ANTIDIARRHOEAL AND ANTIBACTERIAL ACTIVITIES OF METHANOLIC EXTRACTS AND FRACTIONS OF THE PULP AND SEED OF ZIZIPHUS MAURITIANA (CHINEE APPLE)

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DEPARTMENT OF BIOCHEMISTRY,
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MARCH, 2017

DECLARATION

I declare that the work in this dissertation entitled ‘‘ANTIDIARRHOEAL AND ANTIBACTERIAL ACTIVITIES OF METHANOLIC EXTRACTS AND FRACTIONS OF THE PULP AND SEED OF ZIZIPHUS MAURITIANA *(*CHINEE APPLE*).*’’ has been carried out by me in the Department of Biochemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution**.**

ASUGU Martha Mbahi ______________________ ______________________

Signature Date

CERTIFICATION

This dissertation entitled ‘‘ANTIDIARRHOEAL AND ANTIBACTERIAL ACTIVITIES OF METHANOLIC EXTRACTS AND FRACTIONS OF THE PULP AND SEED OF ZIZIPHUS MAURITIANA(CHINEE APPLE).’’ by Mbahi Martha ASUGU meets the regulations governing the award of the degree of M. Sc. Biochemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Prof. I.A.Umar
Chairman, Supervisory Committee ________________ Signature ________________ Date

Prof. D.A. Ameh
Member, Supervisory Committee ________________ Signature ________________ Date

Prof. M. N. Shuaibu
Head of Department ________________ Signature ________________ Date

Prof. K. Bala
Dean, School of Postgraduate Studies ________________ Signature ________________ Date

DEDICATION

I dedicate this dissertation to my parents and siblings who have supported me all the way since the beginning of my studies.

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ABSTRACT

This study evaluated, the antidiarrhoeal and antibacterial activity of the methanolic extracts of seed and pulp of *Ziziphus mauritania.* The crude extracts were prepared by maceration of seed and pulp powder in methanol. The pulp extract was partitioned into n-hexane, ethylacetate, n-butanol and aqueous fractions. The ethylacetate extract was further fractionated by column chromatography into four fractions A-D. The antibacterial activity of the crude extracts, and fractions were evaluated by Agar well diffusion method .The antidiarrhoeal activity of the crude seed and pulp extracts were evaluated using castor oil-induced diarrheal model, charcoal meal test and anti-fluid accumulation test in rats. The result of phytochemical test indicated that tannins, flavonoids, saponin, cyanogenic glycosides, and terpenoids were present in both seed and pulp extracts. The antibacterial susceptibility test showed that the seed and pulp extracts possess inhibitory activity against *Staphyloccus aureus, Eschericial coli and Salmonella* spp. The fractions obtained from the pulp extract also showed varied antibacterial activity, the diameter of the zone of inhibition obtained ranged from 29-11 and 27-10 mm for seed and pulp respectively. In the castor oil-induced model, fluid accumulation test, the methanolic extracts of the seed and pulp produced a significant ($p<0.05$) decline in volume of intestinal contents. Results from the charcoal meal test revealed that all the extract produced a significant ($p<0.05$) anti-motility effect. Based on the findings of this work, the pulp methanolic extract of this plant possess antibacterial and anti-diarrhoeal properties and validates its use in traditional medicine for the treatment of diarrhoea and a number of ailments caused by microorganisms.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of varience
CCL_4	Carbon Tetrachloride
CD4	Cell Differentiation
CDC	Center for Disease Control
CIPRO	Ciprofloxacine
Conc.	Concentration
DMSO	Dimethylsulphuroxide
DNA	Deoxyribonucleic acid
E. coli	*Eschericia coli*
HDL	High density lipoprotein
HIV	Human Immunodeficiency Virus
HL-60 cells	Human Promyelocytic Leukemia Cells
LDL	low density lipoprotein
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
ºC	Degree Celcius
ORT	Oral Rehydration Therapy
RBC	Red Blood Cell
Rf value	Retention Value
RNA	Ribonucleic acid
S. aureus	*Staphylococcus aureus*

SD	Standard deviation
Spp	Species
SPSS	Statistical analysis of variance
TLC	Tin Layer Chromatography
TLC	Thin layer chromatography
tRNA	Transfer Ribonucleic acid
UNESCO	United Nations Educational Scientific and Cultural Oganization.
UNICEF	United Nation Children Education Fund.
UV light	Ultra Violet light
WHO	World Health Organization
Z.mauritiana	*Ziziphus mauritiana*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Since the beginning of human civilization, medicinal plants have been used for their therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Shaikh and Hatcher, 2005).

According to the World Health Organization "a medicinal plant" is any plant, which in one or more of its organs contains substances that can be used for the therapeutic purposes or which, are precursors for the synthesis of useful drugs(WHO, 2001). This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. The term "herbal drug" determines the part/parts of a plant (leaves, flowers, seeds, roots, barks, stems, etc.) used for preparing medicines (Anonymous, 2007). Furthermore, WHO defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products(WHO,2004). Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo*et al.,* 2005).

The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1998). Modern pharmacopoeia still contains at least 25% of drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being and the bioprospecting of new plant-derived drugs. The growing recognition for medicinal plants use is due to several reasons, including escalating faith in herbal medicine (Kala, 2005). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998).

The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemicals in them (Ayodele, 2005). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento*et al.*, 2000).

Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering with the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way, the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host. So the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important. The instant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges (Kala *et al.*, 2006); therefore they are more prone to extinction. Conversely, because information on the use of some plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing socioeconomic changes (Kala, 2000).

Phytochemical screening, evaluation of antimicrobial properties and antidiarrhoeal status of medicinal plants are today recognized as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents (Adjanahoun*et al.,* 2001). Plants which are observed to be efficacious and frequently prescribed may contain compounds that are potential drug candidates and could rightly be recommended for further examination (Tor-Anyiin*et al.,* 2003).

The growing resistance of pathogenic organisms against theconventional antibiotics formerly recognized for their efficiency is today a real problem of public health (Nascimento*et al.,* 2000). In human pathology, microorganisms are responsible for many infections including respiratory tract diseases (pneumonia, bronchitis), skin, wound and mucous infections, sinusitis, endocartidis, osteomyelitis, syphilis, gonorhea, tuberculosis, food poisoning and carbuncles to mention a few. They are also the germs frequently met during surgical wound infections which are often provoked by the use of intravascular catheters or by the spread of bacteria from another source of infection (Landolo*et al.,* 2002). *Staphylococcus aureus* for instance, one of the most pathogenic among the species of *Staphylococcus* is responsible for almost 25 % of septicemias met in hospitals (Moyen*et al.*, 2003). Generally, the treatment of infections caused by microorganisms is long and expensive. The antimicrobial properties of plants have been investigated by a number of researchers worldwide thorough biological evaluation of plants extracts it is vital to ensure their efficacy and safety. These factors are of importance if plant extracts are to be accepted as valid medical agents for the treatment of infectious diseases especially in the light of the emergence of drug resistant microorganisms (Nascimento*et al.*, 2000).

Diarrhoea is a common gastrointestinal disorder characterized by an increase in stool frequency and a change in stool consistency (Farthing, 2002). It remains one of the major health threats to populations in the tropical and subtropical poor countries. According to the World Health Organization estimates that 1.7 billion cases occur each year (760 000 in children less than 5 years of age), and that approximately 5 million deaths are due to diarrhoea annually (2.5 million in children less than 5 years of age) (WHO, 2013). Bacteria and some parasitic organismshave long been implicated in the incidence of diarrhoea (WHO, 1999). Diarrhoea is a common symptom of gastrointestinal infections caused by a wide range of pathogens, including bacteria *(Escherichia coli*, *Shigella*, *Campylobacter, Vibriocholerae*), viruses (rotavirus), and protozoa (*Cryptosporidium*). However, a handful of other organisms are also responsible for most acute cases of childhood diarrhoea. Among these, rotavirus is the leading cause of acute diarrhoea, and responsible for about 40% of all hospital admissions due to diarrhoea among under five children worldwide (CDC, 2008).

Evidence from report of several experimental studies shows that plant material with antimicrobial activity also possesse significant antidiarrhoeal activity particularly in infectious diarrhoea. In both cases, these activities have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids (Longanga Otshudi *et al*., 2000; Umer *et al*., 2013; Getnet, 2015).

*Ziziphusmauritiana*Lam belongs to the family *Rhamnaceae*. It is widely grown in mild-temperate, rather dry areas, of both hemispheres and is adapted to warm climates. *Ziziphusmauritiana*can grow either as shrublets, shrubs or trees with thornybranches and are used as a hedge to form defensivefences for animals. It is often called merely jujube, Chinese date, Indian plump (Morton, 1987). In Northern Nigeria it is called "Magarya" in Hausa or "Huhue" in bura. The plant finds various uses in traditional medicine for instance; the pulps are applied on cuts and ulcers; are employed in pulmonary ailments and fevers; the dried ripe pulp is a mild laxative. The seeds are sedative and are taken sometimes with butter, to halt nausea, vomiting and abdominal pains in pregnancy. Mixed with oil, they are rubbed on rheumatic areas. The leaves are helpful in liver trouble, asthma and fever. The bitter, astringent bark decoction is taken to halt diarrhoea and dysentery and relieve gingivitis. A root decoction is given as a febrifuge, taenicide and emmenagogue, and the powdered root is dusted on wounds. Juice of the root bark is said to alleviate gout and rheumatism (Morton, 1987). The root is also used in the treatment of epilepsy (Msonthi and Magombo., 1983). The dried root is also used to treat diarrhoea in Northern parts of Nigeria (Personal communication). The leaves are applied as poultices and are helpful in liver troubles, asthma andfever (Michel, 2002).The hepatoprotective activity of ethanol extract of *Ziziphus mauritiana*leaf against CCl_4 - induced liver damage in rats and the antidiarrhoeal activity of the methanol root extract were reported (Dahiru*et al.,* 2005, 2006). The antioxidant activity of the aqueous extract of *Ziziphus mauritiana leaf* has also been reported(Dahiru and Obida, 2008).

1.2 Statement of Research Problem

Diarrhoea is one of the major factors that contribute significantly to high child morbidity and mortality in many developing countries (Onyango and Angienda, 2010). Among children under 5 years old, about 9% of all deaths occur due to diarrhoea and children of under 3 years' experience an average of three episodes per year. Annually, at least 1,500 million episodes of diarrhoea occur and at least 1,600 young children die each day or about 580,000 each year (Kumar and Subita, 2012; UNICEF, 2013).An estimated 1.5million children under the age of 5 die from diarrhoea each year more than AIDS, malaria, measles combined according to joint UNICEF-WHO reports (2009).

Although many different antibacterial agents are available in the field of medicine, yet many of these agents are increasingly being incapacitated by microorganisms through the evolution of different mechanisms that amount to resistance to these drugs (Walsh, 2000).In developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also are often with adulterations and side effects (Shariff, 2001).

1.3 Justification of the Study

Phytomedicine has demonstrated its contribution to the reduction of excessive mortality, morbidity and disability due to diseases such as malaria, tuberculosis, sickle-cell anemia, diabetes, mental disorders and microbial infections (Okigbo*et al.*, 2005). It has played a key role in world health care with about 80% of Africans depending on it (WHO, 2004). It hasassisted in reducing poverty by increasing the economic well-being of communities and developed health system by increasing health coverage to the people. Substantial amount of foreign exchange has been earned by exporting medicinal plants to other countries. In this way, indigenous medicinal plants play significant role in the economy of a country (Ghani, 2003).

