IN VITRO AND *IN VIVO* ANTIDIARRHOEAL EFFECTS OF STEM BARK EXTRACT OF *FAIDHERBIA ALBIDA*

BY

MAHMOUD, SULEIMAN JADA

(M.TECH/BC/09/0275)

MARCH, 2014

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THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, SCHOOL OF PURE AND APPLIED SCIENCES, MODIBBO ADAMA UNIVERSITY OF TECHNOLOGY, YOLA. IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF TECHNOLOGY IN BIOCHEMISTRY (M.TECH BIOCHEMISTRY)

MARCH, 2014

DECLARATION

I hereby declare that this thesis was written by me and it is a record of my own research work. It has not been presented before in any previous application for a higher degree. All references cited have been duly acknowledged.

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MAHMOUD, SULEIMAN JADA **DATE**

DEDICATION

I dedicate this work to my parents, Alh. Mahmoud H. Jada and Haj. Amina Mahmoud Jada.

APPROVAL

This thesis entitled " *In vitro* and *In vivo* antidiarrhoeal effects of stem bark extract of *Faidherbia albida"* meets the regulations governing the award of masters of Technology of the Modibbo Adama University of Technology, Yola and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

The *In vitro* and *In vivo* antidiarrhoael effects of stem bark extract of *Faidherbia albida* were investigated. The phytochemical analysis of the crude extract and the most active

fraction of the crude revealed the presence of secondary metabolites like tannin, saponin and alkaloids. The *in vitro* antimicrobial activity of the crude methanolic extract of the stem bark of *Faidherbia albida* was carried out using agar disc diffusion method and the result showed highest activity on *Salmonella typhi* with zone of inhibition of 12.0 ± 0.17mm compared to *E. coli* (11.00 ± 0.12 mm) and *shigella spp* (10.0 ± 0.35 mm). The crude methanolic stem bark extract of *Faidherbia albida* was fractionated using column chromatography. Among the fractions, Fraction III (Ethylacetate/methanol) showed highest activity against the test organism ranging from 14.0 ± 0.06 to 23.0 ± 0.21mm. Thin layer chromatography (TLC) of most active fraction was carried out revealing only one spot with Rf value of 0.89. The spot was scraped and tested on *E.coli* with zone of inhibition of 10.0 ± 0.34mm. Minimum inhibitory concentration of crude extract is 80mg/ml on *E. coli* and *Salmonella typhi* ,100mg/ml on *Shigella spp* while the MIC of most active fraction is 60mg/ml on *E. coli* and *Salmonella typhi* and 80mg/ml on *Shigella spp*. The effect of extract and that of cirprofloxacin has no significance difference ($P<0.05$). The *in vivo* analysis of both the crude extract and the most active fraction of the crude extract was carried out using castor oil-induced diarrhoea model and the result showed positive with 500 mg/kg body weight of the crude maintaining maximal inhibition, while 250 and 500mg/kg body weight of the most active fraction maintaining maximal inhibition throughout the period of the study.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER ONE
INTRODUCTION

1.0 Background of the Study

Diarrhoea is characterized as rapid movement of faecal matter through intestine resulting in poor absorption of water, nutritive elements and electrolytes producing abnormal frequent evacuation of watery stool (Lakshnimanarayana *et al.,* 2011*).* It is often caused by enterotoxins which are produced by bacteria such as *Escherichia coli, Salmonella typhi, Clostridium difficile, Campylobacter jejuni and Vibrio chlorae* (Fernando *et al.,* 2010). These bacteria are commonly infested either by means of polluted water or consumption of contaminated food and by physical contact like handshake (Lakshnimanarayana *et al.,* 2011*).*

Diarrhoea is one of the main causes of high mortality rate in developing countries where over five million children under the age of five die annually from severe diarrhoeal diseases (WHO, 1996). Diarrhoea is mostly common in overpopulated area couple with poor hygiene. It is a major contributor to malnutrition and causes rapid dehydration in infant and elderly people, which could lead to death if not treated (WHO, 1995).

Antidiarrhoeal activity involved the process which may provide a symptomatic relief for diarrhea, while substances that are involved in the process are known as antidiarrhoeal agents. Antidiarrhoeal property of medicinal plants have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, sterols and reducing sugars (Longanga *et at*., 2000).

Faidherbia albida belong to the family; *Mimosaceae* and is widely used in folk medicine in Africa. It is common and widely distributed across Senegal to Northern Nigeria, and extending from Sub-Saharan Africa to Egypt and in East Africa Southward to the Transvaal (Eggeling and Dale., 1952). The common names of the plant include winter thorn and apple-ring acacia. The Hausa people of Northern Nigeria called it “Gawo” while in Fulfulde it is called “Chaski”.

The plant is a large tree, 8 – 15m high in Senegal (Dalziel, 1937) and up to 25m in Nigeria (Keay *et al.,* 1964). The bark is grey, rough deeply fissured, and becomes scaly with age and rich in tannins (Dalziel, 1937). The leaves are bipinnate, blue green with 3 – 12 pairs of pinnae carrying 6 – 23 pairs of leaflets up to 12mm long x 5mm wide, partly overlapping.

Contrary to all other native “acacias” albida sheds its leaves during rainy season and keeps them throughout the dry season.

Barks and roots of the plant, alone or mixed with other components, are common ingredients of traditional medicinal preparation for external or internal usage. These preparations are prescribed for respiratory infections, sterility, digestive problems, dysentery, backache, malaria, fever, heart and circulatory problems, dental infections and deafness (Fagg and Barnes, 1990). Soap is made from the wood ash, which also has depilatory action. Pods can be used as fish bait. Seeds are eaten during famine but require long and elaborate preparation. *Faidherbia albida* has religious significance amongst some tribes, e.g. as a graveyard tree (Fahn *et al.*, 1986).

An infusion or decoction of the plant is made with other plants in Senegal to treat “diangara cayor”, an inclusive term covering many diseases (Tijjani *et al.*, 2008). In Tanganyika and West Africa, a decoction of the plant is taken for diarrhoea and as anti-emetic in fever (Wickens, 1969). The bark in decoction is used to cleanse new wounds, having an action akin to that of potassium permanganate, in the treatment of kidney pains, and mixed with other drugs for madness (Tijjani *et al.*, 2008). In Nigeria, infusion (tea) of the plant is taken for fever, cough and to assist in child birth (Singha, 1965). The Fulanis in Nigeria use it as a portion for chest pain (Jackson, 1973).

1.1 Statement of the Problem

Diarrhoeal disease represents an economic burden for the developing countries. In many nations more than a third of the hospital beds for children are occupied by patients with dirrhoea (WHO, 2009). These patients are often treated with expensive intravenous fluids and sometimes ineffective drugs. Therefore, studies that will bring solutions to this problem will be of great benefit to the world and especially the developing countries.

1.2 Justification of the Study

The *in vivo* antidiarrhoeal property of the plant in rat was reported by Tijani *et al.*, (2008) using aqueous crude extract but based on the available information no work has been done to test the effect of the plant on the causative organisms (*Escherichia coli, Salmonella typhi, Shingella spp*) of diarrhoea. This work was therefore designed to evaluate both the *in vitro* and *in vivo* antidirrhoeal effects of the crude methanolic stem bark extract of *Faidherbia albida* since some components dissolve more in organic solvent than non organic

solvent and also to partially separate the components of the extract in order to pave way in finding the active ingredients responsible for the suspected efficacy if any.

1.3 Aim(s) of the Study

To evaluate the *In vitro* and *In vivo* antidiarrhoeal effects of stem bark extract of *Faidherbia albida.*

1.4 Objectives of the Study

i.) To determine the phytochemical components present in the stem bark of *Faidherbia albida*.

ii.) To determine the effect of the stem bark extract of the plant on *Escherichia coli*, *Salmonella typhi*, *Shingella spp*

iii.) Partial separation of different components of the crude extract.

iv.) To determine the effect of the different fractions of the crude on *Escherichia coli*, *Salmonella typhi*, *Shingella spp*

v.) *In vivo* antidiarrhoeal analysis of crude extract and most active component in rats

CHAPTER TWO

LITERATURE REVIEW

2.0 Diarrhoea

Diarrhoea is an increase in the frequency of bowel movements, an increase in the looseness of stool or both. According to the World Health Organization (2009), Diarrhoea is defined as three or more watery or loose bowel movements in a 24 hour period. It is a common complaint for all age group. Diarrhoea is caused by increased secretion of fluid into the intestine, reduced absorption of fluid from the intestine or rapid passage of stool through the intestine (Bushen and Guerrant, 2003).

2.1.0 Etiology of Diarrhoea

In previous years, pathogenic organism could be identified in the faeces of only about 25% of patient with acute diarrhea. Today, using new technologies, experienced laboratories can identify pathogens in about 75% of cases seen at a treatment facility and up to 50% of milder cases detected in the community (Wilson, 2005).

The organism most frequently associated with diarrhea in young children in developing countries include Rotavirus, enterotoxagenic *Esherichia coli, shigella* specie, *Campylobacter jejuni* and *Cryptosporidium* (Patel *et al.,* 2009).

A number of other pathogens such as Norwalk agent, enteric adenoviruses, *Vibrio cholera* and *Entamoeba histolytica* their importance as causes of acute diarrhea in children in developing countries is either minimal or not yet well defined (Mitchel, 2002).

2.1.2 Pathophysiology of Diarrhoea

There are numerous causes of diarrhea, but in almost all cases, this disorder is a manifestation of one of the four basic mechanisms to be involved in the pathogenesis of a given case.

Osmotic Diarrhoea

Absorption of water in the intestine is dependent on adequate absorption of solutes. If excessive amounts of solutes are retained in the intestinal lumen, water will not be absorbed

and diarrhea result (Kent and Banks, 2010) osmotic diarrhea typically results from one of two situations.

- Ingestion of a poorly absorbed substrate: the offending molecule is usually a carbohydrates or divalent ion. Common examples include mannitol or sorbitol, epson salt (M_gSo_4) and some antacids (M_gOH_2) (Gonzales *et al.*, 2004).
- Malabsorption: inability to absorb certain carbohydrate is the most common deficit in this category of diarrhea, but it can be result virtually any type of malabsoption. A common example of malabsorption afflicting many adults humans and pets is lactose intolerance resulting from a deficiency in the brush border enzyme lactase (Macgillivray *et al.*, 2013).

Secretory Diarrhoea

Large volumes of water are normally secreted into the small intestinal lumen, but a large majority of this water is efficiently absorbed before reaching the large intestine (Strasinger and Dilorenzo, 2008).

Diarrhoea occurs when secretion of water into the intestinal lumen exceeds absorption. Many millions of people have died of the secretory diarrhoea associated with cholera (Farthing, 2006) the responsible organism *Vibrio cholera*, produce cholera toxin which strongly activates adenly cyclase causing a prolonged increase in intracellular concentration of cyclic AMP within crypt enterocytes (Longstreth *et al*., 2006) This changes result in prolonged opening of the chloride channels that are instrumental in secretion of water from the crypts, allowing uncontrolled secretion of water. Additionally, cholera toxin affects the enteric nervous system resulting in an independent stimulus of secretion (Longstreth *et al.*, 2006).

Inflammatory and Infectious Diarrhoea

The epithelium of the digestive tube is protected form insult by a number of mechanisms constituting the gastrointestinal barriers but like many barriers it can be breached. Disruption of the epithelium of the intestine due to microbial or viral pathogens is a very common cause of diarrhea in all species (Viswanathan *et al.*, 2009). Destruction of the epithelium results not only in exudation of serum and blood into the lumen but often associated with widespread destruction of absorption epithelium. In such cases, absorption of water occurs very inefficiently and diarrhoea results. Examples of pathogens frequently

associated with infectious diarrhea include *Salmonella*, *Norovirus* and *crytosporium* (Mitchell, 2002).

