ANALYSIS OF THE CRUDE EXTRACTS OF JATROPHA CURCAS FOR PHYTOCHEMICALS AND THEIR ANTIMICROBIAL ACTIVITIES AGAINSTCLINICAL BACTERIAL ISOLATES

BY

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Declaration

I declare that the work in this dissertation entitled Analysis of the crude extractsof *Jatropha curcas*for phytochemicals and their antimicrobial activities against clinical bacterial isolates acquired from some hospitals in Zaria, Kaduna State, has been carried out by me under the supervision of Professor C.M.Z. Whong and Professor S.E. Yakubu in the Department of Microbiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Zainab Ya'u IBRAHIM	**Signature**	**Date**

Certification

This dissertation entitled ANALYSIS OF THE CRUDE EXTRACTS OF JATROPHA CURCAS FOR PHYTOCHEMICALS AND THEIR ANTIMICROBIAL ACTIVITIES AGAINS CLINICAL BACTERIAL ISOLATES by Zainab Ya'u IBRAHIM meets the regulations governing the award of the degree of Master in Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Dedication

This work is dedicated to my beloved husband Mal. Nasir Bello and my kids Zakariyya, Almustapha, Halima, Aisha, Hafsah and Muhammad Bello. You mean the world to me.

Acknowledgements

I am grateful to Allah Almighty for giving me the ability to carry out this work and for always being there for me.I owe a special appreciation to my able supervisors Prof. C.M.Z. Whong and Prof. S.E. Yakubu who contributed immensely to the success of this work. I am also grateful to my lecturers Dr. E.E Ellah, Dr. M.S. Aliyu, Dr. M.B. Sulaiman just to mention a few. I must appreciate the efforts of my collegues for their immense contribution to the success of my Laboratory work. These include Mal. Adamu Shitu, Hajiya Hajara Ladan, Hajiya Amina Muhammad, Mal. Danlami Dalhatu and Mal. Sani Shika.

To my beloved parents, my dear husband, my lovely children, my sisters, brothers and Mahmood I say thank you and may Allah in His infinite mercies reward you abundantly.

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Abstract

This study investigated the use of *Jatropha curcas* as a medicinal plant used in treating many microbial infections. The lack of scientific standardization for the use of some herbal preparations, as well as possible therapeutic alternatives againstantibiotic resistant bacterial infections indicates a strong need for continuous effort to validate the use of plant material as alternative theraphy regimens with similar or higher antibiotic beneficial properties. The present study describes the phytochemical activities and antibiotic properties of *Jatrophacurcas*extracts and fractions against clinical isolates of *S. aureus, E. coli*, *P. aeruginosa* and *Bacillus* spp. The phytochemical analysis was carried out on aqueous and ethanolic extracts of stem bark, root bark and leaves of the plant using standard methods. The antibacterial potency was initially determined by the agar well diffusion method for both crude extracts and fractions of *jatropha curcas*followed by quantitative evaluation of antibacterial activity by Minimum inhibitory concentration and Maximum bactericidal concentration. The probable chemical compounds present in the plant fractions were identified using Gas Chromatography/Mass Spectrometer analysis. Carbohydrates, saponins, flavonoids and tannins, cardiac glycosides, triterpenes, alkaloids were observed to be present. Root extract of *Jatropha curcas* was observed to be more effective than the stem bark and leave extracts due to its ability to inhibit all the test bacteria. It recorded the highest antibacterial activity with mean inhibition zone diameter ranging from 2.67±0.00-15.33±0.00 mm against *S. aureus* with ethanolic root and 1.4±0.00-14.7±0.00 mmwith aqueous root.The aqueous root extract also recorded mean inhibition zone diameter ranging from 10.0±0.00-17.5±000 mm against *Bacillus* spp and 6.50±0.00-10.0 mm with ethanolic root. A mean inhibition diameter also ranging from 0.00-6.00±0.00 mm with ethanolic root and 0.00-6.50±0.00 mm with aqueous root were also recorded against*P. aeruginosa*and root ethanol hexane recorded the highest activity with fractions on *S. aureus* isolate with mean inhibition zone of 31.7 mm ± 0.1 followed by fraction of ethanolic root ethyl acetate with mean inhibition zone of 26.8 mm± 0.1 with the lowest from root ethanol aqueous fraction with 1.56 mm± 0.1 at a concentration of 100 mg/ml respectively.The highest antibacterial activity of the fractions for *Bacillus* spp was recorded with root ethanol hexane fraction

which had a mean inhibition zone of 39.5 mm ±0.1 and the lowest mean inhibition zone of 13.5 mm ±0.1 was recorded for root ethanol aqueous fraction both at a concentration of 100mg/ml. Acetic acid, linoleic acid ethyl ester, oleic acid methyl ester, 1,3-propanediol, furan-2-ethyl tetrahydro-5-methoxy-2-methyl, hexadecanoic acid, octadecanoic acid, phytol, decanoic acid methyl ester, cic-Z-alpha bisabolene epoxide, octane 2,3,4 trineth, butanedial, di-2-ethylhexyl chlorofornate, phenol, propane 1,1,3 triethoxy, pentafluoropropionic acid dodecyl ester, 2-napthalenemethanol, E-11-tetradecanol,trimethylsilyl etherwere identified chemical compounds with most antibacterial activities. This study has proven the effectiveness and efficacy of *Jatropha curcas* in inhibiting the growth of *S. aureus, E. coli, P. aeruginosa* and *Bacillus*spp pathogenic bacteria of clinical origin.

CHAPTER ONE

1.0Introduction

1.1 Background of study

Historically, plants have provided a source of novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is twofold in the development of new drugs. They may become the basis for the development of a medicine as a natural blue print for the development of new drugs or a phytomedicine to be used for the treatment of diseases (Iwu, 1993). Plants are one of the most important sources of medicine. Plant derived compounds (phytochemicals) have been attracting much interest as natural alternatives to synthetic compounds. Extracts of plants used for the treatment of various diseases forms the basis for all systems of medicine. However, ethnobotany is not much developed when compared to modern system of medicine, mainly because of the lack of proper scientific substantiation in this field. Mostly the pharmacological activity of medicinal plants resides in its secondary metabolites, which are comparatively smaller molecules in contrast to the primary molecules such as proteins, carbohydrates and lipids.Widespread use of drugs is leading to the development of resistance against them in the pathogen and also the side effects associated with them is urging people not to use them. Therefore, there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious disease from medicinal plant (David, 2009; Cordell, 2000).

Medicinal plants remain the feasible source of new compound for the drug development process.The natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to man and it can help to reduce expensive and limited supply of synthetic chemicals. The main advantage of plant products over the synthetic compounds in the treatment of diseases is seen in the eukaryotic system and have no deleterious effect in higher plants and animals including man (Krishnakumar*et al*., 1997).

1.2 *Jatropha curcas*

*Jatrophacurcas*is called physic nut or Purging nut in English, *fula*in fulfulde,*binidazugu* in hausa*, lapalapa* in Yoruba*, etookpa* in Efik and *oru-ebo* in Edo (Burkill, 1994). *Jatropha* is a large genus comprising over 170 species. Commonly occurring species in India are: *J. curcas, J. giandulifera, J. gossypifolia, J. multifida, J. nana and J. podagrica. J.curcas*is the commonest species in Nigeria,mainly grown for bio-diesel because of its high oil content (48%). It is a shrub or small tree with smooth grey bark, which exudes whitish coloured watery latex when cut. *Jatrophacurcas*is a medicinal crop that belongs to the family Euphorbiaceae and has a long history of cultivation in tropical America, Africa, and Asia (Ravindranath*et al*., 2004).

The seed kernels contain a high amount of oil between 58 and 60% (v/w) (Aderibigbe*etal.*,1997), and serve as a source of biodiesel currently being used in

India, Thailand, and other South East Asian countries. The seeds are reported to contain high protein, lectin, saponin, phytic acid, and toxic compounds (Martinez-Herrera *et al.*,2006), as well as a wide range of phytochemicals to which its antimicrobial effect is possibly attributable (Arekemase, 2011; Namuli*etal*., 2011).

It has also been reported that all parts of *Jatrophacurcas*can be used for a wide range of purposes. Extracts from various parts of *Jatrophacurcas*, such as seeds and leaves, have been shown to have molluscicidal, insecticidal, and fungicidal properties (Nwosu and Okafor, 1995; Liu *etal.*,1997; Solsoloy and Solsoloy, 1997; Rug and Ruppel, 2000). *Jatrophacurcas* seed extracts were found to inhibit the growth of *Colletotrichummusae*myceliathat cause anthracnose disease in bananas (Thangavelu*et al*., 2004).The chemicals responsible for those effects were suggested to be phorbol esters in the extract. Gübitz*etal*., (1999) and Goel*et al*., (2007) also stated that some derivatives of phorbol esters are known to have antimicrobial and antitumour properties, as well as molluscicidal and insecticidal effects.*JatrophacurcasL*. is becoming a very useful economic resource both in agriculture, phytomedicine development and development of new lead compounds (Saetae and Suntornsuk, 2010; Mkoma and Mabiki, 2012).

*Jatrophacurcas*grows well particularly in the Northern part of Nigeria and can be used to provide cheap source of antibiotic and disinfectant due to its antimicrobial activities that have been reported (Igbinosa, *et al.*, 2009). Traditional medicine using

plant extracts continues to provide health coverage for over 80% of the world's population, especially in developing countries (WHO, 2002).

Previous studies have reported that*J.Curcas*exhibits antimicrobial activity (Aiyela-agbe*etal.*,2007; Igbinosa*et al*., 2009; Ekundayo*et al*., 2011; Namuli*et al*., 2011). The search for new antibacterial drugs of natural origin is urgently needed in the light of growing cases of microbial resistance to the available synthetic antibiotics (Iwu*et al*., 1999; Wurochekker*etal*., 2008; Krishnaiah*et al*., 2009).

1.3 *Staphylococcus aureus*

*Staphylococcus aureus*a Gram-positivecoccus, which is a member of the firmicutes, frequently found in respiratory tract and on the skin. It is catalase positive and reduces nitrate. It is among the common causes of skin infections like abscesses, respiratory infection like sinusitis and food poisoning. It also produces protein toxins and express cell-surface proteins that bind and inactivate antibodies. *S. aureus*is also implicated in many other illnesses, from minor skin infections such as pimples, impetigo, boils, cellulitis, scalded skin syndrome, ear infection to life threatening diseases like pneumonia, meningitis, osteomyelitis, toxic shock syndrome and bacterial sepsis. It is still one of the five most common causes of hospital acquired infections like postsurgical wound diseases and a host of others. Although, *S. aureus* is susceptible to most *B*-lactam antibiotics like penicillin, there have been reported increase in resistance to antibiotics in infections caused by the organism (Cramer, 2013).*Staphylococcal* strains have evolved mechanisms to inhibit most antibiotics

action such as acquisition of the VanA gene, ribosomal mutations, modifying enzymes and active efflux of the drugs out of the bacteria (Ashton, 2013).

1.4*Escherichia coli*

Escherichia coli is a Gram negative, facultative anaerobe which appeares rod shape under the microscope. It is commonly found in the lower intestine of warm-blooded animals as a normal flora. Most strains are harmless, but some serotypes can cause serious food poisoning in their host. Faecal oral transmission is the major route through which pathogenic strains of the bacterium cause diseases. The pathogenic strains can cause gastroenteritis, urinary tract infections and neonatal meningitis. Although it is susceptible to many antibiotics, *E. coli* have shown strong resistance with streptomycin and other antibiotics to which it was earlier susceptible to (Schrag*et al., 1999*).

1.5*Pseudomonas aeruginosa*

Pseudomonas aeruginosa is also a common Gram negative rod bacterium that causes disease in plants and animals, including humans. It is citrate, catalase and oxidase positive. It is found in soil, water, skin as normal flora and most man-made environments. It uses wide range of organic material as food. In animals its versatility enables this organism to infect damaged tissues or those with reduced immunity. Symptoms of infections by this organism are inflammation and sepsis, but colonizations in critical body organs such as lungs, urinary tract and kidneys can be fetal. It can also cause pneumonia, septic shock, gastrointestinal infections. The

biofilms of *P. aeruginosa* protects the bacteria from adverse environmental factors thus helping it in antibiotic resistance, it also has some chromosomally encoded antibiotic resistant gene and low permeability of the bacterial cellular envelops.

1.6*Bacillus* spp

*Bacillus*spp are Gram positive rods often arranged in pairs or chains with rounded or square ends and usually with a single endospore.The endospores are generally oval or sometimes round or cylindrical and are very resistant to adverse conditions.Most species are motile and resist to penicillin(Salisbury *et al*., 2013).*Bacilluscereus* are implicated mostly in gastrointestinal intoxication, severe cases of meningitis,pneumonia and recurrent bacteremia with *Bacillussubtilis* have been reported by Slima*net al.* (1987).Although most spp are susceptible to antibiotics but some like *Bacillus cereus* and *Bacillus subtilis*have plasmid mediated resistance to antibiotics(Salisbury *et al*., 2013).

1.7 Statement of Research Problems

The traditional treatment and control of diseases by the use of available medicinal plants by rural communities will continue to play significant roles in medical health care implementation in the developing countries (Ekundayo *et al.*, 2011). The use of synthetic medicines as therapeutic agents however is limited, due to various challenges such as poor drug solubility, stability, adsorption and high toxicity. In addition, some of these drugs are expensive and generally unavailable to citizens of developing countries,

especially those residing in the rural areas (Sule *et al.*, 2011). The shortfalls in the use of chemotherapeutic agents as control agents of bacterialdiseases, further encourages the use of plants as a form of alternative medicine.

The drug resistant bacteria and fungal pathogens further complicate the treatment of infectious diseases in immune compromised AIDS and cancer patients. Multi-drug resistant organisms are associated with nosocomial infections (Hidron *et al.*, 2008).

Thus infectious diseases represent a critical problem to health and they are the main cause of high morbidity and mortality rates worldwide (WHO, 2012). Until recently, research and development efforts have provided new drugs in time to treat bacteria that developed resistance to older antibiotics. As bacterial antibiotic resistance continues to exhaust the supply of effective antibiotics, a global public health disaster appears likely.

Substitutes from the nature to the antibiotics are becoming the prime need of the society in the present and in future.

Today, the ongoing emergence of multi-drug resistant bacteria and the infectious diseases caused by them are serious global problems (Albuquerque *et al.*, 2007). Thus, there is an urgent need for novel antimicrobials and/or new approaches to combat these problems (Liu *et al.*, 2000). Antibiotics are the most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to

emergence of drug resistant bacteria. In addition, the present poor efficacies of synthetic antibiotics is partly attributable to widespread production of substandard and adulterated forms. Antibiotics that work today may not work tomorrow. Antibiotic mechanism includes inhibition of cell wall synthesis, cell membrane function, protein and nucleic acid synthesis, and inhibition of specific enzyme system. Therefore, drug synergism between known antimicrobial agents and bioactive plant extracts is a novel concept andhas been recently reported. Therefore, combination therapy is often profitable for patients with serious infections caused by drug-resistant pathogens (Davis *et al.*,2003).

1.8 Justification

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. This problem can be reduced by controlling the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. From centuries, plants have been a valuable source of natural products for maintaining human health (Kapoor, 2014). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The use of plant extracts and phytochemical, both with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency and many plants have been claimed for their exciting anti-microbial activity (Cowan, 2015). The use of and search for drugs derived from plants have accelerated in

recent years. Ethnopharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. While 25 to 50% of current pharmaceuticals are derived from plants, with some used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. Many of plant compounds are currently available as unregulated botanical preparations and are used by the public, there is need therefore, to study these plants and establish their safety(Kumar, 2014). Medicinal plants remain the feasible source of new compound for the drug development process. *Jatropha curcasL*. is becoming a very useful economic resource both in agriculture, phytomedicine development and development of new lead compounds (Saetae and Suntornsuk, 2010). Widespread use of drugs is leading to the development of resistance against them in the pathogen and also the side effects associated with them is urging people not to use them. Therefore there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious disease from medicinal plant (Cordell, 2000).

1.9 Aim

The aim of this study is to determine the phytochemical compounds and antibacterial activityof *Jatrophacurcas* against some clinical bacterial isolates.

1.10Specific Objectives

The specipic objectives for this research were to:

1. determine the phytochemical properties of crude aqueous and ethanolicextractsof stem bark, leaves and root of *Jatrophacurcas.*
2. confirm the clinical isolates of *Staphylococcus aureus, Escherichia coli, Bacillus* spp*, and Pseudomonas aeruginosa* using both conventional and microgen kit.

3. fractionate the crude and ethanolic extracts of stem bark, leaves and root of Jatropha curcas

4. determine the effect of crude extracts and fractions against clinical isolates of *E. coli, S. aureus, Bacillus* spp *and Pseudomonas aeruginosa.*
5. determine the MIC and MBC of crude extracts of*Jatrophacurcas* against clinical isolates.
6. determine the active compounds in *Jatrophacurcas* by Gas Chromatography/mass spectrometer.

CHAPTER TWO

2.0Literature Review

2.1 History of Medicinal Plants

The world have a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value (Sandhu and Heinrich, 2005; Gupta, 2011).

Human beings have used plants for the treatment of diverse ailments for thousands of years (Junaid *et al.*, 2006; Sofowara, 2008). According to the World Health Organization, most populations still rely on traditional medicines for their psychological and physical health requirements (Rabe and Van Stoden, 2000), since they cannot afford the products of Western pharmaceutical industries (Salie *et al.*, 1996), together with their side effects and non-availability of healthcare facilities (Griggs *et al*., 2001). Rural areas of many developing countries still rely on traditional medicine for their primary health care needs. These medicines are relatively safer and cheaper than synthetic or modern medicine (Iwu *et al.*, 1999; Idu *et al*., 2007; Ammara *et al.*, 2009). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines.(Maheshwari *et al.*, 1986; Van Wyk *et al.*, 2000).

Herbal medicines are in great demand in both developed and developing countries as sources of primary health care owing to their wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are reportedly safe and promises toovercome the resistance dilemma developed by the pathogens as such herbal mixtures exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai and Roy, 2004; Tapsell *et al.,* 2006). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources (Fabricant and Farnsworth, 2001). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava*et al.*, 1996; Mahesh and Sathish, 2008).

2.2 Natural Antibiotic Properties of Plant Secondary Metabolites

The plant chemicals are classified as primary or secondary metabolites. Primary metabolites are widely distributed in nature, occurring in one form or another in virtually all organisms. In higher plants such compounds are often concentrated in seeds and vegetative storage organs and are needed for physiological development because of their role in basic cell metabolism. Primary metabolites obtained from higher plants for

commercial use are high volume-low value bulk chemicals (e.g. vegetable oils, fatty acids, carbohydrates etc.) Achten et al., 2010.

Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute important sources of microbicides, pesticides and many pharmaceutical drugs. For a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing important roles in the human society to combat diseases (Wink *et al.,* 2005).

Secondary metabolites (compounds) have no apparent function in a plant's primary metabolism, but often have an ecological role, as pollinator attractants, represent chemical adaptations to environmental stresses or serve as chemical defense against micro-organisms, insects and higher predators and even other plants (allelochemics). Secondary metabolites are frequently accumulated by plants in smaller quantities than the primary metabolites (Karuppusamy**,** 2009; Sathishkumar and Paulsamy, 2009).

In contrast to primary metabolites, they are synthesized in specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result, secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products than the primary metabolites (e.g. steroids, quinines, alkaloids, terpenoids and flavonoids), which are used in drug manufacture by the pharmaceutical industries. These are generally obtained from plant materials by steam distillation or by extraction with organic or aqueous solvents and the molecular weight are generally less than 2000. Some biologically active plant compounds

have found application as drug entities or as model compounds for drug synthesis and semi-synthesis (Paulsamy, 2009).

A survey of current pharmaceutical products for medical uses revealed that, of the total prescription drugs dispensed, 25% are plant derived (Farnsworth and Morris, 1976; Ogundipe *et al.,* 1998). Plant compounds are highly varied in structure. Many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives. However, there have been increased attention on extracts and biologically active compounds isolated from plant species used in herbal medicine, due to the side effects and the resistance that pathogenic micro organisms build against the synthetic antibiotics (Essawi and Srour, 2000). New compounds inhibiting microorganisms such as benzoin and emetine have been isolated from plants (Cox, 1994). Of the various pharmaceuticals used in modern medicine, aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine and tubocurarine serve as examples of drugs discovered through observations of traditional medical practices (Gilani and Rahman, 2005). Eloff (1999) stated that the antimicrobial compounds from plants may inhibit bacteria by different mechanisms than the presently used antibiotics and may have clinical value in the treatment of resistant microbial strains.

Plant constituents may be extracted and used directly as therapeutic agents or as starting materials for drug synthesis or as models for pharmacologically active compounds in drug synthesis. The general research methods include proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemo

pharmacological investigations, toxicological and clinical studies, standardization and use of active moiety as the lead molecule for drug design (Wink *et al.,* 2005).

2.3 Antimicrobial Activity of Medicinal Plants

Medicinal plants have always been considered as source of healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantages of these medicinal plants are numerous (Kalemba and Kunicka, 2003). In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for many years (Barbour *et al.,* 2004).

Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, as well as viral and microbial infections (Koshy *et al*., 2009). Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The harmful microorganisms can be controlled with synthetic drugs and this has resulted in the emergence of multiple drug resistant bacteria and created alarming clinical situations in the treatment of infections. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents (Madunagu *et al*., 2001; Koshy *et al*., 2009).

Antimicrobial studies have shown that Gram-negative bacteria showed a higher resistance to plant extracts than Gram-positive bacteria (Gokhale and Wadhwani, 2015). This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. More specifically, Gram negativebacteria have an outer membrane that

is composed of high density lipopolysaccharides that serve as a barrier to many environmental substances including antibiotics (Paz *et al.*, 1995; Kudi *et al*., 1999; Palambo and Semple, 2001). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Onwuliri and Dawang**,** 2006; Mahesh and Sathish, 2008).

The Indian flora offers great possibilities for the discovery of new compounds with important medicinal applications in combating infection and strengthening the immune system. The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains (Eloff, 1999). There is, thus continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents (Barbour et al., 2004).

2.4 TheRole of Antibiotics in Bacterial Infectious Diseases Treatment

Antibiotics are the mainstay of bacterial treatment (Archer and Ronald, 2001). The goal of these drugs is to kill the invading bacteria without harming the host. Antibiotic effectiveness depends on its mechanism of action, drug distribution, site of infection, immune status of the host and resistance factors of the mico organism (Archer and Ronald, 2001).

Antibiotics work through several mechanisms. Some (such as vancomycin and penicillin) inhibit formation of bacterial cell walls. Erythromycin, tetracycline, and chloramphenicol interrupt protein synthesis. Still others inhibit bacterial metabolism (sulfa drugs) or

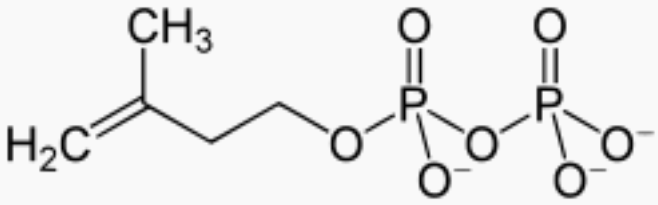
interfere with DNA synthesis (ciprofloxacin, rifampin) and/or cell membrane permeability (polymyxin b) (Conte *et al.*, 2002).

When antibiotics were discovered in the 1940s, they were incredibly effective in bacterial infection treatment. Over time, many antibiotics have lost effectiveness against common bacterial infections because of increasing drug resistance (Perez *et al.,* 1990; Okeke *et al.,* 2005). Bacteria may be naturally resistant to different classes of antibiotics.

2.5 Plant Derived Antimicrobials

2.5.1. Terpenoids

The essential oils of plants are secondary metabolites that are highly enriched with compounds known as terpenes. These compounds are based on an isoprene structure with the general formula of $C_{10}H_{16}$ (Cowan, 1999). Terpenes and terpenoids constitute a very large family of compounds. The structures of terpenoids are diverse and range from relatively simple linear hydrocarbon chains to highly complex ring structures (Back and Chappell, 1996). Terpene hydrocarbons may occur as monoterpenes (C_{10}), diterpenes (C_{20}), triterpenes (C_{30}), tetraterpenes (C_{40}), hemiterpenes (C_5) and sesquiterpenes (C_{15}). Terpenes that contain an additional element (usually oxygen) are termed terpenoids (Cowan, 1999). Triterpenes have been found to be strong inhibitors of HIV-1 reverse transcriptase *in vitro* (Bessong *et al*., 2004).

