ANTIBACTERIAL ACTIVITIES OF COMPOUNDS ISOLATED FROM SELECTED MEDICINAL PLANTS FROM NANDI COUNTY, KENYA

Kimutai Nicholas Kogo

A research thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy (PhD) in Microbiology of Masinde Muliro University of Science and Technology

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DECLARATION

This thesis is my own original work prepared with no other than the indicated sources and has not been presented elsewhere for a degree or any other award.

Signature_____ Date_____

Kimutai Nicholas Kogo,

(SMB/ H /03/15)

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology, a thesis entitled "**Antibacterial activities of compounds isolated from selected medicinal plants from Nandi county, Kenya"**

Signature……. Date……...

Prof. Charles Mutai (PhD),

Department of Medical Laboratory Science,

Masinde Muliro University of Science and Technology.

Signature……. Date……..

Dr. Philip A. Ogutu (PhD),

Department of Biological Sciences,

Masinde Muliro University of Science and Technology.

DEDICATION

This thesis is dedicated to my mother Maria, my wife Sheila and my son Nickson who encouraged me throughout my studies.

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ABSTRACT

Microbial infections are becoming a major public health problem due to the emergence of multi-drug resistant bacterial strains. Traditional healers have long used plants to treat bacterial infections. Globally, 80% of the people depend on traditional medicine as primary health care to treat different human ailments. Hence, there is an increased interest in ethno pharmacological approaches to identify compounds from plants that can be used to treat bacterial infections. However, the efficacy of most of these plant extracts and their compounds have not been determined. Questionnaires were used to ascetain plants that are used to treat bacterial infections and disesases. Fresh plant parts were collected from the field, dried and the extracts obtained using methanol. The extracts were screened for antibacterial activities against selected strains of bacteria using disc diffusion and broth microdilution methods. Active plant extracts were further used for isolation of compounds and to determined the antibacterial activities of crude and isolated compounds. Bioguided fraction isolation using column chromatography was employed to isolate compounds. Antibacterial activities of the isolated compounds was determined as indicated above for plant extracts. Structural elucidation of the bioactive compoundsn was done using nuclear magnetic resonance (NMR). Thirty three (33) medicinal plants distributed within 24 botanical families were found to be used against bacterial infections. Majority of medicinal plants were used to treat pneumonia 11 (33.3%), wounds 10 (30.3%) and diarrhea10 (30.3%), followed by skin diseases 9 (27.3%). Other remedies used fewer plants species. Seventeen (17) medicinal plants were screened for antibacterial activities basing on the frequency of their use. The extracts from *O. rochetiana, A. lahai, L. calastachys* and *C. myricoides* were active against 60% of the test microorganisms and were considered for further tests. Ethyl acetate extracts (EAE) were the most active extracts from all the the plant extract selected except hexane (HE) extracts of *L. calastachys*. Antibacterial activities of fractions from active successive extracts were determined. Antibacterial activities of *O. rochetiana* and *A. lahai* were the most active against majority of bacteria with highest inhibition zone of 14 mm against MR. *S. aureus.* Two phenolic compounds, *Cis* 4″-*O*-acetyl martinoside (2) and 4-*β*-D-glucopyranosylferullic acid (5); one terpenoid, *β*-amyrin tetradecanoate (4) and two fatty acids, Cis oleic acid (3) and 5-(2,5-dimethylhexyl)1-isopentyl 3-hydroxy-2-methylpentanedioate (1) were obtained from the selected medicinal plants for the first time. *Cis* 4″-*O*-acetyl martynoside (2) was bactericidal against *S. aureus, P. aeruginosa,* MR. *S. aureus* with MBC's of 12.5, 25.0 and 50.0 mg/ml respectively. This is due to electron donating groups(-OH and -OCH_3) which are remarkable in influencing the activity by breaking down the bacterialcell membrane of bacteria leading to lysis. The unsaturated fatty acid (oleic acid(3) was also more active against bacteria than saturated fatty acid. This is probably because of their ability to penetrate the cell membrane of bacteria causing lysis or growth inhibition. Moreover, Oleic acid (3) has a *cis*-type double bond and is adsorbed in the cell membrane easily due to their bent structure. Synergism enhanced activity with combination of compounds (**5**), (**3**) and (**2**), having the highest inhibition zone of 20.03 mm and 20.17 mm, against two resistant bacteria MR*S. aureus* and ESBL. *E.coli.*. The antibacterial activities reported were in tandem with the reported uses by herbalists.With the structures determined the compounds could be synthesis for use as phytomedicines.

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ACRONYMS/ABBREVIATIONS

ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
DMSO	Dimethylsulfoxide
CC	Column Chromatography
^{13}C-NMR	^{13}Carbon Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
CLSI	Clinical Laboratory Standard Institute
CMR	Centre for Microbiology Research
HIV	Human Immunodeficiency Virus
^{1}H-NMR	^{1}Hydrogen- Nuclear Magnetic Resonance
^{13}-NMR	^{13}Carbon- Nuclear Magnetic Resonance
KEMRI	Kenya Medical Research Institute
NMR	Nuclear Magnetic Resonance
MBC	Minimum Bactericidal Concentration
MHA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentration
NCCLS	National Committee for Clinical Laboratory Standard
PBP	Penicillin Binding Proteins
SDA	Sabouraund Dextrose Agar
SPSS	Statistical Package for Social scientists
TLC	Thin Layer Chromatography
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Traditional herbal remedies and medicinal plants use in primary health care is widely practiced in Kenya and other parts of the world. Nature has provided majority of drugs presently in use. For instance, more than 80% of the people in the world depend on traditional medicine for primary health care (Srilekha *et al*., 2017). The existence of the antibiotic resistant microorganisms has led to the search for new potential effective plants and plant constituents against pathogenic microorganisms (Marasini *et al.* 2015). An example of antibiotic resistant microorganism is Methiciline Resistant *Staphylococcus aureus* (MRSA) that causes deaths in many countries including United States (Lakhundi and Zhang, 2017). Natural products from plants may therefore serve as an alternative source of substances for the treatment of such and other resistant bacteria (Atanasov *et al*., 2015). The medicinal value of these plants lies in their secondary metabolites. Some of the most important bioactive constituents of the medicinal plants are alkaloids, anthraquinones, flavonoids, and phenolic compounds (Cushine *et al.,* 2014).

The properties of the phytochemical ingredients explains the antibacterial activities observed in some plants. However, there are differences in the phytochemicals present in plants. This is due to many reasons, including; geographical variation that has some effect. Affects on the level of active medicinal compounds of some species of plants, climate, soil, or season of the year (Morsy, 2014). Moreover, biochemical profiles of plants harvested at different times/seasons and locations may vary greatly. This makes the composition of the

biologically active compounds of these medicinal plants to vary in their antimicrobial activities hence the need to further screen even those that have been screened elsewhere in the world. For instance, the best harvesting moment for all polyphenolic classes of *Pistacia latifolia* and some classes of *Cistus incanus* and *Pistacia lentiscus* is July. This is because environmental parameters positively correlated with the polyphenols of *C. incanus* and *P. latifolia* (Antonella *et al*., 2020). This is the reason why many researchers in the world move all over in order to obtain samples of plants to analyse and evaluate their activities with the aim of getting novel drugs. Therefore, studies on antimicrobial activities should be done even on those plants that have been investigated previously due to the variations in phytochemicals for instance those in Nandi county (Kimutai *et al*., 2016).

The other reason is to isolate bioactive compounds to be used directly as drugs or to produce compounds of novel or known structures as lead compounds for semi-synthesis to produce compounds of higher activity as well as to use part or whole plant as a remedy. This is because majority of the pharmaceuticals that are currently in use originated from plants (Yuan *et al*., 2016). The most common approach to choosing plants for pharmacological studies is ethnopharmacology. Since different plant components can be used to treat diarrhoea, tuberculosis, fever, bronchitis and cholera, among others, they have been shown to act as an important source for the development of new chemotherapeutic medication that can be beneficial for the treatment of bacterial infections (Shahadat *et al.*, 2018).

The discovery of new plant species and the functional explanation of their bioactive molecules are the main goals for the continuously evolving technical phytochemical science. Bacterial species include *Escherichia coli* and *Staphylococcus aureus. E. coli* causes many infections such as septicemia and lung infection, biliary blood, skin and meningitis as well as a series of dietary diseases that occur in the form of diarrhoea from a medical standpoint while *S. aureus* causes wound infections, toxicity, pneumonia and toxic shocks (Patricia *et al.,* 2015).

Antimicrobial effects of various plant extracts on certain pathogens have recently been reported. For instance, in Kenya, 75%-90% of rural communities have been reported to use expertise in ethnomedicine and medicinal plants as a complementary to or as a substitute to modern medicines for managing diseases as recommended by local health systems. Indigenous people are a valuable asset to ethnobotanical research owing to their understanding of native plants and their applications, (Kimutai *et al*., 2019). The high cost of manufactured prescription drugs and/or inaccessibility to the Western medical health care system has contributed to over-reliance on traditional medicine. On the other hand, while standard healthcare facilities are accessible, traditional medicine is regarded from a cultural perspective in the treatment of various diseases as an efficient and acceptable system (Jeruto *et al*., 2015).

Different factors such as the Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) pandemic, poor hygiene, overcrowding and resistance to conventional medicine are the causes of increased bacterial infections. Therefore, natural products obtained from higher plants can provide a new source of antimicrobial agents, possibly with unigue or different mechanisms of action. For example,

traditional Chinese medicines are a valuable source for novel antibacterial agents (Anani *et al.*, 2015).

With the increase in the number of people infected with the human immunodeficiency virus (HIV), intensive research has been carried out into the plant derivatives that may be effective, particularly for use in developing countries with little access to conventional HIV/AIDS treatment medicines (Salehi *et al.*, 2018). There is a rise in the number of medical scientists in Kenya and around the world employed in pharmaceutical industries, which indicates the increasing interests in ethnobotanical research. Although these pharmaceutical plants are widely used, so far, only a small number of them have been studied extensively. New antimicrobial phytochemicals are therefore urgently needed which can be used as an alternative to conventional antimicrobial interventions. The purpose of this study was to evaluate antibacterial activities of compounds derived from selected medicinal plants used in Nandi county, Kenya to treat pathogenic infections caused by bacteria.

1.2 Statement of the problem

In the past decade there has been significant increase in the prevalence of microorganisms resistant to antibacterials present in conventional drugs such as tetracycline, sulfonamides and chloramphenicol. This is by enteric bacteria such as *Shigella dysentriae.* This resistance contributes to the increase in morbidity, mortality and health care costs. Additionally, currently used drugs are costly and unaffordable to low income earners. Therefore, medicinal plants present new therapeutic options for the treatment of bacterial infections. However, some of the plants that are utilized by the local people in form of concotions have not been screened scientifically for efficacy and safety. Moreover, the

concotions used are whole extracts that are made up of several phytochemicals that might reduce the efficacy and/or lead to undesirable reactions. Therefore, synergism of isolated and elucidated compounds are important in the elimination of the resistant pathogens especially those that resist the drugs that are used most frequently during treatment.

1.3 Justification of the study

Traditional medicine has remained the most affordable and easily accessible source of treatment among the resource poor communities. The efficacy of both extracts and compounds of most of the plants used among traditional communities in Kenya have not been established. Hence, there is a lot of undiscovered potential in plants. Plants and natural products remain a reservoir of potentially useful chemical compounds, not only as drugs but also as unique templates that could serve as a starting point for synthetic drugs. Over 50% of all modern clinical drugs in use originated from natural product hence, natural products play an important role in the drug development programme in the pharmaceutical industries. For example, the drug quinine is isolated from *Cinchona succurubra* plant that is used all over the world to treat malaria. In addition, it is widely acknowledged that many current drugs were discovered on the basis of their indigenous use. Such indigenous knowledge also needs to be documented before it is lost forever with the passing on of the older generation. It is justifiable to search for alternative therapy in natural products from plants because they have been known for many years as a source of therapeutic agents. Therefore,there is need to identify the active compounds, isolate and characterize them in order to provide scientific validation of phytocompounds as well as development and formulation of phytomedicines. This study therefore, determined the

antibacterial activities of both the extracts and phytocompounds isolated from selected medicinal plants from Nandi county.

1.4 Objectives

1.4.1General objective

To determine antibacterial activities of extracts and individual compounds isolated from four selected medicinal plants from Nandi county, Kenya.

1.4.2 Specific objectives

i. To identify medicinal plants used in the treatment of bacterial infections in Nandi county.
ii. To determine the antibacterial activities of the crude extracts from the selected medicinal plants.
iii. To isolate bioactive compounds from the active crude extracts of selected medicinal plants.
iv. To determine the antibacterial activities of the isolated compounds.
v. To structurally characterize antibacterial bioactive compounds isolated from the selected medicinal plants.

1.5 Research questions

i. Which plants are used in the treatment of bacterial infections in Nandi county?

ii. Do the crude extracts from the selected medicinal plants have antibacterial activity?

iii. What are the active compounds present in the active crude extracts of selected medicinal plants?

iv. Do the individual or combined isolated compounds possses antibacterial activity?

v. What are the structures of the validated bioactive compounds?

1.6 Hypothesis

i. Crude extracts of the selected medicinal plants have antibacterial activities.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background on bacterial infections

Bacteria are microscopic unicellular organisms. A bacterial infection is proliferation of pathogenic bacteria on or inside the body. Pathogenic bacteria are bacteria that cause disease (Ryan *et al*., 2014). Pathogenic bacteria can infect any part of the body. For instance, urinary tract infections are caused by *E. coli*, *S. aureus*, *K. pneumoniae* and *E. faecalis,* (Odoki *et al*., 2019). Bacteria are transmitted to humans through living and non living vectors such as; water, air and food. The modes of transmission of bacterial infections are contact, airborne and droplets, (Tortora *et al*., 2016). Symptoms vary depending on the type of the bacterial infection and the area of the body infected. Infectious bacteria cause various diseases by among other things production of toxins, which can damage tissue, (Nash *et al*., 2015).

Dispite the success in the discovery of antibiotics, infectious diseases caused by bacteria still remain the second leading cause of death in the world. For instance according to (WHO, 2016), over 17 million people die anually due to these infections. This is caused by lower respiratory tract infections and diarhoeal infections that lead to 3.0 million, 1.4 million and1.3 million deaths respectively in the year 2016 worldwide. Similarly, the number of tuberculosis death toll in the same year was 1.1 milllion (W.H.O, 2016). Secondary bacterial infections is one of the causes of deaths in the world especially with outbreak of corona virus diseases (Covid-19) This is because of the association of COVID-19 illness and secondary bacterial infections (Sharrifipour *et al*., 2020).

According to the British study, resistant pathogens claim 700,000 lives each year world wide these deaths are majorly due to antibiotic resistant bacteria (Mohammed and Fatima, 2020).

2.2 Pathogenic bacteria

Pathogenic bacteria are categorized as gram positive or gram negative depending on their ability to take the purple crystal violet dye when subjected to the gram-staining procedure (Metlay *et al*., 2020). Some of the most common bacterial infections caused by pathogenic bacteria include; Salmonella, *Escherichia coli*, *Mycobacterium tuberculosis*, Methiciline Resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumoniae*, (Brundage and Shanks, 2020).

2.2.1 Gram-negative bacteria

Gram negative bacteria do not retain the crystal violet because their cell wall is composed of a single layer of peptidoglycan surrounded by a membranous structure **(**outer membrane). The outer membrane of gram-negative bacteria contains thin peptidoglycan layer which does not retain the purple crystal violet stain. It also produces endotoxins, which are toxic to animals (Kaplan *et al*., 2012).

2.2.1.1 *Escherichia coli*

It causes abdominal pains and diarrhea due to toxins. It is the leading cause of acute kidney failure in children and elderly persons. It also causes abdominal cramps, vomitting and bloody diarrhoeae due to the toxins (Pormohammad *et al.*,2019).

2.1.1.2 Extended-spectrum beta lactamase positive (ESBL) *Escherichia coli*

This is a strain of *Escherichia coli* that contain resistant genes to Esbl (Extended-spectrum beta-lactamase-positive) drugs such as penicillins, cephahlosporins and aztreonam. ESBL-producing *E. coli* has led to the increased incidence of urinary tract infections (Wang *et al.*,2017).

2.2.1.3 *Shigella sonnei*

This is a rod (bacillus) shaped, non-motile, non-spore-forming bacterium, that are non-capsulated (Hirose *et al.*,2005). The bacteria are able to survive in contaminated environments as well as the acidity of the human gastro-intestinal tract. It causes shigellosis (Ranjbar and Farahani, 2019).

2.2.1.4 *Pseudomonas aeruginosa*

It's a bacterium which can cause zoonotic diseases (Picozzi *et al*, 2014). It is an opportunistic pathogen of immunocompromised individuals. *P. aeruginosa* infects the pulmonary tract, urinary tract, burns, wounds, and other blood infections. There is emergence of antibiotic resistance by *P. aeruginosa* in intensive care units which is a serious concern in health care units (Pachori *et al*., 2019).

2.2.1.5 *Klebsiella Pneumoniae*

This is a bacterium found in the normal flora of the mouth, skin, and intestines (Ryan, 2014). It causes pneumonia in humans and animals. There are some strains that are developing resistance by hydrolyzing a broad spectrum of β-lactam antibiotics including penicillins, cephalosporins, carbapenems and monolactam antibiotics (Garbati and Godhair, 2013).

2.2.1.6 *Salmonella typhi*

Salmonella typhi is a gram-negative bacterium that infects the intestinal tract and the blood of animals. The disease is referred to as typhoid fever. Antimicrobial resistance has emerged in *Salmonella enterica* to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole and fluoroquinolone due to chromosomal mutations, (Crump *et al.*, 2015).

2.2.1.7 *Citrobacter freundii*

This is a non-sporing and rodshaped bacterium belonging to the family enterobacteriacea. It causes urinary tract infections, and is found in wounds and respiratory tract. The bacterium is resistant to ampicillin and cephalosporins, (Ranjan *et al*., 2013).

2.2.2 Gram-positive bacteria

Gram-positive bacteria retain the purple crystal violet dye when subjected to the gram-staining procedure. Their cell wall consists of several layers of peptidoglycan that makes it thick (Kaplan *et al*., 2012).

2.2.2.1 *Staphylococcus aureus*

It is a coccus shaped bacterium is that frequently found as part of the normal skin flora on the skin and nasal passages. They appear as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often hemolytic, when grown on blood agar (Zhang, *et al*., 2017). They infect most parts of human body causing respiratory and intestinal diseases with some causing serious food poisoning, (Mostafa *et al*., 2018).

2.2.2.2 Methiciline resistant *Staphylococcus aureus* (MRSA)

MRSA is any strain of *Staphylococcus aureus* that has developed resistance to beta-lactam antibiotics, which include; methicillin, dicloxacillin, nafcillin and cephalosporins. This is a bacterium responsible for several difficult-to-treat infections in humans (Timothy and Foster 2017). It is also called multidrug resistant *Staphylococcus aureus* and oxacillin resistant *Staphylococcus aureus* (ORSA) (Lakhundi and Zhang, 2018).

2.2.2.3 *Enterococcus faecalis*

Enterococcus faecalis is bacterium can cause a variety of infections, including urinary tract infections, bacteremia, endocarditis, and meningitis. It also has the ability to colonize the gastrointestinal tract of hospitalized humans for long periods (Yang and Lu 2020). This influences the development of drug resistance to the most common anti-enterococcal antibiotics which makes the treatment of these infections a challenge, (Farman *et al*., 2019).

2.3 Bacterial infections in Kenya

In Kenya, like other African countries, bacterial infections is endemic and resistance of bacteria to readily available and cheap drugs such as chloramphenicol is increasing. For example, typhoid kill about 45,000 Kenyan children every year, nearly one in every three children who fall sick with it (Malatya and Ochieng, 2014). This is due to use of counterfeit antibiotics that contain none or little of the active ingredients, hence accelerate resistance. The government therefore has found it necessary to provide free treatment of some bacterial infections such as Tuberculosis (TB) in health facilities (World Report 2007).

2.4 Bacterial control through chemotheraphy

Antibiotics are medications that fight bacterial infections. They work by distrupting growth and proliferation processes of bacteria. The discovery of antibiotics began in the year 1929 by Alexander Fleming, and since then antibiotics are being discouvered, (Finberg *et al*., 2004). Antibiotics are categorized into the following classes; Aminoglycosides, Betalactams, Macrolides, Sulfoamines and Tetracycline.

2.4.1 Aminoglycosides

Aminoglycosides are medicinal and bacteriologic category of traditional gram-negative antibacterial medications that contain an amino-modified glycoside (sugar). This a class of antibiotics that inhibit protein synthesis by binding to bacteria 30S ribosomal subunit causing misreading of the genetic code and therefore inhibit translation (Castanheira *et al.,* 2020). Examples include; gentamicin, tobramycin, amikacin, plazomicin, streptomycin, neomycin, and paromomycin (Hood-Pishchany *et al*., 2020).

2.4.2 Beta-lactams

A beta-lactam (β-lactam) is a four-membered lactam ring. It is named as such because the nitrogen atom is attached to the β-carbon atom relative to the carbonyl. The simplest β-lactam possible is 2-azetidinone. Beta-lactam antibiotics are among the most commonly prescribed drugs, grouped together based upon a shared structural feature, the beta-lactam ring, (Har and Solensky, 2017). Beta-lactam antibiotics inhibit the formation of peptidoglycan cross-links in the bacterial cell wall. Examples include; ampicillin,

amocxillin, amikacin, benzathine, cafoperazone,ceftazidime and ceftolazane (Wivagg *et al*., 2014).

2.4.3 Macrolides

These are antibiotics having a macrolide ring to which one or more deoxy sugars, may be attached (Gary, 2009). The lactone rings are usually 14, 15 or 16-membered ring. The mechanism of action involves inhibition of bacterial protein biosynthesis, by associating with the ribosome reversibly (Soichiro and Bruce 2020). It's mode of action is mainly bacteriostatic, but can also be bactericidal in high concentrations, (Martin, 2009). Examples of drugs in this class include azithromycin, chloramphenicol and erythromycin (Corey *et al*., 2016).

2.4.4 Sulfonamides

The original antibacterial sulfonamides are synthetic antimicrobial agents that contain the sulfonamide group. They act by inhibiting enzyme the dihydropteroate synthetase (DHPS) in the folic acid biosynthetic pathway inhibiting folic acid production hence cell death. However, resistant genes for sulfonamides in *Bacillus spp.*, *Pseudomonas* and *Shigella* have developed from manure as sources of agricultural soil pollution (Wang *et al*., 2014). Examples of Sulfonamides include quinolones.

2.4.5 Tetracycline

Tetracycline is a broad-spectrum antibiotic produced by the *Streptomyces* genus of Actinobacteria. It is also used to produce several semi-synthetic derivatives, which together are known as the tetracycline antibiotics and are used against many bacterial infections. It is a protein synthesis inhibitor, (Shutter and Akhondi, 2020). They inhibit bacteria by

preventing aminoacyl-tRNA molecule binding to mRNA ribosome complex. They do this mainly by binding reversibly to 30S ribosomal subunit in the messenger RNA, stopping cell growth and translation (Chopra and Roberts, 2005). Resistance to tetracyclines can arise through drug efflux, ribosomal protection proteins, 16S rRNA mutation, and drug inactivation through the action of a monooxygenase (Zakeri, 2008).

2.5 Molecular basis of antibiotic action

The molecular basis of antibiotic action is well understood and the molecular targets are well known which are classified based on the part of the cell that is damaged or interfered with. The process starts when the antibiotic targets the cell functions inhibiting bacterial cell growth (Vandevelde *et al*., 2016). This is a complex process that involves from the physical interactions of the molecules and the specific target that includes biochemical, molecular and structural changes acting on many cellular targets such as the RNA synthesis, DNA replication, protein and cell wall synthesis. RNA synthesis through the DNA-dependent RNA polymerase which mediates the transcription process for gene expression that leads to cell growth, is an attractive site for antibiotics, (Khameneh *et al*., 2016). For instance, Kanaymycin that inhibits synthesis of RNA, by using stable connection with high affinity β-subunit in the RNA/DNA channel leading to the separation of active site. Hence, inhibiting the initiation of transcription and blocking the path of ribonucleic acid chain growth, (Chopra *et al*., 2002).

DNA replication takes place when DNA gyrase (topoisomerase) that control the topology of the DNA by catalyzing the cleavage pattern and DNA binding which is important for DNA synthesis and mRNA transcription. Quinolones can prevent DNA replication by forming a complex quinolone topoisomerase DNA cleavage, leading to death of bacteria,

(Nikaido, 2009). The cell wall synthesis consists of peptidoglycan biosynthesis that involves three stages; the first stage takes place in the cytoplasm where low molecular weight precursors are synthesised. The second stage involves membrane bound enzymes that catalyse the cell wall synthesised while the third stage is where the antibiotics act by preventing β-lactams and polymerization of glycan synthesis of cell wall enzymes that act on transpeptidases, (Vandevelde *et al*., 2016).

Some antibiotics affect protein synthesis where translation occurs in three stages namely; initiation, elongation and termination. The ribosomes are made up of two subunits 50s and 30s subunits which are normally targeted by antibiotics thus inhibiting protein synthesis. For example the macrolites act by blocking access chain of amino acyl tRNA-ribosomes, spectinomycin interferes with stability of peptidyl tRNA binding to the ribosome. Streptomycin, kanaymycin and gentamycin acts on 16S rRNA that is part of 30S ribosomal sub-units, (Wang *et al.,* 2006).

Cytoplasmic membrane which is a diffusion barrier composed of lipids, proteins and lipoproteins can be interfered with by antibiotics like daptomycin that inserts into the cytoplasmic membrane of bacteria in calcium dependent fashion forming ion channel triggering the release of potassium ions. Several other antibiotics can cause the disruption of membranes or can be categoriezed as either cationic, anionic and neutral agents, (Anderson and Hughes, 2010). The latest antibiotic is platensinmycin which inhibits ketoacyl synthase 1 /11 (Fab F /B) which is the key enzyme in the production of fatty acids, necessary for bacterial cell wall membrane synthesis, (Wang *et al.*, 2014).

2.6 Mechanisms of antibacterial activity and resistance

Antimicrobials are substances that kill or inhibit the growth of microbes such as bacteria, fungi, parasites or viruses (Catteau *et al*., 2018). Antimicrobials that are used to treat infections caused by bacteria are called antibacterials.

2.6.1 Mechanisms of action of antibacterial agents

Antibacterials act by either killing (bactericidal) or slowing growth (bacteriostatic) of susceptible bacteria. They exhibit this through the following ways; damaging the pathogen through inhibition of cell wall synthesis, protein and nucleic acid synthesis, disruption of membrane structure and function as well as blockage of metabolic pathways through inhibition of key enzymes (Khameneh *et al.,* 2019). However, some of these mechanisms have been resisted by bacteria but there are several strategies that have been suggested to overcome the resistance of antibiotics by bacteria. Some of the strategies include; combination with other molecules with the failing antibiotics, which restores activity, (Rana *et al.,* 2018). The possible molecules are phytochemicals that have shown potential activities against bacteria. The phytochemicals can act on their own or in combination with others or with antibiotics to promote activities against bacteria, (Shakeri *et al*., 2018).

2.6.2 Mechanisms of resistance to antibacterial agents

Resistance is the ability of the microorganism to survive at a given concentration of an antimicrobial agent at which the normal population of the microorganism would be killed. Antibacterial resistance was recognized after the discovery of diseases that were once thought to be controlled by antibiotics being resistant to the same antibiotic (W.H.O.,

2014). Antibiotic resistance in bacteria can be divided into the following major groups based on the mechanisms involved. Resistance developed due to presence of an enzyme that inactivates the antibiotic (Beta-lactamases) or modifies the antibiotics (Aminoglycosides), modification of the antibiotic target, reduced permeability and active efflux (Blair *et al*., 2015). The occurrence of the resistant bacteria has caused the existing antibacterial drugs to become less effective or even ineffective, (W.H.O.,2014). Therefore, tracking resistant bacteria may improve the use of antibiotics, and the development of new antibiotics and new diagnostic tests (CDC, 2013). The use of molecular-based methods may offer a promising means to confirm identity and therapeutics, even if principally limited to detection of resistant determinants rather than susceptibility tests (Tanja *et al*., 2015). Susceptible population of bacteria may become resistant to antimicrobial agents through mutation and natural selection, or by acquiring genetic information that encodes resistance from other bacteria through transfer of genes by mechanisms such as conjugation, transduction and transformation. Acquired resistance results from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA sometimes occur by use of antibiotic pharmaceuticals, (Wang *et al*., 2020).

The spread of antibiotic resistance among bacteria may also be mediated by horizontal transfer of plasmids. Plasmids carry genes which encode antibiotic resistance which may result in co-resistance to multiple antibiotics (Haruka, *et al*., 2018). These plasmids can also carry different genes with diverse resistance mechanisms to unrelated antibiotics, but because they are located on the same plasmid, resistances to more than one antibiotic is transferred. Alternatively, cross-resistance to other antibiotics within the bacteria results when the same resistant mechanism responsible for resistance to more than one antibiotic

may occur, especially through desinfection, (Jin *et al.*, 2020). The genetic changes caused by mutation or acquisition of resistant genes through horizontal gene transfer, can cause change in the site of drug action and therefore hindering the action of the antibiotics, (Haruka, *et al*., 2018).

WHO (2001) found that infectious diseases are the leading cause of death worldwide, and rapid resistance development is a growing threat even in developed countries.

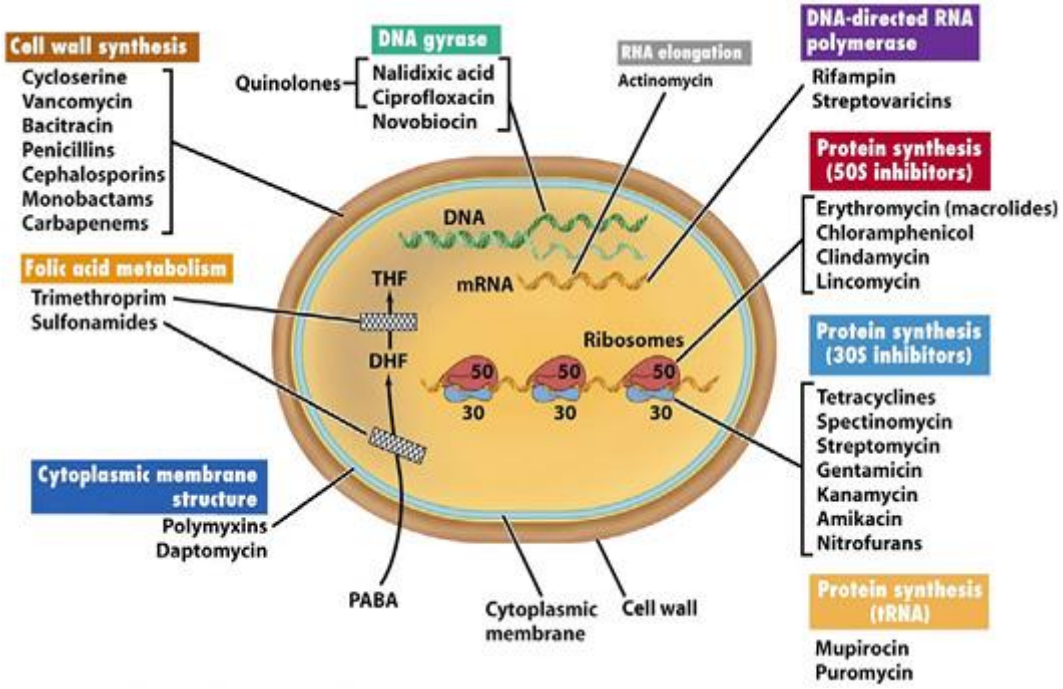


Figure 2. 1: Schematic representation of antibiotic class and mechanism of antibiotic resistance in bacteria (Adopted from Labnotesweek 4, 2013).

Plant extracts and their compouds act as an alternative to this problem but few compounds have been isolated and characterized so far.

2.7 Use of plants and natural products in chemotherapy of bacteria

For a number of years, plants have been an important natural source of natural products of medicinal value for people's health, particularly in the last two decades, with more extensive studies in natural therapies.

Some of the compounds isolated from plants their class, antimicrobial activities, dosage and minimium inhibitory concentration (MIC) commercially available to consumers are listed in Table 2.1.

Table 2. 1: Some commercially available plant products with antibacterial activity

Scientific name of plant	Compound/Class of compound	Active against(Bacteria)	Dosage and MIC
Piper nigrum	Piperine (Alkaloid)	Fungi, *Lactobacillus*, *E. coli* and *E. faecalis*	100µg/Ml
Rhamnus purshiana	Tannins (phenolic compound)	*S. aureus* and *P. aeruginosa*	Capsule 425, 450 mg
Matricaria chamomilla	Anthemic acid	*M. tuberculosis*, *S. typhimurium*, *S. aureus*	-
Syzygium aromaticum	Eugenol (Terpene)	*H. pylori*, MRSA, MSSA, *P. aeruginosa*	Capsule 500 mg (150-300 µg/Ml)
Vaccinium spp.	Fructose	*S. aureus*, *S. epidermidis*, and *P. aeruginosa*	Capsule 500 mg
Allium sativum	Ajoene, (Alkaloid)	*E. coli*, *K.* pneumoniae, *S.aureus* and *L. plantarum*	Tablet, (100 and 160 µg /ml)
Hydrastis canadensis	Hydrastine (Alkaloid)	*E. coli* and *K. pneumoniae*	Solution, 500 mg per dosage
Glycyrrhiza glabra	Glabrol (phenolic compound)	*S. aureus*, *M. tuberculosis* and *E. coli*	Capsule 450 mg (2-128 µg/ml)
Quercus rubra *Allium cepa*	Quercetin (phenolic compound)	*S. aureus*, *H. pylori* and *E. coli*	Capsule 500, 650 mg (19-75 µg/mL)
Hypericum perforatum	Hypericin, (phenolic compound)	*S. aureus*, *P. aeruginosa* and *E. coli*	Tablet 450 mg, (64-80 mg/mL)
Thymus vulgaris	Caffeic acid (phenolic compound)	*E. coli*, *E. aerogenes*, *S. aureus*, *P. aeruginosa*	Capsule 450 mg (5-10 µg/ml)
Thymus vulgaris	Thymol (Terpenoid)	*S. aureus*, *P. aeruginosa* and *E. coli*	Capsule 450 mg (5-10 µg/ml)
Piper nigrum	Piperine (Alkaloid)	Fungi, *Lactobacillus*, *E. coli* and *E. faecalis*	100µg/Ml
Rhamnus purshiana	Tannins (phenolic compound)	*S. aureus* and *P. aeruginosa*	Capsule 425, 450 mg
Matricaria chamomilla	Anthemic acid	*M. tuberculosis*, *S. typhimurium*, *S. aureus*	
Syzygium aromaticum	Eugenol (Terpene)	*H. pylori*, MRSA, MSSA, *P. aeruginosa*	Capsule 500 mg (150-300 µg/Ml)

(Khameneh *et al*., 2019)

2.7.1 Documentation and the use of medicinal plants

Medicinal plants would be the best source of a variety of drugs (Ekeigwe, 2019). In Asia, parts of Latin America, and Africa. The use of these herbal care is widely used by most people. For instance, 80% of people in the previously mentioned territories are projected to use these solutions as the effects are reported to be minimal (Gude, 2013). Traditional medications are used by the same proportion of people from developed countries. Therefore, it is important to study plants in order to understand their properties, their safety and their efficacy (Kimutai *et al*., 2015). A significant number of plants have been studied as an inclusive medicine system for protecting and managing microbial illnesses due to their antimicrobial properties. In treating human pathogenic diseases the use of herbal extracts with antimicrobial potential can be extremely important. For instance, secondary metabolites including flavonoids and alkaloids are used as a medicinal agents as well as tannins, saponins and terpenoid (Cushine *et al.,* 2014). These bioactive substances become integrated medical supportive and alternative medicines into the healthcare system (Yuan *et al.*, 2016).

Previous studies done have showed that there are more medicinal plant species in certain plant families than had been thought. For example, an analysis in *Apillapampa* showed the distribution of medicinal plants in 80 botanical families. A fourth of these species are Asteraceae (85 species; 25%) and Fabaceae (27 species; 8%), Solanaceae (22 species; 6%), Lamiaceae (14 species; 4%) and Scrophulariaceae (10 species; 3%). Asteraceae has been popularized for their the wide range of the bioactive compounds found in them. The bitter chemicals they contain makes people to use them (e.g. sesquiterpene lactones) associated with members of this group. (Laurella *et al.,* 2017).

In Africa, it is projected that 75% of the population are still dependent on traditional tional healthcare methods and medicinal plants. This is because African herbal medicine use has a long and rich tradition which incorporates the use of medicinal plants in the treatment of diseases (James *et al*., 2018).

In developing countries including Kenya, the goal of integrating the use of medicinal plants throughout healthcare systems. This has been done through incorporation of traditional healers into healthcare management and regulation following the WHO resolution of 2003. This move was intended to document and ensure the safety and efficacy of traditional medicines and remedies in these countries WHO (2014). Nevertheless, there is a need to ensure that herbal cures are developed and scientifically justified so that they are included into health systems as an alternative to conventional medicine.

In Kenya 10,000 plant species, especially those within the pastoral communities such as Maasai, Samburu and the Kalenjins, have been recorded as medicinal (Kipkore *et al*., 2014). In the Maasai community, herbal knowledge is prevalent. Families often have their own health care. Beers and other wild fruits, for example, are used to complement the diet, mostly by females and boys, before feeding (Nankaya *et al.*, 2019).

In Mt. Elgon, Elgeiyo Marakwet County, and Nandi, ethnobotanical studies showed a large number of plants are use communities in these regions. It was reported that the families of a large number of herbs used come from Asteracea, followed by the Euphobiacea. A research conducted in Elgeiyo Marakwet county revealed that the medicinal plantsfrom Laminaceae family were the highest species, followed by Asteraceae and Euphorbiaceae

respectively. In Nandi the bulk of medicinal plants used have been reported in Mt. Elgon and in Bungoma county (Gabriel *et al*., 2014, Okello *et al*., 2010).

The aforementioned studies dealt with the ethno-botany studies. It is therefore necessary to determine the antimicrobial activities in order to validate the ethnobotanical studies. In addition, many plants have been widely utilized and detailed toxicity evaluations are not easily available which can lead to serious abnormalities (Kimutai *et al*., 2017, Alam *et al*., 2014). The alarming rate at which the tropical rainforest of Africa is being destroyed to pave way for agriculture, settlement and the industries, is posing grave conservation threat to medicinal plants in their natural environment. Consequently, the behavior of medicinal and other endemic plants has been dietarified, some of them for useful components not yet documented and analyzed (Gaoue *et al*., 2017).

Ethnobotanical studies on the use of Kalenjin medicinal plants carried out in Aldai division in South Nandi district showed their value in the treatment of various virus, bacteria and fungi-related diseases (Kimutai *et al*., 2017). Availability of conventional medicine is not enough in the Nandi county is not adequate as there is only one county referal hospital and a handful of medical facilities. This explains why several species of plants from various families obtained from forests continue to be used to treat a range of medical conditions and related conditions.

2.7.2 An overview of some Kalenjin medicinal plants

Plants that have been studied and used by the Kalenjin community include the *Amaranthus spp.* (Chepkerta, Nandi) a weed that grows on farms and is distributed in Africa, Asia, Oceania and the Americas. The leaves and roots are used as decoction (internal) for management of malaria, diarrhoea and fungal infections among the Kalenjin and is also

taken as a vegetable while the Mbeere and Ameru use them to treat diabetes (Ambuko *et al*., 2017). The flour made from *Amaranthus spp* is sold in supermarkets and is mainly used by diabetics.

Ekebergia capensis (Teldet, Nandi) infusion is used for treatment of colds and malaria. A study conducted in Nandi showed that *E. capensis* being very active against Methicillin resistant *Staphylococcus* aureus with an zone of inhibition of 14.7 mm (Kimutai *et al.*, 2015). *Kalanchoe spp.* has been used to alleviate as an abortitifacient pain and and *Acacia spp.* (Chebitet, Nandi) is mainly used as a stabilizer in the treatment of diarrhoea in HIV patients; this is attributed to the presence of the tannins (Cherotich, 2015). *Croton megalocarpus* (Tebeswet, Nandi) is used for treating pneumonia and is reported to contain compounds that can be used to treat cancer, the main compound is crotonin, (Wabai *et al*., 2018). *Kigelia africana* (Ratinwet, Nandi) fruits and barks are boiled in water and taken orally as a laxative in treating stomach ailments (Bello *et al*., 2016).

The ripe fruits of *Kigelia africana* are used to ferment local beer (Pusaa) and it's known to treat stomach ailments, unripe fruits because they are poisonous. The ethanolic stem bark extract of *K. africana* has been found to possess antibacterial and antifungal activity against *S. aureus* and *C. albicans* (Kimutai *et al.,* 2015). *Eheretia cymosa* (Mororwet, Nandi) the Maasai people use the roots to treat brucellosis while in Ethiopia, crushed roots are soaked in water are used for the treatment of stomach ailments and the root juice is applied to wounds (Julia *et al*., 2015). Studies on this plant showed bacteriostatic activity against *P*. *aeruginosa* (Kimutai, *et al.*, 2015).

The study of secondary metabolites has led to the isolation of drugs for the treatment of human diseases. For instance, it has been found that there are over 119 chemical

substances in plants that are considered very important drugs that are used in many countries (Tesso, 2005). Some of the successes in the development of drugs from plants is the isolation of antimalarial drug quinine which is an alkaloid from the bark of Cinchona plant (*Cinchona succurubra*) and artemisinin which is a terpenoid from artemesia plant, (*Artemisia annua*) (Sisimwet, Nandi). An antibacterial Salicin (Salicylic acid) was isolated from the bark of *Salix salva* (white willow) (Tesso, 2005). Conventional medicine is costly, often inaccessible and unaffordable. This alludes to ethnobotanical uses of many of these plants, majority of which have not even undergone initial phytochemical investigations. According to the WHO, approximately 25% of modern drugs used in the United States have been derived from plants that were discovered on the basis of their indigenous use (Petrovska, 2012).

Therefore, medicinal plants used traditionally by the people of Nandi people of Kenya for the treatment of microbial infections and other diseases need to be investigated for their antimicrobial activities. Information on their use is available both in literature and from the traditional practitioners but their scientific data on their activities is either scanty or not available.The findings may aid in eradicating multi-drug resistant bacteria that pose serious problems in the medical field especially in the intensive care unit (Ngemenya *et al.*, 2019).

2. 7.3 Medicinal Plants commonly used in Nandi County and their local names.

Urtica massaica (stinging nettle) (siwot) is clinically used in treatment of symptoms associated with enlarged prostate (non-cancerous) and is also used for diabetes, (Wabai *et al*., 2018). *Senna didymobotrya* (Senetwet) is used for the treatment of wounds, burns and skin diseases and also used for smoothening the guard for fermenting milk (mursik) the active constituents are anthraquinones. A Terpene was also isolated from the leaves of

Croton macrostachys (Tebeswet) following the active extracts of ethyl acetate against *Salmonella typhi* with a zone of inhibition of 16mm (Jackie *et al.*, 2016).

Adenia gummifera (Chepnyaliltet) is used in Nandi county to treat tuberculosis (TB) and Leprosy. In South Africa it is used to manage HIV/AIDS (De Wet *et al*., 2012). *Zanthoxylum gillettii* (Sagawatiet) bark is also used to for the management of tuberculosis. In addition, it is used for treatment of cold, stomachache, rheumatism and urinary tract infections in Ivory coast (kokwaro, 2003). *Plantago palmate* (Msiririet), *Zeheneria minutifolora* (Senetwet), *Acacia lahai*, *Leucas calastachys* (Ngechepchiat, Nandi), *Rubia cordifolia* (Chepsaleitet) *Prunus africana* (Tendwet) *and Erithrinia abyssinica* (Kakaruet) are used for the treatment of *Pneumonia* in Nandi county (Kimutai *et al.*, 2019). The barks of *Erythrina abysinicca* is boiled and taken for the management of STDI's. It is also used in other African folklore medicine, for the treatment of gonorrhea, (Jeruto, *et al.*, 2015).

Diarrhoea in Nandi couty is treated using *Albicia coriaria* (Musengertet, Nandi), *Cythula schimperiana* (Namgwet), *Plantago palmate, Cuttia abyssinica* (Turmanyat), *Senna didymobotya, Ficus cycomorus* (Mogoiwet), *Spermacose princeae* (Senetwet), *Rubia cordifolia* (Senetwet, Nandi) and *Syzygium cordatum* (Lamaiywet). *Albicia coriaria* is also used to treat the same in Nyanza (Jeruto *et al.*, 2015). Furthermore, *Syzygium cordatum* (Chepsaleitet) is also used to treat other human ailments such as gastro interstinal ailments, wounds, STDIs and tuberculosis (Maroyi Alfred, 2018). *Cythula schimperiana* is also used in other countries for treatment of syphilis and Tuberculosis in Madagascar and Rwanda respectively (Bisi-Johnson *et al*, 2015). Wounds are treated using

a number of plants including; *Lactuca glandulifera, Asparagus racemosus* (Chesibaiyat), *Vangueria volkensii* (Kimolwet), *Toddalia asiatica* (Kipkoskosit), *Zanthoxyllum gilleti, Solanum macrantha* (Sikawet), *Urtica mossaica and senecio discifolius* (Chemamaiyat). The fact that *Solanum macrantha* is used to treat wounds in Nandi county, it has been reported to have antimicrobial activities. (Olayemi *et al.*, 2011).

2. 7.4 Synergistic effects of phytochemicals and antibiotics

A phenomenon in which one compound enhances the individual activity of another compound in combination and vice versa, is referred to as synergism. The use of plant extracts as antimicrobial agents especially those that are mixed (synergism), can reduce the risk of resistance due to the action that makes the microbe adaptability difficult and are reported to have minimum side effects (Hanna *et al.*, 2012). The phenomenon of synergistic effect is often crucial and important to bioactivity in plant extracts and in some cases the activity may be lost in purified compounds.

Combination of two or more compounds is generally superior to the use of single compounds especially for the serious infections caused by antibiotic resistant bacteria (Muroi *et al.*, 2004). Studies on synergism of antibiotics with plant extracts has been documented in literature, especially on resistant bacteria including *Staphylococcus aureus* resistant to methicillin (MRSA) with promising results (Doughari, 2012). For example, the antibiotic activity of gentamicin against *Pseudomonas aeruginosa* was enhanced in the presence of croton Zehntneri essential oil. An association of anacardic acid and totarol with methicillin inhibited strains of *S. aureus* resistant to methicillin (MRSA) (Muroi and Kibe, 2005).

Catechins from green tea extract and Methylxanthines in combination with gentamicin was effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Bazzaz *et al.,* 2016). Synergism interaction was also found between penicillin and eugenol ,ampicillin and eugenol. Verbascoside, *Lemon verbena* extract and caffeine in combination with gentamicin against drug-resistant *Staphylococcus aureus* and *Escherichia coli* clinical isolates has also yielded a promising effect (Bazzaz *et al.,* 2018). Combination of gentamicin and chloramphenicol could be improved by use of plant material against (MRSA).

The improvement of antibiotics activity is probably due to accumulation of inhibitory concentrations of the active compounds at the target sites or due to additional inhibitory effect of the tested plant material (Hannan, *et al.*, 2012). Synergism is of great significance for treatment of diseases that are caused by resistant pathogens (Bessa *et al.,* 2015). However, in some few cases, a reduction in the activity of one component in the presence of the other can be obtained. This phenomenon is refered to as antagonism.

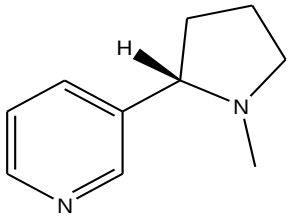
2.8 Secondary metabolites from medicinal plants

The property of the medicinal plants to combat the microbes is due to the presence of the phytochemical compounds which disrupt microbial processes or structures that differ from those of the host and antibiotics. Phytochemicals have exhibited promising results in overcoming the emergence of antibiotic resistance in bacterial pathogens (Barbieri *et al*., 2017). Phytochemicals can act on their own or in combination with others or antibiotics. Phenomenon called synergism and which promote activities against bacteria, (Shakeri *et al*., 2018).

