ANTIBACTERIAL ACTIVITY OF *Vernonia amygdalina* LEAVES ON SELECTED ISOLATES

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CERTIFICATION

Mr. Ajisere, Adam Temitope, a postgraduate student in the Department of Microbiology, majoring in Medical Microbiology, has satisfactorily completed the requirements for course work and research for the degree of Master in Science (M.Sc) in Microbiology.

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External examiner Date

DECLARATION

I adam temitope AJISERE hereby declare that this research titled " Antibacterial activity of *Vernonia amygdalina* leaves extract on selected isolates" was researched and carried out by me under the supervision of Prof. O.Adedayo. All sources used are adequately referenced

_____ _____

Adam temitope AJISERE Date

16/27MMI004

DEDICATION

This research is solely dedicated to almighty Allah who has makes it easy from the beginning to the end.

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I wish to sincerely express my unreserved gratitude to God for His amazing grace and strength granted to me to complete this research work.

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Abstract

- The re-emergence of eradicated diseases, the ineffectiveness of the much cheriched old antibiotics and the "acclaimed success rate" of traditional medicinal preparations has reawaken interest in antimicrobial phytochemicals to curtail human infection affecting the entire world population. This study has been designed to screen *Vernonia amygdalina* for pharmaceutically bio-active compounds, which may be employed to check microbial diseases responsible for increase in both mobidity and mortality in this region. The crude powdered plant extract was soaked in n-hexane to defat and then later soaked in ethanol for extraction. This was then basified and acidified. The partial purification was by bio-assay directed fractionation of the different extracts using solvents of varied polarity. Two partially purified chromatographic fractions were obtained and screened against the bacterial strains. The agar well diffusion method was used to test the activities of the extracts on *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Bacillus subtilis*. The 750 µg of fraction one (Rf = 0.9 in dichloromethane : methanol 12 : 2) showed inhibition zone of 17 mm against *Escherichia coli* while 750 µg of fraction two (Rf = 0.6), showed 15 mm inhibition of zone against *Escherichia coli*. The basified and methanolysed extract showed zone of inhibition of 14 mm on *Staphylococcus aureus.* When compared with standard antibiotics disc, *V. amygdalina* leaves extracts shows to competes favorably and promising when purified. However, the result of the study suggest that *Vernonia amygdalina* can possibly be exploited for antibacterial drug developments which could be used to treat *Staphylococcus aureus* and *Escherichia coli* infections.

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CHAPTER ONE

INTRODUCTION

1.0

The emergence and re-emergence of multi-drug resistant organisms have become of paramount concern to the world health system. Complications arising from either the use or inappropriate use of presently used antibiotics are also of great concern not just to the pharmaceutical industry alone but to the health system at large. This could be as a result of certain compound that was used in the synthesis of these drugs, drug-drug interactions and constant evasion to these drugs by microbes through possession and modification of certain defense mechanisms against these antibiotics.

As resistance to the then effective and present drug therapy increases with other medically important side effects accompanied with the use of these present antibiotics, scientists have channeled their research views towards the discovery of alternative medicine which could stand the test of time with less side effects. This as seen in the discovery of plant with high biological activities and less-desirable side effect by previous scientists, have given rise to several studies on medicinal plants with the view to obtaining plant derived compounds which could be used in the synthesis of drug compounds of choice in the pharmaceutical industry (Das *et al*., 2010).

Medicinal plants are plants with pharmacological properties which have the potentials to render inactive the activities and products of pathogenic microorganism in the host system. They have been found to be cheap and alternate source of medicine and the acclaimed success rate of their use in folk medicine have generated the interest of microbiologist and other scientists around the globe.

Medicinal plants have formed the basis of health care throughout the world and have remained relevant in both developing and the developed nations of the world for various chemotherapeutics purposes. The use of plant derived natural compounds as part of herbal preparations for alternate source of medicaments continues to play major roles in chemotherapy especially in third world countries (Joy *et al*., 1998). Several studies carried out have shown that medicinal plants could provide better control than currently used conventional drugs (Rates, 2001 ; Roja and Rao, 2000) .

Vernonia amygdalina, a member of the *Asteraceae* family, is a widely used local vegetable in Nigeria, Uganda and many other African countries. It grows in a range of ecological zones in Africa and the Arabian Peninsula (Bonsi *et al*., 1995). The leaf is commonly called "bitter leaf" in English, "Olubu or Onugbo" in Igbo, "Ityuna" in Tiv, "Oriwo" in Edo, "Ewuro" in Yoruba, "Chusar-doki" in Hausa, "Etidot" in Efik, "Omubirizi" or "Omululuza" in West and Central Uganda; "Olusia" in Luo, Kenya. It is used in various food preparations and in ethnomedicine for the treatment of malaria and gastrointestinal infections. It is a shrub of 2-5 m tall with petiolate leaves of about 6.0mm wide. It is up to 20 cm long and its bark is rough. The bitter taste of the leaf has been attributed to the presence of anti-nutritive principles like saponins, alkaloids, tannins and glycosides. There have been several reports on its antimicrobial, antiplasmodial, antitumor, antioxidant and antihelminthic properties (Jisaka *et al*., 1993; Izevbigie, 2003; Farombi, 2003).

The use of herbal medicine for the treatment of diseases caused by bacteria and fungi gave rise to the development of natural antibiotics (Akinyemi, 2000). Plants are important sources of medicines and play a key role in world health. Almost all cultures from ancient times to today have used plants as medicine. Medicinal plants are important to the global economy (Adekunle

and Odukoya, 2006), as approximately 85% of traditional medicine preparations involve the use of plants or plant extracts.

However, in the decades that followed, the development of synthetic drugs from petroleum products caused a sharp decline in the pre-eminence of drugs from live plant sources (Dahiya and kumar., 2008).

But with the recent trend of high percentage resistance of microorganisms to the present day antibiotics (Ibekwe *et al*., 2000), efforts have been intensified by researchers towards a search for more sources of antimicrobial agents, Plants may provide such credible source of antibiotics.

1.1 Research problems and justification

- Lack of affordable antibiotics to treat cases of infection in the rural communities
- Surge in the population growth of antibiotics resistance microbe
- Emergence of new but presently incurable disease world wide
- Ressurection of eradicated disease

1.2 General objective of the study

- To determine the antibacterial effect of *Vernonia amygdalina* leaf extract against various bacteria

1.3 Specific Objectives of the study are to:

- Extract the antibacterial agent from *Vernonia amygdalina* leaves
- Determine the inhibitory potency of *Vernonia amygdalina* leaves extracts against bacteria.
- Determine the stability *Vernonia amygdalina* leaves extracts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Antibiotics

Antibiotics are chemical substance that inhibit the growth of microorganism. They are also a type of antimicrobial drug used in the treatment and prevention of bacterial infections. They may either kill or inhibit the growth of bacteria. A limited bnbvb bnnumber of antibiotics also possess antiprotozoal activity (WHO, 2014). Antibiotics are not effective against viruses such as the common cold or influenza; drugs which inhibit viruses are termed antiviral drugs or antivirals rather than antibiotics (Cassir *et al.*, 2014).

Sometimes the term antibiotic (which means "opposing life") is used to refer to any substance used against microbes, synonymous with antimicrobial. Some sources distinguish between antibacterial and antibiotic; antibacterials are used in soaps and disinfectants, while antibiotics are used as medicine (Elsevier, 2013).

