PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF STRYCHNOS INNOCUA (MONKEY ORANGE) EXTRACTS AGAINST CLINICAL ISOLATES OF SALMONELLA SPECIES AND ESCHERICHIA COLI

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN MICROBIOLOGY

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APRIL, 2018

DECLARATION

I declare that the work in this dissertation entitled "**Phytochemical Screening and Evaluation of Antibacterial Activity of *Strychnos innocua* (Monkey Orange) Extracts against Clinical Isolates of *SALMONELLA* species and *Escherichia coli***" has been performed by me in the Department of Microbiology, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

_____ _____

Hamza Mashi, HARUNA (P14SCMC8001)

Date

CERTIFICATION

This dissertation entitled "**Phytochemical Screening and Evaluation of Antibacterial Activity of *Strychnos innocua* (Monkey Orange) Extracts Against Clinical isolates of *Salmonella* species and *Escherichia coli***" by Hamza Mashi, HARUNA (P14SCMC8001) meets the regulations governing the award of the degree of Master of Science in Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This piece of work is dedicated to Almighty Allah and my late father Alh. Haruna Mashi.

ABSTRACT

The study was carried out to ascertain the phytochemical constituents as well as the antibacterial activity of Methanolic, Ethanolic and Aqueous extracts of stem and root bark of *Strychnos innocua* on *Escherichia coli* and *Salmonella* species. Plant parts were successively extracted using cold maceration method and the phytochemical constituents were determined using method of Trease and Evans. Agar well diffusion method was used in determining the antibacterial activity while broth dilution method was employed in the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC). The inhibitions of the test organisms were measured by measuring the diameter of the zone of inhibition. Column chromatography was used in fractionating the extract with the best antibacterial activity. The phytochemical screening revealed the presence of carbohydrate, glycosides, anthraquinones, cardiac glycosides, saponins, steroids and triterpens, flavonoids, tannins and alkaloids in the plant parts used. The extracts showed good inhibitory activities against *Escherichia coli* and *Salmonella* species. The extracts gave different MICs against the test organisms using the double-fold dilution method, with concentrations ranging between 37.5 to 300mg/ml. The ethanolic extract of the stem bark was the most potent against the test bacteria with a minimum inhibitory concentration (MIC) of 37.5mg/ml for *Escherichia coli* and 75mg/ml for *Salmonella* species. The MBC assay revealed that most of the extracts were bactericidal at the concentrations of 75mg/ml for *Escherichia coli* and 150mg/ml for *Salmonella* species. The study revealed that subfractions of the ethanol extract of the stem bark had antibacterial activity with hexane/chloroform subfraction having best activity.

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ABBREVIATIONS AND SYMBOLS

$Bacl_2$	Barium chloride
CFU/ml	Colony Forming Units per millilitre
CLSI	Clinical and Laboratory Standards Institute
Dw	Dry weight
g	Gram
H_2SO_4	Tetra-oxo-sulphate (VI) Acid
IUPAC	International Union of Pure and Applied Chemistry
KOH	Potassium Hydroxide
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
ml	Millilitres
mm	Millimeters
MR	Methyl-red
mg/ml	Milligram per millilitre
NaOH	Sodium Hydroxide
NRC	National research council
RF	Retention Factor
TLC	Thin Layer Chromatography
TSI	Triple sugar iron
UNAIDS	United nations Programme on HIV/AIDS
USNPGS	United State National Plant Germplasm System
VP	Voges-Proskauer
WHO	World health organisation
%	Percentage
^oC	Degree Celsius
µg	Microgram

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Diseases caused by bacteria, viruses, fungi and other parasites are major causes of death, disability, social and economic disruption for millions of people (UNAIDS, 2008; WHO, 2008). Despites the existence of safe and effective interventions, many people lack access to preventive and treatment care. With the development of new antimicrobial agents, microbes developed the ability to elude the best antibiotics and to counter attack their activity with new survival strategies. Antibiotic resistance occurs at an alarming rate among all classes of mammalian pathogens (Njinga *et al.*, 2014).

Over 9.5 million people die each year due to infectious diseases and majority of the death occur in developing countries (WHO, 2008). With the current increasing trends of multidrug resistance among emerging and re-emerging bacterial pathogens to the available modern drugs or antibiotics, it is necessary that the search for newer antibiotic sources be a continued process (Airamasundari *et al*., 2011) and plants being the cheapest and safer alternative sources of antimicrobials (Shariff and Banik, 2006).

Throughout the ages, humans have relied on nature for their basic needs for the production of food-stuffs, shelters, clothing, means of transportation, fertilizers, flavours and fragrances, and not the least, medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continued to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven

to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and thousands of years (Gurib-Fakin, 2006).

The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2600 BC; among the substances that were used were oils of Cedrus species (Cedar) and *Cupressus sempervirens* (Cypress), *Glycyrrhiza glabra* (Licorice), Commiphora species (Myrrh) and *Papaver somniferum* (Poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. Egyptian medicines report on the use of bishop's weeds (*Ammi majus*) to treat vitiligo, a skin condition characterized by a loss of pigments. Moreover, a drug (b-methoxypsoralen) has been produced from this plant to treat psoriasis and other skin disorders, as well as T-cell lymphoma.

Approximately half of the world's flowering plant species live in the tropical forests. Tropical rain forests continue to support a vast reservoir of potential drug species. The existence of undiscovered pharmaceuticals for modern medicine has often been cited as one of the most important reasons for protecting tropical forests, so the high annual extinction rate is a matter for concern, to say the least (Gurib-Fakin, 2006).

In recent times, the use of plants as a source of novel compounds to combat microbial infections has gained prominence. The necessity to search for plant-based antimicrobials is increasing due to high cost, reduced efficacy and increased resistance to conventional medicine (Sankar *et al*., 2012). As a result, medicinal plant research has now gained a momentum among the scientists of the world. Sabir *et al.* (2007) reported that medicinal plants have been relied upon by over 80% of the world population for their basic health care need. Hence, the evolution of rich heritage of traditional medicine is essential (Padmaa *et al.,* 2008).

Plants represent an immense biodiversity which interact with their environment by producing huge and diverse array of organic compounds and molecules called secondary metabolites. These molecules play a major role in a wide variety of processes and fields like pharmaceuticals, agrochemicals, nutrition, cosmetics and industrial products. For this reason, plants are considered as living factories for biosynthesis of a variety of important biomolecules which help in producing life saving drugs. It was estimated that nearly 25% of prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant compounds (Pankaj *et al*., 2011). For instance, the most popular analgesic drug- Aspirin, most valuable anticancer agents- Paclitaxel and Vinblastine are derived exclusively from plant sources (Pankaj *et al*., 2011).

Most of the plants that are used as medicinal plants contain active constituents which need further screening for other use as antimicrobial substance. Studies have reported that secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids among others that are present in plants might confer to them, antimicrobial activity (Fessenden, 1982). A study conducted by Olonitola *et al.* (2010) indicated that *Balanites aegyptiaca* (Desert date) contain tannins, flavonoids, saponins, triterpenes and glycosides which results in its antimicrobial activity against *Escherichia coli, Salmonella* Typhi, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*. Similarly, Njiga *et al.* (2014) had also shown that *Lannea kerstingii* contains flavonoids, tannins, steroids and triterpenes and this account for its antimicrobial activity against *S. aureus, S. feacalis, B. subtilis* and *K. pneumoniae* among many other researches

Strychnos innocua (Monkey orange) commonly called “Kokiya” in Hausa belongs to the family *Loganiaceae* and genus *Strychnos*. It is a small, straight-stemmed tree 3-14m high, with a smooth, green or yellowish-white, powdery bark; branchlets stout and smooth (Bekele *et al.,* 1993; Orwa *et al.,* 2009). Flowering and fruiting of the plant occur concurrently, starting in the

dry season and extending into the rainy season. It takes about a year from flower fertilization to fruit ripening (Maghembe, 1994). Parts of the plant are used for several uses including food (the sweet-sour fruit pulp is edible), as fodder (leaves are eaten by livestock), as fuel (provides excellent firewood that burns even when wet), as repellant (a mixture of ground roots and oil is rubbed on the skin as a fly repellent) (Katende, 1995). The fruit pulp is a good source of lipid, carbohydrates and mineral elements such as potassium, sodium, magnesium, zinc and calcium and is also rich in vitamin C (Hassan *et al.*, 2014).

Strychnos innocua occurs in savanna forest all over Africa, in open woodland and rocky hills. The tree is found in Angola, Chad, Ghana, Ethiopia, Senegal, Sudan, Kenya, Tanzania, Cameroon, Rwanda, Cote D'Ivoire, Ghana, Niger, Nigeria, Zambia and Zimbabwe (USNPGS, 2006; Orwa *et al.*, 2009). A root decoction of *Strychnos innocua* is taken as a remedy for gonorrhea and fresh roots are used to treat snakebite (Hines and Eckman, 1993). The bark and twigs are pounded, soaked in cold water and the infusion drunk to facilitate birth (Hines and Eckman, 1993). The fruit pulp is used as a remedy for dysentery and as eardrops (Kokwaro, 2009; Orwa *et al.*, 2009).

1.2 Statement of Research Problem

Even though pharmaceutical industries have produced a number of new antibiotics in the last three decades, several diseases and microbial infections particularly those caused by the members of the family *Enterobacteriaceae* have shown considerable resistance to a number of antimicrobial agents, such as penicillin, ampicillin, and flouroquinolones among many others (Okeke *et al.*, 2007). This infers that in the near future, there may not be effective antibiotics with which to treat patients with serious infections. Moreover, drug resistance occurs not only due to the poor quality of the drugs but also due to patient non-compliance and irrational use of

antimicrobial agents as well as spontaneous mutation(s) within the microbial populations (Nester, 2002; Denyer *et al*., 2004).

Despite the existence of safe and effective interventions, many people lack access to preventive and treatment care and there is also an increase in high cost and reduced efficacy to conventional medicines (Sankar *et al*., 2012).

1.3 Justification of the study

Herbal preparations may serve as alternative to orthodox medicine and their scientific evaluation is needed to check their antimicrobial activity and validate their use as alternative medicine in the treatment of infections.

In addition to the fact that *Strychnos* plants serve as a rich source of carbohydrates, protein, fiber, Zinc, Iron and vitamin C, some species have antimicrobial activity. Consequently, scientific evaluation of *Strychnos innocua* to check its antimicrobial activity and validate its use as alternative medicine in the treatment of some bacterial infections cannot be over emphasized.

Medicinal plants have important advantages for therapeutic uses in various ailments among which include their availability besides being economical, effective and safer alternative sources of antimicrobials in the treatment of infections.

1.4 Aim of the study

The aim of this study was to determine the phytoconstituents and evaluate the antibacterial activity of *Strychnos innocua* extracts against clinical isolates of *Salmonella* species and *Escherichia coli*.

1.5 Objectives of the study

The specific objectives of the study were to:

1. extract and determine the phytochemical constituents of *Strychnos innocua* from the stem and root bark;
2. determine the antibacterial activity of methanolic, ethanolic and aqueous crude exracts of *Strychnos innocua* against clinical isolates of *Salmonella* species and *Escherichia coli*;
3. fractionate the crude extract of the *Strychnos innocua* with the best activity and determine the activity of the individual fractions against the clinical isolates;
4. determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the *Strychnos innocua* crude extracts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Medicinal Plants

A medicinal plant is any plant which in one or more of its organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. A number of plants have been used in traditional medicine for many years. Some do seem to work although there may not be sufficient data (double-blind trails, for example) to confirm their efficacy (Sofowora, 2008). Medicinal plants include the following:

i. Plants or plants parts used medicinally in galenical preparations (e.g decoctions, infusions e.t.c) e.g Cascara bark
ii. Plants used for extraction of pure substances either for direct medicinal use or for the hemi-synthesis of medicinal compounds (e.g hemi-synthesis of sex hormones from diosgenin obtained from *Dioscorea* yams)
iii. Food, spice and perfumery plants used medicinally e.g ginger
iv. Microscopic plants e.g fungi, actinomycetes, used for isolation of drugs especially antibiotics. Examples are ergot (*Claviceps purpurea* growing on rye) or *Streptomyces griseus*.
v. Fibre plants e.g cotton, flax, jute used for preparation of surgical dressings (Sofowora, 2008).

2.2 Discovery of New Medicines from Plants

Little work was carried out by the pharmaceutical industry during 1950–1980s; however, during the 1980–1990s, massive growth has occurred. This has resulted in new developments in the area of combinatorial chemistry, new advances in the analysis and assaying of plant materials and a

heightened awareness of the potential plant materials as drug leads by conservationists. New plant drug development programmes are traditionally undertaken by either random screening or an ethnobotanical approach, a method based on the historical medicinal/food use of the plant (Biren and Seth, 2010).

One reason why there has been resurgence in this area is that conservationists especially in the United States have argued that by finding new drug leads from the rainforest, the value of the rainforests to society is proven, and that this would prevent these areas from being cut down for unsustainable timber use. However, tropical forests have produced only 47 major pharmaceutical drugs of worldwide importance. It is estimated that a lot more, say about 300 potential drugs of major importance may need to be discovered which would be worth $147 billion. It is thought that 125,000 flowering plant species are of pharmacological relevance in the tropical forests. It takes 50,000 to 100,000 screening tests to discover one profitable drug. Even in developed countries there is a huge potential for the development of nutraceuticals and pharmaceuticals from herbal materials. For example the UK herbal material medica contains around 300 species, whereas the Chinese herbal materia medica contains around 7,000 species. One can imagine what lies in store in the flora-rich India! (Biren and Seth, 2010).

