ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUND FROM *MAERUA ANGOLENSIS DC*

BY

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BEING A THESIS SUBMITTED TO THE DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY, FACULTY OF SCIENCE, BAYERO UNIVERSITY, KANO. IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER (M.Sc) OF SCIENCE IN ORGANIC CHEMISTRY

NOVEMBER, 2015

CERTIFICATION

This is to certify that the thesis by Adamu Audu Askira has met the requirement for the award of Master of Science Degree in Organic Chemistry

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Dr. KABIR ABDU Date
(Project Supervisor)

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APPROVAL

This thesis entitled Isolation and Characterization of Bioactive Compounds from *Maerua Angolensis* DC Submitted by Adamu Audu Askira has been read and found acceptable by the examiners whose signatures appears below.

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REPRESENTATIVE OF THE SPS BOARD

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(Dr. HARUMA MUSA)
HEAD OF DEPARTMENT

DEDICATION

This work is dedicated to my late father M. Audu Babur Ngulde

ACKNOWLEDGEMENTS

First and foremost I wish to express my thanks to ALLAH (SWT) for sparing my life to perform this research work and to write this thesis. May peace and blessings of Allah be upon his beloved Prophet Muhammad (P.B.U.H). I would also like to express my special appreciations and thanks to my project supervisor Dr. Kabir Abdu who has been a tremendous mentor for me. I thank him for his encouragement on my research and for allowing me to grow as a research chemist. Your advice on both my research as well as my career has been tremendous. I will also like to thank all the lecturers in the Department of Pure and Industrial Chemistry for training rendered to me and for their brilliant advice, suggestions and corrections during my seminar presentation.

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ABSTRACT

The powdered leaves of *Maerua angolensis* DC was air dried and grounded into powder. It was extracted with ethanol to get the crude extract and sequentially partition with aqueous methanol, chloroform and acetone using partition method to obtain obtained the methanol soluble fraction, chloroform soluble fraction and acetone soluble fraction respectively. The extracts were further subjected to phytochemical screening to detect the presence of secondary metabolites using standard procedures. The extracts were tested for antimicrobial activity against clinical bacterial isolates of *Escherischia Coli, staphylocucus aures, Klebsiella pneumonia, proteus mirabilis and pseudomonas aeruginosa* using disc diffusion technique. Sensitivity test result showed that the test isolates were sensitive to acetone extract and aqueous methanol extract. All the test isolates were insensitive to the chloroform extract. The result of phytochemical screening indicates the presence of all the secondary metabolites in the crude ethanolic extract, aqueous methanolic extract and acetone extract while the chloroform extract showed the presence of anthraquinones glycoside, Alkaloids and tannins and absence of phenolic glycosides, Flavonoids and Saponnins. Based on the bioactivity result, the acetone extract which proved to be active was selected for Column Chromatograghy. The column fraction was however recrytallised,preparative TLC and HPLC was carried out to further ascertain the purity of the compound. After the purification process the compound AA-01 was characterized using ^{1}H, ^{13}C NMR, COSY, HSQC and HMBC.Based on the evidence compound was suggested as 4-(5-Oxo-tetrahydro-pyran-3-yl)-butyric acid methyl ester.

CHAPTER ONE

1.0 INTRODUCTION

Medicinal plants are the most productive source of new compounds and drug of natural origin (Harvey*,2008*). Most of the natural products isolated from medicinal plants are the secondary metabolites, which include alkaloids, tannins, flavonoids, steroids, terpenoids, phenylprapanoids (Harvey, 2008) and anthraquinones (Ayo, 2010). Some of the products have nutritive value (Prasad and Bisht, 2011) and antifungal and antibacterial activities. However some possess cytotoxicity (Wang *et al,* 2009). It is clear that plants play a vital role in our lives, with the primary use as being food and medicine. Plants have been reported to contain comparatively high amount of valuable nutrients such as vitamin A and C and other antioxidant micronutrients (Szeto *et al;* 2002.Jimoh*et al;* 2008). In the area of medicinal plants research for instance, it has now possible to isolate and characterize some potent bioactive natural products which may provide asolution to many of our health problems.

Plants have undisputedly played a major role in the treatment of traumas and diseases. This is evident by the historic use of chaulmoogra oil from species of *Hydrocarpusgoerta* which was known to be effective in treatment of leprosy.(Le strange,1977). Such use was documented in the pharmacopoeia of the emperor Shen Nung of China between 2730 to 3000 BC with particular reference to the use of ginseng, a Chinese herbal drug for vitality and increased potency (Le strange, 1977) and Ma-huang, shrub that introduced drug ephedrine to modern medicine (Le stange, 1977). The growing public interest and awareness of natural medicine have led the pharmaceutical industries and academic research pays more attention to medicinal plants (Day *et al;* 1998).

In as much as traditional therapy cannot be over emphasized, the need to employ measures of standardization for these plants of natural origin is warranted. Countries in Africa, Asia, and Latin America, the use traditional medicine was found to help in meeting

some of their primary health care needs. For example, in Africa, up to 80% of the population uses traditional medicine for primary health care (Trease and Evans*, 2002*). The world health organization (WHO), however, noted that use of traditional medicine is spreading in popularity in industrialized countries like in the United States, where about 158 million adults use this as alternative medicine (a field which incorporates traditional medicine but is broader in scope. WHO also notes, "Inappropriate use of traditional medicines or practices can have negative or dangerous effects" and that "further research is needed to ascertain the efficacy and safety" of several of the practices and medicinal plants used by traditional medicine systems.

1.1 Importance of Natural Products to Drug Discovery

For many decades, synthetic chemicals as drugs have been effective in the treatment of most diseases. The pharmaceutical industry has synthesized over 3 million new chemicals in their effort to produce new drugs. Despite their success in developing drugs to treat or cure many diseases, the treatment of certain diseases such as cancer, AIDS, heart disease and diabetes has not been a complete success due to the complexity of these diseases (Benyle:1994).

Over the centuries, people have been living in close association with the environment and relying on its flora and fauna as sources of food and medicine. As a result, many societies have their own rich plant pharmacopeias. In developing countries, due to economic factors, nearly 80% of the population still depends on the use of plant extracts as a source of medicine. Natural products also play an important role in the health care system in developed countries. The isolation of the analgesic morphine from the opium poppy, *Papaver somniferum*,in 1816 led to the development of many highly effective pain relievers (Benyle;1994). The discovery of penicillin from the filamentous fungus *Penicillium notatum* by Fleming in 1929 had a great impact on the investigation of nature as sources of new

bioactive agents (Bennet; 2001). Natural products can also be used as starting materials for semi synthetic drugs. The main examples are plant steroids, which led to the manufacture of oral contraceptives and other steroidal hormones. Today, almost every pharmacological class of drugs contains a natural product or natural product analog.

The investigation of higher plants has led the discovery of many new drugs. So far only a small portion of higher plants has been investigated. Consequently, they still remain a bigreservoir of useful chemical compounds not only as drugs, but also as templates for synthetic analogues(Bennet:2001).

1.2.1 Definitions of Infectious Disease

Human disease can be caused by infectious agents, genetic defects, environmental factors, or a combination of these factors. The infectious disease process begins at the time of infection, when a pathogen enters a host and starts to reproduce (Black: 2005). Many infectious agents, though, are killed by the body's numerous defenses before they can begin to reproduce. Furthermore, an infection may not necessarily produce disease; the infectious agent could be defeated quickly or it could hide somewhere in the body where it cannot be detected. The rapidity of onset and severity of the disease caused by an infectious agent depends on the virulence of the pathogen.

Bacteria were discovered in 1675 byAntony van Leeuwenhoek, but it wasn't until 1876 that a German physician named Robert Koch first demonstrated that specific diseases are associated with particular microorganisms (Black: 2005). Koch developed a set of criteria to show that anthrax, a disease of cattle, was caused by a specific bacterium, named *Bacillus anthracis,* and that tuberculosis was caused by a separate distinct bacterium. Koch presented his discovery of *Mycobacteium tuberculosis* in a lecture in March of 1882. He brought his entire laboratory setup to the lecture hall and demonstrated his procedures for his audience,

inviting them to check his findings themselves (Flint; 2000). His methods were so innovative that his criteria still are useful today in identifying disease-causing agents. Difficulties in applying these criteria can arise, however, for agents that are difficult to grow in culture or where a suitable, susceptible experimental host cannot be found. This is an especially difficult situation, and raises ethical concerns, where humans are the only known host. Robert Koch was awarded the Nobel Prize in Physiology and Medicine in 1905 for his work in tuberculosis (Flint: 2000).

Viruses are particles of nucleic acid (DNA or RNA) surrounded by a protective coat that replicate within specific host cells and can spread from cell to cell. Infectious diseases caused by viruses include the flu, the common cold, AIDS, chickenpox, and hepatitis.

Protozoa are single-celled, motile, eukaryotic organisms, found in the Kingdom Protista, which can be human parasites. A protozoan known as *Plasmodium* (over 170 species), causes malaria, an infectious disease that is one of the world's top killers (Albert: 2002).

Fungi are made of eukaryotic cells (organized nucleus and membrane enclosed organelles). All fungi, with the exception of the yeast group, are multi-cellular organisms that absorb nutrients from the environment. Fungi can cause athlete's foot, sinusitis, skin diseases, and vaginal infections.

Helminths (worms and flukes) are invertebrate animals, some of which are parasitic. *Wuchereiabancrofti* is transmitted to humans by way of the mosquito. The mature adults pass into lymphatic glands, obstructing lymphatic drainage and resulting in a disfiguring condition, known as elephantiasis.

1.2.2 History of Infectious Diseases

Infectious diseases probably have always afflicted humans. Numerous ancient writings describe recognizable infectious diseases that are still with us today. Through the

centuries, these diseases have resulted in significant losses of human life. The scope of human death has at times influenced history; it is thought that the 1918 flu epidemic played a contributing role in ending World War I. (Black: 2005). In modern times, improved sanitation and the development of vaccines and antibiotics have saved many lives. However, despite advances in technology, success in eradicating smallpox, and virtually eliminating many other diseases in developed countries, new and old infectious diseases continue to plaque humans. This is clearly evidenced by the HIV epidemic and the looming potential threat of another influenza pandemic. Sadly, even though we have the technology and vaccines to prevent disease, especially childhood illnesses, these advances are not accessible to many of the children in the developing world. Nearly 1.5 million children continue to die of measles each year for lack of a vaccine that costs less than 12 cents per dose (Black; 2005). The only infectious disease to be considered eradicated. Humans are the only reservoir for the virus. Smallpox is an infectious disease unique to humans, caused by either of two virus variants, *Variola major* and *Variola minor*. The disease is also known by the Latin names *Variola* or *Variolavera*, which is a derivative of the Latin *varius*, meaning spotted, or *varus*, meaning "pimple". The term "smallpox" was first used in Europe in the 15th century to distinguish variola from the great pox (syphilis).

Smallpox localizes in small blood vessels of the skin and in the mouth and throat. In the skin, this result in a characteristic maculopapular rash, and laer, raised fluid-filled blisters. *V. major* produces a more serious disease and has an overall mortality rate of 30–35%. *V. minor* causes a milder form of disease (also known as alastrim, cottonpox, milkpox, whitepox, and Cuban itch) which kills ~1% of its victims. Long-term complications of *V. major* infection include characteristic scars, commonly on the face, which occur in 65–85% of survivors. Blindness resulting from corneal ulceration and scarring, and limb deformities due to arthritis and osteomyelitis are less common complications, seen in about 2–5% of cases.

Smallpox is believed to have emerged in human populations about 10,000 BC. The disease killed an estimated 400,000 Europeans each year during the 18th century (including five reigning monarchs), and was responsible for a third of all blindness. Of all those infected, 20–60%—and over 80% of infected children—died from the disease(Black; 2005). During the 20th century, it is estimated that smallpox was responsible for 300–500 million deaths. In the early 1950s an estimated 50 million cases of smallpox occurred in the world each year. As recently as 1967, the World Health Organization estimated that 15 million people contracted the disease and that two million died in that year. After successful vaccination campaigns throughout the 19th and 20th centuries, the WHO certified the eradication of smallpox in December 1979. To this day, smallpox is the only human infectious disease to have been completely eradicated.

The last cases of smallpox in the world occurred in an outbreak of two cases (one of which was fatal) in Birmingham, England in 1978. A medical photographer, Janet Parker, contracted the disease at the University of Birmingham Medical School and died on 11 September 1978, after which the scientist responsible for smallpox research at the university, Professor Henry Bedson, committed suicide. In light of this accident, all known stocks of smallpox were destroyed or transferred to one of two WHO reference laboratories; the Centers for Disease Control and Prevention (CDC) in the United States and the State Research Center of Virology and Biotechnology VECTOR in Koltsovo, Russia where a regiment of troops guard it. In 1986, the World Health Organization recommended destruction of the virus, and later set the date of destruction to be 30 December 1993. This was postponed to 30 June 1995 (Black; 2005). In 2002 the policy of the WHO changed to be against its final destruction. Destroying existing stocks would reduce the risk involved with ongoing smallpox research; the stocks are not needed to respond to a smallpox outbreak. However, the stocks may be useful in developing new vaccines, antiviral drugs, and

diagnostic tests. virus particles causes the cell to lyse or rupture, releasing the new virus particles which can go on to infect more cells. Fungi and protozoa also can release toxins and enzymes that destroy host tissues. Some protozoa, such as the parasite that causes malaria, directly invade host cells (Black; 2005). Many signs and symptoms of disease are brought on by the host's immune system in response to pathogen invasion. Fever, for example, is an attempt by your body to kill invading microbes that are sensitive to changes in temperature. Sneezing, coughing, vomiting, and diarrhea all are efforts by the body to rid itself of pathogens. virus particles causes the cell to lyse or rupture, releasing the new virus particles which can go on to infect more cells. Fungi and protozoa also can release toxins and enzymes that destroy host tissues. Some protozoa, such as the parasite that causes malaria, directly invade host cells.

