female	37-48	Bloody	Stream	Rural	House wife	Farmer	Primary	No-formal
210	GHM210	Male	25-36	Mucus	Well	Urban	Civil servant	Civil servant	Secondary	Secondary
211	CHM211	Male	13-24	Watery	Stream	Urban	House wife	Father	Primary	Secondary
212	GHM212	Female	0-12	Watery	Bore hole	Urban	Civil servant	Civil servant	Secondary	Tertiary
213	GHM21 3	Male	0-12	Watery	Well	Urban	Civil servant	Civil servant	Secondary	Tertiary
214	GHM214	Male	13-24	Watery	Stream	Rural	House wife	Farmer	No-formal	No-formal
215	GHM215		0-12	Watery	Well	Rural	House wife	Driver	No-formal	Primary
216	GHM216	Female	0-12	Watery	Stream	Rural	House wife	Carpenter	No-formal	No-formal
217	GHM217	Male	13-24	Bloody	Well	Urban	Civil servant	Civil servant	Secondary	Tertiary
218	GHM218	Male	13-12	Watery	Well	Rural	House wife	Mechanics	Primary	Primary
219	GHM219	Male	13-24	Bloody	Well	Urban	Civil servant	Civil servant	Secondary	Secondary
220	GHM220	Female	0-12	Bloody	Stream	Rural	House wife	Printers	No-formal	Primary
221	GHM221	Male	0-12	Watery	Well	Urban	House wife	Brick layer	No-formal	Secondary

222	GHM222	Male	13-24	Watery	Bore hole	Urban	House wife	Printer	Primary	Primary
223	GHM223	Male	13-24	Bloody	Bore hole	Rural	Civil servant	Civil servant	Secondary	Tertiary
224	GHM224	Female	25-36	Bloody	Well	Rural	House wife	Brick layer	No-formal	Secondary
225	GHM225	Female	25-36	Bloody	Stream	Rural	House wife	Famer	Primary	Primary
226	GHM226	Male	13-24	Watery	Bore hole	Rural	House wife	Farmer	No-formal	No-formal
227	GHM227	Male	0-12	Watery	Well	Urban	Civil servant	Mechanics	Secondary	Secondary
228	GHM228	Female	0-12	Watery	Bore hole	Rural	House wife	Farmer	No-formal	No-formal
229	GHM229	Female	0-12	Watery	Bore hole	Urban	House wife	Farmer	No-formal	No-formal
230	GHM230	Male	13-24	Bloody	Stream	Rural	House wife	Farmer	Primary	No-formal
231	GHM231	Female	13-24	Watery	Well	Rural	Civil servant	Driver	Secondary	Secondary
232	GHM232	Female	13-24	Watery	Bore-hole	Rural	House wife	Farmer	No-formal	No-formal
233	GHM233	Female	13-24	Mucus	Bore-hole	Rural	House wife	Farmer	No-formal	No-formal
234	GHM234	Male	0-12	Mucus	Well	Rural	House wife	Carpenter	No-formal	No-formal
235	GHM235	Male	0-12	Mucus	Bore-hole	Rural	Civil servant	Civil servant	Tertiary	Tertiary
236	GHM236	Female	13-24	Watery	River	Rural	House wife	Civil servant	No-formal	Secondary
237	GHM237	Male	25-36	Bloody	Well	Urban	House wife	Farmer	No-formal	No-formal