2.3 Reasons for Increasing Popularity of Medicinal Plants

Medicinal plants are used as a source of drugs for the treatment of various human and livestock health disorders all over the world from ancient times to the present day. They are important natural wealth that provides primary healthcare services to people from all walks of life. They serve as important therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicines. A total of 250,000 species of flowering plants are referred to as medicinal plants and the World Health Organization (WHO) enlisted some 21,000 medicinal

plant species. The global herbal market as at 2014 was worth about US$ 62 billion per annum. The annual growth of herbal market is about 15% and the global herbal market by 2050 is expected to be about US $5 trillion.

Plants are one of the most important sources of medicines. Today the large number of drugs in use is derived from plants, like morphine from *Papaver somniferum*, Aswagandha from *Withania somnifera*, ephedrine from *Ephedra vulgaris*, atropine from *Atropa belladonna*, Reserpine from *Rouwolfia serpentina*, etc. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability (Kavita *et al*., 2014). The high costs of western pharmaceuticals put modern health care services out of reach of most of the world's population, which relies on traditional medicine and medicinal plants to meet their primary health care needs. Even where modern medical care is available and affordable, many people prefer more traditional practices. This is particularly true for First Nations and Immigrant populations, who have tended to retain ethnic medical practices (Aba, 2015). The increased global demand for polyherbal formulations is a reflection of positive impact of consolidated efforts aimed at reviving science of phytopharmacy.

2.4 Plant Constituents of Pharmacological Importance

All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into (1) primary metabolites such as sugars and fats, which are found in all plants; and (2) secondary metabolites—compounds which are found in a smaller range of plants, serving a more specific function (Meskin, 2002). Primary metabolism is associated with fundamental life processes common to all plants. It comprises processes such as photosynthesis, pentose cycle, glycolysis, the citric acid cycle, electron transport, phosphorylation and energy regulation and management. Primary metabolites are produced and converted molecular entities,

needed in anabolic pathways to build, maintain and reproduce the living cell. In catabolic pathways, primary metabolites (and food products) provide the chemical energy and precursors for biosynthesis. Primary and secondary metabolisms are interconnected in the sense that the biosynthesis of accumulating secondary metabolites can be traced back to ubiquitous primary metabolites (Gurib-Fakin, 2006). However, in contrast to primary metabolites, secondary metabolites represent features that can be expressed in terms of ecological, taxonomic and biochemical differentiation and diversity.

The biosynthesis and accumulation of secondary metabolites provide a basis for biochemical systematics and chemosystematics. In addition, the wide molecular diversity of secondary metabolites throughout the plant kingdom represents an extremely rich biogenic resource for the discovery of novel drugs and for developing innovative drugs. Not only do plant species yield raw material for useful compounds; the molecular biology and biochemistry provide pointers for rational drug development (Gurib-Fakin, 2006).

Primary and secondary metabolites can be classified on the basis of their chemical structure into much the same categories of chemical compounds: Carbohydrates, Lipids, Amino acids, Peptides, Proteins, Enzymes, Purine and pyrimidine derivatives. Within such compounds classes, secondary metabolites generally show greater individuality and diversity in their molecular structure than primary metabolites. Certain compound classes also appear to be extraordinarily rich in secondary metabolites, e.g. the structurally diverse groups of alkaloids, phenolics, acetogenins and terpenoids. Ubiquitary primary metabolites belonging to these compound classes seem to be restricted to only a limited number of key compounds functioning as biosynthetic precursors. Most of the plant compounds that have been found to be medicinally useful and interesting tend to be secondary metabolites. Also despite enormous structural

diversity, Nature just uses a few building blocks, e.g. Shikimic acid and Shikimate, to create this chemo-diversity. Shikimic acid accounts for the synthesis of many aromatic amino acids including phenylalanine, tyrosine, tryptophan as well as organic acids like benzoic and gallic acids and aldehydes like vanillin, benzaldehyde (Capasso and Sorrentino, 2005).

The basic building blocks are the acetate (C2), isoprenoid (C5) and phenylpropanoid (C9) units. The acetate unit is used in the polyketide biosynthesis and is particularly well developed in microorganisms. The isoprenoid pathways lead to all terpenoids by coupling two or more C5 units. The terpenoids are found in all organisms. The phenyl-propanoid pathway is most typical for plants and it is based on phenylalanine and tyrosine via cinnamic acid and this pathway leads to amongst others lignin and lignans. In combination with three acetate units the C9 unit leads to the flavonoids and the anthocyanins, well known for their role in flower colour. Quite a number of natural product groups can be constructed from the amino acid phenylalanine, in particular, flavonoids, coumarins, lignans etc. all of which possess a common substructure based on aromatic 6-C ring (C6 unit) with a 3-C chain (C3 unit) attached to the aromatic ring (Capasso and Sorrentino, 2005).

Many reactions can occur to this C9 unit including oxidation, reduction, methylation, cyclization, glycosylation (adding of sugar molecules) and dimerization of all which contribute to the value of natural products as a resource of biologically active molecules enhancing at the same time, to the structural complexity of the molecule with the presence of chirality and functionality (Capasso and Sorrentino, 2005).

The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance (Kavita *et al*., 2014). These secondary metabolites are

compounds that are produced in response to stress that is induced by abiotic (e.g. heat, drought) and biotic (e.g. herbivores, pathogens, humans) factors on the plant (Keeling and Bohlmann, 2006). Many secondary metabolites are "antibiotic" in a broad sense, protecting the plants against fungi, bacteria, animals, and even other plants. Some secondary metabolites are toxins used to deter predation and others are pheromones used to attract insects for pollination. It is these secondary metabolites and pigments that can have therapeutic actions in humans and which can be refined to produce drugs (Meskin, 2002).

2.4.1 Alkaloids

One of the largest groups of chemicals produced by plants is the alkaloids. An alkaloid is a nitrogenous organic molecule that has a pharmacological effect on humans and animals. They are a class of compounds which typically contain nitrogen and have complex ring structures. They occur naturally in seed bearing plants and are found in berries, bark, fruit, roots and leaves. Often, they are bases which have some physiological effect (Biren and Seth, 2010). The name derived from the word alkaline was originally used to describe any nitrogen-containing base (an amine in modern terms). Alkaloids are found as secondary metabolites in plants (e.g. in Vinca and Datura), animals (e.g. in shellfish) and fungi, and can be extracted from these sources by treatment with acids (usually hydrochloric acid or sulphuric acid, though organic acids, such as maleic acid and citric acid are sometimes used) (Biren and Seth, 2010). Usually alkaloids are derivatives from amino acids. Even though many alkaloids are poisonous (e.g. strychnine or coniine), some are used in medicine as analgesics (pain relievers) or anaesthetics, particularly morphine and codeine. Most alkaloids have a very bitter taste (Biren and Seth, 2010).

2.4.1.1 Classification

Alkaloids are generally classified by their common molecular precursors, based on the biological pathway used to construct the molecule. From a structural point of view, alkaloids are divided according to their shapes and origins. There are three main types of alkaloids:

(1) true alkaloids, (2) protoalkaloids, and (3) pseudoalkaloids.

True alkaloids and protoalkaloids are derived from amino acids, whereas pseudoalkaloids are not derived from these compounds (Biren and Seth, 2010).

2.4.1 .2 Uses

Most of the known functions of alkaloids are related to protection. For example, aporphine alkaloid liriodenine produced by the tulip tree protects it from parasitic mushrooms. In addition, presence of alkaloids in the plant prevents insects and chordate animals from eating it. However, some animals adapted to alkaloids and even use them in their own metabolism. Such alkaloid-related substances as serotonin, dopamine and histamine are important neurotransmitters in animals. Alkaloids are also known to regulate plant growth (Aba, 2015).

2.4.2 Glycosides

A glycoside is any molecule in which a sugar group is bonded through its anomeric carbon to another group via glycosidic bond, the sugar group is known as the glycone and the nonsugar group as the aglycone or genin part of the glycoside. The glycone can consist of a single sugar group (monosaccharide) or several sugar groups (oligosaccharide). The sugars found in glycosides may be glucose and rhamnose (monosaccharides) or, more rarely, deoxysugars such as the cymarose found in cardiac glycosides (Biren and Seth, 2010). In plants glycosides are both synthesized and hydrolysed under the influence of more or less specific enzymes. They are crystalline or amorphous substances that are soluble in water or alcohols and insoluble in organic solvents like benzene and ether. The aglycone part is soluble in organic solvents like benzene or

ether. They are hydrolysed by water, enzymes and mineral acids as well as optically active. While glycosides do not themselves reduce Fehling's solution, the simple sugars which they produce on hydrolysis will do so with precipitation of red cuprous oxide. The sugars present in glycoside are of two isomeric forms, that is, α form and β form, but all the natural glycosides contain β-type of sugar (Biren and Seth, 2010).

2.4.2 .1 Classification

The glycosides can be classified by the glycone, the type of glycosidal linkage and the aglycone.

On the Basis of Glycone: If the glycone group of a glycoside is glucose, then the molecule is a glucoside; if it is fructose, then the molecule is a fructoside; if it is glucuronic acid, then the molecule is a glucuronide, etc.

On the Basis of Glycosidic Linkage: The classification is as follows:

1. *O-glycosides*: Sugar molecule is combined with phenol or –OH group of aglycon, for example, Amygdaline, Indesine, Arbutin, Salicin, cardiac glycosides, anthraquinone glycosides like sennosides etc.

2. *N-glycosides*: Sugar molecule is combined with N of the –NH (amino group) of aglycon, for example, nucleosides

3. *S-glycosides*: Sugar molecule is combined with the S or SH (thiol group) of aglycon, for example, Sinigrin.

4. *C-glycosides*: Sugar molecule is directly attached to C-atom of aglycon, for example, Anthraquinone glycosides like Aloin, Barbaloin, Cascaroside and Flavan glycosides, etc.

On the Basis of Aglycone: The various classes according to aglycone moiety are given below:

(i) Anthraquinone glycosides (ii) Sterol or Cardiac glycosides (iii) Saponin glycosides (iv) Cyanogenetic and Cyanophoric glycosides (v) Thiocynate and Isothiocynate glycosides (vi) Flavone glycosides (vii) Aldehyde glycosides (viii) Phenol glycosides (ix) Steroidal glycosides (x) Bitter and Miscellaneous glycosides (Biren and Seth, 2010).

2.4.2 .2 Uses

Many plant glycosides are used as medications (Srivastava and Lambert, 1996). In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by a herbivore (Aba, 2015).

2.4.3 Flavonoids

Flavonoid**s** are a class of plant secondary metabolites that are water-soluble polyphenolic molecules containing 15 carbon atoms. Flavanoids can be visualized as two benzene rings which are joined together with a short three carbon chain. One of the carbons of the short chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring, which can be five or six-membered. Chemically, they have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C). The flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and isoflavonoids. Together with carotenes, flavanoids are also responsible for the coloring of fruits, vegetables and herbs (Aba, 2015).

2.4.3 .1 Classification

According to the IUPAC nomenclature, they can be classified into:

i. *flavonoids* or *bioflavonoids*, derived from 2-phenylchromen-4-one (2-phenyl-1,4-benzopyrone) structure

ii. *isoflavonoids*, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure.

iii. *neoflavonoids*, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure

These three flavonoid classes are all ketone-containing compounds, and as such, are anthoxanthins (flavones and flavonols). This class was the first to be termed bioflavonoids (Aba, 2015).

2.4.3 .2 Uses

Flavonoids have been shown to have a wide range of biological and pharmacological activities *in vitro*. Examples include the study conducted by Aba, (2015) who reported flavonoids to be having anti-allergic, anti-inflammatory, antioxidant, anti-cancer, anti-microbial and anti-diarrhoeal activities. Moreover, Manner *et al*. (2013) reported flavonoids to be having antibacterial activity and Friedman, (2007) had shown flavonoids to be having antifungal and antiviral activities.

2.4.4 Tannins

The name ‘tannin’ is derived from the French ‘tanin’ (tanning substance) and is used for a range of natural polyphenols. Tannins are complex organic, non-nitrogenous plant products, which generally have astringent properties. These compounds comprise a large group of compounds that are widely distributed in the plant kingdom. The term ‘tannin’ was first used by Seguin in 1796 to denote substances which have the ability to combine with animal hides to convert them into leather which is known as tanning of the hide. According to this, tannins are substances which are detected by a tanning test due to its absorption on standard hide powder. The test is known as Goldbeater’s skin test (Biren and Seth, 2010).

Tannins are the most abundant secondary metabolites made by plants, commonly ranging from 5% to 10% dry weight of tree leaves. Tannins can defend leaves against insect herbivores by deterrence and/or toxicity. Contrary to early theories, tannins have no effect on protein digestion in insect herbivores. By contrast, in vertebrate herbivores tannins can decrease protein digestion (Barbehenn and Peter, 2011). Tannins are found in leaf, bud, seed, root, and stem tissues. An example of the location of the tannins in stem tissue is that they are often found in the growth areas of trees, such as the secondary phloem and xylem as well as the layer between the cortex and epidermis. Tannins may help regulate the growth of these tissues.

2.4.4 .1 Classification

The tannin compounds can be divided into two major groups on the basis of Goldbeater's skin test. A group of tannins showing the positive tanning test may be regarded as "true tannins", whereas those, which are partly retained by the hide powder and fail to give the test, are called as "pseudotannins". Most of the true tannins are high molecular weight compounds that are complex polyphenolics and are produced by polymerization of simple polyphenols. They may form complex glycosides or remains as such which may be observed by their typical hydrolytic reaction with the mineral acids and enzymes. Two major chemical classes of tannins are usually recognized based on this hydrolytic reaction and nature of phenolic nuclei involved in the tannins structure. The first class is referred to as "hydrolysable tannins", whereas the other class is termed as "condensed tannins" (Biren and Seth, 2010).