In the prodromal phase, a person experiences mild, nonspecific symptoms. During this time, the agent is continuing to multiply and the person is contagious. This phase is absent in some diseases, which cause a person to feel ill suddenly, without any warning. The clinical phase (also called the invasive phase or acute phase) is the period in which typical disease signs and symptoms are evident (Black; 2005). During this phase, there comes a time when symptoms reach their greatest intensity. Called the "acme," this is the height of the battle between the pathogen that is invading and destroying tissue and the efforts of the body's immune system to contain and obliterate the invader. Fever is usually a component of this phase, during which the patient is most contagious. Once the acme is reached, the number of infectious agents begins to drop and the signs and symptoms start to decrease. This is the decline phase, during which thebody's activities gradually return to normal, the tissues heal, and the individual no longer experiences any symptoms.

1.2.3 Types of Infectious Disease

Infectious diseases are classified by their duration and their location within the body, among other characteristics. “Acute,” “chronic,” and “latent” are terms used to describe the duration of a disease, how quickly the symptoms develop, and how long they last (Black; 2005). The common cold is an acute disease; tuberculosis is a chronic disease; and herpes infections can produce latent infections with recurring attacks interspersed by periods with no symptoms (e.g. cold sores). “Local” and “systemic” refer to the location of a disease within the body. Pathogens that spread from a more localized site, enter the bloodstream and are carried to other tissues can produce a systemic infection. “Primary” and “secondary” characterize the order of infection. A primary infection, usually an acute infection, occurs first in a previously healthy person. Sometimes, when a person is suffering from a primary infection and his or her immune system has been weakened by battling the primary infection, the person can succumb to a secondary infection caused by another agent. Thus, a person who has caught a cold due to a virus may then become ill with an ear infection caused by a bacterium because his/her immune system is incapable of fighting off another agent in its weakened state (Black; 2005).

1.2.4 Transmission of Infectious Diseases

There are many ways that infectious diseases can spread. Pathogens usually have specific routes by which they are transmitted, and these routes may depend on the type of cells and tissue that a particular agent targets. For example, because cold viruses infect the respiratory tract, they are dispersed into the air via coughing and sneezing. Once in the air, the viruses can infect another person who is unlucky enough to inhale air containing the virus particles(Black; 2005). Agents vary greatly in their stability in the environment. Some viruses may survive for only a few minutes outside of a host, while some spore-forming bacteria are extremely durable and may survive in a dormant state for a decade or more. As

noted, some diseases, such as colds, the flu and tuberculosis, spread through the air when an infected person coughs or sneezes. Another route or transmission is through ingestion of contaminated food or water. Water- and food-borne illnesses can be caused by bacteria, viruses, or protozoa(Black; 2005). This risk of contracting these illnesses is greatest with impure and untreated water and undercooked or improperly stored foods. Infectious diseases can also be transmitted through body fluids, such as blood, semen, and saliva. For example, HIV easily can be passed through contact with blood and semen, however, even though the virus has been found in saliva, there are no documented cases where HIV has been transmitted by contact with saliva. Similarly, there are no known cases of transmission of HIV by mosquitoes. Additionally, infectious agents can be passed through blood and blood products during medical procedures, such as blood transfusions. Many hemophiliacs receiving blood products were unwittingly infected with HIV before the responsible agent was identified and blood donations were screened. Other people can be infected as a result of sharing contaminated needles (Black; 2005).

Touching contaminated objects commonly leads to infection, especially among pre-school children. Fecal to oral transmission is a major path for diarrheal diseases, such as rotavirus and Norwalk virus that are widespread in daycare centers. It is also possible to become infected with some agents by touching surfaces such as doorknobs and telephones, especially those located in public places. Pigs and birds, in particular, are known to harbor viruses that can mutate and spread to humans upon contact. Finally, pathogens can be transmitted through insect vectors, such as mosquitoes. Mosquitoes are responsible for the spread of malaria, yellow fever, and West Nile virus, among other diseases. Ticks, which spread Lyme's disease, are another common vector. Many hemophiliacs receiving blood products were unwittingly infected with HIV before the responsible agent was identified and blood donations were screened. Other people can be infected as a result of sharing contaminated

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becomes resistant to the drugs. Once treatment is ceased, virus levels go back to earlier levels. Currently, there are four classes of anti-HIV drugs that block three essential steps in the virus reproductive cycle: the entry phase (where the virus particle fuses with the host cell); the reverse transcription step (where the virus makes a DNA copy of its RNA genome); and the protease step (where a virus protein chops long strands of virus proteins into smaller, functional units (Hahn;2005). These usually are given in combination, as a "cocktail" called "highly active antiviral therapy," or HAART for short. HAART is very effective for many people, at least for a period of time, but the treatment can produce unpleasant side effects and is too expensive for most people in the developing world. Most of all, it is important to remember that HAART is not a cure for AIDS.

1.2.5 Reducing the Spread of Infectious Diseases

Human activities drive emergence of disease and a variety of social, economic, political, climatic, technological, and environmental factors can shape the pattern of the disease and influence its emergence into populations (Hahn; 2005). The most effective method of stemming the spread of infectious disease is through vaccination. Vaccines consist of weakened or killed microbes, or just components of a pathogen, and stimulate the body's natural defenses the immune system to combat infections. Vaccination has eliminated smallpox, nearly eradicated poliovirus from much of the world, and drastically reduced the incidence of childhood infections, such as measles, mumps, and whooping cough, at least in the developed world. Influenza vaccines are available to reduce the occurrence of seasonal flu, although the shot must be given yearly due to the extreme variance of the influenza virus from season to season. Vaccines for other infectious diseases, especially HIV, still are being sought(Hahn; 2005).

Antibiotics are effective for many types of bacterial infections (although they are entirely useless against viruses). But increasingly, bacteria are becoming resistant to the

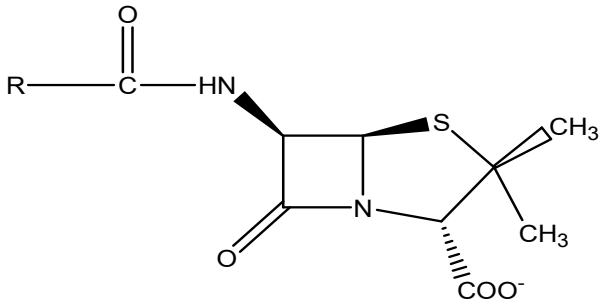
arsenal of antibiotics at our disposal. Very few drugs work well against viruses (anti-viral drugs for influenza and HIV were discussed in the previous two slides). Anti-fungal drugs exist, but their use is limited. There are no vaccines against protozoan parasites, and other medications against them are becoming ineffective. Therefore, protection from insect vectors such as mosquitoes and control of mosquito populations are crucial strategies in containing the spread of insect-borne diseases, such as malaria(Hahn; 2005). Good sanitation, water purification, hand washing, and proper cooking and storage of foods all help to reduce the prevalence of infectious disease. In cases of highly contagious, often fatal diseases, quarantine is employed as a means of preventing the spread of disease through a community. However, regardless of the disease, it is wise to limit contact with other individuals when ill. There are no vaccines against protozoan parasites, and other medications against them are becoming ineffective. Therefore, protection from insect vectors such as mosquitoes and control of mosquito populations are crucial strategies in containing the spread of insect-borne diseases, such as malaria. Good sanitation, water purification, hand washing, and proper cooking and storage of foods all help to reduce the prevalence of infectious disease (Hahn;2005). In cases of highly contagious, often fatal diseases, quarantine is employed as a means of preventing the spread of disease through a community. However, regardless of the disease, it is wise to limit contact with otherindividuals when ill.

1.2.3 β-lactam Antibiotics

β-lactams are the most broadly used antibacterial worldwide because of their comparatively high effectiveness, low cost of production, ease of delivery and minimal side effects (Draw; 2010).These antibaberial inhibits the synthesis of the peptidoglycan layer of bacterial cell walls leading to cell lysis (Bebrone; 2007). Structurally, all β-lactam antibiotic contains a four member β-lactams ring that has a nitrogen containing cyclic amide that is critical for antibacterial activity.

The β-lactam antibiotics comprise six different structural subtypes, including penams (**1**), cephems (**2**), monobactams (**8**), clavams (**3**), penems and carbapenems (6). The penams include benzylpenicillin include classical ceplalosporins such as cephloridine, nitrocefin and ceffatoxine as well as cephaloridine (that is 7-a-methyl-cephalosporins). The monobactams are monocyclic β-lactms and include aztreonams, penems, have a 2,3-double bond, in the fused thiazolidine ring (hence dihydrothiazole), which also have an unsaturated fused five member ring, with carbon in place of sulphur at the 1-position (Bebrone; 2007).These bind to and inhibits the carboxylpeptidase and transpeptidase which are the cell wall synthesizing enzymes, also called the penicillin-binding prpteins,or PBBs, that catalyze the D-ala D-ala Cross linkage of the peptidoglycan wall that surrounds the bacterium. As a result, ther is weakening of the cell wall structure, leading to cell lysis (Nair; 2010).

The first antibiotic that was discovered by Sir, Alexandra Fleming in 1927 named penicillin is also β-lactam. It was not until the early 1940s, through the work of Dr. Florey Chain and Heatley from Oxford University, that penicillin was purified and shown to cure specific bacterial infections.Since that time, many structural derivatives have been developed from penicillin to combat resistance that has risen bacterial.These derivatives commonly referred to as the extended spectrum β-lactams, include antibiotics called the cephalosporins, carbapenems, and monobactams. Over the last 60 years, the β-lactam class of antibiotics were the most widely, representing about 60% of all of the antibiotics used (by weight) in humans and animals medicine. These antibiotics also contain a β-lactam nucleus in their molecular structure by which they can inhibit the synthesis of the peptidoglycan layer of bacterial cell wall (Bebrone, 2007).

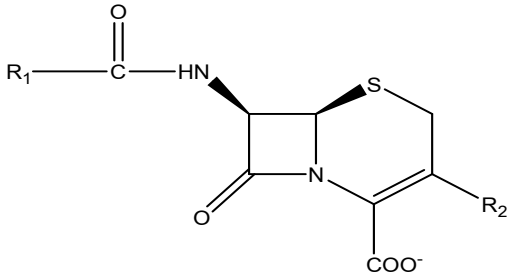


1

R = CH_2-C_6H_5Benzylpenicillin

R = $CH(NH_2)$-C_6H_5 Ampicillin

Fig:-1 Structure of Some Penams Antibiotics

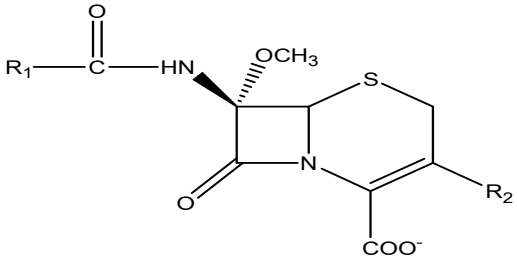


2

R_1=C_3H_2NS-NH_2

R_2=CH_2-S-N_4C-CH_2CH_2-$N(CH_3)_2$

Fig: - 2 Structure of Cephens Antibiotics (Cephalosporins)

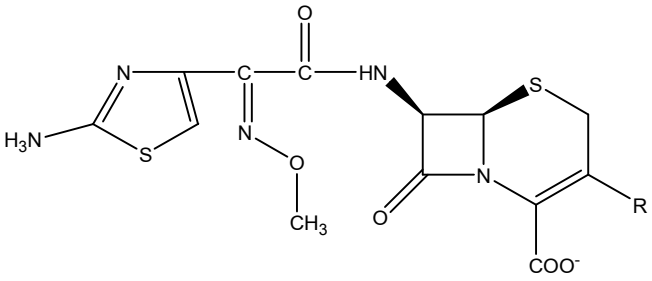


3

R_1=$CH_2CH_2CH_2$-$CH(NH_2)$-COOH

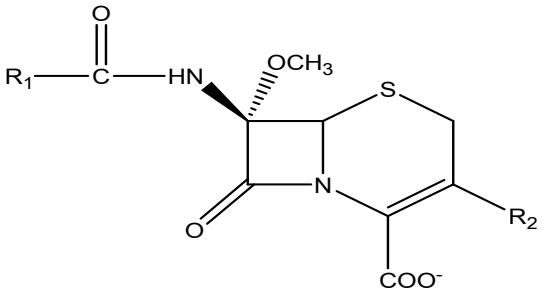
R_2=CH_2-COO-NH_2

Fig:-3 Structure of Cephamycin Antibiotics (Cefoxitin)



4

Fig:-4 Structure of Cefotaxime Antibiotics (Oximino cephalosporin) R=CH_2-O-CO-CH_3

