In Vitro Anti-bacterial Activities of Aqueous, Ethanol and Chloroform Crude Extracts of *Olinia rochetiana*, *Vernonia myriantha* and *Diclipteria laxata*

By

Ayana Erdedo

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Declaration

I declare that this thesis is submitted to the School of Graduate Studies of Addis Ababa University for the Master's Degree in Biology (Applied Microbiology). I would like to prove through my signature below that it is my own independent work and has not earlier been submitted elsewhere by me or anybody else. All authors of the references cited in the current study were duly acknowledged.

Candidate	Date	Signature
Ayana Erdedo	________________	________________

Department of microbial, cellular and molecular biology, Collage of natural and computational science, Addis Ababa university

This is to certify that the thesis prepared by Ayana Erdedo, entitled: “In vitro anti- bacterial activities of aqueous, ethanol and chloroform crude extracts of *Olinia rochetiana*, *Vernonia myriantha* and *Diclipteria laxata*” and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Applied Microbiology fulfils with the regulations of the University and meets the accepted standards with respect to originality and quality.

Approved by Examining Board	**Signature**
Hassen Mamo (PhD) (Advisor)	_________
FassilAssefa (PhD) (Examiner)	_________
Teganu Gelane (PhD) (Examiner)	_________
Asnake Dessalegn (PhD) (Chair of the Department)	_________

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Acronyms

AAU	Addis Ababa University
ANOVA	Analysis of Variance
AST	Antimicrobial Susceptibility Test
ATCC	American Type Culture Collection
BW	Bodyweight
CFU	Colony Forming Unit
CLSI	Clinical Laboratory Standard Institute
EPHI	Ethiopian Public Health Institute
FAD	Food and drug administration
MBC	Minimum Bactericidal Concentration
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MIC	Minimum Inhibitory Concentration
OECD	Organization for Economic Co-operation
SEM	Standard Error of Mean
WHO	World Health Organization

Abstract

With the advent of antibiotics, bacterial diseases which claimed enormous lives in the past have been put under control. However, the emergence and rapid spread of antibiotic-resistant bacterial strains is reversing this success and searching for newer antibacterial agents is currently a top priority. This study was, thus, aimed at assessing the anti-microbial activities of three traditional medicinal plants: *Vernonia myriantha*, *Diclipteria laxata* and *Olinia rochetiana*. The leaves of the three plants and stem-bark of the last one was collected from their natural habitat in Hadiyya, southwest Ethiopia, washed and air-dried in shade and ground into a powder. Each plant powder was soaked in water, ethanol and chloroform in separate Erlenmeyer flasks in a 1:10 solute-solvent ratio and placed on a water bath shaker for 72 hours. The extracts were concentrated in a rotary evaporator and dried on an oven at 35℃ and the aqueous extract was freeze-dried using a lyophilizer. The crude extracts were tested for their *in vitro* antibacterial activities, *in vivo* acute toxicity and phytochemical content. The extracts were tested against selected 3 clinical and 4 standard test bacterial strains by using agar well-diffusion method and the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). The ethanol leaves and stem-bark extracts of *O. rochetiana* inhibited the growth of all bacterial strains at a concentration of 250mg/mL. The inhibition zones, ranged from 20.33±0.57mm for clinical *Pseudomonas aeruginosa* to 25.66±0.57mm for standard *Salmonella typhi* strains. The values for these same extracts were 20.66±2.51mm and 24.33±1.15mm for standard *P. aeruginosa* and *Staphylococcus aureus* strains respectively. The chloroform extract was similarly effective against all of the strains with inhibition zones between 19.00±1.73mm against *P. aeruginosa* and 22.66±2.51mm for *S. aureus.* Comparatively, the ethanol extract of *O. rochetiana* had the lowest MIC (1.95mg/mL) and MBC of 31.25mg/mL against *S. aureus.* The highest MIC (7.81mg/mL) and MBC (62.50mg/mL) were noted against *P. aeruginosa*. On the other hand, chloroform extract of *O. rochetiana* leaf showed the lowest MIC (3.9mg/mL) and MBC (62.5mg/mL) against *S. aureus* and the highest MIC (15mg/mL) and MBC (125mg/mL) were recorded against *P. aeruginosa*. The ethanol extract of *V. myriantha* showed growth inhibition only on *S. aureus* (21.00±1.7mm). While none of *D. laxata* extracts demonstrated any antibacterial activity, only the aqueous extract failed concerning *O. rochetiana* and *V. myriantha*. The ethanol and chloroform extracts of *O. rochetiana* and ethanol extract of *V. myriantha* were not toxic to Swiss albino mice up to dose 2000mg/kg. Both plants tested for terpenoids and glycosides showed positive result, but none for resin. Tannins, phenols, steroids and saponins were detected only in *O. rochetiana*, and flavonoid and alkaloid only in *V. myriantha*. It is worth considering these two plants for future antibacterial discovery studies in light of their potential and safety.

Keywords: Antibacterial activity, Minimum inhibitory concentration, Minimum bactericidal concentration, phytochemicals, crude extract, pathogenic bacteria

1. Introduction

Pathogenic bacteria including pathogenic *Escherichia coli*, Salmonella species, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* are among the common disease-causing bacteria. Bacterial diseases are the major cause of morbidity and mortality in the world, particularly in developing countries.WHO, (2015) reported that foodborne diseases accounted, almost 420,000 deaths in 2015. Among this, foodborne diarrheal disease accounts about 230,000 deaths in 2010. *S. typhi* account 52,000 deaths of all food borne death in the same year. Global emergence of drug resistant bacterial strains has been reported and this challenge limits the effectiveness of current drugs and significantly causing failure in the treatment of infectious diseases (Fackim *et al.,* 2017). The current levels of antibiotic usage often clinically led to increase drug resistance. Resistance of bacteria to antibiotics can be natural or acquired. It is natural upshots of the adaption of the pathogens to the exposures of antimicrobials used in medicine, food, crop production and to disinfections in farms and households.

Infection with antibiotic resistant bacteria may cause severe illness, increased mortality rates, and an increased risk of complications and admission to hospital (Livermore, 2012). According to the European Centre for Disease Prevention and Control, 25,000 people in Europe die each year as a result of resistant infection (European Centre for Disease Prevention and Control, 2011). Antibiotic-resistance leads to an increased amount of healthcare costs. It is estimated that complications associated with antibiotic-resistance cost 9 billion annually in Europe (Oxford and Kozlov, 2013).

As consequence, evaluating the medicinal activities of plants is very important as being a source of natural drugs against pathogens. Medicinal plant extracts provide significant potential for development of new antimicrobial agents since they possess different varieties of phytochemicals. Plants have been used for centuries as remedies of human disease and offer new bioactive chemical compounds as antimicrobial agents and that make them richest bio resources for medicinal systems, modern medicine, for folk medicines, pharmaceuticals and chemicals dignified for synthetic drugs (Srivastava *et al*., 2005). Medicinal use of plants and their products was passed down from generation to generation in various parts of the world throughout the history and has played significant role for the development of different traditional system of

medicine. As it is a sum of knowledge, skill practiced, belief and experiences to different cultures, it is wide spread throughout the world. Its acceptance mainly conditioned by cultural factors and much of traditional, for this reason, it may not be readily transferred from one culture to another (Karunamoorthi *et al.,* 2013).

According to world health organization, medicinal plants forms base for traditional or indigenous healthcare systems used by majority of inhabitants living in third world countries. Indeed, it is reported that more than 3.5milion people depend on plants for human and livestock aliment (Bensassi *et al.*, 2007; Coruh *et al.,* 2007). According to the World Health Organization (WHO), almost 80% of the world's population relay mainly on traditional medicines for their primary healthcare.

In general, many plant species have pharmacological properties as they are known to possess various secondary metabolites (El-Kamale and Amir, 2010). Plants have amazing ability to produce different types of secondary metabolites such as glycosides, saponins, flavonoids, steroids, alkaloids and tripenes which are utilized to combat pathogens (Srivastava *et al.* 2013). Secondary metabolites apart from determining traits of plants complete the functions of plant organs, showing both biological and pharmacological activities of the plant (Hartmann, 2008). They have been investigated as natural resource to treat microbial infections and also subsidize the development of new drugs with specific therapeutic properties.

A vast majority of modern medications were derived from ancient herbal traditions. Many drugs presently prescribed by physicians are either directly isolated from plant or artificially modified version of natural plant products. Natural products are an important source of new antimicrobial agents. Drugs derived from unmodified natural products or semisynthetic drugs from natural source accounts nearly78% of new drugs approved by food and drug administration (FDA) (Sufferdini *et al*., 2006). Almost 25% of drugs in western countries are of plant origin (Payne *et al*., 1991). Studies conducted on pharmacology and phytochemicals from different medicinal plants around the world have led to isolate and screen novel structure of raw materials for new drug discovery (Reuben *et al.,* 2010). Traditional medicine is the most accessible and affordable health care options in most third world countries for the management of various health problems and this knowledge practice has served as clue to scientific investigation of many folklore herbs

for phytochemicals. Anti-microbial agents of plant origin are effective in treatment of infectious disease while simultaneously palliating many of the side effects that are often associated with systematic antimicrobials (Bolla *et al.,* 2011). It has been recognised that they are able to kill or inhibit their growth through inhibition of bacterial cell wall synthesis, DNA replication, transcription and protein synthesis, which are essential for cell growth (Zhou *et al.*, 2015).

2. Statement of the Problem

Plants *Vernonia myriantha, Olinia rochetiana* and *Diclipteria laxata* are commonly used as traditional medicine in Kambata and Hadiyya Zones of southern Ethiopia. The people in these areas use *V. myriantha*, especially its leaves juice, ''*for healing wounds and blood clotting''*. *D. laxata* is also used for treatment of achene and skin allergies whereas *O. rochetiana* is used to treat tooth ache and toothbrush/mouth freshener. However, few studies are available on toxicity, biological activities and phytochemical profile of these plants. Thus, the aim of this study was to investigate the antibacterial activity of aqueous, ethanol and chloroform leaf extracts of the above plants against some selected human pathogenic bacteria. In addition, the antibacterial activity of stem-bark extract of *O. rochetiana* was tested**.** Acute oral toxicity in Swiss albino mice and preliminary phytochemical screening were carried out.