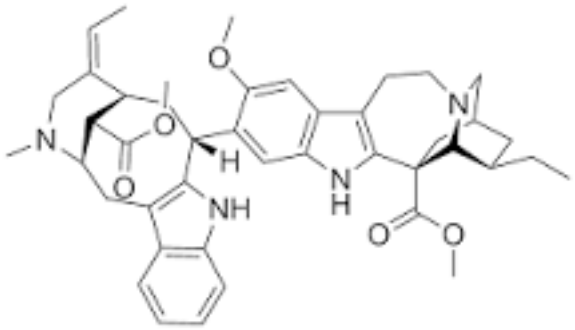


Chemical structure of the terpenoid

2.5.2. Alkaloids

Alkaloids are organic bases containing nitrogen in a heterocyclic ring. Many have pronounced pharmacological activity (Williamson *et al*., 1996). The first medically useful example of an alkaloid is morphine which was extracted from opium poppy *Papaver somniferum* in 1805 (Fessenden and Fessenden, 1982). Some alkaloids have antimicrobial properties (Omulokoli *et al*., 1997; Karou *et al.*, 2006), while others may be useful against HIV infection as well asintestinal infections associated with AIDS (McMahon *et al*., 1995). The mechanism of action of highly aromatic planar quaternary alkaloids such as berberine is attributed to their ability to intercalate with DNA (Cowan, 1999) while indoloquinoline alkaloids such as cryptolepine, cause cell lysis and morphological changes of *Staphylococcus aureus* (Sawyer *et al*., 2013). Berberine is found in roots, rhizomes and stem bark of plants. Extracts and decoctions containing berberine have significant antimicrobial activity against organisms such as bacteria, viruses, fungi, protozoans, helminths and Chlamydia (Birdsall and Kelly, 1997; Karou *et al.*,2006).

Clinically, berberine is used in the treatment of bacterial diarrhoea due to its ability to reduce intestinal secretion of water and electrolytes, induced by cholera toxin, as well as inhibition of some *Vibrio cholerae* and *Escherichia coli* enterotoxins (Karou *et al.*, 2006).

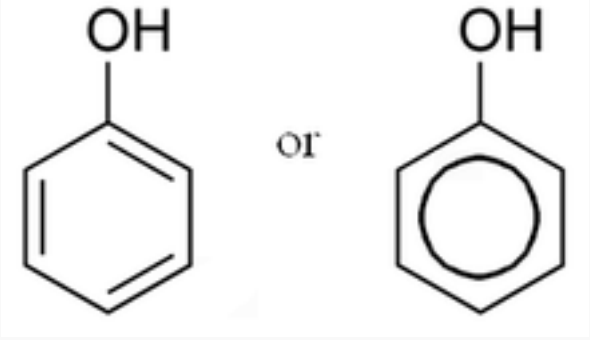


Chemical structure of Alkaloids

2.5.3. Phenolics and polyphenols

Phenolic compounds include a wide range of secondary metabolites found in plants. They possess in common an aromatic ring substituted by one or more hydroxyl groups (Harborne, 1994). The main classes are simple phenols, hydroxybenzoic acids, hydroxycinnamic acids, flavonoids (flavanols, flavones, flavanones, isoflavones and anthocyanins), chalcones, aurones, hydroxycoumarins, lignans, hydroxystilbenes and polyflavans (Chung *et al*., 1998; Krueger *et al*., 2003). The common representatives of a wide group of phenylpropane-derived compounds that are in the highest oxidation state are cinnamic and caffeic acids. Caffeic acid, which is effective against viruses, bacteria, and fungi, is found in common herbs such as tarragon and thyme (Cowan, 1999). Catechin and pyrogallol are both hydroxylated phenols, shown to be toxic to microorganisms. The mechanisms thought to be responsible for phenolic toxicity to

microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with the proteins (Mason and Wasserman, 1987;Alghazeer*et al.*,2012). Eugenol is a well-characterised representative found in clove oil. Phenolic constituents present in essential oils are generally recognized as active antimicrobial compounds. Eugenol, carvacrol, and thymol are phenolic compounds in cinnamon, cloves, sage, and oregano that possess antimicrobial activity. The exact causeeffect relation for the mode of action of phenolic compounds has not so far been determined. However, researches indicated that they may inactivate essential enzymes, reacting with the cell membrane or disturbing material functionality (Zaika, 1988; Eruteya and Odunfa, 2009).



Chemical structure of Phenols

2.5.4. Tannins

Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatine from solution, a property known as astringency (Haslam, 1996). Tannins are found in almost every plant part bark, wood, leaves, fruits and roots. They are divided into two groups namely hydrolysable tanninswhich are based on gallic acid or ellagic acid, and usually occur as multiple esters with D-glucose; and condensed tanninswhich are derived from flavonoid monomers. Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins among others. Both hydrolysable and condensed tannins have been found to be strong inhibitors of HIV-1 reverse transcriptase *in vitro* (Bessong *et al*., 2004).

2.5.5. Flavonoids: flavones and flavonols

Flavonoids are an important group of polyphenols, widely distributed in plant flora. About 4000 flavonoids are known to exist and some of them are pigments in higher plants. Flavones are phenolic structures containing one carbonyl group. The addition of a 3-hydroxyl group yields a flavonol (Fessenden and Fessenden, 1982; Gandhi raja *et al.,*2009). The common flavonoids found in plants are quercetin and kaempferol. Flavonoids are derived from parent compounds known as flavans. Since they are known to be synthesised by plants in response to microbial infection, they have been found to be effective antimicrobial substances against a wide array of microorganisms. Their activity may be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Tsuchiya *et al*., 1996; Gandhi raja *et al.,* 2009).

2.5.6. Saponins

Saponins are a vast group of glycosides, widely distributed in higher plants. They are abundant in many foods consumed by animals and man (Soetan *et al.,* 2006). Saponins are distinguished from other glycosides by their activity in decreasing surface tension. Many saponins have pharmacological properties and are used in phytotherapy and in the cosmetic industry (Sparg *et al*., 2004). Saponins can be classified into two groups based on the nature of their aglycone skeleton. Steroidal saponins are almost exclusively present in the monocotyledonous angiosperms, while triterpenoid saponins occur mainly in the dicotyledonous angiosperms (Soetan *et al.,* 2006). Saponins are believed to form the main constituents of many plant drugs and folk medicine, and are considered responsible for numerous pharmacological properties (e.g. ginseng constituents). They have also been reported to possess antibacterial activity (Sparg *et al*., 2004). The two acylated bisglycoside saponins, Acaciaside A and B, isolated from *Acacia auriculiformis* have been demonstrated to posses antifungal and antibacterial activity (Mandal *et al.,* 2005).

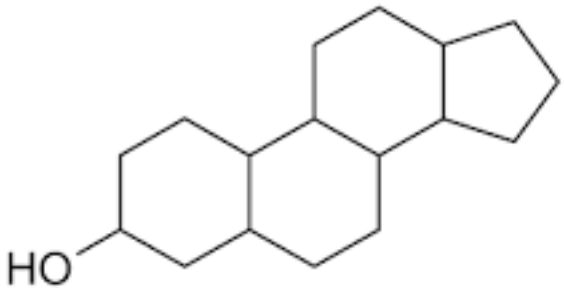
2.5.7. Quinones

Quinones may be defined as aromatic rings with two ketone substitutions. Quinones are characteristically highly reactive. They are responsible for the browning reaction in cut or injured fruits and vegetables which happens because of polymerisation in the presence of oxygen and are an intermediate in the melanin synthesis pathway in human skin (Gokhale

and Wadhwani, 2015). Oxidation and reduction reactions allow an easy switch between diphenol and diketone. Vitamin K is a complex naphthoquinone that possesses antihaemorrhagic activity which may be related to its ease of oxidation in body tissues (Harris, 1963; Gokhale. and Wadhwani, 2015). Quinones may complex irreversibly with nucleophilic amino acids in proteins (Stern *et al*., 1996; Gokhale and Wadhwani, 2015), which leads to inactivation of the protein and loss of function.

2.5.8.Sterols

Plant sterols are C_{28} and C_{29} carbon steroid alcohols 12 that are integral components of plant cell membranes, have been shown to be key components of plant plasma membrane microdomains and may exert similar functions in human cells. These compounds cannot be synthesized by humans and are introduced through the diet where they are found concentrated in plant foods,especially those with are lipid rich (Farnsworth, 1998).



Chemical structure of sterols

2.6 *Jatropha curcas*

Jatropha curcas L. (Euphorbiaceae) or physic nut is an all-purpose, zero-waste perennial plant (Datta, 2007). This plant natively occurs in India, Africa and North America. The genus name *Jatropha* is derived from the Greek *iatrós* (doctor) and *trophé* (food) which implies medicinal use. Natural products isolated from *Jatropha curcas* are found to be the excellent source of synthetic and traditional herbal medicine.

The plant survives with minimum inputs in many parts of the country. *Jatropha*, the wonder plant, produces seed with an oil content of 37%. *J. curcas*is gaining importance commercially as a green fuel source and is being advocated for development of wastelands and dry lands. Currently the oil from *Jatropha curcas* seeds is used for making biodiesel fuel (Fairless, 2007).

2.6.1Description of Jatropha curcas

Jatropha, a succulent perennial shrub or small tree, can attain heights of more than 5 metres, depending on the growing conditions. In each case, the trees are slightly more than two years old. Seedlings generally form a central taproot, four lateral roots and many secondary roots. The leaves, arranged alternately on the stem, are shallowly lobed and vary from 6 to 15 cm in length and width 4). The leaf size and shape can differ from one variety to another. As with other members of this family, the vascular tissues of the stems and branches contain white latex. The branches and stems are hollow and the soft wood is of little value. *Jatropha* is monoecious, meaning it carries separate male and female

flowers on the same plant. There are fewer female than male flowers and these are carried on the apex of the inflorescence, with the more numerous males borne lower down. The ratio of male to female flowers averages 29:1 but this is highly variable and may range from 25-93 male flowers to 1-5 female flowers produced on each inflorescence (Raju and Ezradanum, 2002). It also has been reported that the male-to-female flower ratio declines as the plant ages (Achten *et al.*, 2010), suggesting that fruiting capacity may increase with age.

The unisexual flowers of *Jatropha* depend on pollination by insects, including bees, flies, ants and thrips. One inflorescence will normally produce 10 or more fruits. Fruit set generally results from cross pollination with other individual plants, because the male flowers shed pollen before the female flowers on the same plant are receptive. In the absence of pollen arriving from other trees, *Jatropha* has the ability to self-pollinate, a mechanism that facilitates colonization of new habitats (Raju and Ezradanum, 2002).

The fruits are ellipsoidal, green and fleshy, turning yellow and then brown as they age. Fruits are mature and ready to harvest around 90 days after flowering. Flowering and, therefore, fruiting are continuous, meaning that mature and immature fruits are borne together. Each fruit contains two or three black seeds, around 2 cm x 1 cm in size. On average, the seeds contain 35 percent of non-edible oil. The immature and mature fruits are shown together with the seed. *Jatropha* grows readily from seed which germinate in around 10 days, or from stem cuttings. Growth is rapid. The plant may reach one metre and flower within five months under good conditions (Heller, 1996). The growth is

sympodial, with terminal flower inflorescences and lateral branching, eventually reaching a height of 3 to 5 metres under good conditions. It generally takes four to five years to reach maturity (Henning, 2008).*Jatropha* trees are believed to have a lifespan of 30 to 50 years or more.

Image of jatropha curcas

2.6.2 Medicinal properties of Jatropha curcas

The therapeutic efficacies of many indigenous plants for various diseases had been described by traditional herbal medicinal practitioners. The past decade has seen a considerable change in opinion regarding ethnopharmcological therapeutic application. The presence of various life sustaining constituents in plants has urged scientist to examine these plants with a view to determine potential properties. *J.curcas*is one of the promising ethnomedicinal plants used in Asia and Africa for solving health problems (Lans, 2001). All parts of the plant are used in traditional medicine and active components are being investigated in scientific trials. Several ingredients appear to have promising applications both in medicine and as a plant protectant.

2.6.3 Folk medicine

According to many reports published, *J. curcas* is a proven folk medicine used by many in almost all parts of the world. The leaves of this plant show antileukemic activity and contain aamyrin, b-sitosterol, stigmasterol, and campesterol, 7-keto-bsitosterol, stigmast-5-ene-3-b, 7-a-diol, and stigmast-5-ene- b, 7 b-diol, isovitecxin and vitecxin (Debnath and Bisen, 2008). The seed also contains saccharose, raffinose, stachyose, glucose, fructose, galactose, protein and oils largely of oleic- and linoleic- acids, curcasin, arachidic-, linoleic-, myristic-, oleic-, palmitic-, and stearic-acids (Perry, 1980). The plant is a source of folk remedy for alopecia, anasorca, ascites, burns, carbuncles, convulsions, cough, dermatitis, diarrhoea, dropsy, dysentery, dyspepsia, eczema, erysipelas, fever,

gonorrhea, hernia, incontinence, inflammation, jaundice, neuralgia, paralysis, parturition, pleurisy, pneumonia, rash, rheumatism, scabies, sciatica, sores, stomachache, syphilis, tetanus, thrush, tumors, ulcers, uterosis, whitlows, yaws, and yellow fever. Seed is viewed as aperient; the seed oil emetic, laxative, purgative, for skin ailments. Seeds are also used for treatment of dropsy, gout, paralysis, and skin ailments (Debnath and Bisen, 2008). It is also prescribed homeopathically for cold sweats, colic, collapse, cramps, cyanosis, diarrhoea and leg cramps. It is also reported to be abortifacient, anodyne, antiseptic, cicatrizant, depurative, diuretic, emetic, hemostat , lactagogue, narcotic, purgative, rubefacient, styptic, vermifuge, and vulnerary.

Leaves of physic nut (*Jatropha curcas/gossypifolia*) are boiled and the decoction used to clean sores (Villegas *et al.,*1997; Osoniyi and Onajabi, 2003; Debnath and Bisen, 2008). *J. curcas* latex is applied to external wounds in Perú and Indonesia (Villegas *et al.,*1997). The leaf bath is used for rash, bewitchment and poultices for sores in Trinidad, *J. curcas* leaf and bark contain glycosides, tannins, phytosterols, flavonoids and steroidal sapogenins (Matuse *et al.,*1999; Debnath and Bisen, 2008).

Traditional practitioners from many countries commonly use *J. curcas* for many medical conditions. Mauritians massage ascitic limbs with the oil. Leaves are regarded as antiparasitic, applied to scabies, rheumatism and hard tumors. Cameroon natives apply the leaf decoction in arthritis (Perry, 1980; Debnath and Bisen, 2008). Colombians drink the leaf decoction for venereal disease (Morton, 1981). Bahamans drink the decoction for heartburn. Costa Ricans poultice leaves onto erysipelas and splenosis. Latex applied

topically to bee and wasp stings and is also used to dress sores and ulcers and inflamed tongues. Cubans apply the latex to toothache. Colombians apply the latex to burns, haemorrhoids, ringworm, and ulcers. Barbadians use the leaf as tea for marasmus, Panamanians for jaundice. Root is used in decoction as a mouthwash for bleeding gums and toothache. It is also used for eczema, ringworm, and scabies (Perry, 1980; Debnath and Bisen, 2008). Venezuelans take the root decoction for dysentery. Preparations of all parts of the plant, including seeds, leaves and bark, fresh or as a decoction are used in traditional medicine and for veterinary purposes. The oil has a strong purgative action and is widely used for skin diseases and to soothe pain caused by rheumatism. A decoction of leaves is used against cough and as an antiseptic after birth. Branches are used as a chewing stick in Nigeria (Debnath and Bisen, 2008).

2.6.4 Antimicrobial activities of Jatropha curcas

Traditional uses of plants have paved path for investigations for their bioactive compounds through screening programs. Such studies have helped in the detection of a significant number of therapeutic properties. Parasitological tests were carried out on laboratory bench surfaces using the sap and crushed leaves of *Jatropha curcas* in order to investigate the disinfectant/antiparasitic activities as a first step in providing a cheap, readily available disinfectant and malaria vector control agent. The sap extracted from *J. curcas* was found to exhibit germicidal actions on the growth of common bacteria of *Staphylococcus*, *Bacillus* and *Micrococcus* species, six hours after initial application. Ova

of *Ascaris lumbricoides* and *Necator americanus*incubated in 50% and 100% concentrations of the sap at room temperature showed either no evidence of embryonation after 21 days in the case of *A. lumbricoides*, negation of hatchability in hookworm, or complete distortion in both (Florence *et al.*,2012). The sap also inhibits growth of *Candida albicans* and *Staphylococcus aureus* (Robineau, 1991). Aqueous extracts of physic nut leaves were effective in controlling *Sclerotium* sp., an *Azolla* fungal pathogen (Garcia and Lavas, 1999). Latex has antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiellapneumoniae*, *Streptococcus pyogenes* and *Candida albicans*.

2.7 Bacterial Species Used in this Study

2.7.1*Staphylococcus aureus*

Staphylococci are Gram-positive bacteria, with diameters of 0.5-1.5 μm and grape-like clusters. To date, there are over 32 species and eight sub-species in the genus *Staphylococcus*, many of which preferentially colonize the human body, however *Staphylococcus aureus* and *Staphylococcusepidermidis* are the two most characterised and studied strains (Sievert *et al.*, 2002).

The staphylococci are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. Most species have a relative complex nutritional requirement, however, in general they require an organic source of nitrogen, supplied by 5 to 12 essential amino acids, e.g. arginine, valine, and B vitamins, including thiamine

and nicotinamide (Wilkinson, 1997). Members of this genus are catalase-positive and oxidase-negative, distinguishing them from the genus streptococci, which are catalase-negative, and have a different cell wall composition to staphylococci (Wilkinson, 1997). Staphylococci are tolerant to high concentrations of salt (Wilkinson,1997) and show resistance to heat (Kloos and Lambe 1991). Pathogenic staphylococci are commonly identified by their ability to produce coagulase, and thus clot blood (Kloos and Musselwhite, 1975). This distinguishes the coagulase positive strains, *S. aureus* (a human pathogen),and *S. hyicus* (an animal pathogen), from the other staphylococcal species such as *S.epidermidis*that are coagulase-negative (CoNS).

2.7.1.1Virulence factors of Staphylococcus aureus

Staphylococcus aureus is a major pathogen of increasing importance due to the rise in antibiotic resistance (Lowy, 1998). It is distinct from the CoNS (e.g. *S.epidermidis*), and more virulent despite their phylogenic similarities (Waldvogel, 1990; Projan and Novick, 1997). The species named *aureus*refers to the fact that colonies (often) have a golden colour when grown on solid media, whilst CoNS form pale, translucent, white colonies. To date the *S. aureus* genome databases have been completed for 7 strains, 8325, COL, MRSA, MSSA, N315, Mu50, and MW2 (Horsburg *et al.,* 2002). The average size of the *S. aureus* genome is 2.8Mb (Kuroda *et al.,* 2001). The cell wall of *S. aureus* is a tough protective coat, which is relatively amorphous in appearance, about 20- 40 nm thick (Shockman and Barrett, 1983). Underneath the cell wall is the cytoplasm that is enclosed

by the cytoplasmic membrane. Peptidoglycan is the basic component of the cell wall, and makes up 50% of the cell wall mass (Waldvogel, 1990). It is integral in the formation of the tight multi-layered cell wall network, capable of withstanding the high internal osmotic pressure characteristic of staphylococci (Wilkinson, 1997). Another cell wall constituent is a group of phosphate-containing polymers called teichoic acids, which contribute about 40% of cell wall mass (Knox and Wicken, 1973). There are two types of teichoic acids, cell wall teichoic acid and cell membrane associated lipoteichoic acid; bound covalently to the peptidoglycan or inserted in the lipid membrane of the bacteria. Teichoic acids contribute a negative charge to the staphylococcal cell surface and play a role in the acquisition and localization of metal ions, particularly divalent cations, and the activities of autolytic enzymes (Wilkinson, 1997). Peptidoglycan and teichoic acid together account for about 90% of the weight of the cell wall, the rest is composed of surface proteins, exoproteins and peptidoglycan hydrolases (autolysins). Some of these components are involved in attaching the bacteria to surfaces and are virulence determinants. Finally, over 90% of *S. aureus* clinical strains have been shown to possess capsular polysaccharides (Karakawa and Vann, 1982). Capsule production is reported to decrease phagocytosis *invitro*, and to enhance *S. aureus* virulence in a mouse bacteraemia model, therefore acting as a form of biofilm.

2.7.1.2S. aureus associated infections

*S. aureus*is considered to be a major pathogen that colonizes and infects both hospitalised patients with decreased immunity, and healthy immuno-competent people in the community. This bacterium is found naturally on the skin and in the nasopharynx of the human body. It can cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract, most of which are minor and not life-threatening (Shulman and Nahmias, 1972). Over 4% of patients admitted into one of 96 hospitals in England between 1997 and 1999 for surgery acquired *S. aureus* infection (Aucken *et al.,* 2002). The environment within a hospital also supports the acquisition of resistant *S. aureus* strains. The same study found 81% of the infections were caused by *S.aureus*, and 61% of these were methicillin resistant.

The skin and mucous membrane are excellent barriers against local tissue invasion by *S. aureus*. However, if either of these is breached due to trauma or surgery, *S.aureus* can enter the underlying tissue, creating its characteristic local abscess lesion, and if it reaches the lymphatic channels or blood can cause septicaemia (Waldvogel, 1990). The basic skin lesion caused by an *S. aureus* infection is a pyogenic abscess. However, *S. aureus* can also produce a range of extracellular toxins, such as enterotoxin A-E, toxic shock syndrome toxin- 1 (TSST-1) and exfoliative toxins A and B (Projan and Novick, 1997). Ingestion of enterotoxin produced by *S.aureus* in contaminated food can cause food poisoning (Howard and Kloos, 1990). TSS infections are commonly associated with

menstruating women, particularly those using tampons. The exfoliative toxins are associated with staphylococcal scalded skin syndrome (SSSS). SSSS consists of three entities, toxic epidermal necrolysis, scarlatiniform erythema, and bullous impetigo, all of which damage the epidermal layer of the skin. Infection rates following orthopaedic surgery are 1-2% for total hip arthroplasty, 4% for total knee arthroplasty (Walenkamp, 1990); 2-25% for open fractures and ~1.5% for closed fractures. *S. aureus*has been found to be a common cause of metal-biomaterial, bone-joint and soft-tissue infections (Petty *et al*., 1985; Barth *et al*., 1989). The implantation of biomaterial into the human body, and the damage caused is known to increase the susceptibility to infection and activates host defences, stimulating the release of inflammatory mediators, including oxygen radicals and lysosomal enzymes (Merritt and Dowd, 1987; Dickinson and Bisno, 1989; Gristina, 1994). The fate of a biomaterial surface may be conceptualised as a "race for the surface", involving host cells and bacteria (Gristina, 1987).

Biofilm formation is a two-step process that requires the adhesion of bacteria to a surface followed by cell-cell adhesion, forming multiple layers of the bacteria (Cramton *et al*., 1999). Once a biofilm has formed, it can be very difficult to clinically treat because the bacteria in the interior of the biofilm are protected from antibiotics and phagocytosis (Hoyle and Costerton, 1991). Virulence factors such as proteases are produced once *S. aureus* has colonized a surface (Peterson *et al.*, 1977).

2.7.1.3Treatment of S. aureus infections

The excessive use of antibiotics has led to the emergence of multiple drug resistant *S. aureus* strains (Lowy, 1998). Penicillin was introduced for treating *S. aureus* infections in the 1940s, and effectively decreased morbidity and mortality. However, by the late 1940s, resistance due to the presence of penicillinase emerged (Eickhoff, 1972).

The staphylococci have evolved resistant mechanism to the commonly used antimicrobial agents, such as, erythromycin, ampicillin (Klein and Finland, 1963), and tetracycline (Eickhoff, 1972). In most cases, resistance to antibiotics is coded for by genes carried on plasmids, accounting for the rapid spread of resistant bacteria. Soon after the introduction of methicillin, Jevons (1961) described the emergence of methicillin resistant *S. aureus* (MRSA), which have since spread worldwide as nosocomial pathogens.

Penicillin, a ß-lactam antibiotic works by inhibiting bacterium cell wall synthesis by inactivating the penicillin- binding proteins (PBP). MRSA strains produce a distinct PBP, designated PBP2/, which has a low affinity to ß-lactam antibiotics, hence PBP2/ can still synthesise the cell wall in the presence of the antibiotic (Hiramatsu, 1995). This is the basis for ß-lactam resistance in MRSA strains. PBP2/ are products of the gene *mecA*, which is located in *mec*, foreign chromosomal DNA found in methicillin resistant strains but not in methicillin susceptible strains (Hiramatsu *et al*., 1997). Vancomycin, a glycopeptides has been the most reliable antibiotic against MRSA infections; however, in 1996 the first MRSA to acquire vancomycin intermediate resistance was isolated in Japan

(Hiramatsu *et al*., 1997). Unfortunately, several vancomycin insensitive *S. aureus* (VISA) strains have been reported in the USA, France, Scotland, Korea, South Africa and Brazil (Hiramatsu, 2001). Upon exposure to vancomycin, certain MRSA strains frequently generate VISA strains, called hetero-VISA (Hiramatsu, 2001). VISA resistance appears to be associated with thickening of the cell wall peptidoglycan, and due to an increase in the target for the glycopeptide in the cell wall, therefore requiring more glycopeptide to inhibit the bacteria from growing (Hanaki *et al.*, 1998). All VISA strains isolated appear to have a common mechanism of resistance, which differs from that found in vancomycin resistant enterococci in that enterococcal *van* genes are not present (Walsh, 1993). However, in 2002, the first vancomycin resistant *S. aureus* (VRSA) infection was documented in a patient in the United States (Sievert *et al*., 2002). This strain was shown to carry a *van* gene, suggesting that the resistance determinant might have been acquired through the genetic exchange of material between vancomycin resistant enterococci and *S. aureus*. The spread of vancomycin resistance worldwide is now inevitable, and could potentially result in a return to pre-antibiotic era. Hence, the identification of novel targets on the bacteria seems to be a pre-requisite in the search for new antibiotics and prophylaxis, e.g. vaccines.