Phytochemical screening of the plants extracts is very important in determining the presence of active components in them which have metabolic activities, (Thakkar and Ray, 2014). Phytochemical studies revealed that steroids, terpenoids, tannins, saponins, alkaloids, flavonoids and glycosides contributed to the medicinal value of these medicinal plants, (Ali *et al*., 2018). Since most medicinal plants are being used for treatment of most diseases and ailments due to the presence of these phytochemicals, it is therefore important to conduct phytochemical analysis and document the phytochemicals essential for treatment of particular ailments (Oladotun *et al* , 2018). Though much is known about the chemistry and antimicrobial action of several phytochemicals, few studies are available on the possible mechanisms of action. For instance, antibacterial compounds may target bacterial cell wall (penicillins, cephallosporins), cell membrane (polymixins,) and bacterial enzymes (quinolones and sulfonamides which are bactericidal in nature). Antibiotics also have same mechanisms, for xample there are those which target protein synthesis such as the aminoglycosides, macrolides and tetracyclines are which usually bacteriostatic (Gonelimali *et al*., 2018). Different types of phytochemicals include the folllowing ;

2.8.1 Alkaloids

These are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms due to the presence of double bonds between carbon atom and nitrogen atoms in the pyrrolidine and pyridine rings. This group also includes some related compounds with neutral and even weakly acidic properties (Matsuura and Fett-Neto, 2015).



Pyrrolidine and pyridine rings

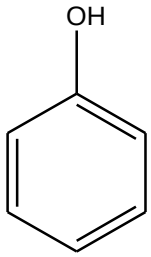
Alkaloid structure contains nitrogen molecule with both pyrrolidine rings and pyridine rings. More than 12,000-alkaloids are known to exist in about 20% of plant species and only few have been exploited for medicinal purposes. The name alkaloid ends with the suffix –ine and plant-derived alkaloids in clinical use include the analgesic morphine and codeine, the muscle relaxant (+)-tubocurarine, the antibiotics sanguinafine and berberine, the anticancer agent vinblastine, the antiarrythmic ajmaline, the pupil dilator atropine, and the sedative scopolamine. Other important alkaloids of plant origin include the addictive stimulants caffeine, nicotine, codeine, atropine, morphine, ergotamine, cocaine, nicotine and ephedrine (Doughari, 2012).

Alkaloids display a good antimicrobial activity against several microorganisms with different mechanisms of action depending on the class. For instance, isoquinalone alkaloids affects microbes by inhibiting nucleic acid synthesis while indolizidine alkaloid in addition to inhibiting nucleic acid synthesis also inhibit dihydrofolate reductase synthesis (Cushine *et al*., 2014). Conessine is one of the recent alkaloid with high antibacterial activities at a concerntration of 20 mg/L against *Pseudomonas aeruginosa*, it works as an efflux pump inhibitor (Siriyong *et al*., 2017). However, some intestinal microbioata have been found to convert alkaloids by using their enzymes and make them inactive. For instance, isoquinoline alkaloids containing a nitro-hexatomic ring such as

palmatine were easily transformed by the intestinal flora *in vitro* into metabolites (Chi-Yu *et al*., 2017).

2.8.2 Phenols

These are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group.



Phenol

Phenols are widely distributed nutrient metabolites in the food environment. They are synthesized as an intervention against pathogens to protect wounded crops. In some instances natural phenols are found in vegetative plants, such as western poison oak to discourage herbivores (Michael, 2003).

Phenolics have been identified as having an inhibitor impact on microbial organisms probably due to lack of iron or hydrogen connections between essential proteins, such as microbial enzymes. Their toxicity to microbes is attributed to their position and to the phenol numbers inhibiting enzymes through sulfur reactions, or by more non-specific interactions with the enzymes. Synthesis and antibacterial activity studies of furan derivatives and phenols have been found to have antimicrobial activities (Sireesha *et al*., 2017). Furthermore, studie shown that pyrogallol-based compounds such as gallic acid and ferulic acid are more potent. This is because they destroy the bacterial cell wall of some

bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, leading to leakage of cellular contents, (Aldulaimi, 2017). The phenols or aromatic copmpounds are categorized according to the number of carbon atoms. Athraquinones are classified phenols or phenolic compounds with 14 carbon atoms while flavonoids have 15 carbon atoms (Harbone, 1998).

2.8.2.1 Anthraquinones

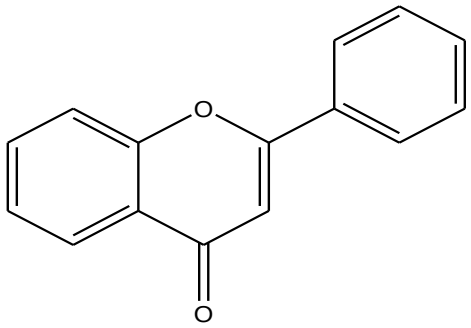
They are also called anthracenedione with the formula $C_{14}H_8O_2$ and are organic aromatic compounds. In some plants (e.g. Senna, Rhubarb, and Cascara buckthorn), fungi, lichens, and insects, anthraquinones occur naturally where they act as simple structures for their pigments. They are accountable for browning reactions in fruits and vegetables that are cut or injured. Natural derivatives of anthraquinone laxate (Harborne, 1998). Anthraquinone glycosides from *Ficus cycomorus* have been shown to have antifungal protection. Their action is by irreversibly combining nucleophilic amino acids in proteins leading to protein inactivation and function loss (Hassan, 2005).

2.9.2.2 Flavonoids, Flavones and flavonols

Flavonoids are polyphenolic compounds possessing 15 carbons atom for example quercetin found in citrus fruit, buckwheat and onions, (Harbone, 1998). Flavones are phenolic structures containing one carbonyl group,where the addition of a 3-hydroxyl group yields a flavonol **.** These are a class phytochemical or constituents active against a wide variety of microorganisms. Studies have found that flavonoids such as naringenin and quercetin exhibit best binding affinity with *Mycobacterium tuberculosis* and inhibit the racemization activity with induced structural perturbation. In addition, they cause cell wall damages in mycobacterial cells (Pawar *et al.,* 2020). Kaempferol isolated from *Alpinia*

calcarata acts by inhibiting efflux pump against Methicilline Resistant *Staphylococcus aureus* at a concerntration of 125 μg/mL being one of the recent most active flavonoid (Randhawa *et al*., 2016).

Plants synthesized flavones such as 2-pheny1-1,4-henzopyrone are produced in response to microbial infection. Their antimicrobials activity against microorganisms have been demonstrated *in vitro*.



2-pheny1-1,4-henzopyrone

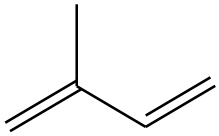
Their mechanisms of action include: inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism and porin on the cell membrane, alteration of the membrane permeability, and attenuation of the pathogenicity (Enwa *et al.*, 2014). This is possibly due to their ability to interact with extracellular and soluble proteins and complex with cell walls of bacteria. The lipophilic flavonoids can also interact with microbial membranes.(Yixi *et al*., 2015).

Conflicting results complicate the other potential mechanism of action of flavones and flavonoids. Flavonoids lacking hydroxyl groups on their *β*-rings are more active against microorganisms than those with the hydroxyl groups. This is because they have an ability to reverse the antibiotic resistance and enhance action of current antibotics such as penicillin G., (Gorniak, *et al.*, 2019). Plants including all fruits, vegetables, herbs and spices contain flavonoids. Examples are catechin found in green tea and phloterine found

in some apples. It has been reported that catechins inactivated cholera toxin *in vivo* and inhibited isolated bacterial glucosyltransferases in *S. mutans,* possibly due to complexing activities (Kumar and Pandey, 2013).

2.8.3 Terpenes

Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers. They may also refer to oxygen derivatives of thsese compounds called terpenoids. Terpenes are normally classified according to the number of isoprene units from which they are derived from;



Isoprene

Preliminary studies have shown that several classes of terpenes have inhibitory antimicrobial activities due to their ability to disrupt membrane by the lipophilic compounds, efflux pump inhibition and mostly through cell membranes breakage. A example is thymol isolated from *Thymus capitatus* leaves with high antibacterial activity against a number of bacteria at a concentration of 8, 10, 6.5, 5 μg/ml, against *E. coli, E. aerogenes, S. aureus, P. aeruginosa* respectively, (Althunibat *et al*., 2016). Terpenes are hydrocarbons resulting from the combination of several isoprene units.

2.8.3.1 Hemiterpenes

Hemiterpenes, have one isoprene unit (5 carbons) an example is isoprene (Figure 4) itself (Valeria *et al*., 2017).

2.8.3.2 Monoterpenes

These are classes of terpenes that contain two isoprene units. They include terpinen-4-ol, thujone, camphor, eugenol, menthol, Carvacrol which have been found to be active against bacteria. For example, Carvacrol was found to be active against *E. coli, E. aerogenes, S. aureus, P. aeruginosa* at a concentration of 8, 8, 7, 7 µg/ml respectively (Miladi *et al*., 2016).

2.8.3.3 Sesquiterpenes

These consist of three isoprene units with molecular formula of C_{15} H $_{24.}$ Those with the highest antibacterial activity are copaenol, cubenol, and torreyol. The presence of an OH group, which is an efficient uncoupler of the bacterial plasma membrane creates instability and breaks the membrane's phospholipid-sterol interactions and is often lethal (Miladi *et al.*, 2016).

2.8.3.4 Diterpenes

Contain twenty carbon atoms (C20) in their skeleton made from four isoprene units. They are classically considered to being resins, for example, taxol an anti-cancer agent.

2.8.3.5 Triterpenes

Contain thirty carbon atoms (C30) made from six isoprene units. Some have shown evidence for antimicrobial activity when mixed. For example, capsaicin is bactericidal to *Helicobacter pylori* (Martinez *et al.*, 2008). Saponins are naturally occurring surface-active glycosides or triterpenoid glycosides, common in a large number of plants for defense against herbivores. Their structure is a composite of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative, (Tian *et al.*, 2017).

2.8.3.6 Tetraterpenes

Are compounds with eight isoprene unit and contain forty carbon atoms (C40). An example is carotenoid which contain vitamin A.

2.8.4 Fatty acids

Are carboxylic acids with a long alipathic chain that is either saturated or unsaturated.

It is that carboxyl group that makes it an acid. If the carbon to carbon bonds are all single, the acid is saturated; if any part of the bond is double or triple the acid is unsaturated and is more active (Zheng et al.,2005).

The antimycotic action of saturated fatty acids increases as the number of carbon atoms in the chain increases up to eleven. The branched-chain fatty acids are less active than straight-chain fatty acids of equal molecular weights (Parsons *et al.,* 2012). It has been reported that the unsaturated fatty acids are more antimycotic than the corresponding saturated fatty acids. Moreover, natural fatty acids are more active than the isomeric fatty acids. This is due to the undissociated molecule, not the anion (Yamamoto 2017). Studies shows that bactericidal action of fatty acids against *Staphylococcus aureus* is due to membrane disruption and that long-chain fatty acids have been found to be more active than the short-chain fatty acids, (Parsons *et al.,* 2012).

2.9 Ethnopharmacological and phytochemical information on four selected medicinal plants

2.9.1 *Acacia lahai* (Fabaceae)

This is a flat-topped tree 15-20 m tall with grey bark and thorny branches. It is commonly known as “Chebitet” by the Nandi community. The bark extracts is traditionally used to

treat coughs and pneumonia (Kimutai *et al*., 2019). Acetone stem bark extracts has been found to be active against *P*. *aeruginosa* with an inhibition zone of 11mm (Cherotich, 2015). Phytochemical screening of bark of acacia species such as *Acacia nilotica* and *Acacia dealbata* reveals the presence of tannins, fatty acids and terpenoids. In addition, straight chain fatty acid, heptacosane -1,2,3-triol and pentacosane dioic acid was isolated from *Acacia nilotica,* (Oliveira *et al*., 2020).

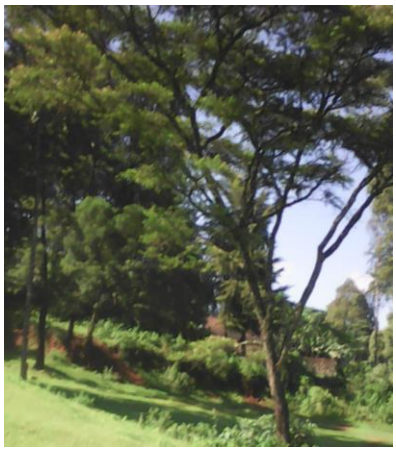


Plate 2. 1: A habit of *Acacia lahai*

(Photo by Nicholas Kogo)

2.9.2 *Cleodendrum myricoides* (Verbanaceae)

This is a shrub that grows to about 4 m tall its roots are traditionally used for the treatment of coughs and skin diseases (Kimutai *et al*., 2019). Phytochemical screening of methanol/dichloromethane root extracts reveals presence of phenols, terpenoids and glycosides (Esatu *et al*., 2015). Earlier studies on chemical constituents of the genus cleodendrum reveals presence of phenyl propanoids (Lee *et al*., 2011). A Phenyl glycoside was isolated from *Cleodendrum myricoides* (Esatu *et al*., 2015).

Plate 2. 2: A *Cleodendrum myricoides* twigs with purple flower (Photo by Nicholas Kogo)

2.9.3 *Leucas calostachy* (Labiatacea)

This is a shrub with hairy stem and ssssile leaves. It grows 1-2m tall and used traditionally to treat wound infections, typhoid and coughs (Kimutai *et al*., 2019). The local name is "Ngechepchat". Phytochemical screening of chlorofoam leaf extracts has been reported to have terpenoids, alkaloids and phenols (Jeruto *et al*., 2011). Antimicrobial activity of leaf methanol extracts was found to be active against *P.aeruginosa* and *C. albicans* with inhibition zones of 11mm and 13 mm respectively. However, liitle has been done to isolate compounds from this plant yet the extracts have good activities against microorganisms (Cherotich, 2015).

Plate 2. 3: *Leucas calastachys* (Ngechepchiat), being dug, (Photo by Nicholas Kogo).

2.9.4 *Olinia rochetiana* (Oliniaceae)

Olinia rochetiana is a tree that grows upto 15 m tall. Nandi people refer to it as "*Museset*". Traditionally the bark is used for the treatment of skin diseases and rheumatism (Kimutai *et al*., 2019, Kokwaro,2003). Methanol leaf extracts has been found to be active against bacteria and fungi (Hailu *et al*., 2012). Phytochemical screening of the genus Oliniceae plants such as *Olinia usambarensis* revealed presence of alkaloids, terpenoids, tannins, anthocyanins, saponins, triterpenoids, flavonoids, caumarins and reducing sugars (Mungweru *et al*., 2016). Compounds reported from the genus inlude olinioside and phenyl glycoside isolated from *Olinia usambarensis* (Deyou *et al.*, 2017).

Plate 2. 4: Mature tree *Olinia rochetiana*

(Photo by Nicholas Kogo)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Nandi county is situated in the western part of Kenya and borders Uasin-Gishu county to the north-east, Kericho county to the south-east, Kisumu county to the south west and; Kakamega county to the north–west (Anon, 2001) (Figure 3.1). The geographical coordinates are 0.1836º N, 35.1269º E. It has six sub-counties namely; Aldai, Tindiret, Nandi Hills, Chesumei, Emgwen, Nandi East and Nandi-North sub-counties. The county is very rich in diverse flora that is suitable for studies related to medicinal plants. All the sub-counties are surrounded by forests. The plants in this area make an integral part of the routine health care systems of the tribes residing in villages. In this couty many people use different plants and plant parts for food, medicine, fodder, festivals and rituals. The local people have knowledge about the uses of plant parts for treatment of various illnesses.

3.2 Plant material

3.2.1 Collection of ethnobotanical data

Ethnobotanical survey was conducted during the period from June 2016 to December 2016 involving twenty six (26) herbalists (18 men and 8 women). Interviews were conducted in local languages using semi-structured quesionnares (Appendix 1). Kiswahili and english was used in situations where one could understand.

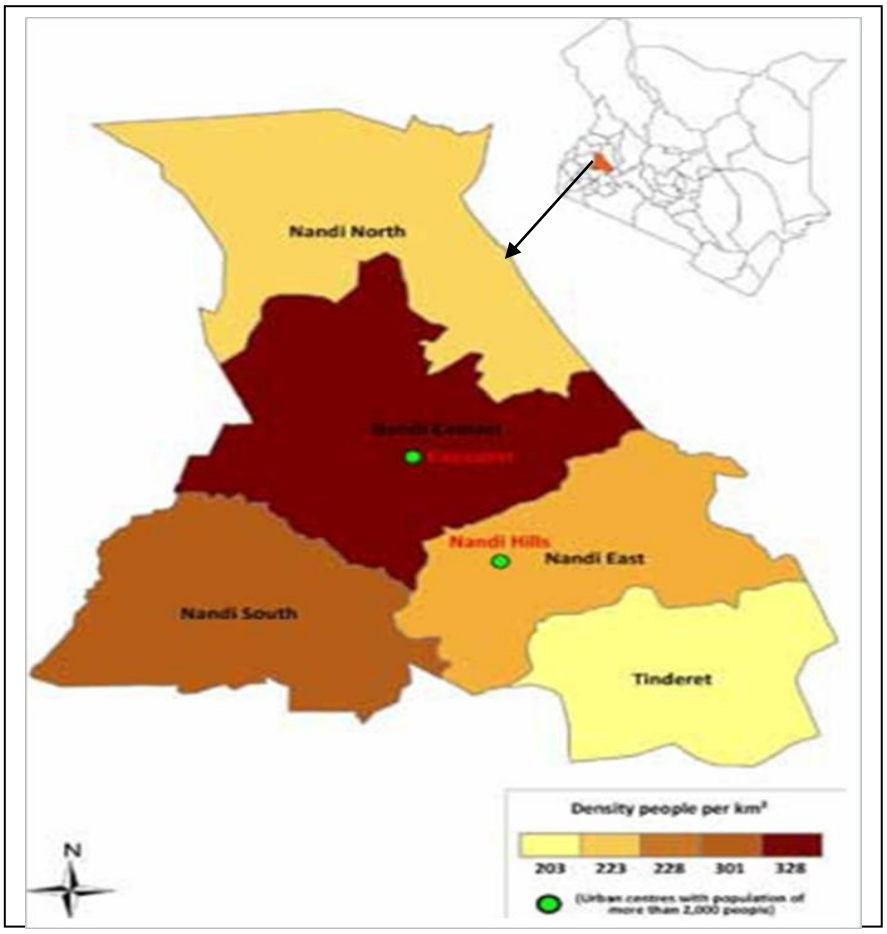


Figure 3. 1: Map of Kenya showing the study area

Source; Google maps, 2014

Key; 1. Map of Kenya-showing the study area

2. Map of the study area (Nandi-county)

This was to obtain information on medicinal plants traditionally used for the management of bacteria. Research Assistants acted as the translators during the conversations between the herbalist and the researcher. After explaining the objectives of the study and seeking their consent, the herbalist were engaged.

The time and place of interviews were arranged according to the schedules of the respondent. The meetings were held at the places of work such as the homes and farms, where they treat the patients. The interviews were conducted in the local Nandi and Kipsigis languages except for a few cases where the respondents could understand Kiswahili and English. The information was recorded immediately or afterwards and appointments were made for more details at a more convenient place arranged with the respondent.

The data collected were to supplement information regarding the local names of the plant species, parts used, preparation, administration, and the disease condition treated was documented, (Kimutai *et al*., 2019). A taxonomist who was conversant with the flora of the area was part of the collection team.

The herbalist or the botanists were used as guides during field trips to identify frequently cited plant species. The alleged antibacterial value of a particular plant was recorded as valid only if it was mentioned by at least 3 independent herbalists. Photographs of plants were taken in the field and collected.

3.2.2 Collection of plant material

The plants were identified by a taxonomist at the University of Eldoret. The voucher specimens were kept at the herbarium in the same university (appendixes 2A and 2B). The information gathered include; plant species, parts used, method of preparation, posology and vernacular names. Thirty three (33) medicinal plants distributed within 24 botanical families were documented (Appendix 2A and 2B). However, seventeen medicinal plants were selected for antibacterial activities basing on ethnobotanical information and literature.

3.2.3 Preparation of plant material

After documentation of 33 medicinal plants (Apendix 2A and 2B), seventeen (17) medicinal plants were selected based on literature and ethnobotanical information from the traditional practitioners. They consisted of barks, whole, roots and leaves that were collected in the morning (8.am -10am) and washed thoroughly with running tap water. Chopped into small pieces and air-dried for two to three weeks at room temperature, by spreading evenly in the open dry area. The dry samples were ground separately into fine powder using a grinder. The powdered samples were packed in black polythene bags and labeled appropriately and stored in air-tight containers.

3.2.4 Exhaustive extraction of 17 medicinal plants

The identification of active plant constituents started with the antibacterial test of crude extracts, (Koehn and Carter, 2005). A sample of the powdered bark, whole, roots and leaves of the respective plants weighing 50 grams were exhaustively extracted with methanol. The extraction was carried out in 250 ml conical flask with 200 ml of the respective solvent added. The extracts were let to stand for 24 hours at room temperature and filtered through Whatman No. 1 filter paper. The solvents were removed using a rotary evaporator and air dried for three days. The percentage yield were calculated by the following equation (Felhi *et al*., 2017): Yield(%)=X/Y x 100, where X=Total mass of extracts obtained and Y=Total mass of dried plant material. The extracts were put in sterile airtight vials weighed and kept in desiccator at 4^{o} C in readiness for use (Eftekhari *et al.,* 2017).

3.2.5 Successive extractions

After preliminary screening of methanol exracts of 17 selected medicinal plants. Four plants namely; *A. lahai, C. myricoides, L. calostachys* and *O. riochetiana.* were selected for further extraction. This was based on their antibacterial activities and literature. Successive extractions were carried out by dissolving one kilogram of finely ground material of *A. lahai* (Appendix 16) in two litres of hexane (HE) for 48 hours. The soaked material was filtered and the filtrate collected in a clean container (appendix 17). The filtrate was then concentrated using rotary evaporator and left to dry in open air for three days, weighed and kept in kept in desiccator at - 4^{o} C in readiness for use. The residue after extraction with hexane was soaked in 2 lires of dichloromethane (DCM) for 48 hours,the soaked materials were filtered. The filtrate was concentrated and solvent recovered by distillation using rotary evaporator. The filtrate was concentrated, dried for three days, weighed and kept in a dessicator. The residue after extraction with DCM was dried and soaked in 2 litres of ethyl acetate(EAE) for 48hours, filtered, concentrated, dried, weighed and kept in a dessicator. The residue after extraction with EAE was soaked in 2 litres of methanol (ME) for 48hours, filtered, concentrated, dried, weighed and extract kept in a dessicator. The extracts were tested for antibacterial activities. The above procedure was repeated with *C. myricoides* (root), *L. calostachys* (root) and *O. riochetiana* (bark) each 1 kilogram.

3.3 Fractionation and isolation of compounds

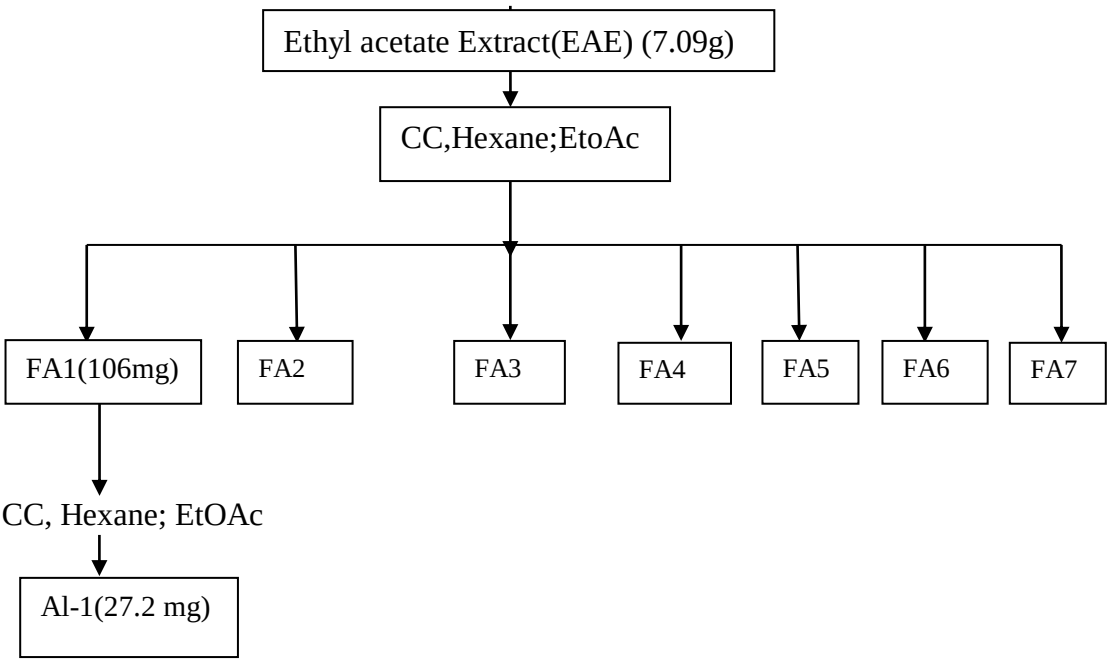
After preliminary screening of sucessive extraction of four (4) selected medicinal plants isolation of the active compounds was carried out using bio-assay guided (Fakhrudin *et al*., 2014) isolation. To obtain pure compounds. The active successive crude extracts EAE

from *Acacia lahai*, *C.myricoides*, *O.rochetiana* and HE from *L.calostachys* were chromatographed over a column of silica gel. This was done by eluting with appropriate solvent systems to fractionate the successive crude extracts into fractions containing compounds according to polarity (from least polar hexane, dichloromethane, ethyl acetate and methanol). The fractions were collected and compounds detected using thin layer chromatography (TLC) using iodine as a visualizing agent, section (3.3.1-3.3.4). Fractions with similar spots on TLC purification were combined. Those that were active were purified by re-chromatography over small silica gel columns and re-crystalized using suitable solvents and the purity also confirmed by TLC (Appendix 20,21and 22). The pure compounds obtained AL-1, CM-1, LC-1, LC-2 and Or-2 were tested against the selected bacteria.

3.3.1 *Acacia lahai* ethyl acetate extracts(EAE)

The bioactive ethyl acetate extracts (EAE) that was active (Appendix 18) was considered for isolation of active compounds. EAE stem bark extract (7.09 g) was adsorbed onto 10 g of silica gel and subjected to column chromatography on a silica gel column. Eluted with n-hexane/ EtOAc (95:5-5:95) followed by EtOAc/ MeOH (99:1, 49:1). Eighty-one eluents were collected in 50 ml aliquots. They were concentrated using a rotary evaporator with similar fractions combined on the basis of TLC profile (Appendix 22). Fractions with similar R_f values on TLC were pooled together to obtain seven fractions. Fraction 4-19 (106g), 20-38 (82g), 39-47(105 mg), 48-55 (1.5 g), 56-69 (58mg), 70-76(71 mg) and 77-81(78mg) had the same Rf values. Hence, were combined and labeled as FA 1, FA 2, FA 3, FA 4, FA 5, FA 6 and FA 7 respectively. which were concentrated and left to dry. They

were later tested for antibacterial activity. FA1(106g) had higher antibacterial activity compared to other tested samples (Table 8) and was further purified by running a small column using hexane: EtOAc (95:5-5:5) that yielded one pure compound Al-1 (white powder) soluble in hexane. Al-1(5) was tested for antibacterial activity and later subjected to spectroscopic analysis (NMR).

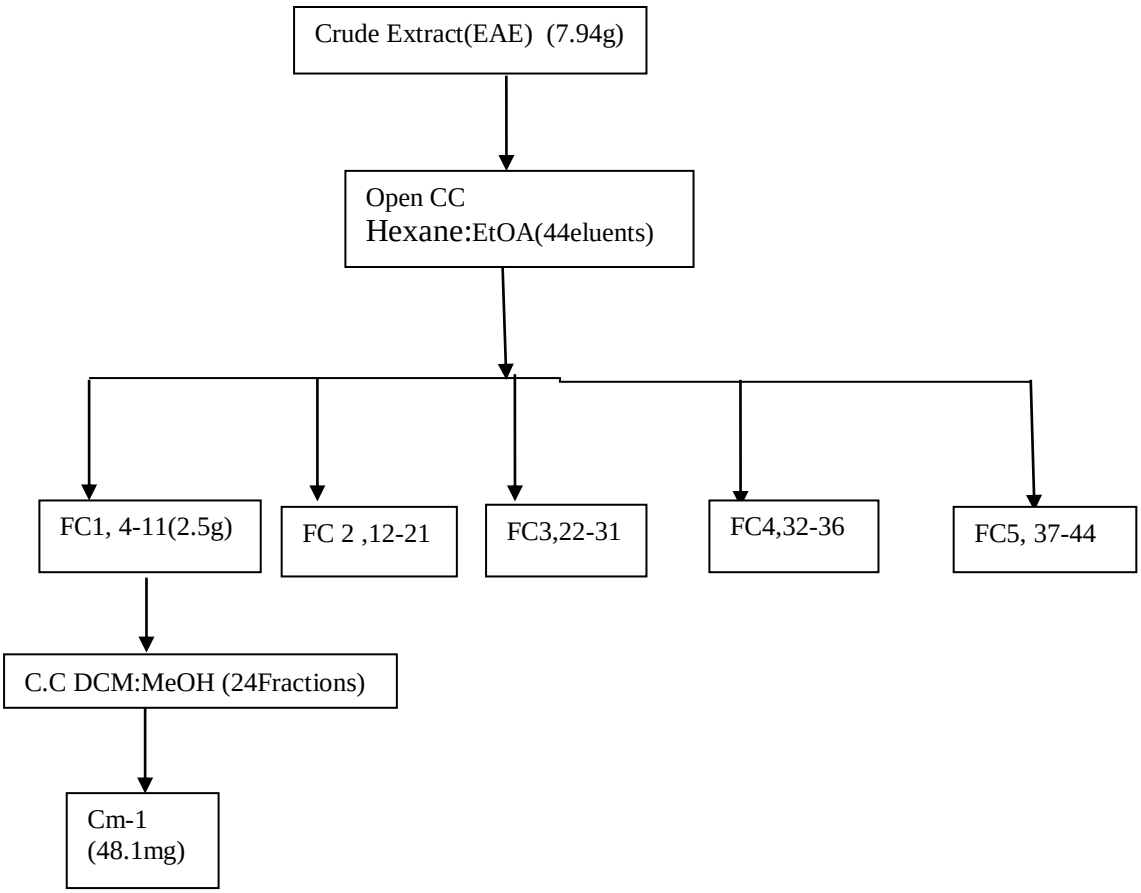


Schemes 3. 1:Isolation of compound Al-1 from ethyl acetate extract (EAE) of *Acacia lahai* barks

3.3.2 *Cleodendrum myricoides* ethyl acetate extracts(EAE)

The ethyl acetate extracts (EAE) was active and was considered for isolation of active compounds. The extracts (7.94 g) was adsorbed onto silica gel. It was subjected to column chromatography (CC) on silica gel column eluted with n-hexane: ethyl acetate (95:5-5:95). Forty-four (44) eluents were obtained. Fractions 4-11(2.54mg), 12-21(60mg), 22-31, 32-36 (1.2g) and 37-44 had similar R_f values (Appendix 21) were each pooled together and labeled as FC1, FC2, FC3 and FC4 (2.8g) and FC5. They were concentrated and left to dry

in a room at 25^{o}C and later subjected to antimicrobial activities. FC 1 had higher antibacterial activity compared to other tested fractions. It was subjected to column chromatography (CC) of dichloromethane: methanol (95:5-5:75) which yielded one pure compound Cm-1(6) (yellow powder) soluble in methanol, which was subjected to antibacterial activities and spectroscopic analysis (NMR).

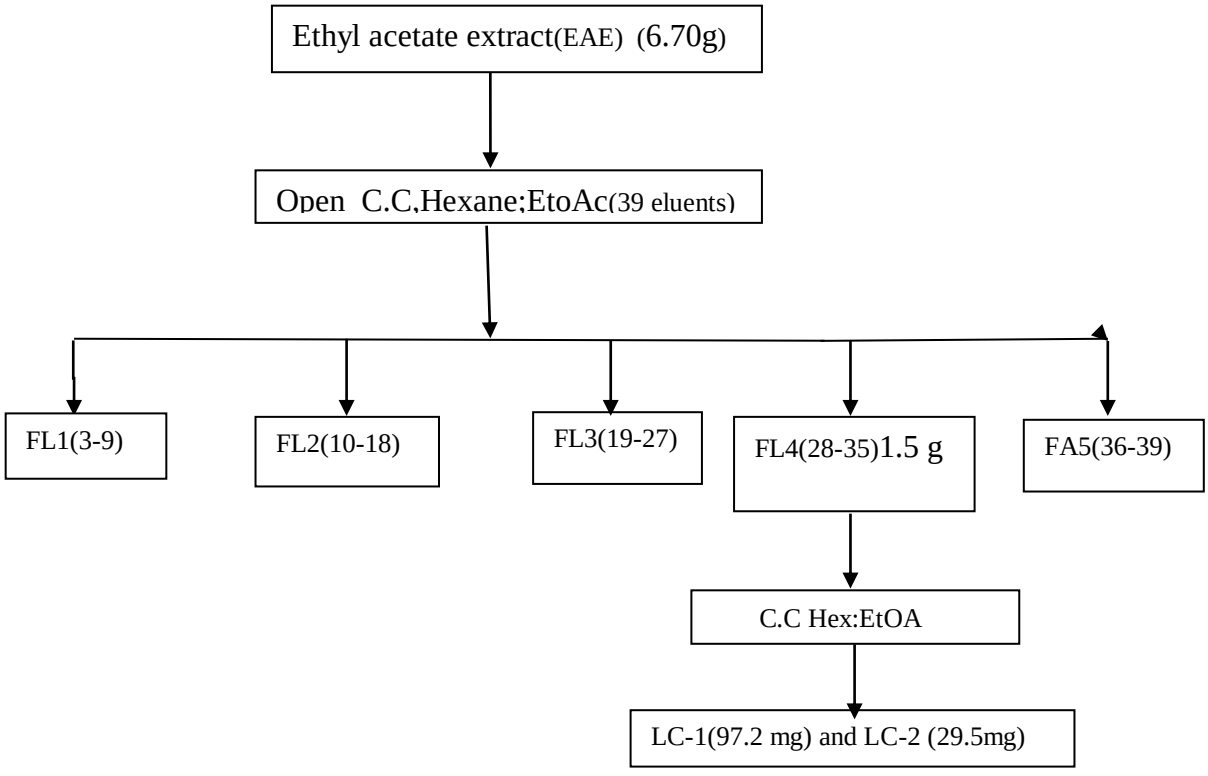


Schemes 3. 2: Isolation of compound Cm-1 from ethyl acetate extract (EAE) of *Cleodendrum myricoides* roots

3.3.3 *Leucas calastachys* hexane (HE)

The hexane extracts (HE) of *Leucas calastachys* that was active (Appendix 19). It was considered for isolation of active compounds. The extract (HE) (6.07g) was adsorbed onto 8.0 g of silica gel. It was subjected to CC on a silica gel column, eluted with n-hexane/

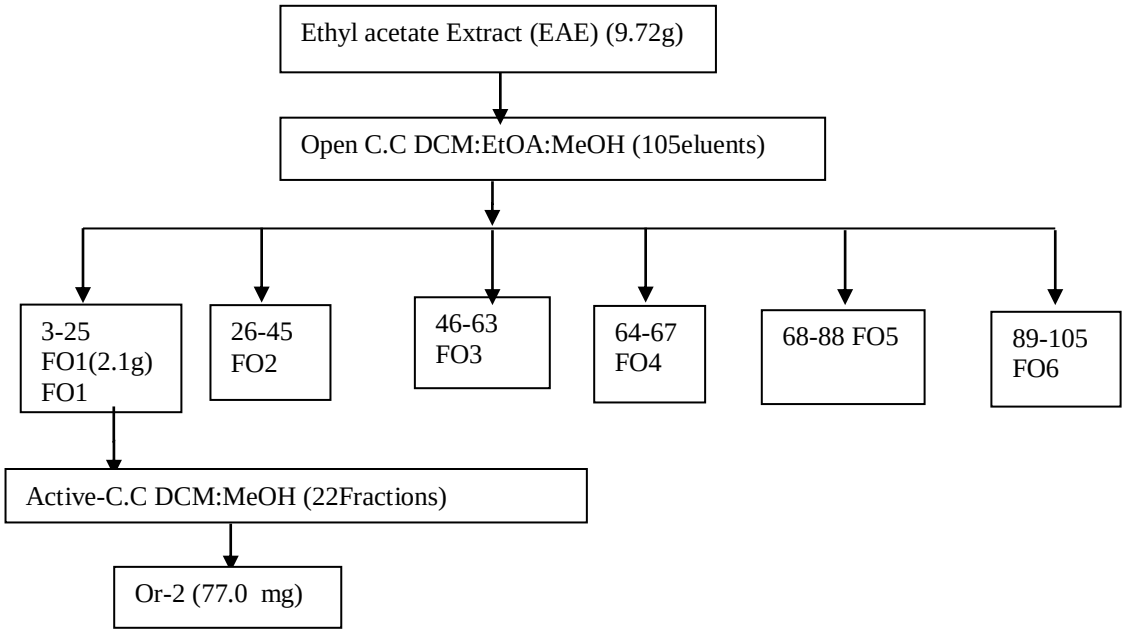
EtOAc (95:5-5: 5) to obtain 39 eluents. The fractions obtained were left to dryness in a room at 25^{o} C and spotted on TLC plate (Appendix 20) to establish the R_f. Fraction 3-9 (2.1g), 10-18 (82g), 19-27(105 mg), 28-35 (1.5 g) and 36-39 (1.8mg) had the same R_f values. Hence, were combined and labeled as FLI, FL2, FL3, FL4 and FL5. They were further tested for antibacterial activities in which fraction FL4 was found to be most active compared to other fractions. It was was further purified using column chromatography (CC) with Ethyl acetate:hexane (95:5-5:85) as eluent. This yielded one white oily compound LC-I(7) and a white solid LC-2(8) both soluble in hexane. They were subjected to antibacterial activities and spectroscopic analysis (NMR). The compounds were subjected to antibacterial activities and spectroscopic analysis (NMR).



Schemes 3. 3: Isolation of compound LC-1 and LC-2 from hexane extract (EAE) of root barks of *Leucas calostrachys* roots

3.3.4 *Olinia rochetiana* ethyl acetate extracts (EAE)

The ethyl acetate extracts (EAE) from *Olinia rochetiana* was active (Table 7). It was considered for isolation of active compounds. The extract (9.72 g) was adsorbed onto silica gel and subjected to column chromatography (CC) on silica gel column eluented with dichloromethane: ethyl acetate (95:5-5:95) to obtain 105 eluents. The eluents were spotted on TLC plate (Appendix 23) to established the R_f values. Fraction 3-25 (2.1g), 26-45 (1.2g), 46-63 (1.4g), 64-67 (80 mg), 68-88 (2.8g), 89-105(1.0g) which had same Rf values were pooled together. They were labelled as FO1, FO2, FO3, FO4, FO5 and FO6. They were tested for antibacterial activities whereby fraction FO1 was found to be the most active compared to other fractions. Fraction FO1 was further purified using column chromatography (CC) on silica gel column eluted with dichloromethane: methanol (95:5-5:65). Twenty two (22) fractions were obtained that yielded compound Or-2 (white powder) soluble in methanol. Compound Or-2(9) was subjected to antibacterial test and spectroscopy.



Schemes 3. 4: Isolation of compound Or-2 from ethyl acetate extract (EAE) fractions of barks of *Olinia rochetiana*

3.4 Structural characterization of compounds isolated from selected medicinal plants

The isolated compounds Al-1(**5**), Cm-1(**6**), LC-1(**7**), LC-2 (**8**) and Or-2(**9**) were weighed and put in eppendofs tubes. Atleast (10mg) were packed in an air free container and sent for NMR analysis in Germany. The following spectroscopic analytical data were obtained for each compound: ^{1}H-NMR, and ^{13}C-NMR. Complete structure determinations were done by the application of high-field, ^{1}H-^{1}H COSY, for proton spin-systems while HMBC and HSQC for long-range spin systems, (Pavia *et al.*, 1996).Chemical shifts of protons and carbons are expressed as *d* values in ppm with reference to TMS (ppm) and spin spin coupling constants (*J*) given in Hertz(Hz). Multiplicity of peaks were given as *d* (doublet), (singlets), *m*(multiplet), *dd*(doublet of doublet) and *dt*(doubletof triplet)

3.4.1 Physical and spectroscopic data of the isolated compounds

3.4.1.1:Fatty acid [5-(2, 5-dimethylhexyl) 1-isopentyl 3-hydroxy-2-methylpentanedioate] AL-1 (1)

White powder (27.2 mg) soluble in methanol,^{1}HNMR (400 MHz, CD_3OD)**:** δ1.96 (*m*, H-2), δ 3.53 (*d*, J=3.6, H-3), δ 1.62 (*m*, H-4) , δ 1.78 (*m*, H-5), δ 1.04 (*d*, J=2.4, H-6), δ 3.45 (*d*, J=4.4, H-1′), δ 2.28 (*m*, H-2′), δ 1.28 (*m*, H-3′), δ 1.62 (*m*, H-4′), δ 1.28(*m*, H-5′), δ 0.97 (*s*, H-6′), δ 1.02 (*d*, J=2.0, H-7′), δ 1.06 (*d*, J=7.2, H-8′), δ 3.59 (*dd*, J=5.2, J=6.4, H-1′′), δ 1.62 (*m*, H-2′′), δ 1.78 (*m*, H-3′′), δ 0.99 (*d*, J=1.2, H-4′′) and δ 1.00 (*d*, J=3.2, H-5′′). ^{13}C NMR(400 MHz, CD_3OD)**:** δ 174.9(C1), δ 37.8(C2), δ 60.8(C3), δ 41.8(C4), δ 173.4(C5), δ 15.7(C6), δ 61.7(C1′), δ 30.9(C2′), δ 26.2(C3′), δ 30.9(C4′), δ 25.9(C5′), δ 12.4(C6′), δ 17.9(C7′), δ 19.2(C8′), δ 54.6(C1′′), δ 41.8(C2′′), δ 26.2(C3′′), δ 22.2(C4′′) and δ 23.4(C5′′)

3.4.1.2 Phenol [*Cis* 4′′-*O*-acetyl martynoside] Cm-1(2)

Yellow powder (48.1 mg) soluble in methanol, ^{1}H-NMR (400 MHz, CD_3OD)**:** δ 7.45(*s,* H-2), δ 7.45 (*d,* H-5), δ 8.53(*d,*j=4.4, H-6), δ 1.25(*m,* H-α), 3.67(*m,* H- *β*), δ 4.16(s, H- OMe), δ 6.24(*d, J*=9.6, H-1′), δ 3.89 (*dt,* H-2′), δ 4.10 (*dd,* H-3′), δ 3.85-3.96 (m, H-4′), δ 3.68 (*dd,* H-5′), δ 3.89(*dt* H-6′), δ 6.24(*d,*J=2.0, H-1′′), δ 3.59(*dd,* H-2′′), δ 3.85-3.96 $^{(a)}$(H-3′′), δ 3.67$^{(a)}$(H-4′′) δ 3.67$^{(a)}$(H-5′′), δ 1.25 (*d, J*=6.4, H-6′), δ 1.81 (*s,* H-OAc),δ 8.53 (*d,* J=7.2, H-2′′′), δ 6.24(*d,* J=9.6, H-5′′′), δ 8.65(*d,*J=9.6, H-6′′′), δ 6.24(*d, J*=9.6, H- α′′′), δ 8.00(*d, J*=2.4, H- *β* ′′′) and δ 3.89(*s,* H-OMe′′′) ^{13}C NMR (400 MHz, CD_3OD)**:** δ 131.5(C1), δ 106.9(C2), δ 150.4(C3), δ 151.9(C4), δ 107.2(C5), δ 122.0(C6), δ 30.9(α), δ 74.2(*β*), δ 56.9(OMe), δ 104.4(C1′), δ 80.5(C2′), δ 79.8(C3′), δ 63.9(C4′), δ 78.2(C5′), δ 63.9(C6′), δ 157.9 (Ac), δ 104.3(C1′′), δ72.9(C2′′), δ 72.9(C3′′), δ 78.4(C4′′), δ 74.2(C5′′), δ 25.6(C6′′), δ 20.9(C7′′), δ 172.3(C1′′), δ 127.2(C1′′′), δ 119.6(C2′′′), δ 151.1(C3′′′), δ 147.2(C4′′′), δ 112.7(C5′′′), δ 120.0(C6′′′), 112.8(C α′′′), δ 141.0 (C *β* ′′′), δ 163.9 (C=0) and 30. 9 (C-OMe).

3.4.1.3 Fatty acid [Cis oleic acid] LC-1(3)

White oily solid (97.2 mg) soluble in hexane, ^{1}H NMR (600MHz, $CDCl_3$): δ 2.34, *t,* (J=5.5, H-2), δ 1.64, t, (J=5.5, H-3), δ 2.15, (*m,* H-4), δ 1.29 (*m,* H-5), δ 1.29 (*m,* H-6), δ 1.26 (*m,* H-7), δ 1.29 (*m,* H-8), δ 5.35, *d* (J=6.0, H-9), δ 5.34, *d* (J=6.0, H-10), δ 2.01 (*m,* H-11), δ 1.29 (*m* H-12), δ 1.29 (*m,* H-13), δ 1.26 (*m,* H-14), δ 1.29 (*m,* H-15), δ 1.28 (*m,* H-16), and δ 0.86, (*t,J*= 6.0, H-17). ^{13}C NMR(600MHz, $CDCl_3$): δ 180.3(C1), δ 34.3(C2), δ 24.8(C3), δ 27.3(C4), δ 29.3(C5), δ 29.2(C6), δ 29.7(C7), δ 29.2(C8), δ 130.2(C9), δ

129.9(C10), δ 29.3(C11), δ 29.7(C12), δ 29.4(C13), δ 29.6(C14), δ 29.3(C15), δ 29.2(C16), δ 29.9(C17) and δ 14.3(C18).

3.4.1.4 Terpenoid [α-amyrin tetracosanoate] LC-2(4)

White solid (38.1 mg) soluble in ethyl acetate, ^{1}HNMR(400MHz, CD_2Cl_2): δ 1.63, (*m,* H-1), 1.08 (*m,* H-1), 1.62 (*m,* H-2), δ 1.90 (*m,*H-2), δ 4. 45 (*dd,* J=8.0,J=4.0,H-3), δ 0. 85 (*m,*H-5), δ 1.45 (*m,* H-6), δ 1.54 (*m,*H-7), δ 1.33(*m,*H-7), δ 1.45 (*m,*H-7) , δ 1.61 (*m,*H-9), δ 1.88 (*m,* H-11), δ 5.19 (*t,* J=4.0, H-12), δ 1.78 (*m,* H-15), δ 0.95 (*m,* H-15), δ 2.02 (*m,*H-16) δ 0.79 (*m,* H-16), 1.95 (*dd* , J=8.0,H-18), δ 1.69 (*m,*H-19), δ 1.02 (*m,*H-19), δ 1.35 (*m,* H-21), δ 1.10 (*m,* H-21), δ 1.38 (*m,* H-22), δ 1.34 (*m,* H-22), δ 0.86 (*s,* H-23), δ 0.84 (*s,* H-24), δ 0.97 (*s,* H-25), δ 0.98 (*s,* H-26), δ 1.14 (*s,* H-26), δ 0.83 (*s,* H-28) δ 0.88 (*s,* H-29), δ 0.87 (*s,* H-30), 1.53(*m,* H-2′), 1.55(*m,* H-3′), 1.21-1.34(m, H-4′- 12′), 1.31(m, H-13′), and δ 0.75 (*t,* J=8.0, H-14′). ^{13}C NMR (400MHz, CD_2Cl_2): δ 38.1(C1), δ 23.8(C2), δ 81.1(C3), δ 38.1(C4), δ 55.8(C5), δ 18.5(C6), δ 31.5(C7), δ 40.2(C8), δ 47.9(C9), δ 37.2(C10), δ 24.1(C11), δ 122.1(C12), δ 145.3(C13), δ 42.1(C14), δ 26.1(C15), δ 27.3(C16), δ 32.8(C17), δ 47.6(C18), δ 47.1(C19), δ 31.3(C20), δ 35.1(C21), δ 37.5(C22), δ 28.1(C23), δ 16.6(C24), δ 15.7(C25), δ 16.9(C26), δ 26.0(C27), δ 28.6(C28), δ 33.5(C29), δ 23.8(C30), δ 171.0(C1′), 34.9(C2′), 23.9(C3′), 26.5-34.5(C4′-12′),32.6(C13′) and δ 14.7(C14′).