Diarrhoea Associated with Deranged Motility

During normal functioning of the intestines, solids and fluid are moved through the gut with peristaltic waves of the smooth muscles within the intestine. This movement is slow and may take 3-5 hours for the mass to move from the pyloric valve at the proximal point of the small intestine to the large intestine. It may take as long as 24 hours for the mass to move from the small intestine to the rectum to be expelled during defecation (Guyton and Hall, 2000)

When the intestine are into functioning normally, motility can be either increased or decreased and both can lead to diarrhoea. Increase motility can be caused by infectious agents, changes within the bowel by inflammatory bowel disease or by irritable bowel syndrome. This increased motility results in faster transport of stool through the bowel so there is less chance for reabsorption of fluid from the large intestine (Bliss, *et al.,* 2006).

On the other hand, decreased motility can lead to diarrhoea. Typically, decreased motility will lead to constipation, which in its most severe form, can allow a large bolus of stool to form in the lower intestine and cause an impaction. The stool behind this bolus may become liquid again due to the action of bacteria on the stool. This results in liquid stool leaking around the bolus and causing diarrhoea. People with altered gut motility and diarrhoea will have low volume, liquid stool and cramping. However disordered motility may be just one factor in a complex mechanism of abnormal gut functioning as seen in infectious diarrhoea, and inflammatory bowel diseases (Bliss, *et al.,* 2006).

2.1.2 Biochemistry of Diarrhoea

Pathogenic mechanism

Microbial agents cause diarrhea by a number of mechanisms, several of which are considered below:-

Viruses:

Viruses, such as rotavirus, replicate within the villous epithelium of the small bowel, causing patchy epithelial cell destruction and villous shortening the loss of normal absorption villous cells and their temporary replacement by immature secretory crypt-live cells causes

the intestine to secretes water and electrolytes (Patel *et al*., 2009) villous damage may also be associated with the loss of disaccharidase enzymes leading to reducing absorption of dietary disaccharides especially lactose (Macgillvary, *et al*., 2013).

Bacteria

- Mucosal adhesion: - Bacteria that multiply within the small intestine must first adhere to the mucosa to avoid being swept away. Adhesion is caused by superficial hair like antigens, termed pili or fimbriae that bind to receptor on the intestinal surface, this occurs for example with enterotxigenic *E. coli* and *Vibrio cholera* (Viswanathan *et al*., 2009)
- Toxin that causes secretion:- *E. coli*, *V. Cholera* and possibly other bacteria e.g. *Salmonella* cause intestinal secretion by producing toxins that alter epithelial cell function, these toxins reduce the absorption of sodium by the villi and may increase the secretion of chloride in the crypts, resulting in net secretion of water and electrolytes (Patel *et al*., 2009).
- Mucosal Invasion: - *Shigella*, *c. jejuni* and enteroinvasive *E. coli* cause bloody diarrhea by invading and destroying mucosal epithelial cells. This occurs mostly in the colon and distal part of the ileum. Invasion is followed by the formation of microabscesses and superficial ulcers and hence the presence of red and white blood cells, or frank blood, in the stool. Toxins produced by these organism cause tissue damage and possibly also mucosal secretion of water and electrolyte (Navaneethan and Giannela, 2008).

2.1.3 Drugs for Treatment of Diarrhoea

Loperamide

Loperamide, a piperidine derivative, is a drug used against diarrhea resulting from gastroenteritis or inflammatory bowel diseases. In most countries it is available generically and under brand names such as lopex, imodium, dimor, fortasec, lopedium, gastro-stop and pepto diarrhoea control (Stokbroek *et al.*, 1973).

Medical uses of loperamide

Loperamide is effective for the treatment of a number of types of diarrhrea (Hanauer 2008)

Mechanism of Action of Loperamide

Loperamide is an opiod–receptor against and acts on the u-opoid receptors in the myentric plexus of the large intestine, by itself it does not affect the central nervous system (Hanauer, 2008). It works similarly to morphine, by decreasing the activity of the myenteric plexus, which in turn decreases the tone of the longitudinal and circular smooth muscles of the intestinal wall (Butler, 2008). This increases the amount of time substance stay in the intestine, allowing for more water to be absorbed out of the faecel matter. Loperamide also decreases colonic mass movements and suppresses the gastrocolic reflex (Katzung, 2004).

Adverse Effect / Toxicity Associated With Loperamide

Adverse drug reactions associated with loperamide include abdominal pain and bloating, nausea, vomiting and constipation. Rare side effects associated with loperamide are paralytic ileus, dizziness and rashes. In high does, loperamide in conjunction with cimetadine, can cause ventricular fibrillation and death (Bichner, 2010). The drug is not recommended for use in hepatic failure since it can precipitate hepatic encephalopathy (Litovitz, 1997).

Pepto Bismol

Pepto–Bismol is a drug use to treat minor digestive system upset. The primary symptoms aided by pepto-bismol are nausea, heartburn, indigestion, upset stomach, diarrhea and other temporary discomforts of the stomach and gastrointestinal tract (Gorbach, 1990).

Mechanism of Action of Pepto-Bismol

Bismuth subsalicyclate (The active ingredient in Pepto-Bismol) is used as an antidiarrhoeal and to treat some other gastro-intestinal diseases (Oligodynamic effect, which relates to killing microbes with small doses of heavy metals) (Sox and Olson, 1989). The means by which this appears to work is still not well documented. It is thought to be some combination of:

- Retarding the expulsion of fluids into the digestive system by irritation tissues, by “coating” them.
- Retarding inflammation/irritation of stomach and intestinal lining.
- Killing some bacteria that causes diarrhea. There is evidence that salicylic acid from hydrolysis of the drug is antimicrobial for *E.coli* (Sox and Olson, 1989).

Adverse Effects / Toxicity Associated With Pepto-Bismol

The bismuth in this medicine may cause severe constipation in children. In addition, it can combine with trace amounts of sulfur in saliva and the gastrointestinal tract, blackening the users tongue and stool. This condition is harmless and subsides within a few days (Pali-scholl and Jensen – Jarolim, 2011).

Children should not take medication with bismouth subsalicylate while recovering from influenza or chicken pox as epidemiologic evidence points to an association between the use of salicylate containing medications during certain viral infections and the onset of Reyes syndrome (Gorbach, 1990) for same reason, it is typically recommended that nursing mother not to use medication containing bismuth subsalicylate (such as pepto-bismol) because small amounts of the medication are excreted in breast milk and pose a theoretical risk of Reye's syndrome to nursing children (Pali-scholl and Jensen – Jarolim, 2011).

Long term use of Pepto-Bismol (greater than 6 weeks) may lead to toxicity due to accumulation of bismuth subsalicyclate (Gorbach, 1990).

2.1.4 Drugs of Choice

Azithromycin

Azithromycin is an azalide, a subclass of macrolide antibiotics. It is derived from erythromycin, with a methyl substituted nitrogen atom incorporated into the lactone ring, thus making the lactone ring 15 membered (Chisholm *et al*., 2009).

Medical Uses of Azithromycin

Azithromycin is used to treat many different infections including acute otitis media, nonstreptococcal bacterial pharyngitis, gastrointestinal infections such as travelers' diarrhea, respiratory tract infections such as pneumonia, *Mycobacterium avium* complex disease such as *Neisseria meningitis* and some sexually transmitted infections (Dajani, 1995). Azithromycin is used as a second line treatment for strep throat and for those allergic to penicillin (Chisholm, *et a.l*, 2009).

Mechanism of Action of Azithromycin

Azithromycin prevents bacteria from growing by interfering with their protein synthesis it binds to the 50s subunit of the bacterial ribosome and thus inhibits translation of mRNA. (Banic, 2011).

Adverse Effects / Toxicity Associated With Azithromycin

Most common side effects are gastrointestinal diarrhea, nausea, abdominal pain and vomiting (Hansen, 2009). Nervousness, dermatologic reactions and anaphylaxis have been reported. As with all antimicrobial agents, pseudomenbranous colitis can occur during and up to several weeks after azithromycin therapy (Baselt, 2008).

Occasionally, patients have developed cholestatic hepatitis or delirium. Accidental intravenous over dosage in an infant caused severe heart block, resulting in residual encephalopathy (Baselt, 2008 and TiIelli *et al.*, 2006).

In 2013, the FDA issued a warning saying that azithromycin can cause abnormal changes in the electrical activity of the heart that may lead to potentially total irregular heart rhythm,

Pharmacokinetics of Azithromycin

Unlike erythromycin, azithromycin is acid-stable so it can be taken orally with no need of protection form gastric acids. It is readily absorbed but its absorption is greater on an empty stomach (Baselt, 2008). Time to peak concentration in adults is 2.1. to 3.2 hours for oral dosage forms and one to two hours after a dose.

Due to its high concentration in phagocytes, azithromycin is actively transported to the site of infection (Noed *et al.*, 2006). During active phagocytosis, large concentrations are released. The concentration of azithromycin in the tissue can be over 50 times higher than in plasma (Hansen, 2009) due to ion trapping and its high lipid solubility (Volume of distribution is too high).

Azithromycin half life allows a large single dose to be administered and yet maintain bacteriostatic levels in the infected tissue for several days (Hansen, 2009).

Ciprofloxacin

Ciprofloxacin is a second generation flouroquinolone antibiotic (Ball, 2008). Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal and abdominal infections including gram negative *E. coli* and gram positive *Staphylococcus aureus* (Laurence *et al*., 2005).

Ciprofloxacin is used alone or in combination with other antibacterial drugs in the empiric treatment of infections for which the bacterial pathogen has not been identified, including urinary tract infections and abdominal infections (Solomkin *et al*., 2010).

Medical Uses of Ciprofloxacin

Ciprofloxacin is used to treat a wide variety of infections including infections of bones and joints, endorcarditis, gastroenteritis, malignant otitis external, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax and Chancroid (Knottnerus *et al*., 2012).

Mechanism of Action of Ciprofloxacin

Ciprofloxacin functions by inhibiting DNA gyarase, a type II topoisomerase and topoisomerase IV (Dilica and Zhao, 1997) enzymes necessary to separate bacterial DNA, thereby inhibiting cell division (Pommier *et al.*, 2010).

Adverse Effect / Toxicity Associated With Ciprofloxacin

Most of the adverse events reported were described as only mild or moderate in severity, abated soon after the drug was discontinued and required no treatment (Saint *et al*., 2000). The most frequently reported drug related events, from chemical trials of all formulations, all dosages, and all drug therapy durations and for all indications of ciprofloxacin therapy were nausea, diarrhoea, abnormal liver function tests, vomiting and rash (De sarro and De Sarro, 2001).

Post marketing surveillance has revealed tendonopathy, tendon rupture and exacerbation of the symptoms of the neurological disorder myasthenia gravis as important serious adverse effects (Corrao *et al*., 2006).

Pharmacokinetics of Ciprofloxacin

Ciprofloxacin when administered over one hour as an intravenous infusion, it rapidly distributes into the tissues, with levels in some tissues exceeding those in the serum. Penetration into the central nervous system is relatively modest, with cerebrospinal fluid levels normally less than 10% of peak serum concentrations (Brown *et al*., 2013). The serum half life of ciprofloxacin is about 4-6 hours, with 50 – 70% of an administered dose being excreted in the urine as metabolized drug. An additional 10% is excreted in urine as metabolites urinary excretion is virtually complete by 24 hours after administration. Dose adjustment is required in the elderly and in those with renal impairment (Bolhnis *et al*., 2011).

Chloramphenicol

Chloramphenicol is considered a prototypical broad spectrum antibiotics, alongside the tetracycline and as it is both cheap and easy to manufacture it is frequently an antibiotics of choice in the developing world (Falagas, 2008). The drug is also known as chlomitromycin is effective against a wide variety of gram positive and gram negative bacteria including most anaerobic organisms (Poulter *et al*., 2010).

Medical Uses of Chloramphenicol

The original indication of chloramphenicol was in the treatment of typhoid, but now almost universal presence of multiple drug resistant *Salmonella typhi* has meant it is seldom used for this indication except when the organism is known to be sensitive (Falagas, 2008). Chloramphenicol may be used as second line agent in the treatment of tetracycline resistant cholera (Holt, 2001).

Because of its excellent blood brain barrier penetration (far superior to any of the cephalosporins). Chloramphenicol remains the first choice treatment for staphylococcal brain abscesses. It is also useful in the treatment of brain abscesses due to mixed organism or when the causative organism is not known (Poulter *et al*., 2010).