Antibiotics revolutionized medicine in the 20th century. Together with vaccination, antibiotics have led to the near eradication of diseases such as tuberculosis in the developed world. However, their effectiveness and easy access have also led to their overuse, prompting bacteria to develop resistance (Gualerzi *et al.*, 2013). This has led to widespread problems, World Health Organisation has declared antimicrobial resistance as a serious threat globally regardless of the country or age (WHO, 2014). Rising antibiotic resistance and the scarcity of new antimicrobials has long been acknowledged (Theuretzbacher and Mouton, 2011 ; Walsh and Toleman, 2012). A major challenge in global health care is the need for novel, effective and affordable medicines to

treat microbial infections, especially in developing countries of the world, where up to one-half of deaths are due to infectious diseases (Awouafack *et al*., 2013 ; Srivastava *et al.*, 2013).

The use of plant-based drugs world-wide is increasing. Through recent researches on herbal plants, there have been great developments in the pharmacological evaluation of various plants used in traditional systems of medicine (Shrestha *et al* 2016; Gajendiran *et al* 2016 ;Agarwal *et al* 2015; Avula *et al* 2015; Srividya *et al* 2012; Dahiya, 2008; 2007; Dahiya and Kumar, 2008). Herbal plant products are emerging all over the world due to the belief that many herbal medicines are free from health and environmental effects. The widespread fear of the side-effects of synthetic drugs often accompanies their single or multiple health benefits (Al Sadhan and Almas, 1999).

Aqueous leaf extracts of *V. amygdalina* have been previously reported to have prebiotic properties. For many years, medicine depended exclusively on leaves, flowers and barks of plants, until the 1970s when synthetic drugs came into use. In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients are extracted, while in traditional medicine, a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food. Plant extracts are potential sources of novel antimicrobial compounds, especially against bacterial pathogens, but it is necessary to scientifically investigate those plants which have been used in traditional medicine to improve the quality of healthcare. Plants extracts are usually composed of many phytochemical constituents (Avula *et al* 2015).

Botany and medicine have been closely linked throughout history. Prior to this century, medical practitioners whether allopath (medical doctors), homeopaths, naturopaths, herbalist or shamans had to know the plants in the area and how to use them since many of their drugs were derived from plants (Das *et al*., 2010). Around 1900, 80% of the drugs were derived from plants.

Many Vernonia species are reported to have antimicrobial properties. Worth mentioning are *V. amygdalina* (Erasto *et al*., 2006 ; Sharma and Sharma, 2010; Adetutu *et al*., 2011), *V. auriculifera* (Hamill *et al*., 2003), *V. cinerea* (Yoga-Latha *et al*., 2009), *V. colorata* and *V. leopoldii* (Mothana *et al*., 2009). *V. hymenolepsis* had shown antibacterial activity against Gram-negative multidrug-resistant bacteria (Noumedem *et al*., 2013). *V. galamensis* demonstrated antimicrobial activity against *E. coli, B. subtilis* and *S. aureus,* and the fungi *S. cerevisiae, Microsporum gypseum* and *Trichophyton mentagrophytes*. Furthermore, the antibacterial activity and mode of action of *V. adoensis* extracts against *S. aureus* and *P. aeruginosa* was reported (Mozirandi and Mukanganyana, 2017).

The development of antimicrobial-resistant bacterial species stems from a number of factors which include the prevalent and sometimes inappropriate use of antibiotics, extensive use of these agents as growth enhancers in animal feed, and increased transboundary passage of antibiotic-resistant bacteria. The problem of antibiotic resistance in humans and animals will continue for a long time (Andersson and Hughes, 2011). Against this backdrop, the development of alternative drug classes to treat such infectious diseases is urgently required (Srivastava, 2013).

Plants have an amazing ability to produce a wide variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins (Das, 2010). These biomolecules are the source of plant-derived antimicrobial substances (Srivastava, 2013). Some natural products are highly efficient in the treatment of bacterial infections. (Fernebro, 2011).

2.2 Search for new active phytochemicals

It is largely recognized that most of the currently available antimicrobials which are mainly synthetic are almost inefficient and most of these agents elicit terrible effects to recipients, For example, Stevens-Johnson syndrome and toxic epidermal necrolysis, and hypersensitivity reactions are associated with the administration of antimicrobials such as sulfonamide and fluoroquinolones, and penicillin, respectively (Ferrandiz-Pulido and Garcia-Patos, 2013 ; Patel *et al*., 2013). All the experts that proposed strategies/solutions to tackle the antimicrobial resistance crisis recognized that development of new and safe antimicrobials is more critical than any other proffered solutions strategies (Huttner *et al*., 2013 ; Roca *et al*., 2015). Many initiatives and programs have been set up by many countries/organizations with the aim of developing new, effective, and safe antimicrobials (Roca *et al*., 2015). For instance, the initiative proposed in 2010 is aimed at developing 10 new, safe, and effective antibiotics by 2020 (Infectious Disease Society of America, 2010). Thus, researchers/scientists are now looking at every ecological niche including soil, plant, animal, and marine for potentially new and safe antimicrobial agent (Laport *et al*., 2009 ; Nasir *et al*., 2015). Unfortunately, the rate at which microorganisms develop resistance outpaces the rate of discovery/development of new drugs (Huttner *et al*., 2013).

The African traditional medicine is the oldest medicinal system and often culturally referred to as the Cradle of Mankind (Van Vuuren, 2008). Traditional herbal medicines have been used to treat infectious diseases for thousands of years in various parts of the world (Cowan, 1999 ; Adebayo and Krettli, 2011). There has been a renewed interest in indigenous medicine worldwide because orthodox medicine is not widespread (Lifongo *et al*., 2014 ; Adebayo and Krettli, 2011). In poor countries, the health care has been sustained by other practices based on cultural alternatives

(Adebayo and Krettli., 2011). In many developing countries, including Nigeria, 80% of patients use indigenous herbal remedies to treat infectious diseases (Lifongo *et al*., 2014 ; Nasir *et al*., 2015 ; Willcox and Bodeker, 2004). Despite the availability of modern medicine in some communities, herbal medicines (medicinal plants) have continued to maintain popularity for historical and cultural reasons, in addition to their efficacy and cheaper cost (Lifongo *et al*., 2014 ; Nasir *et al*., 2015 ; Krettli., 2011) . They also represent sources of potentially important new pharmaceutical substances since all parts of a plant, from roots to seed heads and flowers, are employed in traditional remedies and can, therefore, act as sources of lead compounds. Moreover, molecules from natural products have represented about 80% of drugs that have been put into the market. The use of plant remedies has steadily increased worldwide in recent years as well as the search for new phytochemicals that potentially could be developed as useful drugs for the treatment of infectious diseases (van Vuuren, 2008 and Nasir *et al*., 2015).

Plant with antimicrobial properties undergoes various steps; extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. Some potential active compounds are lost due to the preparation of the extract from the plant sample

2.3 Extraction

Plant extraction is a process that aims to extract certain components present in plants. It is a solid/liquid separation operation: a solid object (the plant) is placed in contact with a fluid (the solvent). The plant components of interest are then solubilized and contained within the solvent. The solution thus obtained is the desired extract.

Therefore, proper actions must be taken to assure that it retain its constituents. If the plant was selected on the basis of traditional uses (Fabricant and Farnsworth, 2001), then it is needed to

prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional 'herbal' drug. The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used in some instances. Extraction with hexane is used to remove chlorophyll (Huie, 2002).

As the target compounds may be non-polar to polar and thermally labile, the suitability of the methods of extraction must be considered. Various methods, such as sonification, heating under reflux, soxhlet extraction and others are commonly used (United States Pharmacopeia and National Formulary, 2002; Pharmacopoeia of the People's Republic of China, 2000; The Japanese Pharmacopeia, 2001) for the plant samples extraction. In addition, plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems.