3.4.1.5 Phenol [4-*β*-glucophyranosylferrulic acid] Or-2(5)

White solid (77.0 mg) soluble in acetone, ^{1}H NMR (400MHz): δ 7.82 (*d,* J=9.2, H-*β*), δ 7.81 (*m,* H-*ɤ*), δ 7.52 (*s,* H-2), δ 7.80 (*m,* H-5), δ 5.56 (*brs,* H-1′), δ 3.38 (*m,* H-2′), δ 3.42

(m, H-3′), δ 3.66 (*m*, H-4′), δ 3.48 (*m*, H-5′), δ 3.89 (*brs*, H-6′a), δ 4.08 (*m*, H-6′b), δ 3.82(s,OCH_3), δ 10.79(s,-COOH) ^{13}C NMR (400MHz): δ 158.2 (Cα), δ 112.5(C*β*),δ 141.7(C*ɤ*),δ 113.0(C2), δ 152.4(C3), δ 150.2(C4), δ 111.8(C5), δ 99.7(C1′), δ 71.5(C2′), δ 70.4(C3′), δ 70.09(C4′), δ 70.2(C5′), δ 61.5(6′a) and δ 60.9(OCH_3)

3.5 Antibacterial assay

3.5.1 Test organisms used

The 10 bacteria used in this study included standard and clinical isolates. These were obtained from the Centre for Microbiology Research (CMR)-KEMRI and Masinde Muliro University of Science and Technology (MMUST). Gram positive bacteria were *Staphylococcus aureus* ATCC 25923, clinical isolate of Methicillin Resistant *Staphylococcus aureus* (MR*SA*) and *Enterococcus faecalis*. Gram negative bacteria were *Escherichia coli* ATCC 25922, ESBL *Escherichia coli, Pseudomona*s *aeruginosa* ATCC 27853, *Citrobacter freundii, Klebsiella Pneumoniae*, *Salmonella typhi* and *Shigella sonnei.*

3.5.2 Preparation of test organisms

This involved sub-culturing of bacterial strains on Muller Hinton agar number CM0337. (Oxoid Ltd, Basingstock, Hampshire, England) followed by incubation at 37ºC for 24 hours to obtain freshly growing strains.

3.5.3 Preparation of McFarland Standard

Precisely 0.5 McFarland equivalent turbidity standards was prepared by adding 0.6ml of 1% barium chloride solution to 99.4ml of 1% sulphuric acid solution and mixed

thoroughly. Two milliliters of the turbid solution was transferred to the tube of the same type that was used to prepare the test and control innocula and kept at room temperature (25ºC). Exactly 0.5 McFarland gives an equivalent approximate bacterial density of $1x10^6$ colony forming unit (CFU)/ml (Ana *et al.*, 2005).

3.5.4 Disc diffusion assay

The antibacterial activities started with the methanol extracts of seventeen medicinal plants. This was followed by sixteen successive extracts of four selected medicinal plants designated HE, DCM, EAE and ME. After preliminary screening of successive extracts fractions for four selected medicinal plants were screened. Finally five isolated compounds designated AL-1, CM-1, LC-1, LC-2 AND Or-2 were screened.

These were determined based on the inhibition zones using disc diffusion method described by Bauer *et al*., 1996. The test microorganisms were sub-cultured for 18-24 hours at 37º C (Bauer *et al*., 1996). Nutrient media for growth of the test microorganisms were prepared as per the manufacturer's instructions, sterilized and left to cool at 25.0ºC. Each of the cultured microorganisms from an overnight growth of the test organism, 4-6 colonies were emulsified and the suspension was adjusted to match the 0.5 McFarland's standard so as to produce inoculated agar with $1x10^6$ colony forming units/ml.

The suspension was inoculated into the respective growth medium using a sterile cotton wool swab. Briefly, 100 mg of each extracts was dissolved in 1ml to come up with a concentration of 100 mg /ml. Ten microliters of each prepared plant extracts, fractions and compounds was measured and impregnated onto 6 mm sterile filter paper disk with a diameter of 6 mm and air dried. The disk was placed aseptically onto the inoculated plates and incubated for 24 hours at 37º C, (Bauer *et al*., 1996).

3.5.5 Determination of Minimum Inhibitory Concentration(MIC)

Broth micro dilution method was used to determine minimum inhibitory concentration (MIC) for the active methanol extracts and isolated compounds against the test microorganisms in disc diffusion test. The method is recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002) now Clinical Laboratory Standard Institute (CLSI). MIC interpretation for the standard drugs Gentamycin was adapted from NCCL's interpretive standards for dilution and disc diffusion susceptibility testing tables. The reference was the 0.5 McFarland turbidometry to achievement of inoculums approximately $1x10^6$ colony forming units (CFU) (Burt and Reinders, 2003).

The tests were performed in 96 well micro-titer plates. Plant extracts (methanol) and isolated compounds were independently dissolved in respective solvents and transferred into micro-titer plates to make serial dilutions ranging from10^1, 10^2, 10^3……..$10.^{10}$ The final volume in each well was 100 µl. The wells were inoculated with 5µl of microbial suspension and plates incubated at 37^0C for 24 hours. Wells that were not inoculated with microbial suspensions served as controls. MIC value was determined as the lowest concentration of the crude extract and compounds in broth medium that inhibited the visible growth of the test microorganism as compared to the control (Motamedi, 2009). All the assays were done in triplicates and the mean values determined.

3.5.6 Determination of Minimum Bactericidal Concentration

Minimum Bactericidal Concentration (MBC) was determined by collecting a loop full of broth from those wells, which did not show any growth in MIC two wells above, and two wells below the lowest MIC value and inoculated on sterile Muller-Hinton agar by

streaking. This was done respectively for active extracts and isolated compounds. The plates were incubated at 37 º C for 18-24 hours. The highest dilution that yielded no colony fraction on a solid medium was considered as MBC (Motamedi, 2009).

3.5.7 Determination of synergistic effects of the most active compounds

The most active compounds were mixed in equal proportions of combinations of two and three and tested for antibacterial activities. For comparison purposes, individual compounds were used.

3.6 Data Analysis

Plant parts for treatment, habit, plant families, yield of extracts and compounds were expressed in percentages. Bioactivity of extracts, fractions, and isolated compounds against bacteria were evaluated using zones of inhibition in millimeters with values expressed as mean ± SD of three replicates. Analysis of variance (ANOVA) and Turkey post hoc test was used to determine significant differences of mean inhibitory zone of each plant extract against all tested bacteria and that of gentamicin (positive control) (Appendix 39). Significant variability between bacteria based on each plant extract was also determined using ANOVA. This was done by comparing average activity of each plant extract and that of gentamicin. The data was presented in form of tables and graphs.

CHAPTER FOUR

RESULTS

4.1 Ethnobotanical information of selected medicinal plants in Nandi county

Thirty three (33) medicinal plants distributed within 24 botanical families were documented (Kimutai *et al*., 2019), (Appendix-2A and 2B). The roots were the most used parts 19 (57.57%), followed by barks 6 (18.18%) while leaves 5 (15.15%), and whole plant was 3 (9.09%). The medicinal plants were mainly trees (45.5%) and shrubs (40.9%) sourced from the wild.

The family with the highest number of species that was used by the Nandi community was Fabaceae with 4 species, (40.0%), followed by Rubiaceae with 3 species, (30.0%), Asteraceae, Euphorbiaceae, Rosaceae and Rutaceae each with 2 species representing (20.0%) while the remaining 18 families had a single species each representing (10.0%). Most plants were used to treat more than one infection or symptom except *Szigium cardatum* that was used to treat diarrhoea only. It was evident that majority of plants were used to treat pneumonia 11 (33.3%), wounds 10 (30.3%) and diarrhea10 (30.3%), followed by skin diseases 9 (27.3%). Other remedies used fewer plants.

4. 2 Yield of exhaustive extracts from 17 selected medicinal plant materials

As shown in Table 4.1, *Olinia rochetiana* bark had the highest percentage yield of 8.50% followed by *Sygium cordatum* with percentage yield of 8.24% while *Plantago palmate* roots had the lowest percentage yield of 0.46%.

Table 4. 1: Masses and percentage yields of exhaustive extracts from selected medicinal plants

S. No.	Plant species	Plant part	Initial weights in grams	Extract weight in grams	% yield
1	*Olinia rochetiana*	Bark	50.0	4.25	8.50
2	*Acacia lahai*	Bark	50.0	2.66	5.32
3	*Adenia gummifera*	Roots	50.0	1.66	3.32
4	*Albicia coriaria*	Roots	50.0	1. 03	2.06
5	*Erythrinia abyssinica*	Bark	50.0	2.21	4.42
6	*Leucas calastachys*	Roots	50.0	0.72	1.44
7	*Cleodedrum myricoides*	Roots	50.0	1.51	3.02
8	*Lactuca glandulifera*	Whole	50.0	0.51	1.02
9	*Urtica mosaic*	Whole	50.0	3.45	6.90
10	*Plantago palmate*	Roots	50.0	0.23	0.46
11	*Cythula schimperiana*	Roots	50.0	1.01	2.02
12	*Zeheneria minutiflora*	Roots	50.0	0.88	1.76
13	*Ficus sycamorus*	Bark	50.0	0.55	1.10
14	*Solanum macranthum*	Roots	50.0	0.92	1.84
15	*Vangueria volkensii*	Bark	50.0	1.22	2.44
16	*Senecio discifolius*	Whole	50.0	0.61	1.22
17	*Sygium cordatum*	Bark	50.0	4.12	8.24

4.3 Yields from sucessive extract of four selected medicinal plants

Dry powdered parts weighing one kilogram each from the selected medicinal plants were succesivelly extracted in order of polarity starting from hexane (HE), dichloromethane (DCM), ethyl acetate (EAE) and methanol (ME). The amount of crude extracts and percentage yields are shown in Table 4.2.

Polar solvent (methanol) achieved the highest quantities of extracts with *Acacia lahai* and *Olinia rochetiana* having the highest percentage yield of 3.51% and 1.84% respectively. The mid polar solvents produced average quantities of the extracts while the less polar solvent (hexane) produced the lowest quantities. For example, *Acacia lahai* bark extracts had a percentage yield of 0.11%, (Table 4.2).

Table 4. 2: Masses and percentage yields of successive extracts of four selected medicinal plants

Plant species	Extraction solvent	Mass in grams	Percentage yield
Acacia lahai	Hexane	1.10	0.11
	Dichloromethane	14.20	1.42
	Ethyl acetate	7.09	0.79
	Methanol	35.14	3.51
Cleodendrum myricoides	Hexane	3.55	0.36
	Dichloromethane	7.74	0.77
	Ethyl acetate	7.94	0.57
	Methanol	10.38	1.04
Leucas calostachys	Hexane	6.07	0.61
	Dichloromethane	5. 01	0.50
	Ethyl acetate	7.24	0.72
	Methanol	9.13	0.91
Olinia rochetiana	Hexane	5.55	0.56
	Dichloromethane	13.74	1.374
	Ethyl acetate	9.72	0.97
	Methanol	18.38	1.84

4.4 Antibacterial assays

Exhaustive crude extracts, sucessive extracts, fractions , isolated compounds and mixed compounds were screened for antibacterial activities against selected bacteria.

4.4.1 Antibacterial activities of exhaustive crude extracts from seventeen (17) selected medicinal plants

Exhaustive crude extracts of 17 selected medicinal plants were screened for antibacterial susceptibility test using disk diffusion methods as described in section 3.5.4. The activities of the test plants were expressed as inhibition zone diameters observed on plates (Appedices 3-5). The tests were done in triplicates and means determined (Table 4.3 and 4.4). Zone of inhibition is the area around the disk without bacterial have growth, enough to be visible due to the effects of the antibacterials. The bigger the diameter the more effective the extract. This is because it can kill or inhibit more bacteria as compared to a

smaller diameter. For example, Plate 2 for *S. aureus* 4a shown by an arrow is bigger than 1a (Figure 4.1).

Plate 1; MR*S. aureus* Plate 2;*S. aureus* Plate 3; *E. faecalis*

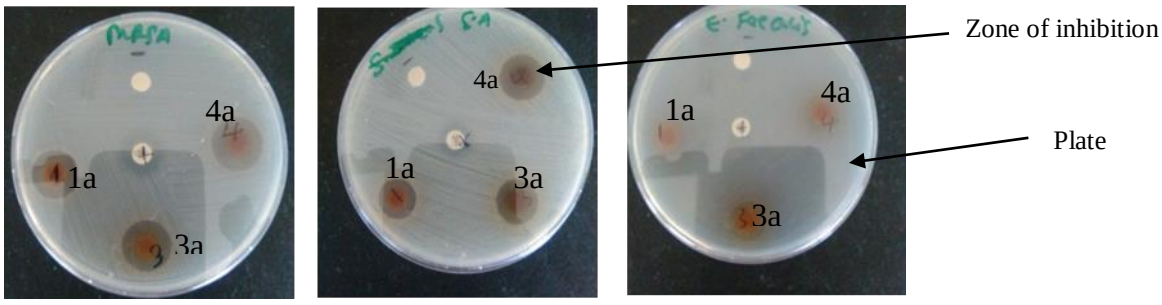


Figure 4. 1: Inhibition zones of exhaustive methanol extracts from selected medicinal plants being assayed for bioactivity as potential antibiotics.

Key; Numerical labelling 1a, 3a and 4a on the plates shows inhibition zones by plant extracts with corresponding serial numbers as indicated on Table 4.3. Other plates are shown in the appendixes 3, 4 and 5.

Table 4. 3: Mean inhibition zones diameters in millimeters of exhaustive (methanol) extracts from selected medicinal plants against test bacteria

S.No.	Plant extracts/ Controls	Average inhibition Zone diameters in millimeter for each *Test microorganism*								
		MR. *Staphylococcus aureus*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Enterococcus faecalis*	*Shigella sonnei*	*Klebsiellapneumoniae*	*Citrobacter freudii*	*Salmonella typhi*
1a	*O. rochetiana*	16.00±0.56	14.00±0.36	20.2±0.80	6.00±0.00	10.1±0.15	6.00±0.00	8.00±0.17	7.01±0.10	6.00±0.00
3a	*A. Lahai*	18.33±0.51	15.30±0.36	17.23±0.55	6.00±0.00	12.17±0.32	6.00±0.00	12.33+0.35	8.51±0.20	6.00±0.00
4a	*A.gummifera*	14.03±0.40	13.30±0.36	19.46±0.61	6.00±0.00	10.33±0.40	6.00±0.00	13.23±0.40	6.00±0.00	6.00±0.00
1	*A. coriaria*	12.10±0.30	10.03±0.71	10.00±0.00	6.00±0.00	7.0±0.50	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
2	*E. abyssnica*	8.00±0.36	11.30±0.36	6.00±0.00	6.00±0.00	7.40±0.43	6.00±0.00	7.27±0.42	6.00±0.00	6.00±0.00
3	*L. Calostcys*	11.63±0.47	12.00±0.26	6.00±0.00	6.00±0.00	8.00±0.40	6.00±0.00	8.33±0.40	6.00±0.00	8.46±0.81
4	*C. myricoides*	10.13±0.39	10.06±0.42	6.00±0.00	6.00±0.00	7.23±0.42	6.00±0.00	8.40±0.50	6.92±0.32	8.93±0.78
5	*L.Glandulifera*	10.23±0.72	7.30±0.46	6.00±0.00	6.00±0.00	Nd	6.00±0.00	7.30±0.36	6.00±0.00	6.00±0.00
+ve	Control	21.20±0.53	23.17±0.21	23.03±0.25	21.1±0.25	21.23±0.45	20.27±0.45	18.90±0.17	21.13±0.25	22.96±0.38
-ve	Control	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Values are means of test replicates ±standard deviation (*n* =3).

Key: Diameter of disk was 6.0mm,+ve -positive control, -ve- negative control(DMSO- Dimethyl sulphoxide), - -No activity, Nd-not done, MR.*S.a* - Methicillin resistant *Staphylococcus aureus.*

Table 4. 4: Mean inhibition zones diameters in millimeters of exhaustive (methanol) extracts from selected medicinal plants against test bacteria

S.No.	Plant extracts/ Controls	Average inhibition Zone diameters in millimeter for each *Test microorganism*								
		MR. *Staphylococcus aureus*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Enterococcus faecalis*	*Shigella sonnei*	*Klebsiellapneumoniae*	*Citrobacter freudii*	*Salmonella typhi*
6	*U. Mossaca*	8.67±0.60	8.17±0.55	6.00±0.00	6.00±0.00	7.30±0.36	6.00±0.00	7.7±0.611	6.00±0.00	7.27±0.76
7	*P. palmate*	7.43±0.35	8.63±0.29	6.00±0.00	6.00±0.00	7.73±0.60	6.00±0.00	6.00±0.00	6.00±0.00	6.87±0.83
8	*C. schim Perianal*	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.96±0.252	6.00±0.00	6.93±0.15	6.00±0.00	6.00±0.00
9	*Z. minuti Flora*	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.90±0.61	6.00±0.00	6.83±0.15	6.00±0.00	6.00±0.00
10	*F. Cycamores*	7.67±0.85	6.90±0.46	6.00±0.00	8.83±0.75	6.00±0.00	7.10±0.26	9.01±0.32	6.00±0.00	7.10±0.30
11	*S. micra Thum*	8.70±1.18	10.00±0.53	6.00±0.00	6.00±0.00	6.00±0.00	7.20±0.27	7.97±0.31	6.00±0.00	7.10±0.36
12	*V. volkensii*	6.00±0.00	6.00±0.00	7.97+0.21	8.13±0.23	8.00+1.1	9.03±0.32	7.90+0.20	6.00±0.00	7.86+0.06
13	*S. discifolius*	6.00±0.00	6.00±0.00	6.00±0.00	8.00+0.36	6.00±0.00	7.80±0.56	9.00±0.36	7.17±0.40	8.83±0.51
14	*S. cordatum*	12.03±0.60	12.07±0.32	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	11.00+0.26	6.87+0.20	6.00±0.00
+ve	Control	21.20±0.53	23.17±0.21	23.03±0.25	21.1±0.25	21.23±0.4	20.27±0.45	18.90±0.17	21.13±0.25	22.96±0.38
-ve	Control	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Values are means of test replicates ±standard deviation (*n* =3).

Key: Diameter of disk was 6.0mm,+ve -positive control, -ve- negative control(DMSO- Dimethyl sulphoxide), - -No activity, Nd-not done, MR.*S.a* - Methicillin resistant *Staphylococcus aureus.*

As shown in figure 4.2, Gentamicin positive control was active against all (100% n=10) tested bacteria and all the plant extracts tested had antimicrobial activity against at least one of the tested bacteria, which was statistically significant (ρ<0.05). For example, extracts from *Vangueria volkensii* and *Acacia lahai*, were active against the highest number of the tested microorganisms (60%). *C. myricoide*, *F.cycamore*, *Olinia rochetiana, Leucas calastachys, Urtica mosaica, Solanum micranthum, Adenia gummifera* and *Senecio discifolius* were active against half (50%) of the test microorganisms. *Cythula schimperiana* and *Zehneria minutiflora* showed antimicrobial activity only against *E. faecalis* and *K. pneumonia* (20%).

Analysis of variance (ANOVA) was used to compare the mean inhibitory zone of each plant extract on all tested microorganis against the mean inhibitory zone of the positive control (Gentamicin). It was observed that there was a significant difference in the mean inhibitory zones of the plant extracts on the test microorganisms ($F_{16, 153}$ =3.028; p=0.000).

All the plants extracts had significantly low mean inhibitory zones compared with Gentamicin (Fig. 4.2) had a mean of 21.40 (sd=2.119) whereas all the plant extracts apart from *Acacia lahai* had mean inhibitory zones of less than 10. *Acacia lahai* had the highest mean inhibition zone of 10.00(SD=5.011) which was attributed to the large average inhibition zones on Methicillin *staphylococcus aureus, Staphylococcus aureus* and *Pseudomonas aeruginosa. Olinia rochetiana* (mean 9.80; sd=5.116) and *Adenia gummifera* (mean 9.90; sd= 4.654) also had comparatively high mean zones of inhibition. This was attributed by the high level of activity on Methicillin *staphylococcus aureus, Staphylococcus aureus, Klebsiella pneumonia* and *Enterococcus faecalis* (Figure 4.3).

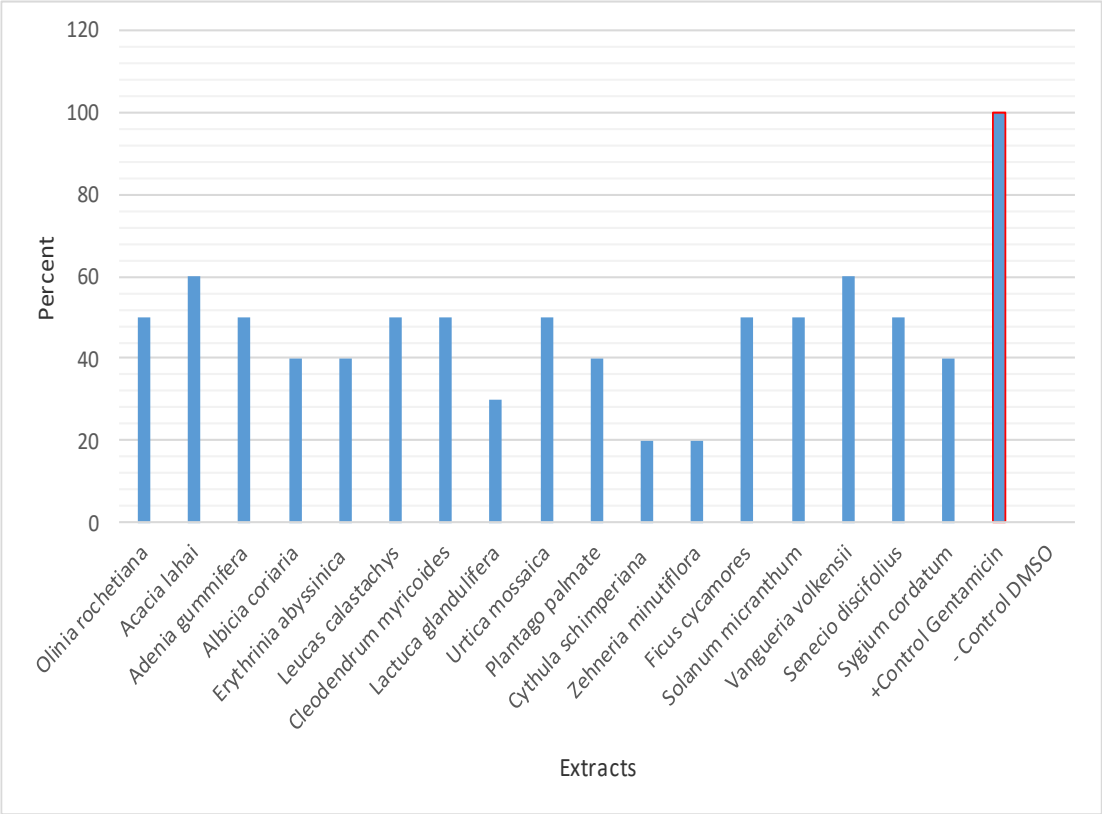


Figure 4. 2:A graph showing comparative antibacterial activity of the exhaustive plant extracts

ANOVA was also used to compare pooled average inhibitory zones of all the plant extracts of each test microorganism. The results showed that the ten (10) microorganisms differed significantly on the level of susceptibility to the plant extracts ($F_{9, 160}$ =6.344; p=0.000). Except ESBL *Escherichia coli*, all the test microorganisms were susceptible to at least one of the plants extracts studied. As shown in figure 4.2, *Klebsiella pneumonia*, Methicillin resistant *S.aureus*, *S.aureus and E. faecalis* had a percentage of 88.24%; n=15, 76.47; n=13, 76.47; n=13 and 70.59; n=12 respectively. They were suscetible to the highest number of the plant extracts (Figure 4.3). *E. coli and C. freudii* were susceptible to only 3(17.65%) and 2(11.76%) of the plant extracts whereas ESBL *Escherichia coli* was not susceptible to any of the plant extracts.

MR*S. aureus* and *Staphylococcus aureus* were among the most susceptible bacteria. This is because all plant extracts except four **(**Table 4.3 and 4.4) were active against the two bacteria with the highest zone of inhibition of (18.33±0.51 mm) and (15.30±0.36 mm) shown by *Acacia lahai* (Plate 1 and 2, Table 4.3).

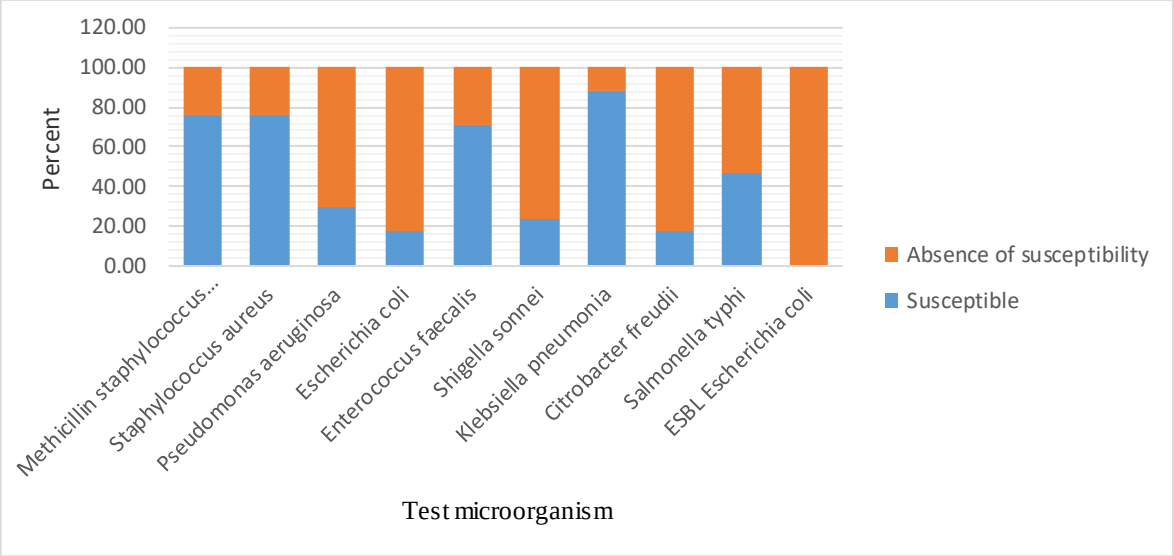


Figure 4. 3:A graph showing percentage susceptibility of bacteria to the exhaustive plant extracts

The Tukey post hoc tests indicated that at p<.05, the mean activity of *Acacia lahai* was significantly higher than the mean activity of *Lactuca glandulifera, Urtica mossaica, Plantago palmate, Cythula schimperiana, Zehneria minutiflora, Ficus cycomores* and *Senecio discifolius*. The results for the multiple comparisons are shown in (Figure 4.4 and Appendix 39).

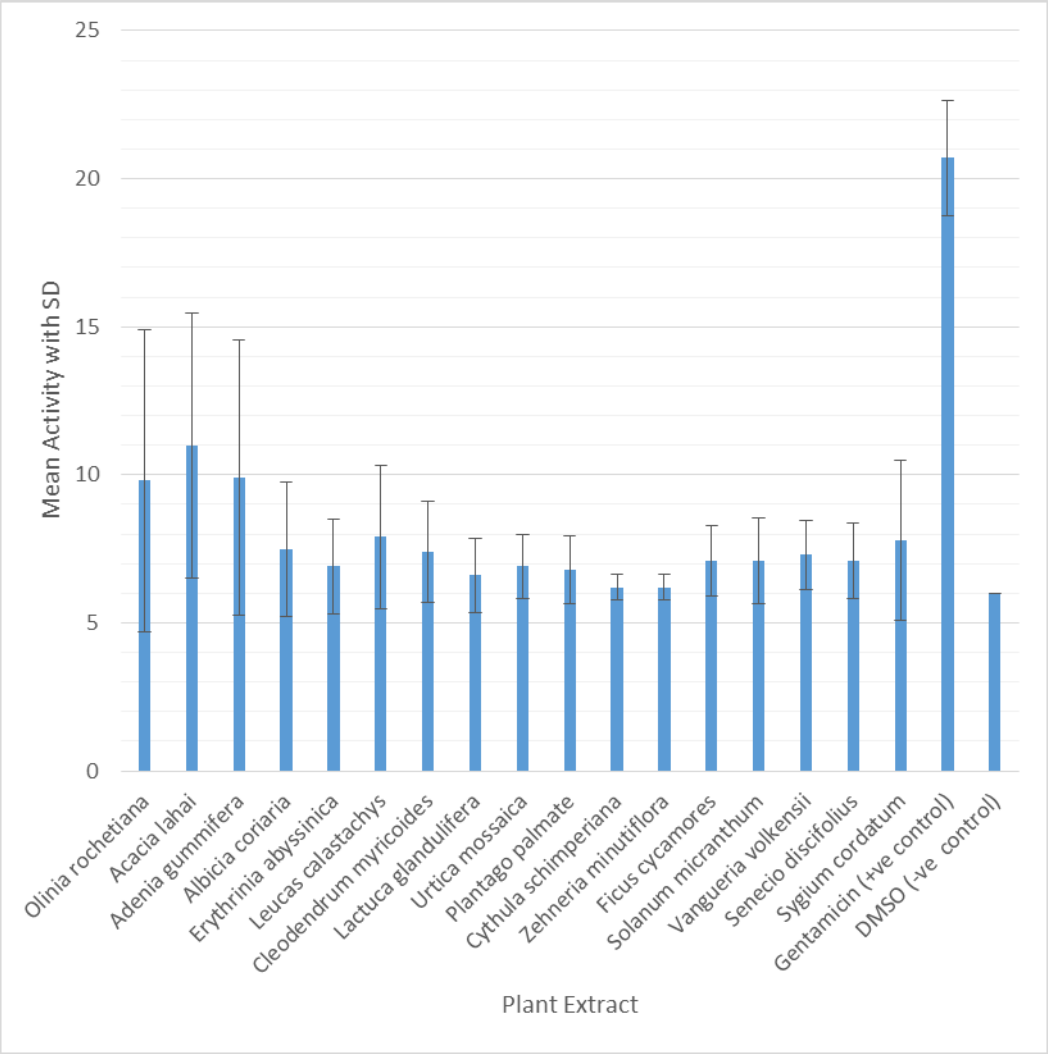


Figure 4. 4: A graph showing Tukey post hoc tests

4.4.2 Minimum inhibitory concentration and Minimum bactericidal concentration of exhaustive extracts from selected medicinal plant

Active exhaustive (methanol) plants extracts namely; *A. lahai*, *A. coriaria*, *A. gummifera*, *C. myricoides*, *L. calostachys* and *O. rochetiana* were screen for MIC and MBC due to their significant activities against various microorganisms (Table 4.3,Figure 4.4 and 4.5).

Table 4. 5: Minimum inhibitory concentration (MC) and Minimum bactericidal concentration (MBC) of active exhaustive extracts from selected medicinal plant against susceptible bacteria.

S. No.	Plant extracts	Mean MIC and MBC in mg/ml for the active plant extracts against sensitive bacteria									
		S. aureus		*P.aeruginosa*		*K.pneumonia*		MR. *S.aureus*		*E. faecalis*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	*A.lahai*	25±0.00	16.67±7.10	12.45±0.00	4.17±1.80	Nd	Nd	4.17±1.80	33.33±14.0	12.5±0.00	41.6±14.00
2	*A. gummifera*	12.5±0.00	-	5.21±1.80	-	25±0.00	-	16.67±7.2	-	12.5±0.00	-
3	*A. coriaria*	12.5±0.0	25.0±0.0	6.25±0.0	25.00±0.00	Nd	Nd	2.08±0.9	-	Nd	Nd
4	*O. rochetiana*	12.5±0.0	-	12.25±0.0	6.25±0.0	Nd	Nd	0.781±0.00	41.67±14.4	20.83±7.2	50±0.00
5	*L. calastachys*	3.15±0.0	66.6±28.80	Nd	Nd	Nd	Nd	12.5±0.0	33.33±14.4	Nd	Nd
6	*C. myricoides*-	66.67±28.9	100±0.00	Nd	Nd	Nd	Nd	50±0.00	100±0.00	Nd	Nd
+ ve	Gentamicin	10.4±3.60	25±0.00	3.13±0.00	16.67±7.20	6.25±0.00	12.5±0.00	0.781±0.00	8.3±3.60	3.13±0.00	6.25±0.00
-ve	DMSO	-	-	-	-	-	-	-	-	-	-

Values are means of test replicates±standard deviation (*n* =3).
Key: Nd- not

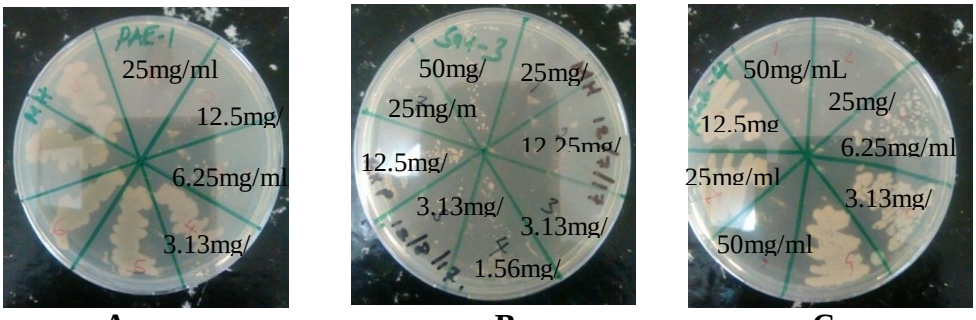
P. aeruginosa and *S. aureus* were among the most susceptible bacteria (Plates A and B). Majority of the selected plant extracts were bactericidal against *S. aureus*, and other selected bacteria with different MBC values. The difference observed between MIC and MBC is that MIC in most cases is higher than MBC. This is because whereas the MIC test demonstrates the lowest level of antimicrobial agent that inhibits growth, the MBC demonstrates the lowest level of antimicrobial agent that results in microbial death. This means that even if a particular MIC shows inhibition, plating the bacteria onto agar might still result in organism proliferation because the antimicrobial did not cause death.

Plate A; *P. aeruginosa* Platete B; *S. aureus*

Figure 4. 5: Minimum inhibitory concentration (MIC) test of plant extracts 1, 3,4(right) and 1-4(left) against *P. aeruginosa* and *S. aureus*

Key; Plants extracts *A. lahai* (1), *A. gummifera* (2), *A. coriaria* (3), *O. rochetiana*(4), (Table 5).

Plate a. *P. aeruginosa* Plate b. *S. aureus* Plate c. MR*S. aureus*



A **B** **C**

Figure 4. 6:Minimum Bactericidal Concentration (MBC) test of active plant extracts

Plate A; beginning from 25mg/ml right at the top represents *A. lahai* extract against *P. aeruginosa* (MBC-6.25mg/ml), the remaining part left of the plate are concentration for *A. gummifera* extracts that were bacteriostatic as there was visible growth at each concentration used. Plate B; starting with concentration of 25mg/ml at the top represent *A. lahai* extracts against *S. aureus* (MBC-3.13 mg/ml), the other parts of the plate belong to *O. rochetiana* which was bacteriostatic. Plate C; represent *A. lahai extracts* against MR*S. aureus* with MBC of 25mg/ml, while O. *rochetiana* extracts left had MBC of 50mg/ml.

4.4.3 Antibacterial activities of successive extracts from four selected medicinalplants

Antibacterial screening for the successive extracts (HE, DCM, EAE and ME) against the selected bacteria was determined using disk diffusion method. The results were recorded and tabulated as shown in table 4.6 and 4.7 as well as plates as shown in Appendixes 6-9

Ethyl acetate extracts(EAE) were the most active of all the extracts used except hexane extracts of *L. calostachys.* For instance, successive extracts of *O. rochetiana* reveals that ethyl acetate extracts were the most active against all the bacteria tested with an inhibition zones above 10.00 mm except against *S. sonnei.* Moreover, ethyl acetate extracts of *C. myricoides* and *A. lahai* extracts were more active than other extracts, (Table 4.6 and 4.7).

Table 4. 6 Mean inhibition zone diameters in millimeters of successive extracts from four selected medicinal plants against test bacteria

Plant species	Solvent	Mean inhibition zone diameters in millimeter for each test bacteria								
		MR.*S. aureus*	*S. aureus*	*P. aeruginosa*	*E. coli*	*E. faecalis*	*S. sonnei*	*K.pneumoniae*	*C. freudii*	*S. typhi*
A. lahai	H	9.07±0.33	7.97±0.46	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	9.23+0.39	6.00±0.00
	D	9.00±0.40	9.63±0.52	8.10±0.29	6.00±0.00	8.10±0.14	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	E	9.93±`0.97	11.20±0.36	8.37±0.31	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	11.23±0.29	6.00±0.00
	M	8.48±0.22	8.13±0.48	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
C. myricoides	H	7.48±0.29	6.00±±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	D	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	8.40±0.45	6.00±0.00	7.13±0.29	6.00±0.00	8.67±0.90
	E	11.37±0.25	10.00±0.62	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	7.87±0.19	6.00±0.00	12.37±0.62
	M	8.67±0.12	8.23±0.25	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	7.93±0.23	6.00±0.00	6.00±0.00
+ve control	G.	21.20±0.53	23.17±0.21	23.03±0.25	21.13±0.25	21.23±0.40	20.27±0.45	18.90±0.17	21.13±0.25	22.96±0.38
-ve control	D	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Values are means of test replicates ±standard deviation (*n* =3).

Key: Diameter of disk was 6.0mm,-ve-negative control, D-DMSO- Dimethyl sulphoxide +ve control, G-Gentamicin, MR.*S. a*-Methicillin resistant *Staphylococcus aureus*, H-Hexane (HE), D-Dichloromethane (DCM), E-Ethyl acetate (EAE) and M-Methanol(ME).

Table 4. 7: Mean inhibition zone diameters in millimeters of successive extracts from four selected medicinal plants against test bacteria

Plant species	Solvent	Mean inhibition zone diameters in millimeter for each test bacteria								
		MR.*S. aureus*	*S. aureus*	*P. aeruginosa*	*E. coli*	*E. faecalis*	*S. sonnei*	*K.pneumoniae*	*C. freudii*	*S. typhi*
L. calostachys	H	10.37±0.50	10.73±0.82	12.07±0.26	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	11.04±0.49	6.00±0.00
	D	6.00±0.00	6.00±0.00	6.00±0.00	6.90±0.06	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	E	7.27±0.21	9.40±0.36	8.00±0.8	7.12±0.70	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	M	7.13±0.49	7.20±0.753	6.00±0.00	7.33±0.45	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
O. rochetiana	H	10.37±1.11	10.33±0.66	6.00±0.00	6.00±0.00	7.03±0.63	6.00±0.00	6.00±0.00	6.00±0.00	6.56±0.29
	D	14.27±0.45	14.02±0.45	11.02±0.29	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.70±0.29	7.20±0.62
	E	18.00±1.00	16.67+0.25	10.67+0.75	8.67±0.76	11.03±0.32	7.000±0.46	11.23±0.12	9.00±0.14	11.73±0.33
	M	18.23±0.50	16.30±0.53	10.13±0.35	8.0±0.31	11.10±0.56	6.00±0.00	6.83±0.12	7.83±0.40	14.61±0.62
+ve control	G.	21.20±0.53	23.17±0.21	23.03±0.25	21.13±0.25	21.23±0.40	20.27±0.45	18.90±0.17	21.13±0.25	22.96±0.38
-ve control	D	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Values are means of test replicates ±standard deviation (*n* =3).

Key: Diameter of disk was 6.0mm,-ve-negative control, D-DMSO- Dimethyl sulphoxide +ve control,G-Gentamicin, MR. *S. a*- Methicillin resistant *Staphylococcus aureus*, H-Hexane(HE), D-Dichloromethane(DCM), E-Ethyl acetate(EAE) and M-Methanol(ME).

4.4.4 Antibacterial activities of fractions from four selected medicinal plants against test bacteria

Antibacterial screening of the fractions from the active successive extracts were obtained as shown in Table 4.8 and 4. 9 as well as plates as shown in appendices 10-13.

There was variation in the activities of the extract fractions due to the distribution of the active constituents within various fractions. This makes it possible to choose the fraction with highest zone of inhibition for purification to obtain pure compound(s). For instance, fractions from *A. lahai* of the stem bark coded FA1 had significant activity against five bacteria namely; MR*S. aureus*, *S. aureus* , *P. aeruginosa*, *E. faecalis* and *C. freudii* with inhibition zone of 13.93±0.42 mm, 14.73±0.79 mm, 11.43±0.45 mm, 12.10±0.16mm, and 13.03±0.26 mm respectively. Hence, this fraction was subjected to purification to obtain pure compounds (Table 4.8).

Fraction coded FO1 of *O. rochetiana* was the most against MR*S. aureus*, *S. aureus* and *C. freudii* and therefore, were considered for purification. All the fractions of O. *rochetiana* had significant activity against *C. freudii,* which is in correlation with the ethyl acetate extracts (EAE) from which the fractions were obtained (Table 4.9).

L. calostachys fractions coded FL4 was most active compared to other fractions against MR*S. aureus*, *S. aureus*, and *E. faecalis* with inhibition zone of 10.37±0.45 mm, 10.23±0.29 mm and 11.63±0.61 mm respectively, (Table 4.9). The hexane (HE) extract from which the fraction were obtained was also active against MR*S. aureus* and *S. aureus* with inhibition zone of 10.37±0.50 and10.73±0.82 mm respectively, (Table 4.9).

Furthermore among *C. myricoides* fractions (FC1-FC5), fraction FC1 was the most active against MR. *Staphylococcus aureus* and *Staphylococcus aureus* with inhibition zones of 12.00+0.62 and 13.33+0.45 mm, (Table 4.8). In addition, fractions FA1, FO1, FO3 were most active against *Staphylococcus aureus* with inhibition zones of 14.73±0.79 mm, 13.87±0.40 mm, 13.27±0.55 mm respectively.

Fraction FA1, FA7, FO2, FO4, FO6 were the only fractions that showed activity against *K. pneumoniaae* with low inhibition zones of less than 10 mm while FA1 and FA7 were active against *P.aeruginosa* with inhibition zone of 11.43±0.45 mm and 10.73±0.42 mm respectively. The other gram negative bacteria were susceptible to one fraction only, (Table 4.8 and 4.9).

Table 4. 8:Mean inhibition zone diameters in millimeters of fractions from four selected medicinal plants against test bacteria

Plant species	Fractions	Mean inhibition zone diameters in millimeter for each test bacteria								
		MR*S. Aureus*	*S. aureus*	*P. aeruginosa*	*E. coli*	E. *faecalis*	*S. sonnei*	*K.pneumoniae*	*C. freudii*	*S. typhi*
A. lahai	FA1	13.93±0.42	14.73±0.79	11.43±0.45	6.00±0.00	12.10±0.16	7.10±0.22	8.00±0.00	13.03±0.26	6.00±0.00
	FA2	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.86±0.37	6.00±0.00	10.80±0.96	6.00±0.00
	FA3	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	FA4	10.00±0.36	11.12±1.01	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	9.11±0.74	11.66±0.70	6.00±0.00
	FA5	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	FA6	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	FA7	11.20±0.71	10.8±0.42	10.73±0.42	6.00±0.00	9.96±0.33	6.00±0.00	7.71±0.21	10.10±0.36	6.00±0.00
C. myricoides	FC1	12.00+0.62	13.33+0.45	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.80+0.29	6.00±0.00
	FC2	8.17+0.40	6.00±0.00	6.00±0.00	6.00±0.00	10.37+0.48	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	FC3	8.23+0.45	8.93±0.31	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	7.90+0.64	6.00±0.00
	FC4	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	7.10±0.29	6.00±0.00
	FC5	10.33+0.51	8.03±0.21	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	7.00±0.36	6.00±0.00
+ve control	GEN.	21.20±0.53	23.17±0.21	23.03±0.25	21.13±0.25	21.23±0.40	20.27±0.45	18.90±0.17	21.13±0.25	22.96±0.38
-ve control	DMSO	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Values are means of test replicates ±standard deviation (*n* =3).

Key:-ve-negative control (DMSO- Dimethyl sulphoxide), +ve control (Gentamicin), MR*.S. a*- Methicillin resistant *Staphylococcus aureus.*

Table 4. 9:Mean inhibition zone diameters in millimeters of extract fractions from four selected medicinal plants against test bacteria

Plant species	Fractions	Mean inhibition zone diameters in millimeter for each test bacteria								
		MR.*S. aureus*	*S. aureus*	*P. aeruginosa*	*E. coli*	E. *faecalis*	*S. sonnei*	*K.pneumoniae*	*C. freudii*	*S. typhi*
L.calastachys	FL1	7.23±0.64	7.21±0.67	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	FL2	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	FL3	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	FL4	10.37±0.45	10.23±0.29	6.00±0.00	6.00±0.00	11.63±0.61	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
	FL5	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
O.rochetiana	FO1	13.57±0.70	13.87±0.40	6.00±0.00	9.13±0.36	7.00±0.36	6.00±0.00	6.00±0.00	12.16±0.60	6.00±0.00
	FO2	9.43±0.91	10.20+0.42	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.75±0.12	13.30±0.4	6.00±0.00
	FO3	6.00±0.00	13.27±0.55	6.00±0.00	6.00±0.00	9.00±0.46	11.10±0.46	6.89±0.63	12.31±0.70	6.00±0.00
	FO4	6.93±0.42	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	12.06±0.64	9.23±0.35
	FO5	10.20±0.36	13.37±0.25	6.00±0.00	6.00±0.00	6.00±0.00	9.93±0.42	6.00±0.00	11.46±0.93	6.00±0.00
	FO6	7.37±0.80	6.00±0.00	6.00±0.00	8.17±0.45	6.00±0.00	6.00±0.00	6.55±0.43	14.03±0.35	6.00±0.00
+ve control	Gent.	21.20±0.53	23.17±0.21	23.03±0.25	21.13±0.25	21.23±0.40	20.27±0.45	18.90±0.17	21.13±0.25	22.96±0.38
-ve control	Dms	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

Values are means of test replicates ±standard deviation (*n* =3). **Key**:-ve-negative control (Dms- Dimethyl sulphoxide), +ve control (Gent.Gentamicin), MR.*S. a*- Methicillin resistant *Staphylococcus aureus.*

4.4.5 Percentage yields of isolated compounds from four selected medicinal plants

Compounds Al-1**(1),** Cm-1**(2),** LC-1**(3),** LC-2**(4)** and Or-2**(5)** were isolated from four selected medicinal plants and percentage yield indicated in table 4.10.

Hexane extracts (HE) of *L. calostachys* produce the highest yield of compound LC-1**(3)**, with a percentage yield of 0.0016%. This shows that it was a major component of this plant. Ethyl acetate extracts EAE) of *C. myricoides* and *Olinia rochetiana* produced compound Cm-1**(2)** and Or-2 **(5)** respectively with a percentage yield of 0.0006% and 0.0008%. *Acacia lahai* produce compound Al-1**(1)** with percentage yield of 0.0004% (Table 4.10). Antibacterial activities of isolated compounds is shown on table 4.11 as well as Appendix 14.