Research has shown that many herbs possess varying degrees of antimicrobial activities. Kaufman *et al.*, (2009) had reported that more than 25% of the prescribed drugs contained at least one active ingredient of plant origin. Despite widespread use of oral rehydration therapies (ORT) and an increased understanding of the pathogenesis of diarrhoea millions of children still die from this disease condition every year(Thapar and Sanderson,2004).Medicinal plants are now very popular in developing countries with knowledge about the safety, efficacy and quality assurance (Calixto, 2000). Therefore there is a need for a continuous search for new effective and affordable plant derived drugs.

1.4 Aim and Objectives

1.4.1 Aim

The aim of this work was to evaluate the phytochemical constituents, antidiarrhoeal and antibacterial activities ofthe methanolic extracts and fractions of thepulp and seed of *Ziziphusmauritiana*.

1.4.2 Specific Objectives

1. To determine the phytochemical composition of thepulp and seeds extractsof Z. *mauritiana.*
2. To determine the antidiarrhoeal activity of the methanolic extracts of pulp and seed of *Z. mauritiana* in rats.

3. To determine the antibacterial activity of the methanolic extracts of thepulp and seed of *Z. mauritiana* against *Staphylococcus aureus*, *Escherichia coli*,and *Salmonella* spp.
4. To fractionate the extracts and determine the fraction with the best antibacterial activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Phytomedicine

Phytomedicine as a major African socio-cultural heritage has been in existence for hundreds of years. It was once believed to be primitive and wrongly challenged by foreign religions dating back during the colonial rule in Africa and subsequently by the conventional or orthodox medical practitioners. Traditional medicine has been the focus for wider coverage of primary health care delivery in Africa and the rest of the world (Elujoba*et al.,* 2005).

Sofowara (1984) defined traditional medicine as the sum total of knowledge or practices whether explicable or inexplicable used in diagnosing, preventing or eliminating a physical, mental or social disease, which may rely exclusively on past experience or observation handed down from generation to generation, verbally or in writing. It comprises therapeutic practices in existence for hundreds of years before the development of modern scientific medicine and is still in use today without much documented evidence of adverse effects. The new health agenda in Nigeria and Africa focuses on the institutionalization of traditional medicine in parallel with orthodox medicine into the national health care scheme in order to move the health agenda forward, since effective health cannot be achieved in Africa by orthodox medicine alone unless it has been complemented with traditional medicine as recorded by Elujoba*et al.,* (2005).

This traditional medicine comprises the use of plant, animal or mineral materials for healing but the focus here is on phytomedicine (plant medicine) type. Furthermore, Iwu*et al*., (1999) reported that the first generations of plant medicine were simple botanical materials employed in more or less crude form. These medicines such as *Cinchona*, *Opium, Belladonna* and *Aloe* were selected based on empirical evidence as gathered by traditional practitioners. The second-generation phytopharmaceutical agents were pure molecules whose compounds differ from the synthetic therapeutic agent only in their origin, for example taxol from *Taxus* spp., quinine from *Cinchona* and reserpine from *Rauvolfia*spp. In the development of third generation of plant medicine, the formulation is based on well-controlled double-blind clinical and toxicological studies with phytomedicine to improve the quality, efficacy, stability and the safety of the preparations (Iwu*et al.*, 1999).

2.2 *Ziziphusmauritiana*

2.2.1 Morphology and distribution

*Ziziphusmauritiana*is a spiny, evergreen shrub or small tree up to 15 m high, with trunk 40 cm or more in diameter; spreading crown; stipular spines and many drooping branches. Bark dark grey or dull black, irregularly fissured. Where climatic conditions are severe, it is commonly a compact shrub only 3-4m tall. Leaves variable, alternate, in 2 rows, oblongelliptic, 2.5-6 x 1.5-5 cm, with tip rounded or slightly notched base; finely wavy-toothed on edges, shiny green and hairless above; dense, whitish, soft hairs underneath. Inflorescence axillary cymes, 1-2 cm long, with 7-20 flowers; peduncles 2-3 mm long; flowers 2-3 mm across, greenish-yellow, faintly fragrant; pedicels 3-8 mm long; calyx with 5 deltoid lobes, hairy outside, glabrous within; petals 5, subspathulate, concave, reflexed. Pulp a drupe, globose to ovoid, up to 6x4 cm in cultivation, usually much smaller when wild; skin smooth or rough, glossy, thin but tough, yellowish to reddish or blackish; flesh white, crisp, juicy, subacid to sweet, becoming mealy in fully ripe pulp. Seed a tuberculate and irregularly furrowed stone; containing 1-2 elliptic brown kernels each 6 mm long (Awasthi and More 2008).

2.2.2 Ethnomedicinal uses

The kernels are reported to have a sedative effect and are recommended as a soporific. They are prescribed to stop nausea and vomiting and for relief from abdominal pain in pregnancy. They are also given as an antidote to aconite-poisoning, and used in poultices and other formulations of wounds healing. The seeds are used for the treatment of diarrhoea. The leaves are eaten with catechu as an astringent. They are regarded as diaphoretic and are prescribed for typhoid in children. They are also used as poultices. A decoction of the bark is used for the treatment of diarrhoea and dysentery. The bark is also used as an astringent in gingivitis (PetoAzam-Ali *et al.;* 2006).

2.2.3 Ayurvedic Therapeutics

In ayurveda, *Ziziphusmauritiana* root is used as bitter and cooling, cures kapha, biliousness and headache. Decoction of roots is used in fever, and as powder applied to old wounds and ulcer. Bark is used as remedy in diarrhoea and cures boils.Leaves are bitter and cooling; cure kapha: biliousness and diarrhoea. They are also considered as antipyretic and anti-obesity. Pulp is mucilaginous, pectoral, styptic, considered to purify blood and aid digestion. They are also cooling aphrodisiac, anodyne, tonic, laxative, and invigorating (Joshi, 2007).

2.2.4 Pharmacological properties

2.2.4.1 *Anticancer activity of the seed extract of Z.maritiana.*

Anticancer potential of seed extract of *Ziziphusmauritiana in-vitro* against different cell lines (HL-60, Molt-4, HeLa, and normal cell line HGF) byMTT assay as well as *in-vivo* against Ehrich ascites carcinoma bearing Swiss albino mice wasinvestigated. The extract was found to markedlyinhibit the proliferation of HL-60 cells. Annexin andPI binding of treated HL-60 cells indicated apoptosisinduction by extract in a dose-dependent manner.The cell cycle analysis revealed a prominent increasein sub Go population at concentration of 20 g/ml andabove*(*Mishra *et al.;* 2010). Agarose gel electrophoresis confirmed DNAfragmentation in HL-60 cells after 3 h incubationwith extract. The extract also exhibited potentanticancer potential *in vivo*. Treatment of Ehrlichascites carcinoma bearing Swiss albino mice withvaried doses 100-800 mg/kg of plant extractsignificantly reduced tumor volume and viable tumorcell count and improved haemoglobin content, RBCcount, mean survival time and percentage life span *(*Mishra *et al.;*2010).

2.2.4.2 ***Antihyperglycemic effect of Petroleum ether, chloroform, acetone, ethanol, and aqueous extracts of pulp ofZizyphus mauritiana***

Petroleum ether, chloroform, acetone, ethanol, and aqueous extracts of pulp of *Zizyphusmauritiana*were tested for antihyperglycemic activity in glucose overloaded hyperglycemic rats. The aqueous extract and the nonpolysaccharide fraction of the aqueous extract of *Ziziphusmauritiana*were found to exhibit significant antihyperglycemic and hypoglycemic activities. The petroleum ether extract was found to exhibit only an antihyperglycemic effect. Treatment of diabetic rats with petroleum ether extract, aqueous extract, and non-polysaccharide fraction of this plant restored the elevated biochemical parameters, glucose, urea, creatinine, serum cholesterol, serum triglyceride, HDL, LDL, hemoglobin, and glycosylated hemoglobin significantly to the near normal level. Comparatively, the non-polysaccharide fraction of the aqueous extract was found to be more effective, followed by the aqueous extract, and the petroleum ether extract. The activity of the non-polysaccharide fraction was comparable to that of the standard drug glibenclamide (Jarald*et al.;* 2009).

2.2.4.3 *Antidiarrhoealeffect of the methanolic leaves extract of Z. mauritiana*

Antidiarrhoeal effect of the methanolic extract as evaluated exhibited a concentration dependent inhibition of the spontaneous pendular movement of the isolated rabbit jejunum and inhibited acetylcholine induced contraction of rat ileum. A dose dependent decrease of gastrointestinal transit was observed with extracts at 25 and 50 mg/kg which also protected mice against castor -oil induced dairrhoea and castor oil induced fluid accumulation, respectively (Dahiru*et al.*, 2006).

2.2.4.4 *Antioxidant or radical-scavengingactivity of methanolic extract of Ziziphus mauritiana.*

*Ziziphusmauritiana*methanolic extract was screened for radical-scavenging effect of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical using colorimetric method. There was an increase in the radicalscavenging effect as the concentration of sample increased (Ndhlala*et al.*,2007).

2.2.4.5 *Hepatoprotective activity of the aqueous extract of Ziziphus mauritiana*

The aqueous extract of *Ziziphusmauritiana*pulp was evaluated for its protective activity against CCl_4-induced induced liver damage. 250, 500 mg/kg pulp extract or 100 mg/kg silymarin (standard) were administered to different groups of rats prior to CCl_4 administration. Both 250 and 500 mg/kg of pulp extract significantly reduced (in a dose-dependent pattern) the levels of enzymes and non-enzymes markers of tissue damage when compared to rats given CCl_4 only. These findings were supported by liver histology and suggested that pulp possessed rich hepatoprotective principles (Dahiru *et al.*, 2010).

2.2.4.6 ***Hypotensive activity of Ziziphus mauritiana***

The extract of *Ziziphusmauritiana*in the dose range 0.4 to 122 mg/kg Pc, caused dose-dependent hypotension in rabbits, similar to that achieved with acetylcholine (ACh). In the presence of increasing doses of atropine, ranging from 4.10 to 4.84 μg/kg PC, hypotension induced in rabbits by acetylcholine (ACh) at 4.10 and *Zizyphusmauritiana*at 22 mg/kg at PC is progressively inhibited (Koffi*et al.;* 2008).

2.2.4.7 ***Positive inotropic and chronotropic of the aqueous extract of Ziziphus mauritiana***

The aqueous extract of *Ziziphusmauritiana*produced positive inotropic and chronotropic effectsin isolated rat heart. These positive effects wereinhibited by the pretreatment of the heart withpropanolol. It was purposed that *Ziziphusmauritiana*contained adrenomimetic substances,which probably act on rat heart via βadrenoreceptors (Traore*et al.;* 2008).

2.2.4.8***Antimicrobial activity of methanolic leaf extract of Ziziphus mauritiana* and *Z. nummularia***

Methanol extracts of *Ziziphusmauritiana*and *Z. nummularialeaves* were investigated for their antimicrobialactivities against nine bacterial species which include onemethicillin resistant strain (*Staphylococcus aureus, Escherichia coli, Klebsiellapneumoniae, Proteus vulgaris, Citrobacterfreundii, Pseudomonas aeruginosa, Streptococcus pyogenes, Listeria monocytogenes, and Bacillus subtilis*) and fourfungal strains, which include two multiresistant species(*Candida albicans, C. tropicalis, C. glabrata, and Aspergillusniger*). Extracts showed significantantimicrobial activity against both Gram positive andnegative bacteria (Kayser and Arndt, 2000).