2.4.4 .2 Uses

Poliovirus, herpes simplex virus, and various enteric viruses are inactivated when red grape juice and red wines are incubated with a high content of condensed tannins. In tissue-cultured cell assays tannins have shown antiviral, antibacterial and antiparasitic effects (Akiyama *et al.*,

2001). Tannins are used as antiseptic due to presence of the phenolic group. Tannin-rich medicinal plants are used as healing agents in a number of diseases. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinnorhoea and diarrhoea (Amritpal, 2011).

The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Tannins are also been used for immediate relief of sore throats, diarrhoea, dysentery, hemorrhaging, fatigue, skin ulcers and as a cicatrizant on gangrenous wounds. Tannins not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more (Aba, 2015).

2.4.5 Saponins

Saponins are glucosides with foaming characteristics. They consist of polycyclic aglycones attached to one or more sugar side chains. The aglycone part, also called sapogenin is either a steroid (C27) or a triterpene (C30). Their foaming abilities are caused by the combination of a hydrophobic (fat soluble) sapogenin and a hydrophilic (water soluble) sugar part. Saponins are a class of chemical compounds found in particular abundance in various plant species. They are amphipathicglycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilictriterpene derivative. Saponins are natural glycosides which possess a wide range of pharmacological properties including cytotoxic activity. They exert a wide range of pharmacological activities including expectorant, anti-inflammatory, vasoprotective, hypocholesterolemic, immunomodulatory, hypoglycaemic, molluscicidal,

antifungal, antiparasitic, hyperglycaenemia, anti-oxidant, anti-cancer, weight loss and serve as natural antibiotics (Podolak *et al.,* 2010).

2.5 The Genus *Strychnos*

Strychnos is a genus of flowering plant, belonging to the family Loganiaceae (sometimes Strychnaceae). The genus includes about 100 accepted species of trees and lianas and more than 200 that are as yet unresolved (The plant list, 2013). The genus is widely distributed around the world's tropics and is noted for the presence of poisonous indole alkaloids in the roots, stems and leaves of the plant of which among the alkaloids are strychnine and curare (The plant list, 2013). The genus *Strychnos* is represented in both the old and new world (Neuwinger, 1994). The old world species contain alkaloids of the Strychnine type while new world species contain substances from the curarine group (Christian, 2005). Several new world *Strychons* species were or still are used to produce curare and similar arrow poisons while some species are used for ethno-medicinal purposes (Neuwinger, 1994). Although pharmacological studies of some species of *Strychnos* showed that muscle relaxant and/or convulsant properties were generally present, antimicrobial, cytotoxic and hypotensive properties among others have also been demonstrated by some of the species and/or alkaloids (Ohiri *et al.*, 1983).

Strychnos species fruits ripen and are harvested from September to December (Akinnifesi *et al*. 2007), a time when intense agricultural labour coincides with low maize stocks and the unavailability of freshly-gathered vegetables (McGregor, 1995). Fresh monkey orange fruit emits a distinct and delicate mixture of complex aroma volatiles, which are perceived by consumers as a mixture of pineapple, apricot, melon, clove, and citrus (Sitrit *et al*., 2003). The ripe monkey orange species has fleshy (Malaisse and Parent, 1985), sweet (Amarteifio and Mosase, 2006) yellow, very aromatic pulp (Mbiyangandu, 1985; Tanto and Haq, 2007) and contains numerous

hard brown seeds (Sitrit *et al*., 2003). There is wide variability in the general description of taste, color, texture and flavour between and within species.

The pulp of the plant *Strychnos innocua* is bright yellow or brown, juicy, sweet and or sour, with few to numerous hard seeds imbedded in the fleshy pulp (Mbiyangandu, 1985; Coates *et al*., 2002). Whole fruit weighs from 145–383g (Mkonda *et al*., 2002) and a single tree produces 300–700 fruits, which translates to approximately 40–100kg fruit weight per tree.

The genus is divided into 12, though it is conceded that the divisions do not reflect evolution of the genus, and all except *Spinosae* are polyphyletic:
(i) *Strychnos* (53 species) (ii) *Rouhamon* (21 species) (iii) *Breviflorae* (32 species) (iv) *Penicillatae* (17 species) (v) *Aculeatae* (1 species) (vi) *Spinosae* (4 species) (vii) *Brevitubae* (18 species) (viii) *Lanigerae* (32 species) (ix) *Phaeotrichae* (1 species) (x) *Densiflorae* (8 species) (xi) *Dolichantae* (9 species) (xii) *Schyphostrychnos* (1 species) (The plant list, 2013).

2.6 Taxonomic / Scientific Classification of *Strychnos innocua*

Kingdom: Plantae

Class: Angiosperms

Subclass: Eudicots

Superorder: Asterids

Order: Gentianales

Family: Loganiaceae

Genus: Strychnos

Species: *Strychnos innocua* (The plant list, 2013).

2.7 Botanical Description of *Strychnos innocua*

2.7.1 Plant

Strychnos innocua is a small, straight-stemmed tree 3-14m height, with a smooth, green or yellowish-white, powdery bark; branchlets stout and smooth (Bekele *et al.,* 1993; Ruffo *et al.,* 2002; Orwa *et al.,* 2009).

2.7.2 Leaves

Simple, alternate, leathery, subsessile or shortly petiolate, obovate, elliptic or oblong-elliptic, 4-15 x 2-9 cm, coriaceous; roundedemarginate or subacute at the apex; widely to very narrowly cuneate or rarely rounded at the base; glabrous to pubescent beneath; venation finely reticulate on both surfaces with 3-7 nerves arising from the leaf base that are prominent beneath; petiole 2-6 mm long (Bekele *et al.,* 1993; Ruffo *et al.,* 2002; Orwa *et al.,* 2009).

2.7.3 Flowers

Greenish-white or yellowish, up to 8 mm long, produced in axillary cymes; stalks short, 2-5 mm long; calyx lobes short and broad (Bekele *et al.,* 1993; Ruffo *et al.,* 2002; Orwa *et al.,* 2009).

2.3.4 Fruits

Globose, 6-10 cm in diameter, with a hard rind, glabrous, bluish-green when young, yellowish or orange when ripe, with a thick woody shell, containing many seeds embedded in a yellowish pulp. Seeds yellowish-white, tetrahedral, stony hard, 1.5-1.8 cm in diameter (Bekele *et al.,* 1993; Ruffo *et al.,* 2002; Orwa *et al.,* 2009).

2.8 Ecology of *Strychnos innocua* plant

Strychnos innocua occurs in savanna forest all over Africa, in open woodland and rocky hills. The tree is found in Angola, Chad, Ghana, Ethiopia, Senegal, Sudan, Kenya, Tanzania, Cameroon, Rwanda, Cote D'Ivoire, Ghana, Niger, Nigeria, Zambia and Zimbabwe (U.S National plant germplasm system, 2006; Orwa *et al.,* 2009).

2.9 Biophysical Limits of *Strychnos innocua* plant

Altitude: 0-1 520 m., Mean annual temperature: 19-31^{0}C, Mean annual rainfall: 400- 800 mm (Ruffo *et al.,* 2002; Orwa *et al.,* 2009).

2.10 Soil types for growing *Strychnos innocua* plant

Occurs on light yellowish-brown to reddish-yellow, gritty sandy clay loams derived from granite and granodiorite rocks. Sometimes found on rocky hills or in moist savanna woodland, *Brachystegia* woodland, more often in coastal lowlands. (Bekele *et al.*, 1993; Ruffo *et al.,* 2002; Orwa *et al.,* 2009).

2.11 Uses of the Plant parts of *Strychnos innocua*

The plant parts contain substances that can be used for different purposes among which include the following:

2.11.1 As Food: The hard woody fruit case is cracked open to expose the yellow seed aril, which is eaten. The seeds are discarded. The aril tastes sweet and is eaten as a substitute for other fruits, especially by children and hunters. Eaten as a snack. If eaten in large amounts it causes stomach upsets due to the strychnine content (Ruffo *et al.,* 2002).

2.11.2 As Fodder: Leaves are eaten by livestock (Katende, 1995, Ruffo *et al.,* 2002).

2.11.3 As Fuel: Provides excellent firewood that burns even when wet (Katende, 1995; Ruffo *et al.,* 2002).

2.11.4 As Timber: The cream or pale yellow hardwood is inclined to split; it is used for tool handles and other small articles (Katende, 1995; Ruffo *et al.*, 2002).

2.11.5 As Repellent: A mixture of ground roots and oil is rubbed on the skin as a fly repellent (Katende, 1995).

2.11.6 As Medicine: A root decoction is taken as a remedy for gonorrhea; fresh roots are used to treat snake bite (Hines and Eckman, 1993; Ruffo *et al.,* 2002). The bark and twigs are pounded, soaked in cold water and the infusion drunk to facilitate birth (Hines and Eckman, 1993). The fruit pulp is used as a remedy for dysentery and as eardrops (Kokwaro, 2009; Ruffo *et al.,* 2002; Orwa *et al.*, 2009).

2.11.7 Other Products: Oil from the fruit pulp and seeds are used in making soaps (Katende, 1995).

Strychnos species (monkey orange) has been identified among the top priority fruit species in Southern Africa through ethnobotanical surveys (Mkonda *et al*., 2002), particularly in dry areas of Zimbabwe such as Binga (Mpofu *et al*., 2014). This fruit tree proliferates in areas with a prolonged dry season, remains dormant when water is un-available and bears fruit in abundance (Mwamba, 2006; NRC, 2008).

Strychnos species has been identified to contribute more than 100% of the recommended daily intake for vitamin C, Fe and Zn; especially for children between four and eight years old and for pregnant women (Ngadze *et al.*, 2016). Nutritionally, the fruits possess potential health benefits attributable to high energy, fiber, minerals (iron and zinc) and vitamin C (Saka and Msonthi, 1994; Bello *et al*., 2008). Because of their wide availability and nutritional composition, monkey oranges have potential for contributing to the alleviation of vitamin and micronutrient deficiencies of the vulnerable rural population, particularly children and women by complementing the monotonous staple food diet. From the literature, there is no scientific

evidence that ripe *Strychnos innocua* pulp contain alkaloids, neither have the fruits been reported to be toxic in their ripe state. Therefore, the loss of toxic alkaloids after fruit ripening may be caused by alkaloid degradation at maturation that renders the fruit consumable by both animals and human as sensory properties, sugar, color and flavor contents also increase (Ruth *et al*., 2017).

Lee *et al*. (2013) reported that fruit sweetness depends highly on sugar composition. It was also shown that the degree of monkey orange ripeness had an effect on sugar composition, thus, taste is dependent on the stage of ripening in addition to environmental factors like soil, geographical location and climatic differences (Ruth *et al*., 2017). Sitrit *et al.* (2003) reported accumulation of sugars and organic acids during ripening and sucrose conversion to glucose and fructose at the onset of ripening for *Strychnos* species.

Strychnos species can tolerate relatively high temperatures and long storage times before deteriorating in quality. Ripening could be either accelerated or reduced by different storage methods. Methods used to accelerate ripening are the mimicking of dark, air tight conditions, such as by burying fruits underground in sand, in sacks with dry hay, dry chicken manure, mealie meal or fine wood ash. Thus, the fruits are kept in the buried environments in order to control the atmospheric conditions that allow the concentration of the ethylene plant hormone that consequently hastens the ripening period, signifying the climacteric nature of the fruit (Barry and Giovannoni, 2007; Sitrit *et al*., 2003)

2.12 Nutritional and Anti-Nutritional Composition of *Strychnos innocua*

Strychnos species depending on species have iron content up to 140mg/100g (Malaisse and Parent, 1985) giving potential to deliver iron when used as a food source by pregnant or lactating

women and children. *Strychnos innocua* fruit pulp is a good source of lipid, carbohydrates and mineral elements such as potassium, sodium, magnesium, zinc and calcium and is also rich in vitamin C (ascorbate) (Bello *et* al., 2008, Hassan *et al.*, 2014). Also reported, *Strychnos innocua* had the highest total carbohydrate content among the genus *Strychnos* ranging from 15.4g/100g dw (Bello *et al*., 2008) to 61g/100g dw (Saka and Msonthi, 1994). Total sugars were 28.2g/100g dw and the most abundant sugar was sucrose (12.9g/100g dw), a disaccharide, followed by the monosaccharides glucose (4.6g/100g dw) and fructose (1.9g/100g dw) (Sitrit *et al*., 2003).

In addition, protein content for *Strychnos innocua* was remarkably wide, ranging from 0.3 g/100g dw (Malaisse and Parent, 1985) to 12.8g/100g dw (Arnold *et al*., 1985) and there is also a notable variability in fat content for all the plant ranging from 0.3g/100g dw (Malaisse and Parent, 1985) to 20 g/100g dw (Hassan *et al*., 2014). Similarly, the fiber content of *Strychnos innocua* was found to be 17.9g/100g dw as reported by Saka and Msonthi, (1994) and the energy values range from 1315.4 kJ/100g (Arnold *et al*., 1985) to 2083.6 kJ/100g (Hassan *et al*., 2014).

The vitamin C content of monkey orange fruits ranged from 34.2mg/100g dw (Arnold *et al*., 1985) to 88mg/100g dw (Amarteifio and Mosase, 2006). Other vitamins assayed were thiamine (vitamin B1) and riboflavin (vitamin B2) (Ruth *et al*., 2017) in which a thiamine content of 2.74mg/100g dw and a riboflavin content of 1.85mg/100 g dw were reported for flesh inside the shell, while a thiamine content of 0.10 mg/100g dw and a riboflavin content of 0.74mg/100g dw were reported for flesh surrounding the seeds (Wehmeyer, 1966). Moreover, the reported variation in moisture content was high for *Strychnos innocua* 60% (Hassan *et al*., 2014) to 91% (Bello *et al*., 2008).