Table 4. 10: Percentage yields of compounds isolated from four selected medicinal plants

S. No.	Compounds	Plant species	Plant part	Weights in g(extract)	Compound weight in µg	% yield
1	Or-2**(5)**	*O. rochetiana*	Bark	9.72	77.0	0.0008
2	Cm-1**(2)**	*C. myricoides*	Roots	7.94	48.1	0.0006
3	Al-1**(1)**	*A. lahai*	Bark	7.09	27.2	0.0004
4	LC-1**(3)**	*L. calastachys*	Roots	6.07	97.2	0.0016
5	LC-2**(4)**	*L. calastachys*	Roots	6.07	29.5	0.0005

4.4.6 Antibacterial activities of isolated compounds from four selected medicinal plants

The results (Table 4.11) indicated that all compounds showed moderate to low activity against different bacteria tested. The compound coded Cm-1 **(2)** from the root bark of *Cleodendrum myricoides* showed the highest activity against MR. *S. aureus, S. aureus, P.*

aeruginosa, C. freudii with inhibition zones of 12.53±0.25mm, 13.23±0.33mm and 12.97±0.53mm respectively. In addition, compound Or-2 **(5)** from stem bark of *O. rochetiana* was also active against MR. *S. aureus, S. aureus, P. aeruginosa, C. freudii* with inhibition zones of 11.36 ±0.7, 11.77±0.29, 8.43±0.46 and 11.30±0.37 respectively.

Table 4. 11: Mean inhibition zone diameters in millimeters of isolated compounds from four selected medicinal plants against selected bacteria

S. No.	Compounds	Mean inhibition zone diameters in millimeter for each test bacteria								
		MR*S. a*	*S. a*	*P. a*	*E. c*	E. *f*	E. *E.c*	*K. p*	*C. f*	*S.t*
1	Or-2 **(5)**	11.36 ±0.71	11.77 ±0.29	8.43± 0.46	6.00± 0.00	7.27± 0.29	6.00± 0.00	6.00± 0.00	11.30 ±0.37	6.00±0. 00
2	Al-1 **(1)**	8.86± 0.33	6.00± 0.00	6.00± 0.00	7.90± 0.54	10.10 +0.43	7.73± 0.34	6.00± 0.00	7.93± 0.37	6.00±0. 00
3	Cm-1**(2)**	10.40 ±0.29	12.53 ±0.25	13.23 ±0.33	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	12.97 ±0.53	6.00±0. 00
4	LC-2**(4)**	8.86± 0.39	10.16 ±0.42	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.70± 0.22	6.00±0. 00
5	LC-1**(3)**	10.83 ±0.45	10.43 ±0.17	9.87± 0.17	6.70± 0.37	6.00± 0.00	7.23± 0.34	6.90+ 0.08	6.00± 0	6.76± 0.19
	+ve control	21.20 ±0.53	23.17 ±0.21	23.03 ±0.25	21.13 ±0.25	21.23 ±0.40	22.17 ±0.35	18.90 ±0.17	21.13 ±0.25	22.96± 0.38
	-ve control	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00±0. 00

Values are means of test replicates ±standard deviation (*n* =3).

Key:-ve-negative control (DMSO- Dimethyl sulphoxide), +ve control (Gentamicin), MR*S. a*-Methicillin resistant *Staphylococcus aureus,S.a-Staphylococcus aureus, P.a-Pseudomonas aeruginosa, E. f- Enterococcus faecalis, K. p-Klebsiella pneumoniae, E.c-Escherichia coli,* E. *E. c*-ESBL *Escherichia coli, S.s-Shigella sonnei, S.t-Salmonella typhi* and *C. f-Citrobacter freudii*

Compound coded LC-1**(3)** obtained from the root bark of *L. calostachys* was active against six bacteria with good antibacterial activities against MR. *S. aureus, S. aureus, and P. aeruginosa* with an inhibition zones of 10.83±0.45, 10.43±0.17, and 9.87±0.17 (Table 4.9). A similar fatty acid coded Al-1 **(1)** of *Acacia lahai* had a significant activity against *E. faecalis* only. Compound LC-2 (**4**) was active against MR. *S. aureus* and *S. aureus* with inhibition zones of 8.86±0.39 and 10.16±0.42.

Compounds Or-2 **(5)**, Cm-1 **(2)** and LC-1 **(3)** were selected for further tests on MIC and MBC against three most susceptible microoganisms basing on their antibacterial activity. Minimum inhibitory concentration (MIC) against each sensitive bacterial strain was determined as well as minimum bactericidal concentration (MBC) (Table 4.12).

The tested compounds showed different MIC values against the selected bacteria with compound Or-2 (**5**), Cm-1(**2**) and LC-1**(3)** showing MIC values of 12.25, 25 and 25 mg/ml respectively, against *S. aureus* , (Table 4.12).

Table 4. 12: Minimum inhibitory concentration and Minimum bactericidal concentrations for the active compounds against susceptible bacteria.

	MIC and MBC in mg/ml for the active compounds against sensitive bacteria						
S.No.	Compounds	*S. aureus*		*P.aeruginosa*		MR. *S. aureus*	
		MIC	MBC	MIC	MBC	MIC	MBC
1	Or-2(**5**)	12.25±0.00	50.00±0.00	Nd	Nd	25.00±0.00	-
2	Cm-1(**2**)	25.00±0.00	12.50±0.00	12.25±0.00	25.00±0.0	12.5±0.00	50.00±0.00
3	LC-1**(3)**	25.00±0.00	50.00±0.00	25.00±0.00	-	6.25±0.00	25.00±0.00
+ ve	GENT.	12.50±0.00	12.50	3.13±000	12.50±0.0	±1.56	±3.125
-ve	DMSO	-	-	-	-	-	-

Values are means of test replicates ±standard deviation (*n* =2).This was done in duplicate due to small quantities of compounds.

Key:-ve-negative control (DMSO-Dimethyl sulphoxide), +ve control (Gentamicin), - No activity (static),Nd-Not determined, MR.*S. a*- Methicillin Resistant *Staphylococcus aureus.*

In addition, compound LC-1was bactericidal at 50mg/ml against *S. aureus*, (plate A). Compound Cm-1(**2**) was also bactericidal against *S. aureus, P.aruginosa* and MR. *S. aureus* with MBC of 12.5, 25 and 50mg/ml. This suggests that this compound could possibly act as a bactericidal agent against this bacterium. Compound Cm-1**(2)** was bactericidal against all the bacteria tested.

Plate A and B, shows MBC of compound LC-1(**3**) and Or-2 against *S. aureus* and MR*S. aureus*

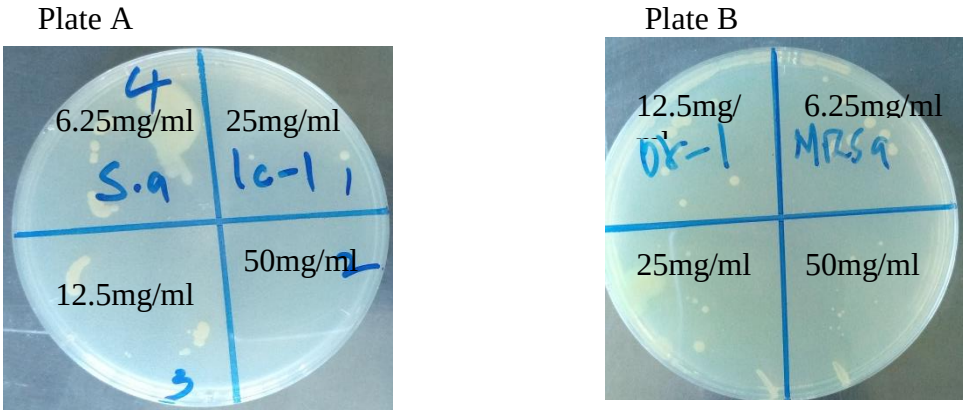


Figure 4.7: Minimum Bactericidal concentration (MBC) test for compound LC-1(3) and Or-2(5) against *S. aureus* and MR*S. aureus*

Compound Or-1 was bactericidal at 50mg/ml as there was no visible growth at that concentration while compound LC-2 was bacteriostatic, as there was growth on the whole plate. (Figure 4.7).

4.4.7: Synergistic activities of most active compounds against selected bacteria

Compounds Or-2 **(5)**, Cm-1**(2)** and LC-1**(3)** were mixed in equal proportions and tested against selected bacteria. The results are shown in Table 4.13 as well as plates in Appendix 15.

The antibacterial activities of the active compounds in a mixture of two and three showed different degrees of inhibition, (Table 4.13). The average diameter of 22.13±0.35 mm were obtained in the following mixture (Or-2+Cm-1+LC-1) against *S. aureus.* Inhibition zones of 20.03±0.25mm was observed in mixture of (Cm-1+Or-2+LC-1) and (Or-2+LC-1) against *P.aeruginosa* while an inhibition zone of 20.00 mm and above was obtained in mixture of (Or-2+Cm-1) against MR*S.aureus* and *S.aureus,* (Or-2+LC-1) against MR*S.aureus, E.coli* and *E. faecalis* and (Or-2+LC-1) against *P.aeruginosa, E.coli*, E. *E.coli* and *E.faecalis* (Table 4.3).

Combination of (Cm-1+Or-2+LC-1) was 18.90±0.17 mm against MR*S. aureus* while a mixture of (Cm-1+Or-2+Cm-1) against *K.pneumoniae*, (Or-2+LC-1) against *K.pneumonia*, *C. freudii* and *S. typhii* had an inhibition zone of 18.03±0.25 mm. The other mixtures had an inhibition zones of between 8-16 mm against the selected bacteria. However, a mixture of (Or-2+Cm-1) and Cm-1 and LC-1 had no inhibitory effect over *P. aeruginosa, E. faecalis, E.coli* and *E. faecalis*, E. *E. coli, K. pneumoniae* and *S. typhii* respectively.

The results show that a mixture of two or three compounds exerted good antibacterial activity against all selected bacterial pathogens than single compounds. The results also show that the greatest synergism was obtained in the mixture of all the three compounds

(Or-2 +Cm-1 +LC-1). This was followed by mixture of Or-2+LC-1 and lowest in mixture between (Cm-1+Or-2) (Table 4.3).

Table 4. 13 :Mean inhibition zone diameters in millimeters showing synergistic effects of most active compounds against selected bacteria

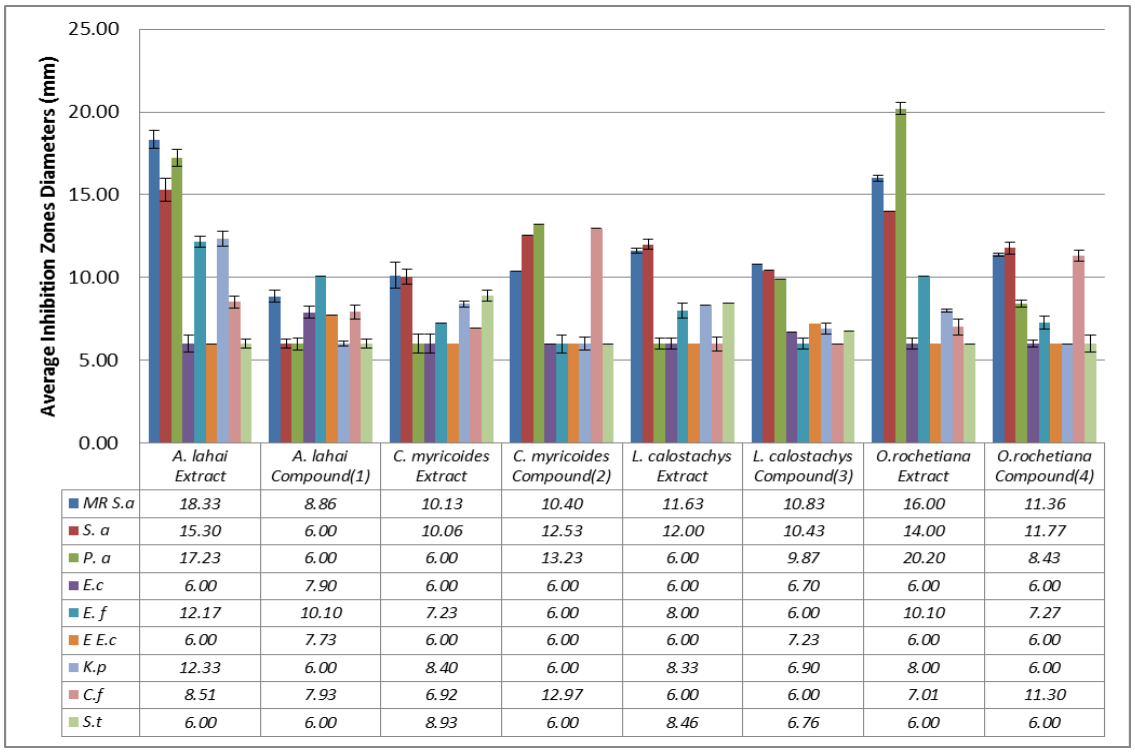
S. No.	Compounds	Mean inhibition Zone diameters in millimeter for each test bacteria								
		MR*S. a*	*S. a*	*P. a*	*E. c*	E. *f*	E. *E.c*	*K. p*	*C. f*	*S.t*
1	Or-2**(3)**+ Cm-1**(2)**	12.20 ±0.30	10.27 ±0.42	6.00± 0.00	6.00± 0.00	6.00± 0.00	10.30 ±0.62	8.17± 0.41	10.77 ±0.85	8.36± 0.61
2	Or-2**(5)**+LC-1**(3)**	20.03 ±0.25	22.23 ±0.31	21.06 ±0.38	19.70 ±0.26	19.77 ±0.31	15.80 ±0.30	17.93 ±0.32	17.86 ±0.35	18.10 ±0.36
3	Cm1**(2)**+ LC-1**(3)**	9.80± 0.26	13.00 ±0.26	13.13 ±0.35	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	13.20 ±0.30	6.00± 0.00
4	Cm-1**(2)**+Or-2**(5)**+LC-1**(3)**	18.80 ±0.30	22.13 ±0.35	20.03 ±0.25	20.27 ±0.25	20.20 ±0.46	20.17 ±0.35	18.03 ±0.25	19.90 ±0.17	20.9± 0.10
	+ve control	21.20 ±0.53	23.17 ±0.21	23.03 ±0.25	21.13 ±0.25	21.23 ±0.40	22.17 ±0.35	18.90 ±0.17	21.13 ±0.25	22.96 ±0.38
	-ve control	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00	6.00± 0.00

Values are means of test replicates ±standard deviation (*n* =3).

Key:-ve-negative control (DMSO- Dimethyl sulphoxide), +ve control (Gentamicin), MR*S. a*-Methicillin resistant *Staphylococcus aureus, S. a- Staphylococcus aureus, P. a- Pseudomonas aeruginosa, E. f- Enterococcus faecalis, K. p-Klebsiella pneumonia, E.c-Escherichia coli,* E. *E. c*-ESBL *Escherichia coli, S.t-Salmonella typhi* and *C. f-Citrobacter freudii*

Key; Compounds are labeled on plates as 1- for Or-2, 2- for LC-1 ,3-for Cm-1,(+) means mixture of compounds.

Isolated compounds and exhaustive plant extracts were compared and the results showed that plant extracts had higher activities against most bacteria than isolated compounds (Figure 4.8). For instance, compound Or-2 **(5)**, cm-1 **(2)** and Al.-1 **(1)** showed an inhibition zone of 11.30±0.37, 12.97±0.53 and 7.93±0.37 against *C. freudii* which was higher than that shown by extracts (Fig 4.8). In addition, all the extracts did not show activity against Esbl. *E. coli* but compound LC-1**(3)** and Al-1(**5**) had inhibition zones of 7.23±0.34 and 7.73±0.34 both being fatty acids. Compound Cm-1 had a good activity against *S. aures* and *P. aeruginosa.* which was higher than exhaustive methanol extract. This can be based on synergism of compounds present in exhaustive methanol extracts. Compound LC-2(**4**) was not included in Figure 4.8 below because *L.calostachys* yieded two compounds, compound LC-1(**3**) was therefore used for comparison.



	A. lahai Extract	A. lahai Compound(1)	C. myricoides Extract	C. myricoides Compound(2)	L. calostachys Extract	L. calostachys Compound(3)	O.rochetiana Extract	O.rochetiana Compound(4)
MR S.a	18.33	8.86	10.13	10.40	11.63	10.83	16.00	11.36
S. a	15.30	6.00	10.06	12.53	12.00	10.43	14.00	11.77
P. a	17.23	6.00	6.00	13.23	6.00	9.87	20.20	8.43
E.c	6.00	7.90	6.00	6.00	6.00	6.70	6.00	6.00
E. f	12.17	10.10	7.23	6.00	8.00	6.00	10.10	7.27
E E.c	6.00	7.73	6.00	6.00	6.00	7.23	6.00	6.00
K.p	12.33	6.00	8.40	6.00	8.33	6.90	8.00	6.00
C.f	8.51	7.93	6.92	12.97	6.00	6.00	7.01	11.30
S.t	6.00	6.00	8.93	6.00	8.46	6.76	6.00	6.00

Figure 4. 8: A graph showing comparative antibacterial activity of the plant extracts and isolated compounds

4.5 Characterization of compounds isolated from selected medicinal plants

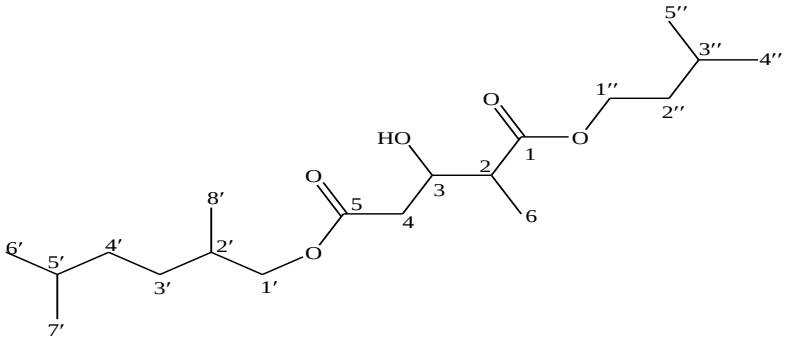
Five compounds namely; [5-(2, 5-dimethylhexyl) 1-isopentyl 3-hydroxy-2-methylpentanedioate] AL-1 (1), [*Cis* 4″-*O*-acetyl martynoside] Cm-1(2), *Cis* Oleic acid, 2 [*β*-amyrin tetradecanoate] LC-2 (4) and [4-*β*-D-glucopyranosylcaffeic acid] Or-2 (5) were isolated from four selected medicinal plant extracts using column chromatography technigues. The structures of these isolated compounds were identified by interpretation of NMR spectroscopic data as discussed below;

4.5.1 Compound isolated from *Acacia lahai*

One compound was isolated from the most active fractions of ethyl acetate extracts (EAE) of *A. lahai* extracts.

4.5.1.1 [5-(2, 5-dimethylhexyl) 1-isopentyl 3-hydroxy-2-methylpentanedioate] AL-1 (1)

The ^{1}H NMR, ^{13}C NMR, ^{1}H ^{13}C HSQC, ^{1}H- ^{1}H COSY and ^{1}H ^{13}C HMBC data is summarized, (Table 4.14). The ^{1}H ^{13}C HSQC, ^{1}H- ^{1}H COSY and ^{1}H ^{13}C HMBC spectra are in Appendixes 23-25.



[5-(2, 5-dimethylhexyl) 1-isopentyl 3-hydroxy-2-methylpentanedioate] AL-1 (1)

Table 4. 14: ^{I}H-NMR (400MHz), ^{13}C-NMR (400 MHz) , ^{I}H-^{I}H COSY and ^{I}H-^{13}C HMBC data of Compound 1 in CD_3OD

Position	Observed H δ(ppm) (*J* in Hz)	C δ(ppm)	COSY	HMBC
1	-	174.9	-	-
2	1.96 (*m*)	37.8	-	-
3	3.53 (*d*, 3.6)	60.8	H/C-4	C-2, C-5, C-6
4	1.62 (*m*) 1.78 (*m*)	41.8	H/C-3	C-3, C-5
5	-	173.4	-	-
6	1.04 (*d*, 2.4)	15.7	-	C-2
1′	3.45 (*d*, 4.4)	61.7	H/C-2′	C-2′, C-8′, C-5
2′	2.28 (*m*)	30.9	H/C-1′	-
3′	1.28 (*m*)	26.2	H/C-4′	C-2′
4′	1.62 (*m*)	30.9	H/C-3′	-
5′	1.28(*m*)	25.9	-	C-4′
6′	0.97 (*s*)	12.4	-	-
7′	1.02 (*d*, 2.0)	17.9	-	-
8′	1.06 (*d*, 7.2)	19.2	-	C-2′
1″	3.59 (*dd*, 5.2, 6.4)	54.6	H/C-2″	C-1, C-2″, C-3″,
2″	1.62(*m*) 1.78 (*m*)	41.8	H/C-1″	C-1, C-1″
3″	1.78 (*m*)	26.2	-	H/C-1″
4″	0.99 (*d*, 1.2)	22.2	-	-
5″	1.00 (*d*, 3.2)	23.4	-	-

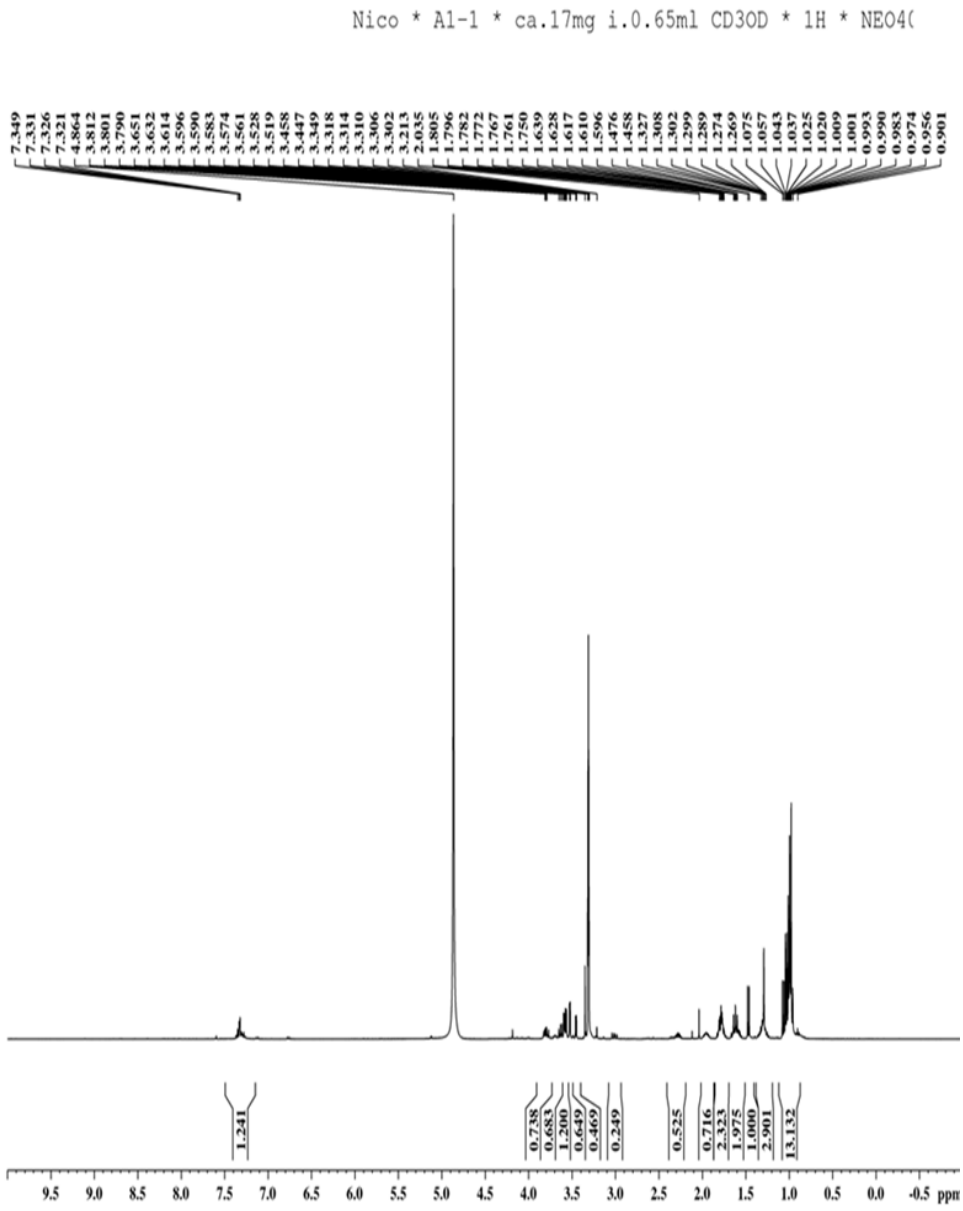


Figure 4. 9:[1]H-NMR spectrum for compound [5-(2, 5-dimethylhexyl) 1-isopentyl 3-hydroxy-2-methylpentanedioate] AL-1 (1)

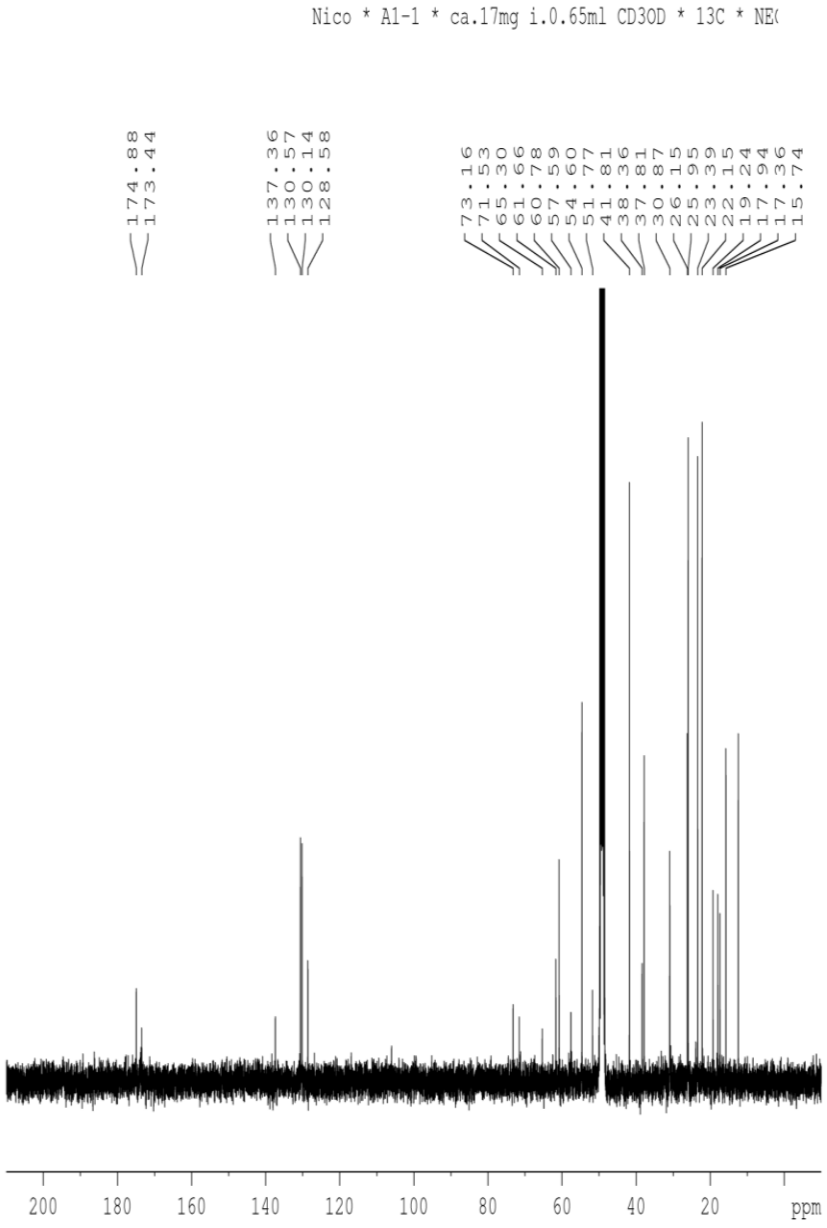


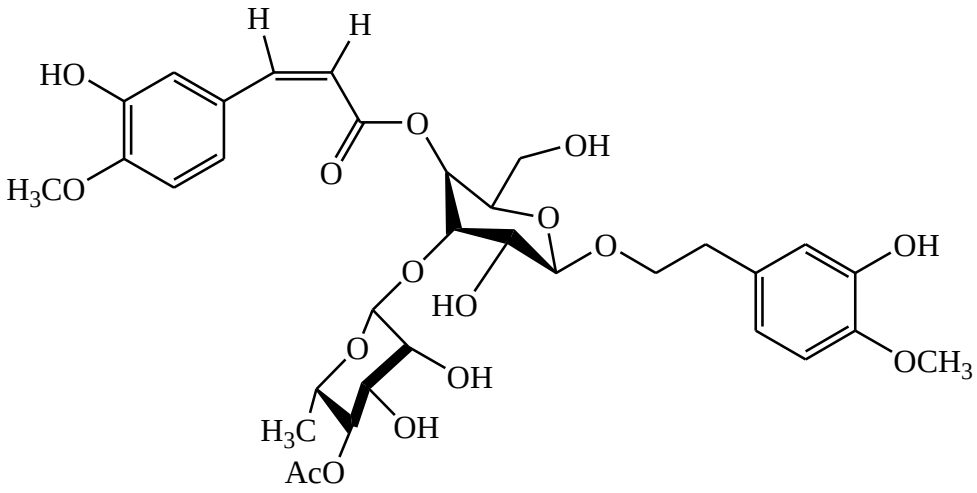
Figure 4. 10: ^{13}C-NMR spectrum for compound 5-(2, 5-dimethylhexyl) 1-isopentyl 3-hydroxy-2-methylpentanedioate AL-1 (1)

4.5.2 Compound isolated from *Cleodendrum myricoides*

One compound [*Cis* 4″-O-acetylmartynoside] Cm-1 (**2**) was isolated from the most active fractions of ethyl acetate extracts (EAE) of *C. myricoides.*

4.5. 2.1 [*Cis* 4″-*O*-acetyl martynoside] Cm-1(2)

The ^{1}H NMR, ^{13}C NMR, ^{1}H ^{13}C HSQC, ^{1}H-^{1}H COSY and ^{1}H ^{13}C HMBC data is summarized, (Table 4.16). The ^{1}H ^{13}C HSQC, ^{1}H- ^{1}H COSY and ^{1}H ^{13}C HMBC spectra are in Appendixes 26-29.



[*Cis* 4″-*O*-acetyl martynoside] Cm-1(2)

Table 4. 15: ^{I}H- NMR ,^{13}C- NMR (400MHz) spectral data in CD_3OD of [*Cis* 4′′-*O*-acetyl martynoside] Cm-1(2) in comparisson with ^{I}H-NMR and ^{13}C- NMR (400MHz) in CD_3OD for 4′′-*O*-acetylmartynoside (Funda *et al*., 2003)

C. No.	H δ (ppm) Observed	C δ (ppm) Observed	C δ (ppm) Reported	H δ (ppm) Reported
Ag. 1	-	131.5	132.9	-
2	7.45(*s*)	106.9	112.8	6.74 (*d*, 2.0)
3	-	150.4	147.6	-
4	-	151.9	147.4	-
5	7.45 (*m*)	107.2	117.1	6.81 (*d*, 8.1, 2.0)
6	8.53 (*d*, 9.6)	122.0	121.2	6.68 (*dd*, 8.2, 2.1)
A	1.25(*m*)	30.9	36.0	2.83 (*d*, 7.3)
B	3.67(*m*)	74.2	72.4	4.05 (*d*, 3.75)
OMe	4.16(s)	56.9	56.5	3.82 (s)
Gl. 1′	6.24(*d*, 9.6)	104.4	104.2	4.28 (*d*, 7.9)
2′	3.89 (*dd*, 9.6, 7.2*)*	80.5	75.6	3.48 (*dd* ,7.9, 9.8)
3′	4.10 (*d*, 7.2*)*	79.8	82.1	3.83 (*d*,9.1)
4′	3.85-3.96 [(a)]	63.9	70.1	4.93 (*d*, 9.0)
5′	3.68 [(a)]	78.2	76.0	3.6-3.4[(a)]
6′	3.89(*d*, 3.54)	63.9	62.4	3.64[(a)]/3.55[(a)]
Rh.1″	6.24 (*d*, 2.0)	104.3	103.0	5.18 *d (*1.6)
2″	3.59 (*m*)	72.9	72.1	3.90[(a)]
3″	3.85-3.96 [(a)]	72.9	72.0	3.65-3.60[(a)]
4″	3.67[(a)]	78.4	75.6	4.83 (*t*, 9.5)
5″	3.67[(a)]	74.2	70.5	3.55-3.50[(a)]
6″	1.25 (*d*, 6.4)	25.6	18.5	1.12 (*d*, 6.21)
OAc	1.81 (*s*)	20.9	172.7	2.07(*s*)
	-	172.3	21.1	-
Ac.1‴	-	127.2	127.6	-
2 ‴	8.53 (*d*, 7.2)	119.6	111.7	7.20 *br.s*
3 ‴	-	151.1	149.4	-
4 ‴	-	147.2	150.9	-
5 ‴	6.24 (*d*, 9.6)	112.7	116.5	6.82 (*d*, 8.2)
6 ‴	8.65 (*d*, 9.6)	120.0	124.4	7.82 (*d*, 8.2)
α‴	6.24 (*d*, 9.6)	112.8	115.1	6.36 (*d*, 15.9)
β ‴	8.00 (*d*, 2.4)	141.0	148.0	7.68 (*d*, 15.9)
C=0	-	163.9	168.3	-
OMe	3.89 (*s*)	56. 1	56.4	3.89s

[(a)]signal pattern unclear due to overlapping

Key: Ag-Aglycone, Ac- Acyl, Rh- Rhamnose, Gl- Glucose

Table 4. 16: ^{I}H NMR (400MHz), ^{13}C-NMR, ^{I}H-^{1}H COSY and ^{I}H-^{13}C HMBC (400MHz) spectral data in CD_3OD for [*Cis* 4′′-*O*-acetyl martynoside] Cm-1(2)

C. No.	H δ (ppm) Observed	C δ (ppm) Observed	COSY Observed	HMBC Observed
Ag. 1	-	131.5		-
2	7.45(*s*)	106.9		C-1, C-3, C-4
3	-	150.4		-
4	-	151.9		-
5	7.44 (*s*)	107.2	H/C-3	C-3, C-4, C-6
6	8.53(*d*, 9.6)	122.0		-
A	1.25(*m*)	30.9	H/C- *β*	C- *β*
β	3.67	74.2	H/C- α	-
OMe	4.16(s)	56.9		C-4
Gl.1′	6.24(*d*, 9.6)	104.4		C-1′′
2′	3.89 (*dd*,*9.6*,7.2)	80.5		C-5′′,C-6′′
3′	4.10 (*d*,7.2)	79.8		-
4′	3.85-3.96 [(a)]	63.9		-
5′	3.68 [(a)]	78.2		-
6′	3.89(*d*,3.54)	63.9		-
Rh.1′′	6.24 (*d*,2.0)	104.3		-
2′′	3.59 (*m*)	72.9		-
3′′	3.85-3.96 [(a)]	72.9		-
4′′	3.67[(a)]	78.4		-
5′′	3.67[(a)]	74.2		-
6′′	1.25 (*d*, 6.4)	25.6		C-3′′,C-4′′,C-5′′
OAc	1.81 (*s*)	20.9		-
Ac1′′′	-	127.2		-
2′′′	8.53 (*d*, 7.2)	119.6		C-6′′′
3′′′	-	151.1		-
4′′′	-	147.2		-
5′′′	6.24 (*d*, 9.6)	112.7	H/C-6′′′	C-3′′′, C-4′′′,C- α′′′
6′′′	8.65 (*d*, 9.6)	120.0	H/C-5′′′	C-4′′′
α′′′	6.24 (*d*, 9.6)	112.8	H/C- *β*′′′	C-1′
β′′′	8.10 (*d*, 2.4)	141.0	H/C- α′′′	C-1′′′
-COOH	-	163.9		-
OMe	3.89(s)	56.1		C-4′′′

[(a)]signal pattern not clear due to overlapping

Key: Ag-Aglycone, Ac- Acyl, Rh- Rhamnose, Gl- Glucose

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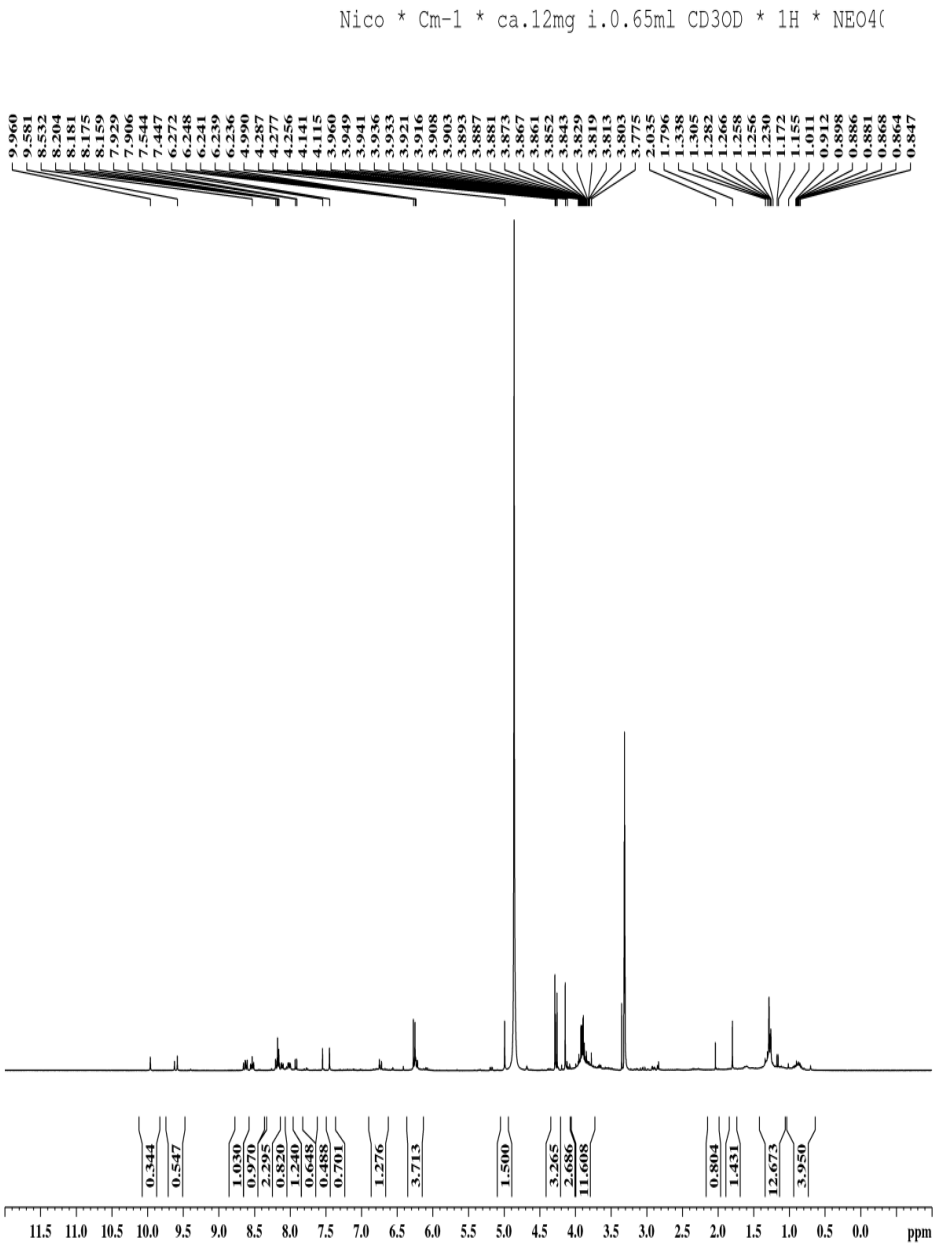


Figure 4. 11:^{1}H-NMR spectrum for compound [*Cis* 4′′-*O*-acetyl martynoside] Cm-1(2)

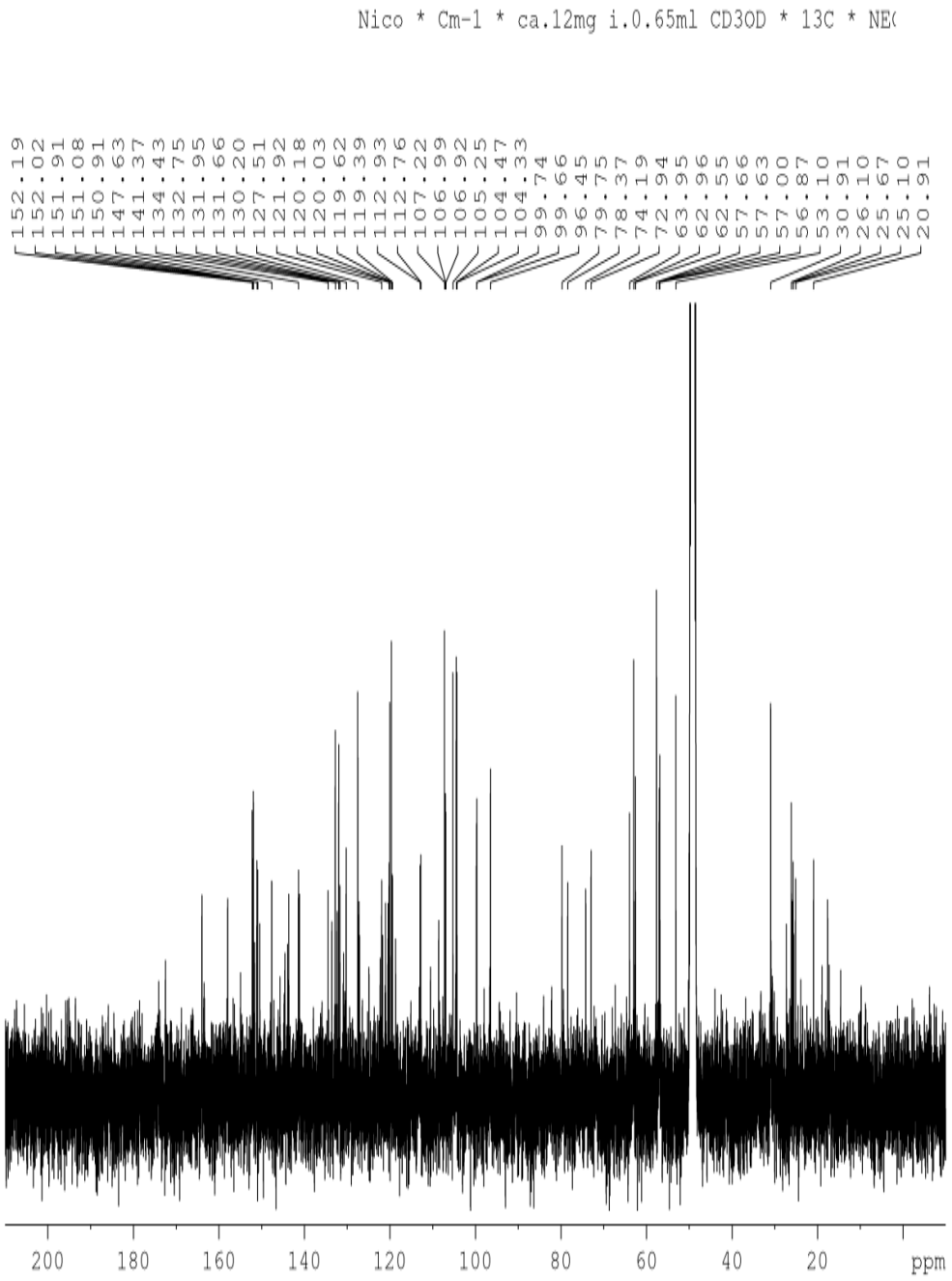


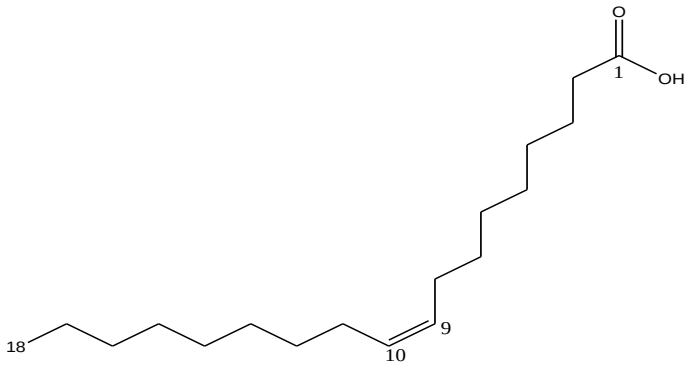
Figure 4. 12: ^{13}C-NMR spectrum for compound [*Cis* 4′′-*O*-acetyl martynoside] Cm-1(2)

4.5.3 Compounds isolated from *Leucas calostachys*

The following two compounds were isolated from the most active fraction of hexane (HE) extracts of *L. calostachys* during purification of an active fraction.

4. 5. 3.1 [*Cis* oleic ac] LC-1(3)

Compound [*Cis* oleic acid] LC-1**(3)** or **(***Cis*-9-decenoic acid**) (3)** was isolated as a white oily solid soluble in hexane. The ^{1}H NMR, ^{13}C NMR, ^{1}H ^{13}C HSQC, ^{1}H- ^{1}H COSY and ^{1}H ^{13}C HMBC data is summarized, (Table 4.18). The ^{1}H ^{13}C HSQC, ^{1}H- ^{1}H COSY and ^{1}H ^{13}C HMBC spectra are in Appendix 29-31.