2.2.4.9 Nutritional value

The pulp is a good source of vitamin C and sugars, and contains appreciable amounts of mineral constituents. A typical analysis of the pulp is as follows: moisture, 81.6; protein, 0.8; fat, 0.3; carbohydrates, 17.0; and minerals, 0.3%; calcium(4 mg/100g), phosphorus(9 mg/100g), iron (1.8 mg/100g); carotene(0.021 mg/100g); thiamine(0.02 mg/100g); riboflavin(0.02 mg/100g), niacin(0.7 mg/100g); and vitamin C(76 mg/100g). Presence of fluoride (0.1- 0.2 p.p.m.) is reported; Pectin content (as calcium pectate) is 2.2-3.4 percent, on moisture-free basis. Fresh pulp contain quercetin. Jujube pulp and leaves are good sources of phenolic compounds (especially rutin and apigenin-7-glucoside for leaves, and catechin and rutin for pulp). Alpha-tocopherol and beta-carotene contents of jujube pulp are very low when compared with pulp rich in alphatocopherol and beta-carotene. The total lipid content of the pulp is very low. The predominant fatty acids in a study of four selected species are oleic acid, linoleic acid, palmitic acid and palmitoleic acid. Palmitic acid is the main saturated fatty acid.Unsaturated fatty acids range from 68.54% to 72.44% of the total fat in the pulp. Therefore the pulp can be recommended by nutritionists to be part of our diet (San and Yildirim, 2010).

2.3 Phytochemistry

Phytochemistry is a distinct discipline somewhere in between organic chemistry, plant biochemistry and closely related to natural products. It deals with a variety of organic substances accumulated in plants. The plant may be considered as a biosynthetic laboratory. Not only their chemical compounds such as carbohydrates, protein, and lipids that are used as food by man, but also a multitude of compounds like glycosides, alkaloids, flavonoids, etc. are used as medicines in various ways and means. The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is considered to be an important step in medicinal plant research. Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals (Banso and Adeyemo, 2007). Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (Adebajo*et al.*, 2003). The phytochemicals contained in medicinal plants are known as active component. There is a reasonable likelihood that medicinal plants with a long history of human use will ultimately yield novel drug prototypes. The most commonly encountered secondary metabolites of plants are saponins, tannins, flavonoids, alkaloids, anthraquinones, cardiac glycosides and cyanogenic glycosides. The pharmacological and other beneficial effects of anti-nutritional factors in plants have been reviewed by Soetan, (2008). The presence of these secondary metabolites in plants probably explains the various uses of plants for traditional medicine.

2.4 Antimicrobial Compounds from Plants

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 2008). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment (Schultes, 2008). Useful antimicrobial phytochemicals can be divided into several categories, described below.

2.4.1 Phenolics and Polyphenols

Phenols are a member of a group of aromatic chemical compounds with weakly acidic properties and are characterized by a hydroxyl (OH) group attached directly to an aromatic ring. The simplest of phenols derived from benzene is also known as phenol and has the chemical formula C_6H_5OH. The presence of phenols is considered to be potentially toxic to the growth and development of pathogens (Okwu and Okwu, 2004). The structural classes of phenolic compounds include the polyphenolic (hydrolysable and condensed tannins) and monomers such as ferulic and catechol (Okwu, 2005). Polyphenols might interfere in several of the steps that lead to the development of malignant tumours, may play a role in inactivating carcinogens and inhibiting the expression of mutagens (Urquiaga and Leighton, 2000).

Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds, which are in the highest oxidation state. Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two OH groups, and pyrogallol has three. The sites and number of hydroxyl groups on the phenol group is related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity.

The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason and Wasserman, 2007). Phenolic compounds possessing a C3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and often cited as antimicrobial as well. Eugenol is a well-characterized representative found in clove oil. Eugenol is considered bacteriostatic against both fungi and bacteria (Michael, 2008).

2.4.2 Quinones

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds, being coloured, are responsible for the browning reaction in cut or injured pulp and vegetables and are an intermediate in the melanin synthesis pathway in human skin (Schmidt, 2004). Their presence in henna gives that material its dyeing properties. The switch between diphenol (hydroquinone) and diketone (quinone) occurs easily through oxidation and reduction reactions. The individual redox potential of the particular quinone- hydroquinone pair is very important in many biological systems. Vitamin K is a complex naphthoquinone. Its antihemorrhagic activity may be related to its ease of oxidation in body tissues (Harris, 2003). Hydroxylated amino acids may be made into quinones in the presence of suitable enzymes, such as a polyphenoloxidase. In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism (Habbal, 2005)

2.4.3 Flavones, flavonoids, and flavonols

Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants which have been shown to exert potent antioxidant activity against the superoxide radical (Middleton*et al.,* 2000). This may be as a result of its antioxidant activity and subsequent inhibitions of low-density lipoproteins (LDL) oxidation known to have been attributed to the dietary and supplemental intake of flavonoids and other micronutrients. Epidemiologic studies indicate an inverse relationship between intake of dietary flavonoids and coronary artherosclerotic disease. Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom. They are known to be synthesized by plants in response to microbial infection and have been found *in vitro* to be effective against a wide array of microorganisms (Kumar *et al.,*2013).

Flavonoids are potent water-soluble super antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anti-cancer activity and protects against all stage of carcinogens. Flavonoids in the body are known to reduce the risk of heart diseases. In terms of anti-cancer activity, they inhibit the initiation, promotion and progression of tumors (Okwu, 2004). In recent times, plant flavonoids have attracted attention as potentially important dietary cancer chemo-protective agents. Some isoflavones act as allele chemicals widely used in insecticides.A number of flavonoids have been shown to suppress carcinogenesis in various animal models (Okwu, 2004).

There is currently considerable interest in these compounds, as they appear to exert a beneficial effect on several key mechanisms involved in the pathogenesis of cancer. The antioxidant property of flavonoids was the first mechanism of action studied, in particular with regard to their protective effect against cardiovascular diseases. Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals, which are possibly involved in DNA damage and tumor promotion (Bravo, 1998).

Flavonoids may also have a beneficial effect through their impact on the bioactivation of carcinogens. Most chemical carcinogens require transformation by phase I metabolizing enzymes into a more reactive form able to bind to DNA. If the resulting mutation is not repaired, it may initiate or promote the carcinogenesis process. The reactive chemical group introduced by phase I enzymes (the original carcinogen) can be detoxified through conjugation by phase II metabolizing enzymes into a water-soluble compound which can then be eliminated from the body. A cancer protective effect from plant-derived foods has been found with uncommon consistency in epidemiologic studies.

However, it has been difficult to identify specific components responsible for this effect. Many phytochemicals have been shown to be biologically active and they may interact to protect against cancer. In recent years, experimental studies have provided growing evidence for the beneficial action of flavonoids on multiple cancer-related biological pathways (carcinogen bioactivation, cell-signaling, cell cycle regulation, angiogenesis, oxidative stress, inflammation). Although the epidemiologic data on flavonoids and cancer are still limited and conflicting, some protective associations have been suggested for flavonoid-rich foods (soy and premenopausal breast cancer; green tea and stomach cancer; onion and lung cancer). Flavonoids lacking hydroxyl groups on their b-rings are more active against microorganisms than are those with the 2OH groups (Chabot *et al.*, 2002); this finding supports the idea that their microbial target is the membrane. Lipophilic compounds would be more disruptive of this structure. However, several authors have also found the opposite effect; i.e., the more hydroxylation, the greater the antimicrobial activity (Sato *et al.,* 2001).

2.4.4 Tannins

"Tannin" is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Their molecular weights range from 500 to 3,000, and they are found in almost every plant part: bark, wood, leaves, pulp, and roots (Scalbert, 2001). They are divided into two groups, hydrolyzable and condensed tannins. Hydrolyzable tannins are based on gallic acid, usually as multiple esters with D-glucose, while the more numerous condensed tannins are derived from flavonoid monomers. Tannins may be formed by condensations of flavan derivatives, which have been transported to woody tissues of plants. Alternatively, tannins may be formed by polymerization of quinone units (Geissman, 2003).

This group of compounds has received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing beverages, especially green teas and red wines, can cure or prevent a variety of illness (Serafini*et al.*, 1994).

Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins (Haslam, 1996). One of their molecular actions is complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation (Haslam, 1996, Stern *et al.*, 1996). Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. The antimicrobial significance of this particular activity has not been explored. There is also evidence for direct inactivation of microorganisms.

Scalbert (2001) reviewed the antimicrobial properties of tannins. He listed 33 studies, which had documented the inhibitory activities of tannins. According to these studies, tannins can be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity (Jones *et al.*, 2012).

2.4.5 Alkaloids

Heterocyclic nitrogen compounds are called alkaloids. The first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy *Papaversomniferum*; the name morphine comes from the Greek Morpheus, god of dreams. Codeine and heroin are both derivatives of morphine. Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae, or buttercup family, are commonly found to have antimicrobial properties. Solamargine, a glycoalkaloidfrom the berries of *Solanumkhasianum*, and other alkaloids may be useful against HIV infection as well as intestinal infections associated with AIDS. While alkaloids have been found to have microbiocidal effects (including against *Giardia* and *Entamoeba*species), the major antidiarrhoeal effect is probably due to their effects on transit time in the small intestine. Berberine is an important representative of the alkaloid group. It is potentially effective against trypanosomes and plasmodia. The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine and harmane is attributed to their ability to intercalate with DNA(Leverinand matron., 1999).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials

3.1.1 Plant Materials

Pulp and seed of *Ziziphus mauritiana* were collected from the wild in Hyera village of Hawul local Government area of Borno State. The plant was identified and authenticated at the Herbarium, Department of Biological Sciences, Ahmadu Bello University,Zaria, Nigeria with the voucher number 295.

3.1.2 Chemical/Reagents

Methanol(Merck-Germany),Loperamide (loe-PER-a-mide.SKG-pharm Nigeria),Chloroform(Sigma-Aldrich,St.Lous,MO,USA) nHaxane(JHD,AR;Lobal Chem,India), Ethylacetate(Qualikems)**,**n-Butanol(Loba Chem,Inidia),TLC silica gel-60 F_{254} pre-coated plates (Merk-Germany), Silica gel-60 for column(60-120µm in size),Sulphuricacid(Sigma-Aldrich,St.Lous,MO,USA),Anisaldehyde(Sigma-Aldrich,St.Lous,MO,USA)

3.1.3 Equipments

Water bath(HHS,Mc Donald Scientific International,), UV Lamp,Glass column (75 by 3.5cm),Dessicator,Whatman filter paper,Laboratory glass wares (Funnel,Conical Flask,Beakers,Measuring Cylinder),TLC tanks (Uni kit®, Shandon, Southern Germany)

3.1.4 Test organisms

The bacterial isolates were obtained from Department of Microbiology Ahmadu Bello University Teaching Hospital Shika, Zaria,Kaduna state. The isolates were sub-cultured on selective and differential media and re-identified using colonial morphology,gram reaction, mortality test, and haemolytic activity and biochemical tests (catalase, citrate, and lactose and sorbitol (Cheesbrough 2002). With wire loop, colonies of culture of bacterial were picked and suspended in 5ml nutrient broth in 10ml bottles and were incubated at $37^{o}C$ for 24hrs for the anti-bacterial test.

3.2. Methods

3.2.1 Preparation of plant extracts.

The dry pulp and seed of *Z. mauritiana* were pulverized using pestle and mortar and sieved with 0.3 mm sieve (Endecott Ltd. London). Two hundred grams of the coarse powdered pulp and seed wereextracted with 90 percent methanol by cold maceration. Methanol was evaporated in water bath at less than $40^{0}C$. Distilled water and DMSO were used to reconstitute the solid extracts to obtain a desired concentration for the studies (El Astal et al., 2005)

3.2.2 Phytochemical screening

The extracts weresubjected to qualitative and quantitative phytochemical screening(Trease and Evans,1983).

