ANTIBACTERIAL ACTIVITY OF *Vernonia amygdalina* LEAF EXTRACTS ON MULTIDRUG RESISTANT ENTERIC BACTERIA

BY

EZEOYE, Onyinye Blessing
MTech/SLS/2018/9288

DEPARTMENT OF MICROBIOLOGY

SCHOOL OF LIFE SCIENCES

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA.

FEBRUARY, 2022

ABSTRACT

Vernonia amygdalina also known as bitter leaf, is a tropical plant that grows in Africa. It has Ethno botanical uses in the treatment of gastroenteritis. This study investigated the antibacterial activity of *V. amygdalina* leaf extracts on four Multi Drug Resistant enteric bacteria; *Klebsiella pneumoniae, Salmonella typhi, Shigella* species and *Serratia marcescens.* Bioactive components from Pulverized leaves of *V. amygdalina* was extracted with water and ethanol using the maceration method. The ethanol extract was further fractionated through the separation funnel technique using n- hexane, chloroform and ethyl acetate. Antibacterial activity of the crude extracts and fractions was carried out using the agar well diffusion method. The Minimum Inhibitory Concentration and the Minimum Bactericidal Concentration were determined using the broth dilution method. Phytochemicals present in the crude extracts include; phenols (18.5 %), flavonoids (0.4 %), tannins (8.7 %), alkaloids (3.0 %), saponins (12.2 %) and steroids (2.8 %). The zones of inhibition by the crude extracts ranged from 16 to 30 mm on all the test organisms at a concentration of 1500 to 3000 mg/ml, while the zones of inhibition of all fractions ranged from 19 to 29 mm on all the test bacteria at 100 to 800 mg/ml concentrations. The MIC ranged from 6.3 to 750 mg/ml while the MBC ranged from 12.5 to 1500 mg/ml. The oral administration of n-hexane and ethyl acetate fractions of *V. amygdalina* to Wister rats, resulted in a significant increase ($p<0.05$) in the Mean capsular volume, Basoplils, Esinophils.Mean Corpuscles Haemoglobin and Red blood cell counts when compared to the control group. Similarly, significant decrease ($p<0.05$) was recorded in Platelet counts, Neutrophils and Lymphocytes levels in all dosed groups, when compared to the control group. The levels of PCV, haemoglobin and total white blood counts were same as that of the control group in rats administered with ethyl acetate fraction while haemoglobin levels was same as that of the control group in rats administered with n-hexane fraction. The levels of AST, ALT, ALP, Creatinine, Urea, Triglycerides and LDL of rats administered with both fractions decreased significantly ($p<0.05$) compared to the control group. The levels of total protein, albumin, cholesterol and HDL increased significantly($p<0.05$) in rats administered with 300 and 600 mg/Kgbw respectively of ethyl acetate and n- hexane fractions. Similarly, total protein, albumin, and HDL increased significantly($p<0.05$) in rats administered with 10 mg/Kgbw of n-hexane fraction. There was no distortion in the kidney and liver architecture of the rats administered with 10 and 300 mg/Kgbw of n-hexane and ethyl acetate fractions. *V. amygdalina* possess potent antibacterial compounds, that are relatively safe and may be useful in developing chemotherapeutic agents for the treatment of infections associated with enteric bacteria.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Traditional medicine is defined by the World Health Organization (WHO), as the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2021). Africa is home to a wide range and assorted medicinal plant life, for thousands of years' herb based treatment has been an important part of the continents traditional medicine (Gouws, 2018). Numerous traditional African medicines are undeniably beneficial in treating disease or maintaining good health and in many parts of rural Africa, traditional healers prescribe medicinal plants because they are easily accessible and affordable health resource available to the local community (Mahomoodally, 2013).

The knowledge of traditional medicine is usually passed orally, from one generation to another, however a number of plants have been used in traditional medicine for many

years without scientific data to back up their effectiveness (Acharya and Anshu, 2018). Examples of some herbs used in traditional medicine includes; *Ocimum gratissimum* (Scent leaf), *Rauvolfia vomitoria* (the poison devil's-pepper), *Afzelia africana* (African Mahogany), *Adansonia digitata*(Bahobab), *Amaranthus spinosus* (spiny pigweed), *Carica papaya* (Paw-Paw) seeds, *Garcinia kola* (bitter kola), *Aloe vera*, Ginger, Garlic, Cloves, *Citrus paradise*(Grapefruit) seeds, *Harungana madagascariensis* (Orange-milk tree), Ginseng, Ginkgo biloba, Elderberry and Turmeric (Ezekwesili and Okaka, 2019; Hill, 2020).

The discovery of antibiotics revolutionized chemotherapy of infectious diseases (Sen and Batra, 2012). However, the emergence of antibiotic resistance has increased greatly in the past few years. In other to curtail this public health problem, researchers have focused on the search of novel antibacterial substance from different sources (Owoade and Raji, 2019). The treatment of human ailments using plant is an antique practice and rapidly gaining attention due to plant availability, biodegradability, low toxicity and cost efficacy (Salami and Agu, 2013). Plants and herbs also possess potential of introducing new templates for modern medicine (Oshilim, 2017).

Vernonia amygdalina is a plant with a characteristic bitter taste, found mostly in tropical Africa (Farobi and Owoeye, 2011). They are members of the *Asteraceae* family that grows as a perineal woody plants slightly smaller than trees, measuring about three meters high, with green leaves which has a characteristic odour (Ijeh and Ejike, 2011). It is a well-known medicinal plant, whose cold concoction is used in the treatment of ailments such as fever, amoebic dysentery, and diarrhea (Du-Bois *et al*., 2019). Its leaves are used in many Nigerian homes in soup preparation and its stem is used as chewing stick (Arekemase *et al.,* 2013).

Enterobacteriaceae also called Enteric bacteria, are a group of bacteria that live in the digestive tract of man and animals, as part of its normal flora of healthy humans, while some of them are regularly pathogenic, others are not pathogenic (Riedel *et al.,* 2019). They are gram negative, facultative anaerobes and can ferment a wide range of carbohydrates, and produce toxins (Oyedum, 2017). In recent times, *Enterobacteriaceae* have developed a rapid resistance to antibiotics, especially the β-lactam type. This is largely due to the enlistment of incessantly expressed solitary genes, that encode competent drug modifying enzymes (Iredell *et al*., 2016).

1.2 Statement of the Research Problem

Alternative sources of antibiotics are a point of focus, as pathogenic microbes are fast gaining resistance to most available antibiotics (Prestinaci *et al*., 2015). Experimental screening of herbal plants, to determine its safety for use is important, as it is depended on by 80% of the world populace for their principal health care (Martins, 2013). There is the need to investigate plants for a better knowledge of their medicinal properties, as many medicinal plants have not been fully evaluated (Alo *et al*., 2012).

1.3 Aim and Objectives of the Study

To evaluate the antibacterial activity of *Vernonia amygdalina* leaf extracts on multidrug resistant enteric bacteria.

The objectives of this study were to:

i. determine the antibacterial activity of the crude extracts and fractions of *Vernonia amygdalina,* leaves ii. determine qualitative and quantitative bioactive constituents of the crude extracts and fractions of *Vernonia amygdalina* leaves.

iii. determine minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of *Vernonia amygdalina* leaves.

iv. determine the Acute and Sub-acute toxicity of the most active fractions of *Vernonia amygdalina* leaves.

1.4 Justification for the Study

An increasing resistance of bacteria to antibiotics, has directed the search for alternative sources for the treatment of bacterial infections (Anyanwu and Okoye, 2017). Plant extracts are being utilized as alternatives to antibiotics because of their availability, cost effectiveness, low toxicity and are environmentally friendly. These places plant at an advantage above other organisms. Hence, the utilization of plant extract as an antibacterial has potential impact in drug development, in coming years (Jaryum *et al*.,
2019). Preceding studies have revealed the antibacterial activities of crude extract of *Vernonia amygdalina* plants against some bacteria, but little study has been done on the fractions of the crude extract of *Vernonia amygdalina* (Alo *et al*., 2012; Salami and Agu, 2013; Zubairu *et al*., 2019). Therefore, this study investigated the antibacterial potentials *Vernonia amygdalina* leaf crude extracts and fractions on *Klebsiella pneumoniae, Serratia marcescens, Shighella* species and *Salmonella typhi*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Medicinal Plants

A medicinal plant is any plant which contains substances that can be used for therapeutic purposes, in one or more of its organs, and can also serve are precursors for the synthesis of useful drugs (Sofowora *et al*., 2013). Medicinal plants possess therapeutic properties or exert valuable pharmacological effect on the human or animal body (Namdeo, 2018). They possess numerous phytochemicals, which have potential as use for drugs, if scientifically confirmed, the content and known pharmacological activity of these substances in medicinal plants is the scientific basis for their use in modern medicine (Ahn, 2017). Plants have important roles in the development of modern medicines, it is estimated that about 65% of modern medicines are directly or indirectly derived from plants (Ardalan and Rafieian-Kopaei, 2013). Medicinal plants are often tough and fibrous, requiring some form of preparation to make them convenient to administer (Yang, 2010). Extraction of medicinal plants is a process of separating active plant materials or secondary metabolites using an appropriate solvent and standard extraction procedure (Abdullahi and Mainul, 2020). Several methods were used in the extraction of medicinal plants such as maceration, infusion, decoction, percolation, digestion and Soxhlet

extraction, superficial extraction, ultrasound-assisted, and microwave-assisted extraction (Azwanida, 2015).

2.2 Uses/Advantages and Efficacy of Medicinal Plants

Medicinal plants have long been the main remedy for treating human diseases, with an estimate that about 25% of modern medicines are made from plants first used traditionally. However, there is inadequate scientific evidence to establish the safety and efficacy of most herbal products (Zhang *et al*., 2015). The first reason for the use of herbals is the belief of people for maintenance of health or to treat certain ailments (Rafieian-Kopaei, 2012). Medicinal plants may provide three main kinds of benefit: health benefits to the people who consume them as medicines; financial benefits to people who harvest, process, and distribute them for sale; and society-wide benefits, such as job opportunities, taxation income, and a healthier labour force (Smith-Hall *et al*., 2012). Medicinal plants are good source of bioactive compounds or phytochemicals, which have been used in the treatment of a wide range of diseases such as diabetes, coronary heart disease, and cancer (Tariq *et al*., 2021). Medicinal plants may provide three main kinds of benefit: health benefits to the people who consume them as medicines; financial benefits to people who harvest, process, and distribute them for sale; and society-wide benefits, such as job opportunities, taxation income, and a healthier labour force (SmithHall *et al*., 2012). Some of the uses of medicinal plants and its uses includes *Vernonia amygdalina,* in the treatment of diarrhea and fever (Du-Bois *et al*., 2019), *Psorospermum febrifugium, Securidaca longipendunculata*, and *Cryptolepis sanguinolenta* are widely used among people living with HIV/AIDS in Uganda (Anywar *et al.,* 2021). *Carapa guianensis* (Aublet), is widely used in Brazilian traditional medicine because of its multiple curative properties against fever, rheumatism, an anti-

inflammatory agent, antibacterial agent, and as an insect repellant (Banerjee *et al*., 2018). Other medicinal plants and their uses are described in Table 2.1.

Table 2.1 Some medicinal plants and their uses

S/N	Botanical name	Common name	Uses	Reference
1	*Azadirachta indica*	Neem	Treatment of sore throat, eczema and stomach ulcers.	
2	*Ocimum tenuiflorum*	Holy Basil	Treatment of Cold and Cough. relieves Stress, and anxiety, possess antimicrobial properties which help in treating stomach infections. Purifies the air and remove the unwanted chemicals from entering the house.	
3	*Mentha spicata*	Mint	improve respiratory health when chewed, improve digestion, and circulation of blood.	Plant décor, (2022)
4	*Aloe Vera*	Aloe Vera	weight loss treatment, for hair growth and shiny hair, asthma, stomach ulcers, bowel diseases, itching, and inflammation	
5	Cymbopogon citratus	Lemon Grass	relieving digestive disorders, congestion, cough, stomach-aches, headaches, cholesterol lowering agent to help in weight loss, enhanced kidney functioning and preventing hair fall.	
6	*Moringa Oleifera*	Drumstick tree	Treatments for Ulcer, inflammation and pain and cures headache	
7	*Garcina Kola*	Bitter Kola	Treatments of colds, coughs and antimicrobial infections	
8	*Ocimum gratissimum*	Scent Leaf	Treatment of diarrhea in form of infusion and for headache	
9	*Adansonia digitata*	Baobab	Possess antimalarial, antimicrobial, antiviral and antidiarrheal effect	Dibia, (2019).
10	*Curcuma longa*	Tumeric	Prevents cancer, pain caused by inflammatory diseases, like arthritis and several skin diseases	

11	*Allium sativum*	Garlic	Anti-bacterial and Anti-parasitic, used in Cancer Prevention. Other include treating Skin infections, Hair loss, acne, asthma, and indigestion.	
12	*Zingiber officinalis*	Ginger	Prevents acid reflux nausea, and vomiting, reduces inflammation during bacterial infections, treatment of heart diseases, cold, and cough	Deering, (2019).

2.3 Toxicity of Medicinal Plants

The safety of medicinal plant is important as large number of them is self-prescribed and is used to treat chronic conditions (Ardalan and Rafieian-Kopaei, 2013). These traditional medicines are consumed for years with apparently little toxicity (AlQathama *et al*., 2020). Medicinal plants are generally considered to be of lower risk compared with synthetic drugs, however, they are not completely free from the possibility of toxicity or adverse effects (Zhang *et al*., 2015). They can be toxic intrinsically or when taken in combination with other preparations (Ardalan and Rafieian-Kopaei, 2013).

2.4 *Vernonia amygdalina*

V. amygdalina is a domesticated plant in tropical Africa, with a characteristic bitter taste, which is as a result of the occurrence of bioactive components such as alkaloids, saponins, glycosides and tanins (Farobi and Owoeye, 2011). This bitter taste is the reason why this plant is also called bitter leaf. *V. amygdalina* belongs to the *Asteraceae* family. It which grows as a perineal shrub, measuring about three meters' high, it has a rough bark with thick dark channels, green leaves which are petiolate and has a characteristic odour (Ijeh and Ejike, 2011).

It is found in many parts of Nigeria and called *Onugbu* in Igbo, *Shuwaka* in Hausa, *Ewuro* in Yoruba and *Edidot* in Efik languages, where it is mostly used in soup preparation, after the bitter taste of the leaves has been washed off. Other uses of *V. amygdalina* includes its use in brewing beer in place of hops (Pieroni, 2005), its use as trado-medical treatment for stomach upset, diarrhea, malaria, intestinal parasites, amoebic dysentery, and schistosomiasis (Du-Bios *et al*., 2019).

Vernonia amygdalina is rich in nutrients, possessing a high protein content (sometimes up to 20%) it also contains a reasonably high lipid content of about 4.7% when compared with other leaves. It is a good source of arabinose, maltose, galactose, glucose and fructose (Ojiemelukwe and Amaechi, 2019). The stem of *V. amygdalina* can be used as chewing stick for teeth cleaning purposes after it has been flayed and it was stated to be very active as it contributed to anticaries, gum healing, haemostasis; to halt the blood flow, antimicrobial action and plaque hindering effect (Nursuhaili *et al*., 2019).

2.5 Phytochemical Components of *Vernonia amygdalina* Leaves

Phytochemicals are chemical primary or secondary chemical metabolic products, which confers protective characteristics to plants (Molyneux *et al*., 2007).

V. amygdalina has been found to contain phytochemicals like flavonoids, alkaloids, Tanins, saponins, steroids, phenols, terpenes, cardiac glycosides, anthraquinone, pylate and oxalate (Ojiemelukwe and Amaechi, 2019). A research by Ekam *et al*. in 2010, reported the occurrence of Flavonoids, alkaloids, tannins, terpenoids, cardiac glycosides and saponins at varying quantities in fraction from ethanol extracts. However, saponins were absent in the chloroform and ethyl acetate fractions, alkaloids were absent the butanol fraction, terpenoids were absent in the benzene chloroform and butanol fraction,

cardiac glucose was absent in the benzene and butanol fractions while flavonoids absent in the benzene fractions. In 2016, a research by Yusmazura *et al*. recorded the occurrence of, alkaloids, saponin, tannin and flavonoids in aqueous extract of *V. amygdalina*, terpenoids and anthraquinone were however absent in the extract. A study by Ali *et al*. (2019) demonstrated the occurrence of alkaloids, tannins, terpenoids, flavonoids, Saponins, steroids and phenols in methanol and aqueous extracts of *V. amygdalina*. A study by Evbuomwan *et al*. (2018), exposed the occurrence of alkaloids, reducing sugars, terpenoids, flavanoids, Saponins, steroids, anthraquinones and cardiac glycosides in ethanol and aqueous extracts of *V. amygdalina*, with the absence tanins were absent in both extracts.