The other modern extraction techniques include solid-phase micro-extraction, supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, solid-phase extraction, and surfactant-mediated techniques, which possess certain advantages. Some of the advantages are the reduction in organic solvent consumption and in sample degradation, elimination of additional sample clean-up and concentration steps before chromatographic analysis, improvement in extraction efficiency, selectivity, and/ kinetics of extraction. The ease of automation for these techniques also favors their usage for the extraction of plants materials (Huie, 2002).

2.4 Identification and characterization of extract

Due to the fact that plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC, could be used to obtain pure compounds that is responsible for the antibacterial activity. Besides that, non-chromatographic techniques such as immunoassay, which use monoclonal antibodies (MAbs), phytochemical screening assay, Fourier-transform infrared spectroscopy (FTIR), can also be used to obtain and facilitate the identification of the bioactive compounds (Nasir *et al*., 2015).

2.4.1 Thin-layer chromatography (TLC) and Bio-autographic methods

Thin Layer Chromatography is a simple, quick, and inexpensive procedure that gives the researcher a quick answer as to how many components are in a mixture. Thin Layer Chromatography is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound. Additional tests involve the spraying of phytochemical screening reagents, which cause color changes according to the phytochemicals existing in a plants extract; or by viewing the plate under the UV light. This has also been used for confirmation of purity and identity of isolated compounds.

Bio-autography is a useful technique to determine bioactive compound with antimicrobial activity from plant extract. Thin Layer Chromatography bioautographic methods combine chromatographic separation and *in situ* activity determination facilitating the localization and target-directed isolation of active constituents in a mixture. Traditionally, bioautographic

technique is used to detect anti-microbial components of extracts chromatographed on a TLC layer. This methodology has been considered as the most efficacious assay for the detection of anti-microbial compounds (Shahverdi, 2007). Bio-autography localizes antimicrobial activity on a chromatogram using three approaches: (i) direct bio-autography, where the micro-organism grows directly on the thin-layer chromatographic (TLC) plate, (ii) contact bio-autography, where the antimicrobial compounds are transferred from the Thin Layer Chromatography plate to an inoculated agar plate through direct contact and (iii) agar overlay bio-autography, where a seeded agar medium is applied directly onto the Thin Layer Chromatography plate (Hamburger and Cordell, 1987; Rahalison *et al*., 1991). The inhibition zones produced on TLC plates by one of the above bioautographic technique will be used to visualize the position of the bioactive compound with antimicrobial activity in the TLC fingerprint with reference to *Rf* values (Shahverdi, 2007). Preparative TLC plates with a thickness of 1mm were prepared using the same stationary and mobile phases, with the objective of isolating the bioactive components that exhibited the antimicrobial activity against the test strain. These areas will be scraped from the plates, and the substance will be eluted from the silica with ethanol or methanol. Eluted samples will be further purified using the preparative chromatography method. Finally, the components will be identified by HPLC, LCMS and GCMS. Although it has high sensitivity, its applicability is limited to micro-organisms that easily grow on TLC plates. Other problems are the need for complete removal of residual low volatile solvents, such as *n*-BuOH, trifluoroacetic acid and ammonia and the transfer of the active compounds from the stationary phase into the agar layer by diffusion . Because Bio-autography allows localizing antimicrobial activities of an extract on the chromatogram, it supports a quick search for new antimicrobial agents through bioassay-guided isolation . The Bio-autography agar overlay method is advantageous in that, firstly it uses very little amount of sample when compared to the normal disc diffusion method and hence, it

can be used for bioassay-guided isolation of compounds. Secondly, since the crude extract is resolved into its different components, this technique simplifies the process of identification and isolation of the bioactive compounds (Rahalison *et al*., 1991).

2.4.2 High performance liquid chromatography

High performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products (Cannell, 1998). Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants (Fan *et al.*, 2006). Natural products are frequently isolated following the evaluation of a relatively crude extract in a biological assay in order to fully characterize the active entity. The biologically active entity is often present only as minor component in the extract and the resolving power of HPLC is ideally suited to the rapid processing of such multicomponent samples on both an analytical and preparative scale. Many bench top HPLC instruments now are modular in design and comprise a solvent delivery pump, a sample introduction device such as an auto-sampler or manual injection valve, an analytical column, a guard column, detector and a recorder or a printer.

Chemical separations can be accomplished using HPLC by utilizing the fact that certain compounds have different migration rates given a particular column and mobile phase. The extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase. Generally the identification and separation of phytochemicals can be accomplished using isocratic system (using single unchanging mobile phase system). Gradient elution in which the proportion of organic solvent to water is altered with time may be desirable if more than one sample component is being studied and differ from each other significantly in retention under the conditions employed.

Purification of the compound of interest using HPLC is the process of separating or extracting the target compound from other (possibly structurally related) compounds or contaminants. Each compound should have a characteristic peak under certain chromatographic conditions. Depending on what needs to be separated and how closely related the samples are, the chromatographer may choose the conditions, such as the proper mobile phase, flow rate, suitable detectors and columns to get an optimum separation.

Identification of compounds by HPLC is a crucial part of any HPLC assay. In order to identify any compound by HPLC, a detector must first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed. UV detectors are popular among all the detectors because they offer high sensitivity (Fan *et al*., 2006). and also because majority of naturally occurring compounds encountered have some UV absorbance at low wavelengths (190–210 nm) (Cannell, 1998). The high sensitivity of UV detection is bonus if a compound of interest is only present in small amounts within the sample. Besides UV, other detection methods are also being employed to detect phytochemicals among which is the diode array detector (DAD) coupled with mass spectrometer (MS) (Tsao and Deng, 2004). Liquid chromatography coupled with mass spectrometry (LC/MS) is also a powerful technique for the analysis of complex botanical extracts (Cai *et al.*, 2002; He, 2000). It provides abundant information for structural elucidation of the compounds when tandem mass spectrometry (MS^n) is applied. Therefore, the combination of HPLC and MS facilitates rapid and accurate identification of chemical compounds in medicinal herbs, especially when a pure standard is unavailable (Ye *et al*., 2007).

The processing of a crude source material to provide a sample suitable for HPLC analysis as well as the choice of solvent for sample reconstitution can have a significant bearing on the overall success of natural product isolation. In the case of dried plant material, an organic solvent (e.g., methanol, chloroform) may be used as the initial extractant and following a period of maceration, solid material is then removed by decanting off the extract by filteration. The filtrate is then concentrated and injected into HPLC for separation. The usage of guard columns is necessary in the analysis of crude extract. Many natural product materials contain significant level of strongly binding components, such as chlorophyll and other endogenous materials that may in the long term compromise the performance of analytical columns. Therefore, the guard columns will significantly protect the lifespan of the analytical columns (Heiu, 2002).