[*Cis* **Oleic acid] LC-1 (3)**

Table 4. 17: ^{I}H-NMR (600MHz), ^{13}C-NMR (600MHz, $CDCl_3$) spectral data of [*Cis* Oleic acid] LC-1(3) in $CDCl_3$ in comparison with ^{I}H-NMR (100MHz), ^{13}C-NMR (399MHz, $CDCl_3$) for *Cis* Oleic acid (Wekesa, 2014)

Posotion	^{13}C –NMR Observed	^{1}H-NMR (*J* values in Hz) Observed	^{13}C-NMR Reported	^{1}H-NMR (J values in Hz) Reported
1	180.3	-	179.9	-
2	34.3	2.34 (*t*,7.5, 5.5,2.5)	34.0	2.34 (*t*, 7.5, 3.5, 1.8)
3	24.8	1.64(*t*,7.5, 5.5,2.5)	24.7	1.64, (*t*,7.5, 3.5, 1.8)
4	27.3	2.15,(*m)*	27.1	2.01, (*m)*
5	29.3	1.29 (*m*)	29.3	-
6	29.2	1.29 (*m*)	29.2	-
7	29.7	1.26 (*m)*	29.7	1.26, (*t*,7.5, 3.5, 1.8)
8	29.2	1.29 (*m*)	29.2	-
9	130.2	5.35(*d*, 6.0, 3.0)	130.0	5.35, (*m*)
10	129.9	5.34(*d* 6.0, 3.0)	129.7	5.34, (m)
11	29.3	2.01 (*m*)	29.2	-
12	29.7	1.29 (*m*)	29.7	-
13	29.4	1.29 (*m*)	29.4	-
14	29.6	1.26(*m)*	29.6	-
15	29.3	1.29 (*m*)	29.5	-
16	29.2	1.28 (*m)*	31.9	1.29, (*m*)
17	29.9	0.86, (*t*, 6.0, 3.0, 2.0)	29.9	1.28, (*m)*
18	14.3		14.1	0.86 (*t*,6.6,3.3,1.5)

Table 4. 18: ^{1}H NMR (600MHz), ^{13}C NMR (600MHz, $CDCl_3$) , ^{1}H- ^{1}H COSY and ^{1}H- ^{1}H ^{13}C HMBC data of [*Cis* Oleic acid] LC-1(3) in $CDCl_3$

Posotion	^{13}C–NMR	^{1}H-NMR (*J* values in Hz)	COSY	HMBC
1	180.3	-	-	-
2	34.3	2.34 (*t*,7.5, 5.5, 2.5)	H/C-3	C-1, C-3
3	24.8	1.64 (*t*,7.5, 5.5,2.5)	H/C-2	C-1, C-3, C-4
4	27.3	2.15(*m)*	-	C-2, C-5
5	29.3	1.29 (*m*)	-	-
6	29.2	1.29 (*m*)	-	-
7	29.7	1.26 (*m)*	-	-
8	29.2	1.29 (*m*)	H/C-11	-
9	130.2	5.35(*d* 6.0,3.0)	H/C-9	-
10	129.9	5.34 (*d* 6.0,3.0)	H/C-9, H/C-10	C-7, C-8
11	29.3	2.01 (*m*)	H/C-12	C-9, C-11, C-12
12	29.7	1.29 (*m*)		C-10, C-13
13	29.4	1.29 (*m*)	-	-
14	29.6	1.29 (*m*)	-	-
15	29.3	1.26 (*m)*	-	-
16	29.2	1.29 (*m*)	H/C-17	C-14
17	29.9	1.28 (*m)*	H/C-16	-
18	14.3	0.86 (*t*, 6.0,3.0,1.8)	H/C-17	C-17

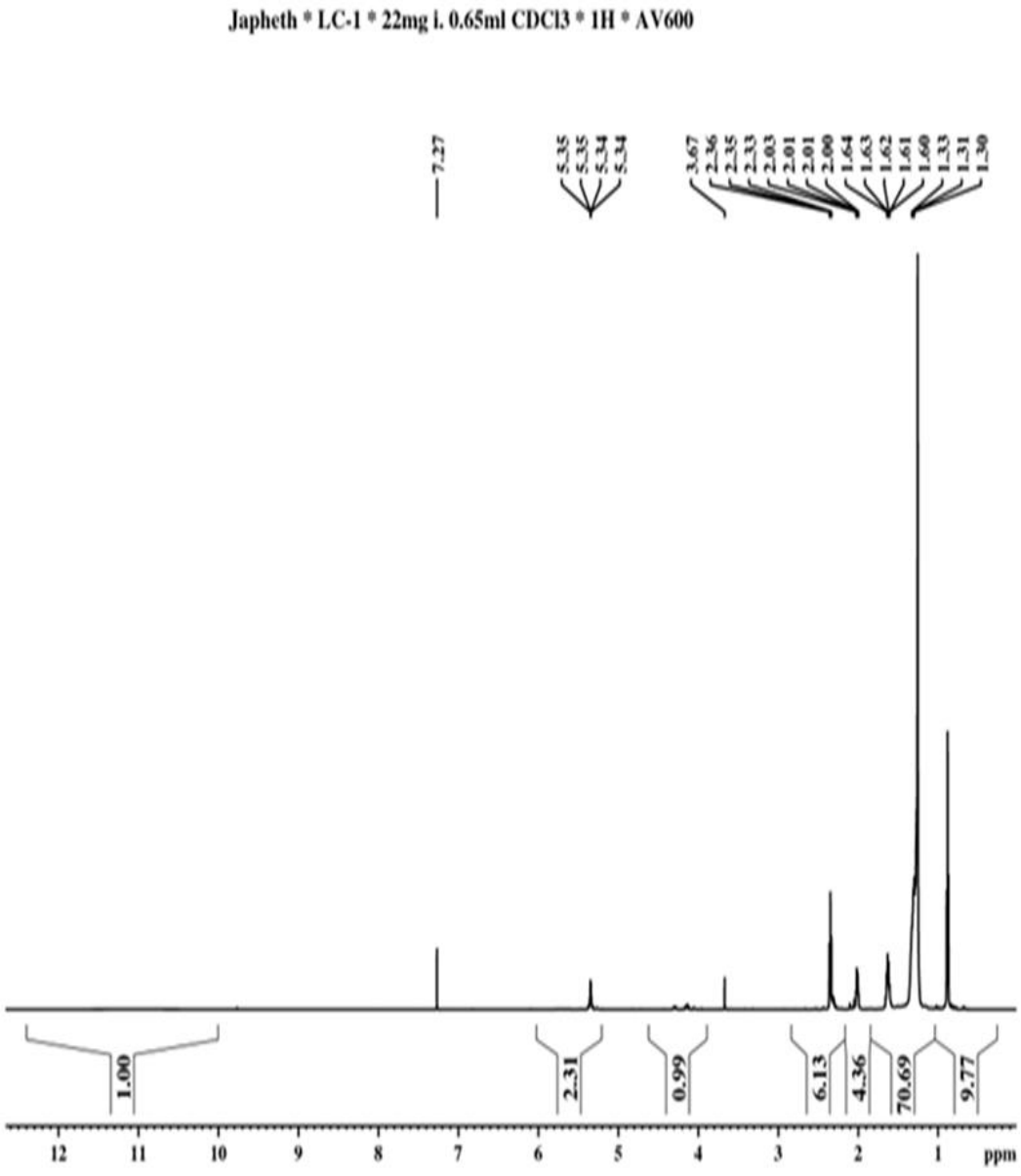


Figure 4.13: ^{1}H-NMR spectrum of compound [*Cis* oleic acid] LC-1(3)

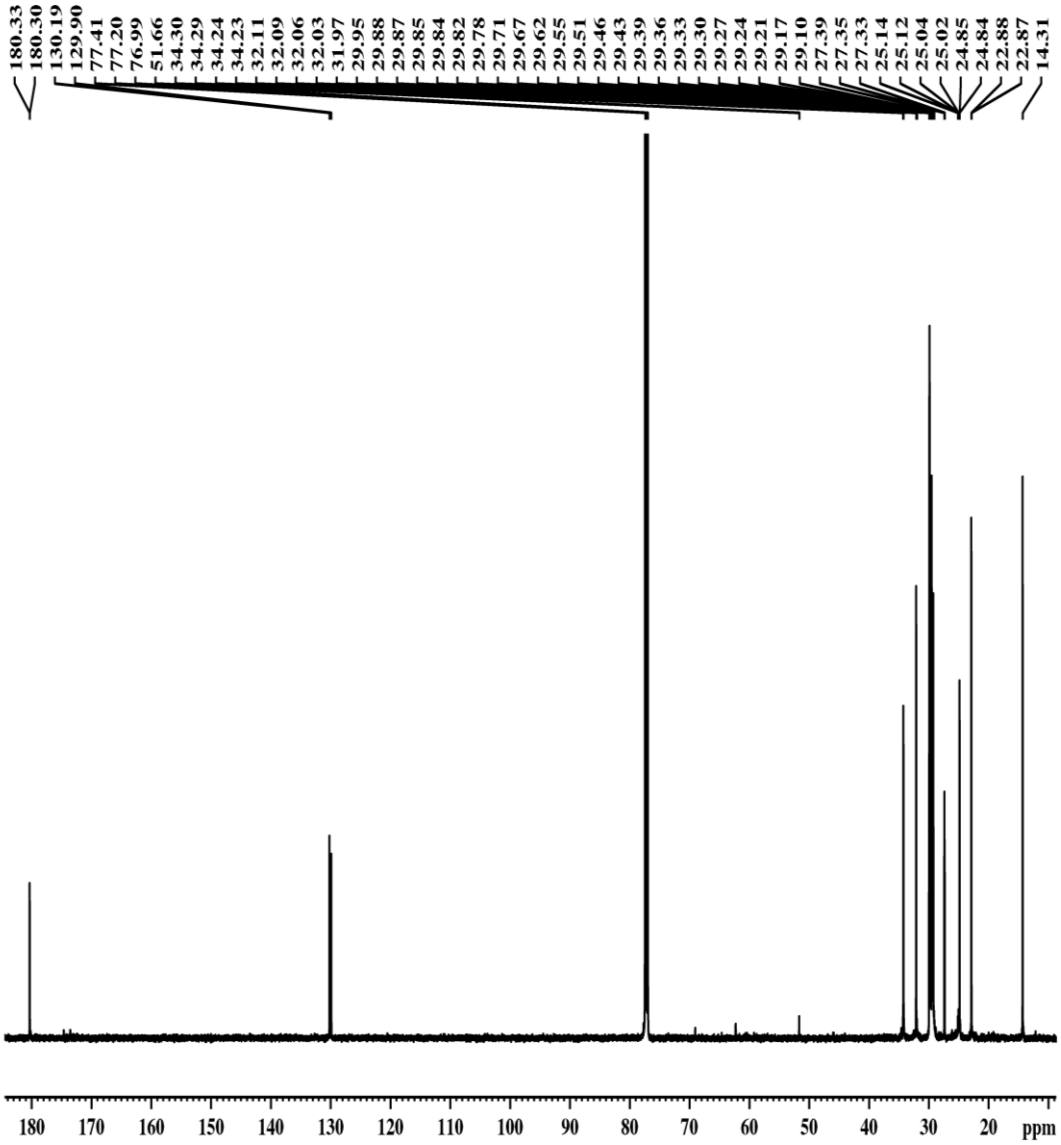
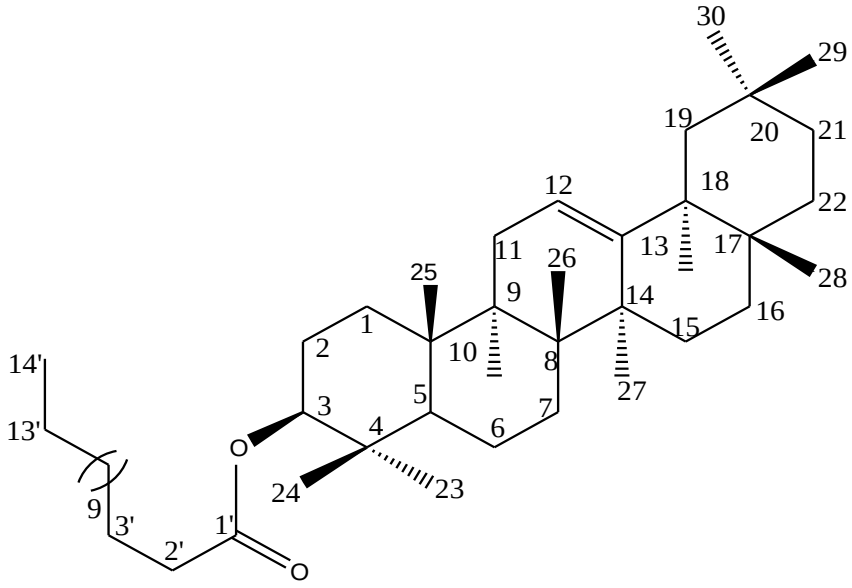


Figure 4. 144:^{13}C-NMR spectrum for [*Cis* oleic acid] LC-1 (3)

4.5.3.2 [*β*-amyrin tetradecanoate] LC-2 (4)

This compound was isolated as a white solid soluble in ethyl acetate (EAE). The ^{1}H-NMR,^{13}C-NMR, ^{1}H ^{13}C HSQC , ^{1}H-^{1}H COSY and ^{1}H ^{13}C HMBC NMR data is summarized in Table 4.20. The ^{1}H, ^{13}C HSQC, ^{1}H-^{1}H COSY and ^{1}H ^{13}C HMBC spectra are in Appendix 32-34.



[*β*-amyrin tetradecanoate] LC-2(4)

Table 4.19: [*β*-amyrin tetradecanoate] LC-2(4) isolated in comparison with spectral data for *β*-amyrin octacosanoate (Machumi, 2011) both in CD_2Cl_2), (400 MHz).

C/No.	^{13}C NMR (Observed) δ_C	^{1}H NMR (*J* in Hz) (Observed) δ_H	^{13}C-NMR δ_C Reported	^{1}H-NMR δ_C Reported (*J* in Hz)
1	38.1	1.63, (*m*),1.08 (*m*)	38.6	1.61, (*m*),1.07 (*m*)
2	21.4	1.62(*m*),1.90 (*m,*)	23.9	1.61(*m*),1.90 (*m,*)
3	81.1	4.45 (*dd*, 8.0,4.0)	80.7	4.46 (*dd*, 10.0,5.8)
4	38.0	-	38.1	-
5	55.8	0.85(*m)*	55.5	0.88(*m)*
6	18.5	1.45 (*m*),1.54 (*m)*	18.6	1.53 (*m*),1.43 (*td* 13.8,3.8,)
7	31.5	1.33(*m*),1.45(*m*)	32.9	1.34(*m*),1.55(*m*)
8	40.2	-	40.2	-
9	47.9	1.61(*m*)	47.9	1.61(*m*)
10	37.2	-	37.2	-
11	24.1	1.88 (*m*),1.61 (*m*)	24.0	1.87 (*m*),1.60 (*m*)
12	122.1	5.19 (*t* ,4.0)	122.1	5.19 (*t* ,3.7)
13	145.3	-	145.5	-
14	42.1	-	42.1	-
15	26.1	1.78 (*m*), 0.95 (*m*)	26.1	1.77 (*td*,13.6,4.6), 0.97 (*m*)
16	27.3	2.02 (*m*) 0.79 (*m*)	27.3	2.01 (*td*,13.6,4.6), 0.80 (*m*)
17	32.8	-	32.8	-
18	47.6	1.95(*dd* , 8.0 ,4.0)	47.6	1.95(*dd* , 13.6,4.6,2.4)
19	47.1	1.69 (*m*),1.02(*m*)	47.1	1.68 (*t*,13.6),1.0 (*ddd*.13.6,4.6,2.4)
20	31.3	-	31.4	
21	35.1	1.35 (*m*),1.10(*m*)	35.1	1.43 (*dd*,13.6,3.9),1.22 (*dd*,13.6,3.2)
22	37.5	1.38 (*m*),1.34 (*m*)	37.5	1.38 (*m*),1.34 (*m*)
23	28.1	0.86 (*s*)	28.2	0.86 (*s*)
24	16.6	0.84 (*s*)	16.9	0.86 (*s*)
25	15.7	0.97 (*s*)	15.7	0.97 (*s*)
26	16.9	0.98 (*s*)	17.0	0.98 (*s*)
27	25.4	1.14(*s*)	26.1	1.14(*s*)
28	28.5	0.83 (*s*)	28.6	0.83 (*s*)
29	33.4	0.88(*s*)	33.5	0.87(*s*)
30	21.5	0.87 (*s*)	23.8	0.87 (*s*)
1′	171.0	-	173.7	-
2′	34.9	1.53(*m*)	35.1	2.27(*td*,7.4, 2.2)
3′	23.9	1.55(*m*)	25.5	1.60(*m*)
4′-25′12′	26.5-34.6	1.21-1.42(*m*)	29.5-30.1	1.26(*s*)-1.31
26′/13′	32.6	1.31(*m*)	32.4	1.26(*m*)
27′-	23.8	1.32(*m*)	23.1	1.29(*m*)
28′14′	14.7	0.75, (*t*, 8.0,4.0,2.0)	14.7	0.88, (*t*, 7.2,3.6,1.8)

Table 4.20: ^{1}H- NMR , ^{13}C- NMR, ^{1}H -^{1}H COSY and ^{1}H-^{13}C HMBC(400MHz, CD_2Cl_2) spectral data of [*β*-amyrin tetradecanoate] LC-2 (4)

C/No.	^{13}C NMR	^{1}H NMR (*J* in Hz)	COSY	HMBC
1	38.1	1.63, (*m*),1.08 (*m*)	H/C-2	C-2,C-3
2	23.8	1.62(*m*),1.90 (*m*,)	H/C-1,C-3	
3	81.1	4.45 (*dd*, 8.0,4.0)	H/C-2,H/C-1	C-1',C-2,C-24,C-24
4	38.1			
5	55.8	0.85(*m)*	H/C-6,H/C-1	C-3, C-7,C-24
6	18.5	1.45 (*m*)	H/C-5	C-5
7	31.5	1.33(*m*),1.45(*m*)		C-5
8	40.2			
9	47.9	1.61(*m*)		
10	37.2			
11	24.1	1.88 (*s*),1.61 (*m*)	H/C-12	C-13
12	122.1	5.19 (*d* ,4.0)	H/C-11	
13	145.3			
14	42.1			
15	26.1	1.78 (*m*), 0.95 (*m*)	H/C-17	C-11,C-17
16	27.3	2.02 (*m*) 0.79 (*m*)	H/C-17	C-15
17	32.8	1.55 (*s*)	H/C-16	C-22
18	47.6	1.95(*dd* , 8.0)		
19	47.1	1.69 (*m*),1.02 (*m*)		C-18, C-20
20	31.3			
21	35.1	1.35 (*m*),1.10 (*m*)	H/C-19	
22	37.5	1.38 (*m*),1.34 (*m*)		C-23
23	28.1	0.86 (*s*)		C-22
24	16.6	0.84 (*s*)		C-2,C-7
25	15.7	0.97 (*s*)		C-8
26	16.9	0.98 (*s*)		C-8.C-14
27	26.0	1.14(*s*)		C-8,C-15
28	28.6	0.83 (*s*)		C-22,C-19
29	33.5	0.88(*s*)	H/C-30	C-19,C-22
30	23.8	0.87 (*s*)	H/C-29	C-19,C-22
1′	171.0			
2′	34.9	1.53(*m*)		C-1′
3′	23.9	1.55(*m*)		
4′-12′	26.5-34.6	1.21-1.42(*m*)		
13′	32.6	1.31(*m*)		
14′	14.7	0.75, (*t*, 8.0,4.0, 2.0)		

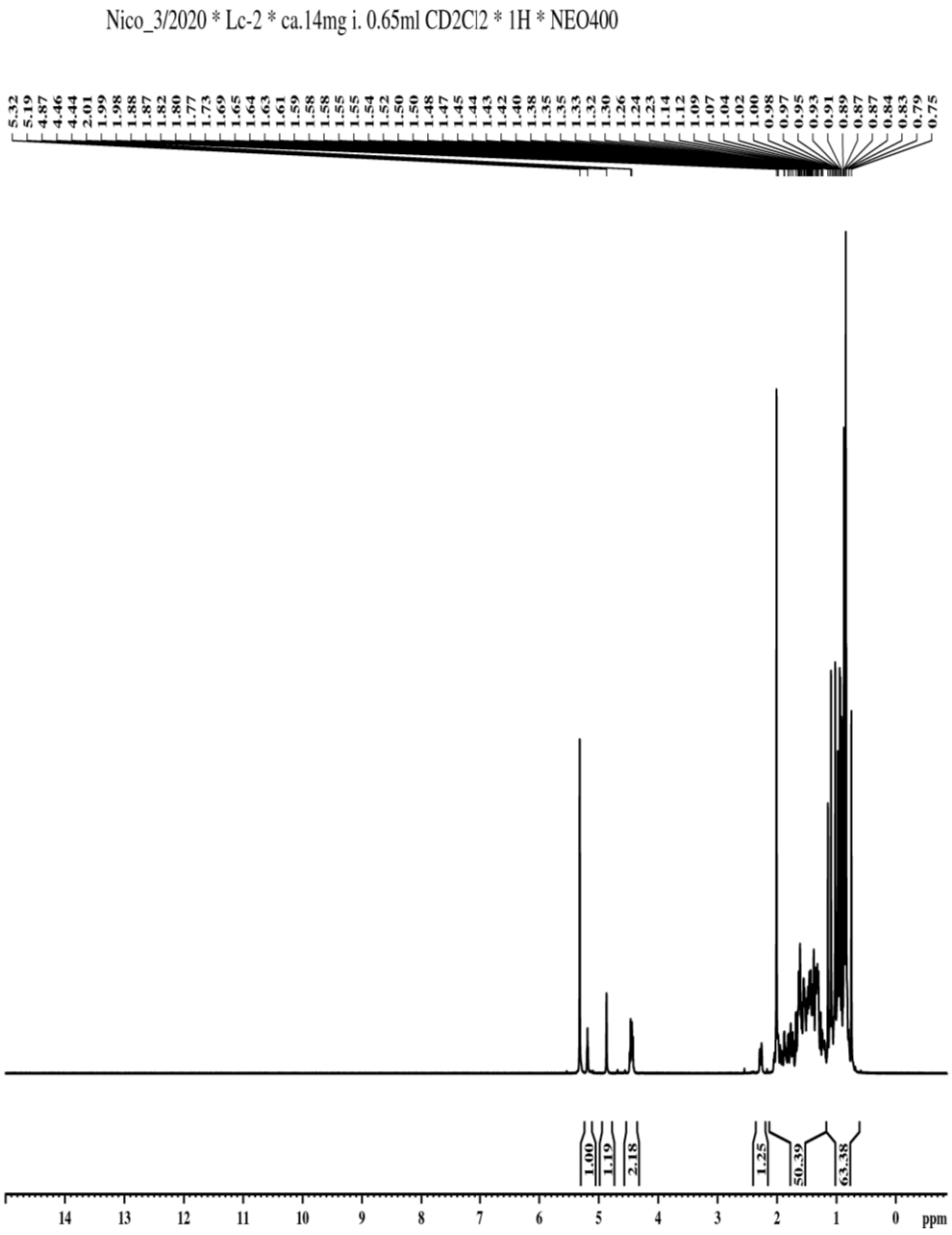


Figure 4.15: ^{1}H-NMR spectrum for [*β*-amyrin tetradecanoate] LC-2(4)

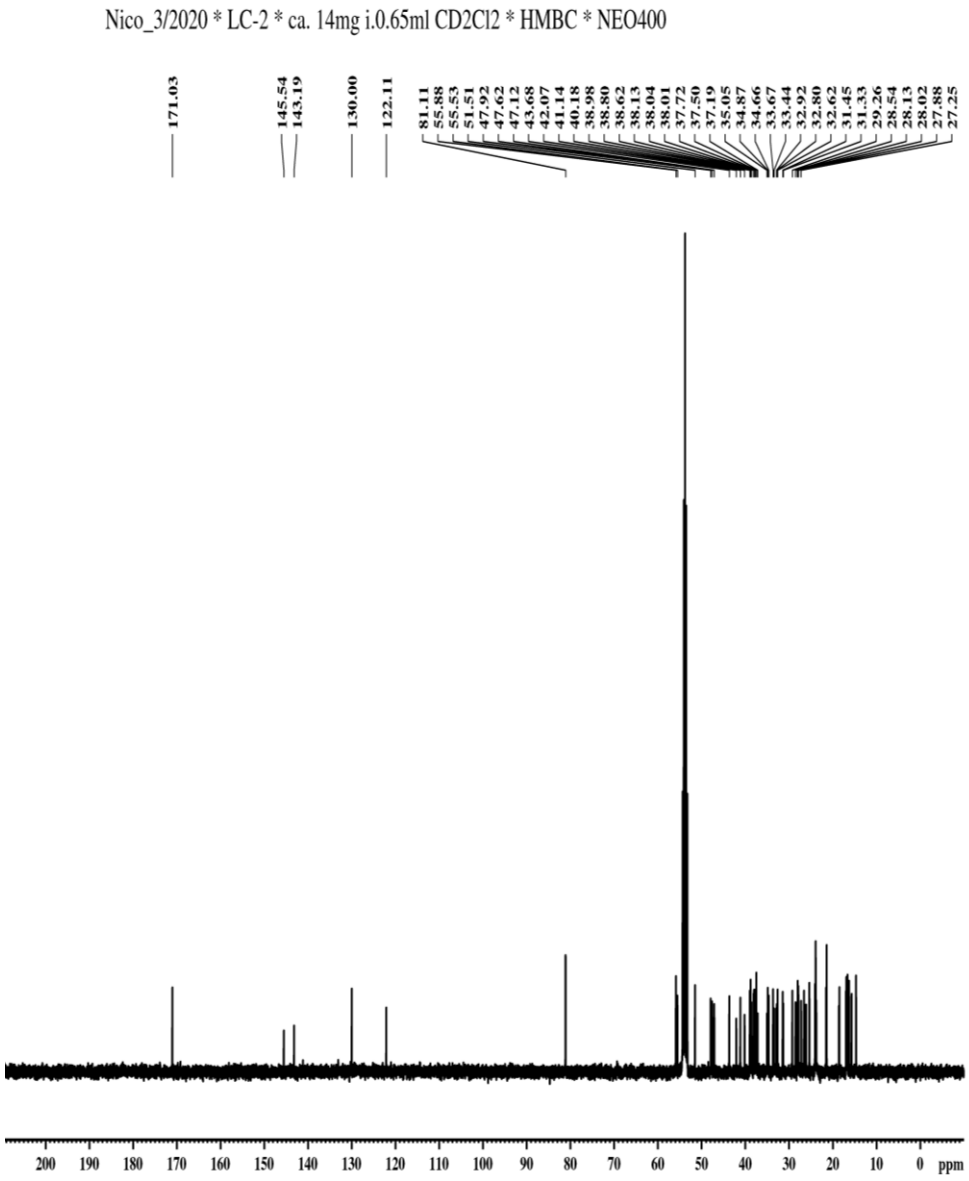


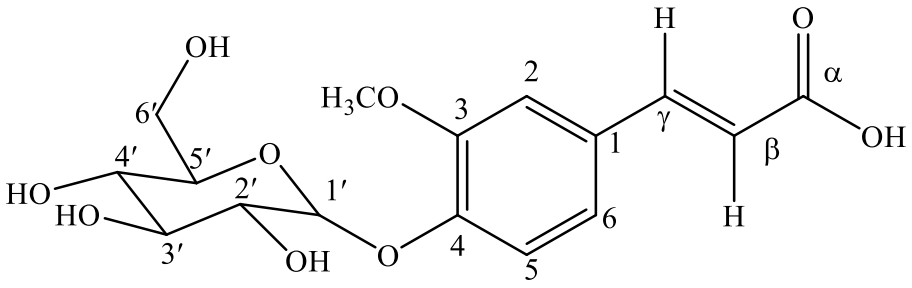
Figure 4.16:^{13}C-NMR spectrum for [*β*-amyrin tetradecanoate] LC-2(4)

4.5.4. Compounds isolated from the stem-bark of *Olinia rochetiana*

The following one compound was isolated from the most active fractions of ethyl acetate (EAE) extracts of *O. rochetiana.*

4.5.4.1: [4-*β*-D-glucopyranosylcaffeic acid] Or-2 (5)

Compound **5** was isolated from the stem-bark of *Olinia rochetiana* as a white solid soluble in acetone. The ^{1}H-NMR,^{13}C–NMR, ^{1}H-^{13}C HSQC , ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC NMR data is summarized in Table 4.21. The ^{1}H-^{13}C HSQC, ^{1}H-^{1}H COSY and ^{1}H ^{13}C HMBC spectra are in the appendix 35-37.



[4-*β*-D-glucopyranosylferrulic acid] Or-2(5)

Table 4.21: ^{I}H NMR (400MHz), ^{13}C NMR , ^{1}H- ^{1}H COSY and ^{I}H- ^{13}C HMBC in (400MHz) DMSO –d_6 of [4-*β*-D-glucopyranosylferrulic acid] Or-2(5) in comparison with ^{I}H NMR and ^{13}C NMR (400MHz) spectral data for 4-*β*-D-glucopyranosyl caffeic acid in DMSO-d_6 (Lee, 2009).

C. No	H δ (ppm) Observed (*J* in Hz)	C δ (ppm) (Observed)	H δ (ppm) Reported (*J* in Hz)	C δ (ppm) (Reported)	COSY (Observed)	HMBC (Observed)
A	-	158.2	-	168.1		-
B	7.82 (*d*, 9.2)	112.5	5.84 (*d*, 12.9)	119.1		*A*
ɤ	7.81 (*m*)	141.7	6.81(*d*,12.9)	141.3		*α, β*
1	-	Ov	-	128.9		-
2	7.52 (*s*)	113.0	7.59 (*d*,1.9)	114.5		C-4,3
3	-	152.4	-	148.4		-
4	-	150.2	-	147.7		-
5	7.80 (*m*)	111.8	7.07(*d*, 8.6)	114.7		C-3, C-4
6	Ov	0v	7.21(*dd*,1.9, 8.6)	124.1		-
1′	5.56 (*brs*)	99.7	4.97(*d*,7.4)	99.9	H/C -2′,4′	C-2′
2′	3.38 (*m*)	71.5	3.15-3.70	77.2	H/C -1′	-
3′	3.42 (*m*)	70.4	3.15-3.70	73.5		C-2′
4′	3.66 (*m*)	70.0	3.15-3.70	69.9	H/C-1′	C-2′
5′	3.48 (*m*)	70.2	3.15-3.70	77.4		-
6′a	3.89 (*brs*)	61.5	3.15-3.70	60.9	H/C -6′b	-
6′b	4.08 (*m*)	-	3.15-3.70	-	H/C -6′a	-
-OCH_3	3.82(*s*)	60.9	3.76	55.8		-
-COOH	10.79(*s*)	-	-	-		

Key: Ov-overlapped

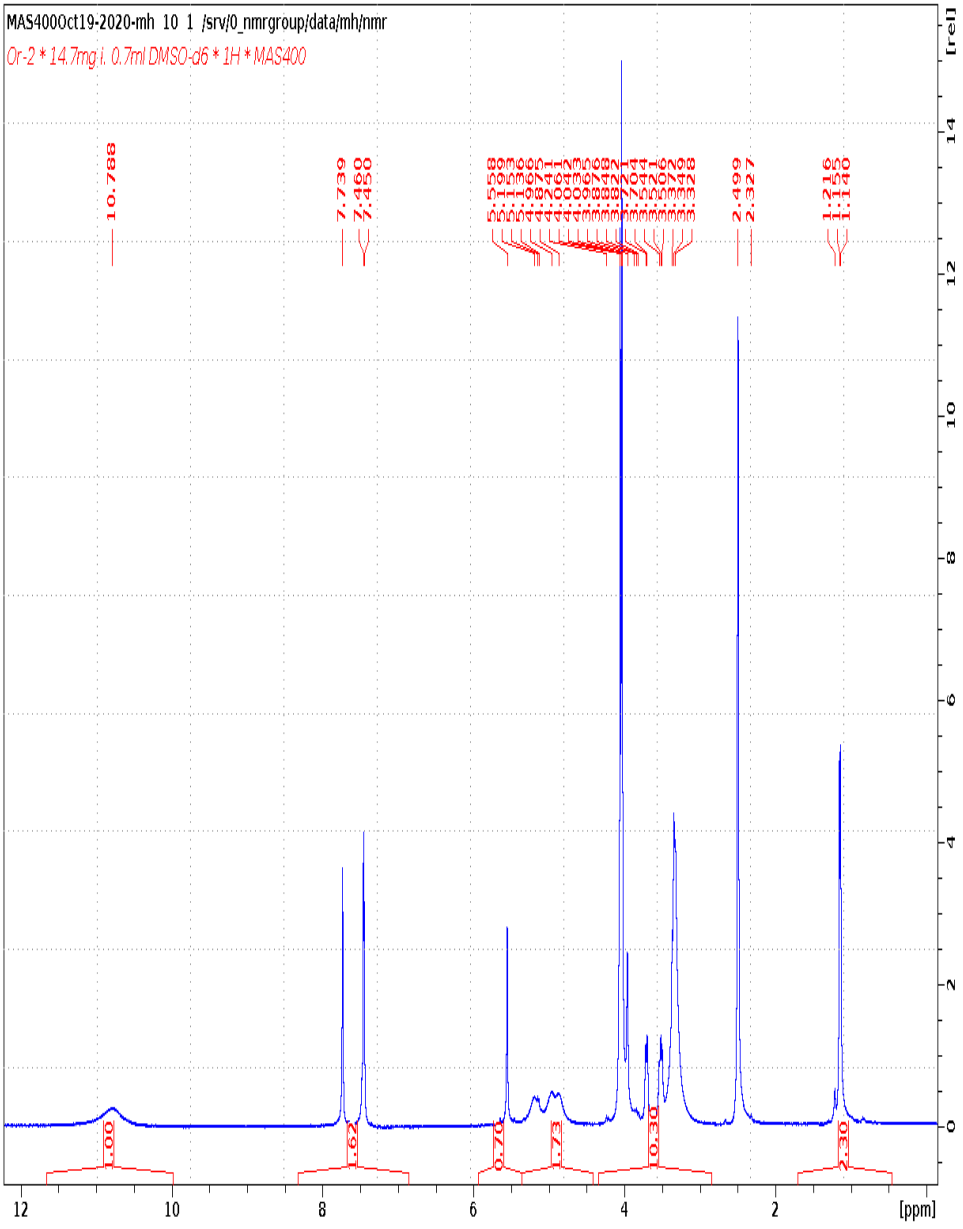


Figure 4.5: ^{1}H-NMR for [4-*β*-D-glucopyranosylferullic acid] Or-2 (5)

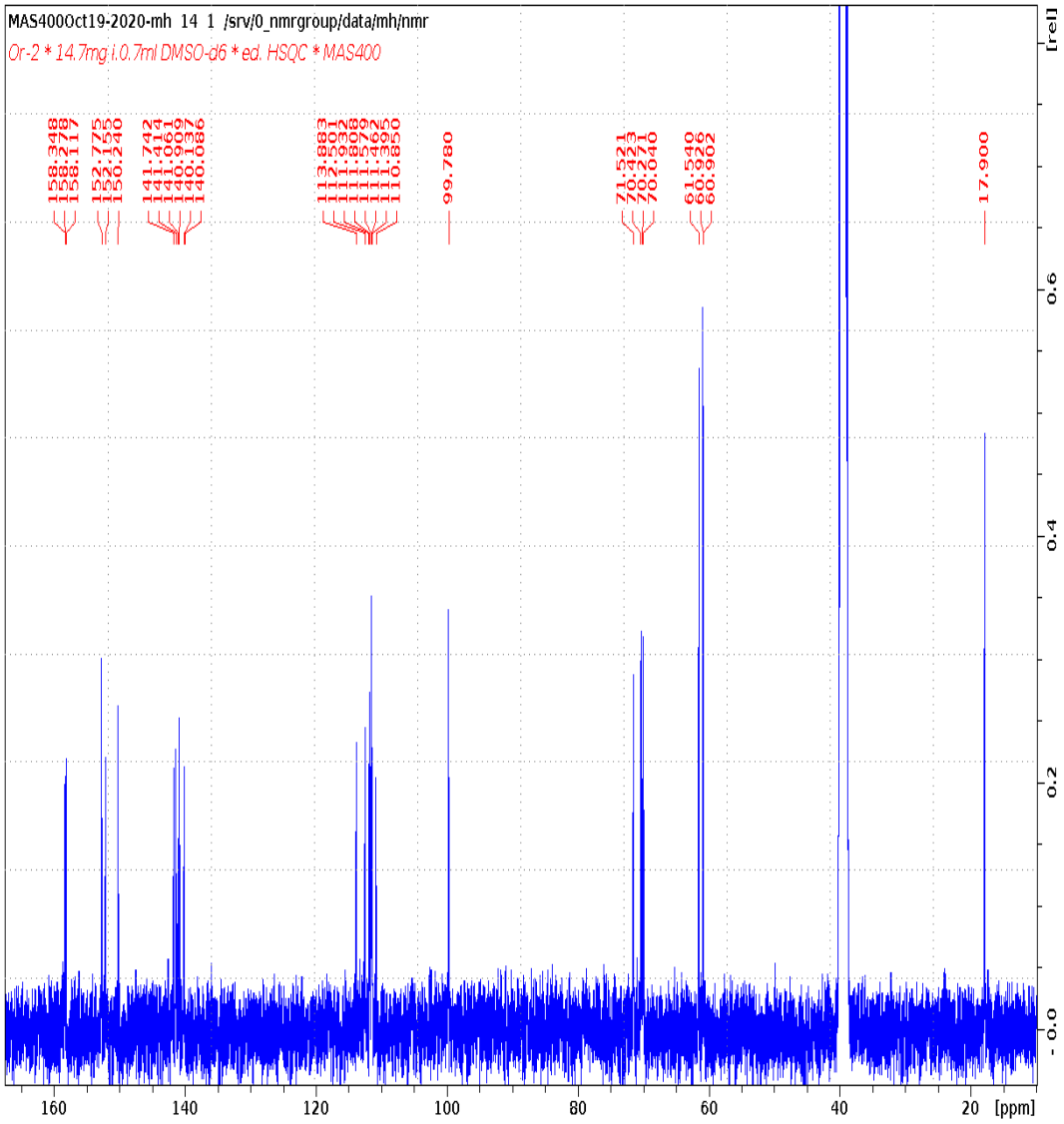


Figure 4.6:^{13}C-NMR for [4-*β*-D-glucopyranosylferullic acid]Or-2 (5)

CHAPTER FIVE

DISCUSSION

5.1 Ethnobotanical information and extraction of selected medicinals from Nandi county

The roots and leaves were frequently used because they have high concentration of exudates stored in them given that its an excretory organ. They are also easy to prepare (Abera *et al.*, 2014). Trees and shrubs are the most frequently used medicinal plant species because they are most abundant compared to other growth forms and people rely more on them (Amuka *et al.*, 2017).

The main methods of preparation were decoction and infusion. However, some plants were prepared using more than one method. Oral administration was the most commonly used method, especially for the internal ailments because injection is one of the modes of administration by traditional practitioners (Tedesse *et al*.,2018). Dosages involved taking 125cm^3 twice a day or thrice a day depending on the efficacy of the drug and the nature of the ailment.

Pneumonia is one of the killer diseases in the county being in the highlands with low temperatures, and Tuberculosis is exacerbated by HIV/AIDS. It is, therefore, necessary to preserve this indigenous knowledge and traditional medicine by proper documentation, identification of plant species, herbal preparation, and dosage. This will assist future studies on the selection of herbal plants to evaluate their phytochemical safety and efficacy.

Exhaustive extraction of plant material were done using methanol. *O.rochetiana* bark had the highest percentage yields while *Plantago palmate* roots had the lowest percentage yield. This suggest that *O.rochetiana* bark had abundant polar compounds than *Plantago palmate* roots, (Table 4.1).

The medicinal plants that had higher antibacterial activities were succesivelly extracted in order of polarity starting from hexane (HE), dichloromethane (DCM), ethyl acetate (EAE) and methanol (ME). On comparing the yields of plant materials on different extraction solvents, the highest yields were observed on methanol extracts while the lowest on hexane extracts. This is due to the high polarity of methanol to extract polar and some of the nonpolar substances compared to other solvents used (Larson *et al*., 2016).

5.2 Antibacterial activities of exhaustive crude extracts, successive extracts and fractions

There was variation in the activity of the exhaustive methanol extracts. This is due to the phytochenmicals contained in the extracted plant materials, (Cushine, 2014). Comparison between plant extracts and gentamicine showed that gentamicine was more active against many bacteria than plant extracts. The plausible reason for this is that, gentamicin is in a pure form while plant extracts are still in crude form and in some cases phytochemicals are antagonistic to each other leading to low activities (Paolo *et al*., 2014).

Gram positive bacteria were more susceptible to plant extracts than gram-negative bacteria. This is because gram positive bacteria have a mesh-like peptidoglycan layer which allow penetration of the extracts (Borges *et al.,* 2015) while gram-negative bacteria have an effective permeability barrier, made of a thin lipopolysaccharide outer membrane, which

restrict the penetration of the plant extracts (Amalya and Sumathy, 2015). Moreover, resistance from gram-negative bacteria against antibiotics like penicillin originates from the secretion of the lactamase enzyme in the periplasmic space between the thin outer membrane and the cytoplasmic membrane (Elisha *et al.,* 2017). This could be the reasons why *E. coli and C. freudii* were susceptible to only 3(17.65%) and 2(11.76%) of the plant extracts. All the plant extracts also had no antibacterial activity against ESBL *E. coli*, this is probably because the bacterium contains resistant genes that resist ESBL drugs and possibly plant extracts. This resistance can be overcome by combination therapy, for example *Indigofera suffruticosa* leave extracts have good antimicrobial activity when combined with Erythromycin against *S. aureus* (Dos santos *et al*., 2015).

The most susceptible gram-positive bacteria to exhaustive crude exctracts were MR.*S. aureus* and *S. aureus.* The probable reason for this apart from being gram positive is because of them being associated with secondary bacterial infections (Tong *et al.*, 2015). MR*S. aureus* is resistant to methicillin but majority of the plant extracts tested were active against the bacterium. This is in agreement with the studies of Venkatadri (Venkatadri *et al*, 2015). MR*S. aureus* and *Staphylococcus aureus* are associated with skin diseases and wound infections (Lakhundi and Zhang, 2018) that are treated by traditional practitioners (Kimutai *et al*., 2019). The two plants therefore, have potential for the control of wound and skin infections. However, *S. discifolius, V. volkensii, Z. minutiflora* and C. *schimperiana* extracts were inactive against these bacterium. However, these plants are used by the traditional practitioners to treat bacterial infections. This may be because these

plants are used to treat symtoms associated with the disease rather than the causative agent of the disease (Bruno *et al*., 2014).

Ethyl acetate(EAE) extract was more active than other solvents used during successive extractions. This probably means that ethyl acetate was able to extract more bioactive compounds from these plants being a mid polar solvent. In addition the compounds in the selected medicinal plants may have been more soluble in it than other solvents used.

There was variations in the activities of fractions against bacteria. *Enterococcus faecalis* was the least susceptible gram-positive bacteria with low inhibition zones on most fractions, this could be due to resistant genes developed by clinical isolates when they colonize intestines for a long time (Farman *et al*., 2019), (Table 4.9). The most susceptible gram negative bacteria was *C. freudii* with all fractions of *O. rochetiana* showing inhibition zones above 11.00 mm. This is in agreement with the studies of Hailu, (Hailu *et al*., 2005). FA1, FA2, and FA4 had inhibition zones of 13.03±0.26 mm, 10.80±0.96 mm and 11.66±0.70 mm against the same organisms respectively. The other gram negative bacteria were susceptible to one fraction only, (Table 4.8 and 4.9), this is because they resist multiple drugs due to their build in abilities to resist drugs (Kaplan, 2012).

Comparison of antibacterial activities of the successive extracts and fractions with those of the crude extracts indicated that the crude extracts of methanol were more active than both sequential extracts and fractions. This indicates that the compounds in plants in many cases are acting synergistically (Hag *et al*., 2019).

5.3 Use of bio-assay guided fractionation in isolation of bioactive compounds

Isolation of the active compounds was carried out using bio-assay guided fractionation and isolation (Fakhrudin *et al*., 2014). This method is still a valuable strategy for the finding of new lead compounds (Pieters *et al*., 2005). This method is commonly used in drug discouvery due to its effectiveness to directly linked the analysed extract and targeted compounds that is followed depending on biological activity (Neelesh and Sapna, 2017). The extract showing desired activity is tested and a defined threshold of expected activity is taken as good to be pursued further for investigation to obtain fraction from multiple major fraction/sub-extracts which is then pursued further to sub-fractions till the activity is demonstrated at the threshold levels. Bioactive fractions of the most active extracts are sepated by column chromatography and evaluated for antibacterial activities. This method has been used to isolate bioactive polyphenols from *Cuspidaria convoluta* leaves (Torres *et al*., 2019).Moreover, this method yieded five bioactive compounds Al-1(1), LC-1(3), LC-2(4) Cm-1 (2) Or-2 (5).

5.4 Antibacterial activities and synergism of isolated compounds

The compound coded Cm-1 **(2)** from the root bark of *C. myricoides* showed the highest activity against *S. aureus, P. aeruginosa,* with inhibition zones of 13.23±0.33mm and 12.97±0.53mm respectively. In addition, compound Or-2 **(5)** from stem bark of *O. rochetiana* was also active against MR. *S. aureus* and *S. aureus,* with inhibition zones of 11.36 ±0.7and 11.77±0.29respectively. These two compounds were structurally elucidated as phenolic glycosides. Phenolic compounds are widely distributed in plants and have beeen found to possess antimicrobial properties (Panzella *et al.,* 2020). The mechanisms by

which the phenolic compounds exert their antibacterial effect involves the interaction with the cytoplasmic membrane, cell wall, nucleic acids and/or energy transport (Xie *et al*., 2015), altering or inhibiting their functions. Furthermore, studies have showed that naringenin and quercetin exhibit cell wall damage properties in mycobacterial cells (Pawar *et al*., 2020).

There was difference in the antibacterial activities of the fatty acid LC-1 **(3)** from *L.calostachys* and AI-1 **(1)** from *A. lahai* . This may be due to their structural differences, compound LC-1 **(3)** is an unsaturated fatty acid while compound Al-1 **(1)** is a saturated fatty acid. The unsaturated fatty acids are more active against bacteria than the saturated fatty acids due to their ability to penetrate cell membrane causing lysis or growth inhibition (Debois *et al*., 2008). The other reason is stearic effect, compound LC-1 **(3**) has a *cis* type double bonds and are adsorbed in the cell membrane easily due to their bent structure (Debois *et al*., 2008). Fatty acids on skin such as oleic acid have been found to be potential antimicrobials (Fischer, 2020).

Compound LC-2(**4**) had significant activity against *S. aureus* only, with an inhibition zone of 10.16±0.42. Terpenoids containing OH group acts as an efficient uncoupler of the bacterial plasma membrane, creating instability and breaking of the plasma membrane hence killing the bacterial cells (Adam *et al.*, 2020, Yang *et al*., 2020).). However, in compound LC-2 (**4**) the hydrogen atom of hydroxyl group at carbon C-3, instead was replaced by an acyl group. This explains the low antibacterial activities observed, (Table 4.12).

The mode of action of the active isolated compounds was determine using broth micro-dilution method. Compound Cm-1(**2**) was found to be bacteridal against all the bacteria tested. This compound was elucidated as a martynoside. Previous studies of on this compound showed activity against *Bacillus subtilis* with a MIC of 7.81 µg/ml, (Pendota, *et al*., 2015). This is in tandem with the studies of Xu *et al*. (2016), who demonstrated that eugenol a phenolic constituent of clove essential oil disrupts the cell wall of *S. aureus*, increasing permeability, causing leakage of cellular substituents and permanent damage to the cell membrane. However, compound Or-2 **(5)** was bacteriostatic against MR. *S. aureus* which was indicated by growth at all concentration tested. despite being a phenol, it is likely to have targeted protein synthesis (Gonelimali *et al*., 2018).

The difference of activity of compound Or-2 (**5**) and Cm-1 (**2**) against MR. *S. aureus* could be due to the structural differences. Compound Cm-1 (**2**) contain two methoxy groups and two sugars while compound Or-2 (**5**) has one methoxy group and one sugar. Phenolic acids with methoxy (-OCH_3) substitutions, for example, ferulic acid have good antibacterial activities (Michael *et al.,* 2019). This could be the reason for better activity of compound Cm-1(**2**) compared to compound Or-2(**5**).

Moreover, the other molecules attached to the compounds such as the number of sugars and hydroxyl groups may have also contributed to their differences in antibacterial activities. For instance, presence of hydroxyl (-OH) group(s) at the *meta*-position of the benzene ring structure appears to enhance the antibacterial activities of the phenolic acid,

(Danica *et al.,* 2019). Syringic acid with one hydroxyl and two methoxy groups was found to have higher activity than that of gallic acid. The antimicrobial activity of hydroxybenzoic acids increased through substitution of a hydroxyl group with a methoxy group, (Sanchez-Maldonado *et al*., 2011).

Synergism of active compounds had better antibacterial activity than single compounds especially against antibiotic resistant bacteria. In addition, mixing of three compounds had better activity than two compounds.This is in agreement with the studies of Natchimuthu *et al*., (2008). Synergism is probably due to accumulation of inhibitory concentrations of the active compounds at the target sites or due to the additional inhibitory effect of the tested compounds against the bacterial cells (Hannan, *et al.*, 2012).

Synergism also demonstrated that a mixture of two different classes of secondary metabolites increases the antibacterial activity. This is probably because they target different sites of the bacteria (Sanhueza *et al.*, 2017). For instance, mixing of a phenolic compound Or-2(**5**) and fatty acid LC-1(**3**) showed greater activity than mixture of two phenolic compounds Or-2 and Cm-1. The activities observed in the mixture of two different classes of metabolites may also be due to different mechanisms of action against bacteria rather than the same class of metabolites having the same mechanisms of action against bacteria.

For example, phenols and phenolic compounds are known to break cell membrane and cause lysis (Tako *et al.,* 2020). In addition, phenolic compounds such as gallic acid and ferulic acid are potent against bacteria (Nzogong *et al*., 2017). This is because they destroy

the bacterial cell wall of some bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, leading to leakage of cellular contents, while fatty acid such as oleic acid damages included cellular components and causes loss of cellular contents (Aldulaimi, 2017).