238	GHM238	Female	37-48	Bloody	Well	Rural	House wife	Civil servant	Primary	Tertiary
239	GHM239	Female	37-48	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
240	GHM240	Male	48-60	Bloody	Stream	Rural	House wife	Driver	Primary	Secondary
241	GHM241	Male	37-48	Mucus	Bore hole	Urban	House wife	Carpenter	No-formal	Secondary
242	GHM242	Male	49-60	Mucus	Well	Urban	Civil servant	Civil servant	Tertiary	Tertiary
243	GHM243	Female	49-60	Mucus	Well	Urban	Civil servant	Civil servant	Secondary	Tertiary
244	GHM244	Male	37-48	Bloody	Bore hole	Urban	Civil servant	Civil servant	Tertiary	Secondary
245	GHM245	Female	49-60	Watery	Stream	Rural	House wife	Painter	No-formal	No-formal
246	GHM246	Female	37-98	Watery	Well	Rural	House wife	Farmer	No-formal	Primary
247	GHM247	Male	25-36	Bloody	Stream	Rural	House wife	Carpenter	No-formal	Secondary
248	GHM248	Female	13-24	Bloody	Well	Rural	House wife	Mechanic	No-formal	No-formal
249	GHM249	Female	0-12	Watery	Well	Urban	House wife	Mechanic	No-formal	Secondary
250	GHM250	Female	13-24	Watery	Stream	Rural	House wife	Brick layer	No-formal	Primary
251	GHM251	Male	25-36	Watery	Stream	Rural	Civil servant	Civil servant	Secondary	Tertiary
252	GHM252	Female	13-24	Watery	Well	Rural	House wife	mechanic	No-formal	Primary
253	GHM253	Female	01-12	Bloody	Borehole	Urban	House wife	Civil servant	No-formal	Tertiary

255	GHM255	Male	13-24	Bloody	Borehole	Urban	House wife	Brick layer	No-formal	Secondary
256	GHM256	Female	13-24	Mucus	Borehole	Rural	Civil servant	Civil servant	Tertiary	Tertiary
257	GHM257	Male	13-24	Mucus	Well	Urban	House wife	Civil servant	No-formal	Tertiary
258	GHM258	Female	0-12	Watery	Stream	Rural	House wife	Farmer	No-formal	No-formal
259	GHM259	Female	13-24	Watery	Borehole	Rural	House wife	Farmer	No-formal	No-formal
260	GHM260	Male	25-36	Bloody	Well	Urban	House wife	Carpenter	No-formal	Primary
261	GHM261	Female	25-36	Bloody	Borehole	Urban	Civil servant	Civil servant	Tertiary	Tertiary
262	GHM262	Male	37-48	Bloody	Borehole	Urban	Civil servant	Civil servant	Secondary	Tertiary
263	GHM263	Female	37-48	Bloody	Well	Rural	House wife	Farmer	No-formal	No-formal
264	GHM264	Male	37-48	Mucus	Stream	Rural	House wife	Carpenter	No-formal	No-formal
265	GHM265	Female	18-24	Watery	Stream	Rural	House wife	Farmer	No-formal	No-formal
266	GHM266	Male	25-36	Watery	Well	Urban	Civil servant	Civil servant	Primary	Tertiary
267	GHM267	Female	13-24	Mucus	Borehole	Urban	Civil servant	Civil servant	Tertiary	Tertiary
268	GHM268	Female	13-24	Watery	Borehole	Rural	House wife	Farmer	No-formal	No-formal
269	GHM269	Female	0-12	Watery	Well	Rural	House wife	Painter	No-formal	Secondary
270	GHM270	Male	15-24	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal

271	GHM271	Male	49-60	Bloody	Borehole	Urban	Civil servant	Civil servant	Tertiary	Tertiary
272	GHM272	Female	37-48	Watery	Well water	Urban	House wife	Civil servant	No-formal	Tertiary
273	GHM273	Female	25-36	Bloody	Well water	Urban	House wife	Brick layer	No-formal	Tertiary
274	GHM274	Male	0-12	Watery	Well	Urban	House wife	Civil servant	No-formal	Secondary
275	GHM275	Female	0-12	Bloody	Borehole	Urban	House wife	Civil servant	No-formal	Tertiary
276	GHM276	Male	0-12	Mucus	Borehole	Urban	House wife	Driver	No-formal	Primary
277	GHM277	Male	13-24	Watery	Stream	Rural	House wife	Farmer	Primary	Primary
278	GHM278	Female	13-24	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
279	GHM279	Female	13-24	Mucus	Well	Rural	House wife	Farmer	No-formal	No-formal
280	GHM280	Female	0-12	Bloody	Well	Urban	Civil servant	Civil servant	Secondary	Tertiary
281	GHM281	Female	0-12	Watery	Borehole	Urban	House wife	Civil servant	No-formal	Tertiary
282	GHM282	Male	0-12	Watery	Well	Rural	House wife	Farmer	No-formal	No-formal
283	GHM283	Female	13-24	Mucus	Borehole	Urban	House wife	Brick layer	No-formal	Primary
284	GHM284	Male	13-24	Watery	Well	Urban	Civil servant	Civil servant	Tertiary	Tertiary
285	GHM285	Male	0-12	Bloody	Tap water	Urban	Civil servant	Civil servant	Tertiary	Tertiary
286	GHM286	Male	13-24	Bloody	Borehole	Urban	House wife	Farmer	Primary	Primary

287	GHM287	Male	13-24	Watery	Borehole	Rural	House wife	Farmer	No-formal	No-formal
288	GHM288	Male	0-12	Watery	Well	Rural	House wife	Farmer	No-formal	No-formal
289	GHM289	Male	0-12	Watery	Stream	Rural	House wife	Carpenter	No-formal	Secondary
290	GHM290	Male	0-12	Bloody	River	Rural	House wife	Farmer	No-formal	No-formal
291	GHM291	Male	0-12	Bloody	Well water	Rural	House wife	Farmer	No-formal	No-formal
292	GHM292	Male	13-24	Mucus	Borehole	Rural	Civil servant	Brick layer	Secondary	Secondary
293	GHM293	Male	13-24	Mucus	Tap water	Urban	House wife	Brick layer	No-formal	Secondary
294	GHM294	Female	0-12	Watery	Tap water	Urban	House wife	Carpenter	No-formal	Primary
295	GHM295	Female	0-12	Bloody	Bore hole	Urban	House wife	Civil servant	Primary	Tertiary
296	GHM296	Male	0-12	Watery	Well water	Rural	House wife	Farmer	No-formal	No-formal
297	GHM297	Female	13-24	Mucus	Well water	Rural	House wife	Farmer	No-formal	No-formal
298	GHM298	Female	13- 24	Bloody	Stream	Rural	House wife	Painter	No-formal	Primary
299	GHM299	Female	13 -24	Bloody	Well water	Rural	House wife	Farmer	No-formal	No-formal
300	GHM300	Female	0-12	Watery	Stream	Rural	House wife	Civil servant	No-formal	Secondary
301	RGH301	Female	0-12	Watery	Stream	Rural	House wife	Farmer	No-formal	No-formal
302	RGH302	Male	13- 24	Watery	Stream	Rural	House wife	Farmer	Primary	No-formal

303	KGH303	Male	13- 24	Bloody	Well water	Rural	House wife	Carpenter	No-formal	Primary
304	RGH304	Male	13- 24	Bloody	Tap water	Urban	Civil servant	Civil servant	Secondary	Tertiary
305	RGH305	Female	0.12	Mucus	Well water	Urban	House wife	Brick layer	Primary	No-formal
306	RGH306	Male	0-12	Mucus	Tape water	Urban	House wife	Civil servant	No-formal	Tertiary
307	RGH307	Male	0-12	Watery	Bore hole	Urban	House wife	Carpenter	No-formal	Secondary
308	RGH308	Male	0-12	Watery	Stream	Rural	House wife	Farmer	Primary	No-formal
309	RGH309	Male	13-24	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
310	RGH310	Female	25-36	Bloody	Well water	Rural	House wife	Farmer	Secondary	No-formal
311	RGH311	Female	13-24	Watery	Bore hole	Rural	House wife	Brick layer	No-formal	Primary
312	RGH312	Female	13-24	Watery	Tap water	Urban	Civil servant	Brick layer	Secondary	Secondary
313	RGH313	Male	25-36	Mucus	Stream	Rural	House wife	Driver	No-formal	Secondary
314	RGH314	Male	13-24	Bloody	Well water	Rural	House wife	Farmer	No-formal	No-formal
315	RGH315	Male	25-36	Mucus	Stream	Rural	House wife	Carpenter	No-formal	Primary
316	KGH316	Female	35-36	Watery	Stream	Rural	House wife	Carpenter	No-formal	Secondary
317	KGH317	Male	37-48	Watery	Well	Urban	House wife	Brick layer	No-formal	Primary
318	KHG318	Female	37-48	Bloody	Bore hole	Urban	Civil servant	Civil servant	Secondary	Tertiary