2.1. Hypothesis

It has been hypothesized that the aqueous, ethanol and chloroform leaves crude extract of *V. myriantha, D. laxata* and steam bark and leaves of *O. rochetiana* showed *in vitro* antibacterial effect on some selected pathogenic bacteria with no toxicity on swiss albino mice *in vivo* test.

3. Objectives of the Study

3.1 General Objective

To evaluate *in vitro* anti-bacterial activity of aqueous, ethanol and chloroform crude leaves extracts of *V. myriantha, D. laxata* and stem-bark and leaves of ***O.*** *rochetiana*

3.2 Specific Objectives

1. To compere aqueous, ethanol and chloroform crude extracts of *O. rochetiana* leaves and steam-bark and leaves of *V. myriantha* and *D. laxata* with selected standard drugs against some selected human pathogenic bacteria.
2. To determine anti-bacterial activity of crude extracts of O. *rochetiana* leaf and stem-bark and leaves of *V. myriantha* and *D. laxata* on selected human pathogenic bacteria.

4. Literature Review

4.1 Medicinal Plants

In different parts of the world Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds used in herbal medicines with acceptable therapeutic use for the development of novel drugs. The beneficial medicinal uses of plant materials typically result from the secondary products present in the plant, although it is not attributed to a single compound but combination of varieties of secondary metabolites. The medicinal action of plant is unique to a particular plant species or group, consistent with the concept that the combination of secondary products in particular plant is taxonomically distinct (Iwu *et al*. 1999).

Africa is considered to be the place of origin of mankind with a rich biological and cultural biodiversity labelled by regional differences in curing practices (Gurib-Fakim, 2006). In many parts of Africa, medicinal plants are the most easily accessible, acceptable and affordable health option preferred by the patients. In African countries unaffordability, inaccessibility and high cost of western forms of medicine forced people to chiefly depend on traditional medicine. Africa is blessed with enormous biodiversity resources and it is estimated to contain nearly 45,000 and out of these 5,000 species are medicinally in use. The reason for this is the fact that plants accumulate important secondary metabolites through evolution as a natural means of surviving in a hostile environmental condition (Manach *et al.* 2004*)*. Because of tropical conditions, Africa has an unfair share of strong ultraviolet rays of the tropical sunlight and numerous pathogenic microorganisms so that African plants accumulate bioactive substances more than plants from the northern hemisphere.

Plants have been used as a source of traditional medicine in Ethiopia from the time immemorial to combat different complaints and human sufferings (Asfaw Debela *et al*., 1999). Ethiopia has tremendous diversity of flora and wide range of potentially useful medicinal plants. Wide verity of climatic and ecological conditions made the country more extensive than available in many other parts of the world (Pankhurst, 2001). Around 80% of Ethiopian population has been

reported to rely chiefly on tradition medicine as a major primary healthcare (Camejo-Rodrgues *et al.,* 2003). As noted by Dawit Abebe (2001), there is a large magnitude of use and interest in medicinal plants in Ethiopia due to acceptability, accessibility and biomedical benefits.

4.2. Test Plant Species

4.2.1. *Olinia rochetiana*

Olinia rochetiana is a shrub, small tree or less often a large tree and evergreen, usually 1.2-16 meters tall, but occasionally reach 27 meters (Fig 1). *O. rochetiana* is a plant included under the family *Penaeaceae* and genus *Olinia*. It is a tree and shrub endemic to some Eastern and southern African countries. It is characterized by quadrangular branches and branch lets, flowers organized in triads, and an inflorescence placement that is axillary and or terminal.

In Ethiopia, the leave of O. *rochetiana* (locally known as "Tife" in Amharigna and Guna in Hadiyyisa) are used traditionally to treat toothache. It was reported by Omino and kowaro, (1993) used as powder, infusion or in the form of ointment for the treatment of eczema, acne and scabies. The Ogiek uses the bark as well as freshly forming leaves for chewing as treatment of colds and chest related conditions, toothbrush/mouth freshener and against pneumonia (Ermias Lukelal *et al*., 2013). The plant has wormicidal activities (Muthee *et al*., 2011).

Figure 1: *O. rochetiana*

4.2.2. *Vernonia myriantha*

The genus Vernonia (Asteraceae family) has more than1000 species growing all over the world with more than 30 species growing in Kenya (Beentje, 1994). It is abundant in Ethiopia and also in neighbouring countries such as Kenya, Somalia, Tanzania, Nigeria, Cameroon and southward to Angola. It is mostly found in Kambata, Hadiya, Gurage, Sidama and Wolayta zones, Southern Ethiopia, East Welega Zone of Oromia Regional State, and West Ethiopia (Moa *et al.,* 2013). Its vernacular name: Reejjichoo in Kambatissa, Barawaa in Hadyissa, Gengorita in Amharic, Reejii in Afan Oromo, Rejicho in Sidamegna and buuzuwaa in Wolaytigna (Fig 2).

The use of different parts of this plant for the treatment of various diseases in traditional or folk remedies throughout the world. Traditionally, it has been used as a medicine for a long time and has similarly used as *V. amygdalina*. In Cameroon, the leaf juice is used as eye drops for the treatment of cataract and in Ethiopia to treat toothache (Mirutse Gidaye *et al.,* 2009). The plant also has application in reproductive health (Namukobe *et al*., 2011).

Figure 2: ***V. mayriantha***

4.2.3. *Dicliptera laxata*

Dicliptera laxata is a member of family *Acanthaceae* and genus *Diclipteria*. *D. laxata* is a scrambling perennial plant or undershrub with 1-5 metres long with stems creeping and rooting at the nodes, ascending above, or erect (Fig 3). The plant is found in high forest, often in deep shade, occasionally in swamp forest, on steep slopes of well-watered ground, at elevations from 1,500-2,300 metres (Hedberg *et al*., 2006). It is distributed in Tropical Africa; in Ethiopia, Kenya, Uganda and Tanzania. The roots are used to treat stomach-aches and coughs (Ruffo *et al.,* 2002). A macerate in hot water is reported to result in a blood-red liquid. A report also described the ethnobotanical use of *D. laxata* by the Meinit people in the Bench Maji Zone, southwest of Ethiopia (Mirutse Giday *et al.,* 2009).

The parts of the herb above the ground are used for headache, using the nasal route of administration. In a study on medicinal plant diversity and the local uses in southern Uganda, the infusion made from the leaves of *D. laxata* has been reported to find application as a poison antidote (Ssegawa and Kasenene, 2007). *D. laxata* is known by its vernacular names ‘Omoro’ in Hadiyisa. A blood red decoction of the aerial part of *D. laxata* is consumed for orofacial inflammation by the local people.

Figure 3: ***D. laxata***

4.3. Test Organisms

4.3.1. *Staphylococcus aureus*

Staphylococcus aureus is a member of family *Micrococcus* and belongs to genus *Staphylococcus*. The *Staphylococcus* genus is subdivided into 32 species and subspecies. *S. aureus* is the most virulent pathogenic bacteria and they are a Gram-positive, non-spore forming spherical bacterium that can be observed as single cells, in pairs or as grape-like irregular clusters. It is facultative anaerobe so can grow under both aerobic and anaerobic conditions.

However, growth occurs at a much slower rate under anaerobic conditions (Stewart 2003). *S. aureus* is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans. The bacterium is characterized as coagulase and catalase positive, non-motile, non-spore-forming and as facultative anaerobic. It grows yellow colonies on nutrient rich media and is referred to as the yellow staphylococci (Winn, 2006).

It has an ability to adapt to different environments and colonize the human skin, nails, nares and mucus membranes. *S. aureus* is a cause of wide range of infections from a variety of skin, wound and deep tissue infections to more life-threatening conditions such as pneumonia, endocarditis, septic arthritis and septicaemia. *S. aureus* may also cause food poisoning, scalded-skin syndrome and toxic shock syndrome, through production of different toxins (Winn, 2006). *S. aureus* produce a heat-stable enterotoxin that has a direct effect upon the central nervous system (Celikel and Kavas, 2008). It is one of the four most common causes of nosocomial infection, often causing postsurgical wound infection and remains an important pathogen, particularly among people who are hospitalized and some of these Staphylococci are resistant to penicillin (Khan *et al.*, 2007).

The fact that *S. aureus* is resistant to multiple classes of antimicrobial agents in the hospital environment is a challenge currently facing clinicians when treating *S. aureus* infection. This resistance stems from a history of over 50 years of recurrent adaptation of *S. aureus* to different antibiotics introduced into clinical practice over the years (Akanbi, 2017). The resistance of *S. aureus* to methicillin is due to the production of penicillin-binding protein (PBP2a), which is encoded by the mecA gene located on the mobile gene element (MGE) of the staphylococcal

chromosome cassette mec (SCCmec), which has a low affinity for beta-lactam antibiotics. Abuse of as well as indiscriminate use of antimicrobials are contributing factors to the spread of resistance. Antibiotic-resistance genes are carried on plasmids and transposons, and can be transferred from one staphylococcal species to another and among other Gram-positive bacteria.

Antimicrobials act on targeting important bacterial functions such as cell wall synthesis, protein synthesis and nucleic acid synthesis (Bingyun, 2018). Several virulence factors bring the ability to cause infection such as enzymes, toxins, adhesion proteins, cell-surface proteins help the bacteria to evade the innate immune defence and antibiotic resistance mediate survival of the bacteria (Zecconi and Scali, 2013).

In the case of severe *S. aureus* disease, the infection may not be explained by the action of a single virulence factor, and it is a number of different factors operating together in the pathogenic process. Besides to that, *S. aureus* may show a multitude of adhesion factors that mediate interactions with host cells and extracellular matrix allowing efficient colonization (Chavakis *et al.,* 2005).