3.2.2.1 Test for alkaloids

To the 0.2gof the extract, 2mls of Picric acid was added. Orange colouration indicated the presence of alkaloids.

3.2.2.2 Test for saponins

The plant extract (0.5 g) was shaken with water in a test tube and washeated to boil. Frothing was observed which was taken as evidence for the presence of saponins.

3.2.2.3 Test for tannins

Extracts (0.5g) was added to10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

3.2.2.4 Test for steroids

Acetic anhydride (2 ml) was added to 0.5 g of methanol extracts of each sample with 2 ml sulphuric acid. A colour change from violet to bluewas an indicationof the presence of steroids.

3.2.2.5 Test for flavonoids

Ethylacetate (10 ml) was added to small portion of the powdered plant material and it was held over steam bathe for 3 minutes. The mixture was filtered and 1ml of dilute ammonia solution was added to 4ml of the filterate and then shaken. A yellow colouration indicates the presence of flavonoids.

3.2.2.6 Test for Anthraquinones

Extracts (0.5g) was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extracts was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink red colour in the ammonical layer indicated the presence of anthraquinones.

3.2.2.7 Test for Phenols

The powdered (0.5g) plant material was boiled with 10ml of sulphuric acid and filtered while hot, 5ml of chloroform was added and shaken. The chloroform layer was then pipetted into another test tube and 1ml of dilute ammonia was added to it. The resulting solution was observed for colour changes to green-blue to indicate the presence of phenol.

3.2.2.8 Test for Cynogenic glycosides

To 0.5g of the extracts10 ml of 50% H_2SO_4 was added and the mixture heated in boiling water for about 15 min. Fehling's solution (10ml) was then added and the mixture boiled. A brick -red precipitate indicate the presence of glycosides.

3.2.2.9 Test for terpenoids.

Exactly 0.5g of the sample was dissolve in water. To it 1ml of acetic acid anhydride was added followed by addition of Conc.H_2SO_4. A change in color from pink to violet showed the presence of terpenoids.

3.2.3 Quantitative test

3.2.3.1 Flavonoids

Ten (10) gram of powdered sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper. The filtrate was transferred into a crucible and evaporated to dryness over a water bath and weighed.(Allen *et al.,* 1974).

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{W_3} \times 100$$

Where W_1 = Weight of beaker

W_2 = Weight of beaker + Evaporated extracts

W_3 = Weight of sample

3.2.3.2Tannins

The powdered sample (0.5g) was placed into 100ml conical flask and 50ml of distilled water was added. The flask was gently heated to boiling for 1 hour, filtered hot and filtrate collected in 50mL volumetric flasks. The residue was washed several times and the combined solution made to the volume with distilled water. To 0, 1, 2, 3, 4 and 5ml of the standard tannic acid and 10mg of the sample solution in a $50cm^3$ volumetric flask, 2.5ml Folin-Denis reagent and 10ml of sodium carbonate solution was added and made up to volume with distilled water. The flasks were allowed to stand for 20 minutes after which optical density(absorbance) was measured at 750nm. The calibration curve was plotted from which the concentration of tannic acid (X) in the sample was extrapolated and tannins content in the sample was calculated (Allen *et al.*, 1974).

$$\text{Soluble tannins (\%)} = \frac{\text{concentration from graph} \times \text{extract volume}}{100 \times \text{sample weight}}$$

3.2.3.3 Saponins

Five (5g) gram of the powdered sample was placed in a $100cm^3$ flask. $60cm^3$ of 50% aqueous methanol was added to the content and boiled under reflux for 30 minutes. The resulting solution was filtered while hot using filter paper into a $100cm^3$ flask. Two (2g) gram charcoal were subsequently added to the filtrate, further boiled and filtered while hot. The extract was allowed to cool and equal volume of acetone was added (to complete precipitation of saponins). The precipitate was separated by decantation and then dissolved in 95% ethanol by boiling, filtered while hot and allowed to cool to room temperature. The filter paper containing the residue was dried in a dessicator and weighed.

% Saponin = $\frac{W2 - W1}{W3}$x 100

Where W_1 = Weight of filter paper

W_2 = Weight of filter paper + Extract

W_3 = Initial weight of sample

3.2.3.4 Glycosides

One (1) gram of the extract was extracted with 10ml of 70% alcohol and the mixture was filtered. 8ml of the filtrate was transferred to 100ml volumetric flask and the volume was completed to the mark with distilled water. 8ml of the mixture was added to 8ml of 12.5% lead acetate (to precipitate resins, tannin and pigments). The mixture was shaken very well and was completed to the volume with distilled water and filtered. 50ml of the filtrate was pipette into another 100ml volumetric flask and 8ml of 4.7% disodium hydrogen phosphate (Na_2HPo_4) solution (to precipitate excess lead) was added, the mixture was made up to the volume with distilled water and mixed. The mixture was filtered twice through a filter paper. Baljet's reagent (10ml) was added to 10ml of the purified filtrate. A blank sample of 10ml of distilled water was added to Baljet's reagent. The two solutions were allowed to stand for one hour (time necessary for colourdevelopment). A blank of 20ml of distilled water was used. The intensity of colour was read at 495nm with a spectrophotometer. The percentage of total glycosides was calculated by referring to the given standard curve.

The percentage of glycosides =$\frac{A \text{ x } 40 \text{ x } 100}{170 \text{ x } 100 \text{ x } 0.04}$ =$\frac{A \text{ x } 100}{17}$

Where A = Absorbance of the sample at 495nm.

3.2.4 Anti-diarrhoeal Studies

3.2.4.1 Animals

Albino wistar rats (110-150g) of either sex were obtained from National Veterinary Research Institute, Vom, Plateau state, Nigeria. They were maintained on standard animal pellets and water *ad libitum*.

3.2.4.2 Castor Oil Induced Diarrhoea

The method of Awouter *et al.,* (1976) was adopted in assaying for the effect of the Z. *mauritiana* extract on castor oil induced diarrhoea. Rats were weighed and grouped into 4 groups (n=5). Group 1 received distilled water, group 2 and 3 were administered 50 and 100 mg/kg extract orally while group 4 received loperamide (2mg/kg) orally. Each animal was then given 0.5 ml of castor oil orally after 30 min the extract was administered and placed in transparent cages to observe for consistency of faecal matter and frequency of defecation for 4hrs.Faeces was collected with an absorbent sheet of paper placed beneath the transparent cages (Mukherjee *et al.*, 1998). The wet faeces (watery stool) were read at the end of the experiment by lifting up the upper part of the cage containing the sheet of paper and animals.

3.2.4.3 Small Intestinal Propulsion

The effect of Z. *mauritiana* on Intestinal Propulsion in rats was tested using the charcoal meal method (Capaso *et al.*, 1976). The rats were fasted for 24 h but allowed free access to water. They were randomized and placed in 3 plastic cages of 5 animals per cage corresponding to 4 groups of animals. Group 1 was administered distilled water orally by orogastric cannula. Groups 2 and 3 were pretreated with 50 and 100 mg/kg extract of *Z.mauritiana*, respectively while group 4 received 2mg/kg of loperamide. After 30 min, each rat was administered 1ml of 5% activated charcoal suspended in 10% carboxy methyl cellulose orally. The rats were anaesthetized 30 min later by inhalation of chloroform and then sacrificed. The small intestine of each animal was carefully inspected and the distance traversed by the charcoal meal from the pylorus was measured. The length of the whole small intestine was also measured. The distance traversed by the charcoal meal from the pylorus was expressed as a percentage of the distance from the pylorus to the ileocaecal junction.

3.2.4.4 Castor Oil Induced Fluid Accumulation

This was determined by the method described by Robert *et al.,* (1976) and Dicarlo *et al.,* (1994). The rats were fasted for 24 h but allowed free access to water. The rats were randomized and placed in 4 cages of 5 rats per cage each. Group 1 was administered distilled water, while groups 2 and 3 were pre-treated with 50 and 100 mg/kg of the extract, respectively and group 4 received 2mg/kg of loperomide. Thirty (30) min later, each rat was administered 2ml castor oil. The rats were anaesthetized 30 min later by inhalation of chloroform. The small intestine from the pylorus to caecum was dissected out and its content expelled into a measuring cylinder and the volume of the fluid was measured.

3.2.5 Bioassay-guided fractionation

Solvent-solvent fractionation was done using the protocol designed by Kupchan*et al*Tson,(1973) and modified version of Wagenen *et al.,*(1993).

The crude extract(28g) was suspended in warm water at 40^{o}C to dissolve. The solution was then partitioned successively using the following solvents n-hexane, ethylacetate,and n-butanol. Shaking the separating funnel, mixture separated into two layers and it was collected separately.

The fractions collected were evaporated to dryness at 40^oC and kept in a tight containers for further analysis.

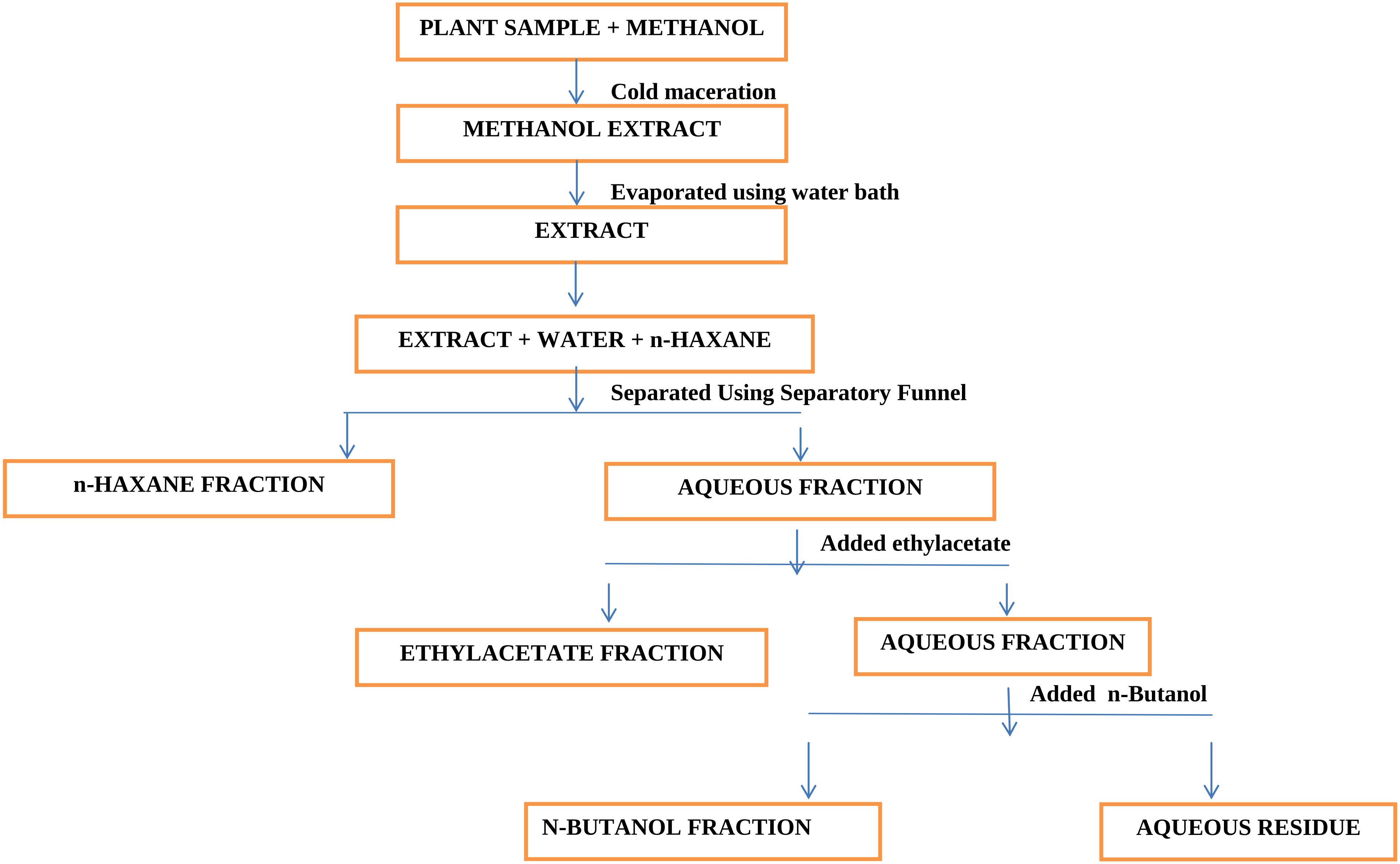

Figure 1: Solvent-solvent Fractionation of Methanolic Extract

3.2.5.1 Column chromatography

silica gel (100g) G(60-120µm mesh size)was carefully packed using wet method in a heavy walled glass tube. The column was allowed to settle for three (3) hours. Four (4)gramsethylacetate fraction pre-absorbed on silica gel was loaded into the packed adsorbent and allowed to stabilize for 3hours before elution began.