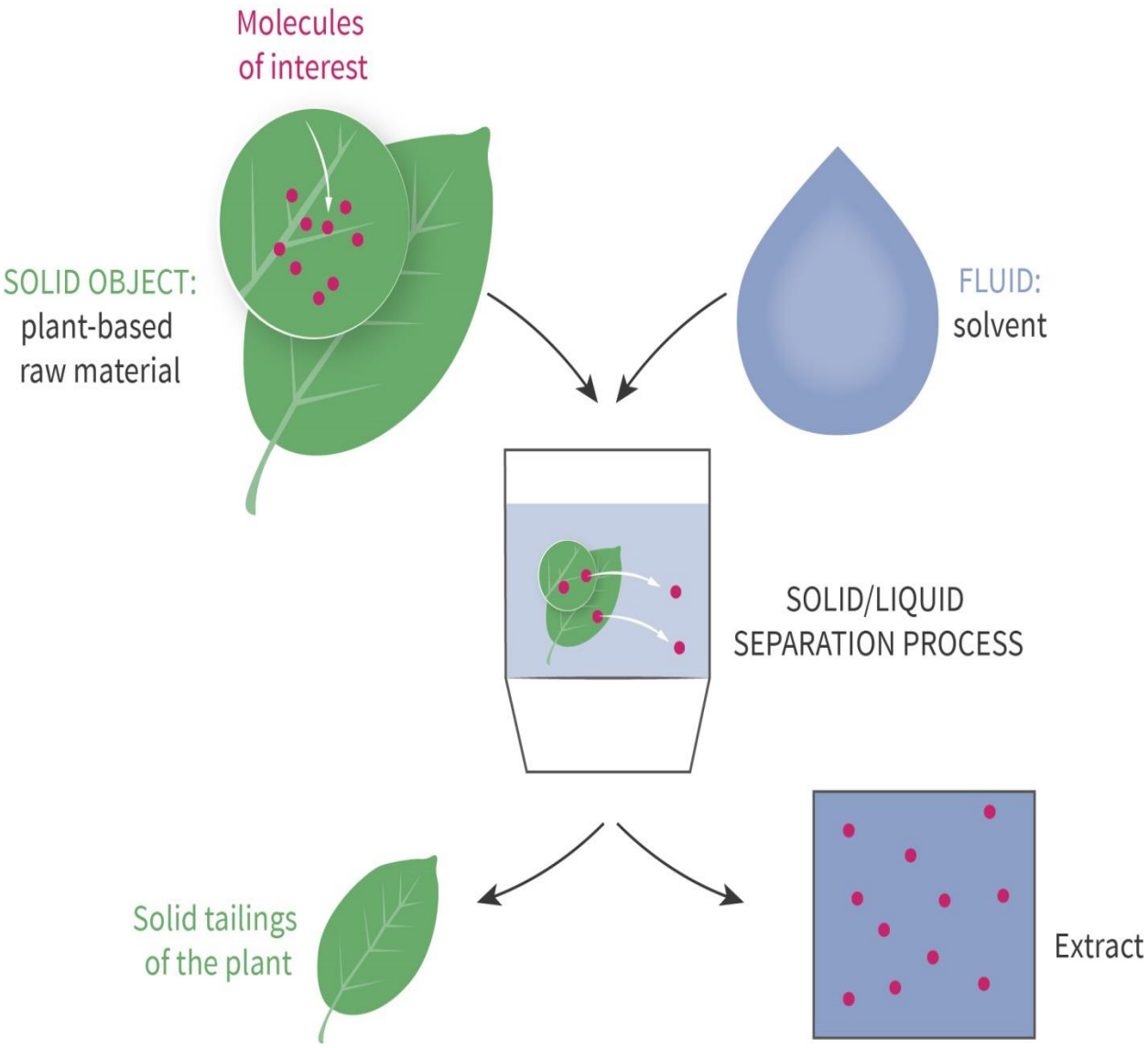

Fig1. Processes in plant extraction (Source: CDC, 2012)

2.5 Research microorganisms

Bacteria that can cause human disease are majorly pathogenic bacteria (Ryan *et al.,* 2014). Although most bacteria are harmless or often beneficial, some are pathogenic, with the number of species estimated as fewer than 100 that are seen to cause infectious diseases in humans. By contrast, several thousand species exist in the human digestive system. One of the bacterial diseases with the highest disease burden is *Staphylococcus* infection, caused by the bacterium *Staphylococcus aureus*, which kills about 2 million people a year, mostly in sub-Saharan Africa. (Schlecht, 2015). Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria such as *Pseudomonas* and *Bacillus* sp and foodborne illnesses, which can be caused by bacteria such as *E coli*, and *Klebsiella* (Ogawa *et al*., 2005). Pathogenic bacteria are also the cause of high infant mortality rates in developing countries (Santosham *et al.,* 2013). Each species has specific effect and causes symptoms in people who are infected. Some, if not most people who are infected with a pathogenic bacteria do not have symptoms. Immuno-compromised individuals are more susceptible to pathogenic bacteria (Santosham *et al.,* 2013). Some pathogenic bacteria cause disease under certain conditions, such as entry through the skin via a cut, through sexual activity or through a compromised immune function. *Staphylococcus aureus* are part of the normal skin microbiota and typically reside on healthy skin or in the nasopharangeal region. Yet these species can potentially initiate skin infections. These infections can become quite serious creating a systemic inflammatory response resulting in massive vasodilation, shock, and death. Other bacteria are opportunistic pathogens and cause disease mainly in people suffering from immunosuppression or cystic fibrosis. Examples of these opportunistic pathogens include *Pseudomonas aeruginosa* (Nash *et al.,* 2015*).*

2.5.1 *Staphylococcus aureus*

Staphylococcus aureus (also known as *golden staph*) is a Gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a member of the normal flora of the body, frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen (Masalha *et al*, 2001). Although *S. aureus* is not always pathogenic (and can commonly be found existing as a commensal), it is a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Despitemuch research and development there is no approved vaccine for *S. aureus*.

Staphylococcus was first identified in 1880 in Aberdeen, Scotland, by surgeon Sir Alexander (1984) in pus from a surgical abscess in a knee joint. This name was later amended to *Staphylococcus aureus* by Friedrich Julius Rosenbach, who was credited by the official system of nomenclature at the time. An estimated 20% to 30% of the human population are long-term carriers of *S. aureus* (Tong, 2015) which can be found as part of the normal skin flora, in the nostrils, and as a normal inhabitant of the lower reproductive tract of women (Senok *et al*., 2009) and (Hoffman, 2012). *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis. It is still one of the five most

common causes of hospital-acquired infections and is often the cause of wound infections following surgery. Each year, around 500,000 patients in hospitals of the United States contract a staphylococcal infection, chiefly by *S. aureus*. Up to 50,000 deaths each year in the USA are linked with *S. aureus* infections (Schlecht *et al*., 2015).

2.5.2 *Pseudomonas aeruginosa*

Pseudomonas is a genus of Gram-negative, Gammaproteobacteria, belonging to the family Pseudomonadaceae and containing 191 validly described species (Euzéby, 1997). The members of the genus demonstrate a great deal of metabolic diversity and consequently are able to colonize a wide range of niches (Madigan and Martinko, 2005). Their ease of culture in vitro and availability of an increasing number of Pseudomonas strain genome sequences has made the genus an excellent focus for scientific research; the best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth-promoting *P. fluorescens* (Lavigne *et al*., 2006). Because of their widespread occurrence in water and plant seeds such as dicots, the pseudomonads were observed early in the history of microbiology. The generic name Pseudomonas created for these organisms was defined in rather vague terms by Walter Migula in 1894 and 1900 as a genus of Gram-negative, rod-shaped and polar-flagellated bacteria with some sporulating species, the latter statement was later proved incorrect and was due to refractive granules of reserve materials (Palleroni, 2010). Despite the vague description, the type species, *Pseudomonas pyocyanea* (basonym of Pseudomonas aeruginosa), proved the best descriptor (Palleroni, 2010).

2.5.3 *Escherichia coli*

Escherichia coli (also known as ***E. coli***) is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of

warm-blooded organisms (endotherms) (Tenaillon *et al.,* 2010). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (CDC, 2012). The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship (Hudault *et al.,*2001). *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards (Russell and Jarvis, 2001).

E. coli and other facultative anaerobes constitute about 0.1% of gut flora, and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination. A growing body of research, though, has examined environmentally persistent *E. coli* which can survive for extended periods outside a host (Ishii and Sadowsky, 2008).

The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *E. coli* is a chemoheterotroph whose chemically defined medium must include a source of carbon and energy. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favorable conditions, it takes up to 20 minutes to reproduce (Tortora, 2010).