319	KHG318	Male	49-60	Mucus	Well	Rural	House wife	Famer	No-formal	No-formal
320	KGH320	Female	49-60	Mucus	Stream	Rural	Civil servant	Civil servant	Tertiary	Tertiary
321	KGH321	Male	37-48	Bloody	Stream	Rural	House wife	Famer	No-formal	No-formal
322	KGH322	Female	25-36	Watery	Well	Rural	House wife	Famer	Primary	Primary
323	KGH323	Female	13-24	Watery	Stream	Rural	House wife	Civil servant	No-formal	Secondary
324	KGH324	Male	0-12	Watery	Well	Rural	Civil servant	Civil servant	Secondary	Tertiary
325	KGH325	Female	0-12	Watery	Stream	Rural	House wife	Farmer	Primary	No-formal
326	KGH326	Female	13-24	Bloody	Well	Urban	Civil servant	Civil servant	Tertiary	Tertiary
327	KGH327	Male	37-48	Mucus	Well	Rural	House wife	Carpenter	No-formal	No-formal
328	KGH328	Male	37-48	Watery	Bore hole	Rural	Civil servant	Civil servant	Secondary	Tertiary
329	KGH329	Male	25-36	Bloody	Tap water	Urban	House wife	Driver	No-formal	Secondary
330	KGH330	Female	0-12	Mucus	Bore hole	Urban	Civil servant	Civil servant	Secondary	Tertiary
331	KGH331	Female	0-12	Mucus	Well	Urban	House wife	Civil servant	No-formal	Primary
332	KGH332	Female	0-12	Bloody	Stream	Rural	House wife	Farmer	Secondary	Tertiary
333	KGH333	Female	0-12	Watery	Stream	Urban	House wife	Civil servant	No-formal	No-formal
334	KGH334	Male	13-24	Watery	Well	Rural	Civil servant	Farmer	No-formal	Secondary

335	KGH335	Male	13-24	Bloody	Stream	Rural	House wife	Civil servant	No-formal	No-formal
336	KGH336	Male	13-24	Watery	Well	Rural	Civil servant	Mechanic	No-formal	Primary
337	KGH337	Female	13-24	Mucus	Well	Rural	House wife	Farmer	No-formal	No-formal
338	KGH338	Female	49-60	Mucus	Well	Rural	House wife	Farmer	No-formal	No-formal
339	KGH339	Female	37-48	Watery	Bore-hole	Urban	Civil servant	Civil servant	Tertiary	Tertiary
340	KGH340	Female	13-24	Watery	Bore-hole	Rural	House wife	Civil servant	No-formal	Tertiary
341	KGH341	Male	25-36	Watery	Tap water	Urban	House wife	Carpenter	No-formal	Secondary
342	KGH342	Male	37-48	Watery	Well	Rural	House wife	Farmer	Primary	No-formal
343	KGH343	Female	0-12	Bloody	Well	Rural	House wife	Farmer	Primary	Primary
344	KGH344	Male	13-24	Mucus	Stream	Rural	Civil servant	Farmer	Primary	No-formal
345	KGH345	Male	25-36	Mucus	Well	Urban	House wife	Farmer	No-formal	No-formal
346	KGH346	Male	37-48	Bloody	Well	Urban	House wife	Brick layer	No-formal	Secondary
347	KGH347	Male	49-60	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
348	KGH348	Male	0-12	Watery	Bore-hole	Urban	Civil servant	Civil servant	Tertiary	Tertiary
349	KGH349	Female	3-24	Bloody	Bore-hole	Rural	House wife	Carpenter	No-formal	Secondary
350	KGH350	Male	25-36	Mucus	Well	Rural	House wife	Farmer	No-formal	No-formal