The antibacterial activity of combined compounds was classified with good efficacy compared to antibacterial activity of extracts (Table 4.3, 4.4 and 4.13). Based on the results, it seems that there is an advantage in mixed compounds to control these types of bacteria because the antibacterial effectiveness of mixed compounds was higher compared to single compounds and extracts (Tables 4.3, 4.9 and 4.11). Synergism is also applied in conventional medicine for the eradication of multidrug resistant bacteria (Gaudereto *et al*., 2020). For instance, the potency of antibiotics was improved when combined with *Jatropha curcas* seeds, (Hag *et al*., 2019).

Exhaustive methanol crude extracts were more active than isolated compounds. This could be because methanol crude extracts contain phenolic compounds such as flavonoids, flavonols and anthraquinones that are active against bacteria (Saavedra *et al*., 2010). Compound Cm-1(**2**) was comparable in activity with the exhaustive methanol extracts and in some cases was active than crude extracts. This supports studies of Saavedra *et al*., (2010) that the activity in methanol extracts is due to presence of phenols. This is because compound Cm-1(**2**) was elucidated as a phenol. Furthermore, compounds present in crude extracts might have been antagonistic to each other leading to low activities (Paolo *et al*., 2014).

The mechanisms of action of compounds depend on their properties some might act on separate targets and some may be a consequence of another mechanism, this has been found to be effective with resistant bacteria such as *K. pneuminia* (Yang *et al.,* 2020). Ideally, combination chemotheraphy for antibiotic resistant bacteria should take advantage of synergistic interaction as this would increase the therapeutic efficacy and lower the risks of resistance especially when pure compounds are combined.The structures of these bioactive compounds were determined using NMR-spectroscopy.

5.5 Characterization of compounds isolated from selected medicinal plants

5.5.1:[5-(2, 5-dimethylhexyl) 1-isopentyl 3-hydroxy-2-methylpentanedioate] AL-1 (1)

Compound **1** was interpreted as the major compound by interpretation of 1D and 2D NMR data, which seemed to represent a mixture of two compounds, which appeared as a white powder. From the ^{13}C-NMR spectrum (Figure 4.10), nineteen major carbon atoms were picked, and by use of HSQC spectrum(Appendix 23), were classified as; six methyl, six methylene (two oxygenated δ_C 54.6, 61.7 ppm), five methine (one oxygenated, δ_C 60.8 ppm) and two shielded carbonyl atoms (δ_C 173.4 and 174.9 ppm). In support, ^{1}H-NMR spectrum (Figure 4.9), showed the presence of six methyls (δ_H 0.90-1.07 ppm), six methylene (two oxygenated at δ_H 3.46, 3.59 ppm) and five methine (one oxygenated at δ_H 3.53 ppm). This data suggested acyclic diester (Chaubal *et al.,* 2006), and careful interpretation of 2D NMR (HMBC and COSY) data correlations were used to determine it's structure.

A doublet representing one of the oxygenated methylene group resonating at δ_H 3.45 (*d,* 4.4) which corresponded to a carbon at δ_C 61.7 ppm was assigned position C-1′ of the

diester skeleton, following its HMBC correlations(Appendix 25) with a methine carbon at δ_C 30.9 (C-2′), methyl group at δ_C 19.2 (C-8′) and carbonyl carbon at δ_C 173.1 ppm (C-5). This assignment was further supported by COSY spectrum(Appendix 24) which indicated a correlation between the oxygenated protons at δ_H 3.45 (*d*, 4.4) (H/C-1′) with the methine proton at δ_H 2.28 (*m*) (H/C-2′).

The second oxygenated methylene group at δ_H 3.59 (*dd*, 5.2, 6.4) ppm was placed at C-1″, following its HMBC correlations with a second carbonyl carbon at δ_C 174.9 (C-1) ppm, a methylene at δ_C 41.8 (C-2″) ppm and an isopropyl methine carbon at δ_C 26.2 (C-3″). The oxygenated protons H/C-1″ appeared as a doublet of doublet because of the COSY correlations with the methylene protons at δ_H 1.62, 1.78 (*m*). An oxygenated methine proton resonating at δ_H 3.53 (*d*, 3.6, δ_C 60.8) ppm was assigned position C-3 through observed HMBC correlations with a methine at δ_C (37.8, C-2), methyl δ_C (15.7, C-6) ppm and the carbonyl at C-5.

The rest of the groups were assigned their respective positions through the same procedure of studying their two-dimensional correlations, (Table 4.14). This led to a conclusion of the structure to be 5-(2, 5-dimethylhexyl) 1-isopentyl 3-hydroxy-2-methylpentanedioate **2**. Prior to this study, a straight chain diester named pentacosane dioic acid dihexadecyl ester was isolated from *Acacia nilotica* (Chaubal *et al.,* 2006). Phytochemical analysis of *Acacia* species also revealed that the contents of saturated fatty acids were found to be higher contents than that of unsaturated ones, accounting for 81.0% and 89.3% of the total fatty acids content in leaves and bark in *Acacia dealbata* (Oliveira *et al*., 2020).

5.5.2 [*Cis* 4′′-*O*-acetyl martynoside] Cm-1(2)

This compound was isolated as a deep yellow powder soluble in methanol. The ^{1}H-NMR spectrum (Fig. 4.11) of compound **2** exhibited the presence of six aromatic protons belonging to ferulic acid moiety (acyl) and aglycone part. The signals belonging to two methoxy group were also identified, one attached to acyl moeity at δ_C (C-4′′′, 147.0) ppm, this was supported by HMBC spectrum(Appendix 28) correlation at (C-6′′′). The second methoxy group was attached to aglycone moeity at δ_C (C-4,151.9.0) ppm, this was also confirmed by HMBC correlation at (C-4).

Two oleifinic protons (*d* each H-α′′′ and H-β′′′) forming an AB spin system with J_{AB} =9.6Hz and 7.2Hz respectively (Table 4.16) indicated a *cis* geometry of the ferulic acid unit. The signals attributed to the acyl part were suggestive of the presence of the 3-hydroxy-4-methoxyphenylalcohol moiety. The compound was assigned α and β positions of the aglycone. This assignment was further supported by COSY spectrum (Appendix 27), which showed a correlation between the α-proton at δ_H 6.24(d, J=9.6) and β-proton at δ_H 8.00(d, J=7.2), and HMBC correlation of β-proton (8.0) ppm with carbonyl. The presence of sugar groups was derived from the presence of a doublet at δ_H 6.24 (*d*, 9.6, δ_C 104.3) ppm for glucose and another doublet at δ_H 6.24 (*d*, 2.0, δ_C 104.3) ppm for rhamnose. The signals were high due to the solvent used (methanol) and attachment of protons to the rings that resulted in shifting of proton signals downfield.

From HSQC spectrum(Appendix 26) of compound **2**, ^{1}H resonances were assigned their corresponding ^{13}C resonance, (Table 16). For example, proton at δ_H 8.00 ppm was found to

be attached to carbons at δ_C 141.0 ppm (C- α‴), while the proton at δ_H 8.53 ppm is attached to δ_C 119.6 ppm assigned position C- 2‴ of the acyl unit. Furthermore, protons at H-5‴ (δ_H 6.24 ppm), H-6 ‴ (δ_H 8.65 ppm), H-2 (δ_H 7.45 ppm), and H-5 (δ_H 7.45 ppm) are attached to carbon C-5 ‴ (δ_C 112.7 ppm), C-6‴ (δ_C 120.0 ppm), C-2 (δ_C 106.9 ppm) and C-5 (δ_C 107.2 ppm) res pectively of both acyl and aglycon units. The same procedure was also followed to assign hydrogen and carbons to sugars (Table, 4.16).

HMBC spectrum, showed methine protons on sugar moiety appearing at δ_H 3.89 which correlated with the carbonyl at δ_C 163.5ppm. In addition, methyl group in rhamnose at δ_H 1.25 (25.6) ppm showed by HMBC correlation with the methine carbons at δ_C 72.9(C-2) and δ_C78.4(C-3) ppm suggested the linkage in that position. HMBC correlation was established between proton at δ_H 6.24 and carbon at δ_C 151.1 (C- 3‴) ppm of acyl. The position of the methoxy group was confirmed to be at C-4‴ of the acyl unit on the basis of HMBC correlation between proton at δ_H 3.89 and carbon at δ_C 147.2 (C-4‴). In addition, H-6′ (δ_H 3.89) of glucose correlated with carbonyl carbon of acyl moiety which shows that glucose unit is linked to an acyl moiety. Moreover, protons at H-2 (δ_H 7.45 ppm) and H-5 (δ_H 7. 45 ppm) correlates with carbon C-1 (δ_C 131.5 ppm), C-3 (δ_C 150.4 ppm), C-4 (δ_C 151.9 ppm) and C-3 (δ_C 150.4 ppm), C-4 (δ_C 151.9 ppm) and C-6 (δ_C 122.0 ppm) of the aglycon.

The downfield chemical shift value of H-3′ of glucose in support of the HMBC correlation of H-1″ of rhamnose and C-1′ (δ_C 104.3 ppm) shows that rhamnose unit is linked to C-3′ position of glucose. Both isomers showed a correlation between H-1″ of glucose with C- α of aglycone in the respective HMBC's confirming the position of the aglycone in the

structure. Signal belonging to the acetyl group (δ_H 1.81, δ_C 172.3 and 20.9 ppm) were observed. Moreover, due to the 2.8 ppm downfield shift of C-4″ (δ_C 78.4) in camparison with that of 4″-*O*-acetyl martynoside suggests the site for attachment of the acetyl group was C-4″of rhamnose.

Several other correlations were assigned in their respective positions as shown in (Table 4.16). ^{1}HNMR and ^{13}CNMR spectroscopic data revealed a close structural similarity to 4″-O-acetyl martynoside (Funda *et al*., 2003), (Table 4.15). This led to a conclusion of the structure to be *Cis* 4″-*O*-acetylmartynoside. Phenolic glycosides have been isolated from the roots of *Cleodendrum myricoides* (Esatu *et al*., 2015). Martynoside has been found to be both antifungal and antibacterial (Pendota *et al*., (2015).

5.5.3 [*Cis* oleic ac] LC-1(3)

The ^{1}H NMR spectrum (Figure 4.13) of the compound **3** revealed the existence of a terminal methyl group at C-18 indicated by a triplet at δ_H 0.88 ppm. According to Pavia *et al*., 1996, a broad multiplet signal at δ_H 5.34-5.35 ppm corresponds to olefinic protons of unsaturated fatty acids. In relation to this observation, the ^{1}H NMR spectrum (Figure 4.13) for compound **7** revealed two olefinic protons appearing at δ_H 5.34 (2H, *d*, 6.0) and 5.35 (2H, *d*, 6.0) ppm which were therefore accounted for unsaturation for the compound and assigned positions C-9 and C-10. Moreover, it is reported that the peak values with J = 6-10 Hz accounts for *cis* while 11-18 Hz represents *trans* geometry (Pavia *et al*., 1996). Thus, the geometry for compound **7** was concluded to be *cis* because the J value obtained is 6.0 Hz. The assignment of the double bond at position C-9/C-10 was also supported by

the chemical shift difference of 0.29 ppm between the two olefinic carbon atoms in the fatty acid (Gunstone *et al.*, 2004).

In addition, ^{13}C NMR spectrum (Figure 4.14) for the compound showed that it contains eighteen carbon signals, one acidic carbonyl (δc 180.3) and two olefinic carbons (δc 130.2; δ_H 5.35 *d*) and (δc 129.90; δ_H 5.34 *d*) ppm one methyl at C-18 and the rest methylenes. From HSQC spectrum (Appendix 29) of compound **3**, ^{1}H resonances were assigned to their corresponding ^{13}C resonance, (Table 4.18). The proton at δ_H 5.35 ppm was found to be attached to the olefinic carbon at δ_C 130.2 ppm (C-9), while the proton at δ_H 0.88 ppm is attached to δ_C 14.3ppm assigned position C-18 of the molecule. Furthermore, protons at H-7 (δ_H 1.26 ppm), H-3 (δ_H 1.64 ppm), H-11 (δ_H 1.29 ppm), and H-5 (δ_H 1.29 ppm) are attached to carbon C-7 (δ_C 29.7 ppm), C-3 (δ_C 24.8 ppm), C-11 (δ_C 29.3 ppm) and C-5 (δ_C 29.3 ppm) respectively. Besides, from the COSY spectrum of compound **7** (Appedix 30), a number of cross peaks were observed including; H-2(δ_H 2.34 ppm) and H-3(δ_H 1.64 ppm) , H-7 (δ_H 1.26 ppm) and H-9 (δ_H 5.35 ppm) , H-11(δ_H 2.01 ppm) and H-16(δ_H 1.29 ppm) among others.

The HMBC spectrum(Appendix 31) supported the assignments of the functional groups in compound 7. For example, the olefinic proton at δ_H 5.35 ppm (H/C-9) showed correlations with the sp^2 hybridized carbon at 129.9 ppm (C-10) and methylene carbon atoms at δ_C 29.7 (C-7) and 29.2 (C-8) ppm. Also, there were observed correlations between the methylene protons δ_H 2.34, *t, J*=5.5 (H/C-2) and 1.64, *t, J*=5.5 (H/C-3) ppm and the carbonyl carbon at δ_C 180.3 (C-1) ppm. There were several other correlations observed from HMBC spectrum, which were helpful in elucidation of compound **7** structure. The spectral data are

in good agreement with that of oleic acid isolated from algae *Spirulina platensis* (Wekesa, 2014) (Table 4.17), hence compound **7** was proposed to be *Cis* oleic acid (*Cis* -9-decenoic acid).

5.4.4 [*β*-amyrin tetradecanoate] LC-2 (4)

The ^{1}H-NMR spectrum (Figure 4.15.) of compound **4** showed nine methyl groups (δ_H 0.75, 0.84, 0.86, 0.97, 0.98, 1.14, 0.83, 0.88 and 0.87) all ppm appearing as singlet's. Signals bearing an ester group at δ_H 4.45 (1 H, *m*, *J* = 8.0 Hz) as well an olefinic protons at 5.19 (1H, *d*, *J* =4.0 Hz) ppm were observed. In addition, the ^{13}C-NMR spectrum **(**Figure 4.16**)** and Table 4.20, displayed more than forty seven signals, and by use of HSQC spectrum(Appendix 32), were classified as; nine methyls, twenty one methylenes, six methines, eight quanternary carbons,one olefinic signal and one shielded carbonyl atom δ_C 171.0 ppm (C-1).

A triplet group resonating at δ_H 1.63 (*m)* which corresponded to a carbon at δ_C 38.1 ppm was assigned position C-1 of compound **4**, following its HMBC correlations (Appendix 34) with a methylene carbon at δ_C 23.8 (C-2), methine carbon at δ_C 81.1 (C-3). This was supported by COSY spectrum (Appendix 33), which indicated a correlation between the protons at δ_H 1.63 (*m*) (H/C-2) with the methine proton at δ_H 0.85 (*m*) (H/C-1). Methine group at δ_H 4.45 (*dd,* 8.0) ppm was placed at C-3, following its HMBC correlations with methylene carbon at δ_C 23.8 (C-2). In addition, carbonyl carbon at δ_C 171.0 ppm showed HMBC correlation with methine group at δ_H 4.45 (dd, *J* = 8.0, 4.0) (H-3), suggesting an acyl group is linked to the oxygen group at C-3. This assignment was confirmed by high downfield shift of carbon 3 appearing at δ_H 4.45 ppm (Reyes, *et al*., 2017).

The proton at δ_H 5.19 ppm was found to be attached to olefinic carbon at δ_C 122.1 ppm (C-12). Furthermore, HSQC spectrum showed protons at H-5 (δ_H 0.85 ppm), H-6 (δ_H 1.45 ppm), H-7 (δ_H 0.94 ppm), H-9 (δ_H 1.61 ppm), H-14 (δ_H 0.95ppm), H-15 (δ_H 1.14 ppm), H-16 (δ_H 2.02 ppm), H-17 (1.55 ppm), and H-19 (δ_H 1.69 ppm) are attached to C-5 (δ_C 55.8 ppm), C-6 (δ_C 18.5 ppm), C-7 (δ_C 31.5 ppm), C-9 (δ_C 47 ppm), C-14 (δ_C 42.1 ppm), C-15 (δ_C 26.1 ppm), C-16 (δ_C 27.3 ppm), C-17 (δ_C 32.8 ppm) and C-19 (δ_C 47.1 ppm) respectively. Besides, from the COSY spectrum (Appendix 33), of compound **4** a number of cross peaks were observed including; H-1(δ_H 1.63 ppm) and H-3(δ_H 4.45 ppm), H-1 (δ_H 1.63 ppm) and H-2 (δ_H 0.87 ppm), H-5(δ_H 0.85 ppm) and H-6 (δ_H 1.45 ppm), H-12 (δ_H 5.19 ppm) and H-14 (δ_H 1.94 ppm) , H-16(δ_H 1.55 ppm) and H-6 (δ_H 0.94 ppm) among others.

The HMBC spectrum supported the assignments of some major groups in compound **4**. For example, the olefinic proton at δ_H 5.19 (*d*, 4.0) ppm showed correlations with the quaternary carbon at δ_C 145.3 ppm (C-13). There were observed correlations between proton at δ_H 4.45 (*m*, 8.0) with carbon at δ_C 38.1 (C-1), 23.8(C-2) ppm and carbonyl carbon. Methylene proton at 1.63 (*t*, 4.0) showed correlation with carbon at δ_C 23.8 (C-2) and 81.1 (C-3) ppm while methylene protons at δ_H 0.85 (*t*) showed correlation with carbon at δ_C 18.5 (C-6) ppm. HMBC correlation between proton at δ_H 1.88 (*m*) (C-11) and carbon at δ_C 122.0 ppm (C-12) and 145.3 (C-13) shows that the two carbon signals are close to (C-11), hence placed at position C-12 and C-13. This was supported by COSY correlation at H-11 (δ_H 1.88) and (δ_H 5.19 ppm). The rest of the groups were assigned their respective positions through the same procedure of studying their two-dimensional correlations, (Table 4.20).

Moreover, ^{13}C-NMR spectra of compounds **4** showed methine and quartenary *sp*^{2} signals (δc 122.1 and 145.3) ppm characteristic of oleanane triterpenes, (Reyes *et al*., 2017). Detailed analyses of these spectra indicated that compound **4** was connected with the alkanoate at position 3 evident by HMBC correlation between H-3 of *β*-amyrin at 4.45 (*dd, J*=8.0,4.0 Hz). The alkanoate was identified to be tetradecanoate. This was confirmed by the presence of an intense peak for multiple methylenes at 34.6, terminal methyl carbon at δ_H 14.75 ppm and that of ester carbonyl at (δ_C 171.0) ppm.

Esters are connected to triterpenoids at carbon 3 (Yue *et al*., 2015). Terpenoids are found in many plants, *β*-amyrin has been isolated from *Leucas aspera* (Alam *et al.,* 2014). The spectral data is in good agreement with that of *β*-amyrin octacosanoate (Table 4.19) previously isolated from *Cleodendrum eriophyllum* (Machumi, 2011), except the presence of fourteen carbon atoms forming an alkanoate in compound **4**, (Table 20). This led to a conclusion of the structure to be *β*-amyrin tetradecanoate **(4).**

5.5.5 [4-*β*-D-glucopyranosylcaffeic acid] Or-2 (5)

The ^{13}C-NMR spectrum (Figure 4.18) for compound **5** revealed the existence of fourteen carbon signals, one acidic carbonyl (δc 158.2) and hydrogenated sp^2 hybridized carbons at δ_C 112.5, 141.7, 113.0 and 111.8 ppm (two oxygenated δ_C 150.2, 152.4ppm). The presence of sugar group was derived from the presence of a doublet at δ_H 5.56 (*d*,7.4, δ_C 99.7) ppm, a typical peak for H/C-1′. In addition, glycosyl protons and carbons appeared in the range of 3.55-3.79 and 61.5-99.7 ppm, respectively.

The ^{1}H-NMR spectrum (Figure 4.17) of the compound **5** showed existence of an aromatic region with signals ranging from δ_H 7.45-7.75 ppm, which from HSQC spectrum(Appendix 35), were attached to sp^2 hybridized carbon atoms at δ_C 112.5- 141.0 ppm. From HMBC spectrum(Appendix 37), the aromatic protons correlated with two oxygenated sp^2 hybridized carbon atoms at δ_C 150.2 and 152.4 ppm and a carbonyl carbon atom at δ_C 158.2 ppm. The COSY spectrum(Appendix 36), revealed correlation between a typical peak for sugar at δ_H 5.60 (*d*, 6.8) ppm and glycosyl protons at 3.66 (*m*) ppm. The information gathered so far suggested a possibility of a ferrulic acid derivative (Deyou *et al.*, 1993). Additionally, the ^{1}H-NMR spectrum showed a signal at δ_H 3.82, which correlated with a carbon at δ_C 60.9 ppm, interpreted for a methoxy group in the structure.

Study of HMBC and COSY correlations revealed that the compound was a glycosylated ferrulic acid, with the sugar group attached at position C-4 of the caffeic acid skeleton. The presence of sugar group was derived from the presence of a doublet at δ_H 5.56 (*d*,7.4, δ_C 99.7) ppm, a typical peak for H/C-1′. In addition, glycosyl protons and carbons appeared in the range of 3.55-3.79 and 61.5-99.7 ppm, respectively.

Careful study revealed that spectroscopic data of the compound **5** was close to that of 4-*β*-D-glucopyranosylcaffeic acid, previously isolated from *Olinia usambarensis*, (Deyou *et al.*, 2017). Therefore, the compound was concluded to be 4-*β*-D-glucopyranosylferrulic acid. Previously isolated from *Sesamum indicum* L. (Pedaliaceae) (Lee, 2009). Total assignments of the protons and carbon atoms of the compound are in Table 21.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The following conclusion were made from the study;

In this research thirty-three (33) medicinal plants distributed in 15 botanical families were identified to be used against bacterial infections in Nandi county. The investigation involved interviewing Traditional practitioners with the aim of getting medicinal plants used in the treatment of bacterial infection in the county.

Seventeen (17) medicinal plants were selected for antibacterial activities based on ethnobotanical information from practical practitioners and literature. The results reported were in tandem with the reported uses by herbalists. This supports and provides a scientific basis for use of these plants in herbal remedies.

The plant extracts were active against gram positive and gram-negative bacteria therefore, considered to have broad spectrum of activity. MIC and MBC values in mg/ml provides the guidance as to doses that can be effectively used for administration. The active compounds are concentrated in extracts and fractions this underlines the importance of testing the activities of different extracts and fractions before reporting that such type of herbal drugs are inactive by simply looking at the results of the crude extracts.

A total of five compounds were isolated from four selected medicinal plants. *L. calostachys* gave two compounds *Cis* Oleic acid (**3)** and *β*-amyrin tetradecanoate (**4**) while the other mediocinal plants gave one each. 5-(2, 5-dimethylhexyl)1-isopentyl 3-hydroxy-2-

methylpentanedioate (**1**) from *A. lahai*, *Cis* 4″-*O*-acetyl martinoside (**2**) from *C. myricoides*, and 4-D-glucopyranosylferullic acid (**5**) from *O. rochetiana.*

The compounds exhibited a wide range of bioactivity against bacterial strains selected. Cis 4″-*O*-acetyl martinoside (**2**) was the most active with high zones of inhibition 13 mm against *S. aureus*, *P. aeruginosa*, *C. freudii*. The other four compounds had inhibition zones of 8-12mm against both gram positive and gram-negative bacteria. The results obtained are in agreement with the therapeutic use of these plants in traditional medicine.

The active compound were investigated for MIC's. *Cis* 4″-O-acetyl martinoside (**2)** was more active against *S. aureus*, *P. aeruginosa*, MR.*S. aureus* with MIC of 25.0, 12.25 and 12.25 mg/ml respectively. 4-D-glucopyranosylferullic acid (**5**) had MIC of 12.5 and 25mg/ml against *S. aureus* and MR.*S. aureus.* Moreover, Oleic acid (**3)** was more active against *S. aureus*, *P.* aeruginosa, MR.*S. aureus* with MIC of 25.0, 25.0 and 6.250 respectively.

Synergism was also determined for the active compounds using disk diffusion method by combining compounds in equal proportions of two and three. Synergism enhanced activity with combination of D-glucopyranosylferullic acid(**5**) + *Cis* oleic acid (**3**)+ *Cis* 5-O-acetyl martinoside (**2**), having the highest inhibition zone of 20.03 mm and 20.17 mm, against two resistant bacteria MR*S. aureus* and ESBL. *E.coli.*

It is interesting to note that whereas ESBL. *E.coli.* was resistant to individual compounds, it was susceptible to a mixture of two and three compounds. An advantage of synergism or combination therapy for isolated compounds against resistant bacteria should be taken into account as this would increase the efficacy and lower the risks of resistance.

The isolated compounds were characterized as 5-(2,5-dimethylhexyl)1-isopentyl 3-hydroxy-2-methylpentanedioate (**1**), Cis 4″-O-acetyl martinoside (**2**), *Cis* oleic acid (**3**), β-amyrin tetradecanoate (**4**) and 4-*β*-D-glucopyranosylferullic acid (**5**). This was the first attempt to extract, isolate, and test extracts, fractions, and *Cis* oleic acid (**3**) and *β*-amyrin tetradecanoate (**4**) of *L .calostachys* from Kenya. In addition,5-(2, 5-dimethylhexyl)1-isopentyl 3-hydroxy-2-methylpentanedioate (**1**), Cis 4″-O-acetyl martynoside (**2**) and 4-β-D-glucopyranosylferullic acid (**5**) were isolated from *A. lahai*, *C. myricoides* and *O. rochetiana* for the first time. 5-(2,5-dimethylhexyl)1-isopentyl 3-hydroxy-2-methylpentanedioate(5), 4-β-D-glucopyranosylferullic acid and D-glucopyranosylferullic (**5**) were also tested for antibacterial activities with range of bacteria for the first time and were active.

6.2 Recommendation

It is recommended that;

Toxicity studies should be carried out to authenticate safe use of the crude extracxts and isolated compounds.

Other parts of the medicinal plants should be investigated for antibacterial activities and if found active isolation of the bioactive compounds to be carried out with the aim of developing phytomedicine.

Studies should be carried out to determine the genes responsible for resistance to the plant phytoconstituents. For instance in, *E.coli* and *S.typhi.*

The active crude extracts and the compounds which displayed potent antibacterial activity, *Cis* oleic acid, *Cis* 4″-O-acetyl martynoside (2) and 4-*β*-D-glucopyranosylferullic acid (5) should be tested for *In vivo* studies and investigated further with the objective of developing phytomedicine.

Synthesis of novel compounds based on elucidated structures of the isolated compounds should be done with the aim of developing and formulating phytomedicines that can be used to treat resistant bacteria.

6.3 Publications

1. Kimutai, Nicholas K., Philip A.Ogutu, Mutai Charles and Jeruto Pascaline, (2019). Ethnobotanical Study of Selected Medicinal Plants used against bacterial Infections in Nandi county, Kenya. *Journal of medicinal plants*. 7(4):103-108
2. Kimutai, Nicholas K., Philip A.Ogutu and Mutai Charles, (2020). Antibacterial activities of Selected Medicinal plants from Nandi county, Kenya. *Asian journal of Microbiology, Biotechnology and Environmental sciences*. 22(3):541-547
3. Kimutai, Nicholas K., Philip A.Ogutu and Mutai Charles, (2021). Antibacterial activities of *Leucas calostachys* extracts. *Asian journal of Plant Sciences*. 20(3):470-476,2021

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APPENDICES

APPENDIX 1: QUESTIONNAIRE

NO……

SECTION A: Use of medicinal plants

Instructions; Answer the questions by marking the appropriate answer ( )

1.Local name of the plant.....

2. Scientific name of the plant…….

3. What is the habit of the plant?

Tree ☐ Shrub ☐ Herb ☐ Climber ☐

Other specify……

4. Where do you obtain the plant?

Forest ☐ Farms ☐ Swamp ☐ River banks ☐

Other specify……

5. At what stage do you harvest the plant?

Seedling ☐ Middle stage ☐ Mature ☐

Other specify……

6. Which part of the plant is used?

Leaves ☐ Stem /Bark ☐ Root ☐ Flower ☐

Fruit

Other specify……

7. What quantities of the plant do you harvest?

1-5 kg ☐ 6-10kg ☐ 10 kg and over ☐

Other specify……

8. Is the plant available?

Very common ☐ Common ☐ Not-common ☐

Other specify……

9. How do you prepare the parts harvested?.....

10. Which disease(s) does the plant treat?.....

12. How effective is the plant

Very effective ☐ Effective ☐ Fairly effective ☐

Other specify……

13. How often do you harvest the plant?

Frequently ☐ Occasionally ☐ Rarely ☐

Other specify……

Appendix X 2A: VOUCHER SPECIMENS

APPENDIX 2B: VOUCHER SPECIMENS

APPENDIX 3: INHIBITION ZONES OF METHANOL EXTRACTS OF SELECTED MEDICINAL PLANT AGAINST GRAM POSITIVE BACTERIA

Plate 4; MR*S. aureus*

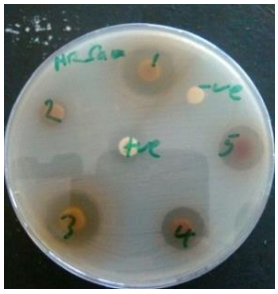


Plate 5; MR*S. aureus*

Plate 6; MR *S. aureus*

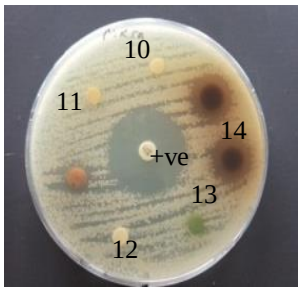


Plate 7; *S. aureus*

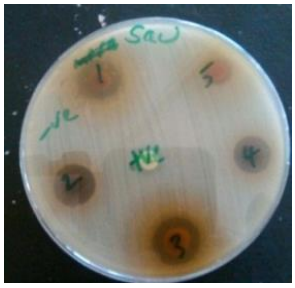


Plate 8; *S. aureus*

Plate 9; *S. aureus*

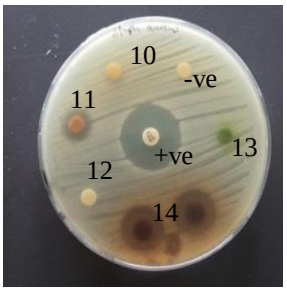


Plate 10; E. *faecalis*

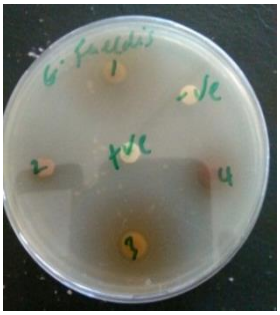


Plate 11; E. *faecalis*

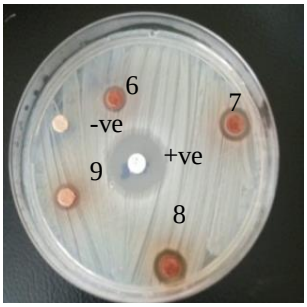
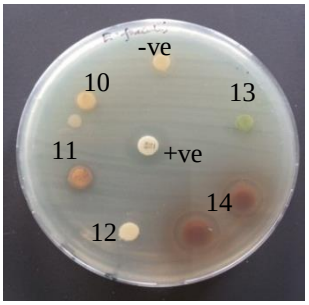


Plate 12; E. *faecalis*



Key; Numerical labelling 1-14 on the plates shows inhibition zones by plant extracts with corresponding serial numbers.

APPENDIX 4: INHIBITION ZONES OF METHANOL EXTRACTS OF SELECTED MEDICINAL PLANT AGAINST GRAM NEGATIVE BACTERIA.

Plate 13; Esbl *E. coli*

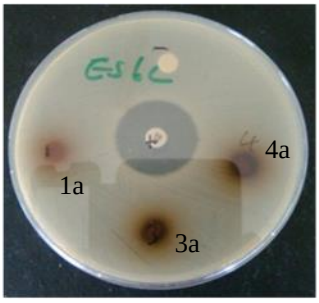


Plate 14; *K. pneumonia*

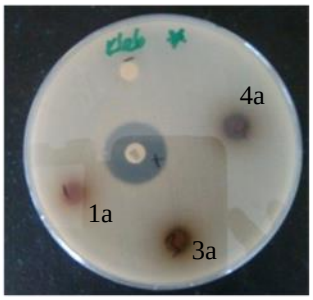


Plate 15; *S. sonnei*

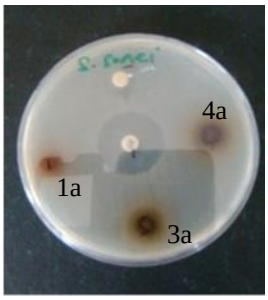


Plate 17; *C. freudii*

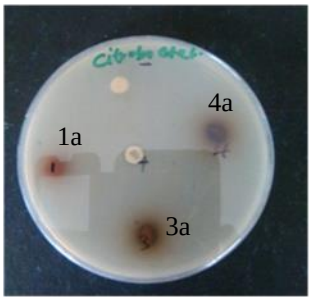


Plate 18; *E. coli*

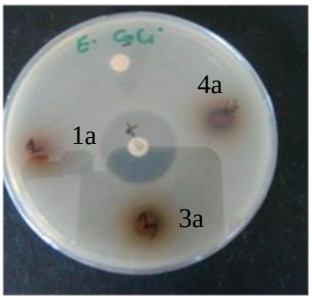


Plate 19; *K. pneumoniae*

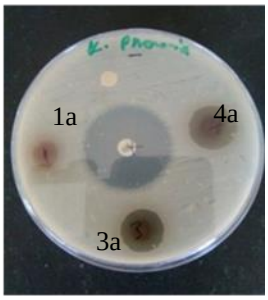
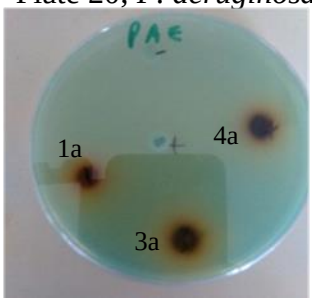


Plate 20; *P. aeruginosa*



Key; Numerical labeling 1a, 3a and 4a on each plate shows inhibition zones by plant extracts with corresponding serial numbers.

APPENDIX 5:INHIBITION ZONES OF METHANOL EXTRACTS OF SELECTED MEDICINAL PLANTS AGAINST GRAM NEGATIVE BACTERIA

Plate 20; *K. pneumoniae*

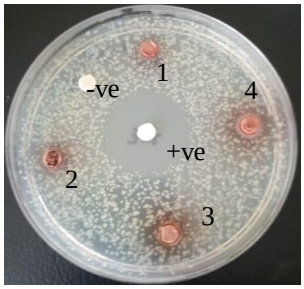


Plate 21; *K. pneumoniae*

Plate 22; *K. pneumoniae*

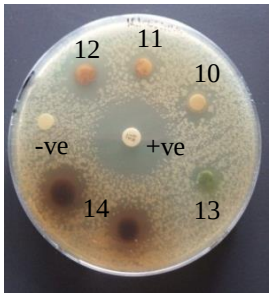


Plate 23; *P. aeruginosa*

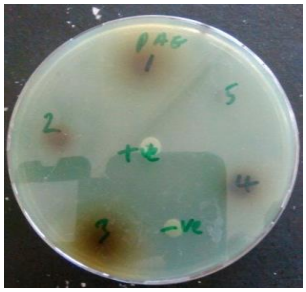


Plate 24; *P. aeruginosa*

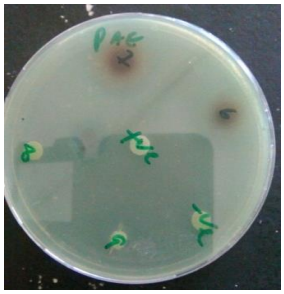


Plate 25; *P. aeruginosa*

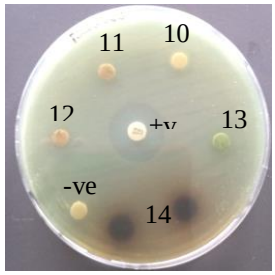


Plate 26; *S. typhi*

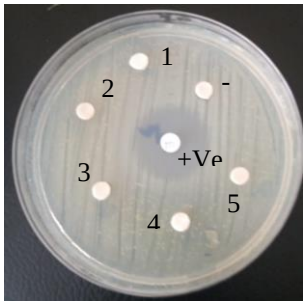


Plate 27; *S. typhi*

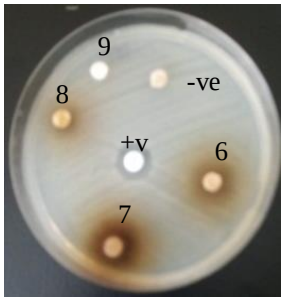
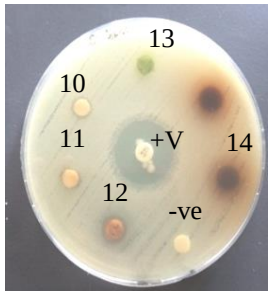


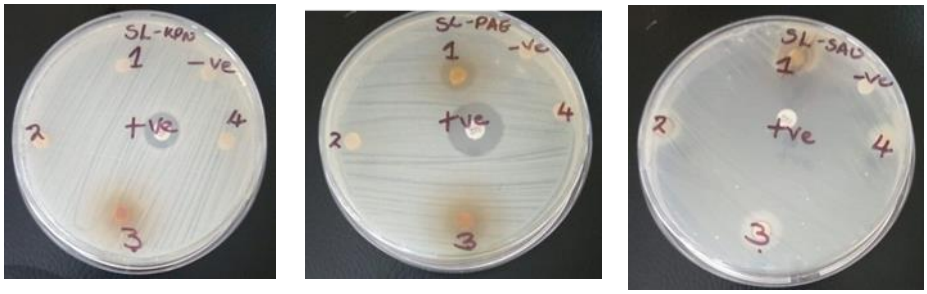
Plate 28; *S. typhi*



Key; Numerical labeling 1-14 on each plate shows inhibition zones of plant extracts with corresponding serial numbers.

APPENDIX 6: INHIBITION ZONES OF SEQUENTIAL EXTRACTS OF *Leucas calostachys*

Plate SL1: *K. pneumonia* Plate SL2: *P. aeruginosa Plate* SL3:*S.aureus*



Key-Hexane extracts, 2- Dichloromethane extracts, 3-Ethyl acetate and 4-Methanol extract

APPENDIX 3B: Inhibition zones of extract fractions of *Leucas calostachys*

Plate FL1-*K. pneumonia* Plate FL2-*S. sonnei* Plate FL3-*E. coli*

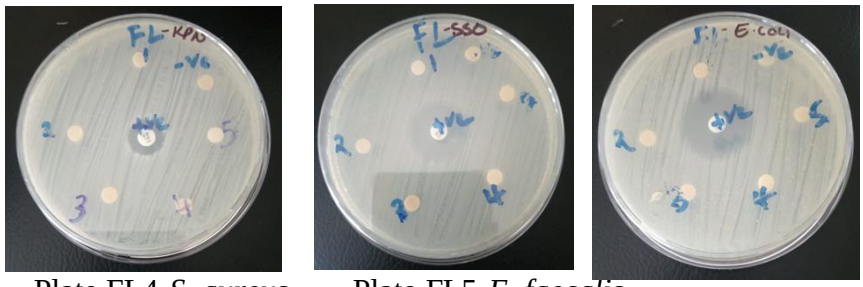
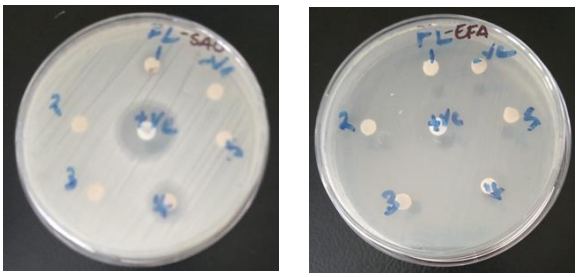


Plate FL4-*S. aureus* Plate FL5-*E. faecalis*



Key: Fractions are labeled 1-5on plate of each organism representing coded number of fractions F-L1-FL-5 for fractions.

APPENDIX 7:INHIBITION ZONES OF SEQUENTIAL EXTRACTS OF *Acacia lahai*

Plate 1SA; *S. typhi*

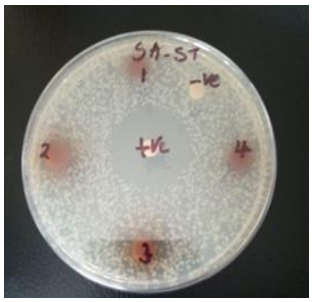


Plate 2SA; *E. coli*

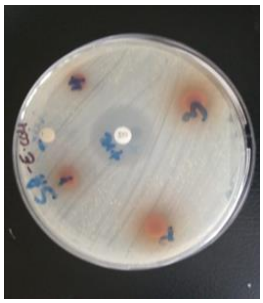


Plate 3SA; *K. pneumonia*

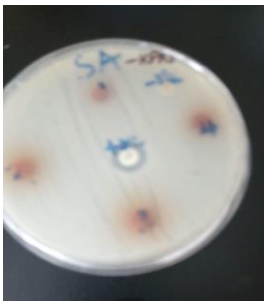


Plate 3SA; *P. aeruginosa*

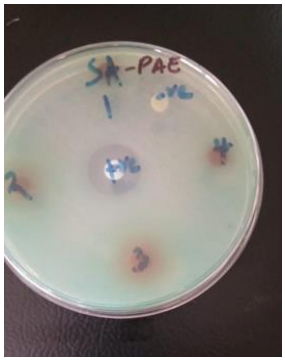


Plate 4SA; *S. aureus*

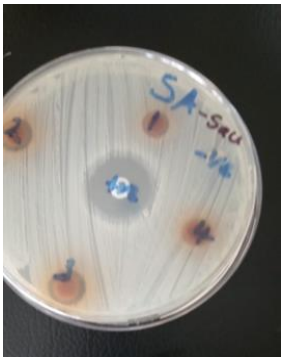
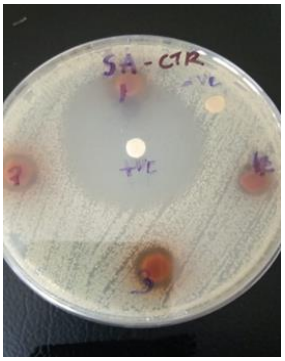


Plate 6SA; *S. sonnei*

Plate 7SA; *E. faecalis*

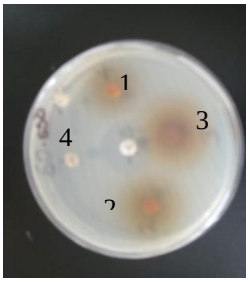
Plate 8SA; *C. freudii*

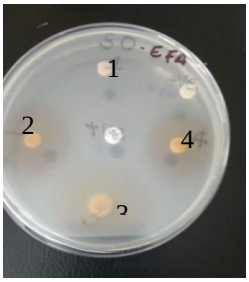


Key-Hexane extracts, 2- Dichloromethane extracts, 3-Ethyl acetate and 4-Methanol extract

APPENDIX 8: INHIBITION ZONES OF SEQUENTIAL EXTRACTS OF *Olinia rochetiana*

Plate SO1-*S. sonnei* Plate SO2-*E . faecalis* Plate SO3-*K. pneumonia*





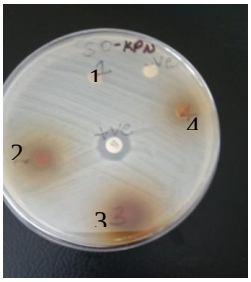
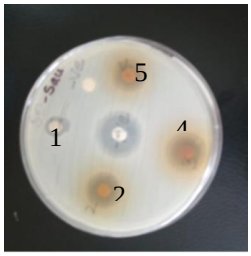
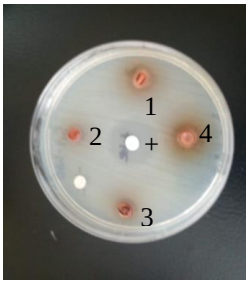


Plate SO4-*S. aureus* Plate SO5-*E. coli* Plate SO6-*P. aeruginosa*





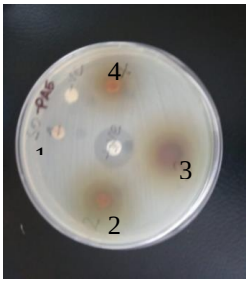
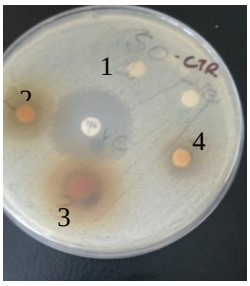
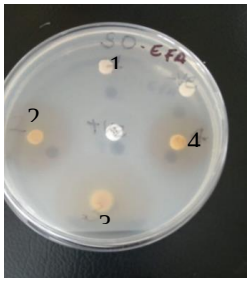
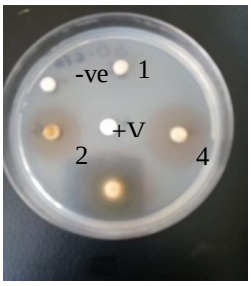


Plate SO7-*C. freudii* Plate SO8-*E. faecalis* Plate SO9-MR*S.aureus*







Key-Hexane extracts, 2- Dichloromethane extracts, 3-Ethyl acetate and 4-Methanol .

APPENDIX 9: INHIBITION ZONES OF SEQUENTIAL EXTRACTS OF *Cleodendrum myricoides* and compound LC-1

Plate CM1; MRS*. aureus*

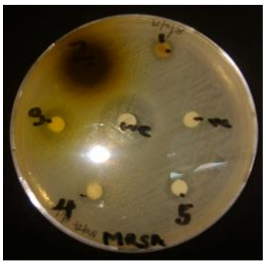


Plate CM2; *S. aureus*

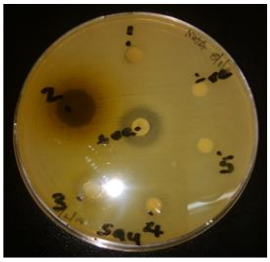


Plate CM3; E*. faecalis*

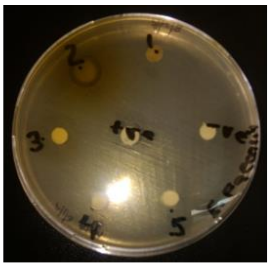


Plate CM 4; P. *auruginosa*

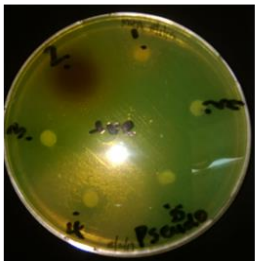


Plate CM5; *S. sonnei*

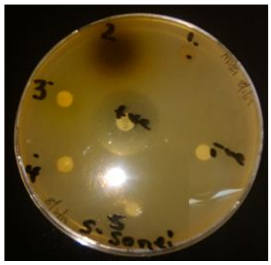


Plate CM6; *E. coli*

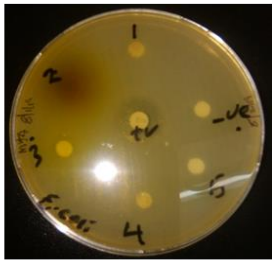


Plate CM7; *S.typhi*

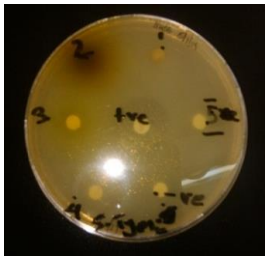
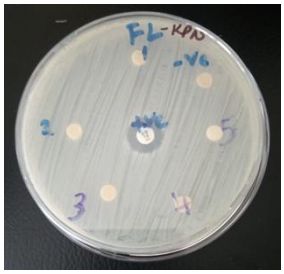


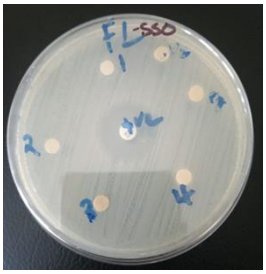
Plate CM8; *K.pneumonia*

Key-Hexane extracts, 2- Dichloromethane extracts, 3-Ethyl acetate, 5- Methanol extracts and 5- Compound LC-1

APPENDIX 10:INHIBITION ZONES OF EXTRACT FRACTIONS OF *Leucas calostachys*

Plate FL1-*K. pneumonia* Plate FL2-*S. sonnei* Plate FL3-*E. coli*





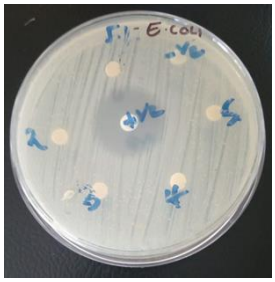
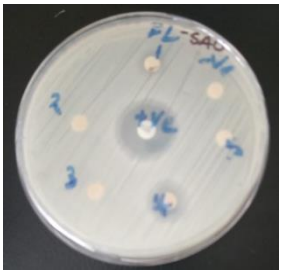
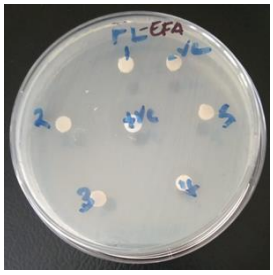


Plate FL4-*S. aureus* Plate FL5-*E. faecalis*

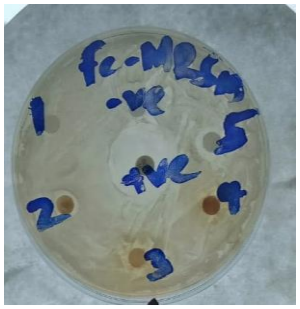




Key: Fractions are labeled 1-5on plate of each organism representing coded number of fractions F-L1-FL-5 for fractions.