Chloramphenicol is also effective against *Enterocucus faecium,* which has led to its being considered for treatment of vancomycin–resistant *Enteroccus* (Falagas *et al*., 2008).

Mechanism of Action of Chloramphenicol

Chloramphenicol is a bacteriostatic drug that stops bacterial growth by inhibiting protein synthesis. Chloramphenicol prevents protein chain elongation by inhibiting the peptidly transferase activity of the bacterial ribosome. It specifically binds to A2451 and A2452 residues in the 23SrRNA of the 50s ribosomal subunit, preventing peptide bond formation (Jardetzky, 1998). While chloramphenicol and the macrolide class of antibiotics both interact with ribosomes, chloramphenicol is not a macrolide. It directly interferes with substrate binding whereas macrolides sterically block the progression of the growing peptide (Wolfe and Hahn 1996, Hahn *et al*., 1997).

Adverse Effect / Toxicity Associated With Chloramphenicol

The most serious adverse effects associated with chloramphenicol treatment is bone marrow toxicity, which may occur in two distinct forms. Bone marrow suppression, which is a direct toxic effect of the drug and is usually reversible and aplastic anemia, which is idiosyncratic (rare, unpredictable and unrelated to dose) and generally fatal (Rich *et al.*, 2001)

Pharmacokinetics of Chloramphenicol

Chloramphenicol is extremely lipid soluble; it remains relatively unbound to protein and is small molecule. It has a large apparent volume of distribution of 100 liters (Feder, 2002) and penetrates effectively into all tissues of the body, including the brains the concentration achieved in brain and cerebrospinal fluid (CSF) is around 30 to 50% even when meninges are not inflamed; this increases to as high as 89% when the meninges are inflamed (Lancaster *et al*., 2000). Chloramphenicol increases the absorption of iron (Harold *et al*., 2006).

Aspirin

Aspirin also known as acetylsalicylic acid, is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti inflammatory medication (Sneader, 2000) salicylic acid, the main metabolites of aspirin, is an integral part of human and animal metabolism. While in humans much of it is attributable to diet, a substantial part is synthesis endogenously (Paterson *et al.*, 2008).

Medical Uses of Aspirin

Aspirin is used in the treatment of a number of conditions including fever, pain, rheumatic fever, and inflammatory diseases such as rheumatoid arthritis, pericarditis and

Kawasaki diseases (U.S Food and Drug Administration, 2012). Lower doses of Aspirin have also shown to reduce the risk of stroke in some circumstances (Seshasai *et al.*, 2012). There is some evidence that aspirin is effective at preventing colorectal cancer, though the mechanisms of this effect are unclear (Algra and Rothwell, 2012).

Mechanism of Action of Aspirin

Suppression of Prostaglandins and Thromboxanes

Aspirin ability to suppress the production of prostaglandins and thromboxanes is due to its irreversible inactivation of the cyclooxygenase, enzymes required for prostaglandin and thromboxane synthesis (Toghi *et al.,* 1992). Aspirin acts as an acetylating agent where an acetyl group is covalently attached to a serine residue in the active site of the enzyme. This makes aspirin different from other NSAIDs such as ibuprofen which are reversible inhibitors (Vane, 1990).

Additional Mechanism

Aspirin has been shown to have at least three additional modes of action. It uncouples oxidative phosphorylation in cartilaginous (and hepatic) mitochondria, by diffusing from the inner membrane space as a proton carrier back into the mitochondrial matrix where it ionizes once again to release protons (Somasundaram, 2000). In addition, aspirin induces the formation of NO – radicals in the body, which have been shown in mice to have an independent mechanism of reducing inflammation. Also salicylic acid and its derivative modulate signaling through a transcription factor complex, plays a central role in many biological processes including inflammation (McCarty and Block, 2006).

Adverse Effect / Toxicity Associated With Aspirin

The main undesirable side effects of aspirin taken by mouth are gastrointestinal ulcers, stomach bleeding and tinnilus, especially in higher doses (Berge and Stevenson, 2004). Aspirin can induce angioedema (Swelling of skin tissues), in some people. In one study, angioedema appeared on to six hours after ingesting aspirin in some patients.

However, when the aspirin was taken alone, it did not cause angioedema in these patients; the aspirin had been taken in combination with another NSAID induced drug when angioedema appeared (Berge and Stevenson, 2004).

Aspirin causes an increased risk of cerebral microbleeds having the appearance on MRI scans of 5 to 10 mm or smaller hypointense (dark holes) patches (Vernooij *et al*., 2009). Such cerebral microbleeds are important, since they often occur prior to ischemic stroke or intracebral haemorrhage, Binswager diseases and Alzhemer's disease (Gorclick, 2009).

Pharmacokinetics of Aspirin

Salicylic acid is a weak acid and very little of it is ionized in the stomach after oral administration. Acetyl salicylic acid is poorly soluble in the acidic conditions of the stomach which can delay absorption of high doses for eight to 24 hours (Ferguson and Boutrus, 1999). The increased PH and larger surface area of the small intestine causes aspirin to be absorbed rapidly there, which in turn allows more of the salicyclate to dissolve (Kaufman and Dubansky 1998). Owing to the issue of solubility, however, aspirin is absorbed much more slowly during overdose and plasma concentrations can continue to rise for up to 24 hours after ingestion (Levy and Tsuchiya, 1999).

2.2 Medicinal Plants

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine (Dubey *et al.*, 2011). Medicinal plants are plants whose extracts can be used directly or indirectly for the treatment of different ailments. Therefore, the use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed (Edward, 2001).

Medicinal plants with a long history of safe and effective use are likely to have a pharmaceutical effect (Tabuti, 2008). In addition, the likeness of a medicinal use being based on pharmaceutical properties rather than on a cultural context increases when this use is repeatedly found in different cultures.

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituent of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and foods; they

are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999, 2001).

Plants provide a source of medicines, which are useful in treatment of various categories of human ailments and conditions. The world health organization (WHO) has estimated that up to 80% of the world's population rely on plants for their primary health care (BCGI, 1995) and also in Nigeria up to 75% of the population patronizes traditional medicine (Omoseyindemi, 2003). More importantly, plants have been the main source of medicine for man before the advancement of science and technology (Schmeltzer and Omino, 2003).

Historically all medicinal preparations were derived from plants, whether in the simple form of plants parts or in the more complex form of crude extracts (Felix and Temitope, 2010). Today a substantial number of drugs are developed from plants which are active against a number of diseases. The majority of these involve the isolation of the active ingredient (Chemical compound) found in a particular medicinal plant and its subsequent modification. In the developed countries 25% of the medicinal drugs are based on plants and their derivatives (Principe, 2005) and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries.

In Northern Nigeria, many indigenous plants are widely consumed as food or home remedies especially in the treatment or management of common diseases (Aliyu *et al.,* 2008). Medicinal plants such *as Anchomanes difformis (Araceae), Anisopus Manni (Asclepiadaceae), pavetta crassipes (Rubiaceae) stachytarpheta angustifolia (vernamaceae) and vernonia blumeodies (Asteraceae)* are continually being utilized as therapeutic agents in formulations for treating disease in the traditional ethonomedicinal system in Northern Nigeria (Aliyu *et al.,* 2008) .

However, environment, atmosphere, pollution, soil, harvesting and handling are some of the factors which may play important roles in contamination of medicinal plants by metals and microbial growth (Ajasa *et al.,* 2004). The importance of medicinal plants and the contribution of phytomedicine to the well being of a significant number of the world population have attracted interest from a variety of disciplines (Biapa *et al.,* 2007).

A big variety of plants have been studied in search of antidiarrhoeal activity and from some them, compounds have been isolated that are responsible for antidiarrhoeal effect. Following are some examples of plants that have been studied for antidiarrhoeal effect.

The aqueous extract of leaves of *Bysocarpus coccineus* was evaluated for antidiarrhoeal activity by Akindele (2006). The result revealed that, the extract produced a decrease in propulsion in castor oil-induced intestinal transit in mice, frequency of defaecation and protected the mice treated with castor oil. Chloroform extract of *A. marmelos* was reported to showed inhibitory activity against castor oil-induced diarrhoea (Mazumder, *et al.,* 2006).

Acanthospermum hipidu DC., Gmelina arborea Roxb., Parkia biglobosa Keay, Vitex dodiana sweet were reported by Agunu (2005), to have pharmacological activity against diarrhoea. A work by Laure *et al.,* (2006) on ethylacetate extract of stem bark of *Cyclicodiscus gabunensis* revealed that, the extract produced a decrease in severe diarrhoea of rats treated with castor oil. The extract also possesses an antienteropooling activity and produced a decrease in intestinal transit.

Aqueous extract of *Shaeranthus senegalensis* causes a dose-dependent protection against castor oil-induced diarrhoea in rats and intraluminal transit motility (Adzu *et al.,* 2004). Nwafor and Bassey (2007) reported that, the ethanolic extract of the leaf of *Carpolobia lutea G.* has the ability to inhibit small intestinal transit time, castor oil-induced diarrhoea and fluid accumulation in rodents. Antidiarrhoeal activity of *Guira senegalensis* was evaluated by Aniagu *et al.*, (2005) which revealed the ability of the plant to reduce the frequency of defaecation as well as the wetness of faecal dropping. The plant extract also produced 100% inhibition of castor oil-induced diarrhoea in mice.

Cassia occidentalis is an annual or perennial plant which is used in several traditional medicines to cure various diseases. This weed has been known to posses antibacterial, antifungal, antidiabetic, anti inflammatory, anticancerous and hepatoprotective activity. A wide range of chemical compounds including cassiollin, apigenin, chrysophanic acid, chrysophanol and chrysoeriol have been isolated from this plant (Yadav *et al.*, 2010).

2.3 Constituents of Plants and Their Mode of Action

Plants produce a good deal of secondary metabolites which have benefited mankind in various ways including treatment of diseases (Elaine *et al.,* 2002). Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edeoga *et al.,* 2005; Mann, 1978). These metabolites serve different purpose

in plants, including growth regulation, defense against predators and infections or they may be waste products. Outside their intrinsic uses in the plant, these secondary metabolites have variously been shown to exhibit interesting biological and pharmacological activities and are important as prophylactics, chemotherapeutics or have served as the starting points in the development of modern medicines (Verpoorte, 1998).

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant (Wink, 1999). This is in contrast to primary products such as carbohydrates, lipids, proteins, heme, chlorophyll and nucleic acids, which are common to all plants and are involved in the primary metabolic processes of building and maintaining plant cells (Kaufman *et al.*, 1999; Wink 1999). Although plant secondary products have historically been defined as chemicals that do not appear to have a vital biochemical role in the process of building and maintaining plant cells, recent research has shown a pivotal role of these chemicals in the ecophysiology of plants (Donald, 2000).

Accordingly, secondary products have both a defensive role against herbivory, pathogen attack and inter plant competition and attractant role towards beneficial organism such as pollinators or symbionts (Kaufman *et al.*, 1999; Wink and Schimmer, 1999). Furthermore, recent work has indicated potential roles of secondary products at the cellular level as plant growth regulators, modulators of gene expression and in signal transduction (Kaufman *et al.*, 1999).

Although secondary products can have a variety of functions in plants, it is likely that their ecological function may have some bearing on potential medicinal effects for humans. For example, secondary products involved in plants defense through cytotoxicity towards microbial pathogens could prove useful as antimicrobial medicines in humans, if not too toxic. Likewise, secondary products involved in defense against herbivores through neurotoxin activity could have beneficial effects in humans (i.e. as antidepressants, sedatives, muscle relaxants or anesthetics) through their action on the central nervous system (Donald, 2000).

To promote the ecological survival of plants, structures of secondary products have evolved to interact with molecular target affecting the cells, tissues and physiological functions in competing microorganism, plants and animals (Wink and Schimmer, 1999). In this respect, some plant secondary products may exert their action by resembling endogenous

metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on human central nervous system, endocrine system etc. (Kaufman *et al* ., 1999).