351	KGH351	Male	37-48	Watery	Well	Urban	House wife	Mechanic	No-formal	Primary
352	KGH352	Male	49-60	Bloody	Bore-hole	Rural	House wife	Driver	No-formal	Secondary
353	KGH353	Female	37-48	Mucus	Stream	Rural	House wife	Farmer	No-formal	Secondary
354	KGH354	Male	25-36	Bloody	Stream	Rural	House wife	Painter	No-formal	Secondary
355	KGH355	Male	37-48	Watery	Bore-hole	Urban	Civil servant	Driver	Secondary	Secondary
356	KGH356	Female	13-24	Watery	Bore-hole	Urban	House wife	Brick layer	No-formal	Secondary
357	KGH357	Female	0-12	Watery	Bore-hole	Urban	House wife	Civil servant	No-formal	Tertiary
358	KGH358	Male	0-12	Water	Well	Rural	House wife	Farmer	No-formal	No-formal
359	KGH359	Female	13-24	Blood	Well	Urban	House wife	Civil servant	No-formal	Tertiary
360	KGH360	Male	13-24	Blood	Well	Urban	House wife	Farmer	No-formal	No-formal
361	KGH361	Male	25-36	Water	Stream	Rural	Civil servant	Civil servant	Secondary	Tertiary
362	KGH362	Male	13-24	Blood	Stream	Urban	Civil servant	Civil servant	Primary	Tertiary
363	KGH 363	Male	13-24	Mucus	Borehole	Rural	House wife	Civil servant	Secondary	Tertiary
364	KGH364	Female	0-2	Water	Stream	Rural	House wife	Carpenter	No-formal	Primary
365	KGH365	Female	0-12	Water	Stream	Rural	House wife	Farmer	No-formal	No-formal
366	KGH366	Female	13-24	Mucus	Borehole	Urban	Civil servant	Civil servant	Tertiary	Tertiary

367	KGH367	Male	49-60	Blood	Well	Urban	House wife	Civil servant	Primary	Tertiary
368	KGH368	Male	37-48	Blood	Stream	Rural	House wife	Farmer	No-formal	No-formal
369	KGH369	Male	25-36	Water	Stream	Rural	House wife	Farmer	No-formal	Primary
370	KGH370	Female	13-24	Water	Well	Rural	House wife	Driver	No-formal	Secondary
371	KGH371	Male	13-24	Blood	Borehole	Rural	Civil servant	Civil servant	Tertiary	Secondary
372	KGH372	Female	0-12	Mucus	Borehole	Rural	Civil servant	Civil servant	Secondary	Tertiary
373	KGH373	Female	0-12	Blood	Borehole	Urban	House wife	Mechanic	No-formal	Secondary
374	KGH374	Male	0-12	Water	Well	Rural	House wife	Farmer	No-formal	No-formal
375	KGH375	Male	0-12	Water	Bore hole	Urban	House wife	Civil servant	No-formal	Tertiary
376	KGH376	Female	0-12	Mucus	Stream	Rural	House wife	Farmer	No-formal	No-formal
377	KGH377	Female	13-24	Blood	Well	Rural	House wife	Painter	No-formal	Primary
378	KGH378	Male	25-36	Mucus	Stream	Rural	Civil servant	Civil servant	Tertiary	Tertiary
379	KGH370	Female	37-48	Bloody	Well	Rural	House wife	Farmer	No-formal	No-formal
380	KGH380	Male	49-60	Watery	Borehole	Rural	House wife	Civil servant	No-formal	Tertiary
381	KGH381	Male	13-24	Bloody	Stream	Rural	House wife	Farmer	No-formal	No-formal
382	KGH382	Female	13-24	Mucus	Stream	Rural	House wife	Farmer	No-formal	Secondary