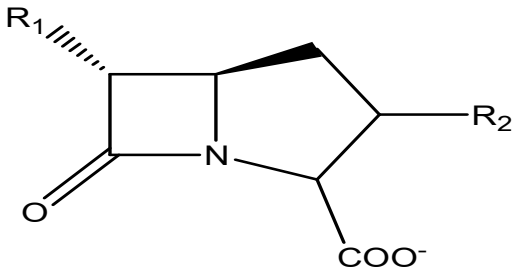


5

R_1=CH_2-$C_6H_5OCH_3$

R_2=S-CO-Ph

Fig:-5 Structure of Oxacephamycins (monolactams)

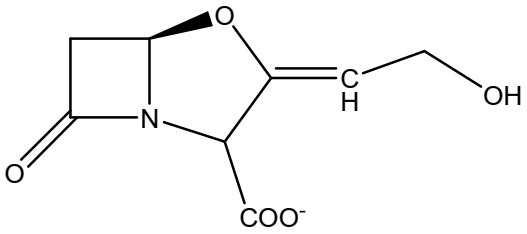


6

R_1=OH-CH_3

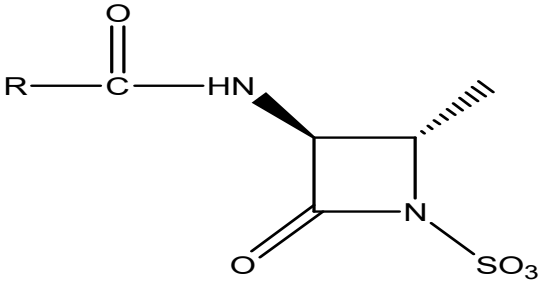
R_2=S-CH_2-CH_2-N-CH-NH_2

Fig:-6 Structure of Carbapenems Antibiotics (Imipenems)



7

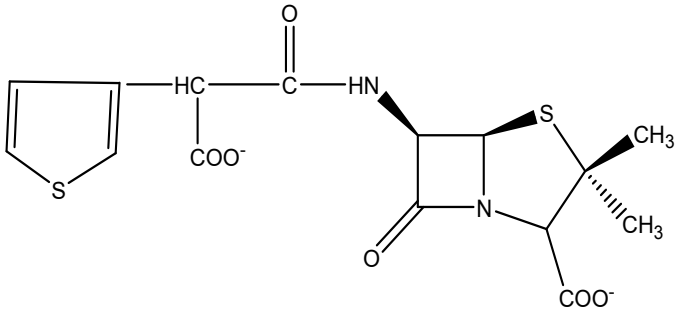
Fig:-7 Structure of Clavulanate (oxapenam)



8

R=N(O-COOH)-C_2SNH-NH_2

Fig: - 8 Structure of Monobactams (aztreonam)



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Fig;-9 StructureofTemocillinAntibiotcs (6-a-methyl penam)

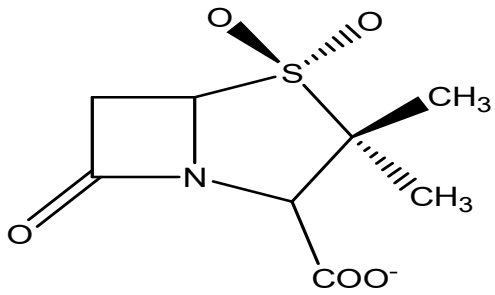

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Fig:-10 Structure of Sulbactam Antibiotics (penamsulphone)

1.3 Aims and Objective

The literature reports suggest that *Maeruaangolensis* finds wide ranging application in the treatment of infectious diseases. The aim of this project is to justify ethnomedical claims through scientific methods. The plant material will be extracted using solvents of different polarity. The resulting extracts will be subjected to phytochemical screening. Natural product compounds responsible for the biological activity will be isolated by means of bioassay guided chromatographic purifications. Antibacterial disc diffusion method will be used to guide the isolation. The structure of the active compounds will be established by means spectroscopic methods involving NMR, IR, UV and Mass Spectrometry.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1MORPHOLOGY *OFMAERUA ANGOLENSIS DC*

Maeruaangolesis DC belongs to the family *Capparidaceae*. It is a medium to big self-planted tree of up to 10m high, growing in bush and rocky areas. It is widespread in the savannah zones of tropical Africa (Burkill, 1997). In Nigeria the plant is commonly called *Mandewa* in Hausa, Legal baali in Fulfulde, and Apci in Kanuri*(*Adam, 1966) the tree is an attractive ornamental for garden planting especially in drier parts. The straggling branches drop at the ends which flowers it is often planted on graves in the Nupe area of northern Nigeria (Burkill, 1997). The wood is hard and brittle.

*Capparisspinosa*L. (*Capparidaceae)* is a particularly common aromatic in the Mediterranean area, and is also important in the commercial preparation of frozen food. The aromatic part of the caper consists of the floral bud, collected immediately prior to blossoming. The plant is not generally cultivated, with wild buds being picked by seasonal workers. These are then stored in salt before packaging takes place. Earlier studies involving *C.spinosa*L. have identified alkaloids, lipids, flavonoids and glucosinolates (Brevard *et al*., 1992), naturally occurring products from the order Capparales, also known as flavor compounds, anticarcinogenic agents and biopesticides (Mikkelsen*et al*., 2000). Also (Bonina*et al*. 2002) had reported that methanolic extract of *C. spinosa*L. was shown to possess strong antioxidant/free radical scavenging effectiveness in different *in vitro* tests. Besides this information, *C. spinosa*L. is a source of phenolic compounds. According to the study of (Argentieri*et al*.2012), Rutin was the dominant phenolic compound in the plant.

Fig:-11 Picture of *Maerua angolensis DC*

2.2 Maeruaoblongifolia(forssk)

Maeruaoblongifolia(Forssk.) A. Rich. is one of the Sudanese medicinal plants named Surreih in Capparaceae family, has been traditionally used to cure various diseases (MadhavaChetty*et al*., 2008). Ethanomedical survey reveals that Murva (*Maeruaoblongifolia*) is used to cure various diseases such as fever, stomach ache, skin infections, urinary calculii, diabetes mellitus, epilepsy, pruritis, rigidity in lower limbs, and abdominal colic .Murva is an important controversial drug used in diseases like anaemia; fever; diabetes; stomach disorders; typhoid; urinary infection and cough (Alice and Asha, 2007). It has botanical description as, a low woody bushy under-shrub sometimes scandent to 2-3 meters high, with a thick root stock and thick leaves, and strongly scented flowers. The root of this plant, which

tastes like coconut pulp, is edible and is eaten with sugar (Boulos, 1999). This plant is widely distributed in grassland with scattered trees (Acacia), deciduous bushland and semi-desert scrub in dry, stony and sandy places. Occurring in savannah woodland from Senegal to Nigeria and in Sudan to the Red Sea and Arabia. The plant survives annual burning by throwing up shoots from its thick rootstock (Boulos, 1999). Arulanandraj*et al.* (2011).

2.3 Medicinal Uses of *MaeruaAngolensis DC.*

The leaves of the plant are used as vegetables and they are rich in crude protein (Tairo*, et al,*2011). The anti – inflammatory *(*Adamu *et al,* 2007) anticonvulsant and anti-diarrheal (Magaji, *et al,* 2008), activities of the aqueous methanolic extract of the stem bark of the plant have been reported (Magaji *et al,* 2008). The leaves of the plant are used in the treatment of convulsion (Stafford *et al,* 2008), dysentery, epilepsy (Adamu *et al;*2007) stomach ulcer, skin rashes (Burkill,1987) and diabetes mellitus (Mohammed *et al,* 2007). The plant is also used in traditional medicine to treat psychosis, ecthyma,epilepsy, jaundice, hepatitis, insomnia, dyspepsia, neurasthenia and liver diseases (Adjanohoun *et al;*1989).*M. angolensis* is claimed to be used in the treatment of arthritis by the Hausa peopleof Northwestern Nigeria (Adamu, et al., (2007).Despite their unpleasant, bitter taste, the leaves are used by African rural tribes during famine periods as a food supplement, and also used as a purgative(Watt *and Brandwijk,*1962). Powdered leaves are used as a fish poison and to treat anorexia and asthenia, while bark extracts and pulped leaves are used to promote the healing of wounds. Decoctions of the leaves are given to children suffering amoebic dysentery or jaundice, and to treat rheumatism, stomach-ache, epilepsy and diarrhoea, while decoctions of the bark are used to treat malaria and as an aphrodisiac. Game and livestock readily browse the foliage.This species, in common with other members of the family, is host to butterflies of the Pieridae family. Instar larvae may defoliate a tree completely, but leaves regrow readily(VonBreitenback, 1965).

2.4 Isolated Compounds From *Capparis Genus.*

*Capparis tomentosa*Lam is a shrub which belongs to the family *Capparidaceae* which has four genera. *C. tomentosa* which belongs to the genus *Capparis* grows in the savanna forest of Western, Eastern and Southern Africa (Irvine, 1961; Marganet, 1965). This plant is found widely in local pharmacopoeias throughout Africa. The plant is used extensively for the treatment of variety of ailments, including mental disorder, snake bites, chest pains, impotency and barrenness (Abbiw, 1990; Burkill, 1965; Watt and Breyer-Braindwijk, 1962). It is also used as a cure for wounds and leprosy (Watt and Breyer- Braindwijk, 1962; Steenkamp, *et al*., 2004). The plant is also known to be very poisonous to livestock and man.

There had been reports of several fatal cases of poisoning from the use of this plant as medicine (Ahmed *et al.,* 1981; White, 1983; Ahmed *et al*., 1993).Judging from the extensive uses to which *C. tomentosa*is put and the fact that the plant is poisonous. Compounds that have been isolated andcharacterized from the genus *Capparis*include Flavonoids, fatty acids, lipids, alkaloids and glucosinolates. Several species of the genus *Capparis* have been reportedto contain glucosinolates. Glucosinolates are uniform class of glucosides with a sulphur atom in its structure and are known to cause goiter. In other studies, glucocapparin was found to be present in the leaves of *Capparis spinosa*, *Capparis flexuos a*and *Capparis linearis*as well as in the seeds of *Capparis ovata* and *Capparis angulata* (Gmelin and Kjaer, 1970; Kjaer, 1955; Cornforth and Henry, 1952). Further investigation of other *Capparis* species resulted in the isolation and detection of glucocoiberin, glicocapparin, sinigrin, glucobrassicin, 4- oxo-heptylglucosinolate and neogucograssicin (Cornforthand Henry, 1952).

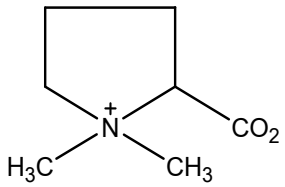

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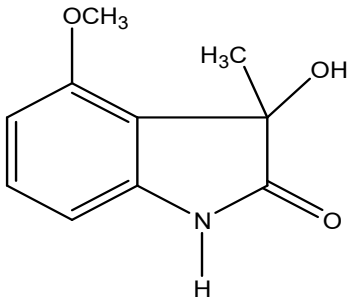
Fig:-12Structure of 2-Carboxy-1, 1-dimethyl-pyrrolidinium



12

Fig:-13Structure of 3-Hydroxy-3-methyl-4-methoxyoxindole

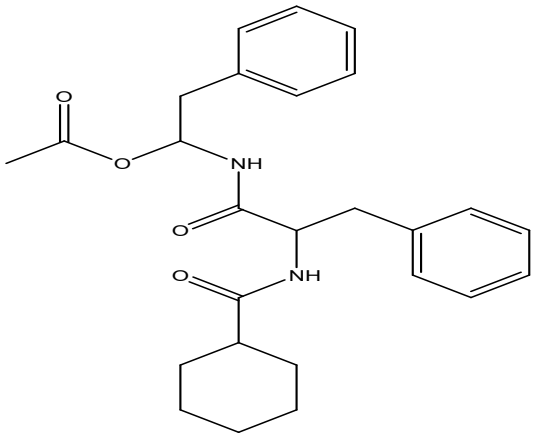


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Fig:-14 Structure of N-Benzoylohenylalanylaninol acetate

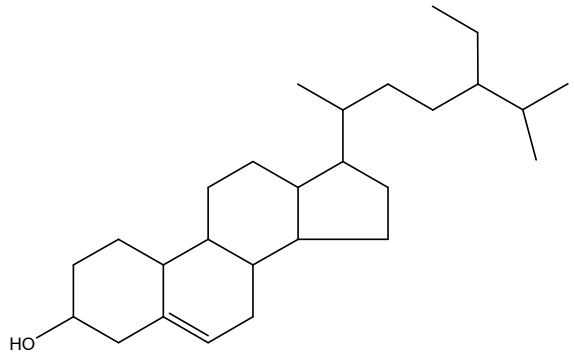

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Fig:-15Structure of24-Ethylcholestan-5-en-3-ol

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 GENERAL PROCEDURE

All solvents used were of analytical grade obtained from Qualikems, LOBO chemie and JDG®. Washed test tubes were used in carrying out phytochemical screening of the plant samples. Column chromatography was performed using silica gel 50-200 mesh. Thin layer chromatography (TLC) was carried out using aluminum precoated silica gel plate. Preparative TLC was done on glass-backed silica gel. The plates were viewed by exposure to iodine vapour or UV lamp at 254 and 365 nm. The spectra of the sample were ran using proton NMR, ^{13}C NMR, COSY, HSQC, and HMBC in Dueterated Chloroform $CDCl_3$ using S400 Varian NMR Spectrometer at the University of Bristol, United Kingdom.