4.3.2 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a common Gram-negative, motile, rod-shaped bacterium that can cause disease in animals and humans. *P. aeruginosa* is a facultative anaerobe, as it is well adapted to multiply in conditions of partial or total oxygen depletion. It can achieve anaerobic growth with nitrate or nitrite as a terminal electron acceptor. It is characterized as citrate, catalase, and oxidase positive. When oxygen, nitrate, and nitrite are absent, it is able to ferment arginine and pyruvate by substrate-level phosphorylation (Schobert and Jahn, 2010). It is found in soil, water, skin flora, and most man-made environments throughout the world and is considered opportunistic in so far as serious infection often occurs during existing diseases and traumatic burns.

When colonization occurs in lungs, the urinary tract, and kidneys, the results can be calamitous (Itah and Essien, 2005). It is not extremely virulent in comparison with *S. aureus* and *Streptococcus pyogenes* even though it is capable of broad colonization and can combine into abiding biofilms (Hoiby *et al*., 2010). One of the most worrisome characteristics of *P.*

aeruginosa is its low antibiotic susceptibility, which is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes. In addition to this intrinsic resistance, *P. aeruginosa* easily develops acquired resistance either by mutation in chromosomally encoded genes or by horizontal gene transfer of antibiotic-resistance determinants.

Development of multidrug resistance by *P. aeruginosa* isolates requires several different genetic events, including acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes (Chuanchuen, 2002). Hypermutation favours the selection of mutation-driven antibiotic resistance in *P. aeruginosa* strains producing chronic infections, whereas the clustering of several different antibiotic resistance genes in integrons favours the concerted acquisition of antibiotic resistance determinants. Some recent studies have shown phenotypic resistance associated to biofilm formation or to the emergence of small-colony variants may be important in the response of *P. aeruginosa* populations to antibiotics treatment (Lebeaux, 2014).

These biofilms are found in the lungs of people with cystic fibrosis and primary ciliary dyskinesia and can cause death (Lebeaux, 2014). *P. aeruginosa* continued to be a major cause of opportunistic nosocomial infections, causing around 9–10% of hospital infections (Lebeaux, 2014). It is also the dominant cause of chronic lung infections contributing to the death of patients with cystic fibrosis (Lebeaux, 2014). A major reason for its prominence as a pathogen is its high intrinsic resistance to antibiotics, such that even for the most recent antibiotics (Alhazmi, 2015). It has the largest bacterial genome sequenced to date with 5570 genes and the largest proportion of regulatory genes with nearly 10% of all genes being devoted to directing the biology of it.

This is consistent with the observation that most of the resistances observed in this bacterium involve regulatory mutations. Infections caused by this pathogen are typically serious and difficult to treat, since it exhibits natural and acquired resistance to many structurally and functionally diverse groups of antibiotics (Alhazmi, 2015). This is due to its relatively low outer membrane permeability, in conjunction with secondary resistance mechanisms like betalactamase production and increased efflux pump activities. *P. aeruginosa,* in hospitalized patients, has the ability to develop multidrug resistance posing serious therapeutic problems and

increases the mortality rate associated with its infection. The basic mechanisms *P. aeruginosa* resist the action of antimicrobial agents through restricted uptake and efflux; drug inactivation and changes in targets (Cai, 2016).

4.3.3 *Escherichia coli*

E. coli is gram-negative rod-shaped bacterium classified under family Enterobacteriaceae. *E. coli* is opportunistic anaerobic bacterium with cell size about 2.0µm long, 0.25-1.0µm in diameter and cell volume of 0.6–0.7µm (Kubitschek, 1990). *E. coli* is a natural inhabitant of the gut of humans, birds and other warm-blooded animals and considered as an indicator of faecal contamination of water.

It is genetically highly adaptable to environmental stresses and has been shown to survive and multiply in the environment (Van-Elsas *et al.,* 2011). Most *E. coli* is normal commensal found in the intestinal tract however some strains are Pathogenic and can be distinguished from normal flora by their virulence factors such as exotoxins. Even though, *E. coli* strains are harmless some serotypes can cause serious food poisoning in their hosts and are occasionally responsible for product recalls due to food contamination.

Although most strains are commensal, pathogenic *E. coli* strains can contain various virulence factors and can be responsible for a variety of infections (Kaper *et al.* 2004). Based on the specific virulence factors, pathogenic *E. coli* can be classified as either extra-intestinal pathogenic *E. coli* or intestinal pathogenic *E. coli*. Extra-intestinal pathogenic *E. coli* strains are usually able to cause infections in anatomical sites outside of the intestinal tract and are associated with urinary tract infections, neonatal meningitis and septicaemia. ExPEC, like commensal *E. coli*, can colonise the intestinal tract without causing gastroenteritis.

E. coli and other facultative anaerobes constitute about 0.9% of gut microbiota and faecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. 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. Due to thin

peptidoglycan layer of cell wall and an outer membrane *E. coli* stains gram negative and it picks up the colour of the counterstain safranin and stains pink during staining process.

The outer membrane surrounding the cell wall provides a barrier to certain antibiotics. Strains those possess flagella are motile. The flagella have a peritrichous arrangement and it also attaches and effaces to the microvilli of the intestines via an adhesion (Darnton *et al.*, 2007)

4.3.4. *Salmonella typhi*

The Salmonellae belong to the family Enterobacteriaceae and genus *Salmonella*. They are gram negative, facultatively anaerobic, non-spore forming rod and predominantly motile enterobacteria with cell diameters between about 0.7 and 1.5 µm, lengths from 2 to 5 µm, and peritrichous flagella. They can be characterized as catalase positive, oxidase negative and reduce nitrates to nitrites (Carr, 2017). Most strains are motile and ferment glucose with production of both acid and gas. They are chemotrophs, obtaining their energy from oxidation and reduction reactions using organic sources. They are also capable of generating ATP with oxygen when it is available and using other electron acceptors or fermentation. They bring major cause of enteric fever and gastroenteritis. Many foods of animal products have been recognized as vehicles for transmitting the organisms to human and to the food processing and preparation environment.

Salmonella species are intracellular pathogens, certain serotypes cause illness. Non typhoidal serotypes can be transferred from animal to human and from human to human. They usually invade only the gastrointestinal tract and cause Salmonella food poisoning; symptoms resolve without antibiotics. They are chemotrophs, obtaining their energy from oxidation and reduction reactions using organic sources. *Salmonella* are major cause of disease in humans, animals, and birds all over the world. *S. typhi* has killed over 600,000 people annually all over the world. (Behailu Bekele 2006). It is a deadly bacterial disease that causes typhoid fever and is transmitted through food and water.

It has become an epidemic in South Asian countries where sanitation is lacking. *S. typhi* usually invades the surface of the intestine in humans but have developed and adapted to grow into the deeper tissues of the spleen, liver, and the bone marrow. Gastroenteritis and typhoid fever in human being are type of disease caused by Salmonella. Typhoid fever is a serious sickness

caused by the bacterium S. *typhi* (Celikel and Kavas, 2008). Typhoid fever is an enteric fever which is potentially fatal, multi systemic disease primarily caused by *S. typhi.* It is a common problem until now in developing world where it harms almost 21.5 million people every year. *S. typhi* has been a major human pathogen until now thriving in conditions of poor sanitation, crowding, and social chaos. Although antibiotics have markedly reduced the frequency of typhoid fever in the developed world, it remains endemic in developing countries (Behailu Bekele, 2006). The route of transmition is oral via food or beverages handled by an individual who chronically sheds the bacteria through stool or, less commonly, urine hand-to-mouth transmission after using a contaminated toilet and neglecting hand hygiene.

4.4. Mechanism of Antimicrobial Resistance

Bacterial resistance is closely associated with the use of antimicrobial agents in clinical practice. Prolonged therapy with antibiotics can lead to the development of resistance in a microorganism that initially is sensitive to antibiotics, but later it can adapt, bit by bit and develop resistance to antibiotics. When an antibiotic attacks bacterium, bacterial cells susceptible to it will die those that have some resistant will survive (Fair, 2014). The emergence of a phenotype resistant to antimicrobial agents depends on degree of resistance expression, capability of a microorganism to tolerate resistance mechanism, initial colonization is some of the factors. Bacterial resistance to antibiotics can be inborn, which is characteristic of a particular bacterium and depends on biology of a microorganism or acquired resistance (Munita, 2016). Acquired resistance occurs from acquisition of inborn genes by plasmids through conjugation, mutation of cellular genes, a combination of these mechanisms.

When resistance determinants are on plasmids, they will spread quickly within the genus and even unrelated bacterial genera, on other hand resistance associated with genes on chromosomes, resistant microorganisms will spread more slowly. Horizontal gene transfer is the main mechanisms of resistance gene transfer in a bacterium by plasmid transfer, transfer by viral delivery, and transfer of free DNA. Genes can be transferred by transduction, conjugation, transformation. Then genes are incorporated into the recipient chromosome by recombination or transposition which results on one or several changes in gene sequence (Munita, 2016). Biochemical resistance mechanisms are one the defence system in bacteria by decreasing antimicrobial uptake, enzymatic modification and degradation and efflux pump.

Membrane proteins that export antibiotics from the cell and maintain their low intracellular concentrations are called efflux pumps. Reduced outer membrane (OM) permeability results in reduced uptake of antibiotics. Single component efflux systems transfer their substrates across the cytoplasmic membrane. Multicomponent pumps found in gram-negative bacteria and together with a periplasmic membrane synthesis protein component and an OM protein (OMP) component transfer substrates across the cell envelope (Munita, 2016).

The outer membrane (OM) of Gram-negative bacteria carries out the essential role of providing an extra layer of protection to the organism without compromising the exchange of material

required for sustaining life. In this double capacity, the OM emerges as an advanced macromolecular advanced. By combining a highly hydrophobic lipid bilayer which enable the OM a selective barrier. The permeability properties of this barrier have a major impact on the susceptibility of the microorganism to antibiotics are essentially targeted at intracellular processes. The existence of drug-resistant strains in a large number of bacterial species is due to modifications in the lipid or protein composition of the OM (Nikaido, 2003).