383	KGH383	Male	0-12	Watery	Well	Rural	House wife	Civil servant	No-formal	No-formal
384	KGH384	Female	0-12	Watery	Well	Rural	House wife	Driver	No-formal	No-formal
385	KGH385	Male	0-24	Watery	Borehole	Rural	House wife	Farmer	No-formal	Secondary
386	KGH386	Female	0-12	Watery	Borehole	Rural	House wife	Farmer	No-formal	No-formal
387	KGH387	Male	13-24	Watery	Well	Urban	House wife	Farmer	No-formal	No-formal
388	KGH388	Female	13-24	Bloody	Well	Rural	House wife	Farmer	No-formal	Secondary
389	KGH389	Female	13-24	Mucus	Stream	Rural	House wife	Civil servant	No-formal	No-formal
390	KGH390	Female	25-36	Mucus	Well	Urban	Civil servant	Civil servant	Secondary	Tertiary
391	KGH391	Male	13-24	Bloody	Tap water	Urban	Civil servant	Civil servant	Tertiary	Tertiary
392	KGH392	Female	13-24	Watery	Bore hole	Rural	House wife	Carpenter	No-formal	No-formal
393	KGH393	Male	13-24	Bloody	Bore hole	Urban	House wife	Carpenter	No-formal	Primary
394	KGH394	Female	13-24	Watery	Tap water	Urban	House wife	Bricklayer	No-formal	Secondary
395	KGH395	Female	13-24	Watery	Borehole	Urban	House wife	Bricklayer	No-formal	No-formal
396	KGH396	Female	0-12	Bloody	Tap water	Urban	House wife	Civil servant	No-formal	Tertiary
397	KGH397	Female	0-12	Watery	Borehole	Urban	Civil servant	Civil servant	Tertiary	Tertiary
398	KGH398	Male	0-12	Watery	River	Rural	House wife	Farmer	No-formal	No-formal

399	KGH399	Female	37-48	Bloody	Well	Rural	House wife	Carpenter	No-formal	Primary
400	KGH400	Female	25-36	Mucus	Well water	Urban	Civil servant	Civil servant	Secondary	Tertiary
401	KGH401	Female	37-48	Watery	Well water	Rural	House wife	Painter	No-formal	Secondary
402	KGH402	Female	49-60	Mucus	Stream	Rural	House wife	Farmer	No-formal	No-formal
403	KGH403	Male	49-60	Bloody	Stream	Rural	House wife	Mechanic	Primary	Secondary
404	KGH404	Female	37-48	Watery	Stream	Rural	Civil servant	Brick layer	Secondary	Secondary
405	KGH405	Female	25-36	Bloody	Well water	Rural	House wife	Farmer	No-formal	No-formal
406	KGH406	Female	13-24	Mucus	Stream	Rural	House wife	Farmer	No-formal	No-formal
407	KGH407	Female	0-12	Watery	Stream	Rural	House wife	farmer	Secondary	Secondary
408	KGH408	Male	0-12	Watery	Stream	Rural	House wife	Farmer	No-formal	No-formal
409	KGH409	Male	13-24	Watery	Stream	Rural	House wife	Carpenter	No-formal	Primary
410	KGH410	Female	25-36	Mucus	Stream	Rural	House wife	Farmer	Primary	No-formal
411	KGH411	Male	13-24	Mucus	Well	Urban	House wife	Brick layer	No-formal	Primary
412	KGH412	Male	13-24	Mucus	Stream	Rural	House wife	farmer	Primary	No-formal
413	KGH413	Female	0-12	Watery	Well	Rural	House wife	farmer	Secondary	Secondary
414	KGH414	Male	13-24	Bloody	Well	Rural	House wife	farmer	No-formal	No-formal