3.2 Collection and Identification of PlantMaterials

The leaves of *Maerua angolensis DC* were collected from Botanical garden of the Department of Biological Sciences, Bayero University Kano. The plant was identified and authenticated by Baha'uddeen Said Adam of the department of plant Biology. It was identified as follows:-Bayero University, Kano Herbarium Accession Number BUKHAN 0335, on 23th March, 2015.

The leaves were air dried under shade grounded into fine powder using pestle and mortar and stored on shelf before using.

3.3Extraction Process of the plant Materials

The powdered leaves of *Maerua angolensis* (300g) was percolated with 96% ethanol (1000 cm^3) for one week with shaking at certain intervals. It was filtered and the residue was repercolated again in 500cm^3 ethanol for one day. The extracts were combined and

concentrated using rotary evaporator (R110) at 40^{o}Cthe crude extract was obtained weighed and labeled (F_1).

3.4 Partitioning Method of Crude Extract (F_1)

The crude extract (F_1) was dissolve in 120cm^{3}of methanol. The solution was the transferred in a separating funnel. This is followed by the addition of 120cm^3 of chloroform and 100cm^{3}of distilled water, after which it was shaken and the pressure released and allowed to stand. Two layers were obtained the aqueous methanol(top) layer and chloroform (bottom) layer which were easily separated. The chloroform layer was concentrated on rotary vapour (R110) at 30^{0}C weighed and labeled (F_2).The aqueous methanol layer (100cm^3) was taken back into separating funnel and partitioned with acetone (100cm^3).It was allowed to stand for 24 hours before it was separated. The acetone soluble fraction was concentrated on rotatory vapor (R110) at 40^{0}C, weighed and labeled as (F_4).The aq. Methanol fraction was also concentrated on rotatory vapour (R110) at 40^{0}C weighed and labeled as (F_3).

3.5 PHYTOCHEMICAL SCREENING

The following tests were carried out as follows:-

3.5.1 Test for phenolic glycosides

Few drops of 1M sulfuric acid were placed on porcelain slab after a small amount of the extract (11mg) was added. Appearance of red colour which disappears upon the addition distilled water indicates the presence of phenolic glycosides (Harbone; 1973).

3.5.2 Test Anthraquinone glycosides

2.5cm^{3}of thesolution of 500 mg/ml of extract was mixed with 1cm^{3}of 0.1M concentrated nitric acid. A yellowish brown colour which rapidly changes to vivid green indicates the presence of anthraquinone glycosides (Tease et al; 1989).

3.5.3 Test for Flavonoids

About 5cm^3 aliquot of 500 mg/ml the solution is made alkaline (pH 10) with 2ml of 2% sodium hydroxide. If a yellow colour is produced, it indicates the presence of Flavonoids compounds (Soforowa; 1993).

3.5.4 Test for Saponins

About 1cm^3 of solution of 500 mg/ml was placed in a test tube followed by the addition of 1cm^3 distilled water corked and shake strongly. The whole tube will be filled with froth that last for several minutes (persistent froth).This is an indication of presence of saponins (Soforowa; 1993).

3.5.5 Test for Tannins

Few drops (2-3cm^3) of 5% ferric chloride solution were added to the solution and the colour was observed. Green-black colouration indicates the presence of tannins (Soforowa; 1993).

3.5.6 Test for Alkaloids

About 1 cm^3 of 500 mg/ml solution of extract in a test tube add solution of potassium permanganate and shake. It decolourized the purple colour of potassium permanganate solution indicating the presence of alkaloids (Harbone; 1973).

3.6 ANTI-MICROBIAL SCREENING

3.6.1 Microorganisms

The strains used for the investigation were gram positive or negative. These includes Escherichia *coli, Staphylococcus aureus, Proteus mirabilis, Klebseilla pneumonae*, and *Pseudomonas auriginosa.* They were obtained from Msc. laboratory of department of microbiology. Bayero University, Kano. It was identified by M. Umar Dorayi a technologist in the laboratory.

3.6.3 Preparation of Sensitivity Disc

Whatman's No. 1 filter paper disc (of 6 mm in diameter) were punched out with the aid of paper punch and placed in Bijour bottles. They were sterilized by autoclaving at 121^{o}C for 15 minutes. The disc was allowed to cool before using.

3.6.2 Solution preparations

Fractions were dissolved in 1 cm^3 dimethyl sulphoxide (DMSO) exactly 60mg of each fraction was dissolved in 1ml of DMSO. Half (0.5cm^3) of the extract was introduced into 50 strerile in Bijour bottles to make 600µg/disc concentration. Half (0.5cm^3) of DMSO was added to remaining stock solution making 1 cm^3, 0.5cm^3 was taken and placed into another bottle containing 50 filter paper discs and labeled 300 µg/disc, 0.5 cm^3 of DMSO was added, another 0.5cm^3 was taken and placed into another 50 filter paper disc and labeled 150µg/disc. With each disc capable of absorbing 0.01cm^3 of the solution. The procedure was employed to prepare 150. 300, 600µg/disc concentrations.

3.6.4 Disc Diffusion Test

Standard inoculants of the isolate were swabbed on to the surface of prepared and solidified Mueller Hinton agar in separate Petri-dishes. The prepared disc of extract were placed onto the surface of the inoculate media at intervals. The plates were incubated at 37^{o}C for 24hrs before taking any observation and measurement of the zones of inhibitions (NCCLS, 2008).

3.7 Column Chromatography.

With the aid of long glass rod clean glass wool was used to hold the silica gel in the column. About 200g of silica gel 50-200 mesh was mixed with 850 cm^{3}of chloroform to make slurry which was added to the column through a funnel from the top of the column. The column was continuously washed with chloroform to make sure the silica gel settles well in

the column. A beaker was placed at bottom of the column so as to collect the eluates that drains in portion.

The acetone fraction (7.40g)of the plant material was mixed with 5.0g of silica gel to make sample mixture. It is allowed to dry. The sample mixture was then carefully poured on top of the silica gel that was previously packed in the column. This was followed by the addition of the small amount (2 g) of silica on top of the column to serve as a protective layer for the slurry. A gradient solvent system between $CHCl_3$and MeOH was used to elute the column as follows; it start with 100% $CHCl_3$ ($1000cm^3$), a mixture of 50% acetone and 50% chloroform ($1000cm^3$) $CHCl_3$, 50% ethyl acetate and 50% chloroform ($1000cm^3$) $CHCl_3$, 50% chloroform and 50% methanol ($1000cm^3$), 100% methanol. Respective fractions were collected in labeled beakers ranging from Ac 1 –Ac 62. The fractions were concentrated and analyzed using thin layer chromatography (TLC). Similar fractions on the basis of their TLC pattern were pooled together (Sharma and Achaya, 1988).

3.8 Thin Layer Chromatography of AC1 to AC60 of Acetone Leaf Extract of *Maerua Angolensis DC.*

Aluminum precoated silica gel plates were used to perform the TLC on the extracts. The solvent which serves as eluate is a mixture of methanol and chloroform in the ratio of 1:3. A set of ten (10) fractions were analyzed on a single plate. After spotting the fractions, the plates were carefully put into the TLC jar containing solvent system (3:1 $CHCl_3$: MeOH). The solvent level must be below the spots on the TLC plate. Similar fractions on the basis of their TLC pattern were pooled together. The fractions Ac-1-03 and Ac-3-02 that gave one spots was assumed to be pure fraction and was taken for spectroscopic analysis i.e.^{13}DEPT–135, ^{1}H NMR, mass and IR spectroscopic analysis.

3.9 Procedure for Preparative TLC

About 20cm^3 of sample was prepared by dissolving 100mg of sample in chloroform/methanol (9:1). A precoated silica plates were used. A line was drawn with pencil horizontally from the bottom at least 10 cm from the end of the plate. The sample was deposited on the horizontal thin line at the bottom of the plate and applying the remaining sample solution repeatedly on the horizontal line drying in between application to ensure uniform sample loading. About 200$cm^3$of eluent was prepared by mixing chloroform/methanol/toluene in the ratio of 95:9:1 respectively. The eluent is now placed in a chamber of preparative TLC made of glass tank. The plate is now placed in the chamber and covered with glass cover. It is allowed to stand for about one hour to allow the eluent to fully carry the sample. After an hour the sample the plate is removed from the tank allowed to dry and visualized under UV light at 254nm and 365nm.The bands are gently marked with a pencil not to remove the silica. A spatula was used to scrape the different bands of the silica from the plates, placed in different beakers and dissolved with chloroform/methanol (9:1). The dissolved silica is allowed to settle and gradually filtered. The filtrate is allowed to evaporate to obtain the desired samples and taken for HPLC analysis.

3.9.1 HPLC Analysis

HPLC analysis was conducted by using solvent system of Water/Methanol HPLC grade buffered with 0.05% formic acid. The detection was achieved at using diode array detector at 220nm and a columntemperature of 40^0C. Analysis wasran following 20minutes method on Hypersil C_{18} column (250 mm x 4mm and 5µM particle size). Samples obtained from column chromatography and the fractions obtained from preparative TLC were analysed.

3.9.2 Procedure for HPLC

Solution of 10mg/ml of each of the column fraction and fractions obtained by preparative TLC were prepared. By auto sampling the machine inject 75µl of sample and run for 20 minute

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results of extraction and fractionation

About 32g of crude ethanol fraction was obtained by percolating 300g powdered leaves of sample (*Maerua angolensis* D.C) in 1000cm^3 of ethanol.The crude ethanol extract was further separated by partitioning and other fractions were obtained as shown in table 1 below:

Table I: Physical properties of the fractions from the leaves extract of *Maerua angolensis D.C.*

Extract/fraction	**Texture**	**Colour**	**Weight(g)**
F_1	Oily	Dark green	32
F_2	Oily	Dark green	12.7
F_3	Oily	Dark brown	10.5
F_4	Oily	Dark brown	7.6

Key:

F_1– Crude ethanolic fraction

F_2 – Chloroform fraction

F_3 – Aqueous methanolic fraction

F_4 – Acetone fraction

The result shows that the all the fractions were observed to be oily in nature However, the colour of ethanol and chloroform fractions were found to be dark green while that of aqueous methanolic fraction and acetone fraction were found to be dark brown in colour.

4.2 Result of phytochemical screening;

The table 2 below gives the results of phytochemical test carried out on all fractions obtained.

Table 2 Result of phytochemical screening

Fractions	Anthraquinone glycosides	Phenolic glycosides	Alkaloids	Flavonoid	Tannin	Saponin
F_1	+ve	+ve	+ve	+ve	+ve	+ve
F_2	+ve	–ve	+ve	–ve	+ve	–ve
F_3	+ve	+ve	+ve	+ve	+ve	+ve
F_4	+ve	+ve	+ve	+ve	+ve	+ve

Key:

F_1 – Crude ethanolic fraction +ve : means present

F_2 – Chloroform fraction ⁻ve: means absent

F_3 – Aqueous methanolic fraction

F_4 – Acetone fraction

The preliminary phytochemical screening of the fractions revealed the distribution of secondary metabolites in the fractions. The crude ethanolic fraction, aq. Methanolic fraction and Acetone fraction showed a positive result for all the secondary metabolites, while the chloroform fraction showed positive result for anthraquinone glycosides,

Alkaloids and Tannins. The chloroform fraction showed the absence of phenolic glycosides, flavanoids and saponins

4.3 Result of antimicrobial screening

Table 3 Inhibitory Activity of extract disc against test isolates.

Organisms	**F_4**(µg/disc)			**F_3**(ug/disc)			**F_2**(µg/disc)		
	150	300	600	150	300	600	150	300	600
Escherichia coli	7	8	12	8	9	10	0	0	0
Staphylococcus aures	0	0	0	0	0	0	0	0	0
Proteus mirabilis	12	13	14	0	0	0	0	0	0
Klebsiella pneumonia	0	0	0	12	14	15	0	0	0
Pseudomonas aeruginosa	8	9	12	7	8	0	0	0	0

Key: F_4 = Acetone extract. F_3 = aqueous methanolic extract.

F_2 = chloroform extract

Sensitivity test result showed that the test isolates were sensitive to acetone extract at 300µg/disc concentrations as follows, *Escherichia coli* (8mm), *Proteus mirabilis* (13mm*), Pseudomonas aeruginosa* (9mm), except *Staphlococcus aures* and *Klebsiella pneumonia* same zone diameter equal to the disc diameter (6mm). *Escherichia coli*, *Klebsiella pneumonia* and *pseudomonas aeruginosa* were sensitive to aqueous methanolic extract with inhibition at 9mm, 14mm and 8mm corresponding to 150µg/disc,300µg/disc and 600µg/disc respectively. All the test isolates were insensitive to the chloroform extract.