Most *E. coli* strains do not cause disease (Han and lee, 2006), but virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and Gram-negative pneumonia. Very young children are more susceptible to develop severe illness, such as hemolytic uremic syndrome, however, healthy individuals of all ages are at risk to the severe consequences that may arise as a result of being infected with *E. coli*. (Lim *et al*., 2017)

2.5.4 *Klebsiella pneumoniae*

Klebsiella is a genus of nonmotile, Gram-negative, oxidase-negative, rod-shaped bacteria with a prominent polysaccharide-based capsule(Ryan and ray, 2004) . *Klebsiella* species are found everywhere in nature. This is thought to be due to distinct sublineages developing specific niche adaptations, with associated biochemical adaptations which make them better suited to a particular environment. They can be found in water, soil, plants, insects and other animals including humans (Brisse *et al*., 2006).

Klebsiella is named after German-Swiss microbiologist Edwin Klebs (1834–1913). Carl Friedlander described *Klebsiella* bacillus which is why it was termed Friedlander bacillus for many years. The members of the genus *Klebsiella* are a part of the human and animal's normal flora in the nose, mouth and intestines. The species of *Klebsiella* are all gram-negative and non-motile. They tend to be shorter and thicker when compared to others in the Enterobacteriaceae family. The cells are rods in shape and generally measures 0.3 to 1.5 µm wide by 0.5 to 5.0 µm long. They can be found singly, in pairs, in chains or linked end to

end. *Klebsiella* can grow on ordinary lab medium and do not have special growth requirements, like the other members of Enterobacteriaceae. The species are aerobic but facultatively anaerobic. Their ideal growth temperature is 35° to 37 °C, while their ideal pH level is about 7.2 (Ristuccia and cunha, 1984). *Klebsiella* species are routinely found in the human nose, mouth, and gastrointestinal tract as normal flora; however, they can also behave as opportunistic human pathogens. *Klebsiella* species are known to also infect a variety of other animals, both as normal flora and opportunistic pathogens.

Klebsiella organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, meningitis, diarrhea, and soft tissue infections. *Klebsiella* species have also been implicated in the pathogenesis of ankylosing spondylitis and other spondyloarthropathies. The majority of human *Klebsiella* infections are caused by *K. pneumoniae*, followed by *K. oxytoca*. Infections are more common in the very young, very old, and those with other underlying diseases, such as cancer, and most infections involve contamination of an invasive medical device (Ristuccia and cunha, 1984).

2.5.5 *Bacillus subtilis*

Bacillus subtilis is a Gram-positive bacterium, rod-shaped and catalase-positive. It was originally named *Vibrio subtilis* by Christian Gottfried Ehrenberg, and renamed *Bacillus subtilis* by Ferdinand Cohn in 1872 (subtilis being the Latin for 'fine'). *B. subtilis* cells are typically rod-shaped, and are about 4-10 micrometers (μm) long and 0.25–1.0 μm in diameter, with a cell volume of about 4.6 fL at stationary phase (Yu *et al.*, 2014). As with other members of the genus *Bacillus*, it can form an endospore, to survive extreme environmental conditions of temperature and desiccation (Madigan, 2005). *B. subtilis* is a facultative anaerobe and had been considered as an obligate aerobe until 1998. *B. subtilis* is heavily flagellated, which gives it the

ability to move quickly in liquids. *B. subtilis* has proven highly amenable to genetic manipulation, and has become widely adopted as a model organism for laboratory studies, especially of sporulation, which is a simplified example of cellular differentiation. In terms of popularity as a laboratory model organism, *B. subtilis* is often considered as the Gram-positive equivalent of *Escherichia coli*, an extensively studied Gram-negative bacterium.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sample collection and preparation

Fresh young leaf of *V. amygdalina* were collected from a farm in Malete, Kwara State, Nigeria. The leaf was identified at the herbarium unit of the department of Plant Biology University of Ilorin, Ilorin, Kwara State. The fresh leaf samples were placed on newsprint paper and air-dried at 40^{o}C to constant weight. Thereafter, the dried leaves were blended using a blender and the powdered sample kept in a clean plastic container until required.

3.2 Extraction

One kilogram (1kg) of powdered leaf sample was weighed and soaked in three (3) liters of n-Hexane for 72hrs to defat the sample, then the defatted sample was soaked in three (3) liters of ethanol for 72hrs.

The n-Hexane and Ethanol sample were filtered using Whatman filter paper No.1, this was carefully done to obtain a clear filtrate. The filtrates were concentrated using rotary evaporator and was labeled n-Hexane extract and defatted ethanolic extract.

3.3 Partial purification of *V. amygdalina* leaves extract

A solution of sodium hydroxide (NaOH) was added to the defatted ethanolic extract, then it was transferred into a separating funnel. Dichloromethane of volume of 2:1 to that of the defatted basify extract was added into the separating funnel for washing. The separating funnel was covered with its glass cork and gently rotated (shaken) while the end part of it (i.e. the control end of it) was slightly opened to allow the free escape of pressure produced during the process of shaking. This was done to ensure adequate mixing between the plant extract and the solvent. After shaking, the separating funnel was carefully clamped on the retort stand to allow the

solvents to separate. Two layers were formed with ethanolic fraction settled at the bottom while dichloromethane fraction settled at the top. The fractions were collected separately and hydrochloric acid (HCl) was added to the dichloromethane fraction to neutralize it.

3.4 Methanolysis

Methanolysis was carried out on the neutralized ethanolic extract of *V. amygdalina.* Two liters (2 L) of methanol was added to the neutralized extract and was transferred into a round bottom flask. Potassium carbonate (K_2CO_3) was added for metabolic processes which lasted for 72 hour at 90^0C . Thereafter, the methanol was removed (using rotary evaporator) and product of methanolysis was dissolved in water to remove the carbonate in the reaction before adding dichloromethane, which was pulled together, dried over Na_2SO_4 and then concentrated to dryness. This extract was then used in running the chromatography.