2.5.1 Alkaloid content

These exist as enormous set of naturally occurring organic compounds, whose structure contains one or more nitrogen atoms (Kurek, 2019). Based on their structure, they are divided into sub major groups, with no uniform classification (Hesse, 2003). These groups include Tropane (Pyrrolidine), Isoquinoline, Pyridine, Pyrrolizidine, Quinoline, Indole, and Purine. Alkaloids can be obtained from most bacteria, fungi, plants and animals (Roberts and Winks, 1998). Asides its protective function to plant (its presence in plants may prevents some insects and animals from feeding from the plants), the role of alkaloids in the metabolism of organisms that synthetize it is not fully understood (Aniszewski, 2007).

Alkaloids have a wide application which includes; It's usage in medicine as narcotics for pain relief, atropine for the management of low heart rate, Tubocurarine used in surgery as muscle relaxants, ephedrine employed in bronchial asthma and to relieve uneasiness of

hay fever, sinusitis, and common colds and quinine used as an antimalarial (Kurek, 2019). In agriculture it is used in the synthesis of pesticides and insecticides with low toxicity (Matolcsy *et al.,* 2002).

Alkaloids possess antibacterial properties, as it has been demonstrated to be active against bacteria like *Esherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella paratyphi A., Salmonella paratyphi B.* and *Salmonella typhi, Bacillus subtilis, Corynebacterium hoff-manii, Staphylococcus aureus, Streptococcus faecalis, Streptococcus pyogenes, Streptococcus viridans* and *Micrococcus lysodicklycus* (Aniszewski, 2007).

2.5.2 Saponin content

Saponins which are produced by plants and some marine organisms, are naturally occurring surface active glycosides which is made up of a steroidal or a triterpenoid aglycone to which one or more sugar chains are joint (Arabski *et al*., 2012). The organic characteristics of saponins as natural non-ionic detergents, which is accountable for its cytotoxic, hemolytic, molluscicidal, anti-inflammatory, antifungal, anti-yeast, antibacterial, and antiviral activities, is determined by its chemical structure (Arabski *et al*., 2012).

Saponins consists of eleven major classes, which are dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes, and steroids. However, oleanane is commonly present in majorly all orders of the Plant Kingdom (Kregiel *et al*., 2017).

It has a bitter taste, has a soap like property as it forms foam when agitated in water which is due to its solubility in fat and water (Shi *et al*., 2004). The presence of saponins in plants

helps protect it from being eaten by animals and microbes (Hartmut, 2006). Saponins have strong antibacterial activity this was demonstrated in a research by Khan *et al*. (2015), where it showed strong activity against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Klebsiella pneumoniae*.

The antibacterial mechanism of saponins is to lyse the bacterial cell, when it binds with cholesterol inside cell, which results in the saponin-cholesterol complex. It also has the ability to upturn the penetrability of the bacterial cell wall, when it binds with the lipid A, in the surface membrane of gram negative bacteria (Muhammad *et al*., 2018). saponins are also used in cosmetic industry, detergent industry, food industry and as adjuvants in development of vaccines (Sun *et al*., 2009; Kregiel *et al*., 2017).

2.5.3 Flavonoid content

These are a class of organic secondary metabolites, produced by most plants. They are made up of polyphenolic compounds having a benzo-γ-pyrone structure (Shashank and Abhay, 2013). They occur in a glyosidic form. Flavonoids have diverse functions in plants which includes; being accountable for the aroma and colour in fruits and flowers, helps to attract pollinators to plant, acts as unique ultra violet light filter, helps in seed dispersal and protects plant from stress (biotic and abiotic) (Panche *et al*., 2016). They also help to protect against plants from being eaten by insects (Pistelli and Giorgi, 2012). The structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization dictates the chemical identity of Flavonoids (Shashank and Abhay, 2013).

Flavonoids possess a general structure made up of a 15-carbon skeleton, which comprise of two phenyl rings (A and B) and a heterocyclic ring (C). around the heterocyclic ring

(C), they vary in structure with all possessing the characteristic C6-C3-C6 carbon skeleton (Ruiz-Cruz *et al*., 2017). Based on the variations on the C group, the are divided into sub groups which are Flavones, Flavonols, Flavanones, Flavanols, Anthocyanidines, and Isoflavones. They possess antioxidant, antibacterial, antiviral and anti-inflammatory properties, and have applications in cosmetic industry and food industry, where it is used as preservative.

Flavonoids exhibits antibacterial activities in the following ways; hindering the synthesis of nucleic acid, hold back the function of the cytoplasmic membrane, impede attachment and biofilm formation, modifying the permeability of the cell membrane, hindering the pirons on the membranes of the cells and weakening of the pathogenicity (Xie *et al*., 2015). Flavonoid compounds have demonstrated abilities in constraining bacterial strains such as *Vibrio cholerae*, *Streptococcus mutans* and *Shigella* species (Enwa *et al*., 2014).

2.5.4 Tannin content

Tannins are secondary metabolic products of mainly plant parts (bark, wood, leaves, seeds, roots, and plant galls), which are water soluble, organic polyphenols (Singh and Kumar, 2019). They are usually segregated inside the vacuole of the plant cells, where they regulate plant growth and also protect plants from insecure and herbivores (Ferrell and Thorington, 2006). Chemically, they are grouped into two major classes hydrolyzable and condensed (Britannica, 2021).

Tannins and its bye-products, possess antibacterial properties, as it was able to ploriferate the development of bacteria such as *Aeromanas, Bacillus, Clostridium, Enterobacter, Helicobacter, Klebsiella, Proteus, Pseudomonas, Shigella, Escherichia, Staphylococcus*, and *Streptococcus* species (Singh and Kumar, 2019). The antibacterial mechanisms of

tannins can be indirect, by dispossessing the bacteria of the substrate needed for growth and hindering bacterial enzymes. It can also be direct by hindering oxidative phosphorylation in the bacterial metabolism (Sieniawska and Baj, 2017). Tannins also have antifungal, antidiabetic, anti-nutrient, anti-inflammatory, anti-obesity and antiviral properties (Muller-Harvey and McAllan, 1992; Kurhekar, 2016; Sieniawska and Baj, 2017) They are aslo useful in the food, leather, wood pharmaceutical and medical industries (Singh and Kumar, 2019).

2.5.5 Terpenoid content

Treprenoids are the largest secondary plant metabolic products, usually present as volatile oils in plants of medicinal values. They are made up of inherently present organic chemicals obtained from Terpenes, a five-carbon isoprene unit (Perveen, 2018). They play a vital part in the progress and growth of plants, physiological processes and its ecology (Yang *et al*., 2020).

They are classified in two ways, first according to the number of isoprene unit which makes up the parent terpene (Ashour *et al.*, 2010) and according to the type and number of cyclic structures they contain (Ludwiczuk *et a*l., 2017). They have antimicrobial, antiinflammatory, antimalarial, antidiabetic, antiaging effects and is used in the hindrance and management of diseases relating to the heart and blood vessels (Yang *et al.*, 2020). Trepenoids mechanism of eliciting antibacterial activity is through the distraction of the bacterial cell membrane (Jasmine *et al*., 2011).

2.5.6 Phenol content

Phenols are compounds comprising of one or more aromatic rings with one or more hydroxyl groups (Dai and Mumper, 2010). They are made up of one of the largest and

extensively disseminated secondary metabolic products of plants (Giada, 2013). These compounds are split into phenolic acids and polyphenols (Minatel *et al*., 2017). They are categorized based on the number of carbons and based on the number of phenolic groups they possess (Vermerris and Nicholson, 2008). They play important roles in plants, some of these roles includes; the biogenesis of lignin and pigment to aid plant development, repulsing and destroying microorganism and other disease causing organism from attacking wounded plants through the secretion of phenolic phytoalexins, production of fragrance that attracts symbiotic insects to the plant, to aid fruit dispersal and pollination and provide Strickland support for plants (Bhattacharya *et al*., 2010).

Phenols possess antioxidants, anticancer properties (Dai and Mumper, 2010). They also possess antibacterial properties, as they have been able to inhibit organisms such as *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*, by connecting and impairing bacterial enzymes necessary for cell wall formation, in a way which will change the resistance of the bacteria (*Staphylococcus aureus*) hereby destroying their cell walls which will lead to the leakage of their cellular constituents (Omar, 2017). Phenols can also impede the formation of biofilm, decrease host ligand adherence and neutralization of bacterial toxins, reduce the fluidity of membrane and hinder the synthesis of nucleic acids (Takó *et al*., 2020).

2.6 Antibacterial Properties of *Vernonia amygdalina*

V. amygdalina has shown great antibacterial properties. Various studies including but not limited to Owoade and Raji (2019), showed that Cold water extracts of *V. amygdalina* leaves extracted employing the maceration method, where inhibitory against bacteria such as *klebsiella pneumoniae, pseudomonas aeruginosa, salmoella typhi, Escherichia coli*,

and *staphylococcus aureus*, at zones of inhibition ranging from 5 to 23 mm using 0.5, 1.0, 1.5, 2.0 and 2.5 g/mL concentrations. The plant extract was inhibitory to *K. pneumoniae* at 2.5 g/mL only with an inhibition zone of 18mm and *S. typhi* was inhibited only at 1.5, 2.0 and 2.5 g/mL with inhibition zones of 5mm, 8mm and 10mm respectively.

Evbuomwan *et al.* (2018), reported the antibacterial efficacy of aqueous and ethanol *V. amygdalina* against *K. pnuemoniae, E. coli, P. aeruginosa, Bacillus subtilis, and S. aureus,* with a zone of inhibition ranging from 6.5 to 11mm at a concentration range of 6.25 to 200 mg/mL.

According to Ogundare (2011), acetone, chloroform and ethanol extracts of *V. amygdalina* were tested against *E. coli, Shigella dysentriae, Bacillus cereus, Staphylococcus aureus, P. aeruginosa, S. typhi, Baccillus subtilis, Proteus vulgaris, Enterobacter aerogenes* and *Clostridium sporogenes* using the disc diffusion technique. All extracts inhibited *Bacillus cereus, Shigella dysentriae* and *Staphylococcus aureus* only, at zones of inhibition ranging from 3 to 14 mm at concentrations ranging from 12.5 to 50 mg/mL. while *E. coli* was only inhibited by the chloroform extract at 20 mm at 50 mg/mL. fractions of *V. amygdalina* (crude methanol extract), was obtained with the aid of column chromatography separation technique with n- hexane, acetone and methanol as solvents of fractionation, was not able to inhibit the growth of *E. coli, S. typhi* and *S. aureus* in a research carried out by Zubairu *et al*. (2019). However, the combination of the acetone and methanol fraction in a 50:50 ratios were able to inhibit the test organisms with zones of inhibition ranging from 20 to 23 mm.

A research by Arekemase *et al.* (2013), revealed that crude ethanol, cold and hot water extract of *V. amygalina*, were effective against *K. pneumoniae, Bacillus subtilis,*

Pseudomonas aeruginosa, E. coli and *S. aureus* at a concentration range of 10 to 30 mg/ml with a zone of inhibition range of 8.2 to 24.4 mm. However, *K. pneumoniae* was inhibited at all concentrations of the ethanol and hot water extract but was only inhibited at 30 mg/ml by cold water extracts. Salami and Agu (2013), reported Ethanol extracts of *V. amygdalina* leaves to inhibit *E. coli, Salmonella* and *Shigella* species (Isolated from stool samples of diarrhea patients) at a concentration of 6.25, 12.50, 25, 50 and 100 g/mL with Salmonella species having the highest inhibition zone of 33 mm at a concentration of 100 g/mL.

Ezenobi *et al*. (2019) showed that ethanol extracts of the plant at 6.25, 12, 25, 50 and 100 mg/disc was effective against *E. coli, K. pnuemoniae* and *Proteus vulgaris*, however only *E. coli* showed susceptibility at 6.25 mg/disc. Jaryum *et al*. (2019), demonstrated that aqueous and ethanol extracts of *V. amygdalina* was able to hinder the progression of *E. coli, S. aureus, P. aeruginosa and proteus vulgaris.* The ethanol extract inhibited all organisms at 800 mg/mL and inhibited only *E. coli and S. aureus* at 400 mg/mL, While the aqueous extract was able to hinder the progression of *E. coli* and *P. aeruginosa* at 800 mg/mL and only *E. coli* at 400 mg/mL. lower concentrations of the extracts used (200, 100 and 50 mg/mL) had no activity on all test organisms.

2.7 Multi Drug Resistant Bacteria

Multi drug resistant (MDR) bacteria are bacteria which are insensitive to two different classes of antibacterial drugs (Jyoti *et al*., 2014). Although other types of MDR (resistant to multiple antifungal, antiviral, and antiparasitic drugs) exists, MDR bacteria is a great menace to public health (National Library of Medicine (NLM), 2022). MDR bacteria are able to ward off attack by antibiotics, which results in ineffective treatment causing persistence and spreading of infections (Jyoti *et al.,* 2014). Common MDR bacteria are;

Multidrug-resistant Gram negative rods (*Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*), Vancomycin-Resistant Enterococci (VRE), Methicillin-resistant *Staphylococcus aureus* (MRSA), Extended-spectrum β-lactamase (ESBLs) producing Gram-negative bacteria, *Salmonella* and Multi-drug-resistant tuberculosis (Boucher *et al*., 2009). Bacteria are able to resist antibiotics as a survival mechanism, they do so employing methods such as decreased cell wall permeability to antibiotics, altering target sites of antibiotic, removing antibiotics by efflux mechanisms and mutation as a response to stress (Stix, 2006; Li and Nikaido, 2009). In a process known as horizontal gene transfer, MDR bacteria are able to transfer DNA that codes for resistance to other bacteria, even those which are not closely related to them, so generations of antibiotics resistant bacteria are produced (Hussain, 2015). MDR can be curtailed by proper use of antibiotic and proper prescription of antibiotic by Clinicians (Wanda, 2018).

2.8 Enteric Organisms

Enteric organisms are bacteria that are usually present as normal flora of the intestinal tract of healthy humans and animals (Todar, 2020). Enteric bacteria are also called *Enterobacteriaceae*, a large family of Gram negative bacteria which belongs to the class *Gammaproteobacteria* and the order *Enterobacterales* (Adeolu *et al*., 2016). They consist of over a 100 species, with many of these species being totally harmless, some of them like *Escherichia coli, Salmomella, Shigella*, and *Klebsiella* species are capable of causing diseases and are commonly found in places with poor hygiene and poor sanitation. Pathogenic Enteric bacteria are also involved in most hospital acquired infections.

Most enteric bacteria are rod shaped, motile because they bear flagella (with few nonmotile ones), reduce nitrate to nitrite, and react negatively to grams' stain (Carroll *et al*.,

2015). They are facultative anaerobes and are capable of fermenting glucose (Todar, 2020). Several isolated strains of *Enterobacteriaceae*, have been found to be resistant to antibiotics, including carbapenems (CDC, 2015) Some enteric bacteria are usually present as contaminant in foods, as a result of poor food handling or transmission by vectors, which are mostly insects (Duedu *et al*., 2017).

2.8.1 *Serratia marcescens*

Serratia marcescens discovered in 1819, by an Italian pharmacist Bizio (Kahnna *et al*., 2013) is a Gram negative, rod shaped measuring 0.9-2 μm long and 0.5-0.8 μm in diameter, facultative anaerobe, motile, non-lactose fermenter with an ability to breakdown citrate to produce pyruvic acid (Aryal, 2019). The bacteria were previously regarded incapable of causing infections, now an opportunistic pathogen accountable for hospital acquired infections (mostly catheter-associated bacteremia) such as those associated with the urinary tract, wound, bones and endocarditis (Herra and Falkiner, 2017).

It's mode of transmission is through contaminated food and water, fomites and the handto-hand mode of health personnel (Ivanova *et al*., 2008). *S. marcescens* produces a red pigment called prodigiosin (Haddix and Shank, 2018) because of its ability to produce red colonies it was used as biomarkers (Kahnna *et al*., 2013). *S. marcescens* is susceptible to aminoglycosides, fluoroquinolones, and third generation cephalosporin. They are resistant to ampicillin, macrolides, and first-generation cephalosporin (Patel, 2021). *S. marcescens* is able to develop resistance to many beta- lactamase antibiotics this is due to

the ability of the organism to produce inducible beta- lactamase (IBL) and Extended spectrum beta lactamase (Simsek, 2021).