2.7.2*Escherichia coli*

Escherichia coli bacteria are Gram-negative and belong to the gamma subclass of proteobacteria. *E. coli*cells are straight rods, motile by flagella or nonmotile. They are

facultative anaerobic, having both a respiratory and a fermentative type of metabolism. Their optimum growth temperature is 37 ºC in pure culture. *Escherichia coli* is the predominant facultative anaerobe of the human colonic flora. The organism typically colonizes the infant gastrointestinal tract within hours of life, and, thereafter, *E. coli* and the host derive mutual benefit (Drasar and Hill, 1974). *E. coli* usually remains harmlessly confined to the intestinal lumen; however, in the debilitated or immunosuppressed host, or when gastrointestinal barriers are violated, even normal "nonpathogenic" strains of *E. coli* can cause infection. Moreover, even the most robust members of our species may be susceptible to infection by one of several highly adapted *E. coli*clones which together have evolved the ability to cause a broad spectrum of human diseases. Infections due to pathogenic *E. coli* may be limited to the mucosal surfaces or can disseminate throughout the body. Three general clinical syndromes result from infection with inherently pathogenic *E. coli* strains: (i) urinary tract infection, (ii) sepsis/meningitis, and (iii) enteric/diarrheal disease.

2.7.2.1Isolation and characterisation

Escherichia coli is the type species of the genus *Escherichia*, which contains mostly motile gram-negative bacilli within the family *Enterobacteriaceae* and the tribe *Escherichia* (Edward and Ewing, 1972; Bettelheim, 1994). *E. coli* can be recovered easily from clinical specimens on general or selective media at 37°C under aerobic conditions *E. coli* in stool are most often recovered on MacConkey or eosin methylene-blue agar,

which selectively grow members of the *Enterobacteriaceae* and permit differentiation of enteric organisms on the basis of morphology (Balows *et al.*,1991). *Enterobacteriaceae* are usually identified via biochemical reactions. These tests can be performed in individual culture tubes or by using test "strips" which are commercially available. Either method produces satisfactory results.

For epidemiologic or clinical purposes, *E. coli* strains are often selected from agar plates after presumptive visual identification. However, this method should be used only with caution, because only about 90% of *E. coli* strains are lactose positive; some diarrheagenic *E. coli* strains, including many of the EIEC strains, are typically lactose negative. The indole test, positive in 99% of *E. coli* strains, is the single best test for differentiation from other members of the *Enterobacteriaceae*.

2.7.2.2 Infections caused by E. coli

Most *E. coli* strains do not cause disease,but virulent strains can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and Gram-negative pneumonia. Uropathogenic *E. coli* (UPEC) is one of the main causes of urinary tract infection.

2.7.3*Bacillus spp*

The genus *Bacillus* is a large and diverse group of bacteria belonging to the family *Bacillaceae,* Phylum Firmicutes. The species in this genus are aerobic or facultatively anaerobic, endospore forming, rod shaped Gram positive bacteria widely distributed in nature, especially in the soil (Harwood, 1989). *B. cereus* group known as the causative agents of food-borne illness belongs to Group I of the genus *Bacillus* (Gibson and Gordon, 1974). Other species in the genus *Bacillus* including *B. subtilis* (Shinagawa, 1990), *B. licheniformis* and*B. thuringiensis* (Beattieand Williams, 1999) and *B. pumilus* are increasingly recognized as food poisoning agents.

The *B. cereus* group comprises six recognised species, *B. cereus*, *B.anthracis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis* (Granum 2001; Jensen *et al.,* 2003; Stenfors Arnesen *et al.,* 2008). In general, species of the *B. cereus* group have a low G+C content of DNA (35%) (Ravel and Fraser, 2005), hydrolyze lecithin and do not ferment mannitol to acid (Fritze, 2004). *B. cereus*, *B. thuringiensis* and *B. anthracis* aremembers of a single species *B. cereus sensu lato.* They are genetically closelyrelated based on genome sequence databoth in the gene content and in synteny(Rasko *etal.*,2004; Didelot *et al*., 2009) with 99% similarity of the 16S rRNA gene sequence.

2.7.3.1 Ultrastructure of the *Bacillus* spore

All *Bacillus* species can form heat stable endospores (Harwood, 1989; Henriques and Moran, 2007). The bacterial endospore is a resting, dormant, tough, non reproductive structure and it is the most resistant living structure known (Atrih and Foster, 2001). Endospores formed by *Bacillus spp*and related aerobic endospore-forming Firmicutes are a strategy to survive during unfavourable conditions.

2.7.3.2 Bacillus spp associated infections

Although anthrax remains the best-known *Bacillus* disease, in recent years other Bacillus species have been increasingly implicated in a wide range of infections including abscesses, bacteremia/septicemia, wound and burn infections, ear infections, endocarditis, meningitis, ophthalmitis, osteomyelitis, peritonitis, and respiratory and urinary tract infections. Most of these occur as secondary or mixed infections or inimmunodeficient or otherwise immunocompromised hosts (such as alcoholics and diabetics), but a significant proportion are primary infections in otherwise healthy individuals. Some of these infections are severe or lethal. Of the species listed in most frequently implicated in these types of infection is *B. cereus,* followed by *B. licheniformis and B. subtilis. B.alvei, B. brevis, B. circulans, B. coagulans, B. macerans, B.pumilus, B. sphaericus, and B.thuringiensis* cause occasional infections. As secondary invaders, *Bacillus* species may exacerbate preexisting infections by producing either tissue-damaging toxins or metabolites such as penicillinase that interfere with treatment (Kramer and Gilbert, 2015).

2.7.3.3 B. cereus as a human pathogen

*B. cereus*belongs to the Hazard group 2 organisms as defined in the European legislation. In 1950 *B. cereus* was first recognized to cause food borne illness. *S. Hauge* isolated in Norway in 1955 *B. cereus*, inoculated it into a sterile vanilla sauce to 106 cfu per ml and consumed it (Hauge, 1955). Severe abdominal pain, diarrhoea and rectal tenesmus followed 13 h after consumingof the contaminated sauce. This allowed Hauge to describe *B. cereus* as a causative agent of food poisoning (Hauge, 1955).

*B. cereus*is the aetiological agent of two major types of foodborne illness, the emetic syndrome and the diarrhoeal syndrome. The diarrhoeal syndrome involves diarrhea and abdominal pain initiating 8 h to 16 h after the ingestion of *B. cereus* in food, followed by toxin production in the small intestine (Granum and Lund 1997). The emetic syndrome is characterized by nausea and vomiting 1 h to 5 h after the ingestion of the heat-stable emetic toxin cereulide, preformed in the food (Granum and Lund, 1997). The dose of the emetic toxin, cereulide, causing acute serious illness in human was reported as 8g of cereulide per kg of body weight (Jääskeläinen *et al.,* 2003b). The outcome of emetic food poisoning may be serious and fatal (Pósfay-Barbe *et al.,* 2008, Pirhonen *et al.,* 2009).

In addition to cereulide, the second toxin of *B. cereus* with known connection to fatal poisoning in human is cytotoxin K (Lund *et al.,* 2000).

Health authorities have not declared *B. cereus* food poisoning as a reportable disease in any country and therefore its incidence is underreported. Food poisoning due to *B. cereus* may be misdiagnosed due to the similarity of symptoms with other types of food

poisoning, for example *Staphylococcus aureus* intoxication and *Clostridium perfringens* (Shinagawa, 1990). Patients with diarrhoeal or emetic syndromes usually do not seek medical care due to the short duration of the symptoms (< 24 h) (Granum, 2011) which adds to underestimation of the reported rate of the illness caused by *B. cereus*. Furthermore *B. cereus* may have been involved in the unclarified outbreaks involving heated food from which no viable bacteria could be isolated.

2.7.4 *Pseudomonas aeruginosa*

Is a common Gram-negative rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P. aeruginosa* is a prototypical "multi drug resistant (MDR) pathogen" that is recognised for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses - especially nosocomial infections such as ventilator-associated pneumonia and various sepsis syndromes.

The organism is considered opportunistic insofar as serious infection is often superimposed upon acute or chronic morbidity - most notably cystic fibrosis and traumatic burns - or found in immune compromised individuals, but the organism does produce a range of clinically-important infections in the immunocompetent and/or in situations where no pre-existing vulnerability is required e.g. hot tub folliculitis. In all infections produced by *P. aeruginosa*, treatment is dually complicated by the organism's resistance profile which may lead to treatment failure and/or expose patients to untoward

adverse effects from advanced antibiotic drug regimens. This dilemma is a central clinical problem in the field of antimicrobial resistance(Balcht and Smith, 1994).

It is citrate, catalase, and oxidase positive. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in hypoxic atmospheres, and has, thus, colonized many natural and artificial environments. It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity.

2.7.4.1 Infections caused by P. aeruginosa

The organism is implicated in infection of wounds and burns, meningitis when introduced by lumber puncture and urinary infections via catheters. It is also involved in respiratory tract infections which can result in pneumonia, may also cause invasive *otitis externa* in diabetic patients. In infants or debilitated persons, *P. aeruginosa* can invade blood stream and result in fetal sepsis (Pier and Ramphal, 2005).

2.8 Antibiotic Resistance

One of the most worrisome characteristics of *P. aeruginosa* is its low antibiotic susceptibility, which is attributable to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes (e.g., mexAB, mexXY etc.) and the low permeability of the bacterial cellular envelopes.In addition to this intrinsic resistance, *P. aeruginosa* easily develops acquired resistance either by mutation in chromosomally

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

Mechanisms underlying antibiotic resistance have been found to include production of antibiotic-degrading or antibiotic-inactivating enzymes, outer membrane proteins to evict the antibiotics and mutations to change antibiotic targets. *P. aeruginosa* can also modify the targets of antibiotic action, for example methylation of 16S rRNA to prevent aminoglycoside binding and modification of DNA. An important factor found to be associated with antibiotic resistance is the decrease in the virulence capabilities of the resistant strain. Such findings have been reported in the case of rifampicin-resistant and colistin-resistant strains, in which decrease in infective ability, quorum sensing and motility have been documented.

Mutations in DNA gyrase are commonly associated with antibiotic resistance in *P. aeruginosa*. These mutations, when combined with others, confer high resistance without

hindering survival. Additionally, genes involved in cyclic-di-GMP signaling may contribute to resistance. When grown in vitro conditions designed to mimic a cystic fibrosis patient's lungs, these genes mutate repeatedly.

2.8.1 Mechanism of action of antibiotics

Different antibiotics have different modes of action, owing to the nature of their structure and degree of affinity to certain target sites within bacterial cells.

1. **Inhibitors of cell wall synthesis**. While the cells of humans and animals do not have cell walls, this structure is critical for the life and survival of bacterial species. A drug that targets cell walls can therefore selectively kill or inhibit bacterial organisms. Examples: penicllins, cephalosporins, bacitracin and vancomycin.
2. **Inhibitors of cell membrane function**. Cell membranes are important barriers that segregate and regulate the intra- and extracellular flow of substances. A disruption or damage to this structure could result in leakage of important solutes essential for the cell's survival. Because this structure is found in both eukaryotic and prokaryotic cells, the action of this class of antibiotic are often poorly selective and can often be toxic for systemic use in the mammalian host. Most clinical usage is therefore limited to topical applications. Examples: polymixin B and colistin.
3. **Inhibitors of protein synthesis**. Enzymes and cellular structures are primarily made of

proteins. Protein synthesis is an essential process necessary for the multiplication and survival of all bacterial cells. Several types of antibacterial agents target bacterial protein synthesis by binding to either the 30S or 50S subunits of the intracellular ribosomes. This activity then results in the disruption of the normal cellular metabolism of the bacteria, and consequently leads to the death of the organism or the inhibition of its growth and multiplication. Examples: Aminoglycosides, macrolides, lincosamides, streptogramins, chloramphenicol, tetracyclines.

4. **Inhibitors of nucleic acid synthesis**. DNA and RNA are keys to the replication of all living forms, including bacteria. Some antibiotics work by binding to components involved in the process of DNA or RNA synthesis, which causes interference of the normal cellular processes which will ultimately compromise bacterial multiplication and survival. Examples: quinolones, metronidazole, and rifampin.

5. **Inhibitors of other metabolic processes**. Other antibiotics act on selected cellular processes essential for the survival of the bacterial pathogens. For example, both sulfonamides and trimethoprim disrupt the folic acid pathway, which is a necessary step for bacteria to produce precursors important for DNA synthesis. Sulfonamides target and bind to dihydropteroate synthase, trimethophrim inhibit dihydrofolate reductase, both of these enzymes are essential for the production of folic acid, a vitamin synthesized by bacteria, but not humans.

Ciprofloxacin

Ciprofloxacin is a fluoroquinolone antibiotic that fights bacteria in the body. Ciprofloxacin is used to treat different types of bacterial infections. Ciprofloxacin is an antibiotic used to treat a number of bacterial infections.This includes bone and joint infections, intra abdominal infections, certain type of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections, among others.For some infections it is used in addition to other antibiotics.It can be taken by mouth or used intravenously.Common side effects include nausea, vomiting, diarrhea, and rash.Ciprofloxacin increases the risk of tendon rupture and worsening muscle weakness in people with the neurological disorder myasthenia gravis. Rates of side effects appear to be higher than some groups of antibiotics such as cephalosporins but lower than others such as clindamycin.It is a second-generation fluoroquinolone with a broad spectrum of activity that usually results in the death of the bacteria. Ciprofloxacin was introduced in 1987.It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system.It is available as a generic medication and not very expensive. It is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, and a type II topoisomerase, topoisomerase IV, necessary to separate bacterial DNA, thereby inhibiting cell division.

CHAPTER THREE

3.0 Materials And Method

3.1 Collection Of Samples

The stem bark, root and leaves of *Jatrophacurcas*were collected from Samaru, Sabon-Gari Local Government area of Kaduna State. These were taken to the herbarium of the Department of Biological sciences of Ahmadu Bello University, Zaria where they were identified as *Jatrophacurcas L*. (voucher No. 22873)belonging to the family *Euphobiaceae.*The stem bark, root and leaves of *Jatrophacurcas* were then washed with tap water, roomdried and ground into fine powder by using mortar and pestle and kept in sterile bottles.

3.1.1 Preparation of plant extract

One hundredgrams (100g) each of the powdered stem bark, root and leaves were added to 300ml each of ethanol and distilled water respectively. Each was allowed to stand for 3days at room temperature (28 ±2ºC), with agitations at intervals. Each extract was sieved through a muslin cloth, filtered through a Whatman (no.1) filter paper, poured unto a clean evaporating dish and placed on a water bath at 50^{o}C until all the solvent evaporated. The dried extracts were stored in a sterile MacCatney bottle and kept in the refrigerator at 4ºC until required for use (Brain and Turner,1975).

3.2 Phytochemical analysis

The extracts were subjected to various phytochemical analysis which identified the chemical constituents present using standard method described by Sofowora (1993). One gram(1g) of each powdered extract was weighed and dissolved in 10ml of sterile distilled water and filtered using WhatmanNo. 1 filter paper. One millilitre each of the filtrate of each dissolved extract, was dispensed into various test tubes and used for the following tests:

3.2.1 Test for carbohydrates

Molisch ‘s test

To one millilitre of each extract in a test tube, 3 drops of Molisch’s reagent was added and concentrated sulphuric acid was added down the side of the test tube to form a lower layer, the result was observed immediately.

3.2.2 Test for Unsaturated Steroid and Triterpines

Liebermann-Bucchard Test

To one millilitre of each extract, 1ml of acetic acid anhydride was added and mixed gently. One (1ml) of concentrated sulphuric acid was added down the side of the test tube to form the lower layer. Colour changes were observed immediately and over a period of 1 h.

3.2.3 Salkwoski Test for Unsaturated Sterols

To one millilitre of each extract, 2 drops of concentrated sulphuric acid was added at the side of the test tube. Immediate colour change at the interface of the extract and sulphuric acid was noted as well as colour changed over a 1h period.

3.2.4Test for Cardiac Glycosides

Keller- Kiliani Test

One gram of each extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. The result was observed for colour changes.

3.2.5Test for Saponin Glycosides

Frothing Test

Tenmillilitre (10ml) of distilled water was added to a portion of each extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 minutes.

3.2.6Test for Tannins

Lead Sub-acetate test

To one millilitre of each extract, 3 drops of lead sub-acetate solution was added. A coloured precipitate indicates the presence of tannins.

3.2.7Test for Flavonoids

Shinoda's Test

One gram (1g) of the extract was dissolved in 2ml of 50% methanol in the heat metallic magnesium chips and 3 drops of concentrated hydrochloric acid was added. The result was observed for colour changes.

3.2.8Test for Alkaloids

Mayer's Test

To test tubes containing 1ml of each extract, 3 drops of Mayer's reagent was added. The result was observed for colour changes.

Dragendoff's Test

To test tubes containing 1ml of each extract, 3 drops of Dragendoff's reagents was added.The result was observed for colour changes.

Wagner's Test

Three drops of Wagner's reagent was added to 1 ml of each extract. The result was observed for colour changes.

3.2.9Test for Free Anthracene Derivatives (Bontrager's Test)

To one gram(1g)of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5 minutes, this was filtered and the filtrate shaken with equal volume of 10% ammonia solution. The result was observed for colour changes (Sofowora, 1993).

3.3 Identification and Characterization of Test Organisms

Clinical isolates of presumptive *S. aureus, E. coli, Bacillus* sppand *Pseudomonas aeruginosa* were obtained from HajiyaGamboSawaba General Hospital, KofarGayan, Zaria city, Kaduna State (HGSH) and Major Ibrahim AbdullahiMemorial Hospital, Sabon-Gari, Zaria, Kaduna State (AIMH). Ethical clearance was obtained from the scientific ethical committee, Kaduna State Ministry of Health as well as the Hospitals. The isolates were then taken to the laboratory, Department of Microbiology Ahmadu BelloUniversity, Zaria, and confirmed using the following tests:

3.3.1 Gram staining and microscopy

Smear of the isolates obtained were fixed on clean grease free slides and stained with crystal violet solution (primary dye) for 60 seconds, rinsed with tap water and drained to

avoid diluting with the mordant. It was further flooded with Gram's iodine solution (mordant) for 30 seconds and rinsed. Then the decolourizer was applied drop wise on the tilted slide until all free colours had been removed and subsequently rinsed with tap water the slides were then flooded with Safranin (secondary dye). The slides were examined under the microscope at ×100 oil immersion objective (Cheesbrough, 2010).

3.3.2 Conventional Biochemical Tests

Biochemical tests were carried out on the clinical bacterial isolates as follows:

Catalase Test:

Catalase is an enzyme that catalyses the decomposition of hydrogen peroxide into oxygen and water. This was done by addition of a drop of the bacterial suspension to a drop of hydrogen peroxide on a clean microscope slide. The appearance of effervescence and bubbling is an indication of a positive reaction. This test was done to identify members of the genus *Staphylococcus* (Cheesbrough, 2010).

Coagulate Test:

A drop of sheep plasma was placed on a grease free slide followed by the addition of a drop of the bacterial suspension in saline. The preparation was rocked and observed for agglutination within two minutes. Appearance of agglutination is indicative of positive reaction. This was done to distinguish *S. aureus* species from other species of the genus *Staphylococcus* (Cheesbrough, 2010).

Citrate Utilisation:

Bacillus spp utilize citrate as its source of carbon. The media was prepared according to manufacturer's instruction by diluting 22.5g of the agar in 1000ml distilled water, boiled and dispensed in bijou bottles in aliquots of 5 mls and sterilized by autoclaving at 121ºC for 15minutes, then slopped and the organism was stabbed at the butt of the slant and incubated at 37 °C **(**Cheesbrough, 2010).

Oxidase Test

This test helps in identifying *Pseudomonas aeruginosa* and *Bacillus* spp. *Pseudomonas* produce an enzyme called Cytochrome oxidase. A piece of filter paper was placed in a clean Petridish and 2 drops of freshly prepared oxidase reagent was added, a colony of the test organism was then smeared on the filter paper using sterile swab stick (Cheesbrough, 2010).

Indole Test

Escherichia coli breaks down the amino acid tryptophan and releases indole. This test was carried out by inoculating the organism in sterile tryptone water and incubated at 37

ºC for 48 h. Thereafter, 0.5ml Kovac's reagent was added and shaken well and observed immediately for colour change.(Cheesbrough, 2010).

Fermentation of Mannitol

Staphylococcus aureus ferments mannitol and is able to grow well on mannitol salt agar. A selective medium containing peptone, lab-lemco powder, mannitol, 70-100g/l sodium chloride, phenol red, pH 7.3 at room temperature and agar was prepared according to manufacturer's instruction by suspending 111 g of the agar in 1000ml distilled water, boiled and sterilised by autoclaving by autoclaving at 121ºC for 15minutes and cooled to 45 ºCbefore dispensing in Petridishes (Cheesbrough, 2010). 0.1 ml of the bacterial suspension was placed on the plates and spreaded with sterile swab stick.

3.4Microgen Staph Latex Agglutination Test

Microgen® Staph Latex Agglutination test is sensitive and specific for the identification of *S. aureus* from primary plate culture in 2 minutes. Microgen® staph contained all auxiliary reagents needed to complete the test which includes positive and negative control, slide cards and plastic sticks for picking of culture. A drop of the reagent was placed on the slide card and a colony of the bacterial isolate was emulcified on it. Agglutination was observed within two minutes.

3.4.1MicrogenS. aureus identification test

The Microgen Staph-ID system comprises a single micro well test strip containing 12 standardized biochemical substrates that have been selected on the bases of extensive computer analysis of published databases for the identification of the genus *Staphylococcus.* The hydrated substrates in each well are reconstituted with a suspension of the organism to be identified prepared in the suspending medium provided. If the individual substrates are metabolized by the organism, a colour change occurs during incubation or after addition of specific reagents. The permutation of metabolized substrate can be interpreted using the Microgen identification system software (MID-60) to identify the organism. Microgen Staph-ID has been developed for the identification of commonly encountered *Staphylococcus* spp.Suspension of the organism was prepared according to MacFarland turbidity standard, 0.1 ml was placed into each of the well and were incubated at 37 ºC for 24 hrs after which it was observed for colour changes.

3.4.2 Microgen GN-ID A Panel for Identification of *E. coli.*

The microgen GN-ID A panel for identification of enterobacteriaceae system comprises a single micro well test strip containing 12 standardised biochemical substrates which have been selected on the basis of extensive computer analysis of published database for the identification of *E. coli* and other enterobacteriaceae. The hydrated substrates in each well were reconstituted with a suspension of the organisms to be prepared in suspending medium (0.5 MacFarland Standard). If the substrates are metabolised by the organism, a colour change occurs during incubation or after the addition of specific

reagents.Suspension of the organism was prepared according to 0.5 MacFarland turbidity standard, 0.1 ml was placed into each of the well and were incubated at 37 ºC for 24 hrs after which it was observed for colour changes.

3.5Antibacterial Susceptibility Test

Mueller Hinton Agar (Titan Biotech Ltd. Bhiwadi- 301 019, Rajasthan, India.) was used for the antibacterial susceptibility testing. It was prepared according to manufacturer's instructions by suspending 38g of medium in 1000ml distilled water, sterilized at 121ºC, and cooled to room temperature prior to dispensing in Petri dishes.

3.5.1 Preparation of extract concentration

This was carried out according to the method described by Srinivasan*et al.,* (2009). Stock solution of the plant extracts were prepared by adding 1g of each crude plant extract in 10ml of 10% dimethylsulphuroxide (DMSO). From each of the stock solutions, 50mg/ml, 25mg/ml and 12.5mg/ml concentrations were prepared using Two-fold serial dilution method. These concentrations were labelled and kept in bijou bottles for subsequent use.

3.5.2 Preparation of turbidity standard

McFarland standard are used as a reference to adjust the turbidity of microbial suspension so that the number of bacteria will be within a given range. Firstly, $BaCl_2$(1%w/v) and H_2S0_4(1% v/v)were prepared by dissolving 1g of $BaCl_2$ in 100ml of sterile distilled water

and 1ml of concentrated H_2S0_4 in 99ml of sterile distilled water respectively to serve as stock solutions for the preparation of the McFarland standard. From the stock solutions, 0.5McFarland scale was prepared by adding 9.95ml of (1%v/v) H_2S0_4 to 0.05ml of (1% w/v) $BaSO_4$ whose density is equivalent to 1.5×10^8 CFU/ml approximate cell density of bacteria. The barium sulphate suspension in 6ml aliquots were transferred in to screw-cap tubes, tightly sealed, and stored at room temperature in order to prevent loss by evaporation. This was subsequently used for comparison with the turbidity of the bacterial inoculum (Cheesbrough, 2010).

3.5.3Standardization of bacterial inocula.