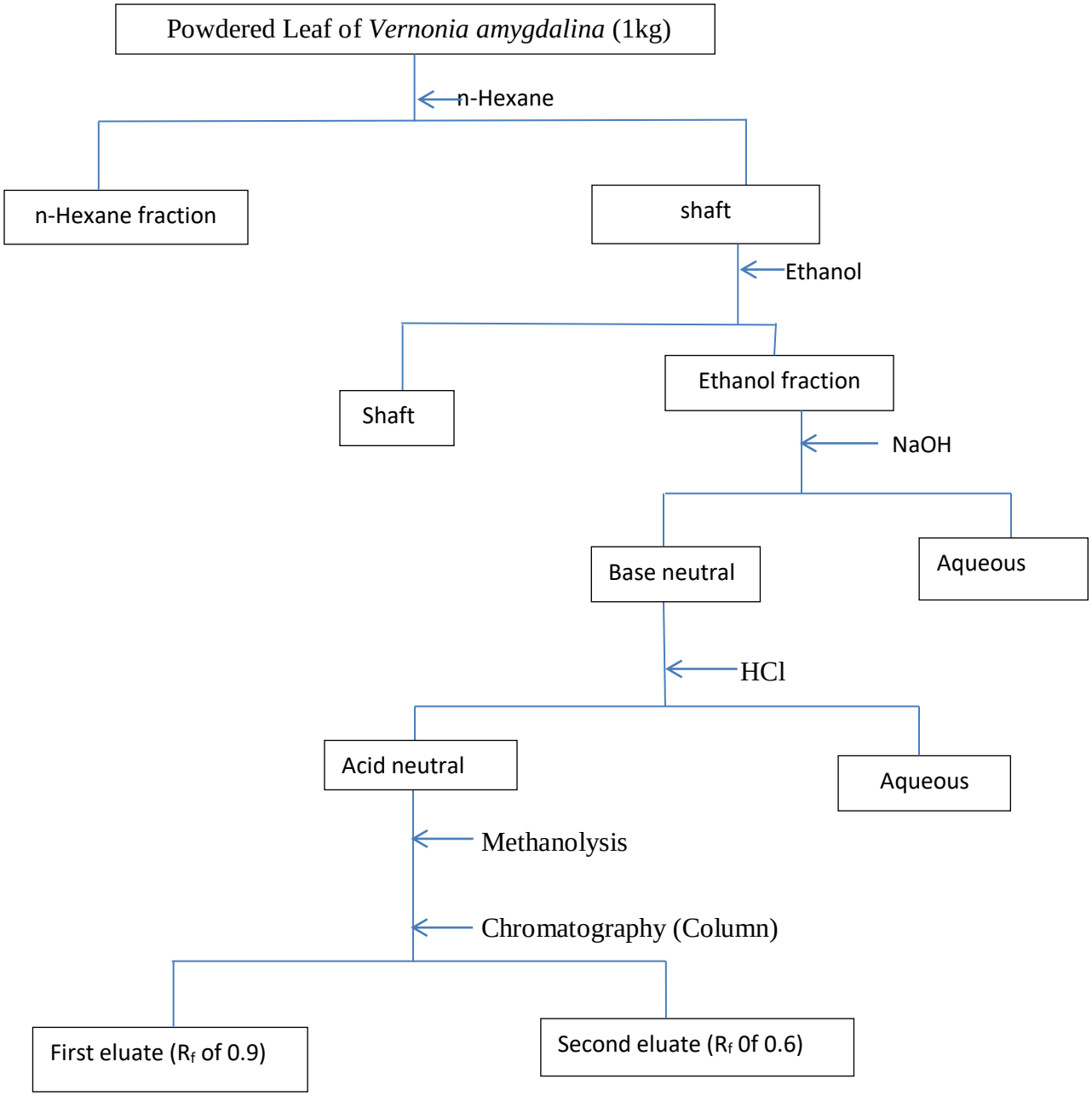

Fig 2: Flow chart of *Vernonia amygdalina* leaves extract treatments.

3.5 Chromatography methods:

3.5.1 Thin layer chromatography

TLC was used to assess the number of fractions that was present in the crude extract before chromatography and the R_f was determined. This was done by preparing the solvent system of Dichloromethane and Methanol of ratio 6:1.

3.5.2 Solvent system determination:

Solvent mixtures of dichloromethane and methanol of ratio 12:3, 12:2 , 8:4, 7:5, 5:5 and hexane and methanol (8:2, 12:4, 11:3) were taken as trials (with dichloromethane being less polar than methano). After trying several ratios of the solvents mixtures, the ratio of 12: 2 of dichloromethane to methanol was shown to be the best because the spots on the TLC was well separated from each other.

Procedure:

A TLC plate was cut to a suitable size and a line was drawn lightly with soft pencil from the bottom end horizontally. A small solution of the plant extract material was spotted on the line marked with pencil on the thin layer plate. Some amount of eluent was poured into a jar which when the plate was placed inside did not touch the spot. The solvent which serve as the mobile phase was used in mobilizing the extract upward and different spot was shown on the plate after few minutes and then viewed under Ultra-Violet light.

3.5.3 Visualization with U-V light

The plate was taken into the U-V light device for observation. Two (2) spots from the sample were observed to be distinct from each other and were marked with pencil. The solvent front of the spot was determined using a meter rule which was used to calculate the retention factors of each of the spots. The retention factor was calculated using the following formula:

3.5.4 Retention factor (Rf) = Distance travel by the sample spot = x

Distance travel by the solvent = y

$$Rf = x/y$$

3.5.6 Column chromatography

The column (20mm) was mounted in a vertical position. A piece of glass wool was blown down the column and little gram of sand was poured gently into the column at a very close distance from the glass wool. Uniform bedding was formed while the sand was being poured inside. Sample to be separated was dissolved in dichloromethane. The column, containing the glass wool and the sand, was washed down with the solvent system (Dichloromethane and methanol). Silica gel was mixed with solvent in a beaker with proper swirling and later, the slurry was poured into the column until the silica solution was fully added. Tapping was done carefully during this process in order to have uniformity on the bed. The bed was allowed to settle and form a uniform level. A little gram of sand was added gently to prevent leakage. The sample was added with the aid of a spatula. The solvent mixture was added and allowed to separate. Two fractions were collected with different color after the elution of the compound.

3.7 Media preparation

Mueller hinton agar (MHA) and nutrient broth were used following the manufacturer's instructions. For Mueller Hinton agar, 38g of the agar was dissolved in one litre of distilled water while 15g per litre of distilled water was used in preparation of nutrient broth. Preparation was done using conical flasks; homogenization was done using hot plate. The homogenized agar and

the broth were sterilized using an autoclave at 121^0C for 15 minutes. The sterilized medium (MHA) was allowed to cool to about 40^0C - 45^0C and then poured into the already sterilized petri dishes aseptically, after which the plates were wrapped with foil paper and placed in a refrigerator for future use.

3.8 Preparation of test organisms

The bacteria isolates used were sub-cultured using sterile nutrient broth and incubated at 37^0C for fourteen (14) hours, after which they were used. Prepared Mueller Hinton agar plates divided with marker were seeded with each of these test organisms and the plant extracts. Streaking was done with the use of cotton swab sticks. Cork borer of 4mm was used to make well on the seeded plates. On each plate containing different test organisms, 100µml (0.1ml) of each of the extract was dissolved in 1ml of DMSO to make a solution, while DMSO without an extract was used as a control. The plates were placed on the bench for few minutes to diffuse and later placed in the incubator at 37^0C for 18-24 hours for observation. The experiment was performed in triplicate and the resulting zones of inhibition were recorded.

CHAPTER FOUR

4.0 RESULTS

The result of the standard antibiotics against the Gram negative bacteria was recorded as shown in Table 1. Tarivid (OFX) shows zone of inhibition of 24 mm, 30 mm and 26 mm against *K. pneumonia*, *P. aeruginosa* and *E. coli* respectively, while nalixidic acid (NA) has no effect against *K. pneumonia* and *E. coli* but zone of inhibition of 20 mm was recorded for *P. aeruginosa*. Reflaxin (PEF) shows zone of 24 mm against *K. pneumonia*, 31 mm against *P. aeruginosa* and 30 mm against *E. coli*. Gentamycin (CN) has a zone of 20 mm against *K. pneumonia*, 22 mm against *P. aeruginosa* and 20mm *E. coli* . Augmentin (AU) was effective only against *P. aeruginosa*, Ciproflox(CPX) shows a zone of 30 mm, 36 mm and 30 mm against *K. pneumonia*, *P. aeruginosa* and *E. coli* respectively. Septrin (SXT) shows a zone of 20 mm against *K. pneumonia*, 22mm against *P. aeruginosa* and 24mm against *E. coli*. Streptomycin (S) has a record of zone of inhibition of 16 mm against *K. pneumonia*, 23 mm against *P. aeruginosa* and 14 mm against *E. coli*. Ampicillin (PN) was not effective against *E. coli* but shows zone of inhibition of 12 mm against *K. pneumonia* and 22 mm against *P. aeruginos.* Although ceporex (CEP) was not effective against *K. pneumonia* but has a zone of inhibition of 18 mm against *P. aeruginosa* and 20 mm against *E. coli*.