Serratia marcescens is implicated in hospital-associated infections, and diarrhea related to HIV/AIDS. It was observed that *Serratia* spp. may be found more normally in the stool samples of patients with diarrhea than in asymptomatic control children (Ochieng *et al.*, 2014). *Serratia* species, particularly *S. marcescens*, has greater affinity for the urinary tract (Bush and Vazquez -Pertejo, 2020).

2.8.2 *Salmonella typhi*

Salmonella typhi is a gram-negative, rod-shaped (with a diameter of about 0.7 to 1.5 μm, and a 2 to 5 μm length), they are non-spore-forming, facultative anaerobic bacterium (Fabrega and Vila, 2013). They are motile organisms, due to the presence of flagella (Ashurst *et al*., 2020). They are able to ferment carbohydrates with the production of gas (Owoade and Raji, 2019). *Salmonella* colonies are colorless on MacConkey agar, this is because it is incapable of fermenting lactose. However, on *Salmonella-Shigella* (SS) agar, xylose-lysine-deoxycholate (XLD) agar, and Hektoen enteric (HE) agar, it grows as colonies with black center, because of its ability to produce hydrogen sulphide. (Farbega and Villa, 2013). They are intracellular pathogens, which have the ability to encroach various cell types (LaRock *et al*., 2015). They can be found in the digestive tracts of humans and animals, in food and water contaminated by faeces of infected individuals or animals (Goldrick, 2003).

Salmonella is a major cause of diarrheal diseases and is one bacterium that accounts for a usual cause of food-borne illness (Anderson and Kendall, 2017). *Salmonella typhi* is the cause of typhoid fever, a type of enteric fever (Wain *et al*., 2015), which is characterized

by symptoms such as fever, stomach pain, constipation, headaches, and mild nausea.(Center for disease control, CDC, 2020).Common Complications of typhoid fever includes gastrointestinal hemorrhage, intestinal perforation, typhoid encephalopathy, and relapse (Ashurst *et al*., 2020).it can be prevented by a good personal and environmental hygiene and vaccination (Marathe *et al*., 2012). In most cases, Antibiotics are used in the treatment of typhoid fever, with Ciprofloxacin (Cipro), Azithromycin (Zithromax) and Ceftriaxone commonly used as routine treatment drugs (Mayo Clinic, 2020).

In the past, ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and streptomycin were used in the treatment of typhoid fever, these antibiotics are no longer used, because the bacteria have developed resistance to them (Zaki and Karande, 2011; CDC, 2019).

2.8.3 *Shigella* species

Named in 1897, after Kiyoshi Shiga, the man who first discovered it, *Shigella* is a Gram negative bacillus bacterium, which is not motile and does not form spore (Yabuuchi, 2002). They are facultative anaerobes, which do not ferment lactose, closely related to *E. coli* genetically (Dockrell *et al*., 2019) and are divided to four main serologic groups A to D (*Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei*) (Southwick, 2019). This bacterium is mainly transmitted through the feacal- oral route, promoted by poor hygiene and sanitation (Yang *et al*., 2005) and causes a kind of food poisoning known as Shigellosis (DerSarkissian, 2020).

The characteristics of Shigellosis includes, watery, blood-stained, or mucoid diarrhea, fever, stomach pains and nausea. Vomiting is often experienced in patients and seizures among young children. (Watkins and Appiah, 2019). There is no vaccine against

Shigellosis (DerSarkissian, 2020), antibiotics like fluoroquinolone, ciprofloxacin, azithromycin, or ceftriaxone are used in its treatment (Williams and Berkley, 2018), although symptoms usually resolve on its own after few days. However, in recent times Multidrug resistant *Shigella* species which are resistant to ampicillin, trimethoprimsulfamethoxazole, fluoroquinolones, azithromycin, and third- and fourth-generation cephalosporin has emerged, and occurs especially in South and East Asia (Watkins and Appiah, 2019). Shigellosis can be prevented through proper sanitation, maintenance of good personal hygiene and adhering strictly to precautionary measures pertaining to food and water.

2.8.4 *Klebsiella pnuemoniae*

K. pneunoniae is a non-motile, bacilli, gram negative bacterium, which is encapsulated, ferments lactose and a facultative anaerobic microbe (Ashurst and Dawson, 2021). It appears mucoid with a pink colouration on MacConkey agar and cream colouration on Nutrient Agar. Though present as normal flora of humans, it causes nosocomial infections and is also associated with ailments such as meningitis, pneumonia, bloodstream infections and wound infection (CDC, 2010). *Klebsiella* is one of the most resistant to antimicrobials amongst the family *Enterobacteriaecae* (Ahmed *et al*., 2004).

Klebsiella pnuemoniae is multidrug resistant, because of the possession of beta-lactamase or due to an acquired extended-spectrum beta-lactamase, infections arising from it is treated using antibiotics such as cephalosporin, aminoglycosides (CDC, 2010; Sanchez *et al*., 2013). Amikacin and Meropenem combination therapy is employed in the management of infections to urinary tract caused by Multidrug resistant *K. pneumoniae* (Yasin *et al*., 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted at the Federal University of Technology Minna, located in Bosso Local Government Area of Niger State, Nigeria. Niger State is situated in the North-Central geopolitical zone of Nigeria with Minna as its capital city. It lies on latitude 3.20° East and longitude 11.30° North and has a land mass of 86,000km^2. The State shares border with other states, which include the Federal Capital Territory (FCT) on the South-East, Zamfara (North), Kebbi (North-West), Kwara (South-West) and Kaduna (NorthEast). The State is composed of the Gwaris, Nupes and Hausas as the dominant ethnic groups. It has a human population of 3,950,249 and most of the inhabitants are civil servants, farmers and traders (Niger state government, 2021).

3.2 Collection and Processing of Plant Materials

Fresh leaves of *Vernonia amygdalina* were obtained from gardens in Maitumbi area of Bosso local government area of Niger state, Nigeria. The leaves were taken to the Department of Plant Biology, Federal University of Technology Minna, Nigeria for identification by a taxonomist. The voucher number was deposited in the herbarium laboratory of the Department of Plant Biology, Federal University of Technology Minna.

The leaves were cleaned with distilled water to eliminate dirt and unwanted particles. The leaves were air-dried for two weeks and pulverized into powder using an electric blender (Opara *et al*., 2012).

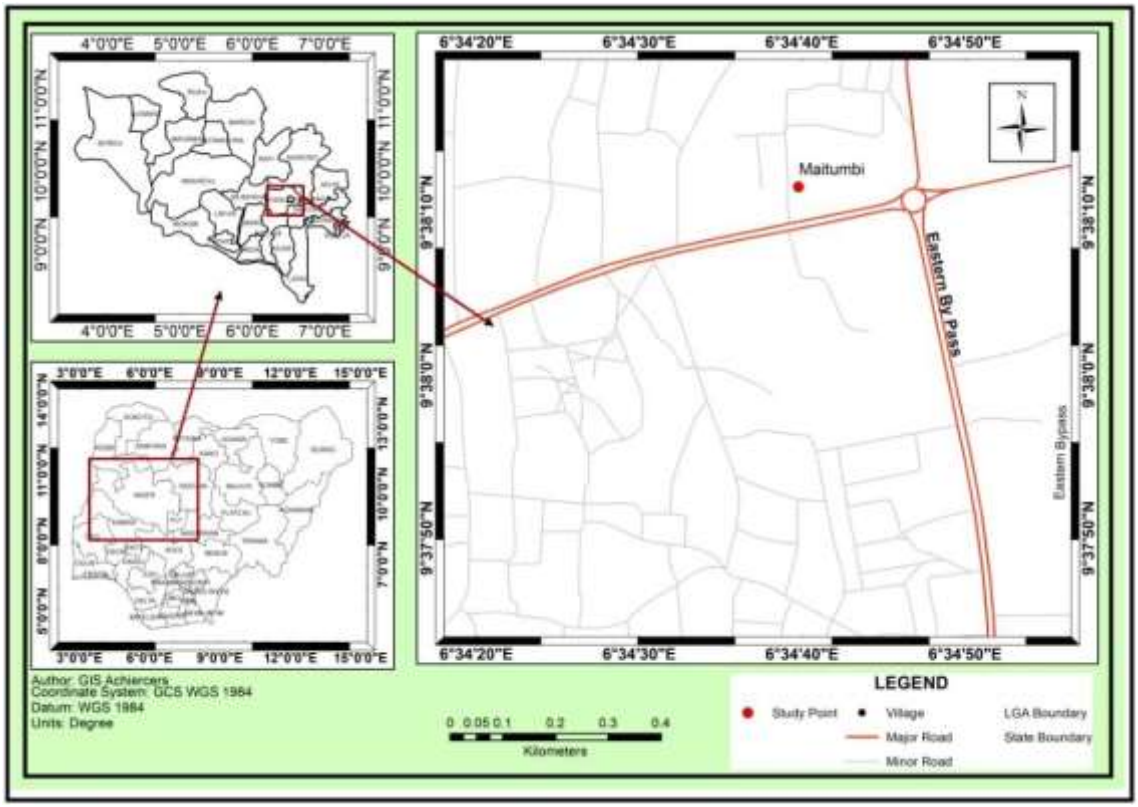

Figure 3.1: Map of Minna, Niger State Showing the Study Area.

Source; GIS Achivers, 2022.

3.3 Preparation of Extracts of *Vernonia amygdalina* Leaves

Five hundred grams (500 g) of powdered leaves each, was macerated in 2.3 liters of cold distilled water and ethanol respectively for 72 hours. Each mixture was filtered using Whattman No.1 (Germany). A rotary evaporator was used to concentrate the filtrate and resulting extract was stored in a refrigerator at 4 ℃, for further use (Azwanida, 2015).

3.4 Fractionation of the Most Active Crude Extract of *Vernonia amygdalina*

The ethanol extract was fractionated with solvents of increasing polarity (n-Hexane, Chloroform and Ethyl acetate), with a separating funnel. Each fraction was concentrated using a rotary evaporator and kept in a sterile bottle at 4 ℃, for further use (Zubairu *et al.,* 2019).

3.5 Source of Bacteria Isolates

The pure culture of the bacterial isolates used (*Klebsiella pneumonia, Serratia marcescens, Shigella* and *Salmonella typhi*) were obtained from Microbiology Laboratory, Federal University of Technology, Minna. The isolates were sub cultured on Muller Hinton agar plates for 24 hours at 37 ℃, they were later sub cultured and stored on agar slants at 4 ℃ until further use. Gram staining and biochemical tests such as the indole, catalase, citrate, urease, motility, methyl-red and sugar fermentation tests were carried out on each bacterium, as confirmatory test (Abalaka *et al.,* 2012).

3.5.1 Standardization of bacteria isolates

Five (5) milliliters of sterile normal saline was dispensed into a test tube and a sterile wire loop was utilized to inoculate the test bacteria into it, turbidity of the solution was adjusted when applicable and compared to 0.5 McFarland standard (Bayot and Bragg, 2020).

3.6 Antibiotic Susceptibility Testing

Kirby bauer disk diffusion method was used to carry out antibiotic susceptibility test on MHA. Suspension of the test organism was prepared in normal saline to the turbidity of 0.5 McFarland standard and streaked onto MHA. Single disc antimicrobial disc of AmoxicillinClavulanic acid (10 µg), Cefixime (5 µg), Ciprofloxacin (30 µg), Gentamicin (30 µg), Amoxil (10 µg), Chloramphenicol (30 µg), Ampicillin (10 µg) and Augmentin (10

µg) were aseptically applied on the surface of the inoculated plates. The plates were allowed to sit for a while at room temperature and then incubated at 37 °C for about 18-24 hours. The diameter of the zone of inhibition around the antibiotic discs was measured and interpreted in accordance with the breakpoints and criteria recommended by the Clinical Laboratory

Standards Institute (CLSI, 2018).

3.7 Antibacterial Activity Determination of Crude Extracts and Fractions of *V.amgdalina* on Bacterial Isolates

Agar-well diffusion method was used to determine the susceptibility of the test bacteria to the leaf extracts and fractions. Sterile Muller Hinton Agar (MHA) plates were prepared and 20 mls of it was dispensed in petri dishes. A sterile cork borer (10 mm) was utilized to bore wells on each MHA plate and base of each well was sealed, by filling with liquefied MHA. The plates were swabbed with 0.5 McFarland standard of test bacteria. 0.5 µL of 1500 mg/ml, 2000 mg/ml, 2500 mg/ml and 3000 mg/ml concentration of the extract were dispensed into each well using a pipette. The plates were left on the work bench, for diffusion of extract. After one (1) hour, the plates were incubated at 37 ℃ for 24 hours. Plates were observed and zones of inhibition around the well were recorded as in millimeters (Evbuomvan *et al*., 2018).

3.8 Phytochemical Analysis of Crude Extracts and Fractions

Phytochemical analysis for qualitative and quantitative detection of alkaloids, steroids, terpenoids, phenols, cardiac glycosides, flavonoid, tannins, and saponins, were performed on the crude extracts and fractions as described by Nduche *et al*. (2019).

3.9 Determination of Minimum Inhibitory Concentration (MIC)

The broth dilution method was employed. Nine (9) milliliters of peptone water, was dispensed into Eight (8) test tubes each. One (1) milliliter of extract of known concentration was dispensed into the first tube, after which a one-fold serial dilution was carried out. A sterile wire loop was used to inoculate test bacteria into each tube asides the test tube which served as control. The test-tubes were incubated at 37 ℃ for 24 hours.

The least dilution that showed no detectible turbidity was considered as minimum inhibitory concentration (Abalaka *et al.*, 2012).

3.9.1 Determination of minimum bactericidal concentration (MBC)

The tubes that displayed no observable turbidity after incubation during MIC, were subcultured on nutrient agar plates and incubated at 37 ℃ for 24 hours. The concentration that showed no observable growth after incubation was recorded as the minimum bactericidal concentration (Oyeleke and Manga, 2008).

3.10 Determination of the Toxicity of the Active Fractions of *Vernonia amygdalina*

3.10.1 Experimental animals

Sixty-four (64) Wister rats of both sexes weighing 102-110 kg, were obtained from National Veterinary Research Institute Vom (NVRI) Jos. The animals were housed in cages at the Animal House of the School of Life Sciences, Federal University of Technology, Minna, Niger State. The Wister rats were feed and allowed to acclimatized for a two weeks' period, preceding experiment.

3.10.2 Acute toxicity study

The acute toxicity study of ethyl acetate and n-hexane fractions of *V. amygdalina*, was carried out using the Lorke's method. This method comprises of two phases. In the first phase four (4) groups of Wister rats which comprised of three (3) rats per group were administered 10, 100 and 1000 mg/kg body weight of each extract, the control group were fed normal saline. Behavioral changes and mortality were looked out for in the Wister rats for a 24 hours' period. The second phase had four groups of rats, with one Wister rat in each group. Each group was administered extract of 1900, 2600 and 5000 mg/kg body weight. The animals were observed for 24 hours for behavioral changes and mortality (Enegide *et al*., 2013).

3.10.3 Sub-acute toxicity study

The sub-acute toxicity study of ethyl acetate and n-hexane fractions of *V. amygdalina*, was carried out by methods descried by Kharchoufa et al. (2020). Four (4) groups of four (4) animals each, were administered extract of 10, 300 and 600 mg/kg body weight orally for twenty-eight (28) days. During this period, the rats were observed daily for weakness, restlessness and signs of toxicity. At the end of the twenty-eight (28) days, the Wister rats were fasted overnight, anaesthetized with chloroform and sacrificed by cervical dislocation. Blood was obtained from the retro orbital sinus for hematological analysis, liver and kidney was also collected for histology test.

3.10.4 Haematological assessment

Experimental animals were analyzed using automated analyzer. Parameters such as complete blood count on the levels of White Blood Cell (WBC), Red Blood Cell (RBC),

Hemoglobin (HB), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Capsular Hemoglobin Concentration (MCHC), Platelets Count (PLC), Neutrophils and Lymphocytes, were measured using standard protocols (Imaga and Bamigbetan, 2013).

3.10.5 Biochemical assessment

Serum of the blood sample collected into the plain tubes was obtained by centrifuging at 3000 rpm for 10 minutes after blood sample was left undisturbed for 3 hours at 4 °C.

Using standard assay kits according to manufacturer's instructions, the concentrations of creatinine, urea, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin were estimated (Pariyani *et al*., 2015).

3.10.6 Histopathological assessment

A section of the liver and kidney tissues were fixed in 10 % formalin and implanted in paraffin wax. Thin sections measuring 4 to 5 microns in chunkiness were made using rotary microtome and stained with haematoxylin-eosin dye. Histological observations were made beneath light according to methods described by Abebe and Gebru (2015).