For inocula standardization, the density of each isolated bacterial culture was adjusted equal to that of 0.5 McFarland standards (1.5×10^8 CFU/ml) by suspending 2 or 3 colonies of each bacterial culture into 2ml of sterile physiological saline as a suspending medium. The physiological saline was prepared by dissolving 8.5g of $NaCl_2$ in 1L of distilled water before steriliseing. To aid comparison, the test organisms and standard were compared against a white background with contrasting black lines (Cheesbrough, 2010).

3.5.4 Susceptibility test of clinical isolates to plant extracts.

The antimicrobial activity of *Jatrophacurcas*crude extract and fractions (Aqueous and Ethanolic) against the test organisms was evaluated using agar well diffusion method of susceptibility test (Srinivasan*et. al.,* 2009*).* Mueller-Hinton agar plates were inoculated with 0.1ml of standardized inoculum of each bacterium (in triplicates) using 0.1ml pipette

and spread uniformly with sterile swab sticks. Wells of 8mm size were made with sterile cork borer into the inoculated agar plates. Using micropipette, 0.1ml volume of the various concentrations; 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml each of the crude extracts and fractions were dispensed into wells of inoculated plates. The prepared plates were then left at room temperature for10minutes, allowing the diffusion of the extracts into theincubation at 37 ºC for 24 h. The diameter of inhibition zones (DIZ) were measured and expressed in millimetres after incubation. The mean values of the diameter of inhibition zones were calculated to the nearest whole number. DMSO was used as negative control. Commercially available standard antibiotic, Ciprofloxacin (10mg) was used as positive control parallel with the extracts. For the antibiotic inhibition zone was interpreted in accordance with CLSI(Clinical Laboratory Standards Institute)(2011) interpretation guidelines.

3.6Determination of Minimum Inhibitory Concentration (MIC)

Extracts that exhibited activity against the test organisms were further assayed for their minimum inhibitory concentrations (MIC). The broth dilution method was employed using Mueller Hinton broth as described by Andrews (2001). Two-fold serial dilutions of each reconstituted extract was made to obtain the following concentrations; 25, 12.5, 6.25 and 3.125mg/ml. Each extract concentration was then inoculated into tubes containing 100µl of active inoculum of standardized bacterial isolates and incubated for 24h at 37ºC.

The MIC was determined as the lowest concentration of the extract that inhibited the organism and results were observed in the form of turbidity.

3.7Determination of Minimum Bactericidal Concentration (MBC)

Minimum Bacteriocidal Concentration (MBC) for each extract was determined from the MIC tubesthat showed no visible bacterial growth by sub-culturing a loopful from each tube on to nutrient agar plate and incubated at 37ºC for 24h. The lowest concentration of each extract that yielded no growth was recorded as the MBC (Andrews, 2001).

3.8 Solvent Fractionation of Crude Plant Extracts

The active crude extract (Root) of *Jatrophacurcas* was fractionated in accordance with the procedures of Venskuttn*et al.*(2009). The extraction solvents were hexane (non-polar), ethyl-acetate, n-butanoland water. The procedure was carried out in a separating funnel in which fractions obtained were evaporated to dryness on a water bath to remove the solvent.

3.9Gas-Chromatography/Mass–Spectrometry (GC-MS) of the Stem Bark, Root and Leaves Extract of *Jatropha Curcas*

To identify the actual phytochemical compounds in the active fractions of the extracts. Each fraction was further analysed by GC-MS inlet port. The results were printed out

from the computer system connected to the GC/MS machine (Amirav*et al.,* 2008). The GC-MS is composed of two major building blocks; the gas chromatograph and the mass spectrometer. GC analysis separates all of the components in a sample and provides a representative spectral output. Each sample was injected into the injection port of the GC device. The GC instrument vapourized the sample and then separated and analysed the various components. Each component ideally produces a specific spectral peak that was recorded on a paper chart electronically. The time elapsed between injection and elution is called the "retention time". The retention time can help to differentiate between component compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimen analysed. The peak is measured from the baseline to the tip of the peak. Mass spectrometry identifies substances by electrically charging the specimen molecules, accelerating them through a magnetic field, breaking the molecules into charged fragments and detecting the different charges. A spectral plot displayed mass of each fragment(Amirav*et al.,* 2008).

3.10 Data Analysis

The data generated are presented in tables and charts and were analysed statistically using the Statistical Package and Service Solution Package (SPSS) version 18. The details are

attached in the appendices. Analysis of Variance (ANOVA) was used to compare means of the plant extracts at different concentrations, the standard strain, and the positive control antibiotics if there is any statistically significant difference in the diameter of zones of inhibition. Subsequently, these were further ranked by the Duncan's multiple range tests

CHAPTER FOUR

4.0RESULTS

4.1 Extraction Yield of Plant Material

After extraction process, the weights of aqueous and ethanolic powdered extracts of root, stem bark and leaves obtained were recorded as root 115.11g, 80.00g, stem bark 90.56g, 75.88g and leaves 89.10g, 60.54g respectively.

4.2 Phytochemical Constituents of Aqueous and Ethanolic Extracts of Plant Material

Table 4.1 is the phytochemical analysis of the leaf,stem bark and root bark extract of *Jatrophacurcas*. The aqueous leaf extract and the ethanolic leaf extract contains carbohydrates, cardiac glycosides, steroid, triterpenes, tannins, flavonoids, alkaloids and glycosides. Saponin is present in the ethanolic leaf extract and absent in the aqueous leaf extract. The ethanolic stem bark extract and the aqueous stem bark extract contains carbohydrates, cardiac glycosides, triterpenes, tannins, flavonoids, alkaloids and glycosides. The ethanolic root extract and the aqueous root extract contains carbohydrates, cardiac glycosides, triterpenes, tannins, flavonoids, alkaloids and glycosides. The ethanolicroot bark extract *Jatrophacurcas*contains steroid and triterpenes which is absent in the aqueous extract.

Table 4.1: Phytochemical Constituents of Aqueous and Ethanolic Extracts of Plants Material

s/n	Constituents/Tests	LEE	LEA	SEE	SEA	REE	REA
1	Carbohydrates	+	+	+	+	+	+
2	Anthraquinone	-	-	-	-	-	-
3	Cardiac glycosides	+	+	+	+	+	+
4	Saponins	-	+	+	+	-	+
5	Steroid	+	+	-	-	-	+
6	Triterpenes	+	+	+	+	+	+
7	Tannins	+	+	+	+	+	+
8	Flavonoids	+	+	+	+	+	+
9	Alkaloids	+	+	+	+	+	+
10	Glycosides	+	+	+	+	+	+

KEY: +=Present, -=Absent, LEE= Leaf Ethanolic Extract, LEA= Leaf Aqueous Extract, SEE= Stem Ethanolic Extract, SEA= Stem Aqueous Extract, REE= Root Ethanolic Extract, REA= Root Aqueous Extract.

4.3 Identification and characterization of test organisms;

4.3.1 Cultural and physiologic properties of clinical isolates

The cultural and physiologic properties of clinical isolates of *Staphylococcusaureus, Bacillus* spp, *Escherichia coli* and *Pseudomonas aeruginosa* are presented on Table 4.2.

Out of 20 (100%) clinical isolates of *Staphylococcusaureus* obtained from the hospitals 10(50%) appeared as golden yellow, slightly raised colonies on Mannitol salt agar, Gram positive, cocci and clustered under the microscope. Catalase positive, coagulase positive, fermentmannitol, positive for latex aggulitinationin conventional biochemical and thus, were identified as *Staphylococcusaureus*.

The result also depicts that out of 10 (100%) clinical isolates of *Pseudomonas aeruginosa*obtained from the hospitals, 2 (20%) appeared as blue –green with fishy odour, smooth,mucoid and flat on cetrimide agar, Gram negative rods under the microscope, motile,both catalase and oxidase positive were identified as *Pseudomonas aeruginosa.*

Also out of 10 (100%) clinical isolates of *Escherichia coli* obtained from the hospitals 6 (60%) appeared greenish with metallic sheen colonies with smooth and raised colonies on Eosin methylene blue agar. Gram negative rods under the microscope, VogesProskauer (VP) negative, citrate negative, methyl red (MR) positive and indole positive as *Escherichia coli*.

It indicates alsothat out of 10 (100%) of clinical isolates of *Bacillus* spp obtained from the hospitals 2 (20%) appeared white, large and opaque colonies on nutrients agar, Gram

TABLE 4.2: CULTURAL AND PHYSIOLOGICAL PROPERTIES OF CLINICAL ISOLATES

Isolate	Colonial Appearance	Gram Reaction	Biochemical Reaction								
			CAT	COA	IND	CIT	VP	MR	OXI	MAN	LA
S. aureus	Golden yellow	Positive cocci	+	+	_	_	_	_	_	+	+
E. coli	Green metallic sheen	Negative rod.	_	_	+	_	_	+	_	_	_
P. aeruginosa	Blue- green pigment	Negative rod	+	_	_	_	_	_	+	_	_
Bacillus spp.	Milky	Positive rod	+	_	_	+	_	_	+	_	_

Key:CAT=Catalase, COA=Coagulase,IND=Indole,CIT=Citrate,VP=Voges Proskauer, MR=Methyl Red, OXI=Oxidase,MAN=Mannitol, LA=Latex agglutination +=Positive, -=Negative.

positive, rods under the microscope, endospore positive, motility positive, catalase positive, oxidase positive, citrate positive were identified as *Bacillus* spp.

4.4 Biochemical Characterization and Identification of Clinical Isolates Using Microgen Kit

Microgen* *S aureus* identification kit and Microgen *E. coli*identification kit

The result of Microgen* *S aureus* and *E. coli* identification kit conducted on both isolates of *S. aureus* and *E. coli* are presented on Table 4.3, where isolates were identified as *Staphylococcusaureus* and *Escherichia coli*.

4.5 Antibacterial Susceptibility Test

The result of antibacterial susceptibility test of clinical isolates of the organisms to different concentrations of aqueous and ethanolic crude extracts of *Jatrophacurcas* as well as the control antibiotic is presented in Figures and Tables:

Fig. 4.1 indicates the susceptibility of clinical isolates of *Staphylococcusaureus* to different concentrations of both Aqueous and Ethanolic crude extracts of roots, stem bark and leaves of *J. curcas.*

LA	C	N	S	T	M	N	MA	T	P	B	B	U	A	PYR	O	MO	L	OR	H_2S	G	X	ONPG	I	UR	VP	CI	TDA	%	OC	FI
+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	77776	SA
-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	-	-	-	-	97.11	6760	EC

Key: LA=Latex agglutination test, C=colony pigmentation, N=Nitrate, S=Sucrose, T=Trehalose M=Mannitol, N=N-acetyl Glucosamine, MA=Mannose, T=Turanose, P=Alkaline Phosphatase, B=Beta Glucosidase, U=Urease, A=Arginine, PYR=Pyrrolidonylarylamidase, O=Oxidase, MO=Motility, L=Lysine, OR=Ornithine, G=Glucose, X=Xylose,I=Indole, UR=Urease, VP=Voges Proskauer, CI=Citrate, OC=Octal Code, FI=Final Identification, +=Positive, -=Negative, SA=*Staphylococcus aureus*, EC=*Escherichia coli*.

At 12.5mg/ml aqueous extract of root and ethanolic stem have the highest activity of 1.4mm±0.1respectively while other extracts with both aqueous and ethanol have no activity. At concentration of 25 mg/ml also RAE have the highest activity with 11.9mm±0.1followed by SEE with 8.5mm±0.1,then REE with 2.67mm±0.1 while the other extracts have no activity. At 50mg/ml aqueous root has the highest activity with 14.7mm±0.1 followed by ethanolic stem with 10.70mm±0.1 then ethanolic root with 10.10mm±0.1 while other extracts record zero activity. At the highest concentration of 100mg/ml aqueous root has the highest activity with 17.7mm±0.1 followed by ethanolic root with 15.3mm±0.1 then ethanolic stem with 13.3mm±0.1 with lowest activity from aqueous stem with a value of 1.2mm±0.1 while leaves has no activity. The control was with a value of 33.7 mm±0.1. The difference was statistically significant, indicating that root extract was more significant than other extracts with P value of <0.05.

Fig. 4.2 represents the varying degrees of susceptibility and resistance of clinical isolate of *E. coli* to different concentrations of both aqueous and ethanolic extracts of root, stem bark and leaves of *J. curcas* where all the extracts showed slight or no activity at all in the isolates. The highest mean inhibition zone was exhibited by aqueous root with a value of 9.25 mm±0.1 at 100 mg/ml concentration with the lowest by ethanolic stem with a value of 2 mm±0.1 at a concentration of 50 mg/ml. the control had a mean inhibition zone of 32.8 mm±0.1.

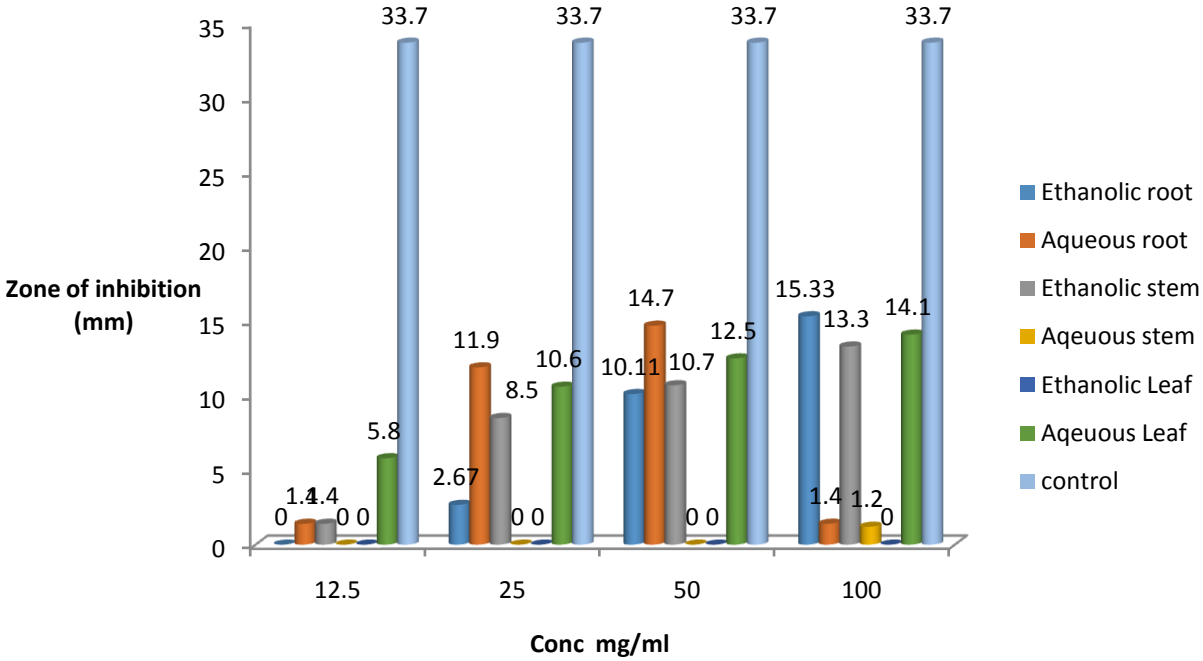


Fig 4.1. Susceptibility of Clinical Isolates of *S. aureus*to different Concentrations of Ethanolic and Aqueous Extracts of *J. curcas.*

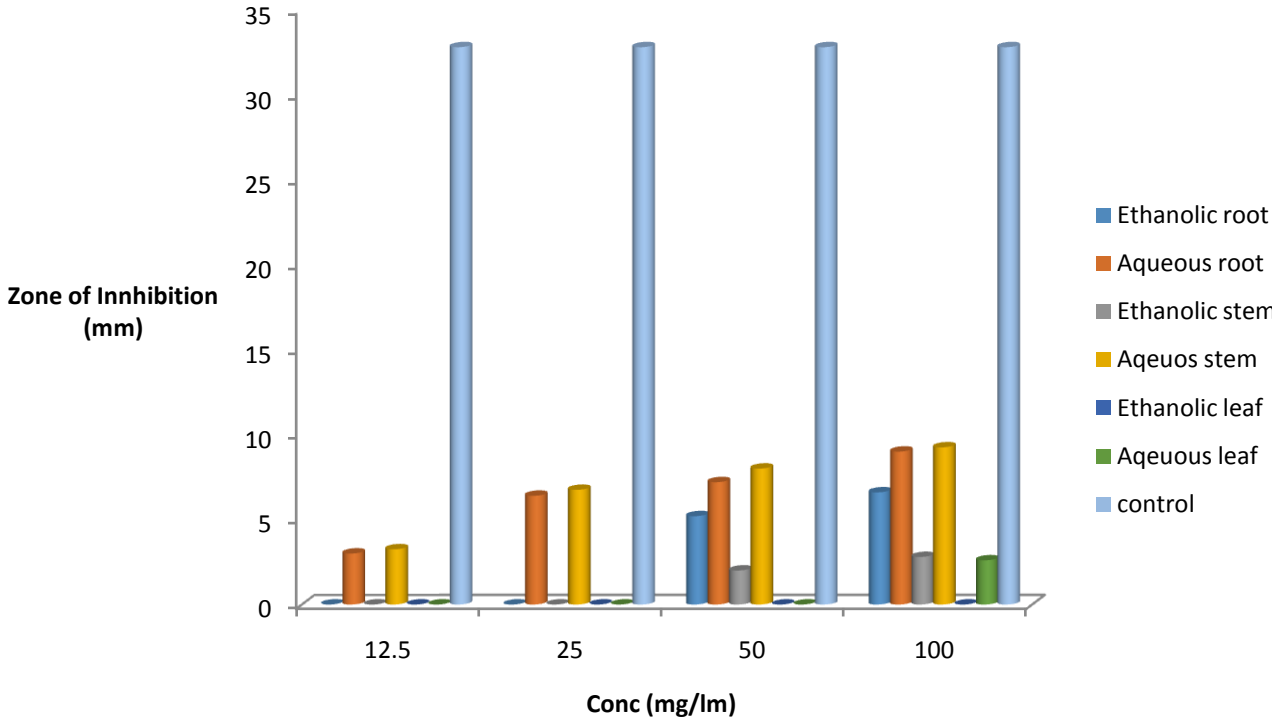


Fig 4.2. Susceptibility of Clinical Isolates of *E. coli*to different Concentrations of Ethanolic and Aqueous Extracts of *J. curcas.*

Fig 4.3 depicts the degrees of susceptibility as well as resistance of the clinical isolate of *Bacillus*sppto stem bark, root and leaves of *J. curcas*. It showed that all the concentration of aqueous root had the highest mean inhibition zone with values ranging from 10mm-17.5mm at concentration of 12.5mg/ml and 100mg/ml respectively, followed by aqueous leaves and then ethanolic root while other extracts had slight or no mean inhibition zone. However, the control had a mean inhibition zone of 38mm$\pm$0. 1.

Fig. 4.4 is the varying degrees of susceptibility and resistance of clinical isolates of *Pseudomonas aeruginosa* to different concentrations of both aqueous and ethanolic crude extracts of root, stem bark and leaves of *J. curcas* as well as Ciprofloxacin usedas the positive control. Thus, different concentrations of both aqueous and ethanolic extracts of leaves and stem bark yield no inhibition on the isolates, while root aqueous and ethanolic shows slight inhibition of the clinical isolates with values of 6.5mm$\pm$0. 1 and 6mm for aqueous and ethanolic roots respectively.

All the results indicates the root extract exhibited the highest activity on all the test organisms with an F= 16.407 and P= 0.01 values respectively.

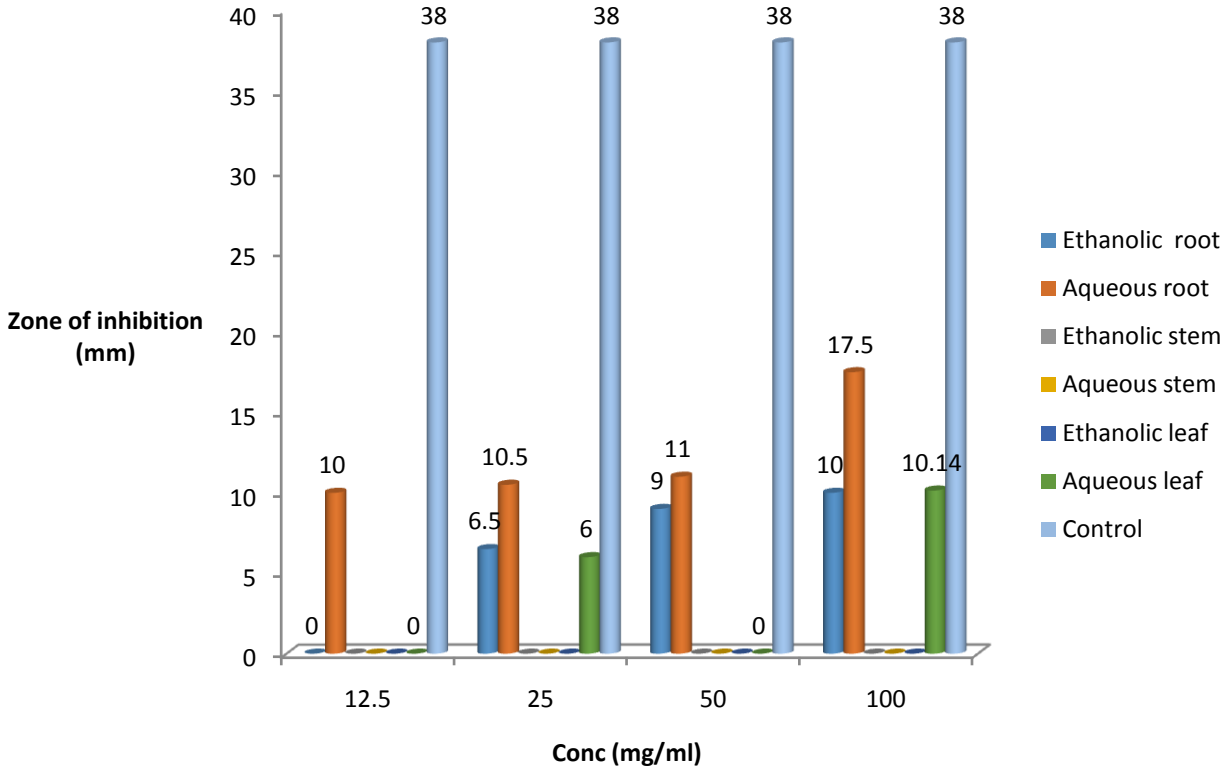


Fig. 4.3. Susceptibility of Clinical Isolates of *Bacillus spp* to different Concentrations of Ethanolic and Aqueous Extracts of *J. curcas.*

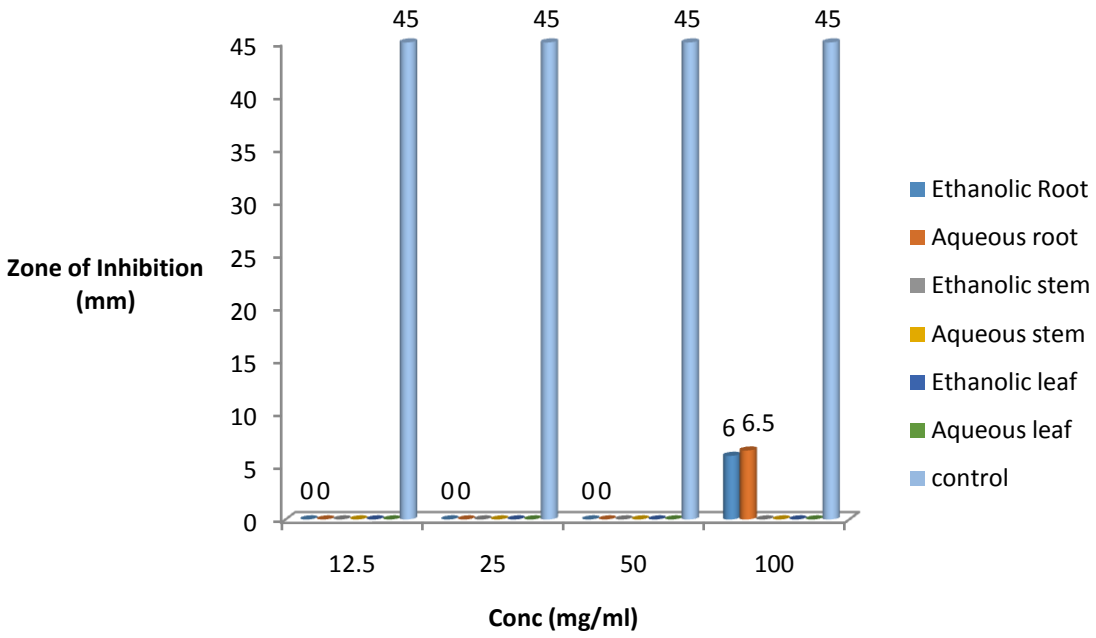


Fig 4.4. Susceptibility of Clinical Isolates of *Pseudomonas aeruginosa*to different Concentrations of Ethanolic and Aqueous Extracts of *J. curcas.*

4.6 Antibacterial Susceptibility of Various Fractions of Root Aqueous and Ethanolic Extract of *J. Curcas* on Clinical Isolates of *S. Aureus* and *Bacillus Spp.*

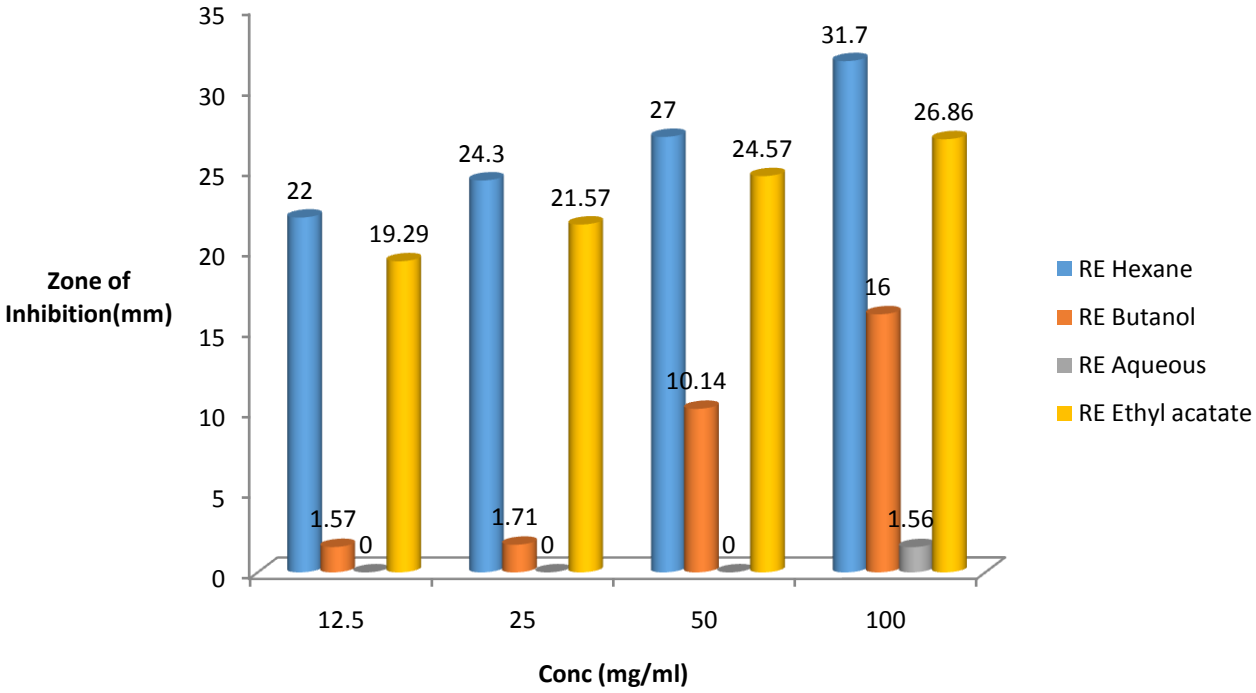
The result of antibacterial susceptibility test of various fractions of root aqueous andethanolic extracts of *J. curcas* to clinical isolates is presented on Figures 4.5- 4.8.