4.4 RESULT OF COLUMN CHROMATOGRAPHY

Table 4:- Result of Column Chromatography

Fraction Number	Eluting Solvent $CHCl_3$:$(CH_3)CO$	TLC Solvent System $CHCl_3$:MeOH	Number of Spots
Ac-1-01	50.0:0.0	,,	1
Ac-1-02	47.5:2.5	,,	-
Ac-1-03	45.0:5.0	,,	-
Ac-1-04	42.5:7.5	,,	-
Ac-1-05	40.0:10.0	,,	-
Ac-1-06	37.5:12.5	,,	-
Ac-1-07	35.5:15.5	,,	2
Ac-1-08	32.5:17.5	,,	-
Ac-1-09	30.0:20.0	,,	-
Ac-1-10	27.5:22.5	,,	2
Ac-1-11	25.0:25.0	,,	-
Ac-1-12	22.5:27.5	,,	-
Ac-1-13	20.0:30.0	,,	-
Ac-1-14	17.5:32.5	,,	-
Ac-1-15	15.0:35.0	,,	2
Ac-1-16	12.5:37.5	,,	-
Ac-1-17	10.0:40.0	,,	1
Ac-1-18	7.5:42.5	,,	-
Ac-1-19	5.0:45.0	,,	2
Ac-1-20	2.5:47.5	,,	-
Ac-1-21	0.0:50.0	,,	-
Ac-2-22	50.0:0.0	,,	-
Ac-2-23	47.5:2.5	,,	-
Ac-2-24	45.0:5.0	,,	-
Ac-2-25	42.5:7.5	,,	2
Ac-2-26	40.0:10.0	,,	2
Ac-2-27	37.5:12.5	,,	-
Ac-2-28	35.5:15.5	,,	-
Ac-2-29	32.5:17.5	,,	2
Ac-2-30	30.0:20.0	,,	2
Ac-2-31	27.5:22.5	,,	-
Ac-2-32	25.0:25.0	,,	-
Ac-2-33	22.5:27.5	,,	-
Ac-2-34	20.0:30.0	,,	-
Ac-2-35	17.5:32.5	,,	-
Ac-2-36	15.0:35.0	,,	2
Ac-2-37	12.5:37.5	,,	-
Ac-2-38	10.0:40.0	,,	2
Ac-2-39	7.5:42.5	,,	-
Ac-2-40	5.0:45.0	,,	1
Ac-2-41	2.5:47.5	,,	-

Fraction Number	Eluting Solvent $CHCl_3$:$(CH_3)CO$	TLC Solvent System $CHCl_3$:MeOH	Number of Spots
Ac-2-42	0.0:50.0	,,	-
Ac-3-43	50.0:0.0	,,	-
Ac-3-44	47.5:2.5	,,	2
Ac-3-45	45.0:5.0	,,	-
Ac-3-46	42.5:7.5	,,	2
Ac-3-47	40.0:10.0	,,	-
Ac-3-48	37.5:12.5	,,	-
Ac-3-49	35.5:15.5	,,	2
Ac-3-50	32.5:17.5	,,	-
Ac-3-51	30.0:20.0	,,	-
Ac-3-52	27.5:22.5	,,	-
Ac-3-53	25.0:25.0	,,	-
Ac-3-54	22.5:27.5	,,	-
Ac-3-55	20.0:30.0	,,	1
Ac-3-56	17.5:32.5	,,	1
Ac-3-57	15.0:35.0	,,	1
Ac-3-58	12.5:37.5	,,	1
Ac-3-59	10.0:40.0	,,	1
Ac-3-60	7.5:42.5	,,	1
Ac-3-61	5.0:45.0	,,	1
Ac-3-62	2.5:47.5	,,	1
Ac-3-63	0.0:50.0	,,	1

Table 5 Result of preparative TLC

Fraction	**R_f Value**	**254nm**	**365nm**	**Weight**
1	0.028	Yellow	Brown	20 mg
2	0.139	White	-	36 mg
3	0.611	Blue	Black	33 mg
4	0.722	Pink	-	27 mg
5	0.917	Orange	Dark brown	29 mg
6	0.944	Blue	Dark brown	42 mg
7	0.972	Light blue	Dark brown	34 mg

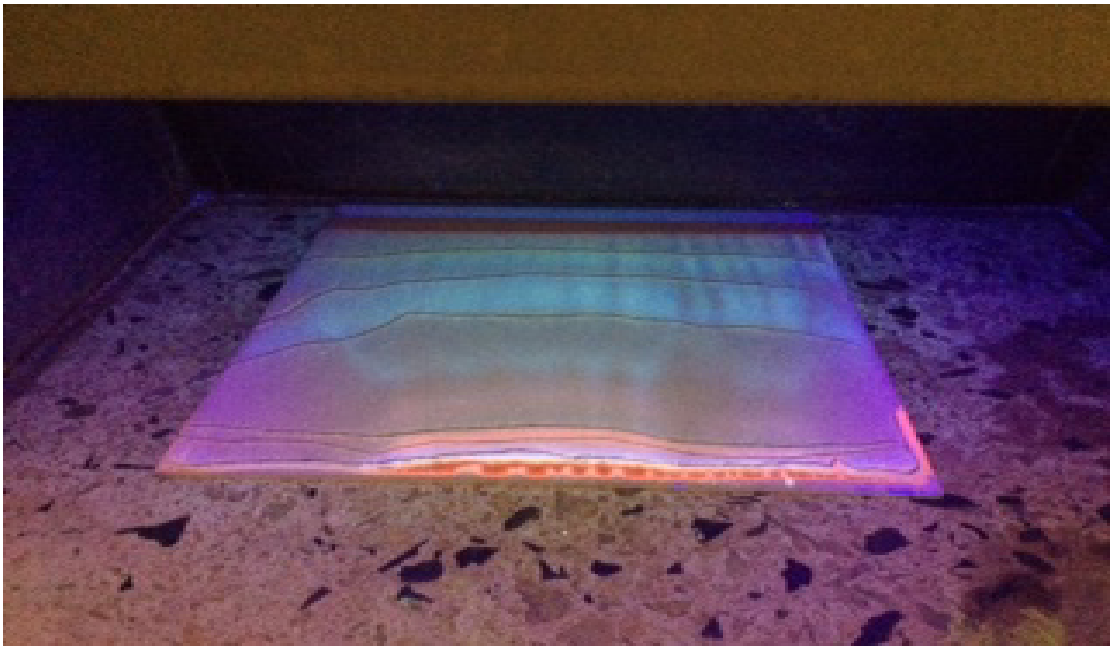


Figure 12 Picture of preparative TLC plate at UV 365nm of Compound AA-01

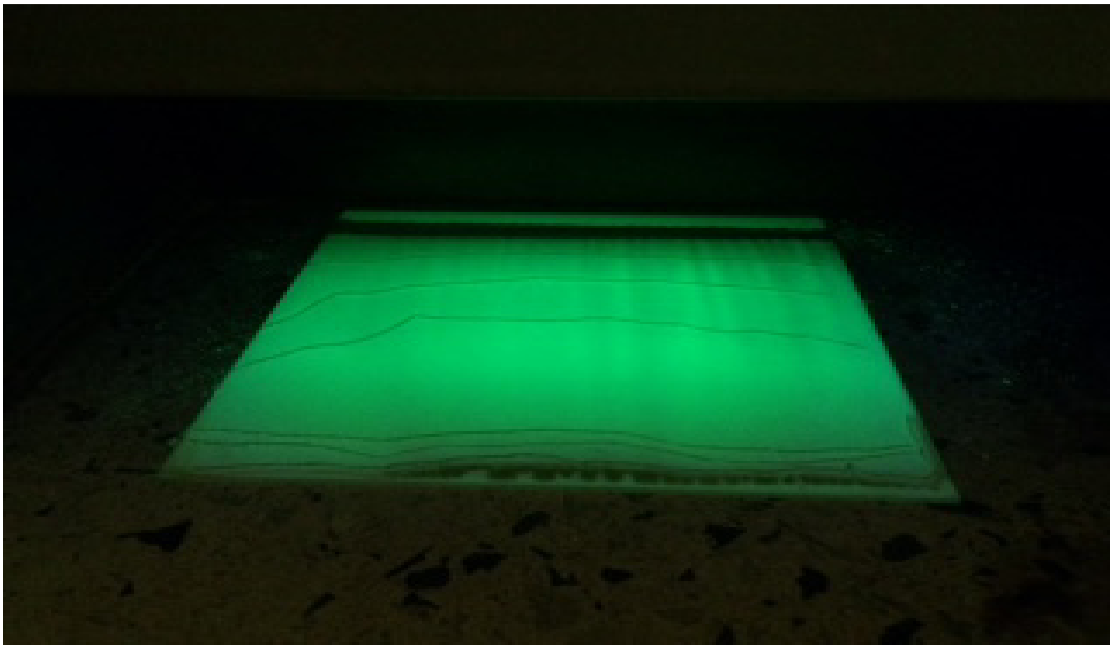


Figure 13 Picture of preparative TLC plate at UV 254nm of Compound AA-01

4.5 SPECTRAL ANALYSIS

The compound was isolated from column fraction Ac-3-03 by further purification a light yellow liquid substance was obtained as pure compound weigh 7.7mg. The ^{13}C reveals

the presence of 1-methyl, 6-methylene, 1-methine and 2 quaternary carbons. The ^{1}H NMR reveals the presence of seven different types of protons at different chemical environment from the chemical shift value (Table 6 Appendix 1, 2). The HSQC ^{1}H-^{13}C correlation, correlate the proton at δ1.28 with carbon at 29.70, δ2.20 with carbon at 19.33 , δ2.20 with carbon 31.00, δ2.57 with carbon at 25.78, δ3.28 with carbon at 46.62, δ3.43 with carbon at 53.00, δ3.41 with carbon at67.30, δ3.68 with carbon at 67.30, δ4.02 with carbon at 77.36.(Appendix 4) However, the ^{13}C spectrum revealed the presence of two carbonyls at δ207.00 and δ166.69 (Table 6, Appendix 3)

The COSY ^{1}H-^{1}H correlation shows linkage between 2.20:3.41, 2.20:3.68, 2.58:2.20, 2.57:4.02, 3.41:2.20, 3.43:3.28, 3.68:3.41, 4.02 with 2.58 (Appendix 5) while, the HMBC ^{1}H-^{13}C correlation across 2 or 3 bonds shows correlation between δ2.20 with carbons at 31.00, 67.30, 206.99. protons at δ2.57 with carbons at 19.27,77.76, 166.72. protons at δ3.28 with carbons at 53.00, 67.30, protons at δ3.43 with carbons at 46.62 65.30 protons at δ3.68 with carbons at 19.33, 25.78,46.98, protons at δ4.402 with carbons at19.33, 25.78, 166.67(Table 6, Appendix 6). Based on the above evidence compound was proposed as 4-(5-Oxo-tetrahydro-pyran-3-yl)-butyric acid methyl ester.

Fig:-16 Structure showing protons position and ^{13}C Chemical shift of pure compound

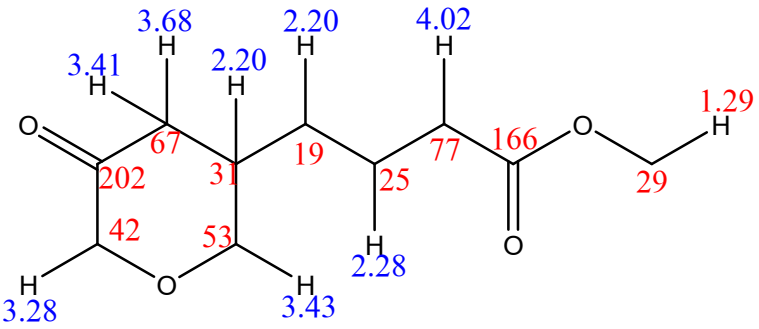


Table 6:- showing position and chemical shift of ^{1}H and ^{13}C with their HMBC

Position	^{1}H NMR	^{13}C NMR	Multiplicity	HMBC
1	3.43	53.50	d	C_2 C_4
2	-	-		
3	3.28	46.62	Singlet	C_1 C_4
4	-	207.00	-	-
5	3.68	67.30	d-d	C_6 C_7 C_2
6	3.41	67.30	d	
7	2.20	30.95	Quinted	C_5 C_4 C_3
8	2.20	19.33	Quinted	
9	2.58	25.78	Sextet	C_7 C_9 C_{10}
10	4.02	77.50	Triplet	C_6 C_7 C_9
11	-	166.69	-	-
12	-	-	-	-
13	1.29	29.71	Singlet	-