The active principles present in a plant are very variable in amount; they may even be entirely absent if, for example, the plant has been grown under very unfavorable conditions or if the plant belonged to a race low in active constituents. On the other hand, the amount of active principles at times may be higher than normal and under such circumstances the plant will have a stronger action (Donald, 2000). Some of the important groups of active constituents of plants are considered together with their mode of action.

Glycosides are substances that are decomposed into a non sugar part and one or several sugars when hydrolyzed by enzymes, by dilute acids or alkalis or by boiling. Their medicinal action is due to the non sugar part of the molecules which are chemically very diverse. The sugar part of the molecules generally influences the solubility in water and hence its absorption by the body (Wink, 1999). Many plant glycosides are of no therapeutic significance, but others are very cardio active and at the same time increase diuresis; they are among the most toxic substances found in plants (Wink, 1999).

A special group comprises the anthraquinone glycosides, these are powerful laxatives. Glycosides derived from salicylic acid comprise another group; their action is febrifuge, anti inflammatory, antiseptic and analgesic they are used in the treatment of rheumatism (Donald, 2000).

Flavonoids are group of colorless or pale yellow glycosides. They strengthen the blood capillaries and prevent the small cutaneous hemorrhages so frequent in the aged. Some of them relieve cramps of the smooth muscles while others improve circulation in the coronary arteries (Wink, 1999). Flavonoids and other phenolic compounds are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity (Okwu, 2001).

Saponins are also glycosides their outstanding physical character is that their aqueous solutions froth greatly. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose or methylpentose, glycosidically linked to a hydrophobic aglycone which may be triterpenoid or steroid in nature (Francis *et al.*, 2002). A

number of studies have shown that saponins from different sources lower serum cholesterol levels in a variety of animals including human subjects (Potter *et al.*, 1993; Matsuura, 2001).

Large mixed micelles formed by the interaction of saponins with bile acids account for their increased excretion when saponins rich foods such as soya bean and chick pea are consumed (Oakenful and Sidhu, 1990). The resulting accelerated metabolism of cholesterol in the liver causes its serum level to go down (Francis *et al.*, 2002).

Saponins are mild laxatives, diuretics and expectorant, like all other glycosides, they are destroyed and lose their activity if their aqueous solutions are boiled (Donald, 2000).

Tannins have the property of precipitating proteins. For this reason they convert animal skins into leather. In the free state and in large doses they irritate the mucosa, in small doses they precipitate small amount of proteins in the cells of the mucosa which are thus rendered impermeable, other irritants are thus prevented from penetrating to the deeper layers of damaged mucosa, hence healing is aided (Wink, 1999). This property also explains the use of tannins as antidiarrheal and in the treatment of certain burns. By analogous process tannins prevent the development of bacteria since the proteins necessary for their nutrition are removed and also their own protein contents are precipitated. Tannins also contract the blood capillaries and so prevent certain haemorrhages. Tannins react with atmospheric oxygen and are converted to inactive substances; they are also destroyed by prolonged boiling in water (Wink, 1999).

Volatile oils are among the most utilized products in popular medicine. They are very volatile especially in steam; their presence is the principal cause of characteristic plant odours. Their medicinal activity is very variable. Some act on the central nervous system. Many increase the secretion of gastric juices and hence increase appetite (Donald, 2000). They aid digestion and regularize intestinal action. When placed on the Mucosa, on wounds or even on intact skin, they can increase the flow of blood especially of leucocytes (hyperemia) (Donald, 2000). Some plants containing volatile oil stimulate secretion of wine; these are used to reduce accumulation of water in the body.

Alkaloids are nitrogenous compounds that have a more or less marked action on the central nervous system and often, also on the peripheral nervous system. Some alkaloids are among the most powerful poisons known (Wick, 1999).

2.4 Biosynthesis and Storage of Plant Secondary Metabolites

Biosynthesis of plant secondary metabolites is organ- cell- or development -specific in almost all higher plant species. In most cases the pathways, and indeed the genes involved in their synthesis, are tightly regulated and may be linked to environmental, seasonal or external triggers (Acamovic and Brooker, 2005). Cellular sites of synthesis are compartmentalized in the plant cell, with the majority of pathways being at least partially active in the cytoplasm.

However, there is some evidence that compounds such as alkaloids, quinolizidiner, caffeine and some tarpenes are synthesized in the chloroplast (Roberts, 1981; Wink and Hartman 1982). The biosynthesis of protoberberine occurs in cell vesicles (Amann *et al.*, 1986) and conine and some amines are synthesized in mitochondria (Roberts, 1981; Wink and Hartmann, 1982).

Although plant secondary metabolites are often detected throughout the plant, their initial site of synthesis is often restricted to a single organ such as roots, fruits or leaves. Thereafter, they can be transported around the plant via the phloem or xylem or by symplastic or apoplastic transport and stored in a number of different tissues. The site of storage often depends on the polarity of the compounds with hydrophilic compounds such as alkaloids, glucosinolates and tannins being stored in vacuoles or idioblasts while lipophilic compounds such as the terpene based essential oils are stored in trichomes, glandular hairs resin ducts, thylakoids membranes or on the curticle (Wiermann, 1981).

For some compounds that are present in the plant as defense barriers e.g. alkaloids, flavonoids, cyanogenic glycosides, coumarins storage may be in the epidermis itself (Wiermann, 1981; Wink, 1993; Wink and Roberts 1998; Harbone, 2001). Storage may be tissue-or cell-specific (Guern *et al*., 1987), with flowers, fruits and seeds being rich sources of many plant secondary metabolites, especially in annual plants. In perennial species plant secondary metabolites are present in high levels in bulbs, roots, bark of the roots and stems.

Plant secondary metabolites may not be the end product of metabolism but may have a regular rate turn over (Barz and Koster, 1981). Plant secondary metabolites containing nitrogen such as alkaloids, cyanogenic glycosides and protease inhibitors are stored by the plant and are metabolized at germination to serve as nitrogen or carbon sources for the

developing seedlings (Wink and Witer, 1985). There is also a turnover of carbohydrate and lipids during germination. Although plant secondary metabolites have been used for thousands of years in human medicine for flavoring as stimulants and hallucinogens, as fragrances in cosmetics and household freshners and as therapeutic agents, their native function in plants remains contentious (Harborne, 2001). Plant secondary metabolites are generally thought to be present in plants primarily for defence purposes and this view has been extended on the basis of some convincing evidence (Harbone, 2001; Ralphs *et al.*, 2004). The proposed functions include defense against grazing herbivores and insects; defense against microorganisms including bacteria, fungi and viruses. Interestingly, plants also regulate the synthesis and storage of plant secondary metabolites so that more of vulnerable tissues such as fruits and young leaves contain higher concentrations of plant secondary metabolites than senescing tissues (Wink, 2004). The physical location of structures such as trichomes, which serve as the sites of synthesis and storage of essential oils, also provides support for a defence role for these compounds. Trichomes are located on the surfaces of leaves and are usually the first point of contact for browsers or insect predators. Tannins are usually located in leaf vacuoles beneath the epidermal surface (Acamovic and Brooker, 2005). The volatile nature of the essential oils or the astringent and bitter taste of tannins and alkaloids respectively can be a clear deterrent to predators (Harborne, 2001 and Ralphs *et al.*, 2004).

2.5 Bioactivity of Plant Secondary Metabolites

The evolution of bioactive defense compounds in plants has produced compounds that may have many other beneficial effects in biotechnology, pharmacy and medicine.

The structures of many plant secondary metabolites have been shaped to interact with many different molecular and cellular targets including enzymes, hormones receptors, neurotransmitter receptor and trans-membrane transporters and can thus mimic a response at the corresponding molecular target (Acamovic and Brooker, 2005). There is hardly any cellular target that some plant secondary metabolic cannot modulate. Thus, plants produce a wide range of bioactive substances and many of these substances are already in widespread use in the pharmacological, medical and agricultural industries while others are under development. In many cases plant secondary metabolite, e.g. the terpeoid essential oils can be more effective than chemically synthesized pure compounds because they are a complex

mixture of components. Their complexity enables the plant secondary metabolites to interact with multiple molecular targets and thus, it is more difficult for target microorganisms or herbivores to develop any effective response because resistance at different targets would be required (Acamovic and Brooker, 2005).

Wounding and infection can trigger several events in plants (e.g. the release of glucosinolates or cyanogenic glycosides), which can work together with secreted enzymes (e.g. p-glycosidases) to assist in protecting the plant from attack. Many antibacterial and antifungal compounds (phytoalexins) are produced in plants as a result of infection and tannins are often produced in response to browsing by herbivores (Baldwin, 1994). However, just as plants evolved defence mechanism for their protection, many animals, microorganism and parasites have evolved parallel mechanisms to overcome these defenses (Hartmann and Witte, 1995).

There is considerable interaction between ingested plant secondary metabolites and tissues, enzymes and other compounds within the animal. The interaction during absorption, deposition and metabolism and excretion are highly dependent on the physico-chemical attributes of the compounds involved and their susceptibility to transformation (Acamovic and Brooker, 2005). When ingested the relevant plant secondary metabolite can pass through the animal unchanged or combine with bile salts and be excreted in the faeces. They can also interact with tissue and other compounds within the gastrointestinal tract, pass through and then excreted. The original compound can be either absorbed directly or transferred within the gastrointestinal tract and then absorbed and deposited (Acamovic and Brooker, 2005). If absorbed the compounds can undergo further transformation, usually to increase hydrophilicity and then excretion via the kidneys in the urine. This process has been clearly demonstrated for glucosinolates, which are transformed and excreted as mercapturic acids after degradation by myrosinase and conjugation with glycine, cystine and glutamic acid under the influence of the relevant amino acid transferases (Timbrell, 1992).

Physiochemical factors that are extremely influential in plant secondary metabolites when ingested are: molecular size and architecture; pH of the environment; hydrophilicity; lipophilicity; charge and polarity (Timbrell, 1992; Harbone, 2001). In general the smaller the molecule and the greater the hydrophilicity, the greater is the likelihood of absroptin of the compound from the GIT when ingested.

Proanthocyanidins, although hydrophilic and water soluble are not absorbed from the gastrointestinal tract and with other tannins they can alter microflora populations, reduce attachment of fungi and bacteria to substrates, increase endogenous loses and damage the gastrointestinal tract in animals (Bento *et al.*, 2005). They have also been shown to interact with parasites within the gastrointestinal tract (Athanasiadon and Kyriazakis, 2004). Hydrolysable tannins undergo ready degradation because of ester linkages to the glucose moiety and the degradation products are absorbed from the gastrointestinal tract and cause toxicity (Cheeke, 1998). Similarly, saponins which are highly hydrophilic and surface active can be absorbed either directly or as micelles. These compounds can affect intestinal parasites as well as the epithelial tissue within the gastrointestinal tract (Johnson *et al.*, 1986).

Alkaloids tend to have high acid dissociation constant and their solubility and thus toxicity (or otherwise) is therefore highly dependent on the pH within the gastrointestinal tract (Harbone, 2001).

Tannins which are produced by many woody plants prevent browsing by ruminants because of their astringent taste and antinutritive properties (Harbone, 2001). However, in animals adapted to these plants, tannin-binding salivary proteins are secreted by the animal (Landu *et al.*, 2000) and many microorganism in the intestinal tract are either resistant to the inhibitory effect of tannins or metabolize the tannins and utilize the energy derived for their own growth.

Microbial enzymes such as gallate decarboxylase and tannin acylhydrolase have been reported to be synthesized in many tannin tolerant microorganisms in response to exposure to tannins (Odonovan and Brokker, 2001). Thus it would appear that plant secondary metabolites are not inactive waste products of plant metabolism but are compounds that are bioactive, which are produced in response to specific signals and provide an important link between the plant, its potential predators and the environment in which they both live (Acamovic and Brokker, 2005).

The effects seen in animals when plant secondary metabolites are ingested are frequently a result of the structural similarity between the plant secondary metabolite and molecules that occur naturally within the animal, such similarities in structure allow the secondary metabolites to interfere in enzyme function and in the synthesis of protein and other essential compounds (Harbone, 2001).