Gradient elution of the solvents (mobile phase) was used starting with n-hexane (100%) as the first eluenting solvent Subsequently the polarity was increased and n-hexane:ethylacetate was gradually added asn-hexane:ethylacetate (8:2),(6:4),(4:6),(2:8) and ethylacetate (100%).

Fractions (100) of 20ml each were collected in beakers and allowed to evaporate at room temperature. The fractions were spotted on TLC plate and visualized with U.V light using p-anisaldehyde and 10% sulphuric acid as the general detecting reagent. Fraction that showed same spot(s), Colour and Rf values were pooled together in one beaker (Nakanishi *et al*., 1998)

3.2.5.2 Preparation of nutrient agar

Nutrient agar base is a general purpose medium used for sensitivity assays and is the basic for other media. 28 g of the powdered agar was weighed and dissolved in 1000ml of distilled water in a conical flask. It was then autoclaved at 121^0C for 15minutes. After sterilization, it will beallowed to cool at roomtemperature and the content will be poured aseptically into a sterile petri-dish and allowed to solidify.

3.2.5.3 Test for antibacterial activity of theextract.

Agar well diffusion and broth dilution techniques were used to test for antibacterial activity in accordance with the method described by Irobi*et al*., (1994). The antibacterial activity of the ethylacetate fraction was tested on*Staphyloccus aureus, Eschericial coli*, and*Salmonella* spp.The isolates werefirst grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (10^6cfu/ml). Two hundred micro liter of the standardized cell suspensions was then be spread on a Mueller-Hinton agar (Oxoid). Wells were bored into the agar using a sterile 6 mm diameter cork borer. 100μl of the crude extract was then introduced into the wells, and was allowed to stand at room temperature for about 2 h and then incubated at 37°C. Controls were set up in parallel using the solvents that was used to reconstitute the extract and the solvent used for extraction. Ciprofloxacin was used as the positive control.The plates were then observed for zones of inhibition after 24 h.

3.2.5.4 Detemination of Minimum Inhibitory Concentration (MIC)

The determination of MIC and MBC of the partially purified ethylacetate fraction was carried out using the method of Akinpelu and Kolawole (2004). Briefly,1.625, 3.25, 6.25, 12.5, and 25mg/ml concentrations of the extracts were prepared and 1 ml was introduced into 2 ml of nutrient broth in test tubes. About 2 ml of the culture diluted to 10^6cell/ml was added and incubated for 24 h at 37°C. The least concentration of the extractsthat did not permit any visible growth in the broth was taken as the MIC.The MIC of the extracts was done for each test organisms in triplicate.

3.2.5.5 Determination of Minimum Bactericidal Concentration (MBC)

Briefly,200µl of broth were taken from the tube with no visible growth in the MIC assay and sub-cultured on freshly prepared nutrient agar plates and incubated at 37°C for 48 h. The MBC was taken as the concentration of the extract that did not show any growth on agar plates.

3.2.6 Statistical Analysis

The results of the experiment were expressed as mean±standard deviation (SD).data were analyzed using analysis of variance (ANOVA) with SPSS Version 20.

Difference between the various extracts was compared using Duncan Multiple Range Test.P value less than 0.05 was taken as significant ($P<0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Screening

4.1.1 Qualitative phytochemical screening of Seed and Pulp of Z. *mauritiana*

The result of qualitative phytochemical screening of the seed and pulp extractsare shown in Table 4.1 below. The phytochemical screening showed the presence of active metabolites such as phenolics, tannins, flavonoids, saponins,cyanogenic glycosides and terpenoids, while, anthraquinones, steroids and alkaloid were not detected.

4.1.2 Quantitative phytochemical screening of Seed and Pulp of Z. *mauritiana*

The result of quantitative phytochemical screening of the seed and pulp extracts are shown in Table 4.2. The seed contain saponins (2.44±0.04mg/g) followed by flavonoids (2.04±0.02mg/g). Phenols had the lowest amount in the seed extract (0.15±0.02mg/g). The pulp contains saponins (2.85±0.02mg/g) followed by Cyanogenic glycosides (1.42±0.02mg/g).

4.2 antidiarrhoeal activity

4.2.1 Antidiarrhoeal activityof Z. *mauritiana*pulp extract

In the castor oil-induced diarrhoeal model for pulp extract (Table 4.3), data from the experiment revealed that the percentage of diarrhoeal inhibition compared to controls was 63.64%, and 61.36% at the doses of 50 and 100mg/kg respectively. The standard drug showed a percentage inhibition of (80.64%). In intestinal fluid accumulation test (Table 4.4), the pulp extract reduced the volume of intestinal fluid. Maximum percentage inhibition of the volume of intestinal contents was observed at 50 mg/kg of the extract, being 27.71% followed by 26.51% at dose of 100mg/kg. However, the differences observed were not statistically significant($p<0.05$). The pulp extract significantly inhibited gastrointestinal transit time of charcoal meal at 50 mg/kg (16.66%) and 100mg/kg (20.33%) compared to the control (Table 4.5).

4.2.2 Antidiarrhoeal Activityof Seed extract of Z. *mauritiana*

Thefindings revealed that 100mg/kg of the seed extract had the percentage (80.70%) diarrhoeal inhibition. The synthetic drug used produced a percentage inhibition of 91.23 %.(Table 4.6)in intestinal fluid accumulation test (Table 4.7), there were no significant differences in percentage reduction of the volume of intestinal fluid of the animals placed on the seed extract. Table 4.8 shows the result of gastrointestinal transit time of charcoal meal. The seed extract significantly inhibited gastrointestinal transit time of charcoal meal by 18.40%, 21.43%and 42.27% at 50mg/kg of extract, 100mg/kg of extract and 2mg/kg of the standard drug respectively.

4.3 Antibacterial activity

4.3.1 Zone of bacterial Growth inhibition by Crude Methanolic seed extract of *Z.mauritiana*

The result of the antibacterial screening of the seed methanolic extract onS*taphyloccus aureus, Eschericial coli, and Salmonella*sppis shown in Table 4.9.It shows zone of inhibition of*Staphyloccus. aureus,*(29.0±0.00,23.5±2.12,20.0±0.00 and 16.0±1.41)mm at 100,50,25,12.5 mg/mlwhile *Salmonella* sppand *Eschericial coli* showed high resistance to the extract at all concentrations used.

Table 4.1: Qualitative Phytochemical Screening of Seed and Pulp methanolic extract of Z. *mauritiana*

Phytochemical	**Seed**	**Pulp**
Phenolics	+	-
Tannins	+	+
Flavonoids	+	+
Alkaloids	-	-
Saponins	+	+
Steroids	-	-
Cyanogenic glycoside	+	+
Anthraquinones	-	-
Terpenoids	+	+

+= Present - = Absent

Table 4.2: Concentration of some phytochemicals in methanolic Seed and Pulp extracts of Z. *mauritiana*

Phytochemicals	**Seed (mg/g)**	**Pulp (mg/g)**
Phenolics	0.15±0.02	-
Tannins	0.30±0.01	0.19±0.02
Flavonoids	2.04±0.02	0.91±0.01
Saponins	2.44±0.04	2.85±0.02
Cyanogenic glycosides	0.22±0.01	1.42±0.02

Values are mean± SD of three replicates.

Table 4.3 Effect of *Ziziphus mauritiana* Pulp Extract on Castor Oil Induced Diarrhoea in Rats

Treatment	**Dose(mg/kg)**	**No. of Watery Diarrhoea**	**% Inhibition**
Control	-	11.00 ± 3.46^{a}	-
Extract	50	4.00 ± 1.15^{b}	63.64
Extract	100	4.25 ± 1.50^{b}	61.36
Loperamide	2	2.13 ± 1.10^{c}	80.64

Values are mean±SD n=5. Values in the same column with different superscript differ significantly at $p<0.05$.

Table 4.4Effect of Methanolic PulpExtract of *Ziziphus mauritiana* on Castor Oil Induced Intestinal Fluid Accumulation

Treatment	**Dose mg/kg)**	**Fluid Volume (ml)**	**% Inhibition**
Control	-	3.32 ± 0.23^{a}	-
Extract	50	2.40 ± 0.42^{b}	27.71
Extract	100	2.44 ± 0.46^{b}	26.51
Loperamide	2	1.40 ± 0.12^{c}	57.83

Values are mean±SD n=5. Results in the same colunm with different superscript differ significantly at $p<0.05$.

Table 4.5 Effect of Methanolic PulpExtract of *Ziziphus mauritiana* on Gastrointestinal Transit in Rats.

Treatment	**Dose (mg/kg)**	**Length of Intestine (cm)**	**Distance Travelled by Charcoal meal (cm)**	**% Inhibition**
Control	-	79.28 ± 8.71^{a}	60.00 ± 6.67^{a}	-
Extract	50	82.10 ± 5.66^{a}	50.80 ± 7.89^{b}	16.66
Extract	100	83.40 ± 4.98^{a}	47.80 ± 9.07^{b}	20.33
Loperamide	2	84.7 ± 4.41^{a}	33.22 ± 3.23^{c}	44.63

Values are mean±SD n=5. Values in the same colunm with different superscript differ significantly at $p<0.05$.

Table 4.6: Effect of *Ziziphus mauritiana* Seed Extract on Castor Oil Induced Diarrhoea in Rats

Treatment	**Dose mg/kg)**	**No. of Watery Diarrhoea**	**% Inhibition**
Control	-	11.40±2.07[a]	-
Extract	50	5.40±1.34 [b]	52.63
Extract	100	2.20±1.10[c]	80.70
Loperamide	2	1.00±0.0.04 [c]	91.23

Values are mean±SD n=5. Values in the same colunm with different superscript differ significantly at $p<0.05$.

Table 4.7: Effect of Methanolic Seed Extract of *Ziziphus mauritiana* on Castor Oil Induced Intestinal Fluid Accumulation

Treatment	**Dose mg/kg)**	**Fluid Volume (ml)**	**% Inhibition**
Control	-	3.44±0.26[a]	-
Extract	50	2.18±0.15 [b]	36.63
Extract	100	2.08±0.13 [b]	39.53
Loperamide	2	1.52±0.36 [c]	55.81

Values are mean±SD n=5. Values in the same colunm with different superscript differ significantly at $p<0.05$.