Fig17:-Suggested structure of the Compound

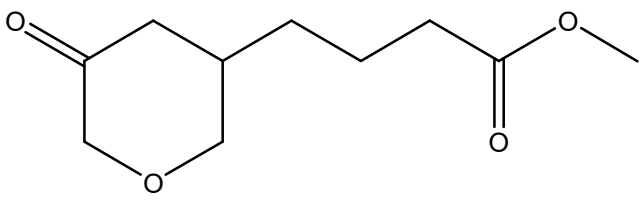


Fig 18 HPLC chromatogram of the column fraction

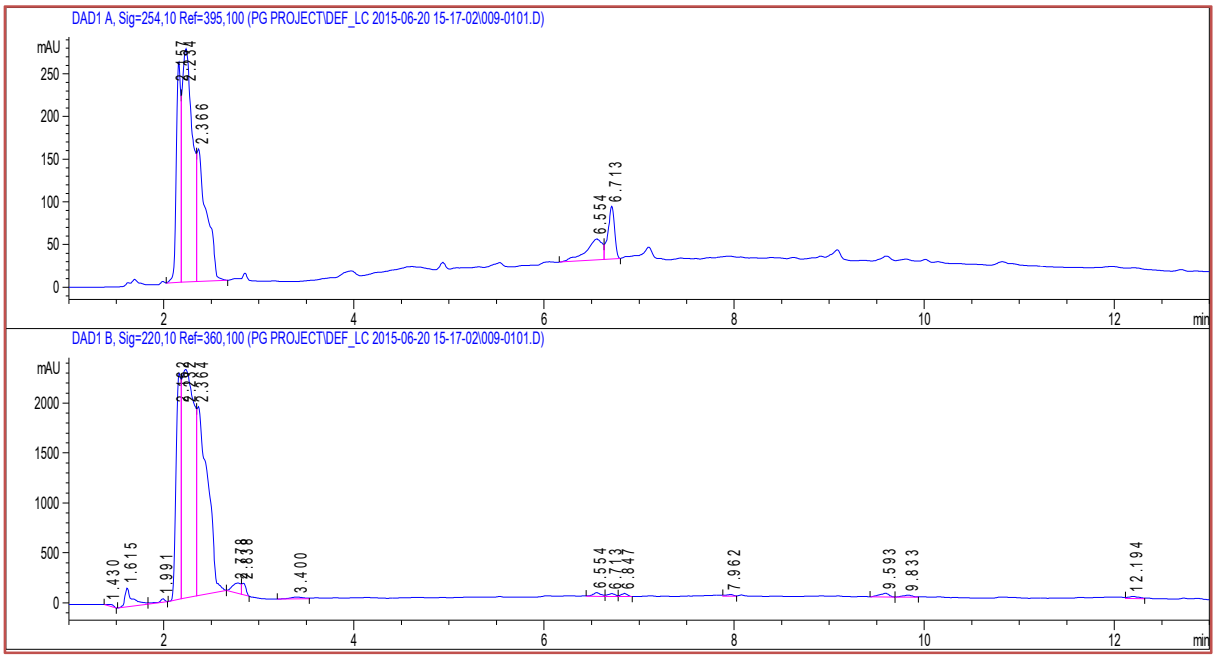


Fig 19 HPLC Chromatogram of Band 4

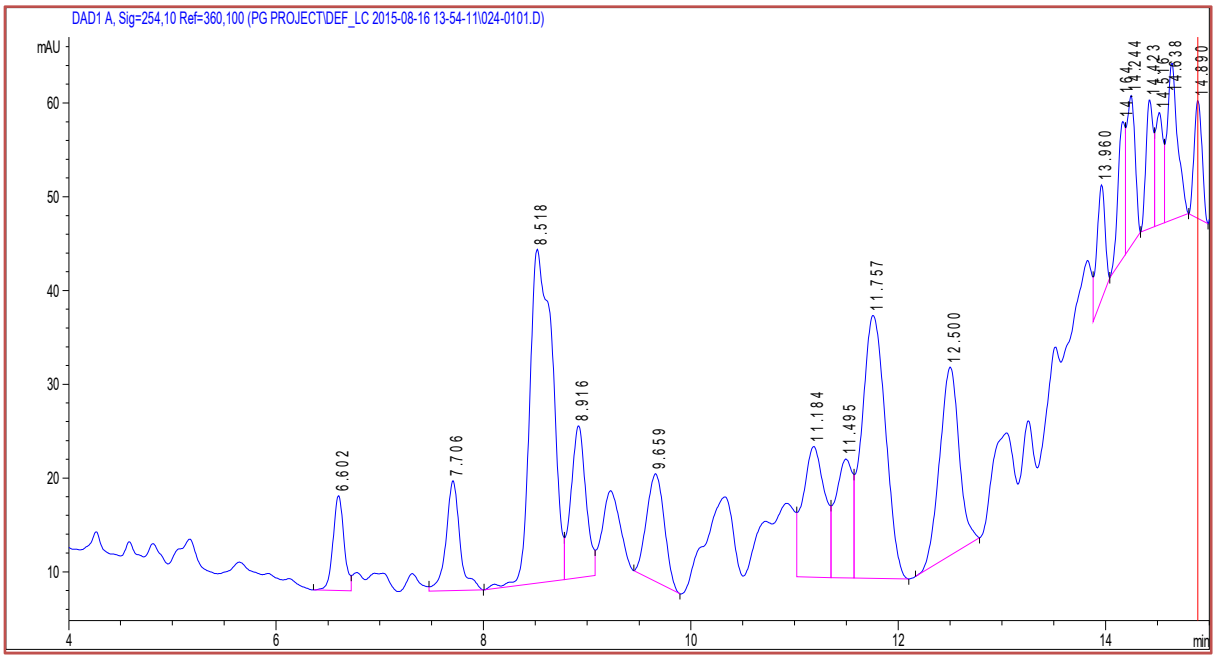


Fig 20 HPLC Chromatogram of band 3

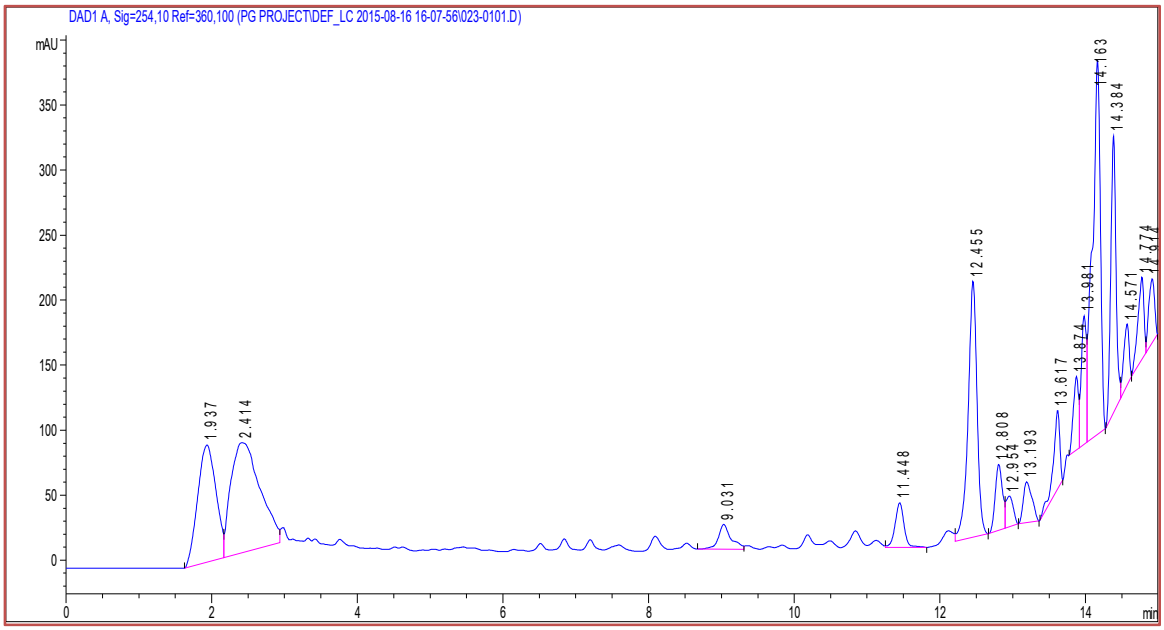
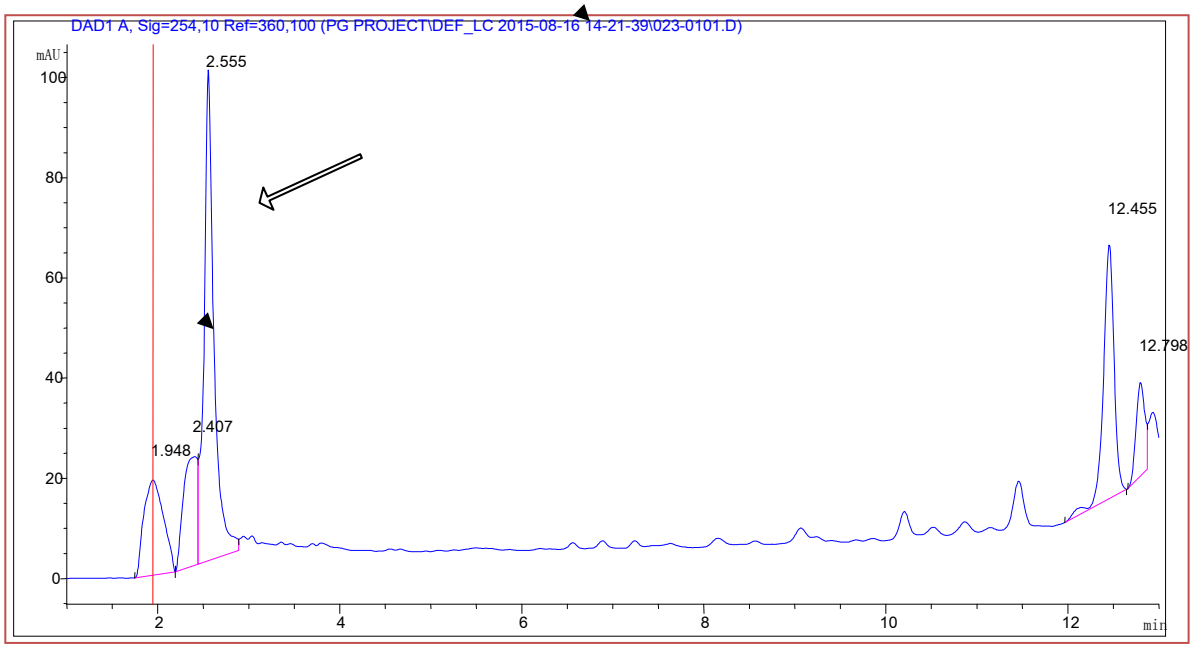


Fig 21 Purification Chromatogram f Band 3, With Arrow Showing Showing Pure Sample Collection At Retention Time 2.555



CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Plants have formed the basis for traditional medicinal systems for thousands of years. Most of the natural products isolated from medicinal plants are the secondary metabolites. Traditional knowledge of medicinal plants has always guided the search for new cure. In spite of the advent of modern drug discovery and screening techniques. In the present study on the leave extract of *Maerua angolensis DC* obtained from botanical garden of Bayero University Kano was analyzed for the first time. The result of the analysis shows the presence of some secondary metabolites in the fractions. Bioactivity guided column chromatography on the acetone fraction of the plant led to the isolation of the pure compound. They are structurally elucidated using 1H, 13C, HSQC and HMBC. The present study confirmed the traditional use of this plant for the treatment of convulsion, dysentery, epilepsy, stomach ulcer, skin rashes, diabetes mellitus, jaundice, hepatitis, insomnia dyspepsia, neurasthenia, liver diseases e.t.c. Based on the results obtained in this study it could be said that *Maerua angolensis DC* Plants powdered leaves contains chemical constituents of pharmacological significance. Hence the compound isolated and characterized was proposed as

5.2 RECOMMENDATION

Considering the efficacy of this plant against certain strains of micro-organism, further work should be carried out to test its anticancer activity of compound.

REFERENCES

Abbiw D.K., (1990). Useful Plants of Ghana.Intermediate publication and Royal BotanicGardens, Kew. p. 22

Adam, (1966).Useful plants of Tropical Africa Vol 1. Royal Botanic Gardens kew (K)

Adamu A, Abdurrahaman EM, Ibrahim H, Abubakar MS, Yero AH (2007).Effect of aqueous methanolic stem bark extract of *Maeruaangolensis* DC on acute and sub-acute inflammation. Nig. *J Pharm.Sci* 6(2); 1-6

Adamu, A, Abdurahaman, E,M., Ibrahim, H., Abubakar, M.S., Magaji, M.G., Yero, A.H., (2007) Effect of aqueous methanolic stem bark *Maeruaangolensis DC* on acute and sub-acute inflammations. Nigeria Journal of Pharmacetical Sciences.Vol. 6, No 2.

Adjanohoun, E., Adjakidje, V., Ahyi, M.R.A (1989). Contrbution aux etdes ethnobotaniquesetfloritiquesenrepubliquepopulaire du Benin. Agence de cooperation culturelleet technique, (A.C.CT.), Paris pp 895

Ahmed OM, Adam SE, Edds GT (1981). The toxicity of Capparistomentosa in sheep and calves. Vet. Hum. Toxicol. (6): 403-409

Ahmed SA, Amin AE, Adam SE, Hapke HJ (1993). Toxic effect of the dried leaves and stem of Capparistomentosa on Nubian goats.DtschTietarztl, Wochenschr. (5): 192-194

Alberts, B., Johnson, A., and Lewis, J., Raff M., Robert, K., and Walter, P., (2002). Molecular Biology of the cell. (4thed). New York. Garlarnd Publishing Inc.

Alice K, Asha S (2007). Medicinal Plants Horticulture Sciences.Series-2; New India, New India publication agency.pp 98

Argentieri M, Macchia F, Papadia P, Fanizzi FP, Avato P (2012). Bioactive compounds from *Capparisspinosa subsp. Rupestris*. Ind. Crop. Prod. 36: 65–69.

Arulanandraj N, Punithavani T, Indumathy S (2011). Effect of Murva (*Maerua Oblongifolia*) on Different Fungal Strains - An *In Vitro* Study. International Journal of Universal Pharmacy and Life Sciences. 1: 163-169.

Ayo R. G. (2010): Phytochemical Constituents and Bioactivities of Extracts of Cassia nigricansVahl; A review, *J. Med. Plants Res* 4(4); 1339 – 1348.

Bebrone, C.(2007) Metallo-β-lactamase (classification, activity, gentic organization, structure, Zinc Coordination) and their superfacial, Biochemical pharmacology, 74, 1686-1701

Bennett, J. W.; Chung, K. T.(2002) Alexander Fleming and the discovery of penicillin. *Adv. Applied Micro*biology. *49*,

Benyhe, S.; Morphine(1994): New aspects in the study of an ancient compound. *Life Science,55,* 969-979.