416	KGH416	Male	13-24	Watery	Bore hole	Rural	Civil servant	Civil servant	Tertiary	Tertiary
417	KGH417	Male	13-24	Mucus	Tap water	Urban	House wife	Brick layer	No-formal	Secondary
418	KGH418	Male	0-12	Mucus	Bore hole	Urban	Civil servant	Civil servant	Secondary	Secondary
419	KGH419	Male	13-24	Bloody	Stream	Rural	Carpenter	Carpenter	No-formal	Primary
420	KGH420	Male	25-36	Mucus	Well	Urban	Civil servant	Civil servant	Tertiary	Tertiary
421	KGH421	Female	37-48	Watery	Well	Rural	House wife	Farmer	No-formal	No-formal
422	KGH422	Female	49-60	Watery	Stream	Rural	House wife	Farmer	No-formal	Primary
423	KGH423	Male	37-48	Bloody	Well	Urban	House wife	Carpenter	No-formal	Secondary
424	KGH424	Male	25-36	Bloody	Well	Urban	House wife	Brick layer	No-formal	Primary
425	KGH425	Male	13-24	Mucus	Borehole	Urban	Civil servant	Civil servant	Secondary	Tertiary
426	KGH426	Male	13-24	Mucus	Well	Rural	House wife	Farmer	Primary	No-formal
427	KGH427	Female	0-12	Bloody	Stream	Rural	House wife	Farmer	Tertiary	Primary
428	KGH428	Female	13-24	Watery	Well	Urban	Civil servant	Civil servant	No-formal	Tertiary
429	KGH429	Male	13-24	Watery	Well	Rural	House wife	Farmer	Primary	Primary
430	KGH430	Male	0-12	Bloody	Stream	Rural	House wife	Civil servant	Secondary	Tertiary
431	KGH431	Male	25-36	Watery	Well	Rural	Civil servant	Driver	No-formal	Secondary

432	KGH432	Male	13-24	Watery	Stream	Urban	House wife	Farmer	No-formal	No-formal
433	KGH433	Male	13-24	Mucus	Borehole	Urban	House wife	Civil servant	Secondary	Tertiary
434	KGH434	Male	0-12	Bloody	Borehole	Rural	Civil servant	Civil servant	No-formal	Secondary
435	KGH435	Female	0-12	Watery	Stream	Rural	House wife	Farmer	Secondary	No-formal
436	KGH436	Female	0-12	Watery	Borehole	Urban	Civil servant	Mechanic	No-formal	Secondary
437	KGH437	Female	13-24	Watery	Tap water	Urban	House wife	Painter	No-formal	No-formal
438	KGH438	Female	13-24	Mucus	Borehole	Rural	House wife	Driver	No-formal	Primary
439	KGH439	Female	13-24	Mucus	Borehole	Rural	House wife	Farmer	No-formal	No-formal
440	KGH440	Female	0-12	Mucus	Stream	Rural	House wife	Farmer	Secondary	Primary
441	KGH441	Female	0-12	Bloody	Stream	Rural	House wife	Farmer	Primary	Primary
442	EUS442	Male	0-12	Watery	Well water	Urban	House wife	Civil servant	No-formal	Primary
443	EUS443	Female	25-36	Watery	Bore hole	Rural	House wife	Brick layer	No-formal	Tertiary
444	EUS444	Male	37-48	Bloody	Tap water	Urban	Civil servant	Civil servant	Secondary	Secondary
445	EUS445	Male	49-60	Bloody	Stream	Rural	Civil servant	Farmer	Secondary	Tertiary
446	EUS446	Male	37-48	Mucus	Stream	Rural	House wife	Driver	No-formal	Primary
447	EUS447	Male	25-36	Bloody	Well water	Urban	House wife	Brick / Layer	No-formal	Primary