Hassan *et al.*(2014) also reported that the fruit pulp on dry weight basis (DW), contains

crude protein (3.97%), crude lipid (20.0%), ash (0. 05%), available carbohydrates (75.53%), calorific value (498.0kcal/100g) and moisture (60.17%Wet Weight). The pulp is also rich in potassium (256.33mg/100g), magnesium (10.67mg/100g), iron (9.77mg/100g), zinc (28.73 mg/100g) and manganese (2.50mg/100g). The pulp contains appreciable concentration of ascorbic acid (17.97 91mg/100g DW). But the pulp also has high content of total oxalate (48.75 % DW) and phytic acid (242.91mg/100g). The levels of various nutrient and mineral elements varied significantly, which indicates the potential of the pulp to be harnessed for diverse application for value addition as health food provided that the anti-nutritional factors are tackled appropriately (Hassan *et al.*, 2014).

Bello *et al*. (2008) also reported *Strychnos innouca* seed to be having Moisture content, Crude fat, Crude fibre, Crude protein, Ash and Carbohydrate values as 8.93 ± 0.97, 1.67g/100g, 13.39g/100g, 15.67g/100g, 1.79g/100g and 71.94g/100g respectively and the levels of total sugar, reducing sugar, starch content and ascorbic acid in the fruit parts were found to be 16.47mg/g, 8.24mg/g, 461.25mg/g and 30.96mg/g respectively. Food high in fiber is often richer in micronutrients (Dhingra *et al*., 2012), thus this might explain the high micronutrient content of *Strychnos innocua*.

But the fruit also contains substances that may be harmful to health when ingested in high quantity and that the non-essential substances are low indicating that little processing is needed before they are consumed (Hassan *et al.*, 2014).

2.13 Medicinal Uses of Some Species of the Genus "*Strychnos*"

Some species of the genus *Strychnos* are having antimicrobial activity among which include the following:

2.13.1 *Strychnos spinosa*

Strychnos spinosa has been used in different places as remedy for many ailments and disease conditions. The antimicrobial activity of *Strychnos spinosa* against *Candida albicans* and *Aspergillus niger* among other microorganisms was reported by Nwozo *et al*. (2010). Other researchers have also reported the anti-plasmodial (Frederich *et al*., 2002; Bero *et al*., 2009) as well as the anti-trypanosomal properties of *S. spinosa (*Nwozo *et al*., 2010).

2.13.2 *Strychnos colubrina*

S.colubrina L. root extracts have a significant antimicrobial activity against the broad spectrum of microorganisms. The antibacterial studies of the extracts against *C. perfringens, S. typhi, B. subtilis* and *S. aureus*, showed the most promising antimicrobial properties indicating the potential discovery of novel drugs from plants (Sudhira, 2015).

2.13.3 *Strychnos potatorum*

The seeds of *Strychnos potatorum* Linn. are used in the treatment of gonorrhea, leukorrhea leukeorrhea, gastropathy, bronchitis, chronic diarrhoea, dysentery, renal and vesicle calculi, diabetes, conjunctivitis, scleritis, ulcers and other eye disease (Kavita *et al*., 2014).

2.14 *Escherichia coli*

Escherichia coli is a Gram-negative facultatively anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm blooded organisms (endotherms) (Singleton, 1999; Tenaillon *et al.*, 2010). Most *Escherichia coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts and are occasionally responsible for product recalls due to food

contamination (Vogt, 2005). The harmless strains are part of the normal flora of the gut and can benefit their hosts by producing vitamin K_2 (Bently, 1982) and preventing colonization of the intestine with pathogenic bacteria (Hudault, 2001). *Escherichia coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards (Russell, 2001).

Escherichia coli and other facultative anaerobes constitute about 0.1% of gut flora (Eckburg *et al.,* 2005) and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination (Feng *et al.,* 2002). The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *Escherichia coli* is a chemoheterotroph whose chemically defined medium must include a source of carbon and energy (Tortora, 2010) and under favorable condition, it takes only 20 minutes to reproduce (Yu *et al.,* 2014).

2.14.1 Metabolism

Escherichia coli can live on a wide variety of substrates and uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate, and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *Escherichia coli* lives together with hydrogen-consuming organisms, such as methanogens or sulphate-reducing bacteria (Madigan, 2006).

2.14.2 Growth Culture

Optimum growth of *Escherichia coli* occurs at 37 °C (98.6 °F), but some laboratory strains can multiply at temperatures up to 49 °C (120 °F) (Fotadar, 2005). *Escherichia coli* grows in a variety of defined laboratory media, such as lysogeny broth, or any medium that contains glucose, ammonium phosphate, monobasic, sodium chloride, magnesium sulfate, potassium phosphate, dibasic, and water. Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen, and amino acids, and the reduction of substrates such as oxygen, nitrate, fumarate,dimethyl sulfoxide and trimethlamine N-oxide (Ingledew, 1984). *Escherichia coli* is classified as a facultative anaerobe. It uses oxygen when it is present and available. It can, however, continue to grow in the absence of oxygen using fermentation or anaerobic respiration. The ability to continue growing in the absence of oxygen is an advantage to bacteria because their survival is increased in environments where water predominates (Tortora, 2010).

2.14.3 Role of *Escherichia coli* in disease(s)

Most *Escherichia coli* strains do not cause disease but virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease (Evans, 2007). Common signs and symptoms include severe abdominal cramps, diarrhoea, hemorrhagic colitis, vomiting, and sometimes fever (Lim *et al.,* 2017). In rare cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and Gram-negative pneumonia. Very young children are more susceptible to develop severe illness, such as hemolytic uremic syndrome, however, healthy individuals

of all ages are at risk to the severe consequences that may arise as a result of being infected with *Escherichia coli* (Lim *et al.*, 2017).

2.14.4 Molecular Mechanisms of Infection

Escherichia coli inhabit the large intestine of humans and other animals as a small but consistent part of the normal mi crobiota. This primary niche also serves as a reservoir for *Escherichia coli* that can cause a variety of enteric and extra-enteric diseases (Eisenstein, 1989). One trait that has been considered important for both normal ecology and pathogenicity is the production of tissue-specific adhesive factors, frequently found in the form of hair-like surface structures called fimbriae. More than 95% of all isolates of *Escherichia coli* express type 1 fimbriae, which are also called mannose-sensitive or common fimbriae (Ofek and Doyle, 1994). Expression of type 1 fimbriae may not be required for maintaining *Escherichia coli* in the colon (McCormick *et al*., 1989), but they do provide *Escherichia coli* with a significant advantage for the transitory colonization of the oropharyngeal portal that is important for the normal fecal/oral transmission of *Escherichia coli* among individual hosts (Bloch *et al*., 1992).

In addition to this essential role in commensal ecology, type 1 fimbriae are also important for the pathogenesis of urinary tract infections (UTIs) (Langermann *et al*., 1997). The d-mannose-sensitive adhesive phenotype of type 1 fimbriae is dependent on the lectin-like activity of a tip-located 30-kDa subunit, FimH (Jones *et al*., 1995). Naturally occurring phenotypic variants of the FimH protein have recently been recognized (Sokurenko *et al*., 1995). All *fimH* alleles studied to date encode subunits that mediate high levels of binding to tri-mannose structures (M_3; α1–3, α1–6-d-mannotriose), but binding to mono-mannose

(M_1) residues among the FimH variants can differ up to 15-fold (Sokurenko, 1997). These adhesive variations are due solely to structural differences in FimH that affect receptor specificity of the lectin but do not affect fimbrial morphology or level of fimbriation (Sokurenko, 1997). Thus, all type 1 fimbriae can be functionally subdivided into either low M_1-binding (M_1L) or high M_1-binding (M_1H) FimH phenotypes.

Escherichia coli exhibiting these two basic phenotypes have been found to predominate in different niches. Most isolates from the large intestine of healthy humans (around 80%) express a distinct M_1L phenotype, whereas most isolates from UTIs (more than 70%) express M_1H FimH variants (Sokurenko, 1995).

2.14.5 Treatment

The mainstay of treatment is the assessment of dehydration and replacement of fluid and electrolytes but administration of antibiotics has been shown to shorten the course of illness (CDC, 2016). The antibiotic used depends upon susceptibility patterns in the particular geographical region. Currently, the antibiotics of choice are fluoroquinolones or azithromycin (Al-Abri *et al.*, 2005).

2.15 Genus *Salmonella*

Salmonella is a genus of rod-shaped (bacillus) Gram-negative bacteria of the *Enterobacteriaceae* family. The two species of *Salmonella* are *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is further divided into six subspecies that include over 2,500 serotypes (Su, 2007). They are chemotrophs, obtaining their energy from oxidation and reduction reactions using organic sources and they are also facultative

anaerobes, capable of surviving with or without oxygen (Fabrega and Vila, 2013).

Salmonella species can be found in the digestive tracts of humans and animals, especially reptiles. *Salmonella* on the skin of reptiles or amphibians can be passed to people who handle the animals (CDC, 2013). Food and water can also be contaminated with the bacteria if they come in contact with the feces of infected people or animals (Goldrick, 2003).

2.15.1 Detection, Culture and Growth Conditions

Most subspecies of *Salmonella* produce hydrogen sulphide which can readily be detected by growing them on media containing ferrous sulfate, such as is used in the triple sugar iron test (Clark and Barret, 1987). Most isolates exist in two phases: a motile and non motile phase and cultures that are non-motile upon primary culture may be switched to the motile phase using a Craigie tube or ditch plate (UK Standards for Microbiology Investigations, 2015).

2.15.2 Role of *Salmonella* in Disease

Salmonella infection, or salmonellosis, is a bacterial disease of the intestinal tract. *Salmonella* infection is generally associated with the ingestion of improperly prepared, previously contaminated food. Meat and dairy products are most likely candidates. Foods containing uncooked eggs can also be a source (Jacquelyn, 2005).

Signs and symptoms of salmonesis include abdominal pain, fever and diarrhea with blood and mucus. They appear 8 to 42 hours after ingestion of organisms and are associated with the organisms invading the mucosa of both the small and large intestine. Fever probably is

caused by endotoxins, toxins that are released from a cell only when lysed. In otherwise healthy adults, salmonellosis last 1 to 4 days and is self-limiting. Infants and elderly or debilitated patients often have more severe and prolonged symptoms. In such cases, antibiotics may be prescribed (Jacquelyn, 2005).

Other serovars of *Salmonella* also cause disease due to their ability to invade intestinal tissue and enter the blood. *Salmonella typhimurium* and *Salmonella paratyphi* cause a somewhat a more serious condition called enterocolitis or enteric fever. Symptoms and bacteremia appear after an incubation period of 1 to 10 days. Enteric symptoms such as fever and chills can last 1 to 3 weeks. Chronic infections of the gallbladder and other tissues are not uncommon (Jacquelyn, 2005).

A carrier state becomes established when after the patient's recovery, organisms from chronically infected tissues continue to be excreted with feces (Jacquelyn, 2005).

2.15.3 Molecular Mechanisms of Infection

Mechanisms of infection differ between typhoidal and nontyphoidal serotypes, owing to their different targets in the body and the different symptoms that they cause. Both groups must enter by crossing the barrier created by the intestinal cell wall, but once they have passed this barrier, they use different strategies to cause infection.

Nontyphoidal serotypes preferentially enter M cells on the intestinal wall by bacterial-mediated endocytosis, a process associated with intestinal inflammation and diarrhoea. They are also able to disrupt tight junctions between the cells of the intestinal wall, impairing the cells' ability to stop the flow of ions, water, and immune cells into and out of the intestine. The combination of the inflammation caused by bacterial-mediated endocytosis and the disruption of tight junctions is thought to contribute significantly to the

induction of diarrhoea (Haraga *et al.*, 2008).

Salmonellae are also able to breach the intestinal barrier via phagocytosis and trafficking by CD 18-positive immune cells, which may be a mechanism key to typhoidal *Salmonella* infection. This is thought to be a more stealthy way of passing the intestinal barrier, and may, therefore, contribute to the fact that lower numbers of typhoidal *Salmonella* are required for infection than nontyphoidal *Salmonella* (Haraga *et al.*, 2008). *Salmonella* cells are able to enter macrophages via macropinocytosis (Kerr *et al.*,2010) Typhoidal serotypes can use this to achieve dissemination throughout the body via the mononuclear phagocyte system, a network of connective tissue that contains immune cells, and surrounds tissue associated with the immune system throughout the body (Haraga *et al.*, 2008).

Much of the success of *Salmonella* in causing infection is attributed to two type III secretion systems which function at different times during an infection. One is required for the invasion of nonphagocytic cells, colonization of the intestine, and induction of intestinal inflammatory responses and diarrhoea. The other is important for survival in macrophages and establishment of systemic disease (Haraga *et al.*, 2008).These systems contain many genes which must work co-operatively to achieve infection.

The AvrA toxin injected by the SPI1 type III secretion system of *S. typhimurium* works to inhibit the innate immune system by virtue of its serine/threonine acetyltransferase activity, and requires binding to eukaryotic target cell phytic acid (IP6) (Mittal *et al.*, 2010). This leaves the host more susceptible to infection.

Salmonellosis is known to be able to cause back pain or spondylosis that can manifest as

five clinical patterns: gastrointestinal tract infection, enteric fever, bacteremia, local infection, and the chronic reservoir state. The initial symptoms are nonspecific fever, weakness, and myalgia among others. In the bacteremia state, it can spread to any parts of the body and this induces localized infection or it forms abscesses. The forms of localized *Salmonella* infections are arthritis, urinary tract infection, infection of the central nervous system, bone infection, soft tissue infection, etc (Choi *et al*., 2010). Infection may remain as the latent form for a long time, and when the function of reticular endothelial cells is deteriorated, it may become activated and consequently, it may secondarily induce spreading infection in the bone several months or several years after acute salmonellosis (Choi *et al*., 2010).