4.5 Impact of Microbial Drug Resistance

As reported by Sharma *et al.,* (2014) about 25% of global deaths is caused by microbial infections. The emergency of different antibiotic resistant bacterial strains, raises the risk of bacterial associated mortality through time. Bacterial infections caused by multidrug-resistant bacteria are a growing problem worldwide associated with greater morbidity and mortality at the same time increases healthcare cost (Prestinaci, 2015). According WHO priority list of pathogens, *P. aeruginosa, E. coli*, *S. aureus* and Salmonella spp. are multi drug resistance strains that causes different bacterial infections in human being (WHO, 2017). About 27 million cases of clinical disease and 200,000 deaths recorded annually due to *S. typhi* by enteric fever. However, antibiotics have markedly reduced the frequency of typhoid fever in the developed world, it remains endemic in developing countries (Behailu Bekele, 2006). *P. aeruginosa* is common cause of hospital acquired infections and it is one of the most crucial pathogenic microorganisms, that cause problems clinically because of its antibiotic resistance nature. Mortality due to *P. aeruginosa* has mortality rates range from 33 to 61% from all patients with *P. aeruginosa* bacteremia.

It was known that *P. aeruginosa* bacteremia is associated with higher mortality than other gram-negative fastidious (Bassetti, 2018). Antimicrobial resistance is a global burning issue and many reports apprise that it is an increasing problem affecting both developed and developing countries. Resistance pattern of pathogenic microbes to common antibiotics reported all over the world (WHO, 2017). Generation of multidrug resistance bacterial strains becomes a growing public health concern globally around 23,000 deaths in the United States and 25,000 deaths in the European Union are caused by drug resistant bacteria. (Li and Webste 2017). *P. aeruginosa* develops resistance against almost all antibiotics by innumerable mechanisms like multi-drug resistance efflux pumps, biofilm formation, resistance genes, aminoglycoside modifying enzymes, and mutations in various chromosomal genes (Blanco *et al.,* 2016). An emergence of multidrug resistance strains and increasing susceptibility to conventional drugs challenges treatment of *S. typhi* in conventional way (Afzal *et al*., 2018).

4.6 Preliminary Phytochemical Screening

Medicinal plants are plants that contain compounds which can be used for therapeutic purposes or helps to synthesize medicinally useful drugs. Phytochemicals are a large group of plant-derived compounds which are responsible for the protection of diseases and obtained from fruits, vegetables, beans, cereals and plant-based beverages (Arts *et al*., 2005). Phytochemicals can be either primary or secondary compounds. Chlorophyll, proteins and common sugars are included under primary metabolites whereas; terpenoid, alkaloids and phenolic compound are secondary metabolites (Krishnagar *et al*., 2007). The most important compounds extracted from medicinal plants are alkaloid, phenols flavonoids, essential oil, tannins, saponins, resins etc. These chemicals can be used as anticancer, antimicrobial, antioxidant, antidiarrheal, anodyne and wound healing activity. Bioactive compounds of plant origin inhibit microorganisms by interfering their metabolic processes, modulating gene expression and signal transduction pathways (Kris-Etherton *et al*., 2002).

As reported by Robber and Huxtable, (1992) medicinal plants produce secondary metabolites as defense against animals, parasites, bacteria, and viruses. Phytochemicals are chemical compounds naturally occurring in plants. Phytochemical screening refers to the extraction and identification of the medically active substances found in plants. As noted by Ahmad *et al.,* (2006) some of the bioactive substances that can be derived from plants are saponins, flavonoids, alkaloids, proteins, tannins, glycosides, terpenes and phenolic compounds.

Phytochemicals display various mechanisms of action such as increasing colonic water and electrolyte re absorption and inhibiting intestinal motility, while some components have been shown to inhibit specific pathogens (Ahmad *et al*., 2006). Saponins are used by the folkloric remedies of Kashmir (India) in treating wounds, help in blood clotting and enteric ulcer problems (Foster and Duke, 2000). This is due to their ability to cause red blood cells to precipitate and coagulate (Maobe *et al*., 2013). Saponins have also being associated with inhibitory effect of growth on microorganisms (Maobe *et al*., 2013). Tannins are also one of the secondary metabolites in plants being glycosides of protocatechuic acids. It has astringent properties which speed up the healing of wounds and inflamed mucous membrane (Njoku and Akumefula, 2007).

In addition to that astringent property makes them practicable in preventing diarrhoea due to their ability to precipitate proteins, mucus and constrict blood vessels (Kokwaro, 2009). This is why traditional healers use plants reach in tannins to treat wounds and burns since they are able to cause blood clotting. This shows how traditional medicinal plants rich in tannins can be used to control bleeding. According to Chung *et al*. (1998), several tannin molecules have been shown to reduce the mutagenic activity of a number of mutagens. As noted by Chung *et al*., (1998) tannin can inhibit growth of fungi, yeast, bacteria and viruses.

Alkaloids have been identified for their functions including analgesic, antiplasmodic and antibacterial activity (Okwu and Josiah, 2006). Flavonoids are known to contain specific compounds called antioxidants which prevent cells from being damaged by effects of free radicals. It also plays a role in heart diseases and neurodegenerative diseases. Flavonoids have also vasodilator activity a property which is useful in improving blood circulation in brain and in Alzheimer disease (Sharma, 2006). Terpenoids have medicinal value such as anticarcinogenic, antimalarial, antimicrobial and diuretics activity (Pichersky and Gershezon, 2002). Terpenoids have also shown a great potential in treatment against pathogenic microorganisms. Terpenoids have exhibited antibacterial activity against *E. coli*, *Staphylococcus, P. aeruginosa* (Piera *et al*., 2011).

5. Materials and Methods

5.1 Collection and Identification of Plant Materials

Healthy, fresh leaves of *Vernonia myriantha*, *Diclipteria laxata,* leaves and stem bark of *Olinia rochetiana* were collected from Hadiyya district, 241km to the southern Ethiopia, from October to January,2017

Identification and authentication of plant specimens was done at the National Herbarium, Department of plant Biology and Biodiversity Management, College of Natural and Computational Sciences, Addis Ababa University. Then the specimens were deposited for future reference with voucher specimen number AE001, AE003 and AE004 for *O. rochetina, V. myriantha* and *D. laxata* respectively**.**

5.2 Extract Preparation

The leaves and stem-bark of the plants were washed with running tape water, disinfected and rinsed with distilled water and dried in shade place at room temperature (25-30°C) for two weeks as described in (Thippeswamy *et al*., 2011). About 1kg of each plant part was powdered by milling and sieved through a fine mesh (Canadian Series sieves with 500µm pores), and the powder were packed in glass bottles and stored at room temperature for further use.

Then,100g powder of each plant was soaked in 1000ml sterile distilled water in Erlenmeyer flasks and placed on shaking water bath for 72 hours according to (Subbarayan *et al.,* 2010). The macerates were first filtered with four-fold muslin cloth. Then, the filtrate was further filtered through Watt man no 1 filter paper (Germany) and were kept at refrigerator for 24 hours and then concentrated by using (BLOCK, CHRIST, ALPHA1‑4) lyophilizer at -14°C with vacuum pressure. The dry and concentrated crude extracts were placed in a small covered bottle at -4°C for further use.

Simultaneously, the same unit (100g) powder of plants were soaked in 1000ml (ethanol 97% and chloroform 99.9%) on separate 500ml Erlenmeyer flascks and placed on shaking water bath at room temperature for 72 hours and filtered through four-fold muslin cloth and then filtered with

Watt man no.1 filter paper (Germany). Ethanol and chloroform filtrate were concentrated in a rotary evaporator (Buchi type TRE121; Switzerland) at a temperature of 45°C and 40°C further dried in oven at 35ºC. Finally, the percentage yield of each extract was preserved in screw caped small bottles at -4ºC for future use. The extraction yield is a measure of the solvent efficiency to extract specific components from the original material.

The percentage yield of crude extract in respective solvent was recorded on Table 1. The percentage yield was calculated according to the formula

$$\% = \frac{Weight\ of\ crude\ extract}{Weight\ of\ sample} \times 100$$

5.3 Test Organisms

5.3.1 Bacterial Strains

Standard (American Type Culture Collection (ATCC) and clinical isolates of different bacterial strains were used for the experiment. These were *E. coli* standard (ATCC 25922) and clinical *E. coli*, *P. aeruginosa* standard (ATCC 2785315) and clinical *P. aeruginosa*, standard *S. aureus* (ATCC25923), clinical *S. aureus* and standard *S. typhi* (ATCC 13311). Both standard and clinical strains were kindly donated by the Ethiopian Public Health Institution (EPHI). The bacterial strains were chemically well identified in the microbiology laboratory of EPHI.

5.3.2 Inoculum Preparation

To obtain pure culture and to avoid contamination the standard and clinical test organisms (*P. aeruginosa*, *S. typhi, E. coli*, *S. aureus*) were streaked on selective media; *S. aureus on* Mannitol salt agar (M2A6B00), *P. aeruginosa* on Pseudomonas agar (Sr102)*, E. coli* on ethylene methylene blue (EMB) (EMLA-1602). Media was prepared according to manufactures guide line and autoclaved at temperature of 121°C**.** After cooling the media to 45° C, it was poured onto pre-labelled sterile petri dishes aseptically and allowed to solidify for an hour. Then, test organisms were streaked on the respective selective agar media using inoculating wire loop following aseptic condition in a Safety Cabinet and incubated for 18-24 hours at 37°C.

The bacterial inoculum of each of bacterium was prepared and standardized by following the guideline of Clinical and Laboratory Standard Institute (CLSI, 2012). Turbidity of the inoculum tube was adjusted visually by either adding bacterial colonies or by adding sterile normal saline solution in comparison with already prepared 0.5 McFarland Standard which is assumed to contain a bacterial concentration of 1-2 $x10^8$ CFU/ml.

5.4 Antibacterial Activity Assay of Plant Extract

5.4.1 Agar Well Diffusion

Bacterial susceptibility to crude extracts was assessed by Agar well diffusion. The aliquot of inoculum of the respective bacteria (1-2 $x10^8$ CFU/ml) were swabed on the sterile MHA plates prepared according to the manufacturer's guideline in 90millimetre (mm) diameter sterile petri dish using a sterile swab.