Aromatic essential oils have been known since antiquity to posses' biological activity, including antibacterial, antifugal, antiviral and antiinflamatory effects. These oils can also be active against higher organisn such as nematodes, helminthes, insects etc. Generally, they have terpenoid structures and their effect is the result of the combination of all their constituents. Some constituents in themselves are bioactive while others may affect physical variables such as absorption rate or bioavailability (Acamovic and Brooker, 2005). One of the well established properties of plant essential oils is their antimicrobial activity. They are active against a wide range of organism, including food spoilage organisms, potentially pathogenic microbes of human, environmental or animal origin and some microorganisms in the gastrointestinal tract of animals (Acamovic and Brooker, 2005). Many essential oils have been tested for pharmacological and toxicological properties and many are used as human medicaments. Essential oils have a bateriocidal effect and could be used to control the digestive microbial ecosystem. However, although the essential oils look promising as alternative to antibiotics, little information is available on the effective dose that can be use in animals (Acamovic and Brooker, 2005).

2.6 Description of the Plant *Faidherbia albida*

The plant *Faidherbia albida* belongs to the family *mimosaceae*. It is a largest tree, 8-15m high in Senegal (Dalziel, 1937) and up to 25m in Nigeria (Keay *et al*., 1964). The roots of the plant can reach aquifers up to 80m below the surface. Young trees have inverted cone-shaped crown, old trees with a hemispherical large canopy. Young branches and twigs are cream coloured to whitish, stipular spines whitish, straight in axillary pairs, somewhat swollen at the base, up to 5 cm long, with a brown tip (Dalziel, 1937). Bark is grey, rough, deeply fissurated and scaly with age. Bipinnate leaves blue -green with 3-12 pairs of pinae carrying 6-23 pairs of leaflets up to 12mm long x 5mm wide, partly overlapping . contrary to all other native '*acacias' albida* sheds its leaves in the rainy season and keep them throughout the dry season, which is made possible by the fact that the species behave as a phreatophyte. Other characteristics are the lack of glands on the petioles, but present between each pair of pinnea. Inflorescence in dense spikes 7-10cm long x 1.5-2cm wide, cream coloured very fragnant (Dalziel, 1937). Pods quite typical bright orange to reddish brown in colour 10-15cm long x 2-3cm wide (hence the vernacular name of 'apple ring acacia', containing 10-20 shinning dark brown seeds) with a small characteristic tubercule. It remains

leafless during the rains and assumes new foliage and flowers after the commencement of the dry season (Dalziel, 1937).

2.7 Medicinal Uses of the Plant *Faidherbia Albida*

Faidherbia albida (Family: *Mimosoidae*) is widely used in African traditional medicine (ATM) for management of fever, diarrhoea and human trypanosomiasis. Acute and sub-acute toxicity profiles of ethanolic stem bark extract of *F. albida* were evaluated in wistar albino rats by Salawu *et al.*, (2010a) and the result suggested that the plant may be considered relatively safe when used sub-acutely. Hypoglycemic effects of methanolic Root bark extract of *Acacia albida* del. was conducted by Salisu *et al* (2009), the results provided evidence that, the extract possessed hypoglycemic activity, which may justify its traditional use in diabetes mellitus.

Barks and roots of the plant, alone or mixed with other components, are common ingredients of traditional medicinal preparation for external or internal usage. These preparations are prescribed for respiratory infections, sterility, digestive problems, dysentery, backache, malaria, fever, heart and circulatory problems, dental infections and deafness (Fagg and Barnes, 1990).

In Northern Senegal as reported by Salawu *et al* (2010a), the leaves of the plant are boiled in water to make a cough mixture. The seeds can be boiled and eaten. The pods may be dried and ground into edible flour. They are said to have been used as fish poison and are worn as charm by African women and children to avert smallpox (Irvine, 1961). According to Fanshawe (1962) in Malawi, the root and bark are used as a poison of stupefy fish. The stem bark exudes a gum which is sometimes collected in Nigeria and used for its emollient and emulsifying properties (Howes, 1983). The Pulaar people of Senegal use this gum as an aphrodisiac to treat ‘impotence’ (Salawu *et al.*, 2010a).

Dalziel, (1982) and Singha, (1986) reported that, in Nigeria, an infusion of the bark is taken for fever, cough and to assist in child birth. The plant is added to a portion to treat chest pain by the Fulanis of Nigeria (Jackson, 1973). A decoction of the bark is used in cleansing fresh wounds in a manner similar to that of potassium permanganate (Salawu *et al.*, 2010a).When Combined with other herbs, it is used to treat ‘madness’ (Salawu *et al.*, 2010b). A decoction of the bark is also used as an emetic in fevers by the Masai people of East

Africa, taken for diarrhea in Tanganyika (Irvine, 1961) and for colds, hemorrhage, leprosy and ophthalmic in West Africa.

According to Salawu *et al* (2010b), a liniment made by steeping the bark is used for bathing and massage in pneumonia. The bark is employed in dental hygiene, strips used as detal floss in Namibia and its extracts is employed in the treatment of toothache. In Northern Nigeria, West Africa, the cattle-rearing nomads take a decoction of the stem bark orally for the management of the sleeping sickness (trypanosomiasis).

Tijani *et al* (2008), reported that the aqueous extract of *F. albida* possesses potent anti-pyretic, anti-inflammatory and anti-diarrheal effects and this pharmacologically justifies its folkloric use in the management of fever, rheumatic inflammatory conditions and diarrhea.

Anti-malarial activity of ethanolic stem bark extract of *Faidherbia albida* in mice was carried out by Salawu *et al* (2010b), and the result suggested that, the extract of the plant possesses significant suppressive effect against early infection, curative effect against established infection and prophylactic effect against established infection and prophylactic effect against residual infection of the parasite at safe doses. Previous studies by Salawu *et al* (2010a), on the safety assessment of plant's stem bark extract showed that its oral median lethal dose was greater than 5000 mg/kg body weight, which suggests that orally administered stem bark extract of the plant is practically non-toxic. This high safety profile may have been responsible for its wide spread use in different ethnotherapeutic interventions.

Tijani *et al* (2009), also reported the trypanostatic as well as anti-haemolytic effects of the extract in *trypanosoma brucei-brucei* – infected rats, thus explaining the basis for its use in folkloric medicine. He also showed that the plant stimulates erythropoiesis in case of anemia.

A previous report on phytochemical screening of *F. albida* revealed the presence of tannins 2 – 28% in the root bark (Irvine, 1961) which are associated with the beneficial effects of various herbs and infusions. Tannins and alkaloids observed to be present in the plant extract have been reported for their anti-diarrheal effects (Yu *et al*., 2000; Al-Rehaily *et al.*, 2001) and may also be partly responsible for other pharmacological properties of the plant.

The plant *Faidherbia albida* is among the twelve medicinal plants whose activity was tested against methicillin – Resistant *Staphylococcus aureus* and the result showed great therapeutic potentials of the plant which might be used as adjunct in the treatment of Methicillin-Resistant *Staphylococcus aureus* and associated infections (Aliyu *et a.,l* 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Materials

3.1.0 Plant Material

The stem bark of *F. albida* was collected around Yolde Pate Ward in Yola South Local Government (coordinates: $9^0$13'48"N $12^0$27'36"E / 9.23000^{0}N 12.46000^{0}E), Adamawa State and was identified and authenticated at the Department of Plant Sciences, Modibbo Adama University of Technology, Yola, Adamawa State.

3.1.1 Microorganisms

The microorganisms *Escherichia coli, Salmonella typhi, Shigella dysenteriae* were collected from microbiology laboratory of Federal Medical Centre Yola and the department of Microbiology Modibbo Adama University of Technology, Yola Adamawa State.

3.1.2 Experimental Animals

Healthy white albino rats of both sexes were used for the study. The rats were obtained from National Veterinary Research Institute Vom, Jos, Plateau State. The rats were kept in well ventilated cages and maintained on a commercial poultry feed and water continuously.

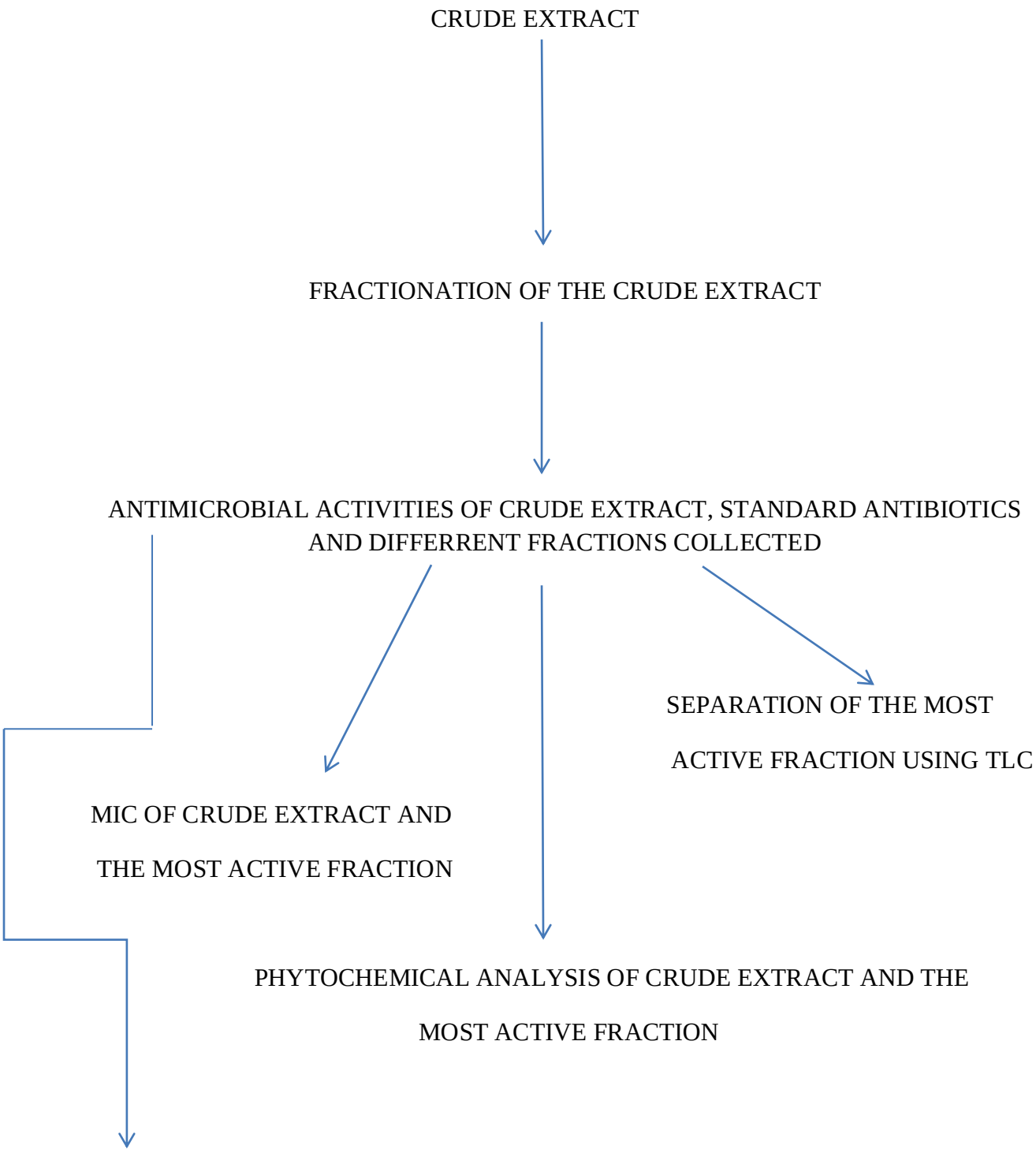
3.1.3 Chemicals/Drugs

Methanol (BDH, Poole England), Ferric chloride (JT Baker Chemical company, USA), Olive oil, Ammonia solution (E. merk Darmstard England), Concentrated tetraoxosulphate (iv) acid(HSR Fiscon PLC, LE 11 ORG, England), Acetic anhydride(E. merk Darmstard England), Hydrochloric acid (JDH, Guandong chemical factory co. Ltd, China), Picric acid (BDH, Poole England), Chloroform (Kermel Company, USA) ,Glacial acetic acid (E. merk Darmstard England), Benzene (Fiscon PLC, LE 11 ORG, England) , Nutrient agar(Titan Biotech. Ltd. Rajastan, India) , Nutrient broth(Lifesafe Biotech,Trade st. Sandiego USA), Silica gel (ADH, Poole England), Ethylacetate (Qualikems company USA),Aspirin(Drugfield pharmaceutical Ltd, Sango-otta, Nigeria), Ciprofloxacin(CIPLA Ltd, Patalganga, India), Azithromycin(Bristol Lab, Ltd, Berkhamsted,Herts HP4,1EG,UK), Chloramphenicol(Bharat parentals Ltd, Gujajat, India) Castor oil(Finest cold drawn bell's UK), Normal saline, Distilled water and Butanol(SLR Fiscon PLC, LE 11 ORG, England).