Table 4.8 Effect of Methanolic Seed Extract of *Ziziphus mauritiana* on Gastrointestinal Transit in Rats.

Treatment	**Dose (mg/kg)**	**Length of Intestine (cm)**	**Distance Travelled by Charcoal meal (cm)**	**% Inhibition**
Control	-	93.4±4.72 [a]	63.88±12.87[a]	-
Extract	50	101.18±4.19 [b]	52.00±9.67 [b]	18.40
Extract	100	103.94±6.07[b]	50.19±9.07 [b]	21.43
Loperamide	2	96.34±9.11[c]	36.88±3.21 [c]	42.27

Values are mean±SD n=5. Values in the same colunm with different superscript differ significantly at $p<0.05$.

Table 4.9 Antibacterial activity of Methanolic Seed Crude Extract of Z.*mauritiana against* test bacterial.

Organism	Concentration of extract (mg/ml)cipro (µg)				
	100	50	25	12.5	Cipro (10)
Staphyloccus aureus	29.0±0.00 ^{d}	23.5±2.12^{c}	20.0±0.00 ^{b}	16.0±1.41^{a}	41.0±1.41^{c}
Eschericial coli	0.00	0.00	0.00	0.00	38.00±0.00^{a}
Salmonella spp	0.00	0.00	0.00	0.00	39.5±0.70^{a}

Values are in mean ±SD of double determinations, values in the same row with different superscripts across the column are significantly different.($p<0.05$)cipro=ciprofloxacin,

4.3.2 Zone of bacterial growth inhibition(mm) by crude methanolpulp extract of *Ziziphus mauritiana*

The result of antibacterial screening of concentration of methanol extract of*Staphyloccus aureus*, *Eschericial coli andSalmonella*sppis shown in Table 4.10.Which showed that the extract inhibited the growth of the isolates at 100mg/ml and 50mg/ml and showed no zone of inhibition at 25mg/ml and 12.5mg/ml, except for *Salmonela* sppwhich showed zone of inhibition at 25mg/ml.

4.4 Yield of fractions obtained fractionation of methanolic extract of the pulp of *Z. mauritaina*

The yield of the methanolic pulp crude extract obtained from solvent-solvent extraction of 50g are as follows n-hexane (2.13g), ethylacetate(2.5g), n-butanol(3.38g), aqueous residue (39.71g) while that of the seed is as follow n-hexane(5.13g), ethylacetate (2.9g), n-butanol (2.9g) aqueous residue(26g).(Table 4.12).

Table 4.10:Antibacterial activity ofMethanol Crude Extract of Pulp of *Ziziphus mauritiana*

Organism	Concentration of extract(mg/ml)/standard drug (µg)				
	100	50	25	12.5	cipro (10 µg)
Staphyloccus aureus	17.00±0.00[b]	11.50±2.12[a]	0.00	0.00	41.00±1.41[c]
Eschericial coli	27.00±1.41[b]	21.00±1.41[a]	0.00	0.00	38.00±0.00[c]
Salmonella spp	19.00±1.41[c]	14.50±0.70[b]	12.00±0.00[a]	0.00	39.00±0.70[d]

Values are in mean ±SD of double determinations, values in the same row with different superscripts across the column are significantly different ($p<0.05$).cipro=ciprofloxacin.

4.2.4 Zone of bacterial growth inhibition (mm) by aqueous, n-butanol, ethylacetate, and n-hexane pulp fraction of the pulp of *Z.mauritiana.*

There was no zone of inhibition was formed on the methanolic aqueous fraction of the pulp of *Z.mauritiana*while The result of n-butanol fraction of the pulp extract of *Z.mauritiana* on the test organismson Table 4.11 showed that *Staphyloccus aureus*was susceptible to the extract with zone of inhibition at 100mg/ml (13.00±0.00)mm, and 50mg/ml(10.00±0.00)mmwhile *Eschericial coli* and *Salmonella* spp were resistant. All the isolate were susceptible to ethylacetate fraction at 100mg/ml 50mg/ml and 25mg/ml and was resistant at 12.5mg/ml *Eschericial coli* which showed no zone of inhibition at 25mg/ml and 12.5mg/ml. The test organisms were resistant to the hexane fraction at all concentration.

4.2.5 Thin layer chromatography of pulp ethylacetate fraction

Thin layer chromatographic seperation of ethylacetate fraction developed in hexane- ethylacetate mixture, ratio 7:3 showed four major spots on plate II.

4:3 Colum chromatographic separation of pulpethylacetate fraction

The fractionation result of ethylacetate fraction using silica gel chromatography (Appendex 1)which yielded one hundred fractions of 20mls each which were later pooled together to four fractions(A-D) based on their TLC profile.

Table 4.11: Antibacterial activity ofaqueous, n-butanol, Ethylacetate, and n-hexane Fractionsof the Pulp of *Z.mauritiana.*

Organism	Concentration of extract/cipro (mg/ml)				
	100	50	25	12.5	cipro (10µg)
			Aqueous Fraction		
Staphyloccus aureus	0.00	0.00	0.00	0.00	38.50±0.70 [a]
Eschericial coli	0.00	0.00	0.00	0.00	36.50.±0.70[a]
Salmonella spp	0.00	0.00	0.00	0.00	40.00±0.00[a]
			n-butanol fraction		
Staphyloccus aureus	13.00±0.00[b]	10.00±0.00[a]	0.00	0.00	38.50±0.70[a]
Eschericial coli	0.00	0.00	0.00	0.00	36.50.±0.70[a]
Salmonella spp	0.00	0.00	0.00	0.00	40.00±0.00[a]
			Ethylacetate Fraction		
Staphyloccus aureus	16.50±0.70[b]	13.00±0.00[b]	10.00±0.00[a]	0.00	38.50±0.70 [d]
Eschericial coli	13.00±1.41[a]	10.00±0.00 [a]	0.00	0.00	36.50.±0.50[c]
Salmonella spp	13.50±0.70[b]	10.50±0.70[a]	10.00±0.00[a]	0.00	40.00±0.00[c]
			n-hexane Fraction		
Staphyloccus aureus	0.00	0.00	0.00	0.00	38.00±0.70[a]
Eschericial coli	0.00	0.00	0.00	0.00	36.50±0.70[a]
Salmonella spp	0.00	0.00	0.00	0.00	41.50±0.70[a]

Values are in mean ±SD of doulble determinations, values with different superscripts across the column are significantly different ($p<0.05$). cipro: Ciprofloxacin.

Table 12: Yield of solvent-solvent Fractionation of the pulp of *Z. mauritiana*.

EXTRACTS/ SOLVENTS USED	YIELD(g)
PULP EXTRACT	
n-hexan	2.13
ethylacetate	2.5
n-butanol	3.38
aqueous	39.71
SEED EXTRACT	
n-hexan5.13	
ethylacetate	2.9
n-butanol	2.9
aqueous	26

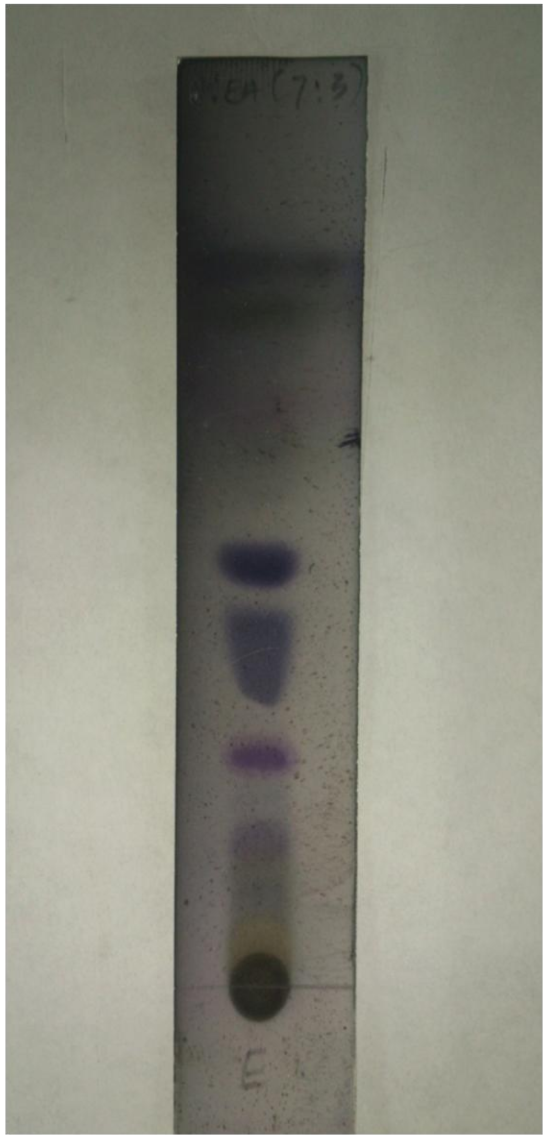

Plate I: thin-layer chromatogram of ethylacetate fraction of methanolic pulp extract of *Z.Mauritana* extract developed in hexane-ethylacetate (7:3).

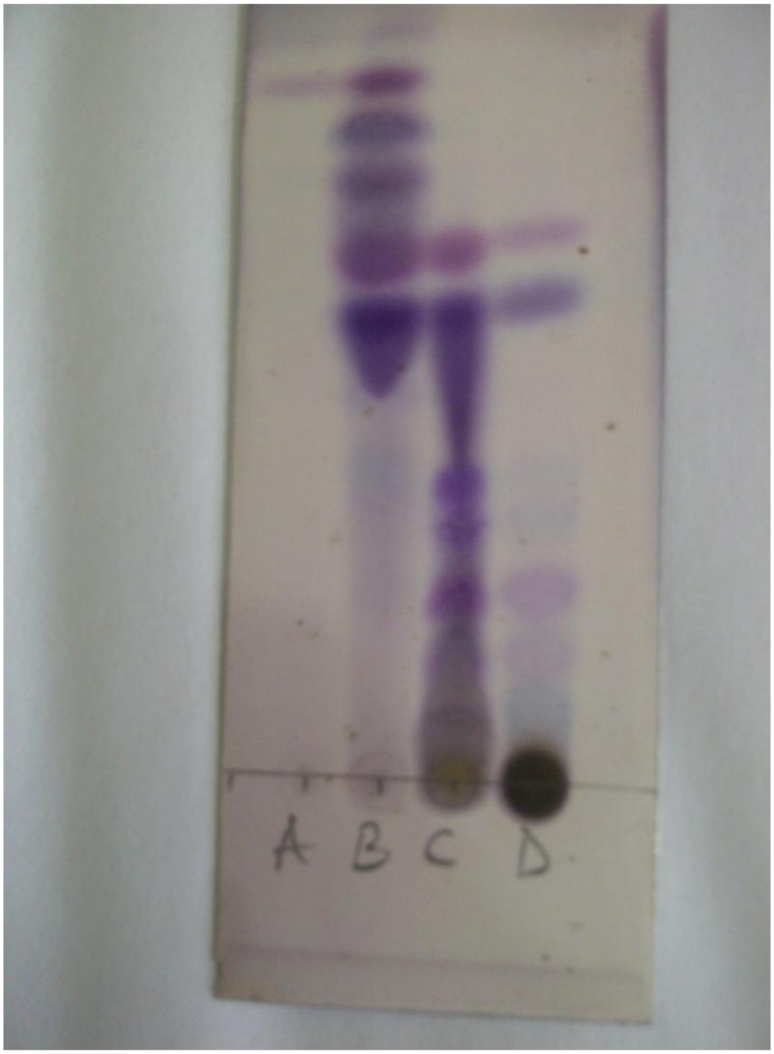

Plate II: thin-layer chromatogram of the fractions obtained from column chromatographic separation of pulp ethylacetate fraction of *Z. Mauritiana* in hexane-ethylacetate (7:3). dectetedwith P-Anisaldehyde in H_2SO_4, A,B,C,D are the pooled fractions.