Fig. 4.5 is the result for the fraction of ethanolic root hexane had the highest activity on *S. aureus*isolate with mean inhibition zone of 31.7mm±0.1 followed by fraction of ethanolic root ethyl acetate with mean inhibition zone of 26.8mm±0.1 then fraction of ethanolic root n-butanol with 16mm±0.1while the lowest mean inhibition zone is with fraction of ethanolic root aqueous with 1.56mm±0.1at a concentration of 100 mg/ml.

Figure 4.6 represents the result of antibacterial activity of various fractions of root ethanol on clinical isolate of *Bacillus*spp where fraction of hexane had the highest mean inhibition zone through out with values ranging from 29-39mm with concentration of 12.5-100mg/ml respectively.

Fig 4.7 shows the result for activity of aqueous root fraction on clinical isolates of *S. aureus* where all fractions had significant activity with the exception of n- hexane fraction. Aqueous fraction had the highest activity with mean inhibition zone range from 19.57-31.71mm at 12.5 and 100 mg/ml concentrations respectively.

Fig 4.8 is the result for activity of aqueous root fraction on clinical isolates of *Bacillus* spp with n-hexane and ethyl acetate fractions having the highest activity with mean inhibition zone range of 18.5±0.1 atconcentration of 12.5mg/ml both and30-30.5mm at 100mg/ml respectively.



Key: Root ethanol

Fig.4.5: Susceptibility of Clinical Isolates of *Staphylococcus aureus*to different Concentrations of Ethanolic root fractions extracts of *J. curcas.*

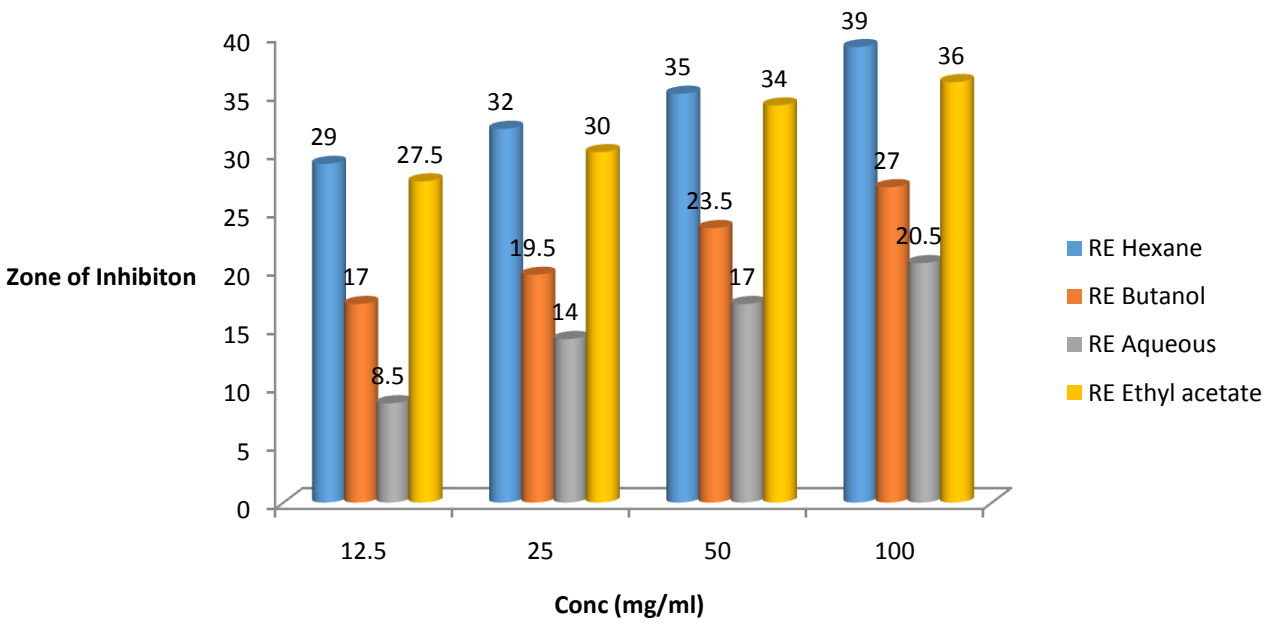


Fig.4.6:Susceptibility of Clinical Isolates of *Bacillusspp* to different Concentrations of fractions of Ethanolic roots s of *J. curcas.*

Key: RE= Ethanolic root

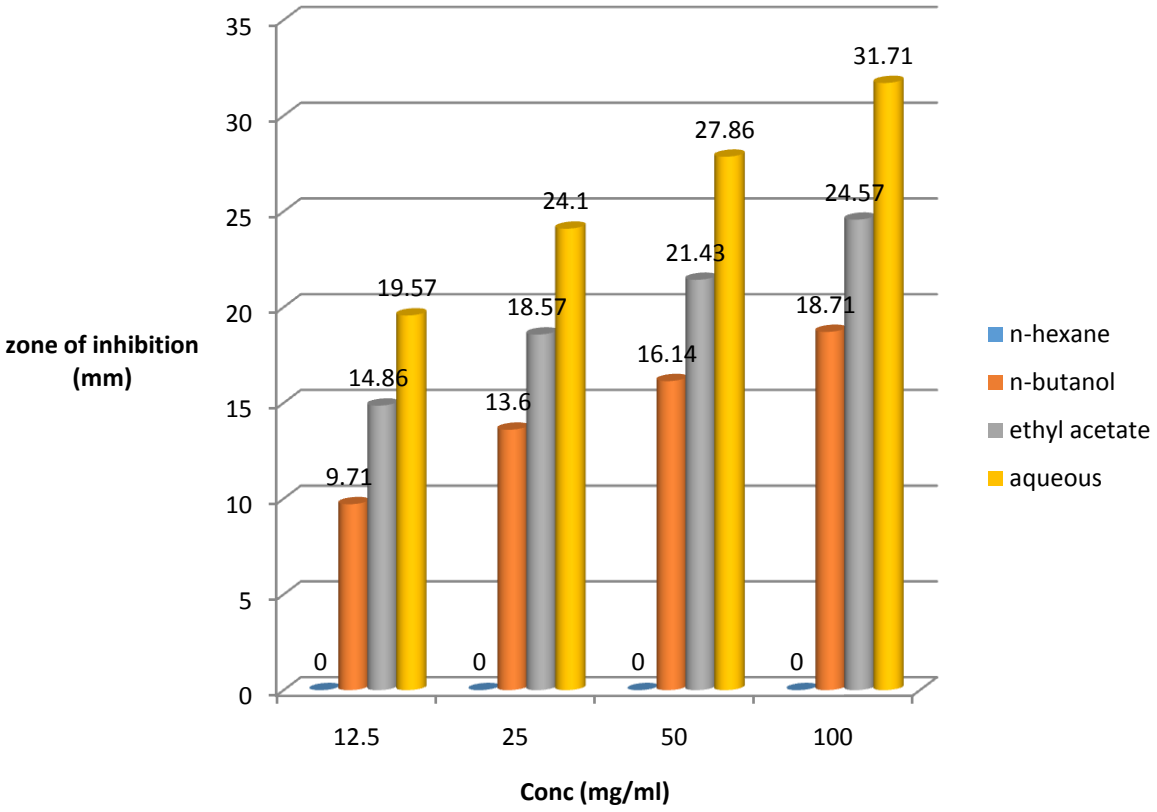


Fig.4.7: Susceptibility of Clinical Isolates of *Staphylococcus aureus*to different Concentrations of fractions of AqueousrootExtracts of *J. curcas.*

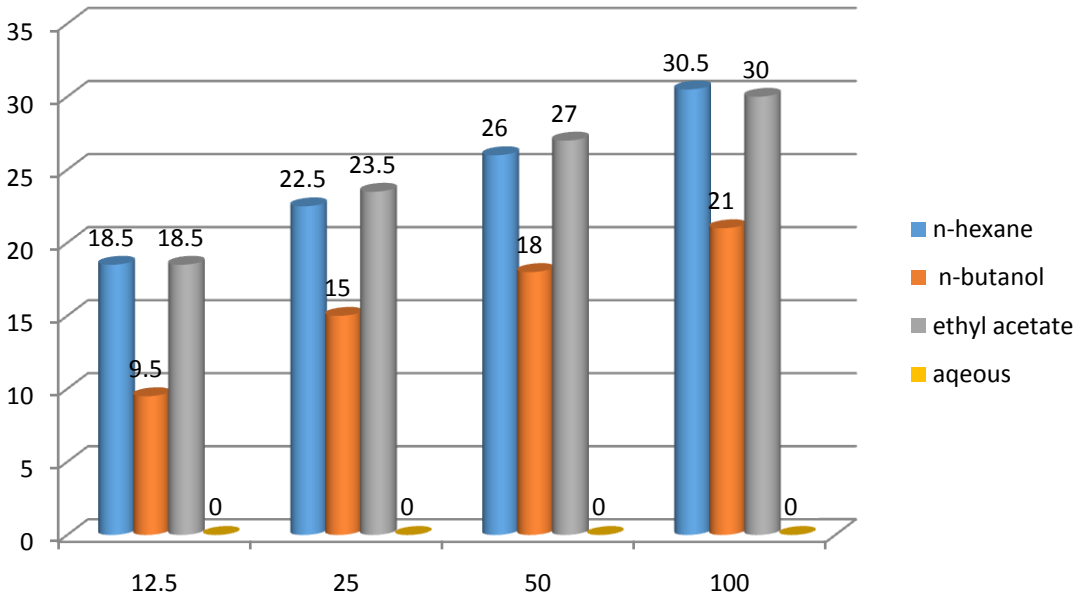


Fig.4.8: Susceptibility of Clinical Isolates of*Bacillus*sppto different Concentrations of fractions of Aqueousroot Extracts of *J. curcas.*

4.7 Minimum Inhibitory (MIC) and Bactericidal Concentrations(MBC) of Crude Aqueous and Ethanolic Extract and Fractions of *JatrophaCurcas* on Clinical Isolates.

The result of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of aqueous and ethanolic extracts of *J. curcas* for*S. aureus* and *Bacillus* spp are presented on the Table 4.4and 4.5.

Table 4.4 represents the MIC and MBC of aqueous and ethanolic extract of *J. curcas* on susceptible clinical isolates of *S. aureus* where root ethanol had the highest value ranges from 3.125-12.5mg/ml for MIC and MBC respectively. With lowest values recorded for ethanolic leaf and aqueous stem with 25mg/ml MIC and 50mg/ml-0 MBC indicating bacteriostatic but not bactericidal actitity respectively.

The MIC and MBC of aqueous and ethanolic extracts of root and leaves of *J. curcas*on susceptible *Bacillus*spp is presented on Table 4.5 where ethanolic root had 3.125mg/ml MIC and 6.25mg/ml MBC, followed by aqueous root with 6.25mg/ml MIC and 12.5mg/ml MBC.

The MIC and MBC results of crude aqueous and ethanolic extracts of *Jatrophacurcas*root fractionson susceptible *S. aureus and Bacillus* sppclinical isolates.

Table4.6 indicates the results for MIC and MBC of and ethanol fraction on susceptible *S.aureus*where all fractions had bactericidal activity with highest by ethyl acetate, n-henane, n-butanol fractions with MIC of 6.25mg/ml and MBC of 12.5mg/ml respectively.

Table 4.4: MIC and MBC of Extracts on*Staphylococcus aureus.*

Sample Code	MIC(mg/ml)	MBC(mg/ml)
R_2A	3.125	6.25
R_2B	3.125	6.25
R_2C	6.25	12.5
R_2D	6.25	12.5
R_2E	3.125	6.25
R_2F	6.25	12.5
R_2G	6.25	12.5
R_2H	3.125	6.25
R_2I	3.125	6.25
R_2J	3.125	6.25
R_1A	25	50
R_1B	25	50
R_1C	25	50
R_1D	25	50
R_1E	25	50
R_1F	25	50
R_1G	25	50
R_1H	25	50
R_1I	12.5	25
R_1J	12.5	25
L_2A	25	50
L_2A	12.5	25
L_2B	25	50
L_2C	6.25	12.5
L_2D	25	50
L_2E	25	50

L_2F	25	50
L_2G	25	50
L_2H	25	50
L_2I	25	50
L_2J	25	0
S_1D	25	0
S_1F	25	0
S_1G	25	0
S_1H	25	0
S_1I	25	0
S_1J	25	0

Key R=root, L= Leaf, S=Stem, A-J=*S. aureus*

Table 4.5: MIC and MBC of extracts on *Bacillus*spp

Sample code	MIC (mg/ml)	MBC (mg/ml)
R_1BC_2	6.25	12.5
R_2BC_2	3.125	6.25
L_2BC_2	12.5	25.0

Key R=root, L= Leaf, BC=Bacillus

Table 4.7 is the MIC and MBC results of ethanolic root fractions on susceptible *Bacillus*spp with ethyl acetate recording highest MIC of 3.125mg/ml and MBC of 12.5mg/ml, the lowest MIC and MBC were with n-butanol fraction with 25mg/ml and 50mg/ml MIC and MBC respectively.

Table 4.8 represent the result of MIC and MBC of aqueous root fraction on susceptible *S. aureus*, n-butanol fraction had an MIC of 6.25mg/ml and MBC of 12.5mg/ml as the highest while aqueous fraction recorded the lowest MIC value of 25mg/ml and MBC of 50mg/ml.

The MIC and MBC result of aqueous root fractions on susceptible *Bacillus spp* is on Table 4.9 where an MIC of 12.5 and MBC of 25mg/ml were recorded for aqueous fraction on both isolates.

Table 4.6: MIC and MBC of EthanolicRoot Fractions on *Staphylococcus aureus.*

Fraction	Isolate	MIC(mg/ml)	MBC
RE n-butanol	A	12.5	25.0
RE Ethyl acetate	A	12.5	25.0
RE Hexane	A	25.0	50.0
RE n-butanol	B	6.25	12.5
RE Ethyl acetate	B	12.5	25.0
RE Hexane	B	25.0	50.0
RE n-butanol	C	6.25	12.5
RE Ethyl acetate	C	12.5	25.0
RE Hexane	C	12.5	25.0
RE n-butanol	D	12.5	25.0
RE Ethyl acetate	D	12.5	25.0
RE Hexane	D	6.25	12.5
RE n-butanol	H	25.0	50.0
RE Ethyl acetate	H	12.5	25.0
RE Hexane	H	12.5	25.0
RE Butanol	I	12.5	25.0
RE Ethyl acetate	I	6.25	12.5
RE Hexane	I	6.25	12.5
RE Butanol	J	12.5	25.0
RE Ethyl acetate	J	12.5	25.0
RE Hexane	J	6.25	12.5

Key; RE= Root ethanol, A-J=*S. aureus*

Table 4.7: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration ofEthanolicRoot fractions on *Bacillus spp.*

Fraction	Isolate	MIC(mg/ml)	MBC(mg/ml)
REA	BC1	6.25	12.5
RE E/A	BC1	3.125	6.25
REH	BC1	6.25	12.5
REB	BC1	25	50
REA	BC2	6.25	12.5
RE E/A	BC2	6.25	12.5
REH	BC2	12.5	25
REB	BC2	12.5	25

Key; RE= Root ethanol, H=heaxane, B= n-butanol EA= Ethyl acetate, A=Aqueous, BC=*Bacillus* spp

Table 4.8:Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Aquoeus Root Fractions on*Staphylococcus aureus.*

Fractions	Isolates	MIC(mg/ml)	MBC(mg/ml)
RAB	A	12.5	25
RAA	A	25	50
RAB	B	6.25	12.5
RAA	B	12.5	25
RAA	C	12.5	25
RAB	D	12.5	25
RAA	H	25	50
RAB	H	25	50
RAH	H	12.5	25
RAE	H	25	50
RAB	I	25	50
RAE	I	25	50
RAA	J	12.5	25
RAB	J	12.5	25

KEY: RAB=Root aqueous butanol, RAA=Root aqueous aqueous, RAH=Root aqueous hexane, RAE=Root aqueous ethyl acetate, A-J=*S. aureus*

Table 4.9:Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Aquoeus Root Fractions on*Bacillus s*pecies

Fractions	Isolate	MIC	MBC
RAA	BC1	12.5mg/ml	25mg/ml
RAA	BC2	12.5mg/ml	25mg/ml

KEY: RAA=Root aqueous aqueous, BC=*Bacillus*

4.9 Gas-Chromatography/Mass-Spectrometry (GC-MS) Analysis of Aqueous and EthanolicCrude Extracts of Stem Bark, Root and Leaves of *Jatrophacurca*s

Tables4.10-4.16represents the interpretation of theprobable chemical compound name, itsformular and structure of stem bark, root and leaves fractions of *jatropha curcas*

Table 4.10 is the result of GC-MS for ethanolic stem bark extract of *J. curcas*where Hexadecane, Hexadecanoic acids and Octadecanoic acid are present.

Table 4.11 indicates the GC-MS result of aqueous stem bark extract of *J. curcas* where Oleic acid, n-hexadecanoic acid and Octadecanoic acids were present.

Table 4.12 is the GC-MS result of aqueous leave extract of *J. curcas* where oleic acid, Nonadecanol and 9-octadecanal are present.

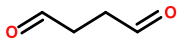
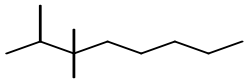
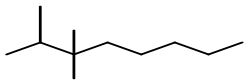
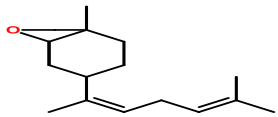
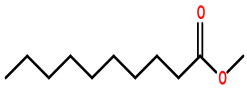
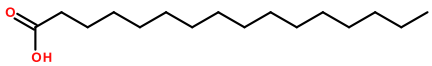
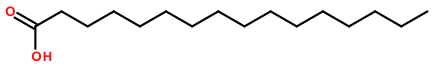
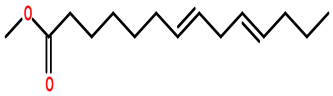
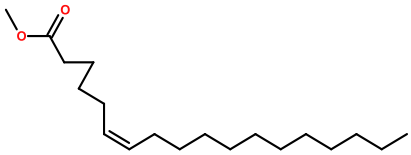
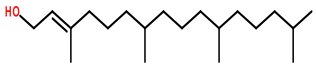
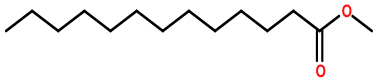
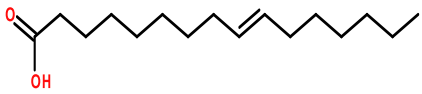
Table 4.13 is the GC-MS result for ethanolic leave extract of *J. curcas* where phenols, oleic aci and phytol were present.

Table 4.14 shows the result for GC-MS of ethanolic root extract of *J. curcas* where propane, oleic acid and oxalic acids were present.

Table 4.15 is the GC-MS result of aqueous root extract of *J. curcas* where acetic acid, oleic acid and furan, 2-ethyltetrahydro-5-methoxy-2-methyl were present.

Table 4.16 indicates the compounds with antimicrobial activity where oleic acids, acetic acids, citric acids and linoleic acids are among the most active.