3.11 Data Analysis

The data generated was subjected to one-way analysis of variance (ANOVA) test. The means were compared using Duncan multiple range (DMRT) test at $p<0.05$ to determine the level of significance. All data were analysed with aid of the statistical package SPSS version 23.0.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Antibiotic susceptibility profile of the test bacteria

As shown in Table 4.1, amongst the various antibiotics used as control, all bacterial isolates were sensitive to Amoxicillin-Clavulanic acid. *K. pneumoniae*, *S. typhii* and *Shigella* species were resistant to Cefixime, Gentamicin, Amoxil, Chloramphenicol and Ampicillin. All other organisms except *Salmonella* species was sensitive to Ciprofloxacin, similarly, only *K. pneumonia*e was sensitive to Augmentin. *S. marcescens* was susceptible to all antibiotics except Augmentin, Ampicillin and Cefixime.

Table 4.1: Antibiotic susceptibility profile of the test bacteria

Test Bacteria	Antibiotics							
	AMC	CFM	CPX	CN	AML	CH	PN	AU
K. pneumoniae	S	R	S	S	R	R	R	S
S. typhi	S	R	R	R	R	R	R	R
***Shigella* species**	S	R	S	R	R	R	R	R
S. marcescens	S	R	S	S	S	S	R	R

KEYS; AMC= Amoxicillin-Clavulanic acid 10µg, CFM= Cefixime 5µg, CPX= Ciprofloxacin 30µg, CN= Gentamicin 30µg, AML= Amoxil 10µg, CH= Chloramphenicol 30µg, PN=Ampicillin 10µg, AU=Augmentin 10µg, S= Susceptible, R= Resistant.

4.1.2 Antibacterial activity of crude aqueous *V. amygdalina* leaf extract

The inhibition zone produced by crude aqueous extract of *V. amygdalina* ranged from 16.00 ± 2.00 to 25.00 ± 0.00 against *K. pneumoniae, S. typhi, Shigella* species and *S. marcescens* (Table 4.2). The highest inhibition zone was produced by 300 mg/ml of the extract, against *K. pneumoniae* while the least zone of inhibition recorded was against *S. marcescens* at 1500 mg/ml.

Table 4.2: Zones of inhibition by crude aqueous extracts of *V. amygdalina* leaf on test bacteria

Concentrations(mg/ml)	*K. pneumoniae*	*S. typhi*	*Shigella* species	*S. marcescens*
1500	$16.00^{a} \pm 2.00$	$19.00^{a} \pm 1.00$	$0.00^{a} \pm 0.00$	$17.50^{a} \pm 0.50$
2000	$16.00^{a} \pm 1.00$	$20.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$18.00^{a} \pm 0.00$
2500	$18.50^{ab} \pm 2.50$	$20.50^{a} \pm 0.50$	$16.50^{b} \pm 0.50$	$16.00^{a} \pm 1.00$
3000	$25.00^{b} \pm 0.00$	$24.00^{b} \pm 1.00$	$21.50^{c} \pm 1.50$	$20.50^{b} \pm 0.50$

Diameter zones of inhibition was measured in millimeters (mm). Values are in mean ± standard deviation of duplicate determinations. Means with the same letter in a column do not differ significantly, according to Duncan's Multiple Range Test (DMRT) at p= 0.05.

4.1.3 Antibacterial activity of crude ethanol *V. amygdalina* leaf extract

The antibacterial effect of crude ethanol extract of *V. amygdalina* against bacterial isolates were concentration dependent (Table 4.3). The 3000 mg/ml of *V. amygdalina* extract, produced the highest zones of inhibition (25.00±0.00, 30.00±0.00, 26.00±1.00 and 29.00±1.00 mm) against *K. pneumoniae, S. typhi, Shigella* species and *S. marcescens* respectively.

Table 4.3: Zones of inhibition by crude ethanol extracts of *V. amygdalina* leaf on test bacteria

Concentration mg/ml	*K. pneumoniae*	*S. typhi*	*Shigella* species	*S. marcescens*
1500	$18.50^{a} \pm 1.50$	$23.50^{a} \pm 1.50$	$17.50^{a} \pm 0.50$	$19.00^{a} \pm 1.00$
2000	$20.50^{a} \pm 0.50$	$26.00^{ab} \pm 1.00$	$20.00^{ab} \pm 0.00$	$23.00^{b} \pm 0.00$
2500	$22.00^{ab} \pm 1.00$	$28.00^{bc} \pm 0.00$	$22.50^{b} \pm 1.50$	$25.00^{b} \pm 0.00$
3000	$25.00^{b} \pm 0.00$	$30.00^{c} \pm 0.00$	$26.00^{c} \pm 1.00$	$29.00^{c} \pm 1.00$

Diameter zones of inhibition was measured in millimeters (mm). Values are in mean ± standard deviation of duplicate determinations. Means with the same letter in a column do not differ significantly, according to Duncan's Multiple Range Test (DMRT) at p= 0.05

4.1.4 Antibacterial activity of *V. amygdalina* leaf fractions

Table (4.4a to c) indicates that n- hexane, chloroform and ethyl acetate fractions of *V. amygdalina* ethanol extracts, had a significant antibacterial activity on the bacterial isolates with varying zones of inhibition at concentrations range of 100 to 800 mg/ml.

Table 4.4a: Zones of inhibition by n-hexane fraction of *V. amygdalina* on test bacteria

Concentration(mg/ml)	*K. pneumoniae*	*S. typhi*	*Shigella* species	*S. marcescens*
100	$20.00^{a} \pm 1.00$	$19.00^{a} \pm 2.00$	$20.00^{a} \pm 2.00$	$20.00^{a} \pm 0.00$
200	$23.00^{ab} \pm 1.00$	$20.00^{a} \pm 2.00$	$22.00^{ab} \pm 1.00$	$21.00^{ab} \pm 2.00$
400	$25.00^{b} \pm 2.00$	$23.00^{a} \pm 2.00$	$25.00^{ab} \pm 0.00$	$25.00^{bc} \pm 1.00$
800	$27.00^{b} \pm 0.00$	$26.00^{b} \pm 1.00$	$26.50^{b} \pm 1.50$	$28.00^{c} \pm 1.00$

Diameter zones of inhibition was measured in millimeters (mm). Values are in mean ± standard deviation of duplicate determinations. Means with the same letter in a column do not differ significantly, according to Duncan's Multiple Range Test (DMRT) at p= 0.05

Table 4.4b: Zones of inhibition by chloroform fraction of *V. amygdalina* on test bacteria

Concentration(mg/ml)	*K. pneumoniae*	*S. typhi*	*Shigella* species	*S. marcescens*
100	$20.00^{a} \pm 2.00$	$18.00^{a} \pm 1.00$	$19.00^{a} \pm 1.00$	$20.00^{a} \pm 0.00$
200	$25.00^{a} \pm 2.00$	$23.00^{ab} \pm 2.00$	$25.00^{b} \pm 0.00$	$22.00^{ab} \pm 2.00$
400	$27.00^{a} \pm 3.00$	$28.00^{b} \pm 2.00$	$27.00^{b} \pm 1.00$	$25.00^{b} \pm 1.00$
800	$25.00^{a} \pm 0.00$	$26.50^{b} \pm 1.00$	$26.50^{b} \pm 1.50$	$26.00^{b} \pm 0.00$

Diameter zones of inhibition was measured in millimeters (mm). Values are in mean ± standard deviation of duplicate determinations. Means with the same letter in a column do not differ significantly, according to Duncan's Multiple Range Test (DMRT) at p= 0.05

Table 4.4c: Zones of inhibition by ethyl-acetate fraction of *V. amygdalina* on test bacteria

Concentration(mg/ml)	*K. pneumoniae*	*S. typhi*	*Shigella* species	*S. marcescens*
100	$20.00^{a} \pm 0.00$	$25.00^{a} \pm 1.00$	$20.00^{a} \pm 2.00$	$25.00^{a} \pm 0.00$
200	$22.00^{a} \pm 1.00$	$28.00^{a} \pm 0.00$	$25.00^{a} \pm 1.00$	$24.00^{a} \pm 2.00$
400	$25.00^{b} \pm 0.00$	$28.00^{a} \pm 3.00$	$25.00^{a} \pm 3.00$	$28.00^{a} \pm 2.00$
800	$27.00^{b} \pm 1.00$	$27.00^{a} \pm 0.00$	$27.50^{a} \pm 1.50$	$29.00^{a} \pm 1.00$

Diameter zones of inhibition was measured in millimeters (mm). Values are in mean ± standard deviation of duplicate determinations. Means with the same letter in a column do not differ significantly, according to Duncan's Multiple Range Test (DMRT) at p= 0.05

4.1.5 Qualitative and quantitative phytochemical components of *V. amygdalina* leaf extracts

Phytochemical screening revealed the presence of phenols, flavonoids, tannins, alkaloids and saponins were existent in all crude extracts and fractions of *V. amygdalina*. Steroid,

terpenoids and cardiac glycosides were not present in the crude aqueous and n-hexane fraction respectively (Tables 4.5a).

Ethyl acetate fraction of the crude ethanol extract of *V. amygdalina* had more concentration of phenols (207. 36±1.56) and flavonoids (4.01±0.11). Saponins (141.37±0.64) and Tannins (109.48±0.72) was more in n-hexane fraction, while Alkaloids (29.68±1.07) was highest in the crude ethanol extracts (Table 4.5b).

Table 4.5a: Qualitative phytochemical components of *V. amygdalina* leaf extracts and fractions

Phytochemical	CEE	CAE	EAF	CF	NHF
Phenols	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Steroids	+	-	+	+	+
Cardiac glycosides	+	+	+	+	-
Terpenoids	+	-	+	+	+

KEYS; CEE= crude ethanol extract, CAE= crude aqueous extract, NHF= n-hexane fraction, CF= chloroform fraction, EAF= ethyl acetate fraction +=Present, -=Absent.

Table 4.5b: Quantitative phytochemical components of *V. amygdalina* extracts and fractions

Phytochemicals (mg/g)	Extracts				
	CEE	**CAE**	**EAF**	**CF**	**NHF**
Phenols	184.86^{d}±0.48	103.47^{a}±1.32	207.36^{e}± 1.56	158.65^{c}± 0.83	136.77^{b}± 0.56
Flavonoids	3.65^{ab} ±0.05	3.95^{b} ±0.06	4.01^{b} ± 0.11	3.53^{ab}± 0.23	3.22^{a} ± 0.22
Tannins	87.43^{d} ±0.26	39.51^{b} ±0.37	32.66^{a} ± 0.14	77.11^{c}± 0.22	109.48^{e}±0.72
Alkaloid	29.68^{d}± 1.07	25.37^{c} ±0.46	17.63^{b} ± 1.29	12.63^{a}± 0.28	20.14^{b}± 0.41
Saponins	122.46^{c}± 0.63	90.04^{a} ±0.29	125.48^{d}±0.55	119.58^{b}±1.16	141.37^{e}± 0.64
Steroids	ND	ND	ND	ND	ND
Cardiac glycosides	ND	ND	ND	ND	ND
Terpenoids	ND	ND	ND	ND	ND

KEYS; CEE= crude ethanol extract, CAE= crude aqueous extract, NHF= n-hexane fraction, CF= chloroform fraction, EAF= ethyl acetate fraction +=Present, -=Absent, ND= not determined. Values are in mean ± standard deviation of duplicate determinations. Means with the same letter in a column do not differ significantly, according to Duncan's Multiple Range Test (DMRT) at p= 0.05

4.1.6 Minimum inhibitory concentration (MIC) of *V. amygdalina* leaf extracts

The crude and fractionated extracts of *V. amygdalina* had different MIC on all bacterial isolates at different concentrations (Table 4.6). n-hexane fraction had the least MIC of 6.3 mg/ml (*K. pneumoniae*) while aqueous extract had the highest MIC of 750 mg/ml recorded against all bacteria. Similarly, the crude ethanol extract, chloroform fraction and ethyl acetate fractions had MICs of 23.50, 100 and 25 mg/ml respectively.

Table 4.6: Minimum inhibitory concentration (MIC) of *V. amygdalina* leaf extract in mg/ml

Bacteria	CEE	CAE	NHF	CF	EAF
K. pneumoniae	156.00	750.00	6.30	400.00	400.00
S. typhi	187.50	750.00	100.00	100.00	200.00
***Shigella* species**	46.90	750.00	12.50	100.00	25.00
S. marcescens	23.50	750.00	50.00	400.00	100.00

KEYS. CEE= crude ethanol extract, CAE= crude aqueous extract, NHF= n-hexane fraction, CF= chloroform fraction, EAF= ethyl acetate fraction

4.1.7 Minimum bactericidal concentration (MBC) of *V. amygdalina* leaf extracts

The MBC of the crude and fractionated extracts of *V. amygdalina* were as shown in Table 4.7. n-hexane fraction had the lowest MBC value against *K. pnuemoniae* (12.5 mg/ml) while aqueous extract had the highest MBC of 1500 mg/ml for all test bacteria. Similarly, the crude ethanol extract, chloroform fraction and ethyl acetate fractions had MBCs of 46.90, 200 and 50 mg/ml respectively.

Table 4.7: Minimum bactericidal concentration (MBC) of *V. amygdalina* leaf extract in mg/ml

Bacteria	CEE	CAE	NHF	CF	EAF
K. pneumoniae	312.5	1500.00	12.50	800.00	800.00
S. typhi	750.00	1500.00	200.00	200.00	400.00
***Shigella* species**	93.80	1500.00	25.00	200.00	50.00
S. marcescens	46.90	1500.00	100.00	800.00	200.00

KEYS. CEE= crude ethanol extract, CAE= crude aqueous extract, NHF= n-hexane fraction, CF= chloroform fraction, EAF= ethyl acetate fraction

4.1.8 Acute toxicity of *Vernonia amygdalina* leaf fractions

At the end of the 24 hours' period of both phase I and II of the acute toxicity, no mortality, weakness, restlessness and any other sign of toxicity was observed in experimental Wister rats.

4.1.9 Haematological assessment

Result of groups exposed to n-hexane faction of *V. amgdalina* shows that the levels of HB compared favorably to that of the control. However, there was a decrease ($p<0.05$) in MCHC, PLC, Neutrophils and Lymphocytes levels in rats administered with n-hexane fraction, when compared to the control. There was an increase ($p<0.05$) in the Mean capsular volume, Mean Corpuscles Haemoglobin, Red Blood Counts, Basophils and Eosinophils levels all treated groups, when compared to the control group. Similarly, an increase in total white blood cell count was recorded in the group administered with 10 mg/Kgbw of n-hexane fraction (Table 4.8a)

Wister rat groups exposed to ethyl acetate faction of *V. amgdalina* shows no significant difference in the haemoglobin, pack cell volume and total white blood counts of the dosed group and the control group. However, there was a substantial increase ($p<0.05$) in the Mean capsular volume, Mean Corpuscles Haemoglobin, Red Blood Counts (at 600 mg/kgbw), Basophils and Eosinophils levels in rats administered with ethyl acetate fraction, when likened to the control group. A substantial decrease ($p<0.05$) was recorded in MCHC, PLC, Neutrophils and Lymphocytes levels in all treatment groups, when compared to the control (Table 4.8b).