On each plate, five equidistant wells were made with a 6mm diameter sterilized cork-borer and finally rim of the agar was swabbed with cotton swab. The corresponding wells were filled with 80µl/well of 250mg/ml crude plant extract. In addition, the commercial antibiotic chloramphenicol 80µl/well and distilled water were used as a positive and negative control respectively. The positive control was selected based on the susceptibility of the bacterium used (CLSI, 2012). Then, the plates were left undisturbed for about 30 minutes at room temperature, in order to give time for pre-diffusion on the inoculated agar. Finally, the plates were incubated at 37^0C for 24 hours.

After incubation, the resulting diameters of zones of inhibition, including the diameter of the well, were measured using a ruler and reported in mm. The experiment was performed in triplicate.

5.4.2 Determination of Minimum Inhibitory Concentration

The crude extracts that showed antibacterial activity by agar well diffusion method was subjected to serial macro broth dilution technique to determine the MIC of the extracts according to CLSI, (2015). Values of the plant extracts against the test organisms were estimated to determine the range of MIC values.

Consequently, the following concentrations were prepared for each extract, using two-fold serialdilution125, 62.5, 31.25, 15.62 7.81, 3.9, 1.9, 0.9 and 0.48mg/ml. The bacterial suspension was prepared according to CLSI guideline (CLSI, 2015) so that the bacterial concentration was made to be approximately 1×10^6 CFU/ml by diluting the 0.5 MacFarland Standard turbidity equivalents to bacterial suspensions in the ratio of 1:150 in the MHB. Briefly, within 15 minutes of standardization and dilution of bacterial suspension, 1ml of diluted bacterial suspension was added to each test tub except the negative control.

After inoculation, the test tubes contain approximately 5×10^5CFU/ml bacteria. The test tube containing only Muller Hinton Broth (MHB) and broth with inoculum (without extract) were included to serve as negative and positive control. Then it was allowed to incubate at 37℃ for 18 to 24 hours. Finally, the presence of growth was indicated by turbidity and absence of growth was confirmed by clear solution at the end of incubation period. The lowest concentration of the extract showing no growth was regarded as the MIC. All the experiments were performed in triplicates for each bacterium to get the average value.

5.4.3 Determination of Minimum Bactericidal Concentration

The MBC is defined as the lowest concentration of antimicrobial agent that killed 99.9% of the final inoculum after incubation for 18 to 24 hours at 37^oC. It was determined by aseptically sub-culturing the contents of test tubes from the MIC results for individual bacterium to antimicrobial free agar as described in different studies (Nikolic *et al*, 2014). In this technique, the contents of all test tubes containing a concentration of test material above the MIC value from each triplicat was streaked using a sterile wire loop on MHA aseptically and incubated at 37ºC for 24 hours. The lowest concentration of the extract which showed no bacterial growth after incubation was MBC. The average value was taken for the MBC of test plant crude extract against each bacterium. It was performed in triplicate to take average.

5.5 Animals Used on the Study

5.5.1 Oral Acute Toxicity Determination

The mice were randomly grouped and each group had five female mice. All mice were housed in standard cages in the animal house and fed a standard pellet diet and tap water *ad libitum.* Then each mouse was measured by using electronics beam balance before the administration of ethanol and chloroform crude extracts of *O. rochetiana* and ethanol extract of *V. myriantha.* Female Swiss albino mice, 5 in each group, were administered with oral dose of 2000mg/kg bodyweight from each crude extract. 0.254g ethanol extract of *O. rochetiaina* leaves and stem bark, each dissolved with 2.542ml distilled water and 0.55ml of it was administered to the mice and chloroform extract of 0.396g *O. rochetiana* leaf was dissolved with 3.962ml distilled water and administered 0.566ml for each mouse and 0.49ml water was administered orally by using oral gavage for negative control group. For 14 days, gross physical and body weight changes were observed.

The physical signs of acute toxicity such as depression, decrease in feeding activities and hair erection after extract administration were observed for 24 hours. The body weights of the mice in each group were measured at the first day before the experimental mice were administered with extracts, day 7 and day 14. The bodyweight of the extract administered mice was measured and their bodyweight changes were compared with mice which were given water. OECD guideline (2001) was used as a reference for oral acute toxicity in mice.

5.6 Preliminary Phytochemical Screening

The crude ethanol extracts of *V. myriantha* leaves, leaf crude ethanol and chloroform extract of O. *rochetiana* and crude ethanol extracts of steam-bark of *O. rochetiana* were screened for the presence of alkaloids, saponins, tannins, steroid, terpenoid, phenol, steroidal glycoside and flavonoids in accordance to standard phytochemical screening methods.

Phenol test:200mg of plant extract mixed with 3ml of distilled water and pipetted 2ml dissolved extract in another test tube and 0.5 ml of 5% $FeCl_2$ (ferric chloride) formation of intense blue colour indicated the presence of phenol (Tiwari *et al.,* 2011)

Test for Triterpenoids: Salkolwski's test was used for triterpenoids test. For this, 200mg extract was treated with chloroform and a few drops of concentrated H_2SO_4, acetic acid was added, and the test tube was shaken well and allowed to stand for some time. The formation of deep red colour at junction of two layers indicated the presence of triterpenes (kumar *etal.*,2009).

Test for saponin: About 200mg of extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent broth. Formation of foam indicated the presence of saponins (Kumar *et al.,* 2009).

Test for tannin: About 200mg of plant extract was treated with few drops of 1% lead acetate and observed for formation of yellowish precipitate confirmed the presence of tannins (Treare and Evans 1985).

Steroid test: 200mg extract was dissolved with 10ml chloroform and 10ml conc. H_2SO_4 by sides of the test tube. Formation of red colour in the upper part and Sulphuric acid layer showed yellow with green fluorescence confirmed the presence of steroid (Gibbs, 1974).

Glycoside test: Plant extract was dissolved in glacial acetic acid heated in steam then allowed to cool and treated with 2 drops of 5% ferric chloride and 2ml conc. H_2SO_2.formation of reddish-brown colour ring was observed at junction of two layers confirmed the presence of steroidal glycosides (Gibbs 1974).

5.6 Data Analysis

Data were analysed by using SAS version 9.1.3 (SAS institute 2003) software. The experimental data were expressed as mean plus-or-minus standard error of the mean (mean±SEM) and statistical significance was considered at 95% confidence interval ($P<0.05$). The statistical differences of the mean zone of inhibition of aqueous, ethanol and chloroform crude extract of the three plants leaves and stem-bark of *O. rochetiana* for individual bacteria was carried out by employing one-way analysis of variance (ANOVA) followed by Tukey test.

5.7 Ethical Considerations

This study was conducted after having obtained ethical clearance from the Institutional Review board of the College of Natural Sciences, Addis Ababa University.

6. Results and Discussion

6.1 Yield of the Extracts

The aqueous extract of *V. myriantha* have the highest percentage yield (18.0%) and followed by *O. rochetiana* ethanol leaf extract (15%). The least yield (5.5%) was obtained from chloroform extract of *O. rochetiana* stem-bark (Table1). From the data, it is possible to conclude that the leaf of V. *myriantha* contained more polar compounds compared to *O. rochetiana* and *D. laxata*. Similarly, the highest (15.0%) yield was obtained from ethanol extracts of *O. rochetiana* leaves whereas the lowest yield (8%) was obtained from stem bark extract. In comparison with other solvents, water yielded more crude extract from leaf and stem-bark of tested plants. In general, the yield obtained from these plants is quite adequate and future work on drug development appears economically feasible (Table1).

Table 1: yields of crude leaves extracts of *V. myriantha, D. laxata* and leaves and stem-bark *O. rochetiana at conc.*1:100 solute to solvent with respective solvent

Plant	Solvent	Yield
O. rocheatiana leaves	C_2H_5OH	15.0
	H_2O	10.5
	$CHCl_3$	9.0
O. rochetiana stem-bark	C_2H_5OH	8.0
	H_2O	12.0
	$CHCl_3$	5.5
V. myriantha leaves	C_2H_5OH	14.0
	H_2O	18.0
	$CHCl_3$	8.0
D. laxata leaves	C_2H_5OH	7.0
	H_2O	10.0
	$CHCl_3$	6.5

Key %: per cent; C_2H_5OH: ethanol; $CHCl_3$: chloroform; H_2O: water

6.2 Antibacterial Susceptibility Result

The extracts showed varying degree of inhibitory effect against *S. aureus*, *E. coli, P. aeruginosa* and *S. typhi* at a concentration of 250mg/mL (Table2). The ethanol and chloroform crude extracts of *O. rochetiana* leaves exhibited significant antibacterial activity against all clinical and standard bacterial strains tested, although the aqueous extract failed. The ethanol leaf extract of this plant exhibited significantly higher inhibitory effect, compared to chloroform crude extract, on both clinical and standard strains.

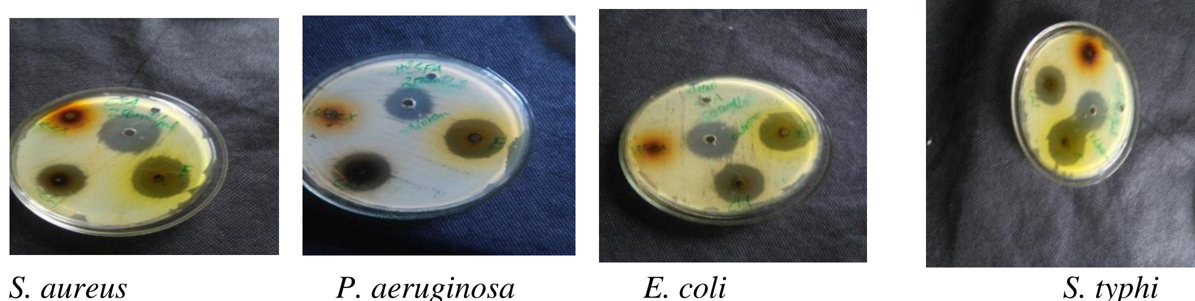
The findings showed that among the tested clinical strains, *S. aureus* was the most susceptible strain and *P. aeruginosa* the least for *O. rochetiana* leaf ethanol extract. Standard *S. aureus* and *E. coli* strains were highly susceptible whereas *P. aeruginosa* again was the least compared to the positive control (chloramphenicol). The inhibitory zone diameter ranged from (20.33±0.57mm) to (22.66±1.15mm) on clinical *P. aeruginosa* and clinical *S. aureus* respectively. The chloroform leaf extract also showed a potent antibacterial growth activity despite its comparatively lower effect than the ethanol extract. Its inhibition zones were (19±1.73 mm) and (22.6±2.51mm) for clinical strains of *P. aeruginosa* and *S. aureus* respectively. The measurements were (18.00mm) and (25.33±0.57mm) respectively, for standard *S. aureus* and *E. coli* (Fig 4).