3.1.4 Equipment

Pestle and mortar, Rotary evaporator(R114, Buchi Switzerland), Water bath(Grant series 60 0622 010 cambridge England),Weighing balance(Diat-o-gram,USA) Filter paper, Funnel, Retort stand, Test tubes, Conical flask, Petri dishes, Autoclave, Pipettes, Incubator, Activated silica gel plate, TLC tank, micro capillary tube and camera.

3.1.5 Experimental Design



3.2 Methods

3.2.1 Extract Preparation

The stem bark was removed from the plant, washed and air-dried for 5-days at room temperature. The stem bark was pounded using pestle and motar, after which the powder (150g) was macerated in methanol (1000ml) and left overnight. The mixture was filtered and evaporated using rotary evaporator. The recovered crude extract after evaporation weighed 14.03g which was kept for future use.

3.2.2 Phytochemical Analysis

Chemical tests were conducted on the crude methanolic stem bark extract of *Faidherbia albida* and and the most active fraction of the crude extract using standard procedures as described by Trease and Evans (1989) and Sofowora (1993),

a.) Test for Tannins

The extract (0.5g) was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% Ferric chloride was added and observed for brownish green or a blue black colouration.

b.) Test for Saponins

The extract (1g) was boiled in 10ml of distilled water in a water bath and filtered. After that, 5ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which the formation of emulsion was observed.

c.) Test for Flavonoids

Exactly 5ml of dilute ammonia solution was added to a portion of the extract followed by addition of Conc. H_2SO_4. Observation of yellow colouration in the extract indicates the presence of flavonoids.

d.) Test for Steroids

Exactly 2ml of acetic anhydride was added to 0.5ml of the extract and then followed with 2ml H_2SO_4. The change of colour from violet to blue or green indicates the presence of steroids.

e.) Test for Alkaloids

The extract (0.5g) was stirred with 5ml of 1% Hcl on the steam bath. The solution was filtered and 1ml of the filtrate was treated with 2 drops of picric acid. The turbidity of the filtrate on addition of picric acid indicates the presence of alkaloids.

f.) Test for Terpenoids

Diluted methanolic extract (5 ml) was mixed with 2ml of chloroform, 3ml of conc. H_2SO_4 was carefully added to form a layer. Observation of reddish brown colouration of the interface that was formed indicates the presence of terpenoids.

g.) Test for Cardiac Glycosides

Exactly 5ml of diluted methanolic extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. After that, 1ml of conc. H_2SO_4 was added to the mixture. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.

h.) Test for Anthraquinones

Specifically, 1g of the extract was mixed with 10ml benzene in a conical flask and the mixture was filtered. To the filtrate, 5ml of 10% Ammonia solution was added and mixed thoroughly. The observation of a pink red or violet colour in the ammonia phase (bottom of the test tube) indicates the presence of anthraquinones.

3.2.3 Antimicrobial Activity Testing

The antimicrobial activity of the crude methanolic stem bark extract of *Faidherbia albida* was determined according to the method described by Emeruwa (1982). Wells were made on the surface of 19ml nutrient agar plates which was seeded with 0.1ml of 10^{-6} test organisms. Aseptically, 0.5ml of crude extract was introduced into the wells made. The plates were allowed to stand on the working bench for 30 minutes after which they were incubated for 24 hours at 37^0C in an incubator. The presence of zone of inhibition was regarded as positive and was expressed in terms of average diameter of the zone of inhibition. Similarly, same procedure was followed in the case of the most active fraction obtained from column

chromatography and the standard antibiotics (Azithromycin, Ciprofloxacin and Chloramphenicol).

3.2.4 Separation of the Crude Methanolic Stem Bark Extract of *Faidherbia Albida*

A slurry was prepared by dissolving 30g silica gel in 100ml methanol: water (1:1) and packed in a column (1.5 x 30cm). The column was loaded with 15ml of the crude extract and sequentially eluted with benzene/methanol (9:1), and acetic acid/methanol (1:1) ethylacetate/methanol (19:1). The fractions were collected separately, concentrated under pressure using rotary evaporator. (Nok *et al.*, 1993).

3.2.5 Minimum Inhibitory Concentration (M I C)

MIC of the crude methanolic stem bark extract of *Faidherbia albida* was determined by dilution of the crude to various concentrations of 40, 60, 80, 100 and 120 mg/ml respectively. Equal volume of crude extract and nutrient broth (1:1) were mixed in a test tube. Specifically, 0.1ml of standardized inoculum ($1.0x10^{-4}$cfu/ml) was added in each tube. The tubes were incubated aerobically at 37^0c for 18-24 hours. Tubes containing extract and growth media without inoculum was used as control. The lowest concentration of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tube was regarded as MIC (NCCLS, 2000). Similarly, same procedure was followed in the case of the most active fraction.

3.2.6 Thin Layer Chromatography

Thin layer chromatography was carried out with activated silica gel plate as stationary phase and a mixture of butanol, acetic acid and water (4:1:5) as solvents for the mobile phase. A thin pencil line mark was made on the plate at about 1cm from one end of the plate known as the starting point. A small amount of the fraction was spotted on the line using micro capillary tube. The spot was allowed to air dry. The plate was put into a TLC tank saturated with the solvent vapor. The plate was allowed to remain in the tank until the solvent is few centimeters away from the top of the plate. The distance moved by the samples and the solvent were noted. Spot visible at day light was documented by photographing.

3.2.7 *In Vivo* Antidiarrhoeal Analysis of the Crude Methanolic Stem Bark Extract of *Faidherbia albida*

The *in vivo* antidirrhoeal activity was evaluated using the castor oil-induced diarrhoea model in rats (Awouter *et al.*, 1978). Five groups of five rats per group were used. The rats were starved for 24 hours prior to the experiment. Normal saline 1ml/kg body weight was given to group 1 (control group) orally. Group 2 received 16mg aspirin/kg orally while group 3, 4, and 5 were treated with 125, 250 and 500mg extract/kg body weight of the crude methanolic stem bark extract of the plant respectively. one hour after the treatment, rats in all groups were given 1ml castor oil/100g body weight orally. The rats in each group placed singly in cages having adsorbent paper beneath and examined for the presence and frequency of wet stole every hour for 4 hours. Absence or delay in production of watery stool was regarded as protective or positive. Similarly, same procedure was followed in the case of the most active fraction.

3.3 Statistical Analysis

Results were expressed as the Mean $\pm$Standard error of mean. Statistical analysis of data was carried out using student's t-test. Differences in mean were considered to be significant when $p<0.05$.

CHAPTER FOUR

RESULTS

4.0 Phytochemical Analysis

The phytochemical analysis of the crude methanolic stem bark extract of *Faidherbia albida* and that of the most active fraction of the crude extract revealed the presence of tannins, saponins and alkaloids. This is summarized in table 4.1 below

Table 4.1: **Phytochemical Analysis of the Crude Methanolic Stem Bark Extract of *Faidherbia albida* and the Most Active Fraction of the Crude Extract (Fraction III)**

	ALK	ANT	GLY	SAP	FLV	TAN
Crude Extract	+	_	_	+	_	+
Fraction III	+	_	_	+	_	+

+= Present - = Absent

ALK=Alkaloids, ANT=Anthraquinones, GLY=Glycosides, SAP=Saponins, FLV=Flavonoids, TAN=Tanins

4.1 Effect of the Crude Methanolic Stem Bark Extract of *Faidherbia Albida* on the Test Organisms.

The *in vitro* antimicrobial activity of the crude methanolic stem bark extract of the plant shows highest activity of 12.0±0.17 mm on *Salmonella typhi* and lowest activity of 10.0±0.34 mm on *Shigella spp*. The result is presented in table 4.2 below.

Table 4.2: **Effects of the Crude Methanolic Stem Bark Extract of *Faidherbia albida* on Test Organisms.**

Test Organisms	Zone of Inhibition(mm)
E. coli	11.0 ± 0.12
Salmonella typhi	12.0 ± 0.17
Shigella spp	10.0 ± 0.34

Value are expressed as mean ± SEM, n = 32

4.2 Effect of Different Fractions of the Crude Extract on Test Organisms.

Among the fractions, fraction III showed highest activity on the test organisms ranging from 14.0±0.06 to 23±0.21 mm. The result is shown in table 4.3 below.

Table 4.3: **Effects of Different Fractions of the Crude Extract on Test Organisms.**

	Zone of Inhibition (mm)		
Test organisms	Fraction I	Fraction II	Fraction III
E. coli	11.0 ± 0.21	21.0± 0.31	23.0±0.21
Salmonella typhi	18.0± 0.06	20.0±0.06	21.0±0.17
Shigella spp.	13.0±0.12	11.0±0.12	14.0±0.06

Values are expressed as Mean ± SEM, n=3

Fraction I = Benzene/methanol

Fraction II = Acetic acid/methanol.

Fraction III = Ethylacetate/methanol.

4.3 Effect of Some Standard Antibiotics Used Against the Test Organisms.

Azithromycin showed highest activity on the test organisms ranging from 17.0±0.11 to 23.0±0.12 mm, Chloramphenicol, 15.0±0.12 to 21.0±0.12 mm and Ciprofloxacin, 12.0±0.08 to 20.0±0.15 mm as shown in table 4.4 below.

Table 4.4: **Effects of Some Standard Antibiotics used against the Test Organisms.**

	Zones of Inhibition (mm)		
Test organisms	**AZM**	**CPF**	**CHL**
E. coli	23-.0± 0.12	17.0 ±0.23	21.0± 0.12
Salmonella typhi	17.0 ±0.11	20.0 ±0.15	15.0 ±0.12
Shigella spp.	17.0 ±0.20	12.0 ±0.08	15.0 ±0.25

Values are expressed as Mean ± SEM, n=3

AZM = Azithromycin, CPF = Ciprofloxacin CHL = Chloramphenicol

4.4 Minimum Inhibitory Concentration (M.I.C)

Minimum inhibitory concentration of crude extract is 80 mg/ml on *E. coli* and *Salmonella typhi* ,100mg/ml on *Shigella spp* while the MIC of most active fraction is 60mg/ml on *E. coli* and *Salmonella typhi* and 80mg/ml on *Shigella spp* as shown in tables 4.5 and 4.6 below.

Table 4.5: **Minimum Inhibitory concentration (M.I.C) of the Crude Methanolic Stem Bark Extract of *Faidherbia albida* on the Test Organisms**

	Concentration of extracts in mg/ml.				
Test organisms	**40mg/ml**	**60mg/ml**	**80mg/ml**	**100mg/ml**	**120mg/ml**
E. coli	+	+	−	−	−
Salmonella typhi	+	+	−	−	−
Shigella spp.	+	+	+	−	−

+= Indicates growth − = Indicates no growth

Table 4.6: **Minimum Inhibition Concentration (M.I.C) of the Active Fraction of the Crude Extract on the Test Organisms**.

	Concentration of extracts in mg/ml.				
Test organisms	**40mg/ml**	**60mg/ml**	**80mg/ml**	**100mg/ml**	**120mg/ml**
E. coli	+	–	–	–	–
Salmonella typhi	+	–	–	–	–
Shigella spp.	+	+	–	–	–

+= Indicates growth – = Indicates no growth

4.5 Thin Layer Chromatography (TLC) of the Most Active Fraction

Thin layer chromatography (TLC) of the most active fraction was carried out revealing only one spot with Rf value of 0.89. The spot was scraped and tested on *E.coli* with zone of inhibition of 10.0±0.34mm.