Table 4.8a: Effect of n-hexane Fraction of *V. amygdalina* on haematological parameters

Parameters	Dose(mg/Kgbw)			
	10	**300**	**600**	**Control**
HB(g/dL)	$12.20^{a}\pm0.60$	$11.65^{a}\pm0.75$	$13.15^{a}\pm0.65$	$12.25^{a}\pm0.35$
PVC(%)	$40.00^{a}\pm1.00$	$41.00^{a}\pm1.00$	$44.00^{b}\pm1.00$	$39.00^{a}\pm1.00$
MCV(fi)	$46.00^{b}\pm1.00$	$45.50^{b}\pm0.50$	$44.00^{b}\pm1.00$	$41.00^{a}\pm1.00$
MCH(pg)	$16.50^{ab}\pm0.50$	$14.50^{a}\pm0.50$	$18.50^{b}\pm0.50$	$14.50^{a}\pm0.50$
MCHC(g/dL)	$42.00^{b}\pm1.00$	$39.00^{ab}\pm1.00$	$37.00^{a}\pm1.00$	$47.00^{c}\pm1.00$
RBC(10^{12}/L)	$6.65^{b}\pm0.25$	$7.05^{b}\pm0.25$	$8.05^{b}\pm0.15$	$5.45^{a}\pm0.25$
PLC(10^{6}/L)	$139.50^{b}\pm1.50$	$129.00^{a}\pm1.00$	$130.50^{a}\pm0.50$	$160.50^{c}\pm1.50$
TWBC(10^{12}/L)	$7.20^{c}\pm0.30$	$5.70^{ab}\pm0.10$	$4.40^{a}\pm0.30$	$5.20^{ab}\pm0.30$
N(%)	$39.00^{c}\pm1.00$	$46.00^{b}\pm1.00$	$50.50^{a}\pm1.50$	$38.00^{c}\pm1.00$
L(%)	$29.50^{a}\pm0.50$	$35.00^{bc}\pm1.00$	$32.50^{ab}\pm0.50$	$38.00^{c}\pm1.00$
E(%)	$30.50^{ab}\pm0.50$	$35.00^{c}\pm1.00$	$33.00^{bc}\pm1.00$	$29.00^{a}\pm1.00$
B(%)	$30.50^{ab}\pm0.50$	$35.00^{c}\pm1.00$	$33.00^{bc}\pm1.00$	$29.00^{a}\pm1.00$

HB= haemoglobin, PCV= Packed Cell Volume, MCV=Mean Corpuscles Volume, MCH= Mean Corpuscles Haemoglobin, MCHC= Mean Corpuscles Haemoglobin Concentration, RBC= Red Blood Count, PLC= Platelet Count, TWBC= Total White Blood Cell Count, N= Neutrophils, L= Lymphocytes, B= Basophils, E=Eosinophils. Values are in mean ± standard deviation of duplicate determinations. Means with the same letter in a column do not differ significantly, according to Duncan's Multiple Range Test (DMRT) at p= 0.05

Table 4.8b: Effect of ethyl acetate fraction of *V. amygdalina* on haematological parameters

Parameters	Dose(mg/Kgbw)			
	10	**300**	**600**	**Control**
HB(g/dL)	$13.60^{a}\pm1.10$	$13.05^{a}\pm0.75$	$13.30^{a}\pm0.40$	$12.25^{a}\pm0.35$
PVC(%)	$40.50^{a}\pm0.50$	$39.50^{a}\pm0.50$	$41.50^{ab}\pm0.50$	$39.00^{a}\pm1.00$
MCV(fi)	$45.00^{b}\pm0.50$	$47.50^{b}\pm0.50$	$45.50^{b}\pm.50$	$41.00^{a}\pm1.00$

MCH(pg)	22.00^{c}±1.00	18.00^{b}±1.00	17.50^{ab}±0.50	14.50^{a}±0.50
MCHC(g/dL)	39.50^{a}±0.50	42.00^{a}±1.00	46.00^{b}±1.00	47.00^{b}±1.00
RBC(10^{12}/L)	6.00^{a}±0.10	5.70^{a}±0.10	7.85^{b}±0.25	5.45^{a}±0.25
PLC(10^{6}/L)	144.00^{a}±1.00	147.50^{a}±0.50	155.00^{c}±1.00	160.50^{b}±1.50
TWBC(10^{12}/L)	6.00^{a}±0.20	5.80^{a}±0.10	5.30^{a}±0.20	5.20^{a}±0.30
N(%)	35.00^{c}±0.00	31.00^{b}±1.00	27.00^{a}±1.00	38.00^{c}±1.00
L(%)	30.00^{a}±1.00	33.00^{bc}±1.00	36.00^{ab}±1.00	38.00^{c}±1.00
E(%)	34.00^{c}±1.00	33.00^{bc}±1.00	29.50^{ab}±0.50	29.00^{a}±1.00
B(%)	34.00^{c}±1.00	33.00^{bc}±1.00	29.50^{ab}±0.50	29.00^{a}±1.00

HB= haemoglobin, PCV= Packed Cell Volume, MCV=Mean Corpuscles Volume, MCH= Mean Corpuscles Haemoglobin, MCHC= Mean Corpuscles Haemoglobin Concentration, RBC= Red Blood Count, PLC= Platelet Count, TWBC= Total White Blood Cell Count, N= Neutrophils, L= Lymphocytes, B= Basophils, E=Eosinophils. Values are in mean ± standard deviation of duplicate determinations. Means with the same letter in a column do not differ significantly, according to Duncan's Multiple Range Test (DMRT) at p= 0.05

4.1.10 Biochemical assessment

Results of groups administered n-hexane faction of *V. amgdalina* shows an increase in the total protein and albumin levels at 10 mg/Kgbw while High Density Lipoprotein (HDL), increased in rats administered 10 and 300 mg/Kgbw of the fraction. There was a decrease ($p<0.05$) in the Aspartate transaminase (AST), Alanine amino transferase (ALT), Total protein, Creatinine, Triglyceride and low density lipoprotein (LDL) when compared with the control. Similarly, there was a decrease in serum Alkaline phosphate (ALP), in the group administered with 600 mg/Kgbw when compared to control ALP, but the ALP of the control group compared favorably Wister rats administered with 10 and 300 mg/Kgbw of n-hexane fraction (Table 4.9a).

Groups exposed to ethyl acetate faction of *V. amgdalina*, shows that there was an increase ($p<0.05$) in the total protein, albumin and cholesterol levels in Wister rats administered

with 600, 300 and 600 mg/Kgbw. while High Density Lipoprotein (HDL), increased in Wister rats administered 300 and 600 mg/Kgbw of the fraction. There was a significant decrease ($p<0.05$) in the Aspartate transaminase (AST), Alanine amino transferase (ALT), Alkaline phosphate (ALP), Total protein, Creatinine, Triglyceride and low density lipoprotein (LDL) when compared with the control group (Table 4.9b).

Table 4.9a: Effect of n- hexane fraction of *V. amygdalina* on the biochemical parameters of Wister rats.

Parameters	Dose(mg/Kgbw)			
	10	**300**	**600**	**Control**
AST(UI/min)	$18.42^{b} \pm 0.48$	$17.53^{ab} \pm 0.70$	$15.78^{a} \pm 0.11$	$21.14^{c} \pm 0.60$
ALT(UI/min)	$22.73^{b} \pm 0.91$	$17.32^{a} \pm 0.58$	$18.14^{a} \pm 0.81$	$27.29^{c} \pm 0.37$
ALP(UI/min)	$67.71^{b} \pm 0.42$	$65.17^{b} \pm 0.79$	$55.03^{a} \pm 0.91$	$66.74^{b} \pm 0.43$
Total protein(g/L)	$24.84^{c} \pm 1.10$	$16.02^{a} \pm 0.19$	$19.83^{b} \pm 0.72$	$18.83^{b} \pm 0.91$
Albumin(g/L)	$13.95^{c} \pm 0.84$	$10.89^{b} \pm 0.36$	$8.15^{a} \pm 0.79$	$9.19^{b} \pm 0.25$
Creatinine(mg/dl)	$4.82^{ab} \pm 0.07$	$4.17^{a} \pm 0.19$	$4.73^{ab} \pm 0.23$	$5.35^{b} \pm 0.29$
Urea (mg/dl)	$40.64^{b} \pm 1.20$	$40.77^{ab} \pm 0.93$	$36.37^{a} \pm 0.53$	$39.61^{b} \pm 0.68$
cholesterol(mmol/L)	$131.78^{c} \pm 1.13$	$126.13^{ab} \pm 1.20$	$123.25^{a} \pm 0.51$	$126.75^{bc} \pm 1.08$
Triglyceride(mmol/L)	$135.04^{a} \pm 0.11$	$144.48^{b} \pm 1.46$	$132.41^{a} \pm 0.48$	$164.99^{c} \pm 0.96$
HDL-C(mmol/L)	$90.59^{c} \pm 0.85$	$98.38^{d} \pm 0.75$	$85.57^{b} \pm 0.76$	$78.58^{a} \pm 0.80$
LDL-C(mmol/L)	$59.63^{c} \pm 0.70$	$52.36^{b} \pm 1.62$	$44.87^{a} \pm 0.96$	$75.79^{d} \pm 1.06$

KEYS; AST= aspartate transaminase, ALT= alanine amino transferase, ALP= alkaline phosphate, HDL-C=high density lipoprotein, LDL-C= low density lipoprotein. Values are in mean ± standard deviation of duplicate determinations. Means with the same letter in a column do not differ significantly, according to Duncan's Multiple Range Test (DMRT) at p= 0.05

Table 4.9b: Effect of ethyl acetate fraction of *V. amygdalina* on the biochemical parameters of Wister rats

Parameters	Dose(mg/Kgbw)			
	10	**300**	**600**	**Control**
AST(UI/min)	$17.40^{b} \pm 0.45$	$15.30^{b} \pm 0.39$	$11.61^{a} \pm 0.96$	$21.14^{c} \pm 0.60$
ALT(UI/min)	$20.83^{a} \pm 0.72$	$18.13^{a} \pm 0.81$	$18.76^{a} \pm 0.78$	$27.29^{b} \pm 0.37$

ALP(UI/min)	$59.69^{b} \pm 0.43$	$64.93^{c} \pm 0.92$	$54.08^{a} \pm 0.86$	$66.74^{c} \pm 0.43$
Total protein(g/L)	$13.50^{a} \pm 0.56$	$20.10^{bc} \pm 0.44$	$22.80^{c} \pm 1.06$	$18.83^{b} \pm 0.91$
Albumin(g/L)	$7.88^{a} \pm 0.98$	$12.94^{c} \pm 0.80$	$9.60^{b} \pm 0.54$	$9.19^{b} \pm 0.25$
creatinine(mg/dl)	$3.99^{a} \pm 0.12$	$4.43^{ab} \pm 0.25$	$5.12^{b} \pm 0.14$	$5.35^{c} \pm 0.29$
Urea (mg/dl)	$44.33^{b} \pm 0.81$	$41.82^{ab} \pm 0.49$	$39.61^{a} \pm 0.68$	$40.61^{a} \pm 1.20$
cholesterol(mmol/L)	$119.18^{a} \pm 1.25$	$130.29^{b} \pm 0.55$	$135.94^{c} \pm 0.91$	$126.75^{b} \pm 1.08$
Triglyceride(mmol/L)	$129.88^{a} \pm 0.17$	$148.47^{b} \pm 0.58$	$152.44^{c} \pm 1.53$	$164.99^{d} \pm 0.96$
HDL-C(mmol/L)	$81.15^{a} \pm 0.20$	$89.09^{b} \pm 1.06$	$95.53^{c} \pm 1.21$	$78.58^{a} \pm 0.80$
LDL-C(mmol/L)	$56.94^{a} \pm 0.11$	$62.79^{b} \pm 0.86$	$55.65^{a} \pm 1.35$	$75.79^{c} \pm 1.06$

KEYS; AST= aspartate transaminase, ALT= alanine amino transferase, ALP= alkaline phosphate, HDL-C=high density lipoprotein, LDL-C= low density lipoprotein. Values are in mean ± standard deviation of duplicate determinations. Means with the same letter in a column do not differ significantly, according to Duncan's Multiple Range Test (DMRT) at p= 0.05

4.1.11 Histopathological assessment

The result analysis for the liver of groups dosed with fractions of n-haxane and ethyl acetate shows hepatic tissue with preserved architecture composed of cords of normal hepatocytes, normal portal tracts and central vein. However 600 mg/kg body weight of ethyl-acetate and n- hexane exhibited mild portal lymphocytic inflammation and mild cytoplasmic vacuolations respectively. Similarly the kidney dosed with fractions of nhaxane and ethyl acetate showed no features of acute or chronic damage, with all renal tissue architecture preserved, as shown in Plates I and II respectively.

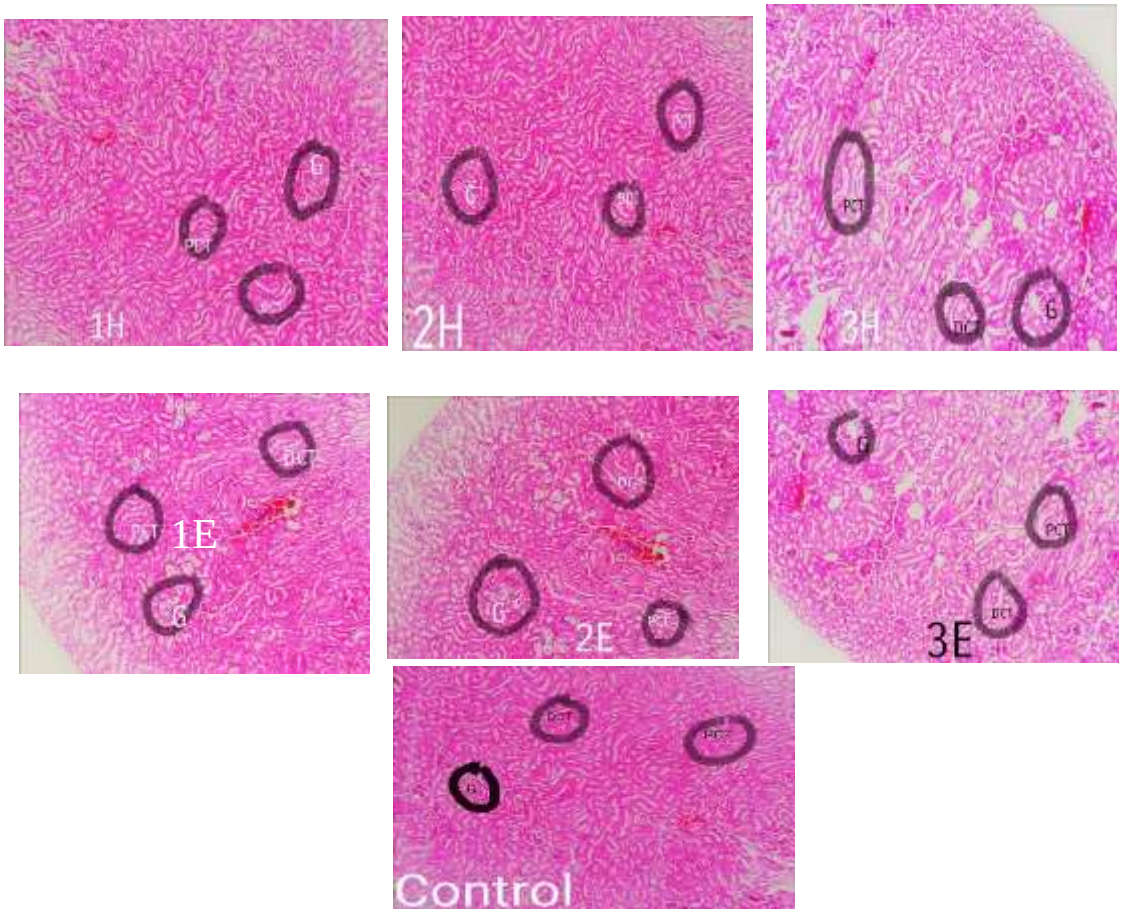

Plate I: micrograph of kidney sections from an Olympus CX21 Microscope showing Control, 1E, 2E and 3E shows 10, 300 and 600mg/kg bw of ethyl-acetate fraction respectively. 1H, 2H and 3H shows shows10, 300 and 600mg/kg bw of n- hexane respectively. G=Glomerulus, PCT= Proximal convoluted tubule, DCT= Distal convoluted tubule.

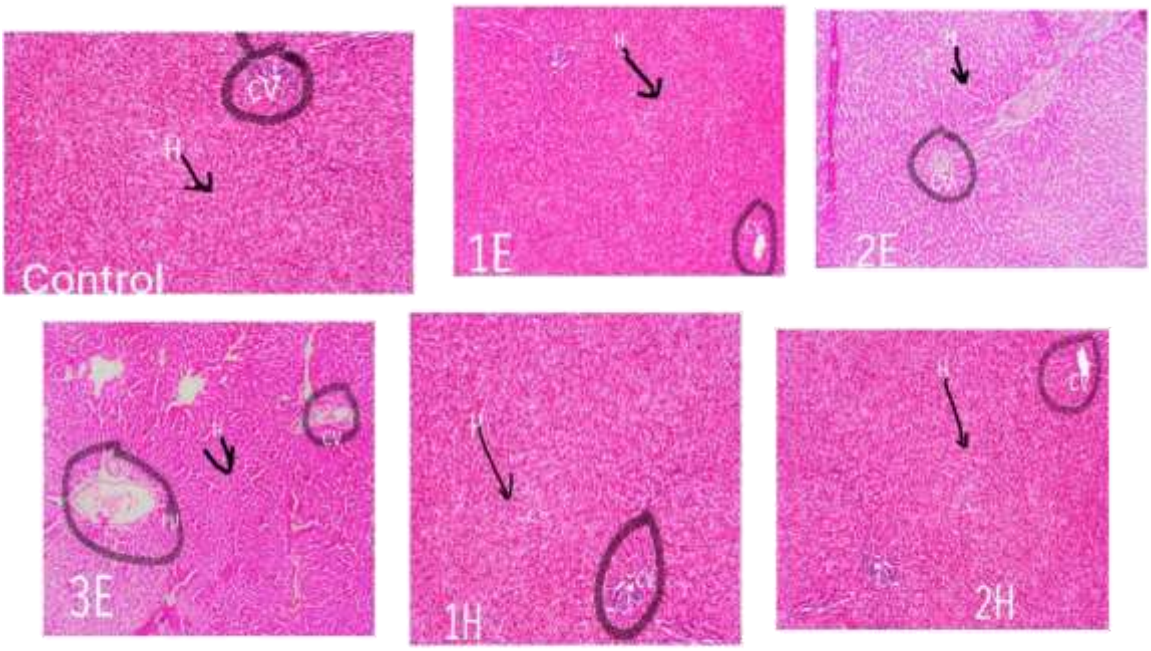

PLATE II: micrograph of liver sections from an Olympus CX21 Microscope showing Control, 1E, 2E and 3E shows 10, 300 and 600mg/kg bw of ethyl-acetate fraction respectively. 1H, 2H and 3H shows shows10, 300 and 600mg/kg bw of n- hexane respectively. CV= Central Vein, H=Hepatocytes.