Table 4.10: Mass Spectrometry of Probable Chemical Compounds inEthanolicExtract Stem Bark of *Jatrophacurcas*

PEAK	IUPAC NAME	CHEMICAL FORMULAE	CHEMICAL STRUCTURE
1.	Butanedial	$C_4H_6O_2$	
2.	Hexadecane	$C_{16}H_{34}$	
3.	Octane, 2,3,4 trimethyl	$C_{11}H_{24}$	
4.	Octane, 2,3,4 trineth	$C_{11}H_{24}$	
5.	Cis-Z-alpha-Bisabolene epoxide	$C_{15}H_{24}O$	
6.	Decanoic acid methyl ester	$C_{11}H_{22}O_2$	
7.	n-hexadecanoic	$C_{16}H_{32}O_2$	
8.	Hexadecanoic acid	$C_{16}H_{32}O_2$	
9.	9,12 octacadienoic acid	$C_{19}H_{34}O_2$	
10.	6-octadecanoic acid	$C_{19}H_{36}O_2$	
11.	Phytol	$C_{20}H_{40}O$	
12.	Tridecanoic acid	$C_{14}H_{28}O_2$	
13.	9-hexanoic acid	$C_{16}H_{30}O_2$	

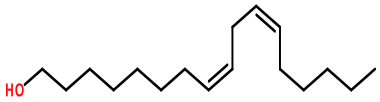
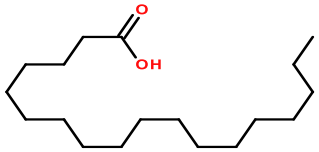
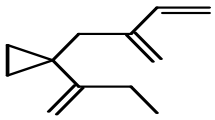
14.	9,12 octadecdien-1-ol(Z,Z)	$C_{18}H_{28}O$	
15.	E-11-Hexadecenoic ethyl ester	$C_{18}H_{34}O_2$	
16.	Octadecnoic acid	$C_{18}H_{36}O_2$	
17.	Octadecanoic acid ethyl ester	$C_{20}H_{40}O_2$	
18.	Cyclopropane	$C_{12}H_{18}$	
19.	2-methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	

Table 4.11: Mass Spectrometry of Probable Chemical Compounds in Aqueous Extract of Stem Bark of *Jatrophacurcas*

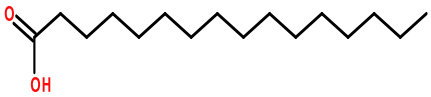
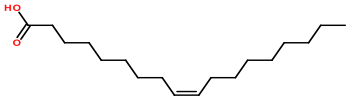
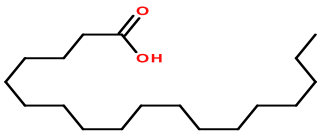
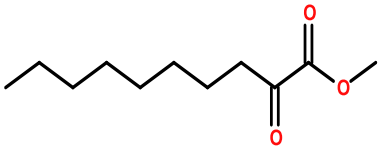
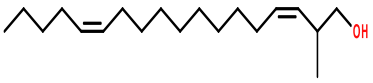
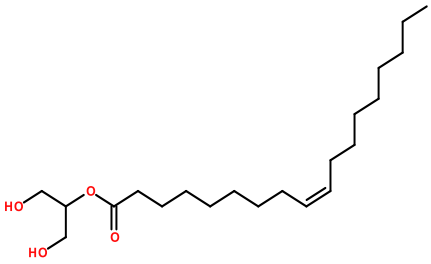
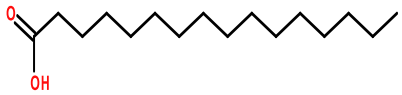
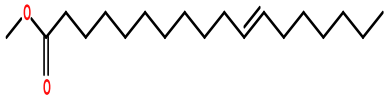
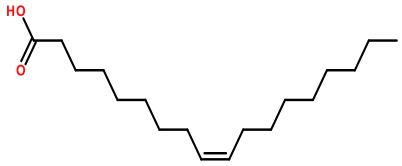
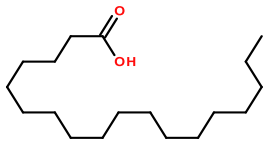
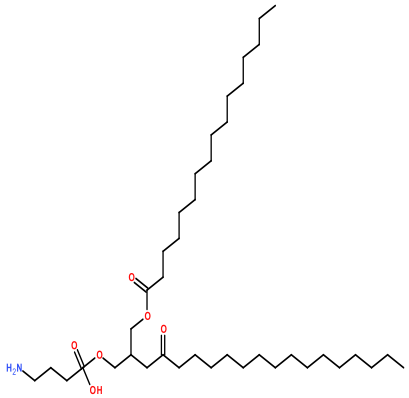
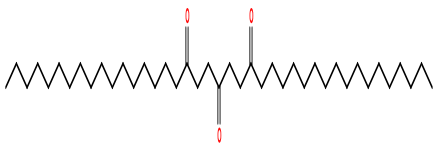
S/N	IUPAC NAME	CHEMICAL FORMULAE	CHEMICAL STRUCTURE
1.	1,3-Propanediol	$C_3H_8O_2$	
2.	n-hexadecanoic	$C_{16}H_{32}O_2$	
3.	11-Octadecanoic acid	$C_{19}H_{36}O_2$	
4.	Oliec acid	$C_{18}H_{34}O_2$	
5.	Octadecanoic acid	$C_{18}H_{36}O_2$	
6.	Decanoic acid,2-oxo,methyl ester	$C_{11}H_{20}O_3$	
7.	2-Methyl-Z,Z-3,13-octadecadinol	$C_{19}H_{36}O$	
8.	9-Octadecenoic acid(Z),2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	

Table 4.12: Mass Spectrometry of Probable Chemical Compounds in Aqueous Extract of Leaves of *Jatrophacurcas*

S/N	IUPAC NAME	CHEMICAL FORMULAE	CHEMICAL STRUCTURE
1.	n-hexadecanoic	$C_{16}H_{32}O_2$	
2.	11-Octadecanoic acid	$C_{19}H_{36}O_2$	
3.	Oliec acid	$C_{18}H_{34}O_2$	
4.	Octadecnoic acid	$C_{18}H_{36}O_2$	
5.	Nonadecanol	$C_{19}H_{40}O$	
6.	Hexadecanoic acid,1-[[[(2-aminoethoxy) hydroxyphosphinyl]oxy]methyl]-1,2-ethanediyl ester	$C_{37}H_{74}NO_8P$	
7.	9-octadecenal	$C_{18}H_{34}O$	
8.	9-octadecena	$C_{18}H_{34}O$	
9.	Octadecanoic acid	$C_{39}H_{76}O_5$	

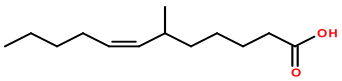
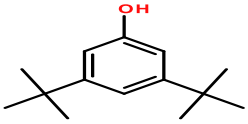
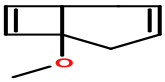
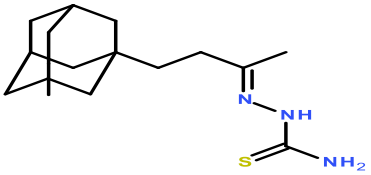
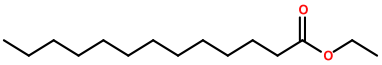
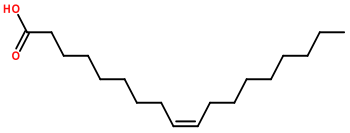
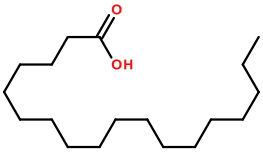
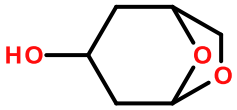
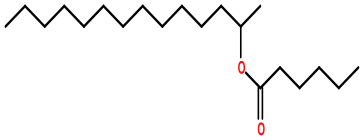
10. Z-8-methyl-9-tetradecenoic acid $C_{15}H_{28}O_2$ 

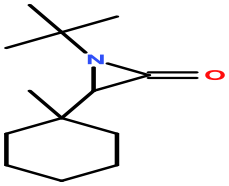
Table 4.13: Mass Spectrometry of Probable Chemical Compounds in EthanolicExtract of Leaves of*Jatrophacurcas*

S/N	IUPAC NAME	CHEMICAL FORMULAE	CHEMICAL STRUCTURE
1.	Phenol, 3,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	
2.	Bicyclo[3.2.0]hepta-2,6-diene, 5-methoxy	$C_8H_{10}O$	
3.	1-[3-(1-Adamantyl)-1-methylpropylidene]thiosemicarbazide	$C_{15}H_{25}N_3S$	
4.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	
5.	Ethyl tridecanoate	$C_{15}H_{30}O_2$	
6.	Phytol	$C_{20}H_{40}O$	
7.	Oliec acid	$C_{18}H_{34}O_2$	
8.	Octadecnoic acid	$C_{18}H_{36}O_2$	
9.	1,6-Anhydro-2,4-dideoxy-.beta.-D-arabo-hexopyranose	$C_6H_{10}O_3$	

10. Hexanoic acid, 2-tetradecyl ester $C_{20}H_{40}O_2$



11. 2-Aziridinone, 1-tert-buttyl-3-(1-methylcyclohexyl) $C_{13}H_{23}NO$



12. dl-2-Ethylhexyl chlorofornate $C_9H_{17}CO_2$

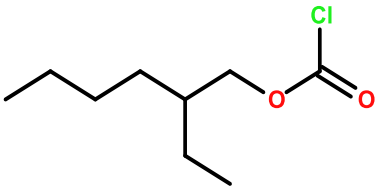
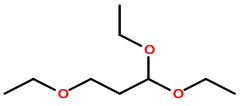
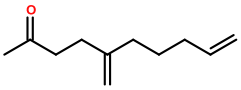
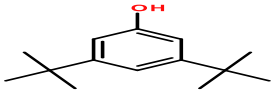
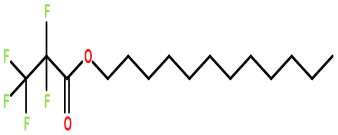
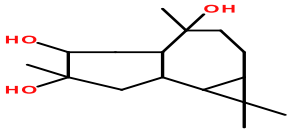
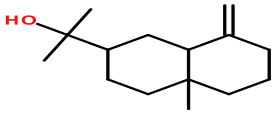
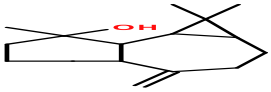
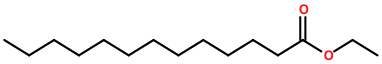
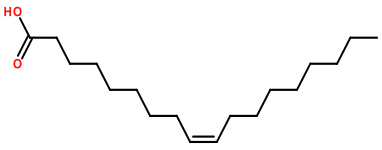


Table 4.14: Mass Spectrometry of Probable Chemical Compounds in EthanolicExtract of Root of*Jatrophacurcas*

S/N	IUPAC NAME	CHEMICAL FORMULAE	CHEMICAL STRUCTURE
1.	Propane, 1,1,3-triethoxy	$C_9H_{20}O_3$	
2.	9-Decen-2-one, 5-methylene	$C_{11}H_{18}O$	
3.	Phenol, 3,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	
4.	Pentafluoropropionic acid, dodecyl ester	$C_{15}H_{25}F_5O_2$	
5.	Perhydrocyclopropa[e]azulene-4,5,6—triol, 1,1,4,6-tetramethyl	$C_{15}H_{26}O_3$	
6.	2-Napthalenemethanol	$C_{15}H_{26}O$	
7.	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene	$C_{15}H_{24}O$	
8.	Hexadecanoic acid	$C_{16}H_{32}O_2$	
9.	Ethyl tridecanoate	$C_{15}H_{30}O_2$	
10.	10-Octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	
11.	Oleic acid	$C_{18}H_3O_2$	

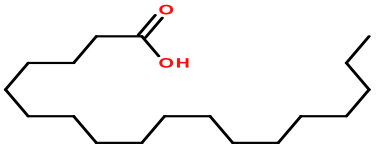
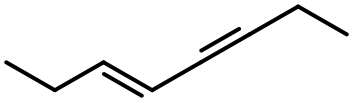
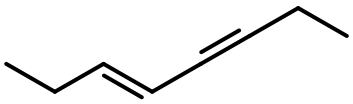
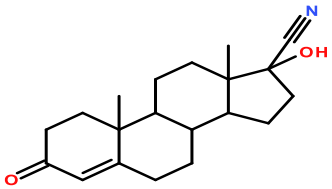
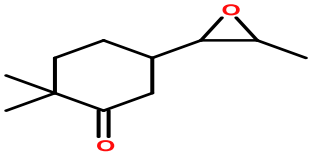
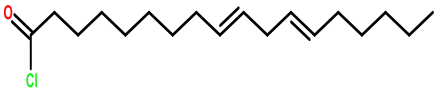
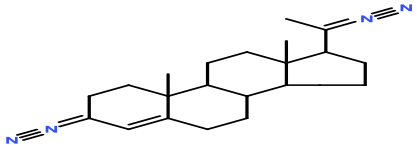
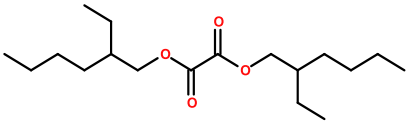
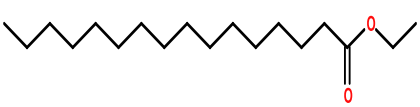
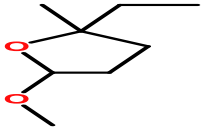
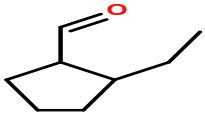
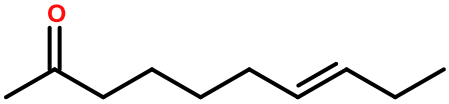
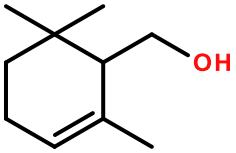
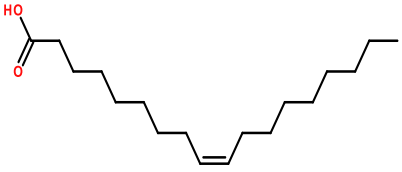
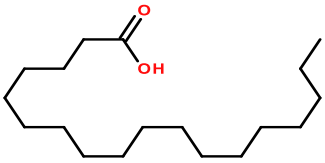
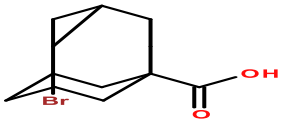
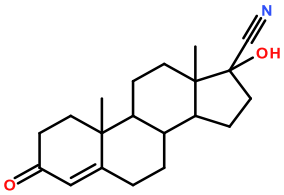
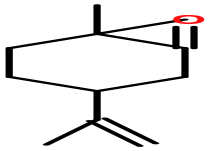
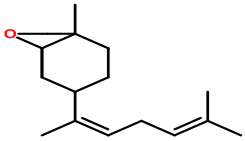
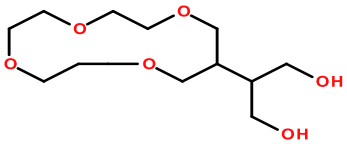
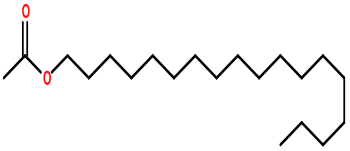
12.	(E)-9-Octadecanoic acid ethyl ester	$C_{20}H_{38}O_2$	
13.	Octadecanoic acid	$C_{18}H_{36}O_2$	
14.	3-Octen-5-yne,(E)	C_8H_{12}	
15.	3-Octen-5-yne,(E)	C_8H_{12}	
16.	Preg-4-en-3-one	$C_{20}H_{27}NO_2$	
17.	Cyclohexanone	$C_{11}H_{18}O_2$	
18.	9,12-Octadecadienoyl chloride	$C_{18}H_{31}C_{lO}$	
19.	9-Octadecenal	$C_{18}H_{34}O$	
20.	Diazoprogesterone	$C_{21}H_{30}N_4$	
21.	Oxalic acid	$C_{18}H_{34}O_4$	
22.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	

Table 4.15: Mass Spectrometry of Probable Chemical Compounds in Aqueous Extract of Root of *Jatrophacurcas*

S/N	IUPAC NAME	CHEMICAL FORMULAE	CHEMICAL STRUCTURE
1.	Furan, 2-ethyltetrahydro-5-methoxy-2-methyl	$C_8H_{16}O_2$	
2.	Cis-2-Ethycyclopentanecarboxaldehyde	$C_8H_{14}O$	
3.	7-Decen-2-one	$C_{10}H_{18}O$	
4.	2-Cyclohexene-1-methanol, 2,6,6-trimethyl	$C_{10}H_{18}O$	
5.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	
6.	Oleic acid	$C_{18}H_{34}O_2$	
7.	Octadecnoic acid	$C_{18}H_{36}O_2$	
8.	E-11-Tetradecanol,trimethylsilyl ether	$C_{17}H_{36}OS$	
9.	3-Bromo-7-methyl-1-adamantanecarboxylic acid	$C_{12}H_{17}BrO_2$	

10.	Preg-4-en-3-one	$C_20H_{27}NO_2$	
11.	Limonene oxide,trans	$C_{10}H_{16}O$	
12.	Decane, 1-chloro	$C_{10}H_{21}Cl$	
13.	Cis-Z-alpha-Bisabolene epoxide	$C_{15}H_{24}O$	
14.	3-(1,3-Dihydroxyisopropyl)-1,5,8,11-tetraoxacyclotridecane	$C_{12}H_{24}O_6$	
15.	Dodacenal	$C_{12}H_{22}O$	
16.	Acetic acid	$C_{20}H_{40}O_2$	

CHAPTER FIVE

5.0Discussion

The result of this study reveals the efficacy of aqueous and ethanolic extracts of root, stem bark and leaves of *Jatrophacurcas* based on *in vitro* evaluation of antibacterial activity of the extracts on clinical isolates of *Staphylococcus aureus, Escherichia coli, Bacillus* sppand *Pseudomonas aeruginosa*. The ethanolic extract of all the plant parts recorded the highest antibacterial activity on the clinical isolates than the aqueous extracts. Although ethanol is polar, but it also has the ability to attract non-polar molecules due to ethyl group of ethanol being non-polar. This contributes to its ability to extract highly polar and non-polar components from the plant material. It has very low toxicity, completely miscible in water, volatile and easily removed from plant material at low temperature (Roy *et al.*, 2014).

The phytochemical screening of *Jatrophacurcas*leaves,stem bark and root extracts recorded the presence of compounds carbohydrates, cardiac glycosides, triterpenes, flavonoids, saponins and alkaloids present in all extracts and responsible for antimicrobial activities as reported in similar studies (Hamid *et al*., 2011).A study conducted by Sharall*et al*., (2013) reported the presence of fatty acids and terpenes responsible for bactericidal activity. Hamid *et al.* (2011) also reported that phenolics, flavonoids, saponins and phorbol esters as antimicrobial compounds in *J.curcas.* Phytochemicals present in the leaves extracts were high but low antimicrobial activity as compared to the stem bark and root extracts was observed, probably due to the fact that different phytochemical compounds exert their antibacterial effects differently from one another.

Generally, the aqueous extracts contained mainly acetic acid, furfural, pyrogallol, and saponins. Pyrogallol has been reported to be an effective antimicrobial agent and its bacterial toxicity is attributed to the three hydroxyl groups present in its structure (Kocacaliskan et al.,2006).

The result disagrees with most studies that showed activity of *J.curcas* on Gram negative organisms, which may be attributed to the fact that cells of Gram negative bacteria are less viable thus less affected by the extracts inhibitoryactivity when compared to those of Gram positive bacteria.Gram negative bacteria also posses efflux system that extrude antibacterial agents out of the cell (Tenovar,2006).In 2011, Arekemase*et al*. (2011) recorded high activity of the plant extract on *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus* spp. So also does Sammy *etal*., (1998).Similar result was also reported by Kalimathu*et al.* in(2010) on *Pseudomonas aeruginosa* and *Staphylococcus aureus* with leaf extract of *Jatrophacurcas.* This study recorded high antibacterial activity on Gram positive*S. aureus* and *Bacillus* spp. While slight or no activity on gram negative *E. coli* and *Pseudomonas aeruginosa* which might be attributed to their difference in cell wall composition.

The antibacterial effects of *Jatrophacurcas* had been previously studied and reported that the extract and latex displayed potent antimicrobial activity against *S. aureus, P. aeruginosa, Bacillus subtilis, Bacillus cereus, E. coli* and host of other bacteria and fungi, giving inhibitory concentration as low as 0.5ml (Arekemase*et al.,* 2011) which confirms the potency of this plant in treating human infections.

The result in this study also shows the root extract of *Jatrophacurcas*was observed to be more effective than each of the extracts due to its ability to inhibit all test bacteria,

this result agrees with Aiyeaagbe*et al.*(2007) who said root extract of *J. curcas*exhibit broad spectrum of activities by inhibiting the growth of bacteria and fungi. A similar result was also reported by Willey *et al.* (2008) that the presence of some secondary metabolites in the root extract of *J. curcas* inhibit micro organisms isolated from sexually transmitted infections.Henrie *et al.*(2009)reported that *J. curcas* root extract disrrupts bacterial cell membrane by increasing membrane permeability and causing leakage of bacterial contents like nucleic acid and amino acids.

Agarwal*et al.*(2012) also states that the presence of metabolic toxins or broad spectrum antimicrobial compounds in *J. curcas* acts against most g Gram positive and some Gram negative bacteria especially in solvents ethanol, ethyl acetate and cold aqueous. In the same vein, Igbinosa*et al.* (2011) reported that the activities of *J. curcas* to both Gram positive and negative micro organisms can be attributed to the presence of phenolic compounds which showed to be powerful antioxidants and free radical scavengers, and those compounds are able to induce reactions of electron transfer which reacts with nitrogen compound in microbial cell like nucleic acid and proteins, this helps as a strong barrier against bacterial infection.

The MIC and MBC assay procedures are frequently used to evaluate some diverse agents such as antibiotics, antiseptics, disinfectants and chemotherapeutic agents (Andrews, 2001). In this study, the MIC and MBC values of both *S. aureus* and *Bacillus* spp with both aqueous and ethanolicextracts of*J. curcas* indicates significant bacteriostatic and bactericidal activities. This implies the strong efficacy of the extracts as stated by Arekemase (2011) that the constituents of the root, stem and latex of *J. curcas* contains phenols, flavonoids and some secondary metabolites that are very

useful in antimicrobial activity. The MIC and MBC effects observed with different concentrations of various extracts against susceptible *S. aureus* and *Bacillus*spp in this study could be attributed to the presence of organic and fatty acids detected in the extracts as shown on Table 1,the interactions of these hydrocarbons with the hydrophobic structures of bacteria had been reported to result in antimicrobial activity(Sikkema*et al*.,1995; Cowan, 1999; Zulfiker *et al*., 2011).

The susceptibility pattern of the root ethanol fractions in this study shows Hexane, n-butanol and ethyl acetate fractions of root ethanol are very effective against both *S. aureus* and *Bacillus* spp with aqueous fractions not active against *S. aureus* but *Bacillus* spp with mean inhibition zone as high as 35mm and 40mm for *S. aureus* and *Bacillus*respectively. So also the root aqueous findings shows very high susceptibility of the isolates to fractions of n-butanol, ethyl acetate and aqueous with *S. aureus* and hexane,n-butanol and ethyl acetate fractions with *Bacillus* spp. This finding indicates that not all the component in crude form of the extract acted on the clinical isolates but rather some of the components.

The main organic compounds detected by GC-MS in both ethanolic and aqueous extracts of *Jatrophacurcas* is on (Tables 4.14-4.20).Oxalic and acetic acids were found to be the major compounds detected in the leaves,stem bark and root extracts. In addition the stem bark also contained citric acid. The fatty acids, which were identified in all but root bark were hexadecanoic acid and 9-octadecanoic acids (oleic acids).

Consequently, this study revealed that *J. curcas* plant extracts of leaves, stem bark and root induced antibacterial action through cell membrane damage and oxalic acid, acetic acid, hexadecanoic acid, citric acid and 9-Octadecenoicacid are found to be

active cell membrane antimicrobial compounds (Namuli*et al*., 2011). The antibacterial activity of 9-octadecanoic acid and hexadecanoic acid against *S. aureus* and *E.coli* has been reported by Pu*et al*.(2010).Similarly, acetic acid and hexadecanoic acid as the main antibacterial compounds have been reported in aqueous extract of *J. curcas* (Feng*et al*., 2010). Solsoly, 1995 and Namuli*et al*., (2011) reported that the presence of acetic acid, hexadecanoic acid ethyl ester, citric acid and decenoic acid as major membrane active antibacterial compounds.The antimicrobial activity of 10-octadecenoic methyl ester, 9,12-octadecadienoic acid and 9-12- octadecanoic acid ethyl ester have been reported by Kalimuthu*et al*. (2010). Yin *et al*. (2011) also found that n-hexadecenoicacid compound present in *J. curcas* leaf extract had antibacterial and antifungal effects.

CHAPTER SIX

6.0Summary and Conclusion

In conclusion, the results obtained in this study clearly demonstrated the effectiveness of aqueous and ethanolic extracts of *Jatrophacurcas* as well as the control (Ciprofloxacin) on Gram positive bacteria of clinical origin.It also revealed the probable chemical compounds in the crude extracts by GC/MS analysis which are responsible for the antibacterial activities. Based on the result of the analysis therefore, the work can be considered as part of an effort to validate the use of *Jatrophacurcas* in traditional medicine as well as source of future discovery of antibacterial drugs.

6.1 Recommendations

In view of the finding in this study, the following recommendations were made.

I. Future work on this study should include further purification of the extracts fractions to be able to isolate the most active compound (s) responsible for antibacterial activity.

II. The plant material should also be tested on fungi to establish its antifungal activities.

III. Further investigation to identify molecular targets in the bacterial cell like spore formation and active sites of enzymes involved in cell division in other to develop compounds in plants to serve as their specific inhibitors.

IV. Government should also help in providing standard research laboratory centers at local, state and federal levels for screening and optimization of quality and safety of traditional medicine.