As reported by Hailu Tadege, (2004) 80% methanol crude extract of *O. rochetiana* at concentration of 100mg/ml revealed inhibitory effect on *S. aureus* (25±00mm), *E. coli* (19±0.8mm) and *P. aeruginosa* (22±1.0mm). Moreover, it was reported that methanol extract at a concentration of 160mg/ml showed growth inhibition with inhibition zones for *S. aureus* (17±0.8mm, 16±0.1mm), *E. coli* (8±00mm, 8±0.5mm) and *P. aeruginosa* (15±1.00mm, 12±1.2mm) on clinical and standard strains respectively (Amuka *et al.,* 2015). Those findings disagreed with the current study. The apparent discrepancy may be related to the solvents used, the age of plant and climate condition of plant collected area.

Table 2: Antibacterial activity of leaves extracts of *O. rochetiana* at conc. of 250mg/mL

Bacterial strain	Inhibition zone (mm)±SEM			
	C_2H_5OH	$CHCl_3$	H_2O	Chlora(0.3mg/mL)
Clinical *E. coli*	$21.66^b±1.88$	$20.33^b±0.57$	-	$29.33^a±1.15$
Standard *E. coli*	$24.33^b±0.57$	$25.33^b±0.57$	-	$31.33^a±1.15$
Clinical *P. aeruginosa*	$20.33^b±0.57$	$19.00^{bc}±1.73$	-	$20.00^a±1.73$
Standard *P. aeruginosa*	$20.66^a±1.15$	$18.00^b±0.00$	-	$21.66^a±3.05$
Clinical *S. aureus*	$22.66^a±1.15$	$22.66^b±2.51$	-	$31.33^a±1.52$
Standard *S. aureus*	$24.33^b±1.15$	$20.00^c±1.00$	-	$31.66^a±1.52$
Standard *S. typhi*	$25.66^b±0.577$	$23.00^c±1.00$	-	$30.66^a±1.15$

Key C_2H_5OH: ethanol; $CHCl_3$: chloroform; H_2O; Chlora: chloramphenicol -: no inhibition zone (the aqueous extract didn't show inhibitory effect on tested bacterial strains.) Treatment mean in the same row having the same subscript have no significant difference (at P-value



S. aureus *P. aeruginosa* *E. coli* *S. typhi*

Figure 4: Antibacterial activity of *O. rochetiana* crude leaves chloroform and ethanol extracts against clinical strains of selected bacteria

The stem-bark of *O. rochetiana* ethanol extract exhibited growth inhibitory effect on both clinical and standard strains of the test organisms. The inhibitory zone diameter of ethanol extract of *O. rochetiana* ranged from (19.00±1.73mm) to (22.66±1.08mm) on clinical *P. aeruginosa* and clinical *S. aureus* respectively. Among all clinical strains tested, *S. aureus* was the most susceptible one and *P. aeruginosa* the least susceptible though it showed relatively significant inhibition compared to the positive control. The least inhibition zone was observed on standard *P. aeruginosa* (16.66±1.15mm) and the highest on standard *E. coli* (20.66±1.15mm). Both aqueous and chloroform crude extracts of stem-bark of *O. rochetiana* did not show any growth inhibitory effect (Table 3). Inability of growth inhibition of aqueous and chloroform extracts might be because of insufficiency of phytochemicals in the crude.

Table 3: Antibacterial activities of crude stem-bark ethanol, chloroform and aqueous extracts of O. *rochetiana* at a conc. of 250mg/mL

	Inhibition zone (mm)±SEM			
Bacterial strains	C_2H_5OH	$CHCl_3$	H_2O	Chlora (0.3mg/mL)
Clinical *E. coli*	18.00^c±0.00	-	-	29.33^a±1.15
Standard E. *coli*	20.66^c±1.15	-	-	31.33^a±1.15
Clinical *P. aeruginosa*	17.33^c±1.15	-	-	21.00^a±1.73
Standard*P. aeruginosa*	16.66^b±1.15	-	-	20.66^a±3.05
clinical *S. aureus*	19.00^c±1.08	-	-	31.33^a±1.52
Standard *S. aureus*	19.33^c±0.57	-	-	31.66^a±1.52
standard *S. typhi*	23.33^c±2.08	-	-	30.66^a±1.15

Key C_2H_5OH: ethanol; $CHCl_3$: chloroform; H_2O: water; Chlora: chloramphenicol; -: no inhibition zone; Treatment mean in the same row having the same subscript have no significant difference at p-value.

However, ethanol crude extract revealed significant growth inhibition effect, the positive control (chloramphenicol) showed highest inhibition zone in comparison to ethanol crude extract.

However, there were significant differences between the activities of either type of the above extracts and that of the positive control ($p<0.0001$). Chloramphenicol, the semisynthetic broad-spectrum antibiotic, showed the highest inhibition zones, 29.33mm±1.15 and 31.33mm±1.15 on clinical *E. coli* and *S. aureus* strains respectively. On the contrary side, both clinical and standard *P. aeruginosa* stains were least susceptible to this antibiotic with inhibition zones of 20.00mm±1.73 and 21.66mm±2.05 respectively, which was almost comparable inhibition zone with the ethanol leaf extract.

Antibacterial assay of clinical and standard bacterial strains with crude extract of *V. myriantha* revealed that only ethanol extract showed significant growth inhibitory effect only on standard and clinical isolates of *S. aureus* having inhibition zones of diameter 22±00mm and 21±1.73mm

respectively (table 5). Aqueous and chloroform crude extracts had no inhibitory effect on all tested bacterial strains. The chloramphenicol, however showed the highest growth inhibition zone (21mm to 31mm) on clinical strains of *E. coli* and *S. aureus* respectively. Also, the standard strains showed nearly equal growth inhibition zones with respective clinical strains.

As reported by Temesgen Orebo (2004), chloroform crude extract of *V. auriculifera* inhibited growth of *S. aureus* (17.66mm), *E. coli* (18.20mm) *S. typhi* (13mm) at concentration of 100mg/mL and methanol extract at the same concentration inhibited *S. aureus* (19.40mm), *E. coli (*20.60mm) and *S. typhi* (14.30mm). Ethanol extract of *V. auriculifera* exhibited antibacterial activity against bacteria in a previous study (Ifeoma and Chukwa, 2011). Ethanol extract of another species of the genus, *V. amygdilina,* showed antibacterial activity similar to results reported by different studies on the same genus (Anbijuwon *et al.*, 2012).

Table 4: Antibacterial activities of crude leaves extracts of *V. myriantha* at a concentration of 250mg/mL

Bacterial strain				
	C_2H_5OH	$CHCl_3$	H_2O	Chloral (0.3mg/mL)
Clinical *E. coli*	-	-	-	$29.33^a \pm 1.15$
Standard *E. coli*	-	-	-	$31.33^a \pm 1.15$
Clinical *P. aeruginosa*	-	-	-	$21.00^a \pm 1.73$
Standard *P. aeruginosa*	-	-	-	$20.66^a \pm 3.05$
Clinical *S. aureus*	$21.00^b \pm 1.73$	-	-	$31.33^a \pm 1.52$
Standard *S. aureus*	$22.00^b \pm 0.00$	-	-	$31.66^a \pm 1.52$
Standard *S. typhi*	-	-	-	$30.66^a \pm 1.15$

Key: C2H5OH: ethanol; CHCl3: chloroform; Chlora: chloramphenicol; - : no inhibition zone; Treatment mean in the same row having the same subscript have no significant difference at (p- value <o. ooo1).

Neither any clinical nor standard bacterial strains were inhibited by the leaf extracts of *D. laxata* 250mg/mL concentration. However, positive control showed highest inhibition zone ranging from (21mm) to (31mm) on clinical *P. aeruginosa* and clinical *S. aureus* respectively. Overall, the extracts showed the lowest activity on standard *P. aeruginosa* and highest against standard *S. aureus*.

For *O. rochetiana* leaf the ethanol extract showed a lower MIC compared to that of chloroform against all bacterial strains. While the MIC against both clinical and standard *S. aureus* strains was 1.95mg/ml for the ethanol extract, it was 7.8mg/mL for the chloroform. Similarly, the ethanol extract exhibited an MIC of 7.8mg/mL against both clinical and standard strains of *P. aeruginosa* which was much higher than what was required for *S. aureus* inhibition. S. *aureus* was the most inhibited test organism with an MIC of 1.95mg/mL of ethanol *O. rochetiana* leaf extract and the least inhibited organism was *P. aeruginosa* (MIC = 7.8mg/mL). Chloroform extract of *O. rochetiana* leaf showed lowest MIC 3.96mg/mL against *S. aureus* and the highest MIC was recorded on *P. aeruginosa* (15.62mg/mL). On other hand, the ethanol extract exhibited an MIC of 3.9mg/ml and the chloroform extract 7.8mg/mL for clinical and standard *E. coli* (Table 5).