4.2 Discussion

4.2.1 Antibiotic susceptibility profile of the test bacteria

All bacterial isolates in this study were multi-drug resistant. Resistance to antibiotics by bacteria are developed at a faster pace than the development of new drugs (Basak *et al*., 2016). The bacterial isolates might have become multi-drug resistant, due to over usage and abuse to the use of antibiotics as most people do not use the antibiotics with a doctors or tend to use it unnecessarily. These bacteria acquire resistance as a survival mechanism and are able to attain multi- drug resistance by decreasing the permeability of the antibiotics into their cell walls, mutation as a response to stress and modifying the antibiotic target sites (Li and Nikaido, 2009). This result is similar to that of Owoade and Raji (2019) and Ezenobi *et al.* (2019), where *K. pnuemoniae* and *S. typhi* were resistant to Ampicillin. Similarly, it is also in line with the study of Ogundare (2011) where *Shigella dysentriae* and *S. typhi* were resistant to Augmentin.

The increasing resistance of bacteria to antibiotics has led to the search for alternative antibacterial agents for the treatment of bacterial infections (Anyanwu and Okoye, 2017). Plant extracts are being considered as an alternative to antibiotics development, due to its availability, cost effectiveness, and low toxicity. The study was aimed at evaluating the antibacterial activity of *Vernonia amygdalina* leaf extracts on multidrug resistant enteric bacteria**.**

4.2.2 Antibacterial activity of crude aqueous *V. amygdalina* leaf extract

The result of this study indicated that crude aqueous *V. amygdalina* leaf extact, had antibacterial activity on all test bacteria (*Klebsiella pneumoniae*, *Salmonella typhi*,

Shigella species and *Serratia marcescens*) in a concentration dependent fashion. This result supports the findings of Owoade and Raji, (2019), however, it disagrees with that of Alo *et al*. (2012). This could be as a result of variation in the extract concentration tested as well as bacterial strains used in these studies. The present study investigated *V. amygdalina* at 1500 to 3000 mg/ml which had antibacterial effect on *S. typhi* and *K. pneumoniae,* as against Alo *et al*. (2012) where the highest concentration investigated was 500 mg/ml and did not have antibacterial effect on *S. typhi* and *K. pneumoniae.*

4.2.3 Antibacterial activity of crude ethanol *V. amygdalina* leaf extract The crude ethanolic extract of *V. amygdalina,* was able to inhibit all test bacteria with zones of inhibition ranging from 17.5 to 30 mm. This finding is similar to that of Salami and Agu, (2013), who reported the antibacterial activity of ethanol extract of *V. amygdalina* on *Shigella* species. However, it is in contrast with the study of Alo *et al*. (2012) and Ogundare (2011), where ethanol extract of *V. amygdalina*, could not inhibit *K. pnuemoniae* and *S. typhi* respectively. The variations in these studies may be due to the climatic conditions in which the plants were grown, which may account for a difference in the bioactive components in the plant.

4.2.4 Antibacterial activity of *V. amygdalina* leaf fractions

Chloroform, n-hexane and ethyl-acetate fractions produced zones of inhibition ranging from 18.00± 1.00 to 29.00 ± 1.00 at 100 to 800 mg/ml against all test bacteria. This is in agreement with the study of Onifade and Agunloye (2019), where ethyl-acetate fraction had antibacterial activity against *K. pneumoniae.* However, it is not in line with the findings of Zubairu *et al*. (2019) and Abubakar *et al*. (2011), where n- hexane and chloroform fractions did not inhibit the growth of *Shigella dysentriae* and *Salmonella*

*typh*i. The discrepancies in these studies might be as a result of the solvent used in crude extraction prior to fractionation (Zubairu *et al*. 2019) alongside technique used in fractionation and antibacterial assay.

4.2.5 Qualitative and quantitative phytochemical components of *V. amygdalina* extracts

The presence of phenols, flavonoids, tannins, alkaloids, saponins, steroids, cardiac glycosides and terpenoids, in the crude extracts of *V. amygdalina* in this study, agrees with the findings Ogundare (2011). However, this report is not in line with Evbuomwen *et al*. (2018), who did not report tannins in ethanol extract of *V. amygdalina*. This present study did not detect steroids and terpenoids, this is in line with the study of Yusmazura *et al*. (2016). However, it differs with the study of Nata'ala *et al*. (2019), where tannins and saponins were not detected. The presence of these phytochemicals revels the efficacy of *V. amygdalina* against enteric bacteria. The disparities in these studies might be as a result of the season in which plant was collected and the geographical variation in the nutritional composition of soil in which the plants were grown. It is worthy of note, that the antibacterial activity exhibited by *V. amygdalina,* may be as a result of the phytochemical components, which it possesses.

The antibacterial activity of *V. amygdalina,* was found to be reliant on the solvent used, in its extraction and also dependent on the concentration of the extract used. Ethanol extract was found to have more antibacterial activity, compared to the aqueous extract. This is in line with the study of Evbuomwan *et al*. (2018), this might be because, ethanol was able to extract more bioactive components from of *V. amygdalina* leaves, compared to the aqueous. The zones of inhibition varied for all extracts and fractions, on all test

bacteria, this may be due to the difference in the chemical composition of the cell wall of each bacterium, which may facilitate slow or fast penetration of the extracts into the cells of that organism.

4.2.6 Minimum inhibitory concentration (MIC) of *V. amygdalina* leaf extracts

Lower Minimum Inhibitory Concentration (MIC) implies high potency of extracts or fractions, as it suggests the minimum concentration required to inhibit the proliferation of the test bacteria. The MIC reported in this study for crude ethanol extract of *V. amygdalina* was lower than that of Alo *et al*. (2012). However, the values were higher compared to the study of Arekemase *et al*. (2013).

4.2.7 Minimum bactericidal concentration (MBC) of *V. amygdalina* leaf extracts

Minimum Bactericidal Concentration (MBC) range (46.9 to 1500 mg/ml) recorded in this study, is in agreement with the findings of Jaryum *et al*. (2019). This finding therefore suggests that extracts of *V. amygdalina* is suitable in tackling diseases caused by the test bacteria.

4.2.8 Acute toxicity of *V. amygdalina* leaf fractions

The absence of mortality, weakness, restlessness and any other sign of toxicity observed in experimental rats at the end of acute toxicity may be an indication that ethyl acetate and n- hexane fractions of *V. amygdalina* is safe when utilized within a very short period of time.

4.2.9 Haematological assessment

Leucocytes are regarded as haematological indicators of immunity, as they defend the body by engulfing invading pathogens and fighting off infections. Similarly, erythrocytes are haematological indicators to access the effects of potential drugs on circulatory

erythrocytes and are significant in the diagnosis of anemia. In the present study, there was an increase ($p<0.05$) in the Mean Corpuscles Volume, Red blood count, Total White Blood Cell Count, neutrophils and eosinophils of the control group compared with groups administered n-hexane and ethyl acetate fractions. Whereas, there was a decrease ($p<0.05$) in MCHC, PLC, neutrophils and lymphocytes levels in groups dosed with nhexane and ethyl acetate fractions, when compared to the control. This is in line with the study of Chike *et al*. (2018), where similar results were recorded for wister rats treated with aqueous extract of *V. amygdalina*. However, the findings of this present study is not in line with the study of Oyedeji *et al*. (2013), where all haematological parameters of treated animals were same as that of the control. The disparity in both studies may be as a result of the concentration of extract administered to the experimental groups and the gender of experimental animals used in the study. The inability of n-hexane and ethyl acetate fractions to lower the erythrocytes, highlights the absence of anemia in treated rats. A decrease in the platelet count suggests that *V. amygdalina,* can induce thrombocytopenia, similarly, a decrease in neutrophils and lymphocytes levels may be as a result of stress induced by *V. amygdalina,* in the systems of the rats during dosing (Bashir *et al.,* 2015).

4.2.10 Biochemical assessment

The biochemical assessment of experimental animals, revealed that there was a decrease in the liver enzymes (aspartate transaminase (AST), alanine amino transferase (ALT), alkaline phosphate (ALP)) in group administered n-hexane and ethyl acetate fractions, when compared with the control group. A decrease in liver enzymes, especially ALT, could be an indication that n-hexane and ethyl acetate fractions of *V. amygdalina,* has hepatoprotective potentials. A decrease in AST may also be an indicator that the n-hexane

and ethyl acetate fractions of *V. amygdalina*, did not induce tissue necrosis. This is in agreement with the study of Kharchoufa *et al*. (2020), where *V. amygdalina*, did not induce tissue necrosis.

There was a decrease ($p<0.05$) in the urea, triglycerides, low density lipoprotein and creatinine levels in group administered n-hexane and ethyl acetate fractions, when compared with the control group. This is in line with the study of Ekpo *et al*. (2017). High levels of creatinine and urea, are markers for possible kidney damage, when they accumulate in biological fluid as a result of damage to the glomerulus (Widyastuti *et al*., 2019).

Rats administered n-hexane, recorded a significant increase ($p<0.05$) in the levels of high density lipoprotein (10 and 300 mg/Kgbw), total protein and albumin at 10 mg/Kgbw compared with the control while those administered ethyl acetate fraction recorded a significant increase ($p<0.05$) in the levels of high density lipoprotein (300 and 600 mg/Kgbw), total protein, albumin and cholesterol at 600, 300 and 600 mg/Kgbw respectively. This is in contrast with the study of Imaga and Bamigbetan, (2013) where a decrease in the lipid profile of experimental animals were recorded. The difference in both studies might be as a result of solvents used in extraction. High levels of lipids may serve as diagnostic indices in conditions such as chronic obstructive jaundice and coronary heart disease (Kharchoufa *et al*., 2020)

4.2.11 Histopathological assessment

Histopathological assessment of the kidney of experimental animals showed that there was no difference between the in group administered n-hexane and ethyl acetate fractions compared to the control, which presented no features of acute or chronic damage, by

displaying renal tissue with preserved architecture composed of normal glomeruli, tubules and interstitium. The histopathological assessment of the liver of experimental animals also showed that there was no difference between the treatment group and the control, which displayed hepatic tissue with preserved architecture composed of cords of normal hepatocytes, normal portal tracts and central vein. Although liver sections of groups treated with 600 mg/kg body weight of ethyl-acetate and n- hexane exhibited mild portal lymphocytic inflammation and mild cytoplasmic vacuolations respectively. This is in agreement with the study of Abebe and Gebru (2015), where leaves of *V. amygdalina*, exhibited no distortion in the kidney and liver architecture.

Vernonia amygdalina, possess potent antibacterial compounds, has very minimal toxic effects and may be useful in developing chemotherapeutic agents for the treatment of infections associated with enteric bacteria.

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATIONS AND CONTRIBUTION TO KNOWLEDGE

5.1 Conclusion

It can be concluded from the findings of this study that:

The crude extracts (1000 to 3000 mg/ml) and fractions (100 to 800 mg/ml) of *Vernonia amygdalina*, leaves have antibacterial activities. The crude extracts and fractions of *Vernonia amygdalina* leaves possess various phytochemical components (phenols, flavonoids, tannins, alkaloids, saponins, steroids, cardiac glycosides and terpenoids) in varying quantities. The minimum inhibitory concentrations (MIC) of *Vernonia amygdalina* leaves ranged from 6.3 to 750 mg/ml and minimum bactericidal concentrations (MBC) ranged from 12.5 to 1500 mg/ml. Ethyl-acetate and n-hexane fraction of *Vernonia amygdalina* leaves exhibited minimal signs of Acute and Sub-Acute toxicity in experimental animals.

5.2 Recommendations

From the study, it can be recommended that;

1. Efforts towards the isolation and purification of the bioactive components present in *Vernonia amygdalina* leaves for the further development of potent antibacterial.
2. Concentration of the crude extracts (1000 to 3000 mg/ml) and fractions (100 to 800 mg/ml) of *Vernonia amygdalina*, leaves can be used in treatment of enteric bacteria.

5.3 Contribution to Knowledge

This research was able to show that *V. amygdalina* exhibits a higher antibacterial activity, when fractionated with ethyl-acetate and hexane, compared to the antibacterial activity it exhibits when it's extracts are not fractionated. Ethyl-acetate and n-hexane fractions contained high percentage of most bioactive components such as phenols, flavonoids, tannis, alkaloids and saponins and the extract exhibited significant inhibitory effects of test on multi drug resistant bacteria at a concentration of 100mg/ml.

The result of this study also contributed that fractionated extract of *Vernonia amygdalina* had significant effect on the hematological and biochemical parameters of experimental rats whereas, it exhibited no damaging effect on the liver which means that the plant is not toxic.

REFERENCES

Abalaka, M. E., Daniyan, S.Y., Oyeleke, S. B., & Adeyemo, S. O. (2012). The antibacterial evaluation of *Moringa oleifera* leaf extracts on selected bacterial pathogens. *Journal of Microbiology Research*, 2(2), 1-4.

Abdullahi, A. R., & Mainul H. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy & Bio allied Sciences*, 12(1), 1–10.

Abebe, M. S & Gebru, G. (2015). Toxic effect of *Vernonia amygdalina Delile* on blood parameters and histopathology of liver and kidney in Rats. *Global Journal of Medicinal Plants Research*, 1(1), 001-008.

Abubakar, B. A., Aliyu, M. M., Mikhail, S. A., Hamisu, I., & Adebayo, O. O. (2011). Phytochemical screening and antibacterial activities *of Vernonia ambigua, Vernonia blumeoides* and *Vernonia oocephala (asteraceae). Acta Poloniae. Pharmaceutical and Drug Research*, 68 (1), 67-73.

Acharya, D., & Anshu, S. (2018). Indigenous herbal medicines: Tribal formulations and traditional herbal practices. Jaipur: Aavishkar Publishers.

Adeolu, M., Alnajar, A., Naushad, A., & Gupta, R.S. (2016). Genome-based phylogeny and taxonomy of the '*Enterobacteriales*': proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., Pectobacteriaceae fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, 66(12), 120-130.

Ahmed, N., Pottinger, P., Drew, L. W., Reller, L. B., Lagunoff, M., & Sterling, C. R. (2004). Sherris medical microbiology (4th ed.). McGraw Hill, 370.

Ahn, K. (2017). The worldwide trend of using botanical drugs and strategies for developing global drugs. *BMB Reports*, 50 (3), 111–116.

Ali, M., Sani, U. D., Waiya, A. S., & Muhammad, S. A. (2019). Phytochemical screening and antibacterial activity of bitter leaf (*Vernonia amygdalina*). *Annals of Microbiology and Infectious Diseases*, 2(4), 01-07.

Alo, M. N., Anyim, C., Igwe, J. C., Elom, M., & Uchenna, D. S. (2012). Antibacterial activity of water, ethanol and methanol extracts of *Ocimum gratissimum, Vernonia amygdalina* and *Aframomum melegueta*. *Advances in Applied Science Research*, 3 (2), 844-848.

AlQathama, A., Ezurike, F. U., Mazzari, L. D. A., Yonbawi, A., Chieli, E., & Prieto, M. J. (2020). Effects of selected Nigerian medicinal plants on the viability, mobility, and multidrug-resistant mechanisms in liver, colon, and skin cancer cell lines. *Frontiers in Pharmacology*, (11), 1-5.

Anderson, C. J., & Kendall, M. M. (2017). *Salmonella enterica* Serovar Typhimurium strategies for host adaptation. *Frontier Microbiology*, 8, 1983.