4.4.1Zone of bacterial growth inhibition(mm) by partially purified pulp ethylacetate fraction of*Z.mauritana* against*Staphyloccus aureus, Eschericial coli and Salmonella* spp.

Table 4.13 shows the zone of inhibition of partially purified ethylacetate fraction ofZ.*mauritaina*pulp extract against *Staphyloccus aureus, Eschericial coli and Salmonella* spp, which showed that the *Eschericial coli* was susceptible to fraction C at all concentrations and resistant at all concentrations to fractions A,B and D.For *Salmonella* sppItshowed resistance to fraction A,B and D at all concentrations but was susceptible to fraction C at all concentrations.

While *Staphyloccus aureus,* was susceptible at all concentrations to fraction C and resistant to fraction B at 12.5mg/ml and 6.5mg/ml while for fractions A and D the bacterials were resistant at all concentrations.

4.4.2 Minimum Inhibitory Concentration (MIC) of Partially Purified pulp ethylacetate Fraction of*Z.mauritaina*

Table 4.14 shows the minimum inhibitory concentration (MIC) of partially purified ethylacetate fraction of methanol extract of *Z.mauritiana.* The fractions exhibited minimum inhibitory concentration (MIC) against all the test isolates. Fraction C exhibited MIC at concentration of 1.56mg/ml and 3.125mg/ml on all the isolates, and then fraction D exhibited MIC at concentration of 12.5mg/ml

Table 4.13: Antibacterial Activity (Zone Of Inhibition(Mm))Of Partially Purified Pulp Ethylacetate Fraction Of*Z.Mauritana* Against *E.Coli, Salmonella*Spp, And *S.Aureus.*

Microorganism	.	Fractions			
		A	B	C	D
Eschericialcoli	50	0.00	0.00	29.50±0.70[b]	0.00
	25	0.00	0.00	26.00±1.41[a]	0.00
	12.5	0.00	0.00	25.00±0.00 [a]	0.00
	6.5	0.00	0.00	24.00±1.41 [a]	0.00
	10 (cipro)	38±0.00[a]			
Salmonela spp	50	0.00	0.00	0.00	19.5±0.70[C]
	25	0.00	0.00	0.00	14.5±0.70[b]
	12.5	0.00	0.00	0.00	10.0±0.00[a]
	6.5	0.00	0.00	0.00	0.00
	10 (cipro)	39.5±0.70[a]			
Staphyloccus aureus	50	0.00	12.00±0.00 [b]	28.00±1.41[d]	0.00
	25	0.00	10.00±0.00[a]	19.00±1.41[c]	0.00
	12.5	0.00	0.00	14.00±1.41[b]	0.00
	6.5	0.00	0.00	10.00±0.00 [a]	0.00
	10 (cipro)	41.00±1.41			

Values are mean ±SD of double determinations, values in the same row with different superscripts across the column are significantly different.($p<0.05$).cipro=ciprofloxacin.

Table 4.14: Minimum inhibitory concentration (MIC) of partially purified ethylacetate fractionsof *Z.mauritiana*

	Concentrations (mg/ml)		
	Organisms		
	Staphylococcus aureus	*Escherichia coli*	*Salmonella* spp
Fractions			
C	3.1251	1.56	-
D	-	-	12.50

4.4.3Minimum Bactericidal Concentration (MBC) of Partially Purified pulp ethylacetate Fractionof *Z.mauritiana*

Table 4.15 shows minimum bactericidal concentration MBC of partially purified ethylacetate fraction of methanol extract of *Z.mauritiana*.. Fraction C had MBC at 6.25mg/ml and 3.125mg/ml Fraction D also exhibited MBC at a concentration of 25mg/ml against all the isolates.

Table 4.15Minimum Bactericidal Concentration (MBC) ofPartially Purified pulp Ethylacetate Fractionsof *Z. mauritiana.*

	Concentrations (mg/ml)		
	Organisms		
Fractions	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella* spp
C	3.125	1.56	-
D	-	-	12.50

CHAPTER FIVE

5.0 Discussion

The Phytochemical composition of the crude methanolic extracts of both seed and pulp were analysed. The result showed that tannins, flavonoids, saponins, cyanogenic glycosides, and terpenoids were present in both the seed and pulp. Anthraquinones and steroids were however, not detected in both seed and pulp. These findings are in agreement with the works of Dahiru *et al.,* (2006) and Das, (2012).

Many common medicinal plants used in the treatment of diseases are said to contain powerful bioactive substances that can protect us against a number of diseases, thus, improving the quality of our health. These bioactive metabolites have been shown to exhibit a number of pharmacological and biochemical roles on living organisms (Enwerem *et al.,* 2003). For instance, Dharmananda (2003) reported that tannins are used for treating disorders such as diarrhoea and dysentery. Shimada (2006) reported that tannins can form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis. Tannins were also reported to possess the ability of reacting with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues (Parekh and Chanda, 2007). These observations therefore may explain why the bitter, astringent bark decoction of *Ziziphus mauritainia* is taken to halt diarrhoea and dysentery and relieve gingivitis. The diuretic and antibacterial activity of plant extracts containing flavonoids have been documented (Rao *et al.*, 1996; Sofowora, 1993). This may also explain why the root decoction *Ziziphus mauritainia* is given as a febrifuge, taenicide and emmenagogue, and the powdered root is dusted on wounds. It has been reported that flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities (Pourmorad *et al*., 2006; Ugwu *et al*., 2013) and they might induce mechanism that affect cancer cells and inhibit tumor invasion (Rafat *et al.*, 2008). The presence of flavonoids may account for the hepatoprotective activity of the ethanol extract of *Ziziphus mauritainia* in CCl_4 -induced liver damage in rats and the antidiarrhoea activity of the methanol root extract as reported by Dahiru *et al.*, (2005) and Dahiru *et al.*, (2006). The antioxidant activity of the aqueous extract of *Ziziphus mauritiana* leaf has also been reported (Dahiru and Obida, 2008). These activities could be attributed to the ability of flavonoids to neutralize and quench free radicals (Ugwu *et al*., 2013; Pourmorad *et al*., 2006; Omale and Okafor, 2008). It can also be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans *et al*., 1995).

Previous studies have shown that the species of *zizyphus(Z.cristi,Z.mauritiana,Z.jujuba)*are very rich in saponins. Saponins are known to have expectorant properties and these have been made use of in treatment of upper respiratory tract inflammation (Trease and Evans, 2002). Saponins are also believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Roa *et al.*, 1996). Saponins have the property of precipitating and coagulating red blood cells (Yadav and Agarwala, 2011). Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Just *et al.*, 1998; Okwu and Emineke, 2006, Liu and Henkel, 2002). Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Yukari *et al*., 1995). Cardiac glycosides have been shown to aid in the treatment of congestive heart failure and cardiac arrhythmia (Okwu, 2001). This may explain why the leaves are helpful in the treatment of cardiac infections along with other ailments such as asthma (Taylor, 2002). Steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). The phytochemical compounds are also responsible for the characteristic odor, pungencies and color of plant, while others give the particular plant its culinary, medicinal or poisonous virtue (Evans, 2002).

The emergence of bacterial strains resistant to a number of antimicrobial agents as well as the potent side effects of modern synthetic drugs demands that a renewed effort be made to seek antibacterial agents of plant origin that are effective against disease causing bacteria resistant to current antibiotics and for use in the development of new chemotherapeutic agents (Eloff, 1998). The first step towards achieving this goal is the *in vitro* antibacterial activity studies of medicinal plants (Samy and Ignacimuthu, 2000).

From the results of the anti-bacterial studies, the methanol extracts of the seed and pulp showed varying degree of anti-bacterial activity. The seed demonstrated maximum anti-bacterial activity against*Staphyloccus aureus* at all the tested concentration. Especially, the seed extract did not show activity against *Eschericial coli* and *Salmonella* spp. The inhibitory activity of ciprofloxacin; the standard commercial anti-bacterial drug which served as a positive control against these bacterialwas more efficacious than for both the seed and pulp extract. These findings are in agreement with previous report in which *Ziziphusmauritiana* demonstrated broad spectrum of antibacterial activity (Goyal *et al.*, 2012; Najafi, 2013). The presence of secondary metabolites may be a plausible explanation for the antibacterial activity observed in this study.

Since the methanol crude extract of the pulp exerted the most pronounced antibacterial activity against the organisms tested, it was subjected to partitioning using water, n-hexane, ethylacetate, and n-butanol. Ethyl acetate fraction of the pulp methanol extract possessed inhibitory activity against all tested organism. Two possibilities that may account for the higher antibacterial activity of this solvent fraction is the nature of biologically active components extracted by the solvent, which may be enhanced in the presence of ethylacetate; and the stronger extraction capacity of the solvent that may have yielded a greater number of active constituents responsible for antibacterial activity (Ghosh *et al.,* 2005). The n-butanol fraction inhibited the growth of *Staphyloccus aureus* at doses of 50 and 100mg/ml. The aqueous fraction had no effect on the tested organisms.

These results concur with the findings of Chung *et al.,* (1998), Achi, (2010), and Goldy and Kalra, (2011). In these reports, the inhibitory effects on pathogenic bacteria were attributed to the presence of bioactive compounds present in plant extracts. For instance, Chung *et al.,* (1998) reported that tannins a plant active metabolite inhibits the growth of many fungi, bacteria and viruses. Saponins a special class of glycosides which have soapy characteristics was reported to be active antifungal agents (Sodipo *et al.,* 1991)Saponins are surface active agents which alter the permeability of the cell wall of organisms thus facilitating the entry of toxic materials or leakage of vital constituents from the cell (Daniyan *et al*., 2010).

saponins as potent antimicrobial agent.. Terpenoids and flavonoids have been reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants (Yusha'u *et al.,* 2008). The mode of antimicrobial action of these plant metabolites may be related to their ability to inactivate microbial enzymes and transport proteins (Tringali, 2005). In view of these established facts, we may posit that the antibacterial activity observed in this study may be as a result of the presence of these phytochemical substances,in the extracts and fractions.

Anti-bacterial activities of the fractions showed that fraction A was not active against all the organism tested. Fraction B showed activity against *Staphyloccus aureus*. Fraction C at all the tested doses demonstrated anti-bacterial activity against *E.coli* and *S.aureus* ranging from 10.00mm- 29.50mm against all the tested pathogens except for *Salmonella* spp. It is possible that component(s) against for *Salmonella* spp is/are lost in fraction C. Fraction D was active against *salmonella* spp only. The fractionation of the ethylacetate pulp extract improved the anti-bacterial activity in Fraction C against all the tested organisms. This result showed that the fractionation process concentrated the active principle in fraction C. Similar results were reported by Mansouri *et al.;* (2001) when evaluating the antibacterial activity of the crude extracts and fractionated constituents of *Myrtus communis*.

Many plant substances have been investigated scientifically for antibacterial activity and a large number of these plant products have shown promising results with respect to inhibiting the growth of pathogenic bacteria. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross resistance with agents already in use may be minimal (Zahner and Fiedler, 1995). Literature is replete with reports on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Samy and Ignacimuthu, 2000; Palombo and Semple 2001; Kumaraswamy *et al.,* 2002). Some of these observations have helped in identifying the active metabolites responsible for such activities and in developing synthetic drugs for the treatment and management of a number of ailments in human beings. The results of this study do not only show the scientific basis for some of the therapeutic uses of the plant in traditional medicine, but also confirms the impact of ethno botanical approach when investigating plants for their antimicrobial properties.