2.15.4 Host Adaptation

Salmonella enterica, through some of its serotypes such as Typhimurium and Enteriditis, shows signs of the ability to infect several different mammalian host species, while other serotypes such as Typhi seem to be restricted to only a few hosts (Thomson *et al*., 2008). Some of the ways that *Salmonella* serotypes have adapted to their hosts include loss of genetic material and mutation. In more complex mammalian species, immune system, which include pathogen specific immune responses, target serovars of *Salmonella* through binding of antibodies to structures like flagella. Through the loss of the genetic material that codes for a flagellum formation, *Salmonella* can evade a host's immune system (Pang *et al*., 2011). In the study conducted by Kisiela *et al.* (2012) more pathogenic serovars of *S. enterica* were found to have certain adhesins in common that have developed out of convergent evolution. This means that, as these strains of *Salmonella* have been exposed to similar conditions such as immune systems, similar structures evolved separately to negate

these similar, more advanced defenses in hosts.

There are still many questions about the way that *Salmonella* has evolved into so many different types but it has been suggested that *Salmonella* evolved through several phases. As Baumler *et al.* (1998) have suggested, Salmonella most likely evolved through horizontal gene transfer, formation of new serovars due to additional pathogenicity islands and through an approximation of its ancestry. So, *Salmonella* could have evolved into its many different serotypes through gaining genetic information from different pathogenic bacteria. The presence of several pathogenicity islands in the genome of different serotypes has lent credence to this theory (Bäumler *et al*., 1998).

2.15.5 Treatment of infection with *Salmonella* species

Antibiotics are usually not given because they tend to induce carrier states and to contribute to the development of antibiotic resistant strains. In patients with enterocolitis, broad-spectrum antibiotics rid carriers of organisms but can activate the disease in some carriers by upsetting the balance of intestinal microflora (Jacquelyn, 2005). Fluoroquinolone (e.g Ciprofloxacin), a broad-spectrum cephalosporin or chloramphenicols are the antibiotics of choice in treating typhoid fever (Jacquelyn, 2005).

2.15.6 Prevention of Salmonellosis and enterocolitis

Prevention defends on the maintenance of sanitary water and food supplies and eradication of organisms from carriers (Jacquelyn, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Identification of the Plant Material

Root and Stem bark of the plant (*Strychnos innocua*) were collected at Dankama Village of Kaita Local Government Area in Katsina State. The samples were taken to the Herbarium Unit in the Department of Biological Sciences, Ahmadu Bello University, Zaria for authentication.

3.1.1 Extraction of the Plant Samples

The samples (stem and root) bark collected were air dried under shade for about three weeks and then separately ground into fine powder using pestle and mortar. About 930g of the powdered stem bark and 226g of the root bark were weighed into 4500ml and 1000ml of methanol respectively and then extracted using cold maceration. The set-ups were kept for two days in tightly sealed vessels at room temperature and were stirred several times daily using sterile glass rod. The mixtures were then filtered through muslin cloth. Further extraction of each of the residue were done for about 3 times until a clear colorless supernatant extracted liquid were obtained indicating that no more extraction from the plant materials was possible. The extracted liquid was then subjected to evaporation in a water bath (40^{o}C) to remove the solvent. The semi-

solid extracts produced were then kept under a ceiling fan to dry. The extracts were then weighed and percentage yield obtained were then calculated. Sequential extraction of the crude methanolic extracts were done with ethanol and then water to obtain ethanolic and aqueous crude extracts of the plant parts respectively. Five grams of each of the extracts were taken and used for susceptibility test and the remaining were used for the phytochemical screening (Olonitola *et al.*, 2010).

3.1.2 Phytochemical Screening of the root and stem bark of *Strychnos innocua*

Standard phytochemical tests were carried out on the crude extracts using the method of Trease and Evans (1983) to determine the presence of carbohydrates, anthroquinone, cardiac glycosides, saponins, steroids and triterpens, flavonoids and alkaloids. About 0.5g of each of the extracts were weighed and put into test tubes and then 15ml of water was then put into each test tube and dissolved to obtain clear extracts of the filtrate.

3.1.2.1 Test for Carbohydrates

Few drops of Molisch's reagent were added to a little quantity of a clear solution of the extract dissolved in distilled water followed by the addition of concentrated sulphuric acid down the side of the test tube so that the acid formed a layer beneath the aqueous layer. The mixture was then allowed to stand for about two minutes and then diluted with five milliliters of water. Formation of red colour at the interphase of the two layers confirmed the presence of carbohydrates (Trease and Evans, 1983).

3.1.2.2 Test for Glycosides

Five milliliters of concentrated sulphuric acid was added to the clear solution of the extract and boiled for about 15 minutes. It was then cooled and neutralized with 20% KOH and then

divided in to two portions. Another part of the extract was dissolved in distilled water; this was used as a control.

3.1.2.3 Test for Phenolic Compounds

Drops of ferric chloride solution were added to one portion and the aqueous portion. Green to black precipitate indicated phenolic aglycone as a result of hydrolysis of the glycoside (Trease and Evans, 2002).

3.1.2.4 Test for Anthraquinones derivatives

About five gram of each plant extract was shaken with ten milliliters benzene, filtered and then five milliliters of 10% ammonia solution was added to the filtrate and stirred. The mixture was then shaken. The presence of a pink, red or violet colour in the ammoniacal (lower) phase indicates the presence of free hydroxyl-anthraquinones (Trease and Evans, 2002).

3.1.2.5 Test for Cardiac Glycosides

About 0.5g of each of the extracts was dissolved in two milliliters of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayed with one milliliter of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of carenolides. A violet ring appeared below the brown ring while in the acetic acid layer; a greenish ring formed just above the brown ring and gradually spread throughout this layer (Trease and Evans, 2002).

3.1.2.6 Test for Saponins

Small quantity of the extracts was dissolved in ten milliliters of distilled water. It was then shaken vigorously for about 30 minutes. A honey comb formed that last for more than 30 minutes indicated saponin (Trease and Evans, 1983).

3.1.2.7 Test for Steroid and Triterpens

Equal volume of acetic anhydride was added to each of the extracts. About one milliliter of concentrated sulphuric acid was then added down the side of the test tubes, a colour change that was observed immediately which indicates the presence of steroids and triterpens. Red, pink or purple colour indicated the presence of triterpenes while blue or blue-green indicated steroids (Trease and Evans, 1983).

3.1.2.8 Test for Flavonoids

Few drops of sodium hydroxide were added to about five milliliters of clear solution of each of the extracts. A yellow colour indicated the presence of flavonoids (Trease and Evans, 1983).

3.1.2.9 Test for Tannins

About five grams of each portion of plant extracts was stirred with ten milliliters of distilled water, filtered and then ferric chloride reagent was added to the filtrates. A blue-black, green or blue-green precipitate was taken as evidence for the presence of tannins (Trease and Evans, 2002).

3.1.2.10 Test for Alkaloids

Few drops of Wagner's reagent were added to a small amount of each of the extracts solution. Whitish precipitate indicated the presence of alkaloids (Trease and Evans, 1983).

3.1.3 Preparation of the Test Concentrations of the Extracts

Stock solution was prepared using three gram of the solid plant extracts dissolved in ten milliliters of normal saline making a stock of 300mg/ml. Serial doubling dilution was then employed to obtain concentrations of 150mg/ml, 75mg/ml and 37.50mg/ml respectively.

3.2 Approval for the Collection of Clinical Isolates

An approval (Appendix I) for the collection of the clinical isolates was obtained from the management of University health services, Ahmadu Bello University, Main Campus, Zaria.

3.3 Collection and Reconfirmation of the Clinical Isolates

Confirmed clinical isolates of *Salmonella* species and *Escherichia coli* were collected from University health services (Sick bay). The isolates collected were sub-cultured several times on MacConkey agar. Discrete colonies were picked and reconfirmed following standard bacteriological procedures. Stock cultures were maintained on nutrient agar slants in a refrigerator at 4°C in the Department of Microbiology, A.B.U Zaria.

3.3.1 Biochemical characterization

Discrete colonies obtained from the reconfirmation of the isolates were subjected to biochemical characterization as described by Purkayastha *et al.* (2010).

3.3.1.1 Indole test

Indole test was carried out by inoculating each of the isolates of *Salmonella* spp *and Escherichia coli* into 1% peptone water and then incubated at 37ºC for 24 hours. After 24 hours of incubation, 3 drops of Kovac's reagent were added, shaken and examined. A positive reaction was indicated by the development of a red colour in the reagent layer above the broth while a negative reaction was indicated by a yellow colour.

3.3.1.2 Methyl red - Voges Proskauer test

Test tubes containing 5ml of MR-VP broth each were inoculated with the colony of the isolates of *Salmonella* spp *and Escherichia coli* using wire loop. The tubes were incubated at 37ºC for

48hrs. After incubation, 3mls of the cultured broth was transferred to a small test tube to which three drops of methyl red indicator was added. Formation of red colour on addition of the indicator signifies a positive methyl red test and a yellow colour signifies a negative test.

To the remaining of the cultured broth, three drops of 40% potassium hydroxide (KOH) were added followed by the addition of five drops of 5% α-naphthol in ethanol. The mixture was shaken and the tubes placed in a sloping position. Development of red colour starting from the liquid-air interface within 15 min indicated a positive VP test and no colour change occured in a VP negative test.

3.3.1.3 Citrate utilization test

This was carried out by inoculating the colonies of each of the isolates of *Salmonella* spp *and Escherichia coli* on Simmons' citrate agar slant and the inoculated slants were then incubated at 37^{o}C for 48hrs and examined for development of a deep blue colour which indicated a positive reaction.

3.3.1.4 Triple Sugar Iron (TSI) test

Tubes of Triple Sugar Iron Agar (TSI Agar) were inoculated by stabbing the butt and streaking the slope. The tubes were then incubated at 37^{o}C for 24 hours. After incubation, the tubes were observed for colour change (from red to yellow), gas production and blackening of the butt. Red slant (Alkaline) indicated lactose or sucrose not fermented, yellow slant (acidic) indicated lactose or sucrose fermented, red butt indicated that glucose was not fermented and yellow butt indicated glucose fermentation. While bubbles or cracks in the medium indicated gas production, blackening of the butt indicated H_2S production.

3.3.1.5 Urease Test

Urease test was carried out by inoculating urea agar slant with each of the colonies of the isolates of *Salmonella* spp *and Escherichia coli* and the slant was then incubated at 37ºC for 24hrs and examined for the development of bright pink or red colour which indicates a positive reaction.

3.3.1.6 Motility Test

This test was carried out by inoculating the motility medium with the colony of the isolates of *Salmonella* spp *and Escherichia coli*. A stab was made with a straight wire to a depth of about one third the total volume of the medium. The cultures were then incubated at 37ºC for 24hrs. If the culture turned cloudy (turbid) after incubation, it means the organism was motile but if growth was restricted to the line of inoculation and the rest of the culture remained clear, then the organism was non-motile.

3.4 Standardization of the test bacteria

The clinical isolates collected were then standardized following bacteriological standard.

3.4.1 Preparation of Mcfarland standard

The Mcfarland standard 0.5 was prepared by mixing 9.95ml of 1% H_2SO_4 and 0.05ml of 1% $BaCl_2.2H_2O$. One percent H_2SO_4 was prepared by mixing 1ml of conc. H_2SO_4 with 99ml of distilled water. On the other hand, 1% $BaCl_2.2H_2O$ was prepared by dissolving 1g of solid $BaCl_2.2H_2O$ in 100ml of distilled water (Olonitola *et al*., 2010).

3.4.2 Standardizing the test bacteria

The inoculum for sensitivity testing was prepared from a broth that has been inoculated and incubated at 37^0C for 24 hours. The turbidity of each of the bacterial suspension was prepared to match 0.5 Mcfarland by placing a loop full of each of the isolates into 5ml of distilled water and comparing the turbidity with that of the 0.5 McFarland standard. This turbidity corresponds to 1.5×10^8CFU/ml of bacterial suspension (Olonitola *et al*., 2010).

3.5 Screening for Antibacterial Activity of the Crude Extracts

The antibacterial activities of each of the crude extracts were determined using standard method as described by Olonitola *et al*. (2010).

3.5.1 Antibacterial Activity of the Extracts against the test bacteria

Within 15mins after adjusting the turbidity of the inoculum suspension to the 0.5 Mcfarland standard (from above in the standardization of the test organisms), one milliliter of each of the standardised suspensions of the isolates were transferred onto the Mueller-Hinton agar plates and spread over the entire surface of the agar with sterile bend glass. A sterile cork borer (6mm) was then use to bore wells in the media and 0.2 ml of the concentrations of each of the crude extracts were aseptically added to the holes in separate plates and the plates were allowed to stand on the bench for one hour before incubating at 37ºC for 24hrs. Antibiotic discs (Amoxicillin 30µg, Gentamicin 30µg and Ciprofloxacin 5µg) were gently placed on another Mueller Hinton agar plate seeded with the isolates to serve as positive control. Zones of inhibition were measured using transparent meter rule to the nearest milliliter and the zones of inhibition of the antibiotic standards were interpreted as susceptible, intermediate or resistant based on CLSI guideline, (2015).

3.6 Fractionation of the Ethanolic Extract

3.6.1 Column Chromatography

The ethanolic extract of the stem bark being the one with the best antibacterial susceptibility test result among the methanolic, ethanolic and aqueous crude extracts of the plant parts was subjected to column chromatography to separate the extract into its component fractions. Wet packing method was used in preparing the silica gel column as described by Ode *et al*. (2011). Silica gel 60-200 mesh was used as the stationary phase while varying solvent combinations of increasing polarity were used as the mobile phase. In the setting up of the column chromatography, the lower part of the glass column was stocked with cotton wool with the aid of glass rod. The slurry was prepared by mixing 50 g of silica gel and 100 ml of n-hexane and was then poured down carefully into the column. The tap of the glass column was left open to allow free flow of solvent into a conical flask below. At the end of the packing process, the tap was closed. The column was then allowed to stand for 24 h to stabilize, after which, the clear solvent on top of the silica gel was allowed to drain down to the silica gel meniscus. The sample was prepared in a ceramic mortar by adsorbing 3.0 g of the extract to 10 g of silica gel 60-200 mesh. The loosened powdery mixture was then gently layered on top of the column. The column tap was opened to allow the eluent to flow at the rate of 40 drops per minute.