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List of Plates

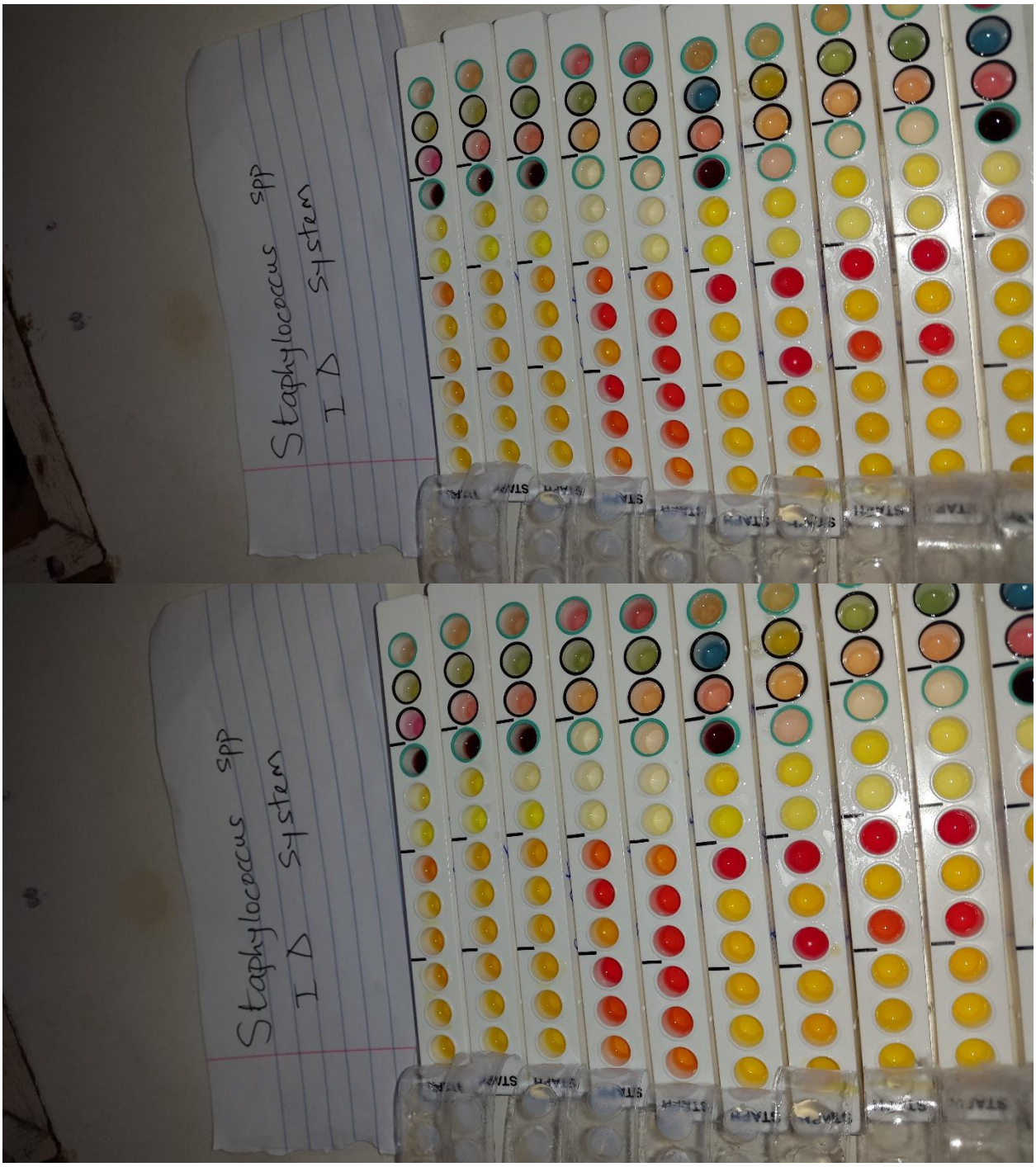


Plate 1: STAPH ID after 24 h incubation

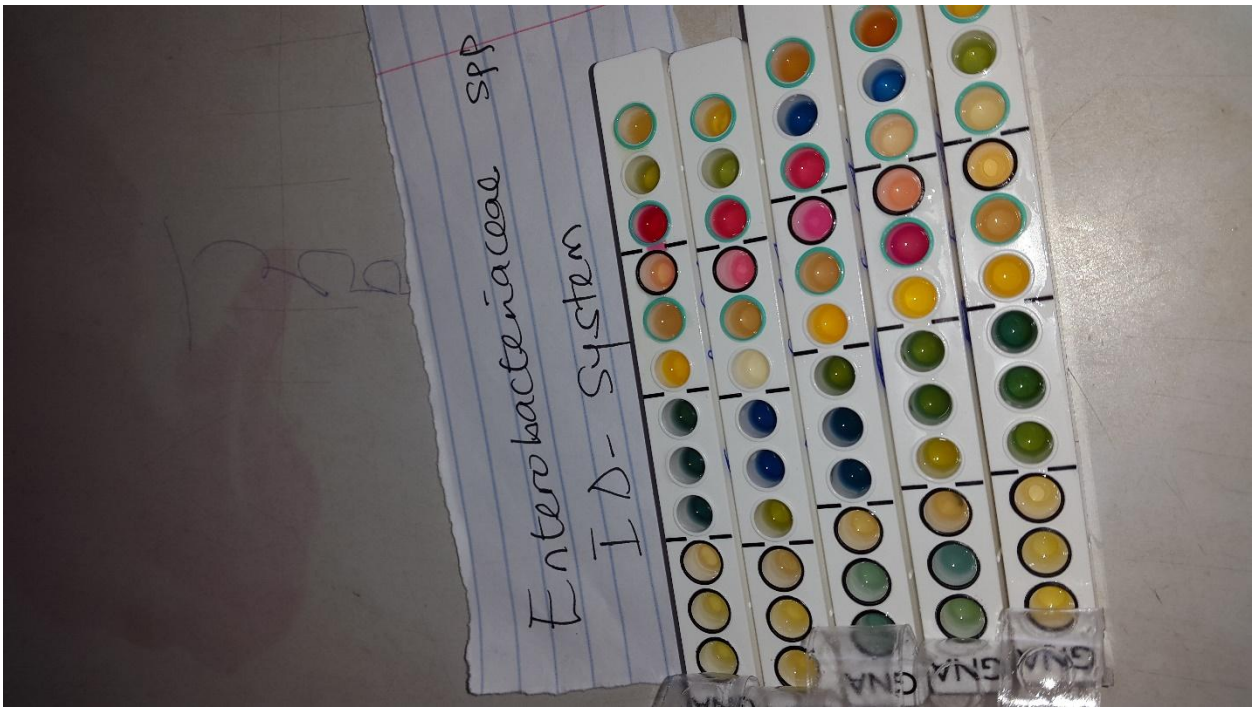


Plate 2: GNA-ID after 24h incubation

Plate 3: Root ethanol antibacterial susceptibility zone of inhibitions of *S. aureus*

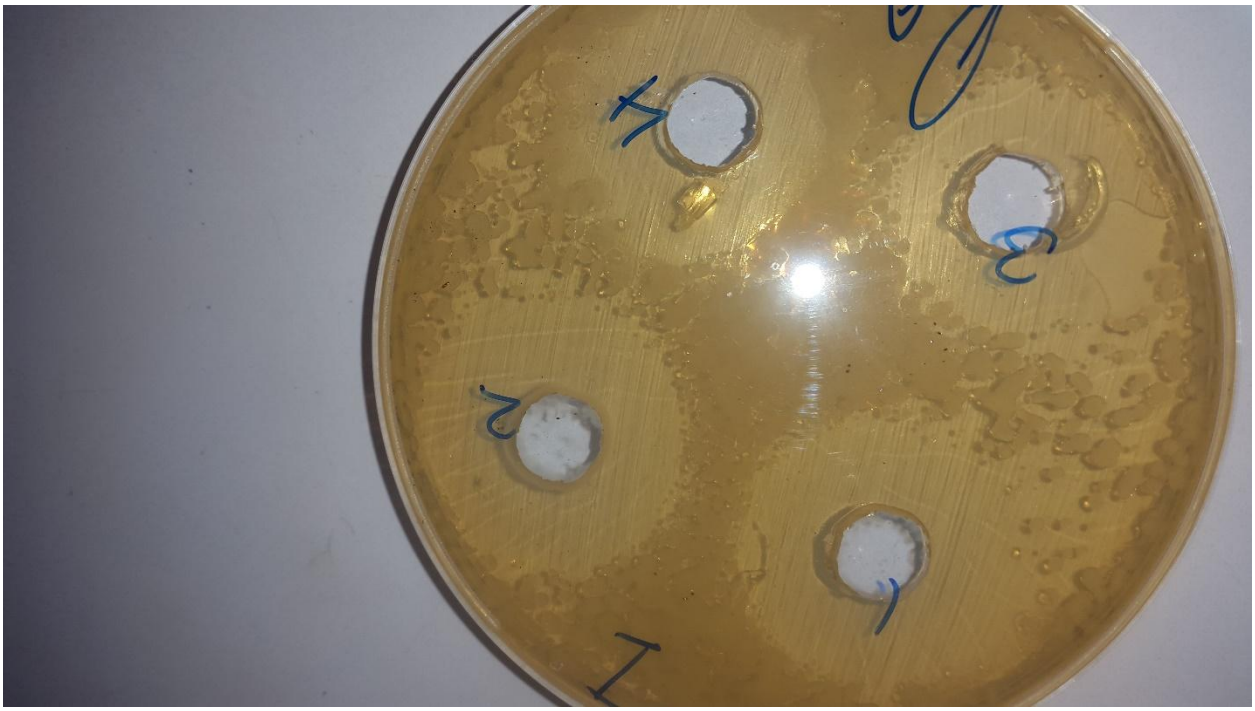


Plate 4: Root ethanol antibacterial susceptibility zone of inhibitions of *Bacillus* spp

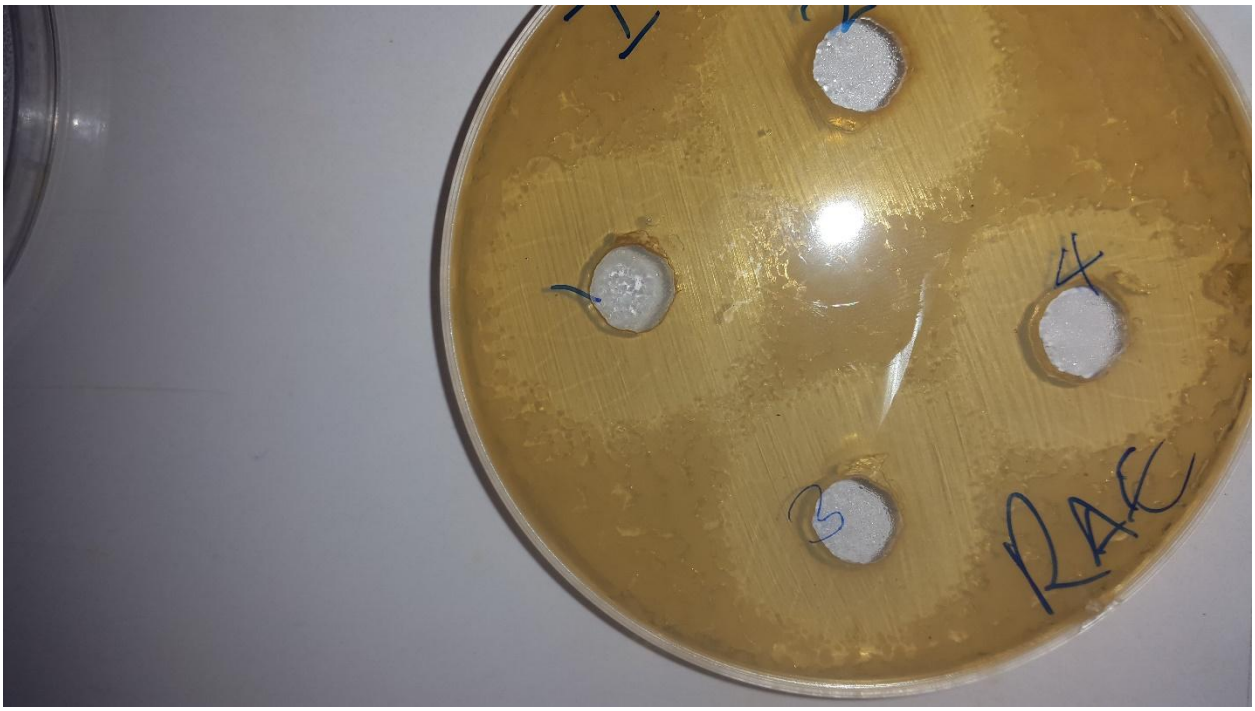


Plate 5: Root aqueous ethyl acetate antibacterial susceptibility zones of inhibition of *Bacillus* spp

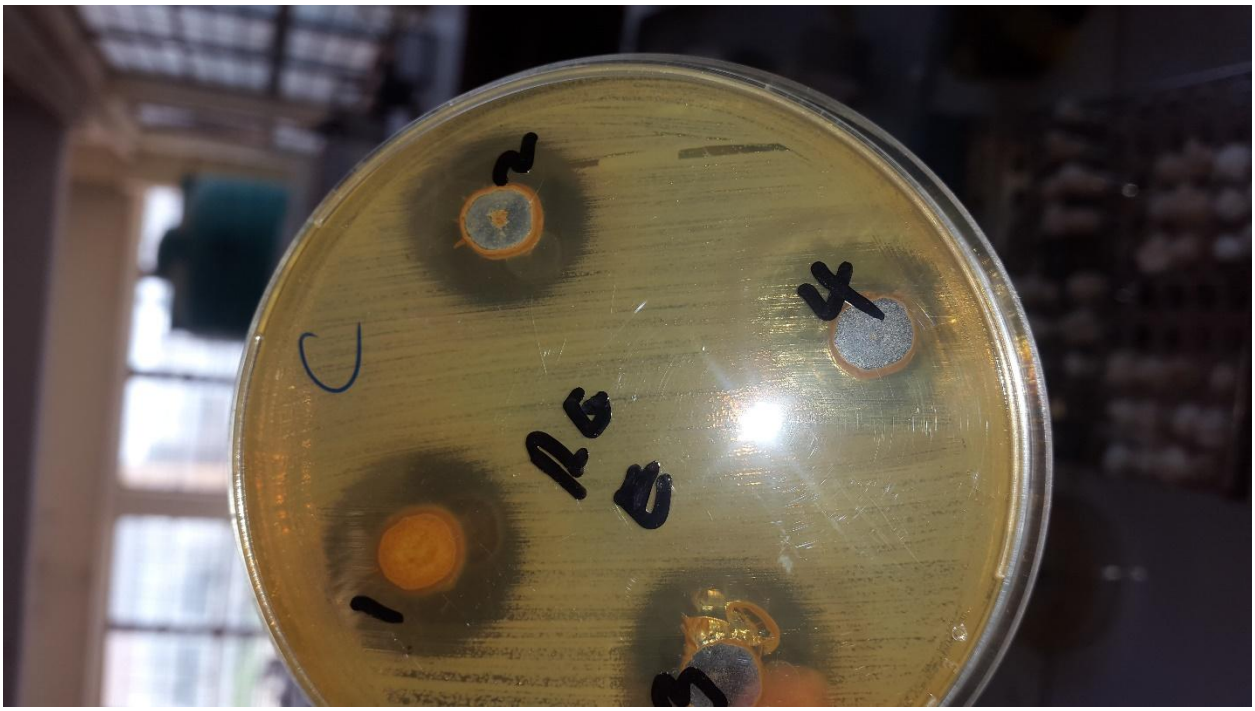


Plate 6: Root ethanol ethyl acetate antibacterial susceptibility zone of inhibitions of *S.aureus*

Appendix I: Ethical Approval

MINISTRY OF HEALTH, KADUNA STATE

All Communication to be addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Telephone: 234-248048
Website: ttp://www/moh.kd.gov.ng.
Email: info@moh.kd.gov.ng.

Independence Way,
P.M/;B 2014
Kaduna.
Kaduna State, Nigeria.

Our Ref: MOH/ADM/744/VOL.1/205

11th February, 2015

To: _____

Ministry of Health Research,
Ethical Clearance.

RE: Phytochemical Anaylsis and Susceptibility Studies of Jatropha Curcas on Some Nosocomial Pathogens

Name of Investigator: - Ibrahim Zainab Yau

Date of Receipt of Application: - 18th November, 2014

Date of Ethical Approval: - 24th November, 2014

Research Period: - March to June, 2015

You are kindly to give researcher maximum cooperation during the period of Research please

However it is mandatory to submit findings to the ministry please.

F.A. Kurah (Mrs)
Secretary to Research Committee

APPENDIX II

Cultural and Physiologic Properties of Clinical Isolates of *Pseudomonas aeruginosa*

Sample ID	Colonial Morphology	Gram Reaction	Cell Morphology	Motility	Catalase	Oxidase
PS1	Blue green pigmentation	Negative	Rod	Positive	Negative	Negative
PS2	Blue green pigmentation	Negative	Rod	Positive	Positive	Negative
PS3	Blue green pigmentation	Negative	Rod	Positive	Positive	Positive
PS4	Blue green pigmentation	Negative	Rod	Positive	Negative	Positive
PS5	Blue green pigmentation	Negative	Rod	Positive	Positive	Positive
PS6	Blue green pigmentation	Negative	Rod	Positive	Negative	Negative
PS7	Blue green pigmentation	Negative	Rod	Positive	Negative	Negative
PS8	Blue green pigmentation	Negative	Rod	Positive	Positive	Negative
PS9	Blue green pigmentation	Negative	Rod	Positive	Negative	Positive
PS10	Blue green pigmentation	Negative	Rod	Positive	Negative	Negative

KEY: PS = *Pseudomonas aeruginosa*

APPENDIX III

Cultural and Physiologic Properties of Clinical Isolates of *Escherichia coli*

Sample ID	Colonial morphology	Gram reaction	Cell morphology	VogesProskauer	Citrate	Methyl red	Indole
A	Green, metallic sheen	Negative	Rod	Negative	Negative	Positive	Positive
B	Green, metallic sheen	Negative	Rod	Negative	Positive	Negative	Negative
C	Green, metallic sheen	Negative	Rod	Negative	Negative	Positive	Positive
D	Green, metallic sheen	Negative	Rod	Negative	Negative	Negative	Positive
E	Green, metallic sheen	Negative	Rod	Negative	Negative	Positive	Positive
F	Green, metallic sheen	Negative	Rod	Negative	Negative	Positive	Positive
G	Green, metallic sheen	Negative	Rod	Negative	Negative	Positive	Positive
H	Green, metallic sheen	Negative	Rod	Negative	Positive	Positive	Positive
I	Green, metallic sheen	Negative	Rod	Negative	Positive	Negative	Positive
J	Green, metallic sheen	Negative	Rod	Negative	Positive	Positive	Positive

KEY: A-J = *E. coli*

APPENDIX IV

Cultural and Physiologic Properties of Clinical Isolates of *Staphylococcus aureus.*

SAMPLE ID	COLONIAL MOPRPHOLOGY	GRAM REACTION	CELL MORPHOLOGY	CELL ARRANGEMENT	CATALASE	COAGULASE	MANNITOL	LATEX AGGLUTINATION	IDENTIFICATION
A	Golden Yellow,Slightly raised	Positive	Cocci	Clusters	+	+	+	+	*S. aureus*
B	Golden Yellow, slightly raised	Positive	Cocci	Clusters	+	+	+	+	*S. aureus*
C	Golden Yellow, Slighty raised	Positive	Cocci	Clusters	+	+	+	+	*S. aureus*
D	Golden Yellow, Slightly raised	Positive	Cocci	Clusters	+	+	+	+	*S. aureus*
E	Golden Yellow, Slightly raised	Positive	Cocci	Clusters	+	+	+	+	*S. aureus*
F	Golden Yellow, Slightly raised	Positive	Cocci	Clusters	+	+	+	+	*S. aureus*
G	Golden Yellow, slightly raised	Positive	Cocci	Clusters	+	+	+	+	*S. aureus*

H	Golden Yellow ,slightly raised	Positive	Cocci	Clusters	+	+	+	+	*S. aureus*
I	Golden Yellow, slightly raised	Positive	Cocci	Clusters	+	+	+	+	*S. aureus*
J	Golden Yellow, Slightly raised .	Positive	Cocci	Clusters	+	+	+	+	*S. aureus*

Key; +=Positive.

APPENDIX V

Cultural and Physiologic Properties of Clinical Isolates of *Bacillus spp.*

Sample ID	Colonial morphology	Gram reaction	Cell morphology	Endospore	Motility	Catalase	Oxidase	Citrate	VP
BC1	Milky	Positive	Rod	Positive	Positive	Positive	Positive	Positive	Positive
BC2	Milky	Positive	Rod	Positive	Positive	Positive	Positive	Positive	Positive
BC3	Milky	Positive	Rod	Positive	Negative	Negative	Positive	Negative	Negative
BC4	Milky	Positive	Rod	Negative	Positive	Positive	Negative	Negative	Negative
BC5	Milky	Positive	Rod	Negative	Negative	Positive	Negative	Negative	Negative
BC6	Milky	Positive	Rod	Negative	Positive	Positive	Negative	Negative	Positive
BC7	Milky	Positive	Rod	Positive	Negative	Negative	Positive	Negative	Positive
BC8	Milky	Positive	Rod	Negative	Negative	Positive	Negative	Negative	Negative
BC9	Milky	Positive	Rod	Negative	Negative	Negative	Negative	Negative	Negative
BC10	Milky	Positive	Rod	Negative	Negative	Negative	Positive	Positive	Negative

KEY: BC = *Bacillus* spp, VP = Voges Proskauer

APPENDIX VI

Biochemical characterization and identification of clinical isolates using microgen kit Microgen* S aureus identification kit

Isolate	L	C	N	S	T	M	N	M	T	P	B	B	U	A	PYR	% Probability	Octal Code	Final identification
A	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	96.55	77756	*S.aureus*
B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	100.0	77776	*S.aureus*
C	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	98.57	77767	*S.aureus*
D	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	100	77776	*S.aureus*
E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	100	77776	*S.aureus*
F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	100	77776	*S.aureus*
G	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	76.21	77676	*S.aureus*
H	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	96.55	77756	*S.aureus*
I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	100	77776	*S.aureus*
J	-	+	+	+	+	-	+	+	-	+	+	+	-	+	-	98.48	36672	*S.aureus*

Key: L=Latex agglutination test, C=Colony pigmentation, N=Nitrate, S=Sucrose, T=Trehalose, M=Mannitol, N= N-acetyl Glucosamine, M=Mannose, T=Turanose, P=Alkaline Phosphatase, B=beta Glusidase, B=beta-Glucuronidase, U=Urease, A=Arginine, PYR=Pyrrolidonyl arylamidase.

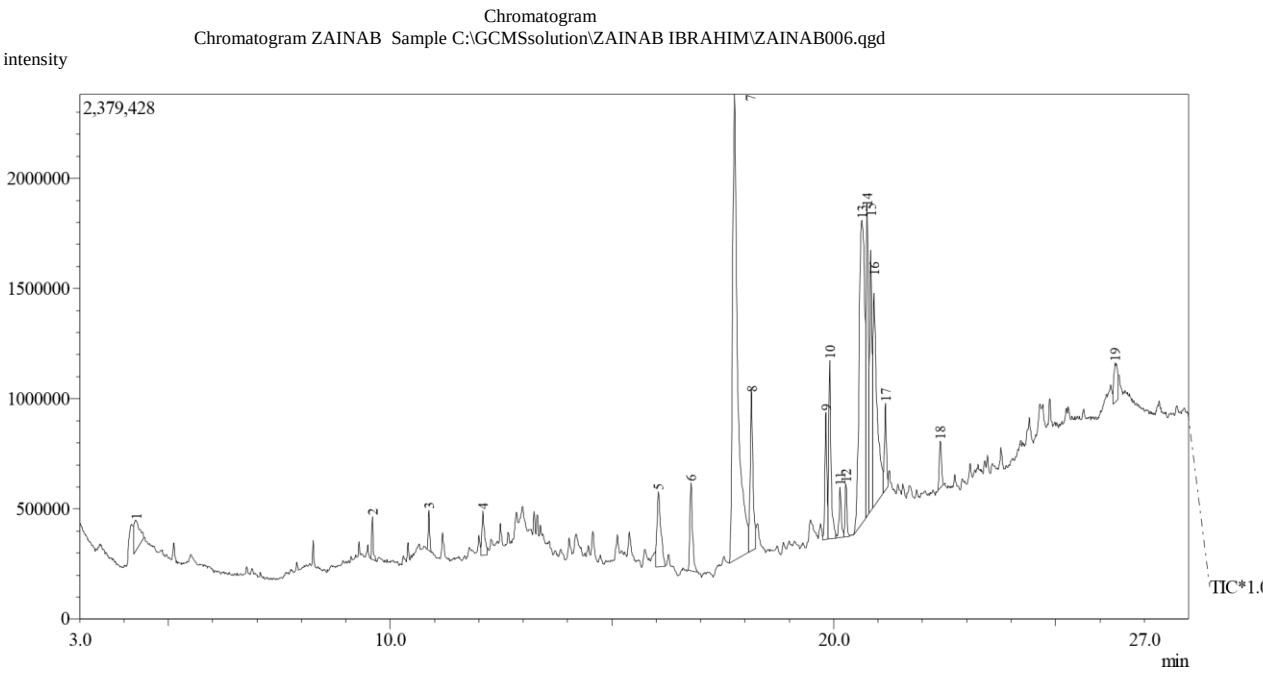
APPENDIX VII

Biochemical characterization and identification of clinical isolates using microgen kit Microgen* *E. coli* identification kit

			Escherichia coli Isolate				
	Reaction	Well number	1	2	3	4	5
	Oxidase		−	−	−	−	−
	Motility		+	+	+	+	+
	Nitrate		+	+	+	+	+
GN A wells	Lysine	1	+	+	+	+	+
	Ornithine	2	+	+	+	+	+
	H2S	3	−	−	−	−	−
	Glucose	4	+	+	+	+	+
	Mannitol	5	+	+	+	+	+
	Xylose	6	+	+	+	+	+
	ONPG	7	+	+	+	+	+
	Indole	8	+	+	+	+	+
	Urease	9	−	−	−	−	−
	V.P.	10	−	−	−	−	−
	Citrate	11	−	−	−	−	−
	TDA	12	−	−	−	−	−
Percentage probability			97.11%	97.11%	97.11%	85%	97.11%
Octal code			6760	6760	6760	4462	6760