Black, J.G., (2005). Microbiology (6thed). John Willey and Son.Inc.

Bonina F, Puglia C, Ventura D, Aquino R, Tortora S, Sacchi A, Saija A, Tomaino A,Boulos L (1999). Flora of Egypt.vol. 1 Al Harara Publishing. Cairo. p.176.

Brandy G. Mac Arthus GS.Fanel P. J. (2007): Epstein – Barr virus and Burkitt Lymphoma J. din Pathol.(2007): 60(12) 1397 – 1402.

Brevard H, Brambilla M, Chaintreau A, Marion JP (1992). Occurrence of elemental sulphur in capers (*Capparisspinosa*L.) and first investigation of the flavour profile. Flavour Fragrance J. 7: 313–321.

Burton GW, Joyce A, Ingold KU (1983). Is vitamin E the only lipidsoluble, chain breaking antioxidant in human blood plasma and erythrocyte membrane? Arch. Biochem.Biophys. 221: 281–290.

Burkill HM. (1987), The useful plants of West Tropical Africa.Whitefriains Press Ltd, Great Britain, Voume 4 page 968

Cornforth JW, Henry A (1952). J. Chem. Soc. pp. 106-109.

Day, C. Dhar M.L, Dhar M. M, Mehrotha, B.N, Dhawan, B. N. (1998): Secreening of Indian plants for biological studies, Ind Journal Exp. Biol. 6: 232 – 247

Draw, S. M and Bonomo, R.A (2010) Three decades of beta- lactamase inhibitors. Clinical Microbiology Reviews, 23, 160-201

F.VonBreitenbach, (1965): The indigenous Trees of Southern Africa.

Flint, S.J. Enquist, L.W,. Krug, R.M., Racaniello, V.R.,andSkalka, A.M., (2000). Principle of Virology: Molecular biology, Pathogenensis and Control. ASM press.

Fried B, Reddy A. Mayer D, (2010): Helminths in Human CarcinOgenesis, Cancer lett 28:305(2): 239 – 249.

Gmelin R, Kjaer A (1970). Phytochem. 9: 601.Irvine FR (1961). Woody plants of Ghana. Oxford University Press,London. p. 46.

Hahn, D.B., Payne W.A and Mauer, E.B (2005). Focus on health (7thed) New York. Mc Graw Hill.

Harborne, J.B. 1973. Phytochemicals Methods.Chapman andHall Ltd., London, pp. 49-188.

Harvey, AL. (2008) Natural product in drug discovery, Drug Discovery Today 13;894- 901

Jimoh F. O. Adedapo A. A. Aliero, A. A, Afolayan A. J. (2008): Polyphenol Contents and Biological Activities of RumexAcklonlamus. Pharm. Biol, 46(5); 333 – 340.

Le – strange, R (1977): A history of herbal plants. Angus Robertson London; 163-184

Leonardi GC. Candilos, Cervello M. Nicolosi D, Raiti F. Travalis, Spandidos D. A, Libra M. (2012): The tumor Microenvironment in Hepatocellular Carcinoma. Int. J. Oncol. 40(6) 1733 – 1747.

Madhava-Chetty K, Sivaji K, Tulasi-Rao K (2008). Flowering plants of Chittoor district-Andhra Pradesh, India. 1 edn.Students offset printers, Tirupati. Pp342

Magaji MG, YeroAH,Malha BB, Maje IM, Musa AM (2008) Preliminary gastro- intestinal studies on aqueous methanolic stem bark extract of *Maeruaangolensis* (cappraraceae).Nigerian jounal of Pharmacetical Science. 7(1); 108-113

Mikkelsen MD, Hansen CH, Wittstock U, Halkier BA (2000). Cytochrome P450 CYP79B2 from Arabidopsis catalyzed conversion of triptophane to indole-3,3-acetaldoxime, a precursor of indole glucosinolates and indole-3 acetic acid. J. Biol. Chem. 275: 33712–33717.

Mohammed A, TankoY,Okasha MA, Sadiq Y, Isa Al (2007) Effect of aqueous methanolic stem bark *Maeruaangolensis* (capparidaceae) extract on blood glucose levelsofstreptozocin-induced diabetic Wister rats. Res. J. Pharmacol. 1(4); 75-78

Nair, D. and Rawat, D.(2010) Extended spectrum β-lactamases in Gram Negative Bacteria. Journal of Global infectious Diseases, 2, 263-274

NCCLS, (2008): National Committee For Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. Wayne, PA, USA.

Pellegrino ML, De Capraris P (2002). *In vitro* antioxidant and *in vivo* photoprotective effects of a lyophilized extract of *Capparisspinosa L.* buds. J. Cosmet. Sci. 53: 321–335.

Prasad K, Bisht G. (2011) Evaluation of nutritive, antioxidant and mineral composition of Pavettaindica Linn. Leaves, Res. J phytochem. 5;54-59.

Sharma, Y. R. and Achaya, R.C (1988) Analytical Methods in Chemistry Kalyani Publishers, New Delhi, Ludhinia, India. Pp 54-56

Sofowora, A. 1993. Medicinal Plants And traditional Medicine InAfrica. Spectrum Books Ltd., Ibadan, Nigeria, 191-289.

Stafford GI, Pedersen ME, Van Staden J, Jager AK (2008) Review on plants with CNS-effects used in traditional South African medicine against mental diseases, Journal Ethnopharmacology. 119;513-537

Szeto, S. Sziwnji S. (2002). *Trend pharma* Sci. 8:148 – 154.

Tairo VE, Njoka JT, Lukhoba WC, Lyaruu, HVM (2011). Nutritive and anti-nutritive qualities of mostly prefferd edible woody parts in selected dry lands of Inringa District, Tanzania Park, *J. Nutr.*10;786-791.

Trease, G.E., Evans, W.C. 1989. Pharmacognosy, 11th edition,Bailliere Tindall, London, pp. 45-50.

Trease, S. E. and Evans W.C (1995).*Text book of pharmocognocy*.13th edition, Bailliere Tin dhall, London 343- 345.

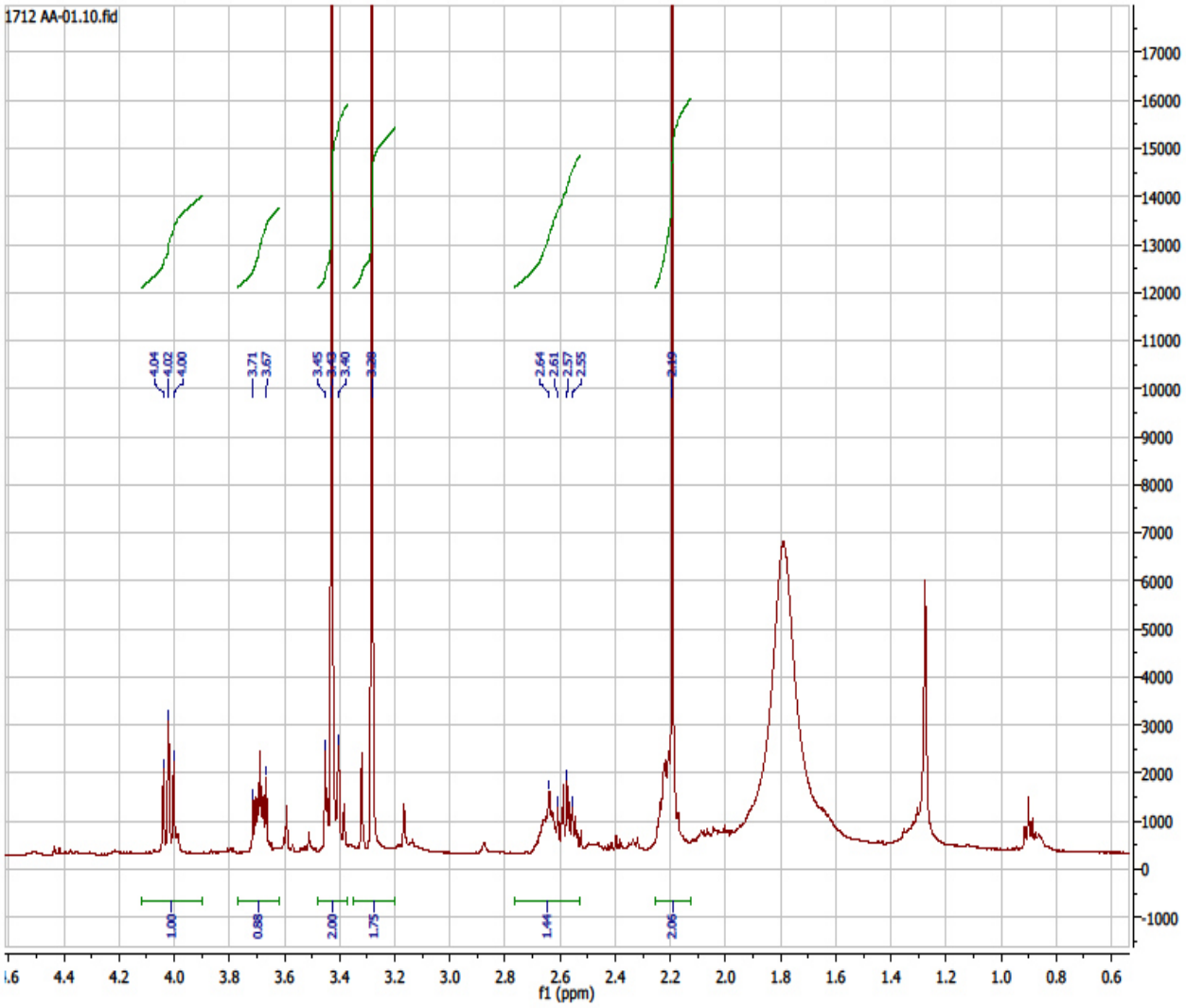
Wang J, Peng Q, Li G (2009). New compounds of natural resources in 2008. African.Journal of Biotechnology, 8(18):4299-4307

Watt and Brandwijk, (1962): Medicinal and poisonous plants of southern and eastern Africa. Pp 32-36

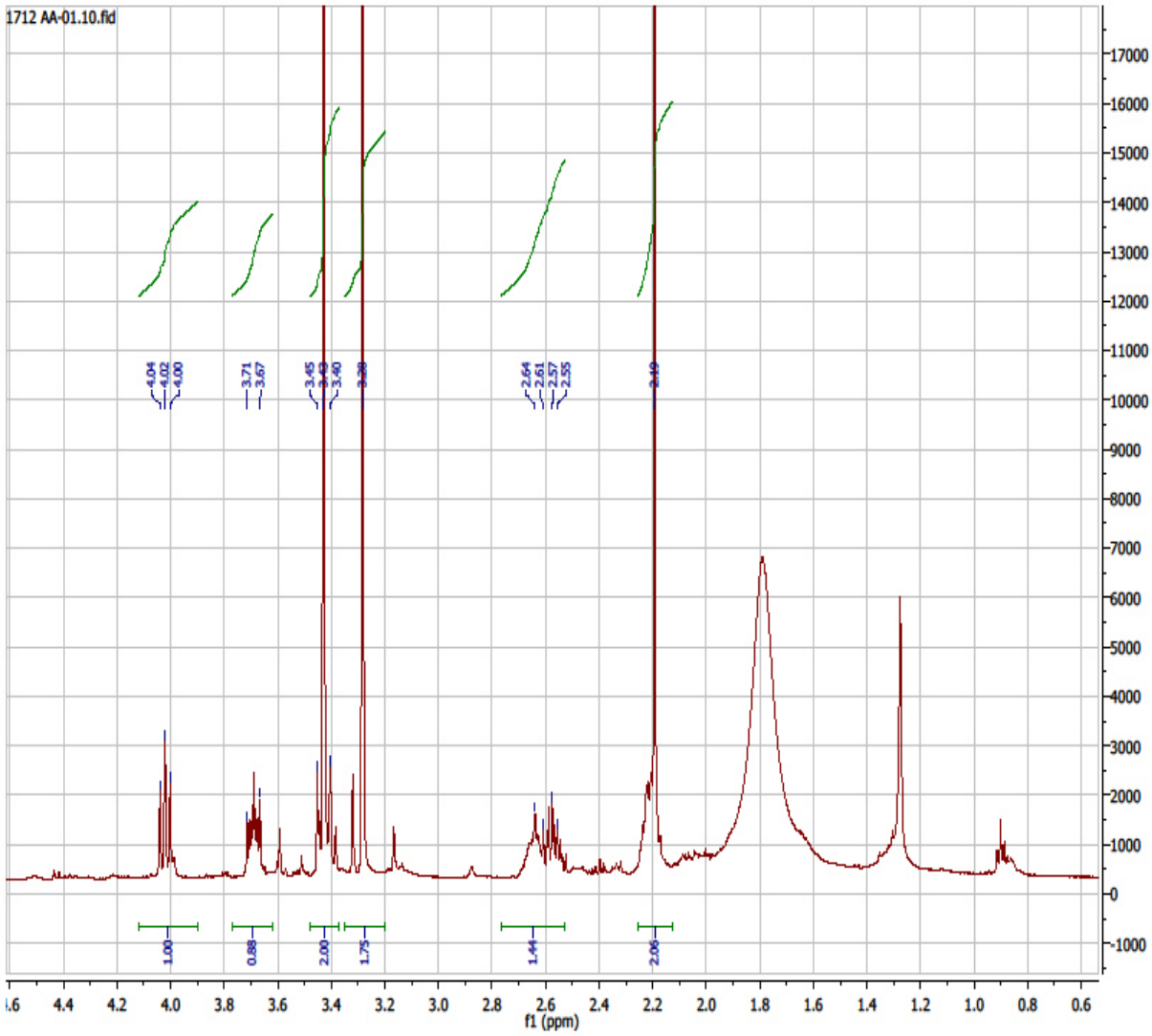
World Health Organization (2006) Accessed 2nd*Cancer fact sheet* No. 297. February June2010. Pp 77-78