APPENDIX 11: INHIBITION ZONES OF EXTRACT FRACTIONS OF *Cleodendrum myricoides*

Plate FC1-MR*S.aureues* Plate FC2-*S.aureues* Plate FC 3-*E. faecalis*



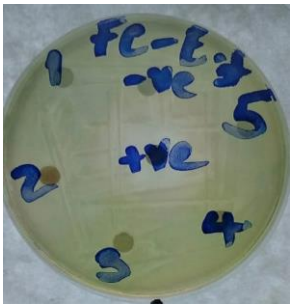


Plate FC 4-*E.coli* Plate FC5- *Esbl* E. *coli* Plate FC6-C.*freudii*



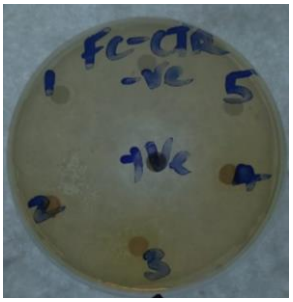
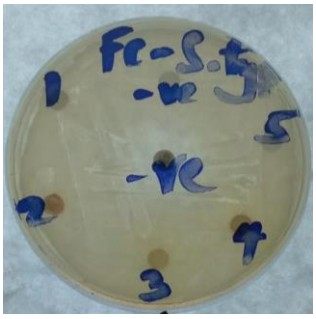
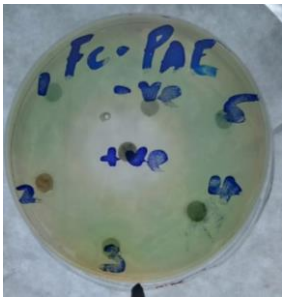


Plate FC7-*S.typhi* Plate FC8-*P.aeruginosa*

Key; Fractions are labeled 1-5 on each plate of organism representing coded number of fractions FC-1-FC-5.

APPENDIX 12: INHIBITION ZONES OF EXTRACT FRACTIONS OF *Olinia rochetiana*

Plate FO1-*E.coli*

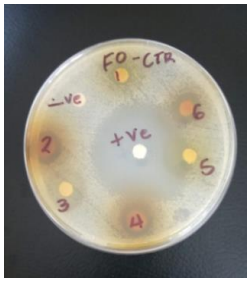


Plate FO 2-*S. typhi*

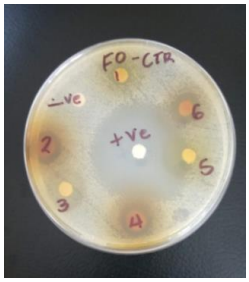


Plate FO 3-*S.sonnei*

Plate FO4-*C. freudii*

Plate FO5-*E.faecalis*

Plate FO6-*S.aures*

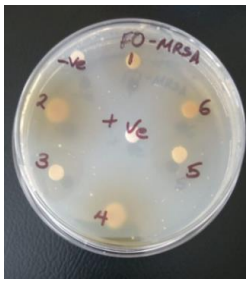


Plate FO 7-*P.aeruginosa*

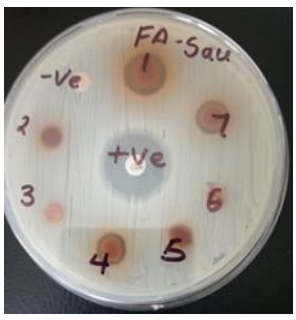
Plate FO8-*K.pneumonia*

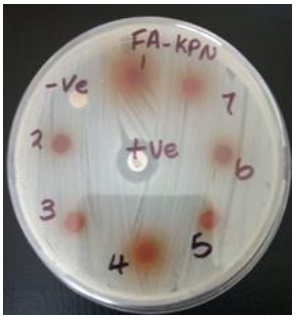
Plate FO9-MR*S.aureus*

Key; Fractions are labeled 1-6 on each organism representing coded number FO1-FO6 for fractions (Table 4.7).

APPENDIX 13: INHIBITION ZONES OF EXTRACT FRACTIONS OF *Acacia lahai*

Plate FA1-*S.aureues* Plate FA2-*K. pneumonia* Plate FA3-*E. coli*





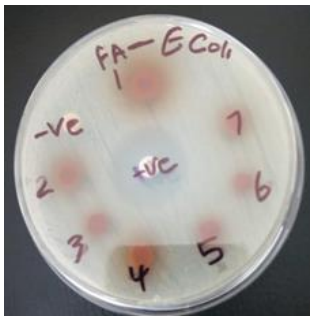


Plate FA4-*S. sonnei* Plate FA5-*E. faecalis* Plate FA 6-*C.freudii*

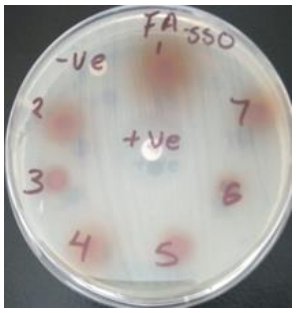
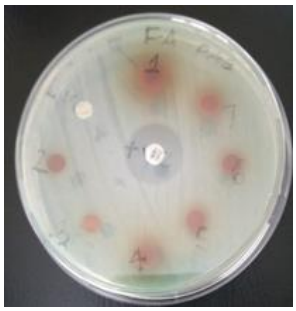
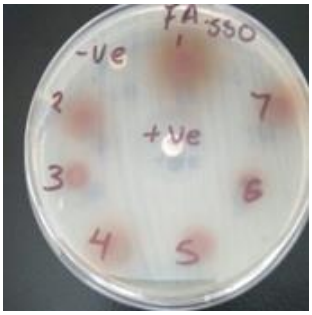


Plate FA7- *P. aeruginosa* Plate FA 8-*C. freudii* Plate FA9-*S. sonnei*





Key; Fractions are labeled 1-7 on each organism representing coded number FA1-FA7 for fractions (Table 4.7).

APPENDIX 14: INHIBITION ZONES OF ISOLATED COMPOUNDS

Plate C1-MR*S.aureus*

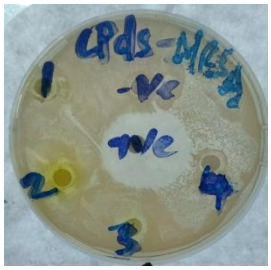


Plate C2-*S.aureus*

Plate C9-*E. faecalis*

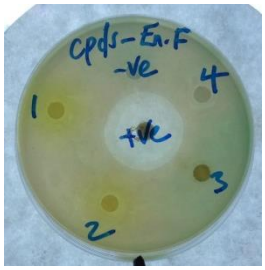


Plate C4-P. *aeruginosa*

Plate C5-K. *pneumonia*

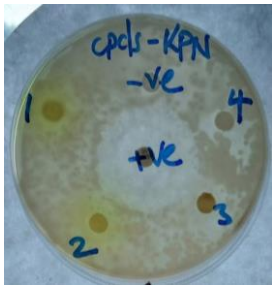


Plate C6-*C. freudii*

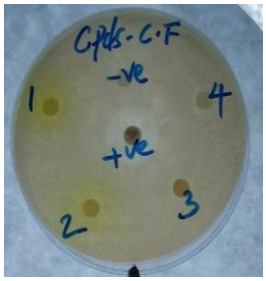


Plate C7-ESBL E.*coli*

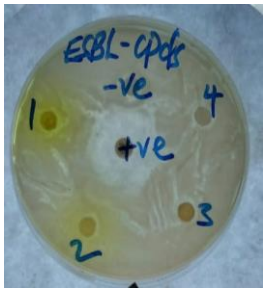


Plate C8- E. *coli*

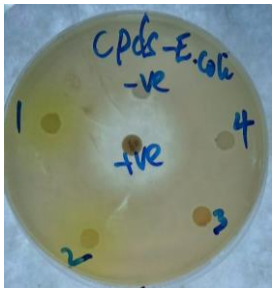
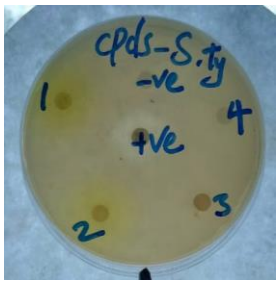


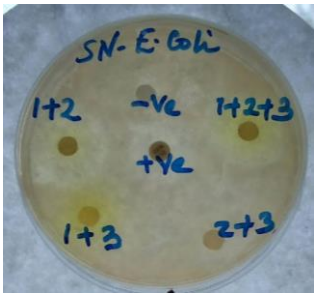
Plate C9-*S.typhi*

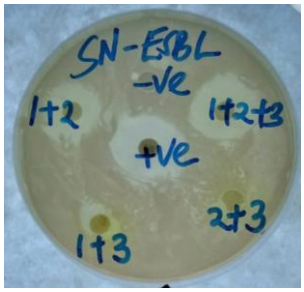


Key: Compounds are labelled as 1for Or-1(9), 2 for LC-2, 3 for Cm-1(6) and 4 for Al-1(5)

APPENDIX 15: INHIBITION ZONES OF MIXED COMPOUNDS

Plate SN1-*E.coli* Plate SN2-ESBL *E .aureus* Plate SN3-*S.aureus*





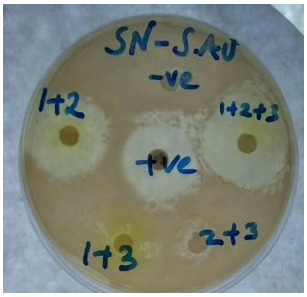
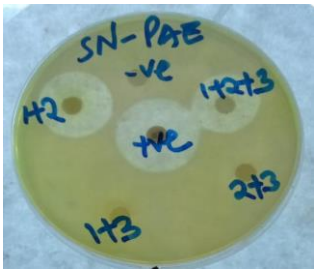
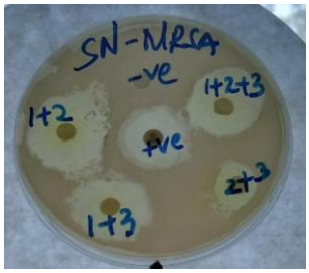


Plate SN4-P.*aeruginosa* Plate SN5-MR*S.aureus* Plate SN6-*E. faecalis*





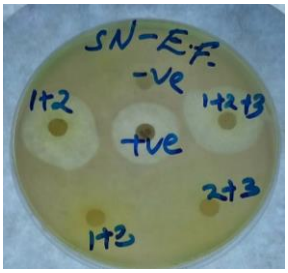
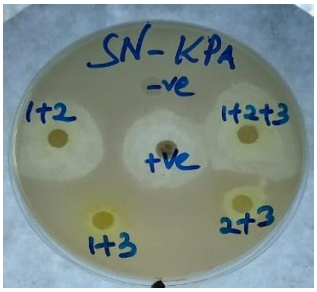
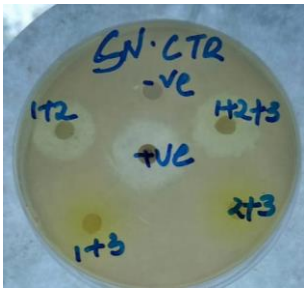
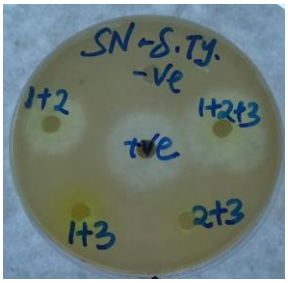


Plate SN7-K. *pneumonia* Plate SN8-C. *freudii* Plate SN9-*S.typhii*







Key; Compounds are labeled on plates as 1- for Or-1, 2- for LC-1 ,3-for Cm-11,(+) means mixture of compounds.

APPENDIX 16: PLANT MATERIALS

From left *O. rochetiana*(bark), *C. myricodes*(roots), *A .lahai* (bark) and *L. Calostachys* (roots) when crushed.

APPENDIX 17: HEXANE FILTRATES.

From left *A .lahai*, *O. rochetiana* (bark),*C.myricodes*(roots),(bark) and *L. calostachys* (roots)

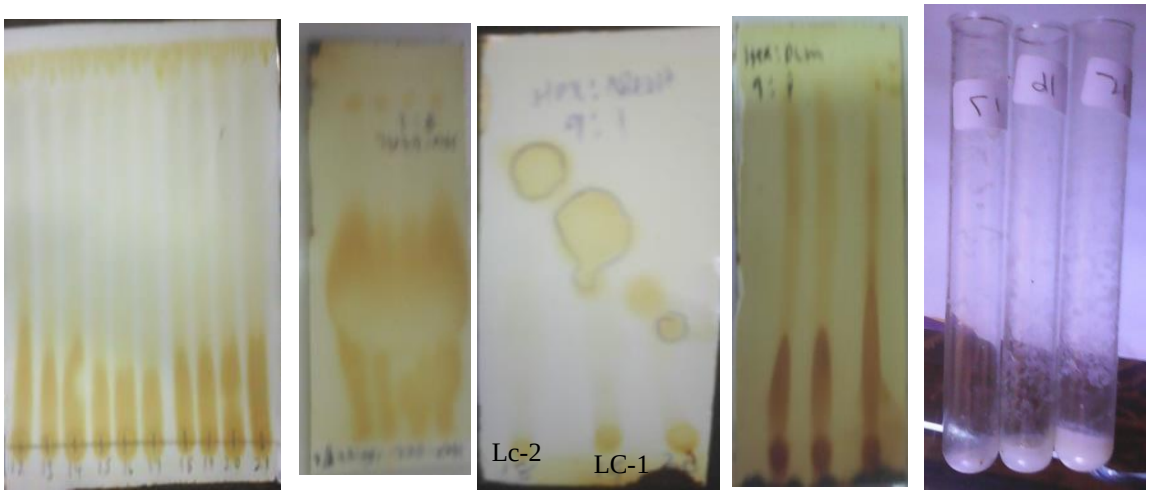
APPENDIX 18: DCM AND ETHYL ACETATE EXTRACTS OF A .lahai.

APPENDIX 19: EXTRACTS OF L . calostachys

hexane, Dcm and ethyl acetate extracts of *L . calostachys*

APPENDIX 20: TLC PLATES OF L. calostachys

Hexane fractions of *L. calostachys* and white oily compound (LC-1) and white solid compound (LC-2)

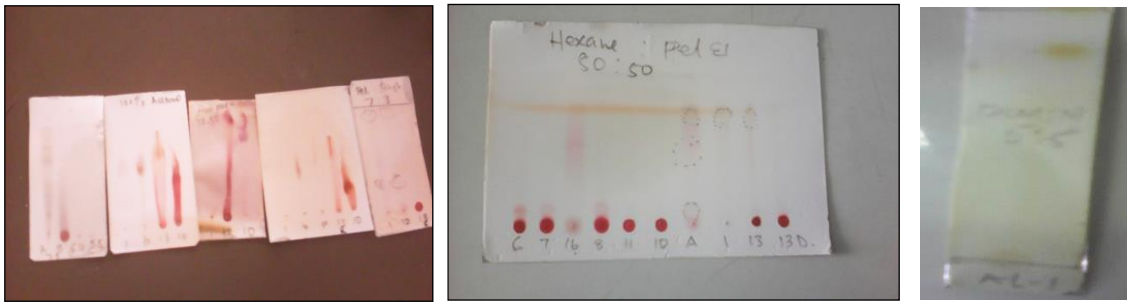


APPENDIX 21:TLC PLATES - C. myricoides

EtOAc fractions of *C. myricoides* and compound Cm-1

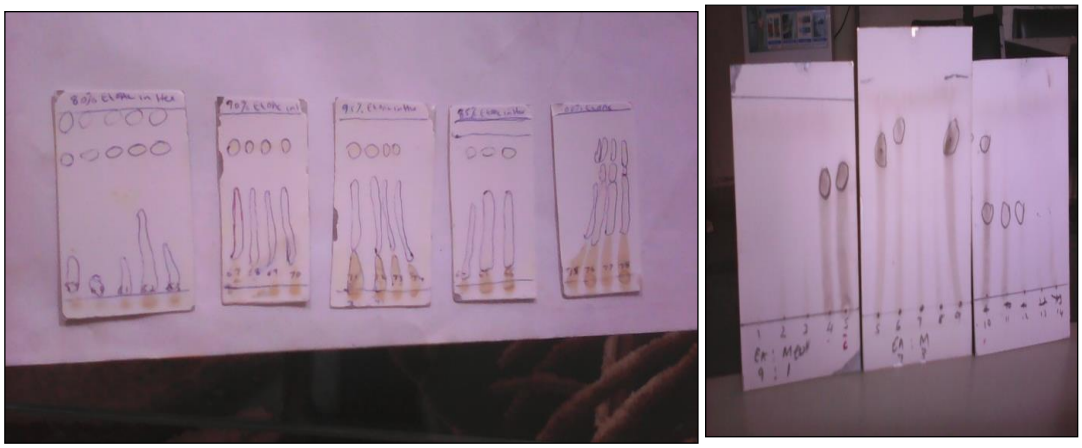
Appendix 4G : TLC PLATES of *A. lahai*

EtOA fractions of *A. lahai* and a single spot on TLC plate showing a pure compound (Al-1)

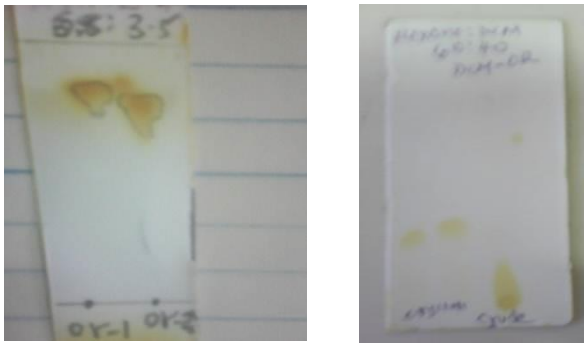


APPENDIX 22: TLC PLATES OF O. rochetiana

for EtOAc fractions of *O. rochetiana*

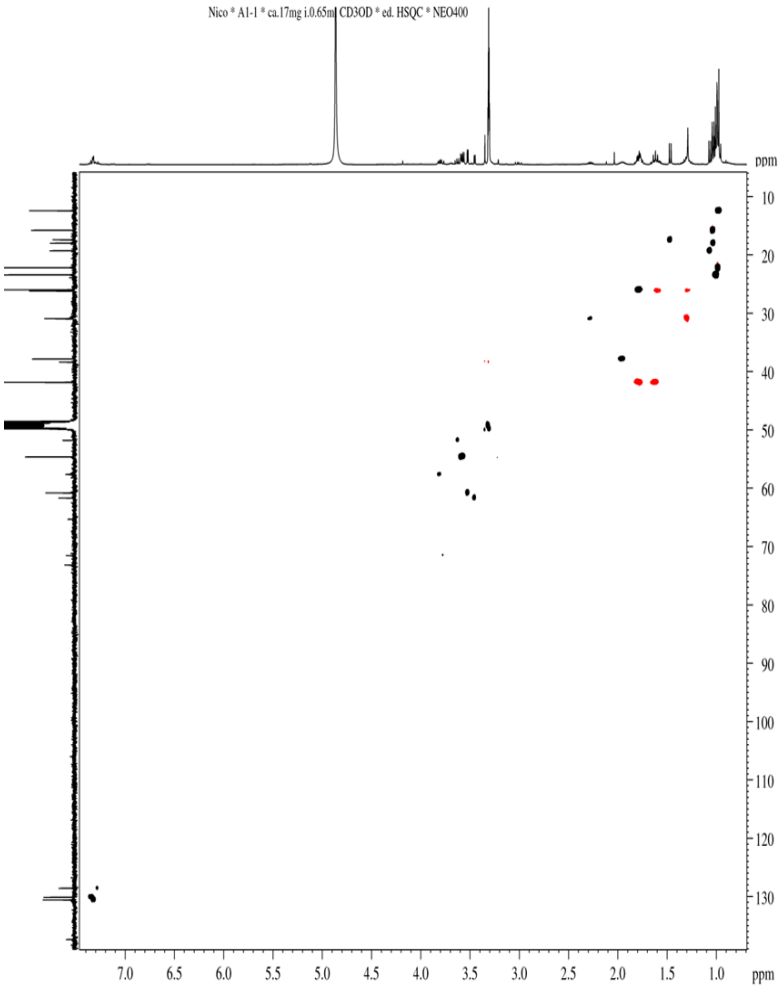


TLC plates showing a from EtOAc of *O. rochetiana*

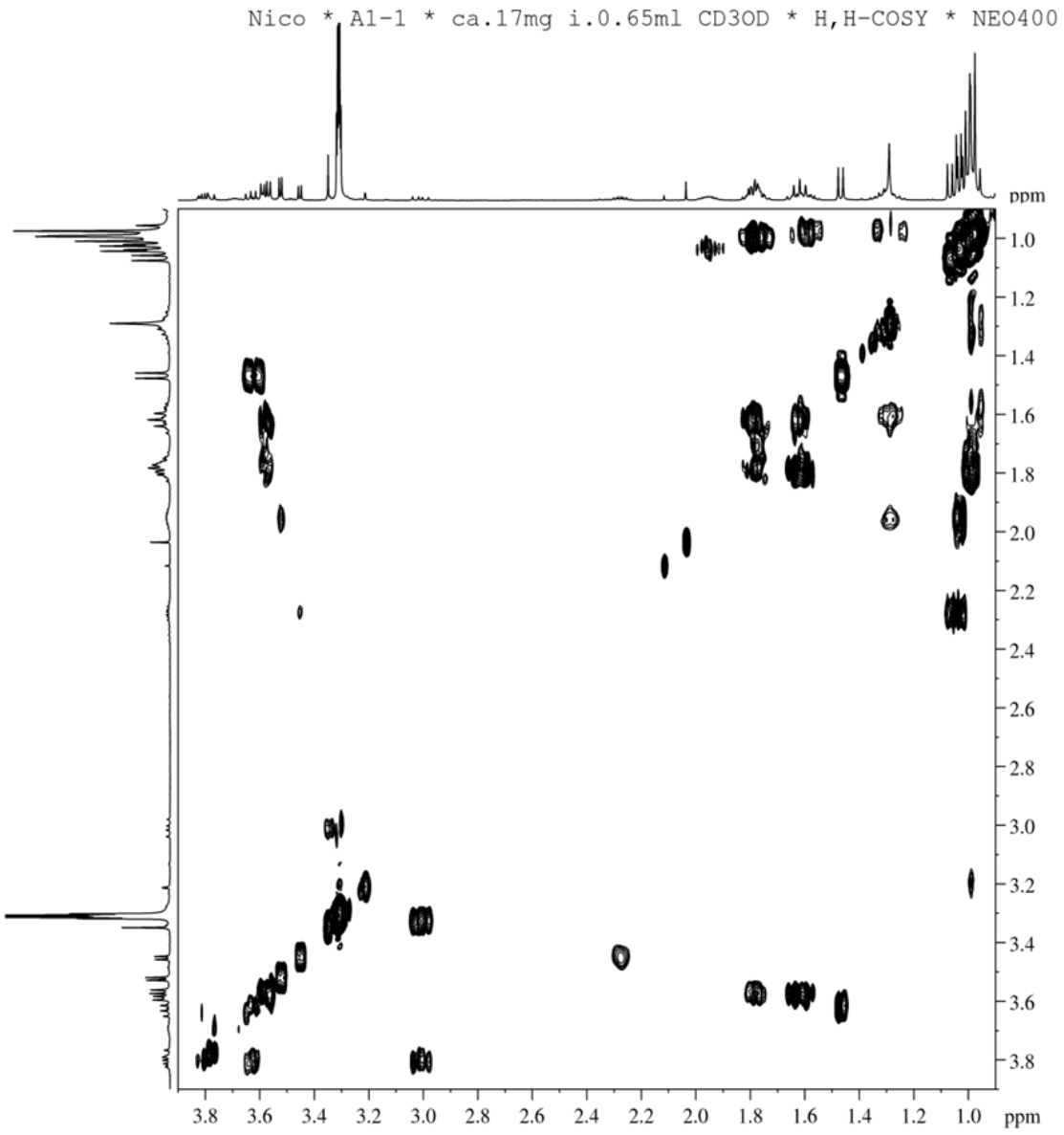


Single spots on TLC plates showing a pure compounds (Or-1 and Or-2) isolated from EtOAc of *O. rochetiana*

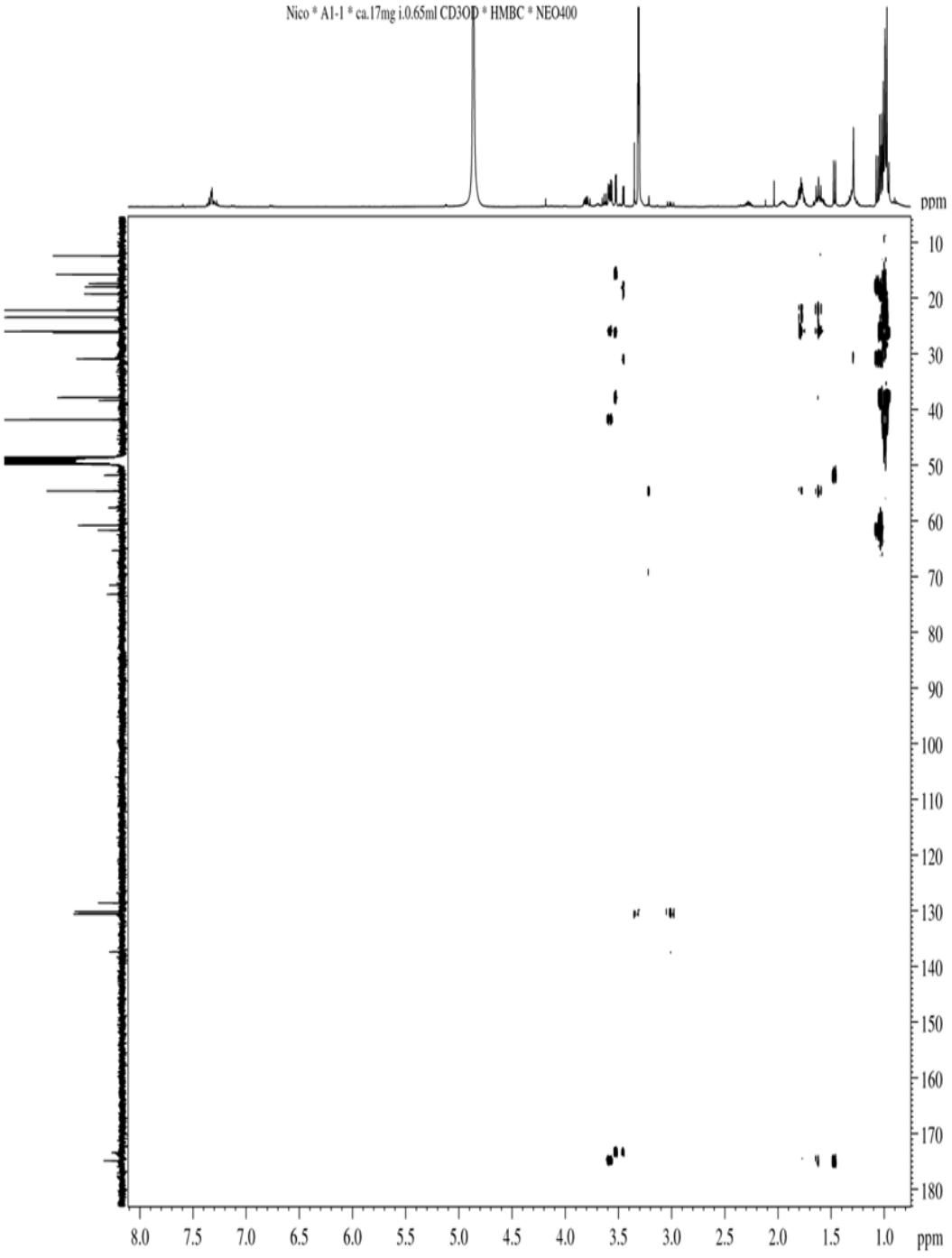
APPENDIX 23: ^{1}H-^{13}C-HSQC spectrum of compound 1



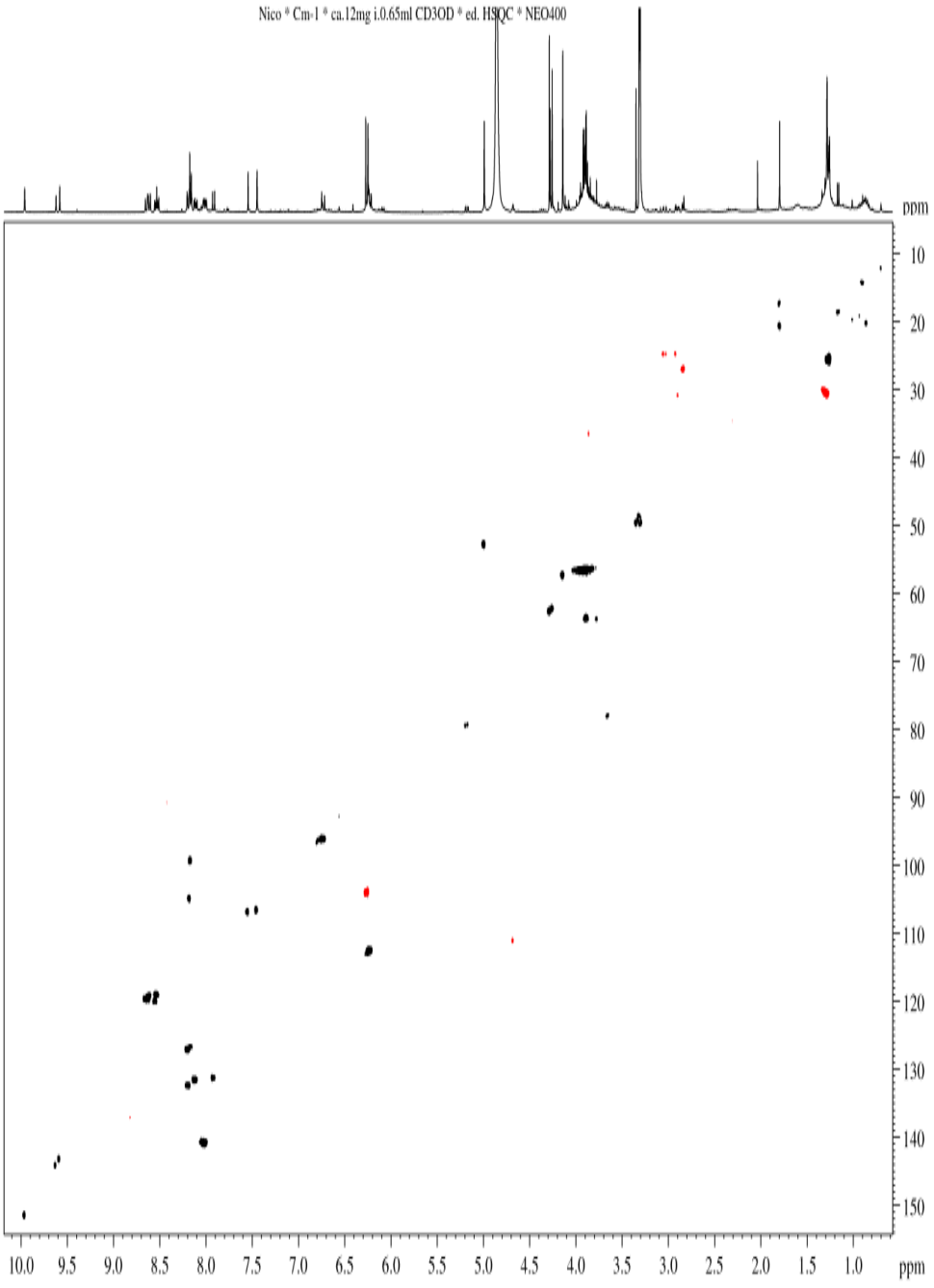
APPENDIX 24: ^{1}H-^{1}H-COSY spectrum for compound 1



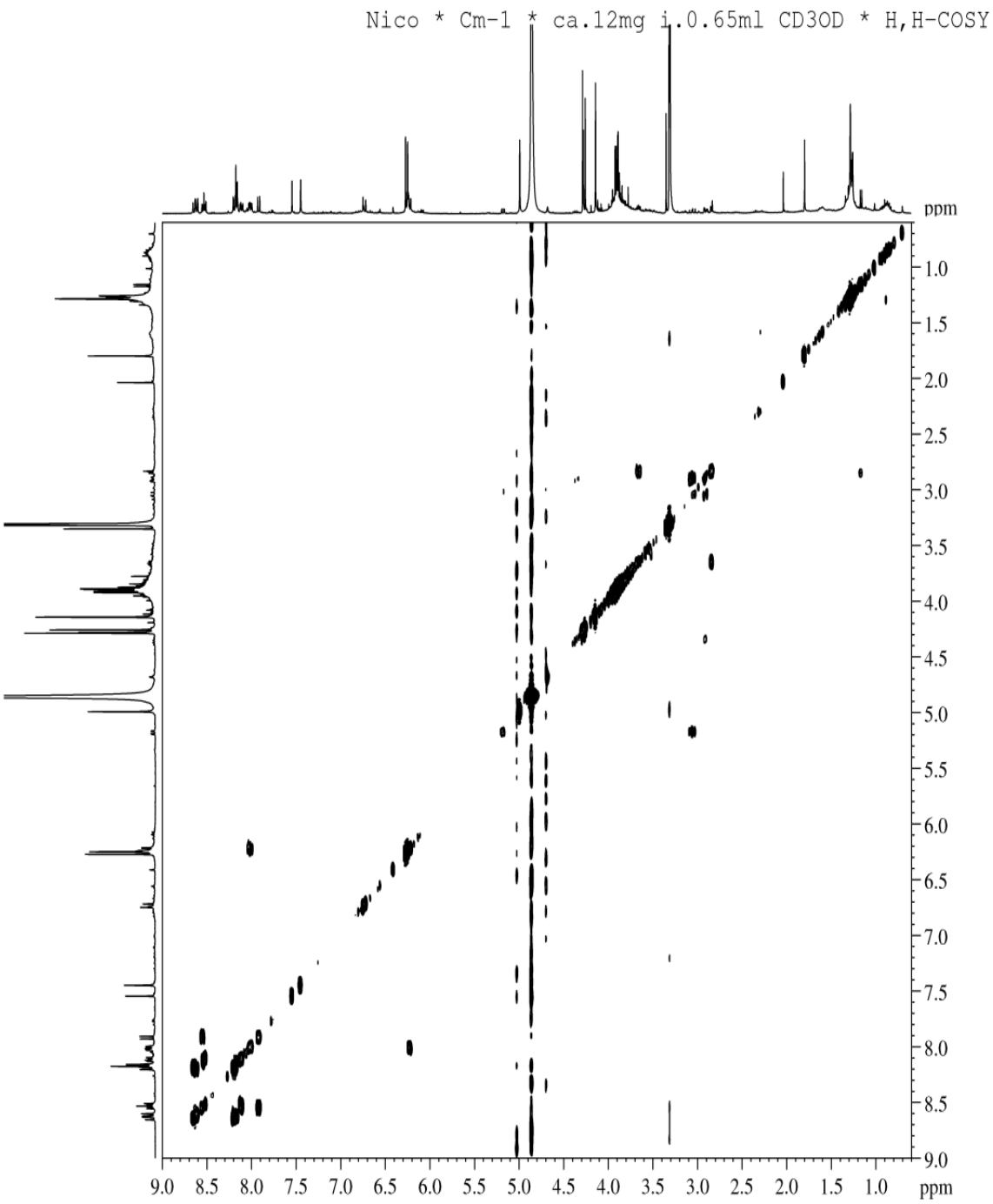
APPENDIX 25: ^{1}H-^{13}C-HMBC spectrum for compound 1



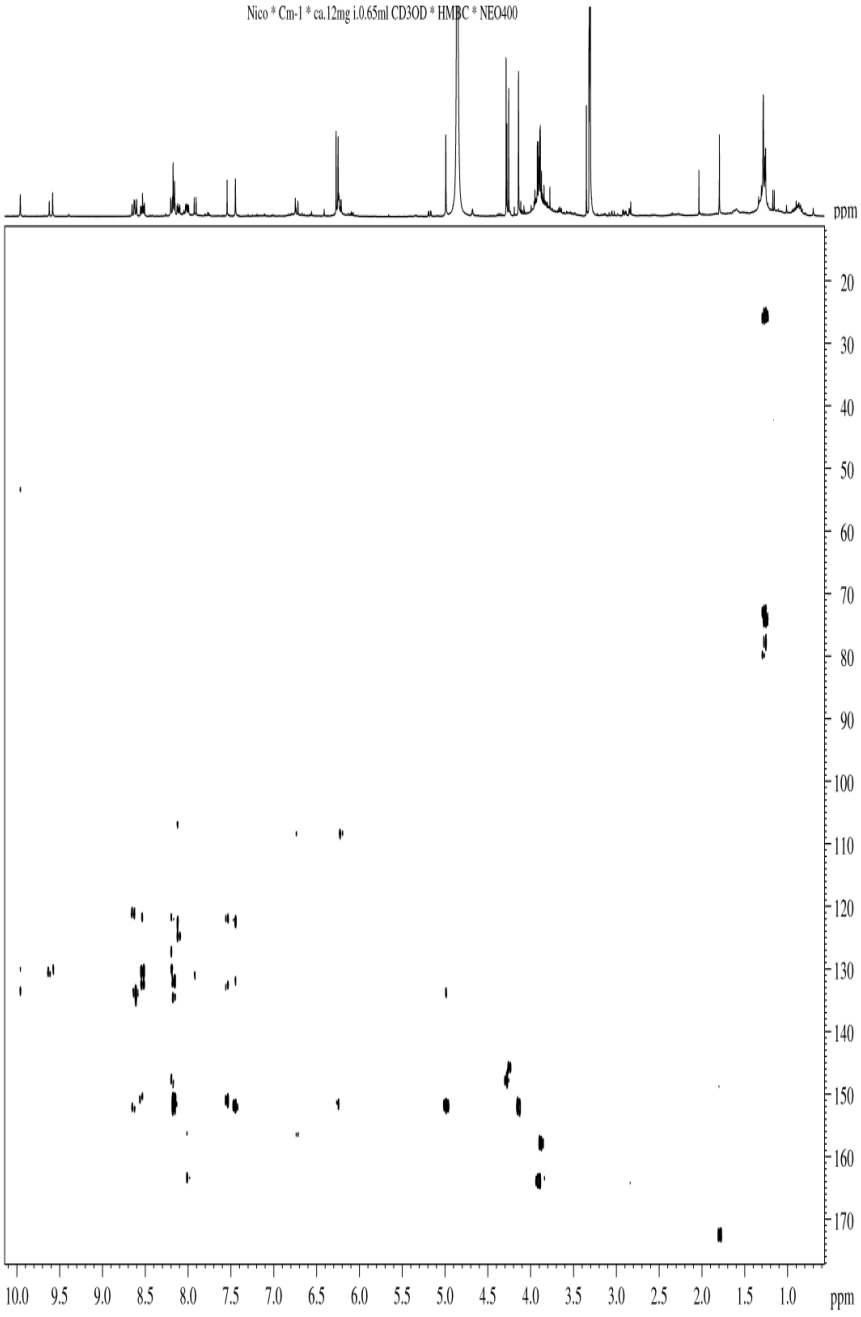
APPENDIX 26: ^{1}H-^{13}C-HSQC spectrum of compound 2



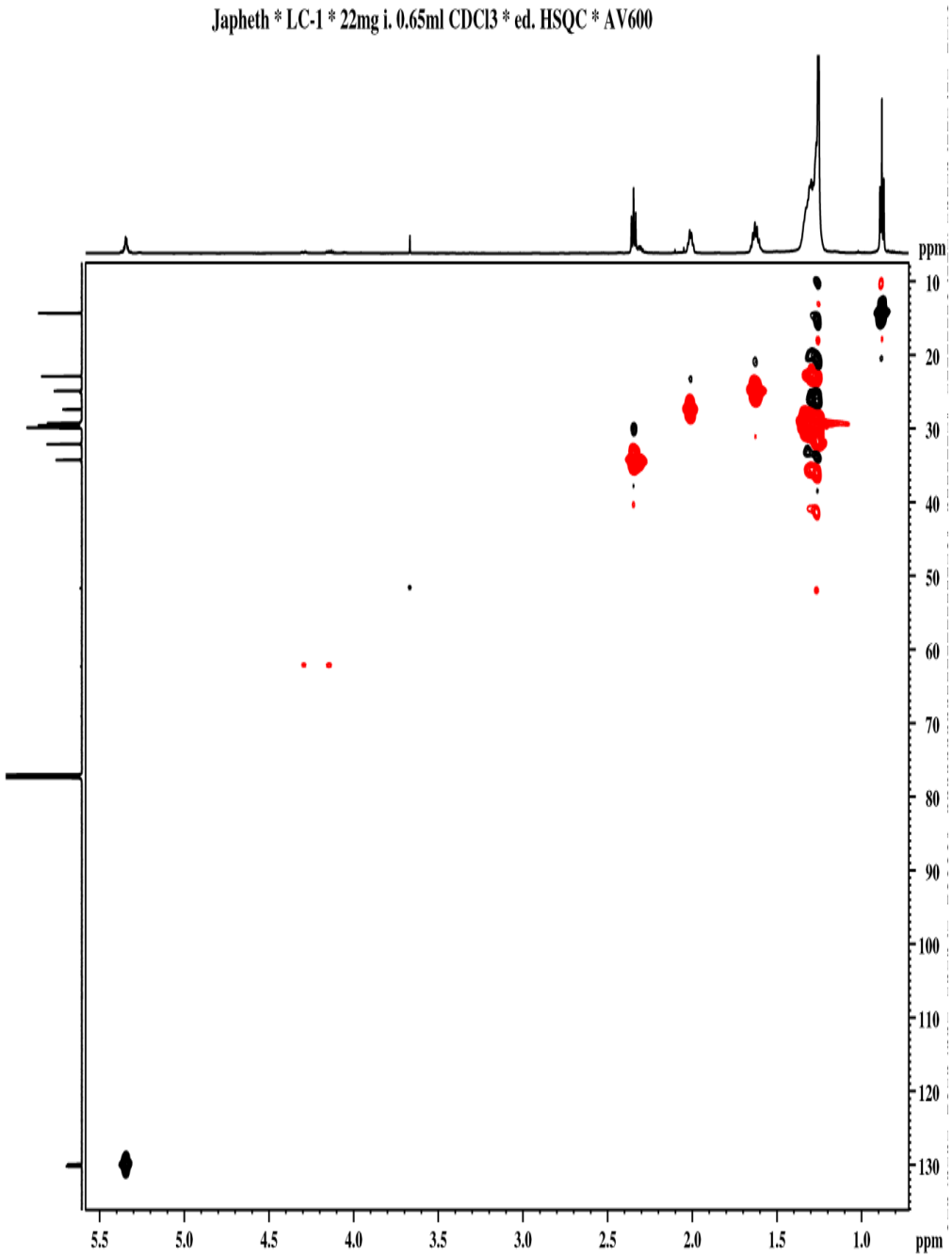
APPENDIX 27: ^{1}H-^{1}H-COSY spectrum of compound 2



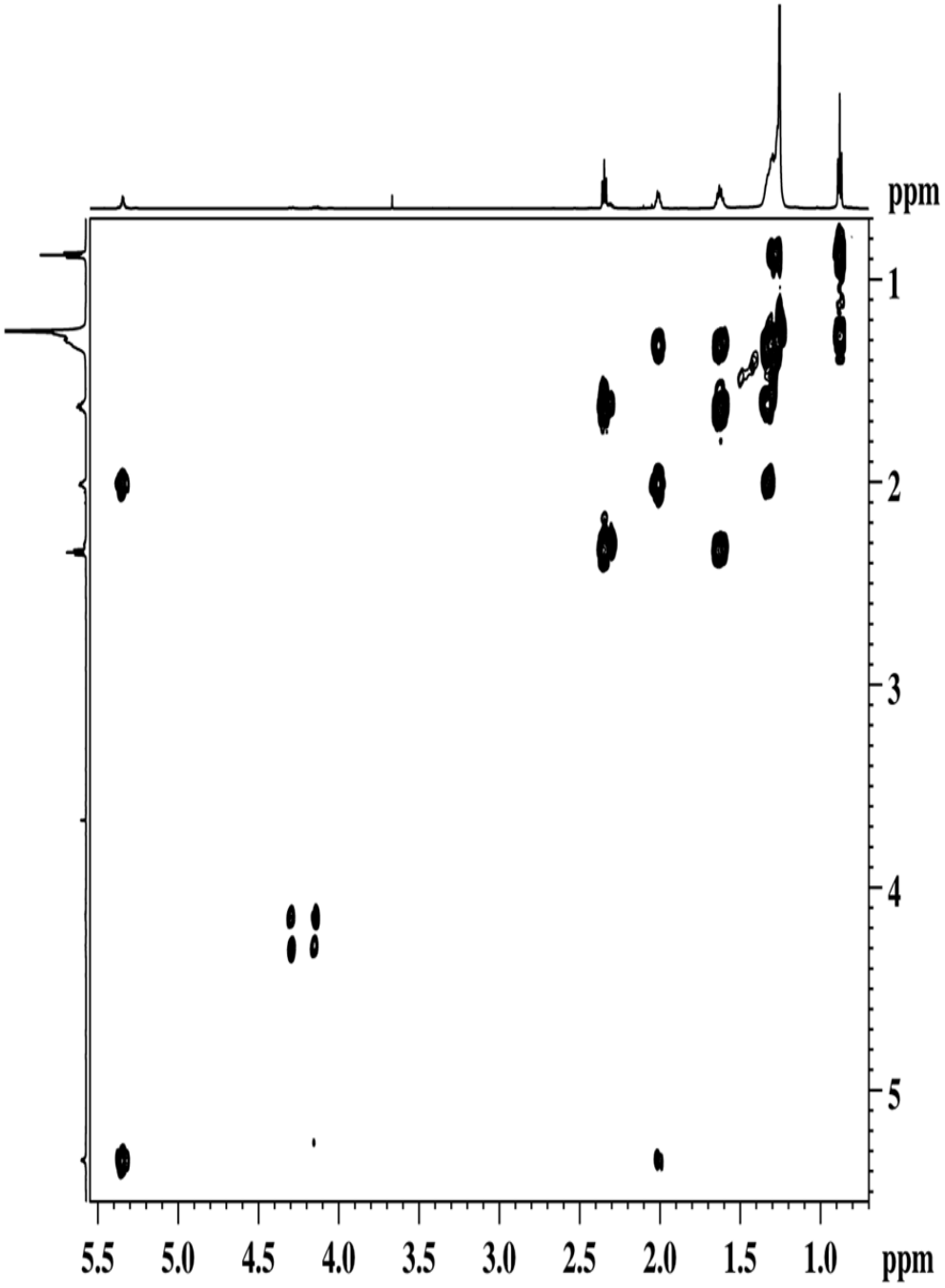
APPENDIX 28: ^{1}H-^{13}C-HMBC spectrum of compound 2