Key: + = positive, − = negative

APPENDIX VIII

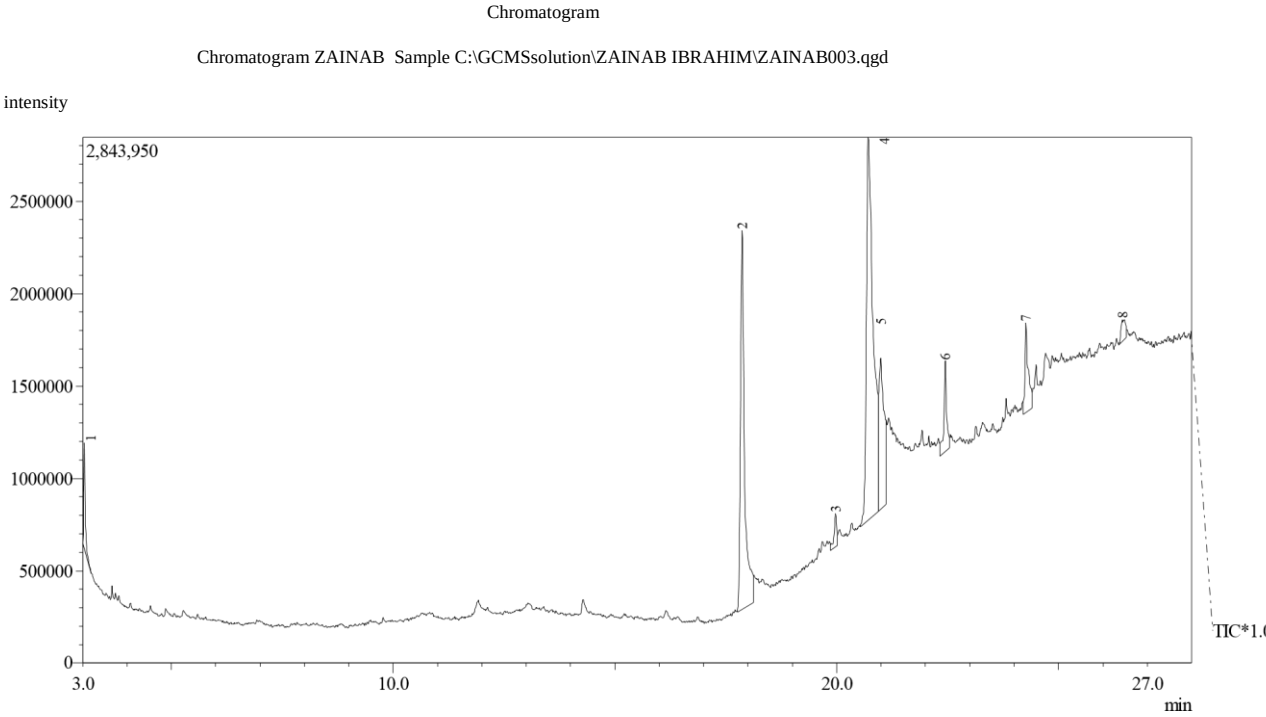


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	4.251	4.225	4.450	1119288	1.76	146122	1.23	7.66	V	
2	9.600	9.558	9.700	436583	0.69	193071	1.62	2.26		
3	10.874	10.833	10.933	390313	0.61	184243	1.55	2.12		
4	12.091	12.042	12.183	810136	1.28	202607	1.71	4.00	V	
5	16.049	15.983	16.200	2012176	3.17	341216	2.87	5.90	V	
6	16.783	16.717	16.933	1651523	2.60	399480	3.36	4.13		
7	17.765	17.642	18.083	17404410	27.41	2110430	17.76	8.25		
8	18.144	18.083	18.242	3212233	5.06	733514	6.17	4.38	V	
9	19.822	19.758	19.867	1805504	2.84	578468	4.87	3.12	V	
10	19.910	19.867	20.050	2845526	4.48	810941	6.83	3.51	V	
11	20.144	20.050	20.225	768074	1.21	228328	1.92	3.36	V	
12	20.274	20.225	20.358	712892	1.12	239872	2.02	2.97	V	
13	20.633	20.442	20.717	11948718	18.82	1375251	11.57	8.69		
14	20.755	20.717	20.800	5194845	8.18	1398094	11.77	3.72	V	
15	20.834	20.800	20.875	4112197	6.48	1184462	9.97	3.47	V	
16	20.909	20.875	21.117	6122759	9.64	968658	8.15	6.32	V	
17	21.165	21.117	21.225	1191919	1.88	394738	3.32	3.02	V	
18	22.403	22.350	22.475	713212	1.12	212927	1.79	3.35		
19	26.344	26.292	26.417	1044797	1.65	179321	1.51	5.83	V	

Fig. 4.9: Gas-Chromatography (GC) analysis of ethanolic stem extract of *J. curcas*

APPENDIX IX

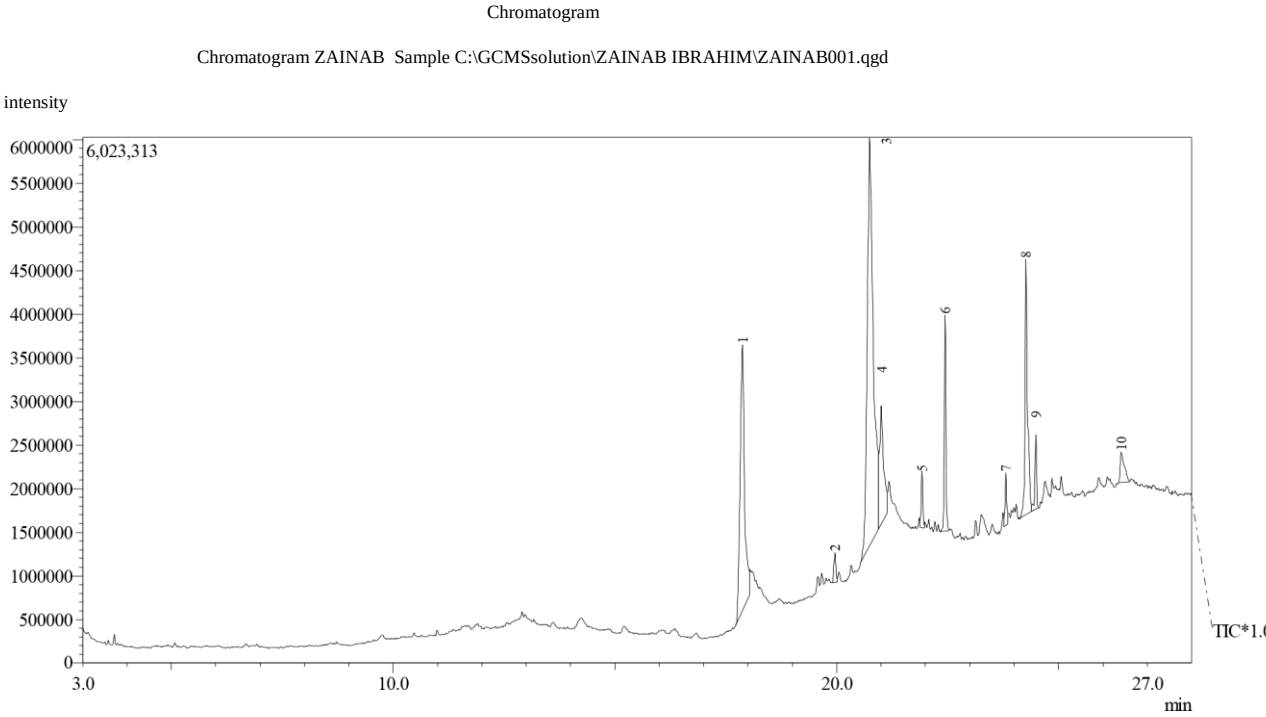


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.038	3.008	3.200	1284185	2.63	571410	8.42	2.25		
2	17.869	17.758	18.125	12988975	26.62	2050019	30.22	6.34	V	
3	19.974	19.858	20.017	754508	1.55	179955	2.65	4.19	V	
4	20.711	20.533	20.933	21721716	44.52	2068343	30.49	10.50	V	
5	20.989	20.933	21.108	6590434	13.51	817892	12.06	8.06	V	
6	22.446	22.325	22.542	2153660	4.41	492011	7.25	4.38	V	
7	24.268	24.208	24.408	2490722	5.10	484911	7.15	5.14	V	
8	26.434	26.350	26.533	811418	1.66	119459	1.76	6.79	V	
48795618				100.00		6784000	100.00			

Fig. 4.10: Gas-Chromatography (GC) analysis of aqueous stem extract of *J. curcas*

APPENDIX X

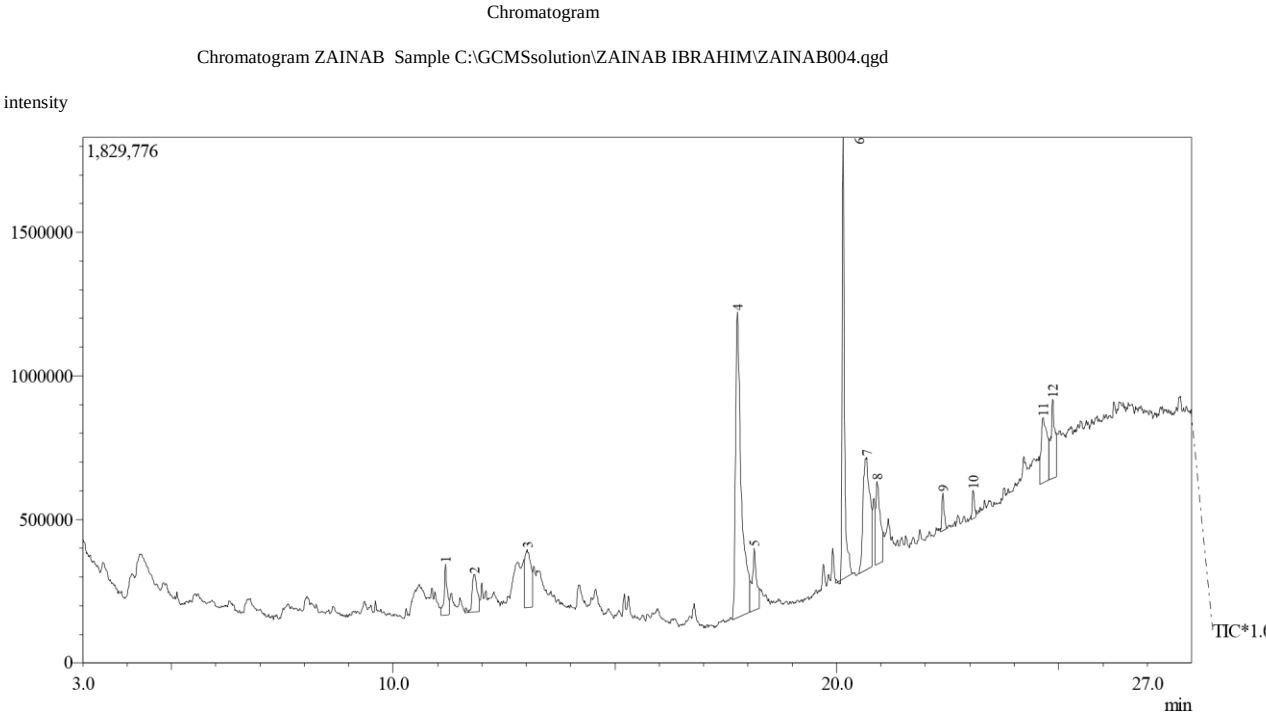


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	17.876	17.725	18.033	20821712		20.74	3048612	17.64	6.83	
2	19.959	19.900	20.008	1064312	1.06	340311	1.97	3.13		
3	20.744	20.542	20.942	44104194		43.93	4680375	27.08	9.42	
4	21.004	20.942	21.142	8811882	8.78	1355422	7.84	6.50	V	
5	21.922	21.883	21.975	1700570	1.69	648463	3.75	2.62	V	
6	22.442	22.317	22.533	6344192	6.32	2473316	14.31	2.57	V	
7	23.813	23.775	23.858	1641850	1.64	602791	3.49	2.72	V	
8	24.267	24.083	24.392	11264404		11.22	2923087	16.91	3.85	V
9	24.488	24.392	24.542	2328271	2.32	859689	4.97	2.71	V	
10	26.412	26.358	26.600	2310786	2.30	350187	2.03	6.60		
100392173					100.00	17282253	100.00			

Fig. 4.11: Gas-Chromatography (GC) analysis of aqueous leave extract of *J. curcas*

APPENDIX XI



Peak Report TIC

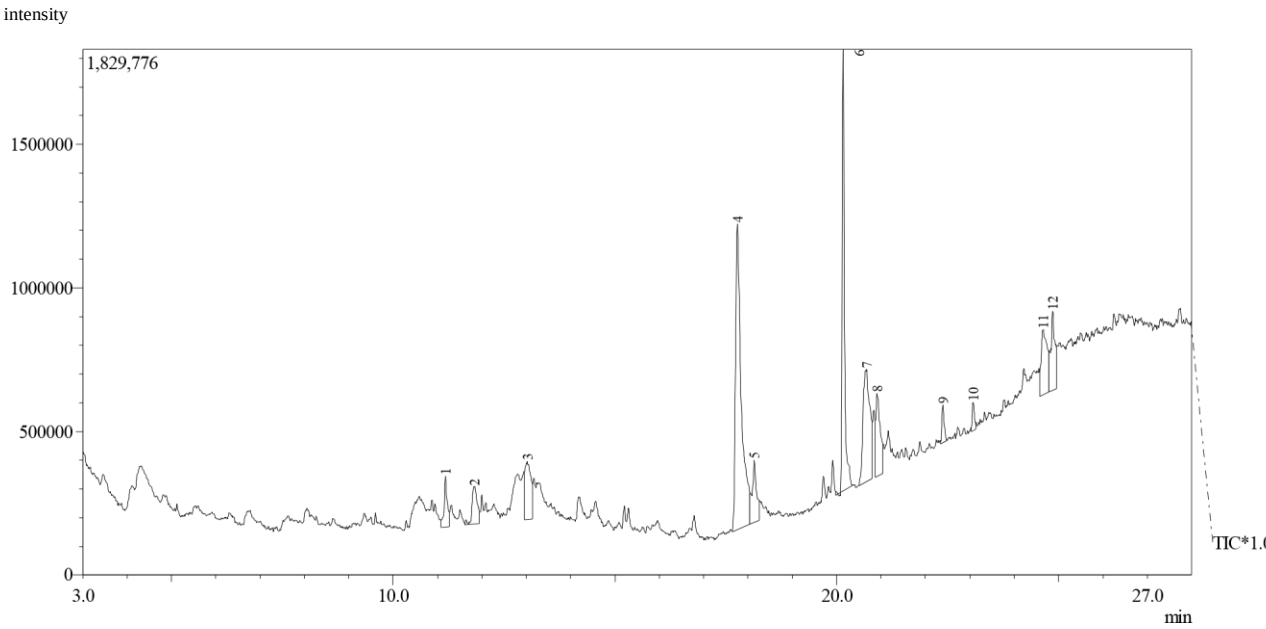
Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	11.179	11.083	11.267	864195	2.73	176168	3.72	4.91	V	
2	11.841	11.617	11.950	1093099	3.46	131115	2.77	8.34	V	
3	13.024	12.958	13.142	1876193	5.94	201545	4.25	9.31	V	
4	17.761	17.642	18.042	9666173	30.59	1063762	22.44	9.09	V	
5	18.141	18.042	18.258	1557385	4.93	217123	4.58	7.17	V	
6	20.144	19.975	20.342	5516696	17.46	1536416	32.41	3.59	V	
7	20.671	20.500	20.800	4286190	13.56	390741	8.24	10.97		
8	20.910	20.867	21.042	2020055	6.39	289557	6.11	6.98	V	
9	22.392	22.342	22.492	459023	1.45	131525	2.77	3.49	V	
10	23.073	23.033	23.133	351468	1.11	99342	2.10	3.54	V	
11	24.654	24.575	24.783	2153727	6.81	227135	4.79	9.48	V	
12	24.869	24.783	24.950	1758840	5.57	275515	5.81	6.38	V	
				31603044	100.00	4739944	100.00			

Fig. 4.12: Gas-Chromatography analysis of ethanolic leaves extract of *J. curcas*

APPENDIX XII

Chromatogram

Chromatogram ZAINAB Sample C:\GCMSsolution\ZAINAB IBRAHIM\ZAINAB004.qgd

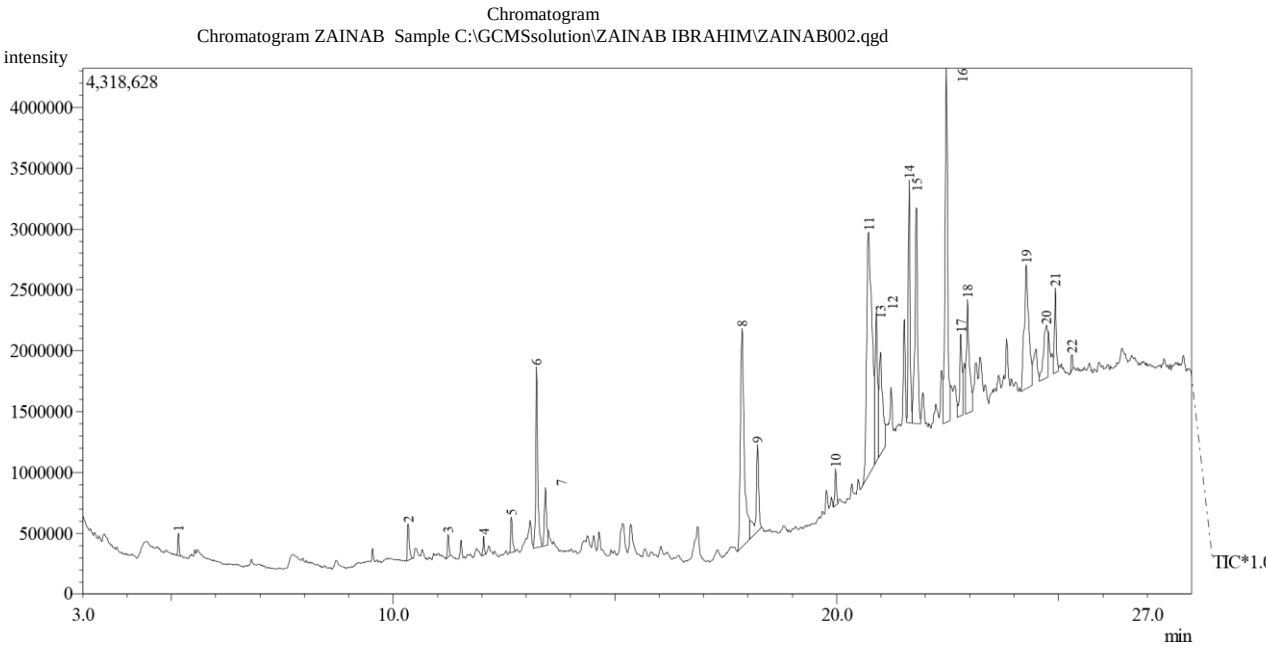


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
13	11.179	11.083	11.267	864195	2.73	176168	3.72	4.91	V	
14	11.841	11.617	11.950	1093099	3.46	131115	2.77	8.34	V	
15	13.024	12.958	13.142	1876193	5.94	201545	4.25	9.31	V	
16	17.761	17.642	18.042	9666173	30.59	1063762	22.44	9.09	V	
17	18.141	18.042	18.258	1557385	4.93	217123	4.58	7.17	V	
18	20.144	19.975	20.342	5516696	17.46	1536416	32.41	3.59	V	
19	20.671	20.500	20.800	4286190	13.56	390741	8.24	10.97		
20	20.910	20.867	21.042	2020055	6.39	289557	6.11	6.98	V	
21	22.392	22.342	22.492	459023	1.45	131525	2.77	3.49	V	
22	23.073	23.033	23.133	351468	1.11	99342	2.10	3.54	V	
23	24.654	24.575	24.783	2153727	6.81	227135	4.79	9.48	V	
24	24.869	24.783	24.950	1758840	5.57	275515	5.81	6.38	V	
				31603044	100.00	4739944	100.00			

Fig. 4.12: Gas-Chromatography analysis of ethanolic leaves extract of *J. curcas*

APPENDIX XIII

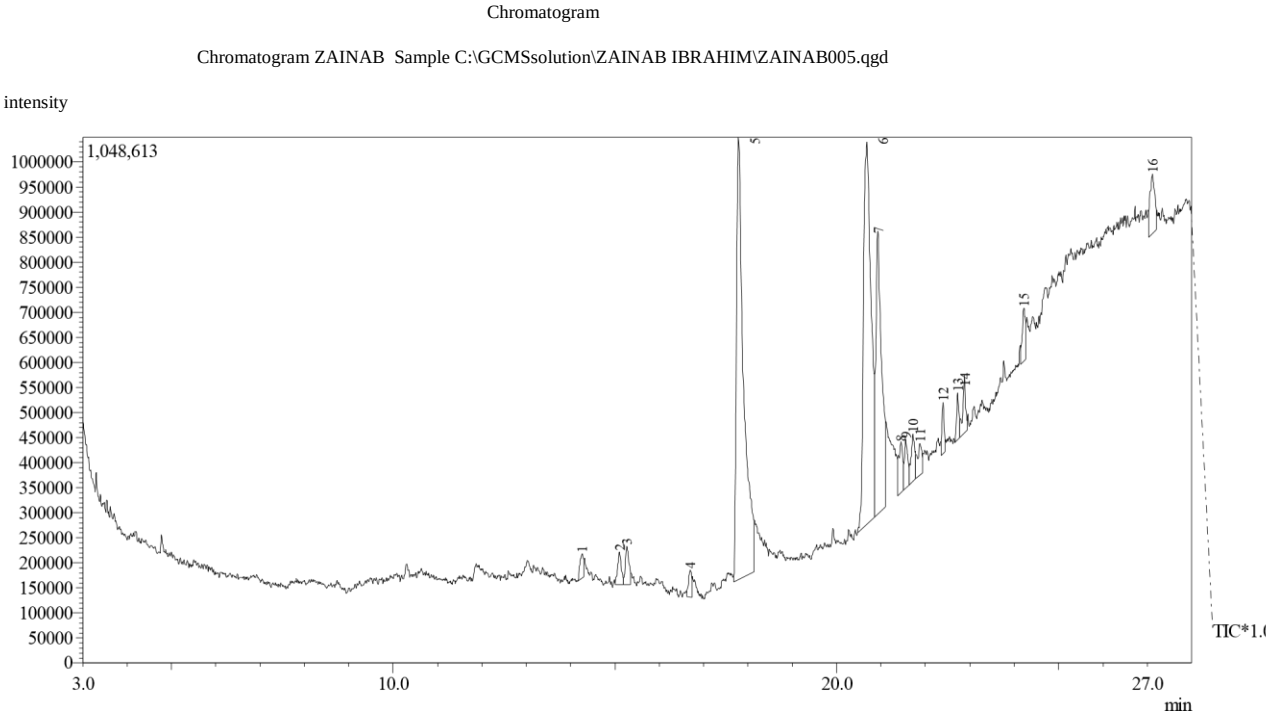


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	5.162	5.125	5.217	365048	0.38	185466	0.90	1.97		
2	10.339	10.300	10.425	914229	0.96	301424	1.47	3.03		
3	11.242	11.192	11.292	482380	0.51	187844	0.91	2.57		
4	12.041	12.000	12.092	354802	0.37	154819	0.75	2.29		
5	12.665	12.617	12.750	795923	0.84	292153	1.42	2.72		
6	13.234	13.158	13.358	4733246	4.97	1482743	7.22	3.19	V	
7	13.433	13.358	13.483	1587608	1.67	477700	2.32	3.32	V	
8	17.867	17.767	18.042	10757814	11.31	1797939	8.75	5.98		
9	18.217	18.042	18.300	3307933	3.48	715274	3.48	4.62	V	
10	19.973	19.925	20.033	814366	0.86	302028	1.47	2.70		
11	20.714	20.583	20.850	16777537	17.63	2003457	9.75	8.37		
12	20.894	20.850	20.942	4864814	5.11	1241229	6.04	3.92	V	
13	20.984	20.942	21.092	4584604	4.82	842178	4.10	5.44	V	
14	21.632	21.575	21.700	6031949	6.34	1996470	9.72	3.02	V	
15	21.795	21.700	21.892	7325013	7.70	1773799	8.63	4.13	V	
16	22.470	22.392	22.558	12087318	12.70	2904015	14.13	4.16	V	
17	22.796	22.717	22.850	2751947	2.89	673005	3.28	4.09	V	

Fig. 4.13: Gas-Chromatography analysis of ethanolic root extract of *J. curcas*

APPENDIX XIV



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	14.261	14.158	14.292	266793	0.88	48655	1.45	5.48		
2	15.098	15.008	15.192	379622	1.25	64569	1.93	5.88	V	
3	15.265	15.192	15.358	443690	1.46	76437	2.28	5.80	V	
4	16.700	16.617	16.733	257061	0.85	54495	1.63	4.72	V	
5	17.785	17.675	18.133	9999380	32.91	881509	26.33	11.34	V	
6	20.679	20.475	20.858	9213767	30.33	763490	22.81	12.07		
7	20.927	20.858	21.100	4995689	16.44	564610	16.86	8.85	V	
8	21.448	21.367	21.508	673557	2.22	101828	3.04	6.61	V	
9	21.557	21.508	21.625	495264	1.63	97427	2.91	5.08	V	
10	21.719	21.625	21.783	594043	1.96	94873	2.83	6.26	V	
11	21.874	21.783	21.933	428475	1.41	63484	1.90	6.75	V	
12	22.395	22.350	22.442	340519	1.12	102439	3.06	3.32	V	
13	22.724	22.658	22.775	344221	1.13	93705	2.80	3.67		
14	22.875	22.775	22.942	521899	1.72	114834	3.43	4.54	V	
15	24.220	24.150	24.267	537235	1.77	106355	3.18	5.05	V	
16	27.121	27.033	27.208	889170	2.93	119180	3.56	7.46	V	

Fig. 4.14: Gas-Chromatography analysis of aqueous root extract of *J. curcas*