Table 1: Susceptibility of Gram negative organisms to standard antibiotics

Standard antibiotics	*Klebsiella Pneumonia*	*Pseudomonas aeruginosa*	*Escherichia coli*
	Zone of inhibition in (mm)		
OFX	24	30	26
NA	0	20	0
PEF	24	31	30
CN	20	22	20
AU	0	18	0
CPX	30	36	30
SXT	20	22	24
S	16	23	14
PN	12	22	0
CEP	0	18	20

Key:

OFX (Tarivid), NA (Nalixidic acid), PEF (Reflaxin), CN(Gentamycin), AU(Augmentin), CPX (Ciproflox), SXT (Septrin), S(Streptomycin), PN (Ampicillin), CEP (Ceporex)

Table 2 shows susceptibility test on Gram positive bacteria (i.e *Staphylococcus aureus* and *Bacillus subtilis*). The result shows that Ciproflox(CPX) has a zone of inhibition of 32 mm on *Staphylococcus aureus* and 33 mm against *Bacillus subtilis* . Erythromycin (E) shows a zone of inhibition of 18 mm against *Staphylococcus aureus* and 14 mm against *Bacillus subtilis*. Levofloxacin (LEV) was recorded with a zone of inhibition of 30 mm against *Staphylococcus aureus* and 32 mm against *Bacillus subtilis*, while gentamycin(CN) was not effective against *Bacillus subtilis* but has a zone of inhibition of 20 mm against *Staphylococcus aureus*. Ampiclox (APX) shows a zone of 14 mm against *Staphylococcus aureus* and not effective against *Bacillus subtilis*. Rifampicin (RD) was recorded to have a zone of inhibition of 20 mm against *Staphylococcus aureus* and 12 mm against *Bacillus subtilis*, but Amoxillin (AMX) was only active against *Staphylococcus aureus* with a zone of inhibition of 19 mm and has no effect against *Bacillus subtilis*. *Staphylococcus aureus* was susceptible to Streptomycin (S) with a zone of inhibition of 24 mm and *Bacillus subtilis* responded to it with a zone of inhibition of 14 mm. Norfloxacin (NB) was active only against *Staphylococcus aureus* but no zone was found against *Bacillus subtilis* while chloramphenicol (CH) shows a zone of inhibition of 22 mm against *Staphylococcus aureus* and 12 mm on *Bacillus subtilis*.

Table **2: Susceptibility of Gram positive organisms to standard antibiotics**

Standard antibiotics	*Staphylococcus aureus*	*Bacillus subtilis*
	Zone of inhibition in (mm)	
CPX	32	33
E	18	14
LEV	30	32
CN	20	0
APX	14	0
RD	20	12
AMX	19	0
S	24	14
NB	16	0
CH	22	12

Key :

CPX(Ciproflox), E(Erythromycin), LEV(Levofloxacin), CN(Gentamycin), APX(ampiclox), RD(rifampicin), AMX (Amoxillin), S (Streptomycin), NB(norfloxacin), CH(Chloramphenicol),

The antibacterial activities of the plant extract on the test organisms were carried out and the zone of inhibition was recorded as shown in Table 3. The hexane, ethanolic and C-2 extract shows no zone of inhibition on the five organisms. Basified extract shows a zone of inhibition of 10 mm against *E. coli*, 11 mm against *K. pneumonia* and 11 mm against *S. aureus*, while methanolysed extract shows zone of inhibition of 10mm against *E. coli* and 11 mm against *S. aureus*. The C-1 shows a zone of inhibition of 12 mm against *E. coli* and zone of inhibition of 10 mm against *S. aureus*, C-2 has a zone of inhibition of 11 mm against *E. coli* and zone of 10 mm against *S. aureus* while C-1a was only active against *S. aureus* with zone of inhibition of 8 mm.

Table 3: Antibacterial activities of *V. amygdalina* fractions

	Diameter of zone of inhibition (mm) at 500µg/ml				
Fractions	*E.coli*	*Klebsiella*	*Pseudomonas*	*Bacillus*	*Staphylococcus*
1(Hexane)	0	0	0	0	0
2(Ethanol)	0	0	0	0	0
3(Basified)	10	11	0	0	11
4(Metholysed)	10	0	0	0	11
5(C-1)	12	0	0	0	10
6(C-2)	11	0	0	0	10
7(C-1a)	0	0	0	0	8
8(C-2a)	0	0	0	0	0

Key :

C-1(Chromatography 1, Rf =0.9),

C-2(Chromatography 2, Rf =0.6),

a (increased in volume per hole)

Table 4 shows the antibacterial activities of partially purified *V. amygdalina* leaves fraction against *Escherichia coli* and *Staphylococcus aureus* with concentration of 750µg/ml. The zone of inhibition of basified fraction was 13 mm against *Escherichia coli* and 14 mm against *Staphylococcus aureus* while the zone of inhibition on methanolysed extract was 14 mm against both the *Escherichia coli* and *Staphylococcus aureus*. The first chromatography extract (C-1) shows a zone of inhibition of 17 mm against *Escherichia coli* and 13 mm against *Staphylococcus aureus*, C-2 has a zone of inhibition of 15 mm against *Escherichia coli* and 12 mm against *Staphylococcus aureus*.

Table 4: Antibacterial activities of partially purified fractions *V. amygdalina* leaves

Fractions	Diameter of zone of inhibition(mm) at750µg/ml	
	Escherichia coli	*Staphylococcus aureus*
3(Basified)	13	14
4(Methanolysed)	14	14
5(C-1)	17	13
6(C-2)	15	12

Key : (3) Basified extract;

(4) Methanolysed extract;

(5) First chromatography extract (Rf=0.9);

(6) Second chromatography extract (Rf=0.6)

4.5 Determination of retention factor

Solvent mixtures;

	Dichloromethane	:	Methanol
Ratio	12	:	2

Rf (a) = $\frac{28mm}{45mm}$ = 0.62

Rf (b) = $\frac{41mm}{45mm}$ = 0.91

CHAPTER FIVE

5.1 DISCUSSION

Vernonia amygdalina leaves extract has been found to be effective against the selected bacteria. The result obtained is supported by previous claims, that *Vernonia amygdalina* is medicinal plant with therapeutic potentials. Sofowora (1993) stated that traditional medicinal plants are a therapeutic resource used by the population of the African continent specifically for health care, which may also serve as starting materials for drug. The use of medicinal plant in developing countries as a normative basis for the maintenance of good health has been widely observed (UNESCO, 1996). Report has shown that phytochemicals are present in *Vernonia amygdalina* leaves for plant normal metabolic processes. Some of these chemicals are often refered to as "secondary metabolites", which include alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoides (Okwu, 2004). The result from this study support the report of Anibijuwon *et al.* (2012) that *Vernonia amygdalina* leaves have bactericidal effect against *S.aureus*. Chromatography extracts recorded high zone of inhibition on *E. coli* than other fractions (Table 3 and 4). This is in line with the report of Muluye *et al.* (2015) which state that chromatography extract are more effective than crude extract. Furthermore, basified fractions were effective against *E. coli, Klebsiella pneumoniae* and *Staphylococcus aureus* but *Pseudomonas aeruginosa* and *Bacillus substilis* were resistant. This maybe as a result of *Pseudomonas aeruginosa* known to have diverse metabolic activity and *Bacillus subtilis* is spores formers having the ability to withstand adverse condition. This is in agreement with the report of Evbuomwan *et al.* (2018). So also, *Pseudomonas aeruginosa* and *Bacillus substilis* indicated no zone of inhibition in all the dilutions examined. This revealed the resistant ability of the organisms (*Pseudomonas aeruginosa* and *Bacillus substilis)* to *Vernonia*

amygdalina extracts. The n-hexane fraction was observed to have no inhibitory effect on the tested organisms. This also could mean that the inhibitory property needed in fraction to inhibit the bacteria isolate was not present in the oil, since the n-hexane as a solvent does not have antimicrobial property.