3.6.2 Elution of the Fractions

Elution of the fractions was done with solvent systems of gradually increasing polarity using hexane, chloroform, ethylacetate and methanol. The following ratios of solvent combinations were sequentially used in the elution process: hexane:chloroform 100:0, 80:20, 60:40, 40: 60 and 20: 80, chloroform:ethylacetate 100:0, 80:20, 60:40, 40: 60 and 20: 80; ethylacetate:methanol

100:0, 80:20, 60:40, 40: 60, 20: 80 and 0:100. A measured volume (200ml) of each solvent combination was collected gradually with a 10 ml syringe and sprayed uniformly each time by the sides of the glass into the column and the eluted fractions were collected in aliquots of 10 ml in test tubes and small amount was taken for phytochemical screening.

3.6.3 Analytical Thin Layer Chromatography (TLC) and Pooling of Fractions

This was done as described by Ode *et al.* (2011). The content of each test tubes were spotted on pre-coated (silica gel F254) glass plates in a small chromatographic tank to separate the different fractions based on their relative mobilities in solvent systems and colour reactions with ultra-violet light. The fractions were concentrated in a rotary vaporator at 40^{o}C, and 210 milibar. The masses of the different fractions were then determined. A strip of the precoated silica gel was then cut out. With 1.0ʎ micro pipette, a spot of the sample was applied on the plate about 1.0 cm from the edge. It was then dried using hot air dryer. The strip was then lowered into a small chromate graphic jar containing the solvent system. The jar was covered with a glass lid. The solvent was allowed to ascend until the solvent front is about ¾ of the length of the strip. The strip was then removed, dried and then sprayed with P-anisaladehyde (general spray) and then air dried by a hot air dryer and then viewed. Other prepared plates were also sprayed using specific sprays, air dried by a hot air dryer and then viewed.

Fractions with the same band profile from the result of the thin layer chromatography were pooled to become the same fractions and the eluents were then subjected to bioassay test.

3.7 Antibacterial Activity of the Individual Fractions

The test isolates were adjusted to 0.5 Mcfarland standard. One milliliter of each of the standardized suspension of the isolates was transferred onto the Mueller-Hinton agar plates and spread over the entire surface of the agar with sterile bent glass rod. A sterilized cork borer

(6mm) was then used to bore wells in the media and 0.2 ml of the various concentrations of each of the individual fractions were aseptically added to the holes in separate plates and the plates were allowed to stand on the bench for one hour before incubation at 37^{o}C for 24hrs.

3.8 Determination of Minimum Inhibitory Concentration of the Crude Extracts

The crude extract which showed significant zone of inhibition was subjected to determination of MIC using standard method of Wariso and Ebong as described by Olonitola *et al*. (2010). The lowest concentration of the crude extracts from the susceptibility test that showed growth inhibition of isolates was serially diluted in the test tubes containing Mueller Hinton broth. The isolates were then inoculated into the test tubes and incubated at 37^{o}C for 24hrs. The MIC was determined as the lowest concentration of the extracts that showed no visible turbidity.

3.9 Determination of Minimum Bactericidal Concentration of the Crude Extracts

A sterilized wire loop was dipped into the test tubes that did not show visible growth (turbidity) in the minimum inhibitory concentration test and a loopful was taken and streaked on a sterile nutrient agar plates. The plates were then incubated at 37^{o}C for 24hrs after which the MBC was taken as the lowest concentration of the extract that did not show any bacterial growth.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of the Crude Extracts of *Strychnos innocua*

The weight of the methanolic extract of the stem bark of *Strychnos innocua* was 61.68g (6.63%), ethanolic extract of stem bark of the plant was 42.75g (4.60%) and aqueous extract of its stem bark was 46.10g (5.00%) as shown in Table 4.1. Likewise, the weight of the methanolic extract of root bark of *Strychnos innocua* was 30.57g (13.53%), ethanolic extract of root bark of the plant was 17.79g (7.87%) and aqueous extract of its root bark was 10.26g (4.54%) as shown in Table 4.1

4.2 Phytochemical Constituents of Stem and Root bark Extracts of *Strychnos innocua*

The Phytochemical screening of the stem and root bark extracts of *Strychnos innocua* revealed the presence of different phytochemical constituents (Table 4.2). The extracts of *Strychnos innocua* contained a number of phytochemicals such as Carbohydrate, glycosides, anthraquinones, cardiac glycosides, saponins, steroids and triterpens, flavonoids, tannins and alkaloids in methanolic, ethanolic and aqueous extracts of the root bark but anthraquinones were absent in methanolic and aqueous extracts of the root bark while glycosides and tannins were absent in aqueous extract of the stem bark.

Table 4.1: Percentage yield of the stem and root bark crude extracts of *Strychnos innocua*

	Weight of plant Material (g)	Weight of extract obtained (g)	Percentage yield (%)
Stem bark			
Methanolic extract	930.00	61.68	6.63
Ethanolic extract	930 .00	42.75	4.60
Aqueous extract	930.00	46.10	5.00
Root bark			
Methanolic extract	226.00	30.57	13.53
Ethanolic extract	226.00	17.79	7.87
Aqueous extract	226.00	10.26	4.54

Table 4.2: Phytochemical Constituent of the Stem and Root bark extracts of *Strychnos innocua*

Secondary metabolite	Name of the test	Stem bark			Root bark		
		ME	EE	AE	ME	EE	AE
Carbohydrate	Molish test	+	+	+	+	+	+
Glycosides	Ferric chloride test	+	+	-	+	+	+
Anthraquinones	Borntrager's test	+	+	+	-	+	-
Cardiac glycosides	Keller-Killiani test	+	+	+	+	+	+
Saponins	Frothing test	+	+	+	+	+	+
Steroid	Lieberman-Burchard test	+	+	+	+	+	+
Flavonoids	Sodium hydroxide test	+	+	+	+	+	+
Tannins	Ferric chloride test	+	+	-	+	+	+
Alkaloids	Wagner's test	+	+	+	+	+	+

Key: '**+**' = Present; '**-**' = Absent; **ME** = Methanolic extract; **EE =** Ethanolic extract; **AE =** Aqueous extract

4.3 Thin Layer Chromatography

The result of the thin layer chromatography of the stem and root bark extracts of *Strychnos innocua* separated by a combination of Chloroform and Methanol in the ratio of 9:1 as mobile phase showed several bands of phytochemical constituents when detected using general spray (P-anisaldehide-sulphuric acid spray) (Appendix II). Specific spray using Aluminum chloride that showed yellow fluorescence under UV lamp is a confirmation for flavonoids as shown in Appendix III.

Appendix IV to Appendix VII showed specific sprays for the confirmation of anthraquinones, phenolic compounds, steroids and alkaloids using Bontragen's, Ferric chloride, Lieberman buchard and Dragendorff sprays which revealed yellow or pink spot; blue-black or green colour; blue, blue-green or violet colour; and orange spot respectively.

4.4 Antibacterial Activities of the Crude Extracts of *Strychnos innocua*

The antibacterial activities of the crude (Methanolic, Ethanolic and Aqueous) extracts of *S. innocua* (stem and root bark) were studied at different concentrations (300 mg/ml, 150 mg/ml, 75 mg/ml and 37.5 mg/ml) against the clinical isolates of *Salmonella* species and *Escherichia coli*. All the crude extracts of the stem and root bark of *S.innocua* showed an increase in zone of inhibition with increase in concentration of the extract on the isolates (Appendix VIII).

Table 4.3 and Table 4.4 showed the sensitivity of the test bacteria and the zones of inhibition values for the standard antibiotics [Ciprofloxacin (5 µg), Gentamicin (30 µg) and Amoxicillin (30 µg)] against the clinical isolates. The test bacteria were found to be more susceptible to the Ethanolic extract of the stem bark with a maximum inhibitory zone of 24 mm, followed by Methanolic extract of the stem bark with inhibition of 23mm while aqueous extracts showed least susceptibility with maximum zone of inhibition of 13 mm. Aqueous extract did not show

any inhibitory activity against the test bacteria at concentrations below 300mg/ml and 150mg/ml as shown in Table 4.3 and Table 4.4 respectively. The results obtained showed that Ethanolic extract of the stem bark had best antibacterial activity against the tested bacteria, followed by Methanolic extract of the stem bark and then Aqueous extract.

Table 4.3: Antibacterial activities of the Methanolic, Ethanolic and Aqueous Extracts of the Stem and Root Bark of *Strychnos innocua* against *Escherichia coli*

Extracts	Concentration of extracts (mg/ml)				Standard Antibiotics		
	300	150	75	37.5	C (5µg)	G (30µg)	A (30µg)
	Zone of inhibition (mm)						
					21(S)	21(S)	14(S)
SBM	23	18	16	12			
SBE	24	21	16	12			
SBA	13	0	0	0			
RBM	14	12	11	8			
RBE	12	12	11	10			
RBA	12	11	0	0			

Key: Stem bark methanolic (SBM), Stem bark ethanolic (SBE), Stem bark aqueous (SBA), Root bark methanolic (RBM), Root bark ethanolic (RBE), Root bark aqueous (RBA)

Ciprofloxacin (C) : Susceptible (S) ≥ 21, Intermediate (I)(16-20), Resistant (R) (≤ 15) for *Escherichia coli*
Ciprofloxacin (C) : Susceptible (S) ≥ 31, Intermediate (I) (21-30), Resistant (R) (≤ 20) for *Salmonella* species
Gentamicin (G) : Susceptible (S) ≥ 15, Intermediate (I) (13-14), Resistant (R) (≤ 12)
Amoxicillin (A) : Susceptible (S) ≥ 18, Intermediate (I) (14-17), Resistant (R) (≤ 13)
(CLSI, 2015).

Table 4.4: Antibacterial activities of the Methanolic, Ethanolic and Aqueous Extracts of the Stem and Root Bark of *Strychnos innocua* against *Salmonella* species

Extracts	Concentration of extracts (mg/ml)	Standard Antibiotics

	300	150	75	37.5	C (5µg)	G (30µg)	A (30µg)
	Zone of inhibition (mm)						
					32 (S)	19 (S)	13 (R)
SBM	17	15	12	10			
SBE	19	17	16	10			
SBA	11	0	0	0			
RBM	13	12	11	10			
RBE	14	13	11	11			
RBA	12	11	0	0			

Key: Stem bark methanolic (SBM), Stem bark ethanolic (SBE), Stem bark Aqueous (SBA), Root bark methanolic (RBM), Root bark ethanolic (RBE), Root bark aqueous (RBA)

Ciprofloxacin (C): Susceptible (S) ≥ 21, Intermediate (I)(16-20), Resistant (R) (≤ 15) for *Escherichia coli*
Ciprofloxacin (C): Susceptible (S) ≥ 31, Intermediate (I) (21-30), Resistant (R) (≤ 20) for *Salmonella* species
Gentamicin (G): Susceptible (S) ≥ 15, Intermediate (I) (13-14), Resistant (R) (≤ 12)
Amoxicillin (A): Susceptible (S) ≥ 18, Intermediate (I) (14-17), Resistant (R) (≤ 13)
(CLSI, 2015).

4.5 Column Chromatography and Pooling of the Fractions

A total of 110 individual fractions were eluted using various combination of solvents by increasing the polarity of the solvents that were used as the mobile phase starting with *n*-hexane : chloroform (5:0, 2:1, 0:5) of which thirty fractions of 10 ml each were collected and combined on the basis of TLC profile to give one major fraction “A” (A: 1–30). Continual elution of the

extract with chloroform : ethyl acetate (4:1, 3:2, 2:3, 1:4) yielded thirty fractions of 10ml each and were combined on the basis of TLC profile to give two major fractions “B” and “C” (B: 31-49, C: 50-57). Elution with ethyl acetate : methanol (5:0, 4:1, 3:2, 2:3, 1:4, 0:5) yielded fifty fractions of 10ml which were combined on the basis of TLC profile to give two major fractions “D” and “E” (D: 58-101, E: 102-110).

The five fractions obtained showed various Retention factor values based on qualitative chromatographic analysis conducted using TLC plate (Table 4.6) of which Fraction “A” has 2 spots (Rf values 0.82, 0.92), Fraction “B” showed 6 spots (Rf values 0.51, 0.62, 0.67, 0.74, 0.81, 0.88), where as 4 spots were found with Fraction “C” (0.48, 0.53, 0.58, 0.77), 6 spots were found with Fraction “D” (Rf values 0.08, 0.19, 0.29, 0.37, 0.48, 0.56) and 1 spot was found in Fraction “E” (Rf value 0.03).

Table 4.5: Retention Factor Values of the Fractions of Ethanolic Extract of the Stem Bark of *Strychnos innocua* based on Phytochemical Test.