4.6 *In Vivo* antidiarrhoeal Analysis of the Crude Extract and Most Active Fraction

Different concentration of crude extract and the most active fraction of the crude extract were used. In the case of the crude, of all the concentrations used only 500mg/kg maintained 100% maximum efficiency throughout the period of study where as in the case of active fraction 250 and 500mg/kg maintained 100% maximum efficiency throughout the period of study. The result is seen in tables 4.7 and 4.8.

Table 4.7: ***In vivo*** **Antidiarrhoeal Effect of Crude Methanolic Stem Bark Extract of** ***Faidherbia albida*** **in Rats**

			Time(h)			
Group	**Treatment**	**Dose(mg/kg)**	**1**	**2**	**3**	**4**
1	Normal Saline	-	0(0%)	0(0%)	0(0%)	0(0%)
2	Aspirin	16	5(100%)	5(100%)	4(80%)	4(80%)
3	Extract	125	0(0%)	0(0%)	2(4%)	3(60%)
4	Extract	250	4(80%)	5(100%)	5(100%)	5(100%)
5	Extract	500	5(100%)	5(100%)	5(100%)	5(100%)

Values are expressed as percentage inhibition, n=5

Table 4.8: ***In vivo*** **Antidiarrhoeal Effect of the Most Active Fraction (Fraction III) of the Crude Extract in Rats.**

			Time(h)			
Group	**Treatment**	**Dose(mg/kg)**	**1**	**2**	**3**	**4**
1	Normal Saline	-	0(0%)	0(0%)	0(0%)	0(0%)
2	Aspirin	16mg	5(100%)	5(100%)	4(80%)	4(80%)
3	Fraction III	125	0(0%)	1(20%)	2(40%)	4(80%)
4	Fraction III	250	5(100%)	5(100%)	5(100%)	5(100%)
5	Fraction III	500	5(100%)	5(100%)	5(100%)	5(100%)

Values are expressed as percentage inhibition, n=5

Fraction III = Ethylacetate/methanol

CHAPTER FIVE

DISCUSSION

The phytochemical analysis of the crude methanolic extract of stem bark of *Faidherbia albida* and the most active fraction of the crude revealed the presence of tannins, saponins and alkaloids (Tables 1 and 2), this findings in contrast with that of Kubmarawa *et al* (2007), who stated that none of these bioactive components are present in the stem bark. The observed difference could be due to environmental changes where the plants were collected or seasonal changes that could have altered the plant components. It could also have been as a result of changes during extraction and / or storage.

According to Heldt (2005) most of these phytochemicals are produced through biosynthesis in the metabolic pathways. The primary metabolites are of major importance to plants (Trease and Evans, 1989). The secondary metabolites are of medicinal value to man and these can equally be obtained from various anatomical structures of plants (Fahn, 1974).

Tannin which is one of the bioactive components found in the stem bark of *Faidherbia albida* is used medically as antidote to poisoning by alkaloids due to their capacity to form insoluble tannates (Bajaj, 2000). Other remedial values of tannins include application on burns to heal the injury and on cuts to stop bleeding, (Davidson 2001). Tannins ability to form a strong 'leather' resistance on the exposed tissues helps in protecting the wounds from being affected further (Akiyama *et al*., 2001). While it stops infection from above, internally tannin continues to heal the wound (Souza *et al.*, 2006). In case of third degree burns using strong tannin sources will not only prevent septicemia, but also help to save life (Davidson, 2001).

Tannin can also be effective in curbing haemorrhages as well as restrict bare swelling. While tannins are proved homeostatic, they are also beneficial when applied on mucosal coating in mouth. Hence, herbs possessing tannins are widely used as mouthwashes, eyewashes, snuff and even as vaginal douches and also to treat rectal disorders (Akerele, 1992). Tannins when applied internally, affect the walls of the stomach and other digestive parts. They sour the mucus secretions and contract or squeeze the membranes in such a manner that secretions from the cells are restricted. The good thing is that tannins anti-inflammatory effect helps to control or curb all indications of gastritis, enteritis, and irritating

bowels disorder. This action is possible by involving lymph static and neutralizing the autolytic enzymes. Conventionally, tannins have also been used to cure diarrhea (Akerele, 1992) Additional advantage of tannin include relief of pain, limitation of secondary infection and epithelialization (Hupkens ,1995 and Halkes, 2001). The presence of tannins in the stem bark extract of *faiherbia albida* support the traditional medicinal used of it in the treatment of diarrhea, relieving of pains and wound healing.

The observed antibacterial effect of crude methanolic stem bark extract of *Faidherbia albida* could be attributed to the presence of tannins, the phytochemical which inhibit bacterial growth and protease activity by damaging the cell and cytoplasm, causing rapid structural destruction (Andrade *et al*., 2006; Cowan *et al*.,1999). One mode of action of plant tannins is to complex with dietary nutrients through hydrogen and hydrophobic effect, as well as by covalent bond formation (Haslam *et al*., 1989; Scalbert *et al*., 1991). Thus their mode of antimicrobial effect may be related to their ability to inactivate microbial adhesion enzymes, cell envelope transport proteins and mineral uptake (Bell *et al*., 1965; Scalbert *et al*., 1991; Min *et al*., 2003).

Saponins are group of naturally occurring plant glycosides, characterized by their strong foam – forming properties in aqueous solution. Many saponins are known to be antimicrobial, to inhibit mold, and to protect plants from insect attack. A large number of the biological effects of saponins have been ascribed to their action on membranes. In fact, their specific ability to form pores in membrane has contributed to their common use in physiological research (EL Izzi *et al*., 1992; Authi *et al*., 1988; Choi *et al*., 2001; Menin *et al*., 2001; Plock *et al*., 2001). Saponins have long been known to have a lytic action on erythrocyte membranes (Authi *et al*., 1988) and this property has been used for their detection. The haemolytic action of saponins is believed to be the result of the affinity of the aglycone moiety for membrane sterols, particularly Cholesterol (Glauert *et al*., 1962), with which they form insoluble complexes (Bangham and Horne, 1962).

The *in vivo* effects of saponins on the reproductive functioning seem to indicate more than a simple permeabilising effect on secretary cell membrane and could possibly be linked to interactions between the basic chemical structure of saponins and steroid hormones (Plock *et al*., 2001). Saponin isolated from different plants and animals have been shown to

specifically inhibit the growth of cancer cells *in vitro* (Marino *et al.,* 1998; Mimaki *et al*., 1998 ; Podolak *et al.,* 1999).

The ability of saponin to lower the serum cholesterol level of animals has also been reported. These favourable effects point to the potential of saponins, including those present in the diet, as a remedy against two of the major health hazards in many countries, obesity and cancer (Francis *et al*., 2002). The observation that saponins have anticancer activity, and can be use in cancer prevention, suggest that the stem bark extract of *Faidherbia albida* has potential as a source of important bioactive molecules for the treatment and prevention of cancer.

Alkaloids are group of naturally occurring chemical compound that contain mostly nitrogen atoms. Alkaloids are very important in medicine and constitute most of the valuable drugs. They have mark physiological effect on animals (Edeoga and Eriata, 2001). Alkaloids such as quinine are used as antimalarials, Vinblastine as antitumor, and reserpine as antihypertensive (David, 2001). Alkaloids are known to have antimicrobial and antiparasitic properties (Neli *et al*., 2011). The presence of alkaloids in the crude methanolic stem bark extract of *Faidherbia albida* might have also contributed to its antimicrobial effect which is reported to act by intercalating into cell wall and/or DNA thereby disrupting the activities of the microorganisms (Neli *et al*., 2011).

The *in vitro* antimicrobial activity of the crude methanolic stem bark extract of *Faidherbia albida* was tested on *E. Coli, Salmonella typhi* and *Shigella spp* and the result showed positive on all the test organisms. The crude extract showed highest activity on *Salmonella typhi* (table 3) suggesting that more of the bioactive components required to act on the organism are contained in the stem bark extract of *Faidherbia albida* and also could have antityphoidal effect since the organism is the causative agent of the disease. The positive result observed is not surprising because of the presence of the bioactive components in the stem bark extract of *Faidherbia albida* as stated by Elujuba, (1997) the antimicrobial activities could be traced to the presence of bioactive component like saponins, tannins and alkaloids.

Fractionation in some cases may result in improved activity but in others may result in loss of activity (Nwodo *et al.,* 2010). In this case fractionation of the crude methanolic stem bark extract of *Faidhebia albida* extract resulted in improved activity because when the

activity of the crude methanolic stem bark extract of *Faidhebia albida* and that of the different fractions collected was compared, there was a significant improvement (Tables 3 and 4). The observation that fraction III had highest activity is a good indication that the active ingredient of the crude methanolic stem bark extract of *Faidhebia albida* is contained in fraction III. This is also in agreement with the view of Nwodo, *et al* (2010) who stated that purification of crude extracts could produce loss or gain of activity depending on the nature of interaction between the constituent compounds of the extract.

The minimum inhibitory concentration of the crude methanolic stem bark extract of *Faidhebia albida* and most active fraction were determined (Tables 6 and 7), this observation further confirmed that the fractionation of the crude extract has increased the activity of the extract. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents (Sen and Batra, 2012).

The zones of inhibition of the crude methanolic stem bark extract of *Faidhebia albida* and that of ciprofloxacin statistically has no significance difference (Tables 3 and 5). This observation may be attributed to the presence of some active compounds or groups in the crude extract with similar mechanism of action to that of the standard drug.

Thin layer chromatography (TLC) of most active fraction was carried out revealing only one spot with Rf value of 0.89. The spot was scraped and tested on *E.coli* still exhibiting the antibacterial effect. This is an indication that the spot contained an active ingredient.

The *in vivo* antidiarrhoael analysis of the crude methanolic stem bark extract of *Faidherbia albida* revealed that at the concentration of 500mg/kg body weight given to the rats maintained a 100% maximum efficiency throughout the period of the study which is in contrast with the view of Tijani *et al* (2008), who stated that it was at 250mg/kg body weight that produced maximal inhibition using the aqueous stem bark extract of *Faidherbia albida*, but the most active fraction of the crude methanolic stem bark extract of *Faidherbia albida* is in agreement with the view of Tijani *et al* (2008) that 250mg/kg and 500mg/kg body weight produced maximal inhibition(Tables 3 and 4).This observation is not surprising because

according to the findings of Gunner (1991) different solvent extracts of some plants may exhibit different pharmacological properties.

The *in vivo* antidiarrhoeal effect of the crude methanolic stem bark extract of *Faidherbia albida* and the most active fraction of the extract were observed in rats using castor oil induced diarrhoea model (Awouter *et al.*, 1978). Several mechanisms have been previously proposed to explain the diarrheal effect of castor oil including inhibition of intestinal Na^+, K^+ ATPase activity to reduce normal fluid absorption (Gaginella and Bass, 1978), activation of adenylate cyclase or mucosal cAMP mediated active secretion (Capasso *et al*., 1994), stimulation of prostaglandin formation (Galvez *et al*., 1993), platelet activating factor and also nitric oxide has been claimed to contribute to the diarrheal effect of castor oil (Mascolo *et al*., 1996). However, it is well evident that castor oil produces diarrhoea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandin which results in the stimulation of secretion (Gaginella *et al.,* 1985). Since the crude methanolic stem bark extract of *Faidherbia albida* and the most active fraction of the extract successfully inhibited the castor oil induced diarrhea, the extracts might have exerted the antidiarrheal action via antisecretory mechanism or the antidiarrheal property may be attributed to the presence of tannins, the phytochemical which is known to reduce the effect through denaturing the protein by the formation of protein tannate, thereby causing the intestinal mucosal more resistant and reduces secretion (Tripathi, 1994). Hence tannins present in the extracts may be responsible for their antidiarrheal activity (Yu *et al*., 2000; Devi *et al*., 2002) and also be partly responsible for other pharmacological properties.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.0 Summary

The *in vitro* and *in vivo* effects of stem bark extract of *Faidherbia albida* were investigated. The phytochemical analysis of the crude extract and the most active fraction of the crude revealed the presence of secondary metabolites like tannin, saponin and alkaloids. The *in vitro* antimicrobial activity of the crude methanolic extract of the stem bark showed highest activity on *Salmonella typhi* with zone of inhibition of 12.0 $\pm$ 0.17mm compared to *E. coli* and *shigella* spp. The crude methanolic stem bark extract of *Faidherbia albida* was fractionated, of these fractions, Fraction III (Ethylacetate/methanol) showed highest activity against the test organisms ranging from 14.0 $\pm$ 0.06 to 23 $\pm$ 0.21mm.