The activities of Standard antibiotics on Gram negative bacteria, ciproflox (CPX) shows to be the most active antibiotic against both Gram positive and Gram negative bacteria (Table 1). Ciproflox was specifically highly effective against *Pseudomonas aeruginosa* with diameter of zone of inhibition of 36 mm while *Klebsiella pneumonia* and *Escherichia coli* had a diameter of zone of inhibition of 30 mm and 30 mm respectively. Although augmentin (AU) was least effective against the Gram negative organisms with no diameter of zone of inhibition against *Klebsiella pneumonia* and *Escherichia coli* ; *Pseudomonas* was susceptible to augmentin (AU) with diameter of zone of inhibition of 18 mm.

In Table 2, Ciproflox (CPX) was the most active antibiotic against the two Gram positive organism that were tested i.e. *Staphylococcus aureus* and *Bacillus subtilis*, but *Bacillus* was the most susceptible organism against the antibiotic while *Bacillus* was resistant to ampiclox (APX) and *Staphylococcus aureus.* This is because it is a broad spectrum antibiotic known for the purpose of treating infections caused by the Gram positive and Gram negative organisms. This is in line with the work of Evbuomwan *et al.* (2018).

Streptomycin had similar effect with methanolyse extract on *E. coli* while ampiclox (APX) has a potency that equals that of base neutral and methanolysed extract on *Staphylococcus aureus* (Table 4). This similarity may be attributable to the level of plant purification.

5.2 Conclusion

This research work has shown that *Vernonia amygdalina* has potential bioactive phytochemicals that may be responsible for its antibacterial activities. It has also proven that bitter leaf extract contained more potent antibacterial substances than conventional antibiotics against *Escherichia coli* and *Staphylococcus aureus*. The results revealed that purified extracts (chromatography extract) showed higher inhibitory effects on most of the test organisms when compared with other extracts, hence appraising chromatography as a better purification method.

5.3 Recommendation

Based on this research the following are recommended;

- More research should be focused on plants as they contain phytochemicals that has antibacterial properties and some are yet to be discovered.
- Purified extract should be used instead of crude extracts.
- *Vernonia amygdalina* could be used to treat *Staphylococcus aureus* and *Escherichia coli* infection.

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Appendix 1

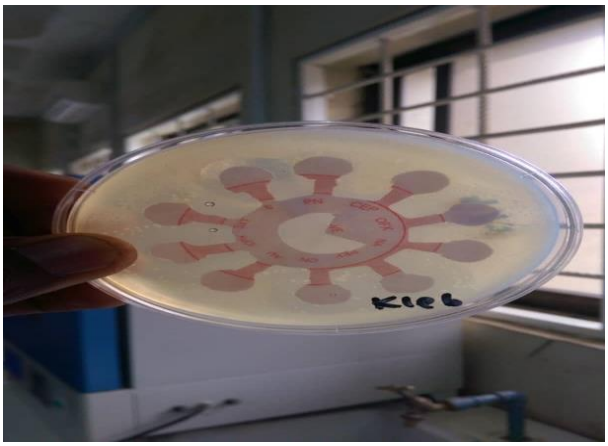

Plate 1: *K. pneumonia* against antibiotics

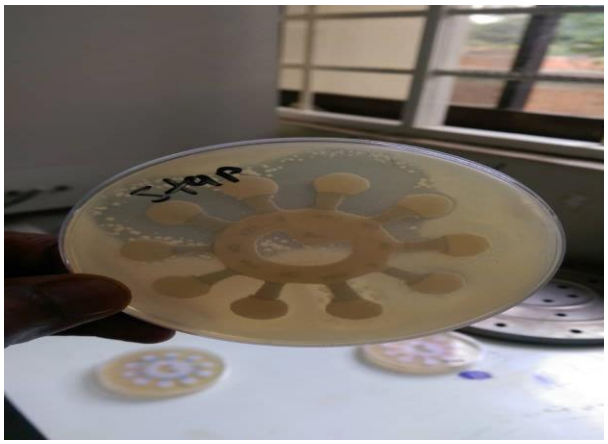

Plate 2*: S. aureus* against antibiotics

Plate 3: *P. aeruginosa* against antibiotics

Plate 4: *B. subtilis* against antibiotics

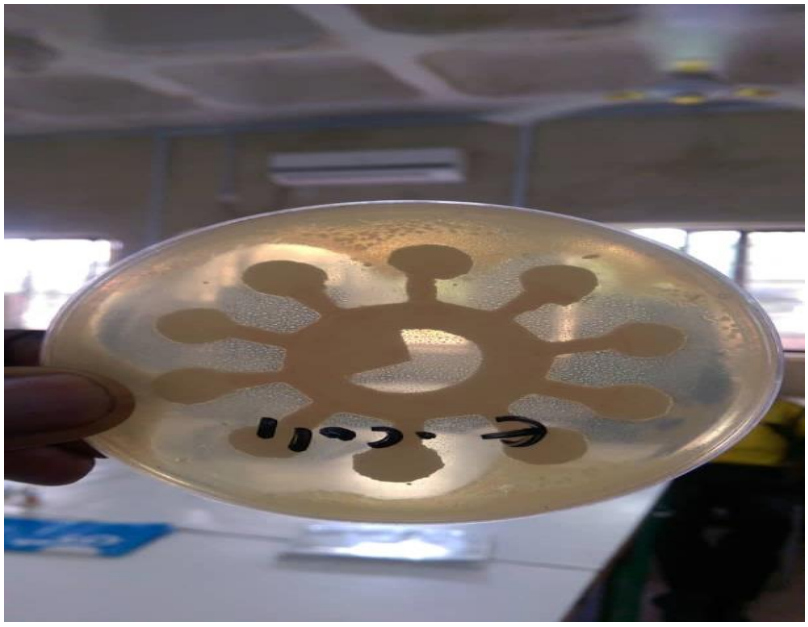

Plate 5 : *E.coli* against antibiotics

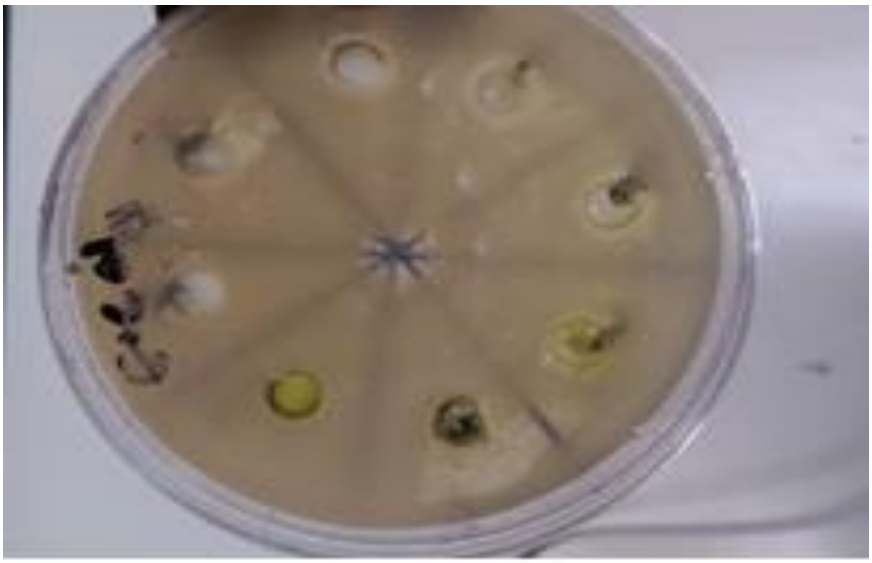

Plate 6: Effect of *V. amygdalina* leaf extract against *E. coli*

Plate 7: effect of *V. amygdalina* leaf against *S. aureus*