Fraction	Spot	Rf value
A	1st	0.82
	2nd	0.92

B	1st	0.51
	2nd	0.62
	3rd	0.67
	4th	0.74
	5th	0.81
	6th	0.88
C	1st	0.48
	2nd	0.53
	3rd	0.58
	4th	0.77
D	1st	0.08
	2nd	0.19
	3rd	0.29
	4th	0.37
	5th	0.48
	6th	0.56
E	1st	0.03

Fraction A: hexane, Fraction B: hexane/chloroform, Fraction C: chloroform/ethyl acetate, Fraction D: ethyl acetate/methanol, Fraction E: methanol

4.6 Susceptibility Test of Clinical Isolates to Individual Fractions

All the subfractions of the ethanolic extract of the stem bark had moderate antibacterial activity with zone of inhibition ranging from 11mm to 15 mm (Table 4.7). Despite the fact that hexane fraction showed no activity when tested against the clinical isolate of *Salmonella* species, it showed moderate activity against the clinical isolate of *Escherichia coli* with zone of inhibition of 14mm. Hexane/chloroform fraction had best activity against all the clinical isolates

(*Escherichia coli* and *Salmonella* species) with zones of inhibition of 15mm and 11mm respectively. The chloroform/ethyl acetate fraction had significant antibacterial activity against *Escherichia coli* and *Salmonella* species with zones of inhibition of 13mm and 12mm respectively. Ethyl acetate/methanol fraction and methanol fraction had moderate activity against *Escherichia coli* and *Salmonella* species with zone of inhibitions of 11mm and 12mm respectively but the two fractions (Ethyl acetate/methanol fraction and methanol fraction) showed no activity when tested against the clinical isolate of *Salmonella* species.

Table 4.6: Susceptibility Pattern of Clinical Isolates to Individual Fractions at 50mg/ml

Fraction	Clinical isolate	Zone of inhibition (mm)

A	*Escherichia coli*	14
	Salmonella species	0
B	*Escherichia coli*	15
	Salmonella species	11
C	*Escherichia coli*	13
	Salmonella species	12
D	*Escherichia coli*	11
	Salmonella species	0
E	*Escherichia coli*	12
	Salmonella species	0

Fraction A: hexane, Fraction B: hexane/chloroform, Fraction C: chloroform/ethyl acetate, Fraction D: ethyl acetate/methanol, Fraction E: methanol

4.7 Minimum inhibitory and Minimum bactericidal concentrations of Aqueous, Methanolic and Ethanolic Extracts of the Stem and Root Bark of *Strychnos innocua* against *Escherichia coli* and *Salmonella* species

Tables 4.8 and Table 4.9 shows the MIC of methanolic, ethanolic and aqueous extract of the stem and root bark of *S. innocua* against *Escherichia coli* and *Salmonella* species. The result revealed that the MIC of the methanolic, ethanolic and aqueous extracts of the stem bark against *Escherichia coli* were 37.5mg/ml, 37.5mg/ml and 300mg/ml respectively. However, the MBC result of the stem bark extract showed that both Methanolic and Ethanolic extracts had 75mg/ml but there was no activity in the aqueous extract.

In addition, the result also revealed that the MIC of the Methanolic, Ethanolic and Aqueous extracts of the root bark against *Escherichia coli* were 150mg/ml, 75mg/ml and 150mg/ml respectively. However, the MBC result showed that only Ethanolic extract had an MBC value of 150mg/ml while Methanolic and Aqueous extracts had no activity.

Moreover, the result revealed that the MIC of the Methanolic, Ethanolic and Aqueous extracts of the stem bark against *Salmonella* species were 150mg/ml, 75mg/ml and 300mg/ml respectively. However, the MBC result revealed that only Ethanolic extract had an MBC value of 150mg/ml while Methanolic and Aqueous extracts showed no activity.

Similarly, the MIC of the Methanolic, Ethanolic and Aqueous extracts of the root bark against *Salmonella* species were 75mg/ml, 75mg/ml and 150mg/ml respectively while the MBC test revealed that only Methanolic and Ethanolic extracts had MBC value of 300mg/ml and 150mg/ml respectively while Aqueous extract showed no activity.

Table 4.8: Minimum Inhibitory and Minimum Bactericidal Concentrations of the Stem and Root Bark Extracts of *Strychnos innocua* against *Escherichia coli*

Extract	MIC (mg/ml)	MBC (mg/ml)
SBA	300	> 300
SBE	37.5	75
SBM	37.5	75
RBA	150	> 300
RBE	75	150
RBM	150	> 300

Key: Stem bark aqueous (SBA), Stem bark ethanolic (SBE), Stem bark methanolic (SBM), Root bark aqueous (RBA), Root bark ethanolic (RBE), Root bark methanolic (RBM).

Table 4.9: Minimum Inhibitory and Minimum Bactericidal Concentrations of the Stem

and Root Bark Extracts of *Strychnos innocua* against *Salmonella* species

Extract	MIC (mg/ml)	MBC (mg/ml)
SBA	300	> 300
SBE	75	150
SBM	150	> 300
RBA	150	> 300
RBE	75	> 300
RBM	75	150

Key: Stem bark aqueous (SBA), Stem bark ethanolic (SBE), Stem bark methanolic (SBM), Root bark aqueous (RBA), Root bark ethanolic (RBE), Root bark methanolic (RBM)

CHPTER FIVE

5.0 DISCUSSION

The need for new, effective and affordable drugs to treat microbial diseases in the developing world is one of the issues facing global health today. Available drugs for the treatment of these diseases are limited by factors ranging from microbe resistance to safety, compliance and cost. The use of medicinal plants for curative purposes is as old as mankind and can be traced from the beginning of civilization. The interaction between man and his environment is such that his food, shelter, the diseases that afflict him and the cure of such diseases are all within the environment. As a result, medicinal plant research has now got a momentum among the scientists of the world. It is evident from the literature that the genus *Strychnos* consists of about 200 species and is known for therapeutic role as antimicrobials (Verpoorte *et al.*, 1983; Mallikharjuna and Seetharam, 2009).

The result of this study reveals the *in vitro* activity of ethanolic, methanolic and aqueous extracts of stem and root bark of *Strychnos innocua* based on evaluation of antibacterial activity against the clinical isolates of *Salmonella* species and *Escherichia coli*.

The phytochemical screening of the stem and root bark extracts of *Strychnos innocua* revealed the presence of carbohydrates, glycosides, anthraquinones, cardiac glycosides, saponins, steroids, flavonoids, tannins and alkaloids in the extracts. These compounds have potentially significant application against human pathogens, including those that cause enteric infections (El-Mahmood *et al*., 2008) and this might be responsible for the antibacterial activities observed in the study which also agree with the findings of (Fessenden, 1982; Olonitola *et al.*, 2010; Kavita *et al.*, 2014; Njiga *et al.*, 2014; Sudhira *et al.,* 2015) that phytochemical constituents present in the plant are responsible for their biological activity. The presence of glycosides moieties like saponins, anthraquinones, cardiac glycosides and flavornoids which are known to inhibit tumor

growth and serve also to protect against gastrointestinal infections are of pharmacognostic importance and give credence to the use of the plant in ethnomedicine (El-Mahmood, 2009). However, this result disagrees with the previous work of Ugoh *et al.* (2013) in which anthraquinones were absent in the aqueous and ethanolic extract of stem bark and also glycosides were absent in methanolic and aqueous extracts of the stem bark of *Strychnos spinosa*. The observed variation might be due to difference in species used and the change in location in which the plants were grown.

The large inhibition zone sizes produced by the plant extracts (stem and root bark extracts of the *Strychnos innocua*) against the test bacteria, especially the ethanolic extract of the stem bark is an indication of the potency of the bioactive components of the plant against all the test bacteria. This agree with the work of Mity and Tom (2015) and Sudhira *et al*. (2015) that reported the antimicrobial activity of crude extracts of *Strychnos* species such as *S. nux-vomica* and *S. colubrina* respectively.

Higher antibacterial activity was recorded for stem bark extract compared to the root bark extract. This might be due to the fact that the phytochemical constituents that might be responsible for the antibacterial activity of the plant parts are produced for protection in response to herbivores, pathogens or competitors and the stem bark is more expose to these factors than the root bark. Consequently, it might produce more active phytoconstituents than the root bark. The variations observed in the potency of the plant parts are in conformity with the reports of Duke (1992) and Yusha'u *et al.* (2008) who reported that antibacterial activity may vary from one plant part to another. It is also in agreement with the work of Bibitha *et al.* (2002) who reported variation in the antibacterial activities of different plant extracts.

The order of the antibacterial efficacy is Ethanol extract > Methanol extract > Aqueous extract. This might be due to the fact that aside being polar, ethanol also has a non-polar end which makes it to dissolve non-polar substances including most essential oils and numerous flavoring, coloring and medicinal agents (Windholz, 1976). This is a clear indication that the solvent system plays a significant role in the solubility of the active principles in the plant and influences the antibacterial activities. This can be explained in terms of the polarity of the compound being extracted by each solvent and in addition to their intrinsic bioactivity, their ability to dissolve or diffuse in the media used in the assay.

This result agrees with the work of Maher *et al*. (2012) which shows that ethanol extract of nine medicinal plant species, namely: *A. membranacea*, *A. hygrophilum*, *A. discoridis*, *G. tournefortii*, *L. varius*, *M. autumnalis*, *P. argentea*, *R. chalepensis* and *U. piluliferа*has revealed a wide antibacterial spectrum against most test bacterial strain than the methanolic extract. These findings are consistent with those obtained in some past studies (Ahmad *et al.*, 1998; Lin *et al.*, 1999; Eloff and McGaw, 2014). In addition, the results obtained indicate that methanol stands as the second effective solvent after ethanol. Similar findings have been reported by Leven *et* al. (1979), Naovi *et al.* (1991), Heisey and Gorham, (1992). However, this result disagrees with the previous work of Ezeigbo *et al*., (2016) who reported that methanol extracts of the *Garcinia kola* and *Cola nitida* showed a wider spectrum of activity than ethanol and aqueous extracts. This might be due to differences in the constituents of the different plants and hence, the difference in extracting the components by the solvents and thereby affecting the activity as earlier addressed by Abayomi (2008).

The antibacterial activity was observed at concentration of 37.5mg/ml and above. The highest zone of inhibition (24mm and 23mm respectively) were recorded at a concentration of 300mg/ml

and the aqueous extract started inhibiting the growth of the clinical isolates at the concentration of 150mg/ml. However, all the extracts did not show inhibition at a concentration lower than 37.5mg/ml. This observation might be due to the fact that the concentration of the phytoconstituents in the extracts at that level was not enough to inhibit bacterial growth. This result corroborates the work of Kanife and Odesanmi (2012) who reported that the ethanol extract of the leaf of *Panicum maximum* had no activity on *Candida albicans* even at concentration of 150mg/ml.

The results also showed that *Escherichia coli* were more susceptible to the extracts than the *Salmonella* species. The effects of the crude extracts correlate with the reports that microorganisms varied widely in their degree of susceptibility (Emeruwa, 1982; Banso and Mann, 2006). This is in agreement with the work of Kubmarawa *et al*. (2009) who reported *Escherichia coli* to be more susceptible to the ethanolic extract of *Ficus platyphylla* than the *Salmonella* spp. It is also in line with the work of Doss *et al*. (2011) who reported activities of some medicinal plants against *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* at 1000mg/ml but no activity against *Pseudomonas aeroginosa* at the same concentration.

From the results of the susceptibility test of the subfractions, it is evident that the subfractions did not have good activity against the clinical isolates as observed from the extracts. This could be justified by the fact that constituents in plant extract or fractions interacting in a synergistic manner may not be highly active when they are not part of a mixture with synergistic compounds (Maurice *et al.*, 2013). This supports the conclusion of Lewis and Ausubel (2006) that plants contain many compounds with low antimicrobial activity and use other mechanisms to combat microbial growth.

All the subfractions obtained from the ethanolic extract fraction of the stem bark had inhibitory action against the clinical isolates of *Escherichia coli* and *Salmonella* spp. Of these subfractions (hexane/chloroform and chloroform/ethyl acetate) were outstanding because they produced the largest zones of inhibition (11-15mm). The fact that the activity of hexane/chloroform subfraction was greater than that of chloroform/ethyl acetate subfraction may be due to the fact that even though they all carry non-polar phytoconstituents, the former has more phytoconstituents (6 bands) than the latter (4 bands) and the amount of the phytoconstituents in the subfraction might be responsible for the biological activity.

Interestingly, the non–polar fractions (Fraction B and Fraction C) had the highest antibacterial activity amongst all the subfractions. However, this finding did not agree with the findings of Negi and Jayaprakasha (2003) and Voravuthikunchai *et al*. (2005).

In this study the MIC values revealed that ethanol and methanol extracts of the stem bark were the most potent against *E. coli* because both ethanolic and methanolic extracts had an MIC of 37.5 mg/ml while aqueous extract had an MIC of 300mg/ml. The MIC of ethanolic, methanolic and aqueous extracts of the root bark on the *Escherichia coli* was 75mg/ml, 150mg/ml and 150mg/ml respectively. Also, the ethanolic extracts of root bark appeared to be the more effective against *Salmonella* species having an MIC value of 75mg/ml followed by methanolic extract with an MIC value of 150mg/ml while the aqueous extracts of both plant parts showed an MIC value of 300mg/ml and 150mg/ml against *Salmonella* species. The MBC assay revealed that most of the extracts were bactericidal at the concentrations of 75g/ml for *Escherichia coli* and 150mg/ml for *Salmonella* species.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The phytochemical screening of the *Strychnos innocua* showed the presence of carbohydrate, glycosides, anthraquinones, cardiac glycosides, saponins, steroids and triterpens, flavonoids, tannins and alkaloids in methanolic, ethanolic and aqueous extracts of the plant.

In vitro activity testing of ethanolic, methanolic and aqueous extracts of stem and root bark of *Strychnos innocua* indicates less activity against the clinical isolates of *Salmonella* species than

that of *Escherichia coli* with inhibition zones diameters of 19mm and 17mm at a concentration of 300mg/ml as against 24mm and 23mm at the same concentration respectively.

Subfractions of ethanolic extract of the stem bark had antibacterial activity with hexane/chloroform subfraction showing best activity with zone of inhibition of 15mm against *Escherichia coli*.

The MIC values revealed that ethanolic and methanolic extracts of the stem bark was the most potent against the clinical isolates with MIC values of 37.5 mg/ml for *Escherichia coli* and *Salmonella* species while ethanolic extract of root bark extract had MIC of 75mg/ml for the test bacteria. Moreover, the MBC assay revealed that most of the extracts were bactericidal at the concentrations of 75mg/ml for *Escherichia coli* and 150mg/ml for *Salmonella* species.