Aniszewski, T. (2007). Biological significance of alkaloids. Alkaloids – secrets of life alkaloid chemistry, biological significance, applications and ecological role. Amsterdam: Elsevier Publisher.

Anyanwu, M, U., & Okoye, R. C. (2017). Antimicrobial activity of Nigerian medicinal plants. *Journal of Intercultural Ethnopharmacology*, 6(2), 145 – 161.

Anywar, G., Kakudidi, E., Byamukama, R., Mukonzo, J., Schubert, A., Oryem-Origa, H., & Jassoy, C. (2021). A review of the toxicity and phytochemistry of medicinal plant species used by herbalists in treating people living with HIV/AIDS in Uganda. *Frontiers in Pharmacology*, 42, 113 - 129.

Arabski, M., Węgierek-Ciuk, A., Grzegorz, C., Lankoff, A., & Wiesław, K. (2012). Effects of saponins against clinical *E. coli* strains and eukaryotic cell line. *BioMed Research International*, 2(2), 34 – 71.

Ardalan, M & Rafieian-Kopaei, M (2013). Is the safety of herbal medicines for kidneys under question? *Journal of Nephropharmacology*, 2(2), 11–12.

Arekemase, M.O., Ganiyu, P.O., & Kabir, I.B. (2013). Assessment of bitter leaf (*vernonia amygdalina*) on some selected pathogenic microorganisms from university of Ilorin teaching hospital. *Journal of Microbiology, Biotechnology and Food Science*, 2 (5), 2360-2365.

Aryal, S. (2019). Biochemical test and identification of *Serratia marcescens*. Retrieved on June 4, 2021 from https://microbiologyinfo.com/biochemical-test-andidentification-of-serratia-marcescens/.

Ashour, M., Wink, M., & Gershenzon, J. (2010). Biochemistry of terpenoids: monoterpenes, sesquiterpenes and diterpenes. *Biochemistry of Plant Secondary Metabolism*, 21, 258–303.

Ashurst J. V, & Dawson A. (2021). *Klebsiella Pneumoniae*. StatPearls, Retrieved on June 15, 2021 from https://www.ncbi.nlm.nih.gov/books/NBK519004/_NBK519004_pubdet_.

Ashurst, J. V., Truong, J., & Woodbury, B. (2020). *Salmonella Typhi*. Retrieved on June 10, 2021 from https://pubmed.ncbi.nlm.nih.gov/30085544/.

Azwanida, N.N. (2015). A Review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal & Aromatic Plants*, 4(3), 103 – 125.

Banerjee, J., Amitava, D., Mithun, S., & Sudipta, S. (2018). Biological efficacy of medicinal plant extracts in preventing oxidative damage. *Oxidative Medicine and Cellular Longevity,* 3(1), 23 – 46.

Basak, S., Singh, P., & Rajurkar, M. (2016). Multidrug resistant and extensively drug resistant bacteria: A study. *Journal of Pathogens*, 1(2), 67 – 79.

Bashir, L., Shittu, O. K., Busari, M. B., Sani, S., & Aisha, M. I. (2015). Safety evaluation of giant african land snails (*Archachatina marginata)* haemolymph on haematological and biochemical parameters of albino rats. *Journal of Advance Medical Pharmaceutical Society*, 3(3),122-130.

Bayot, M. L., & Bragg, B. N. (2020). Antimicrobial susceptibility testing. StatPearls, treasure island, Retrieved on August 2, 2021 from https://www.ncbi.nlm.nih.gov/books/ NBK539714/_NBK539714_pubdet_.

Bhattacharya, A., Sood, P., & Citovsky, V. (2010). The roles of plant phenolics in defense and communication during agrobacterium and rhizobium infection. *Molecular Plant Pathology*, 11(5), 705–719.

Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilvert, D., Rice, L. B., Schedul, M., Spellberg, B., & Bartlet, J. (2009). Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clinical Infectious Diseases*, 48 (1), 1–12.

Britannica, T. Editors of Encyclopaedia. (2021). Tannin. *Encyclopedia Britannica*. Retrieved on May 12, 2021 from https://www.britannica.com/science/tannin.

Bush, L. M., & Vazquez-Pertejo, M. T. (2020). *Klebsiella, Enterobacter*, and *Serratia* Infections. *Msd Manual*, Retrieved on June 10, 2021 from https://www.msdmanuals.com/professional/infectious-diseases/gram-negativebacilli/klebsiella-enterobacter-and-serratia-infections.

Carroll, C. K., Jeffery, A. H., Miller, S., Morse, A. S., Mietzner, T. A., Detrick, B., Thomas, G. M., McKerrow, H. J., & Sakanari, A. J. (2015). *Enterobacteriaecae*. *Jawetz, Melnick, & Adelberg's Medical Microbiology*, McGraw Hill: USA.

Center for Disease Control (CDC) (2010). Healthcare-associated infections (HAIs). Retrieved on June 15, 2021 from https://www.cdc.gov/hai/organisms/klebsiella/klebsiella.html.

Center for Disease Control (CDC). (2019). Antimicrobial resistance. Retrieved on June 10, 2021 from https://www.cdc.gov/drugresistance/biggest-threats.html.

Center for Disease Control (CDC). (2020). Typhoid Fever and Paratyphoid Fever. Retrieved on June 10, 2021 from https://www.cdc.gov/typhoidfever/symptoms.html.

Center for disease control. (CDC). (2015). Facility guidance for control of carbapenemresistant *Enterobacteriaceae* (CRE). National center for emerging and zoonotic infectious disease.

Chike, C. P. R., Njoku, B., Green, K., Akpojotor, P. I., Onyebuenyi, M. O., & Numbara, D. (2018). Effect of ethanolic leaf extract of *Vernonia amygdalina* (bitter leaf) extract on some of the haematological parameters in wistar rats. *Journal of Complementary and Alternative Medical Research*, 4(1), 1-7.

Clinical and Laboratory Standards Institute (CLSI). (2018). performance standards for antimicrobial susceptibility testing.28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Dai, J., & Mumper, J. R. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352.

Deering, S. (2019). Nature's 9 most powerful medicinal plants and the science behind them. Retrieved on February 9, 2022 from https://www.healthline.com/health/most-powerful-medicinal-plantseveningprimrose-oil.

DerSarkissian, C. (2020). What is *Shigella*?. We MD, Retrieved on June 10, 2021 from https://www.webmd.com/food-recipes/food-poisoning/what-is-shigella.

Dibia, P. (2019). 20 most popular medicinal plants in Nigeria and their uses. Retrieved on February 9, 2022 from www.infoguidenigeria.com.

Dockrell, H.M., Goering, R.V, Chiodini L. P., & Zuckerman, M. (2019). Gastrointestinal tract infections. MIM'S Medical Microbiology and Immunology (3rd ed.), Elsevier, 287.

Du-Bois, A., Isaac, T. H., Desmond, O., Acheamponga, F. K., Christian, K. A., Eric, G. O., Nelson, K. D., Emmanuel, A., Lokeswari, P. T., & Elvis, O.A. (2019) Antiinflammatory, anti-nociceptive and antipyretic activity of young and old leaves of *Vernonia amygdalina*. *Biomedicine & Pharmacotherapy*, 111, 1187-1203,

Duedu, K. O., Offei, G., Codjoe, F. S., & Donkor, E. S. (2017). Multidrug resistant enteric bacterial pathogens in a psychiatric hospital in Ghana: implications for control of nosocomial infections. *International Journal of Microbiology*, Retrieved on June 15, 2021 from https://doi.org/10.1155/2017/9509087.

Ekam, V. S., Ebong, P. E., & Umoh, I. B. (2010). Phytochemical screening of activity directed extracts of *Vernonia amygdalina* leaves. *Global Journal of Pure and Applied Sciences*, 16(1), 151-154.

Ekpo, A., Eseyin, A. O., Ikpeme, O. A., & Edoho, J. E. (2017). Studies on some biochemical effects of *Vernonia amygdalina* on rats. *Asian Journal of Biochemistry*, 2(3), 193-197.

Enegide, C., Arome, D., & Ameh, S. F. (2013). A new method for determining acute toxicity in animal models. *Toxicology International*, 20(3), 224–226.

Enwa, F., Omojate. G., Jewo, A., & Eze, C. (2014). Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens – A review. *Journal of Pharmaceutical, Chemical and Biological Sciences*, 2(2), 77-85.

Evbuomwan, L., Chukwuka, E. P., Obazenu, E. I., & Ilevbare, L. (2018). Antibacterial activity of *Vernonia amygdalina* leaf extracts against multidrug resistant bacterial isolates. *Journal of Applied Science and Environment Management*, 22(1), 17-21.

Ezekwesili, J. O., & Okaka, A. N.C. (2019). Herbal medicines in African traditional medicine. *IntechOpen*, Retrieved on November 18, 2021 from https://www.intechopen.com/chapters/ 64851.

Ezenobi, N. O., Chinaka, C. N., & Wonah, M. (2019). In vitro antibacterial activities of crude ethanolic extract of *Vernonia amygdalina* (*Asteraceae*) and *Ocimum gratissimum* (*Lamiaceae*) and their combined effect on some bacterial isolates from sputum. *Journal of Pharmacognosy and Phytochemistry*, 8(2), 1306-1311.

Fabrega, A., & Vila, J. (2013). *Salmonella enterica* serovar *Typhimurium* skills to succeed in the host: virulence and regulation. *Clinical Microbiology Reviews*, 26(2), 308–341.

Farobi, O. E., & Owoeye, O. (2011). Antioxidative and chemopreventive properties of *Vernonia amygdalina garcinia* and *biflavonoid. International Journal of Environmental Research and Public Health*, 8(6), 2533-2555.

Ferrell, K. E., & Thorington, R. W. (2006). Squirrels: The animal answer guide. Baltimore: Johns Hopkins University.

Giada, M. R. (2013). Food phenolic compounds: Main classes, sources and their antioxidant power, oxidative stress and chronic degenerative diseases - A role for antioxidants. *IntechOpen*, Retrieved on June 2, 2021 from http://doi:10.5772/51687.

GIS Achivers. (2022). Maitumbi study area. Retrieved February 10, 2022 from https://m.facebook.com/GISAchivers/

Goldrick, B. (2003). Foodborne diseases more efforts needed to meet the healthy people 2010 objectives. *American Journal of Nursing*, 103(3), 105-106.

Gouws, C (2018). Traditional African medicine and conventional drugs: friends or enemies. Retrieved from theconversation.com, (Accessed 18 November, 2021)

Haddix, P. L., & Shanks, R. M. Q. (2018). Prodigiosin pigment of *Serratia marcescens* is associated with increased biomass production. *Archives of Microbiology*, 200, 989–999.

Hartmut, F. (2006). MetaCyc pathway: saponin biosynthesis. Retrieved on May 15, 2021 from https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=PWY-5203&detail-level=3.

Herra, C. & Falkiner, R.F. (2017). *Serratia marcescens*. Antimicrobial, Retrieved on June 4, 2021 from http://www.antimicrobe.org/b26.asp.

Hesse, M. (2003). Alkaloids: Nature's curse or blessing? *Angewandte Chemie International*, 42(40), 4852-4854

Hill, A. (2020). 9 of the world's most popular herbal medicines. Retrieved on November 20, 2021 from https://www.healthline.com/nutrition/herbal-medicine8.-Valerian.

Hussain, T. (2015). Pakistan at the verge of potential epidemics by multi-drug resistant pathogenic bacteria. *Advance Life Science*, 2(2), 46-47.

Ijeh, I. I., & Ejike, C. C. (2011). Current perspectives on the medicinal potentials of *Vernonia Amygdalina. Journal of Medicinal Plant Research,* 5(7), 1051-1061.

Imaga, N. O., & Bamigbetan, D. O. (2013). In vivo biochemical assessment of aqueous extracts of *Vernonia amygdalina* (Bitter leaf). *International Journal of Nutrition and Metabolism*, 5(2), 22-27.

Iredell, J., Brown, J., & Tagg, K. (2016). Antibiotic resistance in *Enterobacteriaceae:* Mechanisms and clinical implications. *British Medical Journal*, 3(2), 239 – 278.

Ivanova, D., Markovska, R., Hadjieva, N., Schneider, I., Mitov, I., & Bauernfeind, A. (2008). Extended-spectrum β-lactamase-producing *Serratia marcescens* outbreak in a Bulgarian hospital. *The Journal of Hospital Infections*, 70(1), 60-65.

Jaryum, H. K., Gazuwa, S. Y., Mairiga, P. J., & Okeke, C. S. (2019). Antinutritional and antibacterial activity of aqueous and ethanolic extracts of *Vernonia amygdalina* (bitter leaf). *Acta Scientific Nutritional Health*, 3(4), 87-94.

Jasmine, R., Selvakumar, B. N., & Pitchai, D. (2011). Investigating the mechanism of action of terpenoids and the effect of interfering substances on an Indian medicinal plant extract demonstrating antibacterial. *International Journal of Pharmaceutical Studies and Research*, 5, 1832-1837.

Jyoti, T., Shrayanee, D., Zeeshan, F., & Saif, H. (2014). Multidrug resistance: An emerging crisis. *Interdisciplinary Perspectives on Infectious Diseases*, 14, 12 – 26.

Kahnna, A., Khanna, M., & Aggarwal, A. (2013). *Serratia Marcescens*- A rare opportunistic nosocomial pathogen and measures to limit its spread in hospitalized patients. *Journal of Clinical and Diagnostic Research*, 7(2), 243–246.

Khan, H., Khan, A. M., & Abdullah. (2015). Antibacterial, antioxidant and cytotoxic studies of total saponin, alkaloid and sterols contents of decoction of Joshanda: Identification of components through thin layer chromatography. *Toxicology and Industrial Health*, 31(3), 202-208.

Kharchoufa, L., Mohamed, B., Noureddine, B., El Assri, S., Amirou, A., Yamani, A., Mohammed C., Hassane, M., & Mostafa Elachouri. (2020). Acute and subacute toxicity studies of the aqueous extract from *Haloxylon scoparium Pomel (Hammada scoparia (Pomel*)) by oral administration in rodents. *BioMed Research International*, 4(1), 319 – 332.

Kregiel, D., Berlowska, J., Witonska, I., Antolak, H., Charalampos, P., Mirko, B., Babic, L., & Zhang, B. (2017). Saponin-based, biological-active surfactants from plants, application and characterization of surfactants. *IntechOpen*, Retrieved on May 22, 2021 from http://doi:10.5772/68062.

Kurek, J. (2019). Introductory chapter: Alkaloids - Their importance in nature and for human life. *IntechOpen.* Retrieved on May 12, 2021 from https://DOI: 10.5772/intechopen.85400.

Kurhekar, V. K. (2016). Tannins – Antimicrobial Chemical Components. *International Journal of Technology and Science*, 9(3), 5-9.

LaRock, D. L., Chaudhary, A., & Miller, S. I. (2015). Salmonellae interactions with host processes. *Nature Reviews Microbiology*, 13(4), 191–205.

Li, X. Z & Nikaido, H. (2009). Efflux-mediated drug resistance in bacteria: an update". *Drugs*, 69 (12), 1555–623.

Ludwiczuk, A., Skalicka-Woźniak, K., & Georgiev, M. I. (2017). Terpenoids. *Pharmacognosy*, 12, 233–266.

Mahomoodally, M. F. (2013). Traditional medicines in Africa: An appraisal of ten potent African medicinal plants. evidence-based complementary and alternative medicine, Retrieved on February 4, 2022 from https://doi.org/10.1155/2013/617459.

Marathe, A. S., Amit L., Vidya D. N., & Dipshikha, C. (2012). Typhoid fever & vaccine development: A partially answered question. *Indian Journal of Medical Research*, 135(2), 161–169.

Martins E. (2013). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4, 177.

Matolcsy, G., Nadasy, M., & Andriska, V. (2002). *Pesticide Chemistry*. New York: Elsevier.

Mayo Clinic. (2020). Typhoid Fever. Retrieved June 10, 2021 from https://www.mayoclinic.org/diseases-conditions/typhoid-fever/diagnosistreatment/drc-20378665.

Minatel, I. O., Borges, C. V., Ferreira, I. M., Gomez, A. H., Chung-Yen, O. C., & Giuseppina, P. L. (2017). Phenolic compounds: Functional properties, impact of processing and bioavailability. *IntechOpen*, Retrieved on June 2, 2021 from http://doi:10.5772/66368.

Molyneux, R. J., Lee, S. T., Gardner, D. R., Panter, K. E., & James, L. F. (2007). Phytochemicals: the good, the bad and the ugly? *Phytochemistry*, 68 (22–24), 2973–2985.