From the result of castor oil induced diarrhoeal model, the seed and pulp extracts at all the tested doses significantly prolonged the time- of diarrhoeal onset, and decreased the frequency of defecation. In the castor oil induced intestinal fluid accumulation test, treatment of rats with seed and pulp extracts produced a significant reduction in the intestinal fluid accumulation. Results of evaluation of gastrointestinal transit in rats demonstrated that the seed and pulp significantly reduced the intestinal propulsive movement of charcoal meal at all the test doses as compared to the negative control. This study is in line with other studies in which the extract of different plants reduced the frequency of stooling (Mazumder *et al*., 2006; Karthik *et al*., 2011; Billah *et al*., 2013), significantly inhibited the distance travelled by charcoal meal (Zavala-Mendoza *et al*., 2013).

Antidiarrhoeal property of a number of plants have been attributed to antimicrobial activities (DiCarlo *et al.,* 1993; Ilyas *et al.,* 1995), blocking of prostaglandins and presence of secondary bioactive compounds (Longanga-Otshudi *et al.,* 2000; Al-Rehaily *et al.,* 2001; Dahiru *et al.,* 2006). *Ziziphusabyssinica*has been reported to possess activity against *Staphylococcus aureus* (Nyaberi *et al.,* 2010; Gundidza and Sibanda, 1991) which is one of the microorganisms that is implicated in diarrhoea. Tannins are responsible for impeding gastrointestinal motility and can precipitate proteins of the enterocytes, reducing peristaltic movements and intestinal secretions (Almeida *et al*., 1995; Tripathi, 2008; Yadav and Tangpu, 2007), flavonoids are able to inhibit intestinal secretory response induced by prostaglandins E2 and to attenuate NO synthesis (Hamalainen *et al*., 2011; Sanchez de Medina *et al*., 1997; Kim *et al*., 1999, 2004; Messaoudene *et al*., 2011), terpenoids are implicated in attenuation of NO synthesis (Jang *et al*., 2004).

Theresult of phytochemical screening of *Z.mauritiana* pulp and seed revealed the presence of tannins, flavonoids, and terpenoids. Therefore, the seed and pulp extracts may as well have acted through these mechanisms

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The crude methanolic extract of *Z. mauritiana* pulp and seed contain the following phytochemicals phenolics, tanins, flavonoids, saponins, cyanogenic glycosides and terpeniods, the crude and solvent fractions of *Z. mauritiana* pulp and seed possess significant antidiarrhoealand antibacterial activity. These activities may be as a result of varied amount of phytochemical substances evenly distributed in the seed and pulp. The ethylacetate fraction was the most potent.

6.1.2 Recommendation

1. The seed and pulp of *Z. mauritaina* should be used in the treatment of diarrhoea and bacterial infections.
2. Further study is required to determine the different active compounds from *Z.mauritaina* and its full spectrum of efficacy.
3. Further phytochemical screening should be carried out to determine the perticuler constituent responsible for the anti diarrhoeal and anti-bacterial activity.
4. Higher dose should be used to determine the effect on gastrointestinal system.

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APPENDIX

APPENDEX 1: Preparation of stock solution of extract

The stock solution was prepared by dissolving 1.0g of the extract in 10ml of distilled water to get a concentration of 100mg/ml.the stock solution was reconstituted to a graded concentration of 50mg/ml, 25mg/ml and 12.5mg/ml by two fold dilution this was stored at $4^{o}C$.

APPENDEX 2: Fractions Obtained from the Column Chromatography of pulp Ethylacetate Fraction.

Number	Fractions	Eluting Solvent
A	1-14	hexane absolute
B	15-30	8:2
C	31-46	6:4
D	47-64	4:6
E	65-81	2:8
F	82-100	Ethylacetate absolute

PLATES

PLATE III

Plate of antibacterial activity of methanolic extract of *Z. mauritiana* on *E.coli* and *S. aureus.*

PLATE IV

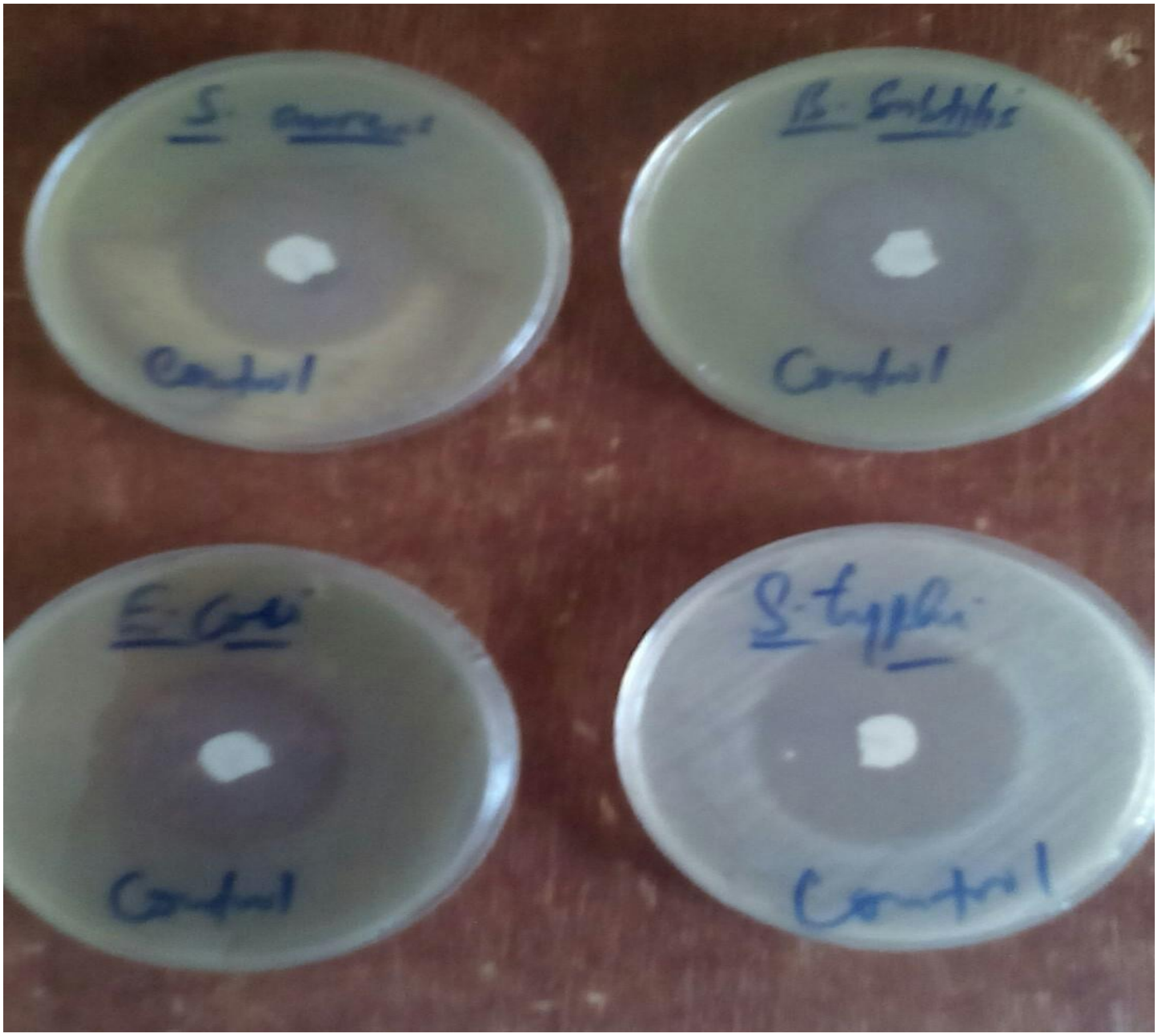
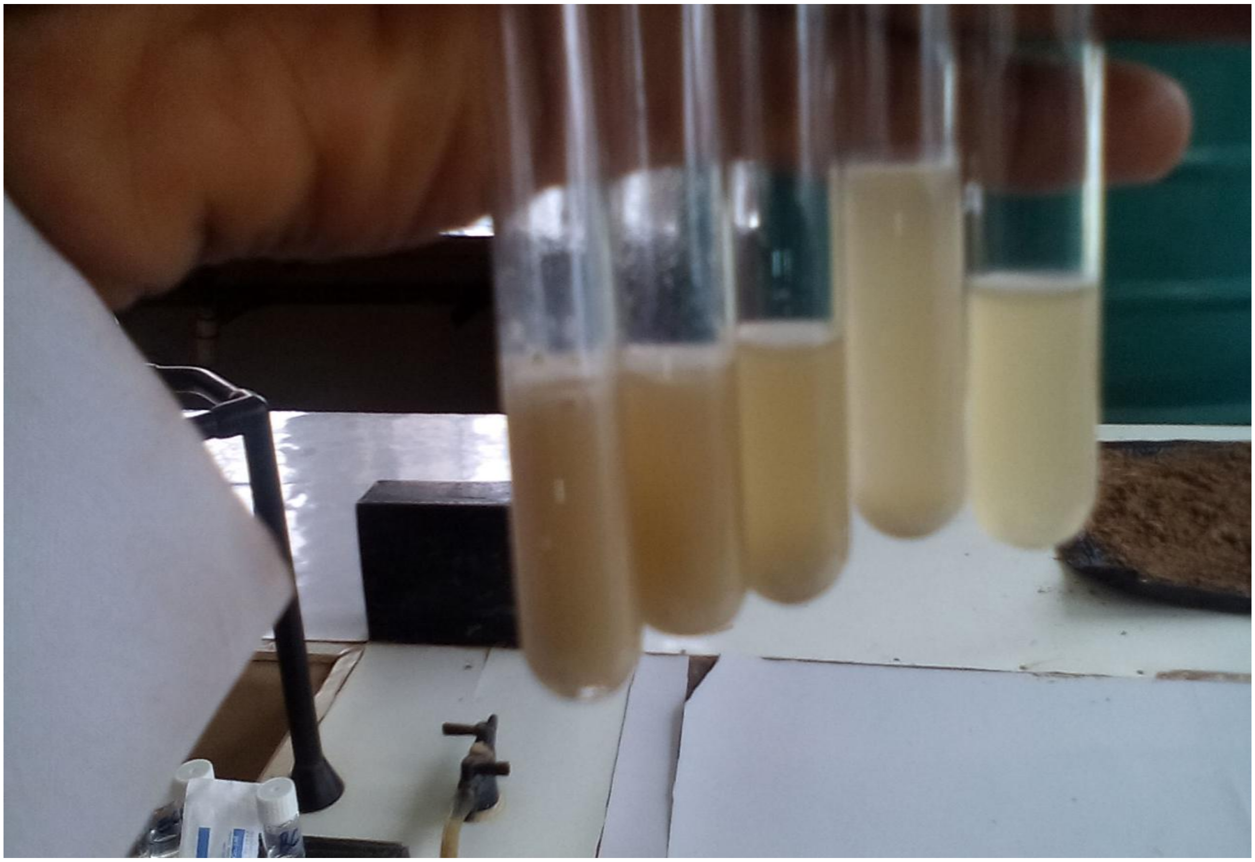


Plate of zone of inhibition of ciprofloxacine against *S. aureus*, *E.coli* and *Salmonella* spp.

PLATE V



Tubes of Minimum Inhibitory Concentration of ethylacetate fraction of *Z.mauritiana.*