6.2 Recommendations

In view of the findings in this study, the following recommendations are made:

1. The stem and root bark of *Strychnos innocua* can be use for the treatment of diarrheal infections.
2. Further study is needed to isolate, purify, characterize and elucidate the structure of bioactive compounds present in extracts with promising antibacterial activity with a view to supplementing conventional drug development.
3. Future study should be carried out to determine the toxicity, side effects and pharmacokinetic of the active constituents of the plant extracts.

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APPENDICES

Appendix I: Approval for the collection of clinical isolates from the University Health Services (Sick bay), Samaru, A.B.U Zaria.

UNIVERSITY HEALTH SERVICES

MAIN CAMPUS CLINIC
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA.

Vice Chancellor: PROFESSOR IBRAHIM GARBA; B.Sc (Hons) Geology, M.Sc (Mineral Exploration) ABU, Ph.D Geology, D.I.C (London), FNMGS
Director: DR. (MRS) N.H. MADUGU; MD (st. Petersborg),MPH (ABU), FMCOG.

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Our Ref:

Your Ref: UHS/ADM/S-8

21st April, 2017

Haruna Hamza Mashi (P14SCMC8001)
Department of Microbiology
Ahmadu Bello University
Zaria.

Dear Sir,

Re: Letter of Introduction

Yours on the above subject was received.

I have been directed to convey you the Director's approval to collect samples in the Medical Laboratory of University Health Services for your project/Dissertation work.

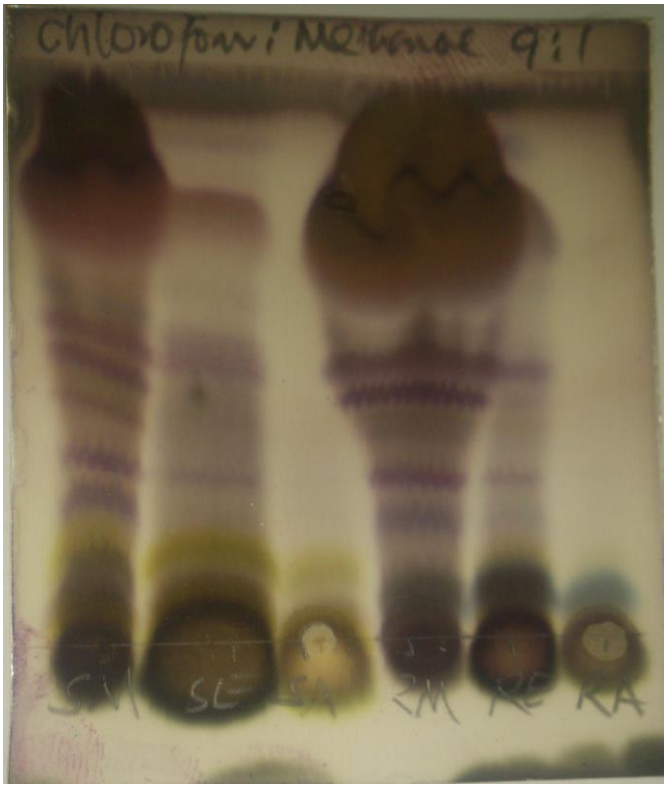
Thank you.

Yours faithfully,

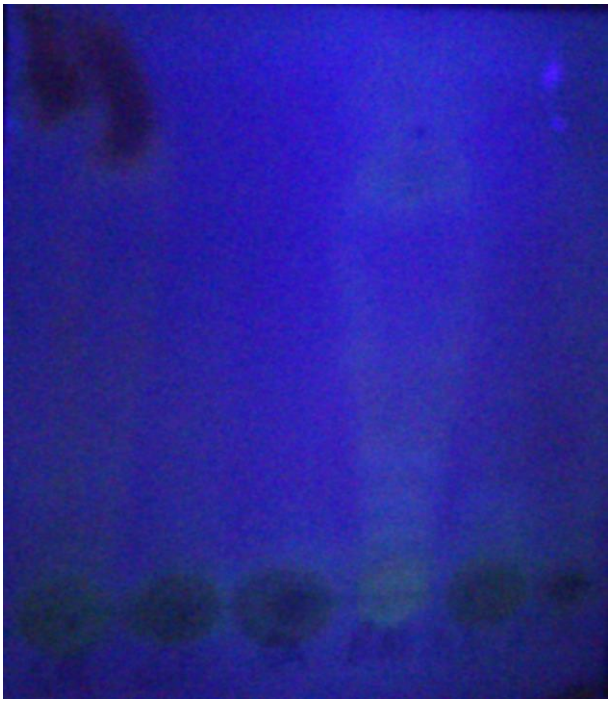
Alh. S.A. Badamasi
For: Admin. Secretary

cc: The Director, UHS
" The Head, Medical Laboratory Unit, UHS
" The SIWES Coordinator, Department of Microbiology, ABU

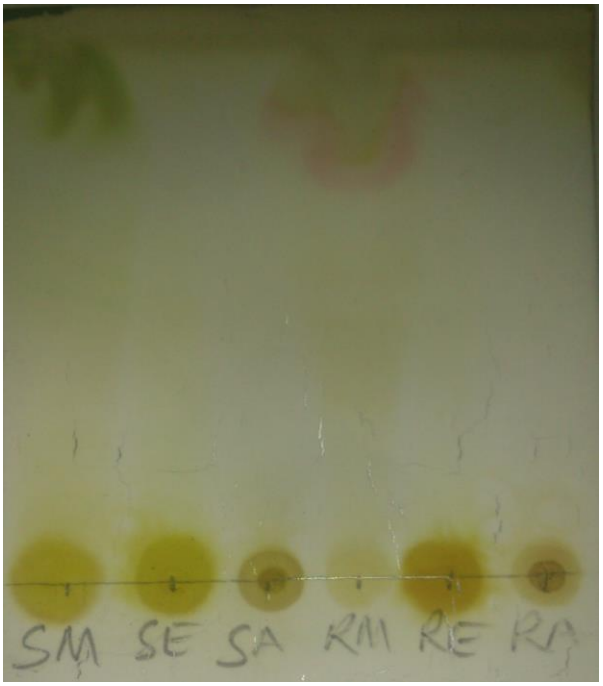
Appendix II: P-anisaldehyde-Sulphuric Acid Spray (General spray)



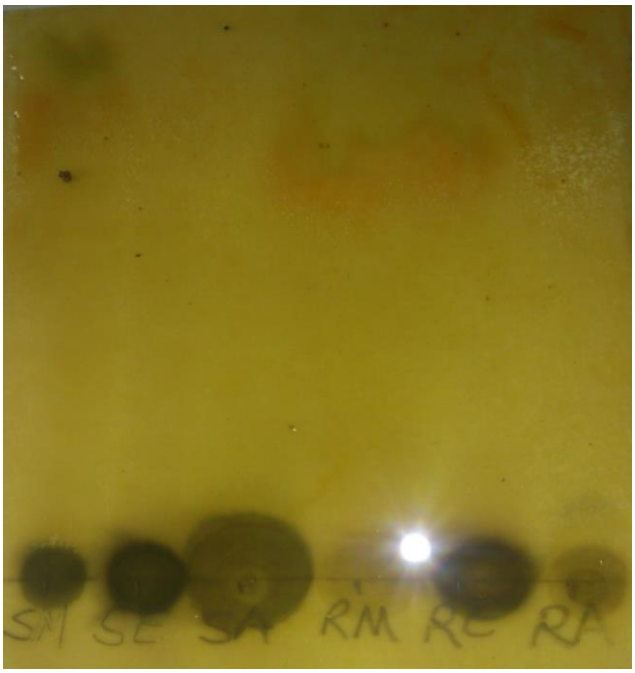
Appendix III: Aluminium chloride spray (for flavonoids)



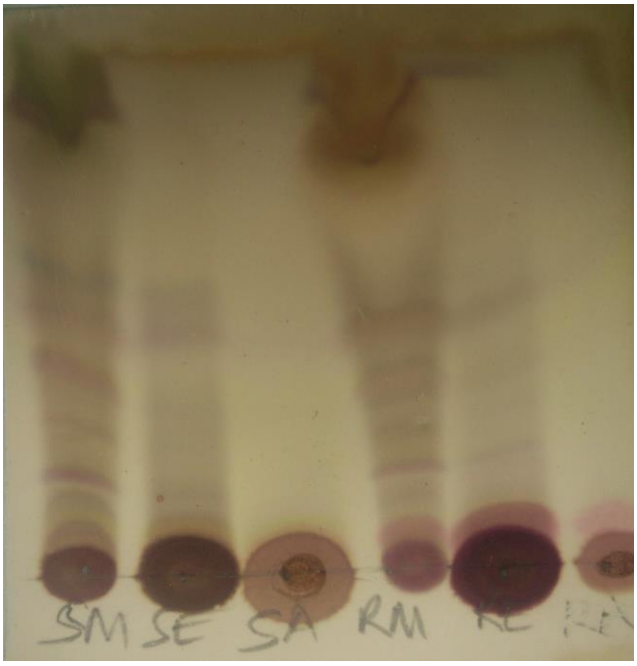
Appendix IV: Bontragens spray (for anthraquinones)



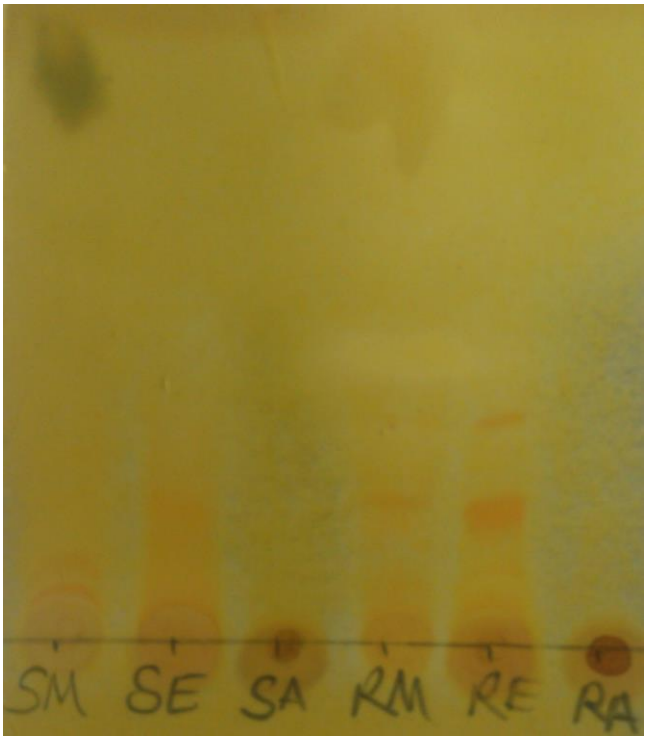
Appendix V: Ferric chloride spray (for phenolic compounds)



Appendix VI: Lieberman buchard spray (for steroids)



Appendix VII: Dragendorff spray (for alkaloids)



Appendix VIII: Plate I-IV: Antibacterial activity of Ethanolic and Methanolic extracts of the stem bark of *Strychnos innocua* against *Salmonella* species and *Escherichia coli* on Mueller Hinton agar using agar well diffusion technique.

Plate I: Antibacterial activity of ethanolic extract against *Escherichia coli*

Plate II: Antibacterial activity of Methanolic extract against *Escherichia coli*

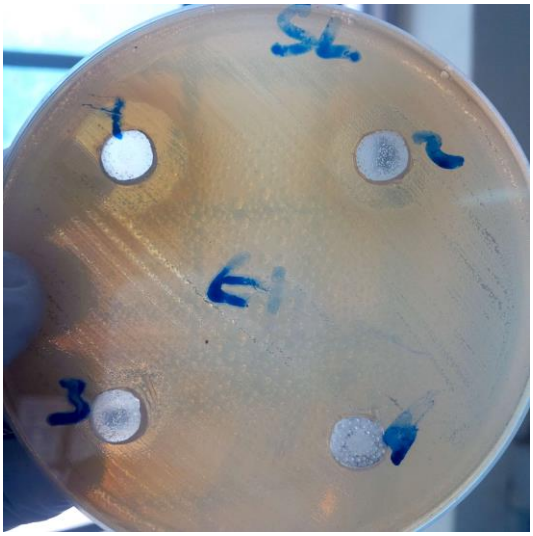


Plate III: Antibacterial activity of ethanolic extract against *Salmonella* species

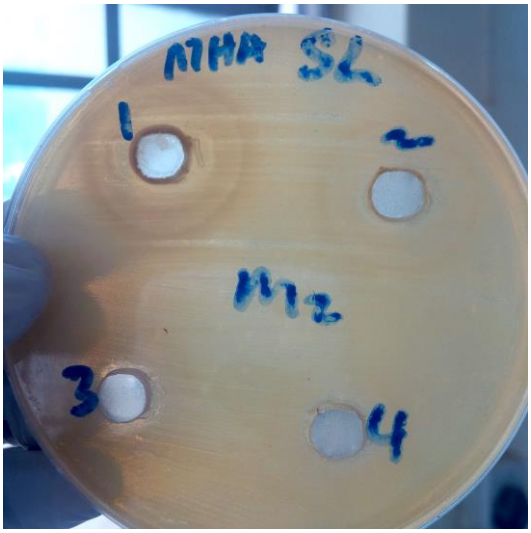


Plate IV: Antibacterial activity of Methanolic extract against *Salmonella* species

Appendix IX: Antibacterial activity of subfraction B (hexane/chloroform fraction) against *Escherichia coli*

Appendix X: Antibacterial activity of subfraction B (hexane/chloroform fraction) against *Salmonella* species


B
SL