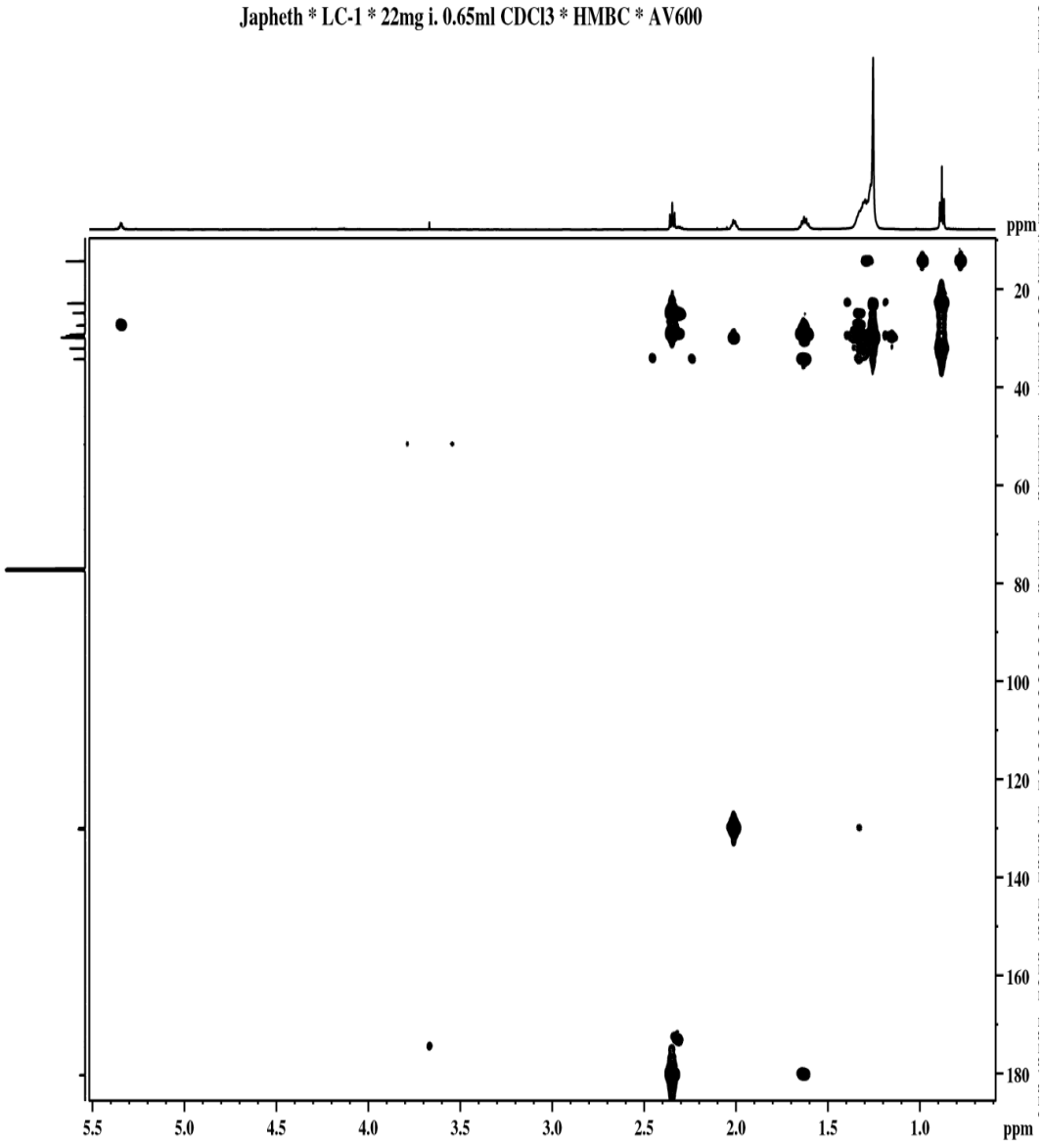
APPENDIX 29: ^{1}H -^{13}C- HSQC spectrum of compound 3



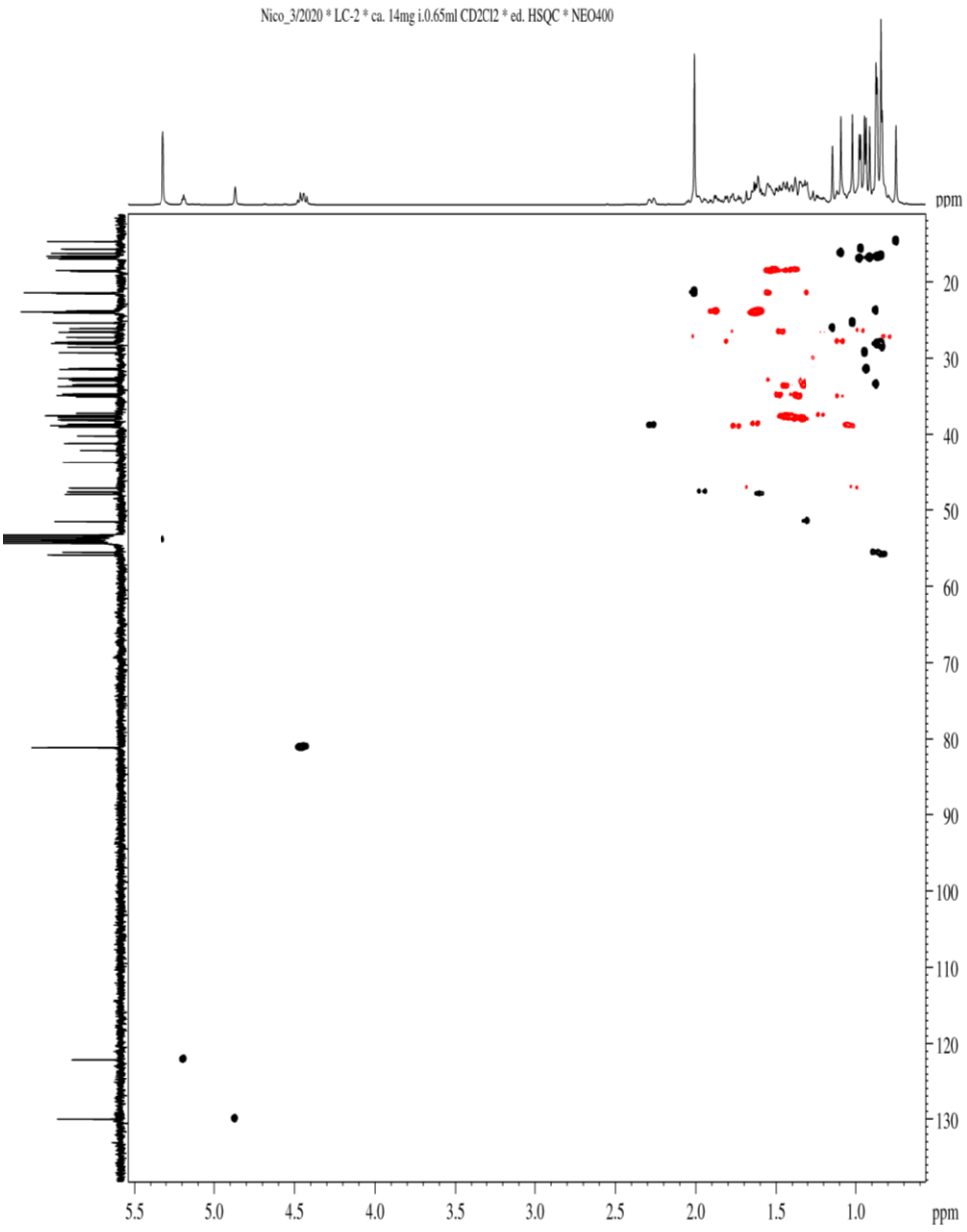
APPENDIX 30: ^{1}H-^{1}H-COSY spectrum of compound 3



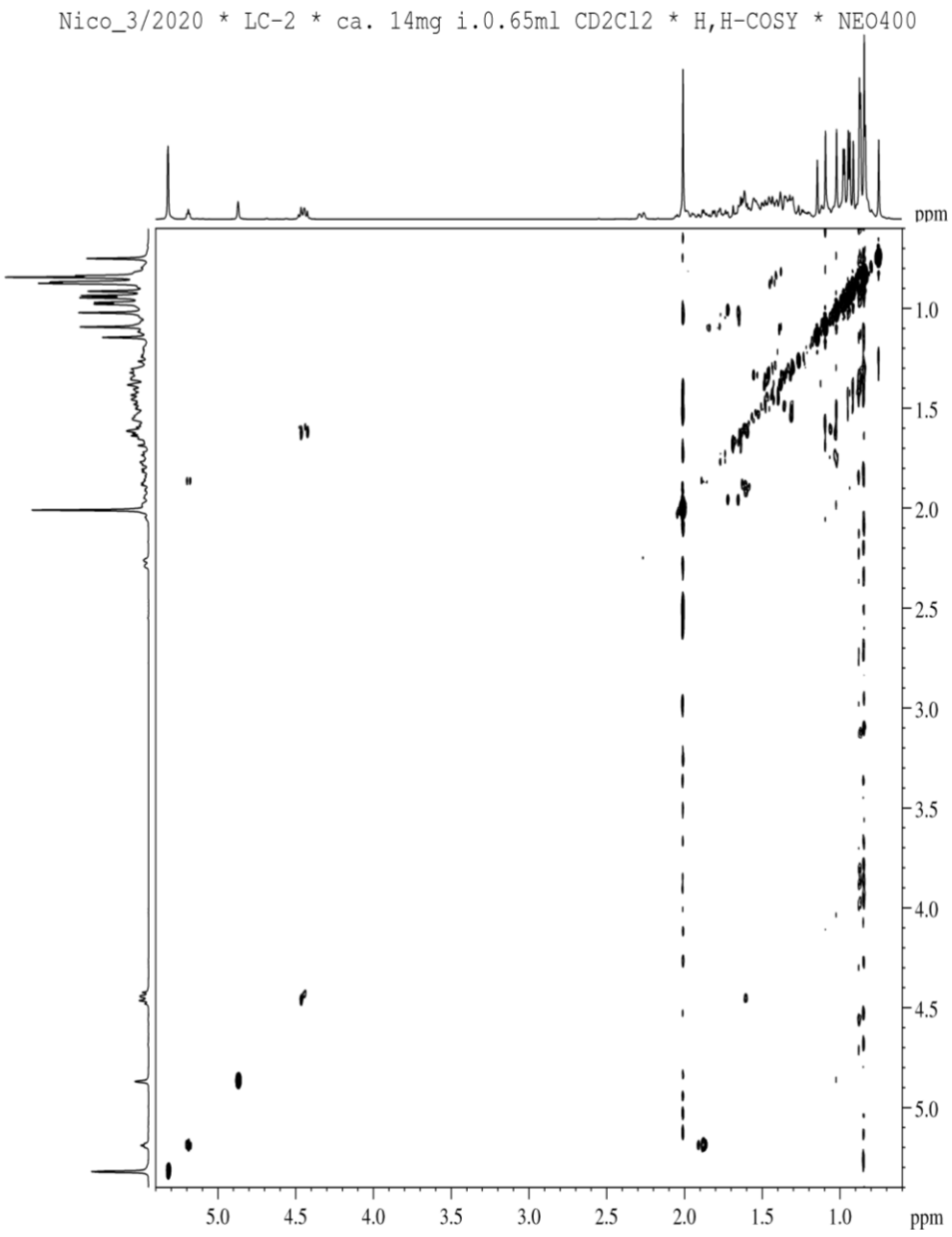
APPENDIX 31: ^{1}H-^{13}C-HMBC spectrum of compound 3



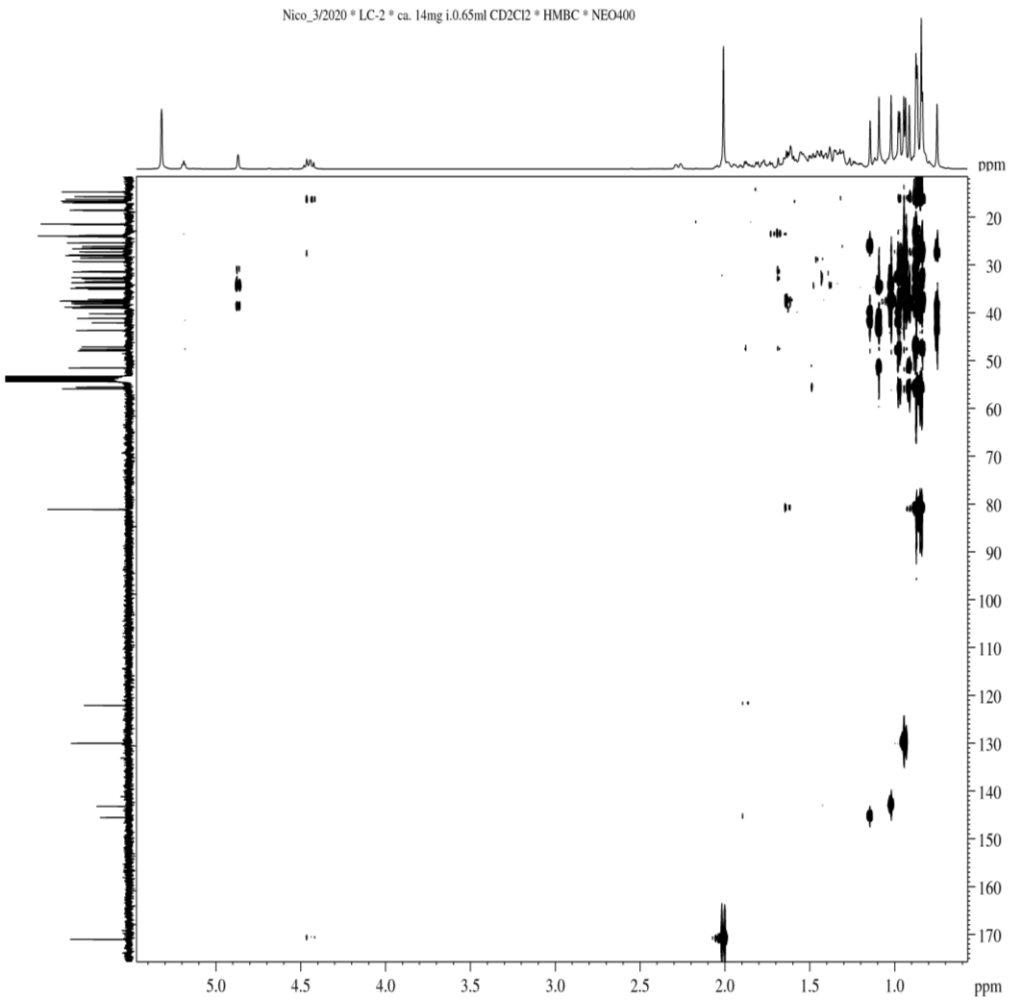
APPENDIX 32: ^{1}H-^{13}C-HSQC spectrum of compound 4



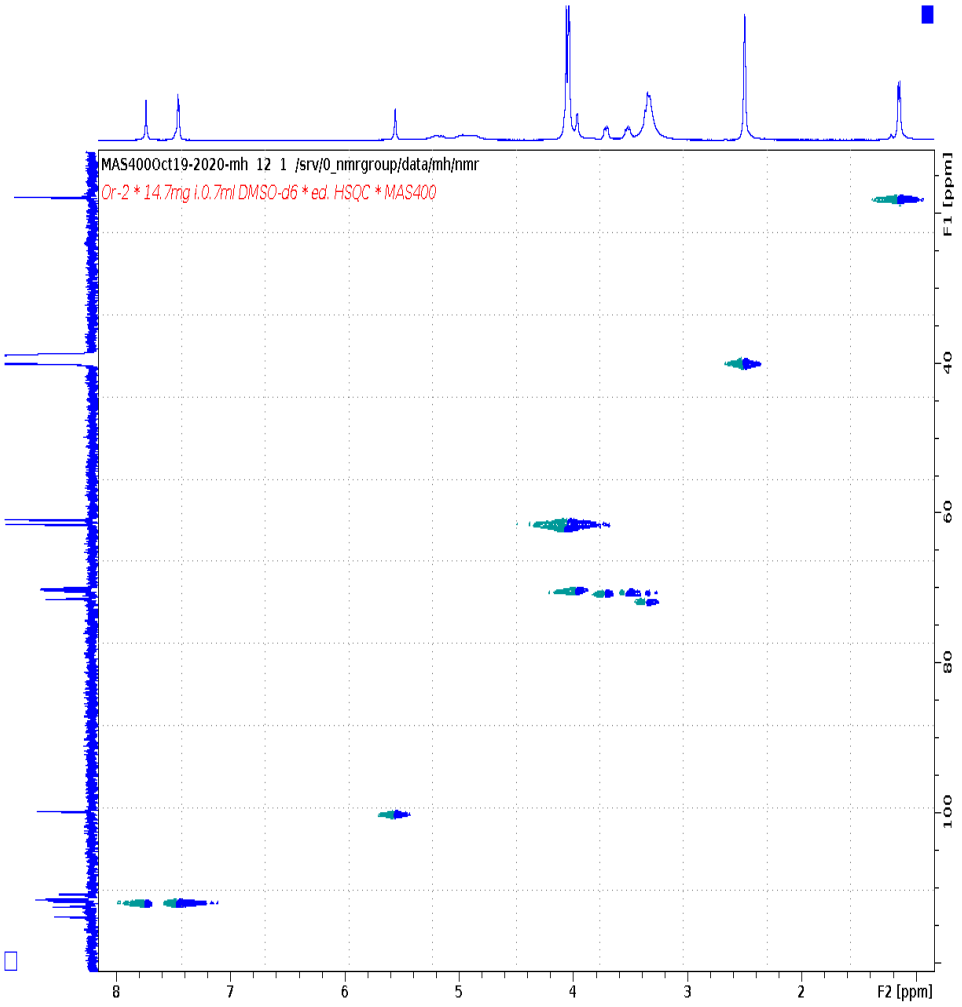
APPENDIX 33: ^{1}H-^{1}H-COSY spectrum of compound 4



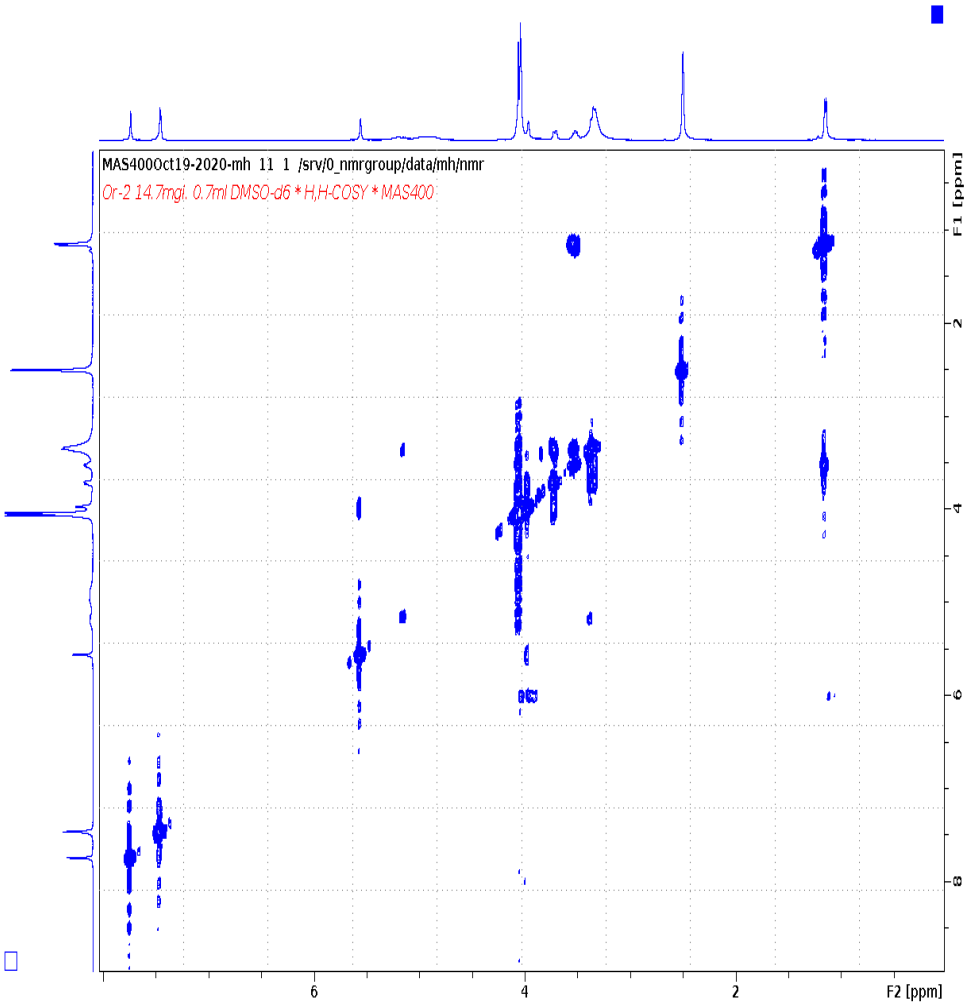
APPENDIX 34: ^{1}H-13C-HMBC spectrum of compound 4



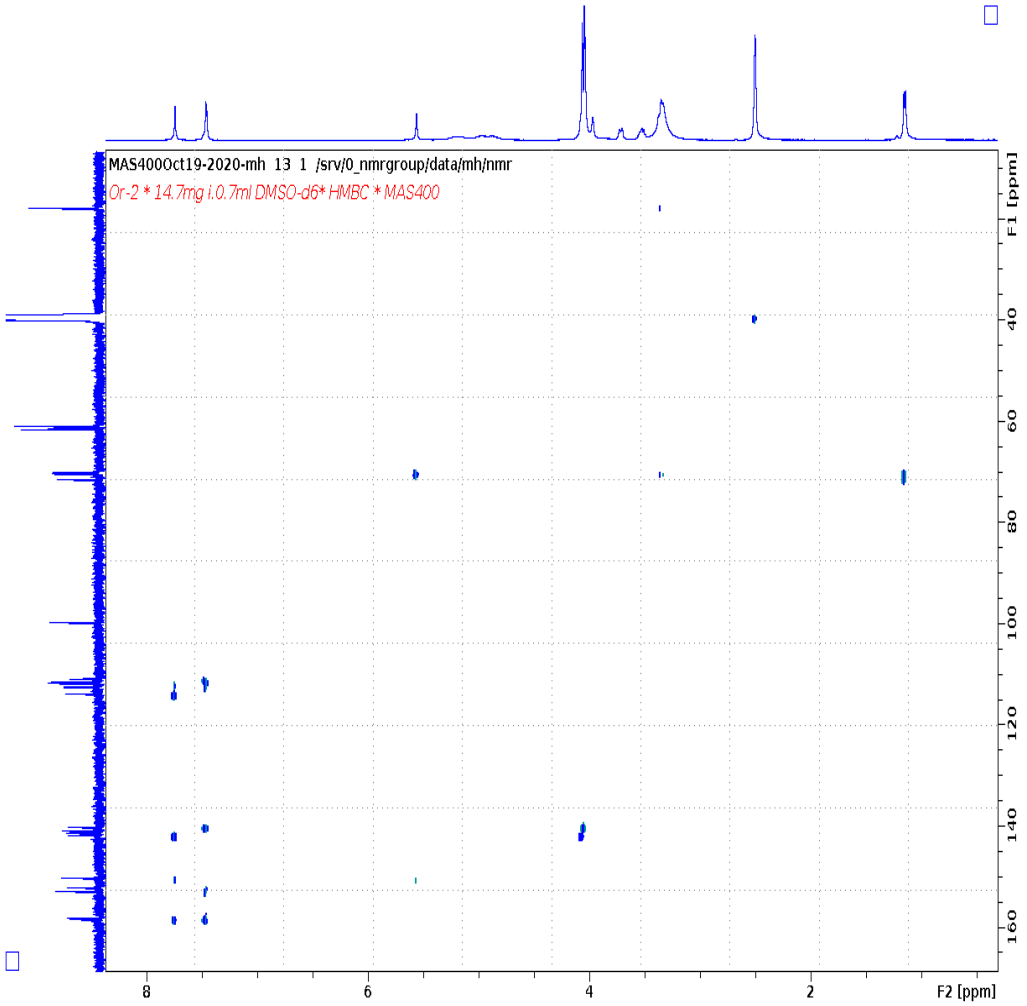
APPENDIX 35: ^{1}H - ^{13}C-HSQC spectrum of compound 5



APPENDIX 36: ^{1}H -^{1}H-COSY spectrum of compound 5



APPENDIX 37: ^{1}H - ^{13}C-HMBC spectrum of compound 5



APPENDIX 38:ANOVA TABLE

Summary of the mean inhibitory zones and *p*-values of the plant extract compared with the positive Control (Gentamicin).

Plant Extract	Mean	Std. Deviation	Sum of Squares	df	Mean Square	F	Sig.
Olinia rochetiana	9.8	5.116	594.05	1	594.05	39.647	.000
Acacia lahai	10.2	4.871	551.25	1	551.25	40.059	.000
Adenia gummifera	9.9	4.654	583.2	1	583.2	45.841	.000
Albicia coriaria	7.5	2.273	871.200	1	871.200	194.561	.000
Erythrinia abyssinica	6.9	1.595	952.2	1	952.2	300.695	.000
Leucas calastachys	8.2	2.741	819.200	1	819.200	169.490	.000
Cleodendrum myricoides	7.9	2.685	884.45	1	884.45	263.142	.000
Lactuca glandulifera	6.6	1.265	994.05	1	994.05	368.926	.000
Urtica mossaica	6.9	1.101	954.818	2	477.409	192.995	.000
Plantago palmate	6.8	1.135	966.05	1	966.05	380.501	.000
Cythula schimperiana	6.2	0.422	1051.25	1	1051.25	530.042	.000
Zehneria minutiflora	6.2	0.422	1051.25	1	1051.25	530.042	.000
Ficus cycamores	7.1	1.197	924.800	1	924.800	354.179	.000
Solanum micranthum	7.1	1.449	924.800	1	924.800	314.083	.000
Vangueria volkensii	7.3	1.160	897.800	1	897.800	349.792	.000
Senecio discifolius	7.1	1.287	924.8	1	924.8	339.722	.000
Sygium cordatum	7.8	2.700	832.050	1	832.050	150.220	.000
Gentamicin	20.7	1.947					

APPENDIX 39: TURKEY POST HOC TEST

Turkey post hoc test

Multiple Comparisons

Dependent Variable: Activity

Tukey HSD

(I) Extract	(J) Extract	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Olinia rochetiana	Acacia lahai	-1.20	1.10	1.00	-5.07	2.67
	Adenia gummifera	-0.10	1.10	1.00	-3.97	3.77
	Albicia coriaria	2.30	1.10	0.79	-1.57	6.17
	Erythrinia abyssinica	2.90	1.10	0.41	-0.97	6.77
	Leucas calastachys	1.90	1.10	0.95	-1.97	5.77
	Cleodendrum myricoides	2.40	1.10	0.74	-1.47	6.27
	Lactuca glandulifera	3.20	1.10	0.24	-0.67	7.07
	Urtica mossaica	2.90	1.10	0.41	-0.97	6.77
	Plantago palmate	3.00	1.10	0.35	-0.87	6.87
	Cythula schimperiana	3.60	1.10	0.10	-0.27	7.47
	Zehneria minutiflora	3.60	1.10	0.10	-0.27	7.47
	Ficus cycamores	2.70	1.10	0.54	-1.17	6.57
	Solanum micranthum	2.70	1.10	0.54	-1.17	6.57
	Vangueria volkensii	2.50	1.10	0.67	-1.37	6.37
	Senecio discifolius	2.70	1.10	0.54	-1.17	6.57
	Sygium cordatum	2.00	1.10	0.92	-1.87	5.87
Acacia lahai	Olinia rochetiana	1.20	1.10	1.00	-2.67	5.07
	Adenia gummifera	1.10	1.10	1.00	-2.77	4.97
	Albicia coriaria	3.50	1.10	0.13	-0.37	7.37
	Erythrinia abyssinica	4.10000*	1.10	0.03	0.23	7.97
	Leucas calastachys	3.10	1.10	0.29	-0.77	6.97
	Cleodendrum myricoides	3.60	1.10	0.10	-0.27	7.47
	Lactuca glandulifera	4.40000*	1.10	0.01	0.53	8.27
	Urtica mossaica	4.10000*	1.10	0.03	0.23	7.97
	Plantago palmate	4.20000*	1.10	0.02	0.33	8.07
	Cythula schimperiana	4.80000*	1.10	0.00	0.93	8.67
	Zehneria minutiflora	4.80000*	1.10	0.00	0.93	8.67
	Ficus cycamores	3.90000*	1.10	0.05	0.03	7.77
	Solanum micranthum	3.90000*	1.10	0.05	0.03	7.77
	Vangueria volkensii	3.70	1.10	0.08	-0.17	7.57
	Senecio discifolius	3.90000*	1.10	0.05	0.03	7.77

	Sygium cordatum	3.20	1.10	0.24	-0.67	7.07
Adenia gummifera	Olinia rochetiana	0.10	1.10	1.00	-3.77	3.97
	Acacia lahai	-1.10	1.10	1.00	-4.97	2.77
	Albicia coriaria	2.40	1.10	0.74	-1.47	6.27
	Erythrinia abyssinica	3.00	1.10	0.35	-0.87	6.87
	Leucas calastachys	2.00	1.10	0.92	-1.87	5.87
	Cleodendrum myricoides	2.50	1.10	0.67	-1.37	6.37
	Lactuca glandulifera	3.30	1.10	0.20	-0.57	7.17
	Urtica mossaica	3.00	1.10	0.35	-0.87	6.87
	Plantago palmate	3.10	1.10	0.29	-0.77	6.97
	Cythula schimperiana	3.70	1.10	0.08	-0.17	7.57
	Zehneria minutiflora	3.70	1.10	0.08	-0.17	7.57
	Ficus cycamores	2.80	1.10	0.47	-1.07	6.67
	Solanum micranthum	2.80	1.10	0.47	-1.07	6.67
	Vangueria volkensii	2.60	1.10	0.61	-1.27	6.47
	Senecio discifolius	2.80	1.10	0.47	-1.07	6.67
	Sygium cordatum	2.10	1.10	0.89	-1.77	5.97
Albicia coriaria	Olinia rochetiana	-2.30	1.10	0.79	-6.17	1.57
	Acacia lahai	-3.50	1.10	0.13	-7.37	0.37
	Adenia gummifera	-2.40	1.10	0.74	-6.27	1.47
	Erythrinia abyssinica	0.60	1.10	1.00	-3.27	4.47
	Leucas calastachys	-0.40	1.10	1.00	-4.27	3.47
	Cleodendrum myricoides	0.10	1.10	1.00	-3.77	3.97
	Lactuca glandulifera	0.90	1.10	1.00	-2.97	4.77
	Urtica mossaica	0.60	1.10	1.00	-3.27	4.47
	Plantago palmate	0.70	1.10	1.00	-3.17	4.57
	Cythula schimperiana	1.30	1.10	1.00	-2.57	5.17
	Zehneria minutiflora	1.30	1.10	1.00	-2.57	5.17
	Ficus cycamores	0.40	1.10	1.00	-3.47	4.27
	Solanum micranthum	0.40	1.10	1.00	-3.47	4.27
	Vangueria volkensii	0.20	1.10	1.00	-3.67	4.07
	Senecio discifolius	0.40	1.10	1.00	-3.47	4.27
	Sygium cordatum	-0.30	1.10	1.00	-4.17	3.57
Erythrinia abyssinica	Olinia rochetiana	-2.90	1.10	0.41	-6.77	0.97
	Acacia lahai	-4.10000*	1.10	0.03	-7.97	-0.23
	Adenia gummifera	-3.00	1.10	0.35	-6.87	0.87
	Albicia coriaria	-0.60	1.10	1.00	-4.47	3.27
	Leucas calastachys	-1.00	1.10	1.00	-4.87	2.87
	Cleodendrum myricoides	-0.50	1.10	1.00	-4.37	3.37
	Lactuca glandulifera	0.30	1.10	1.00	-3.57	4.17
	Urtica mossaica	0.00	1.10	1.00	-3.87	3.87
	Plantago palmate	0.10	1.10	1.00	-3.77	3.97

	Cythula schimperiana	0.70	1.10	1.00	-3.17	4.57
	Zehneria minutiflora	0.70	1.10	1.00	-3.17	4.57
	Ficus cycamores	-0.20	1.10	1.00	-4.07	3.67
	Solanum micranthum	-0.20	1.10	1.00	-4.07	3.67
	Vangueria volkensii	-0.40	1.10	1.00	-4.27	3.47
	Senecio discifolius	-0.20	1.10	1.00	-4.07	3.67
	Sygium cordatum	-0.90	1.10	1.00	-4.77	2.97
Leucas calastachys	Olinia rochetiana	-1.90	1.10	0.95	-5.77	1.97
	Acacia lahai	-3.10	1.10	0.29	-6.97	0.77
	Adenia gummifera	-2.00	1.10	0.92	-5.87	1.87
	Albicia coriaria	0.40	1.10	1.00	-3.47	4.27
	Erythrinia abyssinica	1.00	1.10	1.00	-2.87	4.87
	Cleodendrum myricoides	0.50	1.10	1.00	-3.37	4.37
	Lactuca glandulifera	1.30	1.10	1.00	-2.57	5.17
	Urtica mossaica	1.00	1.10	1.00	-2.87	4.87
	Plantago palmate	1.10	1.10	1.00	-2.77	4.97
	Cythula schimperiana	1.70	1.10	0.98	-2.17	5.57
	Zehneria minutiflora	1.70	1.10	0.98	-2.17	5.57
	Ficus cycamores	0.80	1.10	1.00	-3.07	4.67
	Solanum micranthum	0.80	1.10	1.00	-3.07	4.67
	Vangueria volkensii	0.60	1.10	1.00	-3.27	4.47
	Senecio discifolius	0.80	1.10	1.00	-3.07	4.67
	Sygium cordatum	0.10	1.10	1.00	-3.77	3.97
Cleodendrum myricoides	Olinia rochetiana	-2.40	1.10	0.74	-6.27	1.47
	Acacia lahai	-3.60	1.10	0.10	-7.47	0.27
	Adenia gummifera	-2.50	1.10	0.67	-6.37	1.37
	Albicia coriaria	-0.10	1.10	1.00	-3.97	3.77
	Erythrinia abyssinica	0.50	1.10	1.00	-3.37	4.37
	Leucas calastachys	-0.50	1.10	1.00	-4.37	3.37
	Lactuca glandulifera	0.80	1.10	1.00	-3.07	4.67
	Urtica mossaica	0.50	1.10	1.00	-3.37	4.37
	Plantago palmate	0.60	1.10	1.00	-3.27	4.47
	Cythula schimperiana	1.20	1.10	1.00	-2.67	5.07
	Zehneria minutiflora	1.20	1.10	1.00	-2.67	5.07
	Ficus cycamores	0.30	1.10	1.00	-3.57	4.17
	Solanum micranthum	0.30	1.10	1.00	-3.57	4.17
	Vangueria volkensii	0.10	1.10	1.00	-3.77	3.97
	Senecio discifolius	0.30	1.10	1.00	-3.57	4.17
	Sygium cordatum	-0.40	1.10	1.00	-4.27	3.47
Lactuca glandulifera	Olinia rochetiana	-3.20	1.10	0.24	-7.07	0.67
	Acacia lahai	-4.40000*	1.10	0.01	-8.27	-0.53
	Adenia gummifera	-3.30	1.10	0.20	-7.17	0.57

	Albicia coriaria	-0.90	1.10	1.00	-4.77	2.97
	Erythrinia abyssinica	-0.30	1.10	1.00	-4.17	3.57
	Leucas calastachys	-1.30	1.10	1.00	-5.17	2.57
	Cleodendrum myricoides	-0.80	1.10	1.00	-4.67	3.07
	Urtica mossaica	-0.30	1.10	1.00	-4.17	3.57
	Plantago palmate	-0.20	1.10	1.00	-4.07	3.67
	Cythula schimperiana	0.40	1.10	1.00	-3.47	4.27
	Zehneria minutiflora	0.40	1.10	1.00	-3.47	4.27
	Ficus cycamores	-0.50	1.10	1.00	-4.37	3.37
	Solanum micranthum	-0.50	1.10	1.00	-4.37	3.37
	Vangueria volkensii	-0.70	1.10	1.00	-4.57	3.17
	Senecio discifolius	-0.50	1.10	1.00	-4.37	3.37
	Sygium cordatum	-1.20	1.10	1.00	-5.07	2.67
Urtica mossaica	Olinia rochetiana	-2.90	1.10	0.41	-6.77	0.97
	Acacia lahai	-4.10000*	1.10	0.03	-7.97	-0.23
	Adenia gummifera	-3.00	1.10	0.35	-6.87	0.87
	Albicia coriaria	-0.60	1.10	1.00	-4.47	3.27
	Erythrinia abyssinica	0.00	1.10	1.00	-3.87	3.87
	Leucas calastachys	-1.00	1.10	1.00	-4.87	2.87
	Cleodendrum myricoides	-0.50	1.10	1.00	-4.37	3.37
	Lactuca glandulifera	0.30	1.10	1.00	-3.57	4.17
	Plantago palmate	0.10	1.10	1.00	-3.77	3.97
	Cythula schimperiana	0.70	1.10	1.00	-3.17	4.57
	Zehneria minutiflora	0.70	1.10	1.00	-3.17	4.57
	Ficus cycamores	-0.20	1.10	1.00	-4.07	3.67
	Solanum micranthum	-0.20	1.10	1.00	-4.07	3.67
	Vangueria volkensii	-0.40	1.10	1.00	-4.27	3.47
	Senecio discifolius	-0.20	1.10	1.00	-4.07	3.67
	Sygium cordatum	-0.90	1.10	1.00	-4.77	2.97
Plantago palmate	Olinia rochetiana	-3.00	1.10	0.35	-6.87	0.87
	Acacia lahai	-4.20000*	1.10	0.02	-8.07	-0.33
	Adenia gummifera	-3.10	1.10	0.29	-6.97	0.77
	Albicia coriaria	-0.70	1.10	1.00	-4.57	3.17
	Erythrinia abyssinica	-0.10	1.10	1.00	-3.97	3.77
	Leucas calastachys	-1.10	1.10	1.00	-4.97	2.77
	Cleodendrum myricoides	-0.60	1.10	1.00	-4.47	3.27
	Lactuca glandulifera	0.20	1.10	1.00	-3.67	4.07
	Urtica mossaica	-0.10	1.10	1.00	-3.97	3.77
	Cythula schimperiana	0.60	1.10	1.00	-3.27	4.47
	Zehneria minutiflora	0.60	1.10	1.00	-3.27	4.47
	Ficus cycamores	-0.30	1.10	1.00	-4.17	3.57
	Solanum micranthum	-0.30	1.10	1.00	-4.17	3.57

	Vangueria volkensii	-0.50	1.10	1.00	-4.37	3.37
	Senecio discifolius	-0.30	1.10	1.00	-4.17	3.57
	Sygium cordatum	-1.00	1.10	1.00	-4.87	2.87
Cythula schimperiana	Olinia rochetiana	-3.60	1.10	0.10	-7.47	0.27
	Acacia lahai	-4.80000*	1.10	0.00	-8.67	-0.93
	Adenia gummifera	-3.70	1.10	0.08	-7.57	0.17
	Albicia coriaria	-1.30	1.10	1.00	-5.17	2.57
	Erythrinia abyssinica	-0.70	1.10	1.00	-4.57	3.17
	Leucas calastachys	-1.70	1.10	0.98	-5.57	2.17
	Cleodendrum myricoides	-1.20	1.10	1.00	-5.07	2.67
	Lactuca glandulifera	-0.40	1.10	1.00	-4.27	3.47
	Urtica mossaica	-0.70	1.10	1.00	-4.57	3.17
	Plantago palmate	-0.60	1.10	1.00	-4.47	3.27
	Zehneria minutiflora	0.00	1.10	1.00	-3.87	3.87
	Ficus cycamores	-0.90	1.10	1.00	-4.77	2.97
	Solanum micranthum	-0.90	1.10	1.00	-4.77	2.97
	Vangueria volkensii	-1.10	1.10	1.00	-4.97	2.77
	Senecio discifolius	-0.90	1.10	1.00	-4.77	2.97
	Sygium cordatum	-1.60	1.10	0.99	-5.47	2.27
Zehneria minutiflora	Olinia rochetiana	-3.60	1.10	0.10	-7.47	0.27
	Acacia lahai	-4.80000*	1.10	0.00	-8.67	-0.93
	Adenia gummifera	-3.70	1.10	0.08	-7.57	0.17
	Albicia coriaria	-1.30	1.10	1.00	-5.17	2.57
	Erythrinia abyssinica	-0.70	1.10	1.00	-4.57	3.17
	Leucas calastachys	-1.70	1.10	0.98	-5.57	2.17
	Cleodendrum myricoides	-1.20	1.10	1.00	-5.07	2.67
	Lactuca glandulifera	-0.40	1.10	1.00	-4.27	3.47
	Urtica mossaica	-0.70	1.10	1.00	-4.57	3.17
	Plantago palmate	-0.60	1.10	1.00	-4.47	3.27
	Cythula schimperiana	0.00	1.10	1.00	-3.87	3.87
	Ficus cycamores	-0.90	1.10	1.00	-4.77	2.97
	Solanum micranthum	-0.90	1.10	1.00	-4.77	2.97
	Vangueria volkensii	-1.10	1.10	1.00	-4.97	2.77
	Senecio discifolius	-0.90	1.10	1.00	-4.77	2.97
	Sygium cordatum	-1.60	1.10	0.99	-5.47	2.27
Ficus cycamores	Olinia rochetiana	-2.70	1.10	0.54	-6.57	1.17
	Acacia lahai	-3.90000*	1.10	0.05	-7.77	-0.03
	Adenia gummifera	-2.80	1.10	0.47	-6.67	1.07
	Albicia coriaria	-0.40	1.10	1.00	-4.27	3.47
	Erythrinia abyssinica	0.20	1.10	1.00	-3.67	4.07
	Leucas calastachys	-0.80	1.10	1.00	-4.67	3.07
	Cleodendrum myricoides	-0.30	1.10	1.00	-4.17	3.57

	Lactuca glandulifera	0.50	1.10	1.00	-3.37	4.37
	Urtica mossaica	0.20	1.10	1.00	-3.67	4.07
	Plantago palmate	0.30	1.10	1.00	-3.57	4.17
	Cythula schimperiana	0.90	1.10	1.00	-2.97	4.77
	Zehneria minutiflora	0.90	1.10	1.00	-2.97	4.77
	Solanum micranthum	0.00	1.10	1.00	-3.87	3.87
	Vangueria volkensii	-0.20	1.10	1.00	-4.07	3.67
	Senecio discifolius	0.00	1.10	1.00	-3.87	3.87
	Sygium cordatum	-0.70	1.10	1.00	-4.57	3.17
Solanum micranthum	Olinia rochetiana	-2.70	1.10	0.54	-6.57	1.17
	Acacia lahai	-3.90000*	1.10	0.05	-7.77	-0.03
	Adenia gummifera	-2.80	1.10	0.47	-6.67	1.07
	Albicia coriaria	-0.40	1.10	1.00	-4.27	3.47
	Erythrinia abyssinica	0.20	1.10	1.00	-3.67	4.07
	Leucas calastachys	-0.80	1.10	1.00	-4.67	3.07
	Cleodendrum myricoides	-0.30	1.10	1.00	-4.17	3.57
	Lactuca glandulifera	0.50	1.10	1.00	-3.37	4.37
	Urtica mossaica	0.20	1.10	1.00	-3.67	4.07
	Plantago palmate	0.30	1.10	1.00	-3.57	4.17
	Cythula schimperiana	0.90	1.10	1.00	-2.97	4.77
	Zehneria minutiflora	0.90	1.10	1.00	-2.97	4.77
	Ficus cycamores	0.00	1.10	1.00	-3.87	3.87
	Vangueria volkensii	-0.20	1.10	1.00	-4.07	3.67
	Senecio discifolius	0.00	1.10	1.00	-3.87	3.87
	Sygium cordatum	-0.70	1.10	1.00	-4.57	3.17
Vangueria volkensii	Olinia rochetiana	-2.50	1.10	0.67	-6.37	1.37
	Acacia lahai	-3.70	1.10	0.08	-7.57	0.17
	Adenia gummifera	-2.60	1.10	0.61	-6.47	1.27
	Albicia coriaria	-0.20	1.10	1.00	-4.07	3.67
	Erythrinia abyssinica	0.40	1.10	1.00	-3.47	4.27
	Leucas calastachys	-0.60	1.10	1.00	-4.47	3.27
	Cleodendrum myricoides	-0.10	1.10	1.00	-3.97	3.77
	Lactuca glandulifera	0.70	1.10	1.00	-3.17	4.57
	Urtica mossaica	0.40	1.10	1.00	-3.47	4.27
	Plantago palmate	0.50	1.10	1.00	-3.37	4.37
	Cythula schimperiana	1.10	1.10	1.00	-2.77	4.97
	Zehneria minutiflora	1.10	1.10	1.00	-2.77	4.97
	Ficus cycamores	0.20	1.10	1.00	-3.67	4.07
	Solanum micranthum	0.20	1.10	1.00	-3.67	4.07
	Senecio discifolius	0.20	1.10	1.00	-3.67	4.07
	Sygium cordatum	-0.50	1.10	1.00	-4.37	3.37
Senecio discifolius	Olinia rochetiana	-2.70	1.10	0.54	-6.57	1.17

	Acacia lahai	-3.90000*	1.10	0.05	-7.77	-0.03
	Adenia gummifera	-2.80	1.10	0.47	-6.67	1.07
	Albicia coriaria	-0.40	1.10	1.00	-4.27	3.47
	Erythrinia abyssinica	0.20	1.10	1.00	-3.67	4.07
	Leucas calastachys	-0.80	1.10	1.00	-4.67	3.07
	Cleodendrum myricoides	-0.30	1.10	1.00	-4.17	3.57
	Lactuca glandulifera	0.50	1.10	1.00	-3.37	4.37
	Urtica mossaica	0.20	1.10	1.00	-3.67	4.07
	Plantago palmate	0.30	1.10	1.00	-3.57	4.17
	Cythula schimperiana	0.90	1.10	1.00	-2.97	4.77
	Zehneria minutiflora	0.90	1.10	1.00	-2.97	4.77
	Ficus cycamores	0.00	1.10	1.00	-3.87	3.87
	Solanum micranthum	0.00	1.10	1.00	-3.87	3.87
	Vangueria volkensii	-0.20	1.10	1.00	-4.07	3.67
	Sygium cordatum	-0.70	1.10	1.00	-4.57	3.17
Sygium cordatum	Olinia rochetiana	-2.00	1.10	0.92	-5.87	1.87
	Acacia lahai	-3.20	1.10	0.24	-7.07	0.67
	Adenia gummifera	-2.10	1.10	0.89	-5.97	1.77
	Albicia coriaria	0.30	1.10	1.00	-3.57	4.17
	Erythrinia abyssinica	0.90	1.10	1.00	-2.97	4.77
	Leucas calastachys	-0.10	1.10	1.00	-3.97	3.77
	Cleodendrum myricoides	0.40	1.10	1.00	-3.47	4.27
	Lactuca glandulifera	1.20	1.10	1.00	-2.67	5.07
	Urtica mossaica	0.90	1.10	1.00	-2.97	4.77
	Plantago palmate	1.00	1.10	1.00	-2.87	4.87
	Cythula schimperiana	1.60	1.10	0.99	-2.27	5.47
	Zehneria minutiflora	1.60	1.10	0.99	-2.27	5.47
	Ficus cycamores	0.70	1.10	1.00	-3.17	4.57
	Solanum micranthum	0.70	1.10	1.00	-3.17	4.57
	Vangueria volkensii	0.50	1.10	1.00	-3.37	4.37
	Senecio discifolius	0.70	1.10	1.00	-3.17	4.57

* The mean difference is significant at the 0.05 level.