Ethanol crude extract of *O. rochetiana* leaf had the lowest MBC on both standard and clinical strains of *S. aureus* (31mg/mL*)* and highest MBC was observed on *P. aeruginosa* (125mg/mL). Chloroform extract of *O. rochetiana* leaf exhibited an MBC on both clinical and standard strains of *S. aureus*, *E. coli* and clinical *S. typhi* (62.5mg/mL) whereas the highest MBC (125mg/mL) was shown for *P. aeruginosa*. As noted by Hailu Tadege (2005), 80% methanol crude extract of *O. rochetiana* leaf exhibited MIC values on *S. aureus* 5mg/mL, *P. aeruginosa* 2.5mg/mL and *E. coli* 10mg/mL. Muguweru, (2016) reported that the MIC of *S. typi*, *E. coli* and *P. aeruginosa* were 50mg/ml, 6.25mg/ml and 250mg/ml respectively the same work reported MBC values for *S. typhi*, *E. coli* and *P. aeruginosa* 75mg/mL 12mg/ml and 75mg/mL respectively. In comparison with the current study, higher MIC value was reported for *S. aureus* and *E. coli* but lower for *P. aeruginosa* by same authors. This apparent disagreement may be due to differences in the procedures followed, solvents used and agro-ecology of the plants tested. Alemtsehaye Teka *et al.,* (2015) reported that ethanol crude extract of *O. rochetiana* leaf at concentration ranging from

128μg to 512μg didn't show growth inhibition on standard strains of *E. coli*, *S. aureus* and *P. aeruginosa.*

Ethanol crude extract of *O. rochetiana* steam-bark revealed the lowest MIC value on *S. aureus* (3.9mg/mL), highest on *P. aeruginosa* (15.62mg/mL) and comparatively moderate (7.8mg/mL) was shown on *E. coli*. The most susceptible bacterium was *S. aureus* whereas *P. aeruginosa* was the least. The lowest MBC was observed on both strains of *E. coli, S. aureus* and standard *S. typhi* (62.5mg/mL) whereas the highest MBC was exhibited by both clinical standards of *P. aeruginosa* (125mg/mL).

In comparison with leaf ethanol crude extract, leaf ethanol crude extract exhibited lowest MIC value (1.95mg/mL) on both clinical and standard strains of *S. aureus.* The MIC obtained for the ethanol steam-bark extract was 3.9mg/ml on the same bacterial strains. It is possible to conclude that more bioactive compounds are found on leaf than the steam-bark of this particular plant species. *V. myriantha* leaf ethanol extract had an MIC of 31.25mg/mL and MBC 125mg/mL on *S. aureus,* but it did not show any growth inhibitory activities on the other bacterial strains tested.

Table 5: MIC and MBC of crude ethanol and chloroform extracts of *O. rochetiana* leaves and stem-bark, and *V. myriantha* leaves on pathogenic bacteria

Bacterial strain	**Crude extracts in (mg/mL)**						
		O. rochetina leaf		*O. rochetiana* bark		*V. myriantha*	
	Solvent	MIC	MBC	MIC	MBC	MIC	MBC
Clinical *E. coli*	C_2H_5OH	3.90	62.00	7.8	62.5	ND	ND
	$CHCl_3$	7.81	62.50	ND	ND	ND	ND
Standard *E. coli*	C_2H_5OH	3.90	62.50	7.8	62.5	ND	ND
	CHCl3	7.81	62.50	ND	ND	ND	ND
Clinical *P. aeruginosa*	C_2H_5OH	7.80	62.50	15.62	125	ND	ND
	$CHCl_3$	15.62	125.00	ND	ND	ND	ND
Standard *P. aeruginosa*	C_2H_5OH	7.80	62.50	15.62	125	ND	ND
	$CHCl_3$	15.62	125	ND	ND	ND	ND
Clinical S. *aureus*	C_2H_5OH	1.95	31.25	3.9	62.5	31.25	125
	$CHCl_3$	3.90	62.50	ND	ND	ND	ND
Standard *S. aureus*	C_2H_5OH	1.95	31.25	3.9	62.5	31.25	125
	$CHCl_3$	3.90	62.50	ND	ND	ND	ND
Standard *S. typhi*	C_2H_5OH	3.90	62.50	7.8	62.5	ND	ND
	$CHCl_3$	7.81	62.50	ND	ND	ND	ND

Key C_2H_5OH: ethanol; $CHCl_3$: chloroform: ND ; not done

6.3. Acute Toxicity Test

6.3.1. Acute Toxicity Results of Ethanol and Chloroform Extracts of *O. rochetiana*

The mice which were given crude extracts of the leaf of *O. rochetiana* did not shown any clinical symptoms of toxicity, such as hair erection, loss of appetite, restlessness and there were no mortality and morbidity within 24 hours observation and 14 days follow-up. In current study, the mean BW of the mice treated with 2000mg/kg BW of crude ethanol extracts of leaf of *O. rochetiana* at the first day (D0) was 24.5±0.6mg. The mean BW at D0 significantly increased their BW to 30.8±0.71g on D14 (Table 6). The BW of the mice administered with crude ethanol extracts of leaf of *O. rochetiana* increased by 26.65% on D14.

This increment in the BW of the mice indicates that the ethanol extracts of *O. rochetiana* were not toxic to albino mice at 2000 mg/kg BW. The negative control mice which was administered with water had a significant BW change with 25.8 % at D14 compared to the mean BW of mice at D0. It is known that body weight can be one of the parameters which indicates the extent of oral acute toxicity. Based on the data obtained from the body weight changes in mice from D0 to D14, the BW increment among the ethanol extract of *O. rochetiana,* and water administered groups were not significantly different from each other. From this it is possible to say the ethanol extracts of *O. rochetiana* and water had no significant effect on the body weight of mice at D14. This data revealed that this extract and water are not toxic at the maximum oral dose of 2000 mg/kg BW. Similarly, the mice treated with the chloroform extracts of *O. rochetiana* at 2000 mg/kg BW significantly increased their body weight at D14. As a result, the chloroform extracts of *O. rochetiana* at 2000 mg/kg BW had significant effect on the BW of the mice. It is possible to say that the chloroform extracts of this plant had no toxicity effect on the albino mice at D14. There was no toxicity report of Olinia *rochetian* up to genus level.

Table 6: Acute toxicity of ethanol and chloroform extracts *O. rochetiana* leaves at 2000mg/kg

Crude extract	Solvent	Mean bodyweight			change (%)
		D0	D7	D14	D0 & D14
O. rochetiana leaf	C_2H_5OH	24.5±0.6	27±0.8$^{\#}$a	30.8±0.71*a	27
	$CHCl_3$	26.3 ±0.4	28.2±0.9$^{\#}$b	32.5±0.85*b	24
Negative control	H2O	26.8±0.6	28 ±0.8$^{\#}$c	33.7±0.9*c	252 25.8

Key: BW= Bodyweight; D0= Day 0; D7= day 7; D14= day 14; H2O = water; C_2H_5OH=ethanol; SEM= Standard error of the mean; % = percent a = weight of mice at D0 for C_2H_5OH, b =weight of mice at D0 for $CHCl_3$ *O. rochetiana* c = for water at D0, *=is significantly higher; #= not is significantly higher

6.3.2 Acute Toxicity Test of Ethanol Extract of *O. rochetiana* Stem-bark

None clinical symptoms of toxicity such as hair erection, loss of appetite, restlessness and there were no mortality and morbidity seen during 24 hours observation and 14 days follow up. The mice which were given crude extract of stem bark of *O. rochetiana* followed up for 14 days. In this work the BW of the mice administered 2000mg/kg BW of ethanol crude steam bark of *O. rochetiana* shown significant increment of BW from (25.14±0.32g) to D14 (30.78±0.5 g) Similarly the BW of mice which were treated with 0.2 ml of water significantly increased BW at D14 compared to their corresponding BW at D0 (Table7).

Table 7: Acute toxicity of extracts of *O. rochetiana* stem-bark at 2000 mg/kg

Crude extract	Mean bodyweight (g)±SEM				
	Solvent	D0	D7	D14	% change D0 & D14
Olinia rochetiana Steam-bark	C_2H_5OH	25.14±0.32	28.64±0.73#a	30.78±0.5*a	22.4
Negative control	H_2O	26.8±0.63	28.02±0.98$^{\#b}$	32.76±0.95^{*b}	22.2

Key: BW: Bodyweight; D0= Day 0; D7= day 7; D14= day 14; H_2O = water; C_2H_5OH=ethanol; SEM= Standard error of the mean; % = percent

6.3.3 Acute Toxicity of Ethanol Extract of *V. myriantha* Leaf

The mice which were administered crude extract of the leaf of *V. myriantha* had not shown any clinical symptoms of toxicity such hair erection, loss of appetite, restlessness and there was no mortality within 24 hours. 2000mg/kg body weight of the crude extracts was given which was high dose based on OECD guideline (2001). 0.2mL of water was given to negative control mice. The body weight of the mice which was given crude ethanol extracts *V. myriantha* leaves show significant increase of body weight (26.1±0.87 to 32.14±0.38g as shown in (Table 8).

The mice which were administered with 0.2ml of water also showed significant increase in BW from DO (26.8±0.63g) to D14 (32.0±0.38g).

The percentage body weight change with mice those administered with 2000 mg/kg BW of crude ethanol extracts of *V. myriantha* leaf was 22.6 % at D14 which was almost similar to the percent body eight changes (22.24 %) of the mice given 0.2ml of water. Based on the body weight of the mice as a parameter it is possible to say crude ethanol extracts of *V. myriantha* leaves at 2000mg/kg had no effect on the extract administered mice shown that the extract was not toxic to the mice. (Yusmazura,2016) reported that aqueous extracts of *V. amygdalina* have no toxicity at 2000mg/kg BW and also (Michael, 2017) noted methanolic leaf extract of *V. lasiopus* have no toxicity at 2000mg/kg BW of mice

Table 8: Acute toxicity test result at 2000 mg/kg dose for ethanol extract of *V. myriantha* leaves

Crude extract	**Mean body weight (g)±SEM**				
	Solvent	DO	D7	D14	% change D0 & D14
V. myriantha	C_2H_5OH	26.1±0.87	28.6 ±0.5$^{\#a}$	32±0.38*a	22.6
Negative control	H_2O	26.8±0.63	28 ±0.8$^{\#b}$	32±0.9^{*b}	22.4

Key: D0 = Day before extract administration; D4 = fourth day after extract administration; D14= fourteenth day after extract administration a = weight of mice at D0 for C_2H_5OH, b = weight of mice at D0 for water; * = is significantly higher # =is not significantly higher

6.4 Phytochemical Constituents of the Plants

The preliminary phytochemical screening of leaves of *O. rochetiana* and *V. myriantha,* and steam-bark of *O. rochetiana* revealed the presence of different secondary metabolites. There were metabolites that were detected in all extract types of both plants as well as in none. Some were detected only in either of the plant species or extract type. Terpenoids were detected in both ethanol and chloroform extracts of the leaves of both plants and stem-bark of *O. rochetiana.* Similarly, glycosides were extracted by both solvents, in both plants except in the ethanol extract of *O. rochetiana* stem-bark. On the other hand, while tannins and steroids were detected in both ethanol and chloroform extracts of only *O. rochetiana* leaf and stem-bark; alkaloids and flavonoids were found exclusively in both extracts of *V. myriantha.* A positive test for phenols was obtained on both leaf and stem-bark only for the ethanol extract of O. *rochetiana. Saponins* were recorded only from the ethanol extract of *O. rochetiana* leaf and chloroform extract of *V. myriantha.* None of the extracts of both plants was positive for resins (Table 8).