448	EUS448	Male	13-24	Watery	Bore hole	Rural	House wife	Carpenter	Primary	Secondary
449	EUS449	Female	0-12	Watery	Tap water	Urban	House wife	Farmer	No-formal	No-formal
450	EUS450	Male	0-12	Watery	Stream	Rural	House wife	Painter	No-formal	Primary
451	EUS 451	Male	13-24	Watery	Bore hole	Rural	House wife	Painter	No-formal	Primary
452	EUS452	Female	25-36	Watery	Stream	Rural	House wife	Farmer	No-formal	No-formal
453	EUS453	Female	13-24	Watery	Tape water	Urban	House wife	Brick /Layer	No-formal	Secondary
454	EUS454	Female	13-24	Watery	Well water	Urban	House wife	Carpenter	No-formal	Primary
455	EUS455	Female	0-12	Watery	Well water	Rural	House wife	Mechanic	No-formal	No-formal
456	EUS456	Female	13-24	Watery	Stream	Rural	House wife	Driver	Primary	Secondary
457	EUS457	Male	25-36	Watery	Bore hole	Urban	Civil servant	Civil servant	Secondary	Tertiary
458	EUS458	Male	0-12	Watery	Well water	Rural	Civil servant	Civil servant	Tertiary	Tertiary
459	EUS449	Male	13-24	Watery	Tap water	Urban	Civil servant	Civil servant	Secondary	Tertiary
460	EUS460	Female	25-36	Watery	Bore hole	Rural	Civil servant	Driver	Secondary	Secondary
461	EUS461	Male	25-36	Watery	Well water	Urban	House wife	Mechanic	No-formal	No-formal
462	EUS462	Male	13-24	Watery	Well water	Urban	House wife	Carpenter	No-formal	Primary
	GHM463	Female	37-48	Bloody	Well	Rural	House wife	Farmer	No-formal	No-formal

GHM464	Male	49-60	Bloody	Bore hole	Urban	Civil servant	Brick layer	Secondary	Secondary
GHM465	Male	13-24	Mucus	Stream	Urban	Civil servant	Driver	Primary	Secondary
GHM466	Male	0-12	Watery	Well	Rural	House wife	Carpenter	No-formal	Primary
GHM467	Male	13-24	Watery	Well	Urban	Civil servant	Mechanic	Secondary	Secondary
GHM468	Female	13-24	Mucoid	Well	Rural	House wife	Farmer	No-formal	No-formal
GHM470	Female	25-36	Bloody	Bore hole	Urban	House wife	Farmer	No-formal	No-formal
KGH471	Male	13-24	Watery	Well	Rural	House wife	Farmer	No-formal	No-formal
KGH472	Male	37-48	Mucoid	Stream	Rural	House wife	Farmer	No-formal	No-formal
KGH473	Female	0-12	Watery	Stream	Rural	House wife	Printer	No-formal	Secondary
KGH474	Male	0-12	Mucoid	Well	Urban	Civil servant	Civil servant	Tertiary	Tertiary
KGH475	Male	49-60	Watery	Stream	Rural	House wife	Carpenter	No-formal	Primary
KGH476	Female	25-36	Bloody	Bore hole	Urban	Civil servant	Civil servant	Secondary	Tertiary
KGH477	Male	37-48	Watery	Well	Rural	House wife	Printer	No-formal	No-formal
KHG478	Female	0-12	Watery	Well	Urban	Civil servant	Brick layer	Primary	Secondary
KGH479	Male	13-24	Mucoid	Bore hole	Rural	House wife	Mechanic	No-formal	Primary
KGH480	Female	25-36	Bloody	Tap water	Urban	House wife	Driver	No-formal	Primary