Appendixes

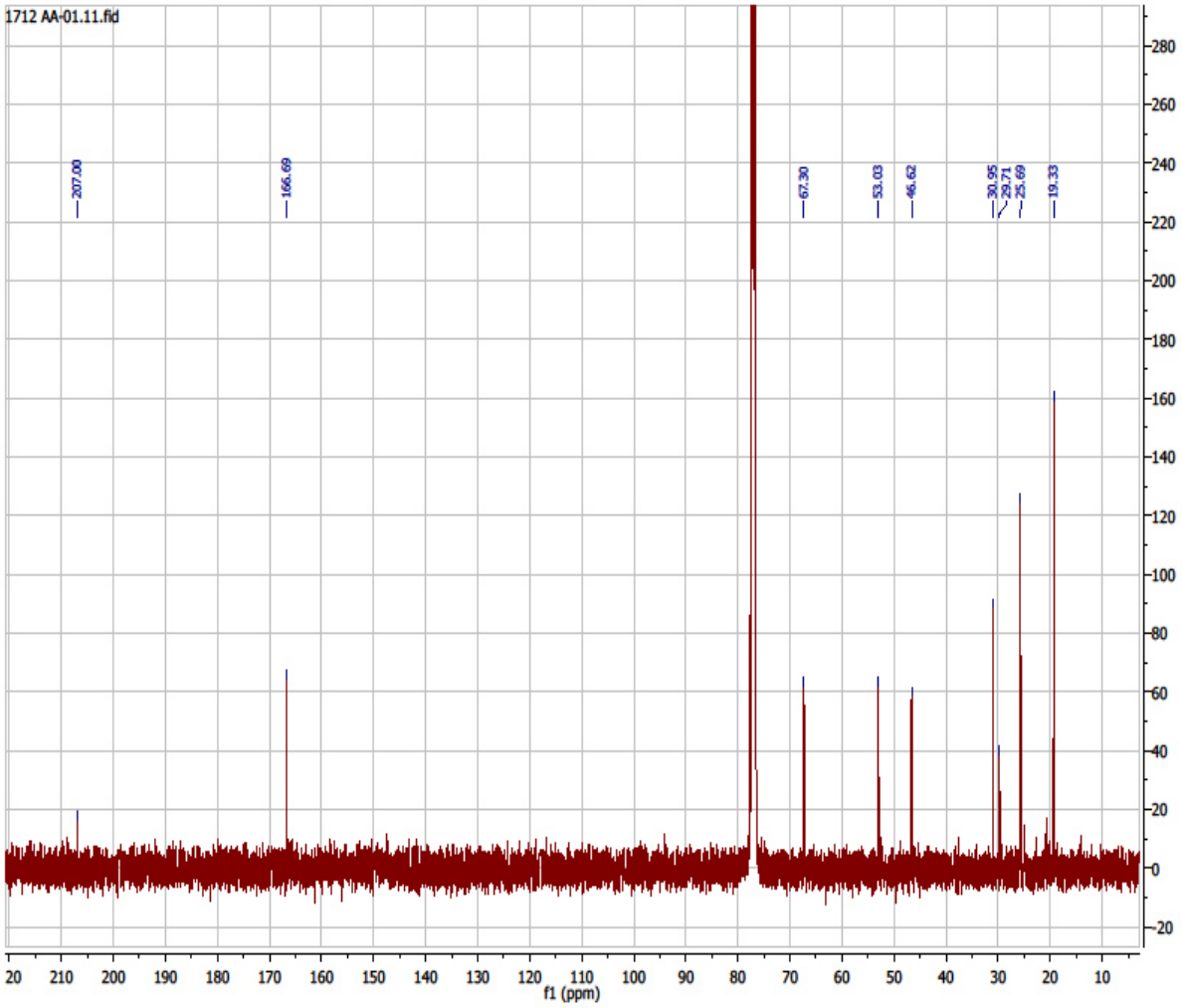
Appendix 1 ^{1}HNMR shows the presence of seven different types of protons at different chemical environment from the chemical shift value.



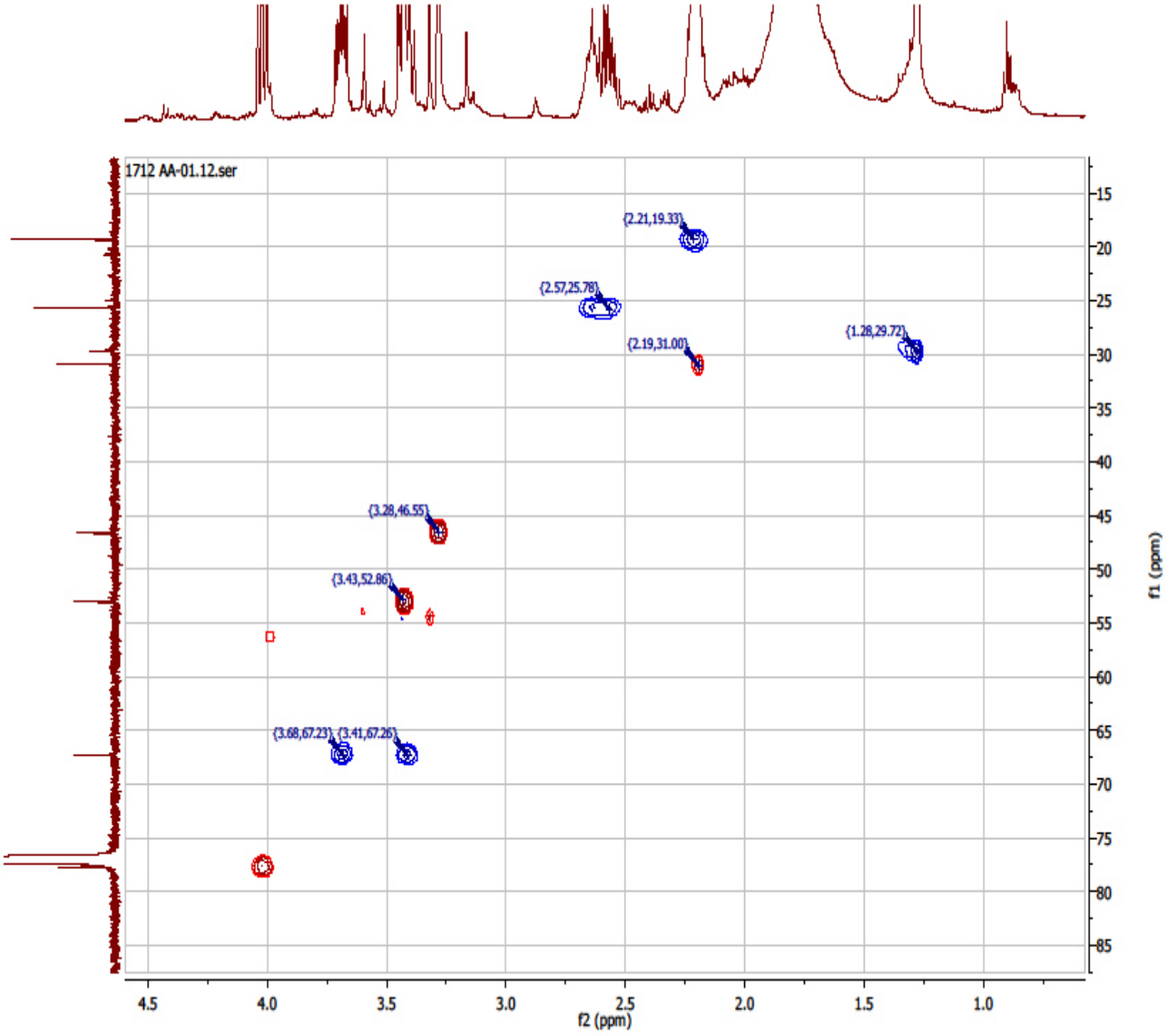
Appendix (ii) Showing expansion of proton NMR



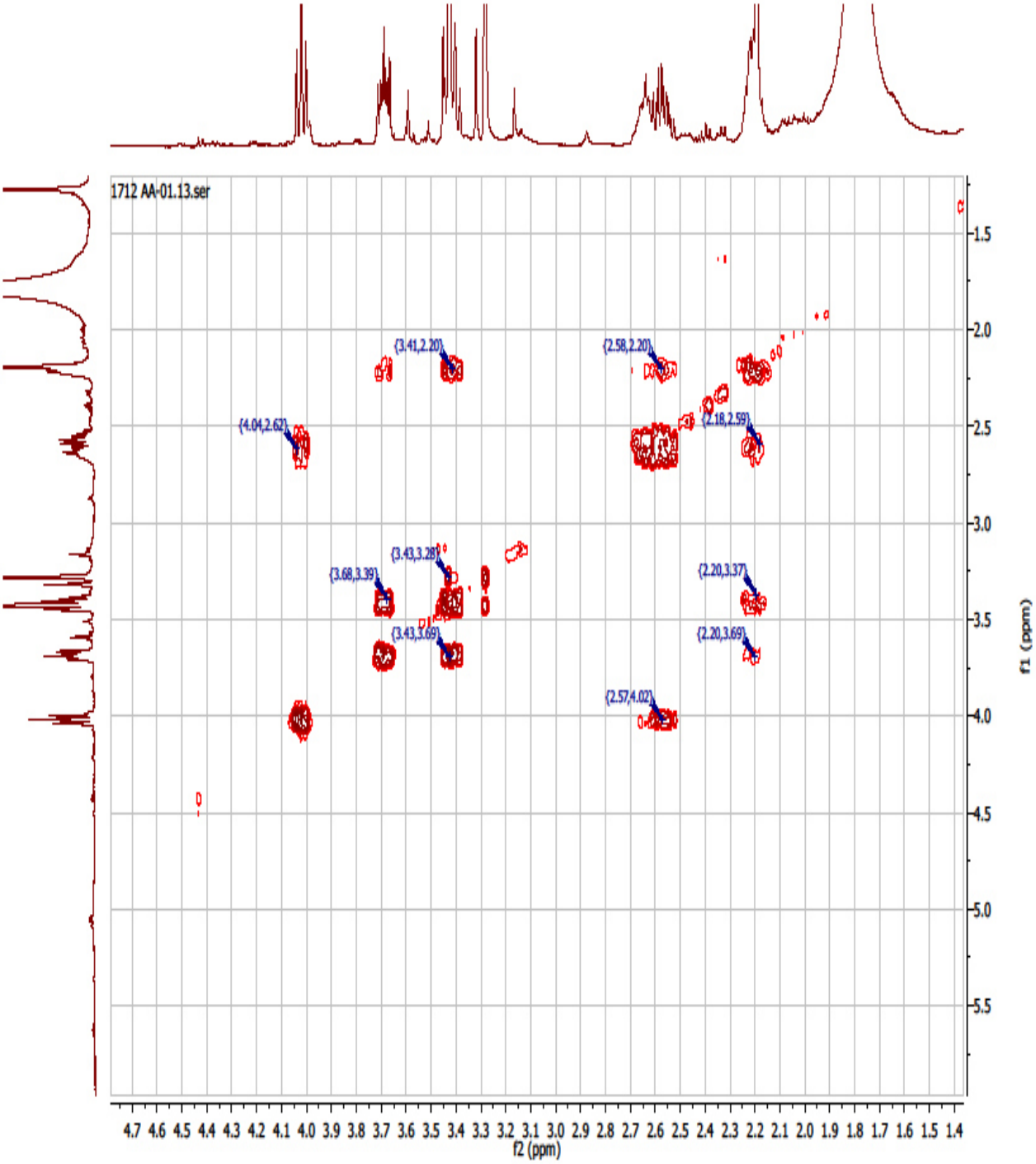
Appendix (iii)^{13}C NMR showing the presence of ten different carbon environments with 1-methyl, 6-methylene, 1-methine and 2-quartenary carbons.



Appendix (IV) HSQC Spectrum showing correlation between proto at δ1.28 with carbon at 29.70, δ2.20 with carbons at 19.33. δ2.20 carbon at 31.00, δ2.57 with carbon at 25.78, δ3.28 with carbon at 46.62, δ3.43 with carbon at 53.00, δ3.41 with carbon at 67.30, δ3.68 with carbon at 67.30, δ4.02 with carbon at 77.30.



Appendix(v) COSY Spectrum showing correlation linkage between the following protons 2.20:3.41, 2.20:3.3.68, 2.58:2.20, 2.57:4.02, 3.41:2.20, 3.43:3.28, 3,68:3.41, 4.02 with 2.58



Appendix (vi) HMBC Spectrum showing correlation across 2 or 3 bonds shows correlation between proton at δ2.20 with carbon at 31.00, 67.30, 206.99, protons at δ2.57 with carbons at 19.27, 77.76, and 166.72 proton at δ3.28 with carbons at 53.00, 67.30, protons at δ3.43 with carbons at 46.62, 65.55, proton at δ3.68 with carbons at 19.33, 25.78, 46.98. Protons at δ4.02 with carbons at 19.33 25.78 and 166.67.