Thin layer chromatography (TLC) of most active fraction was carried out revealing only one spot with Rf value of 0.89. The spot was scraped and tested on *E.coli* with zone of inhibition of 10.0$\pm$0.34mm.

Minimum inhibitory concentration of crude extract is 80mg/ml on *E. coli* and *Salmonella typhi*, 100mg/ml on *Shigella spp* while the MIC of most active fraction is 60mg/ml on *E. coli* and *Salmonella typhi* and 80mg/ml on *Shigella spp.*

The *in vitro* antimicrobial activity of the crude methanolic stem bark extract of *Faidherbia albida* was also compared to some standards antibiotics. The results showed that azithromycin had highest activity ranging from 17.0 $\pm$ 0.11mm to 23.0 $\pm$ 0.12mm against the test organisms but there was no significance difference *($P<0.05$)* between the effect of the crude methanolic stem bark extract of *Faidherbia albida* and that of cirprofloxacin.

The *in vivo* antidiarrhoeal analysis of both the crude extract and the most active fraction of the crude extract proved positive. Among the different concentrations used, it was 500 mg/kg body weight of the crude extract given to the rats that maintained 100% maximum inhibition while in the case of the most active fraction, 250 and 500mg/kg body weight given to the rats maintained 100% maximum inhibition throughout the period of the study.

6.1 Conclusion

In conclusion, the present studies revealed the antibacterial nature of the stem bark of *Faidherbia albida* and the activities of the extract and ciprofloxacin indicate no significance difference suggesting that the stem bark of *Faidherbia albida* possess greater potential to be explored for the development of antibiotics and further established scientifically the basis of using the stem bark by traditional healers for treatment of diarrhoea. The mechanism of action of constituents of stem bark of *Faidherbia albida* may be difficult to speculate: however, many antibacterial agents may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis (Franklin *et al.*, 1987). It is probably that the antibacterial agents in the extract of stem bark of *Faidherbia albida* act via some of the above mechanisms.

6.2 Recommendation

- Further studies should be carried out using other methods other than castor oil to induce diarrhea.
- Studies should also be carried out to identify and elucidate the structure of the bioactive compounds that may be responsible for the observed activity.

6.3 Contribution to Knowledge

Below are the contributions of this study to knowledge

- Fractionation of the crude extract may result in decrease or increase of an activity of the extract as observed in this study.
- The stem bark of *Faidherbia albida* could be used not only for the treatment of diarrhoea but also on other diseases cause by the test organisms used in this study.

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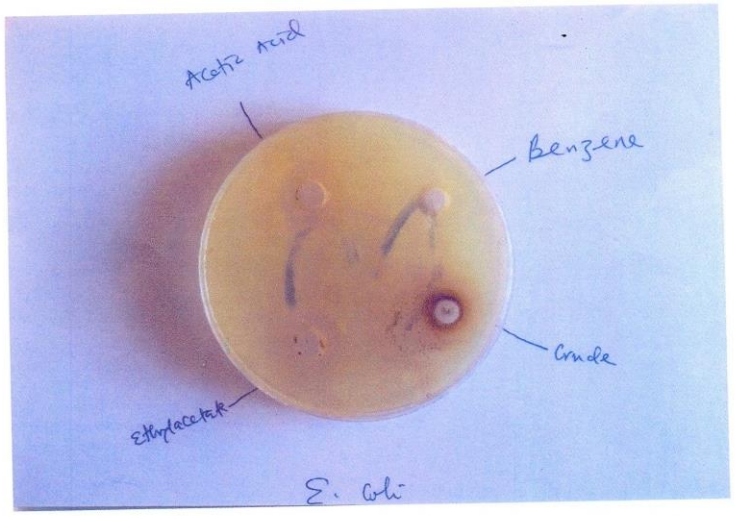
APPENDIX I

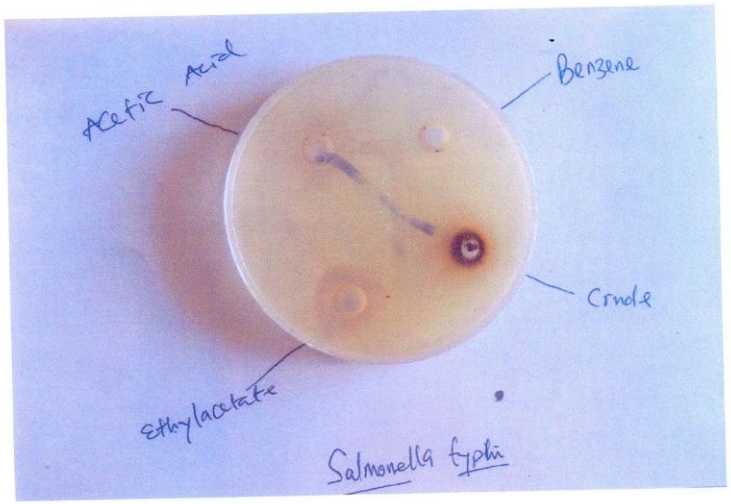
***Faidherbia albida* Tree**

APPENDIX II



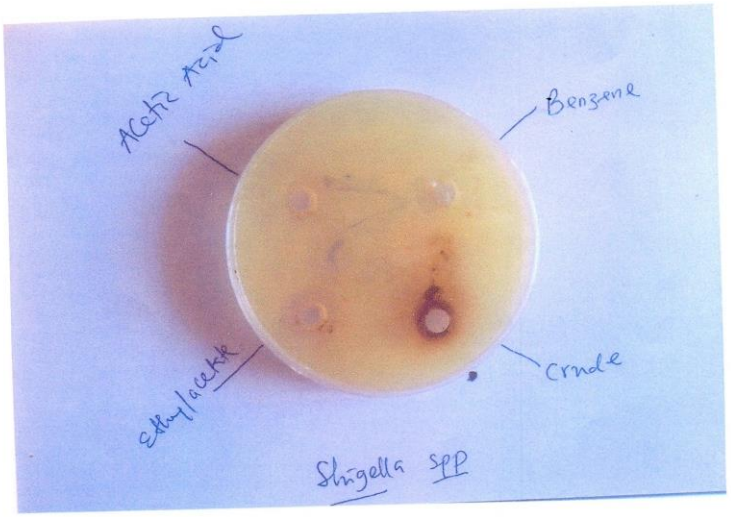
Activity of the Crude Extract and its Different Fractions on *E.coli*

APPENDIX III



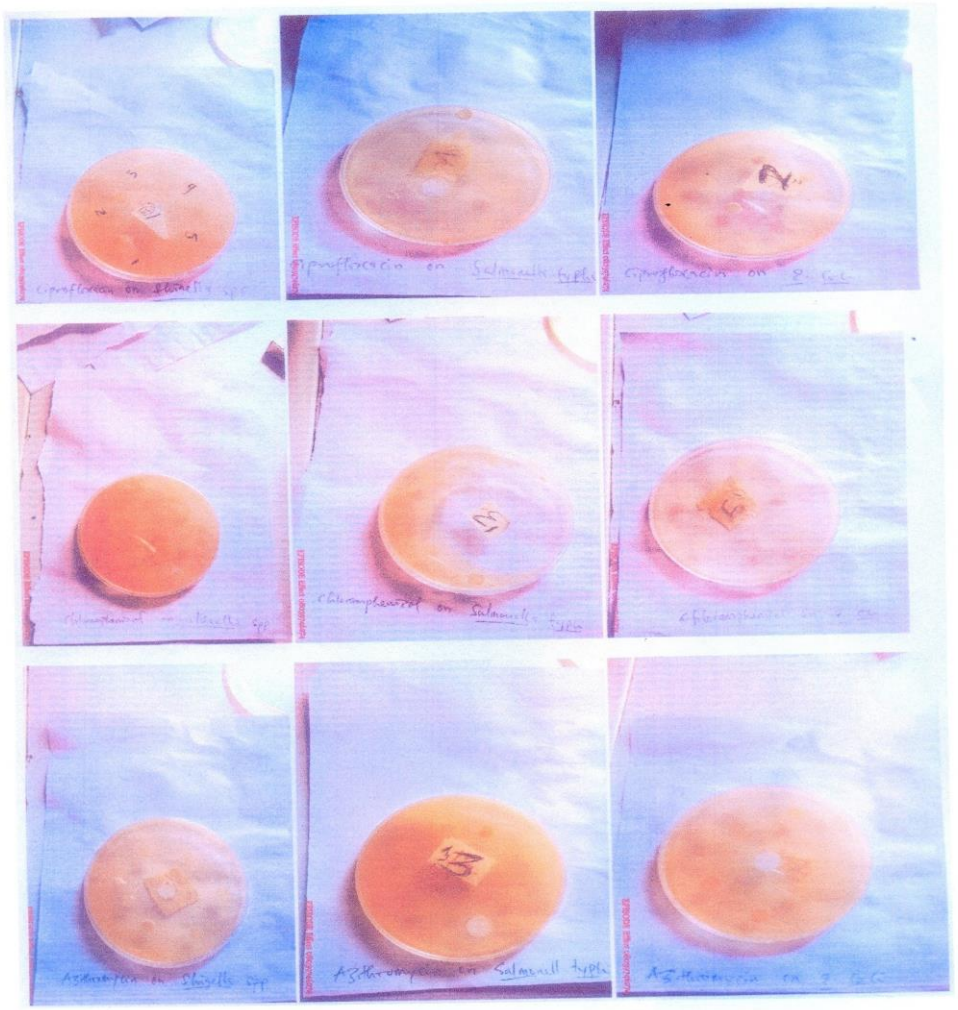
Activity of the Crude Extract and its Different Fractions on *Salmonella typhi*

APPENDIX IV



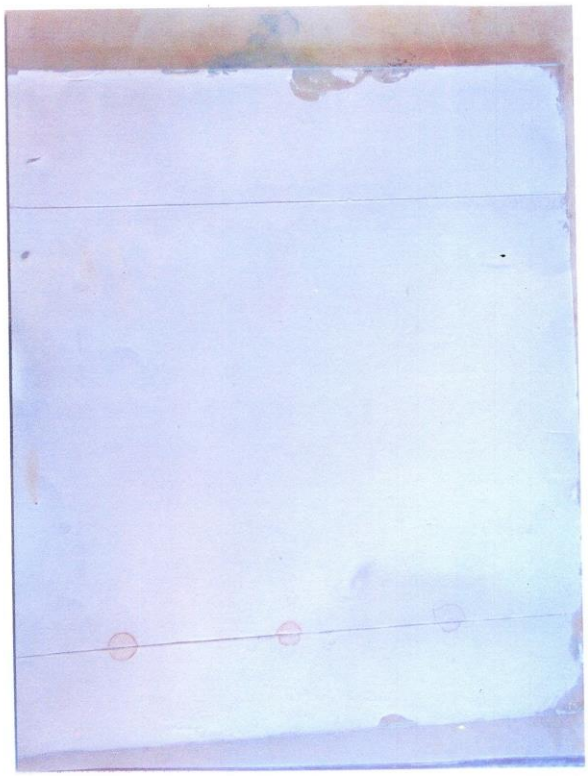
Activity of the Crude Extract and its Different Fractions on *Shigella spp*

APPENDIX V



Activity of the Standard Antibiotics on Test Organisms

APPENDIX VI



Thin Layer Chromatography (TLC) of the Most Active Fraction (Fraction III)