Muhammad, I. K., Abdulatef, A., Jin, H. S., Jun, S. B., Min, Y. K., & Jong, D. K. (2018). Green tea seed isolated saponins exerts antibacterial effects against various strains of gram positive and gram negative bacteria, a comprehensive study in vitro and invivo. *Evidence-Based Complementary and Alternative Medicine*, 2(1), 7542 – 7571.

Muller-Harvey, I., & McAllan, A. B. (1992). Tannins: Their biochemistry and nutritional properties. *Advances in Plant Cell Biochemistry and Biotechnology*, 1, 151-217.

Namdeo, G.A. (2018). Cultivation of medicinal and aromatic plants. *Natural Products and Drug Discovery*, 4(2), 525-553.

Nata'ala, M. K., Fardami, A. Y., Dalhat, M. H., Sirajo, K., Bashiru, I., Usman, M. H., & Mas'ud, I. (2019). Phytochemical screening and antimicrobial analysis of *Vernonia*

amygdalina and *Psidium guajava* stems on bacteria associated with dental caries. *South Asian Journal of Research in Microbiology*, 3(4), 1-8.

National Library of Medicine (NLM) (2022). Drug resistance, multiple. Retrieved on February 10, 2022 from https://meshb.nlm.nih.gov/record/ui?name=Drug%20Resistance,%20Multiple.

Nduche, M. U., Nkaa, F. A., & Onyebinime, A. (2019). Phytochemical screening and antimicrobial activity of *Carica papaya l, Citrus paradisi l, Citrus sinensis l,* and *Vernonia amygdalina del*. *Central Journal of Dairy & Veterinary Sciences*, 9(4), 57.

Niger State Government (2021). This is our Niger State. Retrieved on July 17, 2021 from https://nigerstate.gov.ng/about-niger/.

Nursuhaili, A. B., Nur, A. S., P., Martini, M. Y., Azizah, M., & Mahmud, T. M. M. (2019). A review: medicinal values, agronomic practices and postharvest handlings of *Vernonia amygdalina*. *Food Research*, 3(5), 380-390.

Ochieng, J. B., Boisen, N., Brianna, L., Araceli, S., Ouma, C., Ombok, M., Barry, F., Stine, O. C., & James, P. (2014). *Serratia marcescens* is injurious to intestinal epithelial cells. *Gut Microbes* 5(6), 729–736.

Ogundare A. O. (2011). Antibacterial properties of the leaf extracts of *Vernonia amygdalina, Ocimum gratissimum, Corchorous olitorius* and *Manihot palmate*. *Journal of Microbiology and Antimicrobials*, 3(4), 77-86.

Ojiemelukwe. P., & Amaechi. C. N. (2019). Composition of *Vernonia amygdalina* and its potential health benefits. *International Journal of Environment, Agriculture and Biotechnology*, 4(6), 1836-1848.

Omar, A. A. (2017). General overview of phenolics from plant to laboratory, good antibacterials or not. *Pharmacognosy Reviews*, 11(22), 123–127.

Onifade, A. K., & Agunloye, O. O. (2019). Antibacterial assessment of crude and fractionated extracts of *Vernonia amygdalina* leaf against multiple antibiotic resistant bacteria of wound infection. *Asian Journal of Research in Medical and Pharmaceutical Science*, 7(1), 1-12.

Opara, F. N., Anuforo, H. U., Okechukwu, R. I., Mgbemena, I. C., Akujobi, C. O., & Adjero, A. (2012). Preliminary phytochemical screening and antibacterial activities of leaf extracts of *Terminalia catappa*. *Journal of Emerging Trends in Engineering and Applied Sciences*, 3 (3), 424-428.

Oshilim, A. O. (2017). Comparative study of the effect of bitter leaf extract and antibiotics (gentamycin and amoxicillin) on bacterial species isolated from wound. *Journal of Biotechnology and Pharmaceutical Research*, 6(1), 9-12.

Owoade, R. A., & Raji L. A. (2019). Anti-bacterial effect of cold water extract of bitter leaf (*Vernonia amygdalina*) on some selected microorganisms. *European Journal of Food Science and Technology*, 7(1), 36-43.

Oyedeji K.O., Bolarinwa, A. F., & Akintola, A. M. (2013). Effect of methanolic extract of *Vernonia amygdalina* on haematological and plasma biochemical parameters in male albino rats. *Journal of Dental and Medical Sciences*, 3(5), 64-67.

Oyedum, U. M. (2017). Antibacterial and toxicological effects of leaf extracts of *Eurphorbia heterophylla* on some enteric bacteria. *Global Journal of Pure and Applied Sciences*, 23, 59-67.

Oyeleke, S. B., & Manga, B. S. (2008). *Determination of minimal bactericidal concentration, Essentials of laboratory practical in microbiology*. Minna: Tobest publisher.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, 2(1), 39 – 46.

Pariyani, R., Intan, S. I., Azam, A. A., Abas, F., Khozirah, S., & Mohd, R. S. (2015). Phytochemical screening and acute oral toxicity study of java tea leaf extracts. *BioMed Research International*, 45, 214 – 251.

Patel, P. K. (2021). *Serratia* medication. Retrieved on June 10, 2021 from https://emedicine.medscape.com/article/228495-medication.

Perveen, S. (2018). Introductory chapter: terpenes and terpenoids, terpenes and terpenoids. *IntechOpen*. Retrieved on June 2, 2021 from http://doi:10.5772/intechopen.79683.

Pieroni, A., N (2005). The Cultural History of Plants. Retrieved on June 15, 2021 from https://en.m.wikipedia.org/wiki/Vernonia_amygdalina.

Pistelli, L., & Giorgi, I. (2012). Chapter 2, antimicrobial properties of flavonoids. *Dietary Phytochemicals and Microbes*, Retrieved on May 22, 2021 from http://doi:10.1007/978-94-007-3926-0_2.

Plant décor. (2022). 40 medicinal plants you must grow at your home. Retrieved on February 9, 2022 from www.plantdecors.com/blogs.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health*, 109(7), 309–318.

Rafieian-Kopaei M. (2012). Medicinal plants and the human needs. *Journal of Herbal Medicine Pharmacology*, 1(1), 1–2.

Riedel, S., Hobden, J. A., Miller, S., Morse, A. S., Mietzner, A. T., Detrick, B., Thomas, G. M., Sakanari, J. A., Hotez, P., & Mejia, R. (2019). Enteric gram negative rods

(enterobacteriaceae). Jawetz, Melnic & Adelberg's medical microbiology. twentyEight Edition. New York: McGraw-Hill.

Roberts, M.F. and Wink, M. (1998). Alkaloids: Biochemistry, ecology and medicinal application. Plenum Press, New York. Retrieved on June 15, 2021 from https://doi.org/10.1007/978-1-4757-2905-4.

Ruiz-Cruz, S., Chaparro-Hernández, S., Hernández- Ruiz, L. K., Cira-Chávez, A. L., Estrada-Alvarado, I. M., Ortega, L. E., José de Jesús, O., & Marco, A. M. (2017). Flavonoids: Important biocompounds in food, flavonoids - from biosynthesis to human health. *IntechOpen*, Retrieved on May 28, 2021 from http://doi:10.5772/67864.

Salami, O. O., & Agu, C. G. (2013). Assessment of the antimicrobial activities of *Ocimum Gratissimum* (wild basil) and *Vernonia Amygdalina* (bitter leaf) on some enteric pathogen causing dysentery or diarrhea in patients. *The International Journal of Engineering and Science,* 2(9), 83-96.

Sanchez, G. V., Master, R. N., Clark, R. B., Fyyaz, M., Duvvuri, P., Ekta, G., & Bordon, J. (2013). *Klebsiella pneumoniae* antimicrobial drug resistance, United States, 1998-2010. *Emerging Infectious Diseases*, 19(1), 133–136.

Sen, A., & Batra, A. (2012). Determination of antimicrobial potentialilities of different solvent extracts of the medicinal plant: *Phyllanthus amarus schum* and *Thonn. International journal of green pharmacy*, 6(1), 34 – 79.

Shashank, K., & Abhay, K. P. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal*, 23, 190 – 112.

Shi, J., Konesh, A., Yeung, D., Yukio, K., Gauri, M., & Yueming, J. (2004). Saponins from edible legumes: Chemistry, processing, and health benefits. *Journal of Medicinal Food*, 7(1), 67–78.

Sieniawska, E., & Baj, T. (2017). Chapter 10: Tannins plant metabolites: Their chemistry. *Pharmacognosy*, Retrieved on May 26, 2021 from http://dx.doi.org/10.1016/B9780-12-802104-0.00010-X.

Simsek, M. (2021). Determination of the antibiotic resistance rates of *Serratia marcescens* isolates obtained from various clinical specimens. Retrieved on June 4, 2021 from http://www.njconline.com/.

Singh, A. P., & Kumar, S. (2019). Applications of tannins in industry. In (Ed.), tannins - structural properties, biological properties and current knowledge. IntechOpen, Retrieved on June 15, 2021 from https://doi.org/10.5772/intechopen.85984.

Smith-Hall, C., Larsen, H. O., & Pouliot, M. (2012). People, plants and health: A conceptual framework for assessing changes in medicinal plant consumption. *Journal of Ethnobiology and Ethnomedicine*, 4(3), 45 – 78.

Sofowora, A., Eyitope, O., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional Complementary and Alternative Medicine*, 10(5), 210–229.

Southwick, F.S. (2019). *Shigella* species (bacillary dyentary). Infectious diseases advisor. Retrieved on June 15, 2021 from https://www.infectiousdiseaseadvisor.com/home/decision-support-inmedicine/infectious-diseases/shigella-species-bacillary-dyentary/.

Stix, G. (2006). An antibiotic resistance fighter. *Scientific American*, 294 (4), 80–93.

Sun, H., Xie, Y., & Ye, Y. (2009). Advances in saponin-based adjuvants. *Vaccine*, 27 (12), 1787–1796.

Takó, M., Kerekes, B. E., Zambrano, C., Kotogán, A., Tamás, P., Krisch, J., & Csaba, V. (2020). Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. *Antioxidants*, 9(2), 165 - 189.

Tariq, L., Bhat, B. A., Hamdani, S. S., & Mir R. A. (2021). Phytochemistry, pharmacology and toxicity of medicinal plants. *Medicinal and Aromatic Plants*, 51, 204 – 229.

Todar, K. (2020). Bacterial pathogens of humans. Todar's online textbook of bacteriology. Retrieved on February 23, 2021 from www.textbookofbacteriology.net.

Vermerris, W., & Nicholson, R (2008). *Phenolic Compound Biochemistry*. Netherlands: Springer.

Wain, J., Hendriksen, R. S., Mikoleit, M. L., Keddy, K. H., & Ochiai, R. L. (2015). Typhoid fever. *Lancet*, 385(9973), 1136–1145.

Wanda, C. R. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, 4(3), 482–501.

Watkins, L.F., & Appiah, G.D. (2019). Shigellosis. Retrieved on June 15, 2021 from https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectiousdiseases/shigellosis.

Widyastuti, D. A., Ristianti, A. M., & Sari, I. M. (2019). The study of blood creatinine and urea concentration of Wister rats (*Rattus norvegicus*) to sodium nitrate induction. *Journal ilum kefarmasian Indonesia*, 17(1), 320 – 341.

Williams, P. C. M, & Berkley, A. J. (2018). Guidelines for the treatment of dysentery (shigellosis): A systematic review of the evidence. *Paediatrics and International Child Health*,38(1), 50–65.

World Health Organization. (2021). Traditional, complementary and integrative medicine. Retrieved from https://www.who.int/health-

topics/traditionalcomplementary-and-integrative-medicinetab=tab_1, (Accessed 18 November, 2021).

Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2015). Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Current Medicinal Chemistry*, 22(1), 132–149.

Yabuuchi E. (2002). *Bacillus dysentericus* (sic) 1897 was the first taxonomic rather than Bacillus dysenteriae 1898. *International Journal of Systematic and Evolutionary Microbiology*, 52(3), 1041.

Yang, F., Yang, J., Zhang, X., Lihong, C., Yan, J., Yongliang, Y., Xudong, T., Jing, W., Zhaohui, X., Jie, D., Ying, X., Yafang, Z., Xingye, X., Lilian, S., Shuxia, C., Huan, N., Junping, P., Jianguo, X., Yu, W., Zhenghong, Y., Yumei, W., Zhijian, Yao., Yan, S., Boqin, Q., Yunde, H., Jun, Y., & Qi, J. (2005). Genome dynamics and diversity of Shigella species, the etiologic agents of bacillary dysentery. *Nucleic Acids Research*, 33(19), 6445–6458.

Yang, W., Xu, C., Yanli, L., Shaofen, G., Zhen, W., & Xiuling, Y. (2020). Advances in pharmacological activities of terpenoids. *Sage Journal*, 15(3), 1-13.

Yang, Y. (2010). Theories and concepts in the composition of Chinese herbal formulas". *Chinese Herbal Formulas*, 4(3), 24 – 58.

Yasin, F., Salman, A., Abdul, S. T., Mehr, Z., & Shuja, A. M. (2017). Combination therapy for multidrug-resistant *Klebsiella Pneumoniae* in urinary tract infection. *Cureus*, 9(7), 1503.

Yusmazura, Z., Nurhazirah, Z. A., Nik, F., Nik, H., & Hussin, M. (2016). Phytochemicals and acute oral toxicity studies of the aqueous extract of *Vernonia amygdalina* from state of Malaysia. *Journal of Medicinal Plants Studies*, 4(3), 1-5.

Zaki, S.A., & Karande, S. (2011) Multidrug-resistant typhoid fever: A review. *The Journal of Infection in Developing Countries*, 5, 324-337.

Zhang, J., Igho, J. O., Posadzki, P., & Mohamed E. (2015). The safety of herbal medicine: From prejudice to evidence. *Evidence-Based Complementary and Alternative Medicine*, 3(3), 74 – 91.

Zubairu, A.Y., Mukhtar, M., Saidu, I., Ibrahim, Z., Isah, S., Garga, M. A., & Kebbi, H.S. (2019). Antibacterial activity of methanolic extract of bitter leaf (*Vernonia amygdalina*) from various component fractions using column chromatography. *GSC Biological and Pharmaceutical Sciences*, 07(02), 016–021.

APPENDIX A

Biochemical characteristics of bacterial isolates

BACTERIA	Grams reaction	Shape	Catalase	Citrate	M.R	Indole	Pigment	V.P	Urease	Motility	Sugar fermentation					
											Sucrose	Lactose	Mannitol	Glucose	xylose	H_2S
K. pneumoniae	-	R	+	+	-	-	-	+	+	-	+	+	+	+	+	
S. typhi	-	R	+	-	+	-	-	-	-	+	-	-	+	+	+	+
Shigella species	-	R	+	-	+	-	-	-	-	-	-	-	+	+	-	-
S. marcescens	-	R	+	+	+	-	Rd	+	+	+	+	-	+	+	-	-

KEY; + = positive, - = negative, R= rods, Rd= red.

APPENDIX B

Body Weight of Experimental Rats

Extracts	Weeks				
	0	**1**	**2**	**3**	**4**
G1H	124.46^{a}±2.40	129.99^{a}±2.83	137.39^{a}±3.44	144.31^{a}±3.34	152.86^{ab}±3.58
G2H	123.76^{a}±2.78	133.28^{a}±2.49	144.09^{a}±3.77	155.36^{ab}±2.84	163.41^{bc}±2.60
G3H	123.63^{a}±4.57	130.75^{a}±4.87	137.15^{a}±4.06	147.70^{ab}±3.26	160.32^{abc}±3.35
G1E	120.86^{a}±1.98	127.70^{a}±1.82	135.67^{a}±2.94	143.62^{ab}±2.95	151.36^{a}±3.98
G2E	126.17^{a}±2.40	136.33^{a}±1.79	145.47^{a}±1.27	157.86^{b}±0.88	167.09^{c}±1.10
G3E	123.40^{a}±2.31	131.72^{a}±2.84	139.99^{a}±3.02	149.13^{ab}±4.52	159.36^{abc}±2.96
Control	122.47^{a}±3.69	129.23^{a}±4.14	135.97^{a}±5.01	145.46^{a}±5.38	157.14^{abc}±5.65

KEY; G1H= 10 mg/kg bw of n-hexane fraction, G2H= 300 mg/kg bw of n-hexane fraction, G3H= 600 mg/kg bw of n-hexane fraction, G1E= 10 mg/kg bw of ethyl acetate fraction, G2E= 300 mg/kg bw of ethyl acetate fraction, G3E= 600 mg/kg bw of ethyl acetate fraction