When a different plant species belonging to the genus *Vernonia* is considered, the ethanol extracts of *V. amygdaline* leaf showed a positive result for the existence of alkaloid, saponin and tannin but negative for flavonoids, steroids, phenols and terpenoids which disagree with current study (Anibijuwon *et al.,* 2012).

The preliminary phytochemical screening of *V. auriculifera* from the same genera showed positive test result for the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, and phenolic compounds (Temesgen Orebo, 2015).

Therefore, the phytochemical screening result reveals that the presence of these phytochemical constituents supports the use of *V. myriantha* in traditional medications is probably attributed from these phytochemicals which are responsible for healing properties of wounds and stopping bleeding have been claimed by the peoples in the area of this study.

Table 9: Preliminary phytochemical screening results for *O. rochetiana* and *V. myriantha*

Secondary Metabolites	Plant species, its part and extraction solvent					
	O. rochetiana leaves		*O. rochetiana* stem-bark		*V. myriantha* leaves	
	C_2H_5OH	$CHCl_3$	C_2H_5OH	$CHCl_3$	C_2H_5OH	$CHCl_3$
Phenol	+	-	+	-	-	-
Flavonoid	-	-	-	-	+	+
Tannin	+	+	+	+	-	-
Steroid	+	+	+	+	-	-
Saponin	+	-	-	-	-	+
Terpenoid	+	+	+	+	+	+
Alkaloid	-	-	-	-	+	+
Glycoside	+	+	-	+	+	+
Resin	-	-	-	-	-	-

Key: -: the specific metabolite not detected; +: the specific metabolite detected

Various studies that assessed the antimicrobial activities of the class of phytochemicals listed here in reported the potential of each class of compounds in inhibiting the growth of different microorganisms. Phenolics and polyphenols are among these classes of compounds reported in the literature for having such potential. As noted by Brantner *et al.*, (1996) eugenol, caffeic acid catechol and pyrogallol were detected to have antibacterial and antifungal effect through reaction of sulfhydryl groups or more non-specific reactions with proteins is thought to be the possible mechanism for phenolic effect to microorganisms.

Flavonoids and flavonoid-derived plant natural products have been known to function as antimicrobial agents. As different *in vitro* studies have indicated these molecules are effective antimicrobial substances against a wide spectrum of microorganisms (Kazmi *et al.*, 1994). Intake of tannin comprising beverages, particularly green teas and red wines, was suggested to heal or preclude many varieties of microbial infections (Haslaam, 1996). Tannins can cause complexes of proteins through hydrogen bonding, hydrophobic effects and by formation of covalent bonds. A review on the antimicrobial properties of tannins indicated that they inhibit growth and protease activity in many ruminal bacteria. It is reported that tannins bind to cell coat polymers in all strains (Taylor, 2000).

They also cause morphological changes in the organisms indicating that the cell wall is the main target of tannin toxicity (Serafini *et al.,* 1994). Terpenoids and essential oils are other groups of compounds reported to have antimicrobial activities. Results of many studies indicate that terpenes and terpenoids were active against bacteria and fungi. The diterpenoids and sesquiterpenes obtained from *Salvia sclarea* were found to be active against *S. aureus* and the yeast *Candida albicans*. Two terpenoid constituents capsaicin and petalo stemumol were also shown to have an excellent activity against various strains of bacteria and fungi (Ulubelen *et al*. 1994).

7. Conclusions and Recommendations

The ethanol and chloroform extracts of the leaf of *O. rochetiana* and ethanol extract of *O. rochetiana* stem-bark were active against both clinical and standard strains of pathogenic bacteria (*E. coli, P. aeruginosa, S. typhi* and *S. aureus*). But ethanol extract of *V. myriantha* were active only on clinical and standard strains of *S. aureus* whereas all crude extracts of *D. laxata* were inactive in all the tested pathogenic bacterial stains.

In oral acute toxicity test, crude ethanol and chloroform extracts of the stem-bark and the leaves extract of *O. rochetiana* and the ethanol extract of *V. myriantha* did not show observable clinical signs and death within 14 days for oral dose of 2000 mg/kg BW.

The extracts of both plants did not affect the BW of the mice at highest oral doses of 2000 mg/kg BW within 14 days follow up. Hence the two plants were not toxic to the albino mice at or lower than 2000 mg/kg BW. Preliminary phytochemical screening test from ethanol crude extract of *O. rochetiana* leaves revealed the presence of phenol, tannin, steroid, saponin, terpenoid and glycoside on other hand from the same plant part, chloroform crude extract showed presence of tannin, terpenoid and glycoside. Whereas ethanol extract of stem-bark showed presence of phenol, tannin, steroid and terpenoid.

The presence of these important phytochemicals in the plants is a justification of the plant use in the traditional treatment against various diseases impacting humans and animals. The presence of these phytochemicals in those plants enhances their pharmaceutical and therapeutic potentials. The compounds obtained from leaves of *V. myriantha* showed moderate antibacterial activity on *S. aureus* however it failed to other tested human pathogenic bacterial strains.

Further studies should be carried on structural activity, relationships and mode of action in order to guide to a better apprehension of the relationships between the structures and antimicrobial activities of these secondary metabolites. Phytochemical and biological activity studies should also be conducted on those plants to isolate the specific antimicrobial secondary metabolites.

8. References

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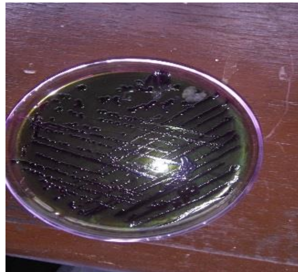
9. Annexes

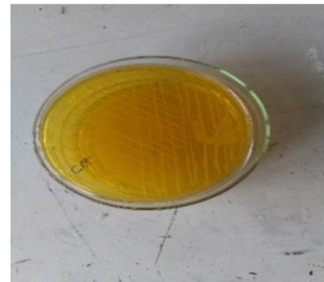
Annex 1: Plant poweder preparation

Annex 2: Pure culture

P. aeruginosa



E. coli



S. aureus

Annex 3: Susceptibility test result of *O. rochetiana*

S. aureus

E. coli

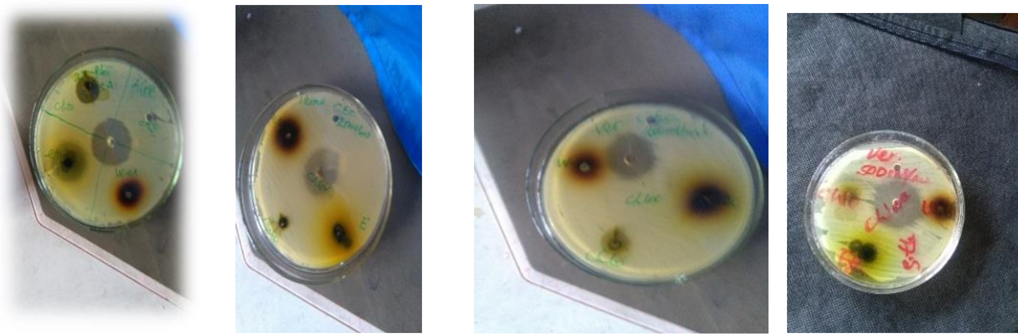
P. aeruginosa

S. typhi

Annex 4: Antibacterial activity with *O. rochetiana* steam bark

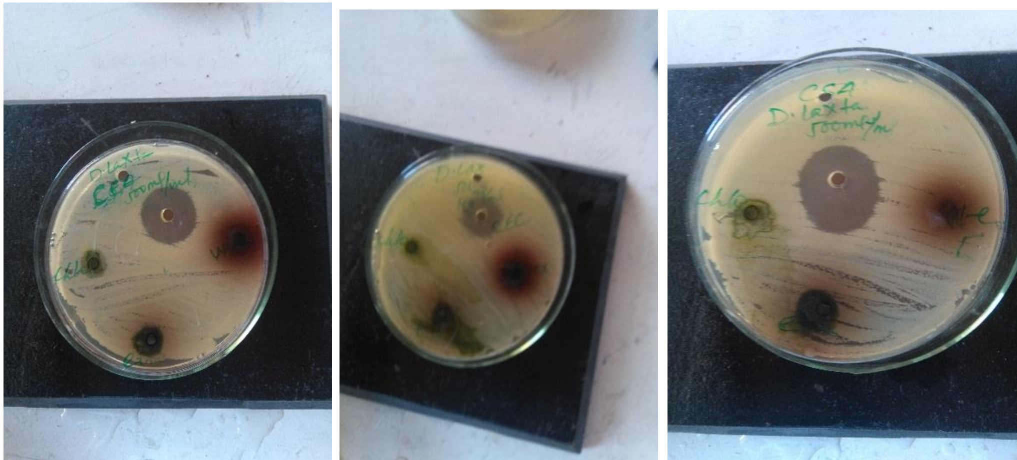
S. aureus *E. coli* *P. aeruginosa* *S. typhi*

Annex 5: Antibacterial activity of *V. myriantha*



S. aureus *E. coli* *P. aeruginosa* *S. typhi*

Annex 6: Antibacterial activity of *D. laxata*



Annex 7: Solvent extraction

Annex 8: Preliminary phythochemical screaning test

Annex 9: MIC detremination test

Annex 10: Toxicity Test